WEST BRANCH SUSQUEHANNA RECOVERY BENCHMARK II

TECHNICAL REPORT
DECEMBER 2020

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Executive Summary

A legacy of abandoned mine drainage pollution (AMD) has impaired over 1,200 miles of waterways in the West Branch Susquehanna River watershed in northcentral Pennsylvania. For over 30 years, numerous remediation projects have been implemented throughout the watershed to improve water quality and biological conditions. Until 2009, there had not been a concerted effort to quantify the effects of remediation at the watershed scale. The initial collaborative effort, the West Branch Recovery Benchmark Project, was developed by Trout Unlimited and was successful in documenting significant improvements in water quality and biological communities. The objective of this project, the West Branch Recovery Benchmark II, was to replicate and expand the original Benchmark project in an effort to document changes in water quality and biological communities since 2009.

The results presented in this report indicate that the West Branch Susquehanna River and many of its historically AMD impaired tributaries are continuing to recover from AMD pollution. The mainstem of the river has maintained a net alkaline condition along its entire length and the upper 26 miles of the river were recently designated as supporting naturally reproducing trout populations. Tributaries with significant AMD remediation efforts completed over the last ten years showed significant improvements in water quality. Many of the tributaries sampled for this project also demonstrated improvement, however those improvements appear to be primarily a result of natural attenuation.

Benthic macroinvertebrate and fish communities also continue to improve throughout the watershed. Increases in pollution sensitive taxa of both benthic macroinvertebrate and fish corroborate that water quality has improved at most sample sites. Several sites throughout the watershed, based on water quality, benthic macroinvertebrate communities, and/or the presence of trout, may warrant further consideration for delisting from Pennsylvania’s list of impaired streams.

Although the improvements documented in this report indicate that the watershed is continuing along a trajectory towards recovery, comparisons with reference site water quality, benthic macroinvertebrates, and trout biomass indicated that most of the historically AMD impaired sites remain distant from a “fully recovered” state. In addition, there are several tributaries that continue to disproportionately contribute acidity to the mainstem of the West Branch Susquehanna River. In order to realize substantial improvements in the watershed, future water treatment and abandoned mine land reclamation will be required. If additional remediation projects are completed, particularly in the severely degraded tributaries noted in this report, it is likely that fish populations will continue to expand in the upper and middle reaches of the river.

Funding and monitoring for the operation and maintenance of existing treatment systems is critical to maintaining and enhancing water quality conditions in the West Branch Susquehanna River watershed. Proper monitoring of these systems will ensure that they continue to function as intended, as failing systems would negatively impact biological communities and offset the recovery of the watershed that has been accomplished to date.

Introduction

The West Branch Susquehanna River originates near the town of Carrolltown, PA and reaches its confluence with the Susquehanna River in Sunbury, PA. The basin drains approximately 7,000 mi^2 of mainly (83%) forested land in northcentral Pennsylvania. The West Branch is a major tributary of the Susquehanna River and drains just over 25 % of the total Susquehanna River watershed and contains nearly 12,000 stream miles of tributaries. The watershed is home to some of the most pristine trout streams in the commonwealth that are among the best strongholds of brook trout (*Salvelinus fontinalis*) in the Mid-Atlantic Region (Fesenmyer et al. 2017; Rummel et al. 2017). However, the area's true economic and ecological potential continues to be negatively impacted as a result of historical coal extraction. Coal mining between the late 1700s and 1970s occurred with little to no regulation and resulted in over 1,200 miles of water polluted by abandoned mine drainage (AMD) and more than 40,000 acres of unreclaimed and scarred mine lands. Rummel and Wolfe (2019) provide a review of the historical impacts of AMD and restoration efforts within the watershed.

Abandoned mine drainage is one of the two main sources (agriculture being the other) of pollution to Pennsylvania's waterways (DEP 2016). AMD is formed as pyrite, a naturally occurring mineral, comes in contact with water and oxygen beginning a chemical reaction that results in the production of iron hydroxide and sulfuric acid. The sulfuric acid produced can drastically lower the pH in a stream to uninhabitable levels for all fish and all but the most tolerant benthic macroinvertebrates. Iron hydroxide, on the other hand, can coat substrate and become dissolved in the water column at low pH. However, iron is not the only metal that can enter streams from an AMD source. Other common metals in AMD impacted streams are aluminum and manganese that are dissolved from the surrounding geology by the sulfuric acid produced in the pyrite reaction. The acidic water and toxic metals found in AMD can negatively influence the growth rate, behavior, and metabolic processes of fish. Additionally, AMD can cause a reduction in the abundance and diversity of aquatic insect populations and the metal precipitates can armor the stream substrate, thereby reducing habitat availability and diminishing the food supply for other aquatic organisms.

In 2009 Trout Unlimited developed the West Branch Susquehanna Recovery Benchmark Project to document and quantify the results from dozens of AMD remediation projects and millions of dollars that have been invested in mine cleanup across the watershed (Trout Unlimited 2011). In partnership with the PA Department of Environmental Protection (DEP), PA Fish and Boat Commission (PFBC), Susquehanna River Basin Commission, and others, Trout Unlimited targeted 90 data collection sites throughout the watershed to collect data on water quality, benthic macroinvertebrates, stream habitat, and fish over a five-month period in 2009. This study noted substantial improvements in water chemistry compared to 2004. The improvements were attributed to AMD treatment and AML restoration, improved mining practices and regulation, and natural attenuation (Trout Unlimited 2011). Natural attenuation is the process in which, over time, the geochemical weathering of pyrite will naturally decrease, reducing the amount of acidity produced from abandoned mine sites.

Since the completion of the initial Recovery Benchmark Project, AMD remediation efforts have continued throughout the West Branch Susquehanna River watershed, including the construction of new passive and active treatment systems and abandoned mine land reclamation. In 2017, Trout Unlimited began work to replicate and expand the 2009 project for the West Branch Susquehanna Recovery Benchmark II. The objective of this study was to document current water quality and biological conditions and identify changes through time in response to the continued efforts to restore the West Branch Susquehanna River watershed to its full ecological potential.

Methods

Sample Site Description & Selection

A total of 110 sample sites were established for data collection in this study (Figure 1). A list of sample sites is provided in Appendix A. Data collected at these sites included water quality, stream flow, habitat evaluations, benthic macroinvertebrate communities, and fishery surveys as described below. Data were collected from 2017-2019. These sites included 78 water quality and 59 macroinvertebrate sample sites that were used in the 2009 Recovery Benchmark I project data collection efforts (referred to as “replicate” sites throughout this report). In addition, 30 sites were added to the current study as “reference” sites. Reference sites were located within the West Branch Susquehanna River watershed, had no listed impairments on the Pennsylvania 303(d) list of impaired and threatened waters (DEP 2020a), and were listed as Class A trout waters by the PFBC. A list of potential reference sites was generated from those criteria and 30 were randomly selected to be sampled using a random number generator.

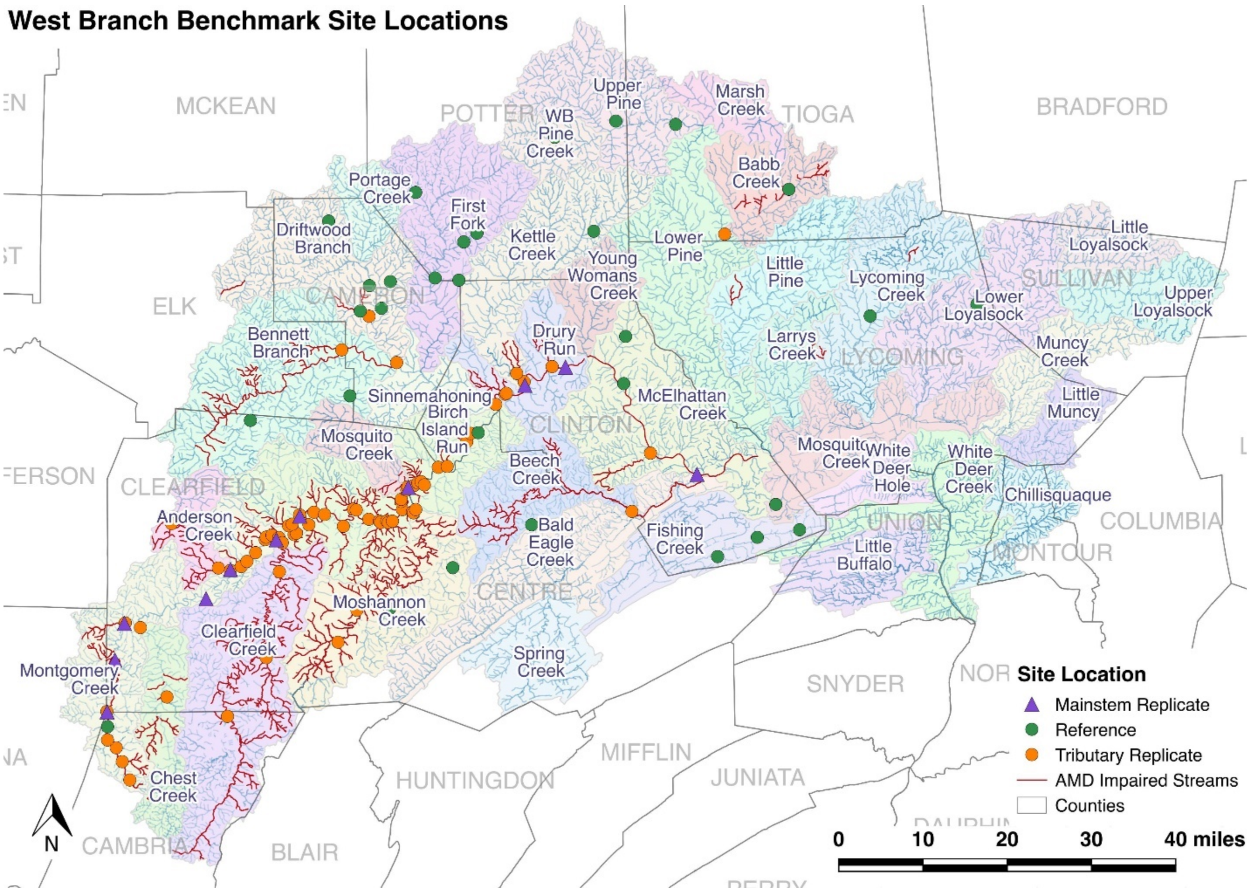

Figure 1. Sample site locations for the West Branch Recovery Benchmark II project.

Replicate sites in 2017 were evaluated for the presence of AMD treatment within their respective watersheds. Analysis of treatment system locations was completed in ArcGIS using publicly available data from the Pennsylvania Spatial Data Access (PASDA) database (PASDA 2020). The data sources used included the abandoned mine lands polygons (DEP 2020c), and coal mining operations (DEP 2020d). Datashed (Datashed 2020) was used to identify passive and active treatment systems within the West Branch Susquehanna River basin. For a few sites, satellite imagery was used to locate treatment systems mentioned in the coal mining operations layer, but not present in Datashed's database. Multiple sources were used to determine the year of treatment system installation (DEP 2006; Cavazza et al. 2012; Datashed 2020); Kelly Williams of Clearfield County Conservation District was also consulted about the Muddy Run passive treatment system. Figure 2 shows the location of AMD remediation within the watershed as identified through these methods. Note that only treatment locations available through the data sources described were included in the analysis. Additional treatment systems and reclamation projects may exist within the watershed. Data was not available to evaluate the effectiveness of the AMD treatment on water quality.

A watershed was determined to have AMD treatment if land reclamation, passive treatment, or active treatment was present upstream of the sampling point in the watershed. From there we examined if the treatment was located within the sample site's HUC-12 or within the smallest watershed unit of the sample site. When grouping sites by treatment, four different groupings were used. Those groups included the following: [1] reference sample sites, [2] any site that has active treatment upstream regardless of other treatment types in the watershed, [3] any site that has passive treatment upstream regardless of other treatment types (excluding sites with active treatment) in the watershed, [4] sites that only have land reclamation present upstream in the watershed, and [5] sites with no known treatment present. These groupings were used in subsequent analyses detailed in this report to compare among treatment types. Appendix B contains a list of sample sites with treatment and details the type of treatment present and the treatment group that the site was placed into for analysis.

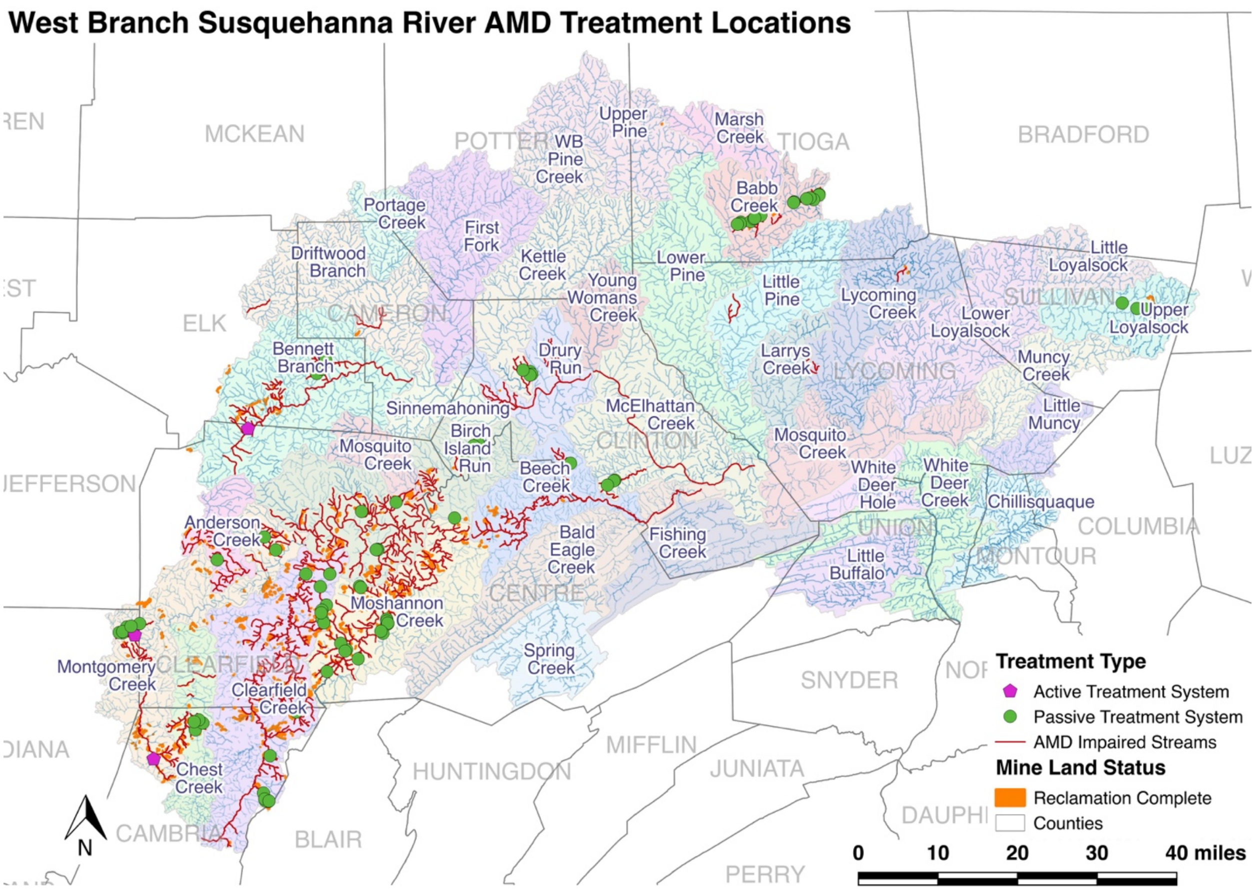

Figure 2. Active and passive treatment systems and land reclamation sites within the West Branch Susquehanna River watershed.

Water Quality/Flows

A total of 108 and 110 sites were sampled for water quality within a 5-day period in May and July 2017, respectively. Flow measurements were made perpendicular to the direction of mid-channel flow and in areas where backwater and obstacles could be avoided. Cross-sectional measurements of depth, velocity at 6/10th of the stream depth, and distance from the bank were taken at approximately 20 locations or at intervals that comprised no more than 10% of the entire flow of the site. Where flows were too large to measure using conventional wading techniques, the existing U.S. Geological Survey (USGS) stream gauge network was used.

Water quality samples were taken from the vertical profile of the main current usually in the center of the stream. In the case of larger tributaries or mainstem river sample locations, 3 to 6 samples from across the sample site were composited. A 500 mL raw water sample, a 250 mL sample fixed with 15-20 drops of HNO^3, and a 250 mL sample filtered through a 0.45 micron filter and then fixed with 15 to 20 drops of HNO^3 were collected from each site. Samples were placed on ice and transferred to a DEP accredited laboratory for analysis of 21 total parameters. Table 1 provides the parameters analyzed by the laboratory. Duplicate and blank samples were also sent to the lab for quality assurance purposes.

Table 1. List of water quality parameters analyzed by DEP accredited laboratory.

Laboratory Water Quality Parameters	
pH	Conductivity (µS/cm)
Alkalinity (mg/L)	Acidity (mg/L)
Total Iron (mg/L)	Total Manganese (mg/L)
Total Aluminum (mg/L)	Sulfate (mg/L)
Total Nickel (mg/L)	Total Zinc (mg/L)
Total Suspended Solids (mg/L)	Total Copper (mg/L)
Dissolved Copper (mg/L)	Total Dissolved Solids (mg/L)
Dissolved Manganese (mg/L)	Dissolved Iron (mg/L)
Dissolved Nickel (mg/L)	Dissolved Aluminum (mg/L)
Chloride (mg/L)	Dissolved Zinc (mg/L)
Hardness (gpg)	

Basic field chemistry was collected at each site using an Oakton multiple parameter meter that measured conductivity, temperature, and pH. Each meter was calibrated daily to the manufacturers' specifications to ensure accuracy.

Loadings (lbs/day) for water quality parameters, at sites that had flow measurements, were calculated. At sites where flow could not be measured, USGS gauges were used. Acidity values were also calculated based on laboratory measured dissolved iron, dissolved aluminum, dissolved manganese, pH, and alkalinity for each site (Hedin 2006). From this calculation, the loadings (lbs/day) were also calculated for acidity incorporating field flow. Measured acidity load (lbs/day) using the lab measured acidity value was also calculated.

Habitat

Habitat was evaluated for 100 meters at 106 sample sites using DEP's *Water Quality Network Habitat Assessment* form (Barbour et al. 1999). All habitat evaluations were completed by the same observer to avoid observation bias in the sampling and ensure that results were comparable. The following twelve parameters: instream cover, epifaunal substrate, embeddedness, velocity/depth regimes, channel alteration, sediment deposition, frequency of riffles, channel flow status, condition of banks, bank vegetative protection, grazing or other disruptive pressure, and riparian vegetation zone width were evaluated at each site. These parameters are explained in greater detail in Appendix C. Each parameter is given a score (from 0 – 20) based on a visual survey of the sample site. The scores from each parameter are summed to obtain an overall habitat score. The habitat scoring system is as follows: “optimal” category scores from 240 to 192, “suboptimal” from 180-132, “marginal” from 120 – 72, and “poor” is a site with a combined score less than 60. The original gaps between these categories were rolled up into the next closest category. For example, anything 181-240 was considered “optimal”; instead of the 192 cutoff above. Gaps in the scores are typically left up to the discretion of the original surveyor, however some sites hadn’t been sampled since 2009 so site details would be difficult to recall.

Benthic Macroinvertebrates

Benthic macroinvertebrate communities were surveyed at 96 of the sample sites between April to June in 2017 and 2018 to be consistent with the original Recovery Benchmark project. These sites included 66 tributaries, 2 mainstem river sites, and 28 reference sites. All benthic macroinvertebrate samples were intended to be collected in 2017, however high water levels prevented the collection of several sites. The remaining sites were collected in 2018. Benthic macroinvertebrates were not collected at several mainstem river sites as water depth precludes sampling at these sites. Surveys were completed by TU personnel who were previously trained by DEP Bureau of Water Standards and Facility Regulation staff in the appropriate protocols.

Benthic macroinvertebrate surveys were completed according to DEP’s Instream Comprehensive Evaluation (ICE) protocols (specifically section C.1.b. *Antidegradation Surveys*) (Chalfant 2007; Chalfant 2015) to replicate methods used in the original Recovery Benchmark Project. In short, benthic macroinvertebrate surveys consisted of a combination of six D-frame efforts in a 100-meter stream section. These efforts were spread out to select the best riffle habitat areas with varying depths. Each effort consisted of an area of 1 m^2 to a depth of at least 4 inches as substrate allowed and was conducted with a 500 micron mesh 12-inch diameter D-frame kick net. The six individual efforts were composited and preserved with ethanol for processing in the laboratory.

Individuals were identified by taxonomists certified by the North American Benthological Society to genus or to the next highest possible taxonomic level. Samples containing 160 to 240 individuals, when available, were evaluated according to the seven metrics comprising the DEP’s Index of Biological Integrity (IBI) (Total Taxa Richness, EPT Taxa Richness, Beck’s Index V.3, Shannon Diversity, Hilsenhoff Biotic Index, ratio of Biological Condition Gradient (BCG) attribute, and Percent Sensitive Individuals). Appendix D contains a description of each of these metrics. Biological metrics were standardized and used to determine if the stream met the Aquatic Life Use (ALU) threshold for coldwater fishes, warmwater fishes, and trout stocked fishes (Figure 3). Functional feeding groups (FFG) were identified for each taxon as well; these include piercers, shredders, filtering collectors, collector gatherers, scrapers, predators, and unknown.

