DEPARTMENT OF ENVIRONMENTAL PROTECTION
Bureau of Water Standards and Facility Regulation

DOCUMENT NUMBER:	391-3200-001
TITLE:	Instream Comprehensive Evaluation Surveys
EFFECTIVE DATE:	Use current date (unless otherwise instructed)
AUTHORITY:	Pennsylvania Clean Streams Law, 35 P.S. §§ 691.1 et. seq., the Federal Clean Water Act, 33 U.S.C.A. §§ 1251 et. seq., 25 Pa Code Chapters 93 and 96.
POLICY:	This guidance provides the established procedures, as federally required, to conduct Instream Comprehensive Evaluation surveys.
PURPOSE:	The guidance was developed, as federally required, to establish and standardize the Departments procedures for conducting Instream Comprehensive Evaluation surveys.
APPLICABILITY:	This guidance applies to DEP staff that are conducting Instream Comprehensive Evaluation surveys.
DISCLAIMER:	The policies and procedures outlined in this guidance are intended to supplement existing requirements. Nothing in the policies or procedures shall affect regulatory requirements. The policies and procedures herein are not an adjudication or a regulation. There is no intent on the part of DEP to give the rules in these policies that weight or deference. This document establishes the framework within which DEP will exercise its administrative discretion in the future. DEP reserves the discretion to deviate from this policy statement if circumstances warrant.
PAGE LENGTH:	59 Pages
LOCATION:	Volume 30, Tab 13

Quality Assurance Work Plan
Instream Comprehensive Evaluation Surveys

July 13, 2005

Project Officer's Signature: _____

Project Officer's Name: Gary Walters

Project Quality Assurance Officer's Signature: _____

Project Quality Assurance Officer's Name: Tony Shaw

July 13, 2005
January 29, 2009

1. **Project Name:** Instream Comprehensive Evaluation Surveys

2. **Project Requested By:** PADEP Bureau of Water Standards and Facility Regulation, Review/Water Quality Monitoring and Assessment Process

3. **Date of Request:** 2005 Survey Year

4. **Date of Project Initiation:** May 2005, July 2008

5. **Project Officer:** Gary Walters

6. **Quality Assurance Officer:** Tony Shaw

7. **Project Description:**

   A. **Objective and Scope Statement:** To investigate and determine possible sources and causes of impairment from point or non-point sources of conventional pollutants and known or suspected in-stream water quality problems through the collection and analysis of biological, physical and chemical data. These surveys are performed to confirm and identify sources and causes of water quality impairments identified by previous Statewide Surface Water Assessment Program screenings and Section 303(d) listed water bodies for non-point source or point source pollution.

   Standardized qualitative and quantitative biological methods and water sampling techniques (Appendix A) are applied to short-term and chronic evaluations of stream impacts from point and non-point sources. Sampling sites are selected, where possible, to delimit the reaches of non-attainment of designated uses.

   B. **Data Usage:** Data are used for listing impaired waterbodies as required by Section 303(d), and to support the compliance and permitting programs by defining the impact of specific discharges or land based activities on receiving waters. Physical, chemical and/or biological data collected during surveys are generally evaluated using non-parametric, classification type analyses designed to display differences or similarities between sampling stations and metric thresholds.

   C. **Monitoring Network Design and Rationale:** Sampling locations are chosen to ensure that data representative of conditions in a given stream reach will be obtained. Factors considered in locating these stations include: watershed land uses, volume and chemical characteristics of known point source wastewater discharges, physiographic and demographic conditions that contribute to non-point source problems and stream hydrology. In flowing water bodies, every effort is made to sample representative, homogeneous low-flow water columns at comparable locations.

