Stressor Analysis to Identify Probable Stressors to the Impaired Benthic Macroinvertebrate Community in the Sand Branch Watershed

Loudoun and Fairfax Counties, Virginia

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List of Abbreviations

BCG	Biological Condition Gradient
CADDIS	Causal Analysis/Diagnosis Decision Information System
CWA	Clean Water Act
DEQ	Virginia Department of Environmental Quality
DMR	Discharge Monitoring Report
DO	Dissolved Oxygen
EPT	Ephemeroptera, Plecoptera, and Trichoptera taxa
FFG	Functional Feeding Group
GIS	Geographic Information System
GP	General Permit
HBI	Hilsenhoff Biotic Index
IP	Individual Permit
IC25	Inhibition Concentration that causes 25% reduction growth or reproduction
LC50	Lethal Concentration that causes 50% mortality
LOEC	Lowest Observed Effect Concentration
LRBS	Log_{10} Relative Bed Stability Index
NOEC	No Observable Effect Concentration
STATSGO	State Soil Geographic database
SSURGO	Soil Survey Geographic database
SWCB	State Water Control Board
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
USEPA	United States Environmental Protection Agency
USGS	United States Geological Service
UT	Unnamed tributary
VDOT	Virginia Department of Transportation
VLCD	Virginia Land Cover Dataset
VPDES	Virginia Pollution Discharge Elimination System
VSCI	Virginia Stream Condition Index
WQS	Water Quality Standards

Units of Measure

°C	degrees Celsius
MSL	above mean sea level
µg/L	micrograms per liter
µS/cm	microsiemens per centimeter
mEq/L	milliequivalents per liter
MGD	million gallons per day
mg/L	milligrams per liter
SU	standard units

1.0 Introduction

The Clean Water Act (CWA) requires that waters in the United States support swimming, sustain aquatic life, and maintain other beneficial uses, like water supply. In order to meet the requirements of the CWA, Virginia has adopted water quality standards (WQS) and assesses water quality monitoring data to determine if waterbodies are meeting the WQS. Waterbodies not meeting standards, i.e. impaired waterbodies, are reported in the biannual 305(b)/303(d) Water Quality Assessment Integrated Report (Integrated Report) or “dirty waters list”.

Sand Branch is a small stream with a drainage area of 1.37 square miles (879.89 acres) and a total stream length of approximately 1.54 miles. It is a tributary to Cub Run, which flows into Bull Run and then into the Occoquan Reservoir. The stream originates in Loudoun County and flows into Fairfax County before its confluence with Cub Run. The watershed is located in an urbanized area, situated directly south of Dulles International Airport, west of Route 28, and north of Route 50. Sand Branch is considered impaired for aquatic life designated use based upon assessment of the benthic macroinvertebrates, meaning there is not a healthy and diverse community. The recreational use is also considered impaired based upon assessment of bacteria. However, that impairment is not addressed in this report because Sand Branch is included in the downstream Occoquan River bacteria TMDL, approved by the United States Environmental Protection Agency (USEPA) on November 15, 2006.

This report discusses the benthic stressor analysis used to identify the cause(s) of the impaired benthic macroinvertebrate community in Sand Branch. The analysis is an investigation of multiple lines of evidence provided by data and scientific literature to identify the most likely stressors resulting in an unhealthy benthic community.

1.1 Applicable Water Quality Standards

Virginia’s WQSs are contained in 9VAC25-260 et seq. and are comprised of three components: antidegradation, identification of designated or beneficial uses, and the criteria (narrative and/or numeric) to ensure beneficial uses are protected. According to Virginia WQS (9VAC25-260-10):

> *“All state waters, including wetlands, are designated for the following uses: recreational uses, e.g., swimming and boating; the propagation and growth of a balanced, indigenous population of aquatic life, including game fish, which might reasonably be expected to inhabit them; wildlife; and the production of edible and marketable natural resources, e.g., fish and shellfish.”*

Water quality criteria can be numerical or narrative. The General Criteria defined in the Virginia WQS (9VAC25-260-20) provides general, narrative criteria for the protection of designated uses from substances that may interfere with attainment of such uses. Section A of the General Criteria states in part:

> *“State waters, including wetlands, shall be free from substances attributable to sewage, industrial waste, or other waste in concentrations, amounts, or combinations which contravene established standards or interfere directly or indirectly with designated uses of such water or which are inimical or harmful to human, animal, plant, or aquatic life.”*

1.2 Aquatic Life Designated Use

One indicator of an impairment of the aquatic life use is a degraded benthic macroinvertebrate community. DEQ administers a biological monitoring program in Virginia that evaluates compliance of the General Standard. Evaluations of monitoring data from this program focus on the benthic (bottom-dwelling) macro (large enough to see) invertebrates (insects, mollusks, crustaceans, and annelid worms) and are used to determine whether a stream segment has a benthic impairment. Changes in water quality generally result in alterations to the quantity and diversity of the benthic organisms that live in streams and other water bodies. Besides being the major intermediate constituent of the aquatic food chain, benthic macroinvertebrates are "living recorders" of past and present water quality conditions. This is due to their relative immobility and their variable resistance to the diverse contaminants within a water body. The community structure of these organisms provides the basis for the biological analysis of water quality.

A multi-metric benthic macroinvertebrate index, the Virginia Stream Condition Index (VSCI), is used to assess the aquatic life use status for wadeable freshwater streams and rivers in non-coastal areas of the state. VSCI scores range from 0 to 100, with higher scores indicating relatively better ecological health. DEQ has set a score of 60 as the threshold for impairment. Scores below 60 indicate an impaired benthic community, while scores above 60 indicate a healthy benthic community.

1.3 Impairment Listing

DEQ performed monitoring on Sand Branch at two monitoring stations (1ASAN000.34 and 1ASAN001.45) at which ambient water quality and biological sampling occurred from 2015 – 2020. The data collected from these two stations were assessed in DEQ’s 303(d)/305(b) Integrated Report. Based upon monitoring data collected at these stations, impairments to the aquatic life and recreational uses were identified.

The aquatic life use impairment, which is the focus of this water quality study, was based upon the VSCI assessment of two biological monitoring events, in the Spring and Fall of 2016 at stations 1ASAN000.34 (at Route 609) and 1ASAN001.45 (at Route 639) that indicates impaired benthic macroinvertebrate communities (Table 1-1). The impaired segment comprises all of Sand Branch, from its headwaters to the confluence with Cub Run. Sand Branch and Cub Run (both highlighted orange in Figure 1-1) were assessed as not supporting aquatic life in the 2020 Integrated Report.

Table 1-1.Sand Branch impaired segments from DEQ's 2020 303(d)/305(b) Integrated Report.

Stream Name	County	VAHUC 6	Impaired Assessment Units	Cause Group Code	First Listed	Length of Impairment (miles)
Sand Branch	Loudoun / Fairfax Counties	PL45	VAN-A22R_SAN01A18	A22R-05-BEN	2018	1.54

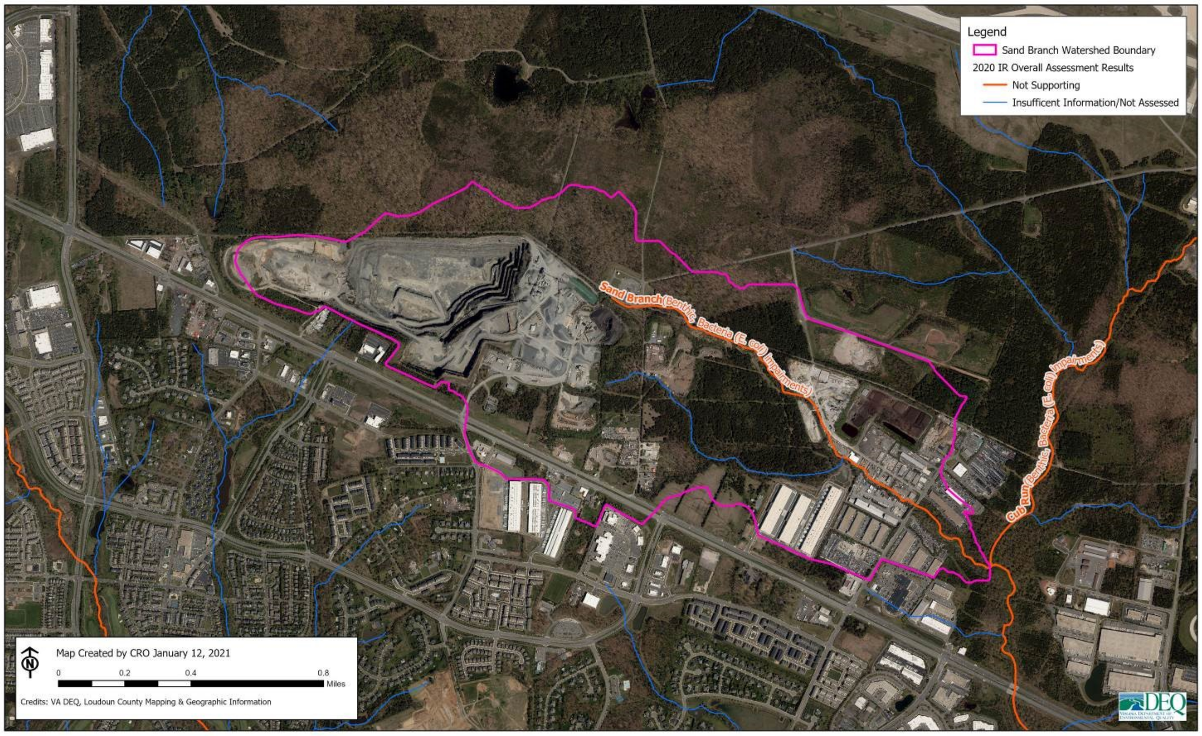


Figure 1-1. Impairment listing for the Sand Branch watershed.

2.0 Benthic Stressor Analysis Approach

Before a TMDL can be developed to address an impaired benthic macroinvertebrate community, a pollutant (or pollutants) must be identified as the probable stressor(s) to the benthic community. A stressor can have direct effects on the organism itself, like dissolved metals or toxic chemicals, or alter habitat and resources resulting in a shift in the macroinvertebrate community.

The benthic macroinvertebrate community at a given location is comprised of a suite of organisms that are adapted to withstand the environmental conditions present at that location. As those environmental conditions change, organisms that are not adapted to those changes will be reduced in numbers or be extirpated, and the macroinvertebrate community will shift to organisms that can withstand the new or changing environmental conditions. The stressor analysis process applied a weight-of-evidence approach to define the probable stressor(s) that explain(s) the shift in the benthic macroinvertebrate community. This approach consisted of an analysis of the water quality chemistry and biological data and USEPA's Causal Analysis/Diagnosis Decision Information System (CADDIS) (USEPA, 2018). From these anlayses, each candidate stressor was categorized as either a non-stressor, possible stressor, or probable stressor. Those pollutants identified as probable stressors are then candidates for development of a TMDL.

Each approach is discussed in-depth in the following respective sections: Section 5.0 (Biological Data Analysis); Section 6.0 (Water Chemistry Data Analysis); and Section 7.0 (Causal Analysis/Diagnosis Decision Information System (CADDIS).

2.1 Candidate Stressors

The first step in the stressor identification analysis is to list potential candidate stressors. DEQ identified these from the listing information, monitoring data, scientific literature, and historic information. Potential stressors include both pollutants that can be targeted through TMDL development and additional contributing factors that can influence and stress benthic communities but that cannot be effectively targeted through TMDL development. The suite of candidate stressors analyzed in the benthic stressor analysis are those known to have effects on macroinvertebrates.

Water quality data collected for the candidate stressors were analyzed to identify if one or more of those parameters may be causing stress to the aquatic community. Candidate stressors were compared to numeric water quality criteria and threshold stressor values, as applicable. The threshold stressor values were developed and published by DEQ (2017) in the "Stressor Analysis in Virginia: Data Collection and Stressor Thresholds" document. The candidate stressors considered in the benthic stressor analysis are shown in Table 2-1 and each are identified as having water quality criteria and/or a stressor threshold.

Table 2-1. Candidate stressors analyzed.

Candidate Stressors Considered					
Candidates With Stressor Thresholds[1,2]:	***pH***	***Dissolved Oxygen (DO)***	Total Phosphorous	Total Dissolved Solids (TDS)	Potassium
	Temperature	Specific Conductivity	Total Nitrogen	Sulfate	***Chloride***
	Sediment[3]	Metal Cumulative Criterion Unit (Metals CCU)	***Individual Metals, Dissolved***	Sodium	
Candidates Without Stressor Thresholds[2]:	Total Suspended Solids (TSS)	***Ammonia***	DO (Saturation)	Turbidity	
Additional Contributing Factors:	Underlying Geology	Land Disturbance	Percent Imperviousness	Degraded Riparian Buffer	

[1] Values published in "Stressor Analysis in Virginia: Data Collection and Stressor Thresholds" (DEQ, 2017)

[2] Parameters with water quality criteria denoted in bold, italicized text. When available, the value was also used in the analysis (Water Quality Standards, 9VAC25-260).

[3] Sediment was evaluated using Log$_{10}$ Relative Bed Stability (LRBS) index and Habitat.

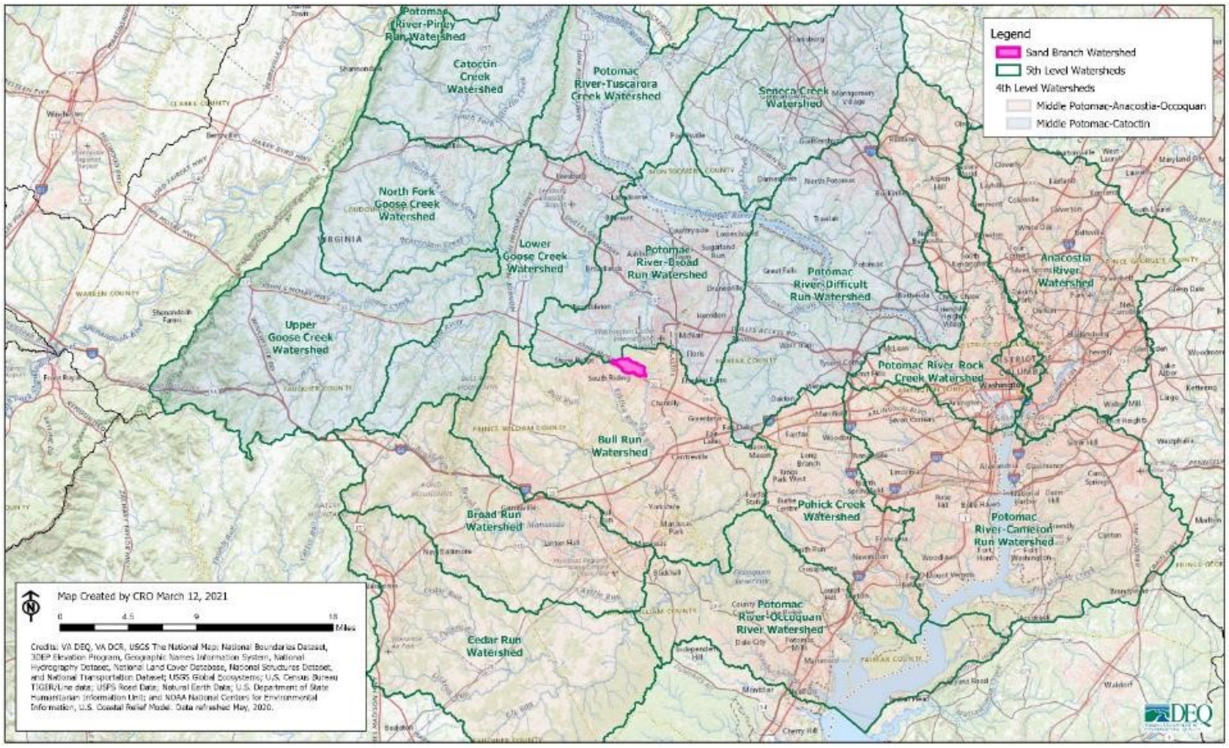
2.2 Contributing Factors

The benthic stressor analysis also considered factors that contributed to the impaired benthic macroinvertebrate community. These are watershed conditions that may exacerbate the impairment, but for which a TMDL cannot be individually developed. Examples of contributing factors can be degraded riparian buffer, hydrologic alternation such as dams or impoundments, current and/or historic land use practices, or the underlying geology. The additional contributing factors considered in this analysis are identified in Table 2-1.

3.0 Watershed Characterization

The Sand Branch watershed, comprising approximately 1.37 square miles, is split between urbanized land uses and forest, with the forested portion mostly on the northern side of the watershed, providing a buffer for the Dulles International Airport.

Sand Branch, totaling a length of approximately 1.54 miles, flows through Loudoun County and a small portion of Fairfax County before its confluence with Cub Run. Cub Run flows into Bull Run, which is a part of the Middle Potomac-Anacostia-Occoquan watershed. Sand Branch and the surrounding 4th and 5th level watersheds are shown in Figure 3-1.



Map Created by CRO March 12, 2021

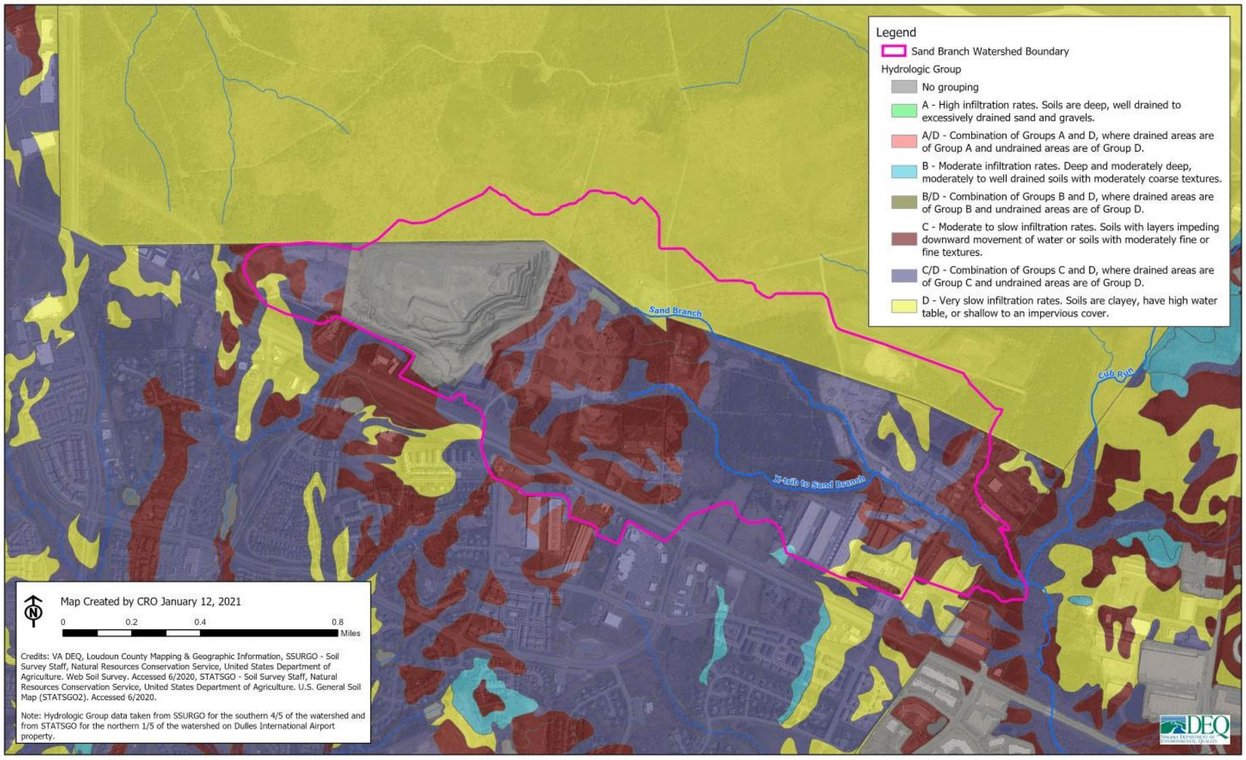
Credits: VA DEQ, VA DCR, USGS The National Map: National Boundaries Dataset, 3DEP Elevation Program, Geographic Names Information System, National Hydrography Dataset, National Land Cover Database, National Structures Dataset, and National Transportation Dataset; USGS Global Ecosystems; U.S. Census Bureau TIGER/Line data; USPS Road Data; Natural Earth Data; U.S. Department of State Humanitarian Information Unit; and NOAA National Centers for Environmental Information, U.S. Coastal Relief Model. Data refreshed May, 2020.

Figure 3-1. Location of the Sand Branch watershed in northern Virginia.

Elevation was calculated using contour spatial data provided by the Loudon County, Office of Mapping and Geographic Information (Z. Irwin, personal communication, August 12, 2020). The Sand Branch watershed elevation ranged between 12 to 388 feet above mean sea level (MSL), with an elevation average of 285 feet. These values include the quarry operation in the headwaters of the watershed, with the lowest elevation of 12 feet MSL being the bottom of the quarry pit.

3.1 Soils

Hydrologic soil group composition in the Sand Branch watershed is identified in Figure 3-2 using the Soil Survey Geographic (SSURGO) database and the State Soil Geographic (STATSGO) dataset. These groups represent the infiltration characteristics of soils, where soils with high infiltration rates are represented by Group “A”, and Group “D” represents soils with the slowest infiltration rates. Soils with slower infiltration rates are more susceptible to higher erosion rates because runoff will flow over the land quicker instead of infiltrating into the ground. The map uses hydrologic group data taken from SSURGO for the southern portion of the watershed and from STATSGO for the northern portion of the watershed on Dulles International Airport property where SSURGO data did not exist.



Map Created by CRO January 12, 2021

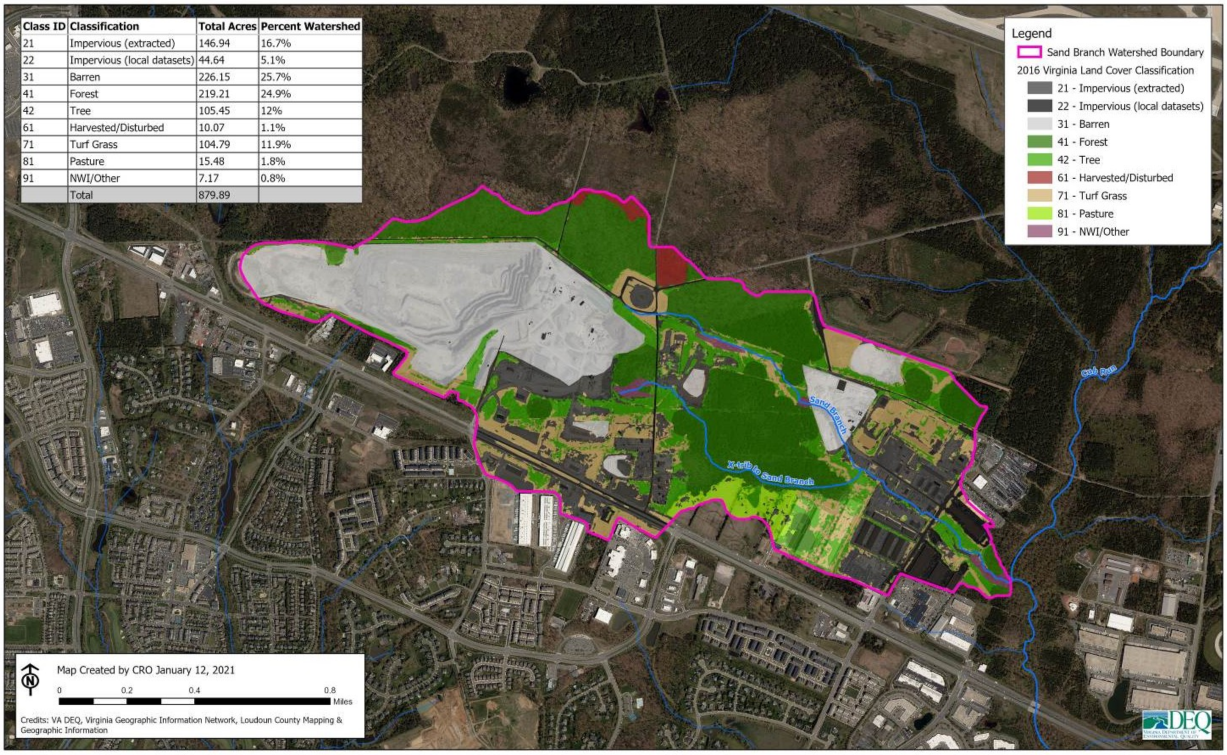
Credits: VA DEQ, Loudoun County Mapping & Geographic Information, SSURGO - Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Accessed 6/2020, STATSGO - Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. U.S. General Soil Map (STATSGO2). Accessed 6/2020.

Note: Hydrologic Group data taken from SSURGO for the southern 4/5 of the watershed and from STATSGO for the northern 1/5 of the watershed on Dulles International Airport property.

Figure 3-2. Sand Branch watershed and area soil hydrologic groups.

3.2 Land Use

The 2016 Virginia Land Cover Dataset (VGIN, 2017) was used to calculate land cover in the Sand Branch watershed (Figure 3-3). The watershed is approximately 36.9% forest/tree, 21.8% developed impervious, 25.7% barren, 13.0% developed pervious and 1.8% pasture.



Class ID	Classification	Total Acres	Percent Watershed
21	Impervious (extracted)	146.94	16.7%
22	Impervious (local datasets)	44.64	5.1%
31	Barren	226.15	25.7%
41	Forest	219.21	24.9%
42	Tree	105.45	12%
61	Harvested/Disturbed	10.07	1.1%
71	Turf Grass	104.79	11.9%
81	Pasture	15.48	1.8%
91	NWI/Other	7.17	0.8%
	Total	879.89	

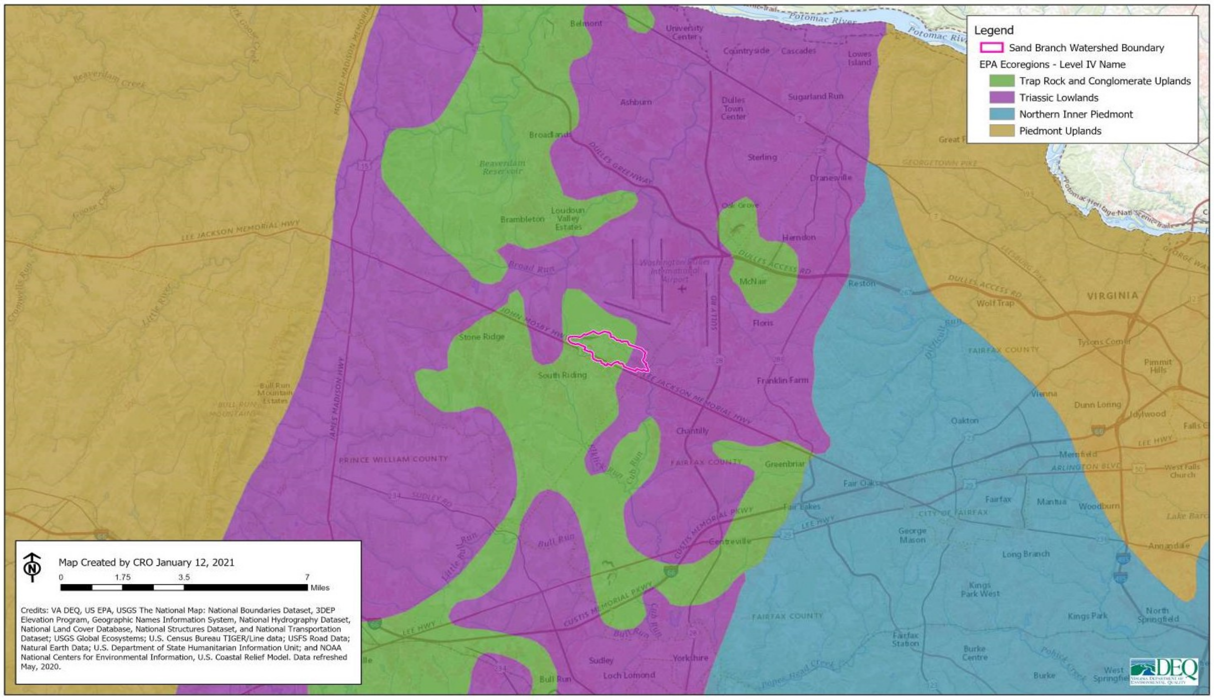
Map Created by CRO January 12, 2021

Credits: VA DEQ, Virginia Geographic Information Network, Loudoun County Mapping & Geographic Information

Figure 3-3. 2016 Virginia Land Cover Data for Sand Branch watershed.

3.3 Underlying Geology

The Sand Branch watershed lies within the Trap Rock and Conglomerates Uplands and Triassic Lowlands ecoregions. The underlying geology of the region is primarily sedimentary rock of the Triassic interspersed with magma intrusions of igneous diabase (trap rock) and heat-altered sedimentary rocks (Woods *et al.,* 1999). This geology naturally produces higher conductivity and higher phosphorus levels in overlying streams than is typical in the Northern Piedmont (Porter *et al.,* 2020). Figure 3-4 depicts the ecoregions surrounding and encompassing Sand Branch's watershed (outlined in pink).



Map Created by CRO January 12, 2021

Credits: VA DEQ, US EPA, USGS The National Map: National Boundaries Dataset, 3DEP Elevation Program, Geographic Names Information System, National Hydrography Dataset, National Land Cover Database, National Structures Dataset, and National Transportation Dataset; USGS Global Ecosystems; U.S. Census Bureau TIGER/Line data; USFS Road Data; Natural Earth Data; U.S. Department of State Humanitarian Information Unit; and NOAA National Centers for Environmental Information, U.S. Coastal Relief Model. Data refreshed May, 2020.

Figure 3-4. Sand Branch watershed area ecoregions.

3.4 Virginia Pollution Discharge Elimination System (VPDES)

DEQ regulates discharges to state waters in accordance with federal and state laws and regulations. DEQ issues Virginia Pollutant Discharge Elimination System (VPDES) permits for point source discharges to surface waters. This includes discharges from municipal and industrial operations which may contain process wastewater water as well as certain types of stormwater. Stormwater which is exposed to industrial activity is regulated under the VPDES program, while Municipal Separate Storm Sewer Systems (MS4) are regulated under the Virginia Stormwater Management Program (VSMP). Under the VPDES and VSMP programs, there are both individual permits as well as general permits. General permits in Virginia are established as regulations, and are issued to categories of activities which have similar operations and nature of the pollutants discharged to surface waters. Discharge permits aim to ensure the Virginia WQS are maintained and protected. Permits may include effluent limits, monitoring requirements, and special conditions to address pollutants that have a reasonable potential to cause or contribute to an instream excursion of a water quality criteria. Facilities conduct monitoring to determine compliance with permit requirements.

As of March 2021, there are seven facilities with VPDES permits and two MS4 permit holders in the Sand Branch watershed (Table 3-1 and Table 3-2). In addition to the discharge monitoring required by the VPDES permit, DEQ Northern Regional Office's Water Compliance staff conducted monitoring at Chantilly Crushed Stone, Inc. (VPDES Permit No. VAG840106) on a quarterly basis, from 2014 – 2018, the discharge of which comprises the headwaters of Sand Branch. This monitoring included many of the parameters being reviewed as potential stressors, including select ions (sulfate, sodium, potassium, chloride), total phosphorus, total nitrogen, ammonia, total dissolved solids, and specific conductance. Water staff also sampled the discharge of two other permitted facilities; three samples between 2016 – 2018 from Superior Concrete (VPDES Permit No. VAG110094) and one sample from Loudoun Composting (VPDES Permit No. VA0091430) in 2016.

The location of the VPDES permitted facilities are shown in Figure 3-5.

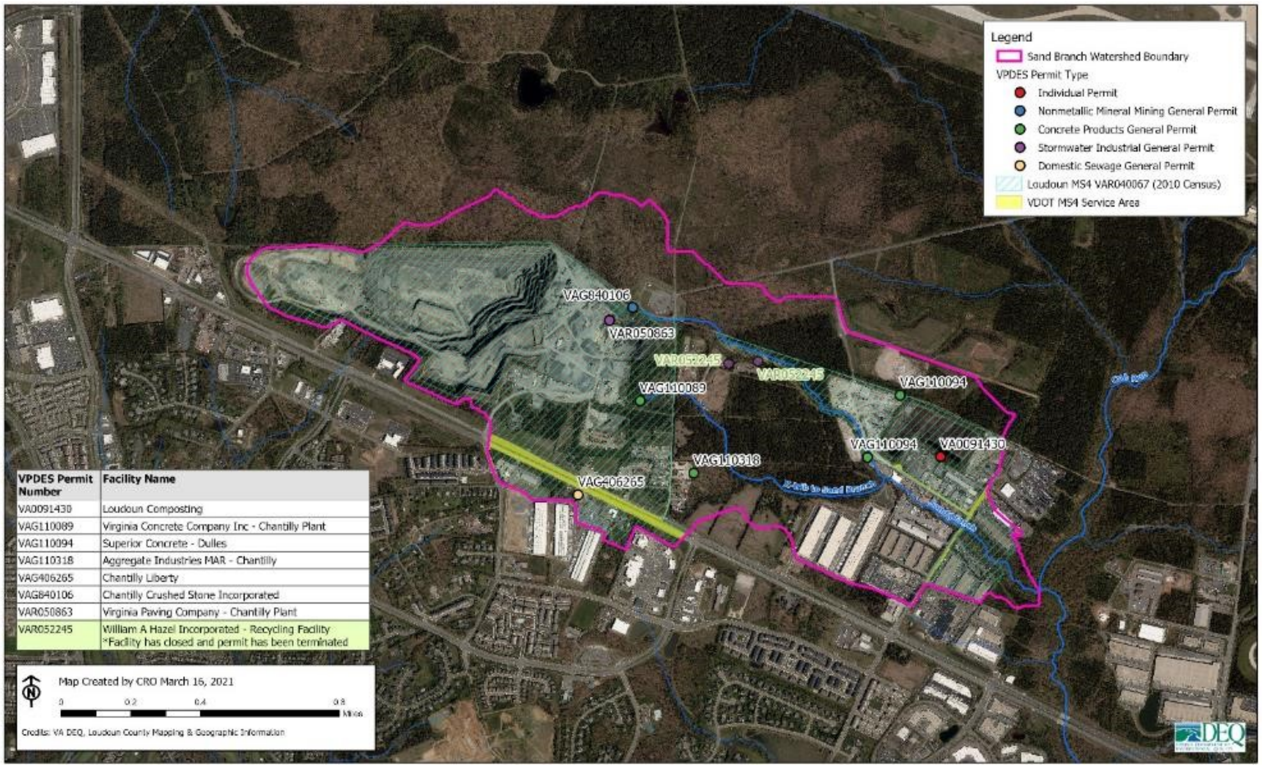
Table 3-1. Permitted VPDES facilities in the Sand Branch watershed.

Permit Number	Facility Name	Receiving Stream[1]	Permit Type[2]
VA0091430	Loudoun Composting	Sand Branch, UT	VPDES IP
VAG110089	Virginia Concrete Company Inc. - Chantilly Plant	Sand Branch, UT	Concrete Products GP
VAG110094	Superior Concrete - Dulles	Sand Branch	Concrete Products GP
VAG110318	Aggregate Industries MAR - Chantilly	Sand Branch, UT	Concrete Products GP
VAG406265	Chantilly Liberty	Sand Branch, UT	Domestic Sewage GP
VAG840106	Chantilly Crushed Stone Incorporated	Sand Branch	Nonmetallic Mineral Mining GP
VAR050863	Virginia Paving Company - Chantilly Plant	Sand Branch, UT	Stormwater Industrial GP
~~VAR052245~~ [3]	~~William A Hazel Incorporated - Recycling Facility~~	~~Sand Branch, UT~~	~~Stormwater Industrial GP~~

[1] UT is an abbreviation for unnamed tributary.
[2] IP denotes an Individual Permit. GP denotes a General Permit.
[3] Facility closed and the VPDES permit was terminated on March 3, 2021.

Table 3-2. MS4 Permit holders in the Sand Branch watershed.

Permit Number	Facility Name	Permit Type
VAR040067	Loudon County	Municipal Separate Storm Sewer System (General Permit)
VA0092975	Virginia Department of Transportation	Municipal Separate Storm Sewer System (Individual Permit)



VPDES Permit Number	Facility Name
VA0091430	Loudoun Composting
VAG110089	Virginia Concrete Company Inc - Chantilly Plant
VAG110094	Superior Concrete - Dulles
VAG110318	Aggregate Industries MAR - Chantilly
VAG406265	Chantilly Liberty
VAG840106	Chantilly Crushed Stone Incorporated
VAR050863	Virginia Paving Company - Chantilly Plant
VAR052245	William A Hazel Incorporated - Recycling Facility *Facility has closed and permit has been terminated

Figure 3-5. Location of VPDES permitted discharges in the Sand Branch watershed.

3.5 Headwater Source

The headwaters of Sand Branch originate from the discharge of a quarry owned by Chantilly Crushed Stone, Inc. (VPDES Permit No. VAG840106). This quarry mines for diabase rock, which is then crushed onsite. Stormwater, which commingles with groundwater infiltration in the mine pit, is collected in a holding pond. After settling, the water is discharged into Sand Branch. The average flow from this discharge was 0.75 MGD, based on the reported flow data from Discharge Monitoring Reports (DMR) submitted monthly for the time period January 1, 2015 to June 30, 2020.

