STREAM BIOLOGICAL ASSESSMENT FOR CHICORY LANE FARM (SPRING 2022)

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Clockwise from upper left: UNT-2 upstream view, <u>Acroneuria</u> stonefly nymph, <u>Tipula</u> crane fly larvae, close-up of riffle habitat, Aeshnidae dragonfly larvae.

Introduction: During the 2022 annual monitoring visit of the conservation easement on Chicory Lane Farm, John Smith discussed his goals with ClearWater staff for enhancing the native riparian ecosystem along the existing unnamed tributaries (UNTs) flowing through the property, as well as his concerns regarding timber harvesting upstream on a neighboring property and the possible effects of this activity on stream health.

Staff Ecologist Suzy Yetter suggested conducting a biological assessment and coarse inventory of the aquatic macroinvertebrate community of the streams flowing through the property as an initial means of meeting these goals. This information can be quite valuable in advancing natural resource objectives, including 1) supporting goals of the property's CAP 106 Forest Management Plan, such as promoting ecological diversity and enhancing ecosystem function, and 2) providing a baseline for comparison to identify natural and/or anthropogenic changes to stream condition. The following is a summary of the methods and results of macroinvertebrate data collected in Spring 2022.

Study Area: The small streams flowing through Chicory Lane Farm drain into Penns Creek and eventually into the Susquehanna River, which is the largest contributor of water and sediment to the Chesapeake Bay. The Pennsylvania Department of Environmental Protection (PA DEP) Stream Designated Use is Cold Water Fishes (CWF), meaning the primary aquatic life use of the streams is to provide habitat for cold water fish species including salmonids (e.g., brook and brown trout). Three sites along two UNTs were identified for future biological assessments: Site 1 occurs upstream on UNT-1; Site 2 is located on UNT-2; and Site 3 is located on UNT-1 downstream of the confluence with UNT-2 (Figure 1).

Methods Summary: Field methods consisted of collecting a sample in riffle habitats using a kick net, transferring the contents to containers, and transporting them back to the lab for sorting and identification. Samples were processed in two phases: 1) a 200-count subsample to determine stream condition; and 2) a timed search of the rest of the sample to obtain a more accurate inventory of the taxa* present. The subsample results were then used to characterize the macroinvertebrate community composition (i.e., richness, diversity, pollution-tolerance, etc.) and calculate specific stream condition measures or 'metrics' to produce an integrity score ranging from 0 to 100, with 100 representing the best attainable condition. The timed search results were then combined with the subsample results to create a more comprehensive taxa list for interpreting ecological results. Refer to the Appendix for additional details on the methods.

* The term 'taxa' (plural) or 'taxon' (singular) is used in place of 'species,' in order to account for differences in taxonomic resolution for identified specimens. Insufficient information exists for most aquatic macroinvertebrates to identify larvae to species level. Hence, the term 'taxa' refers to the lowest identified taxonomic unit representing a distinct type of macroinvertebrate organism.

