Stressor Identification Analysis
for
Greendale Creek and Rich Valley Unnamed Tributary in Washington County

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Prepared for:
Virginia Department of Environmental Quality

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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 Greendale Creek and an unnamed tributary (called Rich Valley Unnamed Tributary in this report) to Fleenor Branch in Washington County, Virginia. 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 115 parameters from 3 monitoring stations totaling over 12,000 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 both of the impaired streams, and dissolved oxygen and total phosphorus were identified as a probable stressor in Rich Valley Unnamed Tributary. As a result, a sediment TMDL should be developed to address the probable stressor and associated benthic impairment in Greendale Creek, and sediment and total phosphorus TMDLs should be developed to address the benthic impairment in Rich Valley Unnamed Tributary.

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 Greendale Creek and Rich Valley Unnamed Tributary in Washington County, Virginia. Greendale Creek flows into the North Fork Holston River near Holston, Virginia. Rich Valley Unnamed Tributary flows into Fleenor Branch, which flows into Cove Creek. Cove Creek empties into the North Fork Holston River near Bruno, Virginia. The impaired segments included in this project are listed in Table 1 and shown in Figure 1.

Table 1. Benthic impairments in Greendale Creek and Rich Valley Unnamed Tributary.

Stream Name	NWBD	Impaired Assessment Units	Cause Group Code	First listed	Length (miles)	Impairment Description
Greendale Creek	TH35	VAS-O12R_GRN01A00	O12R-03-BEN	2010	5.03	Greendale Creek from North Fork Holston confluence east of Rt. 19 bridge, upstream 4.1 miles to Black Hollow Road
Rich Valley Unnamed Tributary	TH40	VAS-O12R_XEO01A12	O12R-04-BEN	2020	0.85	Unnamed tributary to Fleenor Branch near Valley Institute

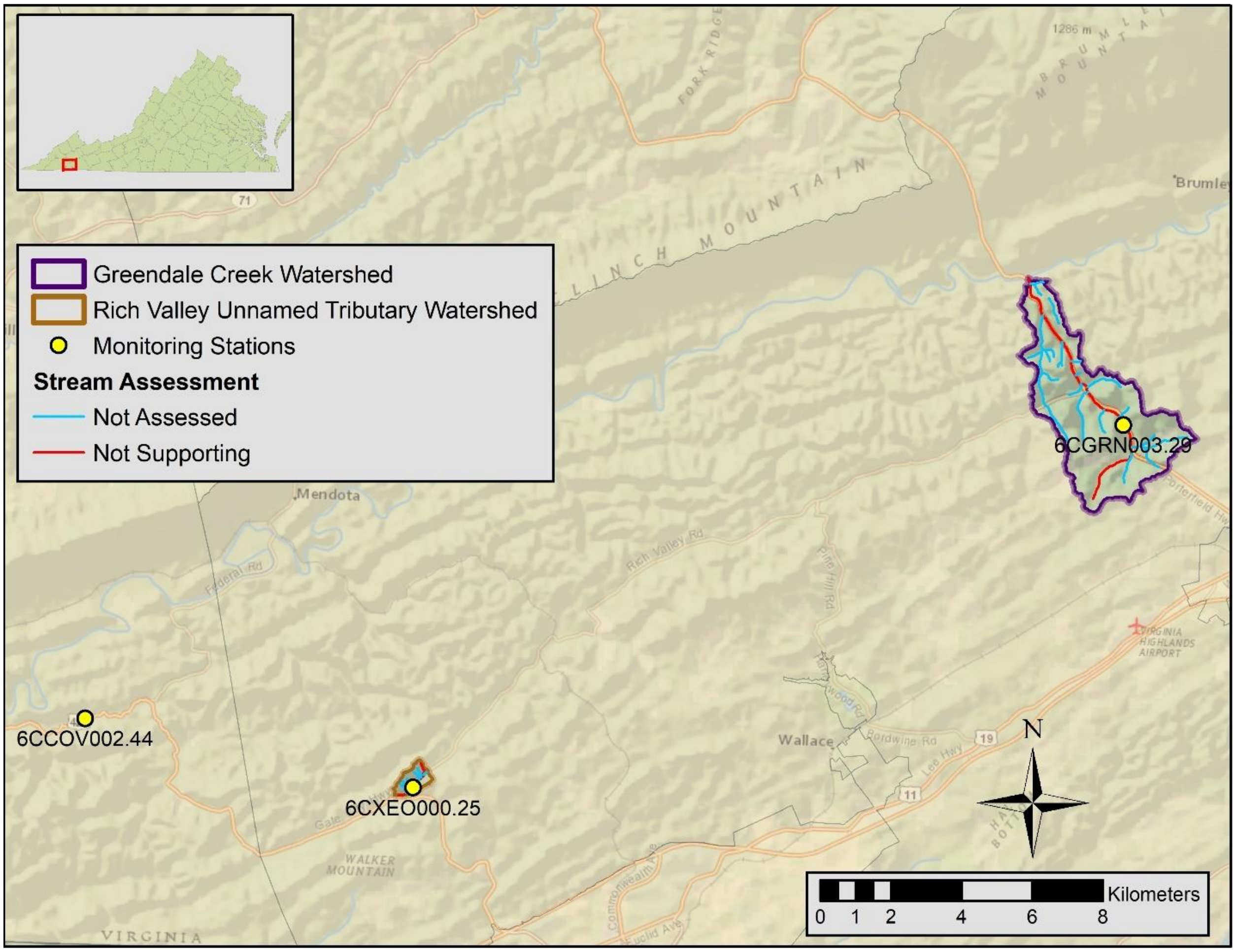


Figure 1. Location of benthic impairments in Greendale Creek and Rich Valley Unnamed Tributary.

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.

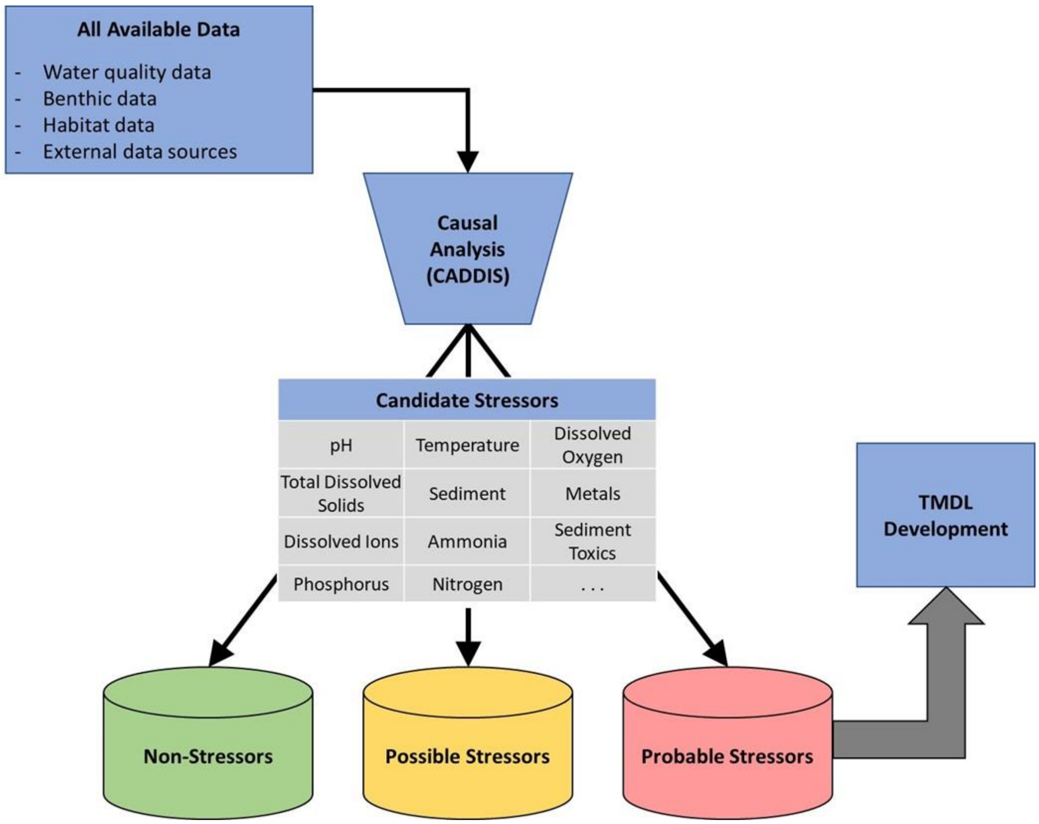


Figure 2. Stressor identification analysis process.

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 Greendale Creek and Rich Valley Unnamed Tributary.

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	
Dissolved Sodium	Nitrogen	
Dissolved Potassium	Phosphorus	
Additional Contributing Factors		
Habitat	Hydrologic Alteration	Instream Flow Conditions
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 3 VDEQ monitoring stations within the Greendale Creek and Cove Creek watersheds (Table 3). Water quality and benthic data were collected from all of these 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, 115 different water quality parameters were measured. In total, over 12,000 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 this project, Cove Creek station 6CCOV002.54 was used as a benthic reference station, and station 6CCOV002.44 was used as a water quality reference station. Since these stations are nearly collocated, they will be referred to as a single station (6CCOV002.44/54).

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
Greendale Creek	Greendale Creek	6CGRN003.29	2007-2018	4	2007-2018	18
Cove Creek	Rich Valley Unnamed Tributary	6CXEO000.25	2009-2019	7	2009-2022	26
	Cove Creek[1]	6CCOV002.44/54[1]	2012	2	2004-2022	32

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

2.1. Benthic Assessments

From 2007 to 2019, VDEQ conducted benthic assessments at stations on Greendale Creek, Rich Valley Unnamed Tributary, and the reference station on Cove Creek. Table 4 and Figure 3 show the average SCI scores for each station. SCI scores averaged 51.5 in Greendale Creek and 54.5 in Rich Valley Unnamed Tributary. Both stations averaged below the SCI threshold of 60, indicating impaired conditions. Benthic scores on Cove Creek were above the SCI impairment threshold, averaging 77.6 and indicating unimpaired conditions. This station will be used as a reference for benthic health and water quality in the stressor analysis.

Table 4. Benthic scores in Greendale Creek and Rich Valley Unnamed Tributary.

Watershed	Stream	Station	Years Sampled	Samples Collected	SCI Average	Assessment
Greendale Creek	Greendale Creek	6CGRN003.29	2007-2018	4	51.5	Impaired
Cove Creek	Rich Valley Unnamed Tributary	6CXEO000.25	2009-2019	7	54.5	Impaired
	Cove Creek	6CCOV002.44/54	2012	2	72.2	Unimpaired

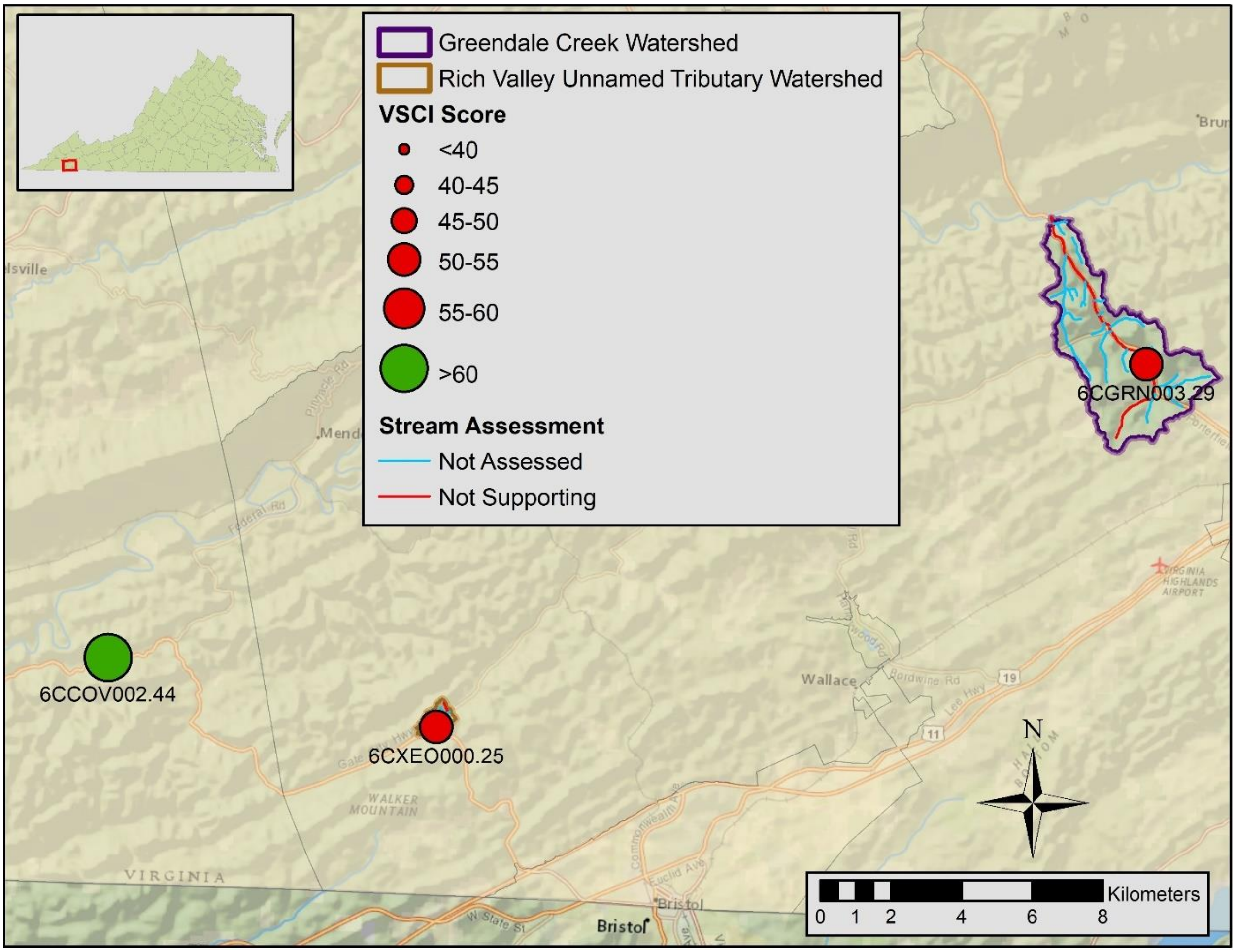


Figure 3. Benthic scores at monitoring stations within the Greendale Creek and Rich Valley Unnamed Tributary Project area.

2.1.1. Temporal and Seasonal Trends in Benthic Data

Figure 4 shows the temporal trends in benthic data from Greendale Creek and Rich Valley Unnamed Tributary, and Figure 5 shows the seasonal trends in benthic data from these streams.

- Greendale Creek – In Greendale Creek, SCI scores averaged 51.5 and ranged from 48.0 to 54.9, indicating a slight impairment. Benthic scores were slightly lower in 2018 than in 2007, and regression analysis revealed that this decrease, while biologically small, was statistically significant ($p<0.05$). There was no discernible seasonal trend. Spring and fall SCI scores were similar, averaging 50.8 in the spring and 52.2 in the fall. Overall, SCI scores in Greendale Creek have consistently indicated a slight impairment over time and across seasons.

- Rich Valley Unnamed Tributary – Benthic SCI scores in Rich Valley Unnamed Tributary averaged 54.5 and ranged from 38.9 to 70.2. Scores were lower in 2017 and 2019 than in earlier samplings in 2009 and 2014 when conditions were above the impairment threshold of 60. Regression analysis showed that decreasing SCI scores over time were not statistically significant at the alpha = 0.05 level, although the trend was significant at the alpha = 0.1 level. SCI scores in Rich Valley Unnamed Tributary also appeared to show a distinct seasonal trend. Spring SCI scores averaged 60.3, while fall scores averaged 46.8. This represents a 22% decrease in SCI scores from spring to fall. This difference was statistically significant at the alpha = 0.1 level (t-test with unequal variance) and might point to stressors such as low flow, low dissolved oxygen, or ammonia, which reach critical levels in the late summer.

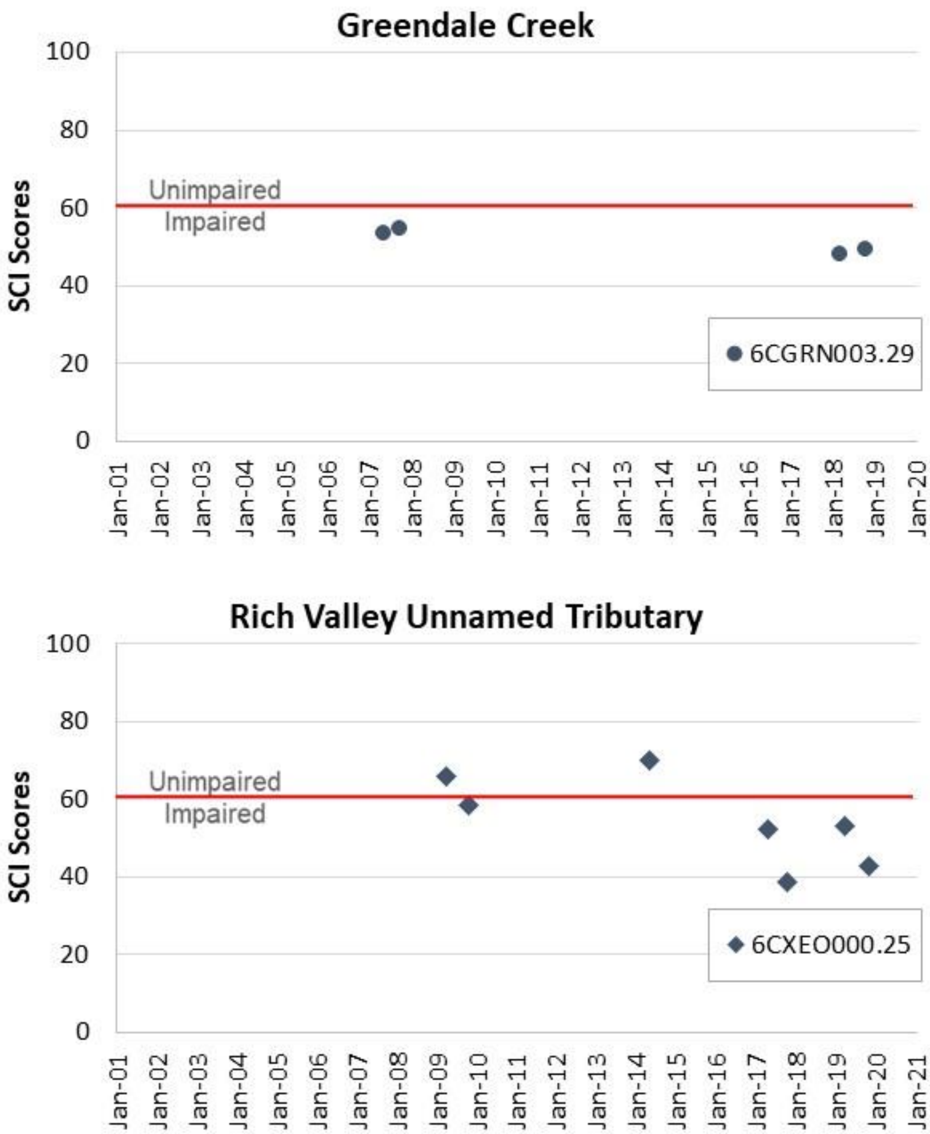


Figure 4. Temporal trends in benthic data for Greendale Creek and Rich Valley Unnamed Tributary.

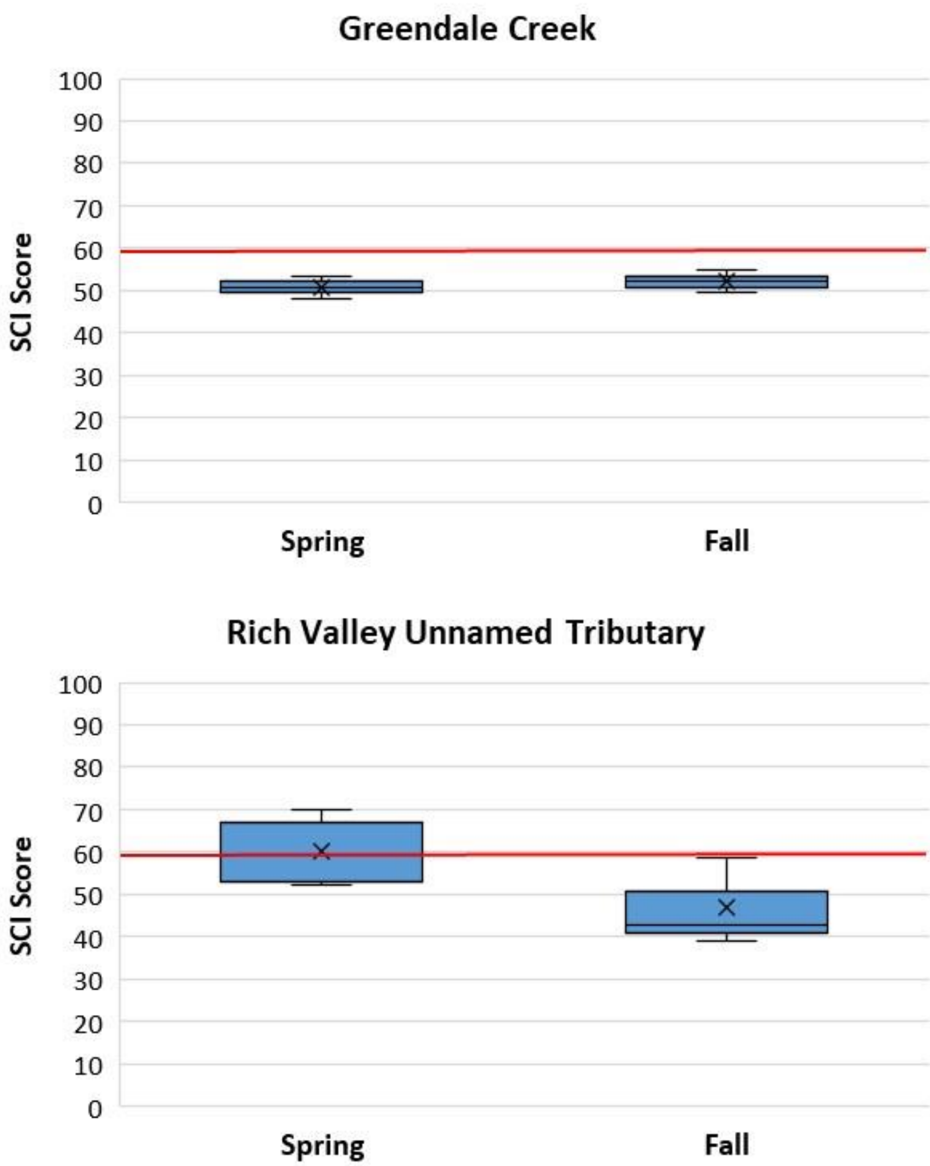


Figure 5. Seasonal trends in benthic data for Greendale Creek and Rich Valley Unnamed Tributary.