   D. **Monitoring Parameters and Their Frequency of Collection:** Sampling locations are listed in the final report for each survey. Both a narrative description and map are provided. In flowing water bodies water samples are collected as grabs at mid-channel, mid-depth unless stream width, hydrology, discharge locations or volumes, or observed biological conditions indicate stratification of flow. Parameters to be analyzed are listed in Table 1, of Section 7E of this document. All water chemistry samples are cooled to

4°C and shipped to the laboratory. Additional parameters may be required based on the specific nature of the water body survey. Biological samples are collected across a transect or throughout a large portion of the water body to ensure inclusion of all available habitat.

E. Parameter Table:

Table 1. Instream Comprehensive Evaluation Survey Parameters

Parameter	Number of Samples	Sample Matrix	Analytical Method Reference[1]	Sample Preservation	Holding Time
pH	Variable	Water	Std. Methods (Potentiometric)	None	Analyze in field
DO	Variable	Water	Std. Methods 421	None	Analyze in field
Specific Conductance	Variable	Water	Std. Methods 205	None	Analyze in field
Temperature	Variable	Water	Std. Methods 212	None	Analyze in field
$BOD_{5\text{-day}}$	Variable	Water	Std. Methods 5210B	Cool to 4ºC	48 hours
Residue, Dissolved at 105ºC	Variable	Water	Std. Methods 209C	Cool to 4ºC	7 days
TSS	Variable	Water	USGS-I-3765	Cool to 4ºC	48 hours
Alkalinity as $CaCO_3$	Variable	Water	Std. Methods 2320B	Cool to 4ºC	14 days
Hardness as $CaCO_3$	Variable	Water	Std. Methods 2340A+B	Cool to 4ºC	24 hours
Acidity, Total hot as $CaCO_3$	Variable	Water	Std. Methods 2310B	Cool to 4ºC	14 days
NH_3-N	Variable	Water	350.1	Field fix with H_2SO_4	48 hours
NO_2-N	Variable	Water	353.2	Cool to 4ºC	48 hours
NO_3-N	Variable	Water	353.2	Cool to 4ºC	48 hours
Kjeldahl N, Total	Variable	Water	351.2	Field fix with H_2SO_4	48 hours
Phosphorus, Total	Variable	Water	365.1	Cool to 4ºC Field fix with H_2SO_4	48 hours
Phosphorus, Dissolved	Variable	Water	365.1	Filter 0.45µ, Field fix with H_2SO_4	48 hours
Phosphorus, Ortho Dissolved	Variable	Water	365.1	Filter 0.45µ, Cool to 4ºC	48 hours
Phosphorus, Orthophosphate, Total	Variable	Water	365.1	Cool to 4ºC	48 hours
Calcium	Variable	Water	200.7 rev 4.4	Field fix with nitric acid to pH<2	6 months

Parameter	Number of Samples	Sample Matrix	Analytical Method Reference[1]	Sample Preservation	Holding Time
Magnesium	Variable	Water	200.7 rev 4.4	Field fix with nitric acid to pH<2	6 months
Cadmium	Variable	Water	200.8 rev 5.4	Field fix with nitric acid to pH<2	6 months
Copper	Variable	Water	200.7 rev 4.4	Field fix with nitric acid to pH<2	6 months
Lead	Variable	Water	200.8 rev 5.4	Field fix with nitric acid to pH<2	6 months
Nickel	Variable	Water	200.8 rev 5.4	Field fix with nitric acid to pH<2	6 months
Zinc	Variable	Water	200.8 rev 5.4	Field fix with nitric acid to pH<2	6 months
Aluminum, Total	Variable	Water	200.8 rev 5.4	Field fix with nitric acid to pH<2	6 months
Aluminum, Dissolved	Variable	Water	200.8 rev 5.4	Filter 0.1µ, Field fix with nitric acid to pH<2	6 months
Iron, Total	Variable	Water	200.7 rev 4.4	Field fix with nitric acid to pH<2	6 months
Manganese, Total	Variable	Water	200.7 rev 4.4	Field fix with nitric acid to pH<2	6 months
Chloride	Variable	Water	300.0	None	28 days
Chromium, Total	Variable	Water	200.7 rev 4.4	Field fix with nitric acid to pH<2	6 months
Mercury, Dissolved	Variable	Water	245.1	Field Filter 0.45µ, fix with nitric acid to pH<2	28 days
Sulfate	Variable	Water	300.0	Cool to 4ºC	28 days
Fecal Coliform Bacteria	Variable	Water	Std. Methods	Cool to 4ºC	30 hours*
Flow	Variable	Water	USGS approved methods	-	Measure in field

