Stressor Identification Analysis for the Middle Fork Holston River and Tributaries

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Prepared for:
Virginia Department of Environmental Quality

October 2021

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Acronyms

BCG	Biological Condition Gradient
CADDIS	Causal Analysis/Diagnosis Decision Information System
CCU	Cumulative Criterion Unit
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
EIS	Environmental Impact Statement
EPT	Ephemeroptera, Plecoptera, Trichoptera
JMU	James Madison University
LRBS	Log Relative Bed Stability Index
MFBI	Modified Family Biotic Index
NWBD	National Watershed Boundary Dataset
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
PEC	Probable Effect Concentration
SCI	Virginia Stream Condition Index
TDS	Total Dissolved Solids
TEC	Threshold Effect Concentration
TOC	Total Organic Carbon
TRV	Toxicity Reference Value
TSS	Total Suspended Solids
TVS	Total Volatile Solids
USEPA	U.S. Environmental Protection Agency
VDEQ	Virginia Department of Environmental Quality

Executive Summary

This Stressor Identification Analysis Report addresses benthic impairments in Byers/Hall Creek, Cedar Creek, Greenway Creek, Tattle Branch, and two impairments on the Middle Fork Holston River (collectively called the MF Holston Project streams). The analysis was conducted in accordance with the U.S. Environmental Protection Agency's (USEPA) Stressor Identification Guidance Document (USEPA, 2000b) using the Causal Analysis/Diagnosis Decision Information System (CADDIS) (USEPA, 2018a). Twenty-one years of data (2000 – 2021) on over 403 parameters from 39 monitoring stations totaling over 19,000 data points were used in the analysis. These data were evaluated according to 18 lines of evidence to categorize candidate stressors as non-stressors, possible stressors, or probable stressors. Based on the evaluation, sediment was identified as a probable stressor in each of the impaired streams. As a result, sediment TMDLs should be developed to address the probable stressor and associated impairments.

1.0 OVERVIEW

1.1. TMDL Development

Section 303(d) of the Federal Clean Water Act and the U.S. Environmental Protection Agency's (USEPA) Water Quality Planning and Management Regulations (40 CFR Part 130) require states to develop Total Maximum Daily Loads (TMDLs) for water bodies that fail to meet designated water quality standards and are placed on the state's Impaired Waters List (VDEQ, 2020). A TMDL reflects the total pollutant loading that a waterbody can receive and still meet water quality standards. A TMDL establishes the maximum allowable pollutant loading from both point and nonpoint sources for a waterbody, allocates the load among the pollutant contributors, and provides a framework for taking actions to restore water quality.

For impairments that result from the violation of numeric water quality standards, a TMDL can be directly developed for the pollutant that violates the standard. Benthic impairments, however, result from violations of the narrative general standard that waters should be free from substances that are harmful to aquatic life. To develop TMDLs that address benthic impairments, the first step is to identify the pollutant(s) causing the impairment. This step is called a Stressor Identification Analysis.

1.2. Benthic Impairments

The Virginia Department of Environmental Quality (VDEQ) contracted Wetland Studies and Solutions, Inc. and James Madison University (JMU) to conduct a stressor identification analysis for benthic impairments in the Middle Fork Holston River (MF Holston) watershed in Smyth, Wythe, and Washington Counties. The five impaired streams (and eight corresponding assessment units) are listed in Table 1, shown in Figure 1, and collectively referred to as the MF Holston Project streams. This project addresses benthic impairments in Byers/Hall Creek, Cedar Creek, Greenway Creek, Tattle Branch, and two reaches of the MF Holston River (Lower Impaired Reach and Upper Impaired Reach).

Table 1. Benthic impairments in the MF Holston Project.

Stream Name	NWBD	Impaired Assessment Units	Cause Group Code	First listed	Length (miles)	Impairment Description
Byers/Hall Creek	TH13	VAS-O05R_ BYS01A94	O05R-01-BEN	2004	0.49	Indian Run to MF Holston
	TH13	VAS-O05R_HAL01A94	O05R-01-BEN	2004	6.91	Headwaters to Byers Creek
Cedar Creek	TH14	VAS-O05R_CED01A94	O05R-01-BEN	2004	5.61	Confluence of East and West Fork Cedar Creek to MF Holston
Greenway Creek	TH14	VAS-O05R_GRW01A02	O05R-02-BEN	2010	5.02	Headwaters to MF Holston
Tattle Branch	TH13	VAS-O05R_TAT01A02	O05R-01-BEN	2004	2.77	Headwaters to Byers Creek
Middle Fork Holston River	TH08	VAS-O03R_MFH05A04	O03R-01-BEN	2010	3.42	Headwaters to confluence with Dutton Branch
	TH13	VAS-O05R_MFH04A00	O05R-05-BEN	2008	9.19	Sulphur Spring Creek to Rt. 91 bridge
	TH13	VAS-O05R_MFH05A04	O05R-05-BEN	2006	3.80	Rt. 91 bridge to Edmondson Dam

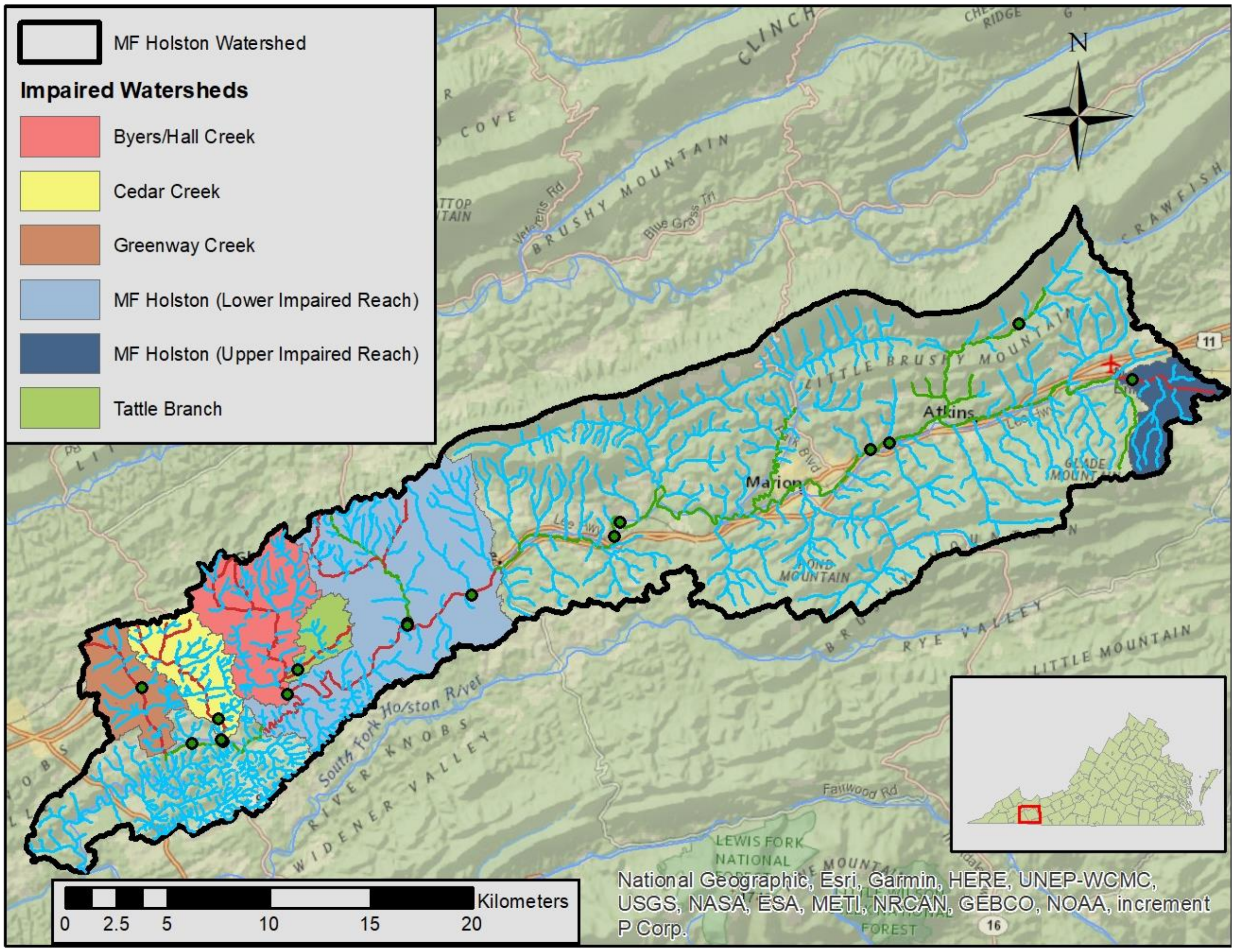

Figure 1. Location of benthic impairments in the MF Holston Project.

1.3. Stressor Analysis Process

Benthic impairments are based on biological assessments of the benthic community. These biological assessments are effective at determining whether a water body is impaired or not, but they do not provide information on the stressor or source causing the impairment. To determine the cause of the impairment, a stressor identification analysis must be conducted. JMU conducted this analysis according to the U.S. Environmental Protection Agency's (USEPA) Stressor Identification Guidance Document (USEPA, 2000b). In short, the stressor identification analysis identifies the pollutant(s) responsible for the benthic impairment through a weight of evidence approach that evaluates all available information on potential candidate stressors (Figure 2). Once the probable stressor(s) is identified, a TMDL can be developed for that pollutant to reduce sources and restore the aquatic life designated use.

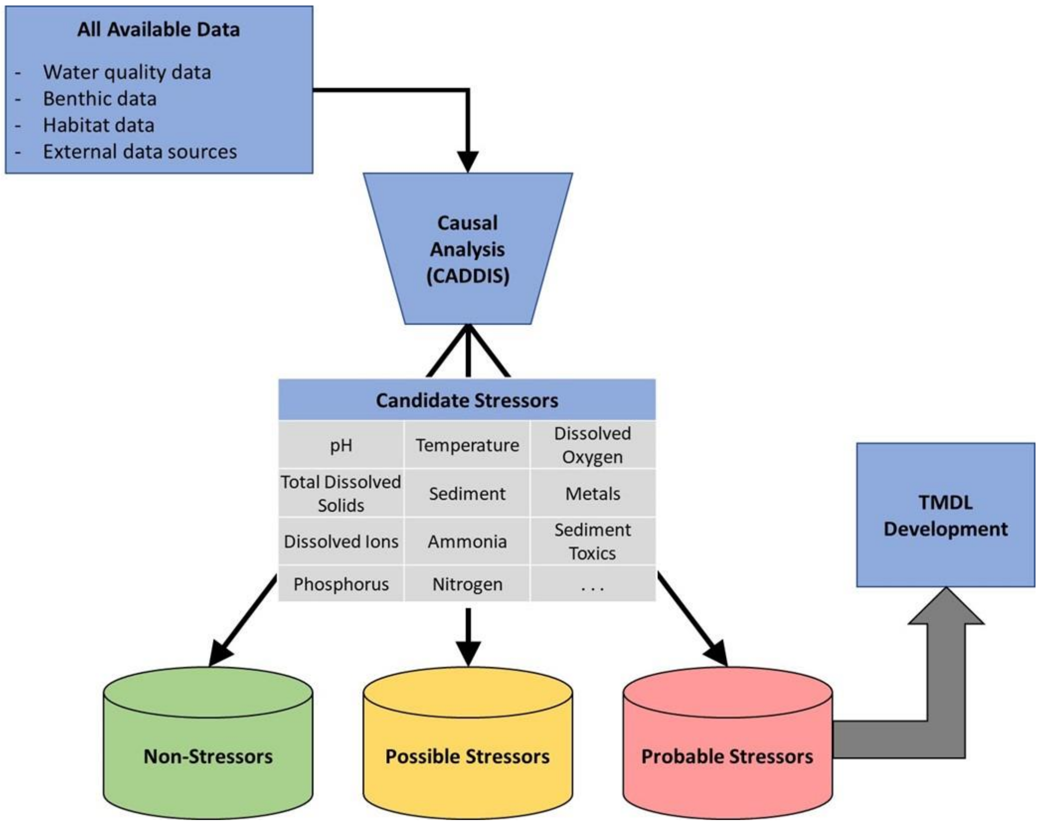

Figure 2. Stressor identification analysis process.

The first step in the stressor identification analysis is to list potential candidate stressors. JMU 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 (Table 2).

The next step is to analyze all of the available evidence to support or eliminate potential candidate stressors. In this step, JMU used the Causal Analysis/Diagnosis Decision Information System (CADDIS) (USEPA, 2018a). The CADDIS approach provides guidance on evaluating various lines of evidence to determine the cause of biological impairments. For this project, JMU used available physical, chemical, and biological data collected throughout the watershed, published water quality standards and threshold values, and available literature from other cases to investigate the potential causes of impairment in each of the impaired streams. Based on the weight of evidence supporting each potential candidate, stressors were then separated into the following categories: non-stressor(s), possible stressor(s), and probable stressor(s).

Table 2. Candidate stressors evaluated in the MF Holston Project.

	Candidate Pollutants	
pH	Dissolved Sulfate	Ammonia
Dissolved Oxygen	Total Dissolved Ions	Dissolved Metals
Temperature	Suspended Solids	Sediment Toxics
Conductivity	Deposited Sediment	Sediment Metals
Dissolved Chloride	Organic Matter	Pesticides
Dissolved Sodium	Nitrogen	Polycyclic Aromatic Hydrocarbons (PAHs)
Dissolved Potassium	Phosphorus	Polychlorinated Biphenyls (PCBs)
	Additional Contributing Factors	
Habitat	Hydrologic Alteration	Existing Dams and Impoundments
Livestock Stream Access	Current Land Use Practices	Imperviousness

Once a probable stressor(s) was identified, a conceptual model was developed to describe the causal pathways linking pollutant sources to the probable stressors and mechanisms of impairment. The pathways in the conceptual model were then evaluated to determine if the existing data support those mechanisms for producing the impairment.

2.0 BIOLOGICAL, PHYSICAL, AND CHEMICAL DATA

For the stressor identification analysis, JMU used biological, physical, and chemical data from 39 VDEQ monitoring stations within the five project watersheds (Table 3). Water quality data was collected from all of these stations, and benthic data was collected from 16 of the stations. These VDEQ stations have been monitored for various parameters, lengths of time, and purposes. Table 3 shows the number of samples and the period of time over which individual stations were monitored. All data collected since 2000 was used in the stressor identification analysis.

For benthic monitoring stations, data include the taxonomic identification (family or genus level) and counts of the collected benthic macroinvertebrates, eight calculated benthic metrics, stream condition index scores (SCI), biological condition gradient scores, and visual habitat assessment scores. For water quality monitoring stations, data include results for various physical and chemical parameters. Across all of the stations and sampling dates, 403 different water quality parameters were measured. In total, more than 19,000 individual data points were compiled and incorporated into the stressor identification analysis.

For some parameters and analyses, the impaired streams were compared to an unimpaired reference. For the MF Holston, the benthic reference was 6CMFH045.83, and the water quality reference was data from this site combined with the closely located 6CMFH045.72 station. For smaller tributaries, Bear Creek was used as the reference. Station 6CBER004.10 was the benthic reference, and 6CBER000.17 was the water quality reference.

Table 3. Benthic and water quality data used in the stressor analysis.

Watershed	Stream	Station	Benthic Sampling		Water Quality Sampling	
			Monitoring Period	Samples Collected	Monitoring Period	Samples Collected
Byers/Hall Creek	Byers Creek	6CBYS000.08	2002-2019	9	2002-2019	21
		6CBYS000.23			2000-2012	85
	Hall Creek	6CHAL000.75			2017	11
		6CHAL002.60			2017	12
	Tattle Branch	6CTAT000.50	2005-2019	7	2005-2019	19
Cedar Creek	Cedar Creek	6CCED000.04	2002	2	2000-2017	15
		6CCED000.14	2012	2	2000-2012	87
		6CCED001.01	2005	1	2005-2017	13
Greenway Creek	Greenway Creek	6CGRW000.09			2005-2019	32
		6CGRW002.31	2008-2019	3	2008-2019	3
MF Holston	MF Holston	6CMFH011.31	2007-2018	7	2000-2018	32
		6CMFH013.21			2000-2021	160
		6CMFH023.41	2005	2	2005	4
		6CMFH026.00	2018	2	2000-2018	44
		6CMFH027.14			2001-2018	27
		6CMFH032.39	2000-2003	2	2000-2003	3
		6CMFH033.40	2008	1	2000-2021	154
		6CMFH040.67			2001-2018	38
		6CMFH045.72[1]			2000-2008	45
		6CMFH045.83[1]	2003-2018	7	2003-2018	18
		6CMFH053.36			2000-2018	27
		6CMFH055.88	2008-2018	3	2007-2018	27
	Dry Branch	6CDRY001.01			2007-2008	12
	Dutton Branch	6CDUT000.14			2017	12
	Nicks Creek	6CNIK000.06			2019	12
	Bear Creek[1]	6CBER000.17[1]			2007-2018	24
		6CBER004.10[1]	2001-2014	7	2001-2014	7

	Hutton Branch	6CHUT000.07	2007	2	2007	2
	Hooks Branch	6CSTA000.05			2007-2008	12
	Hungry Mother Creek	6CHUN001.34			2003-2015	36
		6CHUN004.76			2003-2017	221
		6CHUN006.54			2009-2020	34
	Laurel Springs Creek	6CLRL000.35			2003-2010	24
	Sulphur Spring Creek	6CSUL000.09			2009-2010	12
	Walker Creek	6CWAL000.09			2003-2021	29
	Hutton Creek	6CHTO000.07	2002-2012	5	2002-2012	5
		6CHTO000.24			2000-2017	98
	Plum Creek	6CPLU000.02			2017	12
	Unnamed Tributary to MF Holston	6CXDY000.17			2005-2012	21

[1] This stream/station was used as a benthic and water quality reference.

2.1. Benthic Assessments

From 2001 to 2019, VDEQ conducted benthic assessments at 16 stations within the MF Holston Project area. Table 4 and Figure 3 show the average SCI scores for each station. All benthic scores within Byers/Hall Creek, Tattle Branch, and Cedar Creek were below the SCI impairment threshold score of 60 and were listed as impaired. Greenway Creek was also listed as impaired because 2 of the 3 SCI scores were below 60, even though the average was slightly above 60. On the MF Holston River, there is a lower impaired reach (which encompasses stations 6CMFH023.41 and 6CMFH026.00) and an upper impaired reach (which includes station 6CMFH055.88). Station 6CMFH055.88 was listed as impaired because 2 of the 3 SCI scores were below 60, even though the average was slightly above 60. Below the lower impaired reach (at station 6CMFH011.31) and between the lower and upper impairments (at stations 6CMFH032.39, 6CMFH033.40, and 6CMFH045.83), the MF Holston is unimpaired.

Table 4. Benthic scores in the MF Holston Project area.

Watershed	Stream	Station	Years Sampled	Samples Collected	SCI Average	Assessment
Byers/Hall	Byers Creek	6CBYS000.08	2002-2019	9	59.4	Impaired
	Tattle Branch	6CTAT000.50	2005-2019	7	59.3	Impaired
Cedar Creek	Cedar Creek	6CCED000.04	2002	2	56.7	Impaired
		6CCED000.14	2012	2	52.3	Impaired
		6CCED001.01	2005	1	57.2	Impaired
Greenway Creek	Greenway Creek	6CGRW002.31	2008-2019	3	60.4	Impaired
MF Holston	MF Holston	6CMFH011.31	2007-2018	7	66.8	Unimpaired
		6CMFH023.41	2005	2	54.7	Impaired
		6CMFH026.00	2018	2	57.9	Impaired
		6CMFH032.39	2003	2	66.4	Unimpaired
		6CMFH033.40	2008	1	67.4	Unimpaired
		6CMFH045.83	2003-2018	7	68.0	Unimpaired
		6CMFH055.88	2008-2018	3	60.3	Impaired
	Bear Creek	6CBER004.10	2001-2014	7	67.1	Unimpaired
	Hutton Branch	6CHUT000.07	2007	2	66.0	Unimpaired
	Hutton Creek	6CHTO000.07	2002-2012	5	61.0	Unimpaired

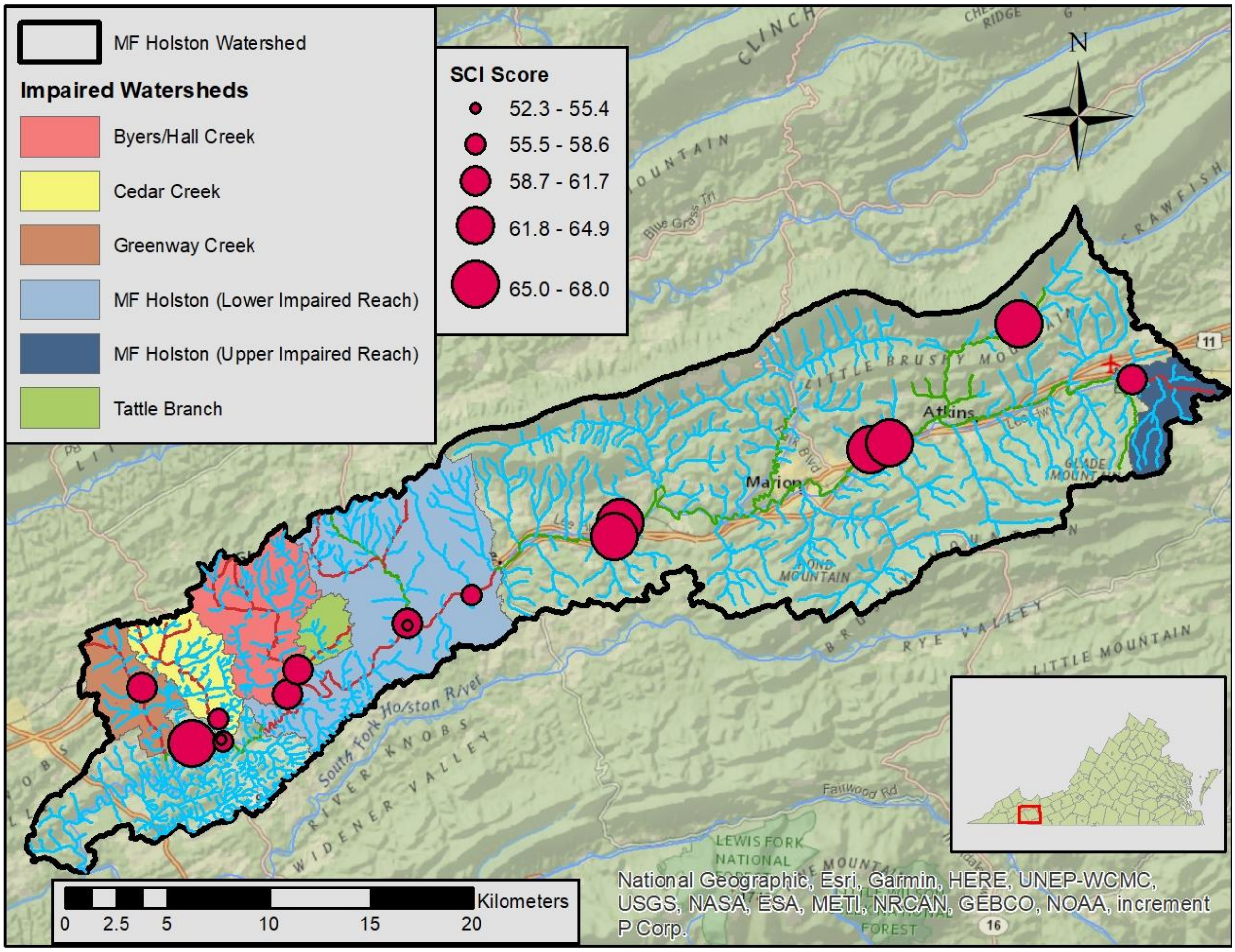

Figure 3. Benthic scores at monitoring stations within the MF Holston Project area.

2.1.1. Temporal and Seasonal Trends in Benthic Data

Figure 4 shows the temporal and seasonal trends in benthic data from the MF Holston tributaries, and Figure 5 shows the temporal and seasonal trends in benthic data from the mainstem MF Holston River.

- Byers/Hall Creek – In Byers/Hall Creek, SCI scores averaged 59.4 and ranged from 52.0 to 66.5, indicating only a slight impairment. Benthic samples collected from 2002 to 2019 showed no general overall trend of increasing or decreasing SCI scores, however, scores appeared to show a distinct seasonal trend. Spring SCI scores averaged 55.0 and were all below the impairment threshold, while fall scores averaged 64.9 and were all above the impairment threshold. This represents an 18% increase in SCI scores from spring to fall.

While this difference was not statistically significantly different due to the low sample number, it might point to stressors that are related to spring high flow (such as nutrients or sediment). Lower springtime scores could also be due to changing habitat or food availability, such as leaf packs that are prevalent in the fall but scarce in the spring.

- Tattle Branch – Benthic SCI scores in Tattle Branch averaged 59.3 and ranged from 37.9 to 74.4. The lowest SCI score of 37.9 was in 2005, and all more recent samples have been above or close to the impairment threshold (57.7 to 74.4). This could point toward an increasing trend over time, but, it is difficult to assess temporal trends in a small dataset. SCI scores in Tattle Branch also appeared to show a distinct seasonal trend. Spring SCI scores averaged 54.1, while fall scores averaged 66.2. This represents a 22% increase in SCI scores from spring to fall. While this difference was not statistically significantly different due to the low sample number, it might point to stressors that are related to spring high flow (such as nutrients or sediment).
- Cedar Creek – Benthic SCI scores in Cedar Creek averaged 55.0 and ranged from 49.5 to 58.4. There were no apparent temporal or seasonal trends in Cedar Creek SCI scores, as scores were relatively consistent throughout the sampling period. Spring SCI scores averaged 53.9, and fall scores were only slightly higher at 56.7.
- Greenway Creek – In Greenway Creek, SCI scores averaged 60.4 and ranged from 55.8 to 66.4. While the average was slightly above the impairment threshold, 2 of the 3 samples had SCI scores below 60. This indicates only a slight impairment at this station. No temporal or seasonal trends were apparent due to relatively consistent SCI scores and a small dataset.
- MF Holston River – Benthic samples were collected from seven MF Holston locations. These stations represent a lower unimpaired reach (6CMFH011.31), a lower impaired reach (6CMFH023.41 and 6CMFH026.00), an upper unimpaired reach (6CMFH032.39, 6CMFH033.40, and 6CMFH045.83), and an upper impaired reach (6CMFH055.88). Within these reaches, SCI scores averaged 66.8, 56.3, 67.6, and 60.3, respectively. Within the lower impaired reach, SCI scores ranged from 52.1 to 63.8, with 3 of the 4 scores below the impairment threshold. Within the upper impaired reach, SCI scores ranged from 50.4 to 72.6, with 2 of the 3 scores below the impairment threshold. There were no apparent

temporal trends in SCI scores within any of these reaches, however, seasonal trends appeared to show lower spring scores than fall scores in each of the reaches except for the upper impaired reach. In the upper impaired reach, spring scores were higher than fall, but this represented only a single spring data point. Within the lower impaired reach, spring SCI scores averaged 54.3 and fall scores averaged 58.3.

In summary, no temporal trends were observed in benthic data. Three streams (Byers/Hall Creek, Tattle Branch, and MF Holston lower impaired reach) showed seasonal trends with benthic SCI scores lower in the spring and higher in the fall. Differences were not statistically significant due to small datasets, but the apparent seasonal trend could point to stressors that are related to spring high flow (such as nutrients or sediment) or changing habitat or food availability.

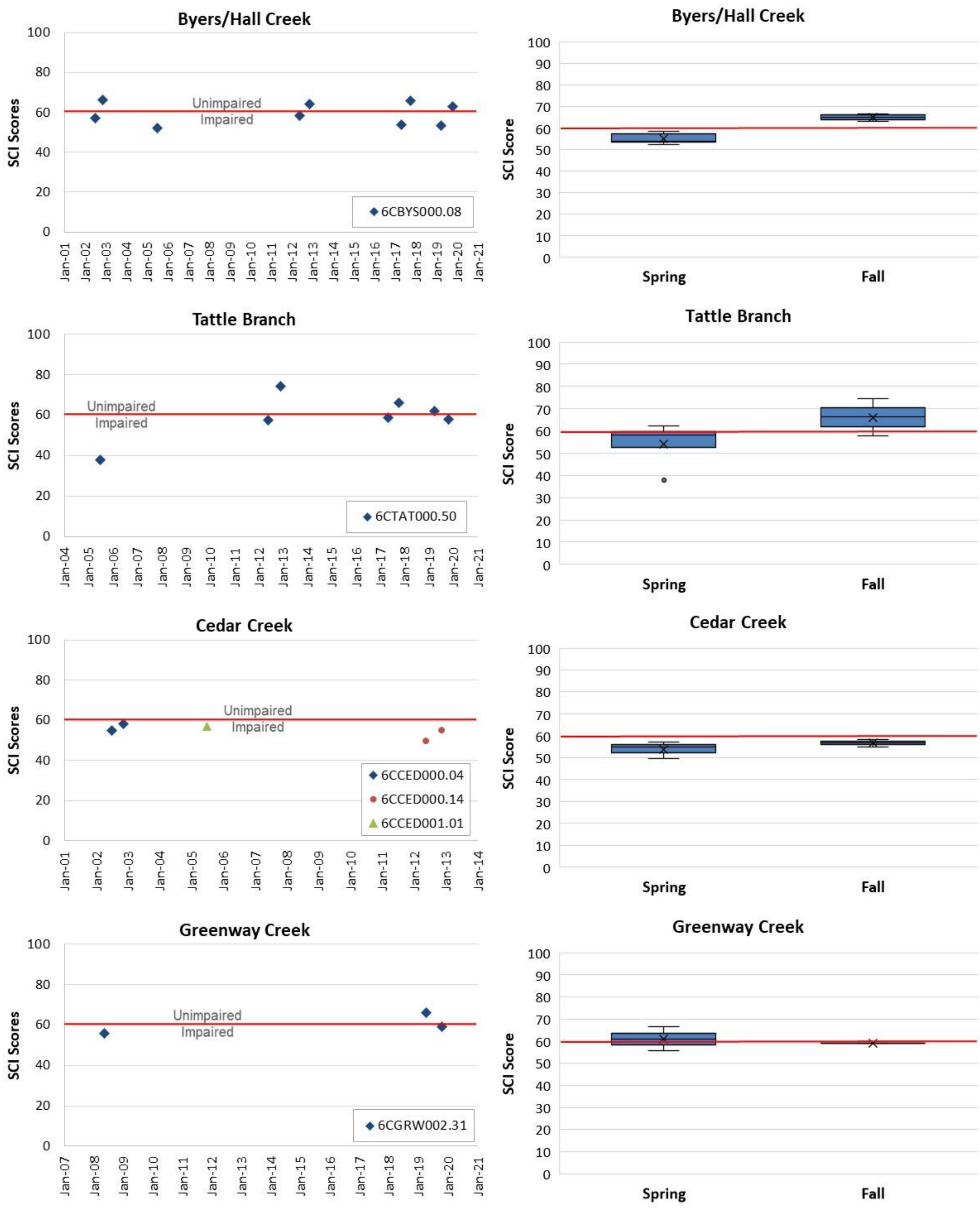

Figure 4. Temporal trends in benthic data for MF Holston tributaries.

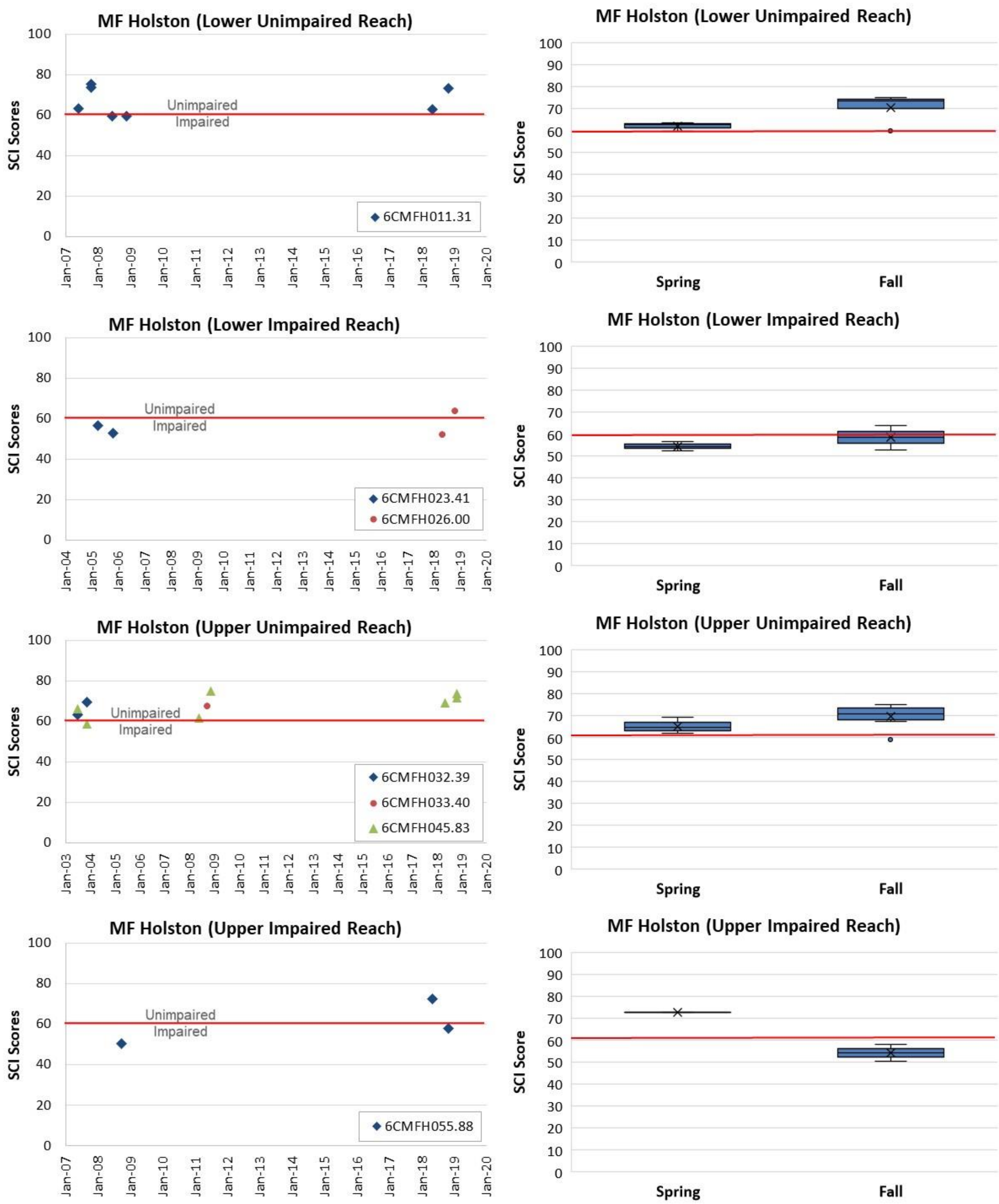

Figure 5. Temporal trends in benthic data for MF Holston.