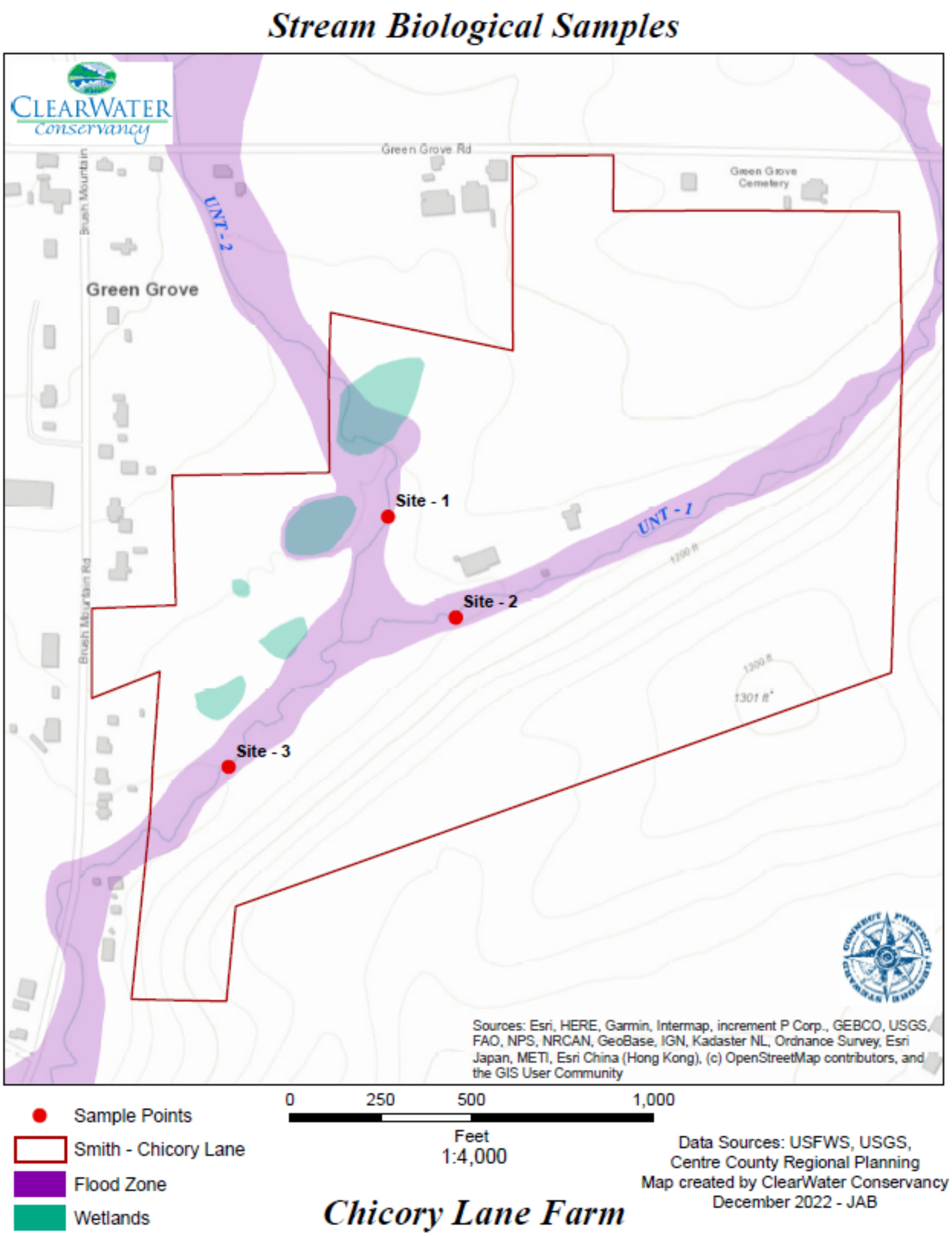
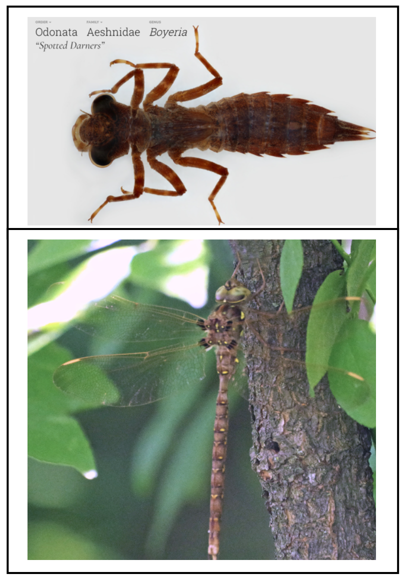


Figure 1. Locations of planned stream sampling locations along unnamed tributaries (UNTs) at Chicory Lane Farm. 2022 samples were collected and results analyzed at Site 1 along UNT-2.

Results Summary: Overall, UNT-2 is in good biological condition and provides excellent instream habitat to support a very diverse group of aquatic macroinvertebrates. A total of 212 individuals were obtained through the subsampling procedure and used to calculate the IBI results. This subsample represented about 15% of the total sample collected; thus, extrapolation suggests ~1413 individuals were most likely collected overall. Subsample results revealed a total of 30 different taxa and 19 different mayfly, stonefly, and caddisfly (EPT) taxa. Over 84% of the individuals collected were pollution-sensitive taxa, and organic enrichment was not detected. Evaluation of the rest of the sample increased diversity to 37 taxa and 23 EPT taxa. The total IBI score was 90.3, which indicates excellent condition. Based on these results we conclude that UNT-2 is ecologically intact and functioning as native, natural habitat to support a healthy macroinvertebrate community consisting substantially of EPT taxa and other sensitive, rare species. Refer to the Appendix for specific details on the subsample and comprehensive sample results, including lists of taxa collected and specific IBI scores.

