APPENDIX A

A-1 Sample Site Location

Multihabitat Sample Sites
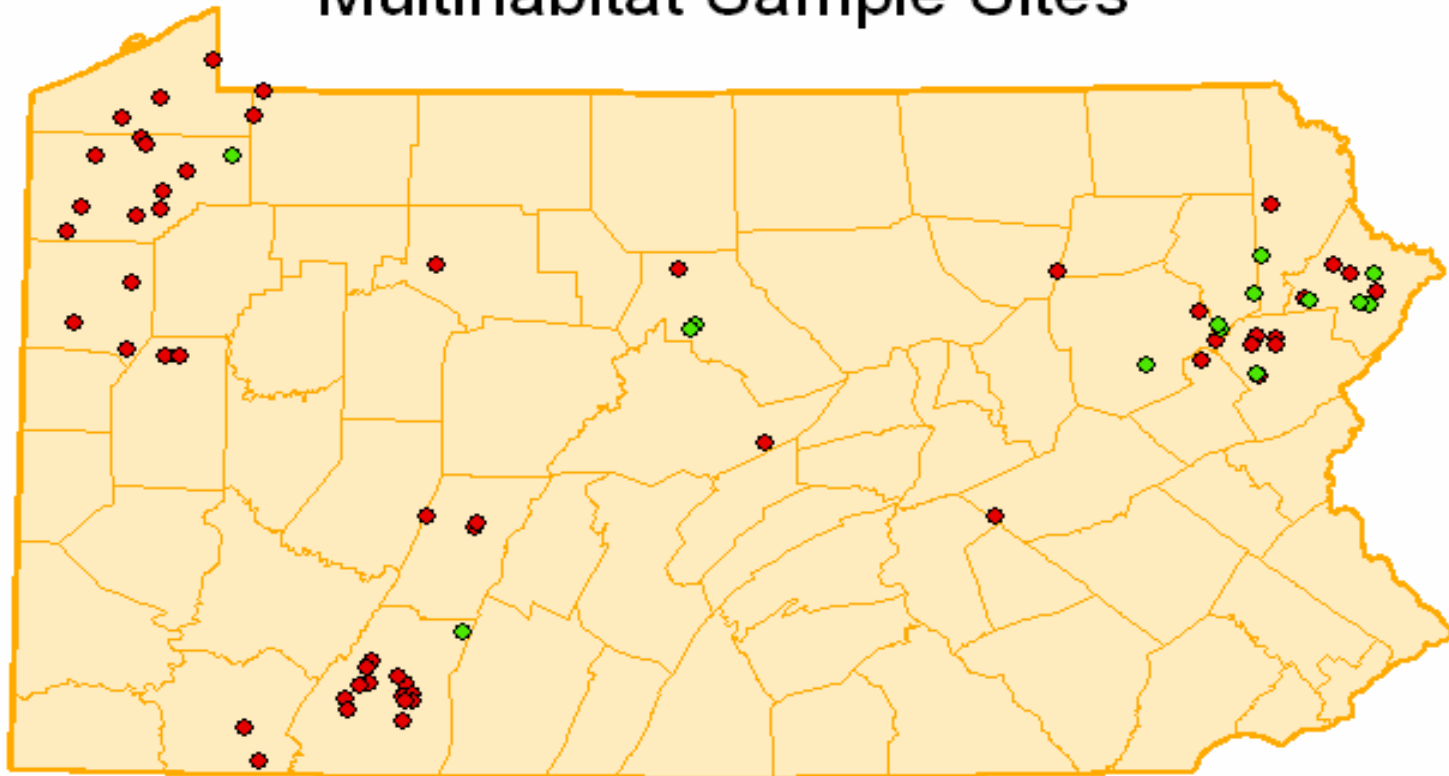
Legend

- ● Reference Sites
- ● Stressed Sites
- ▭ countyline

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

B-1 Habitat Assessment Field Data Sheet
B-2 Stream Habitat Types and Field Sampling Techniques

B-1

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 _____ TIME _____ AM PM	REASON FOR SURVEY

	Habitat Parameter	Condition Category			
		Optimal	Suboptimal	Marginal	Poor
Parameters to be evaluated in sampling reach	**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 not new fall and not 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

B-1 Continued

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) 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

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 4th 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

