Pennsylvania DEP Multihabitat Stream Assessment Protocol

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March 2007

This protocol identifies practical and regionally appropriate field, laboratory, and data analysis procedures needed to evaluate Pennsylvania's low-gradient streams. It also explains how to calculate the Total Biological Score of a stream macroinvertebrate sample and how this can be used to determine the aquatic life use status of that stream. The document that follows is a condensed version of "Pennsylvania DEP Multihabitat Stream Assessment Protocol, March 2007" and was designed for low-gradient waterways that are defined as having pool/glide channel morphology and naturally lack riffles.

Field Methods

All chemical water quality, physical habitat, and aquatic macroinvertebrate data is collected from a sample reach approximately 100 meters in length, during the months of October to May.

If water chemistry samples are taken for total phosphorus and total organic carbon, preserve them with 10% sulfuric acid. Samples analyzed for metals should be preserved with concentrated nitric acid to a pH <2. All samples are kept on ice and should be delivered to the DEP laboratory in Harrisburg, PA within 48 hours of collection.

Habitat Assessment

Physical habitat is documented using the EPA Glide/Pool Prevalence Habitat Assessment Field Data Sheet. This evaluation divides the habitat of the stream and its adjacent land use into ten parameters. Each parameter is scored on a scale of 0 to 20, with a higher score indicating better conditions. Depending on the score, a parameter can fall into one of four categories: Poor, Marginal, Suboptimal, and Optimal.

For the purpose of this protocol, only nine of the ten parameters are used. Channel Sinuosity is not used for low-gradient streams because the range of sinuosity as defined in the data sheet is not applicable to Pennsylvania streams. Thus, total habitat site scores can range from 0-180, with 180 being a perfect score.

Sampling

Aquatic macroinvertebrate samples are collected using a multihabitat sample collection method modified from that described in Barbour et al (1999). Organisms are collected from five different habitat types within the sample reach. Table 1 describes the five habitat types and explains the different sampling techniques. A total of 10 "jabs" are collected within each sample reach. Each jab consists of a 30-inch-long sweep of a 0.3-meter wide area, using a D-frame dip net (500 micron mesh). At least two jabs are made in each of the habitat types present within the sample reach.

Table 1. Stream Habitat Types and Field Sampling Techniques

Habitat Type	Description	Sample Technique
Cobble/ Gravel Substrate	Stream bottom areas consisting of mixed gravel and larger substrate particles; Cobble/gravel substrates are typically located in relatively fast-flowing, "erosional" areas of the stream channel	Macroinvertebrates are collected by placing the net on the substrate near the downstream end of an area of gravel or larger substrate particles and simultaneously pushing down on the net while pulling it in an upstream direction with adequate force to dislodge substrate materials and the aquatic macroinvertebrate fauna associated with these materials; Large stones and organic matter contained in the net are discarded after they are carefully inspected for the presence of attached organisms which are removed and retained with the remainder of the sample; One jab consists of passing the net over approximately 30 inches of substrate.
Snag	Snag habitat consists of submerged sticks, branches, and other woody debris that appears to have been submerged long enough to be adequately colonized by aquatic macroinvertebrates; Preferred snags for sampling include small to medium-sized sticks and branches (preferably < ~4 inches in diameter) that have accumulated a substantial amount of organic matter (twigs, leaves, uprooted aquatic macrophytes, etc.) that is colonized by aquatic macroinvertebrates.	When possible, the net is to be placed immediately downstream of the snag, in either the water column or on the stream bottom, in an area where water is flowing through the snag at a moderate velocity; The snag is then kicked in a manner such that aquatic macroinvertebrates and organic matter are dislodged from the snag and carried by the current into the net; If the snag can not be kicked, than it is sampled by jabbing the net into a downstream area of the snag and moving it in an upstream direction with enough force to dislodge and capture aquatic macroinvertebrates that have colonized the snag; One jab equals disturbing and capturing organisms from an area of ~0.23 m^2 (12" x 30")
Coarse Particulate Organic Matter (CPOM)	Coarse particulate organic matter (CPOM) consists of a mix of plant parts (leaves, bark, twigs, seeds, etc.) that have accumulated on the stream bottom in "depositional" areas of the stream channel; In situations where there is substantial variability in the composition of CPOM deposits within a given sample reach (e.g., deposits consisting primarily of white pine needles and other deposits consisting primarily of hardwood tree leaves), a variety of CPOM deposits are sampled; However, leaf packs in higher-velocity ("erosional") areas of the channel are not included in CPOM samples	CPOM deposits are sampled by lightly passing the net along a 30-inch long path through the accumulated organic material so as to collect the material and its associated aquatic macroinvertebrate fauna; When CPOM deposits are extensive, only the upper portion of the accumulated organic matter is collected to ensure that the collected material is from the aerobic zone
Submerged Aquatic Vegetation (SAV)	Submerged aquatic vegetation (SAV) habitat consists of rooted aquatic macrophytes	SAV is sampled by drawing the net in an upstream direction along a 30-inch long path through the vegetation; Efforts should be made to avoid collecting stream bottom sediments and organisms when sampling SAV areas.
Sand/Fine Sediment	Sand/fine sediment habitat includes stream bottom areas that are composed primarily of sand, silt, and/or clay.	Sand/fine sediment areas are sampled by bumping or tapping the net along the surface of the substrate while slowly drawing the net in an upstream direction along a 30-inch long path of stream bottom; Efforts should be made to minimize the amount of debris collected in the net by penetrating only the upper-most layer of sand/silt deposits; Excess sand and silt are removed from the sample by repeatedly dipping the net into the water column and lifting it out of the stream to remove fine sediment from the sample