Following are descriptions of some of the taxa collected to provide a more ecological interpretation of these results.



Boyeria vinosa (Fawn Darner)—Unlike most dragonflies from the family Aeshnidae, larvae of *Boyeria* occur in small, slowly flowing woodland streams, where they hide under rocks, leaves and submerged wood, feeding on mayflies, caddisflies and other invertebrates or even small fish. Adults prefer shady areas where males patrol the stream edges just before dark, weaving in and out along the bank, often approaching people wading in the stream. Females prefer to deposit eggs on wet wood, either submerged or floating on the water's surface. Here is a link to an interesting website: [Slide 1 (dragonfliesnva.com)](dragonfliesnva.com).

Ephemerella (Spiny Crawler Mayflies)—Larvae occur in all sizes of flowing waters, on a variety of substrates and water currents. This was the most abundant type of macroinvertebrate collected at Chicory Lane UNT-2. As their name implies, they are better at crawling onto rather than clinging to substrate and, as such, they tend to seek protection in rock crevices, woody debris, and various types of vegetation, especially mossy rock substrates like those found in the riffles at Chicory Lane. They are rather intolerant to pollution and represent some of the most famous and popular fly-fishing species in the east (e.g., Sulphur Dun).

Acroneuria (Common Stonefly)—This genus of carnivorous stoneflies can be quite common in clear, cold, forested streams ranging from small headwaters to medium-sized creeks. Larvae are usually found in riffles under large stones or accumulations of leaves and organic debris. They are often crawling through leaf packs in search of prey. Very agile and fast-moving crawlers, common stoneflies breathe through a combination of subcutaneous respiration (through the 'skin') and thoracic gills. This requires fast moving water to obtain sufficient oxygen, and when removed and placed into still waters, they create currents over their gills by raising and lowering their legs, as if they were doing push-ups. Most species require at least 2 years to complete their larval growth and are very sensitive to organic pollution.

Pteronarcys Allonarcys (Giant Stoneflies)—This genus has two subgenera in PA (*P. Allonarcys* and *P. Pteronarcys*), which are very similar appearance. Like *Acroneuria*, they are highly sensitive to pollution and occur in swift-flowing forested headwater streams. Although they are the biggest stonefly genera, they are not predators but primarily shredders that break down leaf material. As such, they are usually found in leaf packs lodged in fast currents. They also require 2-3 years to develop before emerging as adults. There size makes them popular food for fish; fisherman often refer to them as 'salmonflies.'

Rhyacophila (Green Caddisflies)—These caddisflies are quite common in forested headwater streams with good water quality. Caddisflies are an extremely diverse order. The term 'caddis' refers to the habit of most caddisfly larvae of making 'homes' by gluing small pieces of stones, wood, and/or plant material together. *Rhyacophila*, however, belong to the group of free-living caddisflies that roam or drift through the current. They are adept clingers as evidenced by their large hook-like posterior claws. They have no gills and must rely on cold, fast-flowing water to absorb oxygen. These habitat needs combined with their plump, green bodies make them another favorite food for trout. They are the 'green sedges' of fly fishing.

Diplectrona (Net-spinning Caddis)—One of the most widespread and abundant families, the common netspinners (Hydropsychidae) are often associated with agricultural streams or other running waters experiencing the impacts of sediment and nutrient pollution. This particular genus, however, is actually quite intolerant and inhabits small, cool streams with good water quality. Net spinners construct web-like 'net' retreats in the cracks and crevices of rocks. These nets collect and filter food from the current; hence these larvae are known as collector-filterers, a very large and diverse functional feeding group that also includes black fly larvae. Different species construct different-sized mesh nets, allowing them to feed on different food sources and coexist together in the same riffle.