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 this project was Cove Creek (6CCOV002.54). For each impaired stream, average scores for each metric were compared to the reference using a t-test with unequal variances (alpha = 0.05).

- Greendale Creek – In Greendale Creek, Species richness, % Ephem, % 2 Dominant, and MFBI were all significantly lower ($p<0.05$) than in the reference. Greendale Creek averaged 11 total taxa compared to 15.5 at the reference. The % Ephem score in Greendale Creek averaged 20% compared to 85% in the reference. The % 2 Dominant score was 49% compared to 72% in the reference. The amphipod, *Gammaridae,* and the riffle beetle, *Elmidae,* were the two most abundance taxa, accounting for 66% of the community. MacNeil *et al.* (1997) described *Gammaridae* as quickly colonizing and thriving in disturbance prone environments, so their abundance may suggest physical disturbance stressors such as summer drying or storm scouring. *Elmidae* 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. Lastly, the MFBI metric was 74% in Greendale Creek compared to 86% in the reference, a modest, but statistically significant difference. Overall, benthic metrics in Greendale Creek point to a stressor that is eliminating sensitive species and increasing the abundance of a few tolerant species that thrive in disturbance prone and higher suspended solids environments.

- Rich Valley Unnamed Tributary – In the Rich Valley Unnamed Tributary, Species richness, % Ephem, % *Chironomidae,* and % 2 Dominant were all significantly lower ($p<0.05$) than in the reference. Rich Valley Unnamed Tributary averaged 12 total taxa compared to 15.5 at the reference. The % Ephem score in Rich Valley Unnamed Tributary averaged 27% compared to 85% in the reference. The % *Chironomidae* score averaged 80% compared to 92% in the reference. The % 2 Dominant score was 53% compared to 72% in the reference. The midge, *Chironomidae,* and the riffle beetle, *Elmidae,* were the two most abundance taxa, accounting for 63% of the community. 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. Similarly, *Elmidae* 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. Overall, benthic metrics in Rich Valley Unnamed Tributary point to nutrient or sediment enrichment that has reduced sensitive species and allowed sediment tolerant species to thrive.

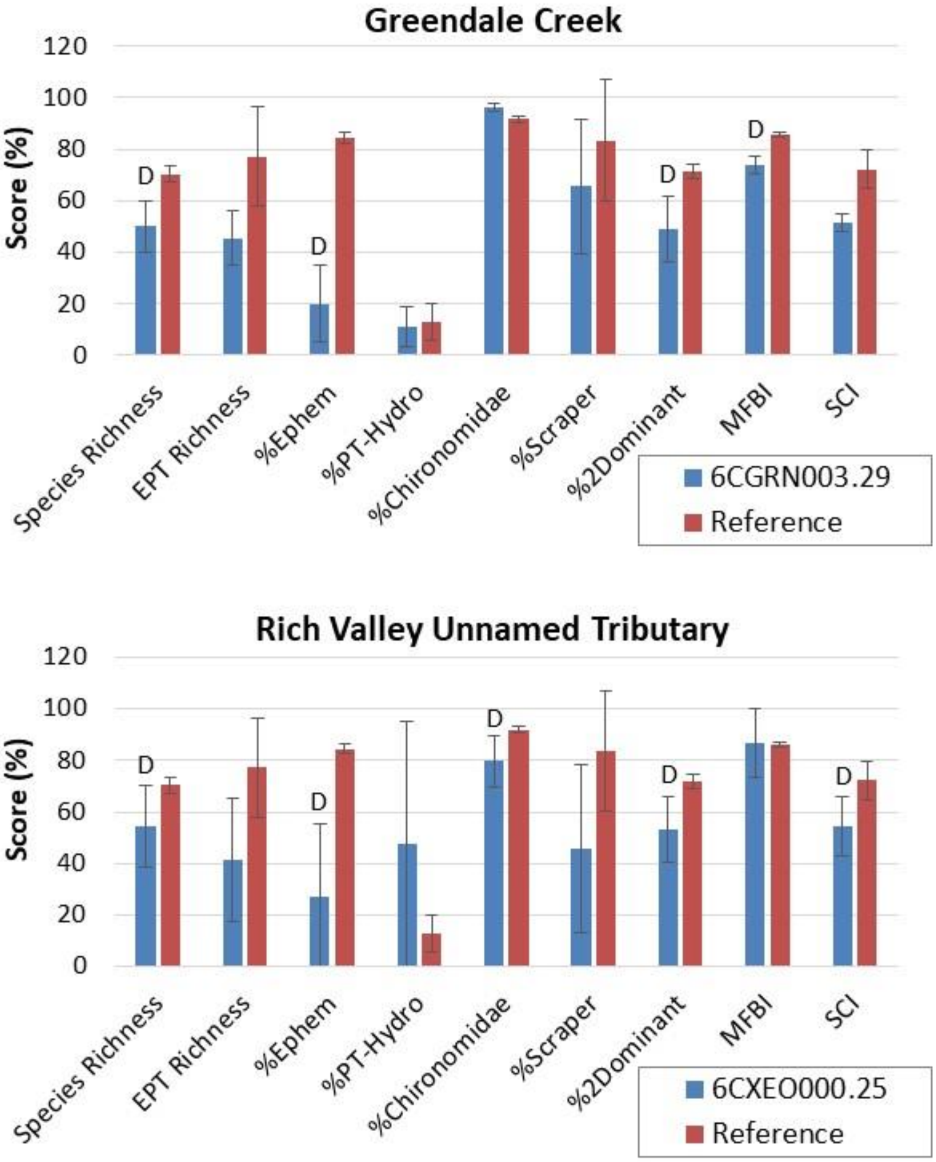


Figure 6. Individual metric scores comprising the stream condition index (SCI) in Greendale Creek and Rich Valley Unnamed Tributary. “D” indicates that the metric was significantly different (alpha = 0.05) from the benthic reference. The benthic reference was Cove Creek (6CCOV002.54).

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 Greendale Creek and Rich Valley Unnamed Tributary to a reference stream, Cover Creek (6CCOV002.54). In the reference stream, the community is dominated by sensitive Ephemeroptera (52%) with moderate representation of Coleoptera (22%) and Diptera (15%).

- Greendale Creek – Taxonomic composition in Greendale Creek was dominated by *Gammaridae* (representing the "Other" category and 36% of the community), Coleoptera (27%), and *Hydropsychidae* (15%). Greendale Creek also had fewer Ephemeroptera than the reference (12% compared to 52% in the reference). As previously mentioned, *Gammaridae* are indicative of disturbance prone environments, and *Elmidae*, the primary Coleoptera taxa, may indicate an early community response to suspended solids as a stressor. *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.
- Rich Valley Unnamed Tributary – The taxonomic composition of Rich Valley Unnamed Tributary is relatively well balanced, indicating only marginal impairment. No taxonomic category represented more than 25% of the community, and sensitive Ephemeroptera and Plecoptera taxa were well-represented, accounting for 17% and 21% of the community, respectively. The most prominent differences from the reference condition were a decrease in Ephemeroptera (17% compared to 52% in the reference) and an increase in Plecoptera (21% compared to 3% in the reference) and Diptera (24% compared to 15% in the reference). The tradeoff between decreasing Ephemeroptera and increasing Plecoptera do not necessarily signal any type of stressor, since both groups are sensitive to pollutants. The increase in Diptera, mostly *Chironomidae*, may signal an increase in sediment, since these organisms are collectors feeding off of deposited organic material.

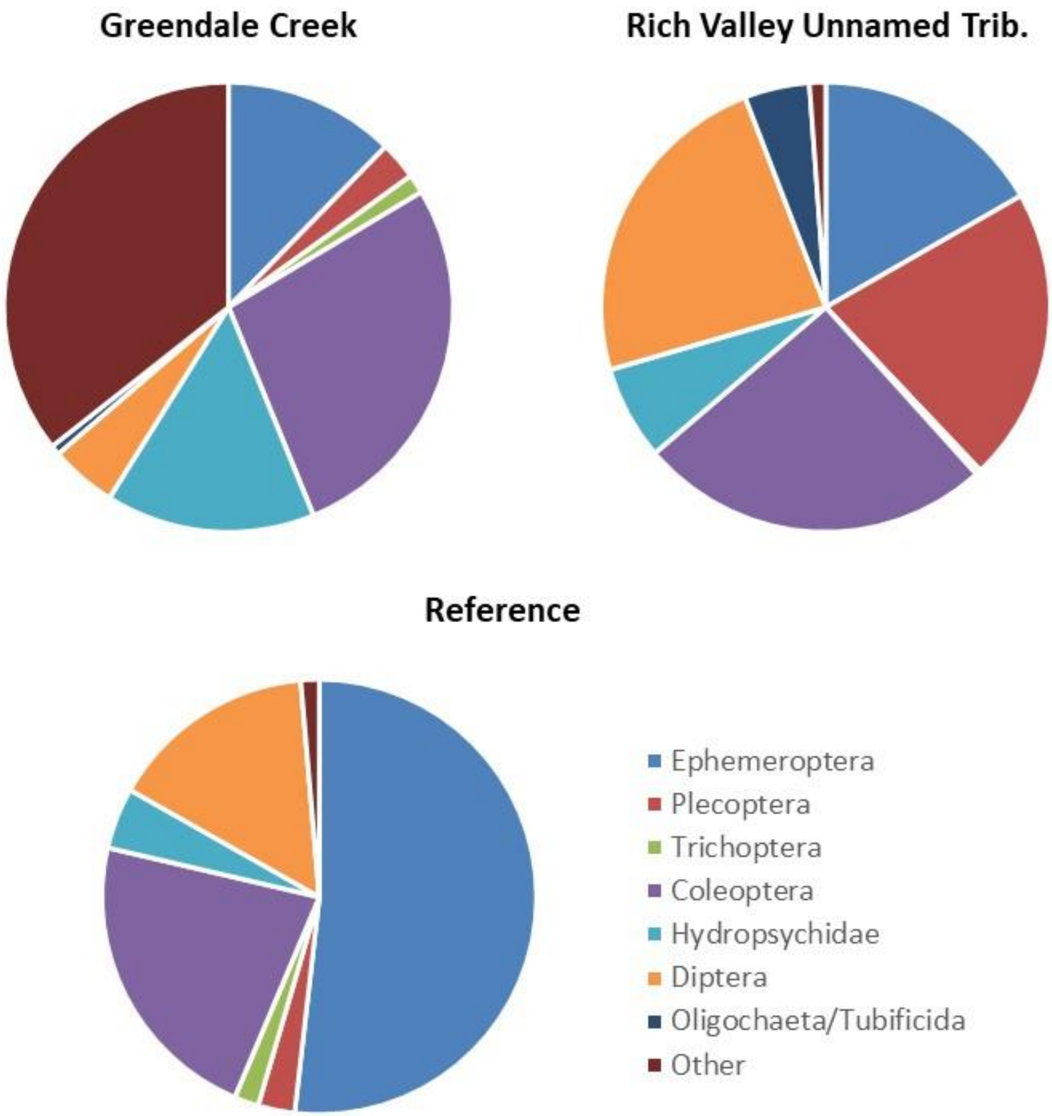


Figure 7. Taxonomic composition of Greendale Creek and Rich Valley Unnamed Tributary compared to a reference.

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 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.

Attribute	Explanation	Stressors Evaluated
I	Historically documented, sensitive, long-lived or regionally endemic taxa	Dissolved oxygen Acidity Alkalinity Specific Conductivity Chloride Sulfate Total Nitrogen and Phosphorus Total Habitat Relative Bed Stability %Imperviousness
II	Highly sensitive taxa	
III	Intermediate sensitive taxa	
IV	Intermediate tolerant taxa	
V	Tolerant taxa	
VI	Non-native taxa	

None of the top three predominant taxa in either of the impaired streams indicated a potential stressor. No BCG scores of 5 were present for these predominant organisms. This means that these species are not expected to increase in abundance in response to a particular stressor, but may be responding to multiple stressors.

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 Greendale Creek, nutrients (nitrogen and phosphorus) were ranked as the top stressor from BCG analysis. For Rich Valley Unnamed Tributary, relative bed stability (RBS), which is associated with sediment enrichment, was the highest ranked stressor. For both streams, nutrients, RBS, and sulfate were the top three stressors.

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

Stream	Station	Predominant Taxa	Diss. Oxy.	Acidity	Alkalinity	Spec. Cond.	Chloride	Sulfate	TN/TP	Total Habitat	RBS	% Imp.
Greendale Creek	6CGRN003.29	*Gammaridae*	4	4	4	4	4	4	4	4	4	4
		Elmidae	3	3	4	3	3	4	3	3	4	4
		Hydropsychidae	3	3	4	4	3	4	4	4	4	4
Rich Valley Unnamed Tributary	6CXEO000.25	*Elmidae*	3	3	4	3	3	4	3	3	4	4
		Chironomidae	4	4	4	4	4	4	4	4	4	4
		Leptophlebiidae	3	4	3	3	2	3	4	3	4	3

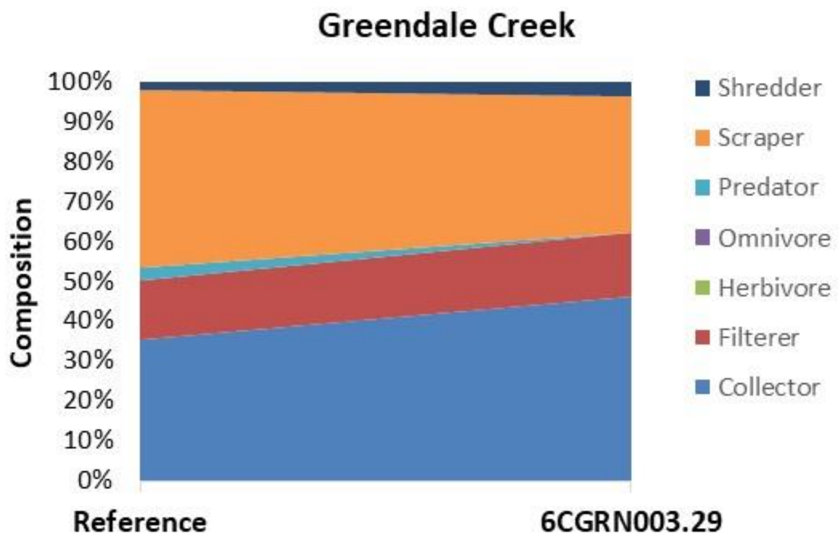
Table 7. Rank of average biological condition gradient scores for each stressor in each impaired stream.

Stream	Station	Diss. Oxy.	Acidity	Alkalinity	Spec. Cond.	Chloride	Sulfate	TN/TP	Total Habitat	RBS	% Imp.
Greendale Creek	6CGRN003.29	10	8	6	4	9	2	1	7	3	4
Rich Valley Unnamed Tributary	6CXEO000.25	9	5	8	4	10	3	2	6	1	7

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 8 shows the composition of functional feeding groups within Greendale Creek and Rich Valley Unnamed Tributary in comparison to a reference stream. In

Greendale Creek, benthic communities shifted to a higher percentage of collectors, while scrapers decreased. Collectors increased by 11%, while scrapers decreased by 11%. 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. The same pattern was observed in Rich Valley Unnamed Tributary, with collectors increasing by 7% and scrapers decreasing by 21%. This pattern is indicative of increased deposited sediment and deposited organic material. In addition, shredders increased by 22% in Rich Valley Unnamed Tributary. This change in functional feeding groups is likely not due to any particular stressor but due to the size and location of the stream. Rich Valley Unnamed Tributary is a very small (1-2 foot-wide) headwaters stream with the monitoring station located in a wooded area. The limited width and overhead tree canopy means that leaves are a readily available food source, providing an accommodating niche for shredders that break up leaf material.



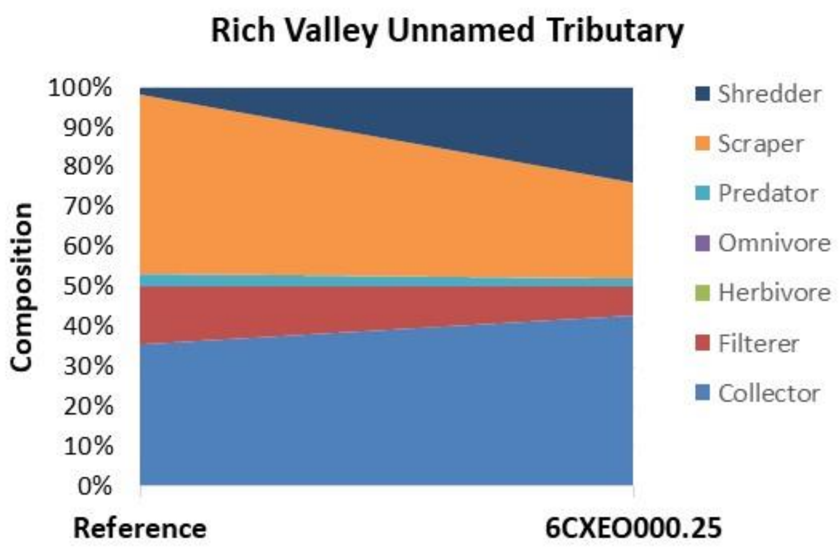


Figure 8. Functional feeding group composition in Greendale Creek and Rich Valley Unnamed Tributary compared to a reference.

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 9 compares the total habitat scores in Greendale Creek and Rich Valley Unnamed Tributary with those from a reference stream (Cove Creek). Median total habitat scores were 148 in the reference, and were 119 and 109 in Greendale Creek and Rich Valley Unnamed Tributary, respectively. Total habitat scores were not statistically lower ($p<0.05$ in a one-tailed t-test with unequal variance) than the reference, but data sets were relatively small (2-7 data points).

Based on VDEQ’s analysis of probabilistic monitoring data (VDEQ, 2017), the colors shown in Figure 9 represent the probability of habitat being a stressor on the aquatic community. Both impaired streams fell in the medium probability range, while the reference was in the low probability range.

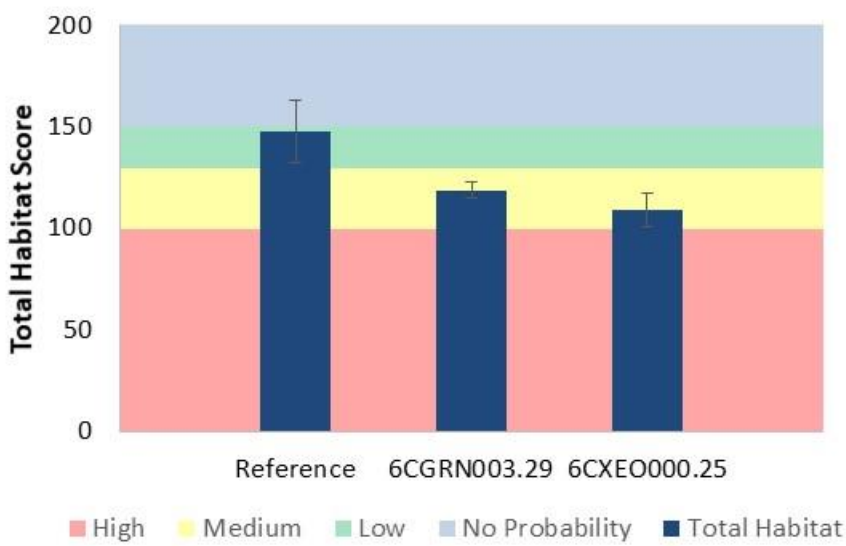


Figure 9. Total habitat scores for Greendale Creek and Rich Valley Unnamed Tributary compared to a reference. Streams with a "D" have statistically lower habitat scores than the reference site. Colors represent the probability that data within that range would be responsible for causing stress.

Figure 10 compares the individual habitat metrics in each impaired stream with metric scores from the reference station. Statistical differences were determined using a one-tailed t-test with unequal variances and alpha = 0.05. Individual metrics are generally categorized into those that indicate degraded bank conditions (bank stability, channel alteration, bank vegetation, or riparian vegetation), those that indicate degraded instream habitat conditions (substrate, embeddedness, or sediment), and those that indicate degraded hydrologic conditions (flow, riffles, or velocity/depth).

- Greendale Creek – In Greendale Creek, habitat metrics for channel alteration 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).
- Rich Valley Unnamed Tributary – In Rich Valley Unnamed Tributary, habitat metrics for substrate, flow, and velocity/depth were significantly lower than the reference. These lower metric scores represent both deposited sediment impacts (substrate) and degraded hydrologic condition (flow and velocity/depth). Rich Valley Unnamed Tributary is a very small stream, and flow conditions may decrease dissolved oxygen and limit ecological health during dry periods.

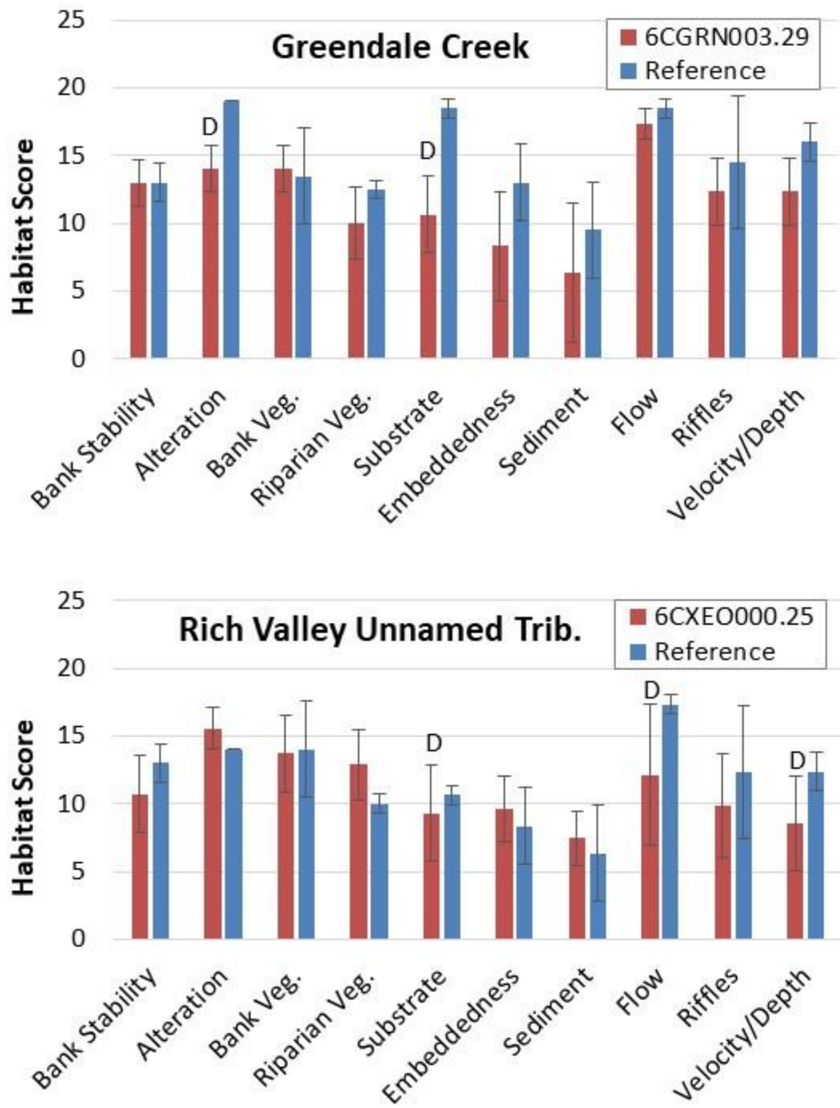


Figure 10. Habitat metric scores for Greendale Creek and Rich Valley Unnamed Tributary compared to a reference. Metrics with a "D" are statistically lower than the reference site.

