pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

**BUREAU OF CLEAN WATER**

# AN INDEX OF BIOTIC INTEGRITY FOR PENNSYLVANIA MULTIHABITAT STREAMS

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## INTRODUCTION

The United States Environmental Protection Agency's Rapid Bioassessment Protocols for use in Wadeable Streams and Rivers (Barbour et al.1999) describes two general approaches to assessing stream macroinvertebrate communities. These approaches are the "single, most productive habitat" approach and the "multihabitat" approach. The single, most productive habitat approach is typically used to assess streams where cobble substrate (riffle/run) is the predominant habitat. The multihabitat approach involves sampling a variety of habitat types instead of sampling a single habitat, such as cobble substrate in riffles and/or runs.

In April of 2002, the Pennsylvania Department of Environmental Protection (PADEP) began developing a macroinvertebrate bioassessment protocol for assessing the Commonwealth's low-gradient streams. Low gradient waterways consist of pool/glide channel morphology and naturally lack riffles. The multihabitat field and laboratory methods described in Barbour et al (1999) were used as a starting point for the project. Water chemistry, physical habitat, and aquatic macroinvertebrates were collected at 77 sampling sites in this study. The project goal was to identify practical and regionally appropriate field, laboratory, and data analysis procedures and to develop an index of biological integrity that accurately reflects the ecological conditions of Pennsylvania's low-gradient streams.

## REFERENCE AND STRESSED SITES

The abiotic conditions of all sample sites were analyzed to determine if the sites should be divided into different bioregions. None of the abiotic conditions investigated provided justification for dividing the sites into different bioregions, therefore, all sample sites will be held to the same criterion when determining if they are reference, non-reference, or stressed. Appendix A-1 contains a map of the sample sites. The 77 sample sites were categorized as reference, non-reference, or stressed based on 15 parameters. All 15 parameters were used as reference site criteria. For sites to be considered reference sites, they had to meet the criteria values of all 15 parameters (Appendix A-2). The first 14 parameters in Appendix A-2 were used to determine if the site was stressed. Sites were considered stressed if they failed any one of the 14 stressed criteria values. For example, if a site had a value of 4.8 mg/l for dissolved oxygen, the site would be considered stressed regardless of other parameter values.

## FIELD METHODS

All chemical water quality, physical habitat, and aquatic macroinvertebrate data is collected from a sample reach approximately 100 meters in length. During development of the protocol, water temperature, pH, dissolved oxygen, and conductivity were measured in the field and a chemical sample was collected from each reach for laboratory analysis. This sample was collected under base flow (non-stormwater runoff) conditions.

| Field | Lab | |
|---|---|---|
| Temperature | pH | Total Organic Carbon |
| Dissolved Oxygen | Alkalinity | Chloride |
| pH | Nitrate-N | Sulfate |
| Conductivity | Total Phosphorus | Iron |

Total phosphorus and total organic carbon samples are preserved with 10% sulfuric acid and samples analyzed for metals are preserved with concentrated nitric acid to a pH <2. All samples are kept on ice and delivered to the PADEP laboratory in Harrisburg, PA within 48 hours of collection.

Physical habitat is documented using the EPA Glide/Pool Prevalence Habitat Assessment Field Data Sheet (Barbour et al. 1999). 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 (indicated as Habitat Parameter 7 in Appendix B-1) is not used because the range of sinuosity as defined in the data sheet is not applicable to Pennsylvania streams. Even the State's most sinuous streams will have low values using this definition. Thus, total habitat site scores can range from 0-180, with 180 being a perfect score (Appendix B-1).

The majority of macroinvertebrate samples were collected from October to May. A small number of samples were collected outside of this period to test the seasonal variability of the protocol. Seasonal variability analysis results are discussed on page 6 and 7.

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. The habitat types and explanations of sampling techniques are described in Appendix B-2. 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.

The biologist first identifies 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.

## LAB 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

A detailed explanation of the laboratory processing procedure is provided in Appendix C. Pollution tolerance values and functional feeding group information are listed in Appendix D.

## METRICS SELECTION

The 200-organism sub-sample data, from 77 samples, was used to calculate values and produce box plots for an initial fifty metrics. Only “truly-aquatic” (hydropneustic) organisms included in the 200-organism sub-samples were used to generate these metric scores. By visually comparing box plots of all fifty metrics and choosing those that could discriminate between minimally disturbed reference and stressed sites, thirteen candidate metrics were selected. An explanation on interpreting box plots can be found in EPA’s RBP manual (Barbour et al. 1999).

The discrimination efficiency (D.E.) of each candidate metric was calculated to better determine how well the metric could distinguish between a reference and stressed site. These values are listed in Table 1 below. The D.E. is the percentage of stressed samples whose scores do not overlap with the interquartile range of reference sample scores. The 25$^{th}$ percentile of the total number of reference samples was used as the threshold for metrics that decrease with pollution. For these metrics, the following formula was used:

D.E. = (the # of stressed samples that fall below the 25$^{th}$ percentile value of the reference distribution / the total # of stressed samples) x 100

The 75$^{th}$ percentile of the total number of reference samples was used as the threshold for metrics that increase with pollution. For these metrics, the following formula was used:

D.E. = (the # of stressed samples that occur above the 75$^{th}$ percentile value of the reference distribution / the total # of stressed samples) x 100

Box plots depicting these two scenarios can be found in Appendix E-1. Those metrics with a D.E. less than 80 were eliminated because of their weak ability to discriminate. Trophic Diversity, % Tolerant Taxa, and % Intolerant Taxa (Hils<5) all had D.E.’s of 76 and were therefore dropped, leaving ten metrics.

**Table 1.** Discrimination Efficiencies of the Thirteen Candidate Metrics

| Candidate Metrics | Discrimination Efficiency (D.E.) |
|---|---|
| EPT | 100 |
| Taxa Richness | 94 |
| # Of Caddisfly Taxa | 94 |
| # Intolerant Taxa (Hils<5) | 94 |
| # Of Mayfly Taxa | 88 |
| Shannon Diversity | 88 |
| Beck4 | 82 |
| Beck3 | 82 |
| % Taxa as EPT | 82 |
| % EPT | 82 |
| Trophic Diversity | 76 |
| % Tolerant Taxa | 76 |
| % Intolerant Taxa (Hils<5) | 76 |

To eliminate redundant metrics that might measure similar attributes, Pearson correlation coefficients were calculated (Appendix E-2). If two metrics were highly correlated ($r^2$ >0.90) the most familiar, easiest to interpret, and/or higher D.E. metric was retained.

This process eliminated two metrics: Beck3 and Number Intolerant Taxa (Hilsenhoff < 5). Beck3 was highly correlated ($r^2$=0.93) with the Beck4 metric. Beck4 had larger values and a tighter reference distribution and therefore was kept. Number Intolerant Taxa (Hilsenhoff < 5) was highly correlated with EPT ($r^2$=0.91); it had the lower D.E. and consequently was dropped.

Percent EPT was then eliminated to avoid having three EPT metrics; this would have created a heavy reliance on those taxa. Percent Taxa as EPT was found to produce high metric scores for streams that should be impaired because of low pH values. This can result from the inclusion of low pH tolerant stoneflies in the metric calculation. To prevent the inapt assignment of attainment status to low pH streams, this metric was eliminated.

The remaining six metrics are the core metrics used to calculate the Total Biological Scores for this protocol.

| | |
|---|---|
| EPT | Beck4 |
| Taxa Richness | # Mayfly Taxa |
| Shannon Diversity | # Caddisfly Taxa |

They are listed and explained in Appendix E-3. Box plots of the raw values for each metric are located in Appendix E-4.

Normalization of Metric Scores and Total Biological Score Calculation
All six core metrics decrease with increasing stress, and therefore were normalized to a scale of 0 to 100 based on the 95$^{th}$ percentile value (least squares estimate) of all samples (n = 77) using the following equation:

Normalized Metric Score = (Observed Value / 95$^{th}$ percentile) x 100

An example of how to calculate metric scores (observed value) and the Total Biological Score of two samples is shown in Appendix F.

## AQUATIC LIFE USE BENCHMARKS

Aquatic life use attainment status of a given sample reach is determined by comparing its Total Biological Score to a use attainment benchmark. If the Total Biological Score of the sample reach is less than the benchmark score, the sample reach is not attaining for aquatic life.