2.1.2. Analysis of Benthic Metrics

The Stream Condition Index (SCI) is a multi-metric index composed of eight individual metrics: species richness, Ephemeroptera Plecoptera Trichoptera richness (EPT richness), % Ephemeroptera (% Ephem), % Plecoptera and Trichoptera minus *Hydropsychidae* (% PT-Hydro), % *Chironomidae*, % scraper, % 2 dominant, and the modified family biotic index (MFBI). Assessing these metrics individually can provide clues to potential stressors, since different stressors may impact the benthic community in different ways. To evaluate individual metrics, impaired streams were compared to an unimpaired reference (Figure 6). The benthic reference for MF Holston sites was 6CMFH045.83, and the benthic reference for all other tributaries was Bear Creek (6CBER004.10). For each impaired stream, average scores for each metric were compared to the reference using a t-test with unequal variances (alpha = 0.05).

- Byers/Hall Creek – In Byers/Hall Creek, EPT richness, % PT-Hydro, and MFBI metrics were all significantly lower ($p<0.05$) than in the reference. Byers/Hall Creek averaged 6.2 EPT taxa compared to 7.9 at the reference. The % PT-Hydro in Byers/Hall Creek was only 7% compared to 38% in the reference. These two metrics indicate a small but significant decrease in the number of sensitive taxa in Byers/Hall Creek. This is also reflected in the lower MFBI metric score, which was developed based on organism sensitivity to organic enrichment. MFBI scores in Byers/Hall Creek averaged 78% compared to 95% in the reference. Reductions in these three metrics indicate that some stressor, possibly organic enrichment, is limiting the abundance of sensitive taxa.

- Tattle Branch – In Tattle Branch, EPT richness, % PT-Hydro, and MFBI metrics were all significantly lower ($p<0.05$) than in the reference. This is consistent with the results of metrics in the broader Byers/Hall Creek watershed. In Tattle Branch, EPT taxa averaged 5.4 EPT taxa compared to 7.9 in the reference. The % PT-Hydro in Tattle Branch was only 11% compared to 38% in the reference. MFBI scores in Tattle Branch averaged 81% compared to 95% in the reference. Similar to Byers/Hall Creek, reductions in these three metrics indicate that some stressor, possibly organic enrichment, is limiting the abundance of sensitive taxa.

- Cedar Creek – In Cedar Creek, EPT richness, % PT-Hydro, and MFBI metrics were all significantly lower ($p<0.05$) than in the reference. This is consistent with the results of metrics in Byers/Hall Creek and Tattle Branch. In Cedar Creek, EPT taxa averaged 5 EPT taxa compared to 7.9 in the reference. The % PT-Hydro in Tattle Branch was only 6.6% compared to 38% in the reference. MFBI scores in Cedar Creek averaged 78% compared to 95% in the reference. Similar to the streams discussed above, reductions in these three metrics indicate that some stressor, possibly organic enrichment, is limiting the abundance of sensitive taxa.

- Greenway Creek – In Greenway Creek, % PT-Hydro and MFBI metrics were significantly lower ($p<0.05$) than in the reference. In Greenway Creek, the % PT-Hydro was only 6.7% compared to 38% in the reference. MFBI scores in Greenway Creek averaged 82% compared to 95% in the reference. Reductions in these two metrics may indicate the same stressor mechanism as in the streams discussed above but to a lesser degree in Greenway Creek, where SCI scores were borderline.
- MF Holston (Lower Impaired Reach) – In the lower impaired reach on the MF Holston, metric scores for species richness, EPT richness, % PT-Hydro, and % 2 dominant were significantly lower ($p<0.05$) than in the reference. Species richness in the lower impaired reach averaged 9.4, compared to 14 in the reference. EPT richness in the lower impaired reach averaged 4.5, compared to 8.5 in the reference. This represents a 33% drop in overall species richness and a 47% drop in EPT richness. While the % PT-Hydro metric was low in the reference condition (6.5%), it was significantly lower in the lower impaired reach (1.2%). This reach also had a greater percentage of the benthic population represented by the two most dominant taxa (70%) than in the reference (50%). This pattern indicates that the stressor in this reach is reducing the presence and abundance of sensitive species but increasing the abundance of more tolerant species. This pattern is consistent with sediment or nutrient enrichment.
- MF Holston (Upper Impaired Reach) – In the upper impaired reach on the MF Holston, the % Ephem metric was the only metric to be significantly lower ($p<0.05$) than in the reference. Ephemeroptera represented 16% of the benthic community in the upper impaired reach and 41% of the community in the reference. All other benthic metrics were similar between the upper impaired reach and the reference. This indicates that the impairment and level of the stressor in the upper impaired reach is very slight.

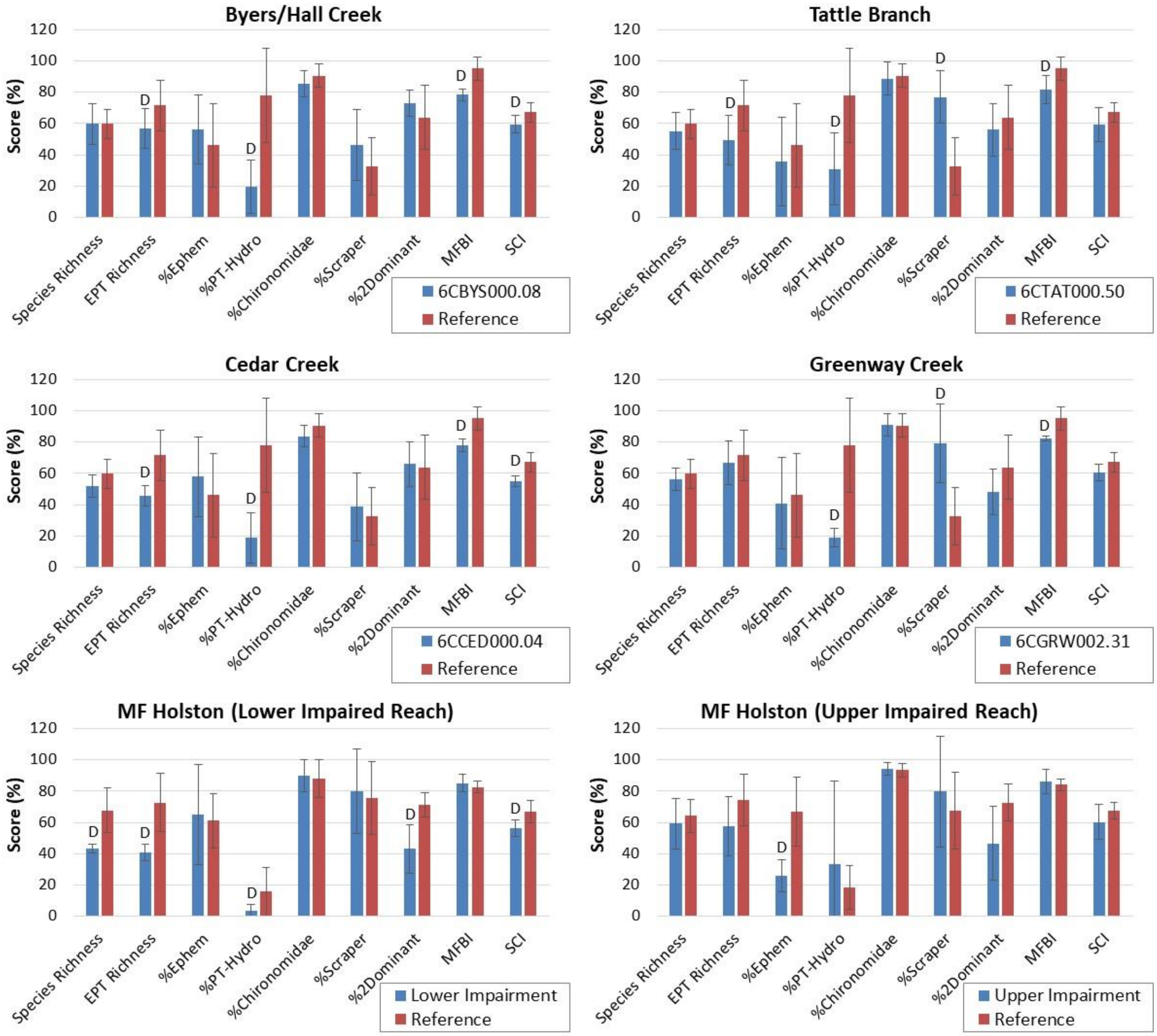

Figure 6. Individual metric scores comprising the stream condition index (SCI) in MF Holston Project streams. "D" indicates that the metric was significantly different (alpha = 0.05) from the benthic reference. The benthic reference for MF Holston sites was 6CMFH045.83, and the benthic reference for all other tributaries was Bear Creek (6CBER004.10).

2.1.3. Analysis of Community Composition

The taxonomic composition of the benthic communities was analyzed to identify shifts in composition at impaired stations that might provide clues to sources or mechanisms of impairment. Figure 7 compares the taxonomic composition in MF Holston Project streams to a reference stream. In the reference stream, taxonomic composition is relatively balanced, but with a high percentage of the community represented by sensitive Ephemeroptera (29%) and Plecoptera (36%) taxa. In impaired streams, the community composition is often less balanced and shifted towards less sensitive taxa.

- Byers/Hall Creek – Taxonomic composition in Byers/Hall Creek was relatively balanced, with no more than 35% of the community represented by any one taxonomic group. This diversity and balance indicate the rather marginal impairment conditions in Byers/Hall Creek. In comparison to the reference, Byers/Hall Creek had fewer Plecoptera (<1% compared to 36% in the reference) and more Coleoptera (17% compared to 8% in the reference) and *Hydropsychidae* (11% compared to 1% in the reference). The decrease in Plecoptera could be due to a lack of riparian vegetation, since most of the Plecoptera are shredders that feed on leaves from the riparian margins. The increase in Coleoptera and *Hydropsychidae* could signal nutrient or sediment enrichment. *Hydropsychidae* are net spinning caddisflies that filter suspended food from the water column, so enriched conditions with more suspended solids and organic matter could cause an increase in this taxon. Most of the Coleoptera were in the *Elmidae* family, which have anal gills covered by a moveable operculum that can protect the gills from suspended solids (Voshell, 2002). This characteristic could give the *Elmidae* a competitive advantage in higher suspended solids environments, so their abundance could indicate an early community response to suspended solids as a stressor.

- Tattle Branch – The taxonomic composition of Tattle Branch is also relatively well balanced, indicating only marginal impairment. The most prominent differences from the reference condition are a decrease in Plecoptera (4% compared to 36% in the reference) and an increase in Coleoptera (36% compared to 8% in the reference). As mentioned above, the lack of Plecoptera could signal a lack of riparian vegetation, which provides a food source for shredders in the Plecoptera taxon. The increase in Coleoptera from primarily the *Elmidae* family could indicate early community shifts in response to increased suspended sediment.

- Cedar Creek – The Cedar Creek taxonomic composition was analyzed at three different stations (6CCED000.04, 6CCED000.14, and 6CCED001.01). At stations 6CCED000.04

and 6CCED001.01, the community was dominated by Ephemeroptera primarily from the family *Baetidae*. These are relatively sensitive mayflies that again point out the very marginal nature of the impairment. In the middle Cedar Creek station (6CCED000.14), the community was more dominated by *Hydropsychidae* (22% compared to 1% in the reference) and Diptera (33% compared to 22% in the reference). *Hydropsychidae* are net spinning caddisflies that filter suspended food from the water column, so enriched conditions with more suspended solids and organic matter could cause an increase in this taxon. The Diptera present in Cedar Creek 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. This shift in community composition indicate that fine sediment and deposited organic matter may be stressors in Cedar Creek.

- Greenway Creek – The most prominent differences in Greenway Creek taxonomic composition are a decrease in Plecoptera (3% compared to 36% in the reference), an increase in Coleoptera (42% compared to 8% in the reference), and an increase in *Hydropsychidae* (14% compared to 1% in the reference). As mentioned above, the lack of Plecoptera could signal a lack of riparian vegetation, which provides a food source for shredders in the Plecoptera taxon. The increase in Coleoptera from primarily the *Elmidae* family could indicate early community shifts in response to increased suspended sediment. The increase in *Hydropsychidae* indicate enriched conditions with more suspended solids and organic matter as a food supply for these filtering caddisflies.

- MF Holston – Benthic community composition in the MF Holston was assessed at a lower and an upper impaired reach in comparison to an unimpaired reference station (6CMFH045.83) (Figure 8). The impaired stations each exhibited less diversity and balance than the reference. In particular, Coleoptera dominated the impaired communities, comprising 53%, 29%, and 49% of the community at stations 6CMFH023.41, 6CMFH026.00, and 6CMFH055.88, compared to 14% in the reference. In each case, most of the Coleoptera were in the *Elmidae* family, which have anal gills covered by a moveable operculum that can protect the gills from suspended solids (Voshell, 2002). This characteristic could give the *Elmidae* a competitive advantage in higher suspended solids environments, so their abundance could indicate an early community response to suspended solids as a stressor.

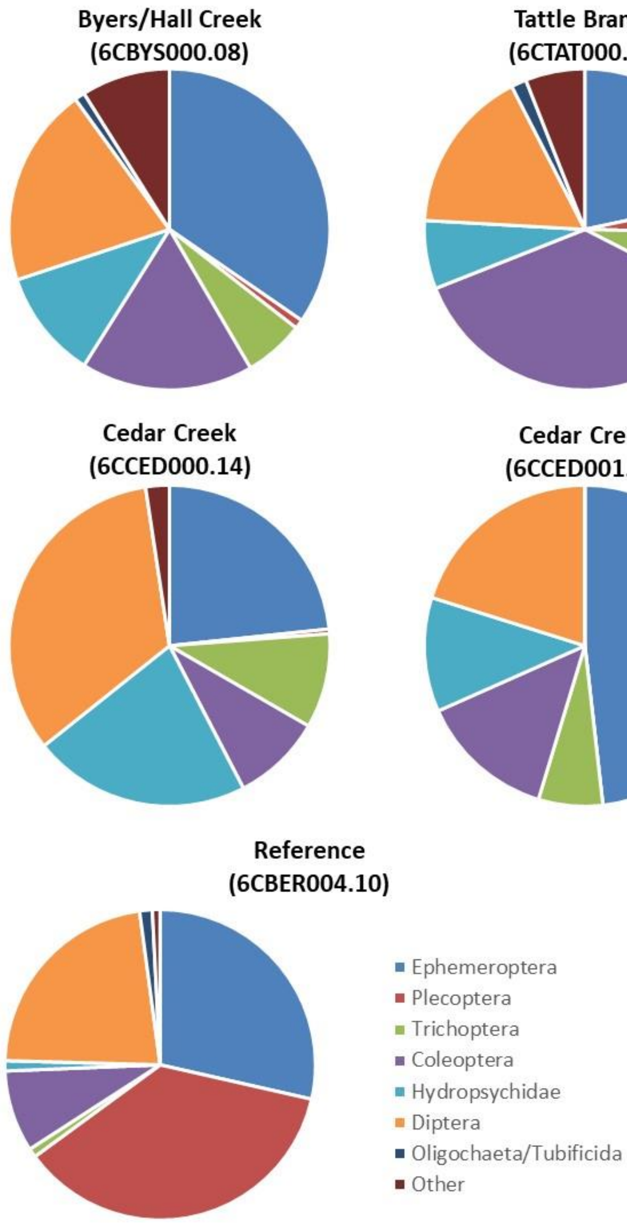

Figure 7. Taxonomic composition of MF Holston tributaries compared to a reference.

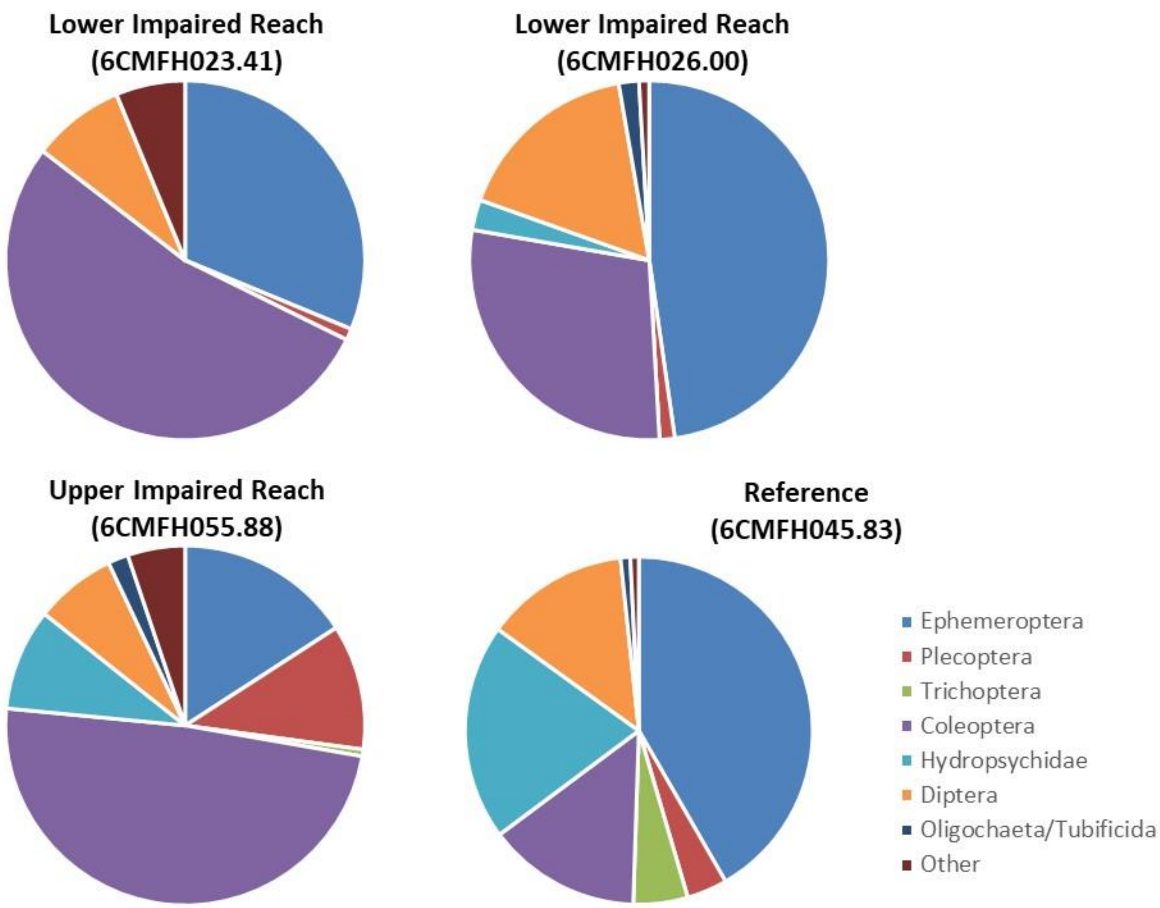

Figure 8. Taxonomic composition of impaired and unimpaired MF Holston stations.

2.1.4. Biological Condition Gradient Analysis

In 2019, Tetra Tech worked with mid-Atlantic region states (including Virginia) to develop a conceptual model of environmental condition called the Biological Condition Gradient (BCG). The BCG model describes environmental conditions by analyzing patterns of pollution tolerance among fish and macroinvertebrates present (Tetra Tech, 2019). The model defined six attributes related to pollution tolerance and scored these attributes across 560 macroinvertebrate taxa for 10 specific stressors (Table 5). Attributes were scored for each taxa and stressor combination based on statistical analysis of regional data and expert consensus. The result is a database that can be useful for stressor analysis.

Using attribute data from the BCG model, taxa present at each of the impaired MF Holston Project streams were assigned attribute scores for each stressor. The average scores and the scores for predominant species were evaluated for each stressor to identify potential stressors that might be indicative of the pattern of organism tolerance observed. Table 6 shows the BCG scores for the three most prevalent taxa at each of the impaired monitoring stations. Attribute scores of 5 indicate tolerant taxa that would be expected to increase in number when the respective stressor is present. Some taxa, like *Chironomidae*, are relatively tolerant to a wide range of stressors and don't show much differentiation with respect to stressor identification. Others, however, show better differentiation and can be indicators of specific stressors.

Table 5. Biological condition gradient attributes and stressors evaluated.

<table>
<tr><th>Attribute</th><th>Explanation</th><th>Stressors Evaluated</th></tr>
<tr><td>I</td><td>Historically documented, sensitive, long-lived or regionally endemic taxa</td><td rowspan="7">Dissolved oxygen
Acidity
Alkalinity
Specific Conductivity
Chloride
Sulfate
Total Nitrogen and Phosphorus
Total Habitat
Relative Bed Stability
%Imperviousness</td></tr>
<tr><td>II</td><td>Highly sensitive taxa</td></tr>
<tr><td>III</td><td>Intermediate sensitive taxa</td></tr>
<tr><td>IV</td><td>Intermediate tolerant taxa</td></tr>
<tr><td>V</td><td>Tolerant taxa</td></tr>
<tr><td>VI</td><td>Non-native taxa</td></tr>
<tr><td colspan="2"></td></tr>
</table>

In each of the streams, one of the top three predominant taxa indicated sulfate as a potential stressor. Either *Baetis* or *Optioservus* were present in high numbers in these streams, and these taxa were assigned a BCG score of 5 for sulfate, indicating that their abundance is expected to grow in the presence of high sulfate conditions. In addition, *Chimarra* was one of the predominant taxa in Cedar Creek, and this taxon is expected to increase in abundance in the presence of high percent imperviousness. Neither of these findings are consistent with watershed and water quality data for these streams. Sulfate levels are consistently low in these streams and imperviousness is relatively low in Cedar Creek.

In addition to analyzing the BCG attribute scores for the top three dominant taxa in each impaired stream, BCG attribute scores of all present taxa were averaged to calculate mean scores for each stressor in each stream. Those scores were then ranked to identify the stressors with the highest scores (Table 7). These represent the stressors that have the greatest likelihood of impact on each stream based on the taxa present and BCG attribute scores for those taxa. For all impaired streams, relative bed stability (RBS), which is associated with sediment enrichment, was a top stressor. In addition, single stations in Cedar Creek and MF Holston showed nutrients (TN/TP) as the top stressor, and another station in MF Holston showed alkalinity as the top stressor. The later finding is inconsistent with water quality data that indicates normal levels of alkalinity in the MF Holston, however, findings of sediment and nutrients are consistent with habitat and water quality data.

Table 6. Biological condition gradient scores for three most prevalent taxa at each impaired station.

Stream	Station	Predominant Taxa	Diss. Oxy.	Acidity	Alkalinity	Spec. Cond.	Chloride	Sulfate	TN/TP	Total Habitat	RBS	% Imp.
Byers/Hall Creek	6CBYS000.08	*Chironomidae*	4	4	4	4	4	4	4	4	4	4
		Baetis	3	4	4	4	4	5	4	4	4	4
		Elmidae	3	3	4	3	3	4	3	3	4	4
Tattle Branch	6CTAT000.50	*Optioservus*	3	3	4	4	4	5	4	4	4	3
		Chironomidae	4	4	4	4	4	4	4	4	4	4
		Elmidae	3	3	4	3	3	4	3	3	4	4
Cedar Creek	6CCED000.04	*Baetidae*	3	3	4	4	4	4	4	4	4	3
		Hydropsychidae	3	3	4	4	3	4	4	4	4	4
		Elmidae	3	3	4	3	3	4	3	3	4	4
	6CCED000.14	*Chironomidae*	4	4	4	4	4	4	4	4	4	4
		Baetis	3	4	4	4	4	5	4	4	4	4
		Chimarra	4	4	4	4	4	4	4	4	4	5
	6CCED001.01	*Baetidae*	3	3	4	4	4	4	4	4	4	3
		Chironomidae	4	4	4	4	4	4	4	4	4	4
		Elmidae	3	3	4	3	3	4	3	3	4	4
Greenway Creek	6CGRW002.31	*Elmidae*	3	3	4	3	3	4	3	3	4	4
		Optioservus	3	3	4	4	4	5	4	4	4	3
		Ephemerella	3	3	3	3	3	3	3	3	4	3
MF Holston	6CMFH023.41	*Elmidae*	3	3	4	3	3	4	3	3	4	4
		Ephemerellidae	3	3	4	3	3	3	3	3	4	3
		Chironomidae	4	4	4	4	4	4	4	4	4	4
	6CMFH026.00	*Optioservus*	3	3	4	4	4	5	4	4	4	3
		Chironomidae	4	4	4	4	4	4	4	4	4	4
		Maccaffertium	4	4	4	3	3	3	4	4	4	4
	6CMFH055.88	*Optioservus*	3	3	4	4	4	5	4	4	4	3
		Elmidae	3	3	4	3	3	4	3	3	4	4
		Perlesta	4	3	4	4	2	4	4	4	4	3

Table 7. Rank of average biological condition gradient scores for each stressor in each impaired stream.

Stream	Station	Diss. Oxy.	Acidity	Alkalinity	Spec. Cond.	Chloride	Sulfate	TN/TP	Total Habitat	RBS	% Imp.
Byers/Hall Creek	6CBYS000.08	10	8	4	5	8	2	3	7	1	6
Tattle Branch	6CTAT000.50	9	10	7	5	8	4	2	6	1	3
Cedar Creek	6CCED000.04	10	8	2	6	9	4	4	6	1	3
	6CCED000.14	9	9	6	2	8	2	1	7	2	5
	6CCED001.01	9	4	2	4	9	4	4	4	1	2
Greenway Creek	6CGRW002.31	10	9	4	5	8	3	2	7	1	5
MF Holston	6CMFH023.41	8	8	1	7	8	5	4	6	2	3
	6CMFH026.00	8	10	3	3	9	5	1	6	1	7
	6CMFH055.88	8	9	7	4	10	3	1	6	2	5

2.1.5. Analysis of Functional Feeding Groups

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 9 shows the composition of functional feeding groups within the MF Holston Project streams in comparison to a reference stream. Two distinct patterns emerged from this analysis. In each of the tributaries (Byers/Hall Creek, Tattle Branch, Cedar Creek, and Greenway Creek), communities shifted to a higher percentage of collectors, while shredders and predators decreased. Collectors increased by 31%, 11%, 25%, and 24% in Byers/Hall Creek, Tattle Branch, Cedar Creek, and Greenway Creek, respectively. This shift in functional feeding group is indicative of increased deposited sediment and deposited organic material. As the amount of deposited organic matter increases, the niche of macroinvertebrates that collect their food from bottom deposits (collectors) expands. To a lesser extent, filterers also increased in each of these streams. Filterers increased by 9%, 7%, 21%, and 7% in these same streams, respectively. An increase in filterers can also indicate an increase in suspended sediment and organic matter. As particulate matter in the water column increases, more food is available for filtering organisms and that feeding niche expands.

The pattern that was observed in the MF Holston was an increase in scrapers and a decrease in filterers. Scrapers increased by 28% and 12% in the lower and upper impaired reaches, respectively. Filterers decreased by 17% and 19% in the same reaches. This shift in functional feeding group points to an increase in nutrients. As nutrients increase, algae growth is spurred and there is an increased availability of food for scrapers.

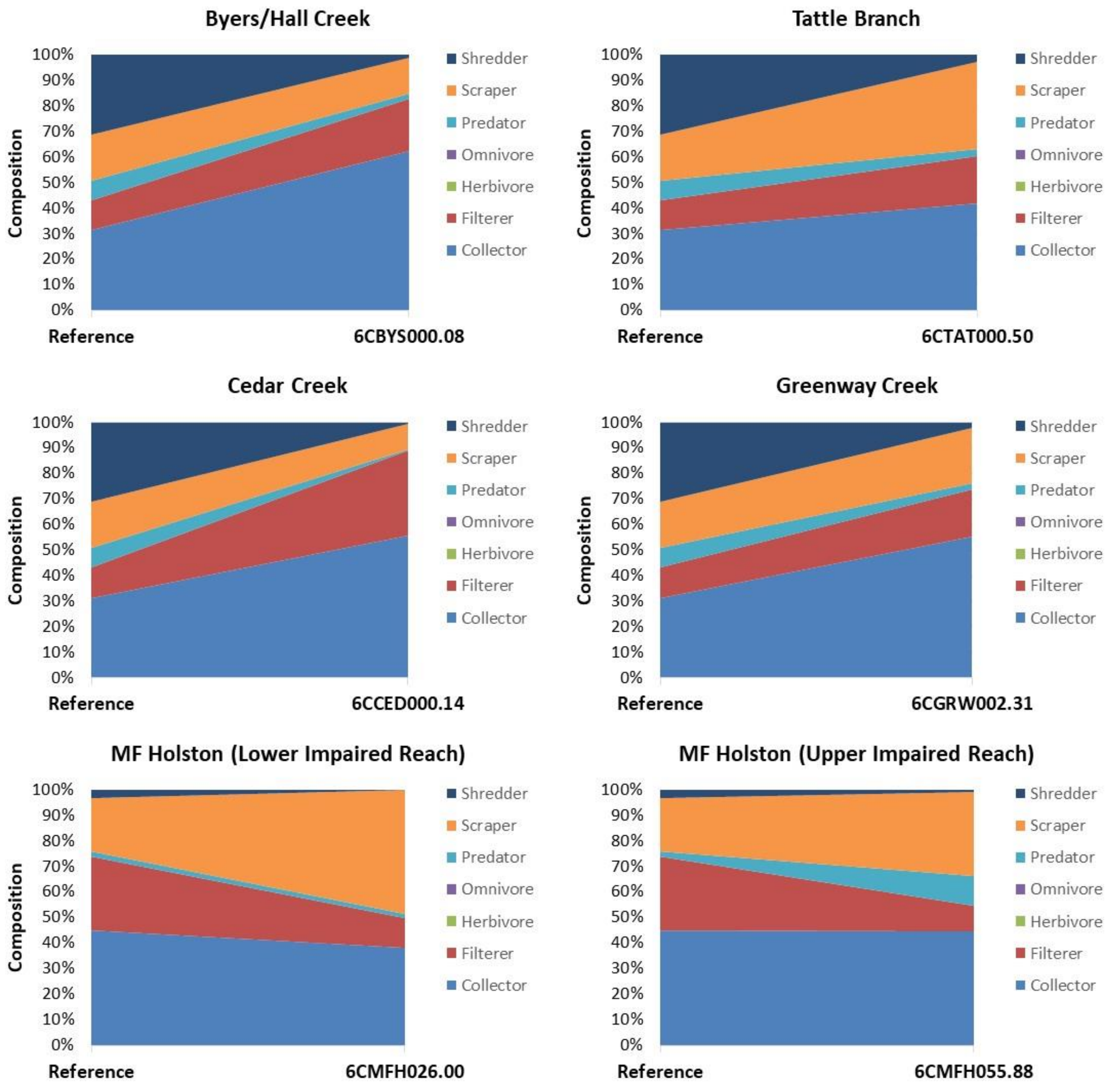

Figure 9. Functional feeding group composition in MF Holston Project streams compared to a reference.

2.2. Habitat Assessment

As part of the Rapid Bioassessment Protocol, a visual habitat assessment is performed at the time of each benthic sample collection. This assessment entails scoring each of a series of habitat components from 0 to 20. These habitat components include bank stability, channel alteration, bank vegetation, riparian vegetation, substrate, embeddedness, flow, riffles, and velocity/depth. The individual scores for each of these measures are then added for a total habitat score. Figure 10 compares the total habitat scores in the MF Holston Project streams with those from a reference stream. While total habitat scores averaged 149 in the small stream reference and 158 in the large stream reference, scores at impaired stations ranged from 108 in Cedar Creek to 142 in Byers/Hall Creek. Total habitat scores were statistically lower ($p<0.05$ in a one-tailed t-test with unequal variance) than the reference in Tattle Branch, Cedar Creek, and the MF Holston upper impaired reach.

Based on VDEQ's analysis of probabilistic monitoring data (VDEQ, 2017), the colors shown in Figure 10 represent the probability of habitat being a stressor on the aquatic community. Tattle Branch, Cedar Creek, and the MF Holston upper impaired reach fell in the medium probability category, while Byers/Hall Creek, Greenway Creek, and the MF Holston lower impaired reach fell in the low probability category.

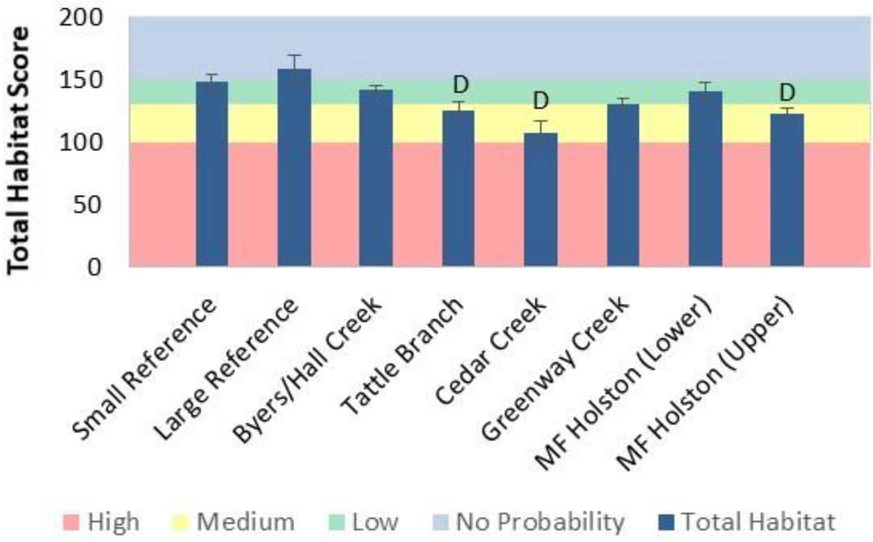

Figure 10. Total habitat scores for MF Holston Project streams. Streams with a "D" have statistically lower habitat scores than the reference site. Colors represent the probability that data within that range would be responsible for causing stress.