1 - EPA methods, unless otherwise specified
*Drinking Water Requirement - Special arrangements can be made with laboratory to meet the 6 hour wastewater holding time.

8. Project Fiscal Information:

Data Collection:

Office -	Planning, file searches, telephone calls & memos to resource persons	1	workday
Field -	Reconnaissance, water and biological sample collection, field observations	3.5	workdays
Taxonomic Identification of Biological Samples:		1.5	workdays

Laboratory Support: Routine chemical/physical/bacteriological/special analyses	2	workdays
Report Preparation: Data synthesis and compilation, analysis and interpretation, recommendations, draft preparation, review/comment, revisions	2	workdays
Subtotal - funded through 106	10	workdays
Regulatory Review: Executive Summary, Preamble, Regulatory Review forms, EQB meeting		N/A
Public Participation: PA Bulletin Notice, Hearings, responsive summaries		N/A
Final Rulemaking: Revisions/regulatory reviews/EQB meeting		N/A
TOTAL	10	Workdays

9. Schedule of Tasks and Products

Instream Comprehensive Evaluation survey work is carried out by the Regional Offices on an on-going basis and stream surveys can be scheduled from May to October.

Date

Activity	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
File Search												
Field Reconnaissance												
Field Sampling												
Lab Work-up												
Report												

10. Project Organization and Responsibility

The following is a list of key project personnel and their corresponding responsibilities, and an organizational chart is included to better define their relationships:

Regional Biologists - sampling operations

Chief, Regional Operations or Planning and Finance - sampling QC

Bureau of Laboratories:

Inorganic Division

Chief, Trace Metals & Sample Receiving Section

Chief, Automated Analysis & Biochemistry Section - laboratory analysis

Organic, Radiation & Biological Division

Chief, Biological Section - laboratory QC

Regional Biologist - data processing activities

Chief, Water Quality Assessment Section - WQ assessment database QC

Regional Liaison; (WQ Assessment Database) - data quality review

Regional Operations Chief & Project Officer - performance auditing

Regional Liaison & Project Officer - systems auditing

Chief, Water Quality Monitoring Section - overall QA

Chief, Water Quality Monitoring Section - overall project coordination

11. Data Quality Requirements and Assessments

Accuracy is determined by routine laboratory protocol, which requires random spiking of samples as described in the Quality Assurance Manual for the PA Department of Environmental Protection Bureau of Laboratories (PaDEP 2008). Precision is determined by collecting field duplicate samples at the rate of 1 in 20 or a minimum of one field duplicate per survey. See Table 2 for data quality information obtained from the laboratory.