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 the medium probability range for stressor effects.

Table 8 shows the results of relative bed stability analysis in Greendale Creek and Rich Valley Unnamed Tributary Project streams. Bottom substrate in both impaired streams was higher in sands and fines (39-59%) than in the unimpaired reference (25%). Embeddedness was much higher in Greendale Creek (73%) than in the reference (38%). Embeddedness was only marginally higher in Rich Valley Unnamed Tributary (48%). LRBS indices were in the high probability range for stressor effects in Greendale Creek (-1.51) and the medium probability range for Rich Valley Unnamed Tributary (-1.07). This indicates that excess sediment may be a stressor in these streams.

Table 8. Log relative bed stability index for Greendale Creek and Rich Valley Unnamed Tributary.

Stream	Station	Date	Slope	% Sand and Fines	Embeddedness (%)	Log Relative Bed Stability Index (LRBS)[1]
Cove Creek	6CCOV002.54	10/15/2012	0.45	25	38	0.32
Greendale Creek	6CGRN003.29	10/1/2007	0.65	59	73	-1.51
Rich Valley Unnamed Tributary	6CXEO000.25	10/22/2009	2.71	39	48	-1.07

[1] Values in blue are in the no probability range for stressor effects. Values in yellow are in the medium probability range for stressor effects, and values in red are in the high probability range.

2.3. Land Cover Assessment

While a more detailed land cover assessment will be part of the Greendale Creek and Rich Valley Unnamed Tributary 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. The Cove Creek reference station was dominated by forest (73%), while the impaired stations were dominated by pasture (42-43%) and forest (32-45%) with a mixture of residential grasses and trees. Impervious areas were 1% in the reference, 2% in Rich Valley Unnamed Tributary, and 4% in Greendale Creek.

Regression analyses were used to compare these land cover trends to benthic SCI scores at the respective stations. While none of the land cover parameters exhibited a statistically significant regression (likely due to a small data set), the strongest correlations with SCI scores were with forest and pasture land covers (Table 10). SCI scores were positively correlated with forest area in the watershed and negatively correlated with pasture. Higher SCI scores were associated with a more forested watershed, and SCI scores decreased as pasture replaced forest in the impaired watersheds.

Table 9. Land cover 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
Cove Creek	6CCOV002.44	0.00%	1.25%	0.00%	73.22%	4.37%	0.77%	0.31%	1.94%	18.04%	0.00%	0.10%
Greendale Creek	6CGRN003.29	0.17%	4.12%	0.00%	32.09%	8.68%	1.61%	1.81%	8.33%	43.18%	0.00%	0.01%
Rich Valley Unnamed Tributary	6CXEO000.25	0.00%	2.13%	0.00%	44.85%	6.96%	0.00%	0.00%	4.15%	41.91%	0.00%	0.00%

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	-0.99598	N	0.99	0.07
Forest	0.985015	N	0.98	0.1
Urban/Res. Trees	-0.96307	N	0.94	0.16
Other	0.963815	N	0.92	0.19
Urban/Res. Grass	-0.84476	N	0.73	0.35
Impervious	-0.81991	N	0.69	0.37
Water	-0.61143	N	0.4	0.57
Harvested/Disturbed	-0.47727	N	0.24	0.67
Scrub/Shrub	-0.15685	N	0.03	0.89
Barren	-	-	-	-
Cropland	-	-	-	-

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. Stressor thresholds were based on VDEQ's analysis of probabilistic monitoring data from across the state (VDEQ, 2017). By comparing the patterns of benthic impairment with concentrations of a variety of water quality parameters, VDEQ established estimated thresholds for impairment. The thresholds represent concentrations where there is no probability of impairment, low probability, medium probability, and high probability of impairment. These thresholds can be useful in determining whether a water quality parameter may be responsible for impairment.

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 streams and the Cove Creek reference (Figure 11). Temperatures obviously vary by season, so ranges are wide when year-round measurements are considered. Neither of the impaired streams had statistically higher temperatures than the reference station ($p<0.05$ in t-test with unequal variances), and neither stream had temperature measurements above the water quality standard. Greendale Creek has a stocked trout designation, so the water quality standard is 21°C. The maximum temperature in Greendale Creek was 16.55°C, well below the standard (Figure 12). In Rich Valley Unnamed

Tributary, the water quality standard is 31°C, and the maximum observed temperature was 20.54°C.

VDEQ also collected diurnal temperature at each of the primary benthic stations during the summer of 2022. Diurnal data were collected at 15-minute intervals for 8 to 14 days at each location. Temperature data during diurnal deployments are shown in Figure 13. Diurnal temperatures exhibited the natural cycle of increases during the day from solar heating and decreases at night. Neither station exceeded the Virginia water quality standard (21ºC in Greendale Creek and 31ºC in Rich Valley Unnamed Tributary) during this late summer critical period. The maximum temperature in Greendale Creek was 16.4ºC, and the maximum temperature in Rich Valley Unnamed Tributary was 21.8ºC. This is an indication that temperature is not likely a stressor in these streams.

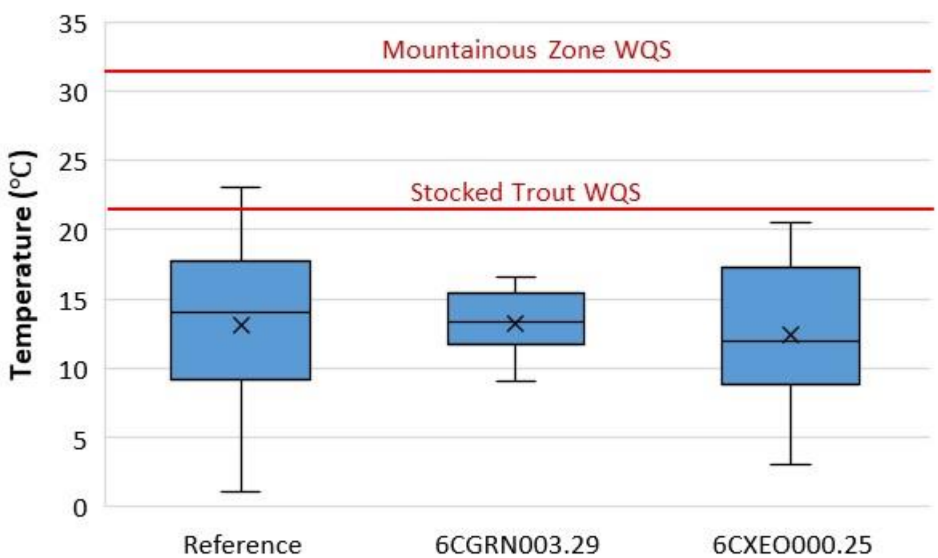
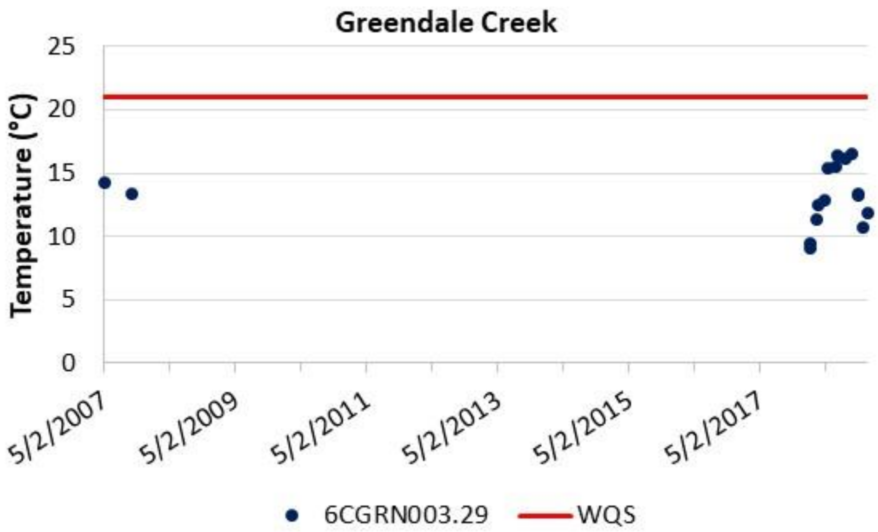


Figure 11. Temperature in Greendale Creek and Rich Valley Unnamed Tributary. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represents the mean. The "D" indicates a statistically significant difference from the reference station. The red line represents the Virginia water quality standard.



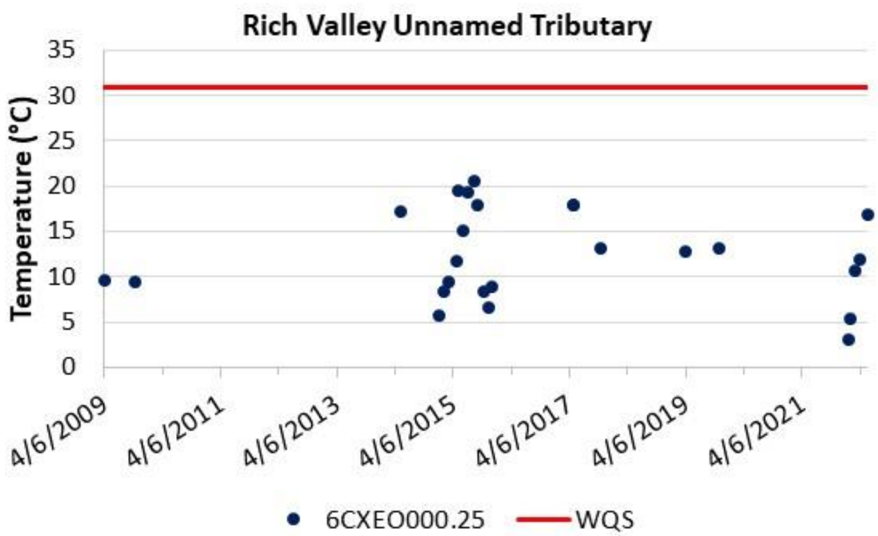
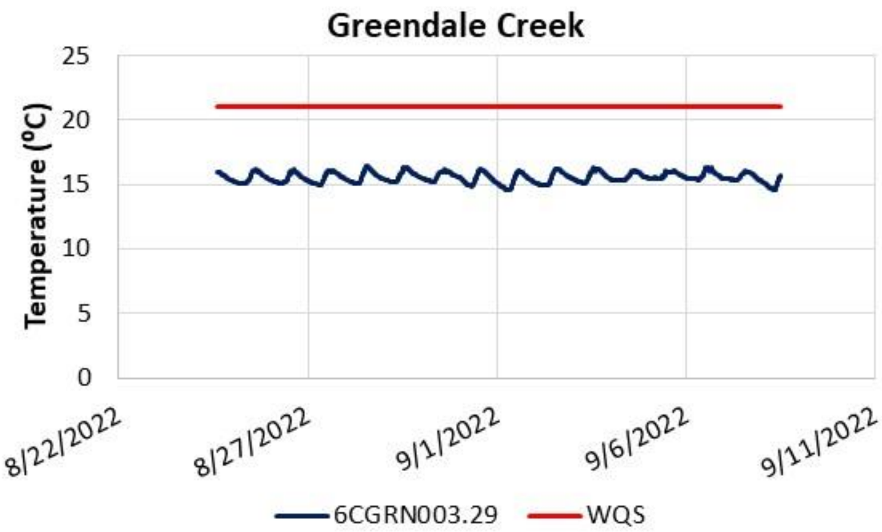


Figure 12. Temperature over time in Greendale Creek and Rich Valley Unnamed Tributary. The red line represents the Virginia water quality standard.



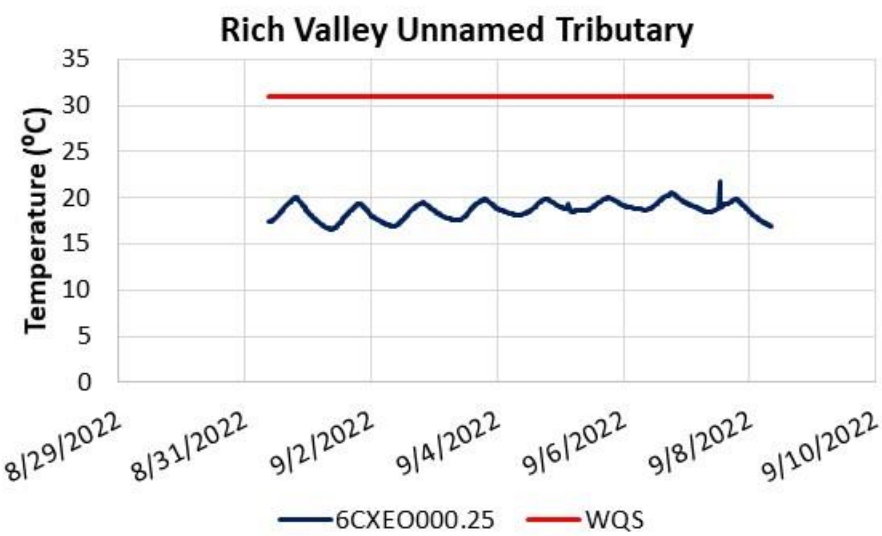


Figure 13. Diurnal temperature in Greendale Creek and Rich Valley Unnamed Tributary. The red line represents the Virginia water quality standard.

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 streams and the Cove Creek reference (Figure 14). Measured pH values were moderately alkaline in each of the streams with averages of 7.86 in Greendale Creek, 7.94 in Rich Valley Unnamed Tributary, and 8.14 in the Cove Creek reference. Both of the impaired streams had statistically lower pH than the reference site ($p<0.05$ in t-test with unequal variance), but median pH values at both sites were within the low probability range for stressor effects. Figure 15 shows the time series of pH values in Greendale Creek and Rich Valley Unnamed Tributary. 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 Greendale Creek or Rich Valley Unnamed Tributary.

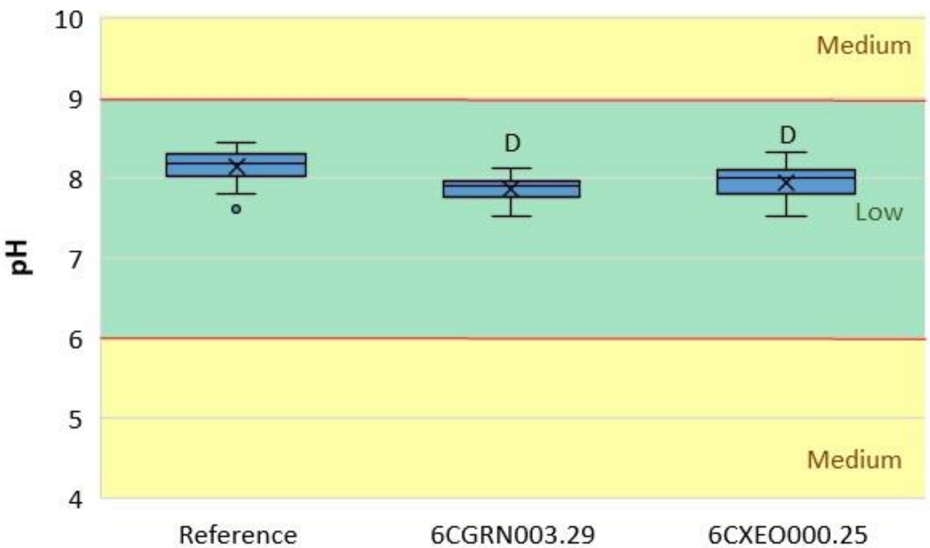
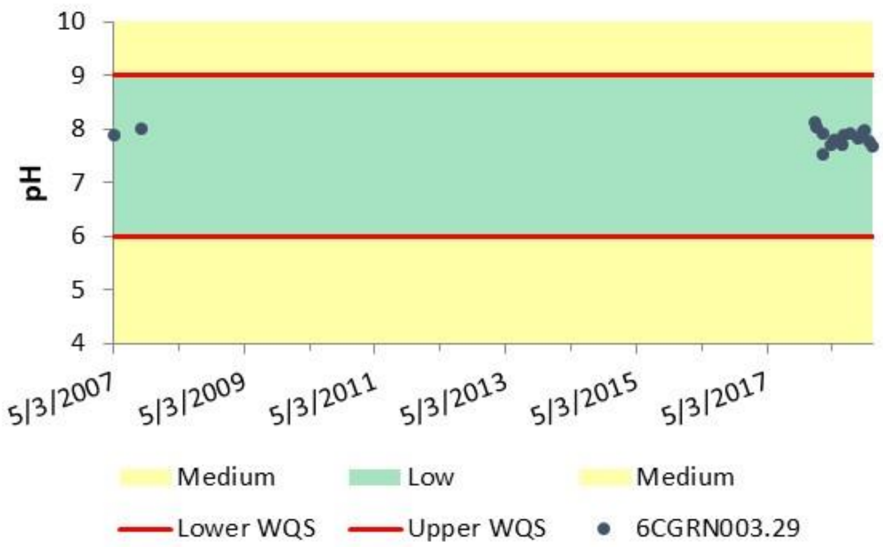


Figure 14. pH in Greendale Creek and Rich Valley Unnamed Tributary. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represents the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile 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.



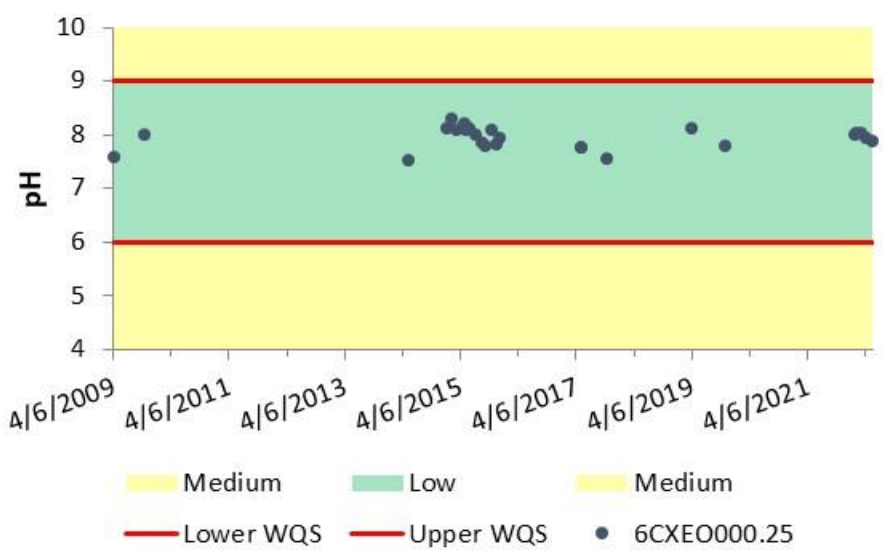


Figure 15. pH over time in Greendale Creek and Rich Valley Unnamed Tributary. The red line represents the Virginia water quality standard. Colors represent the probability that data within that range would be responsible for causing stress.

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 streams and the Cove Creek reference (Figure 16). Dissolved oxygen averaged 10.17 mg/L in Cove Creek, 9.39 mg/L in Greendale Creek, and 9.22 mg/L in Rich Valley Unnamed Tributary. Dissolved oxygen was statistically lower in Greendale Creek and Rich Valley Unnamed Tributary than in the reference ($p<0.05$ in t-test with unequal variance), however, median DO values were in the low probability range for stressor effects in both streams.

Figure 17 shows the time series of DO concentrations in each impaired stream. Greendale Creek had no DO excursions into the high probability range for stressor effects, but three DO values (12%) in Rich Valley Unnamed Tributary were in the high probability range. The minimum DO in Rich Valley Unnamed Tributary was 6.62 mg/L. No DO values were below the average 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 2022. Diurnal data were collected at 15-minute intervals for 8 to 14 days at each location. 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 August through early September, because critical dissolved oxygen levels are more common during the hot and dry summer months.

Dissolved oxygen data during diurnal deployments are shown in Figure 18. Diurnal dissolved oxygen values at both 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 Greendale Creek were very high and exclusively in the low probability range for stressor effects. Dissolved oxygen averaged 9.56 mg/L in Greendale Creek, with a nighttime minimum of 9.24 mg/L. In Rich Valley Unnamed Tributary, DO levels were much lower. Dissolved oxygen averaged 5.49 mg/L in Rich Valley Unnamed Tributary, with a nighttime minimum of 4.70 mg/L. Dissolved oxygen values were exclusively in the high probability range for stressor effects. Nighttime DO dropped below 5 mg/L on 4 out of 8 days, but daily averages were never below the average water quality standard of 5.0 mg/L or the instantaneous water quality standard of 4.0 mg/L.

Figure 19 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. Neither of the streams exhibited large swings of DO indicative of nutrient enrichment and excess algal growth, however, DO was consistently low in Rich Valley Unnamed Tributary, which can suppress the magnitude of diurnal swings. In Greendale Creek, DO was consistently near saturation, fluctuating only between 94 and 99% saturation. In Rich Valley Unnamed Tributary, percent saturation was much lower, fluctuating between 52 and 69%. The pattern of low DO in Rich Valley Unnamed Tributary may indicate nutrient enrichment in addition to other sources of chronic low DO. The monitoring station on this stream is very close to the spring source of the stream and connected wetlands. It is likely that the low DO in Rich Valley Unnamed Tributary is influenced by these contributing factors. Spring-sourced water may be naturally low in DO if the water has not been at the surface for an extended period of time. Wetlands also contribute to low DO because of increased biological activity and stagnant flow conditions. This explanation is further supported by the diurnal data that showed increases in DO during several storm events. Figure 19 compares the patterns of conductivity and DO in Rich Valley Unnamed Tributary. This figure shows that during rain events on 9/5/22 and 9/7/22, conductivity dropped and DO increased. These two days represented the highest peaks in DO in Rich Valley Unnamed Tributary. This emphasizes that when flow in the stream is dominated by the spring source, DO is low, but when surface runoff increases (indicated by lower conductivity), DO also increases.