Figure 11 compares the individual habitat metrics in each impaired stream with metric scores from the respective reference station. Statistical differences were determined using a one-tailed t-test with unequal variances and alpha = 0.05. All streams had one or more habitat metrics that were significantly lower than the reference. Most streams had lower scores for metrics that indicate degraded bank conditions (bank stability, channel alteration, bank vegetation, or riparian vegetation) and degraded instream habitat conditions (substrate, embeddedness, or sediment). Some streams had lower scores for metrics that indicate degraded hydrologic conditions (flow, riffles, or velocity/depth).

- Byers/Hall Creek – In Byers/Hall Creek, habitat metrics for channel alteration, bank vegetation, riparian vegetation, and substrate were significantly lower than the reference. These lower metric scores represent both sediment sources from degraded bank habitat and indications of deposited sediment impacts within the stream channel (substrate metric).
- Cedar Creek – In Cedar Creek, habitat metrics for channel alteration, riparian vegetation, substrate, sediment, and velocity/depth were significantly lower than the reference. Like Byers/Hall Creek, these lower metric scores represent both sediment sources from degraded bank habitat and indications of deposited sediment impacts within the stream

channel (substrate and sediment metrics). In addition, the lower velocity/depth metric indicates degraded hydrologic habitat conditions.

- Greenway Creek – In Greenway Creek, habitat metrics for channel alteration, bank vegetation, riparian vegetation, and velocity/depth were significantly lower than the reference. In this stream, lower metric scores represent degraded bank habitat and hydrologic condition, but do not indicate significant degradation from deposited sediment impacts within the stream channel.
- Tattle Branch – In Tattle Branch, habitat metrics for channel alteration, bank vegetation, and riparian vegetation were significantly lower than in the reference. In this stream, lower metric scores represented only degraded bank habitat and with no significant degradation of in-channel habitat and hydrologic condition.
- MF Holston – In the MF Holston lower impaired reach, habitat metrics were similar to those from the unimpaired reference. Only the riffles metric was significantly lower. At the upstream impaired reach, habitat metrics for bank vegetation, riparian vegetation, sediment, and velocity/depth were significantly lower than the reference. In this reach, the lower metric scores represent sediment sources from degraded bank habitat, indications of deposited sediment impacts within the stream channel, and degraded hydrologic condition.

While a different combination of metrics were reduced across the different stations, all impaired stations had reductions in some habitat metric. In Byers/Hall Creek, Cedar Creek, and the MF Holston upper impaired reach, habitat metrics that indicate degraded bank habitat (like bank stability, channel alteration, bank vegetation, or riparian vegetation) and degraded instream habitat (like substrate, embeddedness, or sediment metrics) were significantly reduced. This could indicate excess sediment as a stressor in these streams. In Greenway Creek and Tattle Branch, bank habitat metrics were reduced, but in-stream habitat metrics were similar to the reference. This indicates that degraded bank condition may impact sediment loads and transport, but those impacts may not have directly resulted in degraded in-stream habitat conditions at that location. In the MF Holston lower impaired reach, only a single hydrologic habitat metric (riffles) was significantly reduced.

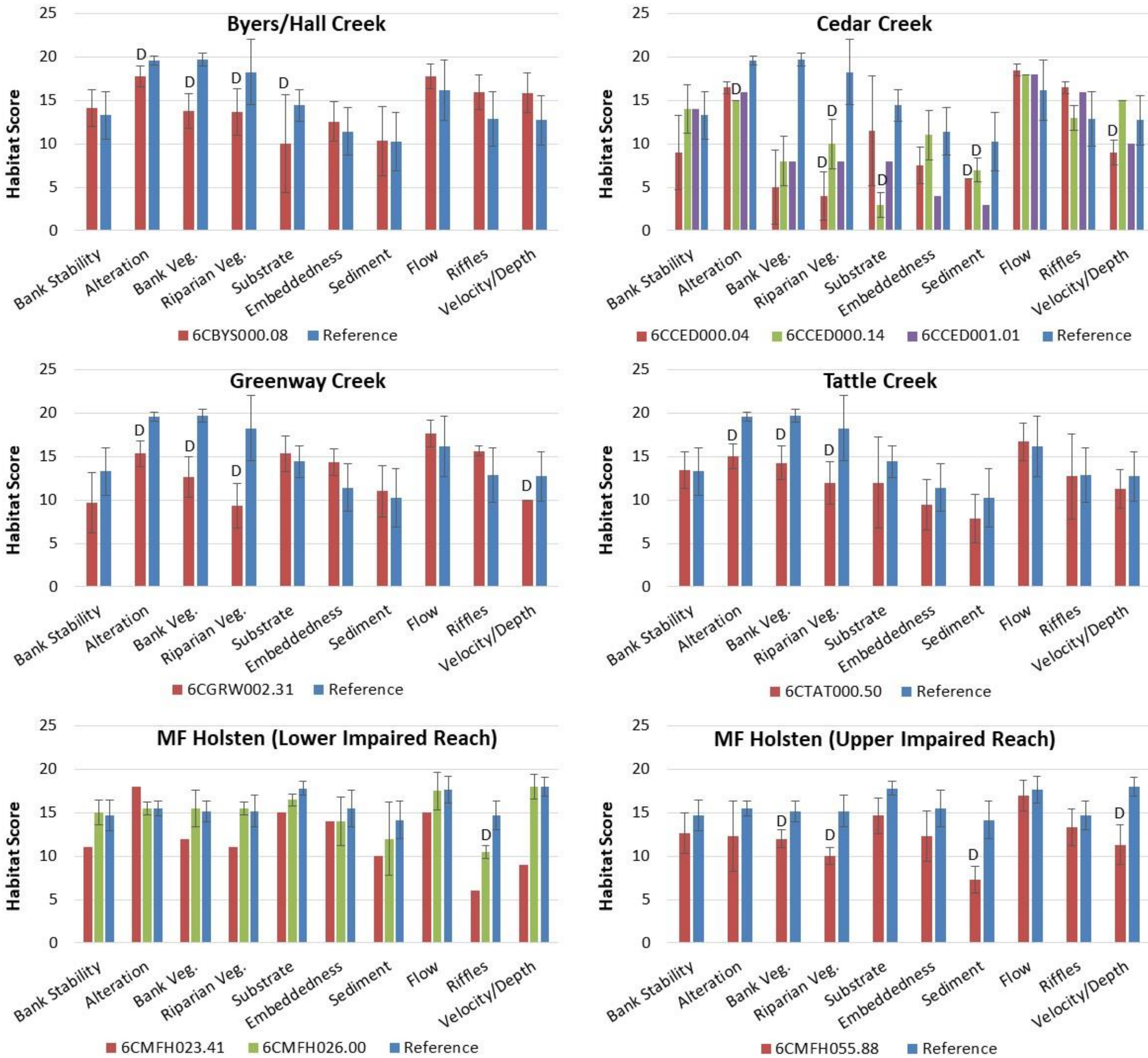

Figure 11. Habitat metric scores for the MF Holston Project streams. Metrics with a "D" are statistically lower than the reference site.

As a part of TMDL monitoring, VDEQ conducted a detailed physical habitat assessment of the impaired streams according to EPA methods for *Quantifying Physical Habitat in Wadeable Streams* (Kaufmann *et al*., 1999). This analysis involved the measurement of channel dimensions and substrate composition at numerous transects within a 150 to 800-m stream reach surrounding the benthic monitoring station. The outcome of this analysis is the calculation of a log 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. 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. In an analysis of streams across the commonwealth, VDEQ has determined that LRBS scores between -1.0 and -1.5 have a medium probability of stressing aquatic life, and LRBS scores <-1.5 have a high probability of stressing aquatic life (VDEQ, 2017). LRBS scores that are too high can also stress benthic macroinvertebrates, and scores >0.5 are also in medium probability range for stress effects.

Table 8 shows the results of relative bed stability analysis in MF Holston Project streams. Relative bed stability analysis was only conducted on one unimpaired tributary (Hutton Branch) and one station on the MF Holston River. Bottom substrate in both streams was a majority sand and fines, and embeddedness was above 50% in both streams. LRBS indices were in the high probability range for stressor effects in Hutton Branch and the no probability range for the MF Holston River. This finding is inconsistent with benthic scores, since Hutton Branch is unimpaired with an SCI score of 66.0.

Table 8. Log relative bed stability index for MF Holston Project streams.

Stream	Station	Date	Slope	% Sand and Fines	Embeddedness (%)	Log Relative Bed Stability Index (LRBS)[1]
Hutton Branch	6CHUT000.07	10/10/2007	1.02	63	85	-2.26
MF Holston	6CMFH023.41	10/21/2005	0.1075	55	60	-0.39

[1] Values in blue are in the no probability range for stressor effects. Values in red are in the high probability range for stressor effects.

2.3. Land Cover Assessment

While a more detailed land cover assessment will be part of the MF Holston Project TMDL Report, the stressor analysis evaluated the potential connections between land cover patterns within the watershed and impaired benthic stations. Table 9 shows the land cover contributing to each of the benthic monitoring stations in the MF Holston Project area. In general, the impaired tributaries were dominated by pasture with a mixture of forest, residential grasses, and residential trees. Impervious areas were greater than 10% in Tattle Branch and Greenway Creek, but were less than 6% in all other watersheds. The MF Holston stations and unimpaired streams were more dominated by forest land cover and less pasture.

Regression analyses were used to compare these land cover trends to benthic SCI scores at the respective stations. A statistically significant regression was observed between natural log transformed SCI scores and several land cover categories (Table 10). SCI scores were positively correlated with forest area in the watershed and negatively correlated with pasture, residential trees, residential grasses, and cropland. The remaining land cover categories were not significantly correlated with benthic health. For those land cover categories with significant regressions, the r^2 values ranged from 0.25 to 0.47, indicating that these land cover categories can account for up to approximately half of the variability in benthic health scores. Figure 12 shows the relationship between SCI scores and forest and pasture land cover within the watershed. As forest land decreases in these watersheds and pasture (along with residential areas) increases, benthic health begins to decline.

Table 9. Land use upstream from each benthic monitoring station.

Stream	Station	Water	Impervious	Barren	Forest	Urban/ Res. Trees	Scrub/ Shrub	Harvested/ Disturbed	Urban/ Res. Grass	Pasture	Cropland	Other
Byers/Hall Creek	6CBYS000.08	0.05%	5.94%	0.66%	19.68%	10.19%	1.35%	1.45%	10.46%	46.56%	3.10%	0.56%
Tattle Branch	6CTAT000.50	0.00%	12.56%	0.06%	10.04%	8.66%	1.94%	0.00%	18.35%	42.04%	6.21%	0.13%
Cedar Creek	6CCED000.04	0.03%	5.14%	0.00%	9.84%	10.27%	0.70%	0.09%	14.07%	56.31%	3.44%	0.09%
	6CCED000.14	0.03%	5.14%	0.00%	9.88%	10.23%	0.70%	0.09%	14.11%	56.26%	3.46%	0.09%
	6CCED001.01	0.04%	5.58%	0.00%	8.98%	10.56%	0.66%	0.11%	15.38%	54.71%	3.89%	0.10%
Greenway Creek	6CGRW002.31	0.00%	11.80%	0.16%	12.79%	8.99%	0.78%	0.15%	15.53%	48.58%	1.00%	0.22%
MF Holston	6CMFH011.31	0.23%	3.49%	0.07%	52.36%	6.98%	0.74%	0.18%	5.16%	29.59%	0.99%	0.20%
	6CMFH023.41	0.20%	3.30%	0.03%	61.60%	6.27%	0.73%	0.09%	4.23%	22.98%	0.42%	0.15%
	6CMFH026.00	0.19%	3.37%	0.03%	63.51%	6.26%	0.74%	0.10%	4.32%	21.00%	0.33%	0.16%
	6CMFH032.39	0.18%	3.11%	0.04%	68.59%	5.73%	0.65%	0.11%	3.84%	17.19%	0.40%	0.17%
	6CMFH033.40	0.17%	3.12%	0.04%	68.84%	5.70%	0.65%	0.11%	3.86%	16.97%	0.37%	0.17%
	6CMFH045.83	0.02%	2.69%	0.05%	68.32%	4.74%	0.69%	0.22%	2.89%	19.66%	0.45%	0.26%
	6CMFH055.88	0.04%	1.76%	0.00%	42.64%	6.70%	0.38%	0.00%	1.36%	44.84%	1.94%	0.35%
Bear Creek	6CBER004.10	0.00%	0.05%	0.00%	99.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.93%
Hutton Branch	6CHUT000.07	0.00%	3.14%	0.00%	62.08%	7.20%	0.73%	0.00%	6.53%	18.77%	1.55%	0.00%
Hutton Creek	6CHTO000.07	0.03%	4.64%	0.00%	22.69%	10.00%	0.50%	0.00%	9.86%	48.16%	3.71%	0.43%

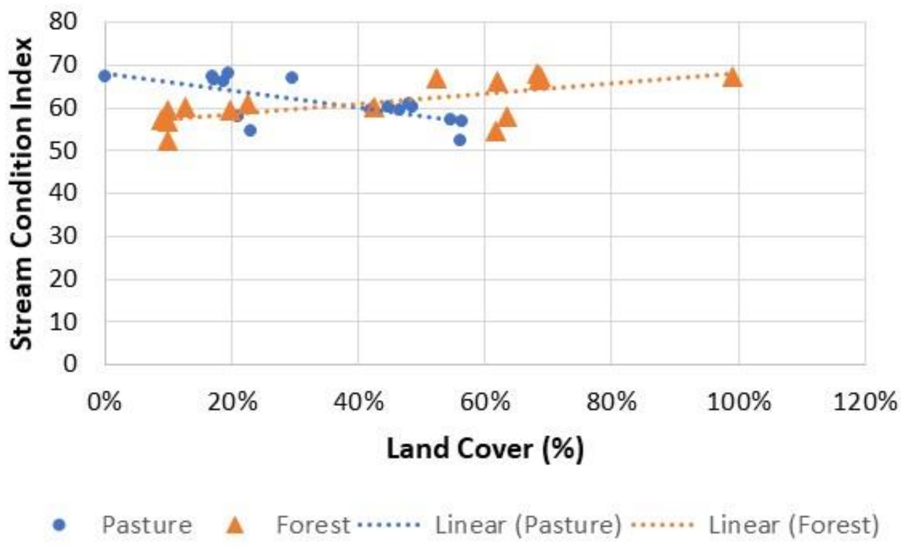

Figure 12. Regression between pasture and forest in the watershed and benthic health.

Table 10. Regression relationship between land cover and stream condition index (SCI) scores.

Parameter	Correlation Coefficient	Regression Significant (Y/N)	r^2	p-value
Pasture	-0.69	Y	0.47	0.0035
Forest	0.67	Y	0.44	0.0053
Urban/Res. Trees	-0.63	Y	0.39	0.0096
Urban/Res. Grass	-0.58	Y	0.32	0.022
Cropland	-0.51	Y	0.25	0.049
Impervious	-0.36	N	0.12	0.19
Other	0.28	N	0.082	0.28
Scrub/Shrub	-0.25	N	0.058	0.37
Water	0.11	N	0.0096	0.72
Harvested/Disturbed	-0.07	N	0.0042	0.81
Barren	-0.05	N	0.0019	0.87

2.4. Water Quality Data Assessment

Water quality data for all of the candidate stressors were evaluated to assess trends and compare to relevant water quality standards and stressor thresholds.

2.4.1. Temperature

VDEQ measures temperature when collecting benthic or water quality samples, so periodic temperature data are available from 2000 to present from each of the impaired tributaries (Figure 13) and from multiple stations on the MF Holston (Figure 14). Temperatures obviously vary by season, so ranges are wide when year-round measurements are considered. Each of the impaired tributaries had stations with statistically higher temperatures than the reference station ($p<0.05$ in t-test with unequal variances), however none of the stations had temperature measurements above the water quality standard of 31°C. Maximum temperatures ranged from 18.7°C in Tattle Branch to 23.3°C in Cedar Creek (6CCED000.14). In the MF Holston River, temperatures generally increased moving downstream, with the lower two stations having statistically higher temperatures than the reference station ($p<0.05$ in t-test with unequal variances). Maximum temperatures ranged from 19.2°C at the reference station (6CMFH045.72/83) to 26.2°C at 6CMFH013.21. Like MF Holston tributaries, no MF Holston River station exceeded the water quality standard of 31°C (Figure 15).

VDEQ also collected diurnal temperature at each of the primary benthic stations during the summer of 2021. Diurnal data were collected at 15-minute intervals for 1 week at each location. Temperature data during diurnal deployments are shown in Figure 16. Diurnal temperatures exhibited the natural cycle of increases during the day from solar heating and decreases at night. No stations exceeded the Virginia water quality standard of 31ºC during this late summer critical period. At most stations, the daytime maximum temperatures were below 25ºC. This is an indication that temperature is not a primary stressor in these streams.

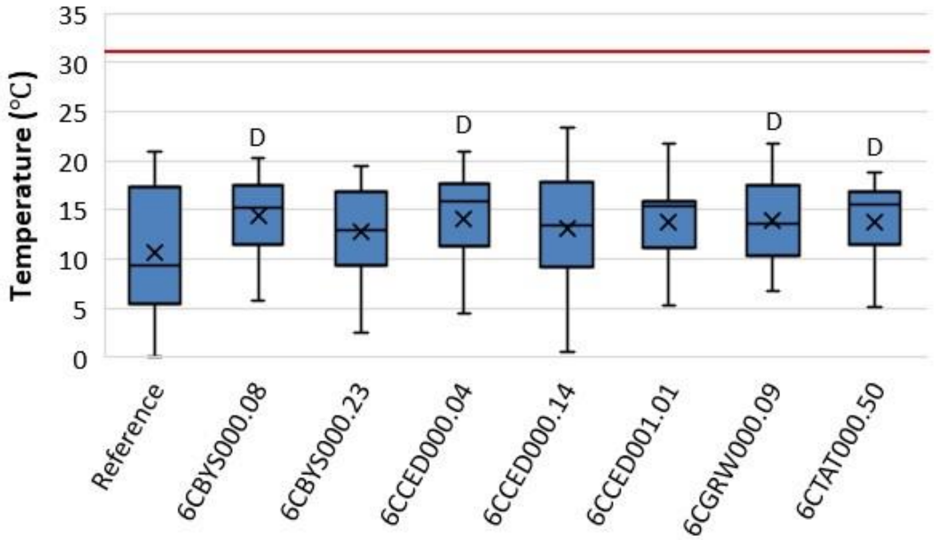

Figure 13. Temperature in MF Holston tributaries. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. The "D" indicates a statistically significant difference from the reference station. The red line represents the Virginia water quality standard.

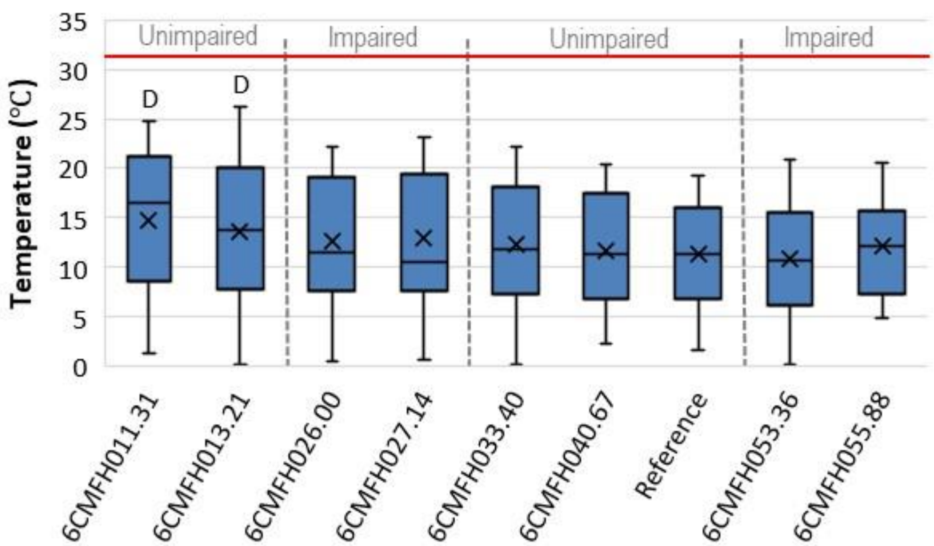

Figure 14. Temperature in the MF Holston River. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. The "D" indicates a statistically significant difference from the reference station. The red line represents the Virginia water quality standard.

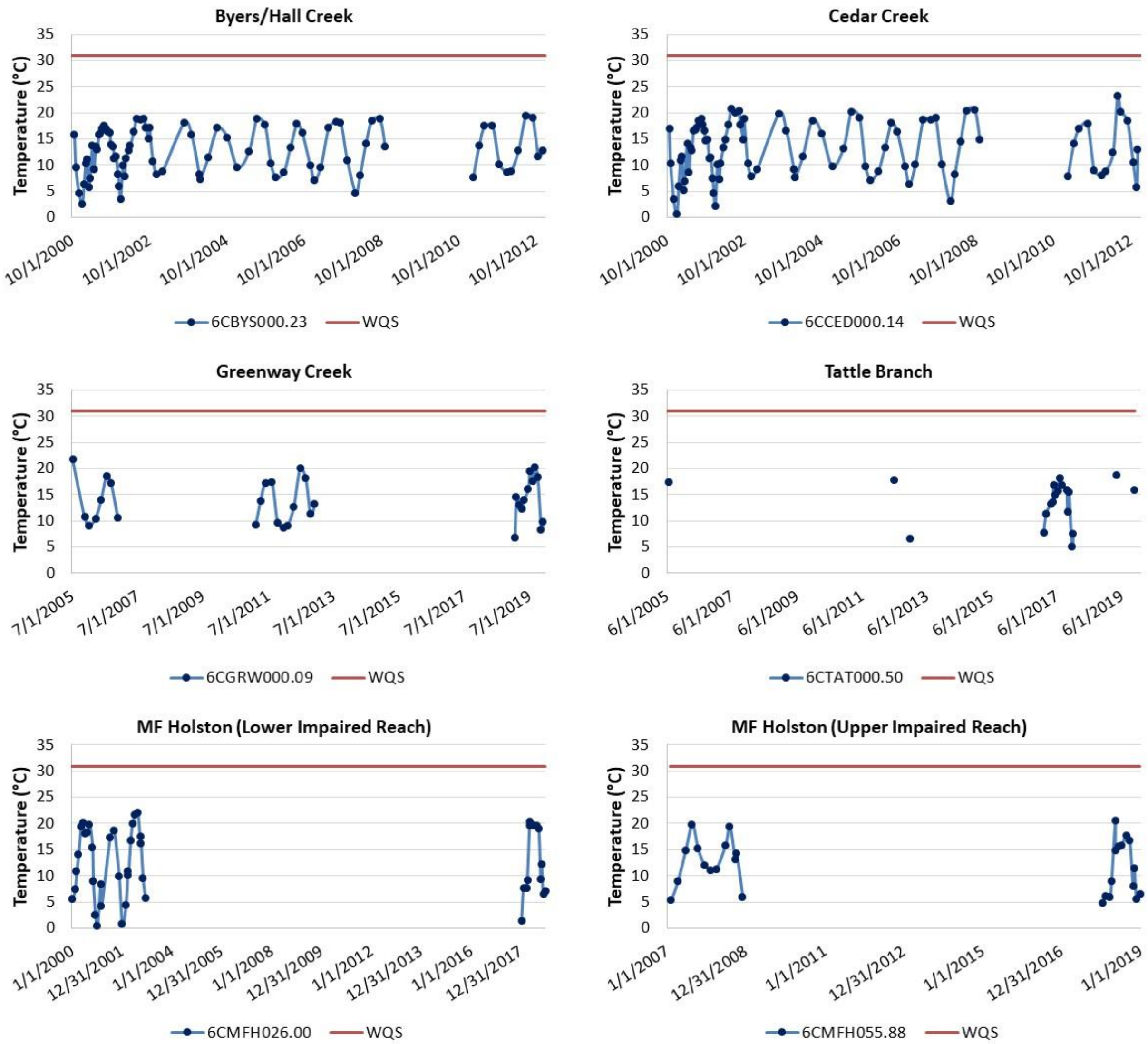

Figure 15. Temperature over time in MF Holston Project streams. The red line represents the Virginia water quality standard.

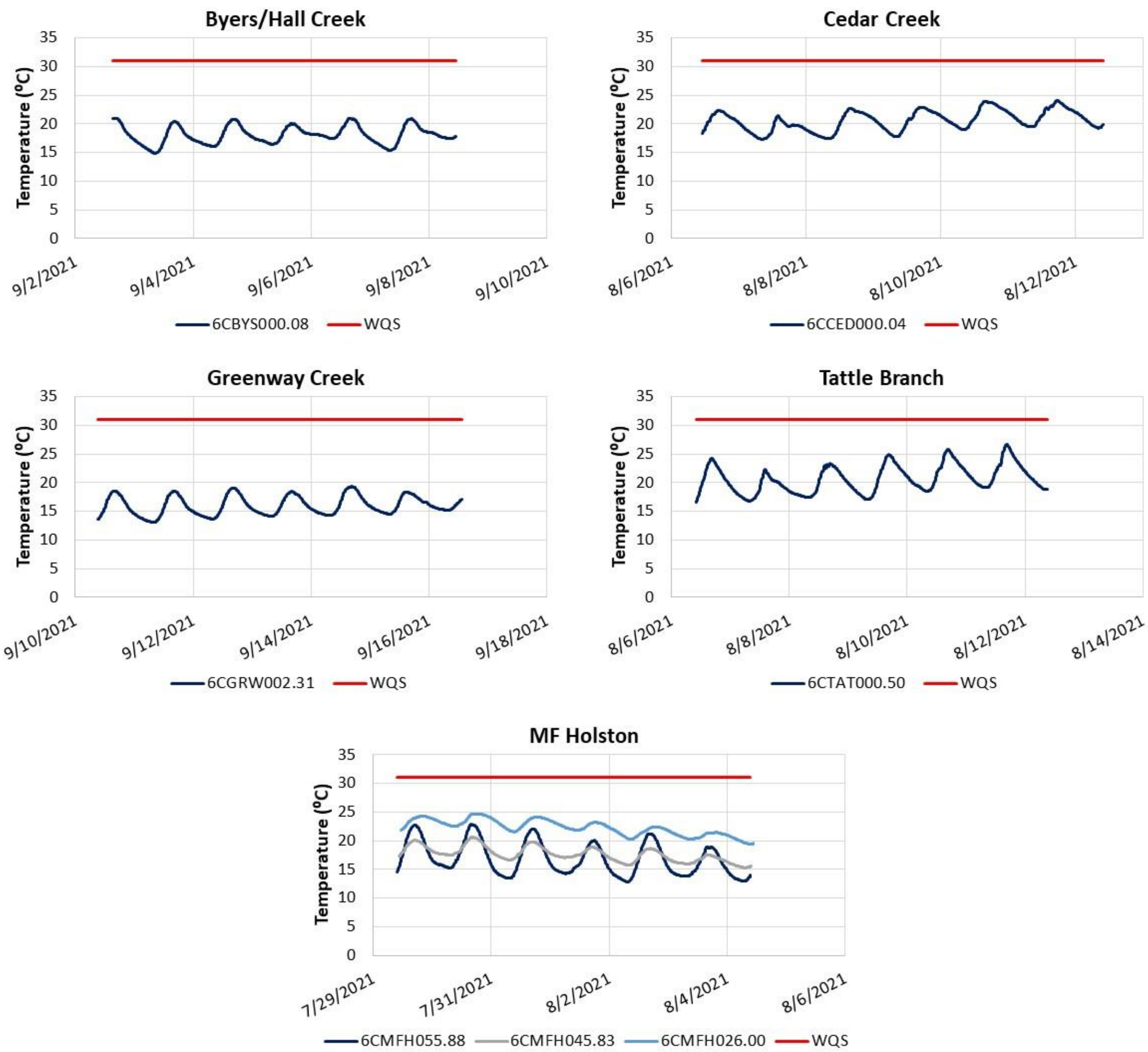

Figure 16. Diurnal temperature in MF Holston Project streams. The red line represents the Virginia water quality standard.

2.4.2. pH

VDEQ measures pH when collecting benthic or water quality samples, so periodic pH data are available from each of the impaired tributaries (Figure 17) and from multiple stations on the MF Holston River. Measured pH values were moderately alkaline in each of the tributaries and averaged from 8.06 in Byers/Hall Creek (6CMFH000.23) to 8.20 in Cedar Creek (6CCED000.04). Each of the tributaries was statistically different from the reference site ($p<0.05$ in t-test with unequal variance), but median pH values at all sites were within the low probability range for stressor effects. pH values in the MF Holston River averaged from 7.80 at station 6CMFH053.36

to 8.20 at station 6CMFH011.31. The two most downstream stations were statistically different from the reference site ($p<0.05$ in t-test with unequal variance), but median pH values at all sites were within the low probability range for stressor effects. Figure 19 show the time series of pH values in MF Holston Project streams. While pH varied over time, all individual samples from all stations were between 6 and 9 and were within the water quality standards. For this reason, pH is not likely a stressor in MF Holston Project streams.

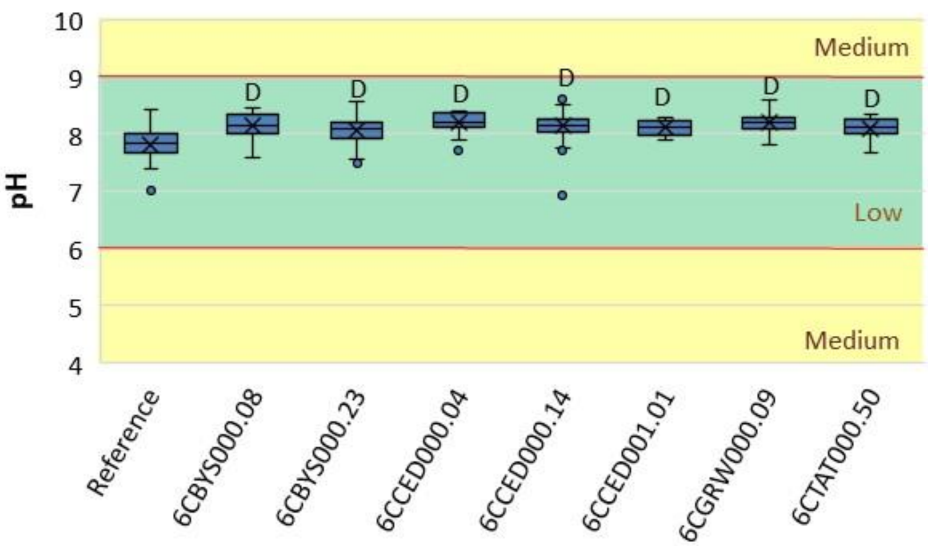

Figure 17. pH in MF Holston tributaries. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station. The red line represents the Virginia water quality standard. Colors represent the probability that data within that range would be responsible for causing stress.

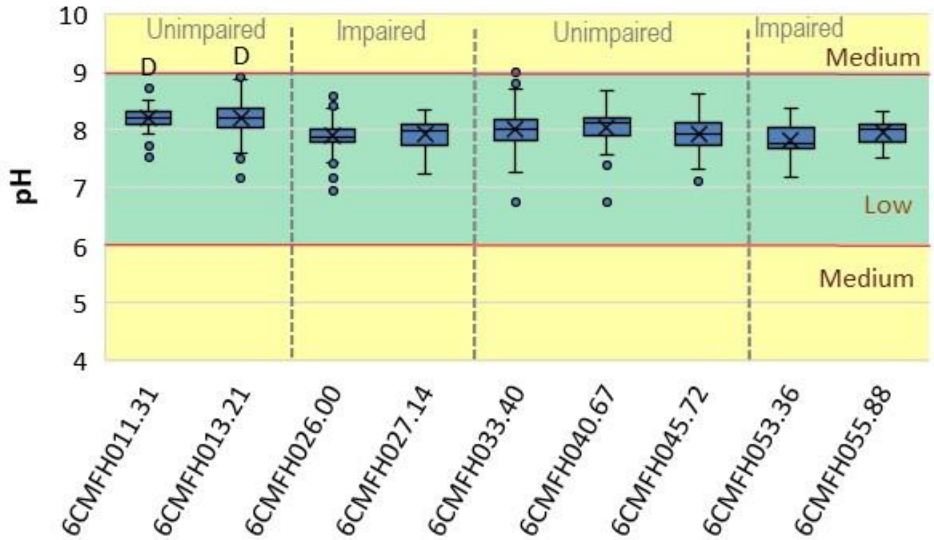

Figure 18. pH in the MF Holston River. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station. The red line represents the Virginia water quality standard. Colors represent the probability that data within that range would be responsible for causing stress.