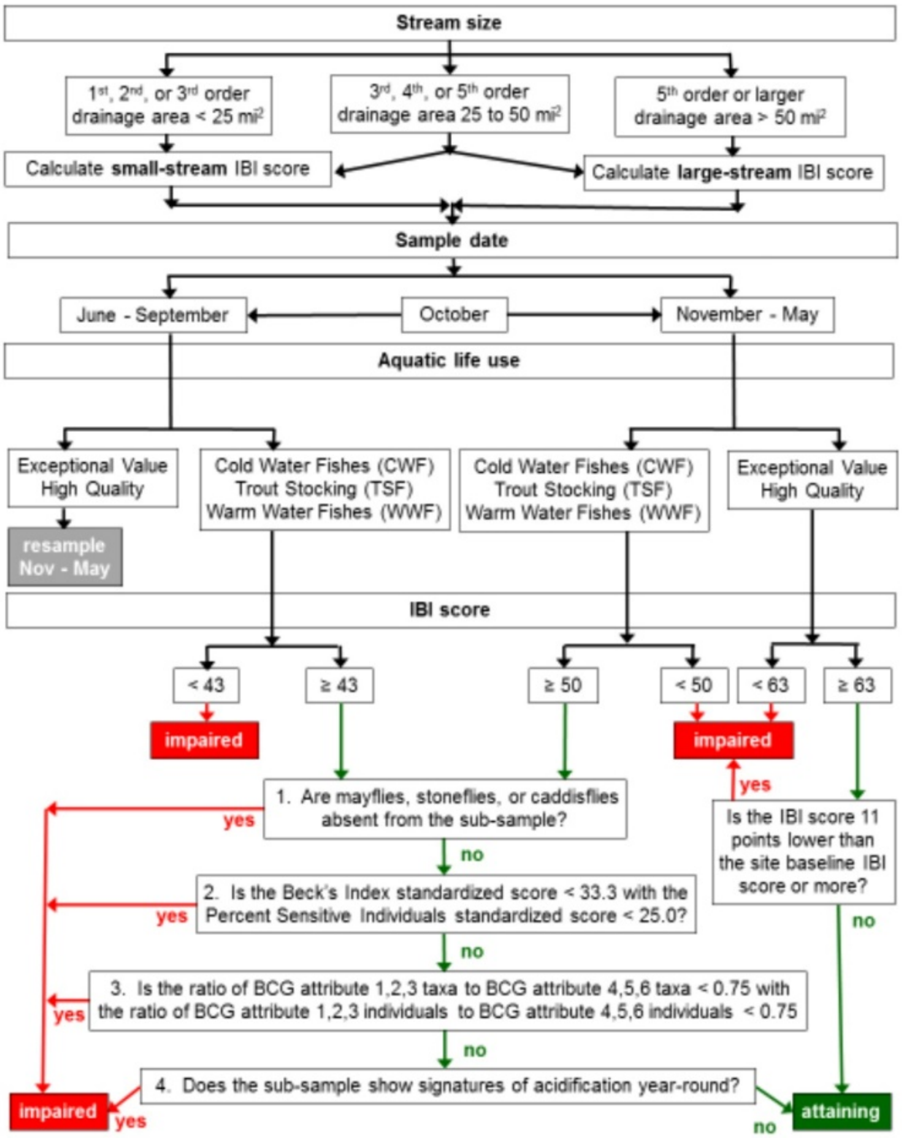

Figure 3. Aquatic life use determination chart for macroinvertebrate sampling (Chalfant 2015).

FFGs were determined and used as ecosystem attribute surrogates (Minshall et al. 1983; Cummins et al. 1981; Cushing et al. 1995; Merritt et al. 1996; Merritt et al. 1999; Merritt et al. 2002; Wagner et al. 2001). These ecosystem attribute calculations were used to examine production to respiration, CPOM to FPOM ratio or riparian linkage, FPOM transport/storage, substrate stability, and top-down control (Table 2). Ecosystem attributes were calculated for all sites collected across all years.

Table 2. Description of ecosystem attribute surrogates using FFGs; calculations and general interpretations (Wagner et al. 2001; Cummins et al. 2005).

Ecosystem Attribute	Abbreviations	FFG Ratio	Criteria Ratios
Autotrophy to Heterotrophy	P/R	$\frac{\text{Scrapers}}{\text{Shredders + Collectors}}$	Heterotrophic <0.75
Coarse particulate organic matter (CPOM) to fine particulate organic matter	CPOM/FPOM	$\frac{\text{Shredders}}{\text{Collectors}}$	Normal shredder associations, functional riparian zone >0.25
Suspended FPOM to deposited FPOM	TFBOM/BFPOM	$\frac{\text{Filtering Collectors}}{\text{Gathering Collectors}}$	FPOM transport greater than normal particulate loading in suspension >0.50
Substrate stability	Stable Channel	$\frac{\text{Scrapers + Filtering Collectors}}{\text{Shredders + Gathering Collectors}}$	Stable substrates abundant >0.50
Top-down control	Pred-Prey interaction	$\frac{\text{Predators}}{\text{All other FFGs}}$	Typical predator prey balance <0.15

Fishery Surveys

PFBC Area 3 staff sampled fish at five historic sample sites and established one new site from 8-16 October 2019 (Figure 4; Table 3) for a total of six survey sites. River conditions precluded sampling efforts in 2017 and 2018. Fishery survey data were collected at 3 additional sites in 2009, however only the sites surveyed in 2019 are discussed in this report. Fish communities were evaluated and water chemistry was measured at each site. Data collection protocols followed those of past surveys (Hollender and Kristine 1998, 1999; Detar and Kristine 2009) using backpack and mini-boom boat electrofishing gear. Detailed description of the electrofishing gear is provided in (Table 4). All fish captured that could be identified at the site were tallied by species and released. Juvenile cyprinids and other unidentifiable fish were preserved and returned to the PFBC laboratory for identification. Identification of preserved fish was confirmed by D. Fischer of PFBC Division of Environmental Services, Natural Diversity Section.

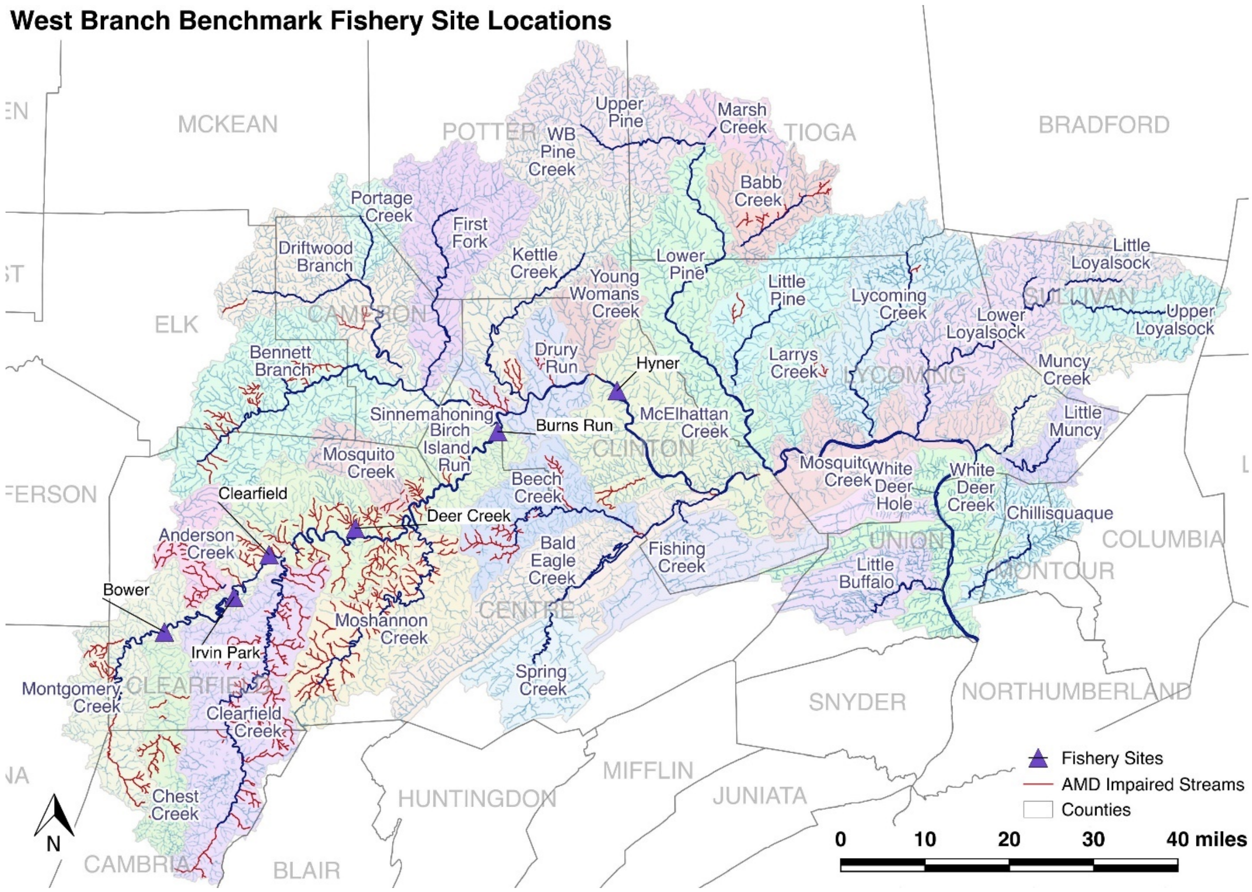

Figure 4. Location of PFBC fishery surveys in 2019 in the mainstem of the West Branch Susquehanna River.

Table 3. West Branch Susquehanna River sample sites in 1998, 2009, and 2019.

Site Name	Latitude	Longitude	River Mile	Section	Sample Date	General Site Description
Bower	40.89694	-78.67722	202.36	4	6/22/98, 6/24/09, 10/9/19	Vicinity of USGS gauge station at T418 bridge.
Irvin Park	40.957672	-78.516746	183.92	4	10/9/19	Irwin Park located downstream of Rt 453 Bridge.
Clearfield	41.031692	-78.435328	173.40	7	6/25/98, 8/10/09, 10/9/19	Vicinity of confluence with Moose Creek in Clearfield.
Deer Creek	41.07762	-78.235962	147.90	8	6/30/98, 7/1/09, 10/8/19	Beginning at SR1009 bridge just upstream confluence with Deer Creek.
Burns Run	41.245573	-77.906943	110.71	8	7/1/98, 7/28/09, 10/16/19	Vicinity of confluence with Burns Run.
Hyner	41.316717	-77.631287	85.52	8	7/2/98, 6/26/09, 10/11/19	About 650 m downstream Rt120 bridge near Hyner.

Table 4. Sampling gear used to capture fish in the West Branch Susquehanna River during 2019.

Gear	Description	Standard Unit of effort
Backpack Electrofishing	PFBC standard Coffelt-type gas powered electrofisher with two 28 cm ring electrodes. Output used ranged 75-100 VAC at 1.3-2.0 A.	Two 100m long sites, along shore in shallow riffle habitat. Two persons netting fish. Fish catches combined for reporting.
Mini-boom boat electrofisher	Smith-Root model 2.5 GPP electrofisher using a single boom with 10 anode droppers and a 4.3m aluminum flat bottom boat. Output used ranged 100-150 VDC, 120 PPS, at 3.0-4.0 A.	Electrofishing runs at two separate sites. Each usually ≥ 20 minutes for variable distances depending on conditions. Pool, run, and riffle habitat. One person netting fish. Sample sites limited by boat launching access and river depth. Fish catches combined for reporting.

Multiple diversity and evenness measures were calculated for the fishery data. Shannon Diversity (Appendix D) was calculated for the 1998, 2009, and 2019 data. Simpson's diversity (Appendix D) and evenness (Appendix D) were also calculated for all three years in order to compare results across all three sampling years. Sorenson's evenness method (Sorenson 1948) was used to compare sites' similarities to each other. This was performed for all gear types and years, as well as combined gear types across years. To combine gear types, fish species totals were added together for sites that had multiple gear types used.

Coldwater fisheries data from historical PFBC data collection efforts since 2009 that were beyond the scope of this project were also included to evaluate changes in trout presence and classification of those streams by the PFBC. Trout biomass data from PFBC was also used to compare replicate sites to reference sites.

Statistical Analyses

A variety of statistical methods were used to compile the results of this study. Descriptions of the specific statistical methods are found within the Results section of this report. In general, parametric and non-parametric tests were used, as appropriate, for comparisons among years, treatment groups, etc. Non-metric dimensional scaling and its associated statistical methods were used to analyze benthic macroinvertebrate communities and is described further in the Results.

Results

Water Quality

Long-term data from USGS gaging stations in the mainstem of the West Branch Susquehanna River document increasing pH (Figure 5), stabilizing sulfate concentrations (Figure 6), and decreasing acidity concentrations (Figure 7) over time. Calcium and magnesium to sulfate ratios have also increased over time (Figure 8).

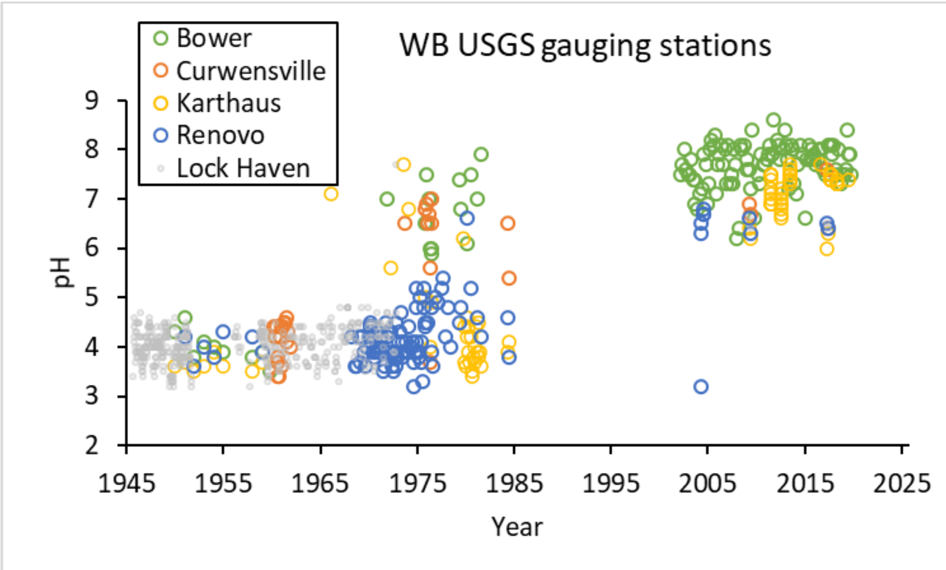

Figure 5. pH over time at USGS gauging stations in the mainstem of the river.

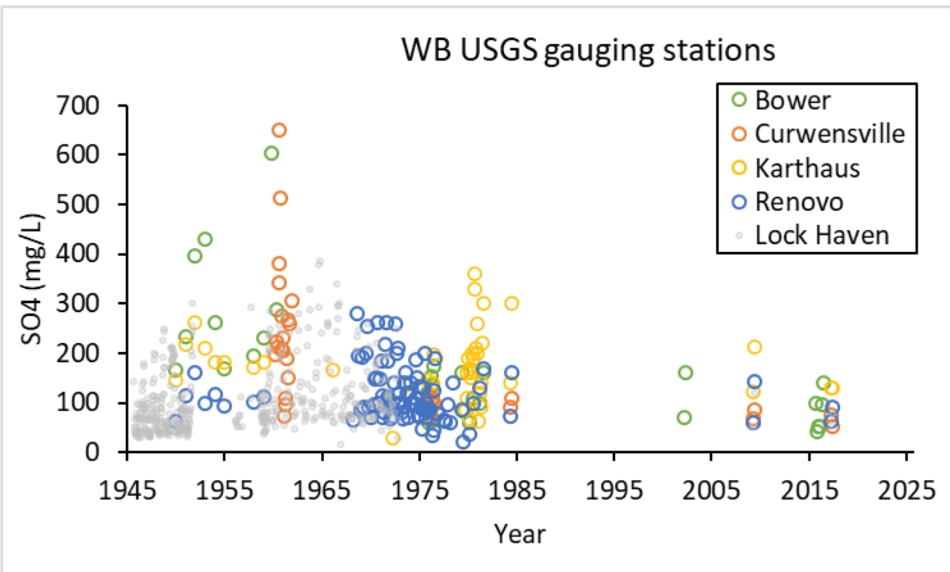

Figure 6. Sulfate concentrations over time at USGS gauging stations in the mainstem of the river.

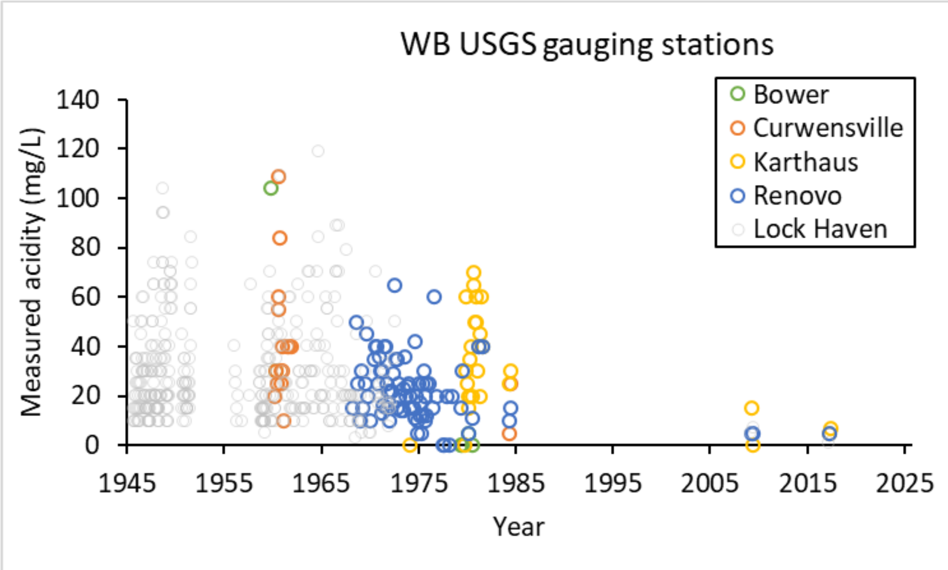

Figure 7. Acidity concentrations over time at USGS gauging stations in the mainstem of the river.

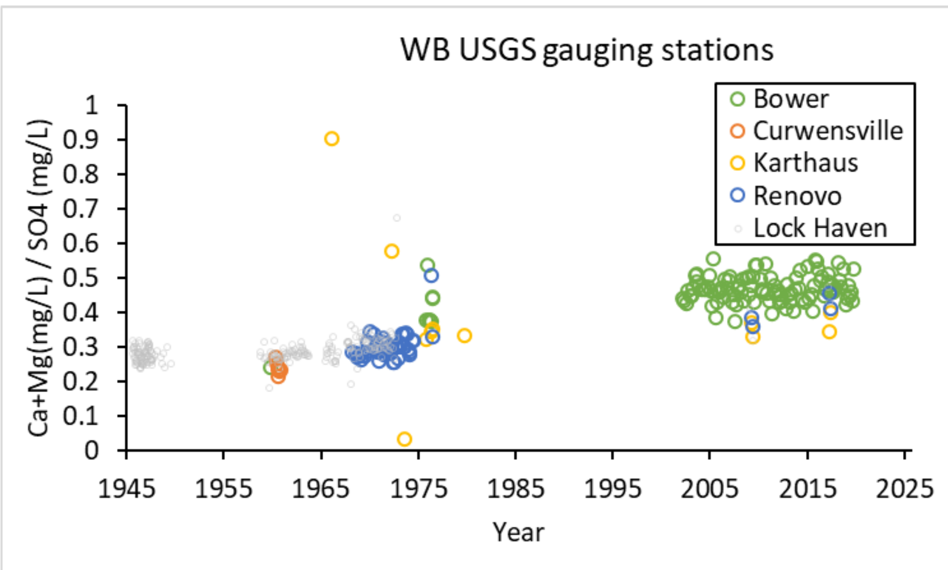

Figure 8. Ca+Mg/SO4 ratio over time at USGS gauging stations in the mainstem of the river.

In the current study, water quality at replicate sites improved from 2009 to 2017. However, there were no statistically significant differences between the two years when comparing all replicate site data using a Kruskal-Wallis test ($p > 0.05$). Mann-Whitney pairwise comparisons of replicate sites between 2009 and 2017 showed statistically significant differences between the following parameters: pH, conductivity, alkalinity, acidity, total iron, total manganese, total aluminum, sulfate, total dissolved solids, dissolved iron, dissolved manganese, dissolved nickel, total nickel, and total zinc. Mean differences of these parameters, standard deviation, and p-values from the pairwise comparisons are provided in Table 5. Statistically significant increases were observed for pH, alkalinity, total iron, and dissolved iron; while conductivity, acidity, total manganese, total aluminum, sulfates, total dissolved solids, dissolved manganese, dissolved nickel, and total nickel, and total zinc significantly decreased between the sample years.

Table 5. Mean difference (standard deviation) of the means of the paired water quality data. Negative values indicate a decrease in the metric from 2009 to 2017. Statistically significant differences ($p<0.05$) are highlighted.

