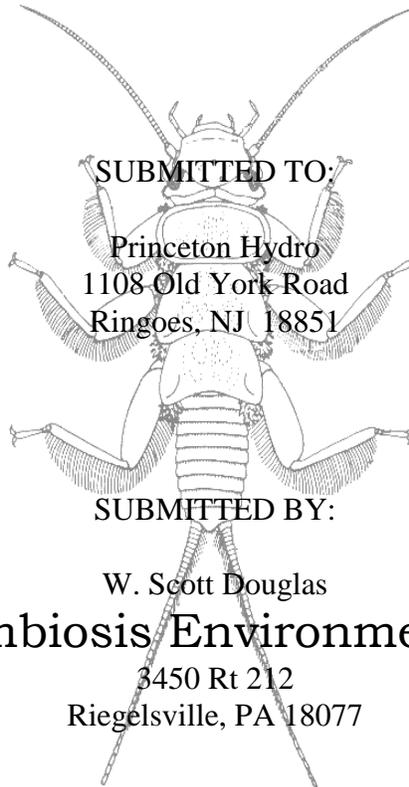


FINAL REPORT

**BIOASSESSMENT
OF
COOKS CREEK
SPRINGFIELD AND DURHAM TOWNSHIPS
PENNSYLVANIA**

December, 2020



SUBMITTED TO:

Princeton Hydro
1108 Old York Road
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SUBMITTED BY:

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PROJECT SUMMARY

Samples of benthic material were collected four times at five sites in the Cooks Creek Watershed from December 2018 to August 2020, encompassing spring, summer, fall and winter seasons. Samples were collected following PADEP protocol for small wadeable streams using kick net and jab techniques. Five separate kick samples and ten jab samples were taken at each location. No field duplicates were taken. After the first sampling period in December of 2018, kick and jab samples were composited in the field. Some processing to remove large leaf litter and other debris was performed in the field prior to placing the collected material into labelled plastic containers with isopropyl alcohol. A habitat assessment following PADEP protocol was performed at each location once during the study period.

Samples were taken to the laboratory by courier. Each samples were emptied into a large gridded sorting tray to subsample. The subsample was transferred to a glass tray to remove organisms from detritus. Each sample was examined carefully under magnification using bottom and top illumination and all organisms removed. If subsamples contained less than 160 organisms, another grid was randomly chosen and the organisms added to the first collection until 200 +/- 40 were removed. If more than 240 organisms were recovered from a grid, the organisms were refloated and resampled until 200 +/- were recovered. Organisms were replaced in 70% isopropyl alcohol prior to identification and enumeration.

Each sample was identified to lowest practical taxon, genus in most cases, using commonly accepted taxonomic references (Peckarsky et al. 1990, Wiggins 1996, Merrit and Cummins 2019, Smith, 2001). Chironomid larvae were counted but not identified to genus. Molluscs were identified to family level in most cases. Exuviae, empty shells, and pieces of larvae without heads were not included in counts. Identified organisms were returned to vials (by order in most cases) and preserved with 70% isopropanol.

The organism identification and enumeration was performed for each sample and recorded on a spreadsheet. Standard metrics; H' diversity (Shannon's), taxa richness, EPT index, EPT: chironomid ratio, percent contribution dominant taxon, BCG attribute ratios, and modified Hilsenhoff biotic index were calculated on each sample. In addition, the metrics of Beck's Index of Biotic Integrity (version 3), modified EPT, Biological Condition Gradient ratios for taxa and individuals, and percent sensitive organisms were calculated as required by the PADEP Freestone Index of Biotic Integrity protocol (PADEP, 2012). A PA Freestone IBI score was calculated for each sample and an Aquatic Life Use determination made for the site and time period.

The communities observed during the spring and winter sampling periods were typical of an Exceptional Value watershed, with overall IBI averages, based on all four sampling events, ranging from a low of 63 at Silver Creek to a high of 77 at Red Bridge (Table S1). Indications of community stress were seen during summer and fall low flow periods at several locations, but primarily at the Kunsman and Silver Creek locations, where the lowest scores for all metrics were observed. This impact is likely due to localized thermal and/or nutrient pollution, stormwater impacts, or some combination of these. Additional more focused monitoring of these two catchments is recommended

as this will potentially identify the sources and extent of the problem, and allow managers to recommend appropriate actions. Indications are that there may be nutrient impacts in the Silver Creek catchment, and stormwater and/or thermal impacts in the Kunsman catchment. On a more positive note, the community at the Red Bridge site, which is the lowest in elevation and contains the catchments of all the other sites, was remarkably resilient and attained the ALU standard during all four seasons. Overall, the Cooks Creek Watershed appears to be attaining the ALU standard for an Exceptional Value watershed.

Table S1. Average community metrics measured during the study at five locations and across four seasons in the Cooks Creek Watershed, Durham and Springfield Townships, Bucks County, PA

	Kunsman	Slifer Valley	Brunswick	Silver Creek	Red Bridge	Average
Taxa Richness	34	30	34	29	31	31
Modified EPT	12	14	13	12	15	13
Beck’s Version 3	14	26	17	17	20	19
Shannon’s Diversity	2.94	2.62	2.78	2.42	2.47	2.64
Hilsenhoff Biotic Index	3.88	3.39	3.71	4.06	2.67	3.54
Percent Sensitive Organisms	35	41	41	22	62	40
PA Freestone IBI	69	76	73	63	77	71

Complete details of the study are included in the following report.

Report Certified by: W. Scott Douglas
W. Scott Douglas, Principal

4/10/2022
Date

PROJECT REPORT
TAXONOMIC IDENTIFICATION OF BENTHIC INVERTEBRATES

I. OBJECTIVE

The objective of this study was to collect, process and identify to lowest practical taxon organisms from samples collected in five locations in each of four seasons. An additional objective was to provide specific community metrics and comparison to reference stations using the latest PADEP Freestone Index of Biotic Integrity.

II. SAMPLE INFORMATION

Location: Springfield Township, PA

Samples:	<u>Client ID</u>	<u>Location</u>	<u>Collection Date</u>
	KNKQ1	Kunsmann, Pleasant Valley	Dec 9, 2018
	KNJQ1	Kunsmann, Pleasant Valley	Dec 9, 2018
	SCKQ1	Silver Creek, Springtown	Dec 8, 2018
	SCJQ1	Silver Creek, Springtown	Dec 8, 2018
	BRKQ1	Brunswick, Springtown	Dec 9, 2018
	BRJQ1	Brunswick, Springtown	Dec 9, 2018
	RBKQ1	Red Bridge, Durham	Dec 9, 2018
	RBJQ1	Red Bridge, Durham	Dec 9, 2018
	SLKQ1	Slifer Valley, Springfield	Dec 9, 2018
	SLJQ1	Slifer Valley, Springfield	Dec 9, 2018
	KN-Comp19	Kunsmann, Pleasant Valley	Oct 20, 2019
	SC-Comp19	Silver Creek, Springtown	Oct 13, 2019
	BR-Comp19	Brunswick, Springtown	Oct 13, 2019
	RB-Comp19	Red Bridge, Durham	Oct 19, 2019
	SL-Comp19	Slifer Valley, Springfield	Oct 20, 2019
	KN-Comp20	Kunsmann, Pleasant Valley	April 5, 2020
	SC-Comp20	Silver Creek, Springtown	April 4, 2020
	BR-Comp20	Brunswick, Springtown	April 4, 2020
	RB-Comp20	Red Bridge, Durham	April 4, 2020
	SL-Comp20	Slifer Valley, Springfield	April 5, 2020
	KN-CompS	Kunsmann, Pleasant Valley	Aug 1, 2020
	SC-CompS	Silver Creek, Springtown	Aug 1, 2020
	BR-CompS	Brunswick, Springtown	July 30, 2020
	RB-CompS	Red Bridge, Durham	July 30, 2020
	SL-CompS	Slifer Valley, Springfield	Aug 1, 2020

Dates received: All samples received the same day as collected

III. METHODOLOGY

A. Sample Collection

Samples were collected using the PADEP Freestone protocols (PADEP, 2012) and in accordance with the QAPP that was approved by PADEP for the project. At each sampling location, a 100 meter transect was identified containing riffle and run habitats. Kick net samples were taken using a D-Net positioned in the stream such that water flow was into the net, then the rocks and debris disturbed by hand upstream as far as could be reached (approximately one square meter) by hand for one minute. Rocks were scrubbed of clinging material and the bottom sediments stirred vigorously. Jab samples were taken at ten locations along the transect by pushing the D-Net under and around stream banks, logs, overhanging rocks, aquatic vegetation and other debris.