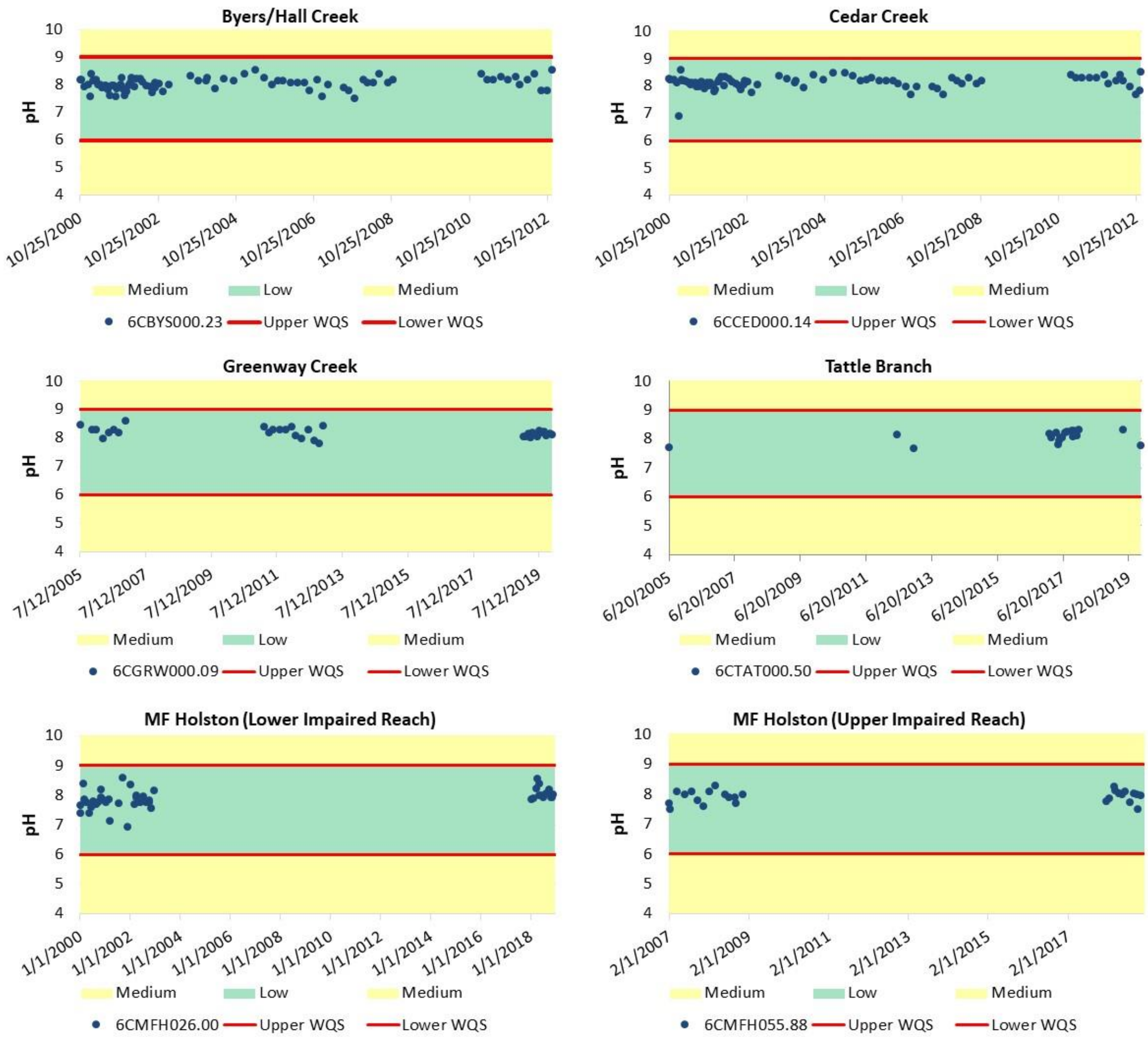

Figure 19. pH over time in MF Holston Project streams. The red line represents the Virginia water quality standard. Colors represent the probability that data within that range would be responsible for causing stress.

2.4.3. Dissolved Oxygen

VDEQ measures dissolved oxygen (DO) when collecting benthic or water quality samples, so periodic DO data are available from 2000 to present in each of the impaired MF Holston tributaries (Figure 20) and various stations on the MF Holston River (Figure 21). Average dissolved oxygen levels in tributaries ranged from 9.49 mg/L in Tattle Branch (6CTAT000.50) to 10.1 mg/L in Byers/Hall Creek (6CBYS000.23). In the MF Holston River, dissolved oxygen averaged from 9.75 mg/L at station 6CMFH055.88 to 10.8 mg/L at station 6CMFH013.21. Median DO values were in the no to low probability range for stressor effects in all of the tributaries and MF Holston stations. Only station 6CMFH055.88 had statistically lower DO than the reference station ($p<0.05$ in t-test with unequal variance).

Figure 22 shows the time series of DO concentrations in each impaired stream. None of the MF Holston tributaries had DO excursions into the high probability range for stressor effects. In the impaired reaches of the MF Holston River, only station 6CMFH026.00 had a single excursion into the high probability range for stressor effects. The minimum DO value observed at this station was 6.43 mg/L. None of the tributaries or MF Holston stations exhibited DO values below the average Virginia water quality standard of 5 mg/L.

In addition to periodic dissolved oxygen measurements, VDEQ collected diurnal dissolved oxygen data at each of the primary benthic stations during the summer of 2021. Diurnal data were collected at 15-minute intervals for 1 week at each station. Diurnal monitoring of dissolved oxygen is important, because critical dissolved oxygen levels are typically encountered just before sunrise. This is due to the combination of oxygen consumption from respiration and the absence of oxygen production from photosynthesis during the night. Diurnal monitoring was conducted in late July through early September, because critical dissolved oxygen levels are also more common during the hot and dry summer months.

Dissolved oxygen data during diurnal deployments are shown in Figure 23. Diurnal dissolved oxygen values at all stations exhibited the natural cycle of increases during the day while plants are photosynthesizing and decreases at night while respiration dominates. Dissolved oxygen levels in Cedar Creek were nearly exclusively in the low probability range for stressor effects, with a nighttime minimum of 7.97 mg/L. In Byers/Hall Creek, DO levels were generally in the low probability range during the day and dropped to the medium probability range at night. Nighttime minimums were still above 7.45 mg/L. In Greenway Creek and Tattle Branch, DO levels were slightly lower and dipped into the high probability range at night on several occasions. The nighttime minimum in Tattle Branch was 6.78 mg/L, and the nighttime minimum in Greenway Creek was 5.06 mg/L, although this value was likely an anomalous instrument malfunction. On 9/14/21, the DO went from 7.09 to 5.06 to 7.08 in three successive 15-minute intervals. This is biologically highly unlikely and likely due to an interference with the optical DO probe during the reading of 5.06 mg/L. Aside from this anomalous value, the nighttime minimum in Greenway

Creek was 6.47 mg/L. None of the tributaries violated the average water quality standard of 5 mg/L or the instantaneous water quality standard of 4 mg/L.

In the MF Holston, diurnal DO at the upstream impaired reach was similar to DO at the unimpaired reference station. Nighttime minima were in the medium probability range for stressor effects and above 7.28 mg/L. At the lower impaired station, diurnal DO was lower, with nighttime DO dropping into the high probability range for stressor effects with a minimum of 6.06. All of the MF Holston stations maintained DO levels above the average water quality standard of 5 mg/L and the instantaneous water quality standard of 4 mg/L.

Figure 24 shows the diurnal dissolved oxygen data expressed as percent saturation. This method of analysis allows the observed DO to be compared with the anticipated DO if the stream were at full DO saturation. Values above 100% mean that the stream is super-saturated with DO, and values below 100% show that oxygen is depleted to varying degrees. Large swings in DO during a day indicate that nutrient enrichment may be driving high levels of photosynthesis by algae during the day and oxygen consumption at night. None of the streams exhibited large swings of DO indicative of nutrient enrichment and excess algal growth. DO maxima were generally near 100% saturation and DO minima were generally near 80% saturation. This represents relatively natural DO cycles without significant nutrient enrichment. Only Greenway Creek and the MF Holston lower impaired reach exhibited larger daily DO swings than the MF Holston reference site. Maximum DO swings were 36% in Greenway Creek and 42% in the MF Holston lower impaired reach, compared to 30% at the MF Holston reference site.

In summary, dissolved oxygen is not likely a stressor in the MF Holston River or the impaired tributaries. Median DO values were in the no to low probability range for stressor effects, and minimum DO excursions at nighttime during hot and dry summer months were well above the average water quality criterion of 5 mg/L at all stations.

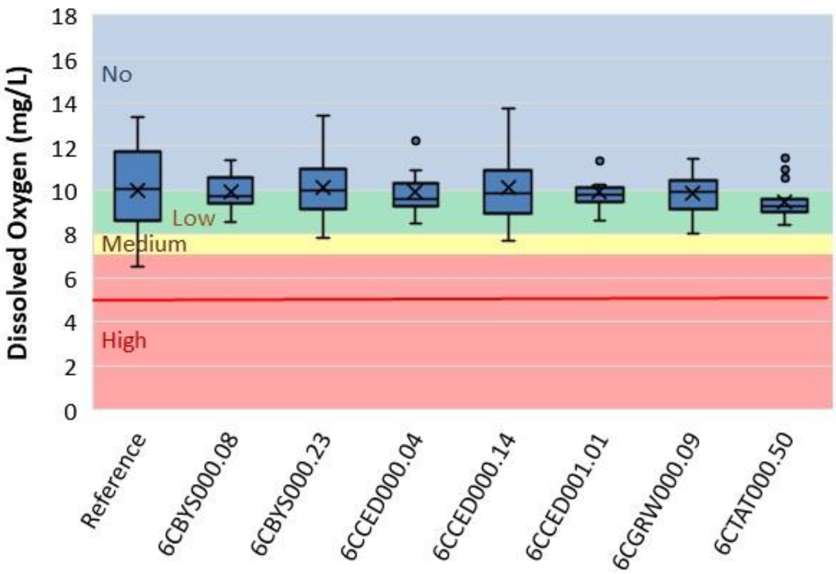

Figure 20. Dissolved oxygen in MF Holston tributaries. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station. The red line represents the Virginia water quality standard. Colors represent the probability that data within that range would be responsible for causing stress.

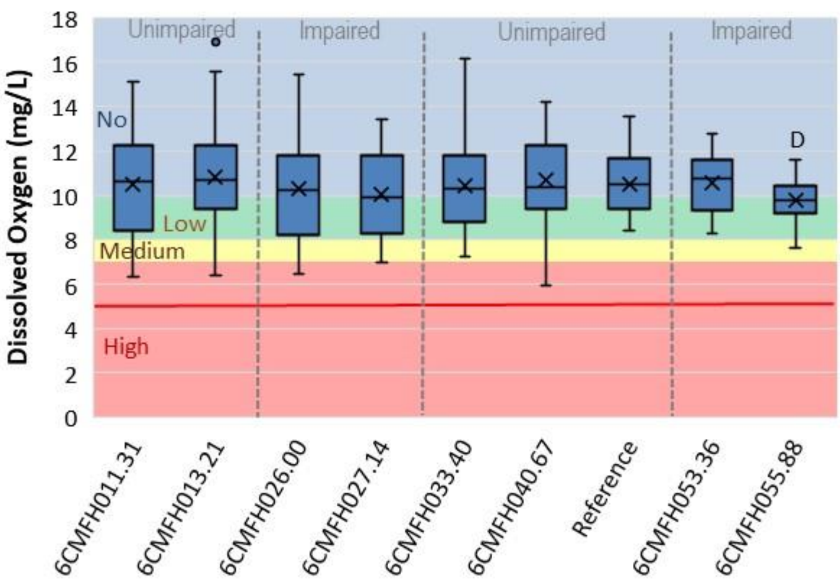

Figure 21. Dissolved oxygen in the MF Holston River. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station. The red line represents the Virginia water quality standard. Colors represent the probability that data within that range would be responsible for causing stress.

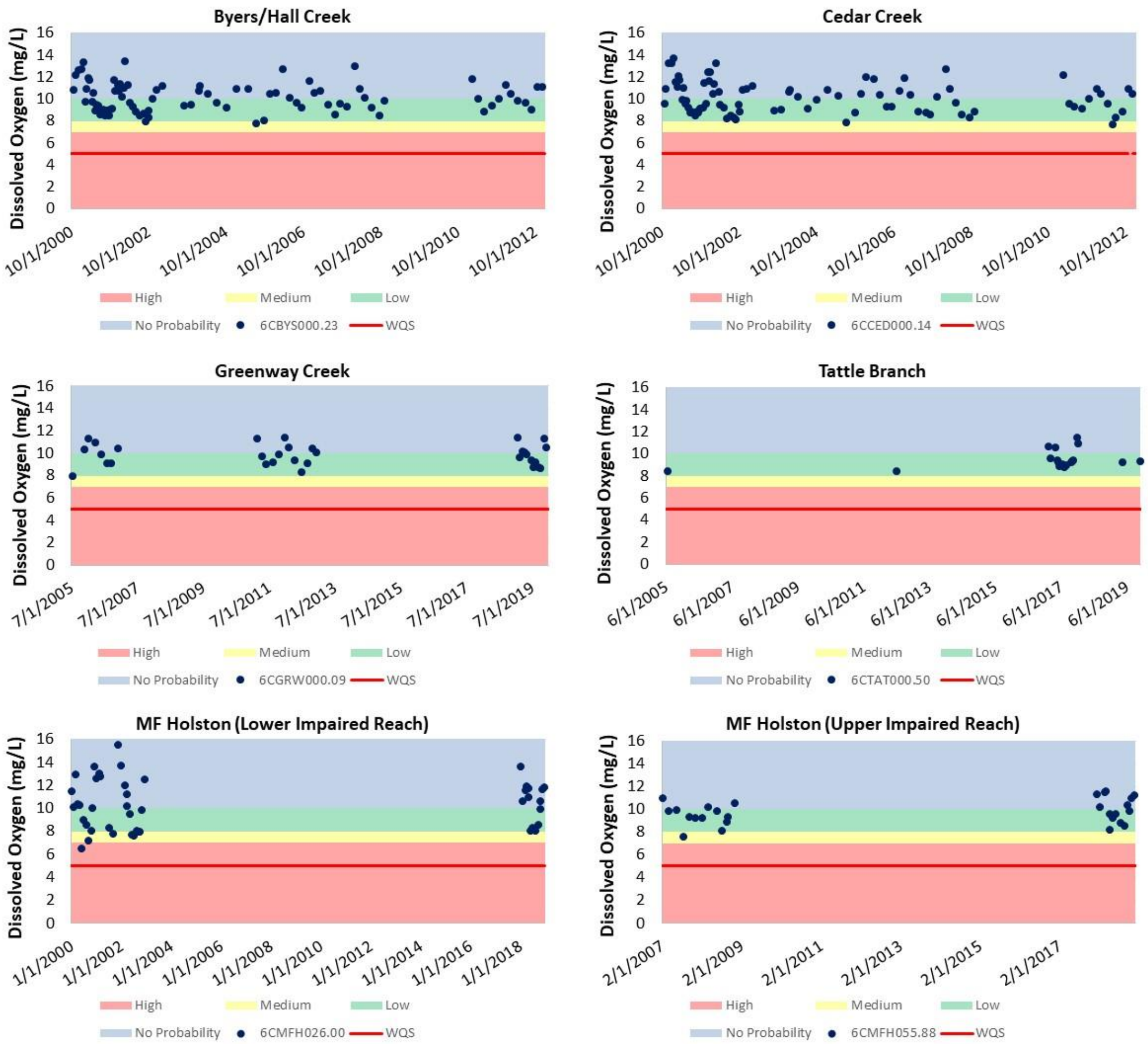

Figure 22. Dissolved oxygen over time in MF Holston Project streams. The red line represents the Virginia water quality standard. Colors represent the probability that data within that range would be responsible for causing stress.

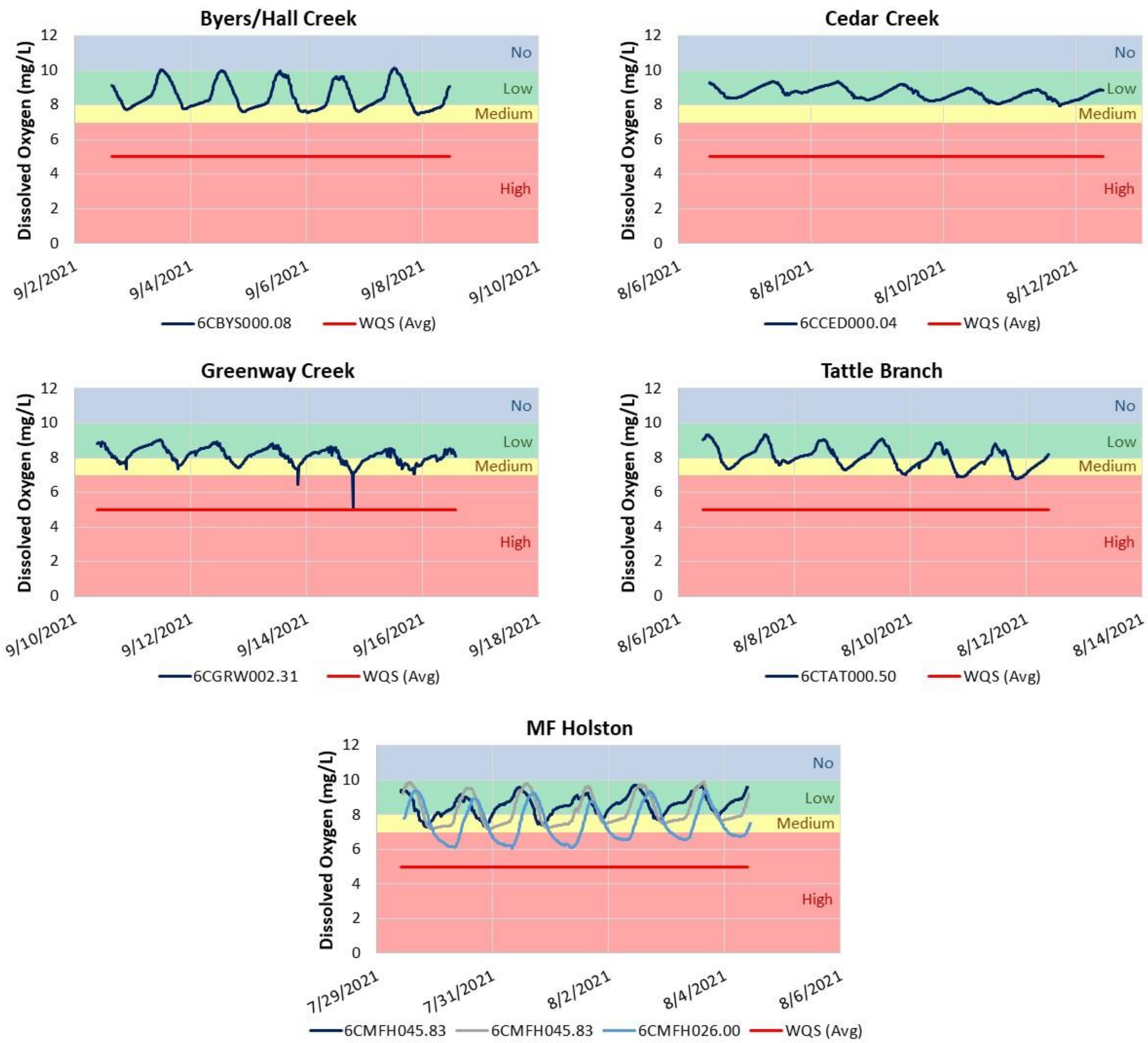

Figure 23. Diurnal dissolved oxygen in MF Holston Project streams. The red line represents the Virginia water quality standard. Colors represent the probability that data within that range would be responsible for causing stress.

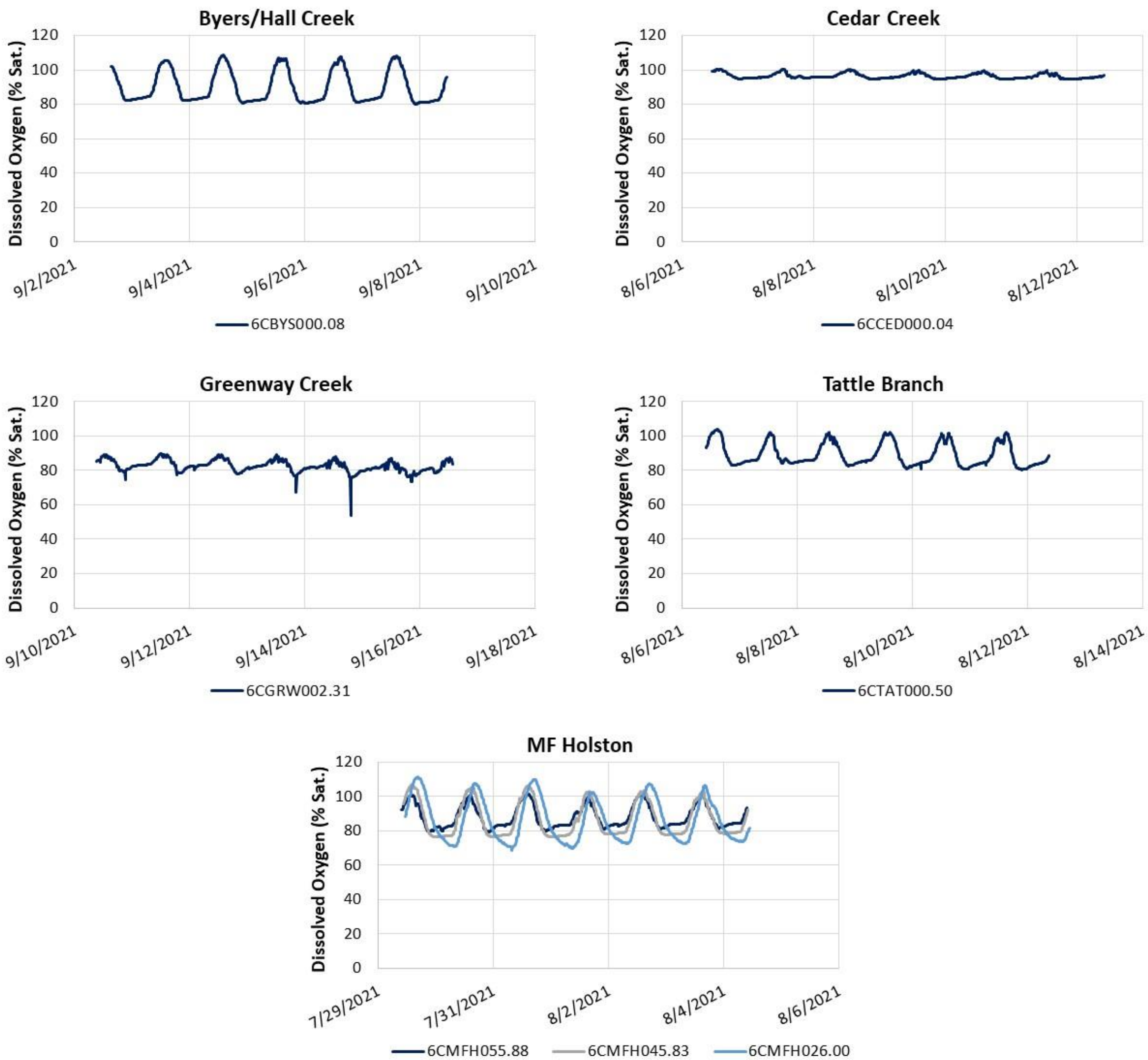

Figure 24. Diurnal dissolved oxygen in MF Holston Project streams expressed as percent saturation.

2.4.4. Conductivity and Total Dissolved Solids

Conductivity is a measure of the electrical potential of water based on the ionic charges of dissolved compounds. For this reason, the conductivity of water is closely related to the total dissolved solids present. VDEQ measures conductivity when collecting benthic or water quality samples, so periodic conductivity data are available from 2000 to present in MF Holston tributaries (Figure 25) and in the MF Holston River (Figure 26). Average conductivity in MF Holston tributaries ranged from 483 uS/cm in Cedar Creek (6CCED000.14) to 567 uS/cm in Tattle Branch. All impaired tributaries had statistically higher conductivity ($p<0.05$ in t-test with unequal variance) than the reference site. The median conductivity in Byers/Hall Creek, two stations on Cedar Creek, and Tattle Branch were in the high probability range for stressor effects, while the median conductivity at one Cedar Creek station (6CCED000.14) and in Greenway Creek was in the medium probability range for stressor effects. In the MF Holston River, conductivity was considerably lower, with values averaging from 206 uS/cm at station 6CMFH053.36 to 384 uS/cm at station 6CMFH011.31. In general, conductivity increased moving downstream in the MF Holston River. Median conductivity in all of the MF Holston River stations was in the no to low probability range for stressor effects, except for the two most downstream stations, where medians were in the medium probability range for stressor effects.

Figure 27 shows conductivity levels over time in each of the MF Holston Project streams. Conductivity varied over time, likely due to rainfall and flow conditions. A majority of conductivity measurements in Byers/Hall Creek (60%) and Tattle Branch (84%) were in the high probability range for stressor effects. In Cedar Creek and Greenway Creek, 29% and 38% of conductivity measurements were in the high probability range, respectively. No conductivity values were in the high probability range at the impaired MF Holston River stations.

In addition to periodic conductivity measurements, VDEQ collected diurnal conductivity data at each of the primary benthic stations during the summer of 2021. Diurnal data were collected at 15-minute intervals for 1 week at each station. Conductivity data during diurnal deployments are shown in Figure 28. In Byers/Hall Creek and Cedar Creek, conductivity remained above 500 uS/cm and in the high probability range for stressor effects for the duration of the diurnal monitoring. In Greenway Creek and Tattle Branch, conductivity was generally above 500 uS/cm with occasional decreases into the medium probability range. Conductivity levels were much lower in the MF Holston River. Conductivity generally increased moving downstream, with values in the no probability range for stressor effect at the upper impaired reach, low probability range at the unimpaired reference station, and medium probability range at the lower impaired reach.

Total dissolved solids (TDS) are closely tied to conductivity, since it is the dissolved ions that transmit electrical current. Figure 29 shows TDS levels in MF Holston tributaries, and Figure 30 shows TDS levels at stations along the MF Holston River. In MF Holston tributaries, TDS averaged from 280 mg/L in Greenway Creek to 331 mg/L in Tattle Branch. Each of the tributaries was statistically higher in TDS (t-test with unequal variance and alpha = 0.05) than the reference, which averaged only 116 mg/L. All tributaries averaged in the medium probability for stressor effects. TDS was considerably lower in the MF Holston than in its tributaries. TDS averaged from 141 mg/L at station 6CMFH055.88 to 221 mg/L at station 6CMFH011.31 and increased consistently moving downstream in the MF Holston River. Stations between miles 33.40 and 11.31 were statistically higher in TDS (t-test with unequal variance and alpha = 0.05) than the reference (6CMFH045.72), but all stations were in the low probability range for stressor effects.

Figure 31 shows TDS levels over time in each of the MF Holston Project streams. In each stream, TDS levels were relatively consistent over time. The majority of samples collected from MF Holston tributaries were in the medium probability range for stressor effects, with two samples from Tattle Branch reaching into the high probability range and two samples from Greenway Creek dropping to the low probability range. In the MF Holston River, TDS levels in all samples except for one (at 6CMFH026.00) were within the low probability range for stressor effects.

In summary, conductivity and TDS are not likely a stressor in the MF Holston River, where values were generally in the no to low probability range for stressor effects and the spatial pattern of impairment along the river did not match the spatial trends in conductivity. In some of the impaired tributaries, however, conductivity and TDS could be a stressor. In Byers/Hall Creek and Tattle Branch, a majority of conductivity values were in the high probability range for stressor effects, but TDS levels were only in the medium probability range.

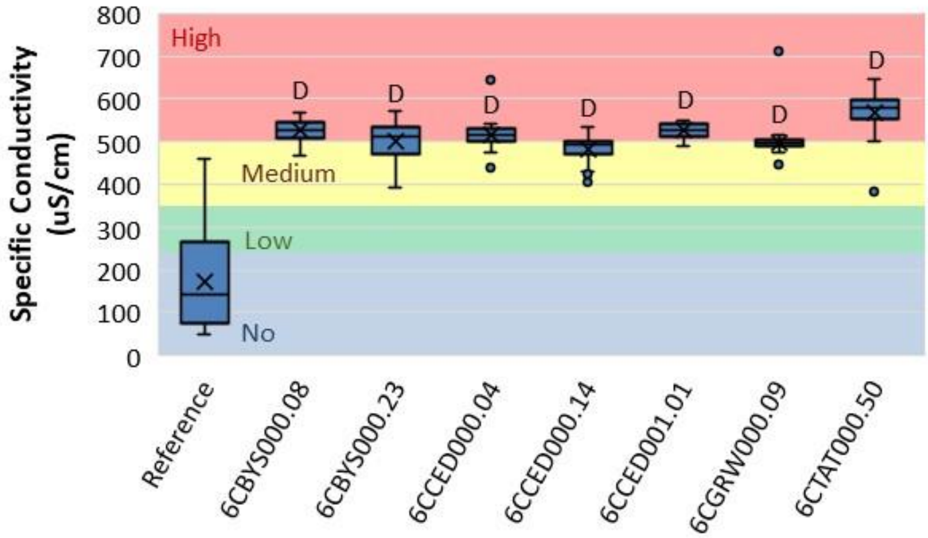

Figure 25. Conductivity in MF Holston tributaries. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station. Colors represent the probability that data within that range would be responsible for causing stress.

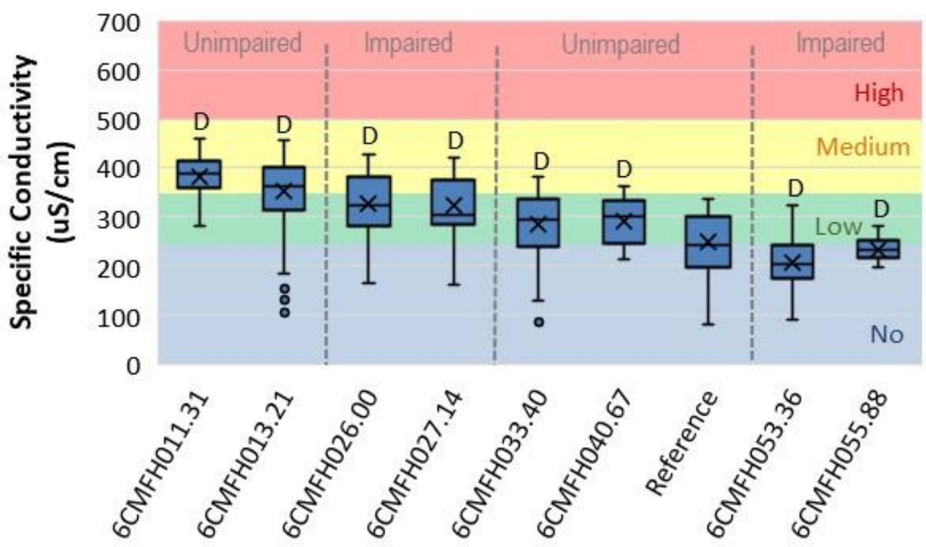

Figure 26. Conductivity in the MF Holston River. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station. Colors represent the probability that data within that range would be responsible for causing stress.

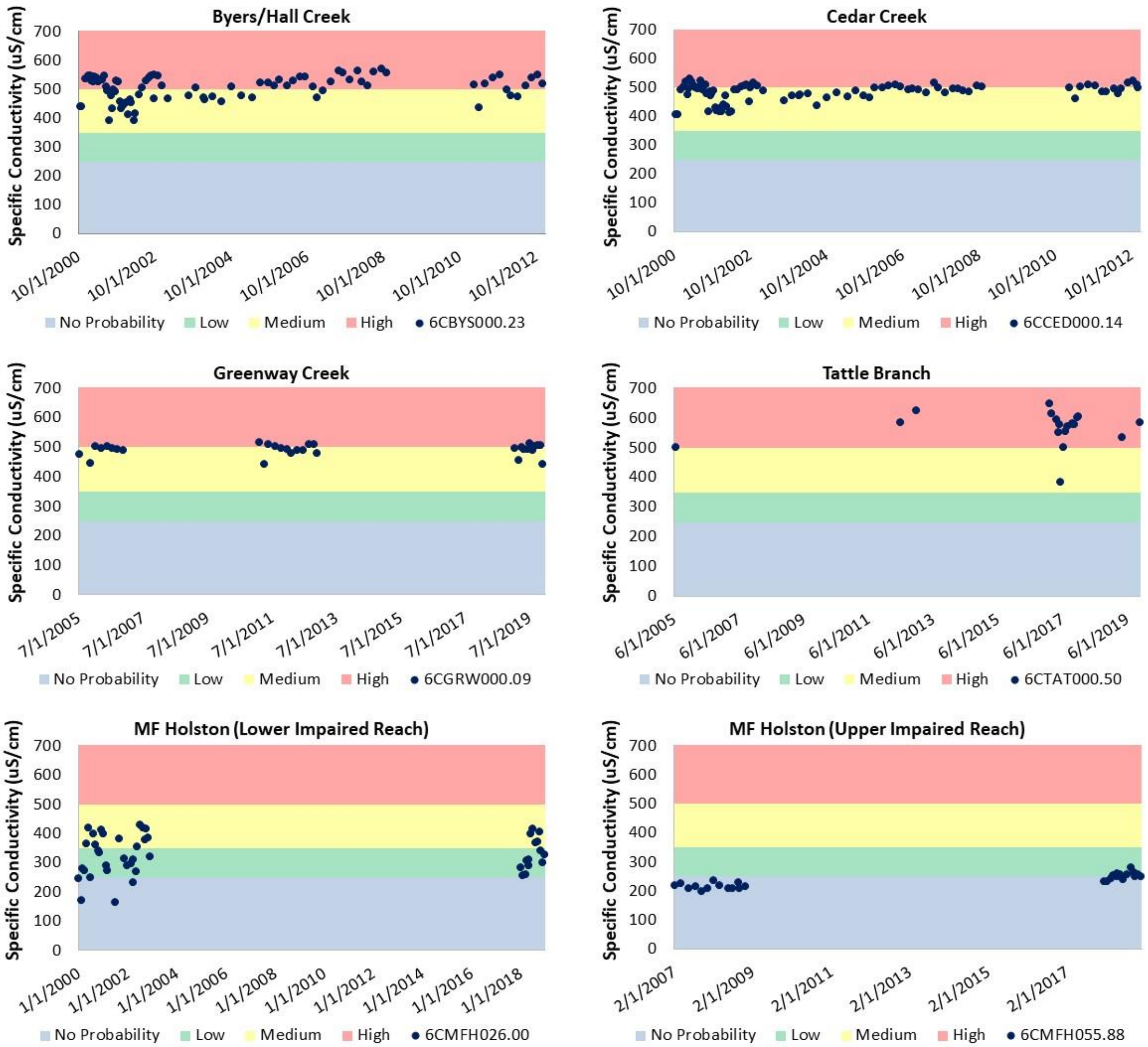

Figure 27. Conductivity over time in MF Holston Project streams. Colors represent the probability that data within that range would be responsible for causing stress.