Metric	2009 vs 2017	p-value
	Mean Difference (SD)	
pH	0.125 (0.163)	0.001
Conductivity (µS/cm)	-44.968 (56.80)	<0.001
Alkalinity (mg/L)	3.672 (3.60)	<0.001
Acidity (mg/L)	-4.6585 (7.54)	<0.001
Total Fe (mg/L)	0.0245 (0.831)	<0.001
Total Mn (mg/L)	-0.6715 (0.519)	<0.001
Total Al (mg/L)	-0.019 (0.421)	0.03
Sulfate (mg/L)	-16.335 (33.05)	<0.001
Total Dissolved Solids	-25.127 (47.33)	<0.001
Dissolved Cu (mg/L)	-0.0005 (0.0006)	0.303
Dissolved Fe (mg/L)	0.1015 (0.767)	<0.001
Dissolved Mn (mg/L)	-0.653 (0.491)	<0.001
Dissolved Al (mg/L)	-0.049 (0.395)	0.117
Dissolved Ni (mg/L)	-0.007 (0.01)	<0.001
Total Ni (mg/L)	-0.008 (0.01)	<0.001
Total Zn (mg/L)	-0.0065 (0.017)	0.003

Currently, Moshannon Creek, Alder Run, Milligan Run, and Cooks Run discharge the largest acidity loadings into the West Branch Susquehanna River, contributing 85% of the acidity load from the spring sample (Figure 9). Summer loadings were slightly different, however stream flow was not collected at Milligan Run, so loadings were not calculated for that site during the summer sample. Water quality results would suggest that Milligan Run continues to contribute high acid loads throughout the year. Sample sites with the greatest improvement in calculated acidity loading include Bennett Branch Sinnemahoning Creek, Muddy Run, Chest Creek (at Mahaffey and Westover), Clearfield Creek (at its mouth and at Dimeling) and Sinnemahoning Creek (Figure 10).

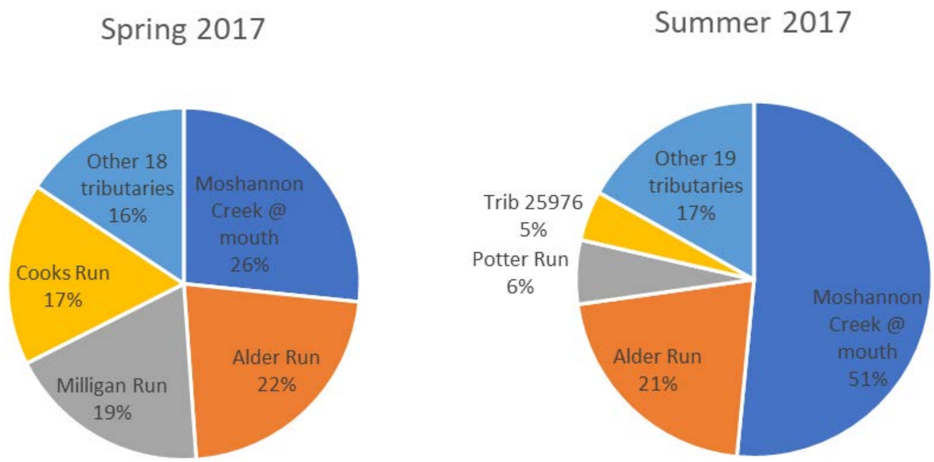

Figure 9. Percent contribution of acidity loading for tributary sites in spring and summer 2017.

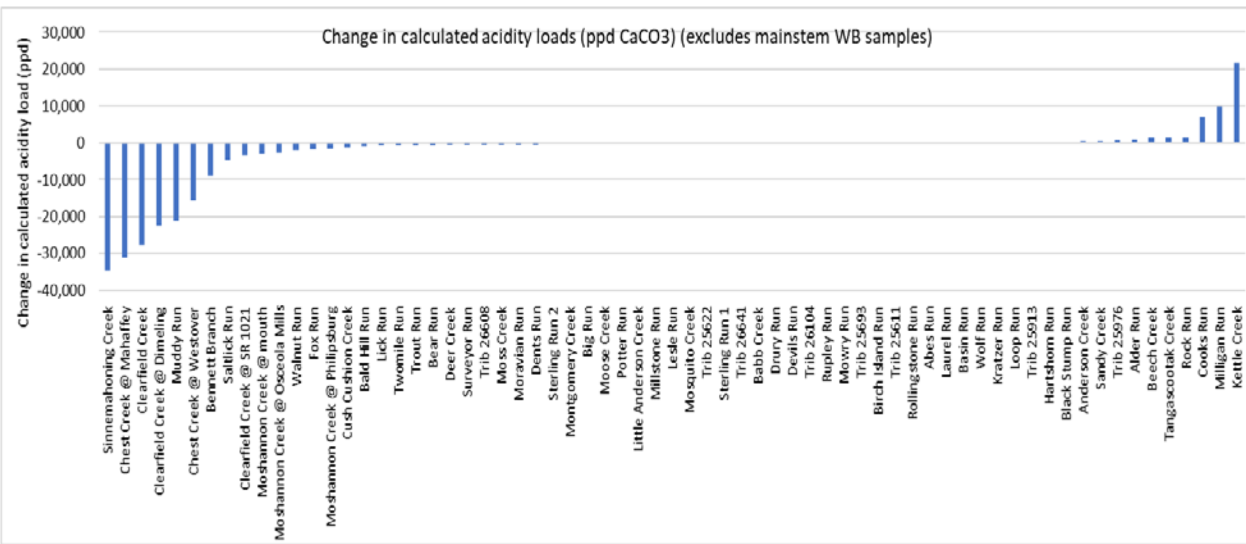

Figure 10. Change in calculated acidity loads (ppd $CaCO_3$). Excludes West Branch Susquehanna River mainstem sites.

The following nine water quality parameters were evaluated for violations of Chapter 93 water quality standards for each sample site (Water Quality Standards 1971): pH, alkalinity, manganese, aluminum, sulfate, total dissolved solids, chlorine, dissolved iron, and dissolved aluminum. Chapter 93 water quality standards are provided in Table 6. The mean (SD) number of parameter violations by season decreased, although not statistically significant, from 3.91 (2.49) and 4.3 (2.7) in 2009 spring and summer, respectively to 3.77 (2.63) and 4.22 (2.77) in 2017 spring and summer, respectively. In 2017, a total of 16 and 21 replicate sites met water quality standards for each of the nine parameters in spring and summer, respectively (Table 7). Comparatively, in 2009, 12 and 13 samples met all criteria in spring and summer, respectively.

Table 6. Chapter 93 water quality standards.

Water Quality Parameter	Chapter 93 Water Quality Standard
pH	From 6.0-9.0 inclusive
Alkalinity	Minimum 20 mg/L
Manganese	Maximum 1.0 mg/L
Aluminum	Maximum 0.75 mg/L
Sulfate	Maximum 250 mg/L
Total Dissolved Solids	Maximum 750 mg/L
Chloride	Maximum 250 mg/L
Dissolved Iron	Maximum 0.3 mg/L
Dissolved Aluminum	Maximum 0.75 mg/L

Table 7. Replicate sample sites meeting Chapter 93 water quality standards by season in 2017.

Site	Spring	Summer
Trib 26622	*x*	
Walnut Run	*x*	
Moss Creek	*x*	*x*
Cush Cushion Creek	*x*	*x*
Chest Creek @ Mahaffey	*x*	*x*
Trib 26641	*x*	
Black Stump Run	*x*	*x*
WB @ Cherry Tree	*x*	*x*
WB @ Burnside (219 Bridge)	*x*	*x*
Chest Creek @ Westover	*x*	*x*
WB @ Lumber City (729 Bridge)	*x*	*x*
WB @ Shawville	*x*	*x*
WB @ 879 Bridge	*x*	*x*
Clearfield Creek @ SR 1021	*x*	
Kratzer Run	*x*	*x*
WB @ Curwensville	*x*	*x*
Fox Run		*x*
Clearfield Creek		*x*
Sinnemahoning Creek		*x*
WB @ McGees Mills		*x*
WB @ Karthaus		*x*
WB @ Lock Haven		*x*
Clearfield Creek @ Dimeling		*x*
WB @ Renovo		*x*
Babb Creek		*x*

Sample sites on the mainstem of the river upstream of Karthaus met DEP Chapter 93 water quality standards in 2017 for both spring and summer samples. Sample sites downstream of this location violated only for low alkalinity. The sample site at Karthaus violated for both low alkalinity and high aluminum concentrations. The following sites violated eight of the nine parameters in 2009 and 2017 for both spring and summer sampling events: Wolf Run, UNT 26104, Rollingstone Run, Rock Run, Potter Run, UNT 25913, UNT 25693, and Milligan Run.

Water quality sample sites were grouped according to the type of AMD treatment with the sample site's watershed as described in the Methods section. Active and passive treatment groups were combined for the water quality comparisons because there were only five samples with active treatment. A Kruskall-Wallis test was used with a posthoc Dunn test (using the Holm method to adjust p-values) to determine if statistically significant differences existed among groups.

Statistically significant differences existed between sites with active and/or passive treatment compared to sites with no AMD treatment. Sites with active and/or passive treatment had significantly higher pH ($p = 0.001$) and alkalinity ($p < 0.001$) and lower acidity ($p < 0.001$) and metal concentrations than sites without AMD treatment. Sites with land reclamation projects had statistically higher pH ($p=0.02$), alkalinity ($p = 0.015$), and lower acidity ($p = 0.023$) than sites without treatment. However, there were no statistically significant differences in metal concentrations between sites with land reclamation and those without AMD treatment. Sites with active and/or passive treatment also had significantly lower conductivity ($p = 0.03$), sulfate concentrations ($p=0.03$), total dissolved solids ($p=0.03$), dissolved nickel concentrations ($p=0.02$), total nickel concentrations ($p=0.019$), and total zinc concentrations ($p=0.009$) than sites with land reclamation projects only. Reference sites had significantly lower conductivity, total iron, total manganese, total aluminum, sulfates, total dissolved solids, dissolved iron, dissolved manganese, dissolved aluminum, dissolved nickel, and dissolved zinc compared to sites with all other treatment groups. Although not statistically significant, pH was generally higher at reference sites compared to sites with active and/passive treatment or land reclamation. Reference site pH was significantly higher than sites without AMD treatment ($p<0.001$).

Natural attenuation at AMD impaired replicate sites was also evaluated. Exponential decay regressions were fit to each monitoring point (1984, 2009, and 2017 data) for each sample site to calculate the decay rate of calculated acidity concentration (Figure 11) and sulfate concentrations per year. Only sites with high correlations to the exponential decay rate ($r^2>0.70$) were used to evaluate sites that had decay rates exceeding the reference for natural attenuation (Figure 12) (Mack and Skousen 2008). Decay constants for calculated acidity and sulfate concentrations and loadings ranged from 0-6% each year.

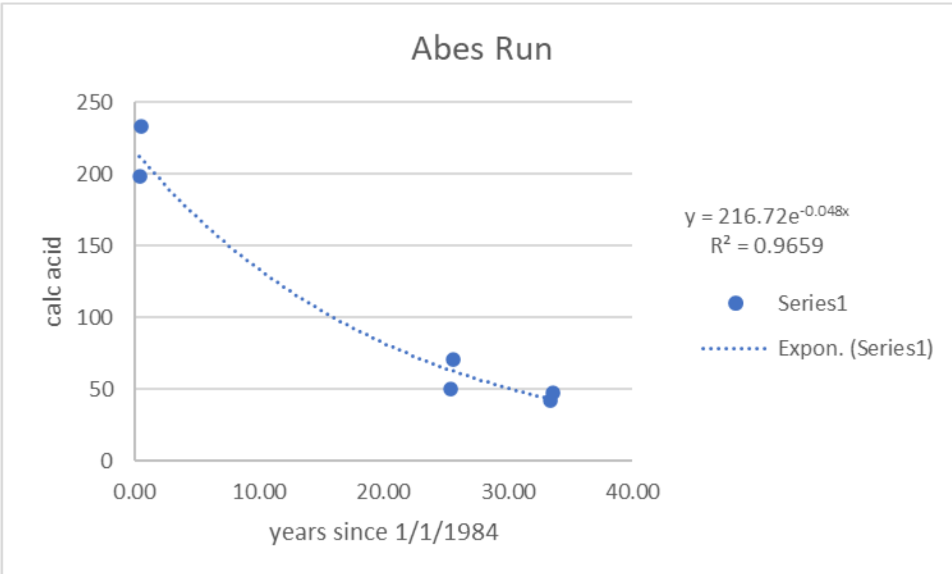

Figure 11. Example exponential decay rate regression for a sample site (Abes Run).

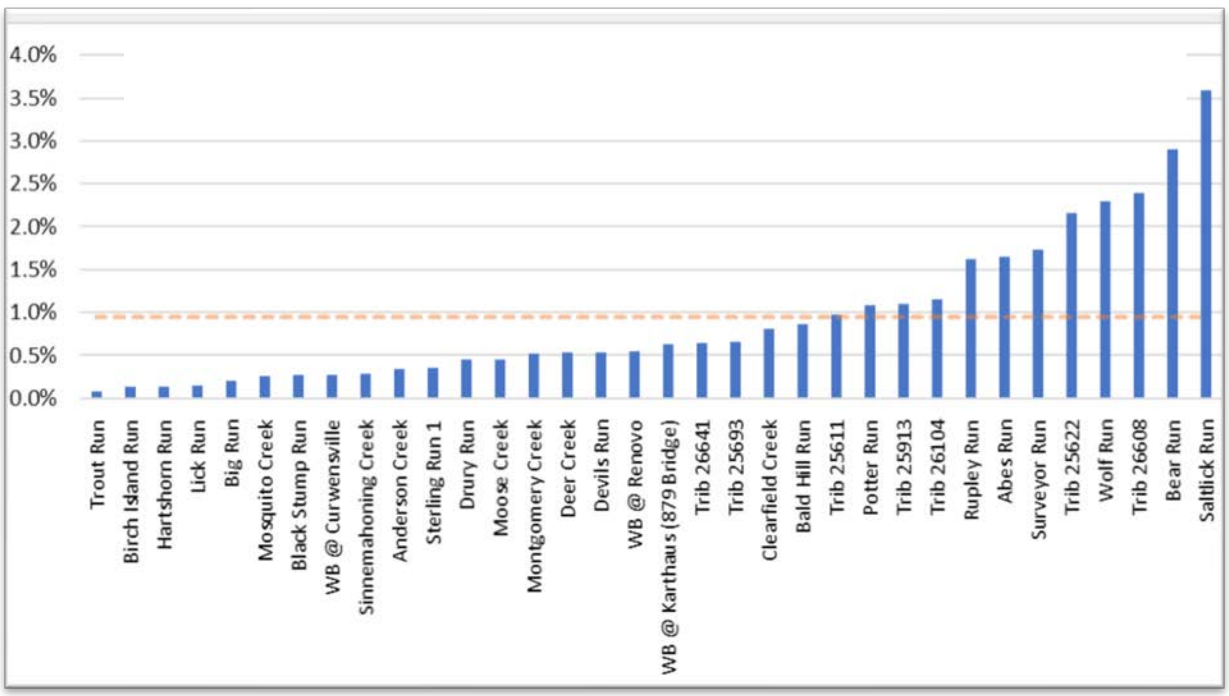

Figure 12. Relative rate of calculated acidity concentration decreases per year for 1984, 2009, and 2017 data. Only decay rates for sites with exponential regressions with an r^2>0.70 are shown. Orange dashed line indicates the natural attenuation reference rate.

Habitat

A total of 106 sample sites were surveyed for habitat in 2017 (77 replicate sites and 29 reference sites). Twenty sites in 2017 were rated as suboptimal, 85 sites as optimal, and one site

(Schreckengast Gap Run) was dry at the time of sampling. One of the reference sites was rated as suboptimal (Waldy Run) missing an optimal rating by only one point. The other 28 reference sites were rated as optimal.

A total of 72 sites were sampled in 2009. Four of the sites were rated as marginal in 2009 (West Branch Susquehanna River at Lock Haven, West Branch Susquehanna River at Renovo, Moshannon Creek at Philipsburg, and Muddy Run). Three of those four sites were rated as suboptimal in 2017 (West Branch Susquehanna River at Lock Haven, West Branch Susquehanna River at Renovo, and Muddy Run) and Moshannon Creek at Philipsburg was rated as optimal in 2017. The remaining sites in 2009 were rated as either optimal (36 sites) or suboptimal (32 sites). Appendix E provides all sites and habitat scores for both 2009 and 2017.

Statistical comparisons among 2009 and 2017 replicate sites and 2017 reference sites were completed using non-parametric statistical tests due to non-normal distributions. Table 8 provides the mean and standard deviation of habitat scores for each metric. Kruskall-Wallis test with Dunn's multiple comparisons test was used to compare habitat scores among the 2009 and 2017 replicate and 2017 reference sites (Figure 13). Statistically significant differences existed between reference sites and both 2009 and 2017 replicates ($p = 0.003$ and 0.024, respectively) with reference sites having higher total habitat scores. A Mann-Whitney pairwise test was not significant between 2009 and 2017 replicate sites ($p = 0.051$), although the mean habitat score increased from 2009 to 2017 (Figure 13).

Table 8. Mean (SD) habitat scores for each habitat parameter.

Metric	2009	2017	Reference
	Mean (SD)	**Mean (SD)**	**Mean (SD)**
Instream Cover	14.73 (4.59)	15.22 (2.43)	16.71 (1.41)
Epifaunal Substrate	14.3 (5.29)	15.81 (2.85)	16.96 (1.23)
Embeddedness	10.79 (5.65)	14.42 (2.34)	14.96 (2.19)
Velocity/Depth Regimes	13.52 (5.48)	16.36 (2.35)	17.21 (0.63)
Channel Alteration	15.85 (4.15)	13.65 (2.53)	14.57 (2.67)
Sediment Deposition	14.96 (4.8)	14.48 (2.53)	14.89 (1.52)
Frequency of Riffles	14.86 (6.07)	15.9 (3.16)	16.93 (1.09)
Channel Flow Status	16.3 (3.12)	17.34 (0.9)	17.25 (1.0)
Condition of Banks	15.62 (4.52)	14.69 (2.95)	15.61 (2.06)
Bank Vegetation Protection	14.65 (5.27)	16.74 (2.29)	17.64 (0.56)
Grazing/Disruptive Pressure	17.62 (3.73)	16.79 (1.52)	17.14 (1.48)
Riparian Zone Width	13.93 (5.98)	15.87 (3.14)	16.93 (1.72)
Total Score	178.3 (34.5)	187.26 (17.42)	196.82 (9.01)

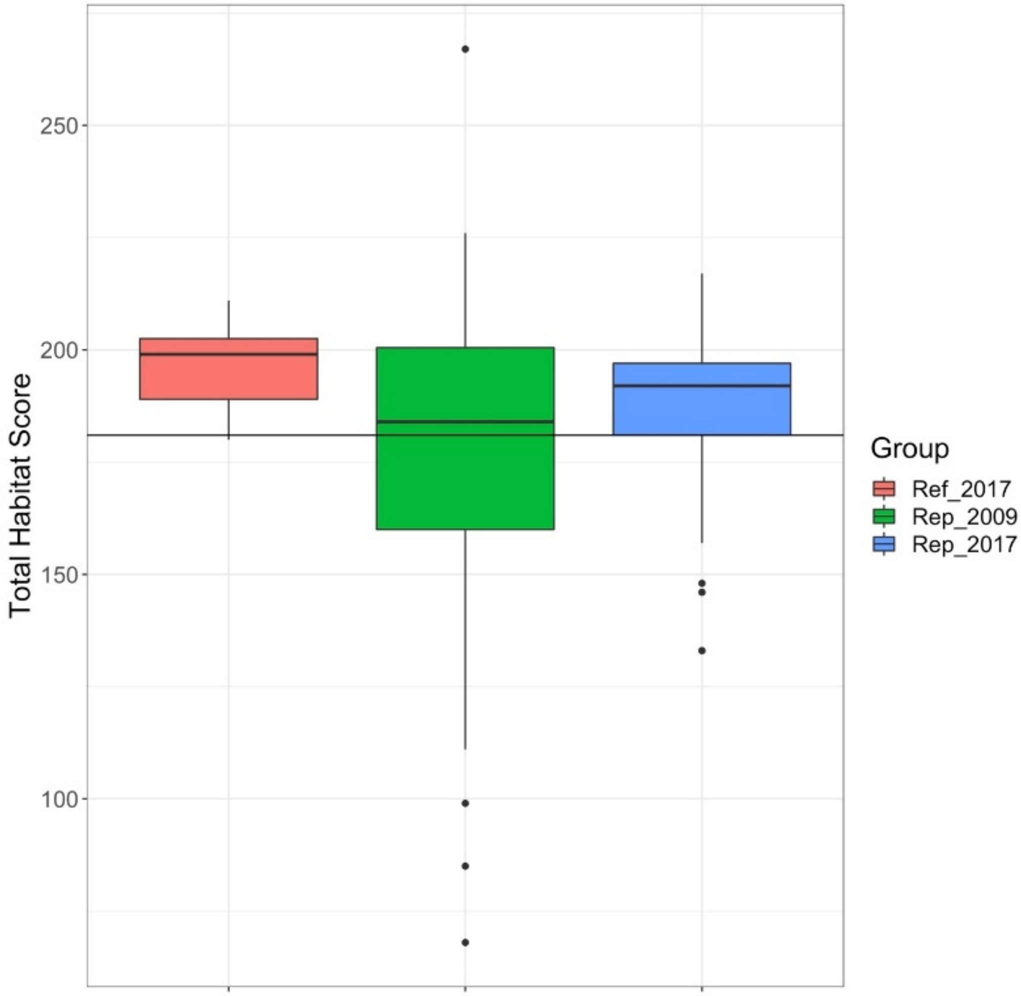

Figure 13. Boxplot of total habitat score between 2009 replicates, 2017 replicates, and 2017 reference sites. Values above horizontal line are considered "optimal" habitat conditions.