First identify which habitat types are present within the sample reach. A minimum surface area of approximately 0.46 m^2 is required for a given habitat type to be sampled. If the total number of jabs (10) is not evenly divisible by the number of habitat types present, the remaining jab(s) are distributed among the most extensive habitat type(s) in the reach. All jabs are combined into several 2-liter largemouth jars and preserved in ethyl alcohol. Typically, the combined 10 jabs will fill three to four 2-liter sample jars about 2/3 full with organic and inorganic material. Sample jars are topped-off with 95% ethanol to ensure adequate sample preservation.

Laboratory Methods

In the laboratory, each composited sample is placed into a 3.5" deep rectangular pan (measuring 14" long x 8" wide on the bottom of the pan) marked off into 28 four-square inch (2" x 2") grids. Using an illuminated magnifying lens, macroinvertebrates are picked from a minimum of four grids, selected at random, to generate a 200-organism (+/- 20%) sub-sample. Additional grids may be selected at random until the sub-sample is obtained. The organisms contained in the 200-organism sub-sample are identified to the lowest practical taxonomic level (usually genus). Some individuals collected will be immature and not exhibit the characteristics necessary for confident identification. If the individual cannot be confidently identified to the proper level, it should be discarded. All pupae are discarded. Certain groups are identified to a higher taxonomic level as follows:

Flatworms (Turbellaria) – Phylum Turbellaria
Segmented worms (Annelida), aquatic earthworms, & tubificids – Class Oligochaeta
Proboscis worms – Phylum Nemertea
Roundworms – Phylum Nematoda
Water mites – "Hydracarina" (an artificial taxonomic grouping of several mite superfamilies)
Midges – Family Chironimadae
Weevils – Family Curculionidae
Sand flies\no-see-ums – Ceratopogonidae
Decapoda, Gastropoda, and Pelecypoda are identified to family

Initial Processing of Raw Macroinvertebrate Sample

1. Fill a five-gallon bucket about 2/3 full with cold water.
2. Decant ethanol from samples by gently dumping the contents of sample bottles into a 500-micron sieve.
3. Gently rinse most of the silt and/or very-fine sand from the sample material in the sieve using an abundance of clean, cold water.
4. Gently transfer the rinsed sample material from the sieve into the five-gallon bucket.
5. Repeat step 2 until approximately ½ of the material contained in a given sample is transferred into the five-gallon bucket.
6. Gently agitate the contents of the bucket and decant the water and a portion of the bucket's contents into a 500-micron sieve.

7. Transfer the contents of the sieve into a clean, white, 3.5" deep rectangular pan (measuring 14" long x 8" wide on the bottom of the pan) marked off into 28 four-square inch (2" x 2") grids.
8. Gently fill the five-gallon bucket about 2/3 full with clean cold water and repeat steps 6 & 7 until all organisms are transferred from the bucket into the pan.
9. Repeat steps 1 through 8 until all of the organisms contained in the sample are transferred to the pan.