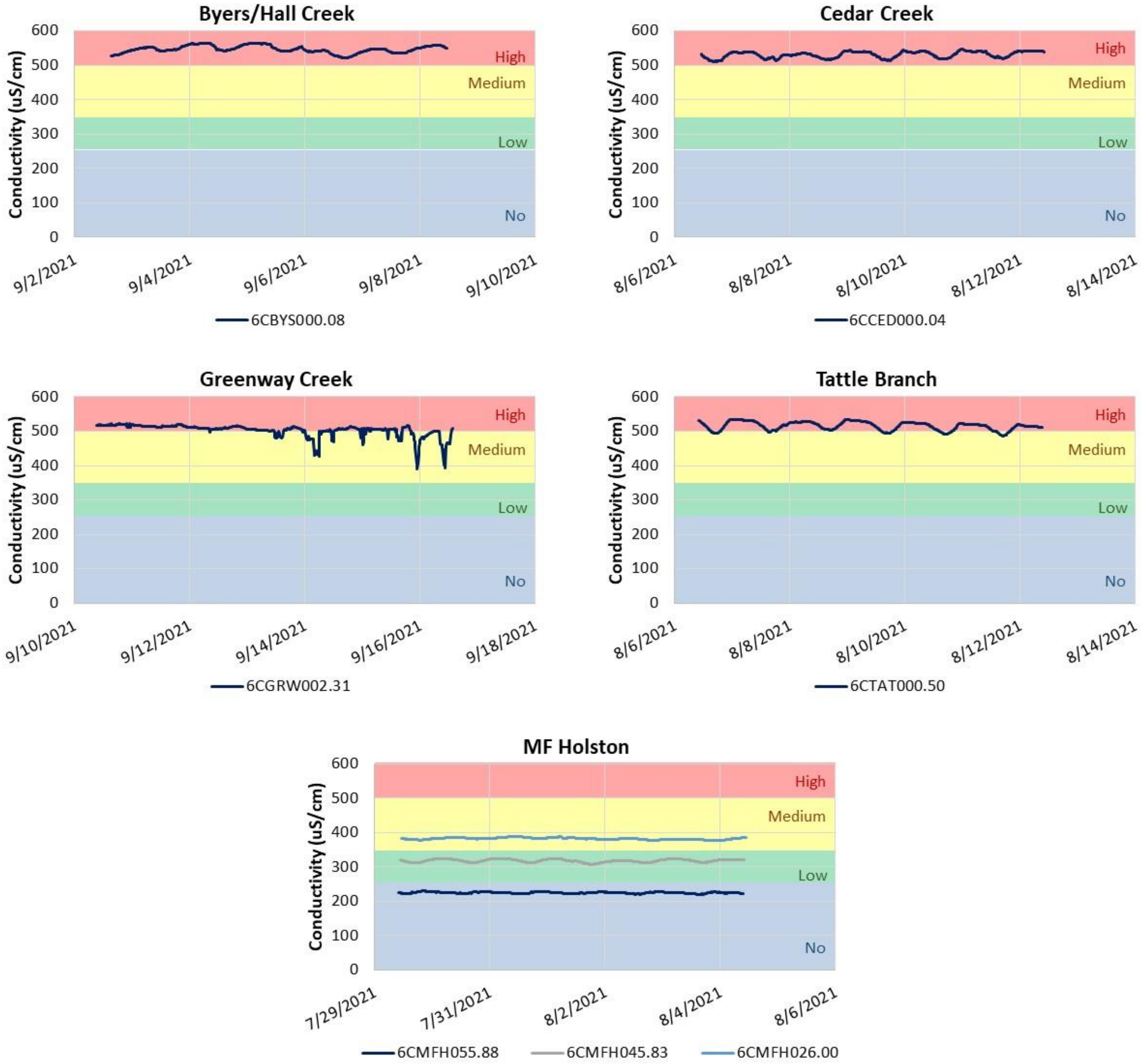

Figure 28. Diurnal conductivity in MF Holston Project streams. Colors represent the probability that data within that range would be responsible for causing stress.

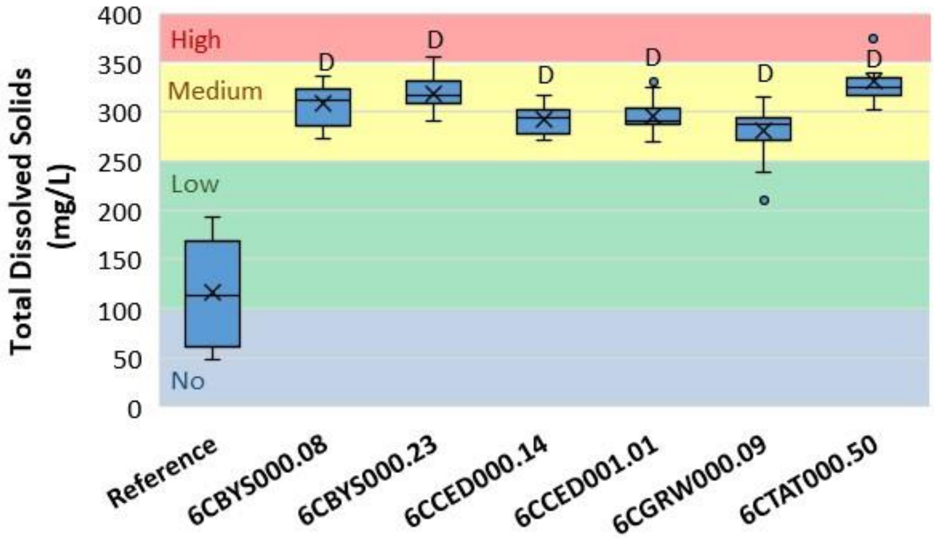

Figure 29. Total dissolved solids in MF Holston tributaries. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station. Colors represent the probability that data within that range would be responsible for causing stress.

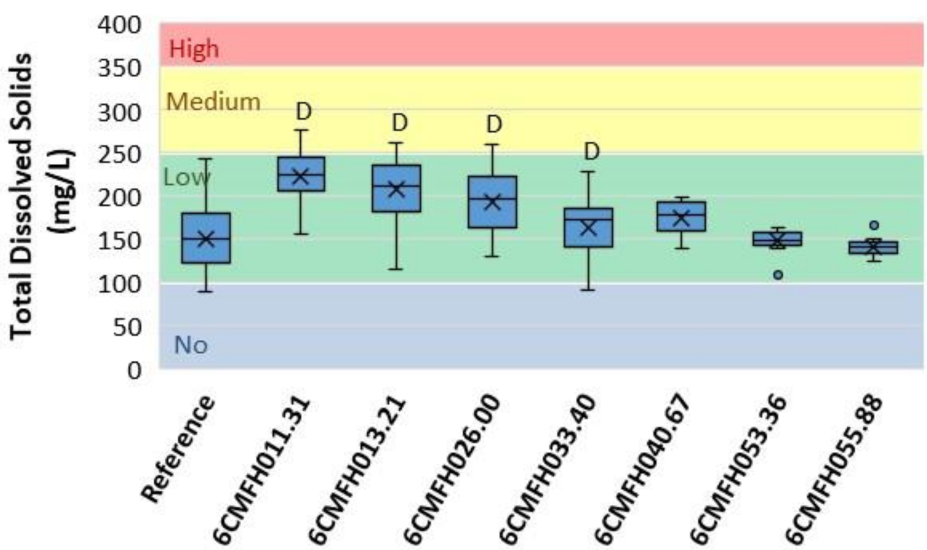

Figure 30. Total dissolved solids in the MF Holston River. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station. Colors represent the probability that data within that range would be responsible for causing stress.

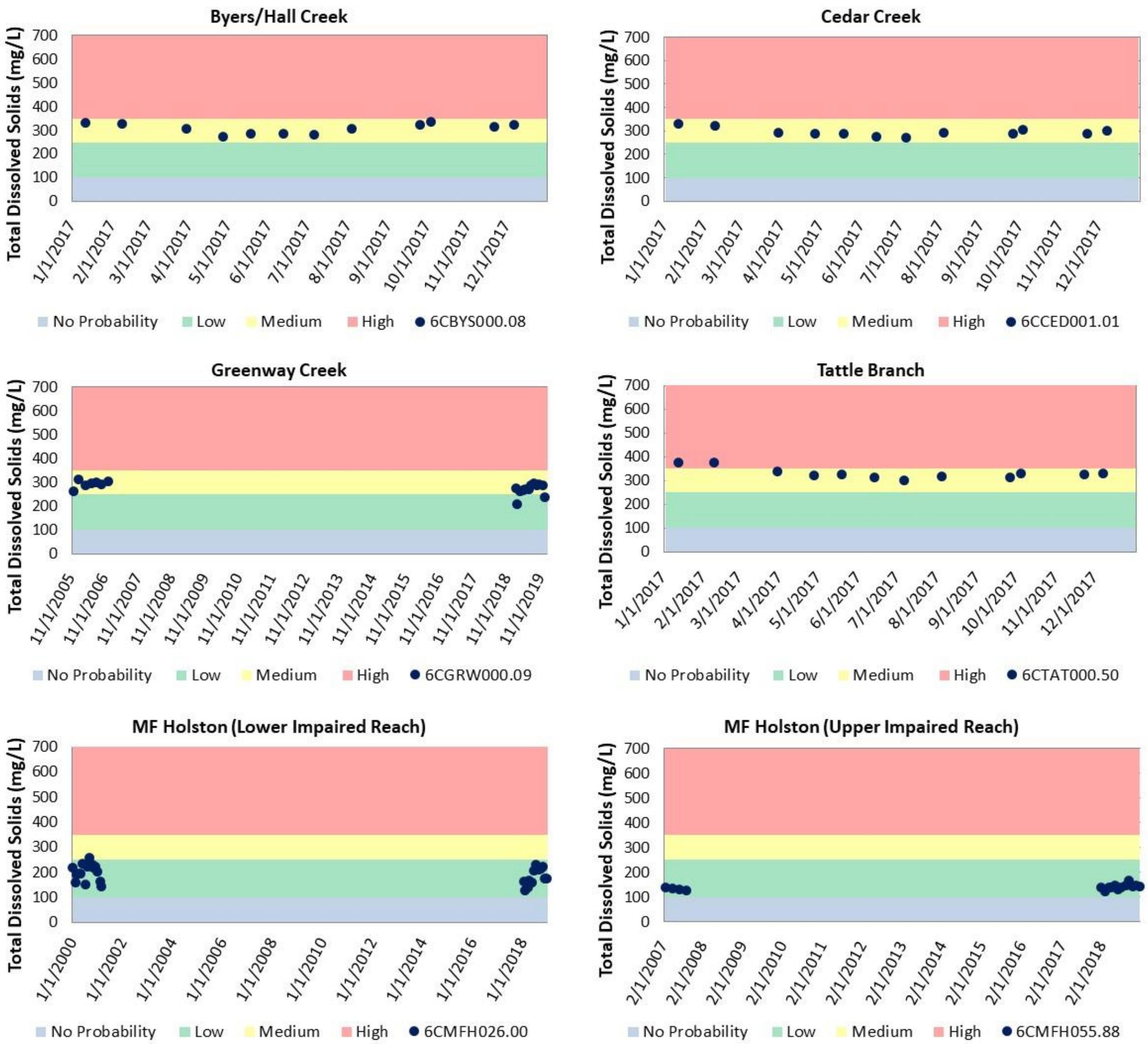

Figure 31. Total dissolved solids over time in MF Holston Project streams. Colors represent the probability that data within that range would be responsible for causing stress.

2.4.5. Dissolved Ions

Dissolved sodium, potassium, chloride, and sulfate were measured in MF Holston Project streams. Figure 32 shows the concentrations of these dissolved ions in MF Holston tributaries, and Figure 33 shows dissolved ion concentrations in the MF Holston River. In MF Holston tributaries, dissolved sodium concentrations averaged from 8.22 mg/L in Greenway Creek to 20.7 mg/L in Tattle Branch. Median dissolved sodium values were in the low probability range for stressor effects in Cedar Creek and Greenway Creek but were in the medium probability range in

Byers/Hall Creek and Tattle Branch. Dissolved sodium concentrations in Tattle Branch averaged in the high probability range, with 42% of values within the high range. Dissolved sodium levels were lower in the MF Holston River. Averages ranged from 2.12 mg/L at station 6CMFH055.88 to 8.46 mg/L at station 6CMFH026.00. Stations 6CMFH011.31 and 6CMFH026.00 were statistically higher than the reference ($p<0.05$ in t-test with unequal variances), and station 6CMFH055.88 was statistically lower than the reference. Median dissolved sodium values were in the no to low probability range for stressor effects at each of the MF Holston stations.

Dissolved potassium concentrations in MF Holston tributaries averaged from 3.10 mg/L in Byers/Hall Creek to 4.03 mg/L in Tattle Branch. Median dissolved potassium values were in the medium probability range for stressor effects in each of the MF Holston tributaries. Dissolved potassium levels were slightly lower in the MF Holston River. Averages ranged from 1.72 mg/L at station 6CMFH045.72/83 to 2.95 mg/L at station 6CMFH055.88. Stations 6CMFH011.31, 6CMFH013.21, and 6CMFH055.88 were statistically higher than the reference ($p<0.05$ in t-test with unequal variances), and median dissolved potassium values at these locations were in the medium probability range for stressor effects. All other stations were in the low probability range.

Dissolved chloride concentrations in MF Holston tributaries averaged from 15.6 mg/L in Greenway Creek to 36.8 mg/L in Tattle Branch. Median dissolved chloride values were in the medium probability range for stressor effects in Tattle Branch and in the low probability range in the remaining MF Holston tributaries. Dissolved chloride levels were lower in the MF Holston River. Averages ranged from 5.45 mg/L at station 6CMFH055.88 to 15.2 mg/L at station 6CMFH026.00. Stations 6CMFH011.31 and 6CMFH026.00 were statistically higher than the reference ($p<0.05$ in t-test with unequal variances), and station 6CMFH055.88 was statistically lower than the reference. Median dissolved chloride values at all MF Holston stations were in the no to low probability range for stressor effects.

Dissolved sulfate concentrations in MF Holston tributaries averaged from 5.78 mg/L in Cedar Creek to 24.5 mg/L in Byers/Hall Creek. Median dissolved sulfate values were in the low probability range for stressor effects in Byers/Hall Creek and in the no probability range in the remaining MF Holston tributaries. Dissolved sulfate levels were comparable in the MF Holston River. Averages ranged from 8.78 mg/L at station 6CMFH055.88 to 11.3 mg/L at station 6CMFH011.31, with concentrations gradually increasing downstream. The three most downstream stations (6CMFH011.31, 6CMFH013.21, and 6CMFH026.00) were statistically higher than the reference ($p<0.05$ in t-test with unequal variances), and median dissolved sulfate values at these locations were in the low probability range for stressor effects. All other stations were in the no probability range.

In summary, the potentially toxic ions chloride, potassium, sodium, and sulfate are not likely to be stressors in the MF Holston River and tributaries. None of the streams had median concentrations of any of the dissolved ions in the high probability range for stressor effects. Several streams had median ion concentrations in the medium probability range for stressor effects, but maximum values were well below water quality criteria or toxic levels reported by Mount *et al.* (2016). Virginia's water quality criteria for chloride is 230 mg/L, which is approximately three times higher than the highest chloride concentration measured in any MF Holston stream. Similarly, measured concentrations were well below toxic levels reported by Mount *et al.* (2016) for sodium (or 460-920 mg/L Na), potassium (78-390 mg/L), or sulfate (96-2400 mg/L).

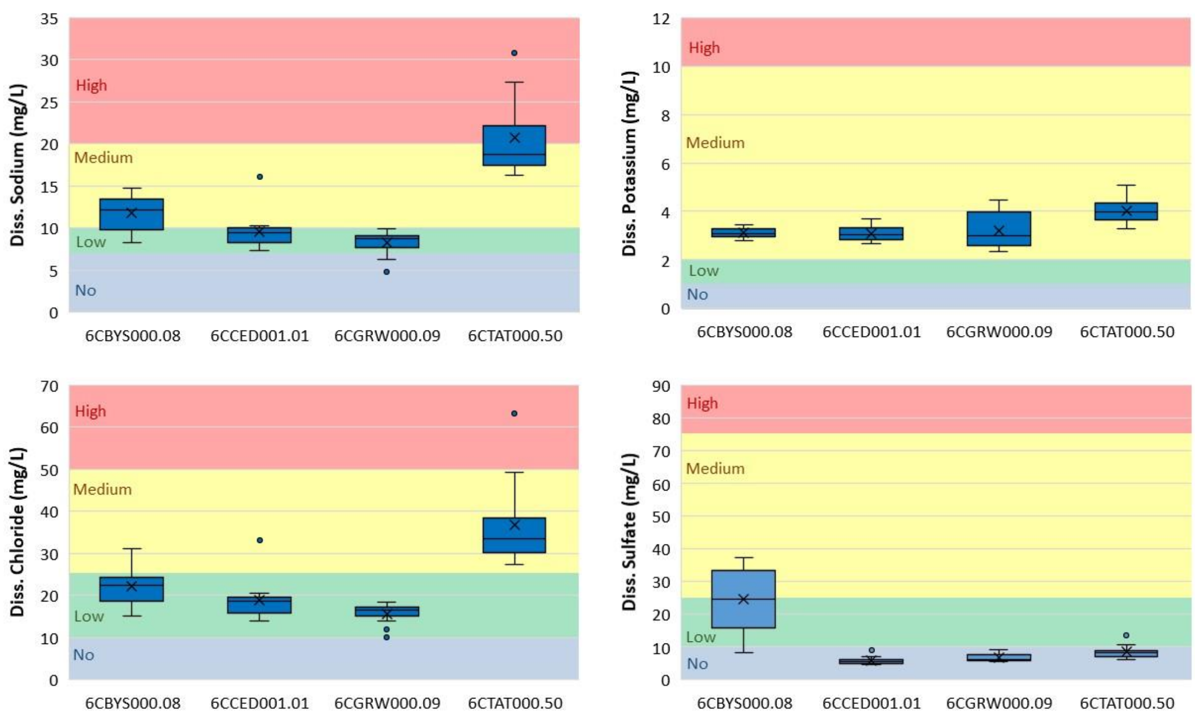

Figure 32. Dissolved ions in MF Holston tributaries. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station. Colors represent the probability that data within that range would be responsible for causing stress.

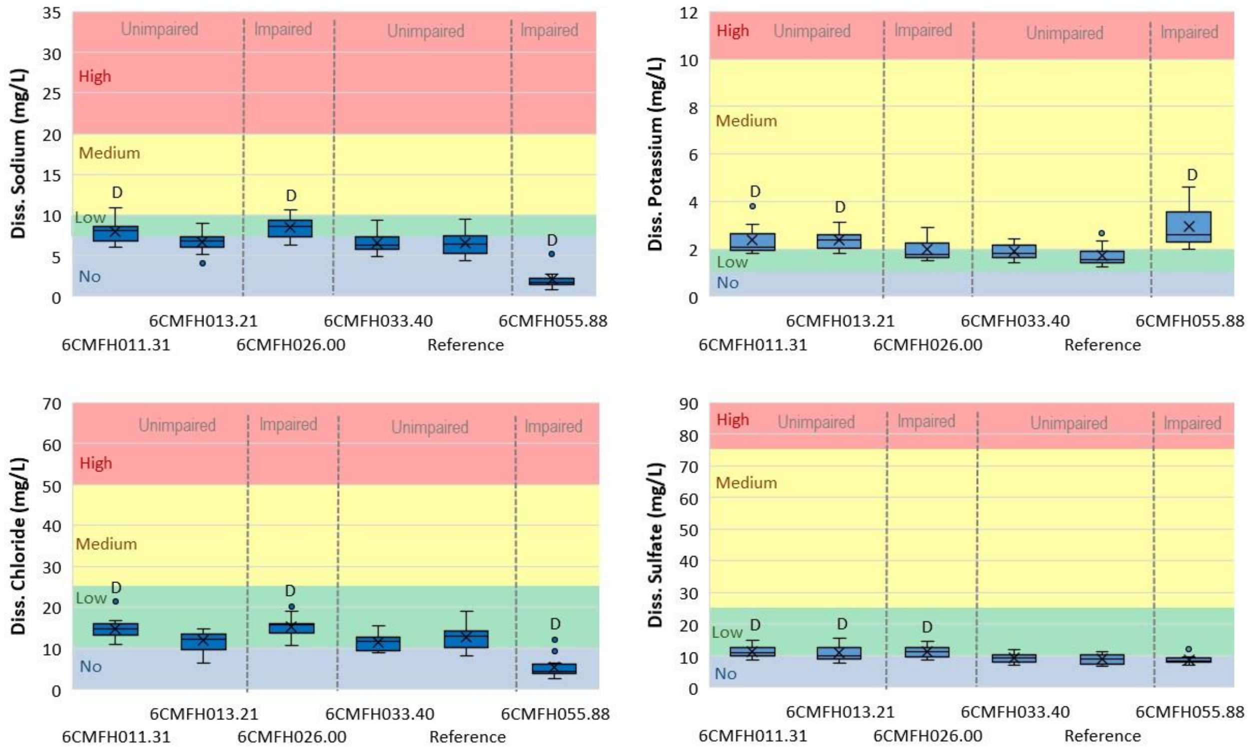

Figure 33. Dissolved ions in the MF Holston River. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station. Colors represent the probability that data within that range would be responsible for causing stress.

2.4.6. Solids

Figure 34 shows total suspended solids (TSS) measured in MF Holston tributaries. In tributaries, TSS concentrations ranged from the detection limit of 3 mg/L in several stations to 87 mg/L in Byers/Hall Creek. Average TSS values ranged from 3 mg/L in the reference to 15 mg/L in Cedar Creek. TSS in each of the impaired MF Holston tributaries was statistically different (p-value <0.05 in t-test with unequal variances) from the reference, however, the reference dataset was relatively small (6 samples) compared to the impaired streams datasets (9 to 56 samples).

Figure 35 shows TSS in the MF Holston River. TSS concentrations ranged from the detection level of 3 mg/L at several stations to 639 mg/L at 6CMFH013.21. Average TSS values ranged from 6 mg/L at 6CMFH040.67 to 37 mg/L at 6CMFH055.88. Using station 6CMFH045.72 as the reference, TSS was statistically higher (p-value <0.05 in t-test with unequal variances) at the upstream impaired station (6CMFH055.88). TSS concentrations were slightly higher in the downstream impaired reach, but differences were not statistically significant. Figure 36 shows the cumulative frequency of TSS concentrations at the impaired MF Holston stations compared to the reference station. In general, each of the impaired stations more frequently had elevated TSS concentrations. For instance, 80% of samples at the reference station were less than 4 mg/L, but the 80th percentile of TSS concentrations at the impaired stations were 16 mg/L, 9 mg/L, and 72 mg/L, at stations 6CMFH026.00, 6CMFH027.14, and 6CMFH055.88, respectively. This means that higher sediment concentrations are more frequent at these impaired stations and indicates that sediment could be a stressor in this river.

In addition to TSS concentrations, turbidity levels were measured in MF Holston tributaries (Figure 37). Among tributaries, turbidity levels ranged from 0.87 NTU in the reference to 66.6 NTU in Byers/Hall Creek. Average TSS values ranged from 2.0 NTU in the reference to 9.7 NTU in Byers/Hall Creek. Turbidity in Byers/Hall Creek and Cedar Creek was statistically different (p-value <0.05 in t-test with unequal variances) from the reference, however, the reference dataset was relatively small (6 samples) compared to the impaired streams datasets (9 to 56 samples).

Figure 38 shows turbidity in the MF Holston River. Turbidity levels ranged from 0.7 NTU at station 6CMFH033.40 to 455 NTU at station 6CMFH013.21. Average turbidity values ranged from 5.6 NTU at 6CMFH011.31 to 25.1 NTU at 6CMFH055.88. Using station 6CMFH045.72 as the reference, turbidity was statistically higher (p-value <0.05 in t-test with unequal variances) at the upstream impaired station (6CMFH055.88). Like TSS, turbidity was slightly higher in the downstream impaired reach, but differences were not statistically significant. Figure 39 shows the cumulative frequency of turbidity levels at the impaired MF Holston stations compared to the reference station. Like TSS, each of the impaired stations more frequently had elevated turbidity. For instance, 80% of samples at the reference station exhibited turbidity of less than 3.6 NTU, while the 80th percentile of turbidity at the impaired stations was 10.7 NTU, 7.4 NTU, and 42.5 NTU, at stations 6CMFH026.00, 6CMFH027.14, and 6CMFH055.88, respectively. This means that higher turbidity is more frequent at these impaired stations and indicates that sediment could be a stressor in this river.

In addition to periodic TSS and turbidity measurements, VDEQ collected diurnal turbidity data at several of the primary benthic stations during the summer of 2021. Diurnal data were collected at 15-minute intervals for 1 week in Byers/Hall Creek, Cedar Creek, and three stations in the MF

Holston River. During the diurnal deployment, turbidity average 4.61 NTU in Byers/Hall Creek, 5.13 NTU in Cedar Creek, 13.3 NTU at the upper impaired reach in the MF Holston River, 5.93 NTU at the lower impaired reach in the MF Holston River, and 1.54 NTU at the unimpaired MF Holston station (Figure 40). Turbidity was generally higher in each of the impaired streams than in the unimpaired MF Holston station. Turbidity did not exceed 3.65 NTU at the unimpaired station, but turbidity peaked as high as 55.1 NTU, 45.6 NTU, 315 NTU, and 40.7 NTU in Byers/Hall Creek, Cedar Creek, MF Holston upper impaired reach, and MF Holston lower impaired reach, respectively. Interestingly, turbidity exhibited a distinct daily pattern in the two impaired MF Holston stations and a lesser degree Byers/Hall Creek. This could be due to periodic discharges from point sources within the watershed or from livestock access to the stream, since livestock retreat to the streams during the hottest portion of the day. In the MF Holston River, diurnal turbidity patterns matched the pattern of impairment, with low levels of turbidity at the unimpaired station and higher turbidity in the upper and lower impaired reaches.

In summary, suspended solids may be a stressor in Byers/Hall Creek, Cedar Creek, Greenway Creek, and the two impaired portions of the MF Holston. Total suspended solids and turbidity were higher at these locations than at an unimpaired reference. These differences were statistically significant for each of the locations with the exception of the downstream impaired reach of the MF Holston River. In addition, the pattern of diurnal turbidity in the MF Holston River matched the spatial pattern of impairment.

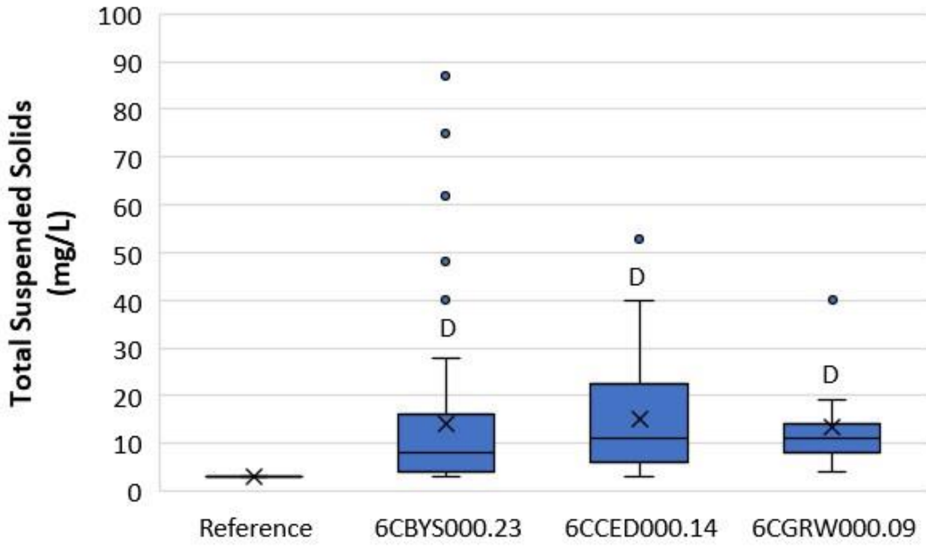

Figure 34. Total suspended solids in MF Holston tributaries. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station.

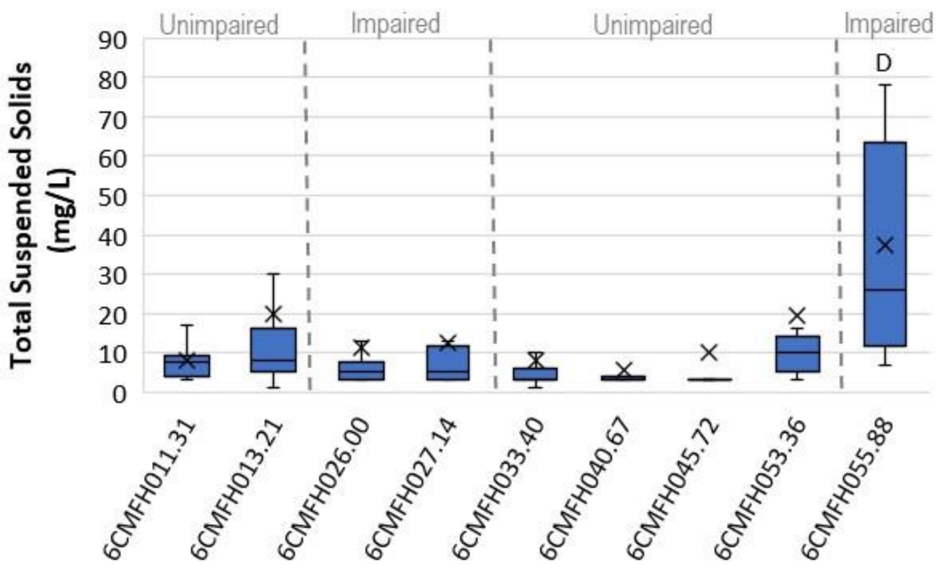

Figure 35. Total suspended solids in the MF Holston River. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Outliers are not show to decrease the scale. The "D" indicates a statistically significant difference from the reference station (6CMFH045.72).

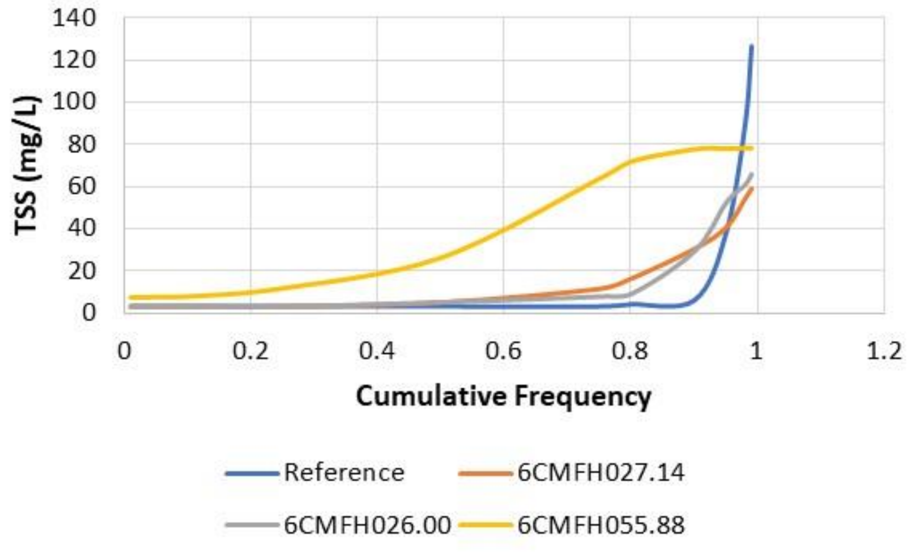

Figure 36. Cumulative frequency of TSS concentrations at MF Holston stations.

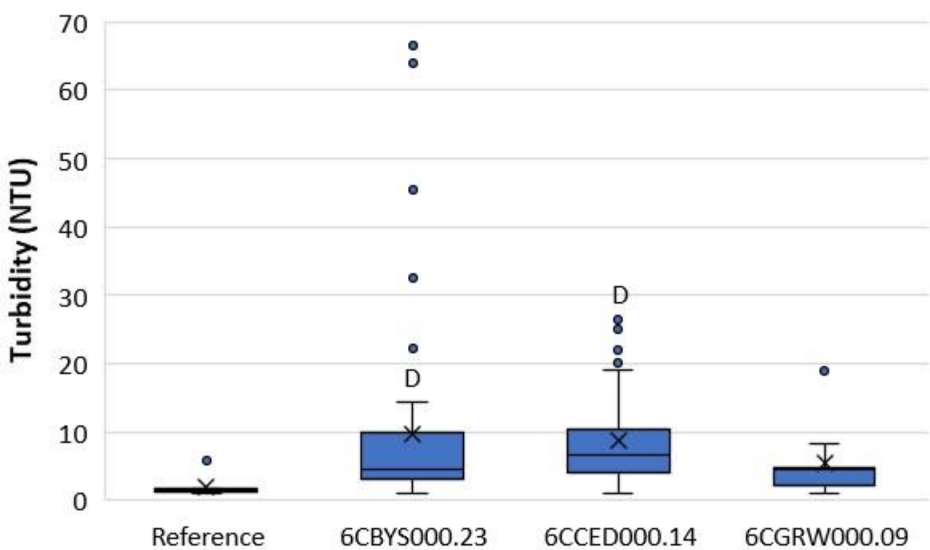

Figure 37. Turbidity in MF Holston tributaries. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station.