4.0 DEQ Sample Collection and Monitoring Stations

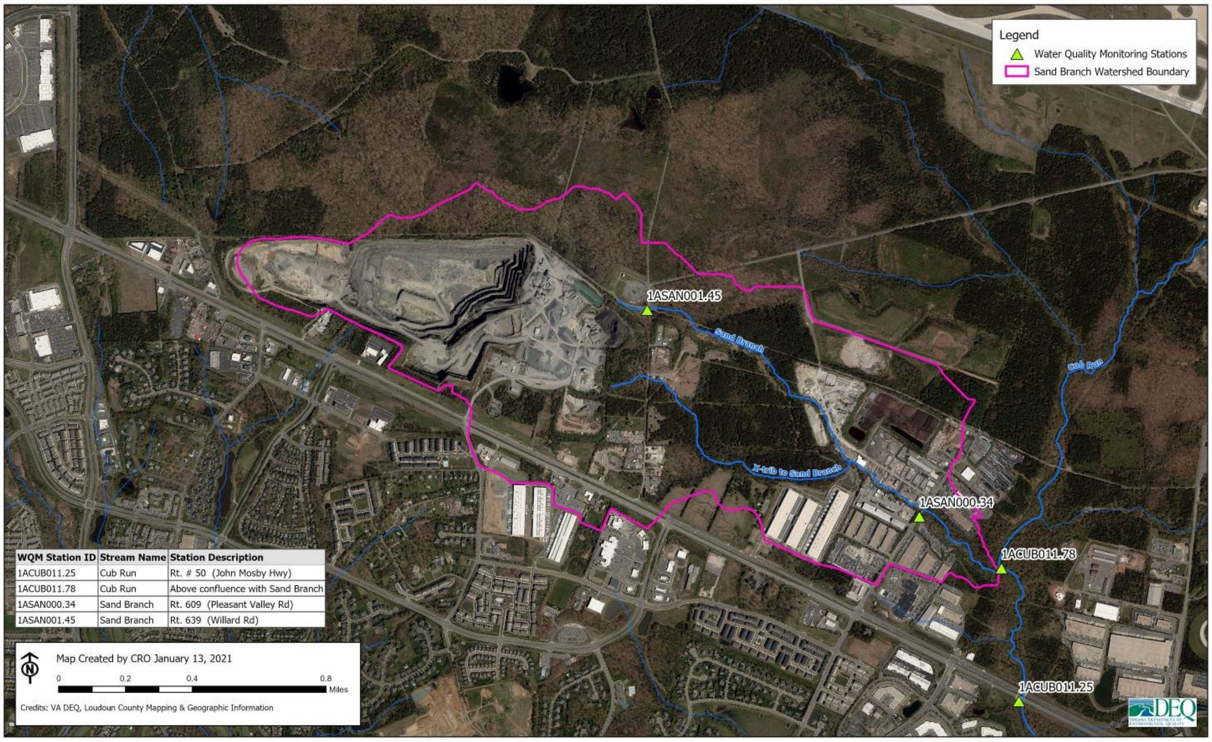
All available DEQ data were examined to investigate likely stressor(s) causing the impairment to the aquatic community. The information considered consisted of data collected specific to Sand Branch and also a reference stream, Licking Run (1ALIL008.29), was used to provide a comparative station for select biological and water quality parameters.

4.1 Sand Branch Monitoring Stations

In preparation for this analysis, monitoring occurred at two monitoring stations located on Sand Branch, one downstream (1ASAN000.34) and one upstream (1ASAN001.45), in effort to help identify potential stressors to the benthic community (Table 4-1, Figure 4-1).

Table 4-1. DEQ monitoring stations in Sand Branch watershed.

Station ID	Location	Monitoring Type
1ASAN000.34	Route 608 (Pleasant Valley Road)	Ambient, Biological
1ASAN001.45	Route 639 (Willard Road)	



WQM Station ID	Stream Name	Station Description
1ACUB011.25	Cub Run	Rt. # 50 (John Mosby Hwy)
1ACUB011.78	Cub Run	Above confluence with Sand Branch
1ASAN000.34	Sand Branch	Rt. 609 (Pleasant Valley Rd)
1ASAN001.45	Sand Branch	Rt. 639 (Willard Rd)

Figure 4-1. DEQ monitoring stations in Sand Branch watershed and its vicinity.

DEQ conducted ambient water quality monitoring at the two monitoring stations, 1ASAN000.34 and 1ASAN001.45, from 2015 – 2020. This included three sampling events for select water column metals at both stations. Metals samples were collected on October 3, 2019, October 31, 2019, and September 17, 2020, representing a variety of flow regimes. Additionally, DEQ staff conducted water quality sampling of select permitted discharges in advance of this stressor analysis to supplement the instream data.

Sampling of benthic macroinvertebrates (biological monitoring) occurred at both monitoring stations, 1ASAN000.34 and 1ASAN001.45, in the fall and spring of 2016 and 2020. Biological sampling typically occurs during low flow to capture colonized aquatic communities. During each biological monitoring, habitat assessments were conducted. As part of these assessments, DEQ observes factors such as epifaunal substrate, embeddedness, velocity/depth regimes, sediment deposition, and riparian vegetative zone width, among others. These categories are given an individual score from 0-20, which are then compiled to produce an overall habitat score of up to 200 points.

The physical aspects of Sand Branch were further characterized at the downstream station, 1ASAN000.34, through a series of measurements taken of the channel to identify its relative bed stability. The resulting value, Log_{10} Relative Bed Stability (LRBS), is based upon a number of metrics that involve the measurement of channel dimensions and substrate composition at numerous transects within a 150 to 800 meter stream reach.

Sampling was done in a manner to capture the full range of flow conditions and a variety of the physical and chemical conditions of the stream. Figure 4-2 presents a flow duration curve for a nearby stream in the Cub Run watershed, Flatlick Branch, which is monitored by the United States Geological Survey (USGS) for both stream flow and water quality parameters. The drainage area in Flatlick Branch at the flow gage is 4.20 square miles. The graph depicts the DEQ sampling events on the flow curve demonstrating the flow regimes captured by the DEQ sampling as influenced by precipitation.

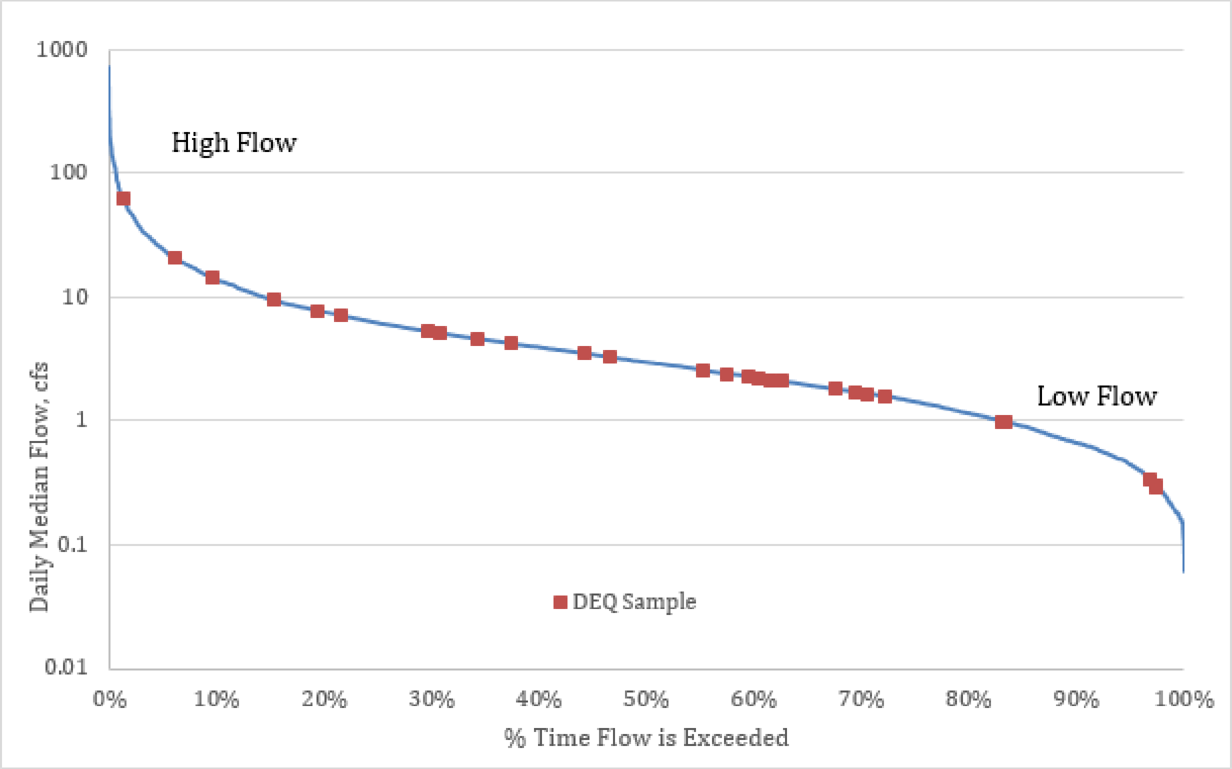


Figure 4-2. Flow duration curve for Flatlick Branch, with DEQ sampling events on Sand Branch, presenting flow regimes captured during ambient sampling.

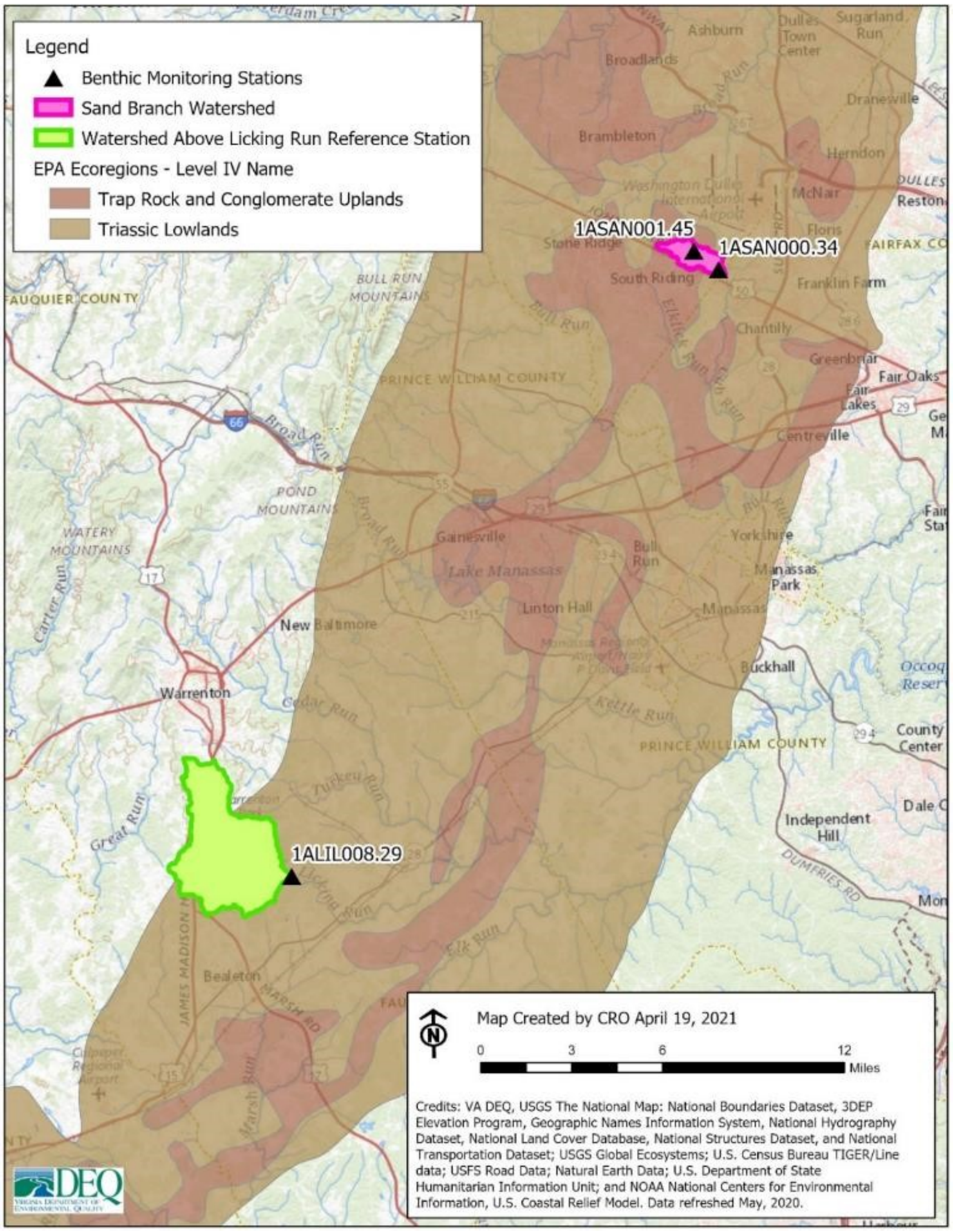
DEQ also deployed a continuous monitoring sonde at 1ASAN000.34 from August 10, 2020 – August 26, 2020 and December 10, 2020 – February 10, 2021. During this period, samples were collected every 15 minutes. These samples were analyzed for a variety of parameters, including dissolved oxygen, pH, specific conductance and temperature. Continuous monitoring allows for trends, such as diurnal patterns, to be observed. Rainfall data from the National Oceanic and Atmospheric Administration's monitoring station at Dulles International Airport, located immediately north of Sand Branch, was used in the analysis of the results of the continuous monitoring sonde.

DEQ considered in this benthic stressor analysis data that was collected on and prior to September 17, 2020, as well as the continuous monitoring data collected December 10, 2020 – February 10, 2021.

4.2 Reference Stream Monitoring Stations

DEQ evaluated 43 benthic monitoring stations within the Triassic Lowlands and/or Trap Rock and Conglomerate Uplands Ecoregion to identify reference stream monitoring station(s) to support identification of stressors of the benthic community in Sand Branch. Of these, only six (6) stations were identified as unimpaired (VSCI score 60 or greater) and deemed suitable as a potential reference station for the benthic stressor analysis. Based upon a review of the water quality data collected at each potential station, the Licking Run monitoring station (1ALIL008.29) was selected as it had the most water quality data (Figure 4-3).

The Licking Run station was used in the data comparison of turbidity, total suspended solids (TSS) and a benthic data comparison of community composition and functional feeding groups. This reference station was deemed unsuitable for a data comparison of specific conductivity or total dissolved solids (TDS) due to the station's limited dataset for those parameters and also its watershed characteristics are more rural in nature and the upper portion of the watershed lies within a different ecoregion. Instead, specific conductivity and TDS data were compared to data collected on Cub Run, to which Sand Branch is a tributary.



Map Created by CRO April 19, 2021

Credits: VA DEQ, USGS The National Map: National Boundaries Dataset, 3DEP Elevation Program, Geographic Names Information System, National Hydrography Dataset, National Land Cover Database, National Structures Dataset, and National Transportation Dataset; USGS Global Ecosystems; U.S. Census Bureau TIGER/Line data; USFS Road Data; Natural Earth Data; U.S. Department of State Humanitarian Information Unit; and NOAA National Centers for Environmental Information, U.S. Coastal Relief Model. Data refreshed May, 2020.

Figure 4-3. Reference Station, Licking Run 1ALIL008.29

5.0 Biological Data Analysis

Benthic macroinvertebrate communities are commonly assessed to reflect water quality in a stream over periods of time, whereas ambient water samples only capture water quality at a specific point in time. DEQ samples these communities to identify stream stressors, both acute and chronic. Compounding stressors often result in decreased diversity, with pollutant tolerant organisms dominating the affected stream reach.

DEQ evaluates biological communities using the VSCI to classify stream health. The VSCI, calculated using a variety of metrics, accounts for taxonomic richness and composition, evenness, tolerance, and trophic group of benthic macroinvertebrates (Table 5-1) (Burton and Gerritsen, 2003). An expected response to disturbance is applied to each metric (identified in Table 5-1) to return a VSCI score. These scores indicate the health of the stream; a VSCI score equal to or greater than 60 is considered non-impaired and a score of less than 60 indicates an impaired community (DEQ, 2021).

Table 5-1. Metrics of the Virginia Stream Condition Index (VSCI).

Metric	**Expected Response to Disturbance**	**Definition of Metric**	**Metric Measures…**
Taxonomic Richness			
Total Taxa	Decrease	Total number taxa observed	Overall variety of macroinvertebrate assemblage
EPT Taxa	Decrease	Total number of *Ephemeroptera*, *Plecoptera*, and *Trichoptera* (EPT) taxa (pollution-sensitive) observed	Prevalence of pollutant-sensitive mayflies, stoneflies, and caddis flies.
Taxonomic Composition			
% PT-H	Decrease	Percent individuals of *Plecoptera*, and *Trichoptera* (PT) taxa (pollution-sensitive) in samples excluding *Hydropsychidae* (pollution-tolerant)	Pollution-sensitive stoneflies and caddies flies without counting pollution-tolerant net-spinning caddis flies
% *Ephemeroptera*	Decrease	Percent individuals of *Ephemeroptera* taxa (pollution-sensitive)	Pollution-sensitive mayflies
% *Chironomidae*	Increase	Percent individuals of *Chironomidae* taxa (pollution-tolerant)	Pollution-tolerant midge larvae
Evenness			
% Two Dominant Taxa	Increase	Percent dominance of two most abundant taxa	Diversity of benthic community
Tolerance			

Metric	Expected Response to Disturbance	Definition of Metric	Metric Measures…
HBI (Family Level)	Increase	Hilsenhoff Biotic Index	Average tolerance to pollution of benthic community, weighted by abundance
Trophic Group			
% Scrapers	Decrease	% of scraper functional feeding group	Macroinvertebrates which graze on substrate- or periphyton-attached algae

DEQ sampled Sand Branch in the spring and fall of 2016 and 2020 at the downstream station (1ASAN000.34) and upstream station (1ASAN001.45), resulting in a total of eight samples. The samples returned an average VSCI score of 26.9 for the downstream sampling location (1ASAN000.34) and 32.9 for the upstream sampling location (1ASAN001.45). The VSCI scores for Sand Branch are consistently below the impaired threshold of 60, with an overall average of 29.91 (Table 5-2).

Table 5-2. VSCI Scores for Sand Branch.

DEQ Monitoring Station	Latitude	Longitude	Monitoring Date	VSCI Score
1ASAN000.34	38.916391666	-77.472136112	03/08/2016	11.4
			08/31/2016	53.3
			03/11/2020	9.6
			09/17/2020	33.4
1ASAN001.45	38.92555556	-77.487	03/08/2016	35.5
			08/31/2016	37.9
			03/11/2020	15.1
			09/17/2020	43.1

The factors driving the low VSCI scores were the following metrics: 1) % *Ephemeroptera*, 2) % *Plecoptera* and *Trichoptera* excluding *Hydropsychidae* (% PT-H), and 3) the *Ephemeroptera, Plecoptera*, and *Trichoptera* (EPT taxa). EPT taxa are typically sensitive to pollutants and water quality stressors.

When averaged, the fall samples tended to have higher VSCI scores (41.92) compared to the spring samples (17.90); however, fall samples score well below the impaired threshold. While seasonal variations are common in benthic communities, extreme variations were observed at 1ASAN000.34, with a 41.95 point difference between the spring and fall samples. This seasonal variation may indicate stressors are more prevalent in the winter and spring, such as sediment and nutrient loads which typically increase with higher spring flows.

DEQ's findings of the downstream and upstream reaches of Sand Branch are discussed separately herein, followed by a summary of the stream health assessments. The taxa identified in samples collected at both stations are provided under APPENDIX A.

5.1 Downstream Station: 1ASAN000.34

At the downstream station, the VSCI scores had a median value of 22.38 and an average value of 26.92. When the years and seasons are reviewed separately, variances are observed between seasons in both years. The spring 2016 and 2020 samples returned VSCI scores of 11.4 and 9.6 respectfully, while the fall 2016 and 2020 samples returned scores of 53.3 and 33.4, respectfully. On average, fall samples had a much higher VSCI score (43.35) than spring samples (10.48).

The scaled metric scores used to calculate the VSCI for 1ASAN000.34 are shown in Table 5-3. Orange shaded values indicate the lowest scores, which are most likely driving the low VSCI scores. Red VSCI scores are below the impairment threshold of 60.

Table 5-3. Scaled metric scores for Station 1ASAN000.34.

VSCI Metric Scores	Spring 2016	Fall 2016	Spring 2020	Fall 2020
% *Ephemeroptera*	0	0	0	0
% PT-H	0	35.75	0	10.21
% *Chironomidae*	4.55	81.82	2.73	80
Total Taxa	22.73	54.55	9.09	45.45
EPT Taxa	0	18.18	0	18.18
% Scrapers	1.76	100	5.29	22.9
% Two Dominant Taxa	3.94	74.88	0	28.9
HBI (Family Level)	58.02	61.36	59.63	61.36
VSCI	11.37	53.32	9.59	33.38

As noted above, mayfly (*Ephemeroptera*) taxa, which decrease with stress, were absent in all spring and fall samples. Taxa included in the second metric (% PT-H), including Stoneflies (*Plecoptera*) and Caddisflies (*Trichoptera*), were also absent in both spring samples; however, a metric score increase was observed in both fall samples. While *Plecoptera* were absent in spring and fall samples, the metric increase was due to the presence of a non-*Hydropsychidae* caddisfly taxa, *Hydroptila*. The presence of this genus resulted in scores of 35.75 and 10.21 respectfully. The absence of this taxa in the spring sample could be attributed to their emergence patterns or their sensitivity to water quality parameters.

Midge larvae (*Chironomidae*), which are more tolerant to pollutants, also influenced the VSCI score. The metric value will increase with a higher percentage of *Chironomidae* present, which results in the metric score to decrease with stress. The spring sample was dominated by taxa belonging to the *Chironomidae* family, while the fall sample contained significantly fewer taxa in this family. The difference resulted in vastly different metric scores, from 4.55 and 2.73 in the spring to 81.82 and 80 in the fall.

The total taxa metric also decreases with stress. This metric increased seasonally in both sampling years, from the spring scores of 22.73 and 9.09 to the fall scores of 54.55 and 45.45. The difference in these scores are a result of only five (2016) and two (2020) families being present in the spring, whereas thirteen (2016) and twelve (2020) families were present in the fall.

Another metric that decreases with stress is the EPT taxa metric. The spring samples both returned a metric score of 0, while the fall samples both increased to 18.18 based on the occurrence of one *Trichoptera* taxa, *Hydroptila*. The lack of mayflies and stoneflies could be attributed to decreased water quality, as *Ephemeroptera* and *Plecoptera* taxa are very sensitive to pollutants.

Also decreasing with stress is the % scrapers metric. The spring 2016 and 2020 samples contained few individuals (*Stenelmis*) from the scraper Functional Feeding Group (FFG), resulting in a score of 1.76 and 5.29. The 2016 fall sample contained more individuals and taxa (*Stenelmis, Physidae, Psephenus*, and *Hydroptila*), which increased the % scraper metric score to 100. The fall 2020 sample also contained more individuals and taxa (*Stenelmis* and *Hydroptila*) than the spring, but far fewer than the fall 2016 sample, resulting in a score of only 22.9. It is important to note that the scraper community mainly consisted of tolerant taxa, with the exception of *Hydroptila*.

The % two dominant taxa metric decreases with stress, where more pollutant-sensitive taxa become diminished and pollutant-tolerant taxa dominate. The spring samples contained mostly *Chironomidae* taxa, where metric values were 3.94 and 0. These values increased to 74.88 and 28.9 in the fall.

The 1ASAN000.34 samples showed high seasonal differences and a severely stressed benthic community. Sensitive Ephemeroptera and Plecoptera taxa were entirely absent from the benthic community.

5.2 Upstream Station: 1ASAN001.45

At the upstream station, the VSCI scores had a median value of 36.71 and an average value of 32.89. The VSCI scores for the upstream station were very similar to each other in 2016 going from 35.48 in the spring to 37.94 in the fall. Similarly to 1ASAN000.34, the VSCI scores in 2020 varied significantly going from 15.09 in the spring to 43.05 in the fall. On average, fall samples had a higher VSCI score (40.5) than spring samples (25.29).

The scaled metric scores used to calculate the VSCI for 1ASAN001.4 are shown in Table 5-4. Orange shaded values indicate the two lowest scores, which are most likely driving the low VSCI scores. Red VSCI scores are below the impairment threshold of 60.

Table 5-4. Scaled metric scores for Station 1ASAN001.45.

VSCI Metric	Spring 2016	Fall 2016	Spring 2020	Fall 2020
% *Ephemeroptera*	0	0	0	0
% PT-H	0	0	0	5.11
% *Chironomidae*	64.55	98.18	11.82	95.45
Total Taxa	22.73	27.27	18.18	36.36
EPT Taxa	9.09	9.09	9.09	18.18

VSCI Metric	Spring 2016	Fall 2016	Spring 2020	Fall 2020
% Scrapers	100	89.85	14.09	95.14
% Two Dominant Taxa	11.82	6.57	6.57	21.02
HBI (Family Level)	75.67	72.59	60.96	73.13
VSCI	35.48	37.94	15.09	43.05

Ephemeroptera taxa were absent from the 2016 and 2020 spring and fall samples, resulting in a score of 0. Taxa included in the second metric, %PT-H, were also absent in the spring and fall samples, except for the 2020 fall sample. The fall 2020 sample contained taxa from the genus *Hydroptila*, resulting in a score of 5.11. While two net-spinning caddisflies were found (*Hydropsychidae*: *Cheumatpsyche* and *Hydropsyche*) in all four samples, these taxa are excluded in the %PT-H metric, resulting in a metric score of 0 for the spring and fall 2016 and the spring 2020 samples. Total absence of *Ephemeroptera* and *Plecoptera* is rare, as Virginia's probabilistic data shows that the 50th percentile of streams in the state have 24.4% *Ephemeroptera* taxa and 13% *Plecoptera* within the community.

Both 2016 and 2020 samples contained fewer *Chironomidae* individuals than the downstream sampling location during each season. The spring sample returned a % *Chironomidae* metric value of 64.55 and 11.82, and the fall sample returned a metric value of 98.18 and 95.45. As previously mentioned, individuals belonging to this family are pollutant-tolerant.

The total taxa metric score increased slightly in 2016, from 22.73 to 27.27. This increase was due to one additional taxa found in the fall, bringing the total from 5 to 6. The total taxa metric score had a similar trend in 2020, doubling from 18.18 in the spring to 36.36 in the fall, which is a result of only four taxa found in the spring versus eight found in the fall.

Samples contained a large number of individuals from the scraper FFG taxa, *Stenelmis*, which resulted in high % scrapers metric scores of 100 (spring 2016), 89.85 (fall 2016), and 95.14 (fall 2020). The spring in 2020 was largely dominated by *Chironomidae,* leaving only a few scrapers in the overall sample. This resulted in a score of 14.09. The % scraper metric score decreases with stress; however the scrapers observed in Sand Branch are more tolerant taxa, like snails and beetle larvae.

The % two dominant taxa was mostly composed of *Stenelmis* and *Chironomidae* in the spring samples, resulting in metric scores of 11.82 and 6.57. The fall metric scores were 6.57 and 21.02, dominated by *Stenelmis* and two *Hydropsychidae* taxa (*Cheumatopsyche* and *Hydropsyche*). 1ASAN001.45 samples were largely dominated by highly tolerant and moderately tolerant taxa, reflecting a severely stressed benthic community. Like the downstream site, sensitive Ephemeroptera and Plecoptera taxa were absent at the upstream location.

5.3 Biological Condition Gradient

The Biological Condition Gradient (BCG) attribution identifies the relative tolerance and sensitivity for taxa commonly found throughout Virginia and surrounding states (Tetra Tech, 2019). The attribution process uses data from several entities (DEQ, surrounding states within the region, counties, etc.) to assign a tolerance value to each taxon using a consensus-based process. The BCG Metric is an average of all stressor specific BCG attribution. The values range from 1 to 5, where 1 indicates that a taxon is highly sensitive to a given stressor pollutant or condition and 5 indicates that a taxon's population will increase in the presence of that stressor (e.g. more pollutant tolerant). A value of 6 is used to indicate the species typically does not naturally occur in the locale or ecosystem. The letter accompanying the 6 attribution denotes intolerant, moderately tolerant, and tolerant characteristics in addition to the non-native status.

Table 5-5 and Table 5-6 present the BCG attribution for the 95th cumulative percentile of all organisms collected at each monitoring station. BCG attributions for all taxa collected are provided in APPENDIX B. The tables provide the response of the identified organism (rows) to the stressor indicator (columns); the taxon’s assigned value is representative of their specific tolerance to 10 individual stressors.

At the downstream station, 1ASAN000.34, 9 of 24 taxa identified in samples collected in 2016 and 2020 comprised the 95th cumulative percentile based upon number of individuals per taxa. The top 5 most abundant taxa were *Chironomidae, Stenelmis, Cheumatophsyche, Hydroptila* and *Hydropsyche.* All 9 taxa of the 95th cumulative percentile have a BCG General Score of 4, 5 or 6t. This grouping, based upon an average of the attribute scores for each of the 10 stressors, indicates that most of the taxa have a high tolerance in the presence of specific conductivity, nutrients and watershed percent imperviousness.

At the upstream station 1ASAN001.45, 5 of 15 taxa identified in samples collected in 2016 and 2020 comprised the 95th cumulative percentile based upon number of individuals per taxa. These 5 taxa were *Stenelmis*, *Chironomidae, Cheumatophsyche*. *Hydropsyche,* and *Hydropsychidae.* All 5 taxa of the 95th cumulative percentile have a BCG General Score of 4 or 5. This grouping, based upon an average of the attribute scores for each of the 10 stressors, indicates that most of the taxa have a high tolerance in the presence of specific conductivity and watershed percent imperviousness.

Table 5-5. BCG attribution for 95th cumulative percentile of benthic macroinvertebrate community sampled in 2016 and 2020 at 1ASAN000.34

Genus Level	**No. of Individuals**	**Functional Feeding Group**	**General Attribute**[1]	**Biological Condition Gradient (BCG) Attribute Assignments for Specific Stressors**									
				DO	**Acidity (pH**[2]**)**	**Alkalinity (pH**[2]**)**	**Specific Conductance**	**Chloride**	**Sulfate**	**Nutrients**[3]	**Total Habitat Score**	**Relative Bed Stability**	**Watershed % Impervious**
Chironomidae (A)	451	Collector	4	4	4	4	4	4	4	4	4	4	4
Stenelmis	73	Scraper	4	4	4	4	5	4	4	4	5	4	5
Cheumatopsyche	68	Filterer	5	4	3	4	5	4	4	5	4	4	5
Hydroptila	37	Scraper	4	3	2	3	5	4	5	5	4	3	5
Hydropsychidae	26	Filterer	4	3	3	4	4	3	4	4	4	4	4
Physidae	25	Scraper	5	5	4	5	5	4	3	5	5	5	5
Corbicula	24	Filterer	6t	4	3	4	5	4	4	5	5	4	5
Hydropsyche	20	Filterer	4	3	3		5	4	5	5	4	4	5
Oligochaeta	11	Collector	5	4	4	3	5	4	4	5	5	5	5
TOTAL	735		**Rounded Average**	4	3	4	5	4	4	5	4	4	5

[1] Characterizes tolerance and other ecological attributes in relation to broad physical and chemical conditions. Lower values indicate greater sensitivity to pollution, higher values indicate greater tolerance to pollution.

2 For pH, responses to acidity and alkalinity were considered independently.

3 For nutrients, although Total Nitrogen (TN) and Total Phosphorous (TP) were analyzed separately, these were assessed as common responses for attribution assignment.

Table 5-6. BCG attribution for 95th cumulative percentile of benthic macroinvertebrate community sampled in 2016 and 2020 at 1ASAN001.45.

Genus Level	No. of Individuals	Functional Feeding Group	General Attribute[1]	Biological Condition Gradient (BCG) Attribute Assignments for Specific Stressors									
				DO	Acidity (pH[2])	Alkalinity (pH[2])	Specific Conductance	Chloride	Sulfate	Nutrients[3]	Total Habitat Score	Relative Bed Stability	Watershed % Impervious
Stenelmis	284	Scraper	4	4	4	4	5	4	4	4	5	4	5
Chironomidae (A)	261	Collector	4	4	4	4	4	4	4	4	4	4	4
Cheumatopsyche	123	Filterer	5	4	3	4	5	4	4	5	4	4	5
Hydropsyche	76	Filterer	4	3	3		5	4	5	5	4	4	5
Hydropsychidae	13	Filterer	4	3	3	4	4	3	4	4	4	4	4
TOTAL	757		**Rounded Average**	4	3	4	5	4	4	4	4	4	5

[1] Characterizes tolerance and other ecological attributes in relation to broad physical and chemical conditions. Lower values indicate greater sensitivity to pollution, higher values indicate greater tolerance to pollution.

2 For pH, responses to acidity and alkalinity were considered independently.

3 For nutrients, although Total Nitrogen (TN) and Total Phosphorous (TP) were analyzed separately, these were assessed as common responses for attribution assignment.

5.4 Community Composition and Functional Feeding Group Analysis

The taxonomic composition and functional feeding groups of the benthic community were analyzed to identify shifts in composition at impaired stations that might provide clues to sources or mechanisms of impairment. Figure 5-1 compares the taxonomic composition in Sand Branch to Licking Run (1ALIL008.29), an unimpaired reference stream within the Trap Rock and Conglomerate Uplands and Triassic Lowlands ecoregion. In the reference stream, taxonomic composition is relatively balanced. Sensitive taxonomic categories, such as *Ephemeroptera, Plecoptera*, and *Trichoptera*, (EPT), each represent at least 10% of the community, and no one category represents more than 25% of the community. In Sand Branch, the community composition is shifted to greater dominance by a few tolerant taxa with less diversity and balance.

At the upstream Sand Branch monitoring station (1ASAN001.45), the benthic community is almost entirely dominated by *Diptera*, *Hydropsychidae*, and *Coleoptera*. All other taxonomic groups combined represented only 1% of the community. The *Diptera* present in Sand Branch were predominantly midges (*Chironomidae*), which could be indicative of nutrient or sediment enrichment. Lawrence and Gressens (2011) showed that Chironomid abundance correlated with increased nutrient enrichment in urban and rural streams. Bjornn *et al*. (1977) demonstrated in artificial mesocosm experiments that increases in fine sediment significantly reduced EPT taxa but were tolerated by Chironomid taxa. *Hydropsychidae* are a net-spinning caddisfly that filter organic material out of the water with their silk-spun nets. High concentrations of *Hydropsychidae* are a reliable indicator of organic or nutrient pollution (Voshell, 2002). The primary *Coleoptera* genera in Sand Branch was the riffle beetle, *Stenelmis*. This beetle clings to rocks and periphyton in fast-moving water and generally feeds by scraping periphyton. Dominance by this taxon could also indicate nutrient enrichment, since it relies on periphyton growth for food.

At the downstream Sand Branch monitoring station (1ASAN000.34), the benthic community was dominated by the same three taxa (*Diptera*, *Hydropsychidae*, and *Stenelmis*). Collectively, these three comprised 86% of the community, with *Diptera* contributing 60%. There was greater contribution from other taxa at the downstream station (14%) than at the upstream station (1%). These additional taxa included a non-Hydropsychid caddisfly taxon (*Hydroptila*), Oligochaete worms, snails, and Asiatic clams. In general, both upstream and downstream Sand Branch locations showed an absence of sensitive species and a dominance by several tolerant taxa that are also indicative of organic matter or nutrient enrichment.

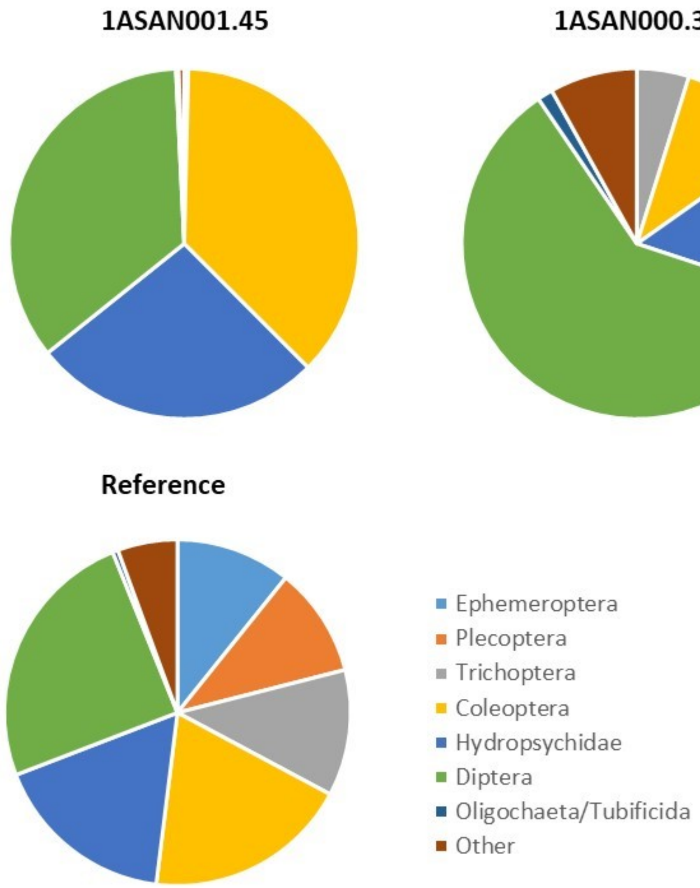


Figure 5-1. Taxonomic composition of benthic community in Sand Branch compared to a reference station (Licking Run, 1ALIL008.29).

The composition of functional feeding groups comprising the benthic community was also analyzed to identify shifts in composition at impaired stations that might provide clues to sources or mechanisms of impairment. Figure 5-2 shows the composition of functional feeding groups within Sand Branch in comparison to the reference stream, Licking Run (1ALIL008.29). Two distinct patterns emerged from this analysis. At the upstream location, shredders and predators were reduced, while scrapers increased in abundance. Scrapers comprised 38% of the population at 1ASAN001.45, compared to only 22% at the reference location. This increase in scrapers is indicative of nutrient enrichment and an abundant production of algae in the periphyton community. As nutrient enrichment fuels algae growth, more food is available for scrapers and the feeding niche expands.

At the downstream station (1ASAN000.34), the same increase in scrapers was not observed. Instead, collectors increased while shredders, predators, and filterers decreased. At this location, collectors comprised 62% of the population, compared to 32% at the reference station, Licking Run (1ALIL008.29). This pattern indicates deposited organic material or sediment enrichment. As upstream periphyton growth is sloughed and sediment is deposited, organically rich sediment provides an increased feeding niche for collectors (like Chironomids) that scavenge deposited material and detritus.