Picking the 200-Organism Sub-sample

1. Remove a reasonable amount of organic material from a randomly selected grid in the 3.5" deep rectangular pan and place it in a large clear glass or plastic dish (sample-picking dish) containing clean water. The sample-picking dish should be placed on top of a white paper towel or piece of paper.
2. Using an illuminated magnifying lens and forceps, grasp individual large pieces of debris from the sample-picking dish, dip them in a deep dish or bowl of cold water (rinse dish), and discard them. Usually after numerous large pieces of debris are discarded, more material from the selected grid can be placed in the sample-picking dish.
3. After the large pieces of debris are removed from the sample-picking dish, move the organic matter away from the front edge of the dish so that there is an area of the dish that is relatively free of debris.
4. Starting with the debris closest to the debris-free area of the sample-picking dish, start moving small allotments of debris into the previously debris-free area so that individual organisms can be clearly detected and transferred from the sample-picking dish to a 3"-diameter petrie dish or similar dish containing clean cold water or ethanol (sub-sample organism dish). Use a hand held counter and keep track of the number of "identifiable" organisms (i.e., organisms in good enough condition to be identified to genus for most taxa) transferred to the sub-sample organism dish.
5. Continue working from the front edge of the sample-picking dish toward the back edge of the dish until all organisms have been transferred from the sample-picking dish to the sub-sample organism dish. Sometimes the water in the sample-picking dish will become cloudy making it hard to see the organisms in the dish. If this happens, carefully pour off the water in the sample-picking dish, being careful not to pour off organisms and debris during the process, and replace it with clean, cold water. It is best to pour off water between steps 2 and 3 above.
6. Use forceps and netting attached to a pipette, pencil, or similar object, to transfer all of the contents of the randomly selected grid to the sample-picking dish and repeat steps 1- 4 above until all organisms have been placed in the sub-sample organism dish.
7. Repeat steps 1-5 above until a minimum of 4 randomly selected grids are processed. All organisms in the 4^{th} grid are to be transferred to the sub-sample organism dish, even if the 200 +/- 20% criterion is already met. If the estimated number of "identifiable" organisms in the sub-sample is less than 160, process additional grids until a minimum of 160 organisms are contained in the sub-sample.

8. If the sub-sample contains more than 240 organisms after picking the fourth grid, place the sub-sample in a clean gridded pan containing a small amount of cold water. Using an illuminated magnifying lens, randomly select grids and transfer all organisms from these grids to a separate container, using a hand-held counter to keep track of the number of "identifiable" organisms transferred. Continue selecting grids and transferring organisms until a sub-sample of 200 +/- 20% is produced.

Metrics

Table 2 describes the six metrics used to calculate Total Biological Scores for samples collected using this protocol.

Table 2. Six Metrics used to Calculate Total Biological Scores of Samples Collected using the Multihabitat Stream Assessment Protocol

Metric	Discrimination Efficiency	Expected Response to Increasing Stress	Metric Description
EPT	100	Decrease	Sum of the total number of taxa found in the Orders Ephemeroptera (Mayfly), Plecoptera (Stonefly), and Trichoptera (Caddisfy) that were sub-sample.
Taxa Richness	94	Decrease	Total number of taxa in the sub-sample.
Beck4	82	Decrease	Pollution weighted taxa richness measure, based on Hilsenhoff Biotic Index Scores (Hils). This is a modified Beck's Index giving taxa with a Hils score of 0 or 1 two points and Hils scores of 2, 3, or 4 are given 1 point.
Shannon Diversity	88	Decrease	This index measures taxa abundance and evenness in the sub-sample by dividing the # of individuals in a taxa by the total # of individuals in the sub-sample and then multiplying by the natural logarithm of this proportion. This is done for all taxa in the sub-sample; the products are then summed and the answer multiplied by -1: $= -\sum_{i=1}^{TaxaRich} (p_i/P) \ln (p_i/P)$
# Mayfly Taxa	88	Decrease	Total number of Mayflies (Ephemeroptera) in the sub-sample
# Caddisfly Taxa	94	Decrease	Total number of Caddisflies (Trichoptera) in the sub-sample

Metric and Total Biological Score Calculations

The following provides a detailed explanation on how to calculate the six metric scores and the Total Biological Scores of two low gradient streams, Saw Creek and Wiconisco

Creek. After the field and lab procedures have been completed, a macroinvertebrate list of 200 +/- 20% organisms will be produced. The following taxa lists are color coded to help distinguish the taxa and information that will be used to calculate the metrics.