Blepharicera (Net-winged Midges)—This is a small but highly specialized family of Diptera (flies) possessing extreme morphological adaptations for living in the cascades and rapids of mountain streams. The most obvious are adhesive discs on the undersides of the abdomen that function as hydraulic suckers. The also have specialized mouthparts for scraping the thin film of algae, bacteria and other organic matter from rock substrates. Blephariceridids are relatively rare and are not often collected in stream samples. This combined with their habitat preferences for clean, cool, well-oxygenated streams makes them good indicators of water quality.

Tipula & Hexatoma (Crane Flies)—Crane flies are a very diverse and species-rich family of >15,000 species, with *Tipula* representing perhaps the most common and diverse genus. Habitat ranges from stones and debris in swift riffle substrates to rich mud and decaying vegetation along stream and wetland edges and even accumulations of algal scum where only a trickle of water remains. Larvae can grow quite large (for a macroinvertebrate) with some species requiring two years to develop. Large spiracles on the end of the abdomen form an open breathing system that is typically surrounded by several tentacle-like lobes. *Hexatoma* are predators that enlarge the muscles at the end of their abdomen in order to wedge themselves between stones in a riffle to hold them in place as they catch and engulf their prey.

Appendix—Detailed Methods and Results:

Methods

Why use PA DEP protocols? Although these biological assessments for Chicory Lane are not meant for official use, following standardized protocols used by state regulatory agencies to determine biological condition and the presence or absence of impairment is quite useful, because it allows us to interpret and compare the results to similar streams throughout the Commonwealth. Specifically, Index of Biotic Integrity (IBI) scores can be calculated from the macroinvertebrate data collected and compared to IBI scores obtained by the Pennsylvania Department of Environmental Protection (PA DEP) for streams monitored throughout the state. Moreover, these protocols represent the most reliable and effective methods available for evaluating stream condition in the region.

Macroinvertebrate Field Sampling: At each site, benthic macroinvertebrate samples are collected following PA DEP protocols for wadeable, freestone, riffle/run habitats (PADEP 2018) where a 12-inch wide x 10-inch high, non-truncated D-frame net with a 500 micron mesh is placed against the stream bottom and a six-kick composite sample is obtained over a 100-meter reach length. All representative varieties of riffle habitats present (e.g., slower flowing, shallow riffles vs. faster flowing, deeper riffles) should be selected within the sampling reach. Approximately one-square meter of substrate immediately upstream of the net is disturbed to an approximate depth of 10 centimeters (as substrates allow) for one minute. This process is repeated six times with all sample material transferred to a sieve bucket (500-micron mesh), composited, and placed into a jar with 95% ethanol. Jars are labeled with site, date, time, and collector information

Additional Field Measures: Ideally, water chemistry will be taken prior to biological sampling, but this was not possible in 2022. The purpose is simply to ensure water quality measures are within normal range by evaluating the standard water quality suite (temperature (°C), pH (standard units), specific conductivity (µS/cm), and dissolved oxygen (mg/L). If biological condition results indicate impairment, parameters with measurements falling out of the normal range can help diagnose the probable source of impairment. If available, these measurements will be taken in the field using handheld meters (YSI Model 63 and Professional Series DO). Each site will also be evaluated for physical instream habitat conditions following PA DEP recommended methods (PADEP 2018). With this method, habitat quality is evaluated over the 100-meter reach by rating and scoring 12 key factors defining habitat criteria for fish and aquatic macroinvertebrates. Scoring for each factor ranges from 1-20 with a total maximum possible score of 240. Data collection forms are for riffle/run high gradient, wadeable, freestone streams and consisted of the following scored parameters: instream cover (fish), epifaunal substrate (macroinvertebrates), embeddedness, velocity/depth regimes, channel alteration, sediment deposition, frequency of riffles, channel flow status, condition of banks, bank vegetative protection, grazing or other disruptive pressures, and riparian vegetative zone width. Note that habitat assessments were not conducted in 2022, but based on observations taken during biological sampling, habitat condition is most likely in the optimal to sub-optimal condition category.