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

D continued

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

D continued

Order (Class)	Family	Taxa	Pollution Tolerance Value [1]	Functional Feeding Group [2]
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)
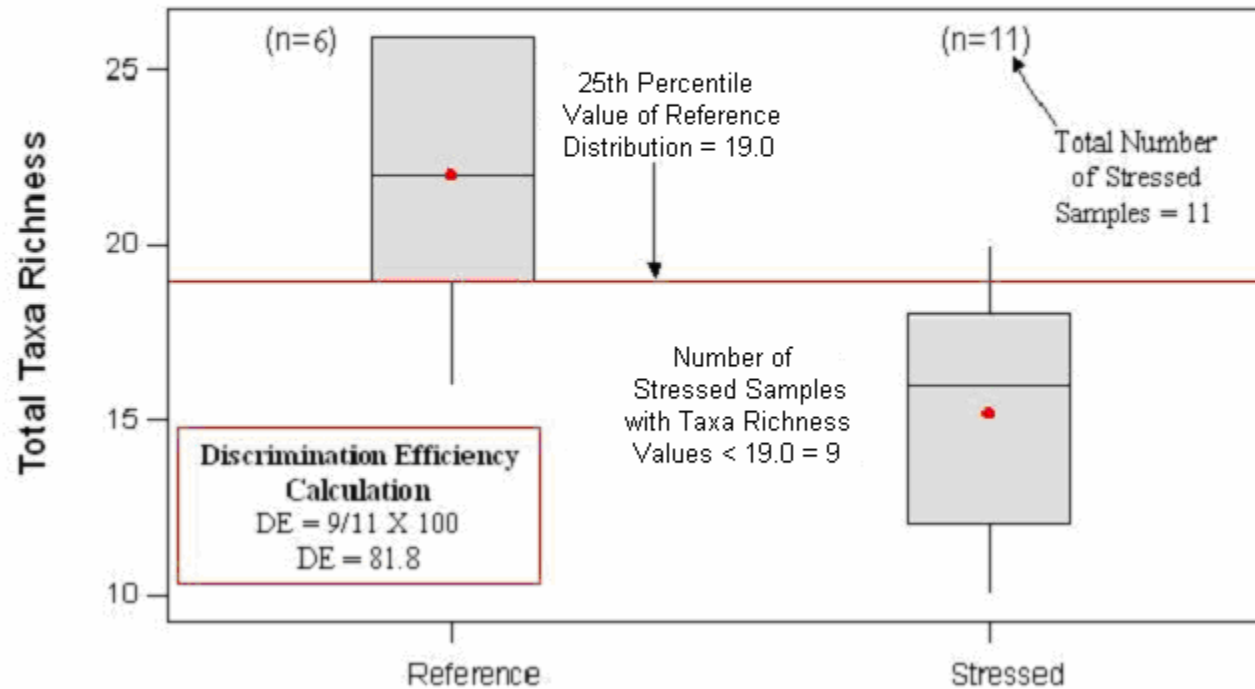
APPENDIX E
Metrics