The 10$^{th}$ percentile of the Total Biological Scores of the reference site dataset (n=16) was used to set the aquatic life use benchmark. Appendix G supports using the 10$^{th}$ percentile value by showing the well-defined separation of the Total Biological Scores of the reference and stressed sites.

**Table 2.** Aquatic Life Use (ALU) Benchmark

| Multihabitat ALU Benchmark |
|---|
| **55** (10$^{th}$ percentile) |

Sites with Total Biological Scores scoring above the benchmark are attaining (Saw Creek, Appendix F) and sites with Total Biological Scores scoring below the benchmark are considered impaired for ALU (Wiconisco Creek, Appendix F).

## PROTOCOL VERIFICATION

The aquatic life use status (reference or stressed) of eighteen low gradient streams was predicted using the chemistry, habitat, and land use criteria listed in Appendix A-2. Ten of the streams were considered impaired and eight attaining, based solely on the abiotic conditions. Macroinvertebrate verification samples were then collected at those eighteen streams to test the accuracy of the field/lab methods and the reliability of the

benchmark. The verification samples were collected between April 12th, 2006 and May 31st, 2006, using the same field/lab procedures described in Appendixes B and C. The Total Biological Scores for all 18 samples were calculated and the aquatic life use attainment status determined using the benchmark set in this protocol. Nine of the ten stressed sites were found to be impaired using the protocol benchmarks. Seven of the eight reference sites had Total Biological Scores exceeding the benchmark. Appendix H-1 lists the metric values and Total Biological Scores of the verification samples. An unnamed Tributary to South Branch Muddy Creek was the only reference sample that did not meet its predicted attainment status. Using this protocol, it had a Total Biological Score of 44, missing attainment status by 11 points. This resulted from the inclusion of a high number of stoneflies in the sub-sample. Randomly selecting more stoneflies would prevent the inclusion of other species in the sub-sample and therefore lower the metric score for Taxa Richness, # Of Mayfly Taxa, and # Of Caddisfly Taxa. This tributary is located in state forest and the macroinvertebrate list otherwise indicates attainment. Kitchen Run was the only stressed stream reach whose verification sample scored above the benchmark. The benchmark was only exceeded by two points. The top three genera in the sub-sample were Simulium, Prosimulium, and Chironomidae, making up 70% of the sub-sample. Four different Ephemeroptera taxa were identified, however, three of the genera contained only one organism. This would inflate the metric scores of EPT, Taxa Richness, and # of Mayfly Taxa. Also, eight of the eighteen taxa identified contained only one organism. This could mask the fact that the sample was dominated by pollution tolerant species.

Overall, the benchmark was 88% affective at identifying ALU attainment and 90% affective in determining ALU impairment. These percentages are very high, indicating the benchmark is accurate in determining the Aquatic Life Use of a sample reach. Appendix H-2 contains box plots of the verification samples verses the reference and stressed sites. These eighteen samples verify the methodology described in this protocol and justify the placement of the aquatic life use benchmark.

## METHOD AND ANNUAL VARIABILITY

Between April 23rd and May 30th, 2003, aquatic macroinvertebrate samples were collected from adjacent stream reaches on three different streams. These paired-samples were used to document method variability. The standard deviation of the Total Biological Scores, calculated as the root mean squared error in an ANOVA, was 10.9. The standard deviation indicates the average variation of the Total Biological Scores in a paired sample. A standard deviation of zero would indicate the sample pairs received the same Total Biological Score. The 90% confidence interval calculated from the standard deviations was 14 for one sample and 9.7 if two samples are collected. This

is relatively high variability, but it may be an overestimate because it was based upon only three paired comparisons. As a rule, variability measures decline as the sample size increases. The annual variability discussed below also indicates this standard deviation based on the three pairs may be an overestimate.

A similar analysis was conducted using paired-sample data collected from four sample reaches during October-May. Two of these reaches were re-sampled one year later, and the remaining reaches were re-sampled two years after the initial data collection effort. The standard deviation (calculated in the same manner described above) of the four sample pairs was used to document long-term variability. The standard deviation of the annual pairs was 6.6 indicating less variability than the paired samples. The 90% confidence interval calculated from the standard deviations was 8.1 for one sample and 5.9 if two samples are collected. This is a more acceptable range of variability. The 3 paired and 4 annual samples all had scores above the attainment benchmark no matter which repeated sample was used in the comparison. This is an indication that at least in this instance the variability was not great enough to affect the attainment/impairment decisions. It would have been a concern if one repeated sample showed attainment and the other impairment creating a lack of consistency. The success of the verification effort is another indication the variability is not creating inconsistencies in attainment/impairment decisions. PADEP will continue to refine the variability estimates with additional surveys in spring 2007. The variability results are summarized in Appendix H-3.

## CONCLUSION

As stated earlier, the project goal was to apply practical and regionally appropriate field, laboratory, and data analysis procedures to the development of an index of biological integrity that accurately reflects the ecological conditions of Pennsylvania's low-gradient streams. Seventy-seven samples collected statewide from low gradient streams, between October and May, were used in developing this protocol. Data analyses did not show any natural differences between the statewide sites that would justify creating separate assessment categories. Therefore, all sites were held to the same criteria when discriminating between reference and stressed sites.

The method used to collect macroinvertebrate samples is modified from the steps described in the EPA document Rapid Bioassessment Protocols for Use in Streams and Wadeable Rivers (Barbour et al 1999). Using a D-frame dip net, ten jabs were distributed between five possible habitat types in each sample reach. The jabs were combined and taken to the laboratory for macroinvertebrate identification. A 200

organism +/-20% sub-sample was identified to the genus level or to the lowest confident taxonomic level.
Six core metrics were chosen from an initial list of fifty metrics, based on how well the metric could distinguish between reference and stressed sites. The resulting six metrics are:

| | | |
|---|---|---|
| EPT | Beck4 | # Of Caddisfly Taxa |
| Taxa Richness | Shannon Diversity | # Of Mayfly Taxa |

Metric scores were then normalized and summed for each sample to produce a Total Biological Score. By visually comparing box plots of the Total Biological Scores of the reference and stressed sites, the 10$^{th}$ percentile value (55) of the reference sites was chosen as the aquatic life use benchmark. This value has an extremely high D.E. of 94. The placement of the benchmark was confirmed by the success of the verification and variability analyses. Although the intra site variability was high, the annual variability was low indicating the protocol can be successfully repeated for low gradient streams. This benchmark of 55 is used as the threshold in determining aquatic life use attainment status for low gradient streams.

## 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. 2$^{nd}$ edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

Merritt, R. W., and K. W. Cummins. 1996. An introduction to the aquatic insects of North America. 3$^{rd}$ edition. Kendall/Hunt Publishing Co., Dubuque, Iowa. p. 862.

Peckarsky, B. L., P. R. Fraissinet, M. A. Penton, and D. J. Conklin. 1990. Freshwater macroinvertebrates of northeastern North America. Comstock Publishing Associates, Ithaca, NY. p. 442.

Rosgen, D. L. 1996. Applied river morphology. Wildland Hydrology, Pagosa Springs, Colorado.

Smith, D. G. 2001. Pennak's freshwater invertebrates of the United States. 4$^{th}$ edition. John Wiley and Sons, Inc., New York, New York. p. 638.