The collected material was placed into a plastic tray containing stream water. The net was carefully rinsed into the tray until no material remained in the net. Jabs and Kicks were kept separate for the first round of sampling, but were composited together at each site for the remaining sampling. The large woody debris, leaves, sticks and rocks were rinsed, examined for clinging organisms and removed. The remaining material was strained through the D-net and placed into a labeled plastic container and preserved with 70% isopropyl alcohol.

B. Sample Preparation

Each sample was placed into a gridded plastic tray for subsampling. Depending on a visual assessment of organism density, either a ¼, 1/8, or 1/16 grid was selected and the material removed from the grid using a spoon and pipette. The material was then placed into a glass tray with tap water and examined using a dissecting microscope. The organisms were removed, counted and placed into a labelled vial containing 70% isopropyl alcohol. In some cases, a duplicate sample was taken in the same manner. If more than 220 organisms were recovered, the subsample was placed into a gridded glass tray and organisms removed from randomly selected grids until the total ranged from 180-220.

C. Taxonomy

Organisms were sorted by taxon and representatives keyed to the lowest practical taxon (usually genus) using one or more of the following keys:

Merritt, R.W., and K.W. Cummins, 2019. *An Introduction to the Aquatic Insects of North America, 5th ed.*, Kendall Hunt Publishing Company, Dubuque, Iowa.

Peckarsky, B. L., P.R. Fraissinet, M.A. Penton, and D.J. Conklin, Jr., 1990. *Freshwater Macroinvertebrates of Northeastern North America*. Cornell University Press, Ithaca, NY.

Smith, D.G., 2001. *Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea, 4th ed.*, John Wiley and Sons, New York, NY.

Wiggins, G. B., 1996. *Larvae of the North American Caddisfly Genera (Trichoptera), 2nd ed.*, University of Toronto Press, Toronto, Canada.

Leeches, flatworms, roundworms, oligocheates and Chironomidae were not keyed further than that grouping. Gastropods were keyed to family. The number of each taxon found in each sample was noted on a bench sheet.

C. Sample Storage

All organisms were stored in separately labeled vials filled with 70% isopropanol. All organisms will be maintained at Symbiosis for 5 years unless alternative arrangements are made.

D. Data Analysis

The total number of organisms present and the number of distinct taxa identified are presented. The functional group of each taxa was determined by using the tables in the Pennsylvania Freestone IBI (PADEP, 2012). Species diversity in each sample was evaluated using Shannon's H' . The number of organisms in the orders Plecoptera, Ephemeroptera, and Trichoptera was determined and compared to the numbers of organisms in the family Chironomidae to provide the EPT to Chironomid ratio. The percent contribution of the dominant taxon was calculated by dividing the number of organisms in the most abundant taxon by the total number of organisms collected. Hilsenhoff's biotic index was conducted using the scores provided in the 2012 version of the Pennsylvania Freestone IBI. Percent sensitive individuals was calculated by summing the individuals with Hilsenhoff tolerance values of 3 or less and dividing by the total number of organisms in the sample. Becks Index (version 3) was calculated by summing the number of taxa with Hilsenhoff tolerance values of 2 or less by a value of 1, 2, or 3 using decreasing weight with increasing tolerance.

An index of biotic integrity (IBI) was calculated for each station using the Freestone Riffle-Run procedure of PADEP (PADEP, 2012). This procedure uses a composite of six metrics (richness, modified EPT, modified Becks, Shannon's diversity, modified Hilsenhoff and percent sensitive organisms) to compare to a standardized reference for the Commonwealth. The average percentage achieved is then compared to Aquatic Life Use scores based on the sample collection time period. In order to evaluate samples taken in the June through October time period, the ratio of sensitive to tolerant Biological Condition Gradient (BCG) scores for taxa and individuals was performed using the PADEP reported attributes. Community stress is indicated if both of these ratios are less than 0.75.

E. Metrics

- **Total Taxa Richness:** A count of the total number of taxa in a sub-sample. This metric is generally expected to decrease with increasing anthropogenic stress to a stream ecosystem, as this will usually lead to the dominance of few pollutant-tolerant taxa.
- **Ephemeroptera + Plecoptera + Trichoptera Taxa Richness (Pollution Tolerance Values 0-4 only):** A count of the number of taxa belonging to the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT), also known as mayflies, stoneflies, and caddisflies, respectively, in a sub-sample. The aquatic life stages of these taxa are considered to be sensitive to many types of pollution and are generally found in higher numbers in healthier streams. This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem.
- **Beck's Index (Version 3):** A weighted count of taxa with pollution tolerance values of 0, 1, or 2, which are considered to be highly intolerant of pollution. This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem.
- **Shannon Diversity:** A measure of taxonomic richness and evenness of individuals across taxa of a sub-sample. This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem.
- **Hilsenhoff Biotic Index:** A community composition and tolerance metric calculated as an average of the number of individuals in a sub-sample, weighted by pollution tolerance values. This metric is expected to increase with increasing ecosystem stress, unlike any of the other five metrics.
- **Percent Sensitive Individuals (Pollution Tolerance Values 0-3 only):** A community composition and tolerance metric calculated as the percentage of individuals with pollution tolerance values of 0 to 3 in a sub-sample. This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem.

These 6 metrics are then standardized and adjusted to a maximum value of 100 if necessary. Thus, the resulting adjusted standardized metric scores can range from maximum values of 100 to minimum values of zero, with scores closer to zero corresponding to increasing deviation from the expected reference condition and progressively higher values corresponding more closely to the biological reference condition. The IBI is then calculated by calculating the arithmetic mean of these adjusted standardized metric values for the six core metrics, resulting in a multimetric index of biological integrity score that can range from 0 to 100.

F. Quality Assurance

All samples are clearly marked with a sample number on arrival. In this case the client ID served as the sample number. This number served as a tracking number for the sample throughout the processing. Taxonomic and enumeration data for each sample was recorded on a separate bench sheet on which the client ID was clearly marked. Each vial for organism

storage was also clearly marked with the client ID.

Data was transferred from the raw data sheets into a Microsoft Excel spreadsheet. Data entry was carefully cross-checked to guard against transcription errors. The spreadsheets were verified by hand calculations.

There were no field duplicates taken, however laboratory duplicates were taken to assess the subsampling process. Kick and jab samples were kept separate during the first sampling period. However, as results were similar for the two sampling methods at each site, subsequent sampling composited the five kick and ten jab samples at each site. In order to better compare the first season with subsequent seasons, an artificial composite was created for the first season by combining the results of the kick and jab samples and recalculating the IBI.

During the first two sampling rounds, the subsampled grid was sorted, keyed and counted in total with no attempt to adjust for density beyond the initial visual assessment. During the third and fourth sampling rounds, the subsamples were reduced using the methods outlined above to bring the total numbers into the preferred range for a 200 organism subsample. In order to ensure comparability between the early and later sampling rounds, the samples from the December 2018 and October 2019 sampling periods were re-sampled by placing all organisms into a gridded glass tray and randomly selecting grids until 200 +/- 20 organisms were removed. In some cases, laboratory duplicates were created by continuing to remove organisms until a second sample of 200+/-20 organisms was obtained.

IV. RESULTS

Site Descriptions and Habitat Assessment

Five stations were selected representing both headwaters and mainstem sites (Figure 1). At each site, a 100 meter transect was selected for habitat assessment and benthic data collection. Data was collected for each transect according to the PADEP sampling protocols (Tables 1 and 2) and entered into datasheets. Since many of the metrics are subjective, all five sites were evaluated by the same investigator. Aerial and ground level photos of each site are provided in Appendix A.