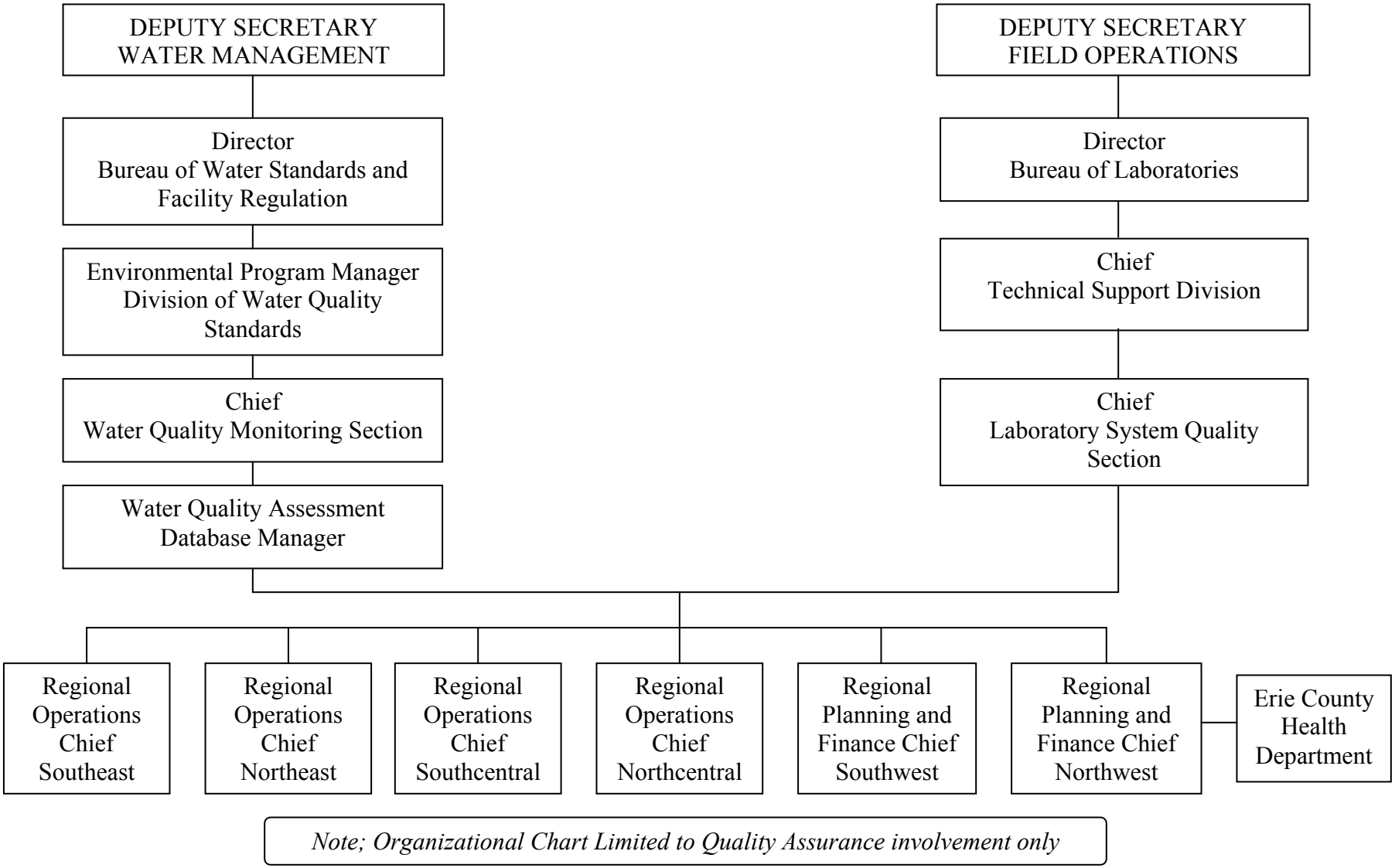
Data Representativeness: Streams studied are divided into representative reaches based upon physiographic and demographic characteristics of the watershed. A sampling station is located in each stream reach. Biological samples are collected along a 100 meter stream transect and chemical grab samples are collected at mid-channel, mid-depth unless stream hydrology or biology indicates a need for composites or depth integrated samples.

Data Comparability: Sampling stations are chosen for physical similarity (i.e., comparable habitat) to help ensure data comparability. Sampling techniques are standardized to insure consistency and repeatability. If circumstances of water body access or hydrology preclude sampling physically similar sites, the differences between stations are assessed using observations of water body and riparian physical characteristics and noted on the field data sheets (Appendix B).