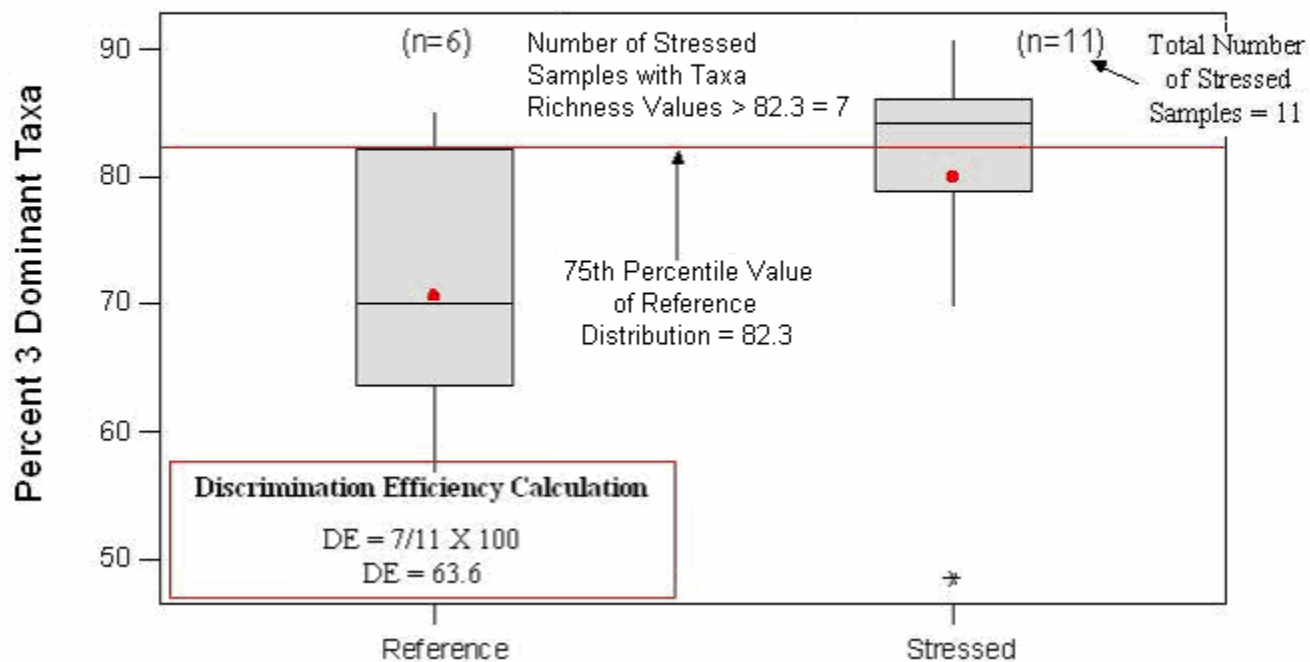Discrimination Efficiency Calculation Example
For Metrics that Decrease with Pollution
(means are indicated by solid circles)

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

6 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 