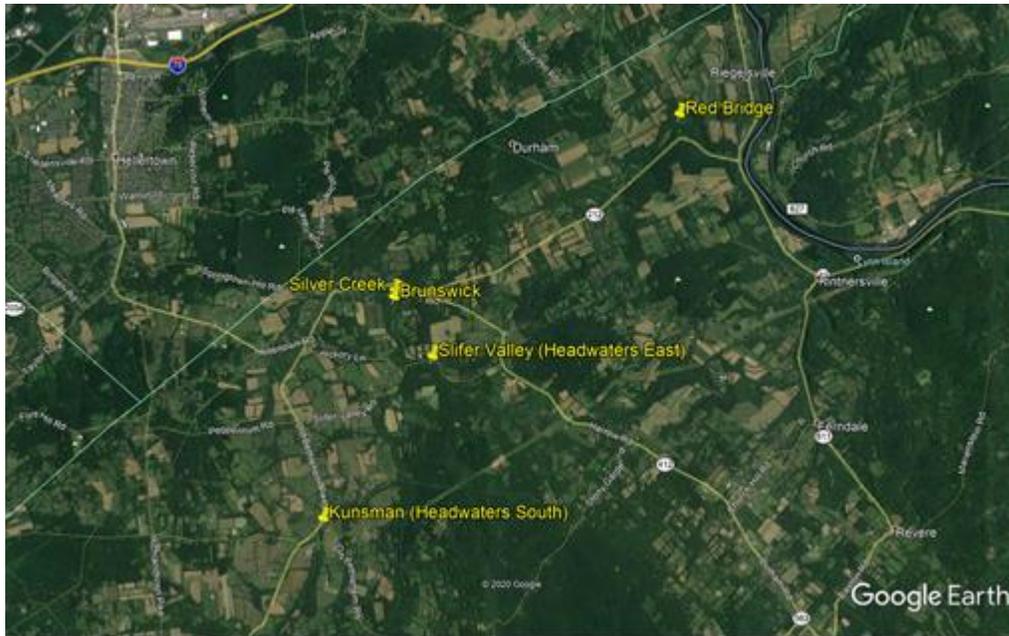


Figure 1. Locations of Sites for Benthic Assessment.

Kunsman, ST-1 – The Kunsman headwater site is located in the village of Pleasant Valley. The catchment is 4,100 acres; primarily agricultural and forested, with 13% residential land use. The transect was just upstream of the iron bridge on Bridge Street, adjacent to a historic gristmill that has been converted into a multifamily residence. Above the transect are extensive riparian wetlands running into secondary growth forest. The transect is approximately 10 feet wide and was 0.5-1 ft deep at the first sampling. Erosion along the bank next to the residence is ongoing and severe. There was a significant amount of exposed bedrock in the transect, with the remaining substrate mostly cobble and gravel. Hydrology was evenly split between riffle, run and pool. The habitat score of 131 was the lowest of all five sites mostly due to the lack of canopy cover and land use in the immediate area.

Slifer Valley, ST-2 – The Slifer Valley site is located on preserved land in a largely undeveloped portion of Springfield Township. The catchment is 1,450 acres; primarily forested and agricultural, with 12.5% residential land use. The transect is just upstream of the bridge over Slifer Valley Rd near the intersection with Walnut Lane. The area is mature oak (*Quercus* spp.), beech (*Fagus* spp.), and ash (*Fraxinus* spp.) forest, with scattered residences upstream. The stream in the transect is approximately 8-10 feet wide and was 0.5-1.0 ft deep at the time of first sampling. Bank erosion is moderate to severe in places, due to the steep topography to the south of the reach. Substrate was mostly cobble and gravel, with some fines in the deep pools. The hydrology was evenly split between riffle, run and pool (one large pool). The habitat score was 157.

Brunswick, ST-3 – The Brunswick site is in a forested area of preserved land near the village of Springtown, just upstream of the confluence with Silver Creek. The catchment is 9,150

acres; primarily agricultural and forest, with 10.5% residential. The area is mature ash, with extensive riparian buffers and good canopy cover. The stream in the transect is approximately 25 feet wide and was 1-2 ft deep at the time of first sampling. Bank erosion is moderate. Substrate mostly boulder and cobble, with some finer grained material in the run. There are pockets of sand indicating stormwater impacts. The hydrology is mostly riffle and run. The habitat score here was 160, similar to Slifer Valley.

Silver Creek, ST-4 – The Silver Creek site is located just upstream of the confluence with the mainstem of Cooks Creek in the village of Springtown. The catchment is 3,150 acres; primarily forested and agricultural, with 21% residential land use, the highest in the study. The area is mature ash and oak. The stream in the transect is 8-10 feet wide and was 0.5-1 ft deep at the time of first sampling. Bank erosion is light. The substrate was mostly cobble and gravel. There are pockets of sand indicating stormwater impacts. The hydrology is primarily riffle. The habitat score was 173, one of the higher scores.

Red Bridge, ST-5 – The Red Bridge site is located near the bottom of the watershed just upstream of the bridge at the intersection of Red Bridge Road and Stout’s Valley Rd, just downstream of the village of Durham. The sampling area is mature oak and sycamore (*Platanus* spp.) with good canopy cover, residences are older, and not close to the creek. The catchment is 19000 acres and representative of the whole watershed, primarily agriculture and forested, with 13% residential land use. The stream at this point is approximately 27 feet wide and was 1-2 ft deep at the time of first sampling. The substrate contains ledges, large boulders and cobble and some gravel. There are pockets of sand indicating stormwater impacts. The hydrology is riffle and run with no pools. The habitat score was 177, the highest recorded.

Table 1. Site Characteristics for Benthic Sampling Locations.

Site ID	Name	Location	Latitude	Longitude	Catchment (acres)	Habitat Score
ST-1	Kunsmen (Headwaters South)	Pleasant Valley	40.517343	75.290948	4,100	131
ST-2	Slifer Valley (Headwaters North)	Springfield Township	40.544228	75.269499	1,450	157
ST-3	Brunswick	Springtown	40.553271	75.277443	9,150	160
ST-4	Silver Creek	Springtown	40.555106	75.277300	3,150	173
ST-5	Red Bridge	Durham Township	40.586668	75.211108	19,000	177

Table 2. Bottom Composition at Sampling Sites.

Site ID	Geology	Bedrock	Boulders	Cobble	Gravel	Sand	Fines
ST-1	Shale	40%	5%	30%	15%	5%	0%
ST-2	Shale	0%	5%	35%	40%	15%	5%
ST-3	Shale	0%	10%	50%	20%	15%	5%
ST-4	Limestone	0%	10%	65%	20%	5%	0%
ST-5	Limestone	0%	20%	40%	20%	20%	0%

Table 3. Hydrology at Sampling Sites, December 2018.

Site ID	Riffle	Run	Pool	Width (ft)	Depth (ft)	Bank Erosion
ST-1	40%	30%	30%	10	0.5-1	Heavy
ST-2	40%	40%	20%	8-10	0.5-1	Moderate-Heavy
ST-3	50%	30%	10%	25	1-2	Moderate
ST-4	75%	25%	0%	9	0.5-1	Light
ST-5	50%	50%	0%	27	1-2	Moderate

Copies of the data sheets are provided in Appendix B.

Benthic Community Assessment

All samples were subsampled and the organisms contained removed and identified. A summary of the organisms found in each sample and their relative abundance over the sampling interval is provided in Table 4. A complete listing of all taxa identified, number of organisms recovered, and all community metrics calculated for each sample are presented in tables C1-C12, Appendix C.

December 2018

The first round of samples was collected in December, 2018. Kick and jab samples were not composited for processing, however a composite was created in the lab after the initial sample was processed and recorded. Further evaluation revealed that this large subsample size may have biased some of the metrics high, making comparisons across sampling periods invalid, therefore the kick and jab samples were later physically combined into a sorting tray and re-sampled to within +/- 40 organisms of the PADEP

Table 4. Summary of Cooks Creek Benthic Invertebrate Taxa Identified by Site (A = Abundant (>25), C = Common (6-25), R = Rare (≤5), across all four sampling periods)

Class	Order	Family	Genus	Sensitivity				Site			
				Hilsen- hoff	BCG (small)	Kunsmann	Slifer Valley	Brunswick	Silver Creek	Red Bridge	
Insecta	Trichoptera	Hydropsychidae	<i>Cheumatopsyche</i>	6	5	A	A	A	A	A	
			<i>Hydropsyche</i>	5	5	A	A	A	A	A	
			<i>Diplectrona</i>	0	2	-	R	-	-	-	
		Lepidostomatidae	<i>Lepidostoma</i>	1	2	C	R	C	C	C	
		Limnephilidae	<i>Pycnopsyche</i>	4	3	R	-	R	R	R	
			<i>Apatania</i>	3	2	-	-	R	R	-	
			<i>Pseudostenophylax</i>	0	3	-	-	-	R	-	
		Hydatophylax	<i>Hydatophylax</i>	2	2	R	C	R	A	R	
			Philopotamidae	<i>Chimarra</i>	4	4	A	A	A	A	A
				<i>Dolophilodes</i>	0	2	-	A	-	A	R
		Glossosomatidae	<i>Glossosoma</i>	0	3	R	C	C	R	C	
			<i>Protoptila</i>	1	2	-	-	R	-	-	
			<i>Culoptila</i>	1	3	-	-	-	-	R	
		Agapetus	<i>Agapetus</i>	0	3	-	-	R	R	-	
			Rhyacophilidae	<i>Rhyacophila</i>	1	2	C	C	C	C	C
		Polycentropodidae	<i>Polycentropus</i>	6	4	R	C	C	R	R	
			<i>Neureclipsis</i>	7	3	C	-	C	R	-	
		Helicopsychidae	<i>Helicopsyche</i>	3	3	C	-	C	-	R	
		Psychomyiidae	<i>Lype</i>	2	2	-	-	-	R	-	
		Leptoceridae	<i>Oecitis</i>	8	3	R	R	R	-	R	
			<i>Triaenodes</i>	6	3	R	R	-	-	-	
			<i>Setodes</i>	2	2	-	-	-	-	R	
		Mystacides	<i>Mystacides</i>	4	3	R	R	R	R	R	
Uenoidae	<i>Neophylax</i>		3	3	C	R	C	C	-		
Molannidae	<i>Molanna</i>	6	2	-	-	-	R	-			
Hydroptilidae	<i>Leucotrichia</i>	6	4	C	-	-	-	-			
	<i>Hydroptila</i>	6	5	C	-	R	-	-			