Saw Creek (20040406-1705-CAM)				
Taxonomic Level	**Taxa Name**	**Number of Individuals**	**Hilsenhoff Score**	**Functional Feeding Group**
Diptera	Chironomidae	109	6	CG
Isopoda	Caecidotea	8	6	CG
Trichoptera	Pycnopsyche	16	4	SH
Ephemeroptera	Eurylophella	4	4	SC
Trichoptera	Platycentropus	2	4	SH
Diptera	Ceratopogonidae	3	6	PR
Bivalvia	Sphaeriidae	3	8	FC
Oligochaeta	Oligochaeta	3	10	CG
Trichoptera	Oecetis	1	8	PR
Hirudinea	Hirudinea	1	8	PR
Ephemeroptera	Stenonema	3	3	SC
Plecoptera	Amphinemura	3	3	SH
Trichoptera	Lype	7	2	CG
Plecoptera	Isoperla	3	2	PR
Plecoptera	Leuctra	5	0	SH
Trichoptera	Diplectrona	3	0	FC
Trichoptera	Wormaldia	1	0	FC
Trichoptera	Rhyacophila	3	1	PR
Trichoptera	Lepidostoma	1	1	SH
Plecoptera	Prostoia	3	2	SH
Trichoptera	Molanna	7	6	SC
Diptera	Simulium	13	6	FC
Diptera	Prosimulium	2	5	FC
Diptera	Pseudolimnophila	1	2	PR
Diptera	Dicranota	11	3	PR
Diptera	Tipula	1	4	SH

Wiconisco Creek (20050525-1030-CAM)				
Taxonomic Level	**Taxa Name**	**Number of Individuals**	**Hilsenhoff Score**	**Functional Feeding Group**
Diptera	Chironomidae	151	6	CG
Isopoda	Caecidotea	1	6	CG
Trichoptera	Platycentropus	1	4	SH
Diptera	Ceratopogonidae	2	6	PR
Bivalvia	Sphaeriidae	3	8	FC
Oligochaeta	Oligochaeta	35	10	CG
Amphipoda	Crangonyx	3	4	CG
Odonata	Calopteryx	1	6	PR
Plecoptera	Leuctra	1	0	SH
Megaloptera	Sialis	1	6	PR
Odonata	Lestes	1	9	PR
Odonata	Ischnura	1	9	PR

EPT

To calculate this metric, sum the total number of Mayfly (Ephemeroptera), Stonefly (Plecoptera), and Caddisfy (Trichoptera) taxa found in the sub-sample:

Saw Creek

Ephemeroptera = 2
Plecoptera = 4
Trichoptera = 9
15

Wiconisco Creek

Ephemeroptera = 0
Plecoptera = 1
Trichoptera = 1
2

Taxa Richness

This metric sums the total number of taxa identified in the sub-sample (count the number of rows in the above tables):

Saw Creek = **26**

Wiconisco Creek = **12**

Beck4

Beck4 is a pollution weighted taxa richness measure, based on Hilsenhoff Biotic Index Scores (Hils). Hilsenhoff's index measures the pollution tolerance of an organism on a scale of 0 to 10, where the organisms' tolerance level decreases with the score. This metric is a modification of Beck's Index; it was chosen because this version works better for low-gradient streams. Therefore, it differs from the Beck's Index used in the 6 D-Frame protocol. For Beck4, taxa with a Hils score of 0 or 1 are given 2 points and Hils scores of 2, 3, or 4 are given 1 point. In the tables, scores of 0 and 1 are highlighted in blue and scores of 2, 3, and 4 are highlighted in purple.

Saw Creek	Wiconisco Creek
Total # of taxa with Hils score of 0 or 1 = **5**	Total # of taxa with Hils score of 0 or 1 = **1**
2 pts. x 5 = **10**	2 pts x 1 = **2**
Total # of taxa with Hils score of 2,3,or4 = 11	Total # of taxa with Hils score of 2,3,or4 = 2
1 pt. x 11 = **11**	1 pt. x 2 = **2**
10 + **11** = **21**	**2** + **2** = **4**

Shannon Diversity

This index measures taxa abundance and evenness in the sub-sample by dividing the # of individuals in a taxa by the total # of individuals in the sub-sample and then multiplying by the natural logarithm of this proportion. This is done for all taxa in the sub-sample; the products are then summed and the answer multiplied by -1.

$$= -\sum_{i=1}^{TaxaRich} (p_i/P) \ln (p_i/P$$

p_i = # of individuals in each taxa
P = total # of individuals identified in the sub-sample
TaxaRich = the total # of taxa in the sub-sample

Saw Creek	Wiconisco Creek
TaxaRich = 26	TaxaRich = 12
P = 217 (sum the Number of Individuals column in the above tables)	P = 201

p_i = this value is listed in the above tables in the Number of Individuals column.