Data Completeness: The following data are collected from each station: water chemistry, semi-quantitative biological data, and physical measurements/observations of riparian land use, stream substrate composition, hydrologic conditions (flow/depth and channel configuration), aquatic habitat, temperature, pH, and dissolved oxygen.

After field reconnaissance is completed, the sample stations are located, so that consideration of point source discharges and changes in the physical attributes of the water body and watershed become an integral part of the assessment. Spatial distribution of sampling stations is arranged so that suspected physical/chemical or biological changes will be detected.

FIGURE 1
QUALITY ASSURANCE ORGANIZATION CHART



Duplicate water samples for chemical analysis are collected at least once on each survey and are concentrated in the effected stream reach. These samples serve the purpose of ensuring data completeness and as a quality assurance check of lab analysis techniques. One field blank is carried on each survey and is opened in the field to serve as a quality assurance check of field sampling techniques. The field blank is prepared by the investigator in the laboratory prior to the trip and consists of 500 ml of deionized distilled water in a 500 ml sample bottle rinsed with deionized distilled water. The field investigator will review sample results and note if target parameters are detected in the field blank and flag samples accordingly in the database. Duplicate sample results will be compared; and if parameter values exceed the laboratory precision, laboratory QA/QC personnel will be notified. Sample custody procedures (Section 13 of this document) are followed to ensure proper processing.

Completeness will be judged on whether the minimum number of samples can be collected in order to make a determination of the attainment of designated aquatic life uses. If data is deemed to be incomplete resampling will be required.

Table 2. Instream Comprehensive Evaluation Survey Parameter Data Quality Assessments

STORET	Parameter	Mean Lab Control Value*	Mean Percent Recovery*	Percent Relative Standard Deviation*
00403	pH	N/A		
00410	Alkalinity, Total as $CACO_3$ (Titrimetric)	243.7 mg/l	99.48	0.7994
00900	Hardness, Total (Calculated)		Data Not Available	
70508	Acidity, Total hot as $CACO_3$ (Titrimetric)	495.3 mg/l	99.07	0.8831
00310	Biochemical Oxygen Demand 5 Day	198 mg/l	99.3	14.7
00515	Residue, Dissolved at 105° C	N/A, varies	109.4	10.66
00530	Total Suspended Solids	N/A, varies	89.5	12.1
00600A	Nitrogen, Total	5.08 mg/l	102	1.72
00610A	Ammonia, Total as Nitrogen	0.97 mg/l	97	1.7
00615A	Nitrite Nitrogen, Total	1.03 mg/l	103	4.36
00620A	Nitrate as Nitrogen	1.01 mg/l	101	8.25
00630A	Nitrite + Nitrate, Total	1.43 mg/l	102	1.12
00625A	Kjeldahl Nitrogen, Total as Nitrogen		Data Not Available	
00665A	Phosphorus, Total as P	0.500 mg/l	100	2.50
00666A	Phosphorus, Dissolved as P	0.500 mg/l	100	2.50
00671A	Phosphorus, Ortho Dissolved	0.391 mg/l	97.8	3.23
70507A	Phosphorus, Total, Orthophosphate as P	0.391 mg/l	97.8	3.23
00916A	Calcium, Total by Trace Elements	5.04 mg/l	101	1.68
00927A	Magnesium, Total by Trace Elements	5.11 mg/l	102	1.96