Macroinvertebrate Laboratory Processing and Data Analysis: Macroinvertebrate field samples are processed in the laboratory following PADEP (2018) protocols. This protocol specifies a sub-sample of at least 200 organisms (i.e., only a small portion of the sample is processed and analyzed). Macroinvertebrate samples are very time consuming to process, as organisms less than 500 microns are difficult to find amongst the debris. Subsampling or sorting a specific proportion of the entire contents collected is a common approach to dealing with this problem. Results for the IBI are calculated from the subsample results. While subsampling works very well for estimating biological condition, it tends to exclude many taxa by chance, resulting in underestimates of diversity and misinterpretations of ecological function. To help correct for this, a timed search was also implemented on the rest of the unsorted sample to retrieve any additional taxa and provide a more comprehensive taxonomic inventory.

Each composited sample is placed into a 3.5” deep rectangular plastic pan 18” long x 13” wide, marked off into (28) 2”x2” grid. Four of the grids are randomly selected and the contents extracted from within four-square inch circular “cookie cutters” placed in the randomly selected grids in the pan using spoons, knives, turkey basters (to remove the liquid contents) and other implements as needed. These extracted contents are then placed into a second pan with the same dimensions and gridding as the original pan. If more than the target 200±40 identifiable organisms are present in the second pan, the grids are randomly selected from the second pan and picked entirely until the target number of organisms (200±40, with 190 to 210 preferred) is reached. The total number of grids selected from each pan is recorded.

All identifiable organisms in the sub-sample are identified under magnification and counted. Taxonomic resolution is primarily to genus-level with the following exceptions: Chironomidae, snails, clams, and mussels (family level); roundworms and proboscis worms to phylum levels of Nematoda and Nemertea, respectively; flatworms and leeches to levels of Turbellaria and Hirudinea, respectively; segmented worms and tubificids to class level Oligochaeta; water mites identified as Hydracarina, an artificial taxonomic grouping.

Data from the 200 ± 40 count subsample are summarized to obtain metric calculations for the benthic index of biotic integrity for wadeable, freestone, riffle/run streams. The following six metrics were selected by PA DEP for inclusion as core metrics in the benthic IBI:

- **Total Taxa Richness** – A count of the total number of taxa in a sub-sample. Generally, this metric decreases with increasing anthropogenic stress, which is reflected in the loss of taxa and increased dominance of a few pollution-tolerant taxa.
- **EPT (Ephemeroptera, Plecoptera, Trichoptera) Taxa Richness (PTV 0 – 4 only)** – A count of the number of taxa belonging to the orders Ephemeroptera (mayflies), Plecoptera (stoneflies), and Trichoptera (caddisflies) in a sub-sample. This metric is expected to decrease in value with increasing anthropogenic stress, reflecting the loss of taxa from these largely pollution-sensitive orders. Most rare taxa collected are EPT taxa.
- **Beck's Index, version 3** – A taxonomic richness and tolerance metric represented as a weighted count of taxa with PTVs (pollution tolerance values) of 0, 1, or 2.This metric is expected to decrease in value with increasing anthropogenic stress and also reflects the loss of pollution-sensitive taxa.
- **Shannon Diversity** – A community composition metric measuring taxonomic richness and evenness of individuals across taxa of a sub-sample. This metric is expected to decrease in values with increasing anthropogenic stress.
- **Hilsenhoff Biotic Index (HBI)** – This community composition and tolerance metric is calculated as an average of the number of individuals in a sub-sample, weighted by PTVs. This metric generally increases with increasing anthropogenic stress, especially increasing organic enrichment.
- **Percent Sensitive Individuals (PTV 0 – 3**) **–** A community composition and tolerance metric representing the proportion of individuals in a sub-sample having a pollution tolerance value of 0, 1, 2, or 3. This metric is expected to decrease with increasing anthropogenic stress, reflecting the loss of pollution-sensitive organisms.

A detailed explanation of IBI metric calculation and scoring is given in detail by PA DEP (2018). In a nutshell, the observed metric value is compared to the standardized metric value, the latter of which is based on the population of metric values calculated from PA DEP's stream monitoring sites across the state. The Standardized Metric Score is simply the percent similarity to the reference condition. That score is adjusted for values above 100% (Table 1).