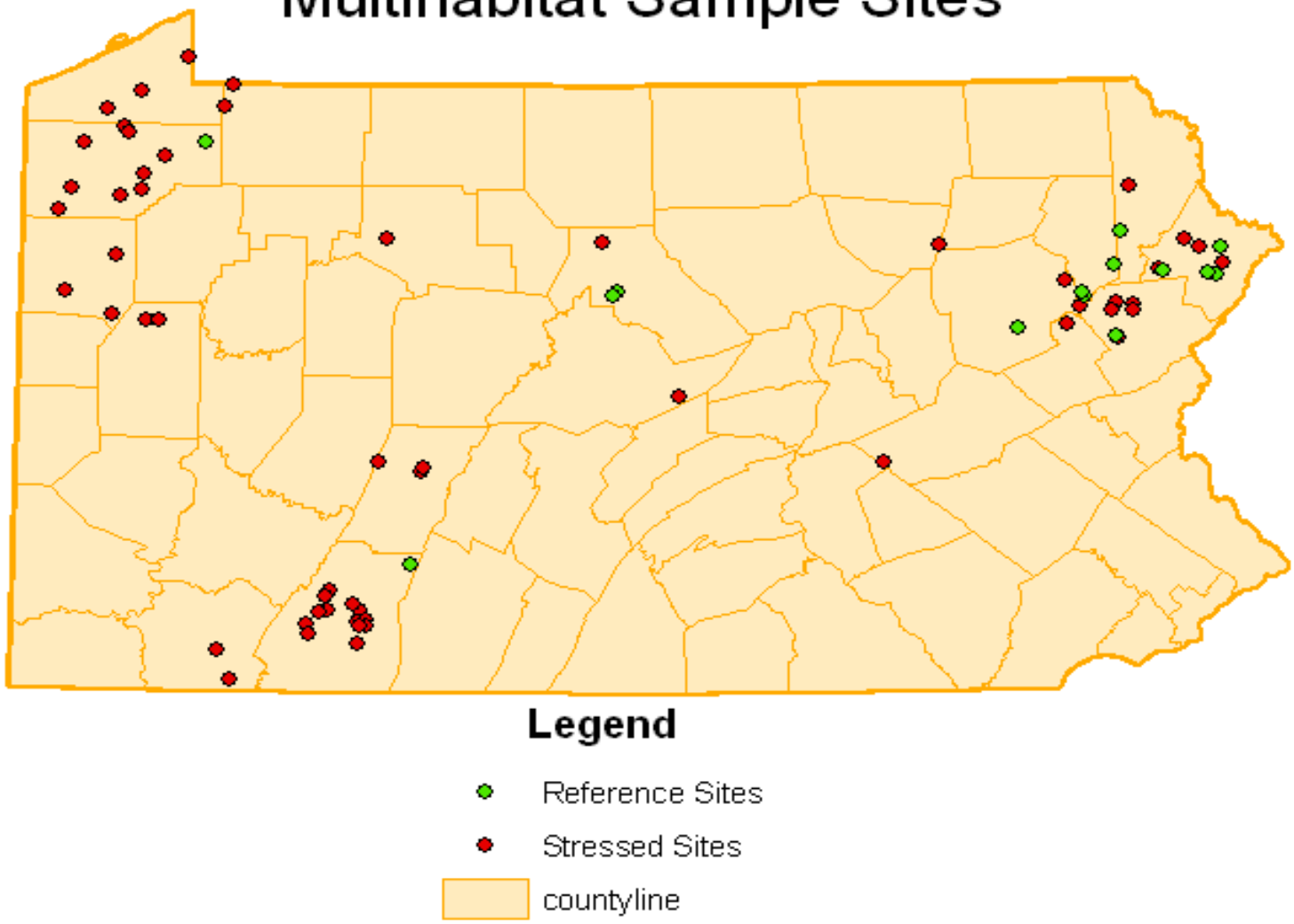
Stewart, K. W., and B. P. Stark. 1988. Nymphs of North American stonefly genera (Plecoptera). The Entomological Society of America, U.S.A. p. 460.

Wiggins, G. B. 1996. Larvae of the North American caddisfly genera (Trichoptera). 2$^{nd}$ edition. University of Toronto Press, Buffalo, NY. p. 457.

# APPENDIX A: SAMPLE SITE LOCATION, AND REFERENCE AND STRESSED SITE CRITERIA

# A.1 SAMPLE SITE LOCATION

# Multihabitat Sample Sites



# A.2 REFERENCE AND STRESSED SITE CRITERIA

| Parameter | Criteria Type | Criteria Values |
|---|---|---|
| Alkalinity (mg/l) | Reference | ≥ 3 |
| | Stressed | < 3 |
| pH | Reference | ≥ 5.3 |
| | Stressed | < 5.1 |
| Total Iron (ug/l) | Reference | ≤ 601 |
| | Stressed | > 1500 |
| Total Phosphorus (mg/l) | Reference | ≤ 0.03 |
| | Stressed | > 0.06 |
| Conductivity (mg/l) | Reference | ≥ 145 |
| | Stressed | > 500 |
| Sulfate (mg/l) | Reference | ≤ 10 |
| | Stressed | > 100 |
| Nitrate (mg/l) | Reference | ≤ 0.56 |
| | Stressed | > 2.00 |
| Chloride (mg/l) | Reference | ≤ 11.7 |
| | Stressed | > 20 |
| Minimum Dissolved Oxygen (mg/l) | Reference | ≥ 7.6 |
| | Stressed | < 5.0 |
| Percent Urban | Reference | ≤ 2% |
| | Stressed | > 5% |
| Percent Agriculture | Reference | ≤ 19% |
| | Stressed | > 50% |
| Percent Forest | Reference | ≥ 81% |
| | Stressed | < 50% |
| Habitat Score | Reference | ≥ 139 |
| | Stressed | < 120 |
| Epifaunal Substrate | Reference | ≥ 16 |
| | Stressed | < 11 |
| Total Organic Carbon (mg/l) | Reference | ≤ 6.3 |

# APPENDIX B: HABITAT DATA SHEET, HABITAT TYPES, AND SAMPLING TECHNIQUES

# B.1 HABITAT ASSESSMENT FIELD DATA SHEET

## HABITAT ASSESSMENT FIELD DATA SHEET—LOW GRADIENT STREAMS (FRONT)

| | | |
|---|---|---|
| STREAM NAME | LOCATION | |
| STATION #_____ RIVERMILE_____ | STREAM CLASS | |
| LAT _____ LONG _____ | RIVER BASIN | |
| STORET # | AGENCY | |
| INVESTIGATORS | | |
| FORM COMPLETED BY | DATE _____<br>TIME _____ AM PM | REASON FOR SURVEY |

Parameters to be evaluated in sampling reach

| Habitat Parameter | Condition Category | | | |
|---|---|---|---|---|
| | Optimal | Suboptimal | Marginal | Poor |
| **1. Epifaunal Substrate/ Available Cover** | Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are <u>not</u> new fall and <u>not</u> transient). | 30-50% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale). | 10-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed. | Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking. |
| **SCORE** | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **2. Pool Substrate Characterization** | Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common. | Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present. | All mud or clay or sand bottom; little or no root mat; no submerged vegetation. | Hard-pan clay or bedrock; no root mat or vegetation. |
| **SCORE** | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **3. Pool Variability** | Even mix of large-shallow, large-deep, small-shallow, small-deep pools present. | Majority of pools large-deep; very few shallow. | Shallow pools much more prevalent than deep pools. | Majority of pools small-shallow or pools absent. |
| **SCORE** | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **4. Sediment Deposition** | Little or no enlargement of islands or point bars and less than <20% of the bottom affected by sediment deposition. | Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20-50% of the bottom affected; slight deposition in pools. | Moderate deposition of new gravel, sand or fine sediment on old and new bars; 50-80% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent. | Heavy deposits of fine material, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition. |
| **SCORE** | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **5. Channel Flow Status** | Water reaches base of both lower banks, and minimal amount of channel substrate is exposed. | Water fills >75% of the available channel; or <25% of channel substrate is exposed. | Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed. | Very little water in channel and mostly present as standing pools. |
| **SCORE** | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |

## HABITAT ASSESSMENT FIELD DATA SHEET—LOW GRADIENT STREAMS (BACK)

Parameters to be evaluated broader than sampling reach

| Habitat Parameter | Condition Category | | | |
|---|---|---|---|---|
| | Optimal | Suboptimal | Marginal | Poor |
| **6. Channel Alteration** | Channelization or dredging absent or minimal; stream with normal pattern. | Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present. | Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted. | Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely. |
| **SCORE** | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **7. Channel Sinuosity** | The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.) | The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line. | The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line. | Channel straight; waterway has been channelized for a long distance. |
| **SCORE** | 20 19 18 17 16 | 15 14 13 12 11 | 10 9 8 7 6 | 5 4 3 2 1 0 |
| **8. Bank Stability (score each bank)** | Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected. | Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion. | Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods. | Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars. |
| SCORE ___ (LB) | Left Bank 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| SCORE ___ (RB) | Right Bank 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| **9. Vegetative Protection** (score each bank)<br><br>Note: determine left or right side by facing downstream. | More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally. | 70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining. | 50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining. | Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height. |
| SCORE ___ (LB) | Left Bank 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| SCORE ___ (RB) | Right Bank 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| **10. Riparian Vegetative Zone Width** (score each bank riparian zone) | Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone. | Width of riparian zone 12-18 meters; human activities have impacted zone only minimally. | Width of riparian zone 6-12 meters; human activities have impacted zone a great deal. | Width of riparian zone <6 meters: little or no riparian vegetation due to human activities. |
| SCORE ___ (LB) | Left Bank 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |
| SCORE ___ (RB) | Right Bank 10 9 | 8 7 6 | 5 4 3 | 2 1 0 |