organisms 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
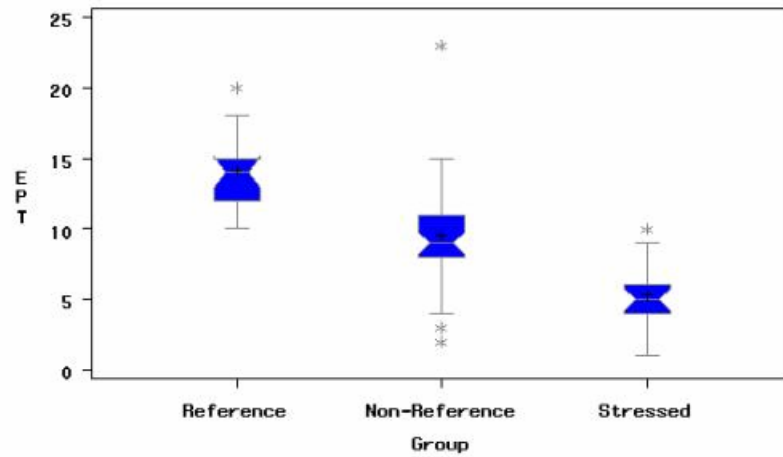
Taxa Richness
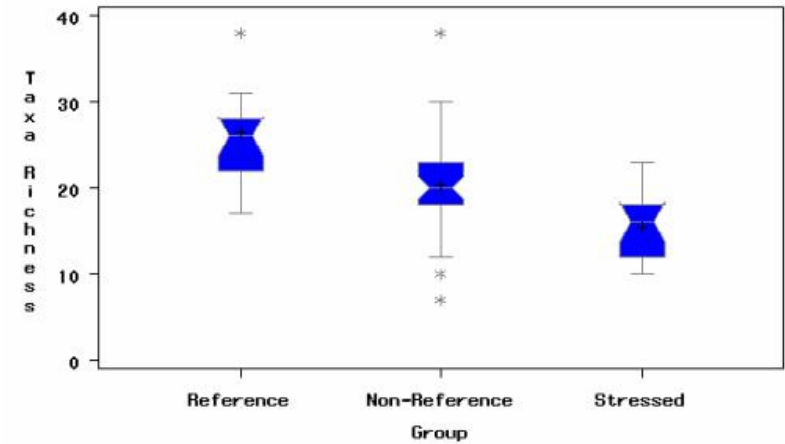
Modified Becks (Beck4)
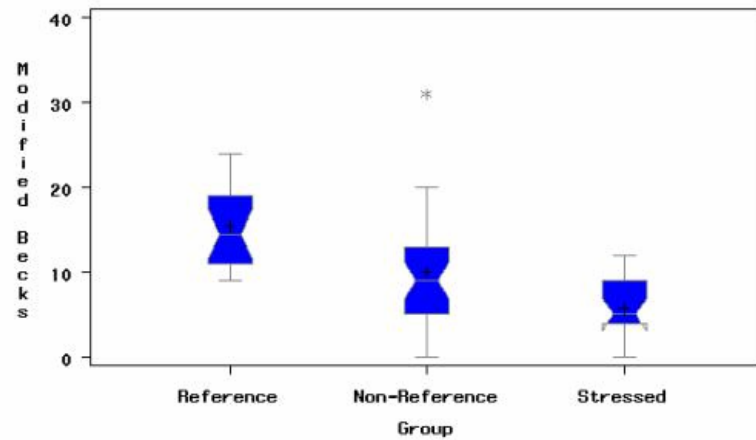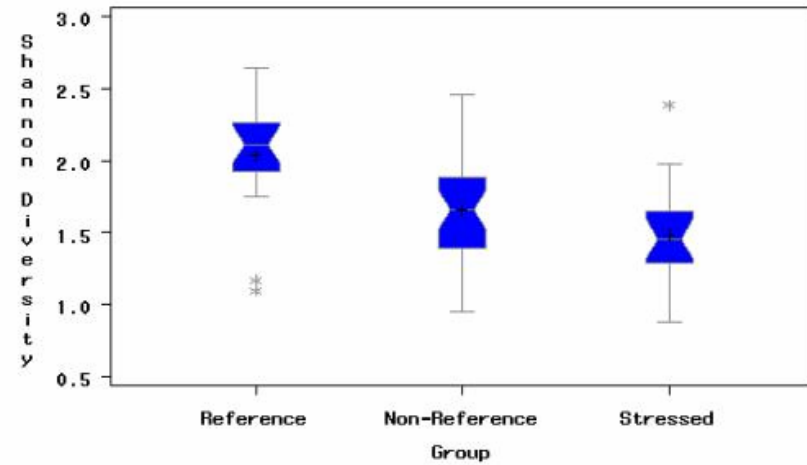