Saw Creek
(109/217) ln (109/217) + (8/217) ln (8/217) + (16/217) ln (16/217)……(1/217) ln (1/217) = -2.12946 * -1 = **2.12946**

Wiconisco Creek
(151/201) ln (151/201) + (1/201) ln (1/201) + (1/201) ln (1/201)……(1/201) ln (1/201) = -0.875322793 * -1 = **0.87532**

Number of Caddisfly Taxa

To calculate this metric, sum the number of Caddisfly taxa present in the sub-sample.

Saw Creek	Wiconisco Creek
Trichoptera = 9	Trichoptera = 1

Number of Mayfly Taxa

Sum the total number of Mayfly taxa identified in the sub-sample.

Saw Creek	Wiconisco Creek
Ephemeroptera = 2	Ephemeroptera = 0

Now that the six metric scores have been calculated, the scores are plugged into the normalized metric score equation: (Observed Value / 95th percentile) x 100. Some metrics may have a normalized score greater than 100 because normalization is based on the 95th percentile values of the statewide dataset. Normalized metric scores above 100 are adjusted to a score of 100. The adjusted metric scores for the six metrics are summed and then averaged to give the Total Biological Score. Tables 3 and 4 below show how to calculate the normalized metric scores and Total Biological Scores for Saw Creek and Wiconisco Creek.

Saw Creek's Raw Metric Scores

EPT = 15
Taxa Richness = 26
Beck4 = 21
Shannon Diversity = 2.12946
Of Caddisfly Taxa = 9
Of Mayfly Taxa = 2

Wiconisco Creek's Raw Metric Score

EPT = 2
Taxa Richness = 12
Beck4 = 4
Shannon Diversity = 0.87532
Of Caddisfly Taxa = 1
Of Mayfly Taxa = 0

Table 3. Total Biological Score Calculation for Saw Creek

Metric	Equation	Observed Value	Normalized Metric Score	Adjusted Metric Score (100 Max)
EPT	(Observed / **17**) x 100	15	88.2	88.2
Taxa Richness	(Observed / **31**) x 100	26	83.9	83.9
Beck4	(Observed / **22**) x 100	21	95.5	95.5
Shannon Diversity	(Observed / **2.43**) x 100	2.12946	87.6	87.6
# Of Caddisfly Taxa	(Observed / **11**) x 100	9	81.8	81.8
# Of Mayfly Taxa	(Observed / **6**) x 100	2	33.3	33.3
Total Biological Score				**78.4**

Table 4. Total Biological Score Calculation for Wiconisco Creek

Metric	Equation	Observed Value	Normalized Metric Score	Adjusted Metric Score (100 Max)
EPT	(Observed / **17**) x 100	2	11.8	11.8
Taxa	(Observed / **31**) x 100	12	38.7	38.7
Beck4	(Observed / **22**) x 100	4	18.2	18.2
Shannon Diversity	(Observed / **2.43**) x 100	0.87532	36.0	36.0
# Of Caddisfly Taxa	(Observed / **11**) x 100	1	9.1	9.1
# Of Mayfly Taxa	(Observed / **6**) x 100	0	0	0
Total Biological Score				**19.0**

Benchmark

The Total Biological Score of a site is then compared to the protocols benchmark. Sites scoring below the benchmark are considered impaired for aquatic life use and sites scoring above are considered attaining for aquatic life.

Table 5. Aquatic Life Use (ALU) Benchmark

Multihabitat ALU Benchmark
55

Therefore, Saw Creek would be documented as attaining for aquatic life use and Wiconisco Creek would be impaired for aquatic life use.

Literature Cited

Barbour, M.T., J. Gerritsen, B.D. Snyder, and J.B. Stribling. 1999. Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates and Fish, Second Edition. EPA 841-B-99-002. U.S. Environmental Protection Agency; Office of Water; Washington, D.C.