**Total Score** _____

## B.2 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 |

# APPENDIX C: LABORATORY PROCESSING PROCEDURE

## 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 are 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.

# APPENDIX D: POLLUTION TOLERANCE VALUES AND FUNCTIONAL FEEDING GROUP DESIGNATIONS

| Order (Class) | Family | Taxa | Pollution[1] Tolerance Value | Functional[2] Feeding Group |
|---|---|---|---|---|
| Insecta | Collembola | Collembola | 9 | CG |
| Ephemeroptera | Ameletidae | Ameletus | 0 | CG |
| Ephemeroptera | Siphlonuridae | Siphlonuridae | 7 | CG |
| Ephemeroptera | Baetidae | Acentrella | 4 | SC |
| Ephemeroptera | Baetidae | Acerpenna | 6 | CG |
| Ephemeroptera | Baetidae | Baetis | 6 | CG |
| Ephemeroptera | Isonychiidae | Isonychia | 3 | CG |
| Ephemeroptera | Heptageniidae | Epeorus | 0 | SC |
| Ephemeroptera | Heptageniidae | Stenacron | 4 | SC |
| Ephemeroptera | Heptageniidae | Stenonema | 3 | SC |
| Ephemeroptera | Ephemerellidae | Drunella | 1 | SC |
| Ephemeroptera | Ephemerellidae | Ephemerella | 1 | CG |
| Ephemeroptera | Ephemerellidae | Eurylophella | 4 | SC |
| Ephemeroptera | Ephemerellidae | Serratella | 2 | CG |
| Ephemeroptera | Caenidae | Caenis | 7 | CG |
| Ephemeroptera | Leptophlebiidae | Habrophlebiodes | 6 | SC |
| Ephemeroptera | Leptophlebiidae | Leptophlebia | 4 | CG |
| Ephemeroptera | Ephemeridae | Ephemera | 2 | CG |
| Ephemeroptera | Ephemeridae | Litobrancha | 6 | CG |
| Odonata | Gomphidae | Gomphus | 5 | PR |
| Odonata | Gomphidae | Hagenius | 3 | PR |
| Odonata | Gomphidae | Lanthus | 5 | PR |
| Odonata | Gomphidae | Stylogomphus | 4 | PR |
| Odonata | Aeshnidae | Aeshna | 5 | PR |
| Odonata | Aeshnidae | Basiaeschna | 5 | PR |
| Odonata | Aeshnidae | Boyeria | 2 | PR |
| Odonata | Cordulegastridae | Cordulegaster | 3 | PR |
| Odonata | Corduliidae | Helocordulia | 2 | PR |
| Odonata | Libellulidae | Sympetrum | 4 | PR |
| Odonata | Calopterygidae | Calopteryx | 6 | PR |
| Odonata | Calopterygidae | Lestes | 9 | PR |
| Odonata | Coenagrionidae | Argia | 6 | PR |
| Odonata | Coenagrionidae | Enallagma | 8 | PR |
| Odonata | Coenagrionidae | Ischnura | 9 | PR |
| Plecoptera | Pteronarcyidae | Pteronarcys | 0 | SH |
| Plecoptera | Peltoperlidae | Tallaperla | 0 | SH |
| Plecoptera | Taeniopterygidae | Taeniopteryx | 2 | SH |
| Plecoptera | Taeniopterygidae | Strophopteryx | 3 | SH |
| Plecoptera | Nemouridae | Amphinemura | 3 | SH |
| Plecoptera | Nemouridae | Ostrocerca | 2 | SH |
| Plecoptera | Nemouridae | Prostoia | 2 | SH |
| Plecoptera | Nemouridae | Nemoura | 1 | SH |
| Plecoptera | Leuctridae | Leuctra | 0 | SH |
| Plecoptera | Capniidae | Allocapnia | 3 | SH |
| Plecoptera | Perlidae | Acroneuria | 0 | PR |
| Plecoptera | Perlidae | Perlesta | 4 | PR |
| Plecoptera | Perlodidae | Clioperla | 2 | PR |
| Plecoptera | Perlodidae | Isoperla | 2 | PR |

| Order (Class) | Family | Taxa | Pollution[1] Tolerance Value | Functional[2] Feeding Group |
|---|---|---|---|---|
| Megaloptera | Sialidae | Sialis | 6 | PR |
| Megaloptera | Corydalidae | Chauliodes | 4 | PR |
| Megaloptera | Corydalidae | Nigronia | 1 | PR |
| Trichoptera | Philopotamidae | Chimarra | 4 | FC |
| Trichoptera | Philopotamidae | Dolophilodes | 0 | FC |
| Trichoptera | Philopotamidae | Wormaldia | 0 | FC |
| Trichoptera | Psychomyiidae | Lype | 2 | CG |
| Trichoptera | Polycentropodidae | Nyctiophylax | 5 | FC |
| Trichoptera | Polycentropodidae | Polycentropus | 6 | FC |
| Trichoptera | Dipseudopsidae | Phylocentropus | 5 | FC |
| Trichoptera | Hydropsychidae | Parapsyche | 0 | FC |
| Trichoptera | Hydropsychidae | Diplectrona | 0 | FC |
| Trichoptera | Hydropsychidae | Cheumatopsyche | 6 | FC |
| Trichoptera | Hydropsychidae | Hydropsyche | 5 | FC |
| Trichoptera | Rhyacophilidae | Rhyacophila | 1 | PR |
| Trichoptera | Glossosomatidae | Glossosoma | 0 | SC |
| Trichoptera | Glossosomatidae | Agapetus | 0 | SC |
| Trichoptera | Hydroptilidae | Hydroptila | 6 | SC |
| Trichoptera | Hydroptilidae | Oxyethira | 3 | CG |
| Trichoptera | Phryganeidae | Ptilostomis | 5 | SH |
| Trichoptera | Brachycentridae | Brachycentrus | 1 | FC |
| Trichoptera | Brachycentridae | Micrasema | 2 | SH |
| Trichoptera | Lepidostomatidae | Lepidostoma | 1 | SH |
| Trichoptera | Limnephilidae | Ironoquia | 3 | SH |
| Trichoptera | Limnephilidae | Apatania | 3 | SC |
| Trichoptera | Limnephilidae | Anabolia | 5 | SH |
| Trichoptera | Limnephilidae | Frenesia | 4 | SH |
| Trichoptera | Limnephilidae | Hydatophylax | 2 | SH |
| Trichoptera | Limnephilidae | Limnephilus | 3 | SH |
| Trichoptera | Limnephilidae | Platycentropus | 4 | SH |
| Trichoptera | Limnephilidae | Pycnopsyche | 4 | SH |
| Trichoptera | Uenoidae | Neophylax | 3 | SC |
| Trichoptera | Sericostomatidae | Psilotreta | 0 | SC |
| Trichoptera | Molannidae | Molanna | 6 | SC |
| Trichoptera | Calamoceratidae | Heteroplectron | 5 | SH |
| Trichoptera | Leptoceridae | Ceraclea | 3 | CG |
| Trichoptera | Leptoceridae | Mystacides | 4 | CG |
| Trichoptera | Leptoceridae | Oecetis | 8 | PR |
| Trichoptera | Leptoceridae | Triaenodes | 6 | SH |
| Lepidoptera | Pyralidae | Parapoynx | 5 | SH |
| Lepidoptera | Pyralidae | Acentria | 5 | SH |
| Coleoptera | Gyrinidae | Gyrinus | 4 | PR |
| Coleoptera | Haliplidae | Peltodytes | 5 | SH |
| Coleoptera | Psephenidae | Psephenus | 4 | SC |
| Coleoptera | Scirtidae | Cyphon | 8 | SC |
| Coleoptera | Scirtidae | Scirtes | 8 | SC |