		<i>Agraylea</i>	8	4	R	-	R	-	-
	Georidae	<i>Geora</i>	0	1	-	-	-	R	-
	Odontoceridae	<i>Psilotreta</i>	0	1	-	-	-	-	R
	Brachycentridae	<i>Micrasema</i>	2	3	R	-	-	R	-
Plecoptera	Nemouridae	<i>Amphinemura</i>	3	3	C	A	A	C	C
		<i>Paranemoura</i>	2	3	-	-	-	-	R
	Perlidae	<i>Agneta</i>	2	3	R	-	R	C	C
		<i>Acroneuria</i>	0	3	-	C	-	-	R
		<i>Attaneuria</i>	3	2	-	-	-	R	R
		<i>Eccopectura</i>	2	2	-	R	-	-	-
		<i>Paragnetina</i>	1	2	-	-	-	-	C
	Capniidae	<i>Paracapnia</i>	1	2	A	A	A	C	A
		<i>Allocapnia</i>	3	3	R	A	A	R	R
		<i>Capnia</i>	1	3	-	R	R	-	-
	Chloroperlidae	<i>Sweltsa</i>	0	3	-	C	R	-	-
		<i>Haploperla</i>	0	3	-	R	-	-	-
	Perlodidae	<i>Isoperla</i>	2	2	-	R	-	R	-
		<i>Diura</i>	2	2	-	C	-	-	R
	Taeniopterygidae	<i>Taeniopteryx</i>	2	3	C	A	C	R	C
	Peltoperlidae	<i>Talloperla</i>	0	1	-	-	-	-	R
Emphemeroptera	Heptageniidae	<i>Stenonema</i>	4	4	A	A	A	C	A
		<i>Stenacron</i>	4	4	-	-	-	-	R
		<i>Epeorus</i>	0	2	C	A	C	-	A
	Leptophlebiidae	<i>Habrophleboides</i>	4	2	R	R	R	R	R
		<i>Paraleptophlebia</i>	4	2	-	-	-	C	-
	Ephemerellidae	<i>Ephemerella</i>	1	3	A	A	A	A	A
		<i>Seratella</i>	2	3	A	R	A	-	C
		<i>Eurylophella</i>	4	3	A	A	A	C	C
	Oligoneuridae	<i>Isonychia</i>	3	3	A	-	A	-	A
	Baetidae	<i>Baetis</i>	6	4	C	C	C	A	A
		<i>Acerpenna</i>	6	3	C	R	C	A	A
	Caenidae	<i>Caenis</i>	7	5	R	R	R	-	R

	Siphonuridae	<i>Ameletus</i>	0	2	C	C	R	R	R
	Neophemeridae	<i>Neophemera</i>	3	NA	-	-	R	-	-
Diptera	Chironomidae		6	5	A	A	A	A	A
	Tipulidae	<i>Antocha</i>	6	5	R	R	C	R	R
		<i>Tipula</i>	3	4	R	C	R	R	R
		<i>Hexatoma</i>	4	5	-	C	-	-	-
		<i>Pseudolimnophila</i>	2	4	-	R	-	-	-
	Simuliidae	<i>Simulium</i>	2	3	C	R	A	C	C
		<i>Prosimulium</i>	6	5	C	A	C	C	R
	Empididae	<i>Hemerodromia</i>	2	3	R	R	R	R	-
		<i>Clinocera</i>	6	4	-	R	R	-	R
		<i>Chelifera</i>	6	4	-	-	-	R	-
	Athericidae	<i>Atherix</i>	2	3	-	-	R	-	R
	Dixidae	<i>Dixella</i>	1	2	R	-	-	-	-
	Chaoboridae		6	NA	R	-	-	-	-
	Tabanidae	<i>Hybomitra</i>	6	5	R	R	-	-	-
		<i>Chrysops</i>	7	5	-	-	-	-	-
	Blephariceridae	<i>Blepharicera</i>	0	1	-	-	-	-	R
Coleoptera	Dryopidae	<i>Helichus</i>	5	4	R		R	-	-
	Elmidae	<i>Stenelmis</i>	5	5	C	R	C	C	A
		<i>Optioservus</i>	4	4	A	C	A	A	A
		<i>Dubiraphia</i>	6	4	A	R	R	R	C
		<i>Microcylloepus</i>	2	4	C	-	R	C	R
		<i>Macronychus</i>	2	4	R	-	C	R	-
		<i>Promoresia</i>	2	3	-	-	-	R	-
		<i>Oulimnius</i>	5	3	-	-	R	-	R
		<i>Acyronyx</i>	2	4	R	-	-	-	-
	Psephenidae	<i>Psephenus</i>	4	4	A	A	C	R	C
	Haliplidae	<i>Peltodytes</i>	5	NA	R	-	R	-	-
	Hydrophilidae	<i>Tropisternus</i>	5	NA	-	-	R	R	-
		<i>Laccobius</i>	5	NA	R	-	-	-	-

Odonata	Aeshnidae	<i>Boyeria</i>	2	3	R	-	R	R	C
		<i>Basiaeschna</i>	2	4	-	-	R	-	-
		Gomphidae	<i>Ophiogomphus</i>	1	3	-	R	R	R
	<i>Argiogomphus</i>		4	4	-	C	R	-	-
	Coenagrionidae	<i>Lanthus</i>	5	2	-	R	-	-	-
		<i>Gomphus</i>	5	4	-	R	-	-	-
		<i>Argia</i>	6	4	C	-	R	-	R
		<i>Nehallenia</i>	7	4	R	-	-	R	-
		<i>Anomalagrion</i>	4	4	-	-	-	-	-
	Calopterygidae	<i>Enallagma</i>	8	4	R	-	R	-	-
		<i>Chromagrion</i>	4	4	R	-	-	-	-
		<i>Hetaerina</i>	6	4	R	R	-	R	R
		<i>Calopteryx</i>	6	4	R	-	-	R	-
	Macromiidae	<i>Macromia</i>	2	4	-	-	R	-	-
	Megaloptera	Corydalidae	<i>Nigronia</i>	2	3	-	R	R	R
<i>Corydalis</i>			4	4	R	R	R	-	R
Sialidae		<i>Sialis</i>	6	5	-	R	-	-	--
Lepidoptera	Pyralidae	<i>Petrophila</i>	5	5	R	-	R	-	-
Hemiptera	Veliidae	<i>Microvelia</i>	9	NA	R	-	-	-	R
		<i>Rhagovelia</i>	9	NA	R	-	-	-	-
Collembola	Gerridae	<i>Gerris</i>	9	NA	R	-	-	-	-
	Isotomatidae	<i>Agrenia</i>	9	NA	-	R	-	R	-
Crustacea	Amphipoda	Gammaridae	<i>Gammarus</i>	4	4	A	R	A	A
	Isopoda	Asellidae	<i>Caecidotea</i>	6	5	C	-	R	R
	Decapoda	Camaridae	<i>Cambarus</i>	6	4	-	R	-	-
Acari	Acariformes	Hydrachnidia	7	4	C	R	R	C	R
Mollusca	Gastropoda	Planorbidae	6	5	R	R	-	-	-
		Vivaparidae	7	4	R	-	-	-	-

		Valvatidae		2	4	R	-	-	-	-
		Physidae		8	5	R	R	-	-	-
	Bivalvia	Sphaeriidae	<i>Pisidium</i>	8	NA	R	-	-	-	R
			<i>Musculium</i>	8	NA	-	-	-	1	-
Platyhelminthes	Tricladida	Planariidae		9	5	C	-	C	-	-
Nematomorpha				9	NA	R	-	R	-	R
Oligochaeta				10	5	R	C	A	A	C

recommended 200 organism subsample. These standardized subsamples provide the final metrics for the results presented in the sections below.