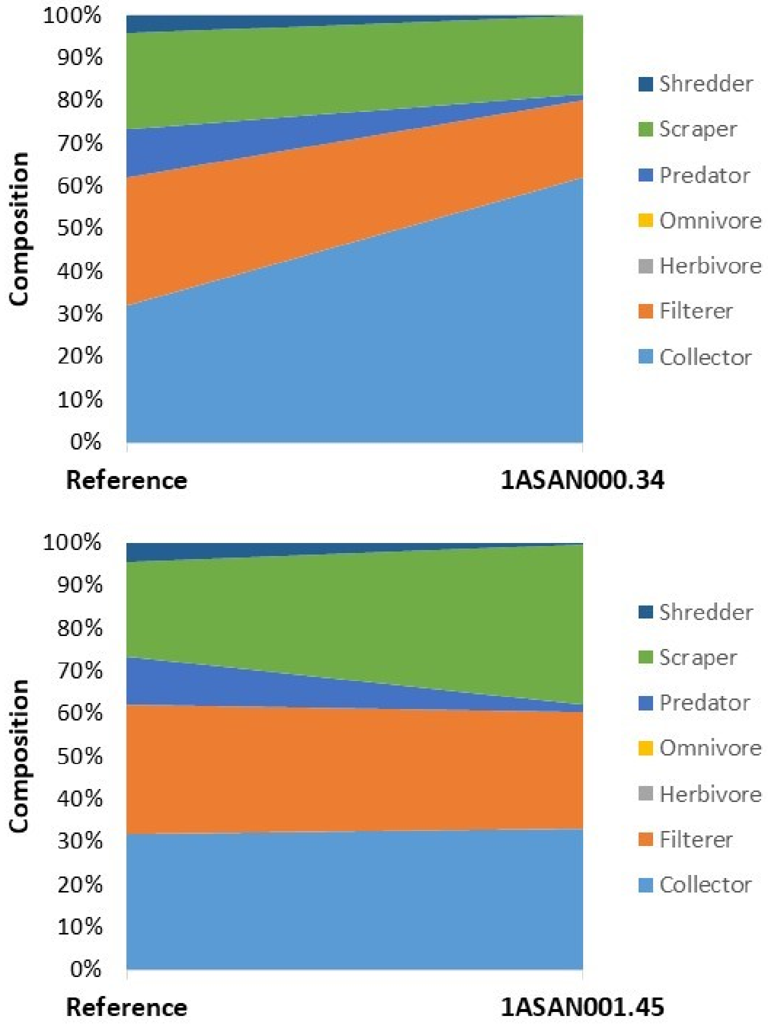


Figure 5-2. Functional feeding groups in Sand Branch compared to a reference station (Licking Run, 1ALIL008.29).

5.5 Ambient Water Toxicity Data Analysis

Toxicity tests are used to identify whether a water sample collected from either a discharge or a waterbody has a toxic effect on test organisms, causing negative biological responses. There are two types of toxicity tests, acute and chronic, which differ by the length of time the test organisms are exposed to the water sample and the type of responses that are measured. Acute tests typically only measure survival, while chronic tests measure sub-lethal responses such as growth and reproduction. Each of these tests include a control sample and dilute the water sample over a specific range to identify the concentration at which toxic responses are exhibited.

In March 2020, water column samples from the downstream monitoring station (1ASAN000.34) on Sand Branch were collected for toxicity testing. Toxicity tests were conducted by EA Engineering, Science, and Technology, Inc. according to USEPA-approved methods (USEPA, 2002a; USEPA, 2002b). Acute and chronic toxicity tests were conducted using the water flea, *Ceriodaphnia dubia*, and the larval fathead minnow, *Pimphales promelas* (Table 5-7). Acute tests were static, non-renewal, 48-hr tests conducted on a sample collected from 1ASAN000.34 on 3/9/2020. Chronic tests were 6 or 7-day, static renewal tests conducted on samples collected from 1ASAN000.34 on 3/9/2020, 3/11/2020, and 3/13/2020. All tests were performed with a moderately hard synthetic freshwater control and a dilution series of 6.25, 12.5, 25, 50, and 100% sample.

There are several endpoints that are used to understand the results of the tests. Survivability of the test organisms is identified as the lethal concentration (LC), which is the concentration when death of the organism occurs. The inhibition concentration (IC) is the concentration at which a biological function, such as growth, is negatively affected. When a number accompanies a LC or IC, such as LC50, this indicates the point at which that percentage of the test population exhibited the toxic effect. For example, LC50 is the percent value at which 50% of the population died. Additionally, No Observed Effect Concentration (NOEC) identifies the highest concentration at which no toxic response was observed, whereas the Lowest Observed Effect Concentration (LOEC) identifies the lowest concentration at which a toxic response is observed. The Chronic Value is an estimate of the threshold of effects. It is determined as the geometric mean of the NOEC and LOEC. All these values are statistically derived using the results of the control sample and the effects measured in each sample dilution.

The test acceptability criteria were met for all tests. Control survival was 95% in the *C. dubia* acute test and 100% in the *P. promelas* acute test. In the *C. dubia* chronic test, the 6-day control survival was 100%, and the mean number of young produced in the control was 25.1 per adult. In the *P. promelas* chronic test, the 7-day control survival was 93%, and the mean biomass was 0.890 mg/organism.

Toxicity test results showed no observed acute toxicity to *C. dubia* or *P. promelas* (Table 5-7). Both species exhibited 100% survival in the 100% sample after 48 hours. No toxicity was observed in the *C. dubia* chronic test, where 6-day survival in the 100% sample was 100% and reproduction was only 2% lower and not statistically different from the control. In the *P. promelas* chronic test, however, toxicity was observed. After 7 days, survival in the 100% sample was only 65% and statistically different from the control survival of 93%. Biomass was also 22% lower than the control and determined to be statistically different. At the 50% sample dilution, survival was 73% and biomass was reduced by 2.7%, but these differences were not statistically different from the control. The resulting NOEC for the test was 50%, indicating that statistically significant toxicity was observed at the 100% sample but not at the 50% dilution. Based on the LOEC and the NOEC, a chronic value of 70.7% was calculated for the test. The IC25 was >100%, since the reduction in biomass did not exceed 25% in the highest concentration, but the IC10 was 63.9%. This indicates that a 10% biomass reduction in larval fish would be expected at an in-stream dilution of 63.9%.

Table 5-7. Results of Sand Branch toxicity testing.

Test Organism	Test Type	Endpoint	Value (%)
Ceriodaphnia dubia (water flea)	48-hr acute test	48-hr LC50	>100
	6-d chronic survival and reproduction test	Survival NOEC	>100
		Reproduction NOEC	>100
		Chronic Value	>100
		IC25	>100
Pimephales promelas (fathead minnow)	48-hr acute test	48-hr LC50	>100
		Survival NOEC	50

Test Organism	Test Type	Endpoint	Value (%)
	7-d chronic larval survival and growth test	Biomass NOEC	50
		Chronic Value	70.7
		IC25	>100
		IC10	63.9

The observation of toxicity in Sand Branch samples points to chemical stressors (as opposed to physical or habitat stressors) as the cause of impaired aquatic life in the stream. While water fleas and fathead minnows are not perfect surrogates for benthic macroinvertebrates, the tests demonstrate that water quality conditions in Sand Branch are toxic to at least some freshwater aquatic species. In fact, these test species are generally more tolerant than many sensitive benthic macroinvertebrate species (e.g., *Ephemeroptera, Plectoptera*, and *Trichoptera*), so observed toxicity likely explains the near absence of sensitive EPT taxa from Sand Branch.

Ancillary water chemistry analyses conducted during the toxicity tests can provide some clues to potential causes of toxicity. Measurements of pH and ammonia showed no indications of those parameters impacting test species health. Dissolved oxygen (DO) levels were low in some of the higher concentration treatments, but never fell below 5.4 mg/L. Low DO in the higher concentrations was most likely due to decomposition of test organisms that died during the exposures. This is common in toxicity tests when significant mortality is observed, and it is consistent with the observed evidence that DO decreased later in the test (Day 6) after organisms died (on Days 3-5). Dissolved oxygen levels in the *C. dubia* test also remained high, indicating that DO decreases were not associated with the Sand Branch samples themselves, but with the conditions (i.e., organism deaths) in the *P. promelas* test.

Lastly, conductivity measurements made on collected samples before the test and in test exposure chambers during the test show a possible cause of toxicity. The Sand Branch samples used for daily renewal of the toxicity test had measured conductivities of 1028-1087 uS/cm, and exposure chambers in the 100% treatment during the test had conductivities of 1008-1122 uS/cm. These conductivity levels are sufficient to cause toxicity to freshwater organisms and exceed benchmarks developed by USEPA (2011) and Cormier *et al.,* (2018). In addition, toxicity models confirm that ion levels and resulting conductivity of Sand Branch samples are adequate to produce toxicity. Using the statistical model developed by Mount *et al*., (1997) to predict the toxicity of major ions to fathead minnows, the average ion composition of Sand Branch was predicted to exhibit 75% mortality in the 100% sample after 96 hours. This is higher than the 35% mortality observed in the toxicity test, but it demonstrates that based on a large database of toxicity results for major ions (Mount *et al*., 1997), the toxicity observed in Sand Branch samples was likely due to the presence of major ions (potassium, magnesium, calcium, sodium, bicarbonate, sulfate, chloride, nitrate) contributing to conductivity and total dissolved solids.

6.0 Water Chemistry Data Analysis

Water quality data collected for the candidate stressors were analyzed to identify if one or more of those parameters may be causing stress to the aquatic community. To help identify potential for stress, freshwater aquatic life water quality criteria were compared to observed values for parameters with numeric criteria. Another evaluation compared parameters, as applicable, to threshold stressor values developed and published by DEQ (2017) in the “Stressor Analysis in Virginia: Data Collection and Stressor Thresholds” document.

Stressor thresholds or concentration/measured ranges linked to probable stress to aquatic life were developed using data collected through DEQ's Freshwater Probabilistic Monitoring Program (Table 6-1). The stressor thresholds are not derived from literature values and are not intended to replace water quality criteria or define TMDL endpoints. Rather, they were derived from empirical data collected using a probabilistic sampling design. The probabilistic approach minimizes bias because it is a statistically designed study and sample sites are selected randomly across a geographical area. The probabilistic monitoring dataset allowed DEQ to determine thresholds ranging from no stress to aquatic life to high probability of stress to aquatic life for the following parameters: dissolved oxygen, pH, total phosphorus, total nitrogen, ionic strength (specific conductivity, TDS, and sulfate, chloride, sodium, and potassium), dissolved metals cumulative criterion unit, total habitat and relative bed stability.

The stress categories were developed by analyzing benthic macroinvertebrate community responses (represented by VSCI scores) through a variety of peer-reviewed statistical techniques: relative risk, relative extent, conditional probability and quantile regression. The results were interpreted into stress categories. "No Stress" to aquatic life means that a parameter range reflects an undisturbed, or background, condition in Virginia. "Low Probability" represents a benthic macroinvertebrate community response that is slightly above background conditions but unlikely to cause a major community shift. The next category is "Medium Probability" and means there is evidence of harm causing a possible shift in benthic communities with changes noticeably above background conditions. The "High Probability" threshold corresponds to values that are among the highest in the Commonwealth and result in degradation of the benthic community (DEQ, 2017).

Table 6-1. Definitions of the probabilities of stress to aquatic life

Probability of Stress to Aquatic Life	Definition
High Probability	Values that are the highest in Virginia, resulting in degradation of the benthic community.
Medium Probability	Noticeable evidence of harm causing a possible shift in benthic communities, changes noticeably above background conditions.
Low Probability	Slightly above background conditions, but unlikely to cause a major benthic community shift.
No Probability	Background conditions.

6.1 pH

Benthic macroinvertebrates require a specific pH range to thrive in aquatic systems. The Virginia WQS establish allowable criteria for pH as between 6.0 and 9.0 standard units in Class III nontidal waters (9VAC25-260-50).

pH measurements in Sand Branch ranged from 7.51 – 8.81 SU, with average and median values of 8.1 SU and 8.12 SU, respectively (n=53). All pH measurements fell into the low probability of stress to aquatic life range and the water quality criteria range, 6.0 SU – 9.0 SU (Figure 6-1). The black lines in Figure 6-1 represent the minimum and maximum water quality criteria for pH, 6.0 SU and 9.0 SU.

At the upstream monitoring station, 1ASAN001.45, the average and median pH values were 8.08 and 8.11, respectively (n=24). At the downstream monitoring station, 1ASAN000.34, the average and median pH values were 8.12 and 8.13, respectively (n=29).

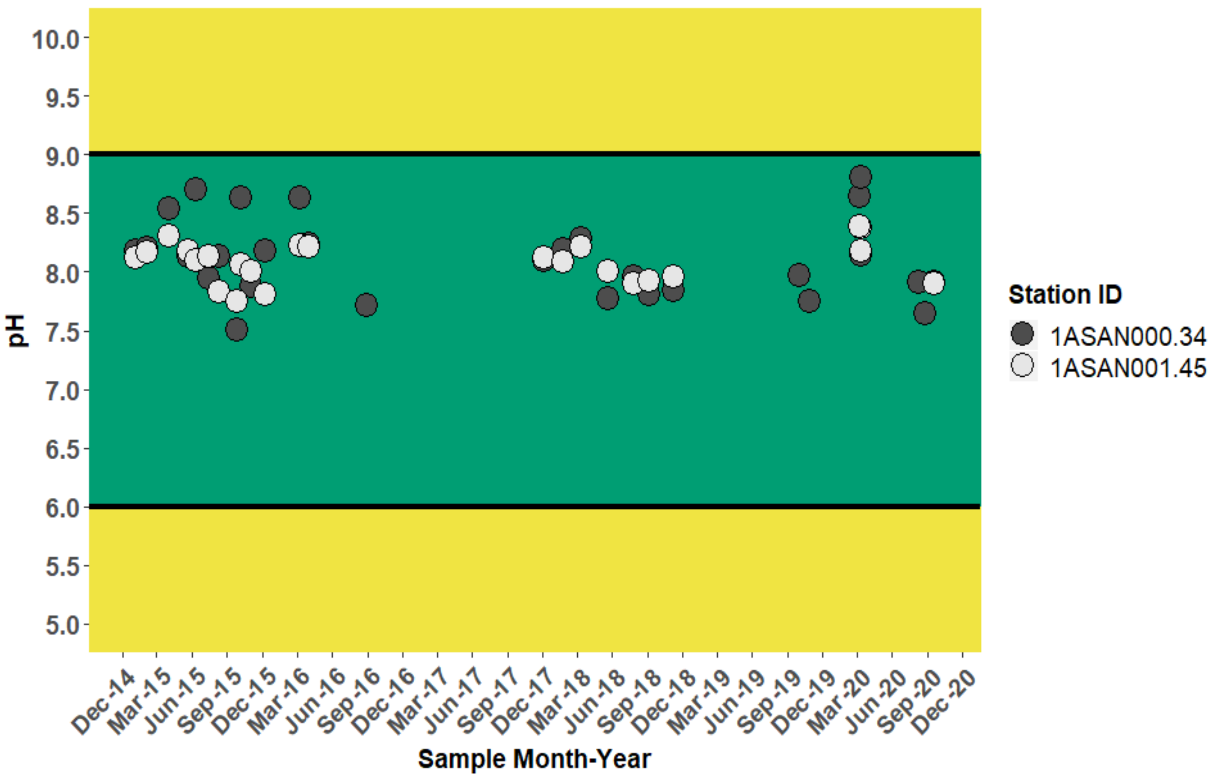


Figure 6-1. pH measurements at 1ASAN000.34 and 1ASAN001.45 from January 2015 – September 2020.

Continuous monitoring occurred from August 10 – 26, 2020 at station 1ASAN000.34. pH measurements were taken at 15 minute intervals. Total rainfall during that timeframe was measured in one hour increments at Dulles International Airport.

pH values ranged from 7.34 – 8.02 SU, with a median of 7.8 SU. pH showed several swings between August 12th – August 13th, when multiple rain events occurred. pH also showed decreases on August 16th, 19th, and August 25th, when rain events occurred. The drop in pH on August 19th was the largest, although the rain event was smaller than events on the 12th, 16th, 22nd, and 25th of August. (Figure 6-2).

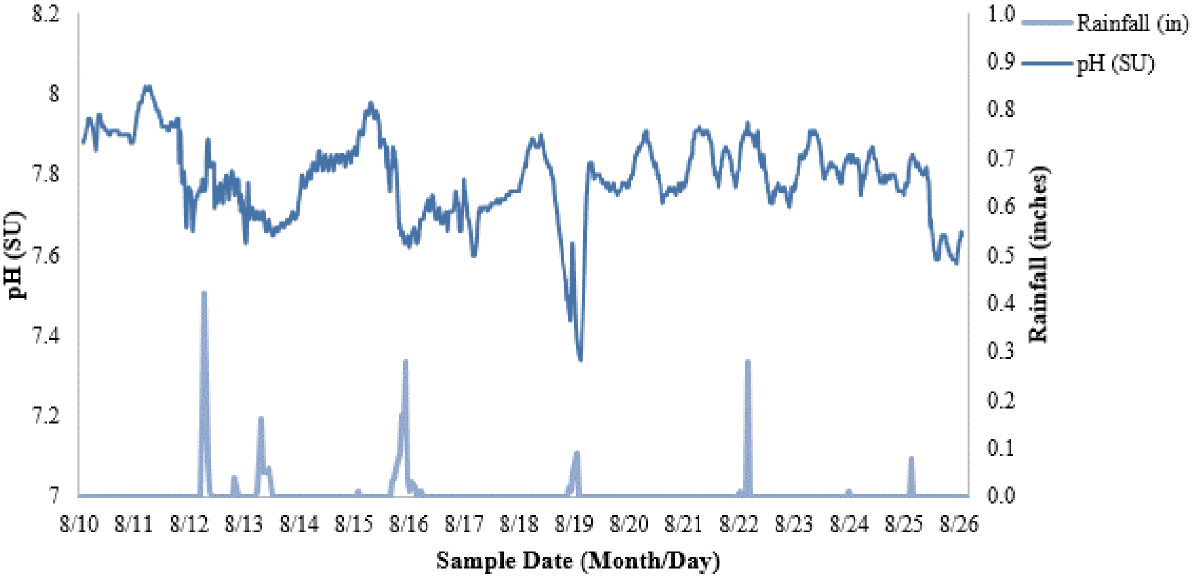


Figure 6-2. Continuous monitoring of pH (SU) at 1ASAN000.34 from August 10 – 26, 2020.

6.2 Dissolved Oxygen

Most organisms require oxygen to sustain life. The amount of oxygen available to aquatic organisms is called dissolved oxygen (DO). Sensitive aquatic organisms require high levels of DO, while more tolerant organisms can withstand lower levels. The Virginia WQS establish a minimum DO of 4 mg/L and a minimum daily average DO of 5 mg/L in Class III nontidal waters (9VAC25-260-50).

DO in Sand Branch ranged from 7.26 - 16.32 mg/L, with an average of 10.32 mg/L (n=53) and a median value of 9.75 mg/L (Figure 6-3). The data measured ranged from no probability of stress to medium probability of stress, with the average and median values indicating a low probability of stress.

All data measured were above the water quality criteria for DO, 4.0 mg/L. At the upstream monitoring station, 1ASAN001.45, the average and median DO were 10.52 mg/L and 10.55 mg/L, respectfully (n=24). The data measured ranged from no probability of stress to medium probability of stress, with the average and median values indicating no probability of stress.

At the downstream monitoring station, 1ASAN000.34, the average and median DO were 10.15 mg/L and 9.39 mg/L, respectfully (n=29). The data measured ranged from no probability of stress to medium probability of stress, with the average and median values indicating a low probability of stress.

The black line at 4.0 mg/L shown in Figure 6-3 represents the water quality criterion for DO.

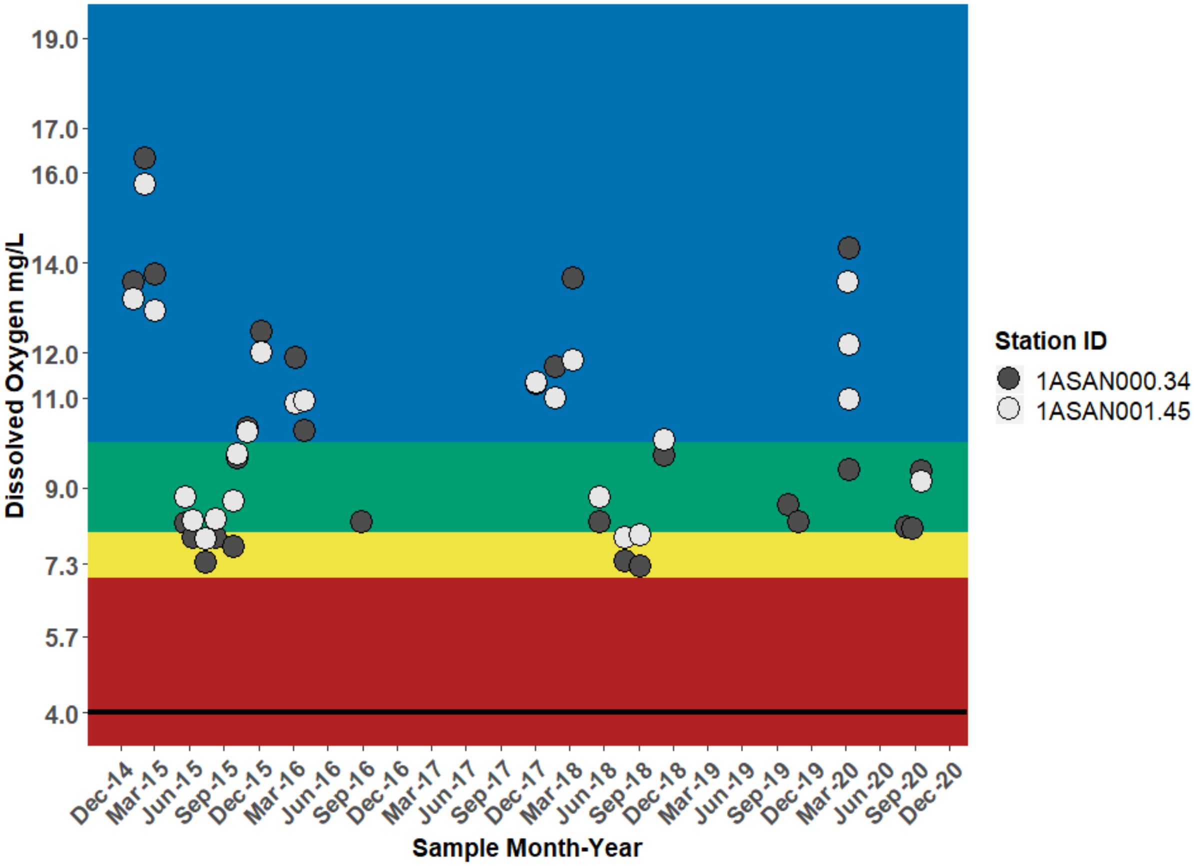


Figure 6-3. Dissolved oxygen measurements at 1ASAN000.34 and 1ASAN001.45 from January 2015 – September 2020.

DO saturation levels above 100% indicate that a stream is supersaturated with DO, while values below 100% indicate oxygen is depleted to various degrees. Additionally, large swings in DO over the course of a day indicate that nutrient enrichment may be driving high levels of photosynthesis by algae during the day and oxygen consumption at night. Because the solubility of dissolved oxygen changes with the instream temperature, DEQ also considered DO saturation levels. DEQ used instream temperature to calculate the percent saturation of DO, when assuming an atmospheric pressure of 1 atm.

At the downstream monitoring station, 1ASAN000.34, the percent DO saturation ranged from 84% - 132%, with an average of 100% and a median of 98%. At the upstream monitoring station, 1ASAN001.45, the percent DO saturation ranged from 76% - 133%, with an average of 104% and a median of 103%. These values indicate that super-saturation does occur in Sand Branch (Figure 6-4).

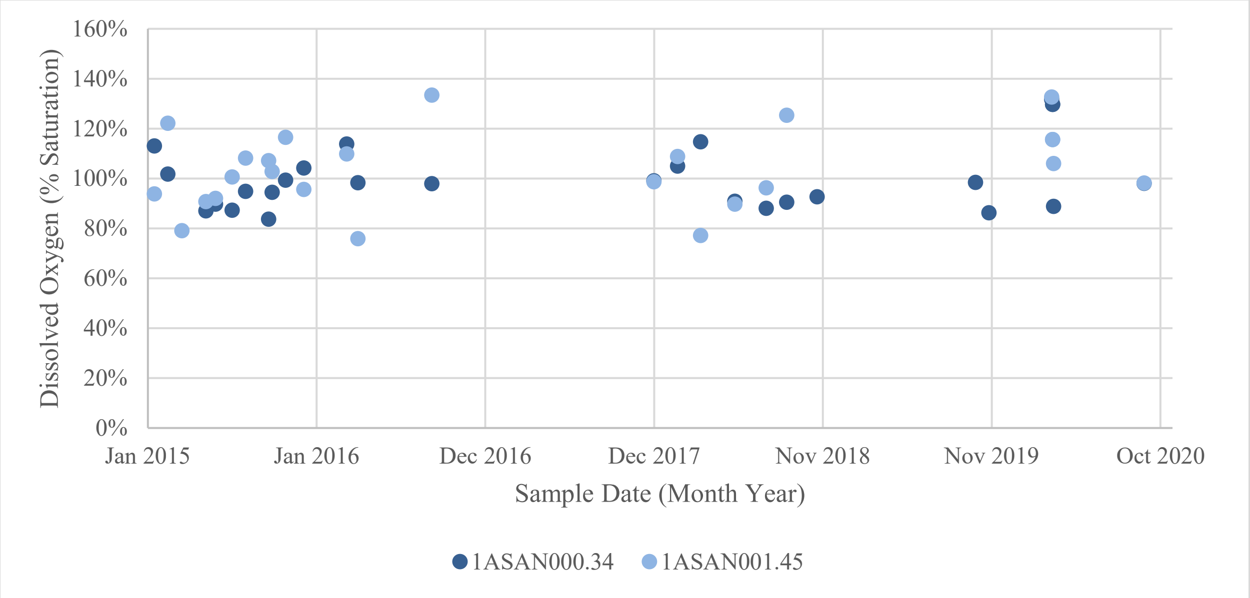


Figure 6-4. Dissolved oxygen saturation at 1ASAN000.34 and 1ASAN001.45 from January 2015 – September 2020.

Continuous monitoring of DO (mg/L) and DO saturation (%) both showed a diurnal pattern. DO ranged from 5.1 – 8.6 mg/L, with a median of 7.5 mg/L. DO saturation ranged from 57.9 – 100.2%, with a median of 86.3%. Both DO and DO saturation showed decreases on August 19th and 25th, which corresponded with relatively small rain events. The largest rain events during this period occurred on August 12th, 16th, and 22nd, which did not appear to cause a significant variation in the DO or DO saturation. (Figure 6-5 and Figure 6-6).

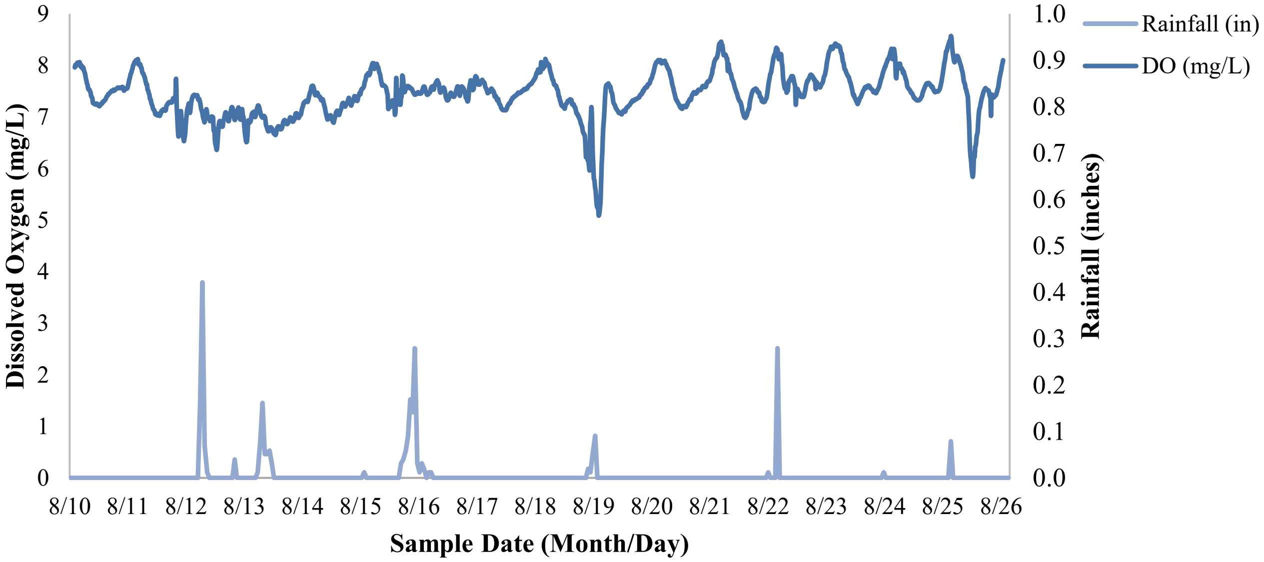


Figure 6-5. Continuous monitoring of dissolved oxygen (mg/L) at 1ASAN000.34 from August 10, 2020 – August 26, 2020.

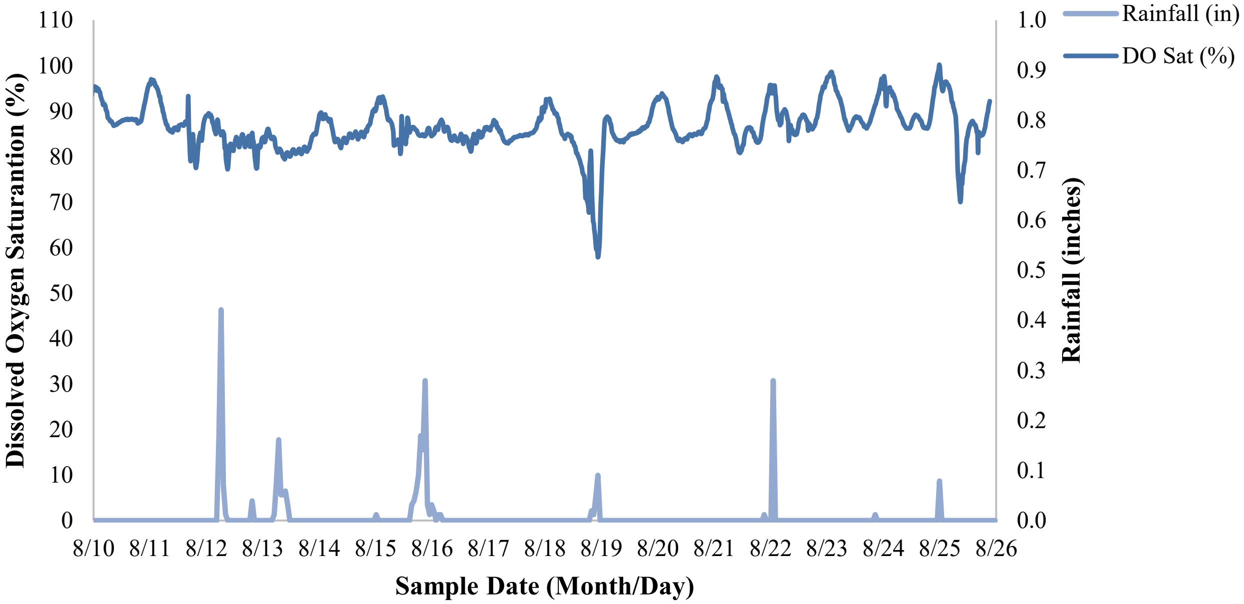


Figure 6-6. Continuous monitoring of dissolved oxygen saturation (%) at 1ASAN000.34 from August 10, 2020 – August 26, 2020.

6.3 Total Phosphorus

Elevated levels of total phosphorus can stimulate algal production and shift aquatic communities. Phosphorus is often the limiting nutrient in aquatic communities, meaning that biota may have a stronger response to phosphorus additions than nitrogen additions (Schindler, 1977). Generally, values above the typical nitrogen to phosphorous ratio of 7.5 seen in algae indicate that phosphorous is the limiting nutrient. In Sand Branch, the ratio of average total nitrogen concentration of 1.72 mg/L to the average total phosphorous concentration of 0.06 mg/L is 29:1. This indicates that phosphorus is likely the limiting nutrient in Sand Branch. In Virginia, there are no numeric water quality criteria for phosphorous in free flowing streams.

Total phosphorus in Sand Branch ranged from 0-0.32 mg/L, with an average of 0.06 mg/L and a median of 0.04 mg/L (n=48) (Figure 6-7). At the upstream station (1ASAN001.45), total phosphorus averaged 0.03 mg/L with a median of 0.02 mg/L in the low probability range for stressor effects. Values were much higher in the downstream station (1ASAN000.34), averaging 0.08 mg/L with a median of 0.05 mg/L. This average fell in the medium probability range for stress effects, and 23% of values fell in the high probability range.

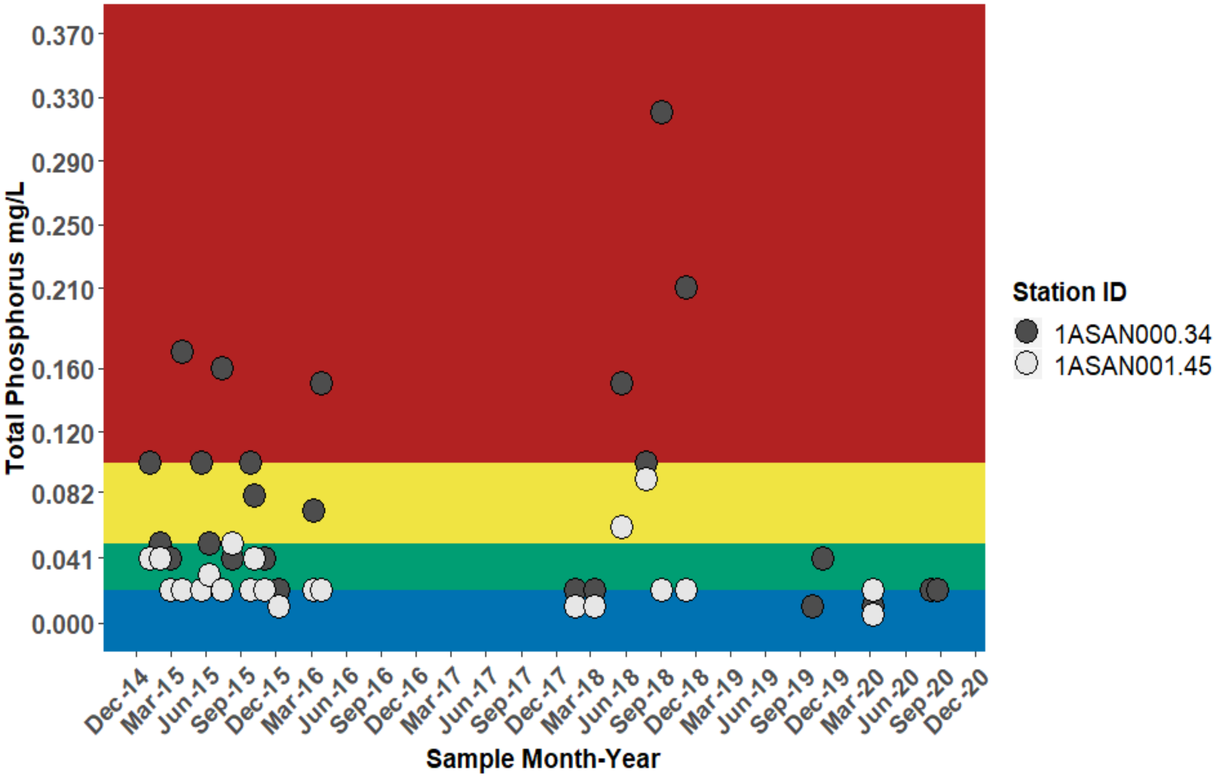


Figure 16. Total phosphorus measurements at 1ASAN000.34 and 1ASAN001.45 from January 2015 – September 2020

While Virginia has not established water quality criteria for nutrients, USEPA has published recommended criteria based on ecoregion (USEPA, 2000a). For the Northern Piedmont ecoregion, the recommended criterion is 0.04 mg/L. Median phosphorus levels at the downstream Sand Branch location are above this value, indicating that nutrients may be elevated.

Within the broader Northern Piedmont Level III Ecoregion, Sand Branch lies specifically within the Trap Rock and Conglomerates Uplands and Triassic Lowlands. The underlying geology of the region is primarily sedimentary rock of the Triassic interspersed with magma intrusions of igneous diabase (trap rock) and heat-altered sedimentary rocks (Woods *et al.,* 1999). This geology naturally produces higher phosphorus levels in overlying streams than is typical for the Northern Piedmont (Porter *et al.,* 2020). For this reason, phosphorus levels in Sand Branch were compared to 38 other benthic monitoring stations within the same geology and collected between July 1999 and September 2020. Among these comparison stations, total phosphorus levels averaged from 0.02 to 0.11 mg/L. The upstream Sand Branch station (1ASAN001.45) represented only the 5th percentile of average phosphorus levels within the ecoregion, while the downstream Sand Branch station (1ASAN000.34) represented the 81st percentile (Figure 6-7). This indicates that there are significant contributions of phosphorus in Sand Branch between the headwaters and downstream portions of the watershed. At the downstream station, phosphorus levels in Sand Branch are high (upper quartile) even among comparison stations within the same geology.