| Order (Class) | Family | Taxa | Pollution[1] Tolerance Value | Functional[2] Feeding Group |
|---|---|---|---|---|
| Coleoptera | Elmidae | Ancyronyx | 2 | CG |
| Coleoptera | Elmidae | Dubiraphia | 6 | SC |
| Coleoptera | Elmidae | Macronychus | 2 | SC |
| Coleoptera | Elmidae | Optioservus | 4 | SC |
| Coleoptera | Elmidae | Oulimnius | 5 | SC |
| Coleoptera | Elmidae | Promoresia | 2 | SC |
| Coleoptera | Elmidae | Stenelmis | 5 | SC |
| Coleoptera | Ptilodactylidae | Anchytarsus | 5 | SH |
| Diptera | Ceratopogonidae | Ceratopogonidae | 6 | PR |
| Diptera | Dixidae | Dixa | 1 | CG |
| Diptera | Dixidae | Dixella | 1 | CG |
| Diptera | Ptychopteridae | Ptychoptera | 8 | CG |
| Diptera | Dolichopodidae | Dolichopodidae | 4 | PR |
| Diptera | Empididae | Chelifera | 6 | PR |
| Diptera | Empididae | Clinocera | 6 | PR |
| Diptera | Empididae | Hemerodromia | 6 | PR |
| Diptera | Tabanidae | Chrysops | 7 | CG |
| Diptera | Tabanidae | Tabanus | 5 | PR |
| Diptera | Tipulidae | Tipula | 4 | SH |
| Diptera | Tipulidae | Antocha | 3 | CG |
| Diptera | Tipulidae | Dicranota | 3 | PR |
| Diptera | Tipulidae | Hexatoma | 2 | PR |
| Diptera | Tipulidae | Limnophila | 3 | PR |
| Diptera | Tipulidae | Ormosia | 6 | CG |
| Diptera | Tipulidae | Pilaria | 7 | PR |
| Diptera | Tipulidae | Pseudolimnophila | 2 | PR |
| Diptera | Simuliidae | Prosimulium | 5 | FC |
| Diptera | Simuliidae | Simulium | 6 | FC |
| Diptera | Chironomidae | Chironomidae | 6 | CG |
| Turbellaria | Turbellaria | Turbellaria | 9 | PR |
| Turbellaria | Planariidae | Dugesia | 9 | PR |
| Nematoda | Nematoda | Nematoda | 9 | CG |
| Gastopoda (Class) | Hydrobiidae | Hydrobiidae | 8 | SC |
| Gastopoda (Class) | Pleuroceridae | Pleuroceridae | 7 | SC |
| Gastopoda (Class) | Lymnaeidae | Lymnaeidae | 7 | SC |
| Gastopoda (Class) | Physidae | Physidae | 8 | SC |
| Gastopoda (Class) | Planorbidae | Planorbidae | 6 | SC |
| Gastopoda (Class) | Ancylidae | Ancylidae | 7 | SC |
| Bivalvia (Class) | Unionidae | Unionidae | 4 | FC |
| Bivalvia (Class) | Sphaeriidae | Sphaeriidae | 8 | FC |
| Bivalvia (Class) | Corbiculidae | Corbicula | 5 | FC |
| Hirudinea (Class) | Hirudinea (Class) | Hirudinea | 8 | PR |
| Oligochaeta (Class) | Oligochaeta (Class) | Oligochaeta | 10 | CG |
| Amphipoda | Crangonyctidae | Crangonyx | 4 | CG |
| Amphipoda | Gammaridae | Gammarus | 6 | CG |
| Amphipoda | Talitridae | Hyalella | 8 | CG |
| Decapoda | Cambaridae | Cambaridae | 6 | CG |
| Isopoda | Asellidae | Caecidotea | 6 | CG |
| Arachnida (Class) | Arachnida (Class) | Hydracarina | 7 | PR |
| Platyhelminthes | Platyhelminthes | Viviparidae | 7 | CG |

[1] Pollution Tolerance Values Range from 0 to 10, tolerance level decreases with the score.
[2] Functional Feeding Groups: Filter/Collector (FC), Predator (PR), Collector/Gatherer (CG), Shredder (SH), and Scraper (SC)