In summary, dissolved oxygen is not likely a stressor in Greendale Creek, but may be a stressor in Rich Valley Unnamed Tributary. In Greendale Creek, DO values were exclusively in the 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 5.0 mg/L. In Rich Valley Unnamed Tributary, diurnal DO values were exclusively in the high probability range for stressor effects, and minimum DO excursions at nighttime were below 5.0 mg/L. This low DO in Rich Valley Unnamed Tributary may be due to a combination of nutrient enrichment and natural contributing factors of proximity to the spring source and connected wetlands.

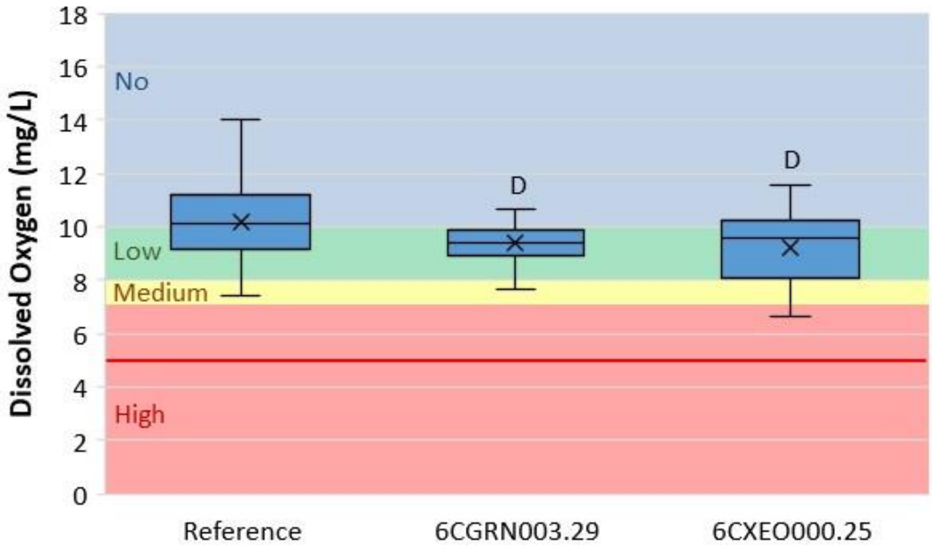


Figure 16. Dissolved oxygen in Greendale Creek and Rich Valley Unnamed Tributary. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represents the mean. Dots represent outliers that are greater than 1.5 times 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.

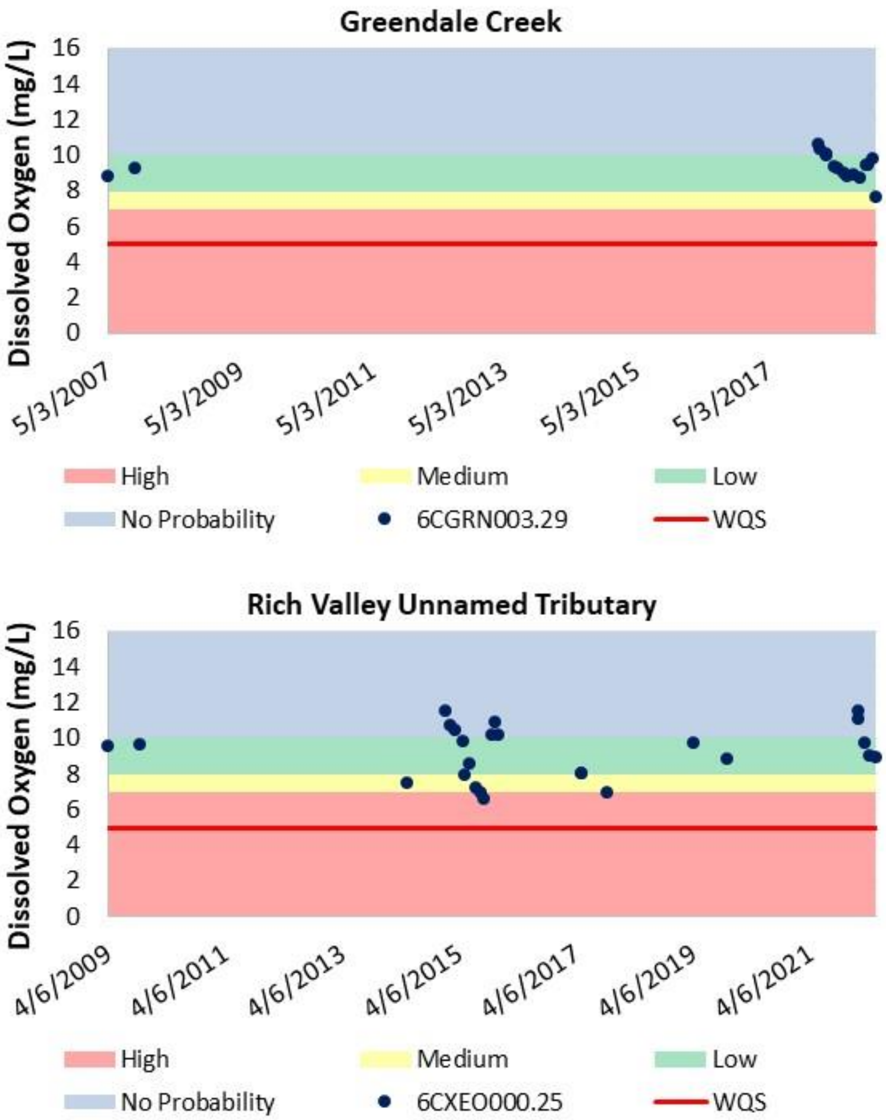


Figure 17. Dissolved oxygen over time in Greendale Creek and Rich Valley Unnamed Tributary. The red line represents the Virginia water quality standard. Colors represent the probability that data within that range would be responsible for causing stress.

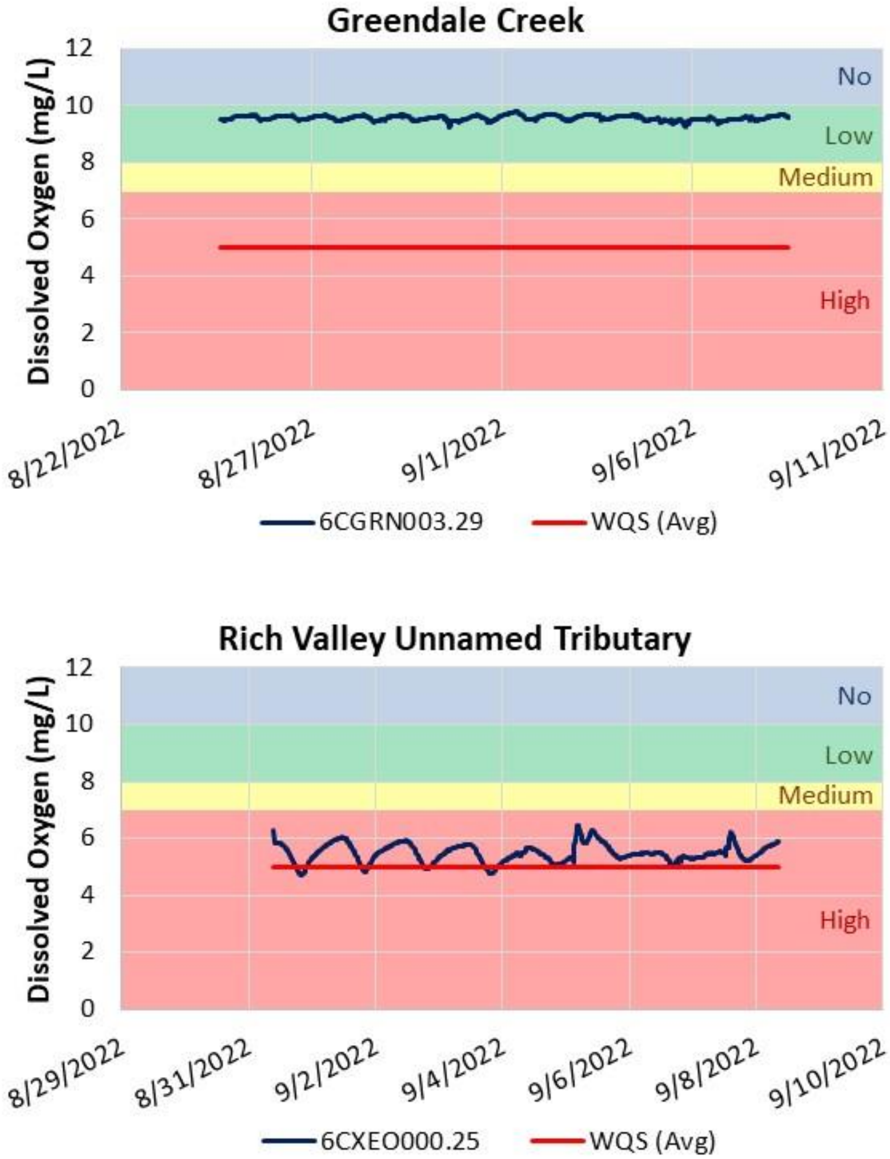
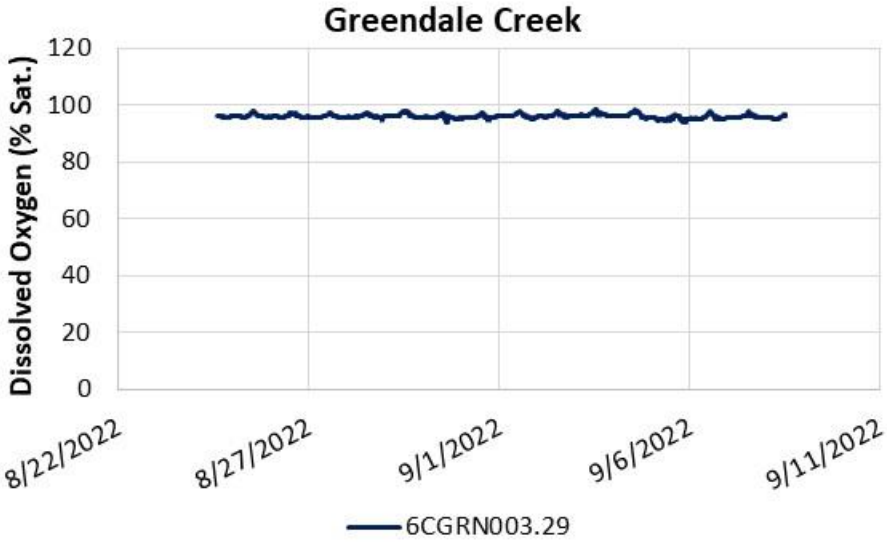


Figure 18. Diurnal dissolved oxygen in Greendale Creek and Rich Valley Unnamed Tributary. The red line represents the Virginia water quality standard. Colors represent the probability that data within that range would be responsible for causing stress.



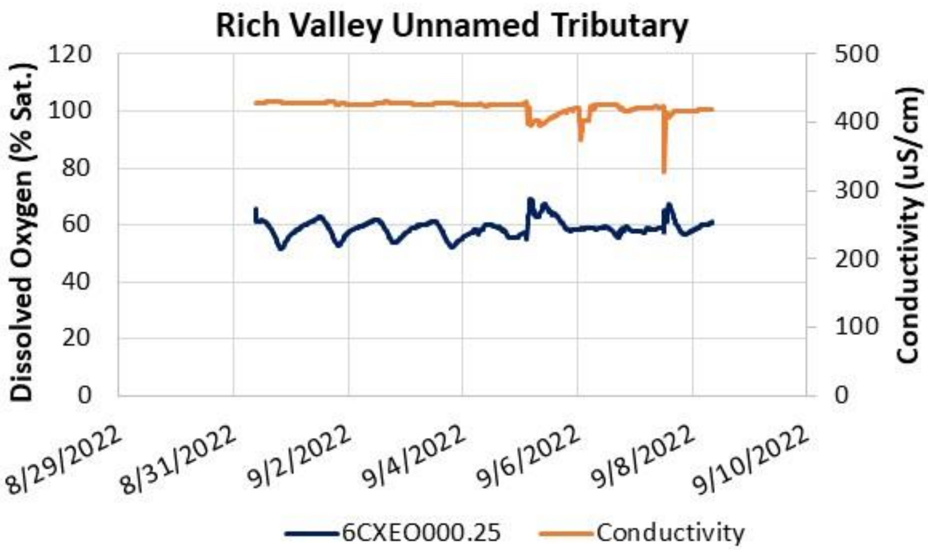


Figure 19. Diurnal dissolved oxygen in Greendale Creek and Rich Valley Unnamed Tributary expressed as percent saturation. The Rich Valley Unnamed Tributary graph also compares conductivity patterns with dissolved oxygen patterns.

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 each of the impaired streams and the Cove Creek reference (Figure 20). Conductivity averaged 439 uS/cm in Greendale

Creek and 402 uS/cm in Rich Valley Unnamed Tributary, compared to 339 uS/cm in the reference. Both impaired streams had statistically higher conductivity ($p<0.05$ in t-test with unequal variance) than the reference site, and median conductivity in both streams was in the medium probability range for stressor effects.

Figure 21 shows conductivity levels over time in Greendale Creek and Rich Valley Unnamed Tributary. Conductivity varied over time, likely due to rainfall and flow conditions. A single conductivity measurement (6%) in Greendale Creek was in the high probability range for stressor effects, and two conductivity measurements (8%) in Rich Valley Unnamed Tributary were in the high probability range.

In addition to periodic conductivity measurements, VDEQ collected diurnal conductivity data at each of the primary benthic stations during the summer of 2022. Diurnal data were collected at 15-minute intervals for 8 to 14 days at each location. Conductivity data during diurnal deployments are shown in Figure 22. In both streams, conductivity was consistently in the medium probability range for stressor effects and did not exceed 451 uS/cm.

Total dissolved solids (TDS) are closely tied to conductivity, since it is the dissolved ions that transmit electrical current. Figure 23 shows TDS levels in Greendale Creek and Rich Valley Unnamed Tributary in comparison to the Cove Creek reference. TDS averaged 236 mg/L in Greendale Creek and 220 mg/L in Rich Valley Unnamed Tributary. These values could not be statistically compared to the reference since only a single TDS measurement (207 mg/L) was available from Cove Creek. Median TDS values in both impaired streams were in the low probability range for stressor effects. Figure 24 shows TDS levels over time in Greendale Creek and Rich Valley Unnamed Tributary. TDS values varied over time, but no values were in the high probability range for stressor effects.

In summary, conductivity and TDS may be stressors in Greendale Creek and Rich Valley Unnamed Tributary. Median conductivity values were in the medium probability range for stressor effects, but median TDS values were in the low probability range.

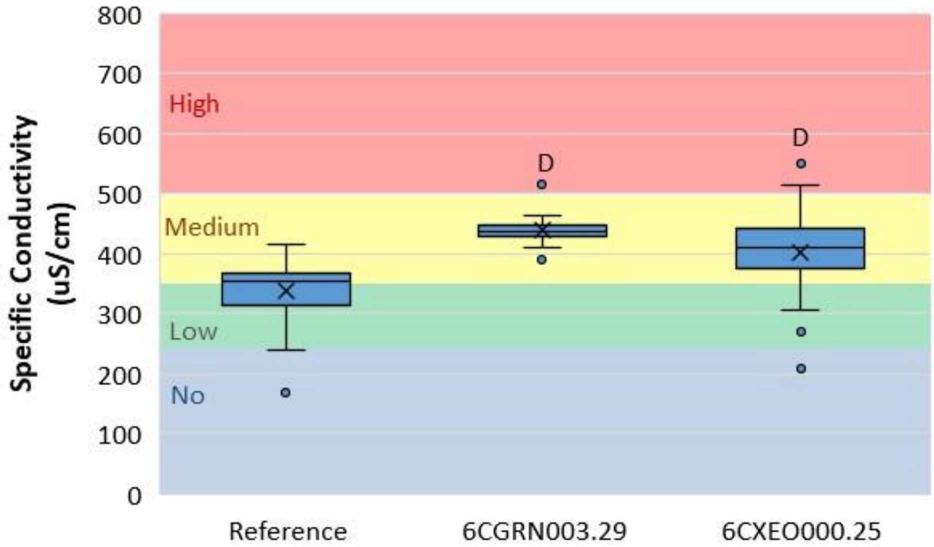
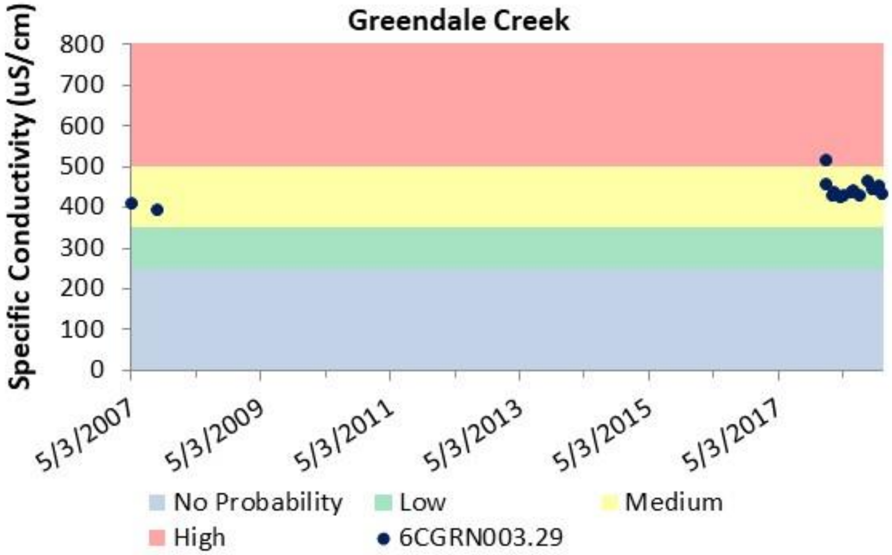


Figure 20. Conductivity in Greendale Creek and Rich Valley Unnamed Tributary. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represents the mean. Dots represent outliers that are greater than 1.5 times 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.



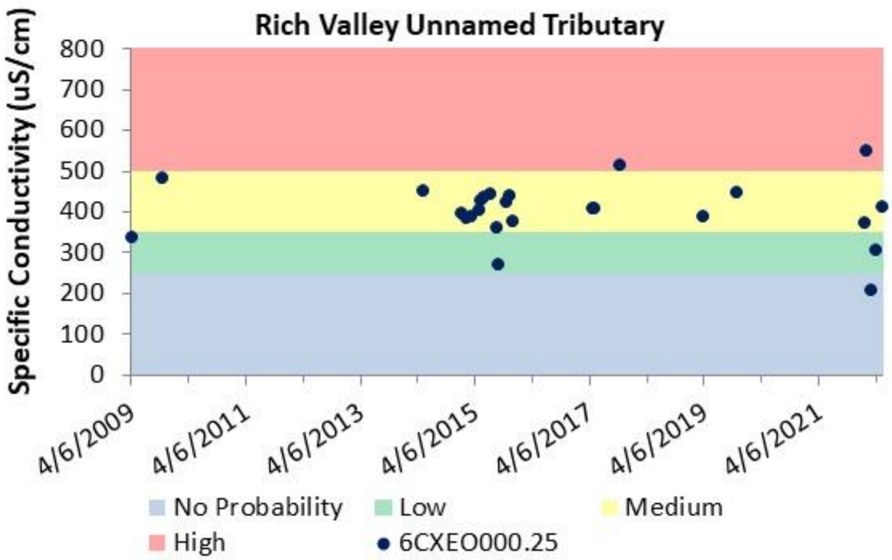
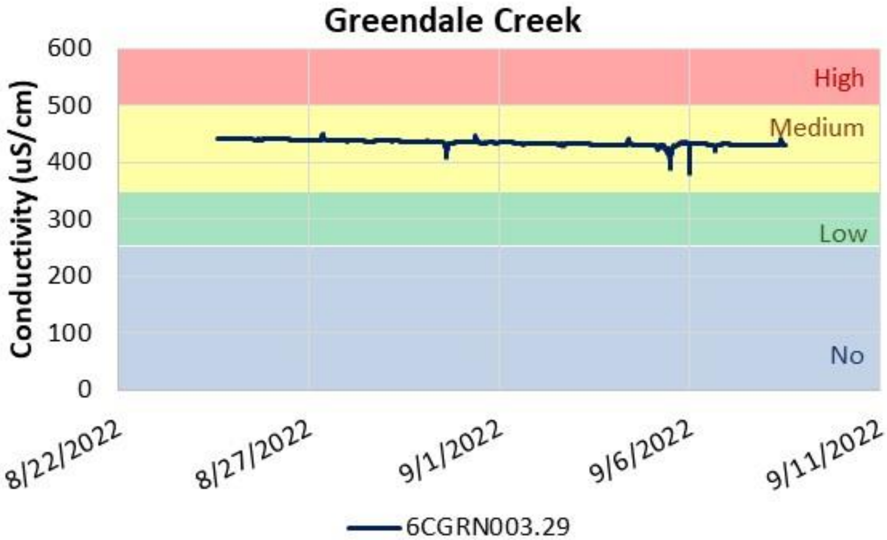


Figure 21. Conductivity over time in Greendale Creek and Rich Valley Unnamed Tributary. Colors represent the probability that data within that range would be responsible for causing stress.



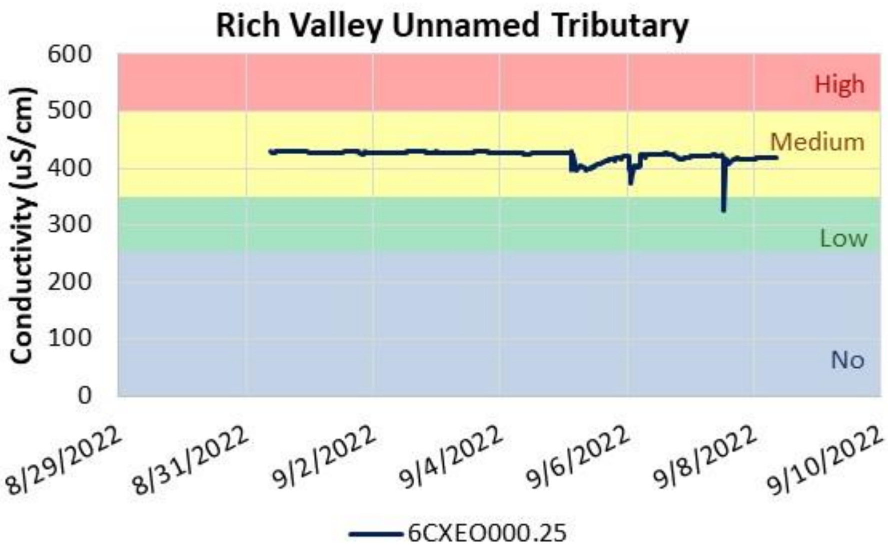


Figure 22. Diurnal conductivity in Greendale Creek and Rich Valley Unnamed Tributary. Colors represent the probability that data within that range would be responsible for causing stress.