Biological (Index of Biotic Integrity) Results

Table 1. Index of Biotic Integrity (IBI) Metric scores for Site 1, UNT-2 samples collected 4/29/2022.

METRIC	Observed Value	Standardized Value	Standardized Metric Score	Adjusted Score
Total Taxa Richness	30	33	90.9	90.9
EPT Richness (0-4 PTV)	16	19	84.2	84.2
Becks Index (V3)	33	38	86.8	86.8
HBI	1.72	1.89	102.1	100
SDI	2.29	2.86	80.1	80.1
% Sensitive Individuals (PTV 0-3)	84.4	84.5	99.8	99.8
IBI SCORE				90.3

The following explanation is intended to help interpret the above biological monitoring results from the context of IBIs and Aquatic Life Use (ALU) designations. PA DEP established use attainment thresholds based on IBI scores for specific stream types, regions and ALU levels. The biological condition required to support a particular ALU can be described in terms of biological condition or tiers along a Biological Condition Gradient (BCG). For example, native brook trout require high water quality or an Exceptional Value (EV) or High Quality Cold Water Fishery (HQ-CWF) ALU indicative of BCG Tier 1, whereas habitat to support a warm water fishery would span a broader range of BCG tiers. Figure 2 illustrates this concept and interpretation.

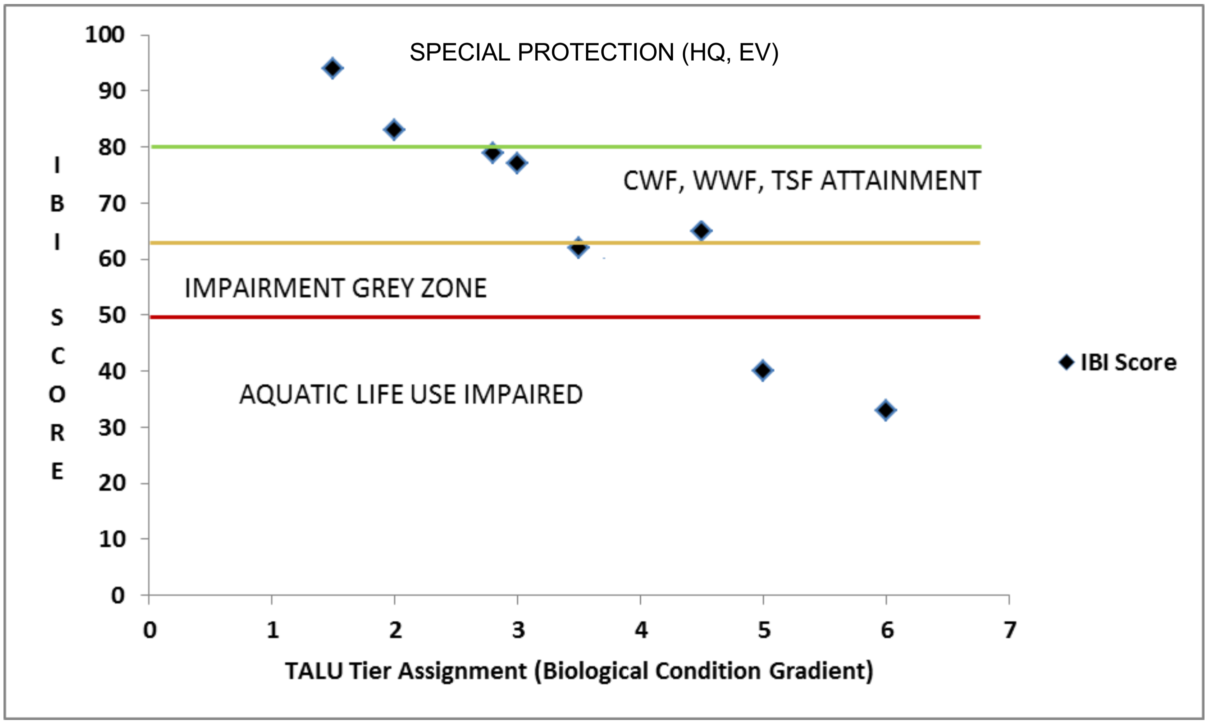


Figure 2. Hypothetical IBI scores plotted against Tiered Aquatic Life Use (TALU) Tiers to inform IBI results. IBI scores ≥ 80 correspond to a TALU tier of approximately 2.5; the established ALU attainment (i.e., IBI score ≥ 63) and impairment (i.e., IBI score < 50) benchmarks correspond to TALU tiers of approximately 3.0 and 4.0, respectively (adapted from PA DEP 2009).