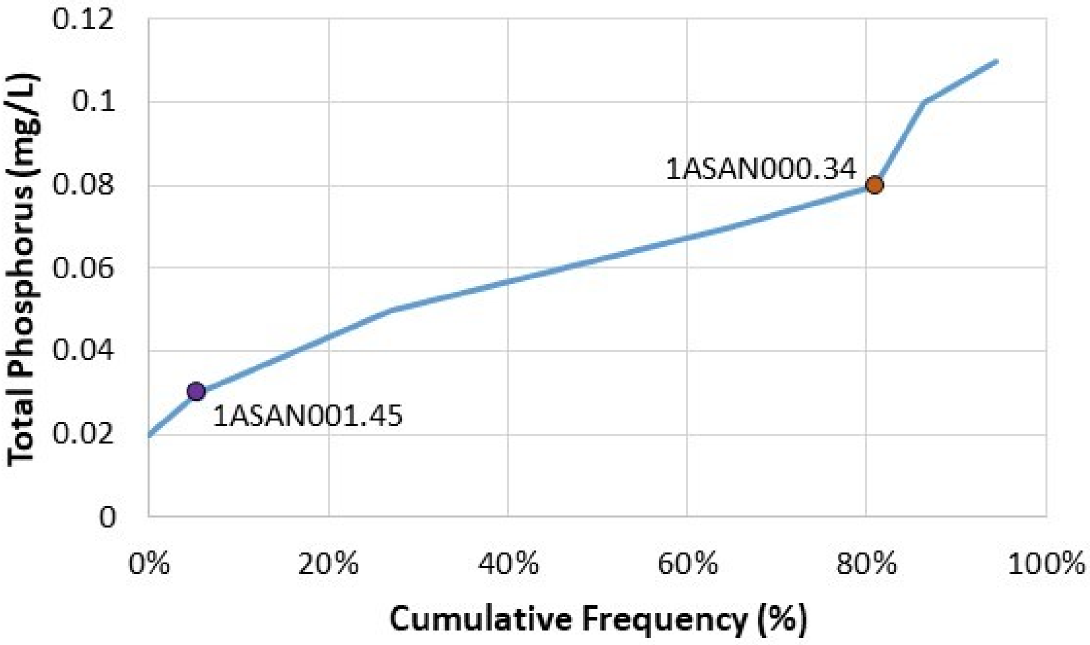


Figure 6-7. Cumulative distribution of average total phosphorus among stations in the Trap Rock and Conglomerate Uplands and Triassic Lowlands ecoregions.

6.4 Total Nitrogen

Total nitrogen is an essential resource for aquatic plants and animals, yet excess nitrogen can over fertilize algae resulting in eutrophication of streams or lakes. Humans may alter natural nitrogen levels through poor agricultural practices, mismanagement of waste, and combustion of fossil fuels. In Virginia, there are no numeric water quality criteria for total nitrogen in free flowing streams.

Total nitrogen ranged from 0.33 - 17.9 mg/L, with an average concentration of 1.72 mg/L and a median concentration of 1.23 mg/L (n= 51) (Figure 6-8). The data measured ranged from no probability to high probability of stress to aquatic life, with the average and median values indicting a medium probability of stress.

At the upstream monitoring station, 1ASAN001.45, the average and median concentration of total nitrogen were 1.22 mg/L and 1.09 mg/L, respectively (n=24). The data measured ranged from no probability of stress to high probability of stress, with the average and median values indicating a medium probability of stress.

At the downstream monitoring station, 1ASAN000.34, the average and median concentration of total nitrogen were 2.16 mg/L and 1.33 mg/L (n=27). The data measured ranged from no to high probability of stress to aquatic life, while the average and median indicated a medium to high probability of stress.

An analysis of the ratios of total nitrogen to total phosphorous can indicate which nutrient is the limiting factor. Based upon an analysis of the average total nitrogen concentration of 1.72 mg/L to the average total phosphorous concentration of 0.06 mg/L, the ratio is 29:1. Values above the ratio of 7.5:1, typically seen in algae, indicates that phosphorous is the limiting nutrient.

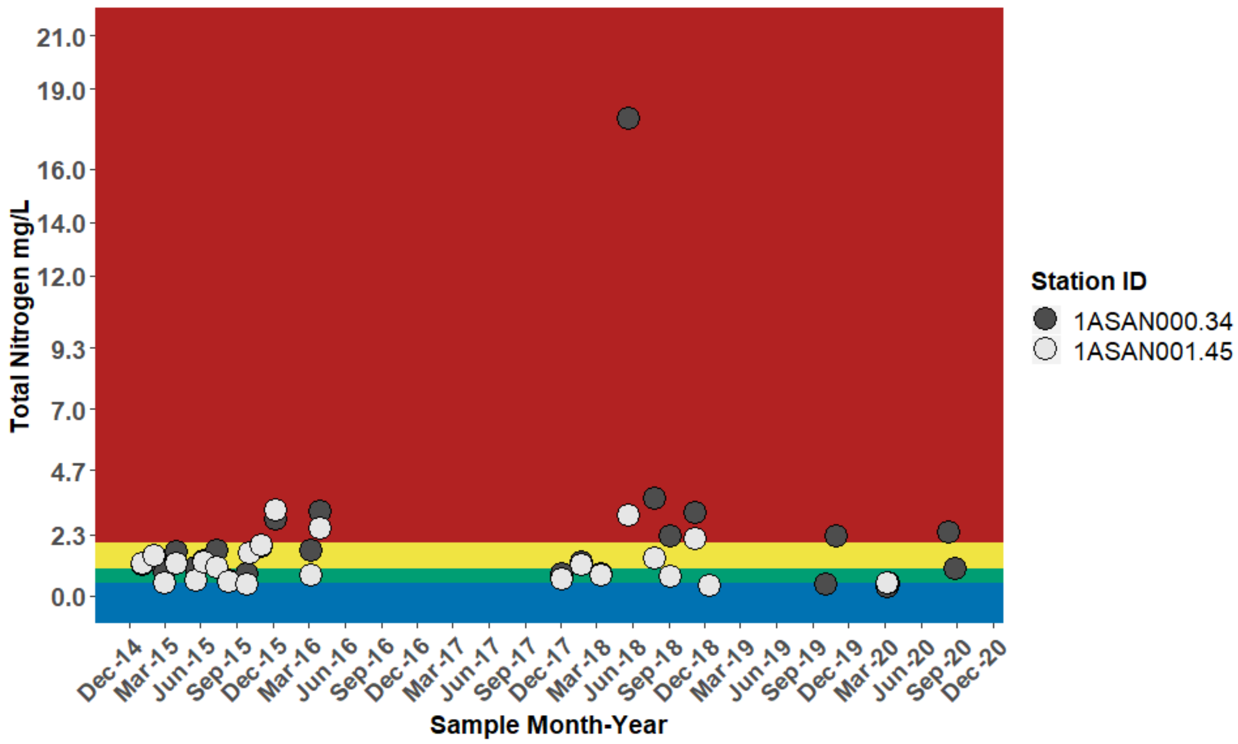


Figure 6-8. Total nitrogen measurements at 1ASAN000.34 and 1ASAN001.45 from January 2015 – September 2020.

6.5 Ammonia

Values for ammonia were compared against the water quality criteria. Stressor thresholds have not been developed for ammonia. Because the water quality criteria for ammonia is dependent on instream pH and temperature, the aquatic life criterion for each monitoring event was calculated using the instream pH and temperature for the corresponding monitoring event. A comparison of the ammonia results with the criteria shows no excursions of the acute criterion and a single sample excursion of the chronic criterion on 5/22/2018 at station 1ASAN000.34 (Table 6-2 and Table 6-3). On this date, stream flows were very high, measured at the 90th percentile flow at the Flatlick Branch flow gauge (USGS 01656903 Flatlick Branch Above Frog Branch at Chantilly, VA), and there were likely many stormwater discharges occurring which may have influenced instream ammonia concentrations. Additionally, an upstream discharger (Loudoun Composting, VA0091430) that has historically demonstrated elevated ammonia concentrations was discharging.

Table 6-2. Ammonia data collected from station 1ASAN000.34 from December 2017 – August 2020.

Monitoring Date	Total Ammonia (mg/L)	Acute Criteria (mg/L)	Chronic Criteria (mg/L)
12/5/2017	0.01 [a]	7.25	1.314
1/23/2018	0.01 [a]	5.94	1.070
3/12/2018	0.03 [a]	5.01	1.089

Monitoring Date	Total Ammonia (mg/L)	Acute Criteria (mg/L)	Chronic Criteria (mg/L)
5/22/2018	1.5	5.86	1.042
7/26/2018	0.06	2.97	0.627
9/6/2018	0.36	3.21	0.657
11/8/2018	0.48	9.19	1.494
10/3/2019	0.05	3.52	0.717
10/31/2019	0.02 [a]	7.55	1.265
3/9/2020	< 0.014 [b]	1.87	0.403
3/11/2020	< 0.014 [b]	6.34	1.130
8/10/2020	0.02 [a]	3.73	0.746
8/26/2020	< 0.014 [b]	6.38	1.087

[a] Analyte detected above the method detection level but below the method quantification limit.

[b] Material analyzed for, but not detected. Value is the limit of detection.

Table 6-3. Ammonia data collected from station 1ASAN001.45 from December 2017 – March 2020.

Monitoring Date	Total Ammonia (mg/L)	Acute Criteria (mg/L)	Chronic Criteria (mg/L)
12/5/2017	0.01 [a]	6.74	1.187
1/23/2018	< 0.008 [b]	6.57	1.165
3/12/2018	< 0.008 [b]	5.75	1.077
5/22/2018	0.06	3.34	0.688
7/26/2018	0.04	3.13	0.650
9/6/2018	0.02 [a]	2.60	0.564
11/8/2018	0.02 [a]	7.13	1.239
12/13/2018 [c]	0.01 [a]	--	--
3/9/2020	< 0.014 [b]	2.94	0.603
3/11/2020	< 0.014 [b]	3.33	0.667

[a] Analyte detected above the method detection level but below the method quantification limit.
[b] Material analyzed for, but not detected. Value is the limit of detection.
[c] pH and temperature data were not collected so acute/chronic criteria cannot be calculated

6.6 Suspended Solids and Deposited Sediment

A stream must have suitable habitat to support a healthy macroinvertebrate community. Sensitive benthic macroinvertebrates require spaces between stream substrate that are free of sediment and stable during storm events. A suite of habitat variables are visibly inspected by DEQ at the time of biological monitoring to calculate a total habitat score (Barbour *et al*. 1999). A total habitat score is an index representing multiple instream and riparian habitat variables that describe the quality of habitat for aquatic organisms. Habitat parameters include substrate composition, flow, riparian quality, and habitat diversity.

Quantitative habitat data is collected as part of the probabilistic monitoring program, which allows DEQ to calculate the Log_{10} Relative Bed Stability (LRBS) index, percent fines, and embeddedness. The LRBS index, developed by USEPA, is the ratio of the observed mean streambed particle diameter to the "critical diameter," which is the largest particle size the stream can move during storm flows (Kaufmann et al. 2007). The index was developed to differentiate between natural and anthropogenic sediment deposition in a watershed.

Excess sediment was investigated as a stressor in Sand Branch by analyzing both the impact of suspended sediment in the water column and the impact of deposited sediment on available habitat. DEQ assessed suspended sediment by analyzing water quality data for total suspended solids and turbidity. Deposited sediment was investigated by analyzing available habitat data and LRBS data.

6.6.1 Total Suspended Solids and Turbidity

Figure 6-9 shows total suspended solids (TSS) measured in Sand Branch. Concentrations ranged from the detection limit of 2 mg/L to 139 mg/L and averaged 19 mg/L at the upstream station (1ASAN001.45) and 30 mg/L at the downstream station (1ASAN000.34). TSS levels at both of these stations were statistically higher than the reference stream, Licking Run (1ALIL008.29) within the same ecoregion (p-value <0.05 in t-test with unequal variances). While high TSS levels are common during runoff events in both impaired and unimpaired streams, TSS is generally low in unimpaired streams under normal (non-storm event) hydrologic conditions.

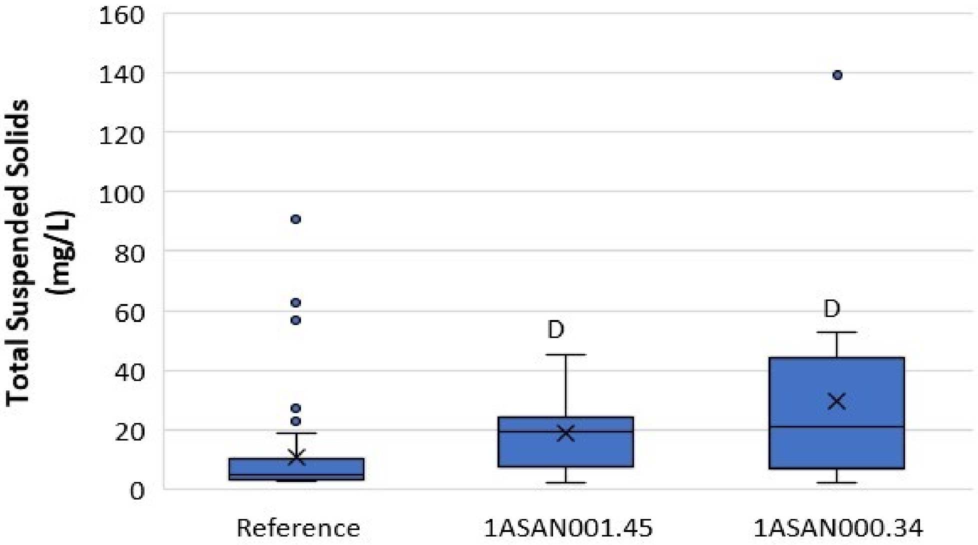


Figure 6-9. TSS in Sand Branch compared to a reference station (Licking Run, 1ALIL008.29). Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station.

A comparative analysis of the cumulative frequency distribution of TSS values between the reference stream monitoring station (Licking Run, 1ALIL008.29) and both Sand Branch monitoring stations shows that the concentration of TSS is elevated more frequently in Sand Branch than the reference stream (Figure 6-10). This indicates that even under typical (non-storm event) hydrologic conditions, Sand Branch carries excess sediment.

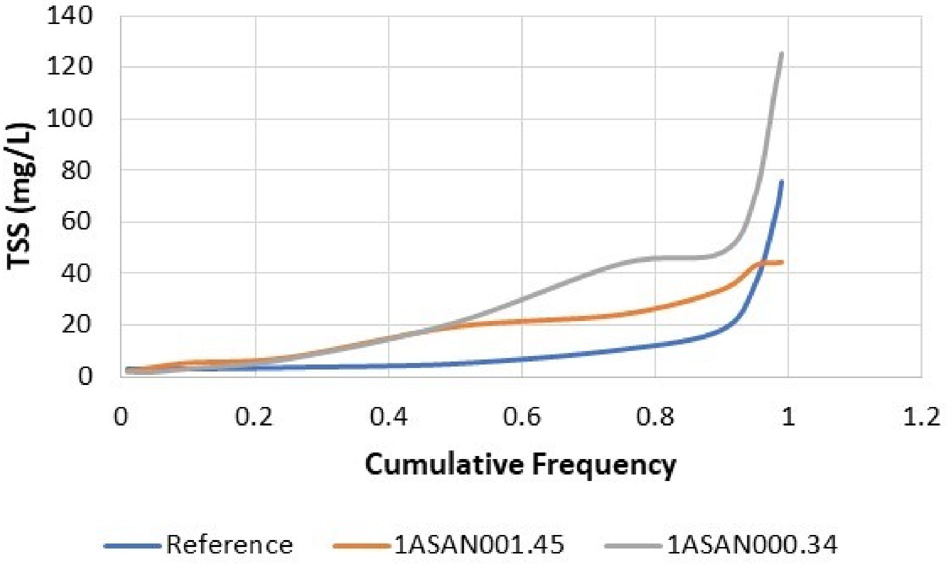


Figure 6-10. Cumulative frequency analysis of total suspended solids in Sand Branch compared to a reference station (Licking Run, 1ALIL008.29).

Turbidity data showed a similar pattern (Figure 6-11). Turbidity averaged 21 NTU at the upstream Sand Branch station and 31 NTU at the downstream station, compared to 16 NTU in the unimpaired reference stream station (1ALIL008.29). Turbidity at 1ASAN000.34 was statistically higher than the reference stream station (p-value <0.05 in t-test with unequal variances), but the difference at 1ASAN001.45 was not statistically significant.

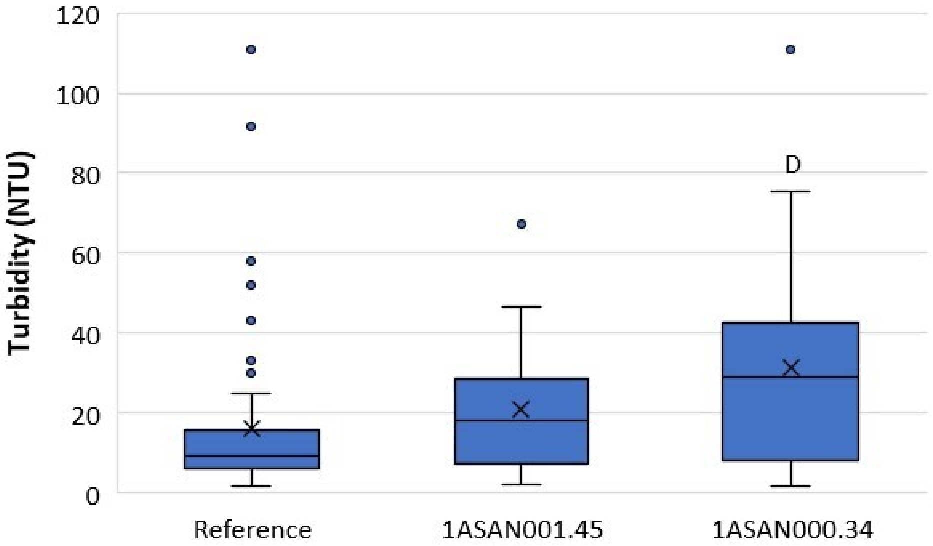


Figure 6-11. Turbidity in Sand Branch compared to a reference station (Licking Run, 1ALIL008.29). Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station.

6.6.2 Habitat Assessment

As part of the Rapid Bioassessment Protocol (Barbour et al. 1999), a visual habitat assessment is performed at the time of each benthic sample collection. This assessment is comprised of a number of habitat components that include substrate, embeddedness, velocity, sediment, flow, channel alteration, riffles, bank stability, bank vegetation, and riparian vegetation. Each of these individual components are scored from 0 to 20, where 20 indicates optimal conditions and 0 indicates poor conditions. Scores between 0 and 5 are deemed very poor, 5 to 10 are marginal, 10 to 15 are suboptimal, and scores over 15 are considered optimal. The individual scores for each of these measures are then added for a total habitat score out of a possible 200. The total habitat score ranges from 0-200, where scores less than 100 indicate sub-optimal habitat and scores greater than 150 indicate optimal habitat.

Total habitat scores in Sand Branch ranged from 101 to 141 (Figure 6-12) and averaged 128 at the upstream station (1ASAN001.45) and 110 at the downstream station (1ASAN000.34). These values place both Sand Branch stations in the medium probability range for stress effects.

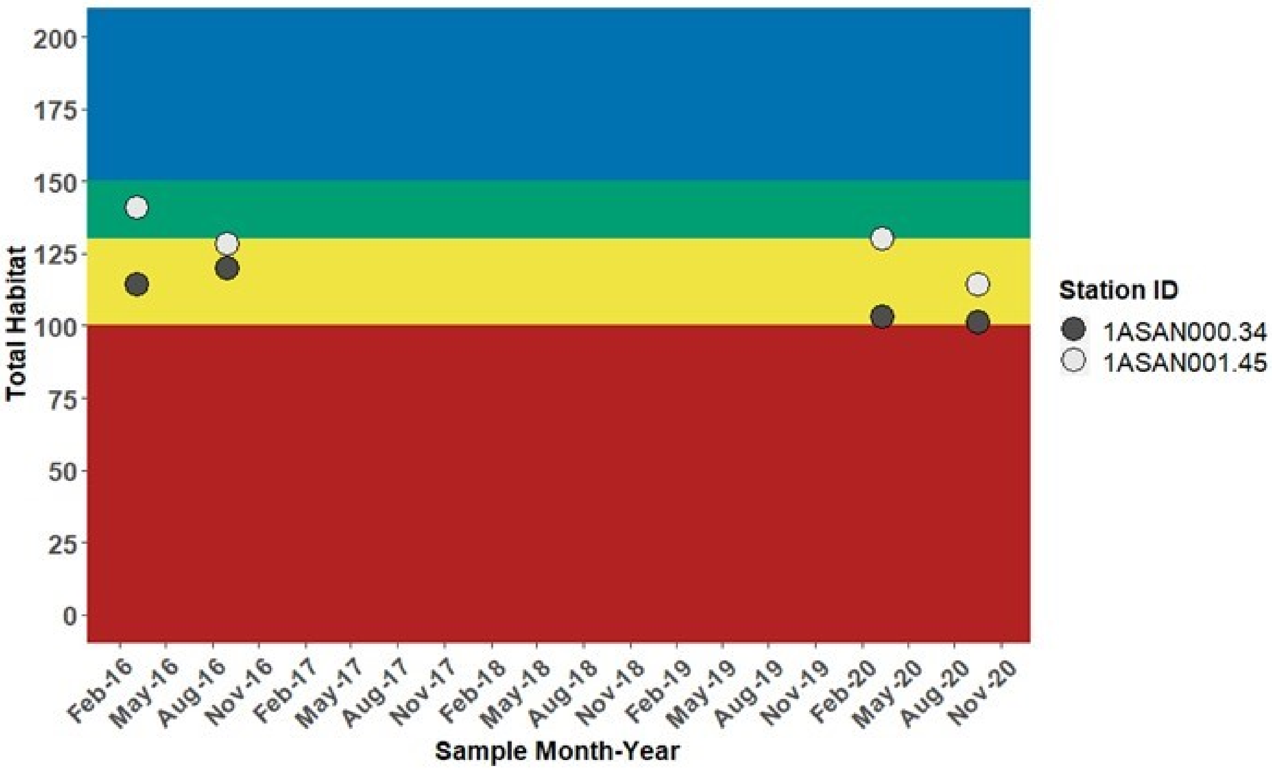
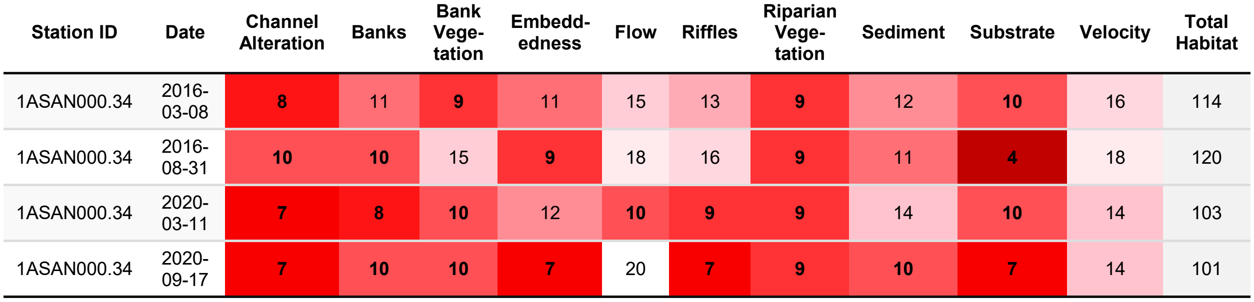


Figure 6-12. Total habitat measurements taken at monitoring stations 1ASAN000.34 and 1ASAN001.45 from March 2016 – September 2020.

Further analysis of the individual metrics that make up the qualitative total habitat score are visible in the heat maps below (Table 6-4 and Table 6-5). The bank stability, embeddedness, and sediment deposition metrics can give evidence of sediment impairment. At the downstream monitoring station (1ASAN000.34), individual habitat metrics were marginal to poor (<11) for channel alteration, banks, bank vegetation, embeddedness, flow, riffles, riparian vegetation, sediment, and substrate. Of these individual metrics, low scores for embeddedness, sediment, and substrate generally imply excess sediment deposition. Low scores for channel alteration, banks, bank vegetation, and riparian vegetation generally indicate that the riparian corridor is highly impacted and provides conditions that favor erosion of the banks and instability of the stream channel. Additional observations that influenced habitat scores included embedded artificial riffle substrate, channelized banks, and dense filamentous algae.

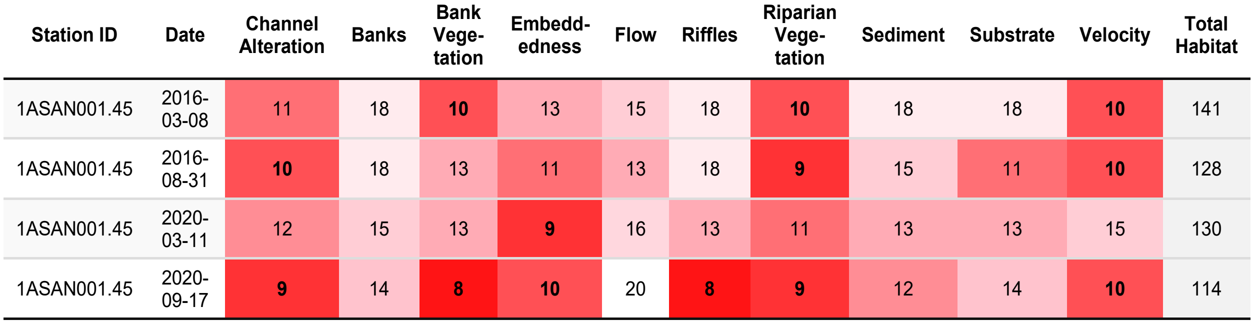
At the upstream station (1ASAN001.45), individual scores were slightly higher. Individual habitat metrics were marginal to poor (<11) for channel alteration, bank vegetation, embeddedness, riffles, riparian vegetation, and velocity. This is similar to the downstream station in that riparian conditions were marginal, however, instream indicators of deposited sediment (embeddedness, substrate, and sediment metrics) were slightly improved. Additional observations that influenced habitat scores included filamentous algae and the presence of clay-like material covering the stream substrate.

Table 6-4. Qualitative habitat measurements taken during biological monitoring at 1ASAN000.34. The lighter red colors represent habitat scores that are optimal or suboptimal and progressively darker red colors represent marginal or poor habitat scores.



Station ID	Date	Channel Alteration	Banks	Bank Vege-tation	Embedd-edness	Flow	Riffles	Riparian Vege-tation	Sediment	Substrate	Velocity	Total Habitat
1ASAN000.34	2016-03-08	**8**	11	**9**	11	15	13	**9**	12	**10**	16	114
1ASAN000.34	2016-08-31	**10**	**10**	15	**9**	18	16	**9**	11	**4**	18	120
1ASAN000.34	2020-03-11	**7**	**8**	**10**	12	**10**	**9**	**9**	14	**10**	14	103
1ASAN000.34	2020-09-17	**7**	**10**	**10**	**7**	20	**7**	**9**	**10**	**7**	14	101

Table 6-5. Qualitative habitat measurements taken during biological monitoring at 1ASAN001.45. The lighter red colors represent habitat scores that are optimal or suboptimal and progressively darker red colors represent marginal or poor habitat scores.



Station ID	Date	Channel Alteration	Banks	Bank Vege-tation	Embedd-edness	Flow	Riffles	Riparian Vege-tation	Sediment	Substrate	Velocity	Total Habitat
1ASAN001.45	2016-03-08	11	18	**10**	13	15	18	**10**	18	18	**10**	141
1ASAN001.45	2016-08-31	**10**	18	13	11	13	18	**9**	15	11	**10**	128
1ASAN001.45	2020-03-11	12	15	13	**9**	16	13	11	13	13	15	130
1ASAN001.45	2020-09-17	**9**	14	**8**	**10**	20	**8**	**9**	12	14	**10**	114

6.6.3 Log_{10} Relative Bed Stability Index (LRBS)

As a part of TMDL monitoring, DEQ conducted a detailed physical habitat assessment of Sand Branch according to EPA methods for Quantifying Physical Habitat in Wadeable Streams (Kaufmann *et al.*, 1999). This analysis is a type of siltation index that involved the measurement of channel dimensions and substrate composition at numerous transects within a 150 to 800 meter stream reach surrounding the downstream benthic monitoring station. The outcome of this analysis is the calculation of a Log_{10} Relative Bed Stability Index (LRBS). The LRBS is the ratio between the observed size distribution of in-stream sediments and the predicted sediment size distribution based on bankfull depth. The index was developed to differentiate between natural and anthropogenic sediment deposition in a watershed.

LRBS values near zero indicate that the stream is stable. Large negative values indicate that the stream is unstable and depositing excess sediment. Large positive numbers, while less common, indicate that the stream is unstable and sediment starved. LRBS scores less than -1.0 are considered sub-optimal, while scores greater than -0.5 are considered optimal. The LRBS index calculated for Sand Branch was -0.02. This indicates relatively stable conditions and based on DEQ's stressor thresholds indicates no probability of stress to aquatic life (Figure 6-13). However, in urban watersheds such as Sand Branch, positive or LRBS values near zero may be the result of flashier storm flow which erodes the banks and removes fine-grain sediment from the reach, armoring the streambed (Hill, 2007).

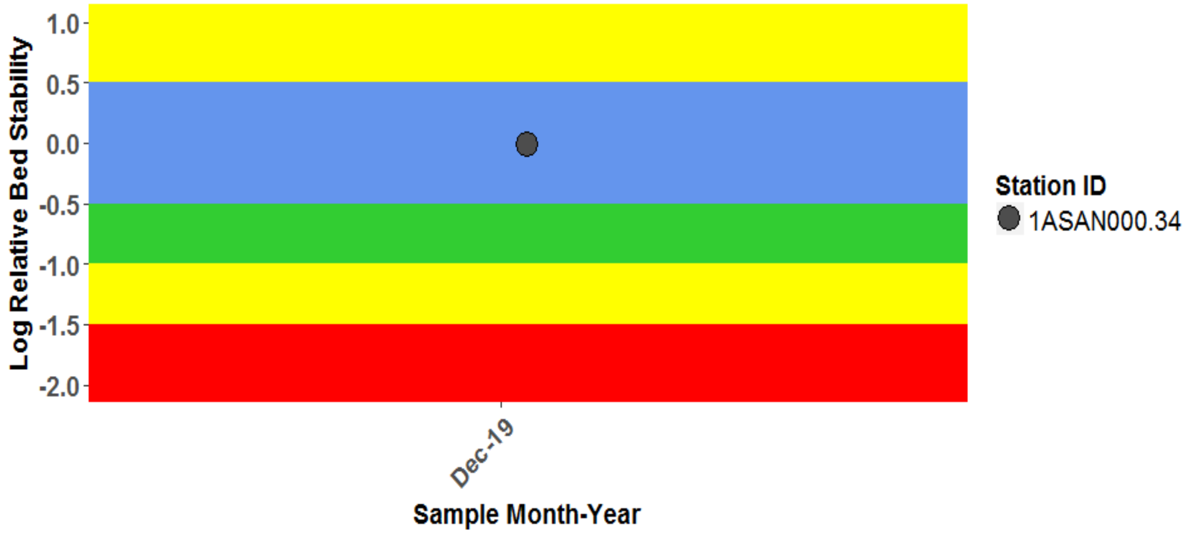


Figure 6-13. Log$_{10}$ Relative Bed Stability (unitless) calculated for downstream monitoring station 1ASAN000.34, sampled on December 17, 2019.

The individual metrics collected and used to calculate the LRBS score provides additional information on the characteristics of the channel substrate. At the Sand Branch study site 1ASAN000.34, approximately 80% of the substrate (43% boulders, cobbles and gravel, 22% hardpan and 15% concrete or asphalt) is comprised of a hardened substrate. Only 12% of the substrate is comprised of sands and fines. This type of substrate tends to be more characteristic of streams located in steeper terrain or in the fall zone between the coastal plain and the piedmont and therefore, could be an indication that the stream is hardening. When these values are compared to data collected through DEQ's Freshwater Probabilistic Program, the values for sands and fines falls in the 12th and 14th percentiles for the Northern Piedmont region and statewide data, respectively. For boulders, cobbles and gravel, Sand Branch falls into the 52nd and 49th percentiles for the Northern Piedmont region and statewide data, respectively. Table 6-6 provides the individual metrics that comprise the LRBS score.

Table 6-6. LRBS Individual Metrics for 1ASAN000.34.

LRBS Metrics	Value
LRBS	-0.02
LRBS Percentile[1] (Northern Piedmont / Statewide)	76th / 79th
Geometric Mean of the Particle Substrate (logged)	1.55
Particle Substrate Percentile[1]	83th / 77th
Slope	0.68
Slope Percentile[1]	58th / 47th
% Sands and Fines	12%
Percentile Sands and Fines[1]	12th / 14th

LRBS Metrics	Value
% Boulders, Cobbles, Gravel	43%
Percentile Boulders, Cobbles, Gravel[1]	52th / 49th
% Hardpan	22%
% Concrete or Asphalt	15%
Average Embeddedness	38%
Percentile Embeddedness	18th / 21th
Streambank incision depth (m)	2.10

[1] Based on DEQ Probabilistic Monitoring data

6.7 Specific Conductivity

Specific conductance is a measure of how well water can conduct an electrical current based on the amount of ions in water. Regional geology has great bearing on the ions in a water body. Therefore, water flowing through materials that easily dissolve into their ionic constituents will most likely have a higher conductivity.

Measurements of conductivity are typically converted to specific conductance (or specific conductivity) at a standard temperature (25ºC) because conductivity changes with temperature. This allows conductivity measurements made at different temperatures to be compared on a common basis. All data presented in this section represents specific conductivity converted to the 25ºC standard, however, the term conductivity may be used to generally refer to the data. While conductivity is a direct measure of electrical conductance, it is an indirect measure of the collective influence of dissolved ions in the water. It is this property of the water that has greater meaning for the analysis of stressors in Sand Branch. As the sum of all dissolved ions in a water increases, so too does the conductivity. It is the chemical action of these ions (both individually and collectively) and not necessarily the conductive property of the water that produces toxic effects on freshwater organisms if above certain thresholds.

Specific conductivity in Sand Branch ranged from 361.0 – 1159.0 µS/cm, with an average of 817 µS/cm and a median of 827 µS/cm (n=55) in periodic measurements (Figure 6-14). The data measured ranged from medium probability to high probability of stress to aquatic life, with the average and median values indicting a high probability of stress.

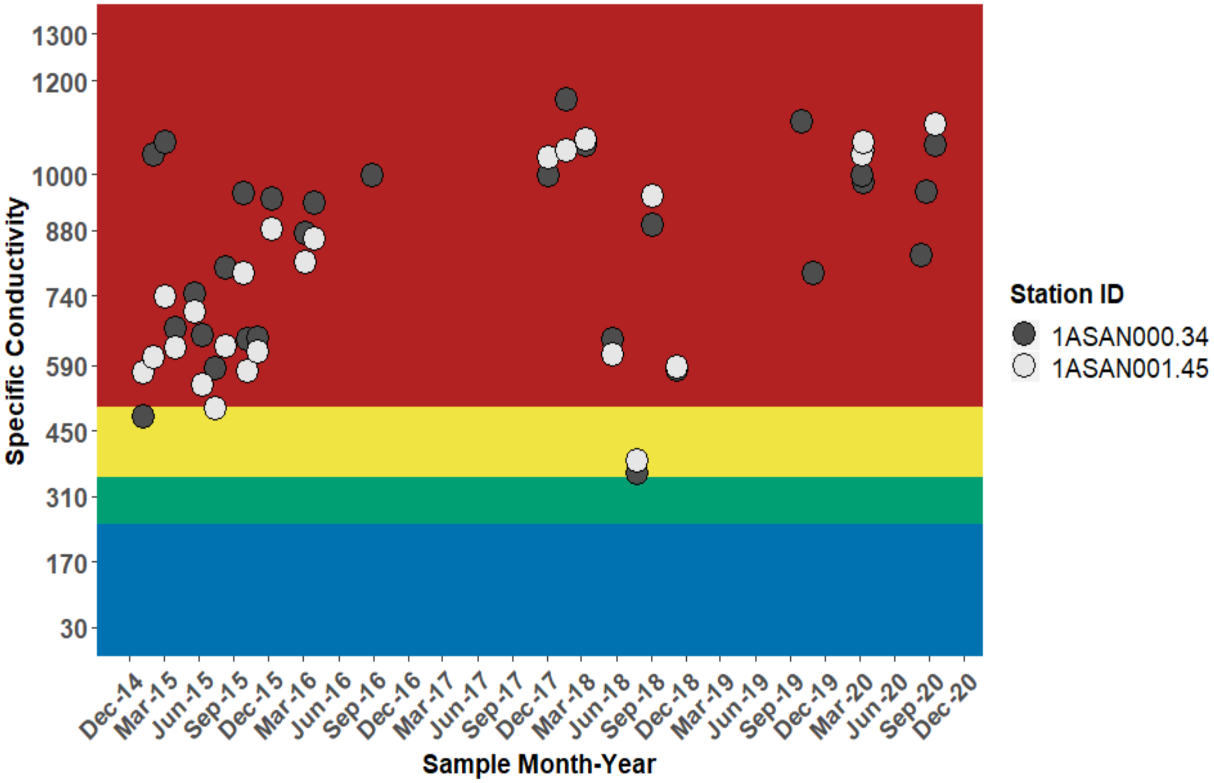


Figure 6-14. Specific conductance (µS/cm) measurements at 1ASAN000.34 and 1ASAN001.45 from January 2015 – September 2020.

These conductivity levels are sufficient to cause toxicity to freshwater organisms and exceed benchmarks developed by USEPA (2011) and Cormier *et al.* (2018). USEPA (2011) developed a field-based aquatic life benchmark for conductivity in Appalachian streams impacted by coal mining. A benchmark of 300 µS/cm was determined to represent the exposure level at which 5% of macroinvertebrate genera are extirpated from streams. Cormier *et al.* (2018) developed a similar criterion chronic concentration (CCC) of 310 µS/cm for ecoregion 69 based on average stream conditions and an annual maximum conductivity threshold of 630 µS/cm. In addition, Pond (2004) showed that on surface mined lands, *Ephemeroptera* taxa decreased significantly at conductivity levels much above 500 µS/cm. In comparison to ecological benchmarks, specific conductivity levels in Sand Branch are consistently high enough to cause toxicity to aquatic organisms and alter the composition of benthic macroinvertebrate communities.