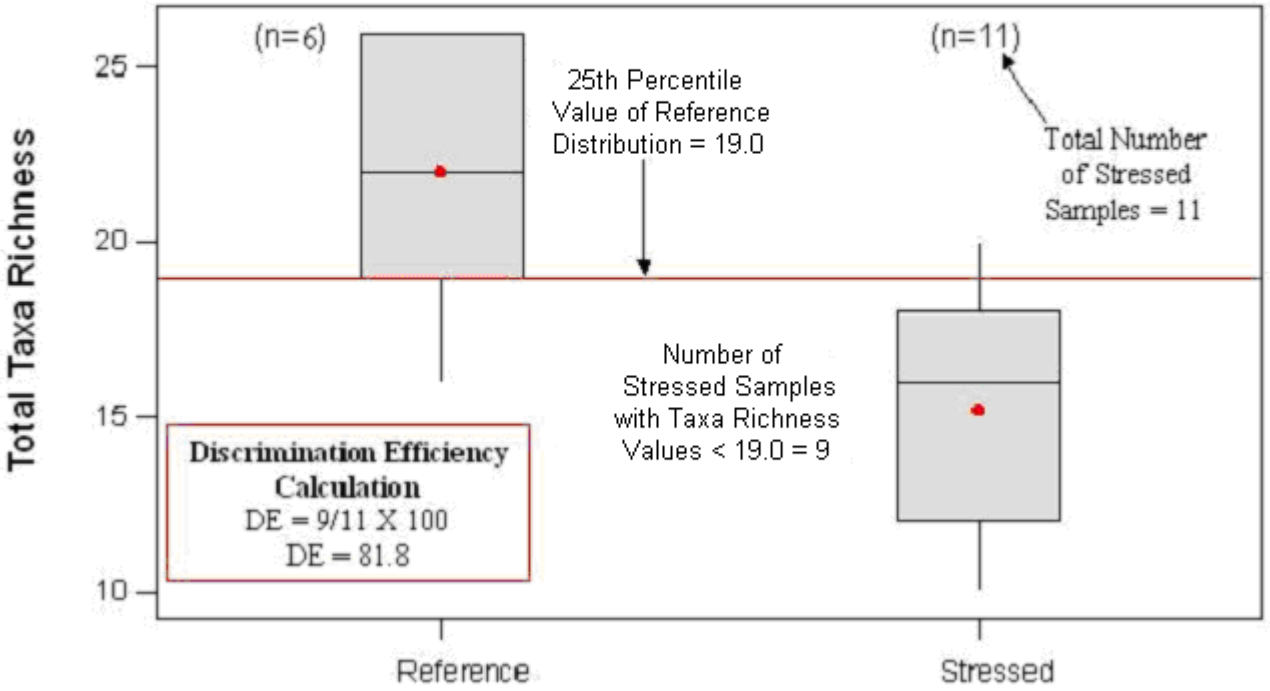
# APPENDIX E: METRIC DETERMINATION

## E.1 DISCRIMINATION EFFICIENCY BOX PLOT EXAMPLE

# Discrimination Efficiency Calculation Example

**For Metrics that Decrease with Pollution**

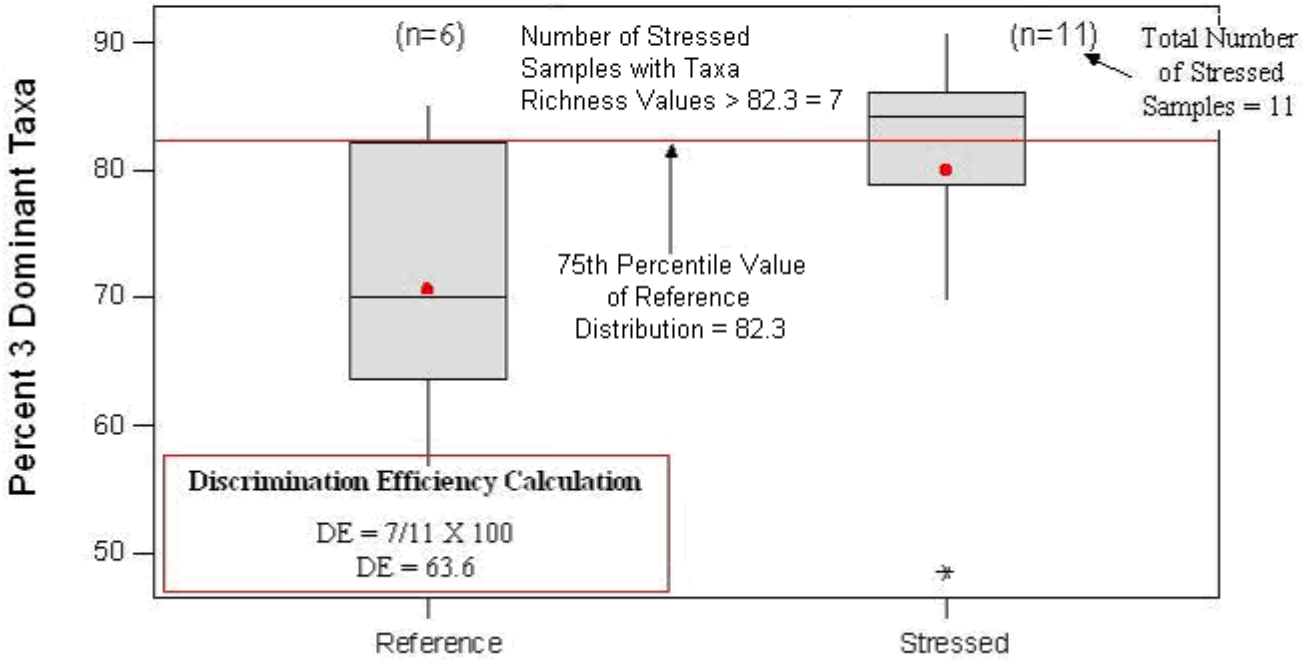
(means are indicated by solid circles)



# Discrimination Efficiency Calculation Example

**For Metrics that Increase with Pollution**

(means are indicated by solid circles)



## E.2 PEARSON CORRELATION COEFFICIENTS

## 13 CANDIDATE METRICS

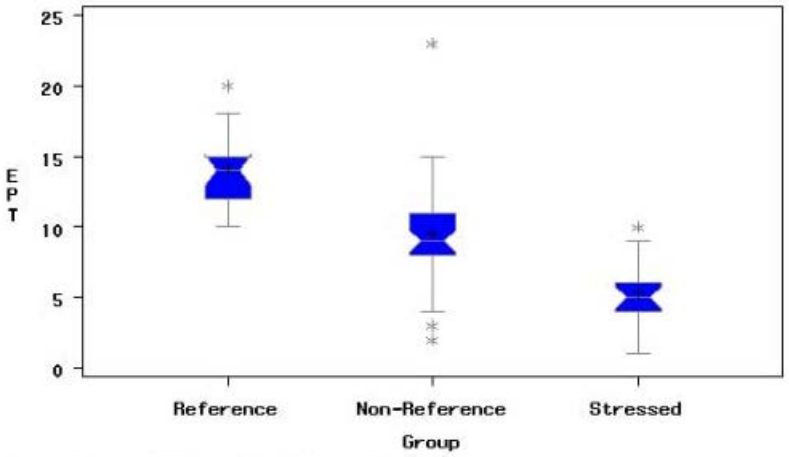
| NAME | EPT | % EPT | #Mayfly Taxa | Shannon Diversity | Taxa Richness | Beck3 | Beck4 | #Intolerant Taxa (Hils< 5) | % Intolerant Taxa (Hils< 5) | #Caddisfly Taxa | Trophic Diversity | % Tolerant Taxa | % Taxa as EPT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| EPT | 1.00 | | | | | | | | | | | | |
| %EPT | 0.60 | 1.00 | | | | | | | | | | | |
| #Mayfly Taxa | 0.74 | 0.42 | 1.00 | | | | | | | | | | |
| Shannon Diversity | 0.65 | 0.61 | 0.41 | 1.00 | | | | | | | | | |
| Taxa Richness | 0.85 | 0.42 | 0.67 | 0.70 | 1.00 | | | | | | | | |
| Beck3 | 0.71 | 0.56 | 0.41 | 0.43 | 0.57 | 1.00 | | | | | | | |
| Beck4 | 0.86 | 0.60 | 0.59 | 0.55 | 0.73 | 0.93 | 1.00 | | | | | | |
| #Intolerant Taxa (Hilsenhoff < 5) | 0.91 | 0.60 | 0.60 | 0.65 | 0.83 | 0.82 | 0.96 | 1.00 | | | | | |
| %Intolerant Taxa (Hilsenhoff < 5) | 0.50 | 0.76 | 0.25 | 0.60 | 0.41 | 0.56 | 0.62 | 0.65 | 1.00 | | | | |
| #Caddisfly Taxa | 0.83 | 0.42 | 0.34 | 0.63 | 0.76 | 0.52 | 0.67 | 0.76 | 0.44 | 1.00 | | | |
| Trophic Diversity | 0.75 | 0.47 | 0.31 | 0.68 | 0.77 | 0.64 | 0.73 | 0.80 | 0.45 | 0.76 | 1.00 | | |
| % Tolerant Taxa | -0.52 | -0.74 | -0.27 | -0.57 | -0.45 | -0.54 | -0.62 | -0.66 | -0.98 | -0.48 | -0.47 | 1.00 | |
| % Taxa as EPT | 0.73 | 0.52 | 0.51 | 0.27 | 0.31 | 0.55 | 0.63 | 0.59 | 0.36 | 0.54 | 0.36 | -0.35 | 1.00 |
| Discriminatory Efficiency (DE) | 100 | 82 | 88 | 88 | 94 | 82 | 82 | 94 | 76 | 94 | 76 | 76 | 82 |

## E.3 SIX CORE METRICS

| 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 Score (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 |

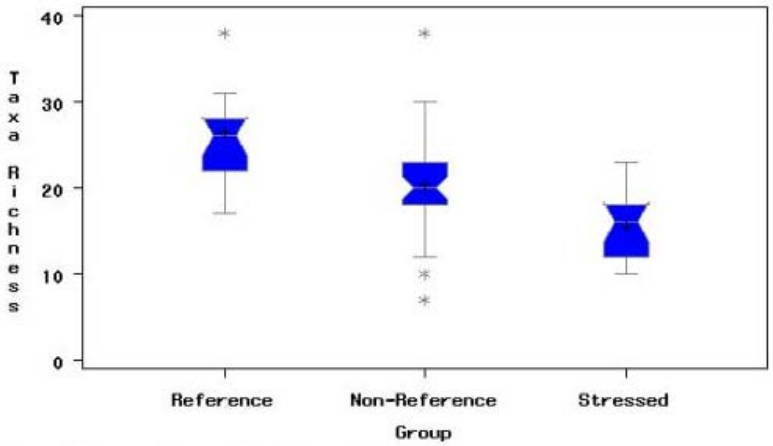
## E.4 BOX PLOTS OF THE SIX CORE METRICS