STORET	Parameter	Mean Lab Control Value*	Mean Percent Recovery*	Percent Relative Standard Deviation*
01027H	Cadmium, Total by Trace Elements	50.5 µg/l	101	1.59
01042A	Copper, Total by Trace Elements	101 µg/l	101	2.00
01051H	Lead, Total by Trace Elements	50.2 µg/l	100	2.08
01067H	Nickel, Total by Trace Elements	99.8 µg/l	99.8	2.02
01092H	Zinc, Total by Trace Elements	99.0 µg/l	99.0	1.62
01105H	Aluminum, Total by Trace Elements	101 µg/l	101	3.03
01106D	Aluminum, Dissolved 0.1 micron filter		Data Not Available	
00945	Sulfate by Ion Chromatography	20.22 mg/l	101.1	3.703
01045A	Iron, Total by Trace Elements	1053 µg/l	105.3	1.629
01055A	Manganese, Total by Trace Elements	100 µg/l	100	2.38
00940	Chloride by Ion Chromatography	9.86 mg/l	98.6	2.51
00951	Fluoride, Ion Chromatography	0.204 mg/l	102	9.65
01034A	Chromium, Total by Trace Elements	208.34ug/l	104.2	2.42
00080	Color	39.54 PT/C	98.9	10.1
718901	Mercury, Dissolved		Data Not Available	
31616	Fecal Coliform		Data Not Available	

* Time period for all laboratory data quality assessments in Table 2 was October 1 to December 14, 2007.

Accuracy is considered acceptable and meeting established criteria when within + or - 20 percent of a known (80-120 percent recovery). Percent Recovery is calculated from the mean analyte recovered for the period, divided by the lab control value. Standard Deviation for the period of observation is calculated in Microsoft Excel using spreadsheet functions for standard deviation and mean.

Standard Deviation

$$S.D. = \sqrt{\frac{\sum_{s=1}^{m} \sum_{i=1}^{n} (y_{is} - M)^2}{(n_y - 1)}}$$

$$M = \frac{\sum_{s=1}^{m} \sum_{i=1}^{n} y_{is}}{n_y}$$

where:

s = series number
i = point number in series s
m = number of series for point y in chart
n = number of points in each series
y_{is} = data value of series s and the ith point
n_y = total number of data values in all series
M = arithmetic mean

Standard Error

$$S.E. = \sqrt{\frac{\sum_{s=1}^{m} \sum_{i=1}^{n} y_{is}^{2}}{(n_y - 1)((n_y)}}$$

12. Sampling Procedures

See attached Instream Comprehensive Evaluation Surveys protocol (PaDEP 2008, Appendix A), and Habitat Assessment Forms (Plafkin et al. 1989, Appendix B). All field collections will be made in accordance with the Bureau of Water Standards and Facility Regulation's Field Procedures, Standard Operating Procedures, Standardized Biological Field Collection Methods (PaDEP 2003), and USGS stream gauging techniques.

13. Sample Custody Procedures

Samples collected in the field are identified by date, time, place, and survey name and are accompanied by a Request for Chemical Analysis Form. Both the form and sample container bear a unique 7 digit identifying number and are transported together (in a shipping cooler) to the DEP Bureau of Laboratories in Harrisburg via contracted courier service.

14. Calibration Procedures and Preventive Maintenance

Meter calibration should be accomplished at the beginning of each sampling effort in accordance with the manufacturer's recommendations. In the case of pH and specific conductance, this is accomplished using a reference standard. Calibration checks should be performed throughout the day if multiple samples will be collected. Results of calibration and the performance of preventative maintenance recommended by the manufacturer must be recorded in an equipment logbook maintained for each piece of equipment. Dates of equipment use, calibration results and operator maintenance activities must be recorded.

15. Documentation, Data Reduction, and Reporting

A. Documentation: Field data is recorded on prescribed field forms (see Appendix C). The biologist responsible for the survey reviews the field forms for completeness and legibility at the completion of each survey. The results of laboratory biological identification are recorded on prescribed forms and initialed by the taxonomist. Field forms and notes, taxonomic forms, survey maps, correspondence, and all other pertinent

information are kept in coded water body files maintained by the Bureau of Water Standards and Facility Regulation.

B. Data Reduction and Reporting: Coded field and laboratory data are transferred to a standard computer database. After the entry is complete, the biologist responsible for the survey reviews a listing of the data for accuracy and completeness. A copy of the verified data listing is initialed, dated, and maintained in the water body file. Further problems with transcription errors are avoided by transferring data from the database to tabulating or analytical programs using verified automated transfer methods. Final survey reports are submitted to the Department's Regional Operations, Permits, and Sewage Planning Chiefs and contain chemical, physical and biological results and conclusions on permit compliance.