6.7.1 Site Comparisons

At the discharge point of Chantilly Crushed Stone, Inc. (VPDES Permit No. VAG840106), which forms the headwaters of Sand Branch, specific conductivity averaged 873 µS/cm with a median of 900 µS/cm (Figure 6-15). In Sand Branch, average and median conductivities were 777 and 737 µS/cm at the upstream station (1ASAN001.45, n=25) and 851 and 914 µS/cm at the downstream station (1ASAN000.34, n=30), respectively. In Cub Run, the average and median conductivities above the confluence with Sand Branch (1ACUB011.78) were 767 and 703 µS/cm. Below the confluence with Sand Branch (1ACUB011.25), average and median conductivities were 739 and 782 µS/cm, respectively. None of these sites differed statistically in a one-way ANOVA with alpha of 0.05 (Figure 6-15). Conductivity varied more within sites based on changing hydrologic conditions than between sites. However, given equivalent hydrologic conditions established by sampling sites on the same day, typical conductivity patterns reveal slight increases in conductivity from the upstream to the downstream Sand Branch stations (approximately 8% increase) and large increases in Cub Run from above to below the confluence with Sand Branch (approximately 75% increase). On 11/19/2019, the Occoquan Watershed Monitoring Laboratory conducted a synoptic survey of conductivities in the Cub Run watershed (Figure 6-16). This survey revealed that the conductivity rose from 479 µS/cm above the confluence with Sand Branch to 837 µS/cm below the confluence (S. Bhide, personal communication, January 28, 2021). On that day, the conductivity of Sand Branch was 1078 µS/cm. This indicates a significant influence of Sand Branch on Cub Run.

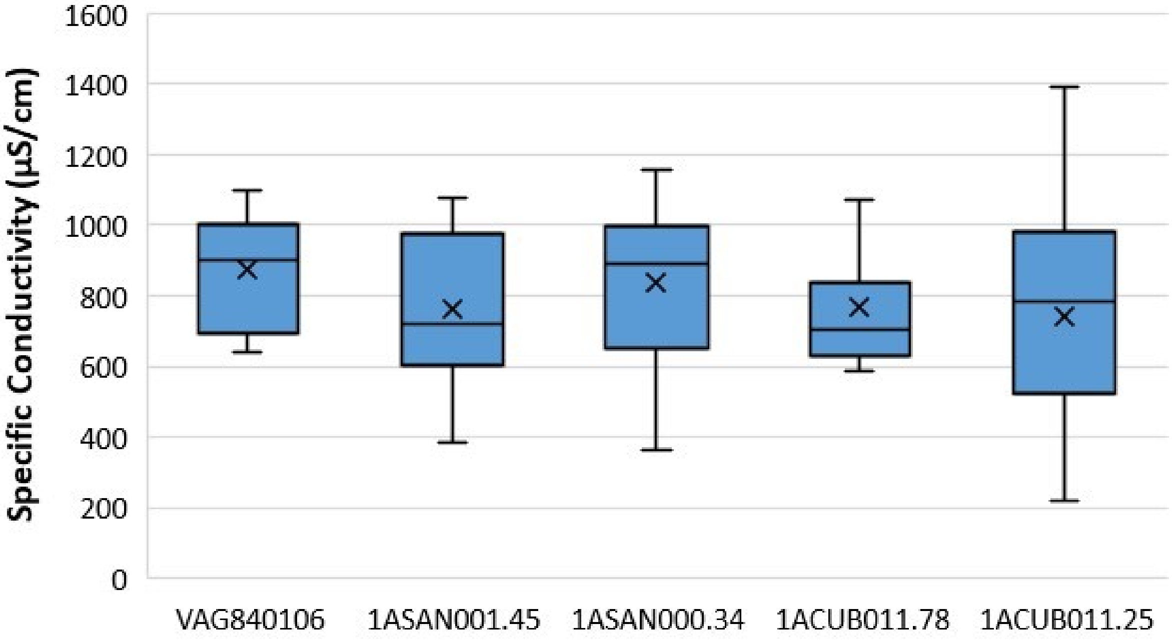


Figure 6-15. Specific conductivity at various stations on Sand Branch and Cub Run and the headwater source discharger.

Figure 6-16. Synoptic survey of conductivity in Cub Run watershed on 11/19/2019 (Occoquan Watershed Monitoring Laboratory, 2019).

6.7.2 Continuous Monitoring

During a two-week period in August 2020 and from December 2020 – February 2021, conductivity was monitored continuously at the downstream station (1ASAN000.34). Specific conductance was measured at 15-minute intervals and total rainfall, measured in one-hour increments, was measured at Dulles International Airport. In August 2020, specific conductivity ranged from 395.2 to 981.3 µS/cm and averaged 852.2 µS/cm (Figure 6-17). In December 2020 to February 2021, specific conductivity ranged from 308 to 3371 µS/cm and averaged 1034 µS/cm (Figure 6-18). During these two time periods, it was obvious that conductivity levels were greatly influenced by storm events. This was evident from correlated precipitation data and stream depth data. During most storm events, conductivity levels would drop precipitously as low conductivity rainwater entered the system. Between storm events, conductivity levels would steadily rise as rainfall driven sources decreased in influence and groundwater and continuous point sources increased in influence. During dry time periods, conductivity levels would typically rise to a baseline level of 900 – 1200 µS/cm. This is an indication that the source of ions to Sand Branch are primarily from underlying geology and continuous point source discharges and not from non-point sources.

However, during winter storm events when deicing salts are added to roadways, conductivity spikes were observed as stormwater from non-point sources introduced additional ions to Sand Branch. Snowfall recorded at Dulles Airport on 12/16/20, 1/25/21, 1/31/21-2/2/21, and 2/7/21 all corresponded to conductivity spikes over 1500 µS/cm. During the largest snowfall of 5.7 inches on 1/31/21-2/2/21, conductivity remained above 1500 µS/cm for more than three days and peaked at 3370 µS/cm.

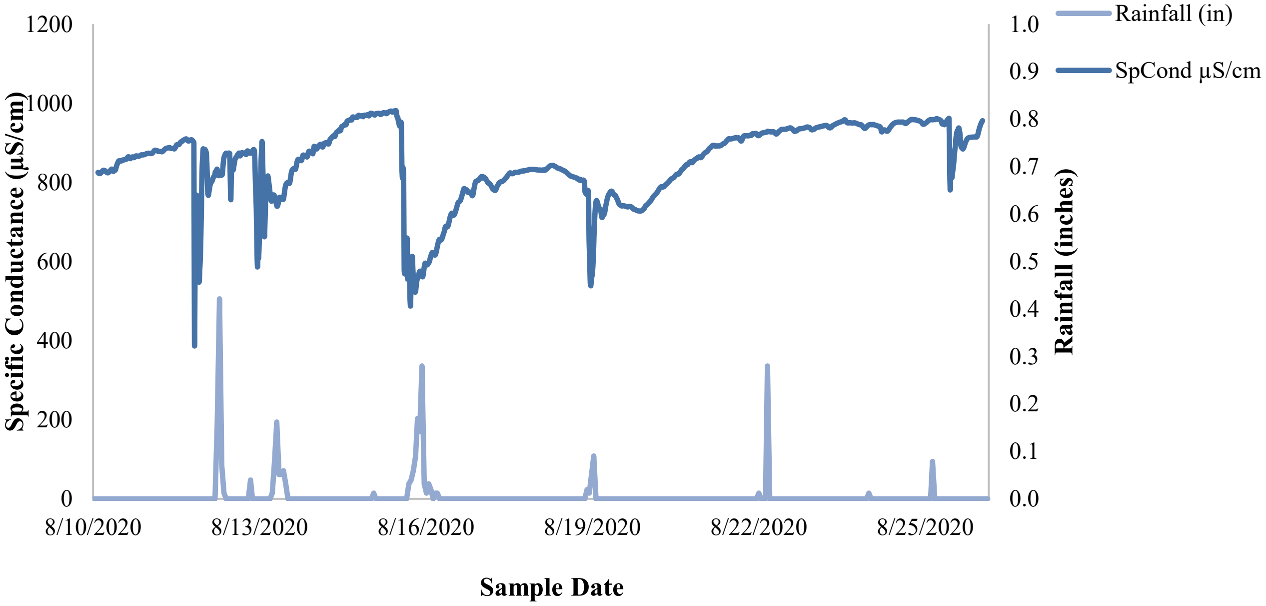


Figure 6-17. Continuous monitoring of specific conductance (µS/cm) at 1ASAN000.34 from August 10, 2020 – August 26, 2020.

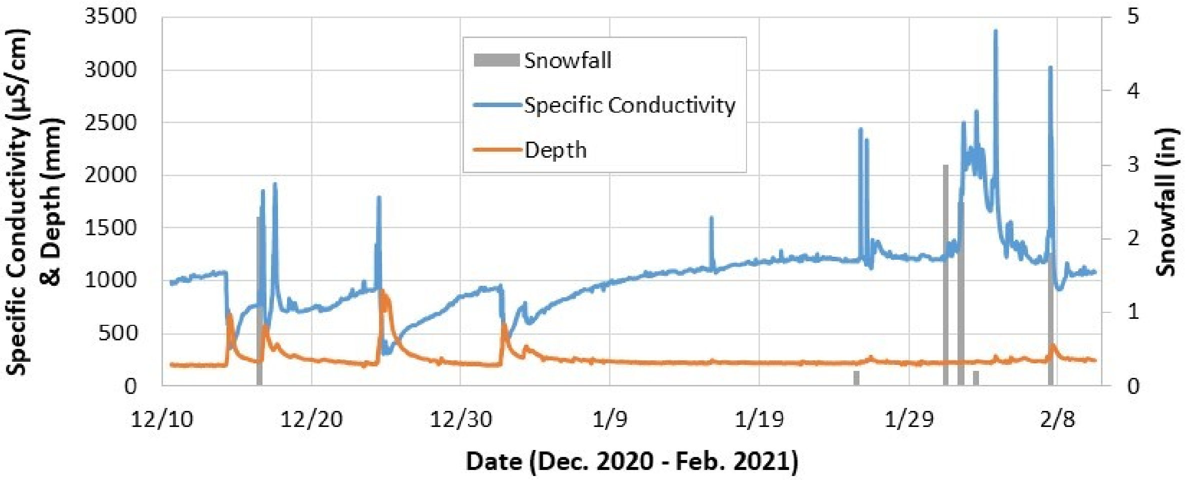


Figure 6-18. Continuous monitoring of specific conductance (µS/cm) at 1ASAN00.34 from December 10, 2020 – February 10, 2021.

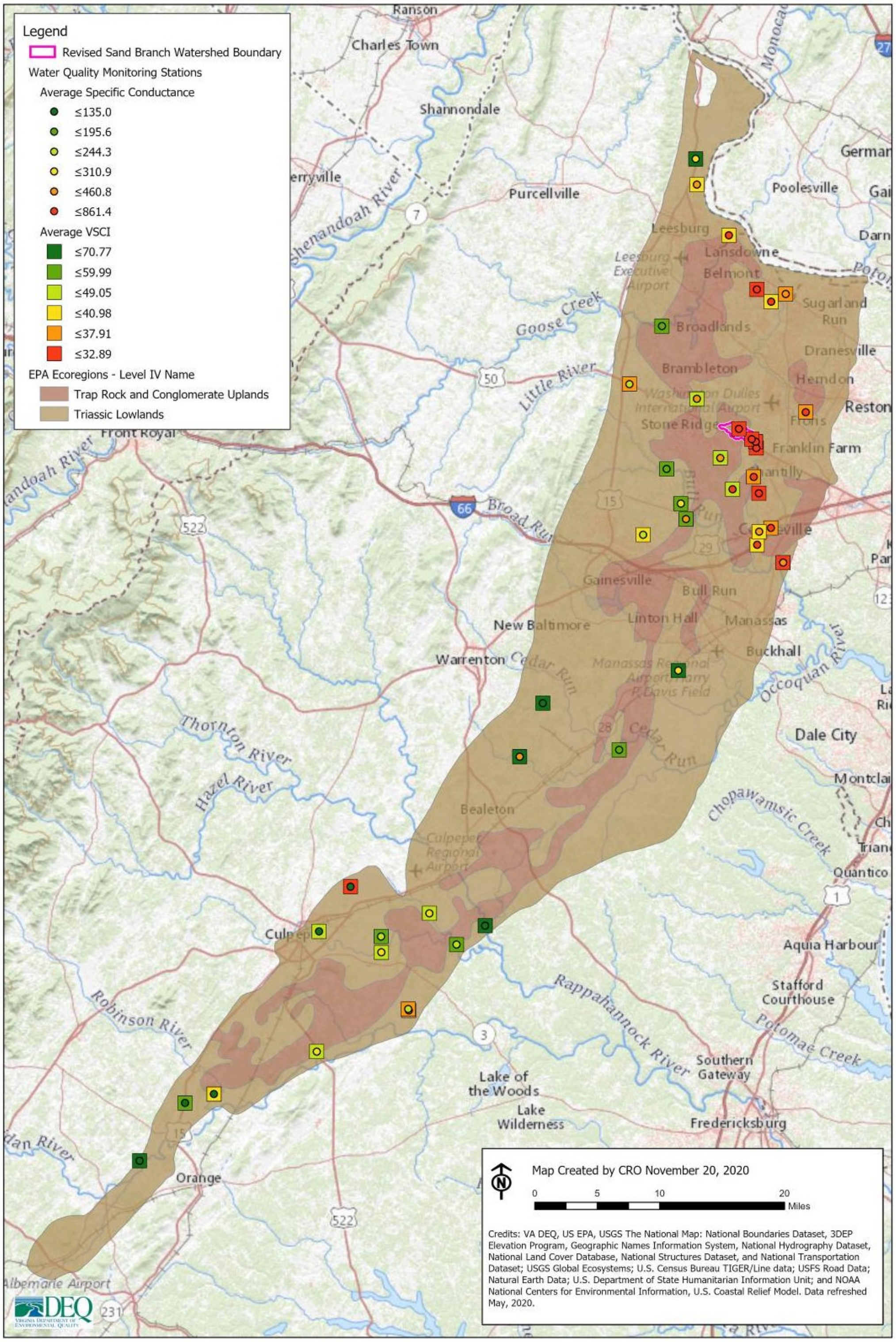
Specific conductance and stream depth were measured at 15-minute intervals. Daily snowfall was recorded at Dulles Airport.

In summary, the pattern observed from continuous conductivity monitoring indicates that Sand Branch maintains a high baseline conductivity level from underlying geology and continuous point source discharges. Non-point sources add to the ion load particularly during winter storm events, when deicing salts are applied in the watershed.

6.7.3 Underlying Geology

The Sand Branch watershed lies within the Trap Rock and Conglomerates Uplands and Triassic Lowlands ecoregions. There are 43 DEQ benthic monitoring stations within this same ecoregion (Figure 6-19). Among these stations, specific conductivity varied widely, averaging from 63.94 to 861.4 µS/cm. The two Sand Branch stations had the highest conductivity of any stations within this ecoregion. Sand Branch station 1ASAN001.45 represented the 98th percentile and 1ASAN000.34 represented the 100th percentile of average conductivity among stations within the ecoregion (Figure 6-20). The next highest conductivity was in Cub Run (95th percentile), to which Sand Branch discharges. While underlying geology in this ecoregion naturally produces higher conductivity than the broader Northern Piedmont region, conductivity levels in Sand Branch are exceptionally high even among stations within the same geology.

VSCI scores among stations in the Trap Rock and Conglomerates Uplands and Triassic Lowlands ecoregions ranged from 23.5 to 70.8. No station with average conductivity above 353 µS/cm exhibited unimpaired conditions of VSCI scores above 60. In fact, VSCI scores were negatively correlated with conductivity (Figure 6-21). The regression of VSCI scores with specific conductivity was statistically significant ($p<0.0001$) with an r^2 of 0.4855. While many other factors may influence biological stream health, this is one indication that specific conductivity may play a significant role within this ecoregion. This may be particularly true for Sand Branch, where conductivity is extremely high even among stations within the same geology.



Credits: VA DEQ, US EPA, USGS The National Map: National Boundaries Dataset, 3DEP Elevation Program, Geographic Names Information System, National Hydrography Dataset, National Land Cover Database, National Structures Dataset, and National Transportation Dataset; USGS Global Ecosystems; U.S. Census Bureau TIGER/Line data; USFS Road Data; Natural Earth Data; U.S. Department of State Humanitarian Information Unit; and NOAA National Centers for Environmental Information, U.S. Coastal Relief Model. Data refreshed May, 2020.

Figure 6-19. Average specific conductivity and stream condition index scores for stations in the Trap Rock and Conglomerates Uplands and Triassic Lowlands ecoregions.

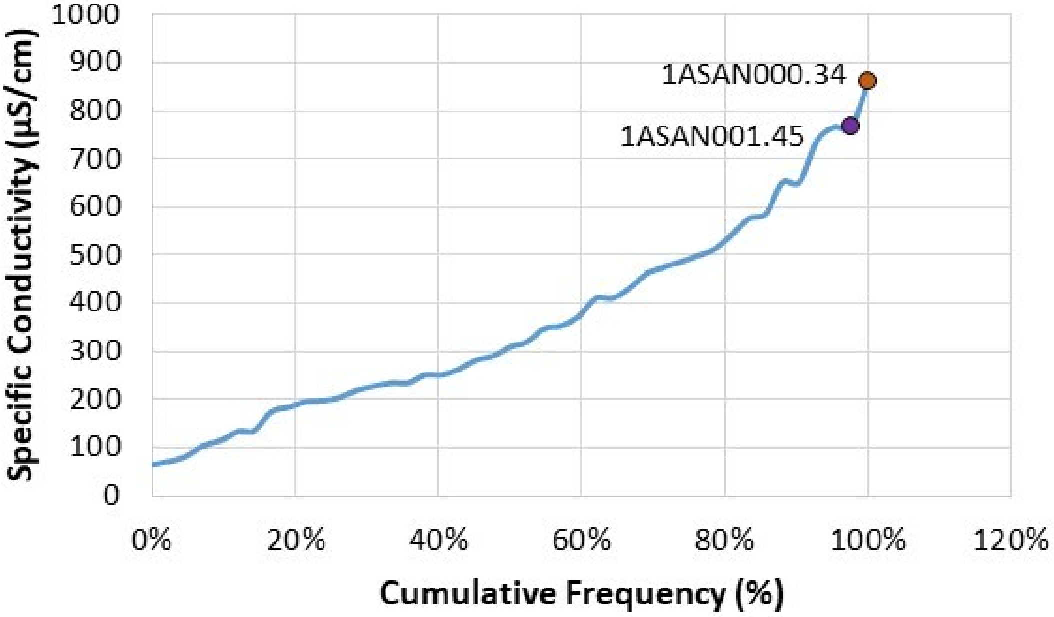


Figure 6-20. Cumulative distribution of average conductivity among stations in the Trap Rock and Conglomerate Uplands and Triassic Lowlands ecoregions.

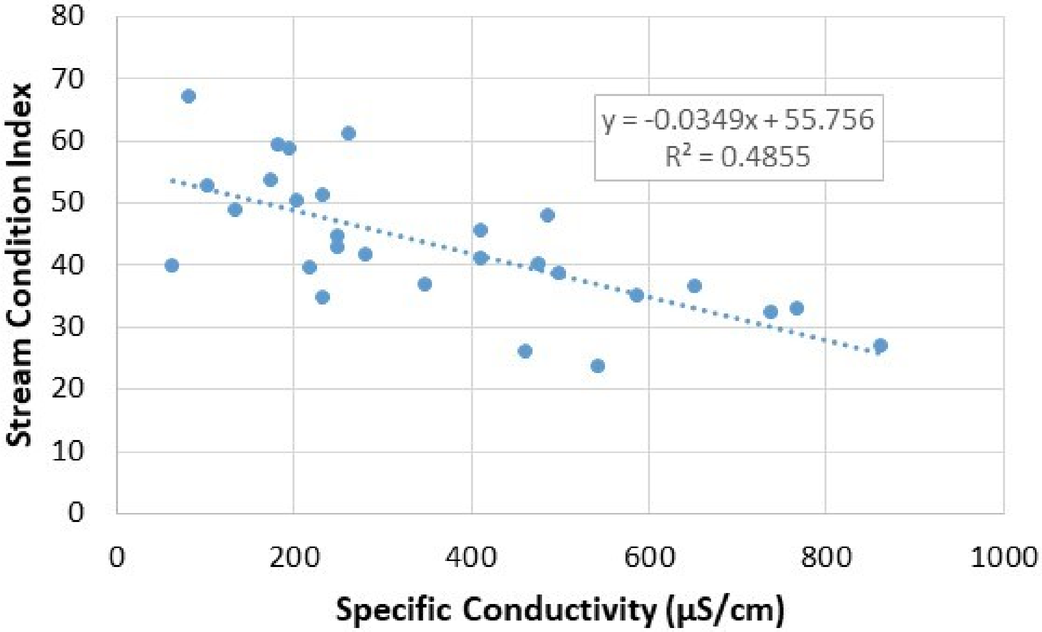


Figure 6-21. Regression of Stream Condition Index and specific conductivity for stations in the Trap Rock and Conglomerates Upland and Triassic Lowlands ecoregions. Stations with fewer than ten conductivity measurements were excluded.

6.7.4 Ion Composition

Conductivity is a direct measure of electrical conductance through the water and an indirect measure of the collective influence of dissolved ions in the water. Because different ions have varying toxicities and may differ in amount, the specific ion composition of Sand Branch, the origination of its headwaters, and Cub Run were analyzed (Figure 6-22 and Figure 6-23). Various cations and anions were measured and compared on a milliequivalent basis to eliminate the influence of charge and atomic mass. The cations calcium (Ca^{+2}), magnesium (Mg^{+2}), sodium (Na^{+}), and potassium (K^{+}) were analyzed, and the anions sulfate (SO_4^{-2}), bicarbonate (HCO_3^{-}), chloride (Cl^{-}), and nitrate (NO_3^{-}) were analyzed. These major ions accounted for most of the ions in these samples, since charge balances were within 10% of neutral for each location, with the exception of the downstream Cub Run station (1ACUB011.25), which had a charge balance of +10.3%. The charge balance for all stations was positive, from +0.1% to +10.3% indicating that there may be additional anions unaccounted for in the analysis, but the contribution of these missing anions is likely small (less than about 10%).

Based on the ion composition analysis, both Sand Branch stations revealed a consistent pattern of ion composition. Cations were comprised of approximately 50% Ca^{+2}, 36% Mg^{+2}, 13% Na^{+}, and 1% K^{+}. Anions were comprised of approximately 66% SO_4^{-2}, 26% HCO_3^{-}, 7% Cl^{-}, and 1% NO_3^{-}. This indicates a primarily calcium or magnesium carbonate and calcium or magnesium sulfate composition, which could come from the dissolution of underlying geology. This pattern of ion composition in Sand Branch was consistent with the pattern of ions in the Chantilly Crushed Stone, Inc. (VPDES Permit No.VAG840106) discharge, which comprises the headwaters of Sand Branch.

There are slight increases in the presence of three ions at the downstream Sand Branch site in comparison to the upstream site. The contribution of sodium increases from 11% at the upstream site to 15% at the downstream site, potassium increases from <1% to 1.5%, and chloride increases from 4% to 9%. The higher contributions of sodium, potassium, and chloride may be due to other watershed sources, such as deicing salts, which increase the contributions of these ions at the downstream station.

The ion composition of Cub Run above and below the confluence with Sand Branch depicts the influence of Sand Branch on Cub Run water quality. Above the Sand Branch confluence on Cub Run (1ACUB011.78), overall ion concentrations were much lower, with cation concentrations averaging 6.7 mEq/L compared to 11.1 mEq/L at the downstream Sand Branch station. Below the confluence with Sand Branch (1ACUB011.25), Cub Run cation concentrations increased to 9.3 mEq/L, reflecting the increased ion load from Sand Branch. Above the Sand Branch confluence, Cub Run had much lower contributions of magnesium (22%) and sulfate (30%) and much higher contributions of sodium (27%), bicarbonate (44%), and chloride (26%). At the downstream Cub Run station, the ion contributions represent a predictable equilibration between Cub Run and Sand Branch flows.

The toxicity of ion mixtures is difficult to determine due to varying concentrations and relative toxicities of the individual constituents as well as the combined osmotic effects of the overall ionic strength. Goodfellow *et al.,* (2000) tried to evaluate the relative toxicity of individual ions and predict the collective effects on freshwater invertebrates and fish. In general, they found that the relative order of toxicity was $K^{+} > HCO_3^{-} \sim Mg^{+2} > Cl^{-} > SO_4^{-2}$, with the toxicities of Na^{+} and Ca^{+2} dependent upon the associated anions. Based on this understanding, the most prevalent ions in Sand Branch (Ca^{+2} and SO_4^{-2}) are not the ions that are most toxic, and those that are most toxic (K^{+} and HCO_3^{-}) are at lower levels in Sand Branch than at the upstream Cub Run location (1ACUB011.78). This does not negate the fact that the overall high ion concentrations and resulting conductivity in Sand Branch may produce combined toxic effects through difficulties in osmotic regulation for freshwater aquatic life.

In summary, the ion composition analysis revealed a calcium/magnesium and carbonate/sulfate dominated solution, which is consistent with the ion composition of the headwaters of Sand Branch, formed by the discharge from Chantilly Crushed Stone, Inc. (VPDES Permit No.VAG840106). Other ions more commonly attributed to urban influences (Na^{+}, K^{+}, and Cl^{-}) are added as Sand Branch moves downstream. The ion composition analysis also revealed that Sand Branch has a significant impact on the composition and water quality of Cub Run below their confluence (1ACUB011.25).

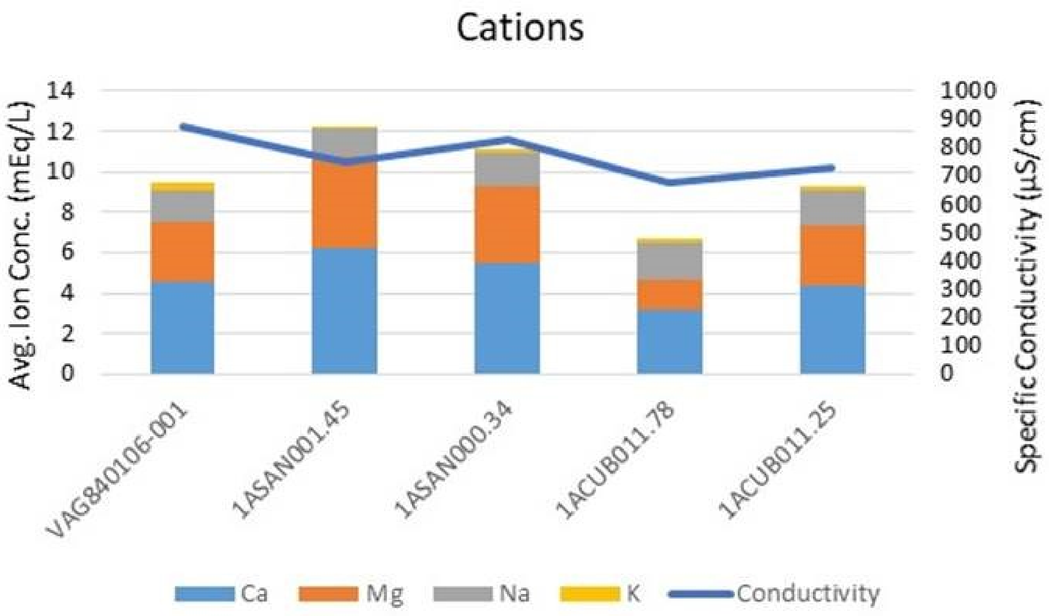


Figure 6-22. Cation composition in Sand Branch, Cub Run, and the headwater source discharger.

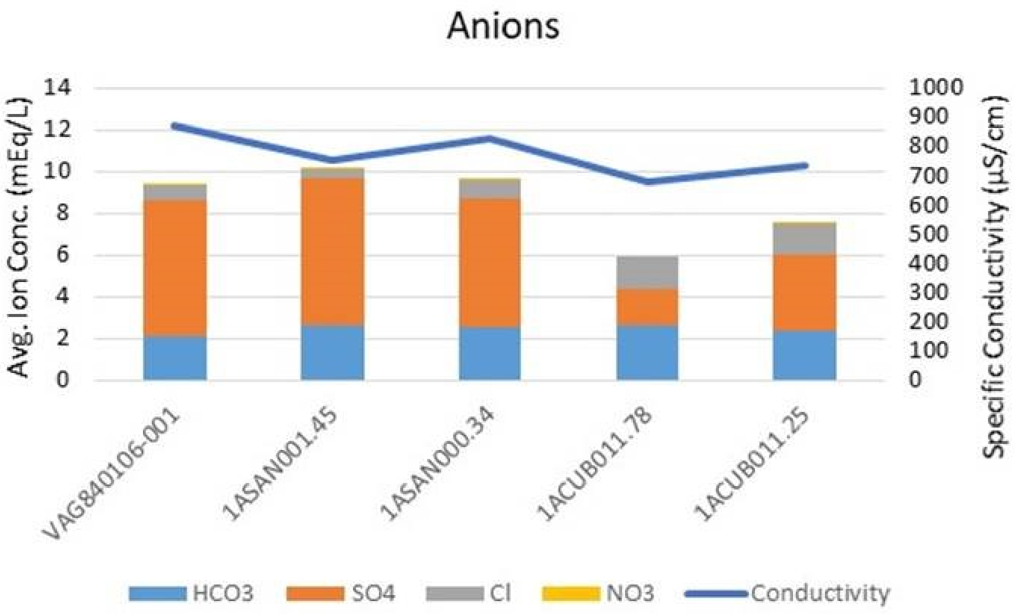


Figure 6-23. Anion composition in Sand Branch, Cub Run, and headwater source discharger.

6.8 Total Dissolved Solids

Total dissolved solids (TDS) is a measurement of the concentration of dissolved ions in a waterbody. Although there are no water quality standards for TDS, EPA Secondary Regulations establish a (non-enforceable) TDS level of 500 mg/L for aesthetic and corrosion prevention purposes (40 CFR §141.208).

Although a TDS level of above 350 mg/L is considered to have a high probability of aquatic stress in Virginia, there are streams with naturally high levels of TDS from underlying geology. However, analyzing a suite of ions that are associated with anthropogenic activities, like sulfate, chloride, sodium, and potassium, can help to understand the cause(s) for elevated TDS concentrations.

TDS measurements in Sand Branch ranged from 290 - 895 mg/L, with an average concentration of 568 mg/L and a median concentration of 576 mg/L (n=51) (Figure 6-24). The data measured ranged from medium to high probability of stress to aquatic life, with the median and average values indicating a high probability of stress.

At the upstream monitoring station, 1ASAN001.45, the average and median TDS concentrations were 556 mg/L and 518 mg/L (n=24). The data measured ranged from low to high probability of stress, with the average and median values indicating a high probability of stress to aquatic life.

At the downstream monitoring station, 1ASAN000.34, the average and median TDS concentrations were 579 mg/L and 600 mg/L (n=27). The data measured ranged from medium to high probability of stress to aquatic life, with the average and median values indicating a high probability of stress.

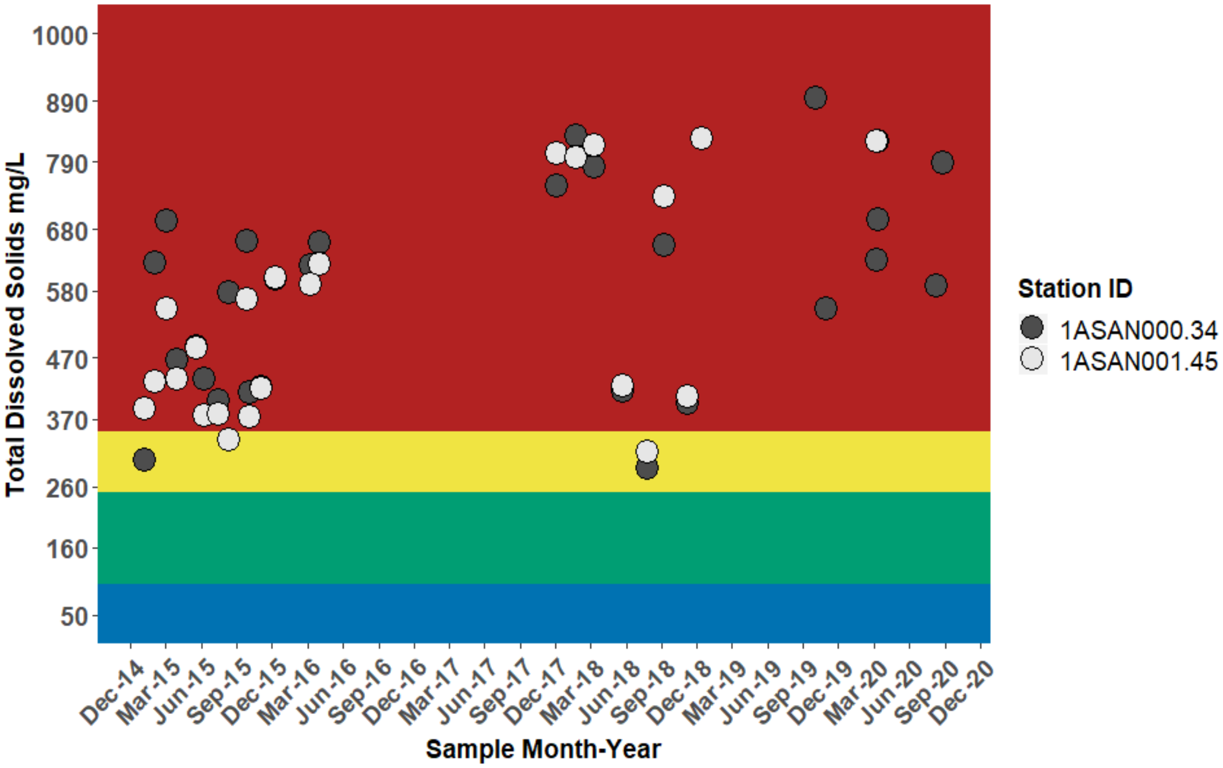


Figure 6-24. Total dissolved solids (mg/L) measurements at 1ASAN000.34 and 1ASAN001.45 from January 2015 – September 2020.

During the period of continuous monitoring in August 2020, TDS (mg/L) was computed from the measurements of conductivity and temperature. The deployed sonde measured conductivity and temperature at 15-minute intervals during the deployment period and computed TDS from these measured values. The computed TDS values were evaluated to understand the relative changes in TDS over the deployment period, especially with regard to precipitation and changes in stream conductivity and TDS associated with storm events. Several rain events occurred during the deployment period, which were measured in total inches of rainfall in one-hour increments at Dulles International Airport. The calculated TDS values decreased during those rain events, with one exception. These decreases in TDS during rain events may indicate that wet weather is diluting the concentration of TDS in Sand Branch.

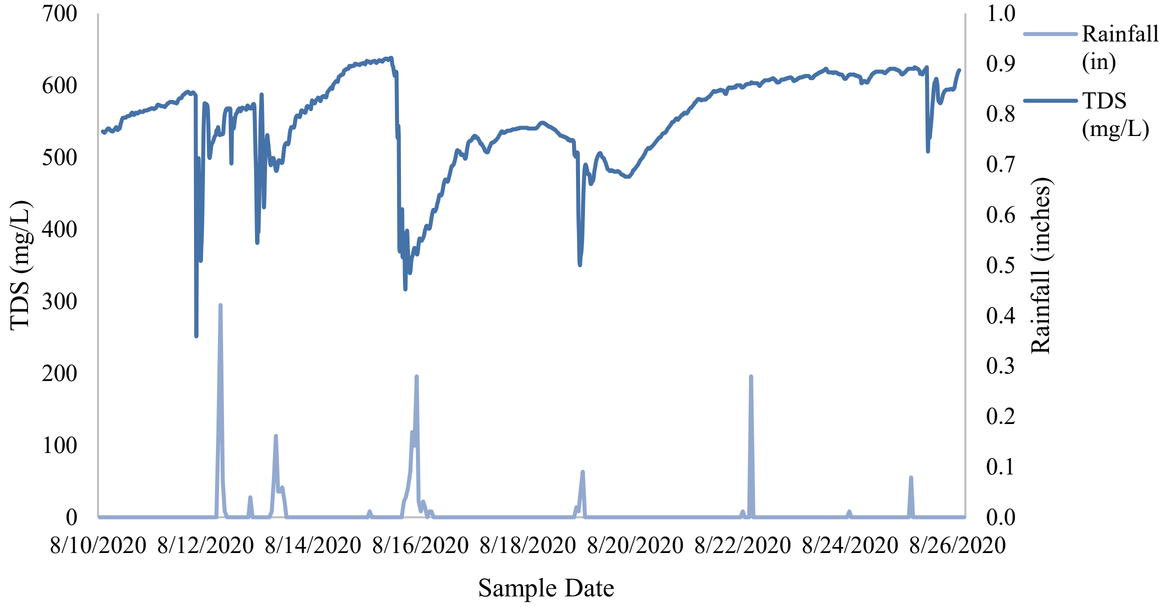


Figure 6-25. Computed TDS values (mg/L) from conductivity and temperature measurements taken during continuous monitoring at 1ASAN000.34 from August 10, 2020 – August 26, 2020.

DEQ evaluated the relationship between TDS and specific conductivity in split samples. Figure 6-26 shows that the two parameters are highly correlated, with an R^2 of 0.92. Based on the strength of this relationship, TDS can be estimated in Sand Branch from measured specific conductivity values. The y-intercept of the regression line was set to 0.055 to represent the typical conductivity of deionized water (0.055 µS/cm).