## EPT



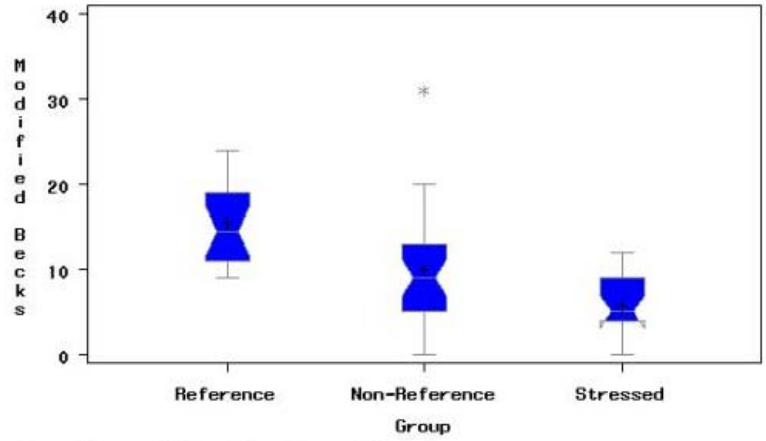

Group Sizes: Min n=17 Max n=37

## Taxa Richness



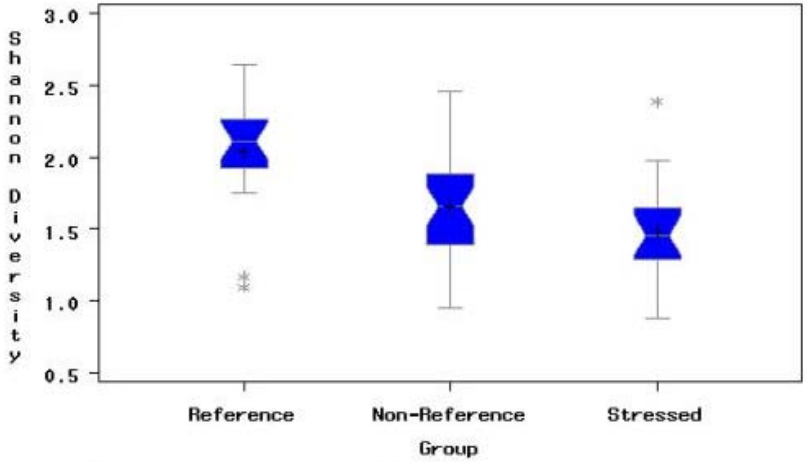

Group Sizes: Min n=17 Max n=37

## Modified Becks (Beck4)



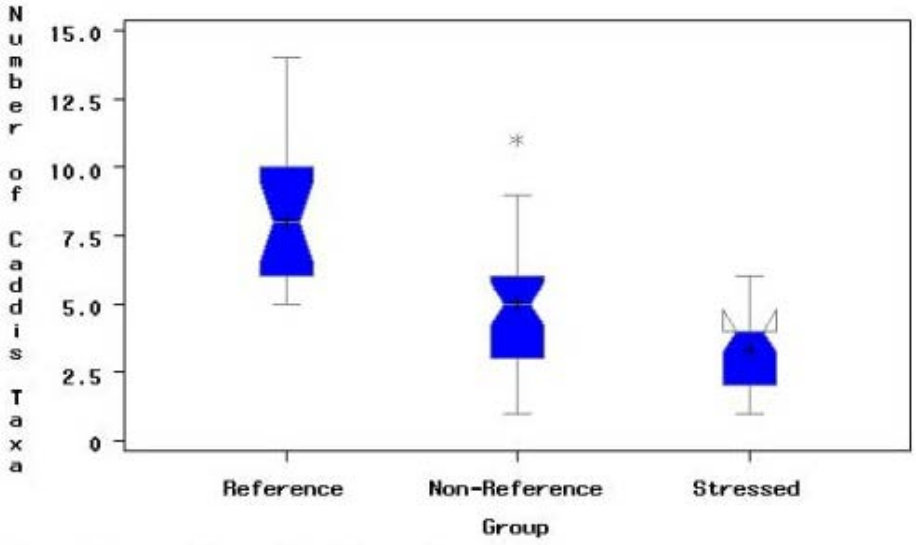

Group Sizes: Min n=17 Max n=37

## Shannon Diversity



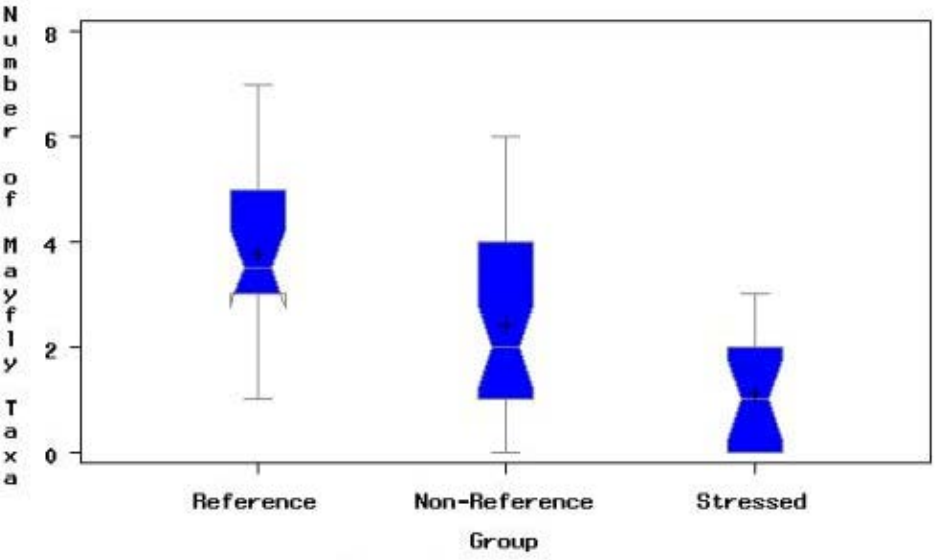

Group Sizes: Min n=17 Max n=37

## Number of Caddisfly Taxa




Group Sizes: Min n=17 Max n=37

## Number of Mayfly Taxa




Group Sizes: Min n=17 Max n=37

# APPENDIX F: METRIC AND TOTAL BIOLOGICAL SCORE CALCULATIONS

This appendix 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 from Appendixes B and C 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
Total # of taxa with Hils score of 0 or 1 = **5**
2 pts. x 5 = **10**

Total # of taxa with Hils score of 2,3,or4 = 11
1 pt. x 11 = **11**

**10** + **11** = **21**

Wiconisco Creek
Total # of taxa with Hils score of 0 or 1 = **1**
2 pts x 1 = **2**

Total # of taxa with Hils score of 2,3,or4 = 2
1 pt. x 2 = **2**

**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

TaxaRich = 26
P = 217 (sum the Number of Individuals column in the above tables)

Wiconisco Creek

TaxaRich = 12
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
Trichoptera = 9

Wiconisco Creek
Trichoptera = 1

**Number of Mayfly Taxa**

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

Saw Creek
Ephemeroptera = 2

Wiconisco Creek
Ephemeroptera = 0

Now that the six metric scores have been calculated, the scores are plugged into the normalized metric score equation: (Observed Value / 95$^{th}$ 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 1 and 2 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 1. Total Biological Score Calculation for Saw Creek**

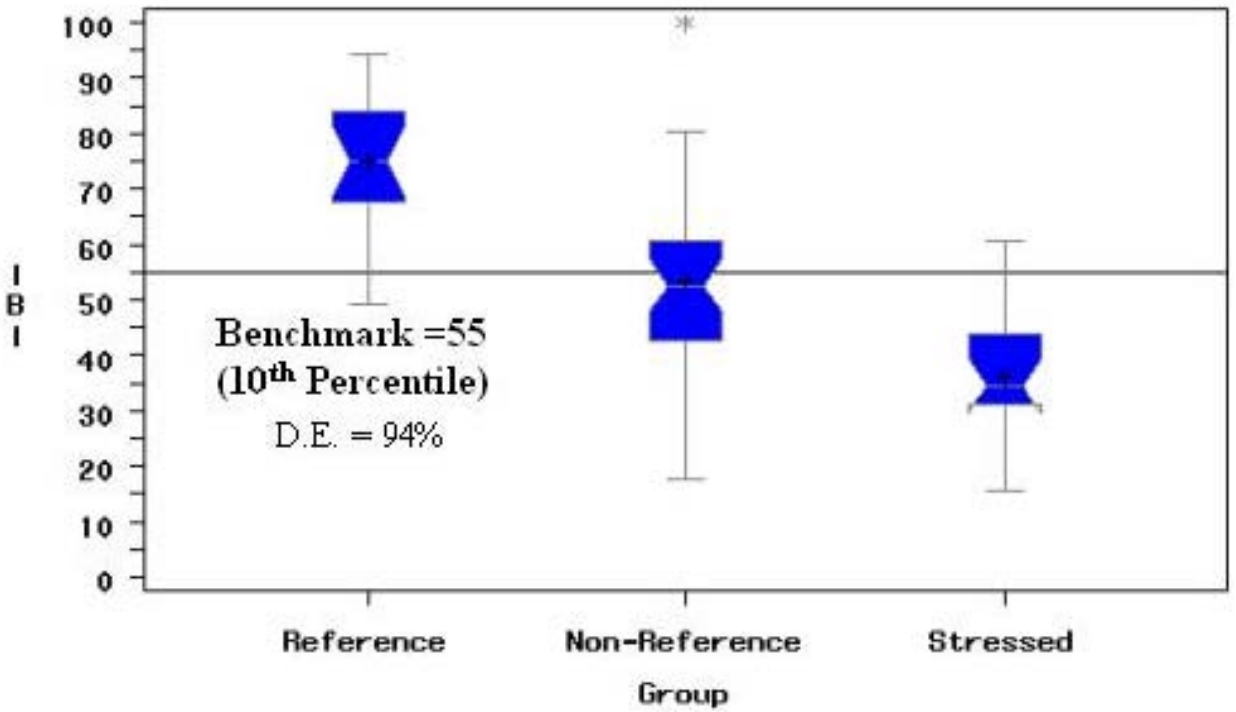
| Metric | Equation | Observed Value | Normalized Metric Score | Adjusted Metric Score (100 Max) |
|---|---|---|---|---|
| EPT | (Observed / **17**) x 100 | 15 | 88.2 | 88.2 |
| Taxa Richness | (Observed / **31**) x 100 | 26 | 83.9 | 83.9 |
| Beck4 | (Observed / **22**) x 100 | 21 | 95.5 | 95.5 |
| Shannon 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 2. 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** |