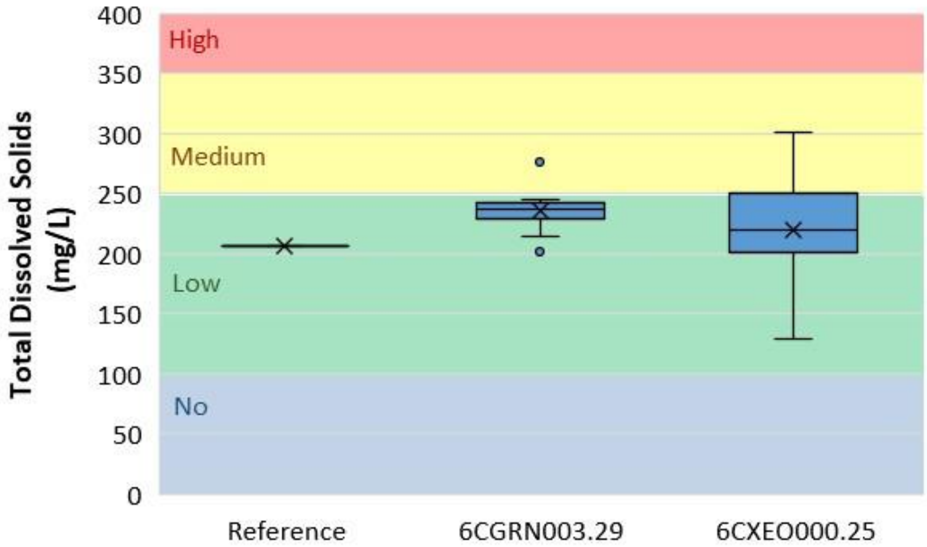
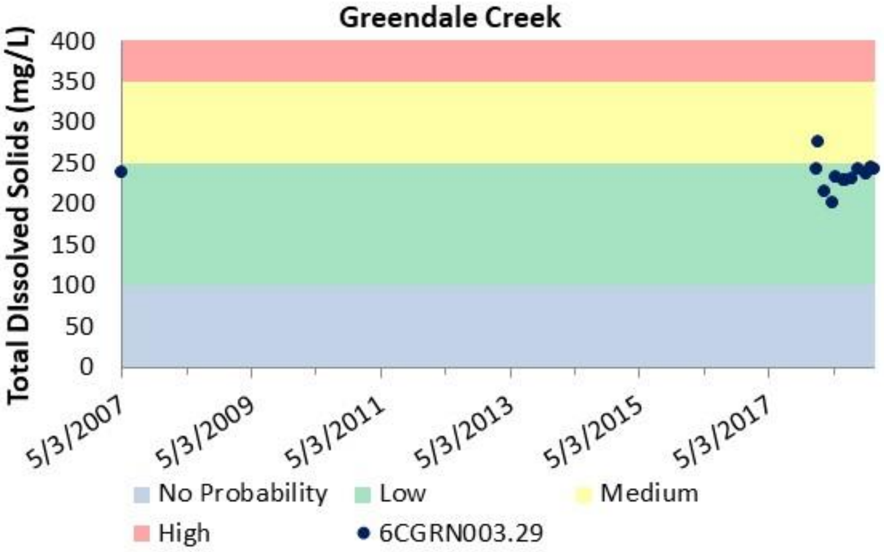


Figure 23. Total dissolved solids in Greendale Creek and Rich Valley Unnamed Tributary. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represents the mean. Dots represent outliers that are greater than 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.



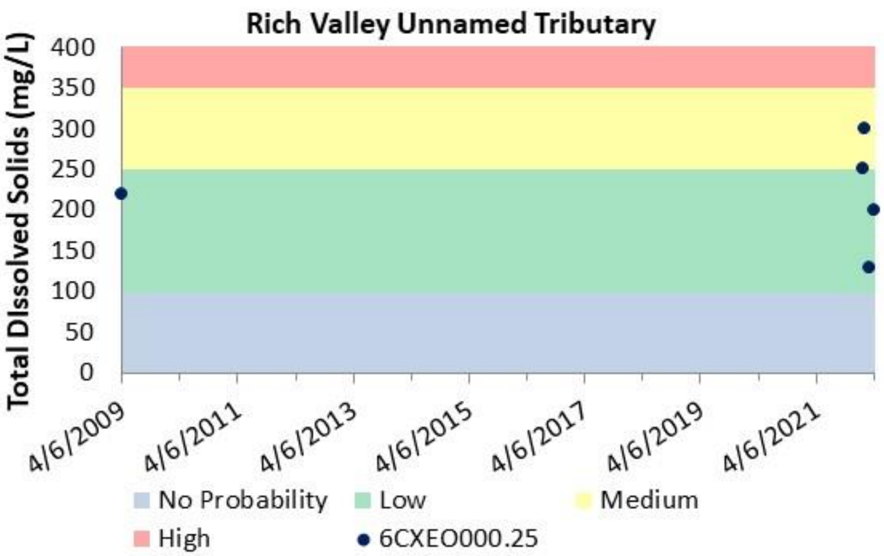


Figure 24. Total dissolved solids over time in Greendale Creek and Rich Valley Unnamed Tributary. Colors represent the probability that data within that range would be responsible for causing stress.

2.4.5. Dissolved Ions

Dissolved sodium, potassium, chloride, and sulfate were measured in Greendale Creek and Rich Valley Unnamed Tributary (Figure 25). Dissolved sodium concentrations averaged 5.69 mg/L in Greendale Creek and 13.0 mg/L in Rich Valley Unnamed Tributary. This was higher than in the Cove Creek reference (3.67 mg/L), but values could not be statistically compared based on a small sample size in the reference. Median dissolved sodium values in both impaired streams were in the no to low probability range for stressor effects. A single excursion into the high probability range was observed in Rich Valley Unnamed Tributary with a maximum concentration of 44.3 mg/L.

Dissolved potassium concentrations averaged 1.94 mg/L in Greendale Creek and 5.85 mg/L in Rich Valley Unnamed Tributary. This was higher than in the Cove Creek reference (1.81 mg/L), but values could not be statistically compared based on a small sample size in the reference. Median dissolved potassium values were in the low probability range for stressor effects in Greendale Creek and were in the medium probability range in Rich Valley Unnamed Tributary. No excursions into the high probability range were observed in either of the two impaired streams.

Dissolved chloride concentrations averaged 11.9 mg/L in Greendale Creek and 24.1 mg/L in Rich Valley Unnamed Tributary. This was higher than in the Cove Creek reference (5.6 mg/L), but values could not be statistically compared based on a small sample size in the reference. Median dissolved chloride values were in the no to low probability range for stressor effects in both of the impaired streams. A single excursion into the high probability range was observed in Rich Valley Unnamed Tributary with a maximum concentration of 81.6 mg/L. This is still well below the Virginia water quality criteria for chloride of 230 mg/L.

Dissolved sulfate concentrations averaged 4.21 mg/L in Greendale Creek and 8.92 in Rich Valley Unnamed Tributary. Unlike the other ions, sulfate was higher in the Cove Creek reference (11.4 mg/L). Median dissolved sulfate values were in the no to low probability range for stressor effects in both impaired streams, and no excursions into the high probability range were observed.

In summary, the potentially toxic ions chloride, potassium, sodium, and sulfate are not likely to be stressors in Greendale Creek or Rich Valley Unnamed Tributary. None of the streams had median concentrations of any of the dissolved ions in the high probability range for stressor effects. Rich Valley Unnamed Tributary had median dissolved potassium concentrations in the medium probability range for stressor effects, but maximum values of all ions 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 either 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).

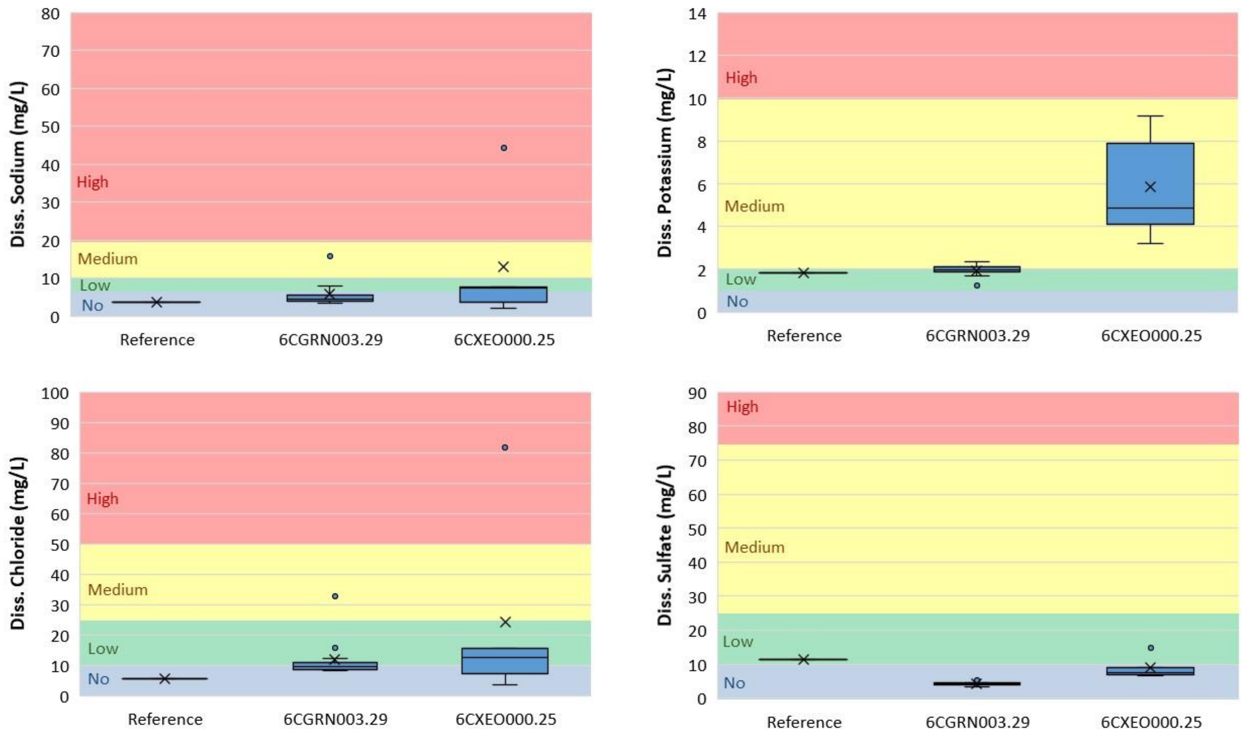


Figure 25. Dissolved ions in Greendale Creek and Rich Valley Unnamed Tributary. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represents the mean. Dots represent outliers that are greater than 1.5 times 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 26 shows total suspended solids (TSS) measured in Greendale Creek, Rich Valley Unnamed Tributary, and the Cove Creek reference. While sufficient data was available for the reference, only a single TSS sample was collected from each of the impaired streams. In those single samples, TSS was 18 mg/L in Greendale Creek and 42 mg/L in Rich Valley Unnamed Tributary, considerably higher than in the reference, which averaged 8 mg/L.

In addition to TSS concentrations, turbidity levels were measured in Greendale Creek and Rich Valley Unnamed Tributary on one occasion (Figure 27). Turbidity was 4.8 NTU in Greendale

Creek and 21.1 NTU in Rich Valley Unnamed Tributary. The reference averaged 6.2 NTU, comparable to the Greendale Creek value, but lower than the Rich Valley Unnamed Tributary value.

In addition to periodic TSS and turbidity measurements, VDEQ collected diurnal turbidity data at the Greendale Creek benthic station during the summer of 2022. Diurnal data were collected at 15-minute intervals for 14 days at station 6CGRN003.29. During the diurnal deployment, turbidity was generally very low in Greendale Creek, averaging 2.2 NTU. One large spike of 137 NTU turbidity was observed on 9/6/22. This was likely due to a storm event, since conductivity measurements during this same time period dropped sharply.

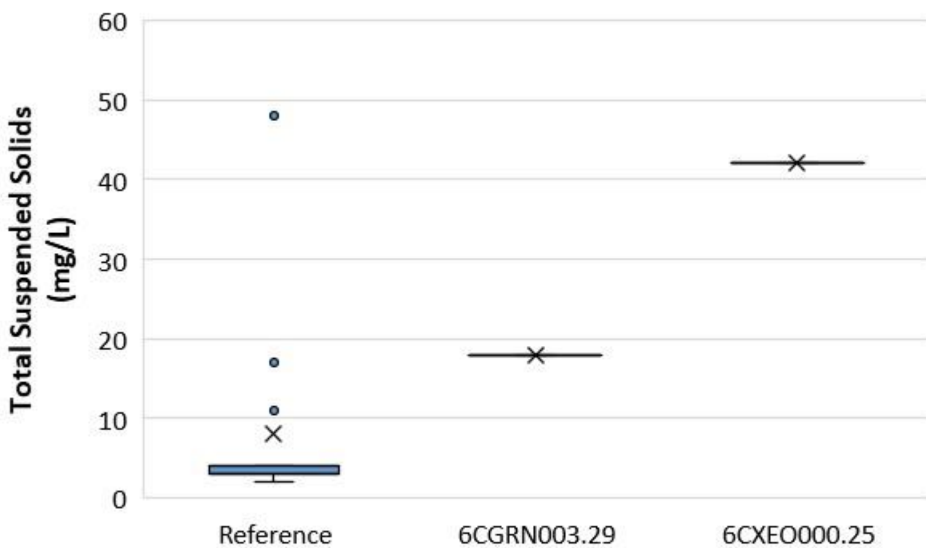


Figure 26. Total suspended solids in Greendale Creek and Rich Valley Unnamed Tributary. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represents the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station.

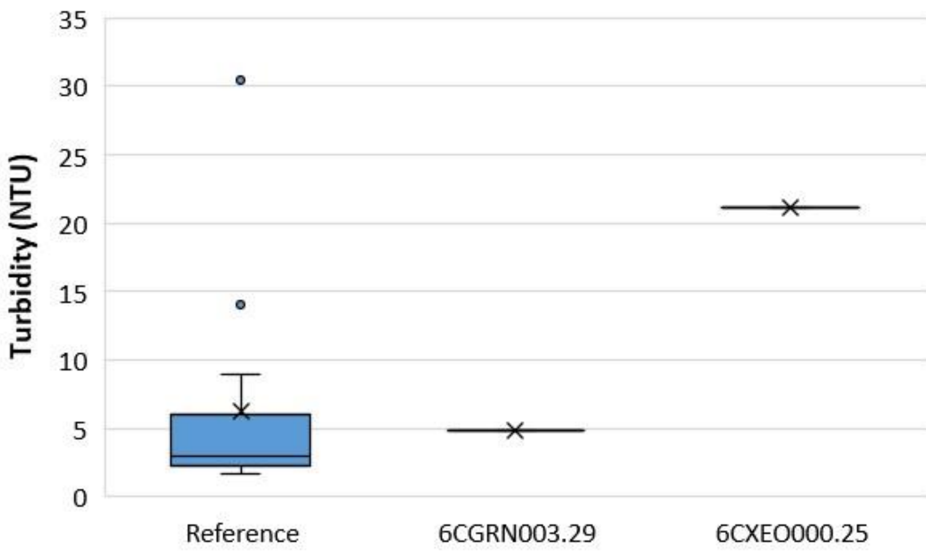


Figure 27. Turbidity in Greendale Creek and Rich Valley Unnamed Tributary. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represents the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. The "D" indicates a statistically significant difference from the reference station.

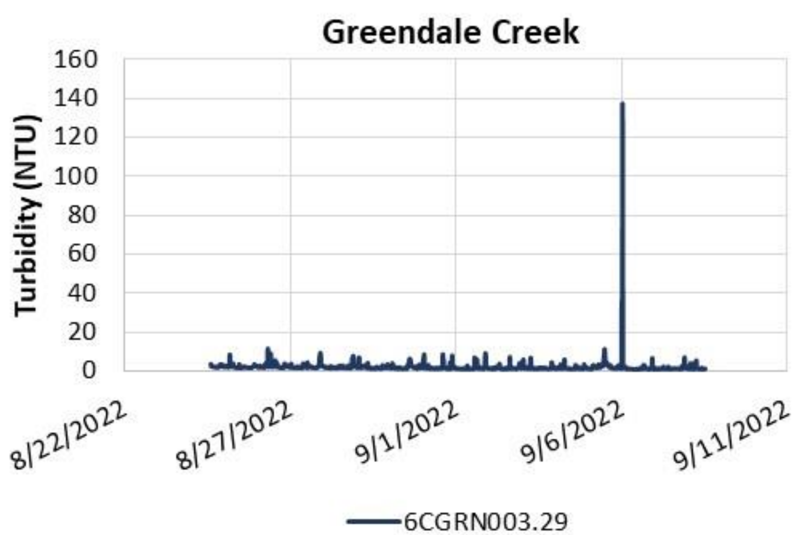


Figure 28. Diurnal turbidity in Greendale Creek.

2.4.7. Organic Matter

Various forms of organic matter were measured in a limited number of samples from Greendale Creek and Rich Valley Unnamed Tributary. 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 values were 60 mg/L in Greendale Creek, 63 mg/L in Rich Valley Unnamed Tributary, and 42 mg/L in the Cove Creek reference.

Total organic carbon (TOC) was also measured in Greendale Creek and Rich Valley Unnamed Tributary. TOC is the mass of carbon in organic form dissolved or suspended in the water column. TOC values were 2 mg/L in Greendale Creek, 6.28 mg/L in Rich Valley Unnamed Tributary, and 1.44 in the Cove Creek reference. In general, measures of organic matter (TVS and TOC) were higher in the impaired streams than in the Cove Creek reference, although values were within a typical range for agricultural streams.

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. Average nitrogen to phosphorus ratios were 93 in Greendale Creek and 12 in Rich Valley Unnamed Tributary, indicating that phosphorus is the limiting nutrient in each stream.

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 29 shows the total phosphorus levels in Greendale Creek, Rich Valley Unnamed Tributary, and the Cove Creek reference. Total phosphorus averaged 0.02 mg/L in Greendale Creek and 0.12 mg/L in Rich Valley Unnamed Tributary. Greendale Creek had similar total phosphorus levels to the reference (0.03 mg/L), and median values were in the no probability range for stressor effects. Rich Valley Unnamed Tributary had statistically higher total phosphorus levels than the reference site (t-test with unequal variance and alpha = 0.05), and median values were in the high probability range for stressor effects.

Figure 30 shows the time series of total phosphorus levels in Greendale Creek and Rich Valley Unnamed Tributary. In Greendale Creek, total phosphorus levels were relatively consistent and all within the no to low probability range for stressor effects. In Rich Valley Unnamed Tributary, total phosphorus levels were much more variable over time, with 50% of samples above the high probability threshold of 0.1 mg/L.

While VDEQ does not have nutrient criteria for freshwater streams, USEPA has published recommended criteria by ecoregion (USEPA, 2000a). Greendale Creek and Rich Valley Unnamed Tributary are in the Ridge and Valley Level 3 Ecoregion, and the recommended total phosphorus criterion based on the 25^{th} percentile of streams in this region is 0.01 mg/L. Greendale Creek barely exceeded this threshold, but Rich Valley Unnamed Tributary was well above this recommended criterion.

In summary, total phosphorus is not likely a stressor in Greendale Creek, where average phosphorus levels were below the reference. Phosphorus could be a stressor in Rich Valley Unnamed Tributary, where median total phosphorus concentrations were in the high probability range for stressor effects and average phosphorus levels were significantly higher than in the reference.

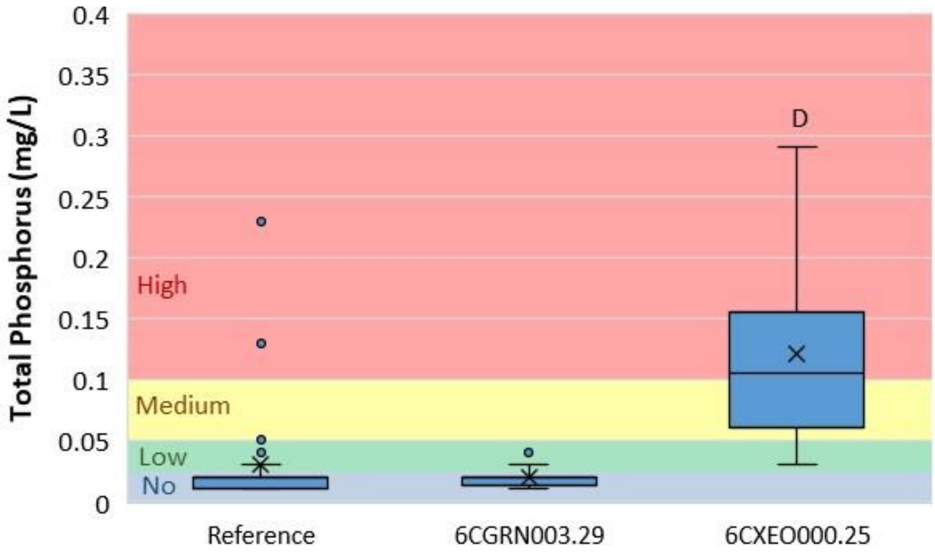


Figure 29. Total phosphorus in Greendale Creek and Rich Valley Unnamed Tributary. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represents the mean. Dots represent outliers that are greater than 1.5 times 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.