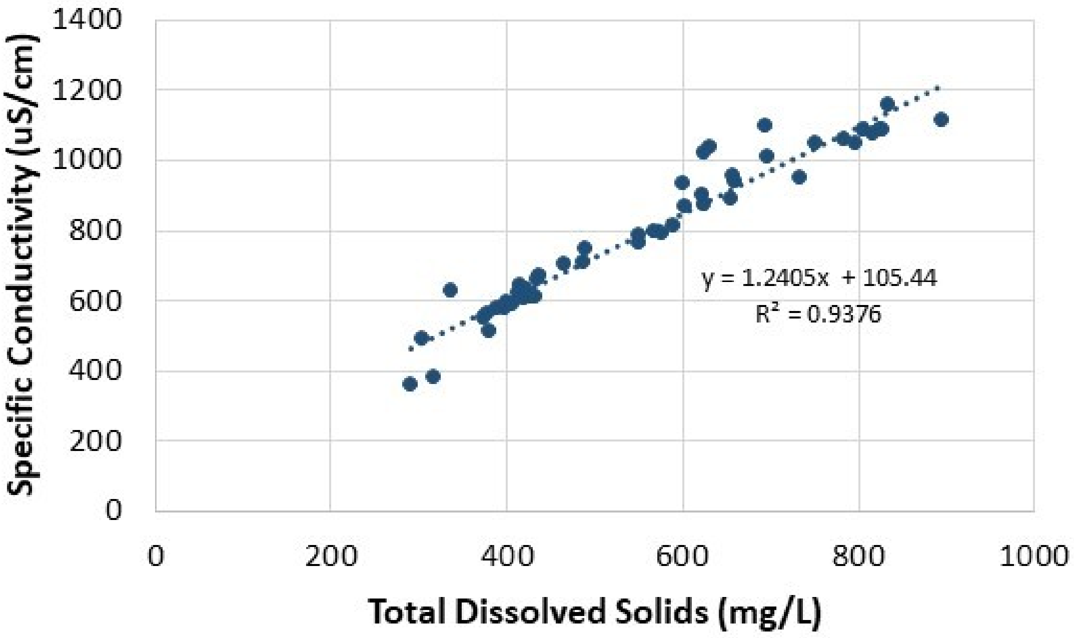


Figure 6-26. Correlation between total dissolved solids and specific conductivity.

6.9 Dissolved Ions

A suite of ions associated with anthropogenic activities were analyzed. These are sulfate, chloride, sodium, and potassium.

6.9.1 Sulfate

Sulfate measurements in Sand Branch ranged from 21 – 463 mg/L, with an average concentration of 239 mg/L and a median concentration of 210 mg/L (n=49) (Figure 6-27). The majority of the data measured fell into the high probability of stress to aquatic life range, with a few data points falling in the low and medium range. The average and median values indicate a high probability of stress.

At the upstream monitoring station, 1ASAN001.45, the average and median sulfate concentrations were 246 mg/L and 220 mg/L (n=23). The data measured indicated a high probability of stress to aquatic life, with the exception of one measurement which fell in the low probability category.

At the downstream monitoring station, 1ASAN00.34, the average and median sulfate concentrations were 232 mg/L and 209 mg/L (n=26). The data measured indicated a high probability of stress to aquatic life, except two measurements which indicated a medium probability of stress.

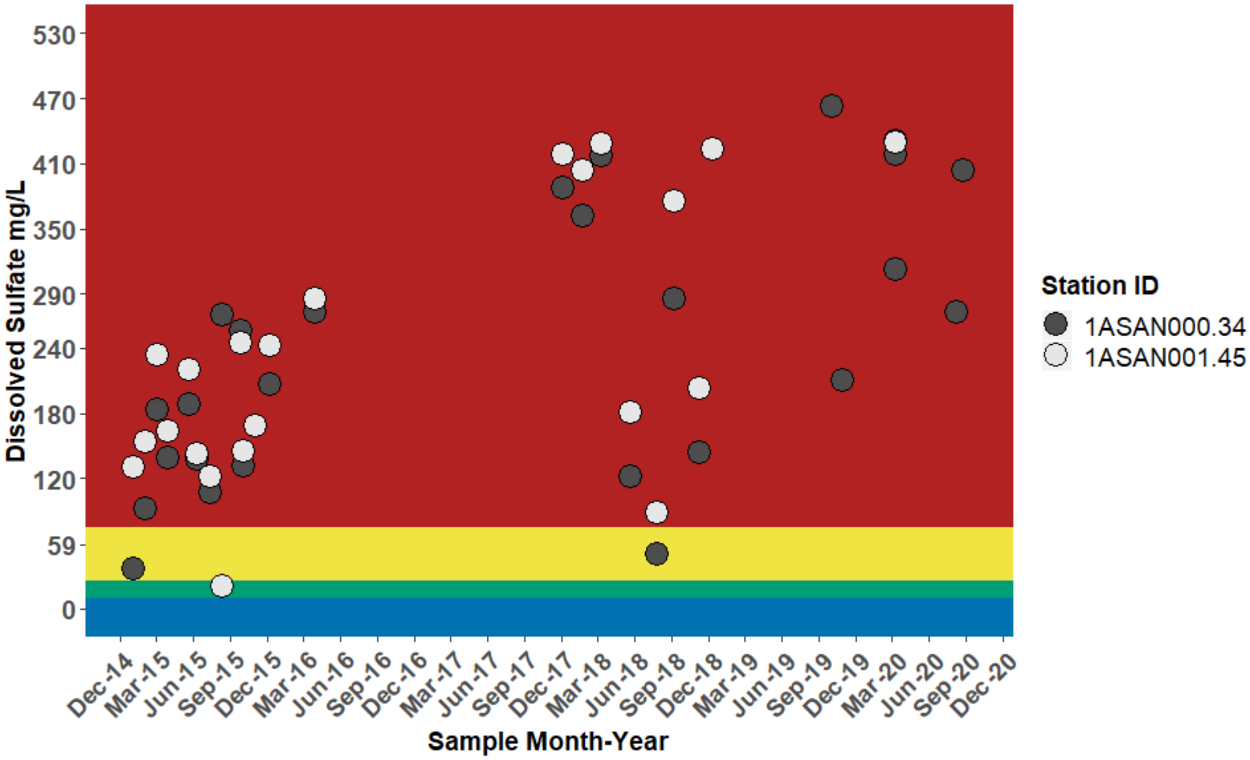


Figure 6-27. Sulfate (mg/L) measurements at 1ASAN000.34 and 1ASAN001.45 from January 2015 – September 2020.

6.9.2 Chloride

Chloride measurements in Sand Branch ranged from 9 – 180 mg/L, with an average concentration of 33 mg/L and a median concentration of 20 mg/L (n=51) (Figure 6-28). The data measured ranged from low to high probability of stress to aquatic life, with the average and median concentrations indicating a medium and low probability of stress, respectively.

At the upstream monitoring station, 1ASAN001.45, the average and median chloride concentrations were 18 mg/L and 14 mg/L (n=24). All data measured indicated low probability of stress to aquatic life.

At the downstream monitoring station, 1ASAN000.34, the average and median chloride concentrations were 46 mg/L and 33 mg/L, respectively (n=27). The data measured indicated a low to high probability of stress to aquatic life, with the average and median concentrations indicating a medium probability of stress.

This analysis indicates that the probability of stress to aquatic life from chloride is greater at the downstream monitoring station than it is at the upstream monitoring station. However, all the measured values are less than the freshwater chronic aquatic life water quality criterion of 230 mg/L.

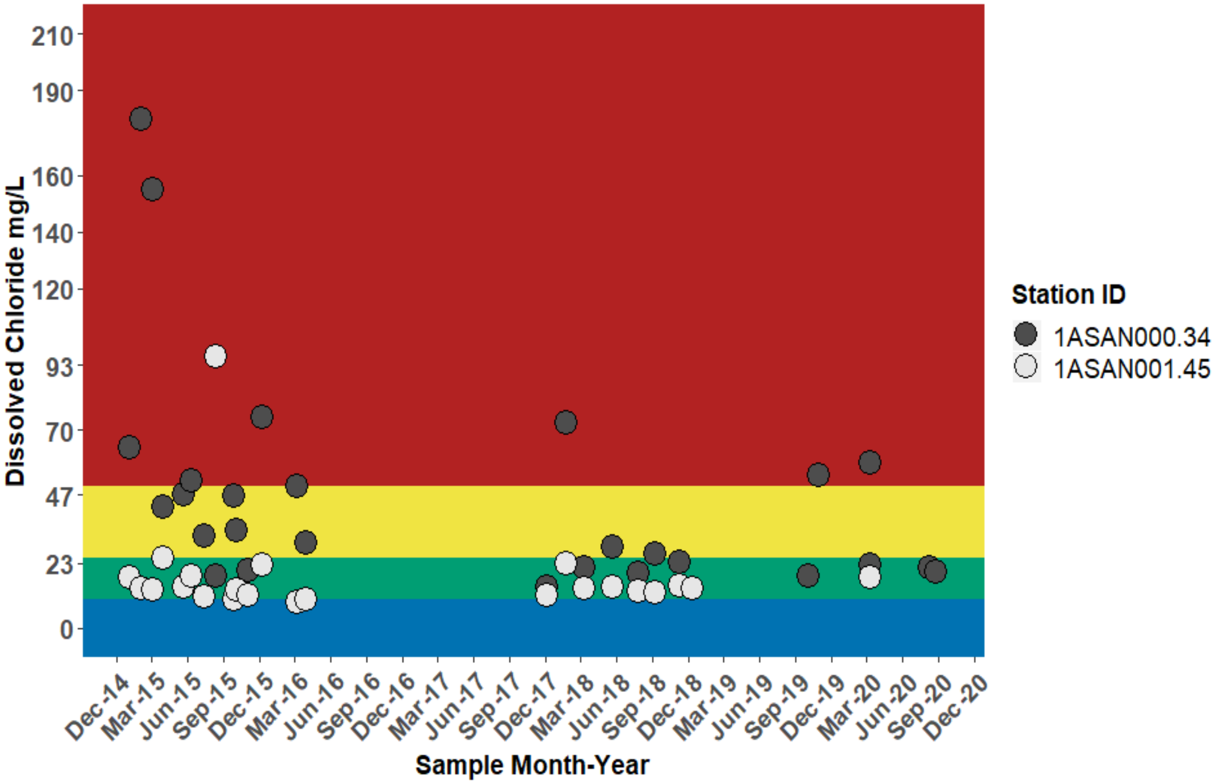


Figure 6-28. Chloride (mg/L) measurements at 1ASAN000.34 and 1ASAN001.45 from January 2015 – September 2020.

6.9.3 Potassium

Potassium measurements in Sand Branch ranged from 1.2 - 21.2 mg/L, with average and median concentrations of 4.2 mg/L and 2.4 mg/L, respectively (n=24) (Figure 6-29). The data measured ranged from low to high probability of stress to aquatic life, with the average and median concentrations indicating a low probability of stress.

At the upstream monitoring station, 1ASAN001.45, the average and median potassium concentrations were 1.7 mg/L and 1.6 mg/L (n=10). The data measured ranged from low to medium probability of stress to aquatic life, with the average and median concentrations indicating a low probability of stress.

At the downstream monitoring station, 1ASAN000.34, the average and median potassium concentrations were 6.0 mg/L and 4.5 mg/L (n=14). The data measured ranged from medium to high probability of stress to aquatic life, with the average and median concentrations indicating a medium probability of stress.

This analysis indicates that the probability of stress to aquatic life from potassium is greater at the downstream monitoring station than it is at the upstream monitoring station.

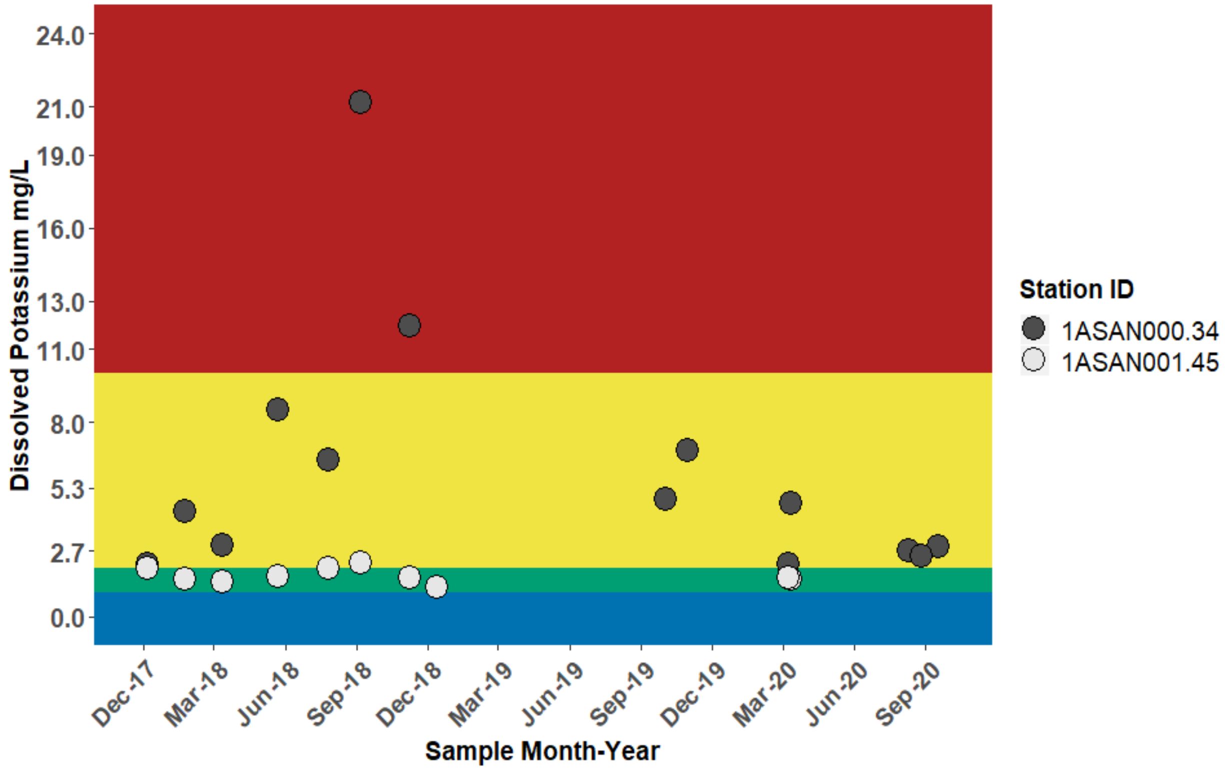


Figure 6-29. Potassium (mg/L) measurements at 1ASAN000.34 and 1ASAN001.45 between December 2017 – September 2020.

6.9.4 Sodium

Sodium measurements in Sand Branch ranged from 24 - 64 mg/L, with an average and median concentration of 36 mg/L and 34 mg/L (n=24) (Figure 6-30). At the upstream monitoring station, 1ASAN001.45, the average and median sodium concentrations were 32 mg/L and 32 mg/L (n=10). At the downstream monitoring station, 1ASAN000.34, the average and median sodium concentrations were 39 mg/L and 34 mg/L (n=14). All data measured, at both monitoring stations, indicate a high probability of stress to aquatic life.

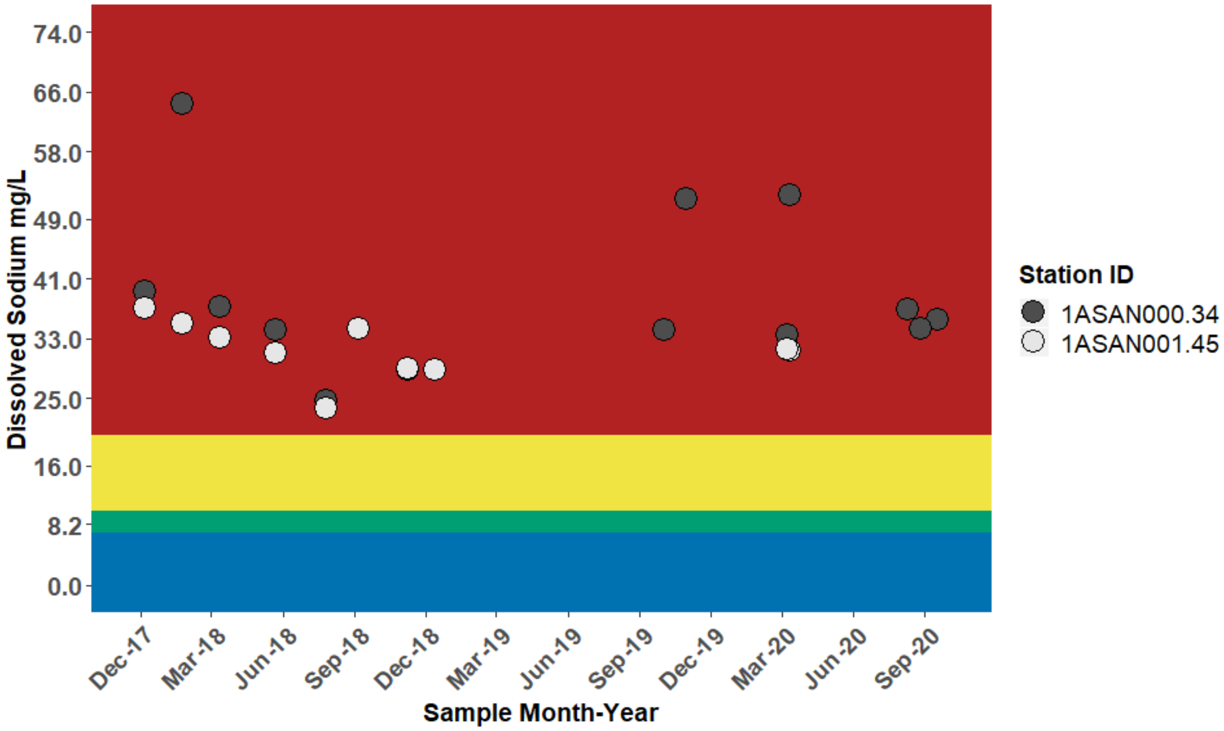


Figure 6-30. Sodium (mg/L) measurements at 1ASAN000.34 and 1ASAN001.45 from December 2017 – September 2020.

6.10 Dissolved Metals

There were three sampling events for metals: October 3, 2019, October 31, 2019 and September 17, 2020. The October 3, 2019 sampling event occurred during base flow conditions in Sand Branch, while the October 31, 2019 sampling event occurred during storm flow conditions. The September 17, 2020 sampling event occurred during typical flow conditions.

6.10.1 Metal Cumulative Criteria Unit

The Metal Cumulative Criterion Unit (Metals CCU) is a measurement that accounts for the additive effect of dissolved metals in the water column by standardizing each dissolved metal's concentration with chronic criterion values established by DEQ. Arsenic, chromium III, copper, lead, nickel, and zinc are included in the calculation, as they are generally considered the most prevalent in the environment. These metal criteria are dependent on the water hardness; therefore, hardness was also included in the calculation.

The Metals Cumulative Criteria Unit (CCU) was calculated from the dissolved metals data collected at the downstream monitoring station (1ASAN000.34) on Sand Branch and ranged from 0.06 - 0.29 (Figure 6-31). The median Metals CCU was 0.08 C (n=3). All three calculated CCU values were in the no probability of stress to aquatic life range.

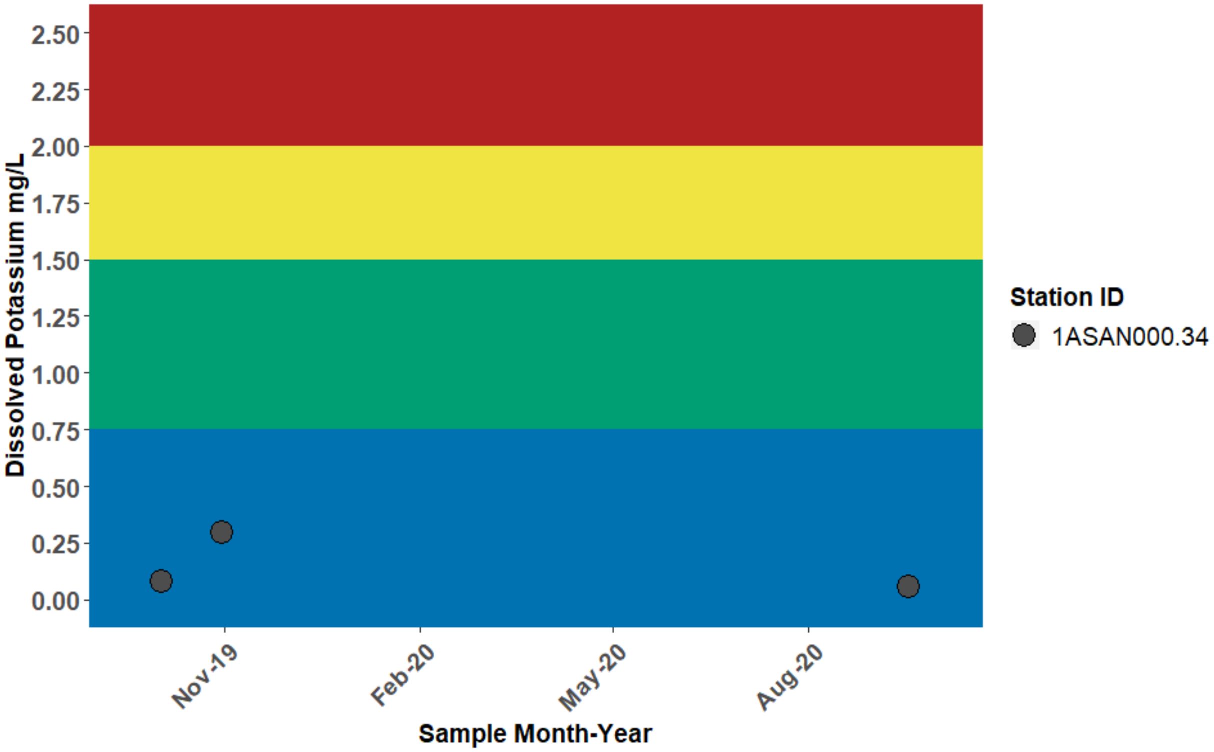


Figure 6-31. Metal CCU (unitless) calculated for downstream monitoring station 1ASAN000.34, sampled October 3, 2019; October 31, 2019 and September 17, 2020.

6.10.2 Individual Water Quality Criteria

A suite of dissolved metals were sampled on three occasions from 2019-2020. The measurements were compared to each of their applicable freshwater aquatic life water quality criteria (Table 6-7 - Table 6-9). With the exception of arsenic and chromium VI, water quality criteria for dissolved metals are hardness dependent, with the criteria formulas allowing a minimum and maximum hardness of 25 mg/L as $CaCO_3$ and 400 mg/L as $CaCO_3$, respectfully. Therefore, using hardness data from each sampling event, criteria were calculated for each metal sampling event.

Values shown for chromium are in the total dissolved form because DEQ monitoring does not differentiate the results into valence states. Chromium primarily occurs in the environment in two valence states, chromium III and chromium VI (USEPA, 2000b). Because Virginia’s WQS for chromium are based on the different valence states, the monitoring results for total dissolved chromium were compared to criteria for the two valence states. However, because it is highly unlikely for one valence state to dominate the total dissolved sample, the comparison should be considered with caution.

Comparison of the measured values against the applicable freshwater quality criteria showed no exceedances (Table 6-10).

Table 6-7. Dissolved metals data collected from 1ASAN000.34 on October 3, 2019[a,b]

Parameter (Dissolved)	Value (µg/L)	Acute Criteria (µg/L)	Chronic Criteria (µg/L)
Arsenic	4.43	340	150

Parameter (Dissolved)	Value (µg/L)	Acute Criteria (µg/L)	Chronic Criteria (µg/L)
Cadmium	0.10	7.10	4.48
Chromium[c]	5.87	1773.3 (III) / 16 (VI)	230.8 (III) / 11 (VI)
Copper	0.43	49.6	29.3
Lead	0.10	409	46.5
Nickel	0.54	589.2	65.6
Selenium	0.76	20	5
Silver	0.02	37.4	-
Zinc	0.30	379.3	382.4

[a] Corresponding calculated acute and criteria freshwater criteria shown. The measured hardness value was 588 mg/L, $CaCO_3$ calculated as dissolved, therefore the water quality criteria maximum hardness of 400 mg/L as $CaCO_3$ was used in the criteria calculations.
[b] A ”-“ indicates there is no criteria for that parameter.
[c] Chromium was measured as total dissolved with no distinction among the valent forms, Cr III and Cr VI.

Table 6-8. Dissolved metals data collected from 1ASAN000.34 on October 31, 2019[a,b]

Parameter (Dissolved)	Value (µg/L)	Acute Criteria (µg/L)	Chronic Criteria (µg/L)
Arsenic	6	340	150
Cadmium	0.10	5.6	3.7
Chromium[c]	12.9	1458.2 (III) / 16 (VI)	189.8 (III) / 11 (VI)
Copper	3.72	39.6	23.9
Lead	0.01	301.8	34.3
Nickel	1.10	481.4	53.6
Selenium	1.34	20	5
Silver	0.02	24.8	-
Zinc	2.19	309.8	312.3

[a] Corresponding calculated acute and criteria freshwater criteria shown. The measured hardness value was 315 mg/L, $CaCO_3$ calculated as dissolved, which was used in the criteria calculations.
[b] A ”-“ indicates there is no criteria for that parameter.
[c] Chromium was measured as total dissolved with no distinction among the valent forms, Cr III and Cr VI.

Table 6-9. Dissolved metals data collected from 1ASAN000.34 on September 17, 2020[a,b]

Parameter (Dissolved)	Value (µg/L)	Acute Criteria (µg/L)	Chronic Criteria (µg/L)
Arsenic	5.24	340	150
Cadmium	0.10	7.1	4.5
Chromium[c]	0.30	1773.3 (III) / 16 (VI)	230.79 (III) / 11 (VI)
Copper	0.41	49.6	29.3
Lead	0.02	409.0	46.5
Nickel	0.34	589.2	65.6
Selenium	0.59	20	5
Silver	0	37.4	-
Zinc	0.59	379.3	382.4

[a] Corresponding calculated acute and criteria freshwater criteria shown. The measured hardness value was 548 mg/L, $CaCO_3$ calculated as dissolved, therefore the water quality criteria maximum hardness of 400 mg/L as $CaCO_3$ was used in the criteria calculations.

[b] A ”-“ indicates there is no criteria for that parameter.

[c] Chromium was measured as total dissolved with no distinction among the valent forms, Cr III and Cr VI.

Table 6-10. Summary of water quality exceedances for individual metals (listed by atomic symbol).

Monitoring Location	Parameter / Sample Exceedance of Water Quality Criteria (Y/N)								
	As	Cd	Cr[b]	Cu	Pb	Ni	Se	Ag	Zn
1ASAN000.34[a]	No	No	No	No	No	No	No	No	No

[a] Results based upon 3 sample events: October 3, 2019, October 31, 2019 and September 17, 2020

[b] Chromium was measured as total dissolved with no distinction among the valent forms, Cr III and Cr VI.

6.11 Temperature

Benthic macroinvertebrates require a suitable instream temperature range to persist in the environment. According to the Virginia WQS, Class III Nontidal Waters (Coastal and Piedmont Zones) streams cannot have temperatures exceeding 32°C (9VAC25-260-50).

Temperature in Sand Branch ranged from 0.5 - 26.6 °C, with an average and median temperature of 15.4 °C and 14.1 °C (n=52) (Figure 6-32). At the upstream monitoring station, 1ASAN001.45, the average and median temperature was 15.4 °C and 14.1 °C (n=25). At the downstream monitoring station, 1ASAN000.34, the average and median temperature was 15.4 °C and 14.2 °C (n=30). All data measured, at both monitoring stations, were below the water quality criterion of 32°C (shown by the black line in

Figure 6-32).

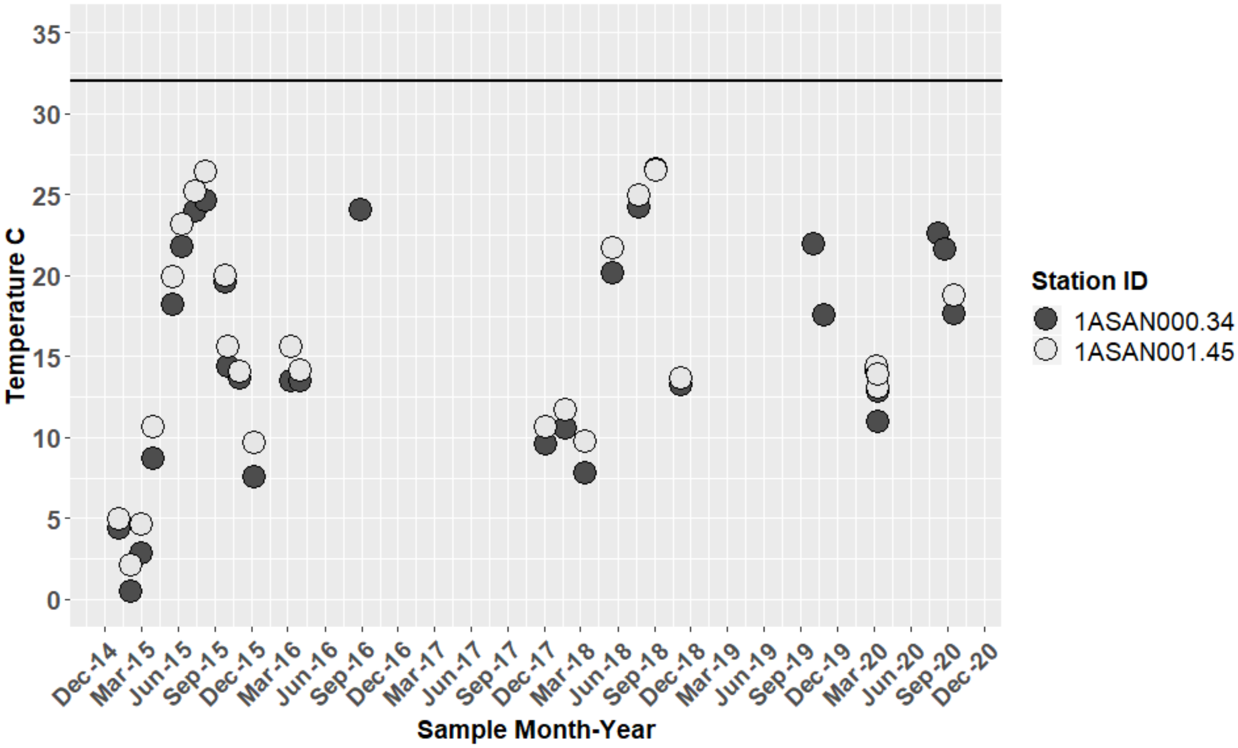


Figure 6-32. Temperature (°C) measurements taken at monitoring stations 1ASAN000.34 and 1ASAN001.45 between January 2015 and September 2020.

Continuous monitoring of temperature, measured at 15-minute intervals, showed a diurnal pattern with a range of 19.9 – 26.1°C, and a median of 22.7°C. Rain events, measured as total rainfall in one-hour increments at Dulles International Airport, did not appear to significantly alter the temperature of Sand Branch (Figure 6-33).

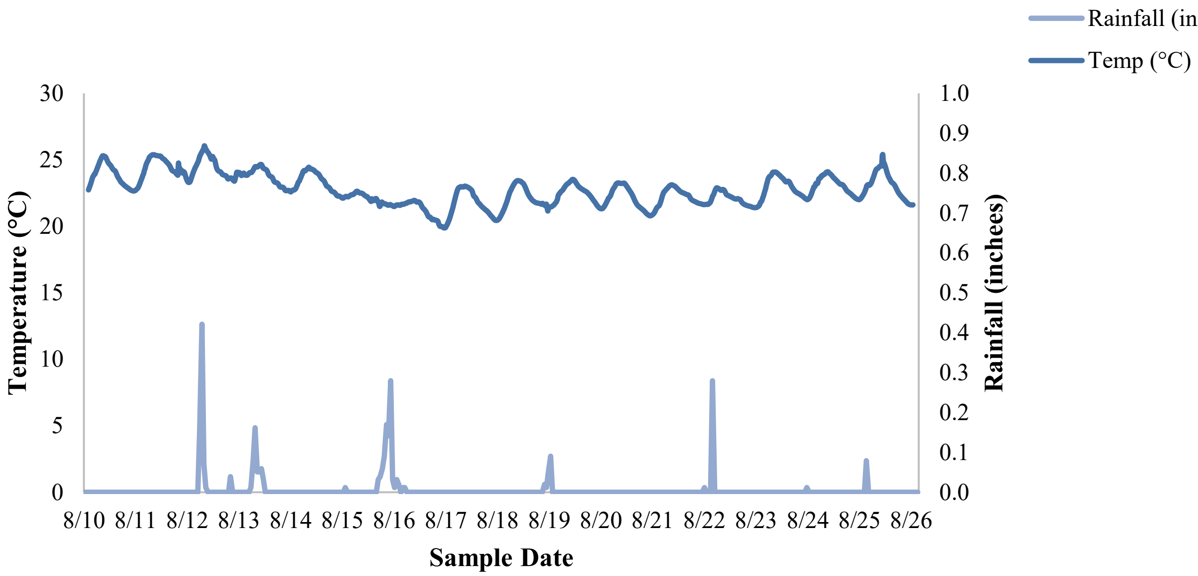


Figure 6-33. Continuous monitoring of temperature (°C) at 1ASAN000.34 from August 10, 2020 – August 26, 2020.

6.12 Summary of Stressor Threshold Comparisons

A summary of the comparison of water chemistry data to stressor threshold values, where developed, is provided in Table 6-11. The table identifies whether the comparison to applicable stressor threshold values indicated there was either no probability, low probability, medium probability or high probability that a particular candidate has potential to cause stress to the benthic macroinvertebrate community.

Table 6-11. Summary of the probability of stress for candidates with stressor thresholds.

Monitoring Location	Parameter												
	pH	DO	TP	TN	SC	TDS	Sulfate	Chloride	Potassium	Sodium	LRBS	Habitat	Metal CCU
1ASAN001.45	Low	No	No	Medium	High	High	High	Low	Low	High	-	Medium	-
1ASAN000.34	Low	Low	Low / Medium	Medium	High	High	High	Low	Medium	High	No	Medium	No
Combined	Low	Low	Low	Medium	High	High	High	Low	Low	High	-	Medium	-

7.0 Causal Analysis/Diagnosis Decision Information System (CADDIS)

This portion of the stressor analysis was conducted according to USEPA's Stressor Identification Guidance Document (USEPA, 2000c) using the Causal Analysis/Diagnosis Decision Information System (CADDIS) (USEPA, 2018). The CADDIS approach provides guidance on evaluating various lines of evidence to determine the cause of biological impairments. In the case of Sand Branch, the analysis used the available data collected from the site, published water quality standards and threshold values, and available literature from other cases to investigate the potential causes of impairment. Table 7-1 shows the lines of evidence suggested by the CADDIS approach, an explanation of the concept, and examples of how these lines of evidence were analyzed in this project. Some lines of evidence were not applicable, such as the analysis of biomarkers, field manipulations, or verified predictions. The majority of the lines of evidence, however, were investigated for this project.

Table 7-1. Lines of evidence used in the causal analysis approach.

Evidence	The Concept	Examples from this Project
Data from the Case		
Spatial Co-occurrence	The biological effect must be observed where the cause is observed, and must not be observed where the cause is absent.	Analysis of water quality and habitat data across stations
Temporal Co-occurrence	The biological effect must be observed when the cause is observed, and must not be observed when the cause is absent.	Analysis of the timing of water quality excursions in relation to the timing of benthic impairment
Evidence of Exposure or Biological Mechanism	Measurements of the biota show that relevant exposure to the cause has occurred, or that other biological mechanisms linking the cause to the effect have occurred.	NA
Causal Pathway	Steps in the pathways linking sources to the cause can serve as supplementary or surrogate indicators that the cause and the biological effect are likely to have co-occurred.	Development and analysis of causal pathways for stressors
Stressor-Response Relationships from the Field	As exposure to the cause increases, intensity or frequency of the biological effect increases; as exposure to the cause decreases, intensity or frequency of the biological effect decreases.	Correlation of water quality data with benthic score
Manipulation of Exposure	Field experiments or management actions that increase or decrease exposure to a cause must increase or decrease the biological effect.	NA
Laboratory Tests of Site Media	Controlled exposure in laboratory tests to causes (usually toxic substances) present in site media should induce biological effects consistent with the effects observed in the field.	Toxicity testing of stream samples
Temporal Sequence	The cause must precede the biological effect.	Analysis of temporal trends in benthic data

Evidence	The Concept	Examples from this Project
Verified Predictions	Knowledge of a cause's mode of action permits prediction and subsequent confirmation of previously unobserved effects.	NA
Symptoms	Biological measurements (often at lower levels of biological organization than the effect) can be characteristic of one or a few specific causes.	Analysis of benthic metrics, Biological Condition Gradient (BCG), community composition, and functional feeding groups
Data from Elsewhere		
Stressor-Response Relationships from Other Field Studies	At the impaired sites, the cause must be at levels sufficient to cause similar biological effects in other field studies.	Water quality comparison with reference stations and stressor probability thresholds
Stressor-Response Relationships from Laboratory Studies	At the impaired sites, the cause must be at levels associated with related biological effects in laboratory studies.	Water quality comparison with VA water quality standards and literature threshold values
Stressor-Response Relationships from Simulation Models	At the impaired sites, the cause must be at levels associated with effects in mathematical models simulating ecological processes.	Confirmation through use of TMDL model
Mechanistically Plausible Cause	The relationship between the cause and biological effect must be consistent with known principles of biology, chemistry and physics.	Development and analysis of causal pathways for stressors
Manipulation of Exposure at Other Sites	Field experiments or management actions at other sites that increase or decrease exposure to a cause must increase or decrease the biological effect.	Confirmation through literature
Analogous Stressors	Agents similar to the causal agent at the impaired site should lead to similar effects at other sites.	Analysis across related parameters
Multiple Types of Evidence		

Evidence	The Concept	Examples from this Project
Consistency of Evidence	Confidence in the argument for or against a cause is increased when many types of evidence consistently support or weaken it.	Weight of evidence approach
Explanation of the Evidence	Confidence in the argument for a candidate cause is increased when a post hoc mechanistic, conceptual, or mathematical model reasonably explains any inconsistent evidence.	Confirmation through use of TMDL model

The applicable lines of evidence were evaluated for each candidate stressor. For each line of evidence, best professional judgement was used to score the candidate stressor on a 3-point positive and negative scale (Table 7-2). This scale represents the strength of the evidence for or against each candidate stressor. A weight of evidence approach was then used to sum the respective scores and classify candidate stressors as either non-stressors, possible stressors, or probable stressors. Candidate stressors were then classified based upon the summed scores. Summed scores for a candidate stressor of ≤0 are classified as a non-stressor, scores from 1-3 are classified as a possible stressor and scores >3 are classified as a probable stressor (Table 7-3).