For the December sampling period, 83 distinct taxa representing 14 orders were identified at one or more of the stations (Table C1-C3, Appendix C). Taxa richness ranged from a low of 21 at Red Bridge to a high of 34 at Brunswick, averaging 29 across all five sites (Table 5). Sensitive taxa at each site ranged from a low of 11 at Red Bridge to a high of 27 at Slifer Valley, with an average of 16. Each of the sites had a high number of sensitive EPT taxa, ranging from a low of 11 at Red Bridge to a high of 17 at Slifer Valley with an average of 14. The relative percentage of sensitive organisms ranged from a low of 39 at Silver Creek to a high of 81 at Red Bridge with an average of 57%. Diversity ranged from a low of 1.79 at Red Bridge to a high of 2.98 at Kunsman, with an average of 2.45. Hilsenhoff biotic index ranged from a low of 1.81 at Red Bridge to a high of 3.54 at Kunsman, with an average of 2.83. BCG Attributes for small streams were calculated for each site, and in three cases the minimum ratio of 0.75 was not met (Kunsman, Brunswick and Silver Creek), however in no case were both ratios below the minimum of 0.75. The Pennsylvania Freestone IBI was calculated for each sample, and they ranged from a low of 64 at Silver Creek to a high of 83 at Slifer Valley.

Table 5. Summary of Results for December 2018 Benthic Assessment

	ST-1	ST-2	ST-3	ST-4	ST-5
	Kunsman	Slifer Valley	Brunswick	Silver Creek	Red Bridge
Taxa Richness	32	32	34	25	21
Modified EPT	14	17	14	12	11
Beck's Version 3	14	27	15	15	11
Shannon's Diversity	2.98	2.59	2.60	2.28	1.79
Hilsenhoff Biotic Index	3.54	2.82	2.69	3.3	1.81
Percent Sensitive Organisms	41	54	62	39	81
BCG attribute ratio of taxa	0.60	1.46	0.74	0.79	1.10
BCG attribute ratio of individuals	0.75	3.93	2.12	0.66	4.48
PA Freestone IBI	73	83	78	64	68

October 2019

The second round of samples was collected in October, 2019. Kick and jab samples were composited in the field. 79 distinct taxa representing 18 orders were identified at one or more of the stations (Table C4-C6, Appendix C). Richness ranged from a low of 24 at Silver Creek to a high of 34 at Red Bridge and Brunswick, averaging 30 across all five sites (Table 6). Sensitive taxa at each site ranged from a low of 12 at Silver Creek to a high of 28 at Red Bridge, with an average of 18. Each of the sites had a high number of sensitive EPT taxa, ranging from a low of 9 at Silver Creek to a high of 17 at Red Bridge, with an average of 12.

The relative percentage of sensitive organisms ranged from a low of 12% at Silver Creek to a high of 53% at Red Bridge with an average of 23%. Diversity was good across all sites, ranging from a low of 2.47 at Silver Creek to a high of 2.81 at Brunswick, with an average of 2.64. Hilsenhoff biotic index ranged from a low of 2.92 at Red Bridge to a high of 4.60 at Brunswick, with an average of 3.97. BCG Attributes for small streams were calculated for each site, and in all cases except Red Bridge at least one of the ratios was less than the target of 0.75. In the case of Kunsman, Brunswick and Silver Creek both ratios were below the target of 0.75. The Pennsylvania Freestone IBI was calculated for each sample, and they ranged from a low of 54 at Silver Creek to a high of 84 at Red Bridge.

Table 6. Summary of Results for October 2019 Benthic Assessment

	ST-1	ST-2	ST-3	ST-4	ST-5
	Kunsman	Slifer Valley	Brunswick	Silver Creek	Red Bridge
Taxa Richness	31	27	34	24	34
Modified EPT	10	11	12	9	17
Beck's Version 3	13	19	16	12	28
Shannon's Diversity	2.72	2.53	2.81	2.47	2.67
Hilsenhoff Biotic Index	4.16	4.31	4.60	4.47	2.92
Percent Sensitive Organisms	20	12	16	14	53
BCG attribute ratio of taxa	0.50	0.86	0.74	0.71	1.13
BCG attribute ratio of individuals	0.26	0.23	0.22	0.23	1.19
PA Freestone IBI	62	60	65	54	84

April 2020

The third round of samples was collected in April, 2020. Kick and jab samples were composited in the field. 71 distinct taxa representing 15 orders were identified at one or more of the stations (Table C7-C9, Appendix C). Richness ranged from a low of 25 at Slifer Valley to a high of 38 at Kunsman, averaging 34 across all five sites (Table 7). Sensitive taxa at each site ranged from a low of 16 at Kunsman to a high of 25 at Slifer Valley and Silver Creek, with an average of 21. Each of the sites had a high number of sensitive EPT taxa, ranging from a low of 13 at Kunsman to a high of 16 at Silver Creek, with an average of 15. The relative abundance of sensitive individuals ranged from a low of 23% at Silver Creek to a high of 61% at Red Bridge with an average of 49%. Diversity was good across all sites, ranging from a low of 2.59 at Silver Creek to a high of 3.11 at Kunsman, with an average of 2.76. Hilsenhoff biotic index ranged from a low of 3.09 at Slifer Valley to a high of 4.06 at Brunswick, with an average of 2.74. BCG Attributes for small streams were calculated for each site, and in all cases either the ratio of sensitive to tolerant taxa or the ratio of sensitive to tolerant individuals (or both) was greater than 0.75. The Pennsylvania Freestone IBI was calculated for each sample, and they ranged from a low of 73 at Silver Creek and Red Bridge to a high of 81 at Brunswick. 1 Based on these results, all sites except Silver Creek were classified as attaining for aquatic life use, cold water fishery, and Exceptional Value.

Table 7. Summary of Results for April 2020 Benthic Assessment

	ST-1	ST-2	ST-3	ST-4	ST-5
	Kunsmann	Slifer Valley	Brunswick	Silver Creek	Red Bridge
Taxa Richness	38	25	35	37	36
Modified EPT	13	14	15	16	15
Beck's Version 3	16	25	23	25	17
Shannon's Diversity	3.11	2.60	2.85	2.59	2.67
Hilsenhoff Biotic Index	3.70	3.09	3.42	4.06	3.48
Percent Sensitive Organisms	47	60	54	23	61
BCG attribute ratio of taxa	0.80	1.5	1.13	0.94	0.89
BCG attribute ratio of individuals	1.13	1.91	1.41	0.46	2.06
PA Freestone IBI	74	78	81	73	73

August 2020

The fourth and final round of samples was collected in late July and early August, 2020. Kick and Jab samples were composited in the field. 79 distinct taxa representing 15 orders were identified at one or more of the stations (Table C10-C12, Appendix C). Richness ranged from a low of 30 at Silver Creek to a high of 35 at Kunsmann, averaging 33 across all five sites (Table 8). Sensitive taxa at each site ranged from a low of 12 at Kunsmann to a high of 31 at Slifer Valley, with an average of 20. Each of the sites had a high number of sensitive EPT taxa, ranging from a low of 10 at Kunsmann to a high of 15 at Red Bridge, with an average of 12. The relative percentage of sensitive individuals ranged from a low of 11 at Silver Creek to a high of 55 at Red Bridge with an average of 34%. Diversity was good across all sites, ranging from a low of 2.34 at Silver Creek to a high of 2.93 at Kunsmann, with an average of 2.73. Hilsenhoff biotic index ranged from a low of 2.46 at Slifer Valley to a high of 4.40 at Silver Creek, with an average of 3.70. BCG Attributes for small streams were calculated for each site, and in six cases the ratios of sensitive to tolerant taxa and individuals was less than the target of 0.75 (only Red Bridge did not have at least one ratio below target). In the case of Kunsmann and Silver Creek both ratios were less than the target of 0.75. The Pennsylvania Freestone IBI was calculated for each sample, and they ranged from a low of 59 at Silver Creek to a high of 83 at Red Bridge.

Table 8. Summary of Results for August 2020 Benthic Assessment

	ST-1	ST-2	ST-3	ST-4	ST-5
	Kunsmann	Slifer Valley	Brunswick	Silver Creek	Red Bridge
Taxa Richness	35	34	32	30	32
Modified EPT	10	13	12	11	15
Beck's Version 3	12	31	15	16	25
Shannon's Diversity	2.93	2.76	2.85	2.34	2.75
Hilsenhoff Biotic Index	4.13	3.35	4.15	4.40	2.46
Percent Sensitive Organisms	33	35	34	11	55
BCG attribute ratio of taxa	0.43	1.00	0.78	0.65	1.07
BCG attribute ratio of individuals	0.50	0.58	0.57	0.14	1.22
PA Freestone IBI	66	78	69	59	83

Quality Assurance Tests

Several tests were performed to evaluate the sampling and subsampling procedures. The questions to be addressed were:

1. Are laboratory subsampling efforts repeatable?
2. Does keeping the kick and jab samples separate provide any additional value?
3. Can kick and jab samples be composited mathematically?
4. Is it necessary to reduce the size of subsamples with more than 240 individuals?