Macroinvertebrate taxa are assigned to a distinct BCG Tier, based on the habitat conditions or range of habitat conditions where they are found, and this information is compiled to determine the proportions of BCG Tier taxa and individuals in a sample. For Cold Water Fisheries (the designated ALU for UNT-1 & UNT-2), IBI scores cannot fall below 50 (IBI score for UNT-2 = 90.3). Mayflies, stoneflies, and caddisflies must be well-represented and contain sufficient numbers of intolerant and sensitive taxa and individuals to result in a high proportion of sensitive individuals associated with the top biological condition gradient tiers (BCG 1, 2, 3). Of the individuals identified from the subsample from UNT-2, 84% belonged to BCG tiers 1, 2, and 3. BCG Tier 1 taxa are considered to be extremely rare and extremely sensitive to anthropogenic stress. They are typically only encountered in <20% of PA DEP 200-ct kick samples and are the first to disappear from the community under stress. The UNT-2 sample contained 2 different BCG 1 taxa. BCG Tier 2 taxa are highly sensitive and rare taxa. The sample collected from UNT-2 contained 7 different BCG Tier 2 Taxa.

References

Pennsylvania Department of Environmental Protection (PADEP). 2018. Water Quality Monitoring Protocols for Streams and Rivers. Office of Water Programs Bureau of Clean Water.

<u>Comprehensive Taxa List (Subsample plus Timed Search of Rest of Sample)</u>

ORDER	FAMILY	TAXON
Ephemeroptera	Ephemerellidae	*Ephemerella*
Ephemeroptera	Ephemerellidae	*Serratella*
Ephemeroptera	Leptophlebiidae	*Paraleptophlebia*
Ephemeroptera	Heptageniidae	*Epeorus*
Ephemeroptera	Heptageniidae	*Maccaffertium*
Ephemeroptera	Baetidae	*Baetis*
Ephemeroptera	Heptageniidae	*Cinygmula*
Ephemeroptera	Ephemerellidae	*Eurylophella*
Plecoptera	Perlidae	*Acroneuria*
Plecoptera	Leuctridae	*Leuctra*
Plecoptera	Nemouridae	*Amphinemura*
Plecoptera	Chloroperlidae	*Sweltsa*
Plecoptera	Chloroperlidae	*Haploperla*
Plecoptera	Perlodidae	*Isoperla*
Plecoptera	Pteronarcyidae	*Pteronarcys Allonarcys*
Plecoptera	Perlodidae	*Remenus*
Trichoptera	Hydropsychidae	*Diplectrona*
Trichoptera	Rhyacophilidae	*Rhyacophila*
Trichoptera	Hydropsychidae	*Ceratopsyche*
Trichoptera	Polycentropodidae	*Polycentropus*
Trichoptera	Psychomyiidae	*Lype*
Trichoptera	Philopotamidae	*Chimarra*
Trichoptera	Hydropsychidae	*Hydropsyche*
Odonata	Gomphidae	*Gomphus*
Odonata	Aeshnidae	*Boyeria vinosa*
Coeloptera	Psephenidae	*Psephenus*
Coeloptera	Elmidae	*Oulimnius*
Coeloptera	Elmidae	*Optioservus*
Diptera	Chironomidae	Chironomidae
Diptera	Tipulidae	*Dicranota*
Diptera	Tipulidae	*Tipula*
Diptera	Blephariceridae	*Blepharicera*
Diptera	Tipulidae	*Hexatoma*
Diptera	Tipulidae	*Antocha*
Diptera	Simuliidae	*Simulium*
Decapoda	Cambaridae	*Cambarus*
Oligochaeta	Oligochaeta	Oligochaeta