Table 7-2. Scoring criteria used to evaluate candidate stressors.

Score	Explanation
+3	The line of evidence **strongly supports** the candidate stressor as the cause of the impairment
+2	The line of evidence **moderately supports** the candidate stressor as the cause of the impairment
+1	The line of evidence **weakly supports** the candidate stressor as the cause of the impairment
0	The line of evidence **does not support or refute** the candidate stressor as the cause of the impairment
-1	The line of evidence **weakly refutes** the candidate stressor as the cause of the impairment
-2	The line of evidence **moderately refutes** the candidate stressor as the cause of the impairment
-3	The line of evidence **strongly refutes** the candidate stressor as the cause of the impairment

Table 7-3. Scheme for classifying candidate stressors based on causal analysis.

Total Score	Classification
<-2	Non-Stressor
-1	
0	
+1	Possible Stressor
+2	
+3	
+4	Probable Stressor
+5	
>+6	

7.1 pH

The total causal analysis score for pH was -24 (Table 7-4), indicating that there is strong evidence that pH is a non-stressor in Sand Branch. All pH values at both sites were in the low probability range for stress effects and were within water quality standards. For these reasons and others explained in Table 7-4, pH was categorized as a non-stressor in Sand Branch.

Table 7-4. Causal analysis results for pH.

Evidence	Score	Explanation
Spatial Co-occurrence	-3	At both Sand Branch locations, VSCI scores were impaired, but pH values were within water quality standards and in the low probability range for stress effects.
Temporal Co-occurrence	-3	At the time of benthic sample collection, pH at both sites met water quality standards.
Causal Pathway	-2	Alkalinity in Sand Branch is relatively high, averaging 138 mg/L as CaCO3, so pH changes are well-buffered.
Laboratory Tests of Site Media	-2	Toxicity was observed in toxicity tests of Sand Branch samples, however, pH measured in those samples were within normal ranges.

Evidence	Score	Explanation
Temporal Sequence	-2	Spring VSCI scores were lower than fall scores; however, no seasonal trend in pH was observed. pH values were consistently in the low probability range for stress effects.
Symptoms	-3	BCG analysis did not identify any of the top predominant taxa as increasing in abundance in the presence of acidity or alkalinity (score of 5).
Stressor-Response Relationships from Other Field Studies	-3	All pH values were in the low probability range for stress effects.
Stressor-Response Relationships from Laboratory Studies	-3	All pH values were within water quality standards.
Consistency of Evidence	-3	Evidence consistently refuted pH as a stressor.
Sum	-24	

7.2 Dissolved Oxygen

The total causal analysis score for dissolved oxygen was -12 (Table 7-5), indicating that there is moderate to strong evidence that dissolved oxygen is a non-stressor in Sand Branch. Dissolved oxygen averaged in the no probability range for stress effects, and all dissolved oxygen values met water quality standards. For these reasons and others explained in Table 7-5, dissolved oxygen was categorized as a non-stressor in Sand Branch.

Table 7-5. Causal analysis results for dissolved oxygen.

Evidence	Score	Explanation
Spatial Co-occurrence	-1	At both Sand Branch locations, VSCI scores were impaired, but DO values averaged in the no probability range for stress effects. During warm summer months, however, DO was consistently in the medium probability range for stress effects.
Temporal Co-occurrence	-1	At the time of benthic sample collection, DO met water quality standards, but was in the medium probability range for stress effects on one occasion.
Causal Pathway	1	Nutrient levels (phosphorus and nitrogen) averaged in the medium probability range for stress effects, so the causal pathway from nutrient enrichment to low DO is intact.

Evidence	Score	Explanation
Stressor-Response Relationships from the Field	1	During critical periods (late summer), DO was lower at the downstream location, where benthic VSCI scores were also lower.
Laboratory Tests of Site Media	-1	Toxicity was observed in toxicity tests of Sand Branch samples; however, DO measured in those samples were within normal ranges.
Temporal Sequence	-3	Spring VSCI scores were lower than fall scores; however, DO was lowest in the late summer during and prior to fall sampling.
Symptoms	-3	BCG analysis did not identify any of the top predominant taxa as increasing in abundance in the presence of low DO (score of 5).
Stressor-Response Relationships from Other Field Studies	-1	At both sites, DO values averaged in the no probability range for stress effects. During warm summer months; however, DO was consistently in the medium probability range for stress effects.
Stressor-Response Relationships from Laboratory Studies	-3	All DO values were above the water quality criteria of 5.0 mg/L (average) and 4.0 mg/L (instantaneous).
Consistency of Evidence	-1	Most evidence weakly refuted DO as a stressor.
Sum	-12	

7.3 Total Phosphorus

The total causal analysis score for total phosphorus was +16 (

Table 7-6), indicating that there is strong evidence that phosphorus is a probable stressor in Sand Branch. Lines of evidence supporting phosphorus as a probable stressor included:

- Average phosphorus levels were in the medium probability range for stressor effects, with excursions into the high probability range.
- Phosphorus levels exceeded EPA-recommended phosphorus criterion for the ecoregion (USEPA, 2000a).
- Phosphorus levels at the downstream station of Sand Branch were in the upper quartile (81st percentile) of phosphorus levels within the Trap Rock and Conglomerates Uplands and Triassic Lowlands ecoregions.
- Seasonal trends of lower spring benthic scores indicate possible nutrient enrichment.
- Daytime super-saturation of dissolved oxygen (as high as 133%) indicates nutrient enrichment.
- BCG analysis identified predominant taxa in Sand Branch that indicate nutrient enrichment.
- Analysis of the benthic community composition and functional feeding group showed an increase in scrapers at the upstream station and a predominance of net-spinning caddisflies that indicate nutrient enrichment.
- Habitat assessments documented observations of thick filamentous algae.

For these reasons and others explained in
Table 7-6, phosphorus was categorized as a probable stressor in Sand Branch.

Table 7-6. Causal analysis results for total phosphorus.

Evidence	Score	Explanation
Spatial Co-occurrence	-1	At both Sand Branch locations, VSCI scores were impaired, but total phosphorus averaged in the low probability range for stress effects at the upstream station and medium probability range for stress effects at the downstream station.
Temporal Co-occurrence	1	At the time of benthic sample collection, total phosphorus levels exceeded the EPA-recommended criterion of 0.04 mg/L for the Northern Piedmont ecoregion on one occasion.
Causal Pathway	2	Super-saturation of dissolved oxygen (as high as 133%) during the daytime indicates that the causal pathway from nutrient enrichment to algal growth to DO impacts is supported. The causal pathway from nutrient enrichment to increased algae growth to altered functional feeding groups and taxa composition is also supported.
Stressor-Response Relationships from the Field	2	Total phosphorus concentrations were much higher at the downstream location, where benthic VSCI scores were lower.
Temporal Sequence	1	Seasonal trends of lower spring benthic scores may indicate nutrient enrichment, as higher spring flows bring increased nutrient loads.
Symptoms	3	BCG analysis identified several of the top predominant taxa (*Cheumatopsyche*, *Hydropsyche, Hydroptila*, and *Oligochaeta*) as increasing in abundance in the presence of nutrient stress (score of 5). Community composition and functional feeding group analysis showed patterns that indicate nutrient enrichment.
Stressor-Response Relationships from Other Field Studies	2	Total phosphorus averaged in the medium probability range for stress effects, with excursions into the high probability range.
Stressor-Response Relationships from Laboratory Studies	3	Total phosphorus levels averaged 0.06 mg/L, above the EPA-recommended criterion of 0.04 mg/L for the Northern Piedmont ecoregion. Average phosphorus levels were also in the upper quartile (81st percentile) of phosphorus levels within the Trap Rock and Conglomerates Uplands and Triassic Lowlands ecoregions.
Mechanistically Plausible Cause	2	Habitat assessments documented observations of thick filamentous algae.

Evidence	Score	Explanation
Consistency of Evidence	1	Most evidence weakly supported phosphorus as a stressor.
Sum	16	

7.4 Total Nitrogen

The total causal analysis score for total nitrogen was +1 (Table 7-7), indicating that there is some evidence supporting nitrogen as a stressor in Sand Branch and other evidence refuting it. Total nitrogen averaged in the medium probability range for stress effects at the upstream station and high probability range at the downstream station. The BCG analysis and analysis of the benthic community composition and functional feeding group also showed patterns of nutrient enrichment. However, those lines of evidence suggest nutrients in general and do not differentiate between phosphorus and nitrogen effects. Analysis of nitrogen to phosphorus ratios showed that phosphorus (and not nitrogen) is the limiting nutrient. In addition, nitrogen levels were below EPA-recommended nitrogen criterion for the ecoregion (USEPA, 2000a). Based on conflicting evidence, nitrogen was categorized as a possible stressor.

Table 7-7. Causal analysis results for total nitrogen.

Evidence	Score	Explanation
Spatial Co-occurrence	2	At both Sand Branch locations, VSCI scores were impaired, and total nitrogen averaged in the medium probability range for stress effects at the upstream station and high probability range at the downstream station.
Temporal Co-occurrence	-2	At the time of benthic sample collection, total nitrogen levels were below the EPA-recommended criterion of 2.225 mg/L for the Northern Piedmont ecoregion.
Causal Pathway	-2	The causal pathway from nutrient enrichment to low DO is intact. The causal pathway from nutrient enrichment to increased algae growth to altered functional feeding groups and taxa composition is supported. However, the nitrogen to phosphorus ratio in Sand Branch averages 29.5. This indicates that phosphorus, and not nitrogen, is the limiting nutrient controlling algae growth.
Stressor-Response Relationships from the Field	2	Total nitrogen concentrations were much higher at the downstream location, where benthic VSCI scores were lower.
Temporal Sequence	1	Seasonal trends of lower spring benthic scores may indicate nutrient enrichment, as higher spring flows bring increased nutrient loads.
Symptoms	3	BCG analysis identified several of the top predominant taxa (*Cheumatopsyche, Hydropsyche, Hydroptila*, and *Oligochaeta*) as increasing in abundance in the presence of nutrient stress (score of

Evidence	Score	Explanation
		5). Community composition and functional feeding group analysis showed patterns that indicate nutrient enrichment.
Stressor-Response Relationships from Other Field Studies	2	Total nitrogen averaged in the medium probability range for stress effects, with excursions into the high probability range.
Stressor-Response Relationships from Laboratory Studies	-2	Total nitrogen levels averaged 1.72 mg/L, which is below the EPA-recommended criterion of 2.225 mg/L for the Northern Piedmont ecoregion.
Mechanistically Plausible Cause	-3	The nitrogen to phosphorus ratio in Sand Branch averages 29.5. This indicates that phosphorus, and not nitrogen, is the limiting nutrient controlling algae growth.
Consistency of Evidence	0	Some evidence supported nitrogen as a stressor, while other evidence refuted nitrogen as a stressor.
Sum	1	

7.5 Ammonia

The total causal analysis score for ammonia was +2 (Table 7-8), indicating that there is some evidence supporting ammonia as a stressor in Sand Branch and other evidence refuting it. Ammonia levels in Sand Branch were above the chronic criterion on 1 out of 13 sampling events. However, excursions occurred only from the chronic criterion in one sample, and ammonia excursions are likely only intermittent. In addition, critical ammonia conditions are typically observed in the late summer, and benthic scores were higher in the fall than the spring. Based on conflicting evidence, ammonia was categorized as a possible stressor in Sand Branch.

Table 7-8. Causal analysis results for ammonia.

Evidence	Score	Explanation
Spatial Co-occurrence	1	At both Sand Branch locations, VSCI scores were impaired, and there was an excursion from the chronic water quality criterion for ammonia in one sample collected at the downstream station.
Temporal Co-occurrence	1	On the week of two benthic sampling dates, an upstream discharger that averages 12.5 mg/L ammonia was discharging.
Causal Pathway	2	The causal pathway from high ammonia levels in a permitted discharge to downstream ammonia levels that were above the chronic water quality criterion in one sample is intact.
Stressor-Response Relationships from the Field	1	Total ammonia concentrations were higher at the downstream location, where benthic VSCI scores were lower.

Evidence	Score	Explanation
Laboratory Tests of Site Media	-2	Toxicity was observed in toxicity tests of Sand Branch samples, however, ammonia measured in those samples was <0.1 mg/L.
Temporal Sequence	-2	Ammonia levels are generally highest in the late summer when water temperatures are highest, however, fall benthic scores were higher than spring scores.
Stressor-Response Relationships from Laboratory Studies	1	1 of 13 ammonia measurements were above the water quality criteria at the downstream station. However, the excursion only occurred from the chronic criterion in one sample, and ammonia excursions are likely only intermittent.
Consistency of Evidence	0	Some evidence supported ammonia as a stressor, while other evidence refuted ammonia as a stressor.
Sum	2	

7.6 Suspended Solids and Deposited Sediment

The total causal analysis score for suspended solids and deposited sediment was +6 (Table 7-9), indicating that there is some evidence that sediment is a probable stressor in Sand Branch. Lines of evidence supporting sediment as a probable stressor included:

- Total habitat scores averaged in the medium probability range for stress effects.
- Seasonal trends in benthic health indicated poorer health in the spring following high spring flows that typically bring higher sediment loads.
- Imperviousness is high in the watershed, providing a causal pathway for increased runoff and instability of benthic substrate.
- BCG analysis identified predominant taxa that indicate sediment-associated stressors.
- Taxonomic community structure indicated shifts to *Dipteran*-dominated communities that prefer sediment and away from *Ephemeroptera, Plecoptera*, and *Trichoptera*, which generally prefer clean substrate.
- Functional feeding group analysis indicated shifts to collectors (at the downstream station) that prefer sediment-rich conditions.
- Total suspended solids (TSS) and turbidity were significantly higher than in the reference stream, Licking Run (1ALIL008.29).

For these reasons and others explained in Table 7-9, suspended solids and deposited sediment were categorized as probable stressors.

Table 7-9. Causal analysis results for suspended solids and deposited sediment.

Evidence	Score	Explanation
Spatial Co-occurrence	2	At both Sand Branch locations, VSCI scores were impaired, and average habitat scores were in the medium probability range for stress effects.

Evidence	Score	Explanation
Temporal Co-occurrence	-1	Habitat scores did not correlate with VSCI scores across Sand Branch sampling dates.
Causal Pathway	1	The causal pathway from high watershed imperviousness to sediment wash off and erosion to embedded substrate is intact.
Stressor-Response Relationships from the Field	1	Habitat conditions and embeddedness were worse at the downstream location, where benthic VSCI scores were lower.
Temporal Sequence	1	Seasonal trends of lower spring benthic scores may indicate sediment enrichment, as higher spring flows bring increased sediment loads.
Symptoms	2	BCG analysis identified two of the top predominant taxa (*Oligochaeta* and *Stenelmis*) as increasing in abundance in the presence of poor habitat conditions (score of 5). Community structure analysis indicated a shift to sediment-tolerant Dipterans, and functional feeding group analysis indicated a shift to collectors that thrive in sediment-rich habitats.
Stressor-Response Relationships from Other Field Studies	2	Habitat scores averaged in the medium probability range for stress effects.
Analogous Stressors	-3	The measurement of relative bed stability was in the no probability range for stress effects.
Consistency of Evidence	1	Most evidence weakly supported sediment as a stressor.
Sum	6	

7.7 Specific Conductivity and Total Dissolved Solids

The total causal analysis score for conductivity and total dissolved solids (TDS) was +31 (Table 7-10), indicating that there is strong evidence that conductivity associated with TDS is a probable stressor in Sand Branch. Lines of evidence supporting TDS as a probable stressor included:

- Conductivity and TDS averaged in the high probability range for stress effects.
- Conductivity levels also exceeded published thresholds for maintaining healthy freshwater aquatic life.
- Conductivity was significantly inversely correlated with VSCI scores across sites within the Trap Rock and Conglomerates Uplands and Triassic Lowlands ecoregions as shown in the regression of VSCI scores and specific conductivity (Figure 6-20).
- Ambient toxicity test demonstrated toxicity of Sand Branch samples, and conductivity levels in those samples were above published thresholds for toxicity.

- Seasonal trends of lower spring benthic scores follow the winter period, where the highest conductivity measurements have occurred due to roadway deicing.
- The causal pathway from underlying geology and quarry discharge to high conductivity and from runoff of roadway deicing salts is supported.
- BCG analysis identified predominant taxa that indicate conductivity effects.

For these reasons and others explained in Table 7-10, conductivity associated with TDS was categorized as a probable stressor.

Table 7-10. Causal analysis results for conductivity associated with TDS.

Evidence	Score	Explanation
Spatial Co-occurrence	3	At both Sand Branch locations, VSCI scores were impaired and conductivity levels averaged in the high probability range for stress effects.
Temporal Co-occurrence	3	At the time of all benthic sample collections, conductivity levels were in the high probability range for stress effects.
Causal Pathway	2	The causal pathway from underlying geology and quarry discharge to high conductivity and from runoff of roadway deicing salts is supported.
Stressor-Response Relationships from the Field	2	Conductivity was significantly correlated with VSCI scores across sites within the Trap Rock and Conglomerates Uplands and Triassic Lowlands ecoregions.
Laboratory Tests of Site Media	2	Toxicity tests demonstrated toxicity of Sand Branch samples, and conductivity levels in those samples were above published thresholds for toxicity.
Temporal Sequence	2	Seasonal trends of lower spring benthic scores follow the winter period, where the highest conductivity measurements have occurred due to roadway deicing.
Symptoms	3	BCG analysis identified several of the top predominant taxa (*Stenelmis, Cheumatopsyche, Hydropsyche, Hydroptila*, and *Oligochaeta*) as increasing in abundance in the presence of high conductivity (score of 5).
Stressor-Response Relationships from Other Field Studies	3	Conductivity averaged in the high probability range for stress effects.
Stressor-Response Relationships from Laboratory Studies	3	Conductivity levels exceeded published thresholds for maintaining healthy freshwater aquatic life (USEPA, 2011; Cormier et al., 2018; Pond, 2004).

Evidence	Score	Explanation
Stressor-Response Relationships from Simulation Models	3	The statistical model of ion toxicity developed by Mount et al. (1997) predicts toxicity based on the ion concentrations exhibited by Sand Branch samples.
Analogous Stressors	2	The analogous stressor of TDS and the individual ions of sulfate and sodium were also in the high probability range for stress effects.
Consistency of Evidence	3	Evidence strongly supports conductivity associated with TDS as a stressor.
Sum	31	

7.8 Dissolved Ions

A suite of ions associated with anthropogenic activities were analyzed. These are sulfate, chloride, sodium, and potassium.

7.8.1 Sulfate

The total causal analysis score for sulfate was +16 (Table 7-11), indicating that there is strong evidence that sulfate is a probable stressor in Sand Branch. Lines of evidence supporting sulfate as a probable stressor included:

- Sulfate averaged in the high probability range for stress effects.
- Sulfate levels also exceeded some published thresholds for toxicity.
- The causal pathway from underlying geology and quarry discharge to high sulfate levels is supported.
- BCG analysis identified predominant taxa that indicate stress effects from sulfate.

For these reasons and others explained in Table 7-11, sulfate was categorized as a probable stressor.

Table 7-11. Causal analysis results for sulfate.

Evidence	Score	Explanation
Spatial Co-occurrence	3	At both Sand Branch locations, VSCI scores were impaired and sulfate levels averaged in the high probability range for stress effects.
Temporal Co-occurrence	1	During one benthic sample collection, sulfate levels were in the high probability range for stress effects.
Causal Pathway	2	The causal pathway from underlying geology and quarry discharge to high sulfate levels is supported.

Evidence	Score	Explanation
Symptoms	2	BCG analysis identified several of the top predominant taxa (*Hydropsyche* and *Hydroptila*) as increasing in abundance in the presence of high sulfate (score of 5).
Stressor-Response Relationships from Other Field Studies	3	Sulfate averaged in the high probability range for stress effects.
Stressor-Response Relationships from Laboratory Studies	1	In an analysis of toxicity to major ions, Mount et al. (2016) reported 2 out of 14 LC50s for *Ceriodaphnia* exposed to $MgSO_4$ that were below the average sulfate concentration in Sand Branch.
Analogous Stressors	2	The analogous stressors of TDS and conductivity were also in the high probability range for stress effects.
Consistency of Evidence	2	Evidence strongly supports conductivity as a stressor.
Sum	16	

7.8.2 Chloride

The total causal analysis score for chloride was +1 (Table 7-12), indicating that there is some evidence supporting chloride as a stressor in Sand Branch and other evidence refuting it. Chloride averaged in the medium probability range for stress effects at the downstream station, but levels were consistently below water quality standards. Chloride levels were in the high probability range for stress effects during two benthic sampling dates, however, BCG analysis did not identify any of the top predominant taxa as increasing in abundance in the presence of chloride. At the upstream station, chloride levels averaged in the low probability range for stress effects. Based on this conflicting evidence and other lines of evidence explained in Table 7-12, chloride was categorized as a possible stressor.

Table 7-12. Causal analysis results for chloride.

Evidence	Score	Explanation
Spatial Co-occurrence	-1	At both Sand Branch locations, VSCI scores were impaired, but chloride averaged in the low probability range for stress effects at the upstream station and medium probability range for stress effects at the downstream station.
Temporal Co-occurrence	2	During two benthic sample collections at the downstream location, chloride levels were in the high probability range for stress effects.
Causal Pathway	1	The causal pathway from high watershed imperviousness and roadway deicing salts to high chloride levels is intact.

Evidence	Score	Explanation
Stressor-Response Relationships from the Field	2	Chloride concentrations were higher at the downstream location, where benthic VSCI scores were lower.
Temporal Sequence	1	Seasonal trends of lower spring benthic scores follow the winter period, where the highest chloride measurements have occurred.
Symptoms	-3	BCG analysis did not identify any of the top predominant taxa as increasing in abundance in the presence of chloride (score of 5).
Stressor-Response Relationships from Other Field Studies	1	Chloride averaged in the low probability range for stress effects but had excursions in the medium and high range.
Stressor-Response Relationships from Laboratory Studies	-3	Chloride levels were all below the Virginia water quality standard for chloride.
Analogous Stressors	1	The analogous stressors of TDS and conductivity were also in the high probability range for stress effects.
Consistency of Evidence	0	Some evidence supported chloride as a stressor, while other evidence refuted chloride as a stressor.
Sum	1	

7.8.3 Potassium

The total causal analysis score for potassium was +1 (Table 7-13), indicating that there is some evidence supporting potassium as a stressor in Sand Branch and other evidence refuting it. Potassium averaged in the medium probability range for stress effects and had excursion into the high probability range, but levels were generally below toxic levels from the literature. Based on this conflicting evidence and other lines of evidence explained in Table 7-13, potassium was categorized as a possible stressor.

Table 7-13. Causal analysis results for potassium.

Evidence	Score	Explanation
Spatial Co-occurrence	-1	At both Sand Branch locations, VSCI scores were impaired, but potassium averaged in the low probability range for stress effects at the upstream station and medium probability range for stress effects at the downstream station.
Temporal Co-occurrence	1	During one benthic sample collection at the downstream location, potassium levels were in the medium probability range for stress effects.

Evidence	Score	Explanation
Stressor-Response Relationships from the Field	2	Potassium concentrations were higher at the downstream location, where benthic VSCI scores were lower.
Temporal Sequence	-1	Seasonal trends of lower spring benthic scores did not appear to correlate with the timing of high potassium levels. Potassium levels were higher in the summer and fall.
Stressor-Response Relationships from Other Field Studies	2	Potassium averaged in the medium probability range for stress effects and had excursions in the high range.
Stressor-Response Relationships from Laboratory Studies	-3	In an analysis of toxicity to major ions (Mount et al., 2016), all LC50s for *Ceriodaphnia* exposed to KCl were well above the potassium levels in Sand Branch.
Analogous Stressors	1	The analogous stressors of TDS and conductivity were also in the high probability range for stress effects.
Consistency of Evidence	0	Some evidence supported potassium as a stressor, while other evidence refuted potassium as a stressor.
Sum	1	

7.8.4 Sodium

The total causal analysis score for sodium was +3 (Table 7-14), indicating that there is some evidence supporting sodium as a stressor in Sand Branch and other evidence refuting it. Sodium averaged in the high probability range for stress effects, but levels were generally below toxic levels from the literature. Based on this conflicting evidence and other lines of evidence explained in Table 7-14, sodium was categorized as a possible stressor.

Table 7-14. Causal analysis results for sodium.

Evidence	Score	Explanation
Spatial Co-occurrence	2	At both Sand Branch locations, VSCI scores were impaired and sodium levels averaged in the high probability range for stress effects, however, these levels are generally below toxic levels from the literature.
Temporal Co-occurrence	1	During one benthic sample collection at both locations, sodium levels were in the high probability range for stress effects.
Temporal Sequence	-1	Seasonal trends of lower spring benthic scores did not appear to correlate with the timing of high sodium levels.

Evidence	Score	Explanation
Stressor-Response Relationships from Other Field Studies	3	Sodium averaged in the high probability range for stress effects.
Stressor-Response Relationships from Laboratory Studies	-3	In an analysis of toxicity to major ions (Mount et al., 2016), all LC50 values for *Ceriodaphnia* exposed to sodium salts were well above the sodium levels in Sand Branch.
Analogous Stressors	1	The analogous stressors of TDS and conductivity were also in the high probability range for stress effects.
Consistency of Evidence	0	Some evidence supported sodium as a stressor, while other evidence refuted sodium as a stressor.
Sum	3	

7.9 Dissolved Metals

The total causal analysis score for dissolved metals was -9 (Table 7-15), indicating that there is moderate to strong evidence that metals are a non-stressor in Sand Branch. Metals values were within water quality standards, and metal CCUs for combined metal effects were in the no probability range for stress effects. For these reasons and others explained in Table 7-15, dissolved metals were categorized as a non-stressor in Sand Branch.

Table 7-15. Causal analysis results for dissolved metals.

Evidence	Score	Explanation
Spatial Co-occurrence	-2	At both Sand Branch locations, VSCI scores were impaired, but metals values were within water quality standards (with the possible exception of Cr VI if >85% of Cr were in that form) and cumulative criteria units were in the no probability range for stress effects.
Stressor-Response Relationships from Other Field Studies	-3	Cumulative criteria units for metals were in the no probability range for stress effects.
Stressor-Response Relationships from Laboratory Studies	-2	All metals values during baseflow and stormflow collections were within water quality standards (with the possible exception of Cr VI if >85% of Cr were in that form).
Consistency of Evidence	-2	Evidence refutes metals as a stressor.
Sum	-9	

7.10 Temperature

The total causal analysis score for temperature was -13 (Table 7-16), indicating that there is strong evidence that temperature is a non-stressor in Sand Branch. All temperature measurements were within water quality standards. For this reason and others explained in Table 7-16, temperature was categorized as a non-stressor in Sand Branch.

Table 7-16. Causal analysis results for temperature.

Evidence	Score	Explanation
Spatial Co-occurrence	-3	At both Sand Branch locations, VSCI scores were impaired, but temperature values were within water quality standards.
Temporal Co-occurrence	-3	At the time of benthic sample collection, temperature at both sites met water quality standards.
Temporal Sequence	-2	Spring VSCI scores were lower than fall scores, however, any potential high temperature excursions would be expected in the late summer.
Stressor-Response Relationships from Laboratory Studies	-3	All temperature values were within water quality standards.
Consistency of Evidence	-2	Evidence consistently refuted temperature as a stressor.
Sum	-13	

7.11 CADDIS Results

The total causal analysis scores for each candidate stressor are shown in Table 7-17. Candidate stressors with causal analysis scores ≤0 were classified as non-stressors, candidate stressors with causal analysis scores of 1-3 were classified as possible stressors, and candidate stressors with scores >3 were classified as probable stressors. The results indicate that sediment, total phosphorus, sulfate, and conductivity from TDS were identified as probable stressors in Sand Branch, with causal analysis scores ranging from +6 for sediment to +31 for conductivity.

These scores provide some indication of the potential degree of impairment from each stressor (TDS > sulfate > phosphorus > sediment). Benthic health in Sand Branch is most impacted TDS. The toxic impacts of high TDS (including sulfate) have nearly eliminated all sensitive species (*Ephemeroptera, Plecoptera*, and non-*Hydropsychid Trichoptera*), and without addressing this stressor, the stream will not likely recover. With TDS addressed, benthic health in Sand Branch may recover to some degree but will likely still remain impaired due to enrichment of phosphorus and sediment. Unless addressed, sediment enrichment will continue to limit available habitat for benthic macroinvertebrates, and nutrient enrichment from phosphorus will continue to fuel algal growth that shifts feeding niches, favors tolerant species, and limits diversity.

Table 7-17. Total causal analysis scores.

Candidate Stressor	CADDIS Score[1]
pH	-24
Temperature	-13
Dissolved Oxygen	-12
Dissolved Metals	-9
Total Nitrogen	1
Chloride	1
Potassium	1
Ammonia	2
Sodium	3
Sediment	6
Total Phosphorus	16
Sulfate	16
Conductivity from TDS	31

[1] Green indicates non-stressors, orange indicates possible stressors, and red indicates probable stressors.

8.0 Benthic Stressor Analysis Conclusions

The benthic stressor analysis considers available data to investigate the potential causes of impairment to the benthic macroinvertebrate community in the Sand Branch watershed. The conclusions from the analysis of the data were considered together through the CADDIS approach. The analysis also considered watershed conditions that may exacerbate the impairment, but for which a TMDL cannot be individually developed.

8.1 Probable Stressors

From the results of the stressor analysis, the potential of each candidate stressor to cause stress and lead to an impaired benthic community was identified. Each candidate stressor was categorized as either a non-stressor, possible stressor, or probable stressor to the benthic macroinvertebrate community (Table 8-1).

Table 8-1. Non-stressors, possible stressors, probable stressors in Sand Branch.

Non-Stressors	Possible Stressors	Probable Stressors
▪ Dissolved Oxygen ▪ Dissolved Metals ▪ pH ▪ Temperature	▪ Ammonia ▪ Chloride ▪ Potassium ▪ Sodium ▪ Total Nitrogen	▪ Conductivity (from TDS) ▪ Sediment ▪ Sulfate ▪ Total Phosphorus

8.2 TMDL Targets

Following causal analysis and the determination of probable stressors, target pollutants for which a TMDL may be developed were selected. TMDL target pollutants are the physical or chemical substances that will be controlled and allocated in a TMDL to result in restored aquatic life (measured by benthic macroinvertebrate health). TMDL targets must be pollutants that are controllable through source reductions, such as sediment, phosphorus, nitrogen, or other substances.

Based on the probable stressors identified through this process (conductivity from TDS, sulfate, total phosphorus, and sediment), TMDLs may be developed to address TDS, phosphorus, and sediment. Development of a TMDL to individually address sulfate in the watershed is not proposed because a TMDL to address TDS will collectively address sulfate as well as the other ions classified as possible stressors (chloride, potassium, and sodium).

Table 8-2. TMDL Targets in Sand Branch watershed.

Stream	TMDL Target
Sand Branch	▪ TDS ▪ Total Phosphorus ▪ Sediment

8.2.1 Total Dissolved Solids

TDS, which collectively addresses conductivity and the ion sulfate, was identified as a probable stressor in Sand Branch. Multiple lines of evidence supported this determination including periodic and continuous conductivity monitoring, toxicity testing, BCG analysis, and causal pathway analysis. High dissolved solids directly impact aquatic life through the possible toxicity of individual ions or the combined toxicity due to osmotic imbalance. This results in a loss of sensitive species and an overall biological impairment of the benthic community. Among DEQ stations within this ecoregion, Sand Branch exhibited the highest TDS levels. During TMDL development, sources causing or contributing to high TDS levels will be identified.

8.2.2 Total Phosphorus

Multiple lines of evidence supported the determination of phosphorus as a probable stressor including periodic phosphorus measurements, diurnal monitoring, seasonal trends, BCG analysis, and functional feeding group analysis. Increased nutrient availability increases algae growth, which directly alters macroinvertebrate feeding niches. Excess food from algae growth encourages the proliferation of a few nutrient tolerant species, which outcompete more sensitive species. This reduces the overall diversity of the benthic macroinvertebrate community and can result in a biological impairment.

8.2.3 Sediment

Sediment was identified as a probable stressor in Sand Branch. Multiple lines of evidence supported this determination including habitat metrics, water quality monitoring, seasonal trends, BCG, taxonomic community structure, and functional feeding group analysis. The increased particulate load acts to biologically impair the stream through two pathways: a change in feeding niches to favor filter feeders and deposit feeders (habitat alteration), and the filling of interstitial spaces that reduces available habitat (sedimentation).

8.3 Contributing Factors

In addition to the probable pollutant stressors identified, several factors were identified that contribute to the impact of the benthic macroinvertebrate community in Sand Branch but for which a TMDL cannot be individually developed. Not all stressors are pollutants amenable to TMDL development; TMDLs can only be developed for pollutants, not pollution in general. Even though these non-pollutant factors influence ecological communities and may be sources of stress, they are not pollutants for which a TMDL can be developed.Table 8-3 presents the likely additional contributing factors to the benthic impairment in Sand Branch.

Table 8-3. Contributing Factors to the Sand Branch Benthic Impairment.

Stream	Contributing Factors
Sand Branch	▪ Underlying Geology ▪ Land Disturbance ▪ Percent Imperviousness ▪ Degraded Riparian Buffer

8.3.1 Underlying Geology

The Sand Branch watershed is situated in the Triassic Lowlands ecoregion, which due to underlying geology, has naturally higher conductivity and higher phosphorus concentrations than the rest of the Northern Piedmont ecoregion (Porter *et al.,* 2020). This contributes to high TDS in Sand Branch through natural groundwater intrusion and from groundwater pumped from the quarry through the discharge. In addition to the contribution of TDS from underlying geology, another contributing factor is exposed rock, stone, and concrete debris in the watershed.

The underlying geology may also contribute to phosphorus in Sand Branch through the same pathways. While this ecoregion has naturally higher phosphorus levels, the levels observed in Sand Branch ranked in the 5th percentile of Triassic Basin stations at the upstream site but 81st percentile at the downstream site. This indicates significant contributions of phosphorus from the watershed between these stations.

8.3.2 Land Disturbance and Percent Imperviousness

Two contributing factors associated with urban areas, land disturbance and percent imperviousness, were identified as additional factors contributing to the stress of the benthic community in Sand Branch. These types of changes in a watershed can alter its natural hydrology (e.g. hydrologic modification) which may result in higher and more variable flows in the stream. Hydrologic modifications can cause shifts in the availability of water, sediment, food supply, habitat and pollutants from one part of the watershed to another, thereby causing changes in the types of biological communities that can be supported by the changed environment. In Sand Branch, the quarry located in its headwaters alters the natural hydrology of the stream due to the discharge creating a more consistent and higher volume of flow where the stream begins.

As watersheds develop and the percentage of impervious surfaces increases, runoff during precipitation events increases. As the amount of runoff increases, peak flows in local streams increase causing streambank erosion and stream bed scouring. This scenario causes unstable habitat conditions for benthic macroinvertebrates and increased sediment loads. Brabec *et al.,* (2002), found that fish and macroinvertebrate diversity decreased when watersheds exceeded 3.6 to 15% imperviousness, and the Sand Branch watershed greatly exceeds this threshold at 22%. The high imperviousness also contributes to high nutrient loads in Sand Branch by providing direct conduits for diffuse nutrient sources to be quickly transported to streams through storm sewer networks.

Land disturbance greatly increases the rate of watershed erosion, leading to increased sediment reaching the stream. Urban and commercial development within the Sand Branch watershed has been prevalent and will likely accelerate with the proposed addition of a large data center.

8.3.3 Degraded Riparian Habitat

The ability for the riparian habitat to support more sensitive benthic macroinvertebrate species is reliant upon the suitability of the riparian buffer and stream channel bottom. Riparian vegetation stabilizes stream banks and reduces bank erosion, which can often be a primary contributor to in-stream sediment loads. Loss of the riparian buffer can lead to increased runoff. This can result in the accumulation of sediment in the channel, covering the substrate with a fine layer of sediment. Alternatively, the runoff may erode the channel substrate, removing desirable habitat. Both effects alter the composition of the benthic community. The Sand Branch watershed has minimal riparian buffer along sections of the stream, with most of the stream corridor in close proximity to developed areas.

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APPENDIX A. Benthic Macroinvertebrate Data Collected in 2016 and 2020

Table A-1. Benthic macroinvertebrate community sampled in 2016 and 2020.

Taxa	**1ASAN000.34**				**1ASAN001.45**			
	2016		**2020**		**2016**		**2020**	
	Spring	**Fall**	**Spring**	**Fall**	**Spring**	**Fall**	**Spring**	**Fall**
VSCI	*11.37*	*53.3*	*9.6*	*33.4*	*35.5*	*37.9*	*15.1*	*43.1*
Antocha		6		2				
Argia	2			1				
Asellidae		1						
Boyeria				2				
Calopteryx				1				
Cambaridae		1						
Cheumatopsyche		6		62	12	73	1	37
Chironomidae (A)	172	33	198	48	80	2	170	9
Chrysops		1						
Corbicula	2	15		7				
Crangonyx					1			
Dubiraphia		3						
Ectopria				1				
Elmidae			2					
Hemerodromia		2				1	4	3
Hydracarina					1			
Hydropsyche			1	19	2	40	3	31
Hydropsychidae				26			1	12
Hydroptila		27		10				3
Lymnaeidae	1	3						
Neoplasta			1	1			2	
Nigronia					1	1		
Oligochaeta	3	8						
Optioservus								2
Physidae		25						
Planorbidae		1						
Psephenus		1				3		7
Simulium								3
Stenelmis		54	2	16	113	87	14	70
Tipulidae	1							3
TOTAL	**181**	**187**	**204**	**196**	**210**	**207**	**195**	**180**

APPENDIX B. Biological Condition Gradient (BCG) Attribute Tables

The attribute for each genus in benthic samples collected in 2016 and 2020 are provided herein.

Table B-1. BCG attribution for benthic macroinvertebrate community sampled in 2016 and 2020 at 1ASAN000.34.