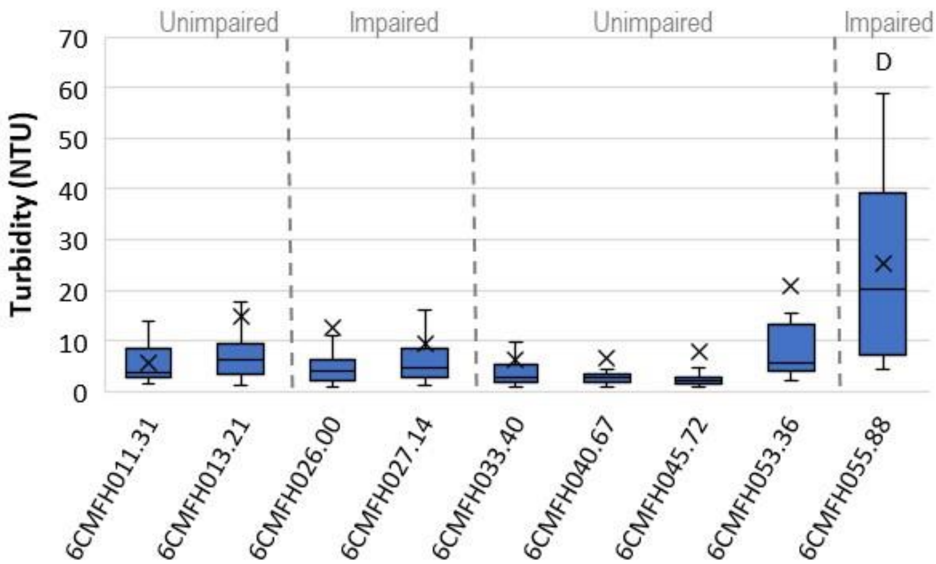

Figure 38. Turbidity in the MF Holston River. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Outliers are not show to decrease the scale. The "D" indicates a statistically significant difference from the reference station (6CMFH045.72).

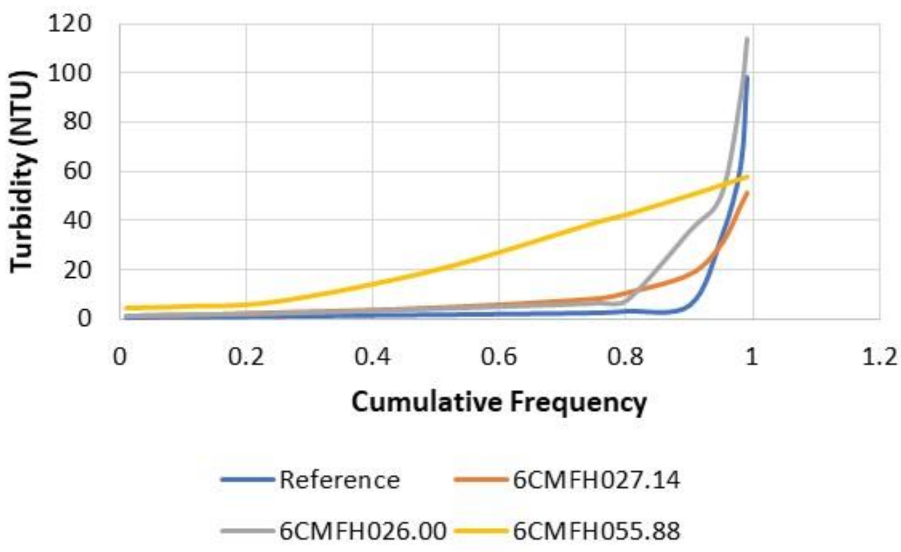

Figure 39. Cumulative frequency of turbidity at MF Holston stations.

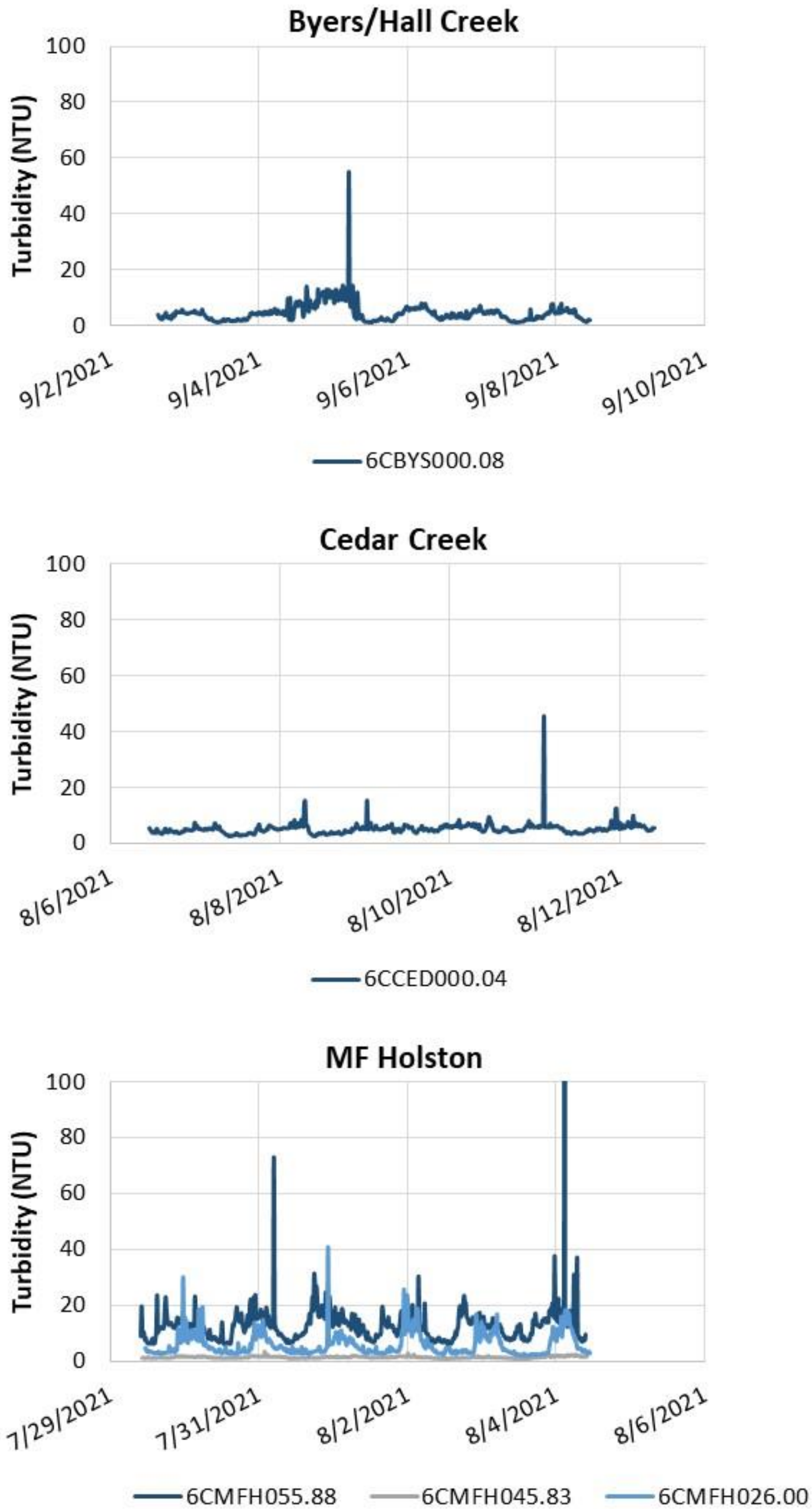

Figure 40. Diurnal turbidity in MF Holston Project streams.

2.4.7. Organic Matter

Various forms of organic matter were measured in MF Holston Project streams. The measurement of total volatile solids (TVS) captures the mass of suspended or dissolved solids in the stream that volatilizes when heated to 550°C. At this temperature, only inorganic material remains, so TVS represents the organic fraction. TVS levels in MF Holston Project streams are shown in Figure 41. TVS values averaged 68 mg/L in Byers/Hall Creek, 65 mg/L in Cedar Creek, 52 mg/L in the MF Holston lower impaired reach (6CMFH026.00), and 59 mg/L in the MF Holston upper impaired reach (6CMFH053.36). In the smaller streams (Byers/Hall Creek and Cedar Creek), TVS values were not statistically different from the small stream reference. In the MF Holston River, TVS values at stations 6CMFH013.21, 6CMFH026.00, and 6CMFH027.14 were statistically higher than the large stream reference. This could indicate that organic matter may be a stressor in the MF Holston River, however, station 6CMFH013.21, which was statistically higher than the reference, is within an unimpaired reach of the river. In addition, TVS values at these stations are much lower than at the unimpaired small stream reference.

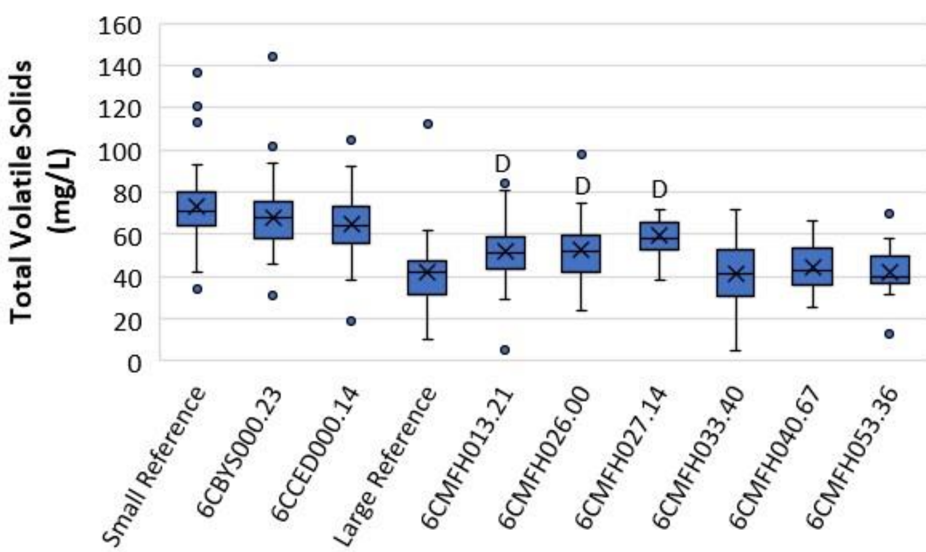

Figure 41. Total volatile solids in MF Holston Project streams. The "D" indicates a statistically significant difference from the respective small or large stream reference.

2.4.8. Nutrients - Phosphorus

Nitrogen and phosphorus are the primary nutrients of concern in freshwater. These nutrients are necessary to support healthy ecosystems, but excess nutrients can lead to eutrophication. Excess nutrients spur algae growth and can change the benthic community composition. An overabundance of algae can reduce oxygen levels, leading to further changes in community composition and eventually hypoxic conditions. The initiation of this eutrophication process is not reliant upon the total nutrient availability, but upon the availability of the limiting nutrient. The typical ratio of nitrogen to phosphorus in algae is 16:1 (Redfield, 1958) on a molar basis, or 7.2:1 on a N to P mass basis. So, ratios of nitrogen to phosphorus concentrations above 7.2 indicate that phosphorus is the limiting nutrient and ratios below 7.2 indicate that nitrogen is the limiting nutrient. In MF Holston tributaries, average nitrogen to phosphorus ratios range from 17 to 72, indicating that phosphorus is the limiting nutrient. In the MF Holston River, ratios range from 11 to 28, similarly indicating that phosphorus is the limiting nutrient.

Over time, VDEQ has measured various forms of phosphorus (total and dissolved orthophosphate, and total and dissolved phosphorus). While these various forms signal the availability of nutrients for biological uptake, total phosphorus is used in the stressor analysis to identify the potential for nutrient enrichment. Figure 42 shows the total phosphorus levels in MF Holston tributaries, and Figure 43 shows the total phosphorus levels in the MF Holston River. In MF Holston tributaries, total phosphorus averaged from 0.05 mg/L in Cedar Creek (6CCED001.01) to 0.17 mg/L in Byers/Hall Creek (6CBYS000.08). All of the MF Holston tributary stations except for 6CCED001.01 had statistically higher total phosphorus levels than the reference site (t-test with unequal variance and alpha = 0.05). In the MF Holston River, total phosphorus averaged from 0.02 mg/L at the reference location (6CMFH045.72/83) to 0.11 at station 6CMFH055.88. All stations were statistically higher in total phosphorus than the reference site (t-test with unequal variance and alpha = 0.05) except for station 6CMFH053.36.

Median total phosphorus values were in the low probability range for stressor effects in Cedar Creek, Greenway Creek, and Tattle Branch but were in the high probability range in Byers/Hall Creek. In the MF Holston River, median total phosphorus values were in the medium probability range for stressor effects at all stations except for the reference station and stations just upstream (6CMFH053.36) and downstream (6CMFH040.67) from the reference, where medians were in the no to low probability range. In the MF Holston River, the pattern of total phosphorus concentrations generally reflected the impairment status, with the highest levels occurring in the upper impaired reach and the lower impaired reach and lower levels occurring in the unimpaired reaches.

Figure 44 shows the time series of total phosphorus levels in MF Holston Project streams. All streams had individual samples above 0.1 mg/L total phosphorus and in the high probability range for stressor effects. These excursions were much more prevalent in Byers/Hall Creek and impaired sections of the MF Holston River than in the other tributaries. Excursions above 0.1 mg/L represented 55% of samples in Byers/Hall Creek, 39% of samples at 6CMFH026.00, and 32% of samples at 6CMFH055.88. All other streams had total phosphorus excursions above 0.1 mg/L less than 15% of the time.

While VDEQ does not have nutrient criteria for freshwater streams, USEPA has published recommended criteria by ecoregion (USEPA, 2000a). The MF Holston Project streams are in the Ridge and Valley Level 3 Ecoregion, and based on this designation, the recommended total phosphorus criterion based on the 25th percentile of streams is 0.01 mg/L. All of the impaired streams exceeded this recommended criterion, while the small stream reference station did not.

In summary, while all impaired streams exceeded the EPA recommended phosphorus criterion, total phosphorus concentrations were the highest in Byers/Hall Creek and in the impaired MF Holston stations. Phosphorus could be a stressor in these streams since median total phosphorus concentrations were in the high probability range for stressor effects in Byers/Hall Creek and in the medium range in the MF Holston River. Phosphorus is not likely a stressor in the other tributaries, where median values were in the low probability range for stressor effects.

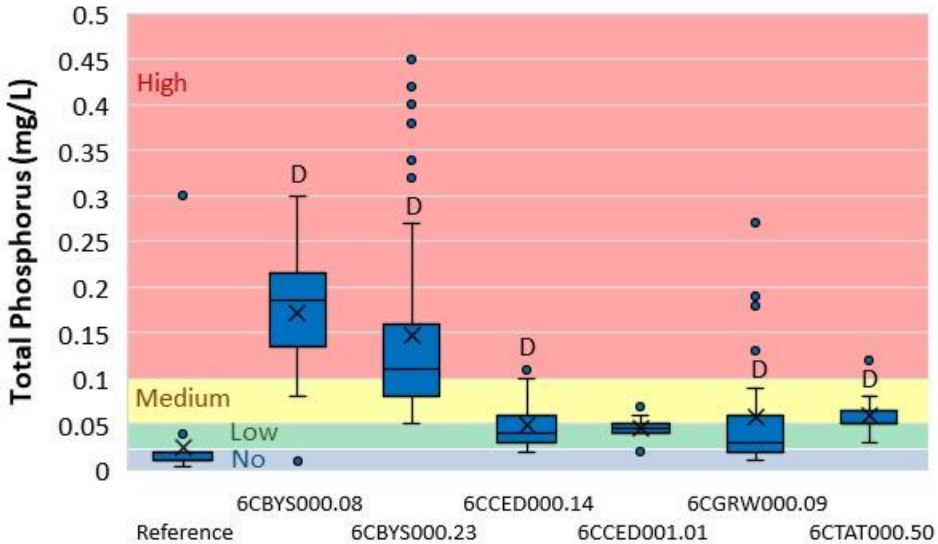

Figure 42. Total phosphorus in MF Holston tributaries. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station. Colors represent the probability that data within that range would be responsible for causing stress.

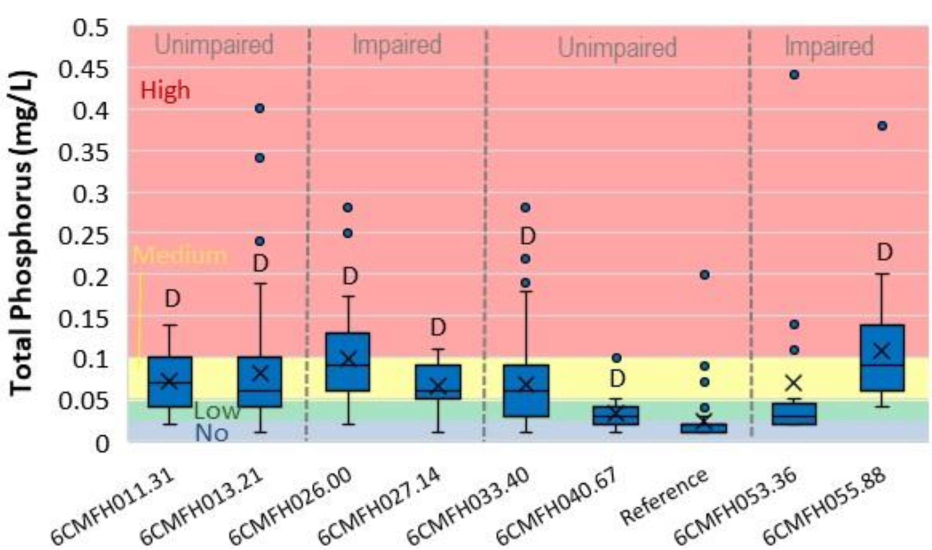

Figure 43. Total phosphorus in the MF Holston River. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station. Colors represent the probability that data within that range would be responsible for causing stress.

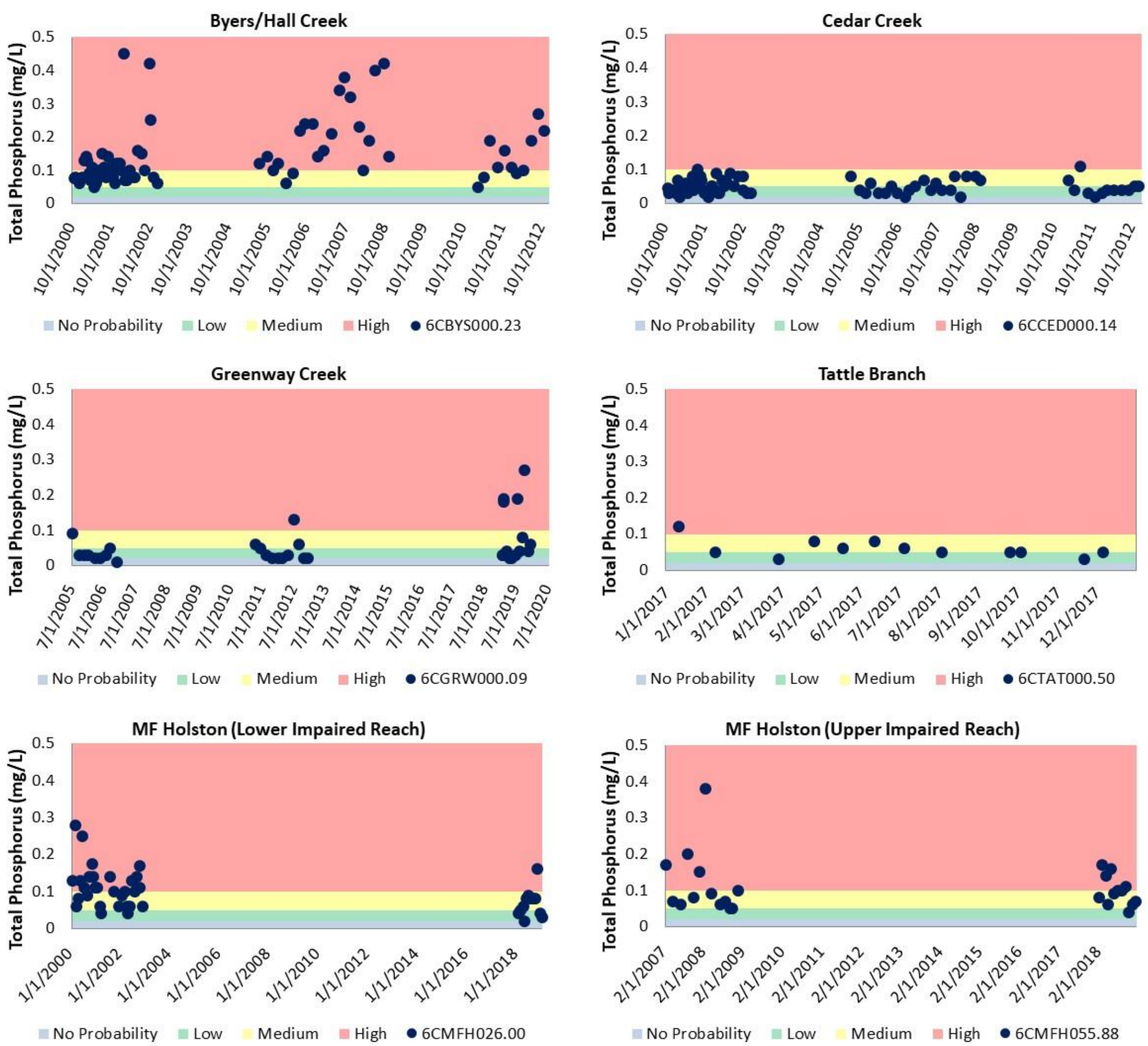

Figure 44. Total phosphorus over time in MF Holston Project streams. Colors represent the probability that data within that range would be responsible for causing stress.

2.4.9. Nutrients - Nitrogen

Over time, VDEQ has measured various forms of nitrogen (total and dissolved nitrite, total and dissolved nitrate, total and dissolved ammonia, total Kjeldahl nitrogen, and total nitrogen). While these various forms signal the availability of nutrients for biological uptake, total nitrogen is used in the stressor analysis to identify the potential for nutrient enrichment. Figure 45 and Figure 46 show the total nitrogen levels in MF Holston tributaries and the MF Holston River, respectively. In tributaries, total nitrogen averaged from 2.7 mg/L in Greenway Creek to 3.4 mg/L in Tattle

Branch. All of the tributaries were statistically higher in total nitrogen than the reference (t-test with unequal variance and alpha = 0.05), and all averaged in the high probability range for stressor effects. In the MF Holston River, total nitrogen levels were lower. Total nitrogen averaged from 0.89 mg/L at the MF Holston reference (6CMFH045.72/83) to 1.7 mg/L at the most downstream station (6CMFH011.31). All of the MF Holston stations averaged in the medium probability range for stressor effects, except for 6CMFH045.72/83 and 6CMFH040.67, which averaged in the low probability range. In general, total nitrogen increased moving downstream, with the exception of station 6CMFH055.88, which was higher in nitrogen than the next two downstream stations. The pattern of nitrogen concentrations across MF Holston stations was somewhat consistent with the location of impairments, with the central unimpaired reach having the lowest total nitrogen levels and increased nitrogen levels in the upstream and downstream impaired reaches. However, the most downstream reach of the MF Holston, which is unimpaired, had the highest total nitrogen levels.

Figure 47 shows total nitrogen concentrations over time in each of the MF Holston Project streams. Total nitrogen concentrations varied modestly over time and by season. Concentrations in MF Holston tributaries generally fell within the high probability range for stressor effects with an occasional drop into the medium probability range. Total nitrogen concentrations in the MF Holston River generally fell in the low to medium probability range, with a single excursion into the high probability range. In most streams, peak total nitrogen levels were observed in the winter to spring time frame.

While VDEQ does not have nutrient criteria for freshwater streams, USEPA has published recommended criteria by ecoregion (USEPA, 2000a). The recommended total nitrogen criterion based on the 25^{th} percentile of streams is 0.399 mg/L for the Ridge and Valley Level 3 Ecoregion. Each of the MF Holston tributaries and the MF Holston River greatly exceeded this value with averages from 0.89 to 3.4 mg/L.

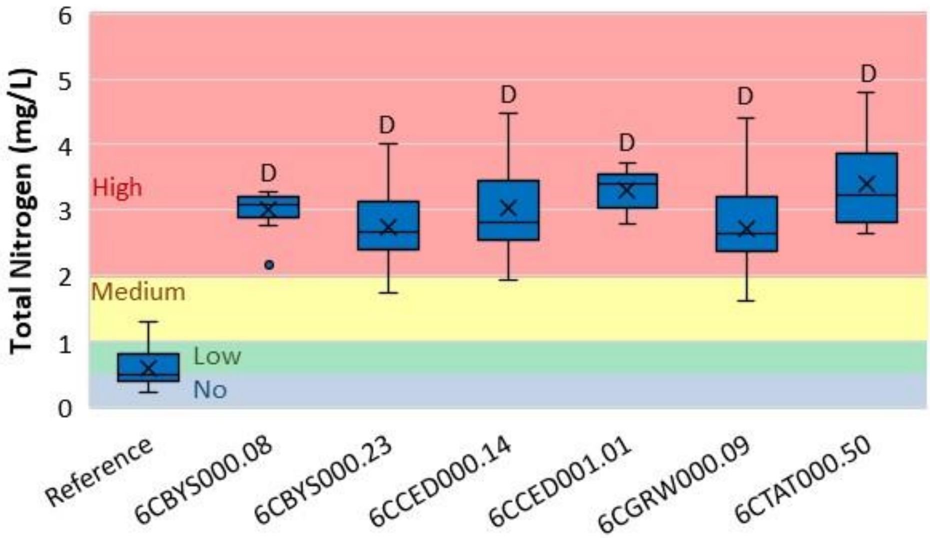

Figure 45. Total nitrogen in MF Holston tributaries. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. Colors represent the probability that data within that range would be responsible for causing stress.

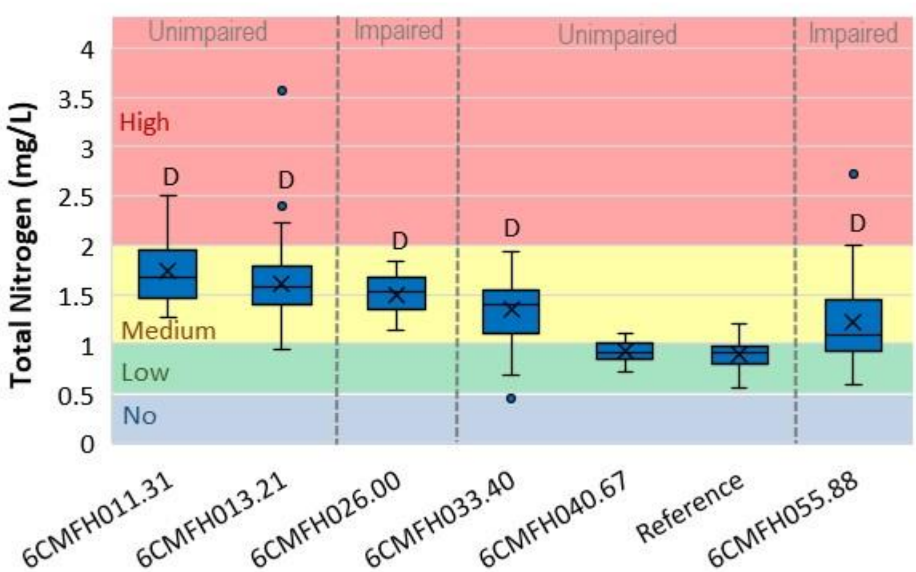

Figure 46. Total nitrogen in the MF Holston River. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represent the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. Colors represent the probability that data within that range would be responsible for causing stress.

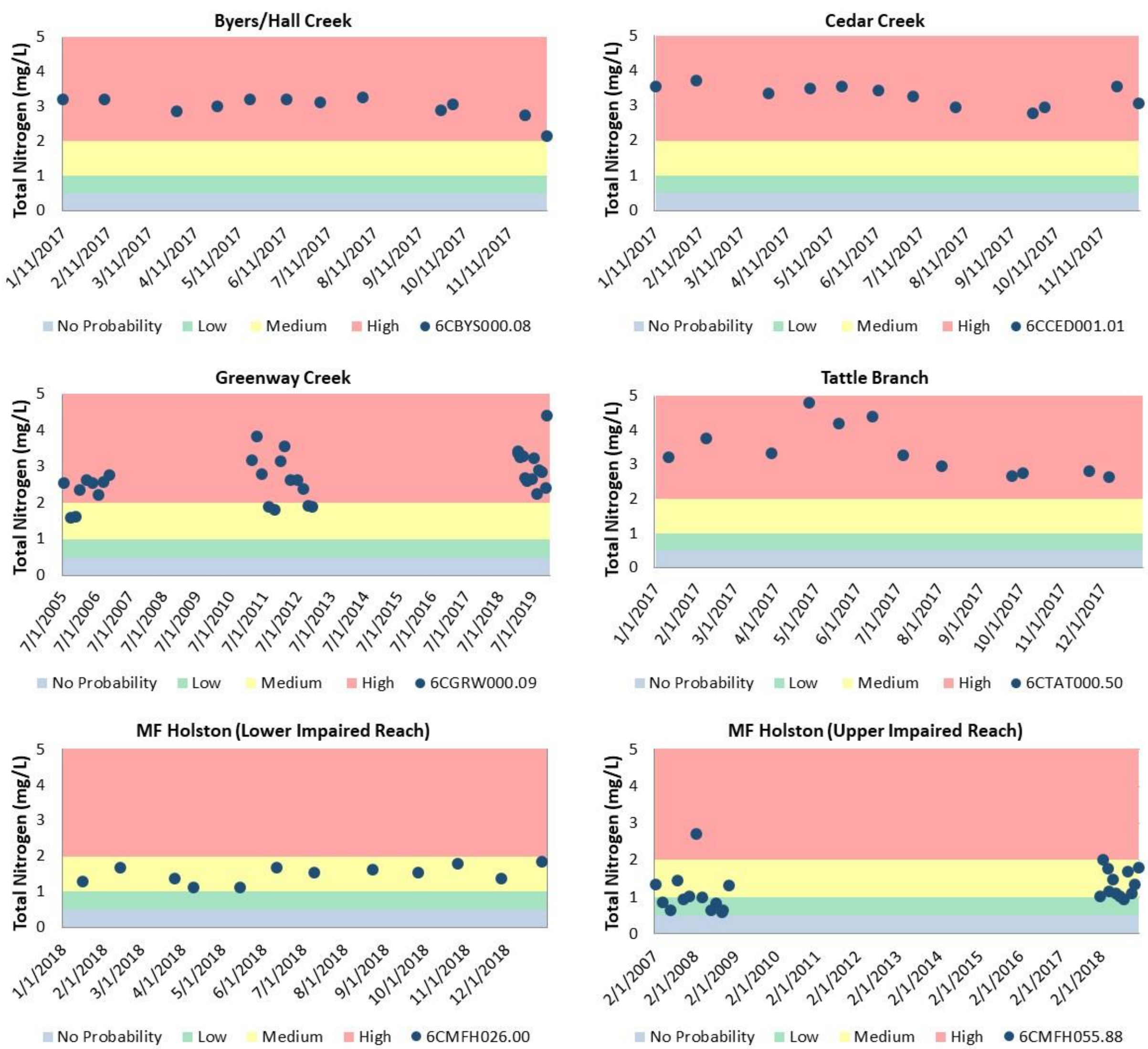

Figure 47. Total nitrogen concentration over time in MF Holston Project streams. Colors represent the probability that data within that range would be responsible for causing stress.

2.4.10. Ammonia

Ammonia is a reduced form of nitrogen that can be toxic at certain temperatures and pHs. Figure 48 shows the ammonia levels in each of the MF Holston Project streams (except for Tattle Branch) along with the relevant water quality standards. The water quality standard for ammonia is dependent upon pH and temperature, so it varies with each sample. None of the samples at any of the stations had ammonia levels above or even close to the water quality standard. Ammonia levels averaged from the detection limit of 0.04 mg/L in Byers/Hall Creek, Cedar Creek, and Greenway

Creek to 0.09 mg/L in the MF Holston lower impaired reach. In the three MF Holston tributaries with ammonia data, 93 to 100% of ammonia samples were at or below the detection limit. In the MF Holston River, 49% and 77% of ammonia samples were at or below detection within the lower and upper impaired reaches, respectively. The maximum observed ammonia level at any of the stations was 0.42 mg/L at station 6CMFH053.36. Even at this maximum level, it was well below the calculated chronic water quality criterion of 4.08 mg/L. For this reason, ammonia is not likely a stressor in any of the MF Holston Project streams.