To address these questions, the following tests were done:

1. Several subsamples were taken in duplicate and the results compared.
2. Kick and Jab samples were processed separately during the December 2018 sampling effort and the results compared.
3. Kick and Jab samples were composited mathematically to produce a third set of data for bioassessment and the results of all three compared.
4. Several samples were assessed before and after reducing the counts to 200+/- 40 as per PADEP protocol.

The results of these efforts produced the following:

Duplicate samples were taken in two ways; either by taking a second random subsample from the sorting tray, or by taking a second random subsample from a picked subsample that had more than twice the requisite number of individuals. Regardless of the technique used, while the duplicates did differ slightly for individual metrics (see appendix D), the ALU determinations were the same.

There appears to be no consistent trend between the kick and job samples across the five sites. In some cases, the job samples were better than the kick, but at other sites there was either no appreciable difference in community structure, or the kick was better than the job. This might argue to eliminate one or the other method, but because different organisms were present in the kick and the job at each site, it was decided that future collections would composite kick and job samples. See Appendix D.

Reducing the size of the subsamples into the range of 200 +/- 40 does appear to change the metric values, but not the conclusions. However, all subsamples with more than 240 organisms were refloats and rekeyed to ensure comparability of results across sampling periods. Comparison tables are provided in Appendix D. The adjusted results are reported here.

The results of QA tests and calculations are presented in Appendix D.

Aquatic Life Use Assessment by Station

Kunsman (Headwaters South), ST-1

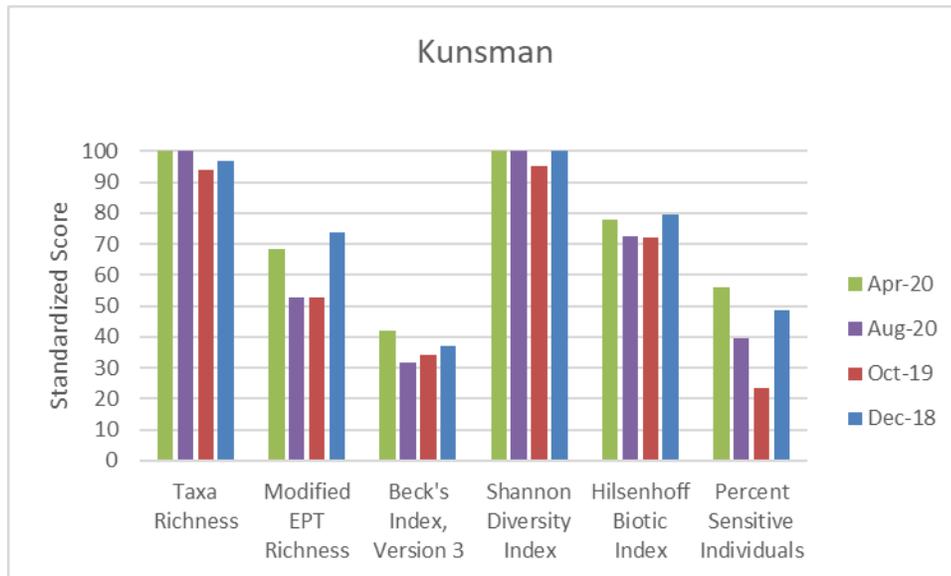


Figure 2. Standardized bioassessment scores from Kunsman Site, ST-1 Pleasant Valley December 2018 – August 2020.

Table 9. Aquatic Life Use Assessment of Kunsman Site, ST-1, Pleasant Valley.

Metric	Spring	Summer	Fall	Winter
PA Freestone IBI Score	74	66	62	73
Caddisflies, Mayflies, Stoneflies Present?	Yes	No	Yes	Yes
Becks Index Standardized Score \geq 33.3?	Yes	No	Yes	Yes
% Sensitive Individuals Score \geq 25?	Yes	Yes	No	Yes
BCG attribute ratio of taxa \geq 0.75?	Yes	No	No	No
BCG attribute ratio of individuals \geq 0.75?	Yes	No	No	Yes
Aquatic Life Use Assessment	Attaining	Marginally Attaining	Marginally Impaired	Attaining

The community metrics for all four sampling periods were standardized according to PADEP guidelines (Figure 2) and used to calculate the PA Freestone Index of Biotic Integrity. The Freestone IBI for the four sampling periods ranged from a low of 62 in October to a high of 74 in mid-April (Table 9). Three out of four IBI scores exceed the minimum Aquatic Life Use standard of 63 recommended by PADEP for Exceptional Value watersheds and were less than 11 points lower than the spring baseline. However, during the summer and fall months the community appears to be stressed due to a reduction in the number of sensitive taxa and individuals. Of particular note is the failure to meet the Biological Condition Gradient (BCG) attribute during summer and fall. The relative abundance of sensitive taxa does not fully recover until the spring.

The severe bank erosion, presence of large amounts of bedrock outcropping and lack of canopy cover are reflective in the relatively low habitat score at this site (Table 1) and this certainly contributes to stress which may result in loss of stoneflies, an increase in tolerant individuals and a decrease in heat intolerant taxa during the low flow periods of summer and fall. This is further evidenced by the fact that winter and spring sampling periods are fully attaining the Aquatic Life Use standard; this indicates that the system does recover when temperatures and flow are moderate. On average, this site shows the lowest percentage of sensitive EPT taxa of all five sampling locations. While this may be a result of less than ideal habitat, additional water quality monitoring is warranted in this catchment, to further

evaluate this result. Efforts should be undertaken to stabilize the steep bank and reduce downstream sedimentation, which may result in an increase in sensitive taxa. Given this, we consider the Kunsman site to be marginally Attaining the ALU standard.

Slifer Valley (Headwaters East), ST-2

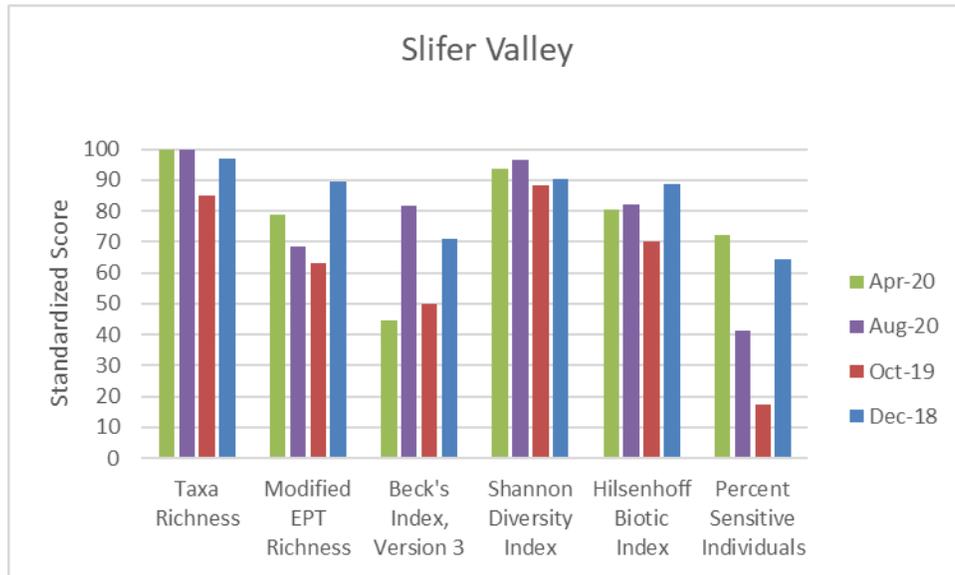


Figure 3. Standardized bioassessment scores from Slifer Valley Site, ST-2, Springfield Township December 2018 – August 2020.

Table 10. Aquatic Life Use Assessment of the Slifer Valley Site, ST-2, Springfield Township.