Benthic Macroinvertebrates

There were statistically significant increases in IBI score, total taxa richness, EPT taxa richness Beck's index, and Shannon diversity from 2009 to 2017/2018 macroinvertebrate surveys among the 59 replicate sites that were sampled in both 2009 and 2017/2108 (Table 9). There were no statistically significant differences in Hilsenhoff biotic index or percent sensitive individuals (Table 9). Most (51 of 59) replicate sites showed increases in IBI score from 2009 to 2017/2018. Total taxa richness increased in all but two sites (UNT 25913 and Milligan Run). Thirteen sites showed no change in EPT taxa richness and the remaining 43 sites showed increasing EPT taxa richness. Beck's Index saw decreases at five of the sample sites and no change at 10 sample sites. There were increases in Beck's Index in the remaining 44 sites. The Hilsenhoff index and percent sensitive individuals saw the least amount of positive change. Decreases in Hilsenhoff scores and percent sensitive individuals were seen at 33 and 29 sites, respectively. The Hilsenhoff colors are inverted in Table 10 due to increases in this index indicating higher numbers of pollution tolerant taxa. Shannon diversity increased at the majority of the sample sites, with only 11 of 59 sites decreasing in Shannon diversity while diversity increased at the remaining 48 sites. Table 9 summarizes the changes in biological metrics at each sample site.

Table 9. Mean (SD) macroinvertebrate metric scores by sample year. Statistically significant (p<0.05) differences are highlighted.

Metric	2009 Mean (SD)	2017/2018 Mean (SD)	Test Stat (#), p-value
IBI Score	32.95 (17.41)	43.75 (19.02)	120.5, <0.001
Taxa Richness	8 (6.00)	17.88 (11.65)	10.5, <0.001
EPT Richness	2.18 (2.93)	4.57 (4.67)	43.5, <0.001
Beck's Index	4.43 (5.5)	8.76 (8.18)	91, <0.001
Hilsenhoff	4.64 (1.72)	4.74 (1.55)	723, 0.3068
Shannon Diversity	1.45 (0.73)	2.03 (0.71)	209, <0.001
Percent Sensitive	29.28 (30.6)	25.53 (25.61)	890, 0.3167

Table 10. Change in biological metrics from 2009 to 2017/2018. Sites highlighted in green denote an increase and those in red denote a decrease. Color scheme is inverted for Hilsenhoff index due to increases indicated more pollution tolerant taxa are present.

Site	IBI	Taxa Richness	EPT Richness	Beck's Index	Hilsenhoff	Shannon Diversity	Percent Sensitive
Lesle Run	4.5	10	2	4	3.38	2.24	-76.2
Fox Run	14.4	12	5	12	0.3	1.53	-49.3
Walnut Run	6.6	9	2	2	0.08	0.01	-2.7
Moss Creek	11.5	23	5	8	1.28	0.42	-36.4
Cush Cushion Creek	16.3	16	6	9	0.59	0.95	3.8
Bear Run	5.2	8	1	8	0.59	0.8	-34
Chest Creek @ Mahaffey	4.4	23	0	0	1.35	0.79	-31.5
Hartshorn Run	18.8	16	5	9	2.75	1.04	1
Trib 26641	24.9	18	4	7	0.55	1.44	9.4
Montgomery Creek	12.8	2	2	4	-1.71	0.14	20
Trib 26608	33.4	26	3	11	0.65	2.1	10
Wolf Run	19.4	5	0	1	-2.75	1.08	22.2
Clearfield Creek	34.6	10	4	2	-3.76	0.37	94.2
Abes Run	5.5	5	1	2	0.05	0.25	-1
Trib 26104	1	1	0	5	0.05	-0.2	-2.1
Lick Run	3.5	7	4	5	1.44	0.57	-30.8
Devils Run	4.5	16	3	6	2.74	0.54	-32.6
Trout Run	-1.4	12	4	5	2.82	0.59	-55
Millstone Run	5.7	7	1	2	0.42	0.47	-7.4
Bald Hill Run	15.2	10	2	7	1.04	1.1	5.8
Moravian Run	-3	4	0	3	2.14	1.55	-71.4
Deer Creek	-0.7	6	0	-3	1.2	0.67	-20
Big Run	9.1	14	0	0	-0.01	0.39	-1.5
Sandy Creek	8.1	3	1	0	-0.2	0.57	10
Alder Run	11.7	5	1	3	-0.68	0.88	2.6
Rollingstone Run	2.5	1	0	3	-0.33	-0.13	3.8
Mowry Run	12.4	9	1	11	-0.78	0.27	-5.2
Basin Run	9.9	6	1	0	-1.21	0.39	6.2
Rock Run	3.7	1	1	2	-0.54	-0.02	2.1
Potter Run	3.5	3	1	3	0.43	0.1	0.5
Trib 25913	-5.4	-2	0	0	1.07	-0.33	-1.3
Rupley Run	6.3	1	2	4	-1.56	-0.56	21.6
Moshannon Creek	-4.5	6	1	-1	1.3	0.39	-38.1
Trib 25693	15.1	5	2	3	-1.34	0.94	6.5
Mosquito Creek	-0.9	12	1	0	2.34	0.42	-27.8
Laurel Run	7.6	5	0	0	0.47	1.04	0

Site	IBI	Taxa Richness	EPT Richness	Beck's Index	Hilsenhoff	Shannon Diversity	Percent Sensitive
Trib 25622	35.6	14	4	6	-2.84	1.9	28.1
Saltlick Run	8.8	4	2	1	0.19	0.42	12.5
UNT 25611	12.9	8	3	4	0.41	0.96	-0.8
Sterling Run	9.1	18	2	5	0.5	0.74	-24
Loop Run	20.8	2	0	-2	-4.67	-0.15	76.3
Birch Island Run	9.9	6	1	6	-2.34	-1.19	38
Black Stump Run	4.2	8	-1	1	-0.42	-0.1	11.8
Sinnemahoning Creek	-11.2	7	-3	-9	0.88	-0.75	-13.3
Cooks Run	3.1	3	0	0	0.4	0.42	0
Milligan Run	1.2	-1	0	0	-0.44	0.12	0
Drury Run	20	12	4	7	-0.2	0.87	9.7
Tangascootack Creek	22.6	33	10	16	1.23	0.89	-28.6
WB @ Cherry Tree	10.9	19	2	5	2.92	0.7	-3.4
Chest Creek @ Westover	19.1	21	6	9	-0.2	0.46	-19.2
WB @ Shawville	30.4	22	11	16	-0.89	0.38	5.7
Clearfield Creek @ SR1021	18	8	0	0	-0.28	2.2	0
Bennett Branch	51.9	33	15	17	-0.33	1.82	24.3
Dents Run	29.5	13	7	14	-0.58	1.08	16
Sterling Run	34.5	33	13	23	-0.04	0.87	-12.8
Two Mile	5.5	2	2	3	-0.11	0.09	3
Kratzer Run	5.3	11	1	7	-1.1	-0.84	-8.2
Beech Creek	-15.1	4	-1	-6	2.07	-0.22	-40.8
Babb Creek	18.2	27	9	18	1.48	1	-42.6

Five replicate sample sites were found to be meeting the IBI criteria for attaining life use designations by the DEP that were not attaining life use designations in 2009 (Table 11). In addition, one reference site (UNT 55220 to Fishing Creek) was found to be not attaining life use criteria.

Table 11. Sites attaining life use according to IBI score in 2017/2018.

Stream Name	IBI Score	Attaining in 2009?
Tangascootack Creek	80.9	No
Bennett Branch	80.8	No
Sterling Run_71	83.8	No
Black Stump Run	65.5	No
Sterling Run_45	78.6	No

Macroinvertebrate community composition was examined using non-metric multidimensional scaling (NMDS) (Bray and Curtis 1957) to compare between 2009, 2017, and reference data. NMDS was also used to separate macroinvertebrate community composition among five levels of AMD treatment (reference sites, active treatment, passive treatment, land reclamation, and no treatment) based on GIS analysis as described in the Methods section. Groupings were decided by order of appearance of a treatment type. For instance, the first group includes any site with active treatment upstream regardless of other types of treatment present in the watershed. From there, passive treatment, without influence from active treatment, was a group determining factor regardless of other treatment types. The final treatment group was any site that had land reclamation in the watershed. There were two additional groups as well, one for sites with no treatment and one for reference sites. Water quality parameters were fitted to 2017 NMDS plots using an environmental fit with the length of the vector arrow indicating the strength of the trend. A metal index was used to incorporate all metals analyzed at the lab. To do this, the metals' drinking water maximum allowable concentration (MAC) was used to standardize each metal. The measured metal concentration was divided by the MAC for each metal and then were summed for each sample. The metal index calculation is described in detail in Abdullah (2013) and Goher et. al (2014).

PERMANOVA (Anderson 2014) results comparing 2009 and 2017 replicates to the refences sites demonstrated statistically significant difference among each of the three groups (Table 12). In reference to the NMDS plots, when comparing the 2009 and 2017 replicates the difference is likely due to dispersion while differences between both 2009 and 2017 replicates and reference sites are likely due to both centroid location and dispersion (Figure 14). Compared to the 2009 replicate data group the 2017 replicate data group is shifting toward the reference condition (Figure 14).

Table 12. PERMANOVA results for comparisons between sample year and reference sites.

Comparison	DF	Sum of Squares	F.Model	R^2	p-value	Adjusted p-value
2009_rep vs 17_18_rep	1	1.461	4.656	0.0362	0.001	0.003
2009_rep vs 17_18_ref	1	5.452	19.212	0.1898	0.001	0.003
17_18_rep vs 17_18_ref	1	3.916	14.292	0.1320	0.001	0.003

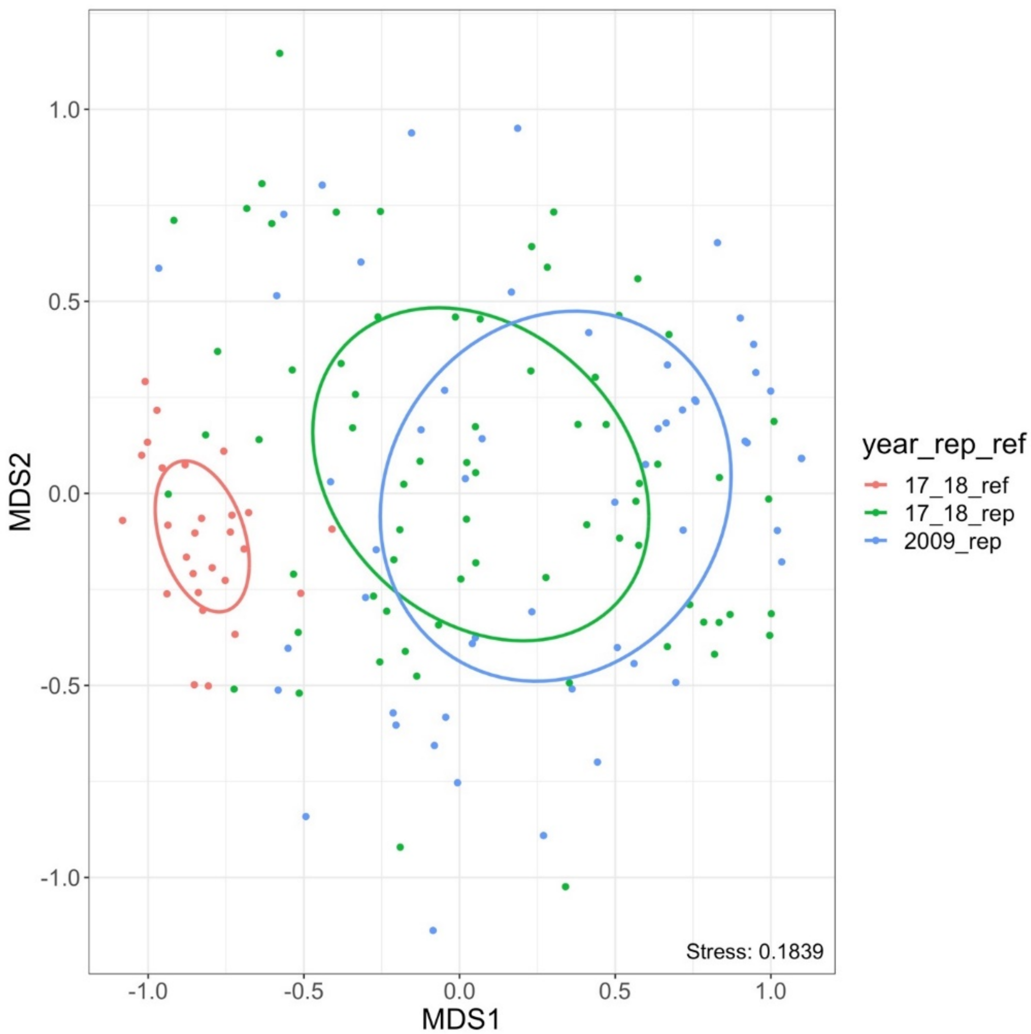

Figure 14. NMDS plot of 2009 and 2017 replicate sites and 2017 reference sites macroinvertebrate community and abundance data.

For the 2017/2018 benthic macroinvertebrate community and abundance data grouped by treatment, NMDS indicated that reference sites were a significantly different cluster than the other four treatment groups and that passive treatment sites (group 2) were significantly different than untreated sites (group 5) (Table 13, Figure 15). PERMANOVA may confound the results of centroid location versus dispersion (Warton et al. 2012), however in Figure 15 reference sites occupy a visibly different space and a much tighter cluster than the other groups so dispersion and centroid location are both likely to be significantly different. For passive treatment versus no treatment, it is more difficult to determine if the groups are occupying different centroid locations. It is possible that this comparison only differs in dispersion. Water quality parameters were fitted to Figure 15 using an environmental fit with the length of the vector arrow indicating the strength and direction of the trend. Most untreated sites (group 4) had the highest values of metals (mg/L), acidity (mg/L), and sulfate (mg/L) (Figure 15).

Table 13. PERMANOVA results for the treatment groupings of 2017 benthic macroinvertebrate community and abundance data.

pairs	Df	SumsOfSqs	F.Model	R2	p.value	p.adjusted
TrtG_3 vs TrtG_2	1	0.62185	1.47870	0.02988	0.049	0.49
TrtG_3 vs TrtG_1	1	0.48862	1.1878	0.03808	0.201	1
TrtG_3 vs TrtG_4	1	0.59144	1.46461	0.03619	0.091	0.91
TrtG_3 vs TrtG_5	1	3.03054	9.15733	0.14973	0.001	0.01
TrtG_2 vs TrtG_1	1	0.46369	1.06130	0.03921	0.348	1
TrtG_2 vs TrtG_4	1	0.95343	2.25961	0.06064	0.004	0.04
TrtG_2 vs TrtG_5	1	2.65442	7.85142	0.14057	0.001	0.01
TrtG_1 vs TrtG_4	1	0.67932	1.66815	0.08935	0.031	0.31
TrtG_1 vs TrtG_5	1	1.12513	4.02661	0.11833	0.001	0.01
TrtG_4 vs TrtG_5	1	2.31600	7.66030	0.16417	0.001	0.01

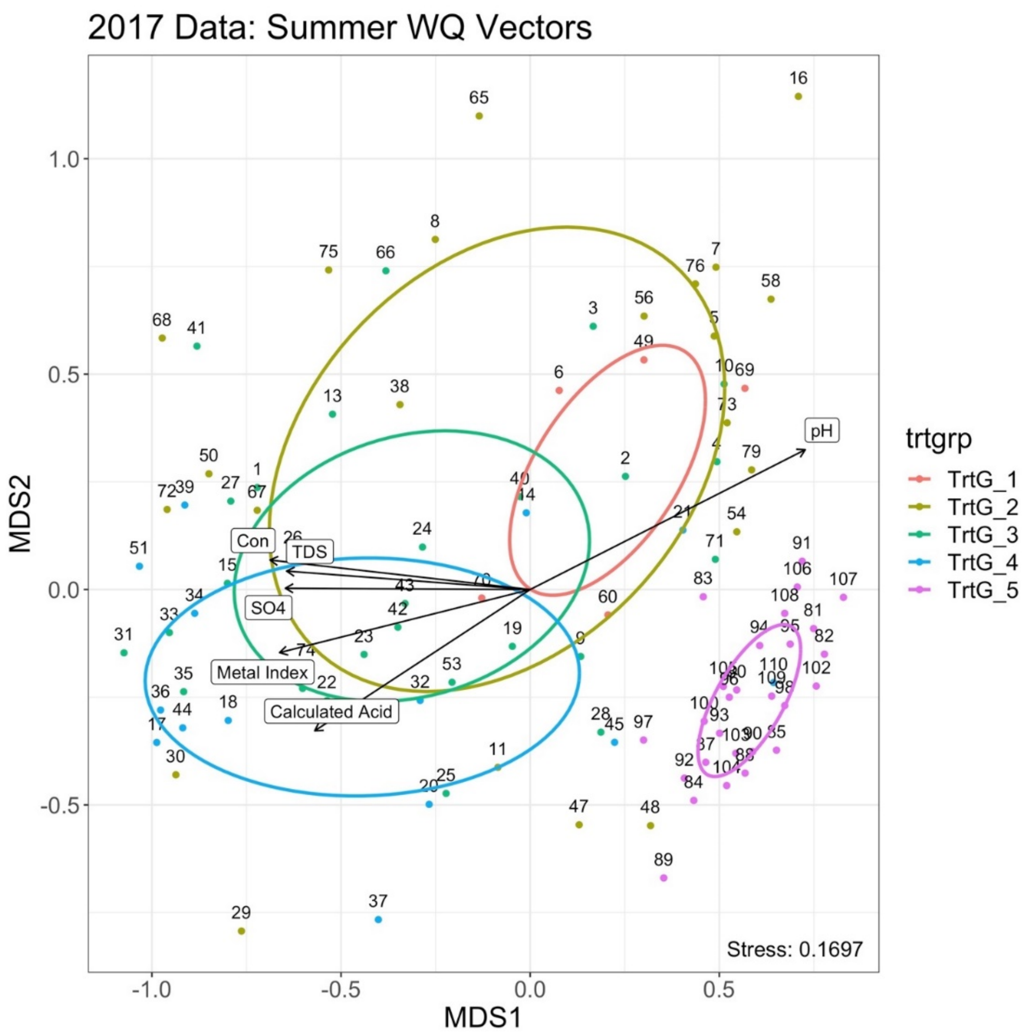

Figure 15. NMDS of macroinvertebrate community and abundance data of treatment groups and reference sites showing relation to water quality parameters in 2017.

Functional feeding group distributions showed a higher percent composition of shredders and scrapers at the reference sites compared to the replicate sites (Figure 16). There was a significantly higher percentage of scrapers at reference sites compared to both replicate site groupings (Table 14). There was also a significantly higher percentage of shredders in the replicate sites from 2017 and the reference sites compared to 2009 replicates (Table 14).

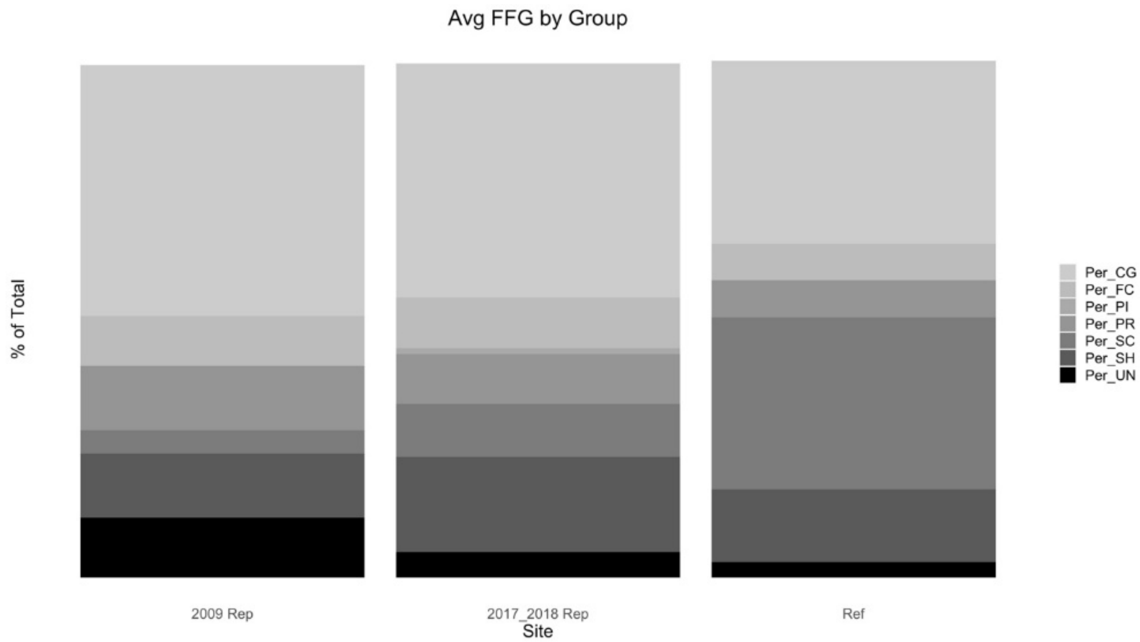

Figure 16. Mean functional feeding group composition for the two replicate groups and the reference group.

Table 14. Kruskal Wallis results for replicate and reference site group comparisons of percent composition of each functional feeding group. CG – Collector/Gatherer, FC – Filtering Collector, PR–Predator, SC–Scraper, SH–Shredder, PI–Piercer, and UN–unknown.