# APPENDIX G: MULTIHABITAT AQUATIC LIFE USE BENCHMARK

# Multihabitat Aquatic Life Use IBI



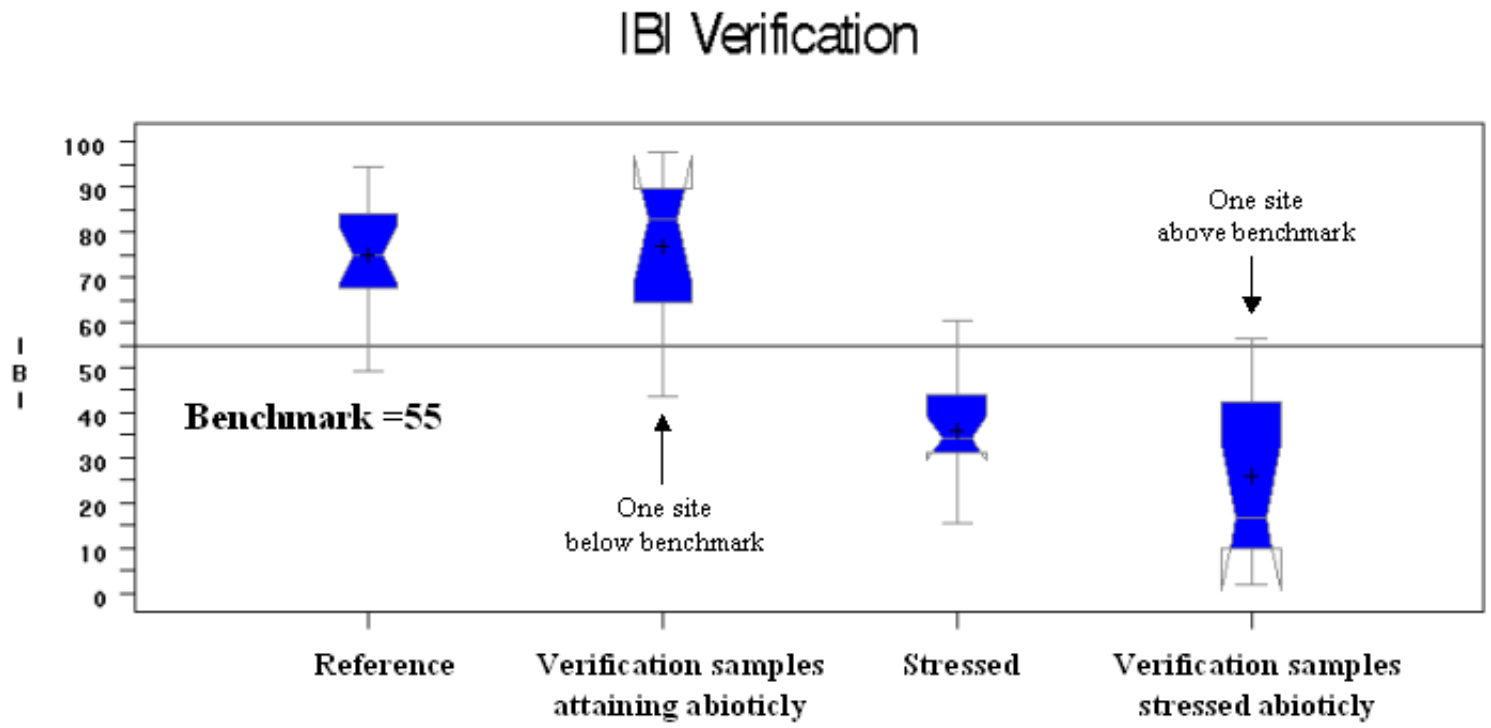

Group Sizes: Min n=17 Max n=37

# APPENDIX H: PROTOCOL VERIFICATION AND VARIABILITY

# H.1 METHODOLOGY AND BENCHMARK VERIFICATION RESULTS

| Total Biological Score | Benchmark | Predicted status | Stream Name | EPT | Taxa Richness | Beck4 | Shannon Diversity | # Of Caddisfly Taxa | # Of Mayfly Taxa |
|---|---|---|---|---|---|---|---|---|---|
| 93 | 55 | attaining | Beaver Run | 100 | 100 | 100 | 96 | 60 | 100 |
| 87 | 55 | attaining | White Deer | 86 | 88 | 80 | 100 | 75 | 70 |
| 83 | 55 | attaining | North Run | 100 | 80 | 86 | 82 | 50 | 100 |
| 82 | 55 | attaining | Mud Run | 88 | 80 | 95 | 89 | 60 | 83 |
| 98 | 55 | attaining | Sugar Run - Upper | 100 | 100 | 100 | 100 | 90 | 100 |
| 64 | 55 | attaining | Sugar Run - Lower | 65 | 60 | 68 | 69 | 40 | 83 |
| **44** | **55** | **attaining** | **Unt S Br Muddy Creek** | **41** | **50** | **36** | **83** | **30** | **17** |
| 65 | 55 | attaining | Beaver Run (Clearfield) | 71 | 50 | 64 | 66 | 50 | 83 |
| 53 | 55 | impaired | Tobyhanna | 59 | 50 | 55 | 62 | 50 | 50 |
| **57** | **55** | **impaired** | **Kitchen Run** | **59** | **60** | **36** | **80** | **40** | **67** |
| 43 | 55 | impaired | Kinzua Creek | 47 | 50 | 27 | 43 | 50 | 33 |
| 6 | 55 | impaired | Stump | 0 | 20 | 0 | 21 | 0 | 0 |
| 2 | 55 | impaired | Pentz Run | 0 | 10 | 0 | 4 | 0 | 0 |
| 18 | 55 | impaired | Juniata River | 6 | 40 | 5 | 41 | 0 | 17 |
| 16 | 55 | impaired | Reisinger Run | 12 | 30 | 9 | 24 | 20 | 0 |
| 41 | 55 | impaired | Muddy Creek | 35 | 60 | 36 | 52 | 30 | 33 |
| 15 | 55 | impaired | Pentz Run - Upper | 12 | 30 | 5 | 20 | 10 | 17 |
| 10 | 55 | impaired | North Fork Beech | 6 | 20 | 18 | 20 | 0 | 0 |

## H.2 BOX PLOT OF VERIFICATION SITES


IBI Verification
IBI
100
90
80
70
60
50
40
30
20
10
0
Benchmark =55
One site
below benchmark
One site
above benchmark
Reference
Verification samples
attaining abioticly
Stressed
Verification samples
stressed abioticly

## H.3 PROTOCOL VARIABILITY ANALYSE

| Variability Analysis | Number of Sample Pairs | Standard Deviation of Paired Samples | 90% Confidence (+/-) of IBI | Aquatic Life Use Attainment Status (Number of Sample Reaches) | |
|---|---|---|---|---|---|
| **Overall Method** | | | **One - *Two Samples** | **Upstream Reaches Attaining** | **Downstream Reaches Attaining** |
| Each pair consists of one sample collected from two adjacent stream reaches on the same day | 3 | 10.9 | 14.0 – 9.7 | 3 | 3 |
| **Overall Method Long-Term** | | | | **Initial Reaches Attaining** | **Reaches Attaining 1 to 2 Years Later** |
| Each pair consists of samples collected during the October-May sample collection window one or two years apart | 4 | 6.6 | 8.1 – 5.9 | 4 | 4 |

* The variability if two repeated samples are taken at a site.