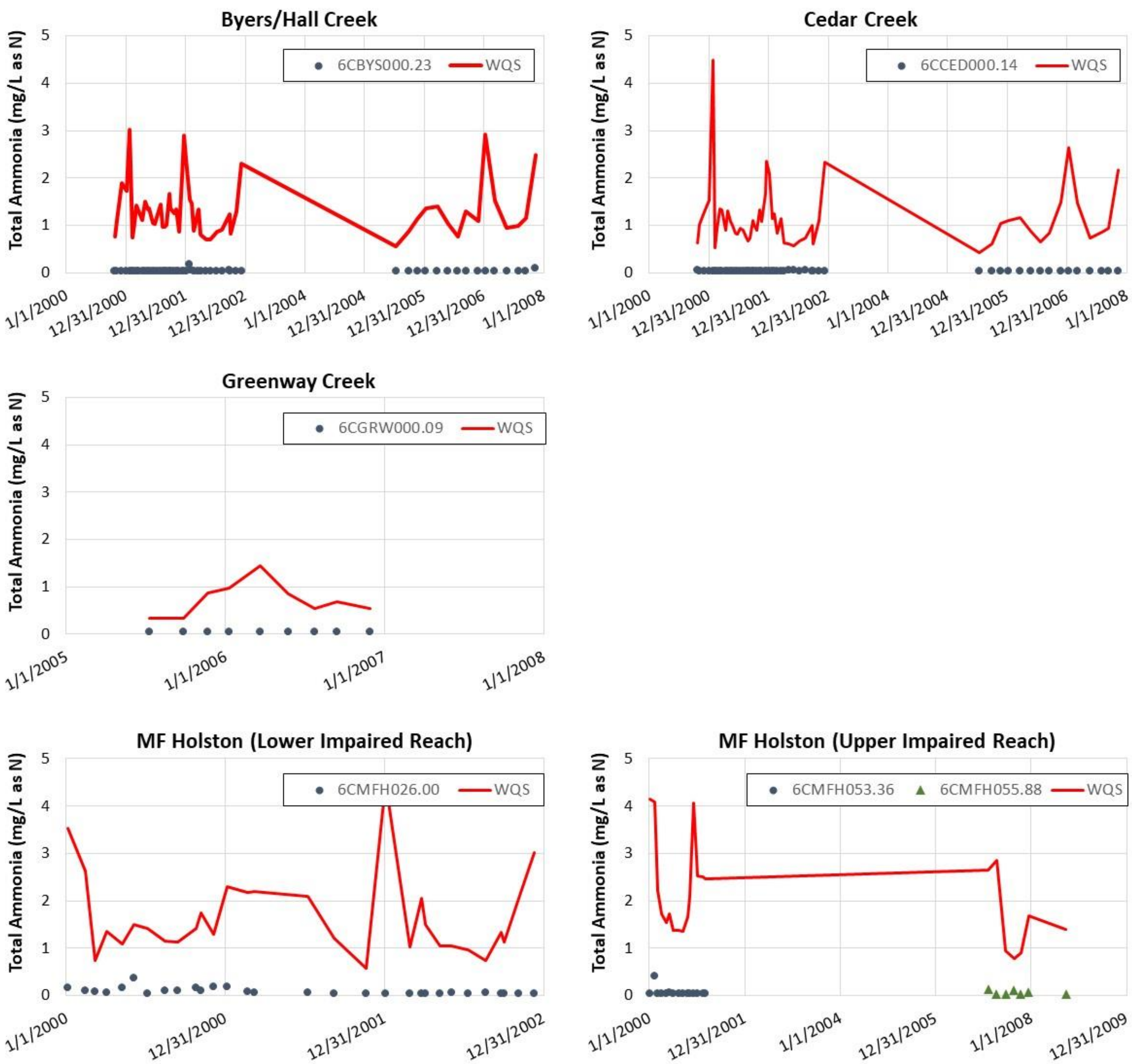

Figure 48. Ammonia levels in MF Holston Project streams. The red line represents the calculated water quality standard based on temperature and pH at the time of sampling.

2.4.11. Dissolved Metals

Dissolved metals were measured in Byers/Hall Creek, Cedar Creek, and at four MF Holston stations on at least one occasion. Table 11 shows the range and average values of eight metals in each stream along with the associated water quality standard (9VAC25-260-140). Virginia's water quality standards for dissolved metals depends upon the hardness of the water (except for arsenic and selenium), so standards were calculated specifically for each stream based on hardness values measured at the time of sampling. All average dissolved metals concentrations were below the respective water quality standards, indicating that these metals do not pose a risk to aquatic life.

For toxic metals that do not have chronic water quality criteria for aquatic life use in Virginia (aluminum, antimony, barium, beryllium, silver, and thallium), toxicity reference values (TRVs) were obtained from the literature. TRVs are threshold values below which toxic freshwater effects are not expected. Table 12 shows the range and average values of these six metals at each of the stations along with the associated TRVs. None of the stations exceeded TRVs in any of the samples, indicating that these metals are not expected to pose a risk to aquatic life.

To investigate the combined effects of dissolved metals, a criterion unit was calculated for each sample as the ratio of measured values to the chronic water quality criterion. In cases where the measured value was censored at the detection limit, half the detection limit was used for the criterion unit calculation. The criterion unit values for each of the eight metals subject to Virginia water quality standards were then summed to obtain a cumulative criterion unit (CCU) for each sampling event. The cumulative criterion unit represents the additive effect of the metals in total. A value greater than one indicates that the combined effects of the metals acting additively could be toxic. The CCUs ranged from 0.12 in Byers/Hall Creek to 0.25 at 6CMFH011.31 (Table 13). The CCU values calculated for the MF Holston Project streams fall into the range of no probability of causing stressor effects, according to VDEQ's stressor threshold analysis (VDEQ, 2017).

To investigate the combined effects of dissolved metals that do not have chronic water quality criteria for aquatic life in Virginia, a toxicity reference value (TRV) quotient was calculated for each sample as the ratio of measured values to the literature-based TRV. In cases where the measured value was censored at the detection limit, half the detection limit was used for the TRV quotient. The TRV quotient values for each of the six metals were then summed to obtain a TRV index for each sampling event. The TRV index is similar to the CCU and represents the additive effect of the metals in total. A value greater than one indicates that the combined effects of the metals acting additively could be toxic. The TRV index values ranged from 0.42 at 6CMFH033.40 to 0.47 at 6CMFH011.31 (Table 13). All of the TRV index values were below 1.0, indicating that these six dissolved metals are not likely a stressor to the benthic community.

Based on comparison to individual water quality standards, literature-based toxicity reference values, cumulative criterion units, and TRV indices, dissolved metals are not likely a stressor in Byers/Hall Creek, Cedar Creek, or the MF Holston River.

Table 11. Average dissolved metals concentrations and corresponding water quality standards for MF Holston Project streams.

Metal	Water Quality Standard[1] (ug/L)	Average (Range)[2] in ug/L					
		Byers/Hall Creek	Cedar Creek	MF Holston (6CMFH011.31)	MF Holston (6CMFH013.21)	MF Holston (6CMFH23.41)	MF Holston (6CMFH033.40)
Arsenic	150	0.56	0.84	0.69	0.49 (0.18-0.81)	0.1	0.15
Cadmium	1.2 (0.97-1.5)	0.05	0.05	0.05	0.05 (0.05-0.05)	0.05	0.05
Chromium	130 (100-170)	0.26	0.24	0.05	2.8 (0.16-5.5)	0.6	0.55
Copper	16 (13-21)	0.5	0.34	0.85	0.67 (0.5-0.84)	0.4	0.5
Lead	22 (16-31)	0.05	0.05	0.05	0.05 (0.05-0.05)	0.05	0.05
Nickel	35 (28-47)	0.28	0.26	0.55	0.42 (0.25-0.58)	0.3	0.2
Selenium	5	0.25	0.25	0.51	0.25 (0.25-0.25)	0.25	0.25
Zinc	210 (170-280)	1.6	1.7	2.5	0.58 (0.5-0.65)	1.2	1.3

[1] Water quality standards for all metals except for arsenic and selenium are hardness based, so standards varied with individual samples.

[2] No range information is given for sites with only a single metals sample.

Table 12. Average dissolved metals concentrations and corresponding toxicity reference values for MF Holston Project streams.

Metal	Toxicity Reference Value (ug/L)	Average (Range)[1] in ug/L					
		Byers/Hall Creek	Cedar Creek	MF Holston (6CMFH011.31)	MF Holston (6CMFH013.21)	MF Holston (6CMFH23.41)	MF Holston (6CMFH033.40)
Aluminum[2]	1000 (600-1300)	2.6	2	2.3	1.8 (1.2-2.5)	3.7	0.8
Antimony[3]	30	0.05	0.05	0.25	0.075 (0.05-0.1)	0.05	0.05
Barium[4]	1700	48	43	62	-	45	-
Beryllium[5]	5.3	0.05	0.05	0.05	0.025	0.05	-
Silver[3]	0.12	0.05	0.05	0.05	0.05 (0.05-0.05)	0.05	0.05
Thallium[3]	40	0.1	0.1	0.05	0.1 (0.1-0.1)	0.1	0.1

[1] No range information is given for sites with only a single metals sample.
[2] Toxicity reference value was based on pH, hardness, and dissolved organic carbon as specified in USEPA, 2018b.
[3] Toxicity reference value from USEPA, 1987.
[4] Toxicity reference value from Golding et al., 2018.
[5] Toxicity reference value from USEPA, 1980.

Table 13. Cumulative criterion units and toxicity reference value index scores for dissolved metals in MF Holston Project streams.

Stream	Station	Date	CCU[1]	TRV Index[2]
Byers/Hall Creek	6CBYS000.23	10/1/2002	0.12	0.46
Cedar Creek	6CCED000.14	10/1/2002	0.13	0.46
MF Holston	6CMFH011.31	7/1/2008	0.25	0.47
	6CMFH013.21	10/3/2000	0.19	0.42
	6CMFH013.21	6/3/2002	0.17	0.43
	6CMFH023.41	3/21/2005	0.16	0.46
	6CMFH033.40	10/3/2000	0.16	0.42

[1] Cumulative criterion unit (CCU) is the sum of the dissolved metal concentration to water quality standard ratio for each metal. Values in blue are in the no probability range of stressor effects.
[2] Toxicity reference value (TRV) index is the sum of the dissolved metal concentration to toxic threshold value ratio for each metal.

2.4.12. Sediment Toxics - PAHs

Polycyclic aromatic hydrocarbons (PAHs) are a class of chemicals that occur naturally in coal, oil, and gasoline and can be generated when organic fuels are burned. PAHs in the aquatic environment are commonly associated with oil or fuel leaks or spills, but PAHs can also be elevated in urban areas from the runoff of deposited fossil fuel combustion byproducts. Many PAH compounds are toxic and can adversely impact benthic aquatic communities when they build up in sediments. PAHs were analyzed in sediments collected from the MF Holston River (6CMFH023.41) on 3/21/2005. A total of 17 PAH compounds were analyzed, and 11 were below detection. For those that were above detection limits, none exceeded threshold effect concentrations (TEC) or probable effect concentrations (PEC) reported by MacDonald *et al.* (2000). As a result, PAHs are likely a non-stressor in the MF Holston River.

2.4.13. Sediment Toxics - PCBs

Polychlorinated biphenyls (PCBs) are a group of man-made chlorinated organic compounds that were widely used in electrical equipment and other applications from the 1930s to 1970s. While their manufacturing has been banned in the US for decades, these compounds are extremely persistent in the environment and can continue to produce toxicity in aquatic sediments. PCBs were analyzed in sediments collected from the MF Holston River (6CMFH023.41) on 3/21/2005. A total of 121 individual or co-eluting PCB congeners were analyzed, and all but 4 were below detection. PCBs 105, 132+153, 135+144, and 180+193 were measured at 0.056, 0.205, 0.343, and 0.077 ug/kg, respectively. These values summed with other congener values are well below the probable effect concentration (PEC) of 676 ug/kg reported by MacDonald *et al.* (2000), so PCBs are likely a non-stressor in the MF Holston River.

2.4.14. Sediment Toxics - Pesticides

A total of 82 different pesticide compounds were analyzed in the sediments of the MF Holston at station 6CMFH023.41 on 3/21/2005. None of these compounds were found at levels above the detection limit. While this is only a single sample at a single location, it suggests that toxic pesticides in the sediments are a non-stressor.

2.4.15. Sediment Toxics - Metals

A total of 16 metals were measured in the sediments of Byers/Hall Creek, Cedar Creek, and the MF Holston River. Levels of the eight more toxic metals were compared to threshold effect concentrations (TECs) and probable effect concentrations (PECs) from MacDonald *et al.* (2000).

TECs are levels below which toxic effects are unlikely, and PECs are levels above which toxic effects are likely. Selected metals with published effect thresholds are shown in Table 14. All metal concentrations in sediments of all streams were below both the TEC and PEC values. This indicates that sediment metals are likely a non-stressor in these streams.

Table 14. Metals concentrations in sediments from MF Holston Project streams.

Metal	TEC[1] (mg/kg)	PEC[2] (mg/kg)	Concentration (mg/kg)		
			Byers/Hall Creek (6CBYS000.23)	Cedar Creek (6CCED000.14)	MF Holston (6CMFH023.41)
Arsenic	9.79	33	<5	<5	<5
Cadmium	0.99	4.98	<1	<1	<1
Chromium	43.4	111	14.6	17.9	15.3
Copper	31.6	149	7.4	5.6	10.5
Lead	35.8	128	14.1	12.2	17.9
Mercury	0.18	1.06	<0.1	<0.1	<0.1
Nickel	22.7	48.6	6.9	6.6	11.8
Zinc	121	459	46.5	34.8	74.3

[1] TEC is the consensus-based Threshold Effect Concentration from MacDonald *et al*., 2000.
[2] PEC is the consensus-based Probable Effect Concentration from MacDonald *et al*., 2000.

2.4.1. Water Quality Regressions

To investigate the potential role of various water quality parameters impacting the benthic macroinvertebrate community, SCI scores at each station were regressed against water quality parameter values at those sites. Table 15 shows the results of these regressions ordered from most significant to least significant. The only parameters that exhibited a statistically significant regression were habitat and conductivity. As habitat scores increased across stations, benthic scores increased, and as conductivity increased, benthic scores decreased. The regression relationship between habitat and benthic health was highly significant (p=0.002) with an r^2 of 0.55. This indicates that roughly half of the variability in benthic scores can be explained by habitat scores. The regression relationship between conductivity and benthic health was also significant (p=0.02) but slightly weaker, with an r^2 of 0.45. No other regressions of SCI scores with water quality parameters were statistically significant.

Table 15. Regression relationship between water quality parameters and stream condition index (SCI) scores.

Parameter	Regression Significant (Y/N)	r^2	p-value
Habitat	Y	0.55	0.002
Conductivity	Y	0.45	0.02
Nitrogen	N	0.31	0.06
Total Dissolved Solids	N	0.29	0.07
Dissolved Oxygen	N	0.27	0.08
Temperature	N	0.19	0.15
pH	N	0.17	0.18
Total Volatile Solids	N	0.58	0.24
Total Suspended Solids	N	0.18	0.26
Chloride	N	0.14	0.32
Turbidity	N	0.14	0.32
Potassium	N	0.07	0.46
Sodium	N	0.04	0.59
Phosphorus	N	0.01	0.75
Sulfate	N	0.006	0.85
Ammonia	N	0.04	0.87

3.0 CAUSAL ANALYSIS

JMU conducted this stressor identification analysis according to EPA's Stressor Identification Guidance Document (USEPA, 2000b) using the Causal Analysis/Diagnosis Decision Information System (CADDIS) (USEPA, 2018a). The CADDIS approach provides guidance on evaluating various lines of evidence to determine the cause of biological impairments. In the case of the MF Holston Project, JMU 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 in each of the impaired streams. Table 16 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 laboratory experiments. The majority of the lines of evidence, however, were investigated for this project.

Table 16. 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 temporal trends in benthic data
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.	NA
Temporal Sequence	The cause must precede the biological effect.	Analysis of temporal trends in benthic data
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, 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.	Confirmation through literature

Multiple Types of Evidence		
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

For each impairment and for each potential candidate cause, the applicable lines of evidence were evaluated. For each line of evidence, the candidate cause was scored on a 3-point positive and negative scale (Table 17). This scale represents the strength of the evidence for or against each candidate cause. A weight of evidence approach was then used to sum the respective scores and classify candidate causes as either non-stressors, possible stressors, or probable stressors. If the summed scores for candidate causes were ≤0, the cause was classified as a non-stressor. If scores were 1-3, the cause was classified as a possible stressor. If scores were >3, the cause was classified as a probable stressor (Table 18).

Table 17. 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 18. Scheme for classifying candidate causes based on causal analysis.

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

3.1. Temperature

Table 19 shows the causal analysis results for temperature across MF Holston Project streams. Total causal analysis scores ranged from -13 to -18, indicating that there is strong evidence that temperature is a non-stressor in these streams. No violations of the temperature standard were observed at any of the benthic monitoring stations even during summertime diurnal monitoring, when critical conditions should be observed. For this reasons and others explained in Table 19, temperature was categorized as a non-stressor.

Table 19. Causal analysis results for temperature as a stressor.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	-3	-3	-3	-3	-3	-3	In each impaired stream, SCI scores were impaired, but temperature values were within water quality standards.
Temporal Co-occurrence	-3	-3	-3	-3	-3	-3	At the time of benthic sample collection, temperature at all sites met water quality standards.
Stressor-Response Relationships from the Field	-3	-3	-3	-3	-3	-3	Temperature was not significantly correlated with benthic health across sites.
Temporal Sequence	-3	-1	0	-3	-2	1	Temperatures are generally highest in the late summer, however, fall benthic scores were higher than spring scores in all streams except for Greenway Creek and the MF Holston upper impaired reach.

Stressor-Response Relationships from Laboratory Studies	-3	-3	-3	-3	-3	-3	All temperature values were within water quality standards.
Consistency of Evidence	-3	-3	-3	-3	-3	-2	Evidence consistently refuted temperature as a stressor.
Sum	**-18**	**-16**	**-15**	**-18**	**-17**	**-13**	

3.2. pH

Table 20 shows the causal analysis results for pH across MF Holston Project streams. Total causal analysis scores ranged from -25 to -21, indicating that there is strong evidence that pH is a non-stressor in these streams. All pH values in all streams were within water quality standards and were in the low probability range for stressor effects. For these reasons and others explained in Table 20, pH was categorized as a non-stressor.

Table 20. Causal analysis results for pH as a stressor.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	-3	-3	-3	-3	-3	-3	In all streams, SCI scores were impaired, but pH values were within water quality standards and in the low probability range for stressor effects
Temporal Co-occurrence	-3	-3	-3	-3	-3	-3	At the time of benthic sample collection, pH at all sites met water quality standards.
Causal Pathway	-2	-2	0	0	-2	-2	Alkalinity in Byers/Hall Creek, Cedar Creek, and MF Holston is relatively high, averaging from 99 to 234 mg/L as $CaCO_3$, so pH changes are well-buffered. No alkalinity data were available for Greenway Creek and Tattle Branch.
Stressor-Response Relationships from the Field	-3	-3	-3	-3	-3	-3	pH was not significantly correlated with benthic health across sites.
Temporal Sequence	-3	-1	-1	-3	-1	-3	Spring SCI scores were lower than fall scores in Byers/Hall Creek, Tattle Branch, and MF Holston lower impaired reach, however, no seasonal trend in pH was observed. pH values were consistently in the low probability range for stress effects. In the MF Holston upper impaired reach fall SCI scores were lower than spring scores, but no seasonal trend in pH was

							observed. In all other streams, distinct seasonal trends were not identified in SCI scores or pH.
Symptoms	-2	-2	-2	-2	-2	-2	Biological Condition Gradient analysis did not identify any of the top predominant taxa as increasing in abundance in the presence of acidity (score of 5).
Stressor-Response Relationships from Other Field Studies	-3	-3	-3	-3	-3	-3	All pH values were in the low probability range for stress effects.
Stressor-Response Relationships from Laboratory Studies	-3	-3	-3	-3	-3	-3	All pH values were within water quality standards.
Consistency of Evidence	-3	-3	-3	-3	-3	-3	Evidence consistently refuted pH as a stressor.
Sum	**-25**	**-23**	**-21**	**-23**	**-23**	**-25**	

3.3. Dissolved Oxygen

Table 21 shows the causal analysis results for dissolved oxygen across MF Holston Project streams. Total causal analysis scores ranged from -20 to -11, indicating that there is strong evidence that DO is a non-stressor in these streams. All DO values in all streams were above water quality standards, and median DO values were in the no to low probability range for stressor effects. For these reasons and others explained in Table 21, DO was categorized as a non-stressor.

Table 21. Causal analysis results for dissolved oxygen as a stressor.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	-2	-2	-1	-1	1	-2	In each of the impaired streams, SCI scores were impaired, but DO values averaged in the no to low probability range for stress effects. During diurnal monitoring in the warm summer months, however, nighttime DO barely dropped into the high probability range for stress effects in Greenway Creek and Tattle Branch and consistently dropped into the high probability range in the MF Holston lower impaired reach.
Temporal Co-occurrence	-2	1	-2	-2	-2	-2	At the time of benthic sample collection, DO met water quality standards and was in the no to low probability range for stress effects in all streams, except for Cedar Creek, where one occasion was in the medium probability range.

Causal Pathway	2	-2	-2	-2	1	1	Median nutrient levels (total phosphorus) were in the high probability range for stressor effects in Byers/Hall Creek and medium range in the MF Holston, so the causal pathway from nutrient enrichment to low DO is intact in these streams. In all other streams, median phosphorus levels were in the no to low probability range, so the causal pathway from nutrient enrichment to low DO is not intact.
Stressor-Response Relationships from the Field	-3	-3	-3	-3	-3	-3	DO was not significantly correlated with benthic health across sites.
Temporal Sequence	-3	-1	0	-3	-2	1	DO is generally lowest in the late summer, however, fall benthic scores were higher than spring scores in all streams except for Greenway Creek and the MF Holston upper impaired reach.
Symptoms	-3	-3	-3	-3	-3	-3	Biological Condition Gradient analysis did not identify any of the top predominant taxa as increasing in abundance in the presence of low DO (score of 5), and DO was the lowest ranked stressor.
Stressor-Response Relationships from Other Field Studies	-2	-2	-1	-1	1	-2	Median DO values were in the no to low probability range for stress effects in each of the impaired streams. During diurnal monitoring in the warm summer months, however, nighttime DO barely dropped into the high probability range for stress effects in Greenway Creek and Tattle Branch and consistently dropped into the high probability range in the MF Holston lower impaired reach.
Stressor-Response Relationships from Laboratory Studies	-3	-3	-3	-3	-3	-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	-2	-2	-2	-2	-1	-2	Most evidence refuted DO as a stressor.
Sum	**-18**	**-17**	**-17**	**-20**	**-11**	**-15**	

3.4. Conductivity and Total Dissolved Solids

Table 22 shows the causal analysis results for conductivity and total dissolved solids across MF Holston Project streams. Total causal analysis scores ranged from -14 to -12 in the MF Holston River, indicating that there is strong evidence that conductivity is a non-stressor in this stream. In MF Holston tributaries, however, total causal analysis scores ranged from 1 to 3, indicating that conductivity is a possible stressor. Median conductivity values were in the no to low probability range for stressor effects in the MF Holston River but were in the high probability range in Byers/Hall Creek, Tattle Branch, and two Cedar Creek stations. In Greenway Creek and one Cedar Creek station, median conductivity values were in the medium probability range for stressor effects. As an analogous stressor, total dissolved solids showed similar results. Lastly, conductivity was also significantly correlated with benthic SCI scores across sites. For these reasons and others explained in Table 22, conductivity and total dissolved solids were categorized as non-stressors in the MF Holston River and possible stressors in Byers/Hall Creek, Cedar Creek, Greenway Creek, and Tattle Branch.

Table 22. Causal analysis results for conductivity and dissolved solids.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	3	2	2	3	-2	-2	In Byers/Hall Creek and Tattle Branch, median conductivity levels were in the high probability range for stressor effects. In Cedar Creek, median conductivity levels were in the medium to high range. In Greenway Creek, median conductivity levels were in the medium range. In the MF Holston and at unimpaired stations, median conductivity levels were in the no to low probability range for stressor effects.
Temporal Co-occurrence	-1	1	-1	-1	-2	-2	At the time of impaired benthic sample collections, conductivity levels were in the high probability range for stress effects 100% of the time in Greenway Creek and Tattle Branch, 60% of the time in Cedar Creek, 40% of the time in Byers/Hall Creek, and 0% of the time in the MF Holston. However, SCI scores above 60 were reported in Byers/Hall Creek, Greenway Creek, and Tattle Branch when conductivity levels were in the high probability range for stressor effects.
Stressor-Response Relationships from the Field	2	2	2	2	2	2	Conductivity was significantly correlated with SCI scores across sites.
Temporal Sequence	-3	-2	-2	-3	-3	-1	Spring SCI scores were lower than fall scores in Byers/Hall Creek, Tattle Branch, and MF Holston lower impaired reach, however, conductivity was generally higher in the fall after the dry summer months. In the MF Holston upper impaired reach, fall SCI scores were lower than spring scores, but conductivity levels were generally low at this location. In all other streams, distinct seasonal trends were not identified in SCI scores, while conductivity was generally higher in the fall.
Symptoms	-3	-3	-3	-3	-3	-3	Biological Condition Gradient analysis did not identify any predominant taxa that increase in abundance in the presence of high conductivity (score of 5), and conductivity did not rank as the top stressor in any of the streams.

Stressor-Response Relationships from Other Field Studies	3	2	2	3	-2	-2	In Byers/Hall Creek and Tattle Branch, median conductivity levels were in the high probability range for stressor effects. In Cedar Creek, median conductivity levels were in the medium to high range. In Greenway Creek, median conductivity levels were in the medium range. In the MF Holston, median conductivity levels were in the no to low probability range for stressor effects.
Analogous Stressors	1	1	1	2	-2	-2	The analogous stressor of total dissolved solids (TDS) was in the medium probability range for stress effects in MF Holston tributaries and low probability range in the MF Holston.
Consistency of Evidence	0	0	0	0	-2	-2	Evidence strongly refutes conductivity as a stressor in the MF Holston, but evidence is inconsistent in MF Holston tributaries.
Sum	**2**	**3**	**1**	**3**	**-14**	**-12**	

3.5. Dissolved Ions

3.5.1. Sodium

Table 23 shows the causal analysis results for dissolved sodium across MF Holston Project streams. In all of the streams except Tattle Branch, total causal analysis scores ranged from -17 to 0, indicating that there is moderate to strong evidence that dissolved sodium is a non-stressor in these streams. In Tattle Branch, the total causal analysis score was +3, indicating that dissolved sodium is a possible stressor. Average sodium levels were in the high probability range for stressor effects in Tattle Branch, medium probability range in Byers/Hall Creek, and no to low probability range for all other streams. While sodium levels were elevated in Tattle Branch, the levels were still well below toxic thresholds reported by Mount *et al.* (2016). For this reason and others explained in Table 23, dissolved sodium was categorized as a possible stressor in Tattle Branch. Dissolved sodium was categorized as a non-stressor in all other streams.

Table 23. Causal analysis results for dissolved sodium.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	1	-1	-2	2	-2	-3	In Tattle Branch, dissolved sodium values at impaired stations averaged in the high probability range for stressor effects. In Byers/Hall Creek, dissolved sodium averaged in the medium

							probability range. In all other impaired streams, dissolved sodium levels were in the no to low probability range for stressor effects.
Temporal Co-occurrence	1	0	-1	2	-1	-1	At or around the time of benthic sampling, dissolved sodium levels were in the medium probability range for stressor effects in Tattle Branch, low to medium range in Byers/Hall Creek, and low range in Greenway Creek and the MF Holston.
Stressor-Response Relationships from the Field	-3	-3	-3	-3	-3	-3	Dissolved sodium was not significantly correlated with benthic health across sites.
Temporal Sequence	-1	-1	-1	-1	-1	-1	Seasonal trends of lower spring benthic scores did not appear to correlate with the timing of high sodium levels.
Stressor-Response Relationships from Other Field Studies	1	-1	-2	2	-2	-3	Median dissolved sodium values were in the medium probability range for stressor effects in Byers/Hall Creek and Tattle Branch. In all other impaired streams, median dissolved sodium levels were in the no to low probability range for stressor effects.
Stressor-Response Relationships from Laboratory Studies	-2	-2	-2	-2	-2	-2	In an analysis of toxicity to major ions (Mount et al., 2016), all LC50s for Ceriodaphnia exposed to sodium salts were well above the sodium levels in MF Holston project streams.
Analogous Stressors	3	2	2	3	-2	-2	The analogous stressor of conductivity was in the high probability range for stressor effects in Byers/Hall Creek and Tattle Branch, medium to high range in Cedar Creek, medium range in Greenway Creek, and no to low probability range for stressor effects in the MF Holston.
Consistency of Evidence	0	-1	-1	0	-2	-2	Evidence strongly refutes sodium as a stressor in the MF Holston and to a lesser degree Greenway Creek and Cedar Creek, but evidence is inconsistent in Byers/Hall Creek and Tattle Branch.
Sum	**0**	**-7**	**-10**	**3**	**-15**	**-17**	

3.5.2. Potassium

Table 24 shows the causal analysis results for dissolved potassium across MF Holston Project streams. Total causal analysis scores ranged from -10 to 0 in Cedar Creek and the MF Holston River, indicating that there is weak to moderate evidence that dissolved potassium is a non-stressor in these streams. In Byers/Hall Creek, Greenway Creek, and Tattle Branch, however, total causal analysis scores ranged from 2 to 3, indicating that dissolved potassium is a possible stressor. Median dissolved potassium values were in the no to low probability range for stressor effects in the MF Holston, but were in the medium probability range in MF Holston tributaries. While tributary dissolved potassium values were elevated, concentrations were still well below toxic thresholds reported by Mount *et al.* (2016). For these reasons and others explained in Table 24, dissolved potassium was categorized as a non-stressor in Cedar Creek and the MF Holston River and a possible stressor in Byers/Hall Creek, Greenway Creek, and Tattle Branch.

Table 24. Causal analysis results for dissolved potassium.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	2	2	2	2	-1	2	In the MF Holston lower impaired reach, median dissolved potassium values were in the low probability range for stressor effects. In all other impaired streams, median dissolved potassium levels were in the medium probability range for stressor effects.
Temporal Co-occurrence	2	0	2	2	1	2	At or around the time of benthic sampling, dissolved potassium levels were in the low to medium probability range for stressor effects in the MF Holston lower impaired reach and medium probability range for all other streams, except Cedar Creek (where no potassium data was collected at or around the time of benthic sampling).
Stressor-Response Relationships from the Field	-3	-3	-3	-3	-3	-3	Dissolved potassium was not significantly correlated with benthic health across sites.
Temporal Sequence	-1	-1	-1	-1	-1	-1	Seasonal trends of lower spring benthic scores did not appear to correlate with the timing of high potassium levels.
Stressor-Response Relationships from Other Field Studies	2	2	2	2	-1	2	Median dissolved potassium levels were in the low probability range for stressor effects in the MF Holston lower impaired reach and in the medium probability range for stressor effects in all other MF Holston project streams.
Stressor-Response Relationships from Laboratory Studies	-2	-2	-2	-2	-2	-2	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 MF Holston project streams.
Analogous Stressors	3	2	2	3	-2	-2	The analogous stressor of conductivity was in the high probability range for stressor effects in Byers/Hall Creek and Tattle Branch, medium to high range in Cedar Creek, medium range in Greenway Creek, and no to low probability range for stressor effects in the MF Holston.
Consistency of Evidence	0	0	0	0	-1	0	Evidence moderately refutes potassium as a stressor in the MF Holston lower impaired reach, but evidence was inconsistent in other MF Holston project streams.
Sum	**3**	**0**	**2**	**3**	**-10**	**-2**	

3.5.3. Chloride

Table 25 shows the causal analysis results for dissolved chloride across MF Holston Project streams. In all of the streams except Tattle Branch, total causal analysis scores ranged from -21 to -12, indicating that there is strong evidence that dissolved chloride is a non-stressor in these streams. In Tattle Branch, the total causal analysis score was +1, indicating that dissolved chloride is a possible stressor. Median chloride levels were in the medium probability range for stressor effects in Tattle Branch, but were in the no to low probability range for all other streams. While chloride levels were elevated in Tattle Branch, the levels were still well below the water quality standard of 230 mg/L. For this reason and others explained in Table 25, dissolved chloride was categorized as a possible stressor in Tattle Branch and a non-stressor in all other streams.

Table 25. Causal analysis results for dissolved chloride.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	-1	-1	-2	2	-2	-3	In Tattle Branch, median dissolved chloride values at impaired stations were in the medium probability range for stressor effects. In all other impaired streams, median dissolved chloride levels were in the no to low probability range for stressor effects.
Temporal Co-occurrence	-1	0	-1	2	-1	-1	At or around the time of benthic sampling, dissolved chloride levels were in the medium probability range for stressor effects in Tattle Branch and no to low range in Byers/Hall Creek, Greenway Creek and the MF Holston.
Stressor-Response Relationships from the Field	-3	-3	-3	-3	-3	-3	Dissolved sodium was not significantly correlated with benthic health across sites.
Temporal Sequence	-1	-1	-1	1	-1	-1	In Tattle Branch, chloride levels were highest in the winter prior to lower spring SCI scores. In other streams, seasonal trends of lower spring benthic scores did not appear to correlate with the timing of high chloride levels.
Symptoms	-3	-3	-3	-3	-3	-3	Biological Condition Gradient analysis did not identify any predominant taxa that increase in abundance in the presence of high chloride (score of 5), and chloride was one of the lowest ranked stressors based on average BCG score.

Stressor-Response Relationships from Other Field Studies	-1	-1	-2	2	-2	-3	Median dissolved chloride was in the medium probability range for stressor effects in Tattle Branch. In all other streams, median dissolved chloride levels were in the no to low probability range for stressor effects.
Stressor-Response Relationships from Laboratory Studies	-3	-3	-3	-3	-3	-3	Chloride levels were all well below the Virginia water quality standard for chloride.
Analogous Stressors	3	2	2	3	-2	-2	The analogous stressor of conductivity was in the high probability range for stressor effects in Byers/Hall Creek and Tattle Branch, medium to high range in Cedar Creek, medium range in Greenway Creek, and no to low probability range for stressor effects in the MF Holston.
Consistency of Evidence	-2	-2	-2	0	-2	-2	Evidence strongly refutes chloride as a stressor in all MF Holston project streams except for Tattle Branch, where evidence was inconsistent.
Sum	**-12**	**-12**	**-15**	**1**	**-19**	**-21**	

3.5.4. Sulfate

Table 26 shows the causal analysis results for dissolved sulfate across MF Holston Project streams. Total causal analysis scores ranged from -16 to -2, indicating that there is weak to strong evidence that dissolved sulfate is a non-stressor in these streams. In each stream, median dissolved sulfate values were in the no to low probability range for stressor effects. Values were also below toxic thresholds reported by Mount *et al.* (2016). For these reasons and others explained in Table 26, dissolved sulfate was categorized as a non-stressor.

Table 26. Causal analysis results for dissolved sulfate.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	-1	-3	-3	-3	-2	-3	In all streams, SCI scores were impaired, but median dissolved sulfate levels were in the no to low probability range for stressor effects.
Temporal Co-occurrence	1	0	-2	-2	-2	-2	At or around the time of benthic sampling, dissolved sulfate levels were in the low to medium probability range for stressor effects in Byers/Hall Creek and no to low probability range for all other streams, except Cedar Creek (where no sulfate data was collected at or around the time of benthic sampling).