Comparison	FFG	Z	p-value
ref-rep_09	CG	-1.972	0.097
ref-rep_17		-2.968	0.009
rep_09-rep_17		-1.214	0.225
ref-rep_09	FC	2.65	0.024
ref-rep_17		2.132	0.066
rep_09-rep_17		-0.721	0.471
ref-rep_09	PR	-0.48	1
ref-rep_17		-0.789	1
rep_09-rep_17		-0.38	0.704
ref-rep_09	SC	8.225	<0.001
ref-rep_17		6.444	<0.001
rep_09-rep_17		-2.459	0.014
ref-rep_09	SH	2.822	0.009
ref-rep_17		-0.124	0.901
rep_09-rep_17		-3.804	<0.001
ref-rep_09	UN	-1.25	0.423
ref-rep_17		0.612	0.541
rep_09-rep_17		2.391	0.051
ref-rep_09	PI	0	1
ref-rep_17		-1.426	0.308
rep_09-rep_17		-1.808	0.212

Ecosystem attributes were calculated as described in the methods and Table 2 using the FFG results described above. There were no differences in top-down predator control among any of the Kruskal-Wallis comparisons. The majority (146/ 156; 93.6%) of the sites were described as heterotrophic, however the 10 sites that were described as autotrophic were all reference sites. Statistical results from Kruskal-Wallis comparisons for each parameter are provided in Table 15. Replicate groups from both 2009 and 2017/2018 were significantly more heterotrophic than the reference sites. The linkage between the riparian zone and shredders is described by the CPOM/FPOM parameter. There was a significantly higher link in the 2017/2018 replicate and references sites compared to the 2009 replicate group. There was also significantly more transported FPOM in the reference sites compared to both the 2009 and 2017/2018 replicate groups. There was no significant difference in transported FPOM between both replicate groups. Significantly more stable substrates were available in the reference sites compared to both the 2009 and 2017/2018 replicate groups. There was no significant difference in the substrate stability between the replicate groups.

Table 15. Kruskal-Wallis comparisons for ecosystem attributes.

Parameter	Comparison	Z score	p-value
Auto_hetero	2009-Ref	-8.31	<0.001
Auto_hetero	2009-2017/2018 Rep	-2.7	0.007
Auto_hetero	2017/2018 Rep vs Ref	6.35	<0.001
CPOM_FPOM	2009-Ref	-3.54	0.001
CPOM_FPOM	2009-2017/2018 Rep	-3.14	0.003
CPOM_FPOM	2017/2018 Rep vs Ref	1.13	0.26
Predator Control	2009-Ref	0.48	1
Predator Control	2009-2017/2018 Rep	-0.37	0.71
Predator Control	2017/2018 Rep vs Ref	-0.79	1
FPOM Dominance	2009-Ref	-3.08	0.006
FPOM Dominance	2009-2017/2018 Rep	-0.58	0.56
FPOM Dominance	2017/2018 Rep vs Ref	2.7	0.01
Substrate Stability	2009-Ref	-6.12	<0.001
Substrate Stability	2009-2017/2018 Rep	-1.39	0.16
Substrate Stability	2017/2018 Rep vs Ref	5.17	<0.001

Fishery Surveys

Twenty-four total fish species were captured in 1998, 29 in 2009, and 31 in 2019. Species gained in 2019 compared to other years included brook trout (*Salvelinus fontinalis*, I), American eel (*Anguilla rostrata*, T), white crappie (*Pomoxis annularis*, T), black crappie (*Pomoxis nigromaculatus*, M), fantail darter (*Etheostoma flabellare*, M), banded darter (*Etheostoma zonale*, I), and greenside darter (*Etheostoma blennioides*, I). Letters after scientific names represent the tolerance of each respective species (T=tolerant, M=intermediate, I=intolerant) according to (Plafkin et. al 1989; Meador and Carlisle 2007; Barbour et al. 1999). The American eel is considered endangered (Casselman et al. 2017), and only one individual was only captured in 2019 at the Irvin Park site. Fish species present in 2009 that were not present during 2019 were brown bullhead (*Ameiurus nebulosus*, T), common carp (*Cyprinus carpio*, T), yellow bullhead (*Ameiurus natalis*, T), and yellow perch (*Perca flavescens*, M).

Of the species captured in 1998, 6 were intolerant, 12 were moderate, and 6 were tolerant species. In 2009, 7 species were intolerant, 13 were moderate, and 9 were tolerant species. Finally, in 2019, 10 were intolerant, 14 were moderate, and 7 were tolerant species. Appendix F provides a list of all species captured, abundance, and their pollution tolerance category. Data were non-normally distributed, so a Kruskal Wallis test was used to compare percent tolerant, intolerant, and moderate composition across years. Comparisons of percent intolerant individuals between 1998 and 2019 showed a statistically significant increase from 1998 (1.89±2.95) to 2019 (21.8±26.75) in percent intolerant individuals ($H=-2.39$, $p=0.049$; Figure 17). However, there were no other statistically significant differences when comparing percent intolerant, tolerant, and moderate species between years. Comparisons on abundance were not completed due to variance in flow conditions between sample years and associated sampling efficiency variation.

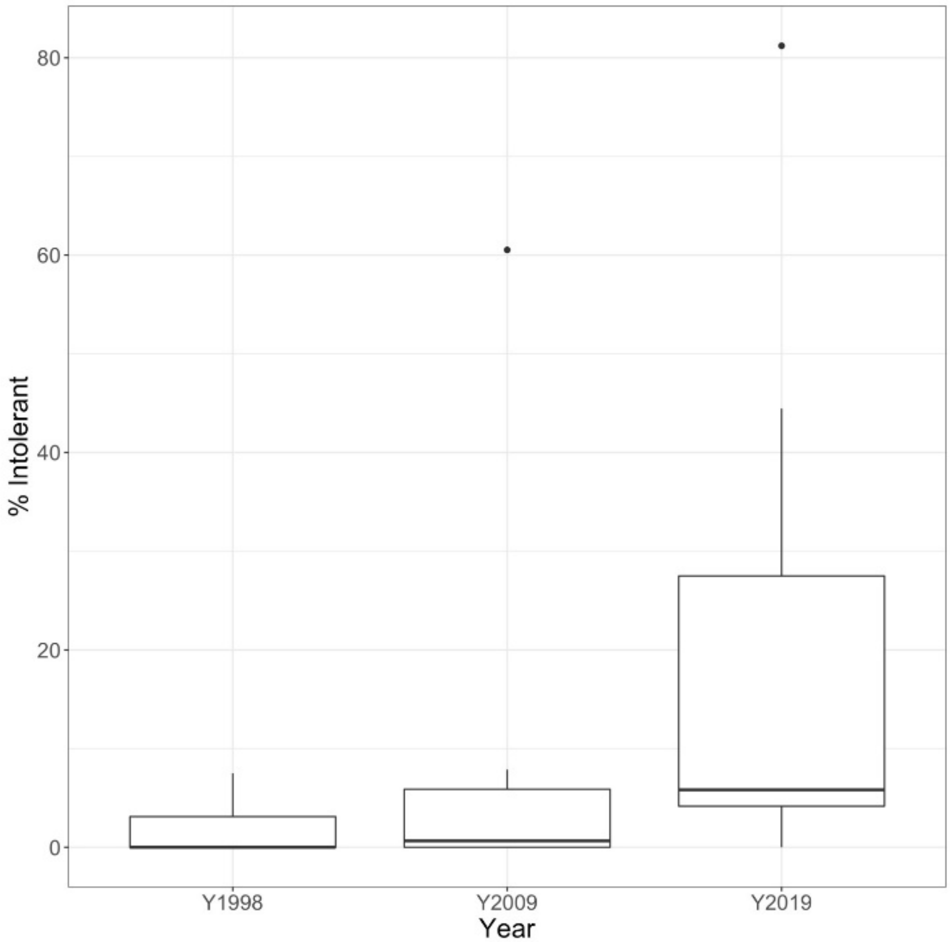

Figure 17. Boxplot of percent intolerant species on mainstem river sample sites.

A Kruskal-Wallis test was used to compare Simpson diversity and evenness among years and a one-way analysis of variance was used to compare Shannon diversity among years. A description of these metrics is provided in Appendix D. There were no statistically significant differences in Shannon diversity (1.973, p=0.163), Simpson diversity (0.496, p=0.78), or evenness (3.75, p=0.153) across sampling years (Figure 18). Even though there were no statistically significant differences in Shannon diversity, a general increasing trend could be seen in 2009 (mean ± SD; 1.62±0.26) when compared to 1998 (mean ± SD;1.16±0.58). A decrease from 2009 Shannon diversity was observed in 2019 (mean ± SD; 1.57±0.59), however, the 2019 mean was greater than the 1998 mean. Means (with standard deviation) for Simpson diversity were 0.74±0.17 in 1998, 0.71±0.09 in 2009, and 0.68±0.23 in 2019. Means (with 95% confidence intervals) for evenness were 0.81±0.12 in 1998, 0.71±0.1 in 2009, and 0.67±0.19 in 2019.

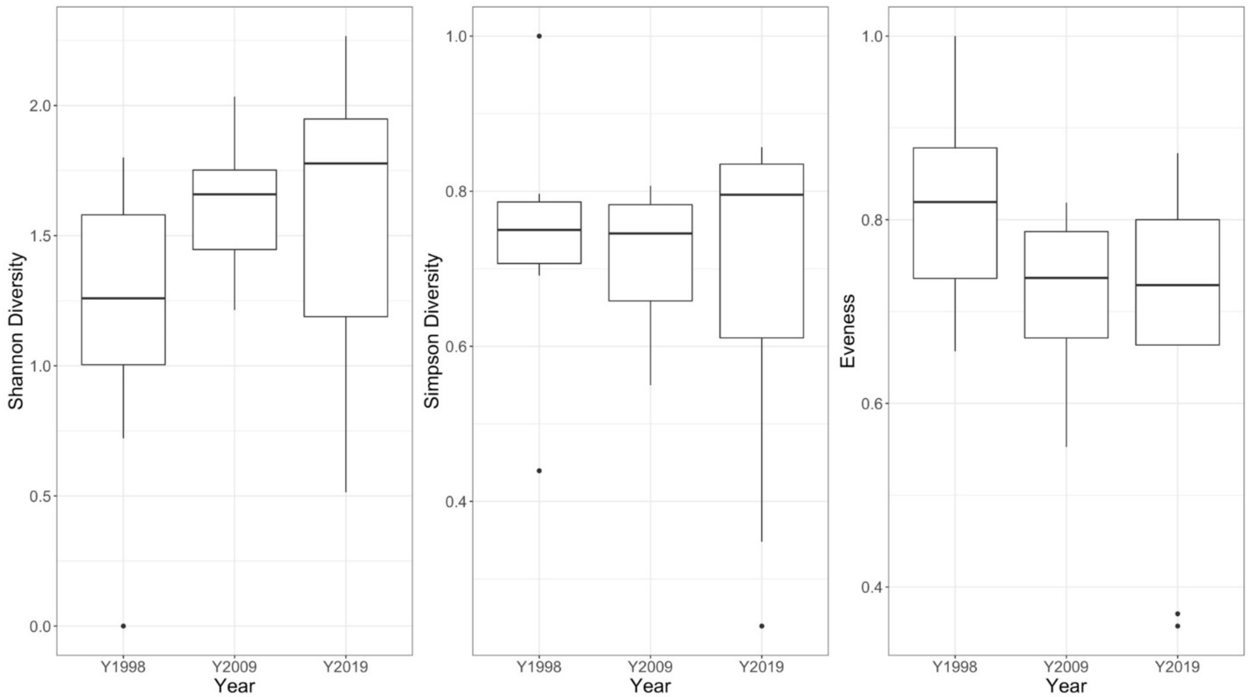

Figure 18. Boxplot of Shannon Wiener diversity, Simpson diversity, and evenness metrics for all mainstem river sample sites by sample year.

Sorenson's index of similarity sites combining backpack and boat sites by year can be found in Appendix G. Range of Sorenson's similarity values for site comparisons was 0-0.75. The greatest similarity was seen between Irvin 2019 and Burns 2009 with a value of 0.75. Hyner 1998 shared no similarity with Irvin 2019, Clearfield 1998, Burns 1998/2009, and Hyner 2019.

Data collected by PFBC and partners on both the mainstem of the river and its tributaries that were beyond the scope of this project were compiled to evaluate the coldwater fishery in relation to historic AMD issues in the watershed. Since 2009, approximately 630 miles of Class A trout streams and approximately 2,800 miles of streams supporting natural trout reproduction have been added throughout the entire West Branch Susquehanna River watershed (Figure 19). The majority of these additions were made through the PFBC's Unassessed Waters Initiative, which aims to document trout presence in streams previously lacking fishery surveys. Of the sites sampled as part of this project, 12 sections of streams and the mainstem of the river have been added as supporting natural reproduction (approximately 200 stream miles, including Class A tributaries) with several supporting Class A trout fisheries (approximately 56 stream miles) since 2009 (Table 16). The majority of those sections are currently listed as AMD impaired by the DEP (Table 16).

Table 16. Replicate sample sites designated as supporting wild trout populations since 2009.

TU ID	Site_Name	303d Impaired?	WT Miles added
2	Fox Run	Yes	6.11
3	Walnut Run	Yes	2.67
4	Moss Creek	Yes	5.44
45	Sterling Run 1	No	12.7
9	Hartshorn Run	Yes	4.81
54	Tangascootack Creek	UPS North Fork	15.81
56	WB @ Cherry Tree	Yes	9.78
57	WB @ Burnside	Yes	23.17
23	Surveyor Run	Yes	4.26
26	Deer Creek	Yes	13.98
71	Sterling Run 2	No, some tributaries	22.89
73	Kratzer Run	Yes	17.09
Total:			138.71

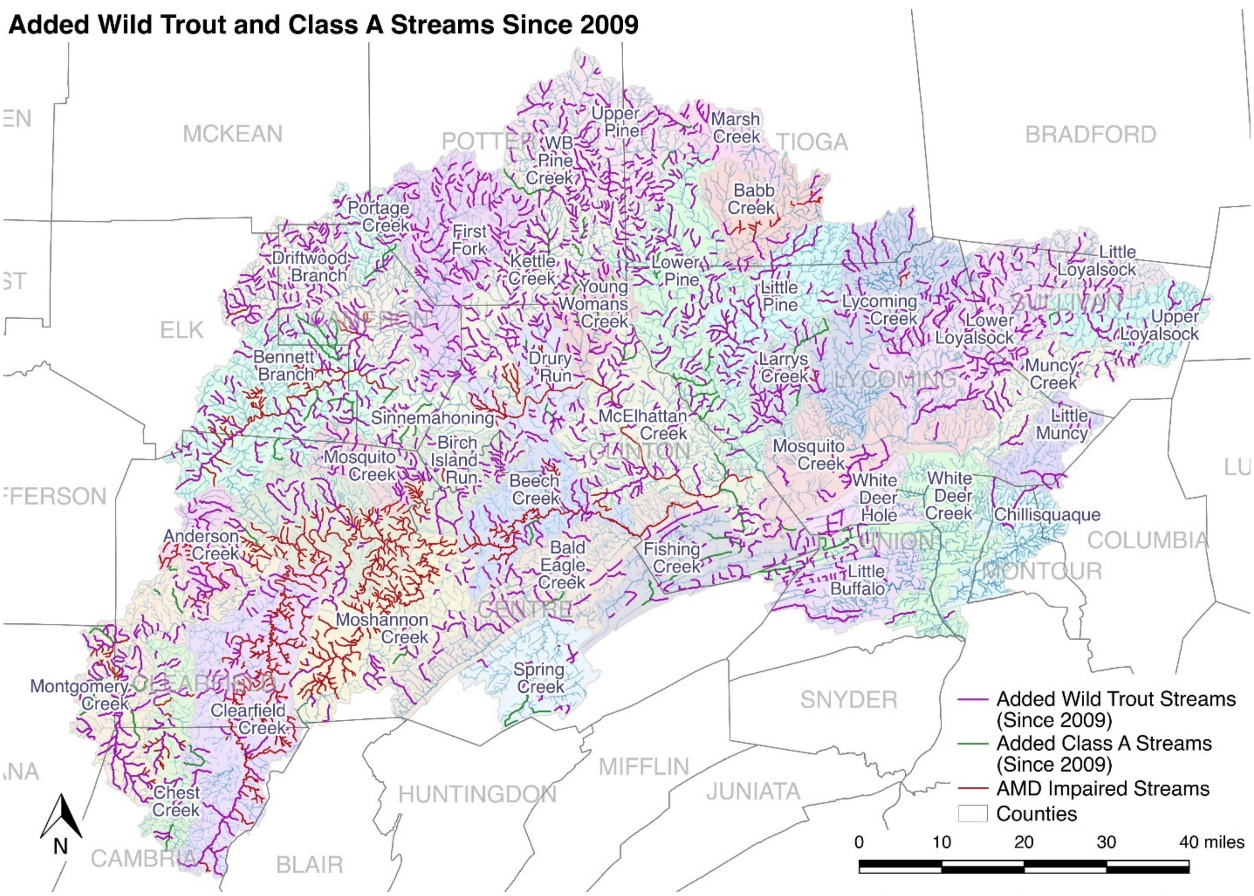

Figure 19. Map of streams that have been classified as either supporting natural trout reproduction or Class A trout fisheries by the PFBC in the West Branch Susquehanna River watershed since 2009.

Trout biomass data from the PFBC from 2010-2018 was compiled for the replicate and reference sites in the current study and compiled for comparisons. Data were non-normal and data transformation failed, so a non-parametric Wilcoxon Mann-Whitney test was used for group comparisons for each type of trout biomass. Some sites had multiple samples taken across multiple years. Trout biomass in reference tributaries ranged from 0-71.68 kg/ha for all brook trout, 0-23.52 kg/ha for brown trout, and 0-95.2 kg/ha for combined trout species. Biomass ranges in replicate tributaries were significantly lower ($p < 0.001$), with the exception of brown trout biomass ($p = 0.298$) compared to reference sites. Values for biomass ranged from 0-20 kg/ha for brook trout, 0-59.99 kg/ha for brown trout, and 0-61.34 kg/ha for combined trout species. Among reference tributary samples, one had no trout, 20 had only brook trout, and 7 were a mix of brook and brown trout. And among replicate tributary samples, 17 had no trout, 14 had only brook trout, 9 had only brown trout, and 12 were a mix of brook and brown trout. Figure 20 shows trout biomass in replicate and reference sites.

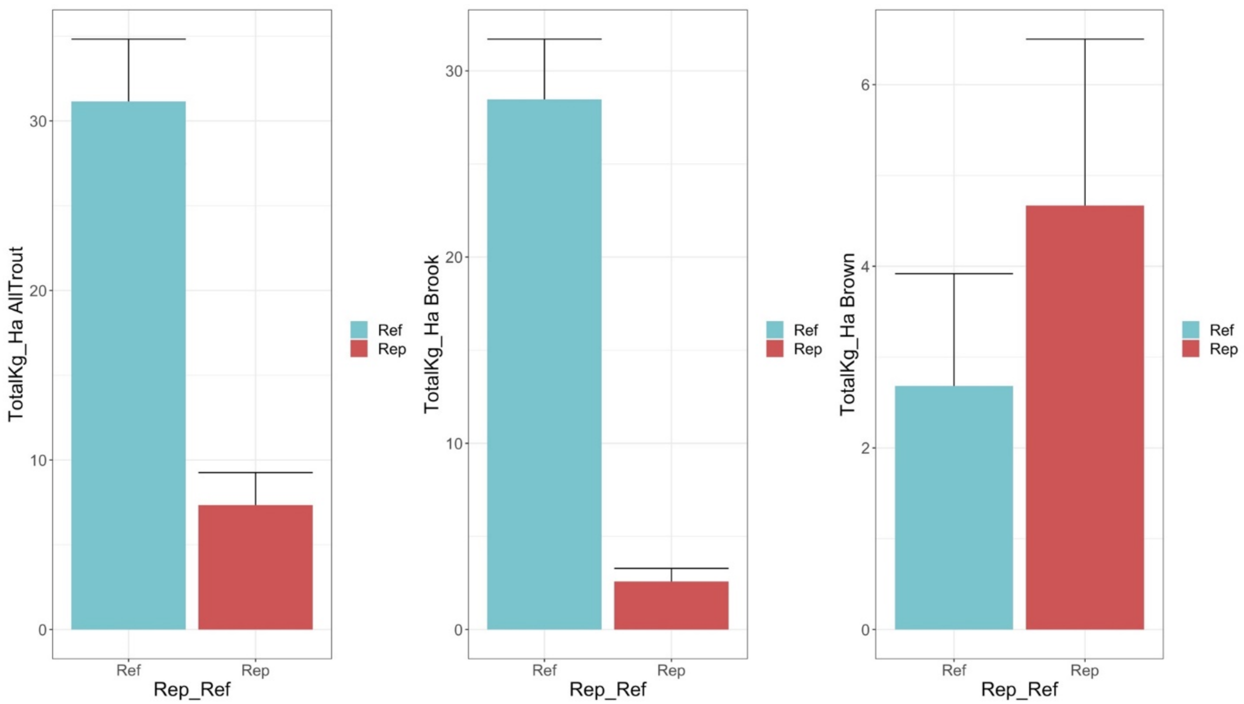

Figure 20. Mean biomass across all years and sites for replicate and reference sites sampled for trout biomass from 2010-2018.

Discussion

Water quality in the mainstem of the West Branch Susquehanna River has changed dramatically over the past 60 years. The mainstem of the river had low pH along its entire course in the 1960's (Federal Water Quality Administration 1968). Throughout the 1970s and 1980s, predominantly or intermittently acidic conditions along with high metal concentrations existed along the river's course. The historic conditions of the river are discussed in greater detail in the West Branch Susquehanna Recovery Benchmark Project technical report (Trout Unlimited

2011). As documented by the first recovery benchmark project and the results of the current project, neutral pH, low metals, and net alkaline conditions exist along the entire mainstem of the river. The most notable improvements in the river's water quality occurred between the 1980s and 2009 due to substantial investments in AMD remediation (Trout Unlimited 2011).