Metric	Spring	Summer	Fall	Winter
PA Freestone IBI Score	78	78	60	83
Caddisflies, Mayflies, Stoneflies Present?	Yes	Yes	Yes	Yes
Becks Index Standardized Score ≥ 33.3 ?	Yes	Yes	Yes	Yes
% Sensitive Individuals Score ≥ 25 ?	Yes	Yes	No	Yes
BCG attribute ratio of taxa ≥ 0.75 ?	Yes	Yes	Yes	Yes
BCG attribute ratio of individuals ≥ 0.75 ?	Yes	No	No	Yes
Aquatic Life Use Assessment	Attaining	Attaining	Marginally Impaired	Attaining

The community metrics for all four sampling periods were standardized according to PADEP guidelines (Figure 3) and used to calculate the PA Freestone Index of Biotic Integrity. The Freestone IBI for all four sampling periods ranged from a low of 60 in October to a high of 83 in December (Table 10). Three out of four IBI scores exceed the minimum Aquatic Life Use standard of 63 recommended by PADEP for Exceptional Value watersheds, however the October sampling period was more than 11 points lower than the spring baseline. In addition, the community present in the fall was lower in sensitive individuals. This is not unexpected during the low flow fall periods, which is why PADEP does not recommend sampling Exceptional Value watersheds during the fall. Because of this, it is likely that the Slifer Valley site is achieving the Aquatic Life Use standard.

Brunswick, ST-3

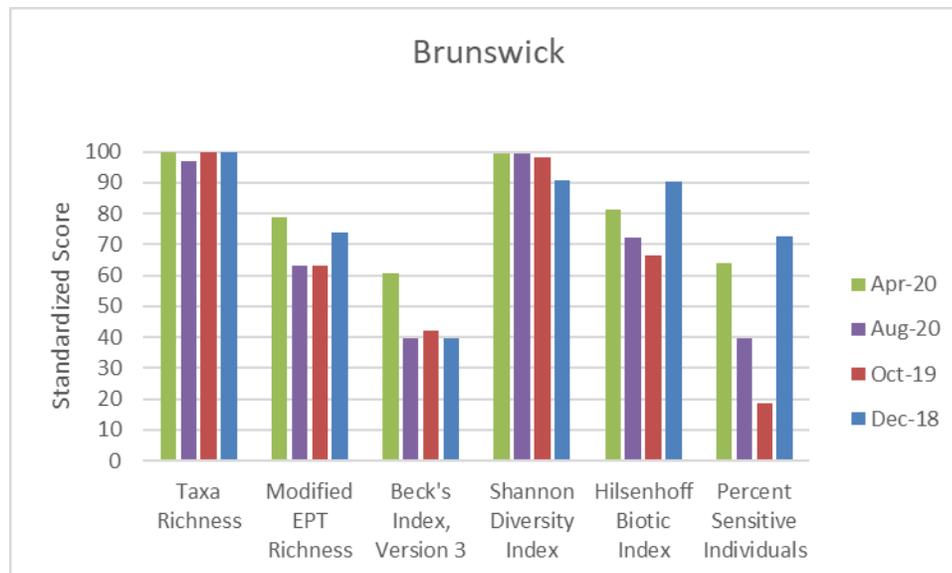


Figure 4. Standardized bioassessment scores from Brunswick Site, ST-3, Springtown December 2018 – August 2020.

Table 11. Aquatic Life Use Assessment of Brunswick Site, ST-3, Springtown.

Metric	Spring	Summer	Fall	Winter
PA Freestone IBI Score	81	69	65	78
Caddisflies, Mayflies, Stoneflies Present?	Yes	Yes	Yes	Yes
Becks Index Standardized Score ≥ 33.3 ?	Yes	Yes	Yes	Yes
% Sensitive Individuals Score ≥ 25 ?	Yes	Yes	No	Yes
BCG attribute ratio of taxa ≥ 0.75 ?	Yes	Yes	No	No
BCG attribute ratio of individuals ≥ 0.75 ?	Yes	No	No	Yes
Aquatic Life Use Assessment	Attaining	Marginally Attaining	Marginally Attaining	Attaining

The community metrics for all four sampling periods were standardized according to PADEP guidelines (Figure 4) and used to calculate the PA Freestone Index of Biotic Integrity. The Freestone IBI for all four sampling periods ranged from a low of 65 in August to a high of 81 in April (Table 11). All four IBI scores exceed the minimum Aquatic Life Use standard of 63 recommended by PADEP for Exceptional Value watersheds. However, note that the IBI score in the summer and fall months was more than 11 points lower than the spring baseline of 81. This may indicate stress during the summer months. While PADEP does not recommend sampling Exceptional Value streams during the summer and early fall, the ALU standard for these months in other streams is relaxed to 43 but requires the interpretation of relative abundance and diversity of sensitive taxa.

During the summer and fall, the community does not lose caddisflies, mayflies or stoneflies, however the percentage of sensitive individuals present does drop below 25% in the fall. In addition, the ratio of BCG sensitive to tolerant taxa and individuals drops as well, with fewer sensitive individuals in the summer, and fewer individuals and taxa in the fall. The number of sensitive taxa does not recover until spring. The reason for this observation is unclear. The habitat at this site is good, with excellent buffers and canopy cover. However, water quality monitoring in the watershed does indicate that this catchment area does exhibit higher water temperatures than desired during the summer months. Despite this issue, it is likely that the Brunswick site is achieving the Aquatic Life Use standard.

Silver Creek, ST-4

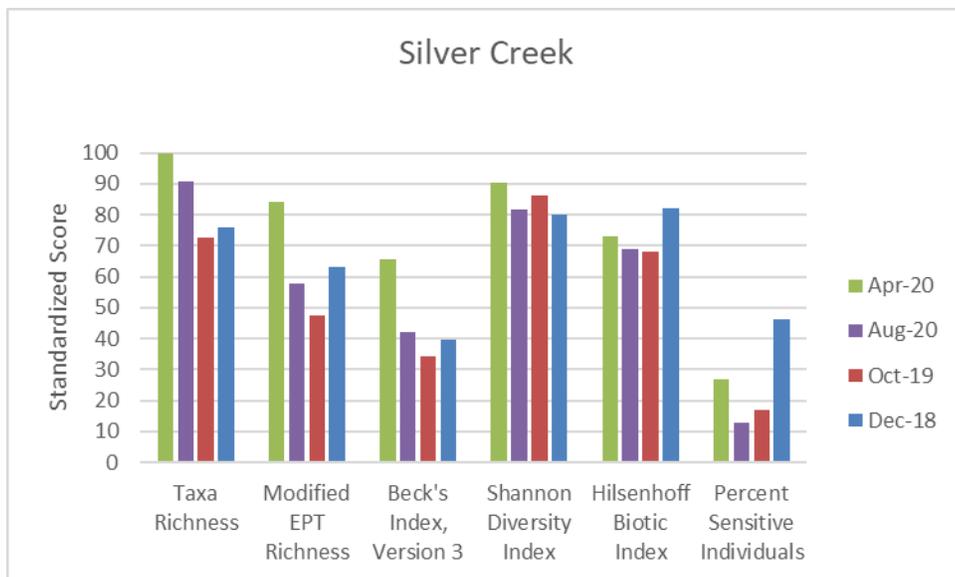


Figure 5. Standardized bioassessment scores from Silver Creek Site, ST-4, Springtown December 2018 – August 2020.

Table 12. Aquatic Life Use Assessment of Silver Creek Site, ST-4, Springtown.

Metric	Spring	Summer	Fall	Winter
PA Freestone IBI Score	73	59	54	64
Caddisflies, Mayflies, Stoneflies Present?	Yes	Yes	Yes	Yes
Becks Index Standardized Score ≥ 33.3 ?	Yes	Yes	No	Yes
% Sensitive Individuals Score ≥ 25 ?	Yes	No	No	Yes
BCG attribute ratio of taxa > 0.75 ?	Yes	No	No	Yes
BCG attribute ratio of individuals > 0.75 ?	No	No	No	No
Aquatic Life Use Assessment	Attaining	Marginally Impaired	Marginally Impaired	Attaining

The community metrics for all four sampling periods were standardized according to PADEP guidelines (Figure 5) and used to calculate the PA Freestone Index of Biotic Integrity. The Freestone IBI for all four sampling periods ranged from a low of 54 in October to a high of 73 in April (Table 12). In two cases the IBI scores failed to achieve the minimum Aquatic Life Use standard of 63 recommended by PADEP for Exceptional Value watersheds. The scores for these two sampling periods are also more than 11 points lower than the spring baseline of 73. If one relaxes the ALU standard to 43, which is recommended for most streams during summer and fall (PADEP, 2012), then the standards are met, but further evaluation of relative abundance and diversity of sensitive taxa is necessary. While all three important water quality indicator groups of mayflies, stoneflies and caddisflies were present during both sampling periods, the percentage of sensitive individuals as well as the Beck's index (fall only) were lower than needed to achieve the ALU standard. The ratio of sensitive to tolerant taxa and the ratio of sensitive to tolerant individuals based on BCG attributes also failed to achieve 0.75 both in summer and fall (Table 10). This indicates that the community in this region is stressed at least part of the year.