Shannon Diversity

E-4 Box Plots of the Six Core Metrics Continued

Number of Caddisfly Taxa

Group Sizes: Min n=17   Max n=37

Number of Mayfly Taxa

Group Sizes: Min n=17   Max n=37
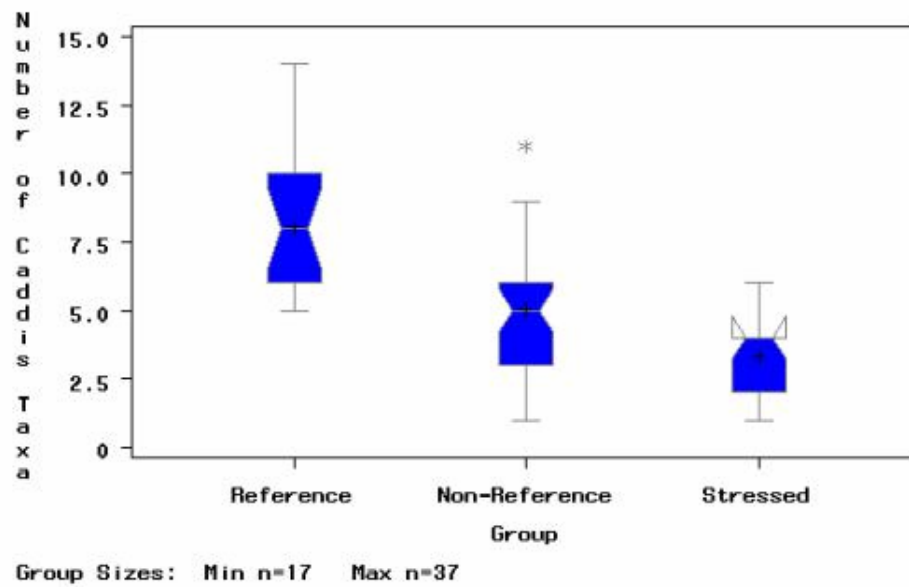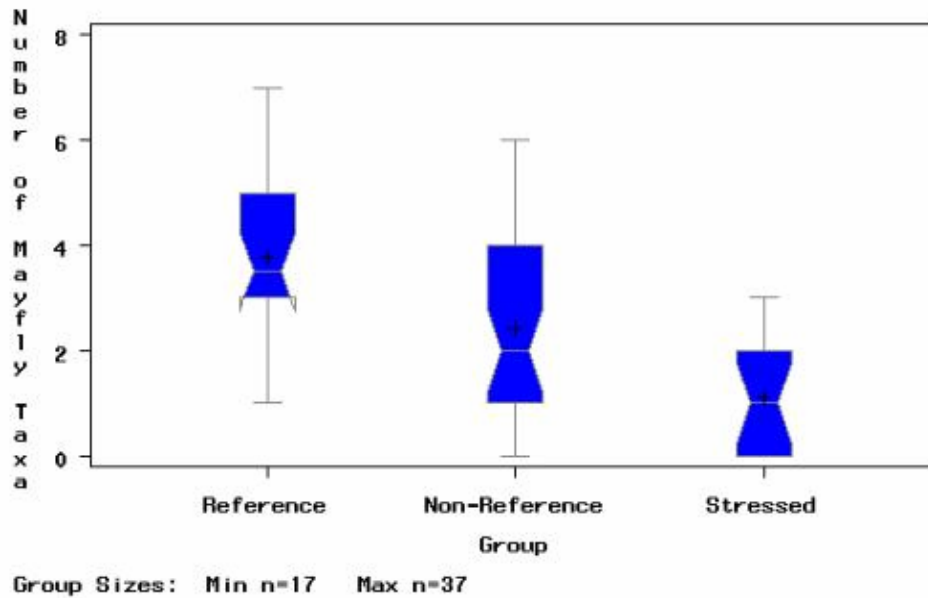
APPENDIX F