Long-term monitoring data at USGS gauging stations along the mainstem of the river corroborate these results, documenting increases in pH, reductions in acidity, and generally stabilizing sulfate concentrations over time. Results from these data also demonstrate increasing calcium and magnesium to sulfate ratios, suggesting that improvements in water chemistry are due to (at least in part) AMD water treatment since treatment typically adds calcium and magnesium and sulfate concentrations remain relatively stable over time. For example, data from the mainstem of the river near Renovo show that while pH has increased from approximately 4.0 to roughly 6.5 over the last 30 years, sulfate concentrations at that site have not dramatically decreased. This result at one of sites located downstream of most AMD impairments suggests that these changes are largely due to AMD treatment (which would raise pH but not change sulfate concentrations) and not natural attenuation (which would raise pH and lower sulfate concentrations).

The first West Branch Recovery Benchmark Project documented large improvements between 1984 and 2009 (Trout Unlimited 2011). Those improvements were attributed to a combination of natural attenuation, improvements in mining methods and regulations, and AMD water treatment efforts within the watershed. Similar large-scale improvements in water chemistry were absent in the current study. This may be due to a shorter time scale between sampling, fewer treatment sites being installed throughout the watershed, decline in the effectiveness of older systems from a lack of maintenance, variability of other environmental variables, additional environmental stressors in the watershed (e.g., changes in land-use), or other factors not considered in this project. The results of this project demonstrate that an overall trend towards recovery has continued at the watershed scale with increasing pH and alkalinity and decreasing metal concentrations, conductivity, and acidity concentrations since 2009. However, at the sample site/stream reach level, results are highly variable, with some sites showing increases in AMD related parameters (i.e. getting worse) while other sites demonstrate substantial improvements since 2009.

Moshannon Creek, Alder Run, Milligan Run, and Cooks Run continue to discharge large loads of acidity to the West Branch Susquehanna River. Moshannon Creek acidity loads to the West Branch have decreased over the past 50 years, however the creek remains net acidic with low pH. Alder Run and Cooks Run acidity loadings have not changed substantially over the past 35 years. However, a large reclamation project with substantial alkaline addition was completed in the Cook's Run watershed following the 2017 sampling period of this project (DEP 2019). Collaborative efforts are currently underway to evaluate the impact of that restoration project on water quality and biological conditions in the Cook's Run watershed. The acidity loading in Milligan Run has increased since the 2009 sampling. This is due to the KC204 AMD mine pool stabilization project that diverted AMD from the Kettle Creek watershed into Milligan Run in 2010 (Hedin Environmental 2011).

Conversely, Bennett Branch of Sinnemahoning Creek, Muddy Run, Chest Creek, Clearfield Creek, and Sinnemahoning Creek showed the greatest improvements in acidity loading since 2009. Each of these sites have had AMD restoration projects completed upstream of the sample site since the 2009 data collection. A large active treatment facility on the Bennett Branch of Sinnemahoning Creek was completed in 2013 (Beam 2019). In addition, on Dents Run (a tributary to the Bennett Branch), lime dosers were installed in 2012 and three passive treatment systems were constructed in 2008 (Baker et al. 2012). These systems collectively contribute to the improved water quality for both the Bennett Branch of Sinnemahoning Creek and the mainstem of Sinnemahoning Creek. In Muddy Run, a passive treatment system was installed in 1998, however the site was rehabilitated in 2009 and data from the Clearfield Creek Watershed Association suggests that system is effectively treating water (CCWA 2008, 2009, 2014; Kelly Williams, Clearfield County Conservation District, personal communication). Chest Creek has had some land reclamation projects completed, however the stream at both sample points is not currently listed by the DEP as impaired due to AMD. Clearfield Creek improvements may be attributed to a combination of several passive treatment systems completed within the watershed in recent years. These include multiple systems on Morgan Run completed in 2008, 2012, 2013, and 2016, a system on Long Run that was completed in 2009, and a system on the mainstem of Clearfield Creek completed in 2011.

The reduction in the number of water quality parameters in violation of Chapter 93 water quality standards provides further evidence of water quality improvements throughout the watershed. The sample sites that meet Chapter 93 water quality standards should be further evaluated for potential delisting from the impaired waterways list (see Long-term Monitoring section).

Results from grouping sampling sites into treatment categories demonstrated that sites with some form of treatment (active and/or passive treatment and/or land reclamation) had significantly improved water quality compared to replicate sites without AMD treatment. Sites that only had land reclamation present upstream of the sample point did show improvements in water quality over sites without AMD treatment. However, these results indicate that land reclamation alone may not be removing metals effectively from the stream, which could impair aquatic life recovery and prevent these streams from reaching their full restoration potential (DEP 1998). Since land reclamation projects may encompass a wide variety of techniques, further evaluation would be needed on a case-by-case basis determine the effectiveness of the treatment in addressing water quality issues. Sites with active and/or passive treatment did show significantly lower metal concentrations than sites with land reclamation alone, indicating that these water treatment techniques may be more efficient at treating AMD impaired water with higher metal concentrations than just land reclamation (DEP 1998). Elevated sulfate concentrations and total dissolved solids on sites with land reclamation compared to sites with active and/or passive treatment are likely due to the land disturbance associated with land reclamation projects.

Although sites with some form of treatment demonstrated improvements in water quality, overall water quality at these sites are still significantly below the reference conditions. It is evident from the results of this study and others (Rose 2013) that the effectiveness of treatment is highly variable with some systems functioning at high efficiency and other failing. Data was lacking on the majority of the treatment sites in this study to attempt to determine the effectiveness of restoration projects. Future efforts should be made to better quantify the effectiveness of AMD restoration and remediation efforts for both water quality and biological conditions. In addition, the presence of a treatment system within the watershed upstream of the sample sites in this project does not automatically indicate that all sources of AMD in the subwatershed are being treated. Other small tributaries or polluted groundwater may be present in the watershed that are not currently being treated. Finally, other impairments or disturbances (agriculture, surface runoff, development, etc.) may exist in these subwatersheds that may diminish water quality and were beyond the scope of this project.

Natural attenuation of AMD impaired waters also has a role in water quality improvements in the watershed. Decay constants for calculated acidity and sulfate concentrations and loadings for sites in this study range from 0-6% per year, which is within the literature values for natural attenuation rates of 3-5% per year (Mack and Skousen 2008; Perry and Rauch 2013). Robust acidity decay relationships were available for only a subset of the streams sampled as part of this project, and the majority of streams were improving at a rate consistent with natural attenuation, suggesting slow rates of improvement for most streams. However, the rate of improvement for some streams was much higher than what is expected from natural attenuation alone. The three streams with the highest rate of improvement (Saltlick Run, Bear Run, and UNT 26608) each have passive treatment systems present upstream of the sample point. Other streams exceeding the rate of natural attenuation included Wolf Run, UNT 25622, Surveyor Run, and Abes Run each have land reclamation projects within their subwatersheds.

Water quality issues appear to be the main limiting factor in the replicate sites sampled in this study. Results from the habitat surveys show that habitat is generally not the main limiting factor among most of the sites that were surveyed, with the majority of sites rating in the optimal or suboptimal habitat categories. However, habitat scores in replicate sites were significantly lower than those in reference sites. This result suggests that habitat issues may need to be addressed in the future if water quality reaches levels appropriate to sustain aquatic life. Many studies underscore the need to approach watershed restoration at an ecological scale, instead of focusing on one restoration need (Palmer et al. 2005; Palmer et al. 2007; Palmer et al. 2014).

Biological community results from this study provide additional insight to improvements within the West Branch Susquehanna River watershed (Wallace and Webster 1996; Chovanec et al. 2003). Benthic macroinvertebrate community results showed significant increases in most biological metrics (IBI, total taxa richness, EPT taxa richness, Beck's Index, and Shannon Diversity) between 2009 and 2017/2018. The Hilsenhoff biotic index and percent sensitive individuals were the only metrics that were not significantly higher in 2017. Both of these indices use pollution tolerance values that are primarily based on nutrient pollution rather than pollution due to AMD (Hilsenhoff 1987; Bode et al. 1996), which may explain the lack of change in these metrics. Total taxa richness increased at all sites with the exception of two (UNT 25913 and Milligan Run). UNT 25913 has no treatment within its watershed and water quality on Milligan Run degraded between 2009 and 2017 due to the KC204 mine pool stabilization project. Five of the replicate sample sites met the IBI criteria for attaining life use by the DEP. Tangascootack Creek, Bennett Branch of Sinnemahoning Creek, and Black Stump Run were among those meeting IBI criteria and each of the sites have passive treatment upstream of the sample site. These sites should be further evaluated for delisting (see Long-term Monitoring section). The remaining two streams that met IBI criteria where the two Sterling Runs, one of which has been delisted by DEP and the other has no evidence of mining within its watershed and is not currently on the 303(d) list of impaired waters in Pennsylvania.

The results from the NMDS analysis indicate that macroinvertebrate communities at replicate sites in both 2009 and 2017/2018 were significantly different than communities in reference sites. There was a slight shift of the macroinvertebrate community in 2017/2018 towards the reference condition, however the variability (dispersion) of the sites support the water quality results that some sites are further along in their recovery from AMD than others. The 2017/2018 sites were also grouped by treatment type for the NMDS analysis. The reference sites were significantly different than the other four treatment groups. In addition, sites with passive treatment were significantly different than sites without AMD treatment, separating along the vectors of pH and metal concentrations. The high dispersion of sites within the treatment groups suggest that not all treatment is equally effective. For example, macroinvertebrate communities for some sites with passive treatment were more closely related to the reference condition, while others were more closely related to sites without AMD treatment. These results may be useful to determine which treatment sites may not be effectively treating water quality. Replicate sample sites that were most similar in benthic macroinvertebrate communities to the reference group were in various treatment categories, suggesting that the similarity may be due to other factors that were not included in this study. More detailed data collection would be needed to determine possible reasons that these sites are most similar to the reference communities.

The FFG composition of benthic macroinvertebrate samples revealed that replicate sites have fewer shredders and scrapers than reference sites. This supports previous findings that decomposition rates in impacted AMD is impaired (Hogsden and Harding 2012). In addition, the majority of sites were heterotrophic, which was expected because many of the sites were small tributaries that receive most energy from allochthonous sources (Vannote et al. 1980). However, several reference sites were described as autotrophic. The lack of autotrophic conditions in replicate sites is likely related to depressed decomposition rates in the replicate sites (Hogsden and Harding 2012).

Higher stream flows and increased precipitation in 2017 and 2018 compared to 2009 conditions may explain the higher CPOM_FPOM scores as higher flows and surface runoff would increase allochthonous material in the streams. There was also significantly more transported FPOM in the reference sites compared to both the 2009 and 2017/2018 replicate sites. The higher numbers of grazers/scrapers present in the reference sites may explain this result. Finally, reference sites had significantly higher substrate stability than replicate sites. A decrease in the amount of stable substrates at replicate sites could explain the lack of macroinvertebrate recolonization at those sites (MacCausland and McTammany 2007).

The fishery in the mainstem of the river also showed modest improvements since 2009. The increase in fish species diversity and the percent pollution intolerant species at survey sites may be attributed to improving water quality conditions. However, similar to the water quality results, improvements in the fish community were moderate compared to the improvements from 1999 to 2009.

An American eel was documented in the fishery surveys in 2019. Dams downstream of Harrisburg, PA have prevented eel migration upstream to the West Branch Susquehanna River. In 2016, an eel ladder was installed on the Conowingo dam and eels/elvers were also captured and transported to locations upstream in the Susquehanna River basin (Reily and Minkkinen 2016). One of the stocking locations was located on the West Branch Susquehanna River, upstream of Lock Haven, PA (Reily and Minkkinen 2016). Recapture of eels was recorded from 2005-2017 within the West Branch Susquehanna River upstream of Renovo to upstream of Clearfield, PA and also in the headwaters of Clearfield Creek and some tributaries within this stretch of the river (Henning and Wiley 2018).

The designation of nearly 26 miles of the mainstem of the river, from its headwaters downstream to the confluence of Cush Creek, as supporting natural trout reproduction is a testament to the improved conditions in this region of the watershed. The presence of a wild trout fishery is attributed to the cumulative improvements in water quality from active and passive treatment systems and land reclamation in this region. This region was once devoid of life and the impairments to this region were thought to be insurmountable. The 1972 Scarlift report for the West Branch Susquehanna River stated that “conditions in the study area are such that no more than 30 miles of stream between Barnesboro and Bower could possibly be restored for fishing and recreational use under the most ideal abatement, treatment costs for which could easily range from $20 to $30 million. This is completely unrealistic in terms of the Federal Water pollution Control Act benefit values for this reach” (Commonwealth of Pennsylvania 1972).

In addition to the mainstem of the West Branch Susquehanna River, many additional tributaries within the watershed have been designated as supporting natural trout reproduction since 2009. However, not all of these additions may be attributed to improvements in water quality. In 2009, PFBC launched its Unassessed Waters Initiative (PFBC 2013) to document trout presence/absence in streams that did not have fishery survey data. Many of the waters in the West Branch Susquehanna River waters have been surveyed by PFBC and its partners through this initiative. Therefore, without historical fishery data for many of these sites, it is impossible to determine if a trout fishery was always present at these sites and was documented through the Unassessed Waters Initiative, or if conditions have improved and trout have recolonized these areas. It is also possible that some streams that have been listed as AMD impaired by DEP were incorrectly classified as not every stream reach in the watershed was sampled to determine impairment.

However, the results of this study can attribute trout populations in tributaries that were sampled as part of this project to improvements in water quality from AMD restoration. Of the streams that have been designated as supporting natural trout reproduction and were sampled as part of this study, Kratzer Run and Deer Run both have land reclamation and passive treatment projects, Tangascootack Creek has numerous passive treatment systems, and Fox Run, Walnut Run, Moss Run, Hartshorn Run, Surveyor Run, and Sterling Run each have land reclamation projects within their subwatersheds.

The presence of trout populations in historically AMD impaired waters in the West Branch Susquehanna River watershed is encouraging. However, biomass comparisons showed that brook trout biomass was lower in replicate sites than reference sites. This may be an indication that water quality or other environmental factors (e.g., water temperature, detrimental land uses) could be suppressing the biomass in replicate sites through decreased reproduction, limited habitat availability, population isolation, or other mechanisms. Further study would be required to determine the causes and underlying mechanisms suppressing biomass in these streams.

Conclusion

The results of this West Branch Susquehanna Recovery Benchmark II project indicate that the river and many of its historically AMD impaired tributaries are continuing to recover from AMD pollution. Improvements throughout the watershed were documented in water quality, benthic macroinvertebrate communities, and fish communities. The mainstem of the river has maintained a net alkaline condition from its headwaters downstream to Lock Haven. The upper 26 miles of the river was recently designated as supporting naturally reproducing trout populations.

Water quality in the tributaries also continued along a trajectory of improvement since the 2009 surveys were completed. However, improvements from 2009 to 2017 were less dramatic than those reported in the first Recovery Benchmark Report that documented improvements between 1984 and 2009. On the West Branch Susquehanna River watershed scale, water quality improvements in most tributaries appear to be primarily a result of natural attenuation. Although, tributaries with significant AMD remediation efforts completed over the last ten years showed significant improvements in water quality, greater than the rate of natural attenuation.

Benthic macroinvertebrate and fish communities also continue to improve throughout the watershed. Increases in pollution sensitive taxa of both benthic macroinvertebrate and fish corroborate that water quality has improved at most sample sites. Several sites throughout the watershed, based on water quality, benthic macroinvertebrate communities, and/or the presence of trout, may warrant further consideration for delisting from Pennsylvania’s list of impaired streams.

Comparisons with reference site water quality, benthic macroinvertebrates, and trout biomass indicated that most replicate sites remain distant from a “fully recovered” state. In addition, there are several tributaries that continue to disproportionately contribute acidity to the mainstem of the West Branch Susquehanna River. In order to realize substantial improvements in the watershed, future water treatment and abandoned mine land reclamation will be required. If additional remediation projects are completed, particularly in the severely degraded tributaries noted in this report, it is likely that fish populations will continue to expand in the upper and middle reaches of the river.

Ongoing operation and maintenance of existing treatment systems (including actively monitoring treatment systems to ensure they are effectively treating water) and funding to conduct operation and maintenance is critical to maintaining and enhancing water quality conditions in the watershed. Treatment systems that are found to be ineffectively treating AMD should be re-evaluated and rehabilitated. Proper monitoring of these systems will ensure that systems continue to function properly, as failing systems would negatively impact biological communities and offset the recovery of the watershed accomplished to date.

Long-Term Monitoring

As previously described, monitoring is a crucial component to ensuring the long-term success of AMD treatment and the continued recovery of the West Branch Susquehanna River and its watershed. The success of recovering the West Branch Susquehanna River relies on the cumulative effects of smaller scale projects like individual passive treatment systems in small catchments. Therefore, large-scale restoration projects such as this require monitoring at several geographic scales. Regular snapshots of the status of water quality and biological communities at the watershed scale similar to this project are necessary to evaluate restoration effectiveness, identify and prioritize future restoration efforts, guide large scale restoration goals, and implement an adaptive management strategy. At the project scale, routine water quality monitoring plans for individual AMD treatment systems are essential to provide data at regular intervals on the effectiveness of the treatment, the detection of any issues within the system, and feedback on when maintenance of the system is needed.

The largest data gap noted in this study was the lack of data regarding AMD treatment system effectiveness. While some AMD treatment systems have routine monitoring completed on a regular basis by state and local agencies, non-governmental organizations, watershed groups, and others, many do not. However, most, if not all, AMD treatment systems have an operation and maintenance plan associated with them which includes a monitoring plan. Although resources such as Datashed are in place to store and compile these data, they appear to be underutilized. In order to fully understand continued AMD impacts in the watershed and implement an adaptive management strategy for its recovery, it is critical to identify failing and declining, as well as fully functioning, treatment systems.

Future efforts to address this need should include a thorough inventory of existing AMD treatment systems and land reclamation projects and effectiveness monitoring at all project sites. At a minimum, treatment systems should be sampled at high and low flow to identify systems that are not performing according to their designed treatment capacity. Ideally, all treatment systems would have routine monitoring according to the system's operation and maintenance plan completed at regular intervals. However, the monitoring component of projects is often the most difficult component to fund since most grants and funding programs operate on a 2- or 3-year basis from project inception to project completion. It is critical that funding for AMD restoration projects include a robust monitoring component to ensure that the project is effective well beyond the funding cycle.

An aspect to monitoring the effectiveness of AMD treatment systems and recovery of the watershed that has not been used to its full potential is the use of a citizen-based monitoring program. Public participation in monitoring efforts has increased drastically as state and federal agencies have developed protocols for citizen science monitoring of stream conditions and water quality (Nerbonne and Vonracek 2003; Newman et al. 2012). As a member-based organization with approximately 300,000 members and supporters, Trout Unlimited has the ability to successfully organize and lead citizen science efforts. In Pennsylvania, Trout Unlimited volunteers and other citizens have monitored water quality in response to the expansion of shale gas development in the commonwealth (Williams et al. 2016). A similar effort could be used to monitor passive treatment systems to assist in the identification of failing systems, treatment effectiveness monitoring, and identification of potential problems (clogging, leaking ponds, etc.).

Citizen science could be a cost-effective way to collect meaningful AMD-related data, as well as encourage a sense of ownership to local citizens in the AMD impacted areas of the watershed. As part of this project, Trout Unlimited has developed a “Monitoring Guide for Abandoned Mine Drainage and Passive Treatment Systems” as a resource to begin using citizen science in this manner. The guide is meant to be provide an overview of monitoring approaches and considerations for AMD-related monitoring for both in-stream and treatment system sampling and will be publicly available.

In-stream monitoring should also be included in AMD-related monitoring efforts in the West Branch Susquehanna River watershed. Although large-scale efforts, such as this study, may not be feasible on an annual basis, routine monitoring at a selection of these sites annually with a probabilistic sample design would produce an improved long-term dataset. Reference sites should be included for in-stream monitoring plans to provide perspective on the recovery of these streams. The results of this study included several streams that have shown dramatic improvements over the past 10 years. The streams identified in this report as meeting Chapter 93 water quality standards, meeting benthic macroinvertebrate IBI requirements for attaining life use, or have been documented to support naturally reproducing trout populations should be further evaluated for potential delisting from the PA impaired waters list.

Recommendations

- Improve upon and continue the collaboration of government agencies, non-government organizations, private industry, philanthropy, and other partners so that new AMD treatment and land reclamation projects may be cost-effectively and successfully implemented.
- Ensure abandoned mine cleanup remains a priority for funding programs. Reauthorize the Abandoned Mine Land Fund of the 1977 Surface Mine Control and Reclamation Act, which expires in September 2021.
- Establish and secure funding sources to support the long-term operation and maintenance of all treatment systems. Ensure that monitoring occurs so that issues may be detected before they become problematic and to identify when maintenance is needed.
  - Inventory passive treatment systems and associated operation and maintenance plans and execute monitoring to regularly evaluate effectiveness of systems.
  - Rehabilitate systems that are not effectively treating AMD.
- Protect the water quality and biological improvements from new sources of potential impairment.
  - Identify other potential limiting factors and address those in streams recovering from AMD so they become fully capable of supporting biological communities.