While the habitat quality at this sampling site was relatively high, it is well documented that the water quality of the headwater tributaries draining the village of Springtown contain high nitrate and occasional high bacteria counts due to underperforming poor septic systems (cite Rivers Conservation Plan). There is also a trout hatchery that discharges untreated effluent directly to Silver Creek. It is possible that during the low flow periods of summer and fall,

that there is insufficient dilution of these impacts and that there are localized temporary impacts to the benthic community. It should be noted that the benthic community at Silver Creek had the poorest average metric values across the five sampling locations for five out of seven metrics, which further illustrates that this catchment is experiencing unique stressors that the other catchments are not. In addition to the problems of nutrient input from septic systems, this drainage area is predominantly limestone, and likely is groundwater influenced, more so during low flow periods of summer and fall than in winter and spring. It is noted that the BCG attribute ratio for individuals is less than desired even in winter and spring, which may be further indication of water quality impacts from groundwater or nutrient pollution. We believe the catchment area for this site should continue to be monitored closely. It is likely that Silver Creek is only marginally Attaining the ALU standard.

Red Bridge, ST-5

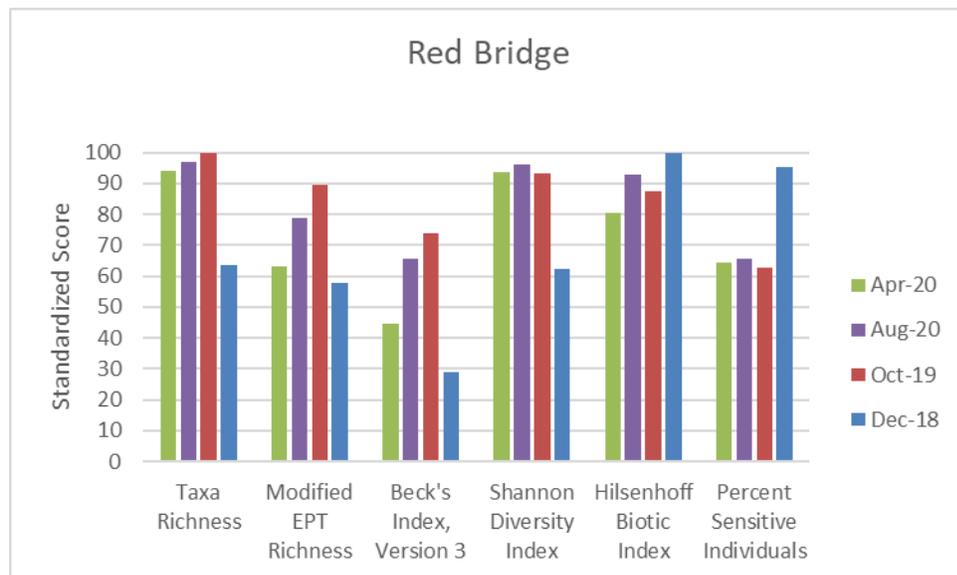


Figure 6. Standardized bioassessment scores from Red Bridge Site, ST-5, Durham December 2018 – August 2020.

Table 13. Aquatic Life Use Assessment of Red Bridge Site, ST-5, Durham.

Metric	Spring	Summer	Fall	Winter
PA Freestone IBI Score	73	83	84	68
Caddisflies, Mayflies, Stoneflies Present?	Yes	Yes	Yes	Yes
Becks Index Standardized Score \geq 33.3?	Yes	Yes	Yes	Yes
% Sensitive Individuals Score \geq 25?	Yes	Yes	Yes	Yes
BCG attribute ratio of taxa $>$ 0.75?	Yes	Yes	Yes	Yes
BCG attribute ratio of individuals $>$ 0.75?	Yes	Yes	Yes	Yes
Aquatic Life Use Assessment	Attaining	Attaining	Attaining	Attaining

The community metrics for all four sampling periods were standardized according to PADEP guidelines (Figure 6) and used to calculate the PA Freestone Index of Biotic Integrity. The Freestone IBI for all four sampling periods ranged from a low of 68 in December to a high of 84 in October (Table 13). All four IBI scores exceed the minimum Aquatic Life Use standard of 63 recommended by PADEP for Exceptional Value watersheds, and none were less than 11 points lower than the spring baseline. All additional metrics were well above the standards. Based on this, we consider the Red Bridge site to be Attaining the ALU standard.

Overall Assessment of the Cooks Creek Watershed

Over the course of the two-year study period, IBI scores ranged from a low of 54 to a high of 84 at the five sites in all seasons (Figure 7). The benthic communities at four of the five sites, excluding Red Bridge, may be experiencing stress from transitory water quality impacts as a result of nutrient pollution, thermal pollution and/or stormwater impacts during the summer and early fall. This is particularly true of the Kunsman and Silver Creek sites which showed some of the lowest community metric scores in this study (Table 14). It is recommended that additional, more targeted sampling be performed in these catchments in order to determine the nature and extent (and potential sources) of these impacts. On a positive note, these impacts are localized and temporary as witnessed by the fact that the scores improved in the winter and spring and that the Red Bridge site, which contains the other four sites in its catchment area, did not show similar impacts. If one only considers the mid-April and late December sampling periods (as recommended for Exceptional Value watersheds), then all five sites attained the Aquatic Life Use standard for Exceptional Value Watersheds. It is therefore reasonable to state that the Cooks Creek Watershed is attaining the PA Aquatic Life Use standard for an Exceptional Value watershed.

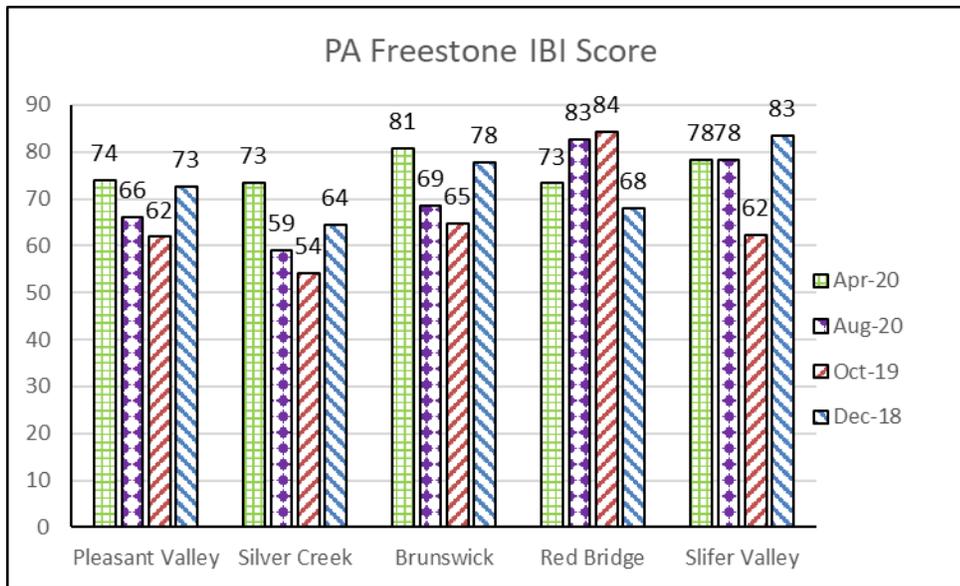


Figure 7. Pennsylvania Freestone Index of Biotic Integrity for Stations in Cooks Creek Watershed Dec 2018 – August 2020.

Table 14. Summary of Benthic Community Assessment metrics for various locations within the Cooks Creek Watershed for the period of Dec 2018 –August 2020.

	Kunsmann	Slifer Valley	Brunswick	Silver Creek	Red Bridge	Average
Taxa Richness	34	30	34	29	31	31
Modified EPT	12	14	13	12	15	13
Beck’s Version 3	14	26	17	17	20	19
Shannon’s Diversity	2.94	2.62	2.78	2.42	2.47	2.64
Hilsenhoff Biotic Index	3.88	3.39	3.71	4.06	2.67	3.54
Percent Sensitive Organisms	35	41	41	22	62	40
PA Freestone IBI	69	76	73	63	77	71

V. SOURCE OF DOCUMENTATION

Copies of all raw data are provided in the appendix.

All original documentation will be stored for a maximum of 5 years at:

Symbiosis Environmental
 3450 Route 212
 Riegelsville, PA 18077

A P P E N D I X

APPENDIX A

Sampling Site

Descriptions

APPENDIX B

Habitat Assessments

Appendix C

Laboratory Benchsheets

and Calculations