Genus Level	**No. of Individuals**	**Functional Feeding Group**	**General Attribute[1]**	**Biological Condition Gradient (BCG) Attribute Assignments for Specific Stressors**										**Total Sample Size**	**No. of Taxa Types**	**Sampling Date**	**VSCI Score**
				DO	**Acidity (pH[2])**	**Alkalinity (pH[2])**	**Specific Conductance**	**Chloride**	**Sulfate**	**Nutrients[3]**	**Total Habitat Score**	**Relative Bed Stability**	**Watershed % Impervious**				
Chironomidae (A)	172	Collector	4	4	4	4	4	4	4	4	4	4	4	181	6	3/8/2016	11.37
Oligochaeta	3	Collector	5	4	4	3	5	4	4	5	5	5	5				
Argia	2	Predator	4	4	3	4	4	4	4	4	4	4	5				
Corbicula	2	Filterer	6t	4	3	4	5	4	4	5	5	4	5				
Lymnaeidae	1	Scraper	5	5	4	4	5	5	4	5	5		5				
Stenelmis	1	Scraper	4	4	4	4	5	4	4	4	5	4	5				
Stenelmis	54	Scraper	4	4	4	4	5	4	4	4	5	4	5	187	16	8/8/2016	53.32
Chironomidae (A)	33	Collector	4	4	4	4	4	4	4	4	4	4	4				
Hydroptila	27	Scraper	4	3	2	3	5	4	5	5	4	3	5				
Physidae	25	Scraper	5	5	4	5	5	4	3	5	5	5	5				
Corbicula	15	Filterer	6t	4	3	4	5	4	4	5	5	4	5				
Oligochaeta	8	Collector	5	4	4	3	5	4	4	5	5	5	5				
Antocha	6	Collector	4	3	2	4	5	4	4	4	3	4	4				
Cheumatopsyche	6	Filterer	5	4	3	4	5	4	4	5	4	4	5				
Dubiraphia	3	Collector	4	4	3	3	4	4	4	4	4	4	4				
Lymnaeidae	3	Scraper	5	5	4	4	5	5	4	5	5		5				
Hemerodromia	2	Predator	5	4	4	4	5	4	5	4	4	4	5				
Asellidae	1	Collector	4	5	5	2	4	4	4	4	5	4	4				
Cambaridae	1	Collector	4	4	4	4	4	4	4	4	4	4	4				
Chrysops	1	Predator	4	4	4	4	3	4	3	4	5		3				
Planorbidae	1	Scraper	5	5	4	3	4	4	3	5	5	4	5				
Psephenus	1	Scraper	4	3	3	4	4	3	4	3	3	3	3				

Genus Level	No. of Individuals	Functional Feeding Group	General Attribute[1]	Biological Condition Gradient (BCG) Attribute Assignments for Specific Stressors											Total Sample Size	No. of Taxa Types	Sampling Date	VSCI Score
				DO	Acidity (pH[2])	Alkalinity (pH[2])	Specific Conductance	Chloride	Sulfate	Nutrients[3]	Total Habitat Score	Relative Bed Stability	Watershed % Impervious					
Chironomidae (A)	198	Collector	4	4	4	4	4	4	4	4	4	4	4		204	5	3/11/2020	9.59
Elmidae	2	Collector	4	3	3	4	3	3	4	3	3	4	4					
Stenelmis	2	Scraper	4	4	4	4	5	4	4	4	5	4	5					
Hydropsyche	1	Filterer	4	3	3		5	4	5	5	4	4	5					
Neoplasta	1	Predator	4	2	4	4	5	3	4	3	3	4	4					
Cheumatopsyche	62	Filterer	5	4	3	4	5	4	4	5	4	4	5		196	13	9/17/2020	33.38
Chironomidae (A)	48	Collector	4	4	4	4	4	4	4	4	4	4	4					
Hydropsychidae	26	Filterer	4	3	3	4	4	3	4	4	4	4	4					
Hydropsyche	19	Filterer	4	3	3		5	4	5	5	4	4	5					
Stenelmis	16	Scraper	4	4	4	4	5	4	4	4	5	4	5					
Hydroptila	10	Scraper	4	3	2	3	5	4	5	5	4	3	5					
Corbicula	7	Filterer	6t	4	3	4	5	4	4	5	5	4	5					
Antocha	2	Collector	4	3	2	4	5	4	4	4	3	4	4					
Boyeria	2	Predator	4	4	4	3	4	4	4	4	4	4	4					
Argia	1	Predator	4	4	3	4	4	4	4	4	4	4	5					
Calopteryx	1	Predator	4	4	4	3	4	5	4	5	5		5					
Ectopria	1	Scraper	3	3	4	4	4	3	4	4	3	4	4					
Neoplasta	1	Predator	4	2	4	4	5	3	4	3	3	4	4					

[1] Characterizes tolerance and other ecological attributes in relation to broad physical and chemical conditions. Lower values indicate greater sensitivity to pollution, higher values indicate greater tolerance to pollution.

[2] For pH, responses to acidity and alkalinity were considered independently.

[3] For nutrients, although Total Nitrogen (TN) and Total Phosphorous (TP) were analyzed separately, these were assessed as common responses for attribution assignment.

Table B-2. BCG attribution for the benthic macroinvertebrate community sampled in 2016 and 2020 at 1ASAN001.45.

Genus Level	No. of Individuals	Functional Feeding Group	General Attribute[1]	Biological Condition Gradient (BCG) Attribute Assignments for Specific Stressors										Total Sample Size	No. of Taxa Types	Sampling Date	VSCI Score
				DO	Acidity (pH[2])	Alkalinity (pH[2])	Specific Conductance	Chloride	Sulfate	Nutrients[3]	Total Habitat Score	Relative Bed Stability	Watershed % Impervious				
Stenelmis	113	Scraper	4	4	4	4	5	4	4	4	5	4	5	210	7	3/8/2016	35.48
Chironomidae (A)	80	Collector	4	4	4	4	4	4	4	4	4	4	4				
Cheumatopsyche	12	Filterer	5	4	3	4	5	4	4	5	4	4	5				
Hydropsyche	2	Filterer	4	3	3		5	4	5	5	4	4	5				
Crangonyx	1	Collector	5	5	5	3	4	5	4	5	5		5				
Hydracarina	1	Predator	4														
Nigronia	1	Predator	4	3	4	3	4	4	4	4	3	4	3				
Stenelmis	87	Scraper	4	4	4	4	5	4	4	4	5	4	5	207	7	8/8/2016	37.94
Cheumatopsyche	73	Filterer	5	4	3	4	5	4	4	5	4	4	5				
Hydropsyche	40	Filterer	4	3	3		5	4	5	5	4	4	5				
Psephenus	3	Scraper	4	3	3	4	4	3	4	3	3	3	3				
Chironomidae (A)	2	Collector	4	4	4	4	4	4	4	4	4	4	4				
Hemerodromia	1	Predator	5	4	4	4	5	4	5	4	4	4	5				
Nigronia	1	Predator	4	3	4	3	4	4	4	4	3	4	3				
Chironomidae (A)	170	Collector	4	4	4	4	4	4	4	4	4	4	4	195	7	3/11/2020	15.09
Stenelmis	14	Scraper	4	4	4	4	5	4	4	4	5	4	5				
Hemerodromia	4	Predator	5	4	4	4	5	4	5	4	4	4	5				
Hydropsyche	3	Filterer	4	3	3		5	4	5	5	4	4	5				
Neoplasta	2	Predator	4	2	4	4	5	3	4	3	3	4	4				
Cheumatopsyche	1	Filterer	5	4	3	4	5	4	4	5	4	4	5				
Hydropsychidae	1	Filterer	4	3	3	4	4	3	4	4	4	4	4				

Benthic Stressor Analysis for Sand Branch Watershed

Genus Level	No. of Individuals	Functional Feeding Group	General Attribute[1]	Biological Condition Gradient (BCG) Attribute Assignments for Specific Stressors											Total Sample Size	No. of Taxa Types	Sampling Date	VSCI Score
				DO	Acidity (pH[2])	Alkalinity (pH[2])	Specific Conductance	Chloride	Sulfate	Nutrients[3]	Total Habitat Score	Relative Bed Stability	Watershed % Impervious					
Stenelmis	70	Scraper	4	4	4	4	5	4	4	4	5	4	5	178	11	9/17/2020	43.05
Cheumatopsyche	37	Filterer	5	4	3	4	5	4	4	5	4	4	5				
Hydropsyche	31	Filterer	4	3	3		5	4	5	5	4	4	5				
Hydropsychidae	12	Filterer	4	3	3	4	4	3	4	4	4	4	4				
Chironomidae (A)	9	Collector	4	4	4	4	4	4	4	4	4	4	4				
Psephenus	7	Scraper	4	3	3	4	4	3	4	3	3	3	3				
Hemerodromia	3	Predator	5	4	4	4	5	4	5	4	4	4	5				
Hydroptila	3	Scraper	4	3	2	3	5	4	5	5	4	3	5				
Simulium	3	Filterer	4	4	4	4	4	3	5	5	4	4	4				
Tipulidae	3	Shredder	4	4	5	3	4	4	4	4	4	5	4				
Optioservus	2	Scraper	4	3	3	4	4	4	5	4	4	4	3				

[1] Characterizes tolerance and other ecological attributes in relation to broad physical and chemical conditions. Lower values indicate greater sensitivity to pollution, higher values indicate greater tolerance to pollution.

[2] For pH, responses to acidity and alkalinity were considered independently.

[3] For nutrients, although Total Nitrogen (TN) and Total Phosphorous (TP) were analyzed separately, these were assessed as common responses for attribution assignment.

APPENDIX C. Public Participation

Development of a TMDL, including the benthic stressor analysis, includes a public participation component to inform the public of the effort and to encourage their participation. Their involvement is important as their input and local knowledge helps to identify probable pollutants for which a TMDL may be developed to address the benthic impairments in the watershed. DEQ staff encouraged public participation during benthic stressor analysis portion of the TMDL study to address a benthic impairment in the Sand Branch watershed. This portion of the study consisted of two public meetings and three technical advisory committee (TAC) meetings. A summary of the meetings is presented in Table C-1.

Table C-1. Public Participation during the Benthic Stressor Analysis

Meeting (Date)	Location	Numbers of Attendees	Purpose
1st Public Meeting / 1st TAC Meeting (October 29, 2020)	Virtual	16	Provide an overview of the water quality planning process, the water quality impairments in Sand Branch, and an overview of the TMDL study followed by an opportunity to ask questions and share local knowledge on the watershed.
2nd TAC Meeting (January 23, 2021)	Virtual	12	Provide information on benthic stressor analysis, specifically the analysis of water chemistry data.
3rd TAC Meeting (April 21, 2021)	Virtual	12	Provide information on benthic stressor analysis, specifically the analysis of biological data and CADDIS.
2nd Public Meeting (May 26, 2021)	Virtual	5	Summarize the benthic stressor analysis and initiate the 30-day public comment period on the report. Also to discuss the next phase of the project, developing TMDLs to address the probable stressors to the benthic community.

For the stressor analysis, a joint public meeting and TAC meeting initiated the TMDL study on October 29, 2020 to discuss the benthic impairment and identify data to be used in the stressor analysis. Second and third TAC meetings were held on January 23, 2021 and April 21, 2021, respectively, to discuss the results of the stressor analysis. A Public Meeting was held on May 26, 2021 to present the results of the Benthic Stressor Analysis report. All these meetings were held virtually due to the Governor's declaration of a State of Emergency in response to the COVID-19 pandemic.

After the second Public Meeting, a 30-day public comment period began from May 27, 2021 to June 28, 2021. DEQ received five comments during this period.

Table C-2. Public Comments Received on the Benthic Stressor Analysis

Organization	Contact	Date Received
Chantilly Crushed Stone, Inc.	Edward Hoy	6/28/21
Luck Stone Companies	Mark Williams	6/28/21
Quarry Permit Environmental Training Services, LLC	David Cress	6/28/21
Virginia Concrete Company	Thomas Foley	6/28/21
Virginia Transportation Construction Alliance	Rob Lanham	6/28/21

The public comments and DEQ's response to comments on the Stressor Analysis report are provided in Appendix D. These responses to comments, compiled in a separate document dated August 23, 2021, were shared with the TAC by email dated August 23, 2021.

The version of the benthic stressor analysis report dated May 18, 2021, upon which the public comments were provided, was revised to address minor typographical errors and to add two new appendices, Appendix C (Public Participation) and Appendix D (Public Comments).

APPENDIX D. Public Comments

A public comment period was held from May 27, 2021 through June 28, 2021 on the report entitled *Stressor Analysis to Identify Probable Stressors to the Impaired Benthic Macroinvertebrate Community in the Sand Branch watershed, Loudoun and Fairfax Counties, Virginia* dated May 18, 2021. The report summarizes the results of the benthic stressor analysis conducted to identify probable stressors to the impaired benthic macroinvertebrate community in the Sand Branch watershed. The report concludes that the three most probable stressors to the benthic community are sediment, total dissolved solids and total phosphorus, and that a Total Maximum Daily Load (TMDL) be developed for each of these pollutants.

The public comments voiced similar concerns on the benthic stressor analysis and aspects of TMDL development. Those comments are summarized, in italicized text, and staff responses to each are provided below.

The public comments voiced similar concerns on the benthic stressor analysis and aspects of TMDL development. Those comments are summarized, in italicized text, and staff responses to each are provided below.

1. *Due to the irregular and intermittent characteristics of flow in Sand Branch, the stream is not able to consistently support a benthic macroinvertebrate community, particularly one that includes Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) (EPT) taxa. Local maps identify Sand Branch as having an intermittent, not a perennial, hydrologic regime. Further development of the watershed will lead to more impervious surfaces, reduced riparian buffers and minimal stormwater treatment which will concentrate runoff and cause the stream to be flashier and more intermittent. Complicating the stream hydrology is that pit water discharged from Chantilly Quarry is irregular and does not provide consistent flow except when water from the pit is discharged. The commenters noted that EPA taxa, while not absent, are less abundant in intermittent streams and that the community structure shifts with seasonal fluctuations of flow. Therefore, it is expected that the Virginia Stream Condition Index (VSCI) also fluctuates with the seasons. Commenters recommended that the VSCI be adjusted to account for differences between intermittent and perennial streams.*

   <u>Watershed Characteristics and Hydrology</u>
   Maps depicting watershed characteristics are drawn using the tools and information available at the time of development. Depending on the resources available and timeframe when those maps were completed, the features depicted may not reflect existing conditions, particularly in rapidly developing watersheds. Therefore, it is necessary, particularly in urban watersheds such as Sand Branch, to conduct site visits to verify the desktop review of watershed characteristics.

   Loudoun County conducted a countywide stream assessment in 2009, which included fieldwork to determine extent of stream perenniality. Their study sites included Sand Branch, specifically in the vicinity of DEQ's monitoring station 1ASAN001.45 (Loudoun County email communication dated July 26, 2021 and August 13, 2021). Loudoun County's recorded field observations noted presence of high groundwater table or seeps/springs and identified a moderate presence of benthic macroinvertebrates (including EPT taxa) and concluded the reach was perennial based upon a total score of 30.5 points, which was above the perennial threshold of 25

   Since 2015, DEQ water quality monitoring staff and DEQ biologists have conducted water quality monitoring (32 events, year round) and biological monitoring (four events, spring and fall) on Sand Branch at two stations, one upstream (1ASAN001.45) and one downstream (1ASAN000.34). DEQ water compliance staff has also conducted water quality monitoring of several permitted discharges in the watershed, including that of Chantilly Crushed Stone, Inc.'s discharge from Outfall 001 (VPDES Permit No. VAG840106) from 2014 -2018 (17 events). Based upon staff observations during these monitoring events, flow has been present in the stream channel at Outfall 001 and both monitoring locations sufficient to collect samples. Additionally, a continuous monitoring sonde was deployed by DEQ at 1ASAN000.34 from August 10, 2020 – August 26, 2020 and December 10, 2020 – February 10, 2021, during which time water quality samples were collected and recorded every 15 minutes, exhibiting there was continually sufficient flow for the frequent readings to be taken.

   Discharge Monitoring Report (DMR) data submitted quarterly by Chantilly Crushed Stone, Inc. indicates that discharges occurred during each reporting period since 2006 and the average monthly flows were approximately 0.75 MGD (based upon DMRs submitted from 2015-2020). While the flow in Sand Branch is highly augmented by the operation of the quarry, DEQ's observations and available data support that the upper watershed of Sand Branch experiences a more constant hydrologic source than may otherwise be expected based upon watershed size. Additionally, it's worth noting that the impaired status of Sand Branch is based upon not only the upstream station but also assessment of water quality and biological data collected at the downstream station (1ASA000.34), which is shown in various resources as being perennial. Monitoring at the upstream station (1ASAN001.45) serves to provide a more robust understanding of the water quality and biology characteristics between the upper and lower reaches of the watershed.

DEQ is willing to review any additional information the commenters or other interested stakeholders may have in regards to this topic that can help to further our understanding of this watershed, which is currently based upon known available external resources, data reported in DMRs and field observations of DEQ staff.

Virginia Stream Condition Index and Stream Hydrology

The Virginia Stream Condition Index (VSCI) is a multi-metric macroinvertebrate index used to assess the aquatic life use status for wadeable freshwater streams and rivers in non-coastal areas of the state. DEQ's biological monitoring program evaluates streams where benthic macroinvertebrates are expected to reside. Based upon staff observations of the stream characteristics at both the upstream and downstream monitoring stations, the stream is capable of supporting a benthic community.

VSCI scores have fluctuated by season, however, the pattern of fluctuation does not indicate intermittent flow conditions. Spring VSCI scores have averaged 25.3 at the upstream monitoring station and 10.5 at the downstream station, while fall scores have been much higher, averaging 40.5 at the upstream station and 43.4 at the downstream station. If low VSCI scores were due to drying, scores during the fall would be expected to be lower than spring scores, since late spring and early fall is the typical timing for minimum instream flow. Furthermore, the pattern of higher VSCI scores at the upstream monitoring station and lower scores at the downstream station do not fit with intermittent flow as a stressor. Upstream portions of a watershed are generally more susceptible to drying than downstream portions. Lastly, the pattern of community composition does not necessarily point to intermittent flow as a stressor. Straka et al. (2019)[1] developed a tool to identify intermittent stream conditions based on benthic macroinvertebrate indicators. While the tool was developed in Europe and not completely applicable to Sand Branch, the authors identified specific species that were indicators of intermittent versus perennial flow conditions. EPT taxa were represented in both groups (some species indicators of intermittent flow conditions and some species indicators of perennial flow conditions), so absence of EPT taxa does not necessarily suggest intermittent flow conditions. Interestingly, Hydropsyche sp. was one of the top five strongest indicators of perennial flow conditions. This is likely because Hydropsyche build nets to catch organic matter flowing in the water column. Decreases in flow would limit the effectiveness of this feeding strategy. In Sand Branch, Hydropsyche sp. (and other species of net-spinning caddisflies in the family Hydropsychidae) were one of the most abundant taxa, indicating that intermittent flow conditions are not likely a stressor.

Future Development

Based upon a watershed tour conducted on December 10, 2020, recent aerial imagery and 2016 Virginia Land Cover Data, approximately 60 percent of the roughly 880 acre Sand Branch watershed is developed. Approximately 37 percent of the watershed is forested, with the majority being concentrated in the center of the watershed. Correspondence with Loudoun County staff (email communication on 7/14/2020 and 4/28/2021) shared that approximately 100 acres of the forested tract is proposed to be developed into a datacenter by Amazon.com, Inc. Any project will be subject to the existing laws and regulations in place at the time for the control of construction and post construction stormwater to attenuate and treat stormwater runoff before it enters surface waters.

2. *The potential stressors to the impaired benthic community, as well as the potential source of TDS, has not been thoroughly studied. In the coalfields, streams have higher levels of TDS (up to 1,000 mg/L) and higher VSCI scores (40 to mid-50 range) than the lower VSCI scores seen in Sand Branch. Additionally, the ambient water toxicity test results conducted in March 2020 in which fathead minnows (Pimphales promelas) had a NOEC of 50% and an IC of 63.0% but the water flea (Ceriodaphnia dubia) had no mortality seems to indicate that TDS is not as toxic as identified. Commenters noted a potential source of stressors and/or TDS could be chemicals in firefighting foam, such as PFAS and potassium carbonate, which are used and present in large quantities at Dulles International Airport (IAD)'s firefighting training facility located in the Sand Branch watershed. Further investigation is needed on the potential source of TDS and to identify*

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[1] Straka, M., Polášek, M., Syrovátka, V., Stubbington, R., Zahrádková, S., Němejcová, D., ... & Pařil, P. (2019). Recognition of stream drying based on benthic macroinvertebrates: A new tool in Central Europe. *Ecological Indicators*, *106*, 105486.

the presence of additional stressors, other than TDS and total suspended solids (TSS), causing the unhealthy benthic community.

The benthic stressor analysis for Sand Branch was conducted in accordance with agency policy and practice and the document published by DEQ (2017) in the "Stressor Analysis in Virginia: Data Collection and Stressor Thresholds." The benthic stressor analysis process applied a weight-of-evidence approach, which consisted of an analysis of the available water quality chemistry and toxicity, biological data, physical habitat information and USEPA's Causal Analysis/Diagnosis Decision Information System (CADDIS) (USEPA, 2018)[2], to define the probable stressor(s) that explain(s) the shift in the benthic macroinvertebrate community.

Candidate Stressors Considered

A stressor can have direct effects on the organism itself, like dissolved metals or toxic chemicals, or alter habitat and resources that in turn result in a shift in the macroinvertebrate community. In Virginia, the suite of candidate stressors considered in benthic stressor analyses are those with known effects on macroinvertebrates and are found to be widespread and common stressors to benthic communities in Virginia. This suite (summarized in the table below) was compiled from freshwater probabilistic monitoring data collected by DEQ since 2005 throughout Virginia and supported by scientific literature. Candidate stressors are compared, as applicable, to numeric water quality criteria and stressor threshold values (latter were developed and published by DEQ in 2017).

Table 1. Candidate stressors analyzed and additional contributing factors identified.

Candidate Stressors Considered					
Candidates With Stressor Thresholds[1,2]:	***pH***	***Dissolved Oxygen (DO)***	Total Phosphorous	Total Dissolved Solids (TDS)	Potassium
	Temperature	Specific Conductivity	Total Nitrogen	Sulfate	***Chloride***
	Sediment[3]	Metal Cumulative Criterion Unit (Metals CCU)	***Individual Metals, Dissolved***	Sodium	
Candidates Without Stressor Thresholds[2]:	Total Suspended Solids (TSS)	***Ammonia***	DO (Saturation)	Turbidity	
Additional Contributing Factors:	Underlying Geology	Land Disturbance	Percent Imperviousness	Degraded Riparian Buffer	

[1] Values published in "Stressor Analysis in Virginia: Data Collection and Stressor Thresholds" (DEQ, 2017)

[2] Parameters with water quality criteria denoted in bold, italicized text. When available, the value was also used in the analysis (Water Quality Standards, 9VAC25-260).

[3] Sediment was evaluated using Log_{10} Relative Bed Stability (LRBS) index and Habitat.

While the stressor analysis is robust within the scope of the candidates analyzed, it is not intended to be an exhaustive review of potentially thousands of chemicals and contaminants. Rather, it's an analysis of the most common and thus likely stressors to best allocate and apply limited available staff and resources to conduct these studies. For a specific waterbody, if the analysis of the suite of candidates are inconclusive as stressors, further investigation beyond the standard suite of candidates may be warranted.

For Sand Branch, the analysis identified several stressors contributing to the unhealthy benthic macroinvertebrate community from the suite of candidate stressors. Four probable stressors (sediment, sulfate, TDS and total phosphorous) were identified which are pollutants and can be

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[2] USEPA. 2018. Causal Analysis/Diagnosis Decision Information System (CADDIS). Retrieved: January 17, 2018. Available from: www.epa.gov/caddis. Office of Research and Development, Washington, D.C.

addressed through TMDLs. There were also several contributing factors identified (e.g. underlying geology, land disturbance, percent imperviousness and degraded riparian buffer) that influence and may stress benthic communities, but that cannot be effectively targeted through TMDL development. Because the results of the benthic stressor analysis strongly pointed to several stressors as contributing to an unhealthy benthic macroinvertebrate community, further investigation into additional stressors was deemed unnecessary. Additionally, monitoring of the benthic community and ambient water quality of the impaired surface water will continue through TMDL implementation. If the established endpoints for each of the TMDLs are met, yet the benthic macroinvertebrates community is still impaired, it indicates there are additional stressors to the benthic community which could be further investigated at that time.

Potential Sources of TDS

A benthic stressor analysis is conducted to identify the cause(s) of stress to an unhealthy or impaired benthic macroinvertebrate community. At this phase of a TMDL study that addresses a benthic impairment, the focus is on understanding the existing water quality of the impaired waterbody and identifying probable stressors. Once a pollutant(s) is identified as a probable stressor(s), and a TMDL approach to address the pollutant(s) is selected, the focus then turns to pollutant source identification from nonpoint source and point source (permitted) surface water discharges.

The TMDL study for Sand Branch moved into TMDL development following completion of the benthic stressor analysis which concluded with a public meeting held on May 26, 2021, and public comment period that closed June 28, 2021. Staff are currently reviewing available information to conduct a source assessment for each of the three pollutants (sediment, total phosphorus and TDS) within the Sand Branch watershed. DEQ will accept any information members of the Technical Advisory Committee (TAC) or interested stakeholders have to assist in this source assessment for sediment, total phosphorous or TDS.

Comparison to Coalfield TDS TMDLs

DEQ agrees that the VSCI scores observed in Sand Branch indicate a more severe impairment than is typically observed in coalfield TMDLs; however, caution should be used in making such comparisons, because: 1) Sand Branch is subject to multiple stressors (sediment, sulfate, and phosphorus in addition to TDS), and 2) the toxicity of TDS is site-specific and dependent upon the composition of individual ions. During the second Technical Advisory Committee (TAC) meeting held on January 25, 2021, a representative of the Virginia Department of Minerals, Mines and Energy (DMME) provided a similar observation regarding the levels of TDS seen versus VSCI scores in watersheds impacted by coal mining activities and questioned whether other stressors may also play a role. At that time, the benthic stressor analysis was not completed, and subsequently the completed analysis did identify three probable stressors in addition to TDS (sediment, sulfate, and phosphorus) and four additional contributing factors (underlying geology, land disturbance, percent imperviousness, and degraded riparian buffers). Due to these multiple stressors, Sand Branch would be expected to have a more severely impaired benthic community than watersheds that may be impacted by TDS alone or in combination with fewer additional stressors.

Secondly, care is needed in comparing TDS levels and effects to the benthic macroinvertebrate community in one watershed to another watershed due to the variations that can occur in the specific ion composition of TDS based on the underlying geology of the watershed. Based upon the ion composition of TDS, the toxicity can differ due to varying concentrations and relative toxicities of the individual constituents as well as the combined osmotic effects of the overall ionic strength. Therefore, while the benthic stressor analysis concurs there are additional stressors contributing to the benthic impairment in Sand Branch, a straight comparison of the toxic effects associated with TDS levels in other parts of the state with differing underlying geology can be misleading and limit the understanding of TDS effects in Sand Branch.

Because the composition of ions contributing to the TDS is so important in understanding the toxic effects, DEQ expanded the stressor analysis to include an ion composition analysis and factored this information into the plan for developing the TMDL. Various cations and anions were measured in Sand Branch, the origination of its headwaters, and in downstream Cub Run. Ions were compared on a milliequivalent basis to eliminate the influence of charge and atomic mass. The ion composition analysis of ambient samples collected at the two Sand Branch stations (1ASAN000.34 and 1ASAN001.45) revealed a consistent pattern of ion composition. Cations were comprised of approximately 50% calcium (Ca^{+2}), 36% magnesium (Mg^{+2}), 13% sodium (Na^{+}), and 1% potassium (K^{+}). Anions were comprised of approximately 66% sulfate (SO_4^{-2}), 26% bicarbonate (HCO_3^{-}), 7% chloride (Cl^{-}), and 1% nitrate (NO_3^{-}). This indicates a primarily calcium/magnesium and carbonate/sulfate dominated composition, which could come from the dissolution of underlying geology. This pattern of ion composition in Sand Branch was consistent with the pattern of ions in the Chantilly Crushed Stone, Inc. (VPDES Permit No.VAG840106) discharge, which comprises the headwaters of Sand Branch. Other ions more commonly attributed to urban influences (Na^+, K^+, and Cl^-) are added as Sand Branch moves downstream. The ion composition analysis also revealed that Sand Branch has a significant influence on the ion composition of Cub Run below their confluence. This information on TDS composition is being factored into the TMDL development phase through the use of toxicity testing on laboratory-prepared samples created to match the ion composition of Sand Branch. Toxicity data will then be used to develop a TMDL endpoint for TDS that is specific to Sand Branch.

The lines of evidence supporting TDS and sulfate as stressors were strong, resulting in those being identified as probable stressors. For the other three ions (chloride, potassium and sodium) considered, the lines of evidence were not as strong, but still categorized those pollutants as possible stressors. Focusing on TDS for TMDL development addresses not only sulfate, but also the other three ions, chloride, potassium and sodium. This approach, focusing on the whole instead of individual parts of TDS, will provide greater flexibility in the implementation of the TMDL, including enabling a broader range of management measures to minimize sources of TDS.

Ambient Toxicity Tests
Based upon DEQ's review, the ambient toxicity testing results from Sand Branch are consistent with the conclusions of the benthic stressor analysis given the ecological relevance of the test organisms and the snapshot nature of ambient sampling. Toxicity test results from March 2020 showed no observed acute toxicity to *C. dubia* or *P. promelas* and no chronic toxicity to *C. dubia*. However, test results did show chronic toxicity to *P. promelas,* where survival in the 100% dilution sample was 65% in comparison to the 93% survival rate of the control sample. The NOEC was 50%, which means dilutions of the ambient water above 50% (half ambient sample and half control water) exhibited toxicity. While *P. promelas* and *C. dubia* are common test species for measuring toxicity in waters and wastewaters, they do not fully represent the diversity and sensitivity of aquatic ecosystems that is represented through benthic macroinvertebrate surveys. The water flea is a lentic organism that is typically found in still water like lakes and ponds while the fathead minnow is a fish species that lives in the water column. The benthic macroinvertebrate community, which is the focus of this TMDL, is a complex community of different organisms living on and within the stream substrate. This difference in habitat and species diversity means that single-species toxicity tests may underestimate the community-level effects that are observed through benthic macroinvertebrate surveys. To address this issue and increase the ecological relevance of toxicity testing, the next round of toxicity sampling will include two additional species that are members of the benthic macroinvertebrate community (*Hyalella azteca* and field-collected mayfly and/or snail species).

Secondly, ambient toxicity testing results must be viewed within the context of the grab sampling regime. The toxicity tests were performed on a series of three grab samples collected from Sand Branch during the week of 3/9/2020. This represents a snapshot of time, compared to the continuous exposure of in-stream benthic macroinvertebrates. Levels of TDS and individual ions change continually throughout the seasons, and conductivity levels as high as 3370 uS/cm have been measured during runoff events in Sand Branch. While ambient sampling for one-time toxicity testing is not likely to capture such excursions, in-stream benthic macroinvertebrate communities are subject to these excursions, and the benthic community is shaped by the long-term totality of exposures. This represents another reason why ambient toxicity tests may underestimate the community-level effects that are observed through benthic macroinvertebrate surveys. To address this issue during TMDL development, ambient toxicity testing will be supplemented with toxicity testing of laboratory-prepared samples created to match the ion characteristics of Sand Branch. This will isolate the toxic effects of TDS and avoid the vagaries of grab sampling.

Live Fire Training Facility
DEQ coordinated with the Metropolitan Airports Authority (MWAA) on the Live Fire Training Facility on the Dulles International Airport (IAD) property. By email communication dated July 13, 2021, MWAA stated that they do not use chemicals (e.g. PFAS or potassium carbonate) in their training activities at the Live Fire Training Facility. There are two ponds associated with the facility: a retention pond that collects water from the facility and does not discharge and a detention pond that collects water from around the facility and discharges to Sand Branch. Based upon DEQ review of available information, DEQ's water quality and biological monitoring station at river mile 1.45 (1ASAN001.45, located just downstream of Willard Road), is located above the point at which discharge from the detention pond enters Sand Branch. Additional water quality and biological monitoring occurs downstream of that discharge at DEQ Monitoring Station 1ASAN000.34. Because chemicals are not used at the facility and the discharge point from the facility is located downstream from the upstream monitoring station (1ASAN001.45) that identified an impaired benthic community, PFAS are not a likely stressor to the benthic community in Sand Branch.

3. *The choice of reference stream is critical in determining how the TMDL endpoint concentrations for TDS will be determined. The reference stream currently selected does not receive flow from areas underlain by the Triassic Basin, as occurs in Sand Branch, which may set a standard lower than what is present in the natural environment. Commenters recommended that an alternative to the reference stream approach is to identify if the background source of TDS is from the shallow groundwater table and the deeper underlying aquifer, and thus more natural, by collecting from samples from either an existing well or drilling a well if none exists. They noted this may also point to other sources of potential pollutants.*

   The comparison watershed approach, referred to as AllForX, will only be used to develop TMDL endpoints for sediment and total phosphorous. This approach has been used in other TMDL studies in Virginia. For TDS, DEQ considered three approaches to develop a TMDL threshold: (a) a literature-based approach; (b) a reference watershed approach; and (c) a site-specific approach using toxicity test data for Sand Branch. Each approach is discussed below, and DEQ selected the third approach for this project (site-specific approach using toxicity test data for Sand Branch).

   a. The literature-based approach, which consists of reviewing studies conducted throughout the country, was not selected because those values may have limited applicability to the conditions in Sand Branch. The ion composition of TDS in literature-derived studies varies widely and likely varies from the ion composition seen in Sand Branch. As some ions are more toxic to aquatic species than others, this difference can lead to differences in the resulting TDS thresholds from these studies. This means that literature-based thresholds may have limited applicability to Sand Branch and could overestimate or underestimate in-stream effects.

   b. The reference watershed approach, which uses geographically relevant information to develop the TDS threshold, was considered as this approach has been used by DEQ in the development of endpoints for TDS TMDLs developed in watersheds impacted by coal mining activities in southwestern Virginia. In those TMDLs, the 90th percentile of TDS (or indirectly, conductivity) in a reference watershed similar to the TMDL watershed, but with an unimpaired benthic community, was used to develop the TDS endpoint. However, as noted by the commenters, the challenge in applying a similar approach for the Sand Branch watershed is identifying a watershed(s) in the same ecoregion (Triassic Lowlands and/or Trap Rock and Conglomerate Uplands) with similar land use and an unimpaired benthic community. While a reference station (Licking Run, 1ALIL008.29) was used in the benthic stressor analysis as a comparative station for select biological and water quality parameters, it was identified as not suitable to develop a TMDL endpoint for TDS due to limited data collected on ions and conductivity. Additionally, its watershed characteristics are more rural in nature and the upper portion of the watershed lies within a different ecoregion. Due to the Triassic Lowlands and/or Trap Rock and Conglomerate Uplands Ecoregion being a contributor to the composition of the water quality in those watersheds that lie within it, such as Sand Branch, it is highly desirable for a reference watershed to be entirely within the same ecoregion. Therefore, the short-comings with Licking Run (lack of a robust dataset, some limitations in the comparability of the underlying geology, significantly different land uses) rendered this station less than ideal as a reference station for TDS. While the reference watershed approach is viable, DEQ concluded that for this project there are insufficient candidate reference watersheds and therefore, the reference watershed approach was not adopted for setting a TDS target in this TMDL study.

   c. The third approach considered, and which was selected, is development of a TDS threshold using water column toxicity test results from Sand Branch to establish a site-specific endpoint. This approach uses acute and chronic toxicity testing to identify the point at which toxicity is exhibited in certain test species. Toxicity testing evaluates the response of an organism(s) to a sample. It may consider acute and/or chronic effects including lethality, impaired growth or reproduction. The site-specific approach entails conducting toxicity tests using ambient Sand Branch water samples as well as a laboratory-prepared water sample representing the ion composition of Sand Branch (identified through an analysis of ions in ambient water samples). The use of a laboratory-prepared water sample is important to isolate the effects of TDS from other potential interferences in the ambient samples in order to estimate a toxic threshold for TDS based on the ion composition in Sand Branch. The results of the toxicity tests can be translated to a protective TDS concentration that can be used for TMDL development. This approach is the most robust method to develop a TDS threshold for this TMDL because the threshold will be developed based upon the ion composition specific to Sand Branch and will directly link the threshold value to in-stream toxicity.

Given the approach selected to develop the TMDL threshold for TDS is the site-specific ambient toxicity data approach discussed above under No. 3.c, the threshold will reflect the ion composition of the TDS in Sand Branch. DEQ is also not aware of any existing ground water quality data or well information in the Sand Branch watershed. While this information would characterize the water quality of the groundwater, ground water quality data is not necessary to develop the TMDL threshold for TDS for this project.

The response to No. 2 addresses the concern noted about potential pollutants other than TDS contributing stress to the impaired benthic community.

4. *Virginia Concrete operates a ready-mix concrete plant under a VPDES General Permit in the Sand Branch watershed. In general, the easiest targets to identify as pollutant sources are industrial permit holders. This watershed is more complicated than that, as has been discussed during the Technical Advisory Committee (TAC) meetings, which makes a viable solution that much more difficult.*

   DEQ agrees that this is a complicated watershed with various pollutant sources. As with all TMDLs, DEQ will assess all relevant sources of the TMDL pollutants. This includes point sources, which are included in the wasteload allocation of the TMDL, as well as non-point sources, which are included in the load allocation portion of the TMDL equation. DEQ will accept any information members of the TAC or interested stakeholders have to assist in this source assessment for sediment, total phosphorous or TDS.

5. *Several respondents commented that while this specific TMDL study does not directly impact their organization, this study could establish a standard that may be applied in other TMDL studies statewide.*

   TMDL endpoints that are not based on an existing numeric water quality standard are specific to the watershed for which they were developed. While the methodology used to develop the endpoint could be applied in other watersheds, such as the comparison watershed approach AllForX that has been used in other TMDL studies throughout Virginia, the resulting endpoint itself developed from any such approach will not be applied to other watersheds. The site-specific TDS endpoint that will be developed for this project is specific to the ion composition and toxicity test results of Sand Branch and will not be applied to other watersheds statewide.