Acknowledgements

Funding and support for the West Branch Susquehanna Recovery Benchmark II project was provided by the Richard King Mellon Foundation and a Growing Greener grant from the PA Department of Environmental Protection. Project partners included the PA Department of Environmental Protection, PA Fish and Boat Commission, Susquehanna River Basin Commission, Western PA Conservancy, Clinton County Conservation District, and Clearfield County Conservation District. Trout Unlimited sincerely thanks those who assisted with data collection, data analysis, and interpretation and other support for this project. Specifically, assistance with data analysis from Cole Ecological, Inc. and Hedin Environmental is greatly appreciated.

For more information about Trout Unlimited and its work to conserve, protect, and restore North America's coldwater fisheries and their watersheds, please visit: www.tu.org

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Appendix A – Sample Sites

Site Name	TUID	Lat	Lon	Rep/Ref	WQ		Habitat	Benthics
					SP	SU		
Lesle Run	1	40.60609	-78.7525	Rep	X	X	X	X
Fox Run	2	40.63821	-78.77	Rep	X	X	X	X
Walnut Run	3	40.66255	-78.7839	Rep	X	X	X	X
Moss Creek	4	40.6762	-78.804	Rep	X	X	X	X
Cush Cushion Creek	5	40.72585	-78.8055	Rep	X	X	X	X
Bear Run	6	40.88075	-78.7631	Rep	X	X	X	X
Chest Creek @ Mahaffey	7	40.87262	-78.728	Rep	X	X	X	X
Anderson Creek	8	40.9724	-78.5202	Rep	X	X	X	X
Hartshorn Run	9	40.9791	-78.4951	Rep	X	X	X	X
Trib 26641	10	40.98803	-78.4818	Rep	X	X	X	X
Montgomery Creek	11	41.00336	-78.4618	Rep	X	X	X	X
Trib 26622	12	41.02592	-78.4397	Rep	X	X		X
Moose Creek	13	41.03034	-78.4374	Rep	X	X	X	X
Trib 26608	14	41.03359	-78.4239	Rep	X	X		X
Wolf Run	15	41.02973	-78.408	Rep	X	X	X	X
Clearfield Creek	16	41.02056	-78.4002	Rep	X	X	X	X
Abes Run	17	41.03485	-78.3729	Rep	X	X	X	X
Trib 26104	18	41.03715	-78.3677	Rep	X	X	X	X
Lick Run	19	41.05016	-78.3857	Rep	X	X	X	X
Devils Run	20	41.05288	-78.3772	Rep	X	X	X	X
Trout Run	21	41.06918	-78.3603	Rep	X	X	X	X
Millstone Run	22	41.05175	-78.3387	Rep		X	X	X
Surveyor Run	23	41.07381	-78.3271	Rep	X	X	X	X
Bald Hill Run	24	41.06971	-78.3025	Rep	X	X	X	X
Moravian Run	25	41.04924	-78.259	Rep	X	X	X	X
Deer Creek	26	41.0791	-78.2358	Rep	X	X	X	X
Trib 25976	27	41.07764	-78.2292	Rep	X	X	X	X
Big Run	28	41.06153	-78.2002	Rep	X	X	X	X
Sandy Creek	29	41.05825	-78.1759	Rep	X	X	X	X
Alder Run	30	41.05589	-78.1732	Rep	X	X	X	X
Rollingstone Run	31	41.0583	-78.1587	Rep	X	X	X	X
Mowry Run	32	41.05557	-78.155	Rep	X	X	X	X
Basin Run	33	41.05815	-78.1456	Rep	X	X	X	X
Rock Run	34	41.07828	-78.1224	Rep	X	X	X	X
Potter Run	35	41.09257	-78.1257	Rep	X	X	X	X
Trib 25913	36	41.09612	-78.1253	Rep	X	X	X	X
Rupley Run	37	41.0742	-78.0997	Rep	X	X	X	X
Moshannon Creek	38	41.07258	-78.0971	Rep	X	X	X	X
Trib 25693	39	41.11216	-78.1126	Rep	X	X	X	X

Mosquito Creek	40	41.11796	-78.1099	Rep	X	X	X	X
Laurel Run	41	41.11996	-78.0963	Rep	X	X	X	X
Trib 25622	42	41.12474	-78.0868	Rep	X	X	X	X
Saltlick Run	43	41.12676	-78.0795	Rep	X	X	X	X
Trib 25611	44	41.12131	-78.0718	Rep	X	X	X	X
Sterling Run 1	45	41.15181	-78.0397	Rep	X	X	X	X
Loop Run	46	41.15335	-78.0191	Rep	X	X	X	X
Birch Island Run	47	41.19599	-77.974	Rep	X	X	X	X
Black Stump Run	48	41.2106	-77.9651	Rep	X	X	X	X
Sinnemahoning Creek	49	41.26103	-77.9069	Rep	X	X	X	X
Cooks Run	50	41.27864	-77.8854	Rep	X	X	X	X
Milligan Run	51	41.28029	-77.8826	Rep	X	X	X	X
Kettle Creek	52	41.30023	-77.8414	Rep	X	X	X	
Drury Run	53	41.32668	-77.7767	Rep	X	X	X	X
Tangascootack Creek	54	41.17639	-77.5494	Rep	X	X	X	X
WB @ McGees Mills	55	40.88012	-78.7651	Rep	X	X	X	
WB @ Cherry Tree	56	40.72535	-78.8049	Rep	X	X	X	X
WB @ Burnside (219 Bridge)	57	40.81579	-78.7869	Rep	X	X	X	
Chest Creek @ Westover	58	40.7514	-78.6668	Rep	X	X	X	X
WB @ Lumber City (729 Bridge)	59	40.92282	-78.5764	Rep	X	X	X	
WB @ Shawville	60	41.0671	-78.3597	Rep	X	X	X	X
WB @ 879 Bridge	61	41.0259	-78.414	Rep	X	X	X	
WB @ Karthaus (879 Bridge)	62	41.11706	-78.1091	Rep	X	X	X	
WB @ Westport (above Kettle)	63	41.29425	-77.8397	Rep	X	X	X	
WB @ Lock Haven (Jay Street Bridge)	64	41.13956	-77.4418	Rep	X	X	X	
Clearfield Creek @ SR 1021	65	40.71751	-78.5268	Rep	X	X	X	X
Muddy Run	66	40.82006	-78.4373	Rep	X	X	X	X
Moshannon Creek @ Osceola Mills	67	40.84715	-78.2714	Rep	X	X	X	X
Moshannon Creek @ Philipsburg	68	40.90292	-78.2278	Rep	X	X	X	X
Bennett Branch	69	41.33367	-78.136	Rep	X	X	X	X
Dents Run	70	41.35563	-78.2629	Rep	X	X	X	X
Sterling Run 2	71	41.41384	-78.1995	Rep	X	X	X	X
Twomile Run	72	41.31487	-77.8587	Rep	X	X	X	X
Kratzer Run	73	40.97657	-78.548	Rep	X	X	X	X
Little Anderson Creek	74	41.05397	-78.656	Rep	X	X	X	X
Beech Creek	75	41.0752	-77.5923	Rep	X	X	X	X
Clearfield Creek @ Dimeling	76	40.97004	-78.4069	Rep	X	X	X	X
WB @ Curwensville	77	40.97399	-78.52	Rep	X	X	X	

WB @ Renovo	78	41.32567	-77.7458	Rep	X	X	X	
Babb Creek	79	41.55593	-77.378	Rep	X	X	X	X
Whitehead Run	80	41.47381	-78.1504	Ref	X	X	X	X
Waldy Run	81	41.57831	-78.2931	Ref	X	X	X	X
Noon Branch	82	41.43464	-76.795	Ref	X	X	X	X
Emeigh Run	83	40.69943	-78.8038	Ref	X	X	X	X
Rock Run	84	40.97711	-78.0062	Ref	X	X	X	X
Council Run	85	41.05186	-77.824	Ref	X	X	X	X
Schreckengast Gap Run	86	40.99637	-77.3944	Ref	X	X		
Fields Run	87	41.212	-77.9473	Ref	X	X	X	X
Hagerman Run	88	41.41437	-77.0418	Ref	X	X	X	X
UNT To Gottshall Run (Robbins Run)	89	41.08736	-77.2597	Ref	X	X	X	X
Black Bear Run	90	40.9054	-78.1523	Ref	X	X	X	X
Birch Run	91	41.55764	-77.9504	Ref	X	X	X	X
Sanders Draft Run	92	41.27591	-78.2439	Ref	X	X	X	X
Nickel Run	93	41.63316	-77.2296	Ref	X	X	X	X
Berge Run	94	41.47998	-78.047	Ref	X	X	X	X
Lyman Run	95	41.72276	-77.7705	Ref	X	X	X	X
Right Branch Lushbaugh Run	96	41.47629	-77.9921	Ref	X	X	X	X
UNT 22550 To Fishing Creek	97	41.04307	-77.2048	Ref	X	X		X
Painter Run	98	41.74526	-77.4913	Ref	X	X	X	X
Right Branch Hyner	99	41.37889	-77.6069	Ref	X	X	X	X
Mill Creek	100	41.02998	-77.3026	Ref	X	X	X	X
Saunders Run	101	41.23325	-78.4739	Ref	X	X	X	
Johnson Brook	102	41.75093	-77.6294	Ref	X	X	X	X
Black Stump Hollow	103	41.54236	-77.9809	Ref	X	X	X	X
Ritchie Run	104	41.29718	-77.6112	Ref	X	X	X	X
Square Timber Run	105	41.42773	-78.1711	Ref	X	X	X	X
Long Run	106	41.56081	-77.6806	Ref	X	X	X	X
West Branch Freeman Run	107	41.62797	-78.0919	Ref	X	X	X	X
Tannery Hollow Run	108	41.42291	-78.22	Ref	X	X	X	X
Canoe Run	109	41.46683	-78.1989	Ref	X	X	X	X
Redlick Run	110	41.0784	-78.0919	Rep		X		

Appendix B – AMD Treatment Groupings

Replicate Sites w/ Treatment	***Treatment Type (active, passive, Land reclamation)***	***Treatment Group***
Lesle Run	***Land rec***	Land Rec
Fox Run	***Land rec***	Land Rec
Walnut Run	***Land rec***	Land Rec
Abes Run	***Land rec***	Land Rec
Wolf Run	***Land rec***	Land Rec
Moss Creek	***Land rec***	Land Rec
Chest Creek @ Mahaffey	***Passive***	Passive
	Land rec	
Hartshorn Run	***Land rec***	Land Rec
Trib 26641	***Land rec***	Land Rec
Moose Creek	***Land rec***	Land Rec
Lick Run	***Land rec***	Land Rec
Black Stump Run	***Passive, sat***	Passive
Big Run	***Land rec***	Land Rec
Millstone Run	***Land rec***	Land Rec
Trib 26622	***Land rec***	Land Rec
Surveyor Run	***Land rec***	Land Rec
Bald Hill Run	***Land rec***	Land Rec
Moravian Run	***Land rec***	Land Rec
Trib 25976	***Land rec***	Land Rec
Sandy Creek	***Land rec***	Passive
	Passive, sat	
Rollingstone Run	***Land rec***	Land Rec
Basin Run	***Land rec***	Land Rec
Potter Run	***Land rec***	Land Rec
Mosquito Creek	***Land rec***	Land Rec
Laurel Run	***Land rec***	Land Rec
Trib 25622	***Land rec***	Land Rec
Saltlick Run	***Land rec***	Land Rec
Loop Run	***Land rec***	Land Rec
Drury Run	***Land rec***	Land Rec
Chest Creek @ Westover	***Passive***	Passive
	Land rec	

<table>
<tr><td rowspan="2">Muddy Run</td><td>Land rec</td><td rowspan="2">Passive</td></tr>
<tr><td>Passive</td></tr>
<tr><td rowspan="2">Moshannon Creek @ Osceola Mills</td><td>Land rec</td><td rowspan="2">Passive</td></tr>
<tr><td>Passive, sat</td></tr>
<tr><td rowspan="2">Moshannon Creek @ Philipsburg</td><td>Land rec</td><td rowspan="2">Passive</td></tr>
<tr><td>Passive, sat</td></tr>
<tr><td>Sterling Run 2</td><td>Land rec</td><td>Land Rec</td></tr>
<tr><td>Little Anderson Creek</td><td>Land rec</td><td>Land Rec</td></tr>
<tr><td rowspan="2">Cush Cushion Creek</td><td>Land rec</td><td rowspan="2">Passive</td></tr>
<tr><td>Passive</td></tr>
<tr><td rowspan="2">Beech Creek</td><td>Passive</td><td rowspan="2">Passive</td></tr>
<tr><td>Land rec</td></tr>
<tr><td rowspan="2">Anderson Creek</td><td>Passive</td><td rowspan="2">Passive</td></tr>
<tr><td>Land rec</td></tr>
<tr><td rowspan="3">Sinnemahoning Creek</td><td>Active</td><td rowspan="3">Active</td></tr>
<tr><td>Passive</td></tr>
<tr><td>Land rec</td></tr>
<tr><td rowspan="3">Bennett Branch</td><td>Land rec</td><td rowspan="3">Active</td></tr>
<tr><td>Passive</td></tr>
<tr><td>Active</td></tr>
<tr><td rowspan="2">Dents Run</td><td>Passive</td><td rowspan="2">Active</td></tr>
<tr><td>Active</td></tr>
<tr><td rowspan="2">Kratzer Run</td><td>Passive</td><td rowspan="2">Passive</td></tr>
<tr><td>Land rec</td></tr>
<tr><td rowspan="2">Montgomery Creek</td><td>Passive</td><td rowspan="2">Passive</td></tr>
<tr><td>Land Rec</td></tr>
<tr><td rowspan="2">Deer Creek</td><td>Passive</td><td rowspan="2">Passive</td></tr>
<tr><td>Land Rec</td></tr>
<tr><td>Birch Island Run</td><td>Passive</td><td>Passive</td></tr>
<tr><td rowspan="2">Babb Creek</td><td>Passive</td><td rowspan="2">Passive</td></tr>
<tr><td>Land rec</td></tr>
<tr><td>Tangascootack Creek</td><td>Passive</td><td>Passive</td></tr>
<tr><td rowspan="2">Moshannon Creek</td><td>Land rec</td><td rowspan="2">Passive</td></tr>
<tr><td>Passive</td></tr>
<tr><td rowspan="2">Clearfield Creek</td><td>Land rec</td><td rowspan="2">Passive</td></tr>
<tr><td>Passive</td></tr>
<tr><td rowspan="2">Clearfield Creek @ Dimeling</td><td>Land rec</td><td rowspan="2">Passive</td></tr>
<tr><td>Passive</td></tr>
</table>

Kettle Creek	***Passive***	Passive
Twomile Run	***Passive***	Passive
Alder Run	***Land rec***	Passive
	Passive	
Bear Run	***Passive***	Active
	Active	
	Land rec	
Clearfield Creek @ SR 1021	***Land rec***	Passive
	Passive	
Trib 26622	***Land rec***	Land Rec
Trib 26608	***No Treatment***	No Treat
Trib 26104	***No Treatment***	No Treat
Devils Run	***No Treatment***	No Treat
Trout Run	***No Treatment***	No Treat
Mowry Run	***No Treatment***	No Treat
Rock Run	***No Treatment***	No Treat
Trib 25913	***No Treatment***	No Treat
Rupley Run	***No Treatment***	No Treat
Trib 25693	***No Treatment***	No Treat
Trib 25611	***No Treatment***	No Treat
Milligan Run	***No Treatment***	No Treat
Redlick Run	***No Treatment***	No Treat

Appendix C – Habitat Parameters

Adapted from Shull and Lookenbill 2018.

Instream Fish Cover (riffle/run & low gradient)

Evaluates the percent makeup of the substrate (boulders, cobble, other rock material) and submerged objects (logs, undercut banks) that provide refuge for a variety of fish including both large bodied pelagic species as well as smaller benthic specialists.

Epifaunal Substrate (riffle/run)

Evaluates riffle quality, i.e. areal extent relative to stream width and dominant substrate materials(cobble, boulders, gravel) that are present.(low gradient) –Evaluates the relative quantity and variety of natural structures in the stream, such as large rocks, fallen trees, logs and branches, and undercut banks.

Embeddedness (riffle/run)

Evaluates the extent to which rocks (gravel, cobble, and boulders) and snags are covered or sunken into the silt, sand, or mud of the stream bottom. The rating of this parameter may be variable depending on where the observations are taken. To avoid confusion with sediment deposition (another habitat parameter), observations of embeddedness should be taken in the upstream and central portions of riffles and cobble substrate areas.

Velocity/Depth Regime (riffle/run)

Evaluates the presence/absence of four velocity/depth regimes (fast-deep, fast-shallow, slow-deep, and slow-shallow).Generally, shallow is< 0.5m and slow is < 0.3m/sec.

Channel Alteration (riffle/run & low gradient)

Evaluates the extent of channelization or dredging, but can include any other large-scale changes in the shape of the stream channel that would be detrimental to the habitat. Channel alteration is present when artificial embankments, riprap, and other forms of artificial bank stabilization or structures are present; when the stream is very straight for significant distances; when dams and bridges are present; and when other such changes have occurred.

Sediment Deposition (riffle/run & low gradient)

Estimates the extent of sediment effects in the formation of islands, point bars, and pool deposition. Deposition is typically evident in areas that are obstructed by natural or manmade debris and areas where the stream flow decreases, such as bends.

Riffle Frequency (riffle/run)

Estimates the frequency of riffle occurrence based on stream width and thus the heterogeneity occurring in a stream. For riffle/run prevalent streams where distinct riffles are uncommon, a run/bend ratio is used as a measure of meandering or sinuosity.

Channel Flow Status(riffle/run & low gradient)

Estimates the areal extent of exposed substrates due to water level or flow conditions. The flow status will change as the channel enlarges (e.g., aggrading stream beds with actively widening channels) or as flow decreases as a result of dams and other obstructions, diversions for irrigation, or drought. In riffle/run prevalent streams, riffles and cobble substrate are exposed; in low gradient streams, the decrease in water level exposes logs and snags, thereby reducing the areas of good habitat.

Condition of Banks (riffle/run & low gradient)

Evaluates the extent of bank failure, signs of erosion, or the potential for erosion. The stream bank is defined as the area from the water's surface to the bankfull delineation. Steep banks are more likely to collapse and suffer from erosion than are gently sloping banks, and are therefore considered to be unstable. Signs of erosion include crumbling, unvegetated banks, exposed tree roots, and exposed soil.

Bank Vegetative Protection (riffle/run & low gradient)

Estimates the extent of stream bank that is covered by plant growth providing stability through well-developed root systems. The stream bank is defined as the area from the water's surface to the bankfull delineation. This parameter supplies information on the ability of the bank to resist erosion as well as some additional information on the uptake of nutrients by the plants, the control of instream scouring, and stream shading. This parameter is made more effective by defining the native vegetation for the region and stream type (i.e., shrubs, trees, etc.). In some regions, the introduction of exotics has virtually replaced all native vegetation. The value of exotic vegetation to the quality of the habitat structure and contribution to the stream ecosystem must be considered in this parameter. In areas of high grazing pressure from livestock or where residential and urban development activities disrupt the riparian zone, the growth of a natural plant community is impeded and can extend to the bank vegetative protection zone.

Grazing or Other Disruptive Pressures (riffle/run & low gradient)

Evaluates disruptions to surrounding land vegetation due to common human activities, such as crop harvesting, lawn care, excavations, fill, construction projects, and other intrusive activities.

Riparian Vegetative Zone Width (riffle/run & low gradient)

Estimates the width of natural vegetation from the edge of the stream bank out through the riparian zone. Narrow riparian zones occur when roads, parking lots, fields, lawns, bare soil, rocks, or buildings are near the stream bank. Residential developments, urban centers, golf courses, and rangeland are the common causes of anthropogenic degradation of the riparian zone. Conversely, the presence of "old field" (i.e., a previously developed field not currently in use), paths, and walkways in an otherwise undisturbed riparian zone may be judged to be inconsequential to altering the riparian zone and may be given relatively high score.

Appendix D – Biometric Descriptions

Adapted from Chalfant (2015).

Total Abundance

The total abundance is the total number of organisms collected in a sample or sub-sample.

Dominant Taxa Abundance

This metric is the total number of individual organisms collected in a sample or sub-subsample that belong to the taxa containing the greatest numbers of individuals.

Taxa Richness

This is a count of the total number of taxa in a sample or sub-sample. This metric is expected to decrease with increasing anthropogenic stress to a stream ecosystem, reflecting loss of taxa and increasing dominance of a few pollution-tolerant taxa.

% EPT Taxa

This metric is the percentage of the sample that is comprised of the number of taxa belonging to the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT). Common names for these orders are mayflies, stoneflies, and caddisflies, respectively. The aquatic life stages of these three insect orders are generally considered sensitive to, or intolerant of, pollution (Lenat and Penrose 1996). This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting the loss of taxa from these largely pollution-sensitive orders.

Shannon Diversity Index

The Shannon Diversity Index is a community composition metric that takes into account both taxonomic richness and evenness of individuals across taxa of a sample or sub-sample. In general, this metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting loss of pollution-sensitive taxa and increasing dominance of a few pollution-tolerant taxa.

Simpson's Diversity (Fish)

The Simpson's Diversity Index is a community composition metric that takes into account both taxonomic richness and evenness of individuals across taxa of a sample or sub-sample. This value ranges from 0 to 1, the greater the number the greater the diversity. It represents the probability that two individuals selected randomly from a sample will belong to a different species.

Sorenson's Similarity Index (Fish)

A similarity coefficient used to determine similarities in species composition between sites. The values range from 0 to 1 and in cases of complete similarity the value will be 1. This is designed to evaluate species composition similarities between sites without regard to abundances of each species; so it uses species presence/absence to compare sites.

Evenness

Evenness is a diversity index that measures how equal a community is numerically. This value ranges from 0 to 1; the lower the value the greater the likelihood of a dominant species and indicates lower evenness.