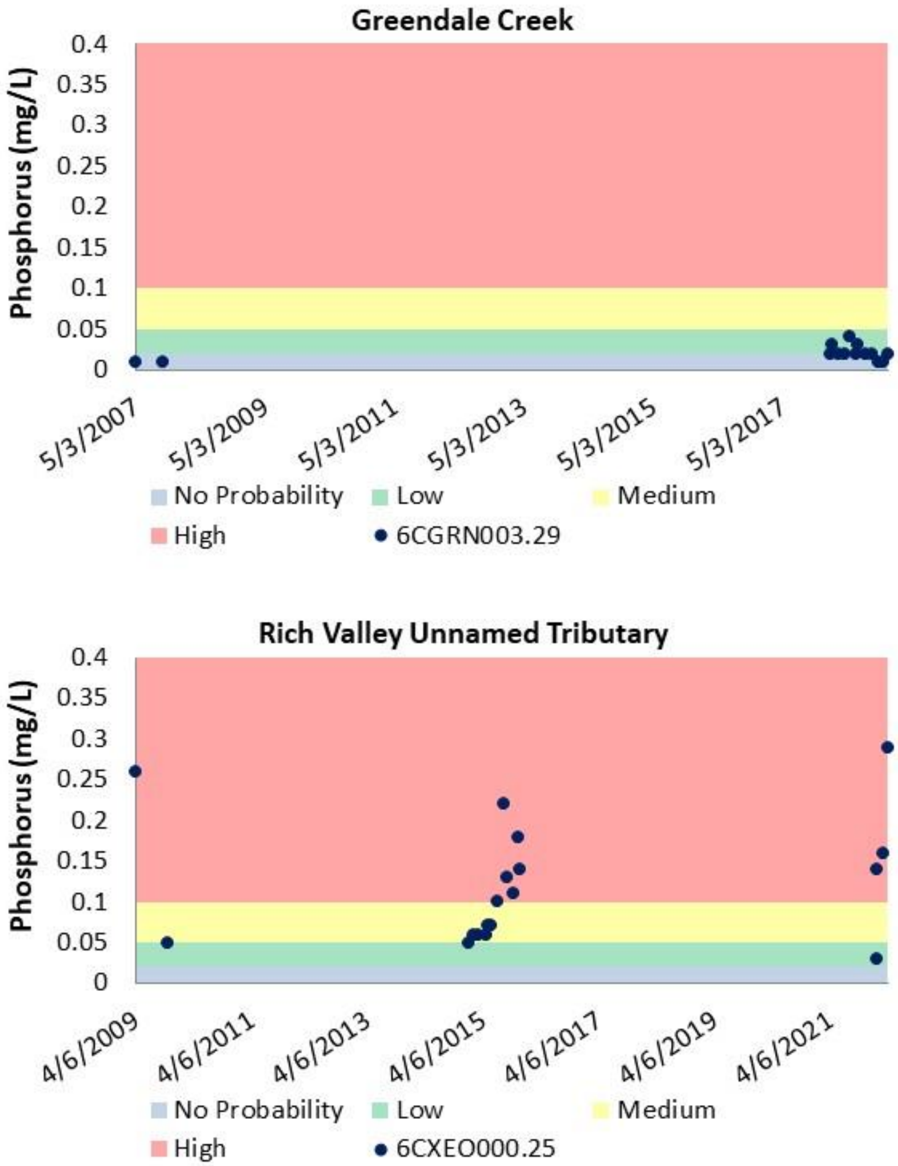


Figure 30. Total phosphorus over time in Greendale Creek and Rich Valley Unnamed Tributary. Colors represent the probability that data within that range would be responsible for causing stress.

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 31 shows the total nitrogen levels in Greendale Creek and Rich Valley Unnamed Tributary. Total nitrogen averaged 1.86 mg/L in Greendale Creek and 1.49 mg/L in Rich Valley Unnamed Tributary. Both impaired streams had statistically higher total nitrogen than the reference (t-test with unequal variance and alpha = 0.05), which averaged 0.96 mg/L. The median total nitrogen value in both impaired streams was in the medium probability range for stressor effects.

Figure 32 shows total nitrogen concentrations over time in Greendale Creek and Rich Valley Unnamed Tributary. Total nitrogen concentrations varied considerably over time and by season. Greendale Creek had 4 excursions (29%) into the high probability range for stressor effects, and Rich Valley Unnamed Tributary had 2 excursions (11%) into the high probability range.

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 25th percentile of streams is 0.399 mg/L for the Ridge and Valley Level 3 Ecoregion. Both of the impaired streams (and the reference) exceeded this value with averages from 0.96 to 1.86 mg/L.

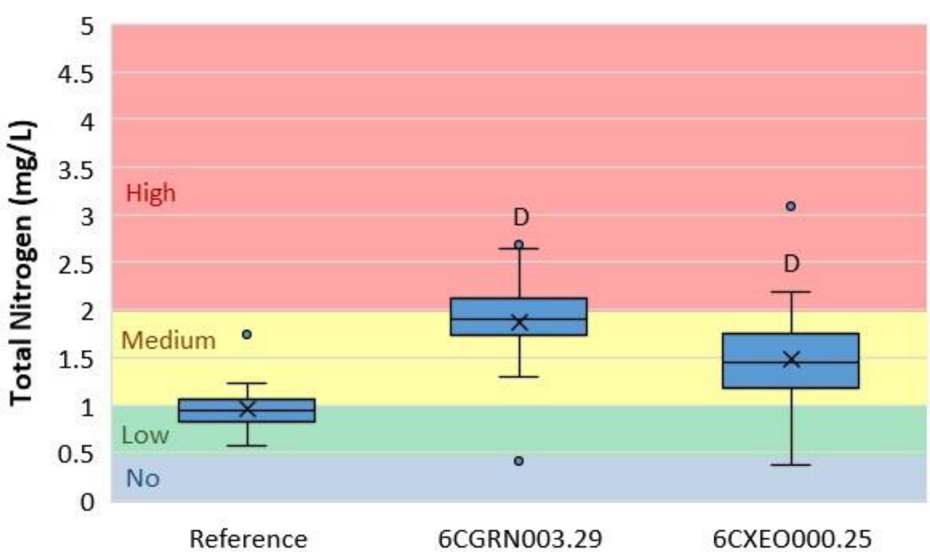
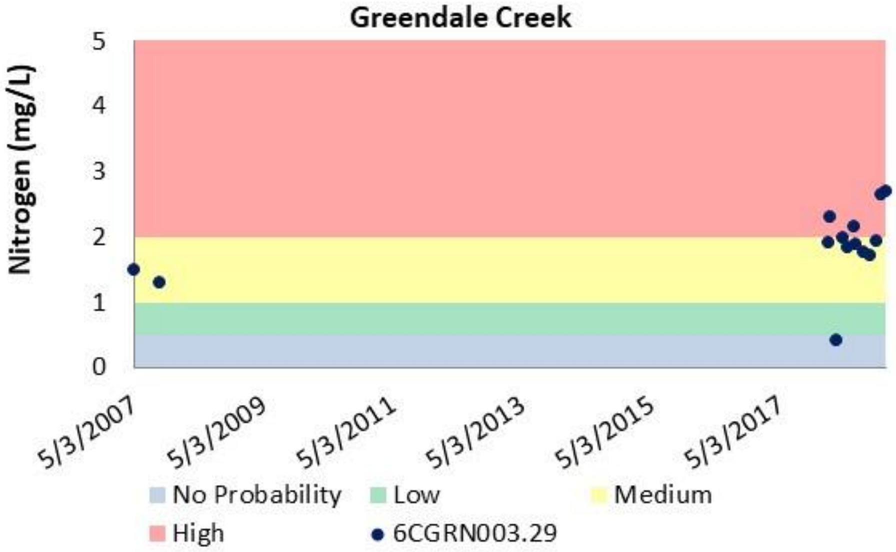


Figure 31. Total nitrogen in Greendale Creek and Rich Valley Unnamed Tributary. Boxes represent the inter-quartile range, whiskers represent minimum and maximum values excluding outliers, lines represent the median, and the X represents the mean. Dots represent outliers that are greater than 1.5 times the inter-quartile range away from the mean. Colors represent the probability that data within that range would be responsible for causing stress.



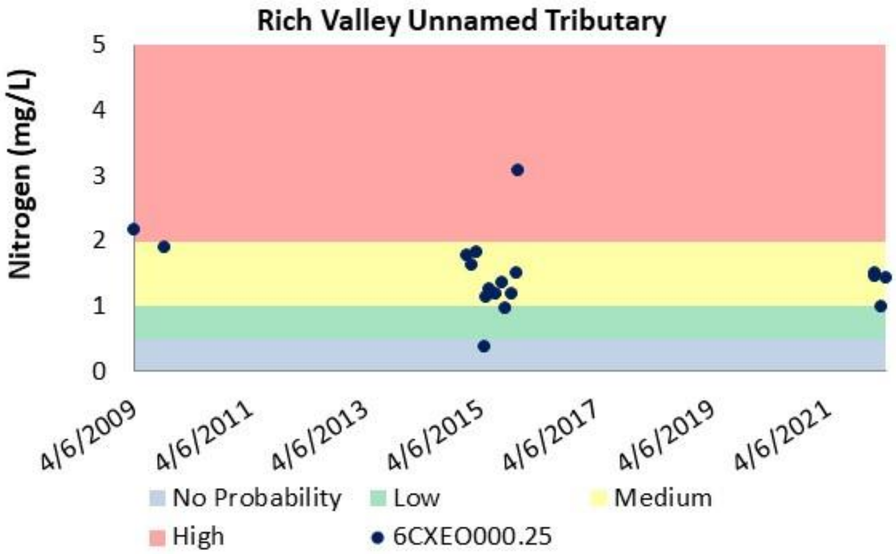
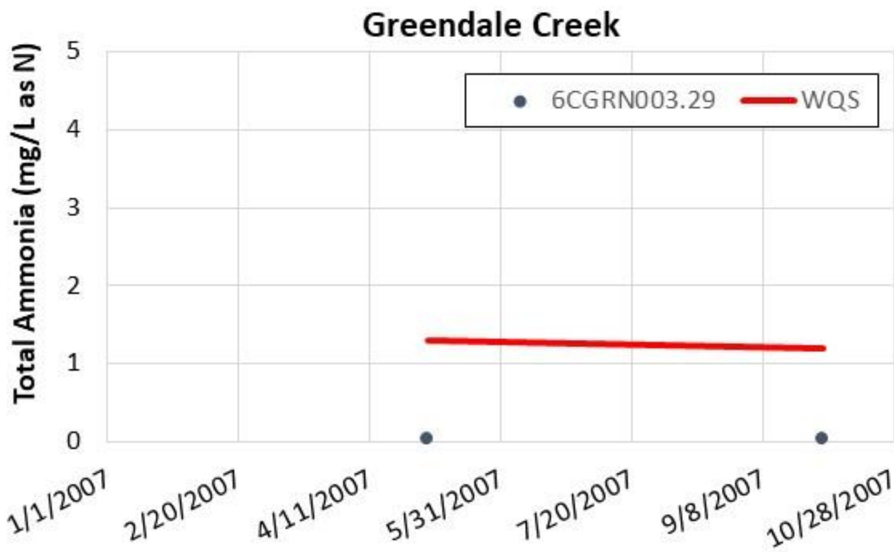


Figure 32. Total nitrogen concentration over time in Greendale Creek and Rich Valley Unnamed Tributary. Colors represent the probability that data within that range would be responsible for causing stress.

2.4.10. Ammonia

Ammonia is a reduced form of nitrogen that can be toxic at certain temperatures and pHs. Figure 33 shows the ammonia levels in Greendale Creek and Rich Valley Unnamed Tributary 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. Only two ammonia samples were collected at each impaired station, but none of the samples at either station had ammonia levels above or even close to the water quality standard. Ammonia levels were below the detection limit of 0.04 mg/L in each sample. For this reason, ammonia is not likely a stressor in Greendale Creek or Rich Valley Unnamed Tributary.



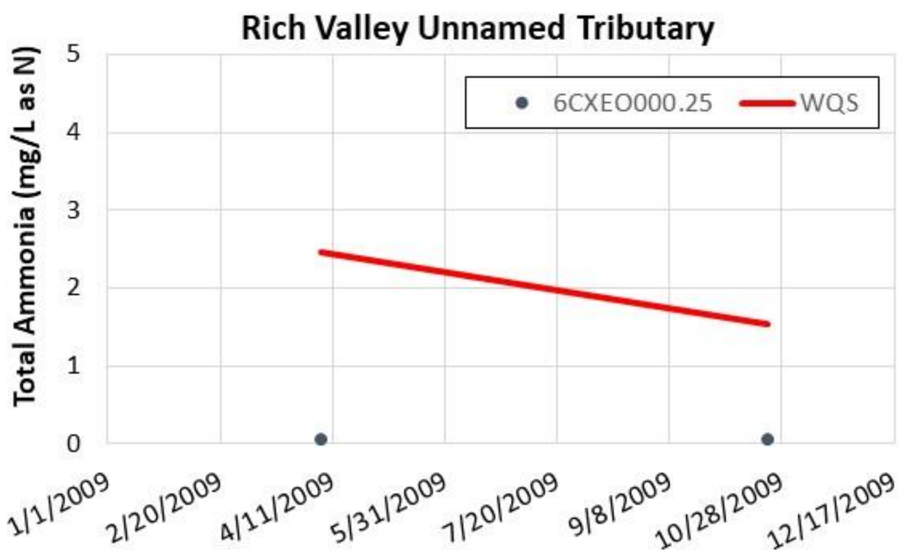


Figure 33. Ammonia levels in Greendale Creek and Rich Valley Unnamed Tributary. The red line represents the calculated water quality standard based on temperature and pH at the time of sampling.

2.4.11. Dissolved Metals

Dissolved metals were measured in both of the impaired streams and the Cove Creek reference. Table 11 shows the measured 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 measured 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 Greendale Creek and the Cove Creek reference to 0.22 in Rich Valley Unnamed Tributary (Table 13). The CCU values calculated for these 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 0.15 in Cove Creek to 0.46 in Greendale Creek and Rich Valley Unnamed Tributary (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 Greendale Creek or Rich Valley Unnamed Tributary.

Table 11. Dissolved metals concentrations and corresponding water quality standards for Greendale Creek and Rich Valley Unnamed Tributary.

Metal	Water Quality Standard Average and (Range)[1] (ug/L)	Concentration (ug/L)		
		Cove Creek (6CCOV002.44)	Greendale Creek (6CGRN003.29)	Rich Valley Unnamed Tributary (6CXEO000.25)
Arsenic	150	0.35	0.1	0.38
Cadmium	1.2 (1.0-1.3)	<0.03	<0.1	<0.1
Chromium	130 (110-150)	4.93	1.5	1.25
Copper	16 (13-18)	0.45	0.3	1.03
Lead	22 (18-26)	<0.02	<0.1	<0.1
Nickel	35 (30-41)	0.16	0.1	0.52
Selenium	5	<0.3	<0.5	<0.5
Zinc	210 (170-240)	<0.4	<1	2.29

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

Table 12. Dissolved metals concentrations and corresponding toxicity reference values for Greendale Creek and Rich Valley Unnamed Tributary.

Metal	Toxicity Reference Value Average and (Range)[1] (ug/L)	Concentration (ug/L)		
		Cove Creek (6CCOV002.44)	Greendale Creek (6CGRN003.29)	Rich Valley Unnamed Tributary (6CXEO000.25)
Aluminum[2]	1000 (740-1300)	1.97	1.8	7.17
Antimony[3]	30	0.05	<0.5	<0.5
Barium[4]	1700	29.4	33.8	16.8
Beryllium[5]	5.3	<0.04	<0.1	<0.1
Silver[3]	0.12	<0.03	<0.1	<0.1
Thallium[3]	40	<0.02	<0.1	<0.1

[1] Toxicity reference values were based on literature values from the references below.
[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 Greendale Creek and Rich Valley Unnamed Tributary.

Stream	Station	Date	CCU[1]	TRV Index[2]
Cove Creek	6CCOV002.44	5/1/2012	0.12	0.15
Greendale Creek	6CGRN003.29	5/3/2007	0.12	0.46
Rich Valley Unnamed Tributary	6CXEO000.24	4/6/2009	0.22	0.46

[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 for 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 - Metals

A total of 16 metals were measured in the sediments of Greendale Creek and Rich Valley Unnamed Tributary. Sediment metals were measured at station 6CGRN003.29 on 5/3/2007 and at station 6CXEO000.25 on 4/6/2009. 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 both streams were below both the TEC and PEC values. This indicates that sediment metals are not likely a stressor in these streams.

Table 14. Metals concentrations in sediments from Greendale Creek and Rich Valley Unnamed Tributary.

Metal	TEC[1] (mg/kg)	PEC[2] (mg/kg)	Concentration[3] (mg/kg)	Concentration[3] (mg/kg)
			Greendale Creek (6CGRN003.29)	Rich Valley Unnamed Tributary (6CXEO000.25)
Arsenic	9.79	33	8.26	5.39
Cadmium	0.99	4.98	<1	<1
Chromium	43.4	111	16.8	19.7
Copper	31.6	149	12	15.5
Lead	35.8	128	20.1	24.2
Mercury	0.18	1.06	<0.1	<0.1
Nickel	22.7	48.6	12.8	14
Zinc	121	459	-	2.75

[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.
[3] Green shading indicates that measured values are below both TEC and PEC values, orange shading indicates that measured values are between TEC and PEC values, and red shading indicates that measured values are above PEC values.

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. No parameters exhibited a statistically significant regression, but data was limited, since only three stations within the watersheds had benthic data. The strongest regression relationship (although not statistically significant at the alpha = 0.05 level) was between relative bed stability and benthic health. This relationship had an r^2 of 0.99, indicating good fit with limited data.

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

Parameter	Regression Significant (Y/N)	r^2	p-value
Relative Bed Stability	N	0.99	0.06
pH	N	0.98	0.09
Conductivity	N	0.94	0.15
Total Volatile Solids	N	0.93	0.17
Nitrogen	N	0.92	0.18
Dissolved Oxygen	N	0.91	0.19
Habitat	N	0.88	0.23
Total Dissolved Solids	N	0.81	0.29
Sulfate	N	0.71	0.36
Ammonia	N	0.63	0.41
Chloride	N	0.45	0.53
Total Suspended Solids	N	0.39	0.57
Sodium	N	0.32	0.62
Total Organic Carbon	N	0.23	0.68
Potassium	N	0.16	0.73
Dissolved Metals	N	0.14	0.75
Turbidity	N	0.09	0.8
Phosphorus	N	0.09	0.81
Temperature	N	0.03	0.89

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. 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. 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. Total causal analysis scores were -13 and -11 for Greendale Creek and Rich Valley Unnamed Tributary, respectively. This indicates 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 reason and others explained in Table 19, temperature was categorized as a non-stressor in both streams.

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

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	-3	-3	SCI scores were impaired, but temperature values in each of the impaired streams were similar to the reference and within water quality standards.
Temporal Co-occurrence	-3	-3	At the time of benthic sample collection, temperature at all sites met water quality standards.
Stressor-Response Relationships from the Field	-1	-1	Temperature was not significantly correlated with benthic health across sites.
Temporal Sequence	-1	1	Seasonal trends of lower fall benthic scores in Rich Valley Unnamed Tributary correlate with the timing of high temperatures in the late summer, but temperatures were well within water quality standards. Seasonal trends were not apparent in benthic scores for Greendale Creek, but

			temperatures were higher in the late summer prior to fall benthic sampling.
Stressor-Response Relationships from Laboratory Studies	-3	-3	All temperature values were within water quality standards.
Consistency of Evidence	-2	-2	Evidence consistently refuted temperature as a stressor in Greendale Creek and Rich Valley Unnamed Tributary.
Sum	**-13**	**-11**	

3.2. pH

Table 20 shows the causal analysis results for pH. Total causal analysis scores were -17 in both Greendale Creek and Rich Valley Unnamed Tributary, indicating that there is strong evidence that pH is a non-stressor in these streams. All pH values 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 in both streams.

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

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	-2	-2	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	At the time of benthic sample collection, pH at all sites met water quality standards.
Causal Pathway	-2	-2	Alkalinity in Greendale Creek and Rich Valley Unnamed Tributary is relatively high, averaging from 157 to 216 mg/L as $CaCO_3$, so pH changes are well-buffered.
Stressor-Response Relationships from the Field	-1	-1	pH was not significantly correlated with benthic health across sites.
Symptoms	-2	-2	Based on average Biological Condition Gradient scores, acidity and alkalinity were ranked relatively low (5th to 8th) as stressors in Greendale Creek and Rich Valley Unnamed Tributary.

Stressor-Response Relationships from Other Field Studies	-2	-2	All pH values were in the low probability range for stressor effects.
Stressor-Response Relationships from Laboratory Studies	-3	-3	All pH values were within water quality standards.
Consistency of Evidence	-2	-2	Evidence consistently refuted pH as a probable stressor.
Sum	**-17**	**-17**	

3.3. Dissolved Oxygen

Table 21 shows the causal analysis results for dissolved oxygen. Total causal analysis scores were -19 in Greendale Creek and +8 in Rich Valley Unnamed Tributary. There is strong evidence that dissolved oxygen is a non-stressor in Greendale Creek but a probable stressor in Rich Valley Unnamed Tributary. In Greendale Creek, median dissolved oxygen levels were in the low probability range for stressor effects, and concentrations were well above average and instantaneous water quality standards. For these reasons and others explained in Table 21, DO was categorized as a non-stressor in Greendale Creek.

In Rich Valley Unnamed Tributary, several lines of evidence supported dissolved oxygen as a probable stressor. These lines of evidence included:

- Diurnal DO monitoring showed consistently low DO and nighttime values below 5.0 mg/L on 4 out of 8 days.
- The timing of low DO in the late summer corresponds to the pattern of lower fall SCI scores.
- Total phosphorus levels were high, indicating that the causal pathway from nutrient enrichment to low DO is intact.

For these reasons and others explained in Table 21, DO was categorized as a probable stressor in Rich Valley Unnamed Tributary.