Stressor-Response Relationships from the Field	-3	-3	-3	-3	-3	-3	Dissolved sulfate was not significantly correlated with benthic health across sites.
Temporal Sequence	-1	-1	-1	-1	-1	-1	Seasonal trends of lower spring benthic scores did not appear to correlate with the timing of high sulfate levels.
Symptoms	2	2	2	2	2	2	Biological Condition Gradient analysis identified several of the top predominant taxa in each impaired stream (Baetis and Optioservus) as increasing in abundance in the presence of high sulfate (score of 5).
Stressor-Response Relationships from Other Field Studies	-1	-3	-3	-3	-2	-3	Median sulfate values were in the no to low probability range for stressor effects in each of the MF Holston project streams.
Stressor-Response Relationships from Laboratory Studies	-2	-2	-2	-2	-2	-2	In an analysis of toxicity to major ions (Mount et al., 2016), all LC50s for Ceriodaphnia exposed to sulfate salts were well above the sulfate levels in MF Holston project streams.
Analogous Stressors	3	2	2	3	-2	-2	The analogous stressor of conductivity was in the high probability range for stressor effects in Byers/Hall Creek and Tattle Branch, medium to high range in Cedar Creek, medium range in Greenway Creek, and no to low probability range for stressor effects in the MF Holston.
Consistency of Evidence	0	-1	-1	-1	-1	-2	Evidence moderately refutes sulfate as a stressor in the MF Holston project streams, except for Byers/Hall Creek where evidence was inconsistent.
Sum	**-2**	**-9**	**-11**	**-10**	**-13**	**-16**	

3.6. Suspended Solids and Deposited Sediment

Table 27 shows the causal analysis results for suspended solids and deposited sediment across MF Holston Project streams. Total causal analysis scores ranged from +7 to +13, indicating that there is moderate to strong evidence that sediment is a probable stressor in these streams. Lines of evidence supporting sediment as a probable stressor in these streams included:

- Total habitat scores and habitat metrics that indicate instream sediment were significantly lower in most streams than in the reference.
- Seasonal trends in benthic health in most streams indicated poor health in the spring following high spring flows that typically bring higher sediment loads.
- Total habitat scores were significantly correlated with benthic health across sites.
- Average Biological Condition Gradient scores ranked sediment-associated stressors as the top one or two stressors in all streams.

- Taxonomic community structure indicated shifts to sediment-tolerant Dipteran or Elmid-dominated communities and away from Ephemeroptera, Plecoptera, and Trichoptera, which generally prefer clean substrate.
- Functional feeding group analysis in most streams indicated shifts to filterers and collectors that prefer sediment conditions and away from shredders and scrapers that prefer clean substrate.
- Total suspended solids and turbidity were significantly higher in impaired streams than in the unimpaired reference.
- The spatial pattern of turbidity in the MF Holston River matched the pattern of benthic impairment.

For these reasons and others explained in Table 27, suspended solids and deposited sediment were categorized as probable stressors.

Table 27. Causal analysis results for suspended solids and deposited sediment.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	2	2	2	0	3	3	At each impaired station except for MF Holston lower impaired reach, SCI scores were impaired, and TSS was significantly higher than in an unimpaired reference. No TSS data were available for Tattle Branch. The spatial pattern of diurnal turbidity in the MF Holston also matched the spatial pattern of impairment.
Temporal Co-occurrence	1	1	1	0	0	0	In Byers/Hall Creek and Greenway Creek, habitat scores correlated with benthic health scores. In Cedar Creek, elevated TSS levels occurred on or around the time of benthic sampling.
Causal Pathway	1	1	0	0	0	1	The causal pathway from degraded banks and riparian area to sediment deposition and degraded instream habitat is intact in Byers/Hall Creek, Cedar Creek, and the MF Holston upper impaired reach. This pathway is incomplete in other impaired streams.
Stressor-Response Relationships from the Field	3	3	3	3	3	3	Habitat scores were significantly correlated with benthic health across sites.

Temporal Sequence	1	0	0	1	1	-1	Seasonal trends of lower spring benthic scores observed in Byers/Hall Creek, Tattle Branch, and the MF Holston lower impaired reach may indicate sediment enrichment, as higher spring flows bring increased sediment loads. In the MF Holston upper impaired reach, seasonal trends were reversed with fall scores lower than spring scores.
Symptoms	3	3	3	3	2	2	Average Biological Condition Gradient scores for relative bed stability were ranked first or second in each of the impaired streams. Community structure analysis in each impaired stream indicated a shift to sediment-tolerant Dipterans or Elmids, and functional feeding group analysis in each of the MF Holston tributaries indicated a shift to collectors and filterers that thrive in sediment-rich habitats.
Stressor-Response Relationships from Other Field Studies	-1	2	1	2	-1	2	Median total habitat scores were in the medium probability range for stress effects in Cedar Creek, Tattle Branch, Greenway Creek, and the MF Holston upper impaired reach. Median total habitat scores were in the low probability range for stress effects in Byers/Hall Creek and the MF Holston lower impaired reach.
Analogous Stressors	0	0	0	0	-2	0	The measurement of relative bed stability was in the no probability range for stress effects in the MF Holston lower impaired reach.
Consistency of Evidence	1	1	1	1	1	1	Most evidence weakly supported sediment as a stressor.
Sum	**11**	**13**	**11**	**10**	**7**	**11**	

3.7. Organic Matter

Table 28 shows the causal analysis results for organic matter across MF Holston Project streams. Total causal analysis scores ranged from -9 to -3, indicating that there is moderate evidence that organic matter is a non-stressor in these streams. In several impaired streams, total volatile solids were lower than in the unimpaired reference. Total volatile solids were also not significantly correlated with benthic health across sites. Dissolved oxygen also remained high in the impaired streams indicating that the decomposition of organic matter was not controlling dissolved oxygen levels. For these reasons and others explained in Table 28, organic matter was categorized as a non-stressor.

Table 28. Causal analysis results for organic matter.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	-2	-2	0	0	1	-1	In Byers/Hall Creek, Cedar Creek and the MF Holston upper impaired reach, total volatile solids were lower than in the respective unimpaired reference stream. Total volatile solids were significantly higher in the MF Holston lower impaired reach than in the large stream reference. No organic matter data were available for Tattle Branch and Greenway Creek.
Temporal Co-occurrence	-1	-1	0	0	0	0	At or around the time of benthic sampling, total volatile solids in Byers/Hall Creek and Cedar Creek were below levels in the unimpaired small stream reference.
Causal Pathway	-2	-2	-2	-2	-2	-2	Dissolved oxygen was high in all streams, so the pathway from organic matter to decomposition to low dissolved oxygen was not intact.
Stressor-Response Relationships from the Field	-2	-2	-2	-2	-2	-2	Total volatile solids were not significantly correlated with benthic health across sites.
Temporal Sequence	-1	0	0	-1	-1	1	Seasonal trends of lower spring benthic scores observed in Byers/Hall Creek, Tattle Branch, and the MF Holston lower impaired reach are inconsistent with organic matter enrichment, since organic matter from primary productivity would increase in the summer and fall. In the MF Holston upper impaired reach, seasonal trends were reversed with fall scores lower than spring scores.
Symptoms	2	2	2	2	-2	-2	Functional feeding group analysis in each of the MF Holston tributaries indicated an increase in filterers that thrive in organic-rich habitats. Filterers decreased in MF Holston impaired reaches.
Stressor-Response Relationships from Other Field Studies	-2	-2	0	0	1	-1	In Byers/Hall Creek, Cedar Creek and the MF Holston upper impaired reach, total volatile solids were lower than in the respective unimpaired reference stream. Total volatile solids were significantly higher in the MF Holston lower impaired reach than in the large stream reference. No organic matter data were available for Tattle Branch and Greenway Creek.
Consistency of Evidence	-1	-1	-1	-1	-1	-1	Most evidence weakly refuted organic matter as a stressor.
Sum	**-9**	**-8**	**-3**	**-4**	**-6**	**-8**	

3.8. Nutrients

3.8.1. Total Phosphorus

Table 29 shows the causal analysis results for total phosphorus across MF Holston Project streams. In Cedar Creek, Greenway Creek, Tattle Branch, and the MF Holston upper impaired reach, total causal analysis scores ranged from -13 to 0, indicating that there is weak to strong evidence that phosphorus is a non-stressor in these streams. In Byers/Hall Creek and the MF Holston lower impaired reach, total causal analysis scores were +3, indicating that phosphorus is a possible stressor in these streams. Median total phosphorus values were in the high probability range for stressor effects in Byers/Hall Creek, medium probability range in the MF Holston River, and low probability range in all other streams. Diurnal DO fluctuations were moderate in most streams, and only the lower MF Holston had fluctuations greater than the reference location. For these reasons and others explained in Table 29, phosphorus was categorized as a possible stressor in Byers/Hall Creek and the MF Holston lower impaired reach. In the remaining streams, total phosphorus was categorized as a non-stressor.

Table 29. Causal analysis results for total phosphorus.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	3	-2	-2	-2	2	2	In the MF Holston, spatial patterns of impairment matched patterns of phosphorus concentrations with lower concentrations in the unimpaired reach and higher concentrations in the impaired reach. In Byers/Hall Creek, impairment co-occurred with high phosphorus levels, but all other streams had phosphorus levels in the low probability range for stressor effects.
Temporal Co-occurrence	1	-2	1	-2	-1	-1	At or near the time of benthic sample collection, total phosphorus levels were in the high probability range for stressor effect on one occasion in Greenway Creek and the MF Holston, and two occasions in Byers/Hall Creek. However, in the MF Holston and one date in Byers/Hall Creek, benthic SCI scores were above the impairment threshold of 60 when phosphorus levels were elevated.

Causal Pathway	-1	-3	-1	-3	1	-3	Diurnal DO measurements in most streams did not show strong fluctuations from daytime to nighttime indicating that the causal pathway from nutrient enrichment to low DO is weak or not intact. Only the MF Holston lower impaired reach had larger daily fluctuations in DO than the MF Holston reference site. Greenway Creek had a one-day diurnal fluctuation larger than the reference, but the minimum value on that day is considered anomalous.
Stressor-Response Relationships from the Field	-3	-3	-3	-3	-3	-3	Phosphorus was not significantly correlated with benthic health across sites.
Temporal Sequence	-2	-1	-1	-1	-2	-1	Spring SCI scores were lower than fall scores in Byers/Hall Creek, Tattle Branch, and MF Holston lower impaired reach, however, seasonal trends in phosphorus concentrations didn't match this pattern. Fall phosphorus levels were generally higher in Byers/Hall Creek and the MF Holston lower impaired reach. In all other streams, distinct seasonal trends were not identified in SCI scores or phosphorus levels.
Symptoms	-1	0	-1	0	2	2	Functional feeding group analysis showed a pattern of increasing scrapers in Tattle Branch and MF Holston, possibly indicating nutrient enrichment. No other streams had functional feeding group patterns that indicated nutrient enrichment. Biological Condition Gradient analysis did not identify any of the top predominant taxa as increasing in abundance in the presence of nutrients (score of 5), but nutrients were the highest ranked stressor in Cedar Creek and the MF Holston.
Stressor-Response Relationships from Other Field Studies	3	-2	-1	-2	2	2	Median total phosphorus concentrations were in the high probability range for stress effects in Byers/Hall Creek, medium probability range in MF Holston, and low probability range in all other streams.
Stressor-Response Relationships from Laboratory Studies	3	1	1	1	2	2	Total phosphorus levels averaged 0.05 to 0.17 mg/L at impaired stations, which is above the EPA-recommended criterion of 0.01 mg/L for the Ridge and Valley ecoregion.
Consistency of Evidence	0	-1	-1	-1	0	0	In Cedar Creek, Greenway Creek, and Tattle Branch evidence weakly refuted phosphorus as a stressor. In the remaining streams, some evidence supported phosphorus as a stressor, while other evidence refuted phosphorus as a stressor.
Sum	**3**	**-13**	**-8**	**-13**	**3**	**0**	

3.8.2. Total Nitrogen

Table 30 shows the causal analysis results for total nitrogen across MF Holston Project streams. The total causal analysis score for the MF Holston upper impaired reach was -1, indicating that there is weak evidence that nitrogen is not a stressor at this location. In the other impaired streams, total causal analysis scores ranged from +1 to +3, indicating that nitrogen is a possible stressor. Median nitrogen levels were in the high probability range for stressor effects in all of the MF Holston tributaries and medium probability range in the MF Holston upper and lower impaired reaches. Total nitrogen levels were also above the EPA-recommended criterion for the Ridge and Valley Ecoregion. While some lines of evidence indicate nutrient enrichment as a stressor, nitrogen to phosphorus ratios showed that phosphorus (and not nitrogen) is the limiting nutrient. In addition, nitrogen was not significantly correlated with benthic health across sites. For these reasons and others explained in Table 30, nitrogen was categorized as a possible stressor in each of the impaired streams, except for the MF Holston upper impaired reach.

Table 30. Causal analysis results for total nitrogen.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	3	3	3	3	2	2	At impaired MF Holston tributary stations, median nitrogen levels were in the high probability range for stressor effects. At impaired MF Holston stations, median nitrogen levels were in the medium probability range for stressor effects. At unimpaired MF Holston stations, nitrogen levels were in the low probability range.
Temporal Co-occurrence	3	3	3	3	2	1	At or around the time of benthic sample collection, total nitrogen levels were in the high probability range in MF Holston tributaries, medium probability range in the MF Holston lower impaired reach, and low to medium range in the MF Holston upper impaired reach.
Causal Pathway	-1	-3	-1	-3	1	-3	Diurnal DO measurements in most streams did not show strong fluctuations from daytime to nighttime indicating that the causal pathway from nutrient enrichment to low DO is weak or not intact. Only the MF Holston lower impaired reach had larger daily fluctuations in DO than the MF Holston reference site. Greenway Creek had a one-day diurnal fluctuation larger than the reference, but the minimum value on that day is considered anomalous.
Stressor-Response Relationships from the Field	-3	-3	-3	-3	-3	-3	Nitrogen was not significantly correlated with benthic health across sites.
Temporal Sequence	-2	-1	-1	1	-2	-1	Spring SCI scores were lower than fall scores in Byers/Hall Creek, Tattle Branch, and MF Holston lower impaired reach, however, no seasonal trend in nitrogen was observed in

							Byers/Hall Creek and MF Holston. Nitrogen was slightly higher in the spring in Tattle Branch. In all other streams, distinct seasonal trends were not identified in SCI scores or nitrogen levels.
Symptoms	-1	0	-1	0	2	2	Functional feeding group analysis showed a pattern of increasing scrapers in Tattle Branch and MF Holston, possibly indicating nutrient enrichment. No other streams had functional feeding group patterns that indicated nutrient enrichment. Biological Condition Gradient analysis did not identify any of the top predominant taxa as increasing in abundance in the presence of nutrients (score of 5), but nutrients were the highest ranked stressor in Cedar Creek and the MF Holston.
Stressor-Response Relationships from Other Field Studies	3	3	3	3	2	2	In MF Holston tributaries, total nitrogen averaged in the high probability range for stress effects. In the MF Holston, total nitrogen averaged in the medium probability range.
Stressor-Response Relationships from Laboratory Studies	2	2	2	2	2	2	Total nitrogen levels averaged 1.2 to 3.4 mg/L at impaired stations, which is above the EPA-recommended criterion of 0.399 mg/L for the Ridge and Valley ecoregion.
Mechanistically Plausible Cause	-3	-3	-3	-3	-3	-3	The nitrogen to phosphorus ratios in MF Holston Project streams ranged from 11 to 72, indicating that phosphorus, and not nitrogen, is the limiting nutrient controlling algae growth.
Consistency of Evidence	0	0	0	0	0	0	Some evidence supported nitrogen as a stressor, while other evidence refuted nitrogen as a stressor.
Sum	**1**	**1**	**2**	**3**	**3**	**-1**	

3.9. Ammonia

Table 31 shows the causal analysis results for ammonia across MF Holston Project streams. Total causal analysis scores ranged from -18 to -10, indicating that there is strong evidence that ammonia is not a stressor in these streams. All samples in all streams were well below the water quality standard for ammonia. Based on multiple lines of evidence explained in Table 31, ammonia was categorized as a non-stressor in each of the impaired streams.

Table 31. Causal analysis results for ammonia.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation

Spatial Co-occurrence	-3	-3	-2	-1	-3	-3	In all streams, SCI scores were impaired, but ammonia values were well above water quality standards. No ammonia data were available for Tattle Branch, but high levels of ammonia in this stream would have been observed downstream in Byers/Hall Creek.
Temporal Co-occurrence	-3	-1	-1	-1	-1	-3	At or around the time of benthic sampling, ammonia levels in Byers/Hall Creek and MF Holston upper impaired reach were well below water quality standards. Ammonia data were not collected at or around the time of benthic sampling in other streams, but all ammonia values were well below water quality standards. No ammonia data were available for Tattle Branch, but high levels of ammonia in this stream would have been observed downstream in Byers/Hall Creek.
Stressor-Response Relationships from the Field	-3	-3	-3	-3	-3	-3	Ammonia was not significantly correlated with benthic health across sites.
Temporal Sequence	-3	-1	0	-3	-2	1	Ammonia levels are generally highest in the late summer when water temperatures are highest, however, fall benthic scores were higher than spring scores in all streams except for Greenway Creek and the MF Holston upper impaired reach.
Stressor-Response Relationships from Laboratory Studies	-3	-3	-2	-1	-3	-3	All ammonia values were well below water quality standards. No ammonia data were available for Tattle Branch, but high levels of ammonia in this stream would have been observed downstream in Byers/Hall Creek.
Consistency of Evidence	-3	-3	-2	-2	-2	-2	Evidence consistently refuted ammonia as a stressor.
Sum	**-18**	**-14**	**-10**	**-11**	**-14**	**-13**	

3.10. Dissolved Metals

Table 32 shows the causal analysis results for dissolved metals across MF Holston Project streams. Total causal analysis scores ranged from -17 to -2, indicating that there is weak to strong evidence that dissolved metals are not a stressor in these streams. In all streams that had dissolved metals data, the CCU was in the no probability range for stressor effects. All metals in all streams were below water quality standards and reference toxicity values. For these reasons and others explained in Table 32, dissolved metals were categorized as non-stressors in MF Holston Project streams.

Table 32. Causal analysis results for dissolved metals.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	-3	-3	0	-1	-3	-3	In all streams (except Greenway Creek and Tattle Branch), SCI scores were impaired, but dissolved metal CCU values were in the no probability range for stressor effects. No dissolved metal data were available for Greenway Creek and Tattle Branch, but high levels of dissolved metals in Tattle Branch would have been observed downstream in Byers/Hall Creek.
Temporal Co-occurrence	-3	-3	0	-1	-1	-1	At or around the time of benthic sampling, dissolved metal levels in Byers/Hall Creek and Cedar Creek were well below water quality standards and published effect thresholds. Dissolved metal data were not collected at or around the time of benthic sampling in other streams, but all dissolved metal values were well below water quality standards. No dissolved metal data were available for Greenway Creek and Tattle Branch, but high levels of dissolved metals in Tattle Branch would have been observed downstream in Byers/Hall Creek.
Causal Pathway	-2	-2	-2	-2	-2	-2	The watersheds of the impaired streams are primarily agricultural and do not contain significant sources of metals, so the causal pathway for metals is absent or incomplete.
Stressor-Response Relationships from Other Field Studies	-3	-3	0	-1	-3	-3	Cumulative criteria units for dissolved metals were in the no probability range for stressor effects. No dissolved metal data were available for Greenway Creek and Tattle Branch, but high levels of dissolved metals in Tattle Branch would have been observed downstream in Byers/Hall Creek.
Stressor-Response Relationships from Laboratory Studies	-3	-3	0	-1	-3	-3	All dissolved metals values were below water quality standards and published effect thresholds. No dissolved metal data were available for Greenway Creek and Tattle Branch, but high levels of dissolved metals in Tattle Branch would have been observed downstream in Byers/Hall Creek.
Consistency of Evidence	-3	-3	0	-1	-3	-3	Evidence consistently refuted dissolved metals as a stressor in streams where data were available.
Sum	**-17**	**-17**	**-2**	**-7**	**-15**	**-15**	

3.11. Sediment Toxics

Table 33 shows the causal analysis results for sediment toxics across MF Holston Project streams. Total causal analysis scores ranged from -11 to -1, indicating that there is weak to strong evidence that sediment toxics (including sediment metals, PAHs, PCB, and pesticides) are not a stressor in these streams. In all streams that had sediment toxics data, all toxics were below published effect thresholds. For this reason, and others explained in Table 33, sediment toxics were categorized as non-stressors in MF Holston Project streams.

Table 33. Causal analysis results for sediment toxics.

Evidence	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)	Explanation
Spatial Co-occurrence	-2	-2	0	-1	-2	-2	In all streams (except Greenway Creek and Tattle Branch), SCI scores were impaired, but sediment toxics (metals, PAHs, PCBs, and pesticides) were below published effect thresholds. No sediment toxics data were available for Greenway Creek and Tattle Branch, but high levels of sediment toxics in Tattle Branch would have been observed downstream in Byers/Hall Creek.
Temporal Co-occurrence	-3	-3	0	-1	-1	-1	At or around the time of benthic sampling, sediment metal levels in Byers/Hall Creek and Cedar Creek were well below published effect thresholds. Sediment metal data were not collected at or around the time of benthic sampling in the MF Holston River, but all sediment toxics (metals, PAHs, PCBs, and pesticides) were well below published effect thresholds. No sediment toxics data were available for Greenway Creek and Tattle Branch, but high levels of sediment toxics in Tattle Branch would have been observed downstream in Byers/Hall Creek.
Causal Pathway	-1	-1	-1	-1	-1	-1	The watersheds of the impaired streams are primarily agricultural and do not contain significant sources of toxics that would accumulate in sediments, so the causal pathway for sediment toxics is absent or incomplete.
Stressor-Response Relationships from Laboratory Studies	-2	-2	0	-1	-3	-3	All sediment toxics that were measured (metals, PAHs, PCBs, and pesticides) were below published effect thresholds. No sediment toxics data were available for Greenway Creek and Tattle Branch, but high levels of sediment toxics in Tattle Branch would have been observed downstream in Byers/Hall Creek. In Byers/Hall Creek and Cedar Creek, only sediment metals data were available.
Consistency of Evidence	-3	-3	0	-1	-3	-3	Evidence consistently refuted sediment toxics as a stressor in streams where data were available.
Sum	**-11**	**-11**	**-1**	**-5**	**-10**	**-10**	

4.0 CAUSAL ANALYSIS SUMMARY

4.1. Probable Stressors

The total causal analysis scores for each candidate stressor are shown in Table 34. 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. Table 35 shows the non-stressors, possible stressors, and probable stressors identified for each impaired stream. The results indicate that sediment was identified as a probable stressor in all of the MF Holston Project streams, with causal analysis scores ranging from +7 to +13.

Table 34. Total causal analysis scores by stream and by candidate stressor. Green indicates non-stressors, orange indicates possible stressors, and red indicates probable stressors.

Candidate Stressor	Byers/Hall Creek	Cedar Creek	Greenway Creek	Tattle Branch	MF Holston (Lower)	MF Holston (Upper)
Temperature	-18	-16	-15	-18	-17	-13
pH	-25	-23	-21	-23	-23	-25
DO	-18	-17	-17	-20	-11	-15
Conductivity/TDS	2	3	1	3	-14	-12
Sodium	0	-7	-10	3	-15	-17
Potassium	3	0	2	3	-10	-2
Chloride	-12	-12	-15	1	-19	-21
Sulfate	-2	-9	-11	-10	-13	-16
Sediment	11	13	11	10	7	11
Organic Matter	-9	-8	-3	-4	-6	-8
Phosphorus	3	-13	-8	-13	3	0
Nitrogen	1	1	2	3	3	-1
Ammonia	-18	-14	-10	-11	-14	-13
Metals	-17	-17	-2	-7	-15	-15
Sediment Toxics	-11	-11	-1	-5	-10	-10

Table 35. Non-stressors, possible stressors, and probable stressors in MF Holston Project streams.

Stream	Non-Stressors	Possible Stressors	Probable Stressors	TMDL Target
Byers/Hall Creek	Temperature, pH, Dissolved Oxygen, Dissolved Sodium, Dissolved Chloride, Dissolved Sulfate, Organic Matter, Ammonia, Dissolved Metals, Sediment Toxics	-Conductivity/TDS -Dissolved Potassium -Phosphorus -Nitrogen	-Sediment	-Sediment
Cedar Creek	Temperature, pH, Dissolved Oxygen, Dissolved Sodium, Dissolved Potassium, Dissolved Chloride, Dissolved Sulfate, Organic Matter, Phosphorus, Ammonia, Dissolved Metals, Sediment Toxics	-Conductivity/TDS -Nitrogen	-Sediment	-Sediment
Greenway Creek	Temperature, pH, Dissolved Oxygen, Dissolved Sodium, Dissolved Chloride, Dissolved Sulfate, Organic Matter, Phosphorus, Ammonia, Dissolved Metals, Sediment Toxics	-Conductivity/TDS -Dissolved Potassium -Nitrogen	-Sediment	-Sediment
Tattle Branch	Temperature, pH, Dissolved Oxygen, Dissolved Sulfate, Organic Matter, Phosphorus, Ammonia, Dissolved Metals, Sediment Toxics	-Conductivity/TDS -Dissolved Sodium -Dissolved Potassium -Dissolved Chloride -Nitrogen	-Sediment	-Sediment
MF Holston Lower Impaired Reach	Temperature, pH, Dissolved Oxygen, Conductivity/TDS, Dissolved Sodium, Dissolved Potassium, Dissolved Chloride, Dissolved Sulfate, Organic Matter, Phosphorus, Nitrogen, Ammonia, Dissolved Metals, Sediment Toxics	-Phosphorus -Nitrogen	-Sediment	-Sediment
MF Holston Upper Impaired Reach	Temperature, pH, Dissolved Oxygen, Conductivity/TDS, Dissolved Sodium, Dissolved Potassium, Dissolved Chloride, Dissolved Sulfate, Organic Matter, Phosphorus, Nitrogen, Ammonia, Dissolved Metals, Sediment Toxics		-Sediment	-Sediment

4.1.1. Sediment

Sediment was identified as a probable stressor in all of the MF Holston Project streams. Multiple lines of evidence supported this determination including habitat metrics, TSS and turbidity data, seasonal trends, biological condition gradient analysis, taxonomic community structure, and functional feeding group analysis (Section 3.6). Based on the observed data and causal analysis, a conceptual model was developed to describe the causal relationships between the sources of sediment in the watershed, increased suspended sediment loads, and the observed loss of benthic macroinvertebrates (Figure 49). In this conceptual model, sources of sediment are derived from point sources, the erosion of watershed soils, the washoff of accumulated sediment on impervious surfaces, the erosion of streambanks, and the resuspension of channel sediments. These sources and other contributing factors lead to an increased particulate load (i.e., suspended sediment) in the stream. The increased particulate load then acts to biologically impair the stream through two pathways: a change in feeding niches to favor filter feeders and deposit feeders, and the filling of interstitial spaces that reduces available habitat. Benthic taxa data provide evidence of these pathways with an observed increase in filter and deposit feeders and a decrease in taxa richness. Habitat assessments also provide evidence of interstitial filling. The combined weight of evidence documented in the causal analysis supports this conceptual model of sediment as a stressor in the MF Holston Project streams. A TMDL developed to reduce sediment loads in the watershed will address the benthic impairments in these streams through the pathways described in Figure 49. In addition, efforts to address several contributing factors that exacerbate the impact of the sediment stressor will also be effective at reducing the impairment.

4.1.1.1. Contributing Factors

Several factors contribute to the impact of sediment in MF Holston Project streams, including crop and pasture management, livestock stream access, imperviousness in the watersheds, and poor riparian vegetation. In most of the MF Holston impaired watersheds, pasture and cropland account for the majority of land cover. Practices on these lands that result in exposed soil increase erosion and can contribute excess sediment to streams. In particular, livestock access to stream banks greatly decreases the stability of the banks and increases bank erosion. Livestock also resuspend channel sediments, increasing suspended solids. Alternatively, best management practices on pasture and croplands that reduce erosion can be beneficial in reducing sediment loads. Agricultural best management practices such as livestock exclusion fencing, off-stream watering, cover crops, riparian buffers, rotational grazing, and others will likely be an important part of implementation plans to fully restore aquatic life in these streams.

While imperviousness is relatively low across the entire MF Holston watershed, some tributaries have significant percentages of imperviousness, such as 12% in Greenway Creek and 13% in Tattle Branch. 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 streambed 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. While the TMDL does not directly address the percentage of imperviousness in watersheds, efforts to reduce imperviousness and increase infiltration can support the TMDL and assist in reducing the impact of sediment. Practices such as rain gardens, green roofs, rain barrels, and pervious pavers can all reduce runoff. Regional planning, zoning practices, and building codes can also be implemented to discourage imperviousness and reduce runoff.

Lastly, poor riparian vegetation is a contributing factor to sediment impairments in MF Holston Project streams. Riparian vegetation stabilizes stream banks and reduces bank erosion, which can often be a primary contributor to in-stream sediment loads. Practices such as riparian plantings, greenways, conservation easements, and regional planning and zoning practices that protect stream corridors can be effective mechanisms for reducing sediment loads from streambank erosion.

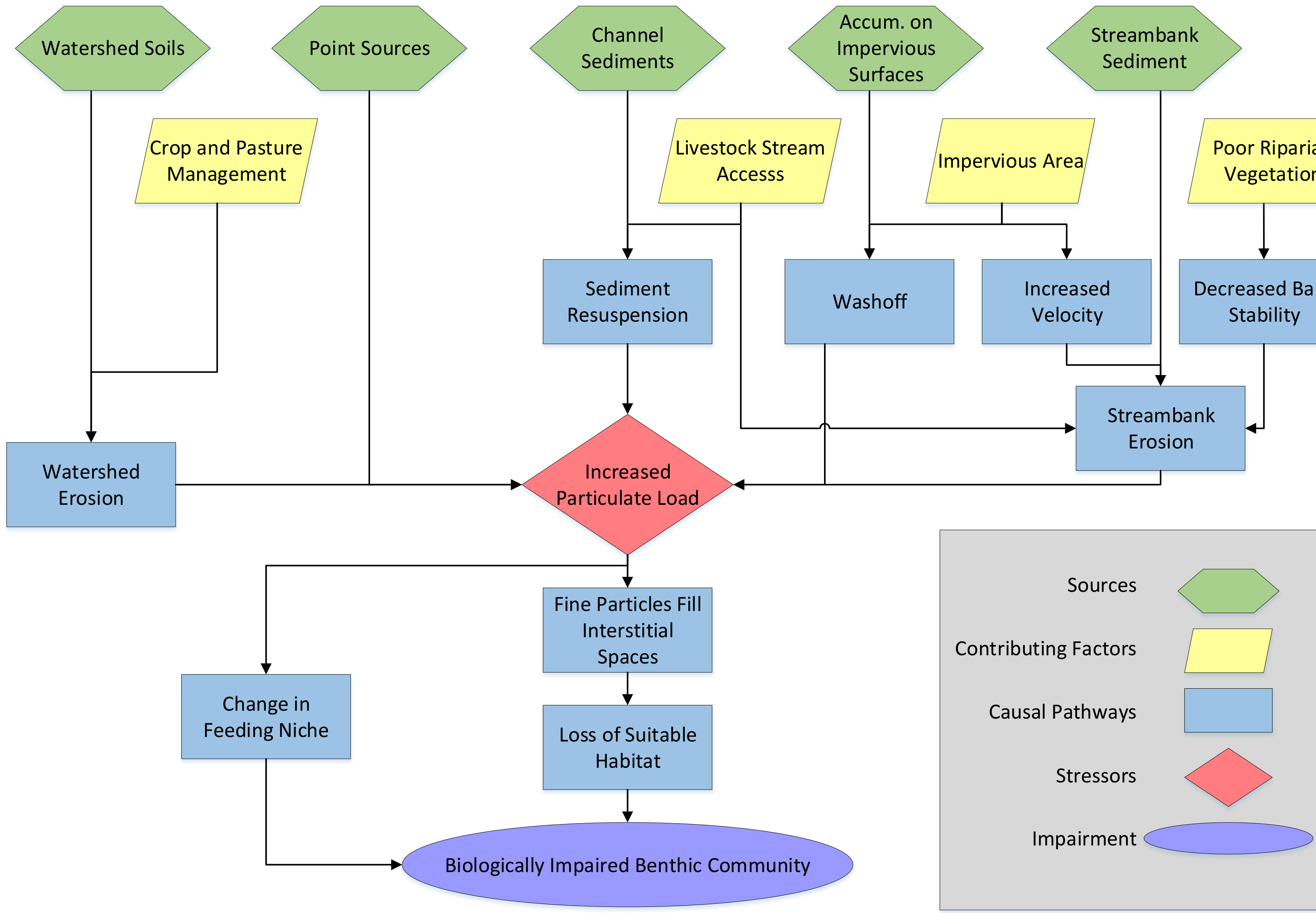

Figure 49. Conceptual model for the causal pathway of sediment impacts on benthic macroinvertebrates in MF Holston Project streams.

4.2. Conclusions

Following causal analysis and the determination of probable stressors, target pollutants for the TMDL were selected. TMDL target pollutants are the physical or chemical substances that will be controlled and allocated in the 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. Physical factors or environmental conditions, such as flow regimes, hydrologic modifications, or physical structures (like dams) cannot be TMDL target pollutants. Even though these conditions influence ecological communities and may be sources of stress, they do not represent substances that originate from point and nonpoint sources, they cannot be quantified, summed, and allocated to respective sources, and they cannot be controlled through source reductions.

TMDL target pollutants were selected by analyzing the causal pathways of probable stressors and identifying the primary substance responsible for controlling the pathway. For each of the MF Holston Project streams, the TMDL target pollutant was sediment.

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