Metric and Total Biological Score Calculations

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

F - Metric and Total Biological Score Calculations

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			Wiconisco Creek		
Ephemeroptera =	2		Ephemeroptera =	0	
Plecoptera =	4		Plecoptera =	1	
Trichoptera =	9		Trichoptera =	1	
	15			**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, organisms 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.

F - Metric and Total Biological Score Calculations

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   P = 201
                    column in the above tables)
  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

F - Metric and Total Biological Score Calculations

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 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**

F - Metric and Total Biological Score Calculations

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

G - Benchmark

Multihabitat Aquatic Life Use IBI

Benchmark =55
(10th Percentile)

D.E. = 94%
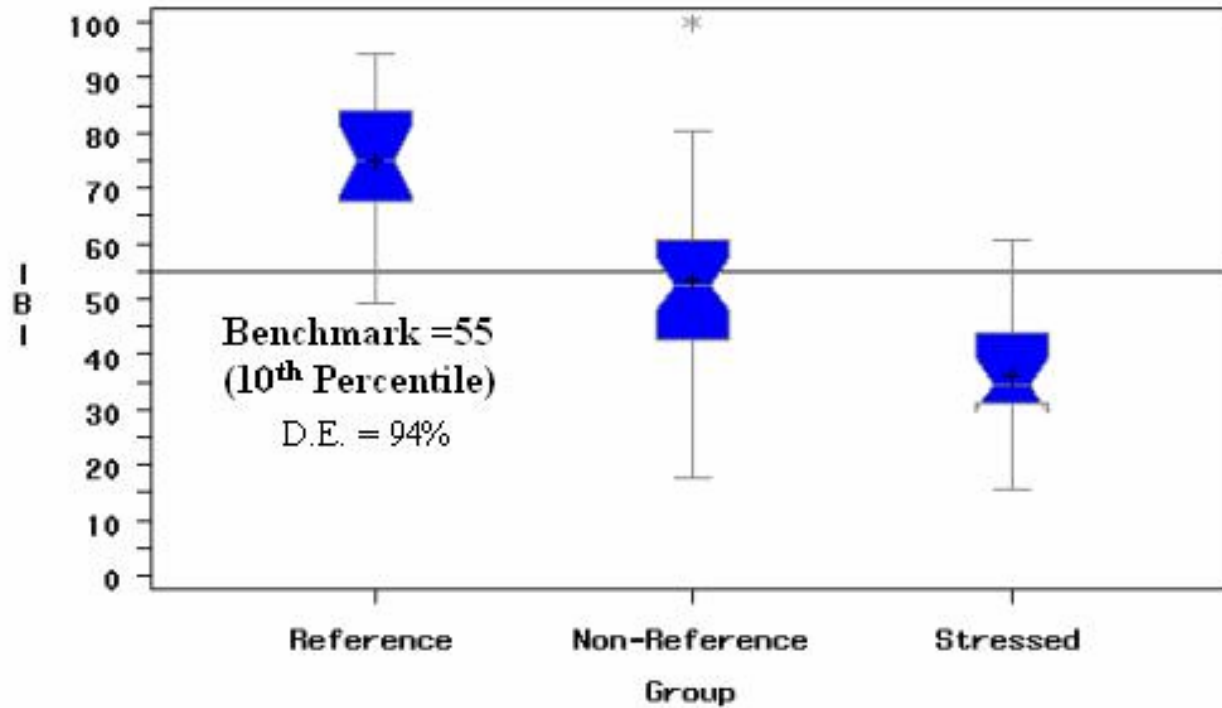
Group

Group Sizes: Min n=17   Max n=37

APPENDIX H
Protocol Verification and Variability

H –1 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 Verification Box Plot

IBI Verification
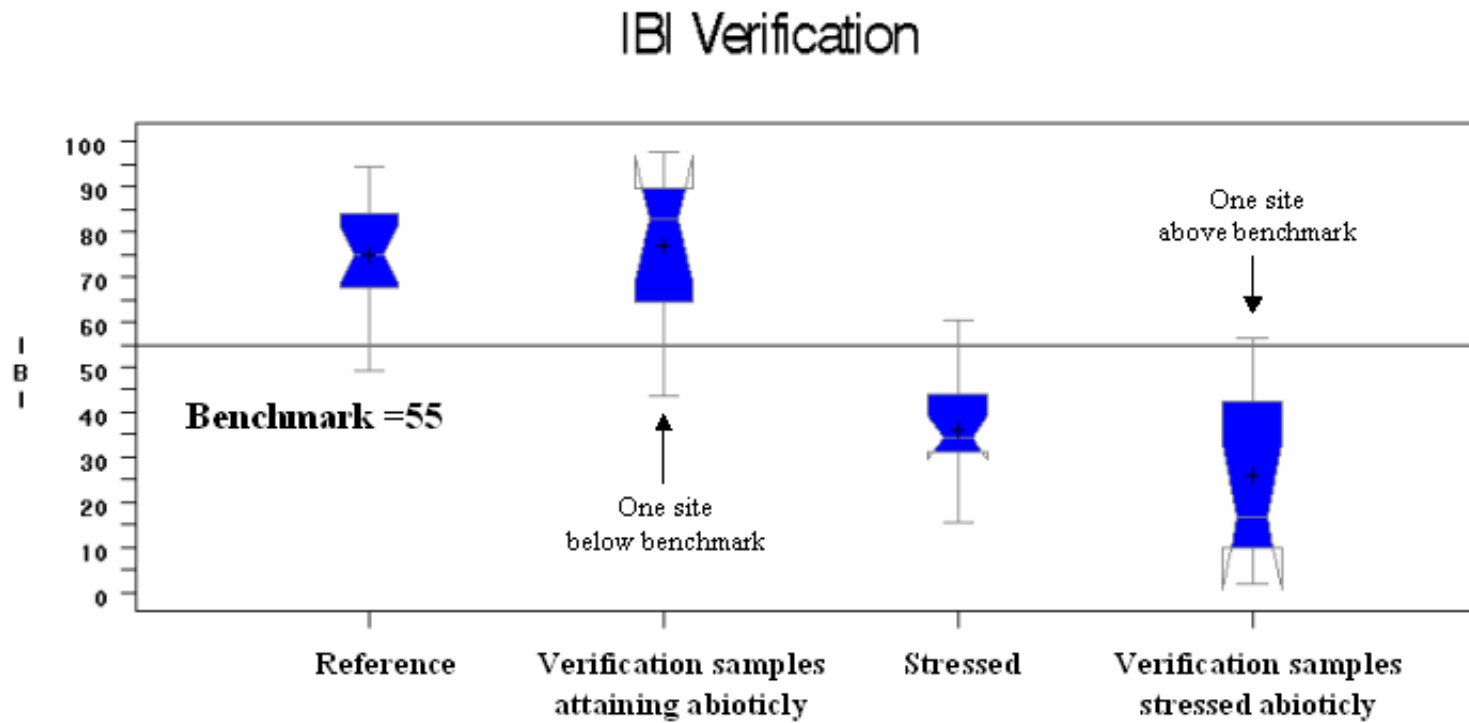
H-3 Protocol Variability Analyses

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.

APPENDIX I
Literature Cited

Literature Cited

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