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

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	-2	1	In each of the impaired streams, SCI scores were impaired, but median DO values were in the low probability range for stressor effects. During diurnal monitoring in Greendale Creek, nighttime DO remained in the low probability range for stressor effects, but nighttime DO was consistently in the high probability range for stressor effects in Rich Valley Unnamed Tributary.
Temporal Co-occurrence	-2	2	At the time of benthic sample collection, DO met water quality standards and was in the low probability range for stressor effects in Greendale Creek. In Rich Valley Unnamed Tributary, however, DO was in the medium probability range for stressor effects on one occasion and high probability range on one occasion.
Causal Pathway	-3	2	Median nutrient levels (total phosphorus) were in the no probability range for stressor effects in Greendale Creek, so the causal pathway from nutrient enrichment to low DO is absent. In Rich Valley Unnamed Tributary, however, median nutrient levels were in the high probability range, so the causal pathway from nutrient enrichment to low DO is intact for Rich Valley Unnamed Tributary.
Stressor-Response Relationships from the Field	-1	-1	DO was not significantly correlated with benthic health across sites.
Temporal Sequence	-1	2	DO is generally lowest in the late summer, and fall benthic scores were lower than spring scores in Rich Valley Unnamed Tributary. No seasonal trend in benthic scores was observed in Greendale Creek.
Symptoms	-2	-2	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 one of the lowest ranked stressors in both streams.
Stressor-Response Relationships from Other Field Studies	-3	2	Median DO values were in the low probability range for stressor effects in both streams, however, diurnal monitoring in Rich Valley Unnamed Tributary showed DO values consistently in the high probability range for stressor effects.
Stressor-Response Relationships from Laboratory Studies	-3	1	In Greendale Creek, all DO values were well above the water quality criteria of 5.0 mg/L (average) and 4.0 mg/L (instantaneous). In Rich Valley Unnamed Tributary, nighttime DO values dropped below 5.0 mg/L on four out of eight days,

			but neither the average (5.0 mg/L) or instantaneous standard (4.0 mg/L) were violated.
Consistency of Evidence	-2	1	Most evidence refuted DO as a stressor in Greendale Creek but supported DO as a stressor in Rich Valley Unnamed Tributary.
Sum	**-19**	**8**	

3.4. Conductivity and Total Dissolved Solids

Table 22 shows the causal analysis results for conductivity and total dissolved solids. Total causal analysis scores were +2 in Greendale Creek and Rich Valley Unnamed Tributary. Some evidence supported conductivity as a stressor in these streams, and other evidence refuted it. Median conductivity values were in the medium probability range for stressor effects, but median total dissolved solids values were in the low probability range. In addition, conductivity was not significantly correlated across benthic stations, and Biological Condition Gradient analysis did not identify conductivity as a top stressor. Due to the conflicting lines of evidence, conductivity and total dissolved solids were categorized as possible stressors in both streams.

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

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	2	2	Impaired stations were significantly higher in conductivity than the reference station, and median conductivity at impaired stations was in the medium probability range for stressor effects.
Temporal Co-occurrence	2	2	At the time of impaired benthic sample collections, conductivity levels were in the medium probability range for stressor effects in Greendale Creek and ranged from the low to high probability range in Rich Valley Unnamed Tributary.
Stressor-Response Relationships from the Field	-1	-1	Conductivity was not significantly correlated with SCI scores across sites.
Symptoms	-2	-2	Conductivity ranked fourth and was not one of the top ranked stressors based on average Biological Condition Gradient scores.

Stressor-Response Relationships from Other Field Studies	2	2	Median conductivity values were in the medium probability range for stressor effects in both of the impaired streams, however conductivity at the reference site was also in the medium probability range.
Analogous Stressors	-1	-1	The analogous stressor of total dissolved solids (TDS) was in the low probability range for stressor effects in both streams.
Consistency of Evidence	0	0	Evidence supporting conductivity as a probable stressor in Greendale Creek and Rich Valley Unnamed Tributary was inconsistent.
Sum	**2**	**2**	

3.5. Dissolved Sodium

Table 23 shows the causal analysis results for dissolved sodium. Total causal analysis scores were -11 and -8 for Greendale Creek and Rich Valley Unnamed Tributary, respectively. This indicates that there is strong evidence that dissolved sodium is a non-stressor in these streams. Median sodium levels were in the no to low probability range for stressor effects in both streams, and maximum sodium levels were well below toxic thresholds reported by Mount *et al.* (2016). For these reasons and others explained in Table 23, dissolved sodium was categorized as a non-stressor in both streams.

Table 23. Causal analysis results for dissolved sodium.

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	-2	-1	SCI scores were impaired, but median dissolved sodium levels were in the no to low probability range for stressor effects. Rich Valley Unnamed Tributary had one excursion into the high probability range.
Temporal Co-occurrence	-2	-1	At or around the time of benthic sampling, dissolved sodium levels were in the no probability range for stressor effects in Greendale Creek on three occasions and in the low probability range in Rich Valley Unnamed Tributary on one occasion.
Stressor-Response Relationships from the Field	-1	-1	Dissolved sodium was not significantly correlated with benthic health across sites.
Temporal Sequence	-1	-2	Seasonal trends of lower fall benthic scores in Rich Valley Unnamed Tributary did not appear to correlate with the timing

			of high sodium levels in the winter. Seasonal trends were not apparent in benthic scores for Greendale Creek, but sodium was generally higher in the winter.
Stressor-Response Relationships from Other Field Studies	-2	-1	Median dissolved sodium levels were in the no to low probability range for stressor effects. Rich Valley Unnamed Tributary had one excursion into the high probability range.
Stressor-Response Relationships from Laboratory Studies	-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 either impaired stream.
Analogous Stressors	1	1	The analogous stressor of conductivity was in the medium probability range for stressor effects in both impaired streams.
Consistency of Evidence	-2	-1	Most evidence moderately refuted dissolved sodium as a probable stressor in Greendale Creek, and most evidence weakly refuted dissolved sodium as a probable stressor in Rich Valley Unnamed Tributary.
Sum	**-11**	**-8**	

3.6. Dissolved Potassium

Table 24 shows the causal analysis results for dissolved potassium. Total causal analysis scores were -10 in Greendale Creek and +1 in Rich Valley Unnamed Tributary. This indicates that there is strong evidence that dissolved potassium is a non-stressor in Greendale Creek and mixed evidence in Rich Valley Unnamed Tributary. In Greendale Creek, median dissolved potassium levels were in the low probability range for stressor effects, and concentrations were well below toxic thresholds reported by Mount *et al.* (2016). Potassium levels in Rich Valley Unnamed Tributary were still below reported toxic thresholds, but median values were in the medium probability range for stressor effects. For these reasons and others explained in Table 24, dissolved potassium was categorized as a non-stressor in Greendale Creek and a possible stressor in Rich Valley Unnamed Tributary.

Table 24. Causal analysis results for dissolved potassium.

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	-2	2	SCI scores were impaired in Greendale Creek, but median dissolved potassium levels were in the low probability range for stressor effects. In Rich Valley Unnamed Tributary, median dissolved potassium levels were in the medium probability range.
Temporal Co-occurrence	-2	1	At or around the time of benthic sampling, dissolved potassium levels were in the low probability range for stressor effects in Greendale Creek on three occasions and in the medium probability range in Rich Valley Unnamed Tributary on one occasion.
Stressor-Response Relationships from the Field	-1	-1	Dissolved potassium was not significantly correlated with benthic health across sites.
Temporal Sequence	0	-2	Seasonal trends of lower fall benthic scores in Rich Valley Unnamed Tributary did not appear to correlate with the timing of high potassium levels in the spring. Seasonal trends were not apparent in benthic scores or potassium levels for Greendale Creek.
Stressor-Response Relationships from Other Field Studies	-2	2	Median dissolved potassium levels were in the low probability range for stressor effects in Greendale Creek and in the medium probability range for stressor effects in Rich Valley Unnamed Tributary.
Stressor-Response Relationships from Laboratory Studies	-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 either impaired stream.
Analogous Stressors	1	1	The analogous stressor of conductivity was in the medium probability range for stressor effects in both impaired streams.
Consistency of Evidence	-2	0	Most evidence moderately refuted potassium as a probable stressor in Greendale Creek, but evidence was inconsistent in Rich Valley Unnamed Tributary.
Sum	**-10**	**1**	

3.7. Dissolved Chloride

Table 25 shows the causal analysis results for dissolved chloride. Total causal analysis scores were -15 and -13 in Greendale Creek and Rich Valley Unnamed Tributary, respectively. This indicates that there is strong evidence that dissolved chloride is a non-stressor in these streams. Median dissolved chloride levels were in the no to low probability range for stressor effects, and maximum concentrations were well below the water quality standard of 230 mg/L. In addition, Biological Condition Gradient analysis identified chloride as one of the lowest ranked stressors. For these reasons and others explained in Table 25, dissolved chloride was categorized as a non-stressor in both streams.

Table 25. Causal analysis results for dissolved chloride.

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	-2	-1	SCI scores were impaired, but the median dissolved chloride level was in the no to low probability range for stressor effects. Rich Valley Unnamed Tributary had one excursion into the high probability range.
Temporal Co-occurrence	-2	-1	At or around the time of benthic sampling, dissolved chloride levels were in the no to low probability range for stressor effects in Greendale Creek on two occasions and in the low probability range in Rich Valley Unnamed Tributary on one occasion.
Stressor-Response Relationships from the Field	-1	-1	Dissolved chloride was not significantly correlated with benthic health across sites.
Temporal Sequence	-1	-2	Seasonal trends of lower fall benthic scores in Rich Valley Unnamed Tributary did not appear to correlate with the timing of high chloride levels in the winter. Seasonal trends were not apparent in benthic scores for Greendale Creek, but chloride was generally higher in the winter.
Symptoms	-3	-3	Chloride was one of the lowest ranked stressors based on average Biological Condition Gradient scores.
Stressor-Response Relationships from Other Field Studies	-2	-1	The median dissolved chloride level was in the no probability range for stressor effects in Greendale Creek and the low probability range in Rich Valley Unnamed Tributary.
Stressor-Response Relationships from Laboratory Studies	-3	-3	Chloride levels were all well below the Virginia water quality standard for chloride.
Analogous Stressors	1	1	The analogous stressor of conductivity was in the medium probability range for stressor effects in both impaired streams.
Consistency of Evidence	-2	-2	Evidence moderately refutes chloride as a stressor in both impaired streams.
Sum	**-15**	**-13**	

3.8. Dissolved Sulfate

Table 26 shows the causal analysis results for dissolved sulfate. Total causal analysis scores were -11 and -12 in Greendale Creek and Rich Valley Unnamed Tributary, respectively. This indicates that there is strong evidence that dissolved sulfate is a non-stressor in these streams. Median dissolved sulfate values were in the no probability range for stressor effects, and maximum concentrations were well 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 in both streams.

Table 26. Causal analysis results for dissolved sulfate.

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	-3	-3	SCI scores were impaired, but the median dissolved sulfate levels were in the no probability range for stressor effects in both streams.
Temporal Co-occurrence	-2	-1	At or around the time of benthic sampling, dissolved sulfate levels were in the no probability range for stressor effects in Greendale Creek on two occasions and in the low probability range in Rich Valley Unnamed Tributary on one occasion.
Stressor-Response Relationships from the Field	-1	-1	Dissolved sulfate was not significantly correlated with benthic health across sites.
Temporal Sequence	0	-2	Seasonal trends of lower fall benthic scores in Rich Valley Unnamed Tributary did not appear to correlate with the timing of higher sulfate levels in the spring. Seasonal trends were not apparent in benthic scores or sulfate levels for Greendale Creek.
Symptoms	1	1	Based on average Biological Condition Gradient scores, sulfate was the second and third ranked stressor in Greendale Creek and Rich Valley Unnamed Tributary, respectively.
Stressor-Response Relationships from Other Field Studies	-3	-3	Median sulfate values were in the no probability range for stressor effects in both of the impaired streams.
Stressor-Response Relationships from Laboratory Studies	-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 either impaired stream.
Analogous Stressors	1	1	The analogous stressor of conductivity was in the medium probability range for stressor effects in both impaired streams.

Consistency of Evidence	-2	-2	Evidence moderately refutes sulfate as a stressor in both impaired streams.
Sum	**-11**	**-12**	

3.9. Suspended Solids and Deposited Sediment

Table 27 shows the causal analysis results for suspended solids and deposited sediment. Total causal analysis scores were +11 and +10 in Greendale Creek and Rich Valley Unnamed Tributary, respectively. This indicates 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 includes:

- Median total habitat scores were in the medium probability range for stressor effects.
- Individual habitat metrics that indicate instream sediment (substrate) were significantly lower in the impaired streams than in the reference.
- At the time of benthic sampling, total habitat scores were significantly correlated with benthic health.
- Average Biological Condition Gradient scores ranked sediment-associated stressors (RBS) as the top stressor in Rich Valley Unnamed Tributary and the third ranked stressor in Greendale Creek.
- Functional feeding group analysis indicated a shift to collectors that thrive in sediment-rich habitats.
- Relative Bed Stability measures were in the high probability range for stressor effects in Greendale Creek and medium range in Rich Valley Unnamed Tributary.

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

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

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	2	2	SCI scores were impaired and total habitat scores were in the medium probability range for stressor effects.
Temporal Co-occurrence	2	2	At the time of benthic sampling, habitat scores correlated significantly with SCI scores.
Causal Pathway	1	1	Individual habitat metrics that indicate instream sediment (substrate) were significantly lower in the impaired streams than in the reference.
Stressor-Response Relationships from the Field	-1	-1	Habitat scores were not significantly correlated with benthic health across sites.
Temporal Sequence	-1	-2	Sediment levels are generally highest in the spring and accompanied by higher spring flows, however, fall benthic scores were lower in Rich Valley Unnamed Tributary and spring and fall benthic scores were consistent in Greendale Creek.
Symptoms	2	3	Average Biological Condition Gradient scores for relative bed stability were ranked first in Rich Valley Unnamed Tributary and third in Greendale Creek. Functional feeding group analysis in both impaired streams indicated a shift to collectors that thrive in sediment-rich habitats.
Stressor-Response Relationships from Other Field Studies	2	2	Median total habitat scores were in the medium probability range for stress effects in both impaired streams.
Analogous Stressors	3	2	The measurement of relative bed stability was in the high probability range for stress effects in Greendale Creek and in the medium probability range in Rich Valley Unnamed Tributary.
Consistency of Evidence	1	1	Most evidence weakly supported sediment as a probable stressor in Greendale Creek and Rich Valley Unnamed Tributary.
Sum	**11**	**10**	

3.10. Organic Matter

Table 28 shows the causal analysis results for organic matter. Total causal analysis scores were -9 in Greendale Creek and +3 in Rich Valley Unnamed Tributary. This indicates that there is moderate evidence that organic matter is a non-stressor in Greendale Creek and mixed evidence in Rich Valley Unnamed Tributary. In Greendale Creek, total volatile solids values were below the detection limit. In Rich Valley Unnamed Tributary, the level of total volatile solids was above the reference station, but still within the normal range for agricultural streams. In addition, dissolved oxygen levels were relatively high in Greendale Creek, meaning that the pathway from organic matter to decomposition to low dissolved oxygen was not intact. But in Rich Valley Unnamed Tributary, this pathway may be intact, because DO levels were consistently low during diurnal monitoring. For these reasons and others explained in Table 28, organic matter was categorized as a non-stressor in Greendale Creek and a possible stressor in Rich Valley Unnamed Tributary.

Table 28. Causal analysis results for organic matter.

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	-1	1	SCI scores were impaired, but total volatile solids were below the detection limit in Greendale Creek. Total volatile solids were higher than the reference in Rich Valley Unnamed Tributary, but were within normal ranges for agricultural streams.
Temporal Co-occurrence	-1	1	At the time of benthic sample collection on a single occasion, total volatile solids were below the detection limit in Greendale Creek. In Rich Valley Unnamed Tributary, total volatile solids were higher than in the reference, but were within normal ranges for agricultural streams.
Causal Pathway	-2	1	Dissolved oxygen was high in Greendale Creek, so the pathway from organic matter to decomposition to low dissolved oxygen was not intact. In Rich Valley Unnamed Tributary, dissolved oxygen was low during diurnal monitoring, so the causal pathway from organic matter to decomposition to low dissolved oxygen may be intact.
Stressor-Response Relationships from the Field	-1	-1	Total volatile solids were not significantly correlated with benthic health across sites.
Temporal Sequence	-1	1	Seasonal trends of lower fall benthic scores observed in Rich Valley Unnamed Tributary were consistent with organic matter enrichment, since organic matter from primary productivity would increase in the summer and fall. In Greendale Creek, no seasonal trends were observed in benthic scores.
Symptoms	-1	-1	Functional feeding group analysis in both of the impaired streams indicated an increase in collectors rather than

			scrapers and filterers. This indicates sediment enrichment rather than organic matter enrichment.
Stressor-Response Relationships from Other Field Studies	-1	1	Total volatile solids were below the detection limit in Greendale Creek. Total volatile solids were higher than the reference in Rich Valley Unnamed Tributary, but were within normal ranges for agricultural streams.
Consistency of Evidence	-1	0	Most evidence weakly refuted organic matter as a probable stressor in Greendale Creek. Evidence was inconsistent in Rich Valley Unnamed Tributary.
Sum	**-9**	**3**	

3.11. Total Phosphorus

Table 29 shows the causal analysis results for total phosphorus. Total causal analysis scores were -11 in Greendale Creek and +15 in Rich Valley Unnamed Tributary. This indicates that there is strong evidence that total phosphorus is a non-stressor in Greendale Creek but a probable stressor in Rich Valley Unnamed Tributary. In Greendale Creek, median total phosphorus was in the no probability range for stressor effects and similar to the reference station. Diurnal DO monitoring also showed high DO with limited fluctuations, indicating that the causal pathway from nutrient enrichment to low DO was not intact in Greendale Creek. For these reasons and others explained in Table 29, phosphorus was categorized as a non-stressor in Greendale Creek.

In Rich Valley Unnamed Tributary, several lines of evidence supported phosphorus as a probable stressor. These lines of evidence include:

- Total phosphorus values were statistically higher in Rich Valley Unnamed Tributary than in the reference.
- Median total phosphorus was in the high probability range for stressor effects.
- Diurnal DO monitoring showed consistently low DO and nighttime values below 5.0 mg/L on 4 out of 8 days. This indicates that the causal pathway from nutrient enrichment to low DO is intact.
- Biological Condition Gradient analysis identified nutrients as the second highest ranked stressor.

- The timing of high phosphorus and low DO in the late summer corresponds to the pattern of lower fall SCI scores.
- Total phosphorus levels were well above the EPA recommended criterion for the ecoregion.

For these reasons and others explained in Table 29, phosphorus was categorized as a probable stressor in Rich Valley Unnamed Tributary.

Table 29. Causal analysis results for total phosphorus.

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	-3	3	In Rich Valley Unnamed Tributary, SCI scores were impaired and total phosphorus was statistically higher than the reference and in the high probability range for stressor effects. In Greendale Creek, SCI scores were also impaired, but total phosphorus was similar to the reference and in the no probability range for stressor effects.
Temporal Co-occurrence	-2	1	At or near the time of benthic sample collection, total phosphorus levels were in the no probability range for stressor effects on all occasions in Greendale Creek. In Rich Valley Unnamed Tributary, total phosphorus levels at or near the time of benthic sampling were in the high probability range for stressor effects on one occasion and medium probability range on two occasions.
Causal Pathway	-2	2	Diurnal DO measurements in Greendale Creek did not show strong fluctuations from daytime to nighttime indicating that the causal pathway from nutrient enrichment to low DO is not intact. In Rich Valley Unnamed Tributary, diurnal DO fluctuations were not extremely large, but DO was consistently depressed, indicating that the causal pathway from nutrient enrichment to low DO may be intact.
Stressor-Response Relationships from the Field	-1	-1	Phosphorus was not significantly correlated with benthic health across sites.
Temporal Sequence	-1	2	In Greendale Creek, no distinct seasonal trends in phosphorus or SCI scores were observed. In Rich Valley Unnamed Tributary, higher fall phosphorus levels coincided with lower fall SCI scores.
Symptoms	2	1	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 Greendale Creek and the second highest ranked stressor in Rich Valley Unnamed Tributary.
Stressor-Response Relationships from Other Field Studies	-3	3	Median total phosphorus concentrations were in the no probability range for stressor effects in Greendale Creek and high probability range in Rich Valley Unnamed Tributary.
Stressor-Response Relationships from Laboratory Studies	1	2	Total phosphorus levels averaged 0.02 in Greendale Creek and 0.12 mg/L in Rich Valley Unnamed Tributary, which are above the EPA-recommended criterion of 0.01 mg/L for the Ridge and Valley Ecoregion.
Consistency of Evidence	-2	2	Evidence consistently refuted phosphorus as a stressor in Greendale Creek and consistently supported it in Rich Valley Unnamed Tributary.
Sum	**-11**	**15**	

3.12. Total Nitrogen

Table 30 shows the causal analysis results for total nitrogen. Total causal analysis scores were +1 in Greendale Creek and +2 in Rich Valley Unnamed Tributary. This indicates that there is mixed evidence for total nitrogen as a stressor in these streams. Median nitrogen levels were in the medium probability range for stressor effects and above the EPA-recommended criterion for the Ridge and Valley Ecoregion. Biological Condition Gradient analysis also identified nutrients as the first or second ranked stressor. While these lines of evidence suggest 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 both streams.

Table 30. Causal analysis results for total nitrogen.

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	2	2	SCI scores were impaired and total nitrogen in both impaired streams was in the medium probability range for stressor effects and statistically higher than in the unimpaired reference.

Temporal Co-occurrence	1	2	At or near the time of benthic sample collection, total nitrogen levels were in the medium probability range for stressor effects on two occasions in Greendale Creek. In Rich Valley Unnamed Tributary, total nitrogen at or near the time of benthic sampling was in the high probability range for stressor effects on one occasion and medium probability range on two occasions.
Causal Pathway	-2	-1	Diurnal DO measurements in Greendale Creek did not show strong fluctuations from daytime to nighttime indicating that the causal pathway from nutrient enrichment to low DO is not intact. In Rich Valley Unnamed Tributary, diurnal DO fluctuations were not extremely large, but DO was consistently depressed, indicating that the causal pathway from nutrient enrichment to low DO may be intact. However, this causal pathway is interrupted for nitrogen because phosphorus was determined to be the limiting nutrient.
Stressor-Response Relationships from the Field	-1	-1	Nitrogen was not significantly correlated with benthic health across sites.
Temporal Sequence	-1	-1	In Greendale Creek, no distinct seasonal trends in nitrogen or SCI scores were observed. In Rich Valley Unnamed Tributary, no distinct seasonal trends in nitrogen were observed, but fall SCI scores were lower than spring.
Symptoms	2	1	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 Greendale Creek and the second highest ranked stressor in Rich Valley Unnamed Tributary.
Stressor-Response Relationships from Other Field Studies	2	2	Median total phosphorus concentrations were in the medium probability range for stressor effects in both impaired streams.
Stressor-Response Relationships from Laboratory Studies	1	1	Total nitrogen levels averaged 1.49 to 1.86 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	The nitrogen to phosphorus ratios in Greendale Creek and Rich Valley Unnamed Tributary ranged from 12 to 93, indicating that phosphorus, and not nitrogen, is the limiting nutrient controlling algae growth.
Consistency of Evidence	0	0	Some evidence supported nitrogen as a stressor, while other evidence refuted nitrogen as a stressor.
Sum	**1**	**2**	

3.13. Ammonia

Table 31 shows the causal analysis results for ammonia. Total causal analysis scores were -11 and -9 in Greendale Creek and Rich Valley Unnamed Tributary, respectively. This indicates that there is strong evidence that ammonia is a non-stressor in these streams. All samples in both streams were well below the water quality standard for ammonia. For this reason and others explained in Table 31, ammonia was categorized as a non-stressor in both streams.