Hilsenhoff Biotic Index

This community composition and tolerance metric is calculated as an average of the number of individuals in a sample or sub-sample, weighted by pollution tolerance values. The Hilsenhoff Biotic Index was developed by William Hilsenhoff (Hilsenhoff 1977, 1987; Klemm et al. 1990) and generally increases with increasing ecosystem stress, reflecting dominance of pollution-tolerant organisms. Pollution tolerance values used to calculate this metric are largely based on organic nutrient pollution. Therefore, care should be given when interpreting this metric for stream ecosystems that are largely impacted by acidic pollution from abandoned mine drainage or acid deposition.

Beck's Biotic Index

This metric combines taxonomic richness and pollution tolerance. It is a weighted count of taxa with PTVs of 0, 1, or 2. It is based on the work of William H. Beck in 1955. The metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting the loss of pollution-sensitive taxa.

Ratio of Biological Condition Gradient (BCG) Attribute

This screening question evaluates the balance of pollution tolerant organisms with more sensitive organisms in terms of taxonomic richness and organismal abundance. By using the BCG attributes to measure pollution tolerance, this screening question serves as a check against the IBI metrics which account for pollution sensitivity based only on PTVs. *This question must be applied to small-stream samples collected between November and May, but does not have to be applied to samples from larger streams and samples collected between June and September.*

Percent (%) Sensitive Individuals

This community composition and tolerance metric is the percentage of individuals with PTVs of 0 to 3 in a sample or sub-sample and is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting the loss of pollution-sensitive organisms.

Appendix E – Habitat Scores by Site

Abbreviations are IC: Instream Cover; ES, Epifaunal Substrate; E: Embeddedness; V: Velocity and Depth Regimes; C: Channel Alteration; S: Sediment Deposition; F: Frequency of Riffles; CF: Channel Flow Status; CB: Condition of Banks; B: Bank Vegetation Protection; G: Grazing or Other Disruptive Pressure; R: Riparian Vegetation Zone Width; and T=total score. Each metric has four categories (optimal, suboptimal, marginal, and poor), cutoffs for each category are 16-20, 11-15, 6-10, and 1-5 respectively. The total score also follows the same four categories, however the cutoffs are 181-240, 121-180, 61-120, and 1-60 respectively. Site 86 was dry at the time of the survey in 2017.

ID	Year	Rep/ Ref	IC	ES	E	V	C	S	F	CF	CB	B	G	R	T
1	2009	Rep	11	12	4	8	15	3	20	16	13	17	17	8	144
1	2017	Rep	11	11	13	13	7	15	12	16	16	15	16	15	160
2	2009	Rep	15	17	12	9	7	14	16	8	3	14	14	13	142
2	2017	Rep	14	18	16	18	13	12	18	17	16	18	18	18	196
3	2009	Rep	14	18	14	17	15	13	16	18	16	16	13	10	180
3	2017	Rep	7	13	9	15	6	12	15	17	13	17	16	8	148
4	2009	Rep	8	12	6	7	18	6	11	8	11	6	20	18	131
4	2017	Rep	12	15	12	18	13	10	17	18	16	18	18	18	185
5	2009	Rep	14	7	14	12	15	7	10	19	18	20	20	16	172
5	2017	Rep	13	16	15	18	12	13	11	19	11	17	18	14	177
6	2009	Rep	14	17	5	18	11	13	18	9	17	17	10	10	159
6	2017	Rep	18	17	17	18	13	17	18	19	16	16	16	12	197
7	2009	Rep	15	16	18	16	19	19	18	15	15	19	18	19	207
7	2017	Rep	17	17	18	18	18	19	17	19	17	19	19	19	217
8	2009	Rep	12	5	8	6	14	14	2	16	15	16	18	5	131
8	2017	Rep	16	16	13	16	11	15	16	17	15	15	11	11	172
9	2009	Rep	19	18	13	19	20	16	19	17	2	7	7	3	160
9	2017	Rep	18	19	17	19	17	17	19	17	17	19	19	19	217
10	2009	Rep	19	13	9	15	20	10	20	19	14	17	17	15	188
10	2017	Rep	16	17	14	18	12	10	17	17	12	17	16	16	182
11	2009	Rep	19	19	18	17	15	20	19	20	20	18	18	5	208
11	2017	Rep	13	18	16	18	11	18	19	19	18	17	17	8	192
13	2009	Rep	13	19	18	14	10	19	18	18	15	10	18	2	174
13	2017	Rep	16	14	17	18	10	17	18	19	18	13	16	8	184
14	2009	Rep	13	17	3	17	12	16	19	18	18	18	14	4	169
15	2009	Rep	16	17	2	13	15	18	19	13	5	4	11	6	139
15	2017	Rep	9	15	15	17	14	7	10	19	3	2	11	11	133
16	2009	Rep	10	13	10	17	16	19	14	19	19	19	19	15	190
16	2017	Rep	13	17	16	17	19	19	19	19	18	19	19	17	212

17	2009	Rep	7	18	14	17	16	19	19	17	15	14	20	1	177
17	2017	Rep	13	16	13	17	13	10	17	15	7	16	17	17	171
18	2009	Rep	19	17	18	6	18	19	16	16	18	18	20	1	186
18	2017	Rep	15	15	11	16	10	14	16	18	15	17	15	3	165
19	2009	Rep	18	18	7	17	14	18	18	19	18	19	19	17	202
19	2017	Rep	16	18	17	17	13	18	18	18	17	18	18	18	206
20	2009	Rep	20	20	18	14	19	19	19	18	20	19	20	10	216
20	2017	Rep	16	17	11	17	17	15	17	17	14	18	18	18	195
21	2009	Rep	18	15	19	16	15	17	14	19	17	18	18	13	199
21	2017	Rep	16	17	16	17	15	15	17	17	13	18	18	18	197
22	2009	Rep	17	18	10	15	19	17	17	18	16	16	19	18	200
22	2017	Rep	17	17	16	18	16	14	17	17	15	17	17	17	198
23	2009	Rep	15	18	5	15	11	18	118	16	11	11	19	10	267
23	2017	Rep	16	16	16	18	12	13	17	17	16	17	18	18	194
24	2009	Rep	17	18	5	17	17	17	19	17	15	13	19	17	191
24	2017	Rep	17	17	13	17	16	16	17	18	12	16	17	16	192
25	2009	Rep	20	20	8	20	20	12	20	12	18	13	20	17	200
25	2017	Rep	15	16	12	17	17	11	17	17	14	17	17	17	187
26	2009	Rep	14	19	10	16	20	16	20	17	20	14	20	14	200
26	2017	Rep	17	17	16	17	13	16	17	17	15	17	17	17	196
27	2009	Rep	8	11	5	17	18	18	16	19	15	5	20	20	172
27	2017	Rep	17	17	16	17	13	16	17	17	16	17	17	17	197
28	2009	Rep	19	18	19	19	19	19	19	20	20	9	16	11	208
28	2017	Rep	16	16	14	17	12	15	17	17	15	16	16	16	187
29	2009	Rep	16	19	16	18	20	16	18	16	19	19	20	20	217
29	2017	Rep	16	16	15	17	14	15	17	17	15	17	17	17	193
30	2009	Rep	20	20	5	20	20	18	1	19	20	19	20	20	202
30	2017	Rep	17	17	14	17	17	16	17	17	16	17	17	17	199
31	2009	Rep	15	18	5	19	20	14	20	20	20	10	20	20	201
31	2017	Rep	17	17	15	16	16	16	17	17	17	17	17	17	199
32	2009	Rep	18	19	17	20	20	19	20	16	15	19	20	17	220
32	2017	Rep	16	16	16	17	13	16	17	17	15	17	17	17	194
33	2009	Rep	15	19	9	18	18	15	19	14	17	10	20	20	194
33	2017	Rep	17	17	16	17	17	16	17	17	16	18	18	18	204
34	2009	Rep	10	11	2	6	11	5	18	10	20	8	20	20	141
34	2017	Rep	17	17	16	17	13	16	17	17	16	17	17	17	197
35	2009	Rep	19	20	10	16	15	15	20	16	17	7	20	16	191
35	2017	Rep	17	17	16	17	12	16	17	17	17	17	17	17	197

36	2009	Rep	15	11	13	5	20	20	16	8	6	19	20	20	173
36	2017	Rep	17	17	17	17	14	16	17	17	17	17	17	17	200
37	2009	Rep	20	20	15	18	20	19	20	14	20	20	20	20	226
37	2017	Rep	17	17	14	17	15	15	17	17	17	17	17	17	197
38	2009	Rep	16	18	7	20	20	14	20	20	20	20	20	20	215
38	2017	Rep	16	17	13	17	14	14	17	17	15	17	17	17	191
39	2009	Rep	17	16	2	3	20	11	16	16	20	16	20	20	177
39	2017	Rep	17	17	16	17	14	16	16	17	15	17	17	17	196
40	2009	Rep	20	15	18	20	15	19	20	14	20	20	20	15	216
40	2017	Rep	17	17	16	17	12	15	17	17	15	17	17	14	191
41	2009	Rep	17	10	7	18	20	16	19	13	20	18	20	20	198
41	2017	Rep	17	16	16	18	18	16	16	18	18	18	18	18	207
42	2009	Rep	4	11	3	8	19	19	17	18	19	19	19	19	175
42	2017	Rep	16	17	11	16	17	16	16	16	7	16	18	18	184
43	2009	Rep	1	11	1	8	19	4	16	16	13	14	20	16	139
43	2017	Rep	17	17	13	17	16	15	17	17	17	17	17	17	197
44	2009	Rep	17	13	7	19	19	18	19	16	19	14	19	19	199
44	2017	Rep	17	17	15	17	17	14	17	17	15	18	18	18	200
45	2009	Rep	19	19	18	19	15	19	19	17	18	17	20	19	219
45	2017	Rep	18	18	18	18	18	15	18	18	16	18	18	18	211
46	2009	Rep	16	13	15	10	15	16	18	15	14	19	20	20	191
46	2017	Rep	16	17	9	17	15	15	17	17	13	17	18	18	189
47	2009	Rep	19	18	16	16	20	20	16	20	20	20	20	20	225
47	2017	Rep	16	16	12	16	17	15	17	17	15	18	18	18	195
48	2009	Rep	18	17	15	18	15	20	20	9	20	20	20	20	212
48	2017	Rep	17	17	13	17	17	14	17	17	13	18	18	18	196
49	2009	Rep	15	6	18	11	15	19	5	18	16	18	18	10	169
49	2017	Rep	11	13	11	15	13	15	15	18	18	18	16	16	179
50	2017	Rep	15	16	14	16	11	14	16	17	15	17	17	17	185
51	2009	Rep	17	18	15	12	15	18	18	15	12	18	19	16	193
51	2017	Rep	15	15	11	16	12	9	16	16	8	17	17	17	169
52	2009	Rep	15	16	14	13	14	17	11	13	14	16	16	15	174
52	2017	Rep	16	18	16	17	13	17	17	17	17	18	18	18	202
53	2009	Rep	14	17	17	9	5	18	18	16	18	4	6	8	150
53	2017	Rep	17	16	17	17	12	17	17	17	17	17	16	16	196
54	2017	Rep	16	18	16	17	11	16	17	18	17	17	17	18	198
55	2009	Rep	15	5	11	5	15	17	1	15	14	18	14	16	146
55	2017	Rep	16	16	11	16	15	11	16	17	13	17	16	16	180
56	2009	Rep	15	16	14	16	14	15	16	17	12	18	14	13	180

56	2017	Rep	16	13	13	16	13	12	10	14	14	16	16	16	169
57	2009	Rep	15	14	17	17	20	15	17		18	17	17	6	173
57	2017	Rep	12	6	16	13	11	12	6	18	13	17	16	17	157
58	2009	Rep	15	19	20	19	20	20	14	20	12	10	9	10	188
58	2017	Rep	17	17	18	18	18	19	17	19	17	19	19	19	217
59	2009	Rep	14	5	15	5	15	16	0	16	11	17	19	18	151
59	2017	Rep	11	3	16	8	15	17	3	19	17	19	19	19	166
60	2009	Rep	17	13	6	17	14	16	13	19	17	18	17	17	184
60	2017	Rep	16	16	17	16	13	17	17	17	16	17	16	18	196
61	2009	Rep	14	5	18	6	16	18	2	17	18	19	19	8	160
61	2017	Rep	16	16	14	16	13	15	16	18	13	18	16	16	187
62	2009	Rep	18	10	9	20	20	14	14	19	19	18	20	14	195
62	2017	Rep	16	16	14	16	12	15	16	17	15	16	16	15	184
63	2009	Rep	11	5	8	4	18	8	2	14	15	19	19	19	142
63	2017	Rep	16	16	14	17	16	14	17	17	16	18	16	18	195
64	2009	Rep	8	1	6	1	1	7	1	14	17	4	4	4	68
64	2017	Rep	8	16	11	6	11	11	13	18	18	8	18	8	146
65	2009	Rep	14	13	12	12	10	17	14	17	13	10	20	19	171
65	2017	Rep	13	15	15	16	12	11	15	18	8	18	18	18	177
66	2009	Rep	2	0	0	3	18	2	10	20	2	2	20	20	99
66	2017	Rep	8	6	6	10	14	8	6	18	6	15	18	18	133
67	2009	Rep	17	19	2	17	15	12	16	20	20	20	20	7	185
67	2017	Rep	16	17	14	16	13	13	17	17	15	17	14	14	183
68	2009	Rep	1	1	2	2	3	2	2	20	20	13	15	4	85
68	2017	Rep	16	16	14	16	13	15	17	17	15	17	16	13	185
69	2017	Rep	17	17	15	17	17	15	17	17	14	18	18	18	200
70	2017	Rep	15	17	15	18	14	14	17	18	18	18	15	15	194
71	2017	Rep	17	18	16	18	13	15	17	17	12	16	12	7	178
72	2017	Rep	15	16	14	17	13	10	17	17	14	16	17	17	183
73	2009	Rep	19	19	10	17	11	12	18	18	8	2	20	12	166
73	2017	Rep	17	17	13	17	11	12	16	18	12	17	16	16	182
74	2009	Rep	18	19	14	14	15	12	18	19	8	3	19	20	179
74	2017	Rep	16	16	12	16	11	13	16	16	14	16	16	17	179
75	2017	Rep	15	16	15	16	13	14	17	17	15	17	16	14	185
76	2009	Rep	19	18	9	17	17	18	16	19	16	19	20	20	208
76	2017	Rep	13	17	17	18	13	17	17	18	16	18	18	16	198
77	2009	Rep	16	6	14	11	11	10	5	17	19	10	13	2	134
77	2017	Rep	16	17	12	16	12	14	17	17	15	15	15	15	181
78	2009	Rep	5	5	5	4	17	7	1	15	14	13	11	14	111

78	2017	Rep	11	5	17	6	11	18	3	17	16	13	16	13	146
79	2009	Rep	16	17	17	15	17	19	17	15	15	19	20	18	205
79	2017	Rep	14	17	16	18	13	14	18	18	17	18	16	16	195
80	2017	Ref	16	17	16	17	11	14	17	17	17	17	17	17	193
81	2017	Ref	17	17	11	17	11	13	17	17	15	18	16	11	180
82	2017	Ref	13	15	16	16	16	13	16	17	14	17	17	17	187
83	2017	Ref	16	17	17	18	13	17	18	18	16	17	18	16	201
84	2017	Ref	17	17	16	17	17	17	17	18	18	18	18	18	208
85	2017	Ref	17	17	15	17	17	15	17	17	16	17	17	17	199
86	2017	Ref	-	-	-	-	-	-	-	-	-	-	-	-	-
87	2017	Ref	17	17	17	17	17	16	18	17	17	18	18	18	207
88	2017	Ref	17	17	16	17	11	16	17	17	17	18	18	18	199
89	2017	Ref	18	12	10	16	13	13	14	13	18	18	18	18	181
90	2017	Ref	17	17	17	17	17	16	17	17	16	18	18	18	205
91	2017	Ref	17	17	14	17	12	14	17	17	16	17	17	17	192
92	2017	Ref	18	18	17	18	18	17	18	18	15	18	18	18	211
93	2017	Ref	17	17	17	17	18	14	15	18	15	18	18	18	202
94	2017	Ref	16	17	11	17	13	13	17	17	17	17	17	17	189
95	2017	Ref	17	18	16	18	11	16	18	18	16	18	18	18	202
96	2017	Ref	18	17	17	18	18	15	18	18	15	18	18	18	208
98	2017	Ref	19	19	16	18	17	17	18	18	8	18	18	18	204
99	2017	Ref	16	17	16	17	16	17	17	16	16	18	16	18	200
100	2017	Ref	13	16	12	18	11	13	14	17	18	18	18	18	186
101	2017	Ref	18	18	16	19	18	16	18	18	15	18	18	18	210
102	2017	Ref	18	16	16	17	12	16	16	17	16	18	16	16	194
103	2017	Ref	17	17	11	17	17	13	17	17	11	18	18	16	189
104	2017	Ref	17	18	15	17	13	14	17	17	16	18	18	18	198
105	2017	Ref	16	17	16	17	13	15	17	18	17	18	15	14	193
106	2017	Ref	17	18	14	17	12	13	17	18	16	16	11	16	185
107	2017	Ref	14	17	12	17	13	13	18	18	16	18	16	13	185
108	2017	Ref	17	17	16	17	16	16	17	18	16	17	17	17	201
109	2017	Ref	18	18	16	17	17	15	17	17	14	17	18	18	202

Appendix F – Fish Species

Numbers below site names indicate the year of sample; 1=1998, 2=2009, 3=2019. TV indicates the species' tolerance to pollution (I=intolerant, M=moderate, T=tolerant).

Common Name	TV	Site Name															
		Bower			Irvin Park	Clearfield			Deer Creek			Burns Run			Hyner		
		1	2	3	3	1	2	3	1	2	3	1	2	3	1	2	3
Brook Trout	I			X													
Brown Trout	I		X	X													
Common Carp	T								X	X						X	
Cutlips Minnow	I	X		X		X					X						
Common Shiner	M															X	
Mimic Shiner	I															X	
Comely Shiner	T		X														
Central Stoneroller	T												X				
Spottail Shiner	M	X		X													
Swallowtail Shiner	M			X							X	X					
Rosyface Shiner	I							X			X			X		X	X
Bluntnose Minnow	T		X	X	X		X	X			X		X			X	X
Blacknose Dace	T		X														
Longnose Dace	M					X	X	X		X							
Creek Chub	T	X	X	X			X			X							
Fallfish	M				X	X	X	X		X	X		X	X		X	X
River Chub	M		X	X	X		X	X			X	X	X	X			
Redhorse species	M		X	X													
White Sucker	T	X		X				X		X						X	
Northern Hog Sucker	M	X	X	X				X		X			X	X		X	X
Yellow Bullhead	T									X			X		X		
Brown Bullhead	T														X	X	
Channel Catfish	M								X	X	X					X	
Margined Madtom	M	X	X	X		X	X	X				X	X	X			X
Rock Bass	M	X	X	X			X	X	X	X	X		X	X	X	X	
Green sunfish	T			X	X		X			X							
Pumpkinseed	M	X	X	X					X	X							

Bluegill	T		X	X	X			X	X		X		X			X	X
White Crappie	T			X	X												
Black Crappie	M			X													
Smallmouth Bass	M	X	X	X	X	X			X	X	X	X	X	X		X	X
Largemouth Bass	M									X	X						
Greenside Darter	I																X
Tessellated Darter	M	X		X	X		X	X		X			X	X		X	X
Banded Darter	I										X			X			X
Shield Darter	M			X	X	X											
Fantail Darter	M													X			X
Yellow Perch	M											X				X	
Sculpin Species	I	X	X	X													
American Eel	T				X												
Total species		11	14	22	10	6	9	11	6	14	12	5	11	10	3	15	11

Appendix G – Sorenson's Similarity Index

	Bower 1998	Bower 2009	Bower 2019	Irvin 2019	Clearfield 1998	Clearfield 2009	Clearfield 2019	Deer Creek 1998	Deer Creek 2009	Deer Creek 2019	Burns 1998	Burns 2009	Burns 2019	Hyner 1998	Hyner 2009
Bower 2009	0.69														
Bower 2019	0.74	0.70													
Irvin 2019	0.25	0.38	0.56												
Clearfield 1998	0.38	0.26	0.34	0.40											
Clearfield 2009	0.33	0.46	0.50	0.56	0.40										
Clearfield 2019	0.38	0.57	0.53	0.50	0.35	0.70									
Deer Creek 1998	0.38	0.43	0.28	0.27	0.17	0.13	0.24								
Deer Creek 2009	0.57	0.53	0.50	0.36	0.32	0.55	0.50	0.53							
Deer Creek 2019	0.22	0.41	0.46	0.48	0.33	0.38	0.52	0.44	0.40						
Burns 1998	0.20	0.27	0.29	0.29	0.36	0.29	0.25	0.18	0.11	0.35					
Burns 2009	0.18	0.42	0.40	0.75	0.31	0.50	0.56	0.31	0.30	0.53	0.33				
Burns 2019	0.40	0.44	0.42	0.42	0.38	0.53	0.67	0.25	0.43	0.55	0.40	0.47			
Hyner 1998	0.11	0.10	0.08	0.00	0.00	0.17	0.14	0.22	0.13	0.13	0.00	0.00	0.15		
Hyner 2009	0.39	0.48	0.41	0.40	0.18	0.32	0.59	0.45	0.55	0.50	0.19	0.43	0.46	0.32	
Hyner 2019	0.31	0.43	0.41	0.50	0.35	0.40	0.64	0.24	0.33	0.52	0.25	0.56	0.76	0.00	0.52