Table 31. Causal analysis results for ammonia.

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	-2	-2	In both streams, SCI scores were impaired, but ammonia levels were well below water quality standards.
Temporal Co-occurrence	-2	-2	At the time of benthic sampling, ammonia levels in Greendale Creek and Rich Valley Unnamed Tributary were measured on two occasions and were well below water quality standards.
Stressor-Response Relationships from the Field	-2	-2	Ammonia was not significantly correlated with benthic health across sites.
Temporal Sequence	-1	1	Ammonia levels are generally highest in the late summer when water temperatures are highest, however, spring and fall benthic scores were consistent in Greendale Creek. Fall benthic scores in Rich Valley Unnamed Tributary were lower than spring scores.
Stressor-Response Relationships from Laboratory Studies	-2	-2	All ammonia values were well below water quality standards.
Consistency of Evidence	-2	-2	Evidence consistently refuted ammonia as a stressor.
Sum	**-11**	**-9**	

3.14. Dissolved Metals

Table 32 shows the causal analysis results for dissolved metals. Total causal analysis scores were -12 in Greendale Creek and Rich Valley Unnamed Tributary, indicating that there is strong evidence that dissolved metals are a non-stressor in these streams. In both streams, the CCU for combined effects was in the no probability range for stressor effects. All metals were also below water quality standards and reference toxicity values in both streams. For these reasons and others explained in Table 32, dissolved metals were categorized as non-stressors in both streams.

Table 32. Causal analysis results for dissolved metals.

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	-2	-2	SCI scores were impaired in both streams, but dissolved metal CCU values were in the no probability range for stressor effects.
Temporal Co-occurrence	-2	-2	At the time of benthic sampling, dissolved metal CCU values were in the no probability range for stressor effects.
Causal Pathway	-1	-1	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	-2	-2	Cumulative criteria units for dissolved metals were in the no probability range for stressor effects.
Stressor-Response Relationships from Laboratory Studies	-2	-2	All dissolved metals values were below water quality standards and published effect thresholds.
Analogous Stressors	-1	-1	Toxic metals in sediments were below published effect thresholds.
Consistency of Evidence	-2	-2	Evidence consistently refuted dissolved metals as a possible stressor.
Sum	**-12**	**-12**	

3.15. Sediment Metals

Table 33 shows the causal analysis results for sediment metals. Total causal analysis scores were -10 in Greendale Creek and Rich Valley Unnamed Tributary, indicating that there is strong evidence that sediment metals are non-stressors in these streams. Sediment metals in both streams were below published effect thresholds. For this reason, and others explained in Table 33, sediment metals were categorized as non-stressors in both streams.

Table 33. Causal analysis results for sediment metals.

Evidence	Greendale Creek	Rich Valley Unnamed Tributary	Explanation
Spatial Co-occurrence	-2	-2	In both streams, SCI scores were impaired, but toxic metals in sediment were below published effect thresholds.
Temporal Co-occurrence	-2	-2	At or around the time of benthic sampling, sediment metal levels in both impaired streams were well below published effect thresholds.
Causal Pathway	-1	-1	The watersheds of the impaired streams are primarily agricultural and do not contain significant sources of toxic metals that would accumulate in sediments, so the causal pathway for sediment metals is absent or incomplete.
Stressor-Response Relationships from Laboratory Studies	-2	-2	All toxic metals in sediment were below published effect thresholds.
Analogous Stressors	-1	-1	Dissolved metals in the water column were well below water quality standards, and CCU values were in the no probability range for stressor effects.
Consistency of Evidence	-2	-2	Evidence consistently refuted toxic metals in sediment as a stressor in both impaired streams.
Sum	**-10**	**-10**	

4.0 CAUSAL ANALYSIS SUMMARY

The total causal analysis scores for each candidate stressor are shown in Table 34. Eight of the fifteen candidate stressors had causal analysis scores ≤0 and were classified as non-stressors in both impaired streams. Four candidate stressors had causal analysis scores of 1-3 in one or more stream and were classified as possible stressors. These included conductivity/TDS in both streams, potassium in Rich Valley Unnamed Tributary, organic matter in Rich Valley Unnamed Tributary, and nitrogen in both streams. Three candidate stressors had causal analysis scores >3 and were classified as probable stressors in one or more streams. Results showed that sediment is a probable stressor in both streams and dissolved oxygen and phosphorus are probable stressors in Rich Valley Unnamed Tributary. To address these probable stressors, sediment TMDLs will be developed for both streams, and a total phosphorus TMDL will be developed for Rich Valley Unnamed Tributary. The dissolved oxygen impairment in Rich Valley Unnamed Tributary will be addressed through the total phosphorus TMDL. Table 35 summarizes the non-stressors, possible stressors, and probable stressors identified for each of the impaired streams.

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	Greendale Creek	Rich Valley Unnamed Tributary
Temperature	-13	-11
pH	-17	-17
DO	-19	8
Conductivity/TDS	2	2
Sodium	-11	-8
Potassium	-10	1
Chloride	-15	-13
Sulfate	-11	-12
Sediment	11	10
Organic Matter	-9	3
Phosphorus	-11	15
Nitrogen	1	2
Ammonia	-11	-9
Dissolved Metals	-12	-12
Sediment Toxics - Metals	-10	-10

Table 35. Non-stressors, possible stressors, and probable stressors in Greendale Creek and Rich Valley Unnamed Tributary.

Stream	Non-Stressors	Possible Stressors	Probable Stressors	TMDL Target
Greendale Creek	Temperature, pH, Dissolved Oxygen, Dissolved Sodium, Dissolved Potassium, Dissolved Chloride, Dissolved Sulfate, Organic Matter, Total Phosphorus, Ammonia, Dissolved Metals, Sediment Metals	-Conductivity/TDS -Total Nitrogen	-Sediment	-Sediment
Rich Valley Unnamed Tributary	Temperature, pH, Dissolved Sodium, Dissolved Chloride, Dissolved Sulfate, Ammonia, Dissolved Metals, Sediment Metals	-Conductivity/TDS -Dissolved Potassium -Organic Matter	-Dissolved Oxygen -Sediment -Total Phosphorus	-Sediment -Total Phosphorus

4.1. Probable Stressors

4.1.1. Sediment

Sediment was identified as a probable stressor in both of the impaired streams. Multiple lines of evidence supported this determination including habitat metrics, relative bed stability measures, seasonal trends, biological condition gradient analysis, and functional feeding group analysis (Section 3.9). 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 34). 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 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 Greendale Creek and Rich Valley Unnamed Tributary. A TMDL developed to reduce sediment loads in these watersheds will address the benthic impairments in these streams through the pathways described in Figure 34. 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 Greendale Creek and Rich Valley Unnamed Tributary, including pasture management, livestock stream access, imperviousness in the watersheds, and poor riparian vegetation. In both watersheds, pasture accounts for 42 to 43% 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 that reduce erosion can be beneficial in reducing sediment loads. Agricultural best management practices such as livestock exclusion fencing, off-stream watering, 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 impaired watersheds (2-4%), the contributions of impervious surfaces to sediment impairment can be significant. 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 the impaired streams. This was not as evident from habitat scores at the benthic stations, but poor riparian vegetation is prevalent throughout other reaches of these 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.

4.1.2. Dissolved Oxygen and Total Phosphorus

In addition to sediment, dissolved oxygen and total phosphorus were identified as probable stressors in Rich Valley Unnamed Tributary. Multiple lines of evidence supported this determination including periodic phosphorus measurements, diurnal dissolved oxygen monitoring, seasonal trends, and biological condition gradient analysis (Section 3.3 and 3.11). Based on the observed data and causal analysis, a conceptual model was developed to describe the causal relationships between the sources of phosphorus in the watershed, increased nutrient loads, decreased DO, and the observed loss of benthic macroinvertebrates (Figure 35). In this conceptual model, sources of phosphorus include point sources, direct deposit from livestock, and runoff of manure, fertilizers, and other diffuse sources. These sources and other contributing factors lead to increased nutrient load in the stream, which can act to biologically impair the stream through two pathways. Increased nutrient availability increases algae growth, which can directly alter macroinvertebrate feeding niches and competition or indirectly limit sensitive species through oxygen decreases as algae respire or are decomposed. Low DO during diurnal monitoring provided evidence of these pathways. The combined weight of evidence documented in the causal analysis supports this conceptual model of phosphorus and dissolved oxygen as stressors in Rich Valley Unnamed Tributary. A TMDL developed to reduce phosphorus loads in the watershed will address the benthic impairments in these streams through the pathways described in Figure 35. In addition, efforts to address several contributing factors that exacerbate the impact of nutrient enrichment and low DO will also be effective at reducing the impairment. Some of the contributing factors, however, are natural and the result of the location of the benthic monitoring station. Since these cannot be completely addressed, achievement of benthic standards at this location may not be fully realized.

4.1.2.1. Contributing Factors

Several factors contribute to the impact of nutrient enrichment and low dissolved oxygen in Rich Valley Unnamed Tributary. Pasture accounts for 42% of the watershed, and the management of manures on these lands plays a large role in determining the loading of nutrients from the watershed. Practices that limit application, avoid overcrowding, maintain turf health, and utilize buffer zones can be instrumental in reducing nutrient loads from agricultural lands. Livestock access to stream corridors also greatly impacts the direct loading of nutrients to the stream, so livestock exclusion fencing and off-stream watering can be used to eliminate this source of nutrients and bacteria.

Other contributing factors based on the location of the benthic monitoring station also influence the dissolved oxygen levels observed in Rich Valley Unnamed Tributary. The watershed upstream of the benthic monitoring station is only 93 acres, which is very small for a perennial stream. The stream at this location would probably not be a perennial stream if it were not for a spring located just 250 m upstream from the benthic station. The close proximity of the spring may influence the dissolved oxygen level of the stream. Depending upon the amount of time since the spring water was at the surface, dissolved oxygen could be lower than saturation and take some time to reach equilibrium with the atmosphere. In addition, there is a series of wetlands between the spring and the benthic monitoring station. The stagnant conditions in wetlands limit reaeration, and the ample biological growth in wetlands contribute to oxygen depletion. These natural wetland conditions likely contribute to the low dissolved oxygen in Rich Valley Unnamed Tributary at the benthic station. Since these contributing factors (small watershed, spring source, and connected wetlands) cannot be completely addressed, achievement of benthic standards at this location may not be fully realized. However, efforts to reduce phosphorus will help to increase dissolved oxygen and improve benthic conditions through the pathways identified in Figure 35.

4.2. TMDL Targets

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. Sediment was selected as a TMDL target pollutant in both impaired streams, and total phosphorus was selected as a TMDL target pollutant in Rich Valley Unnamed Tributary to address the combined effects of the dissolved oxygen and total phosphorus stressors.

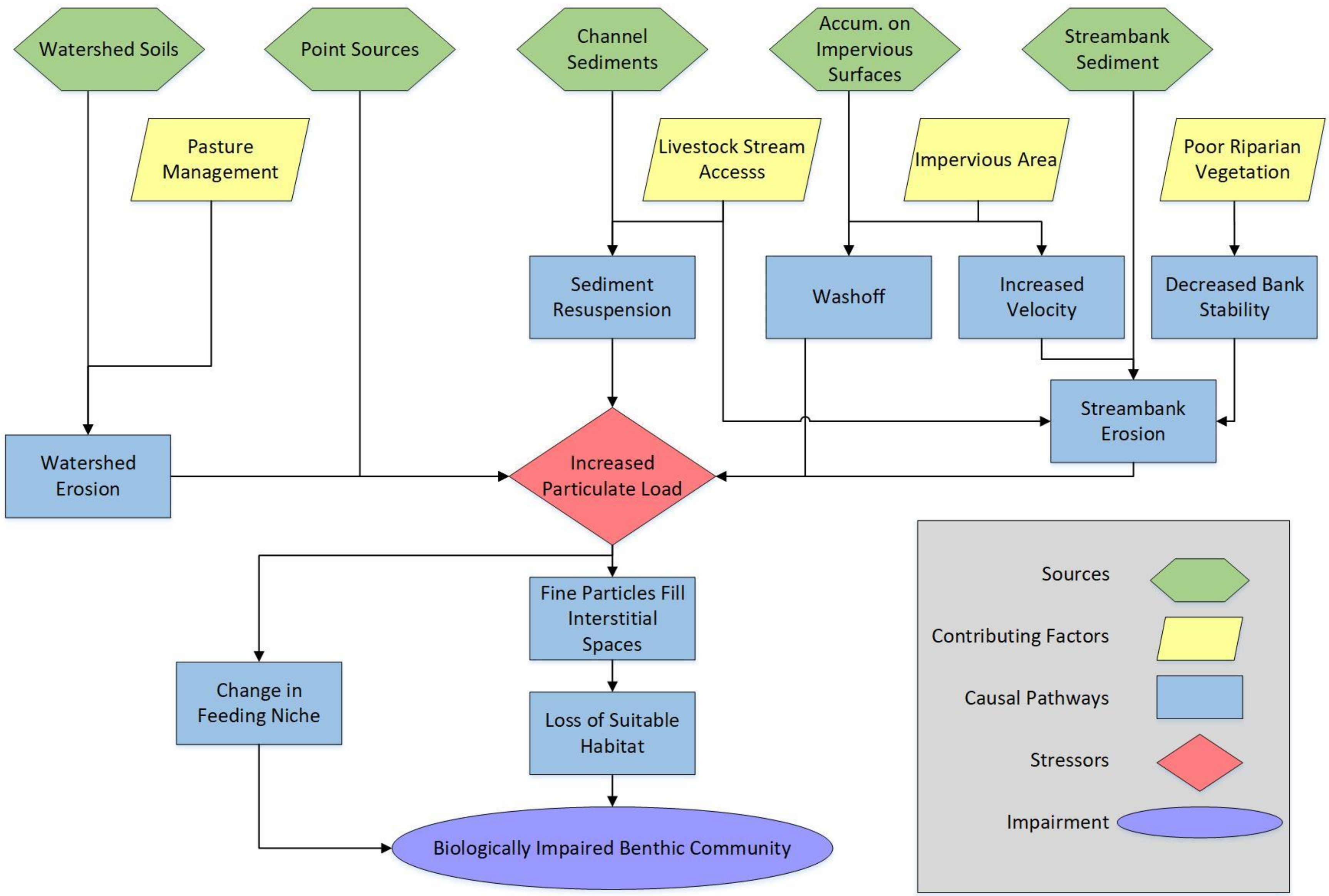


Figure 34. Conceptual model for the causal pathway of sediment impacts on benthic macroinvertebrates in Greendale Creek and Rich Valley Unnamed Tributary.

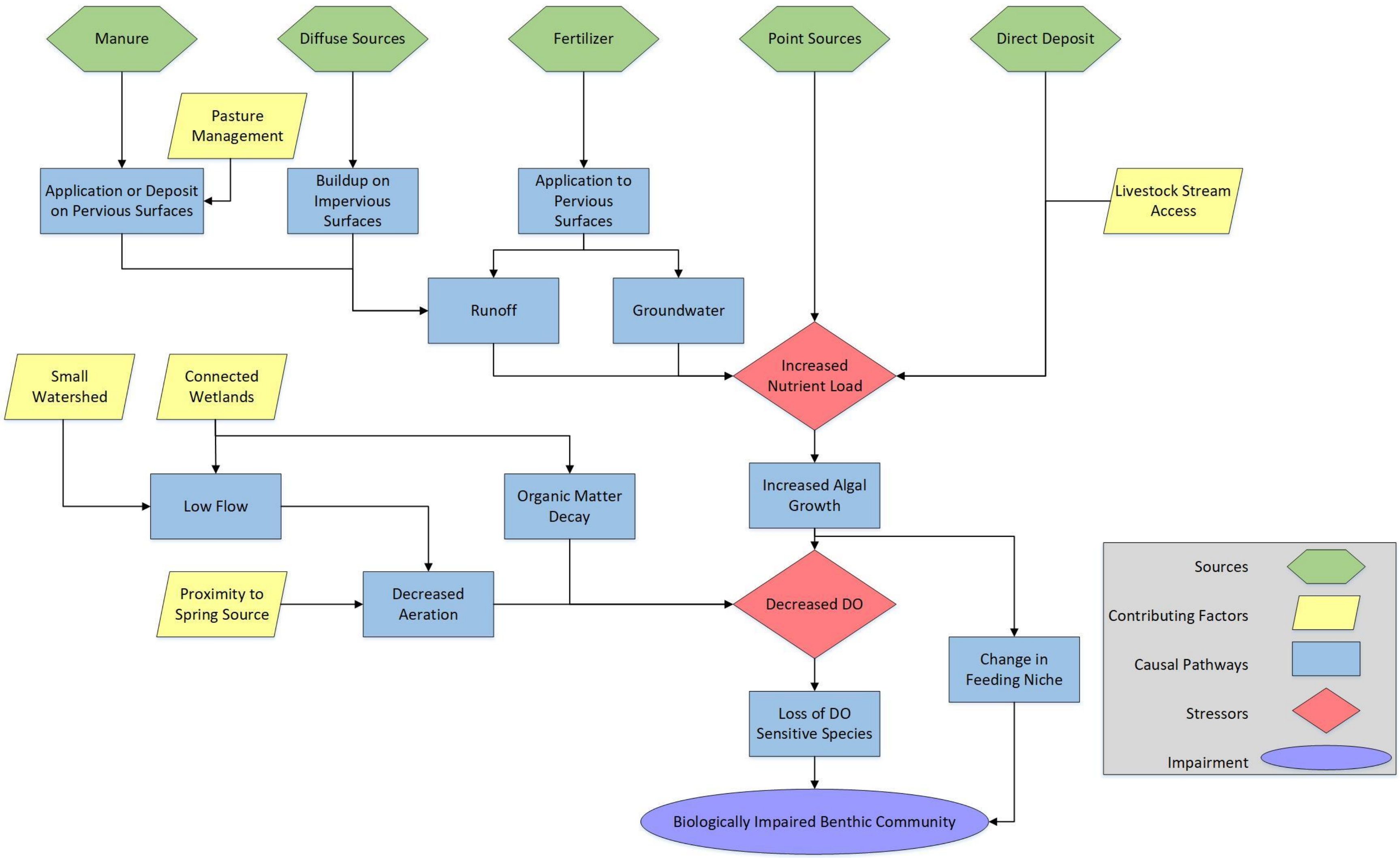


Figure 35. Conceptual model for the causal pathway of phosphorus and dissolved oxygen impacts on benthic macroinvertebrates in Rich Valley Unnamed Tributary.

5.0 REFERENCES

Bjornn, T.C., M.A. Brusven, M.P. Molnau, J.H. Milligan, R.A. Klamt, E. Chacho, and C. Schaye. 1977. *Transport of Granitic Sediment in Streams and its Effects on Insects and Fish*. University of Idaho, Forest, Wildlife and Range Experiment Station, Moscow, ID.

Brabec, E., S. Schulte, and P.L. Richards. 2002. Impervious surfaces and water quality: a review of current literature and its implications for watershed planning. *Journal of Planning Literature*, 16(4):499-514.

Golding, L. A., K. McKnight, M. Binet, M. Adams, and S.C Apte. 2018. Toxicity of dissolved and precipitated forms of barium to a freshwater alga (*Chlorella sp*. 12) and water flea (*Ceriodaphnia dubia*). *Environmental Toxicology and Chemistry*, 37(6): 1632–1642.

Kaufmann. P.R., P. Levine, E.G. Robison, C. Seeliger, and D.V. Peck. 1999. *Quantifying Physical Habitat in Wadeable Streams*. EPA/620/R-99/003. U.S. Environmental Protection Agency, Washington, D.C.

Lawrence, J.M. and S.E. Gressens. 2011. Foodweb response to nutrient enrichment in rural and urban streams. *Journal of Freshwater Ecology*, 19(3):375-385.

MacDonald, D.D., C.G. Ingersoll, and T.A. Berger. 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. *Archives of Environmental Contamination and Toxicology*, 39:20-31.

MacNeil, C., J.T. Dick, and R.W. Elwood. 1997. The trophic ecology of freshwater *Gammarus* spp. (Crustacea: Amphipoda): problems and perspectives concerning the functional feeding group concept. *Biological Reviews*, 72(3), 349-364.

Mount, D.R., R.J. Erickson, T.L. Highland, J.R. Hockett, D.J. Hoff, C.T. Jenson, T.J. Norberg-King, K.N. Peterson, Z.M. Polaske, and S. Wisniewski. 2016. The acute toxicity of major ion salts to Ceriodaphnia dubia: I. Influence of background water chemistry. *Environmental Toxicology and Chemistry*, 35(12): 3039-3057.

Redfield, A. C. 1958. The biological control of chemical factors in the environment. *American Scientist*, 46(3): 230A-221.

Tetra Tech. 2019. *Biological Condition Gradient (BCG) Attribute Assignments for Macroinvertebrates and Fish in the Mid-Atlantic Region (Virginia, West Virginia, and Maryland)*. Tetra Tech, Burlington, VT.

U.S. Environmental Protection Agency. 1980. *Ambient Water Quality Criterion for Beryllium*. EPA 440/5-80-024. U.S. EPA Office of Water Regulations and Standards, Washington, D.C.

U.S. Environmental Protection Agency. 1987. *Quality Criteria for Water - Update #2*. EPA 440/5-86-001. U.S. EPA Office of Water Regulations and Standards, Washington, D.C.

U.S. Environmental Protection Agency. 2000a. *Ambient Water Quality Criterion Recommendations: Rivers and Streams in Nutrient Ecoregion XI.* EPA-822-B-00-020. U.S. EPA Office of Water, Washington D.C.

U.S. Environmental Protection Agency. 2000b. *Stressor Identification Guidance Document*. EPA-822-B-00-025. U.S. EPA Office of Water, Washington D.C.

U.S. Environmental Protection Agency. 2018a. *Causal Analysis/Diagnosis Decision Information System (CADDIS)*. Retrieved: January 17, 2018. Available from: www.epa.gov/caddis. Office of Research and Development, Washington, D.C.

U.S. Environmental Protection Agency. 2018b. *Final Aquatic Life Ambient Water Quality Criteria for Aluminum*. EPA-822-R-18-001. U.S. EPA Office of Water, Washington, D.C.

Virginia Department of Environmental Quality. 2017. *Stressor Analysis in Virginia: Data Collection and Stressor Thresholds.* Virginia Department of Environmental Quality, Richmond, VA.

Virginia Department of Environmental Quality. 2020. *Virginia Water Quality Assessment 305(b)/303(d) Integrated Report 2020*. Virginia Department of Environmental Quality, Richmond, VA.

Voshell, J.R. 2002. *A Guide to Common Freshwater Invertebrates of North America*. The McDonald &Woodward Publishing Company, Blacksburg, VA.