16. Data Validation

The protocol for validation of chemical data is found in the Quality Assurance Manual for the PA Department of Environmental Protection Bureau of Laboratories (PaDEP 2008). A log is maintained of field instrumentation calibrations, performance, and repairs. Taxonomy of questionable organisms is verified by cross checking with other taxonomists. Database fields are validated through error checking routines and automatic exclusion of data outside of specified ranges. Records of analyses used in the assessment of survey data are maintained in the water body file. At a minimum, this includes a copy of the data used in the analytical program, a copy of the analytical program, the program output, normality testing (if parametric tests are used), and a rationale for eliminating outliers or creating data subsets. The outputs shall be initialed and dated by the analyst.

17. Performance and Systems Audits

An auditor accompanies each individual on at least one survey per season to insure adherence to protocols. The auditor shall also select, at random, water body files to verify that data documentation is accurate and complete, and that appropriate analytical techniques are used. The auditor will maintain records for each individual to include: (1) date of audit; (2) a list of protocols for which the individual was evaluated; and (3) any deficiencies noted.

18. Corrective Action

Errors are detected through verification of data by the biologist responsible for the survey and/or taxonomist, in-house review of reports, and audits. These can be traced to an individual through the initialed documentation within the water body files. When problems are noted, the individual is notified, provided with the appropriate protocol and training, and reevaluated before performing the task again. The auditor shall maintain the records of any corrective actions on the Department's Employee performance evaluation system.

19. Reports

Reports concerning ongoing performance and systems audits, data quality assessments, and significant quality assurance problems will be included in the quarterly grant status report to EPA and will be provided to the Department's Quality Systems Program Manager and the Chief of the Division of Water Quality Standards.

APPENDIX A

BUREAU OF WATER STANDARDS AND FACILITY REGULATION INSTREAM COMPREHENSIVE EVALUATION SURVEYS (MARCH 2005)

SURVEY PROTOCOL

INSTREAM COMPREHENSIVE EVALUATION SURVEYS

I. PURPOSE:

This survey protocol is intended to assess the aquatic life uses of Pennsylvania's wadeable waters and will be applied to those riffle/run dominated stream segments previously assessed by the Statewide Surface Water Assessment Program's (SSWAP) Biological Screening Protocol. Assessments of low gradient, limestone, and non-wadeable streams will be based on protocols developed for those stream types.

This Instream Comprehensive Evaluation Survey protocol will target streams with the following assessment needs - those streams identified as:

- Attaining aquatic life uses but may be "at risk" of impairment;
- Impaired but needing more intensive follow-up assessment because the source or cause of impairment could not be clearly determined by the SSWAP Biological Screening Protocol, other assessment methods, or during future assessment cycles;
- Needing more detailed field information for TMDL support;
- Candidates for impairment delisting from the PA CWA Section 303(d) list; or
- Unimpaired waters in need of confirmation.

While the SSWAP biological screening protocol was effective in determining impairment/non-impairment conditions for most streams, it was not rigorous enough to adequately assess streams with Antidegradation aquatic life uses (High Quality and Exceptional Value). Those streams with Antidegradation aquatic life use designations that were not effectively assessed by the SSWAP biological screening will be reassessed by the Aquatic Life Special Water Quality Protection Survey protocol specifically designed for Antidegradation evaluations.

This new protocol describes a more intensive field survey and water quality assessment approach than that used in the biological screening protocol. Once a waterbody has been identified as needing an Instream Comprehensive Evaluation Survey, the biologist must design a study plan that will effectively assess the nature of impairment, "at risk" conditions, or other questions relating to use attainment status. The survey must consider previous assessment results and station locations. Further, because these survey results will replace existing data entries derived from aquatic surveys using different field methods of varying levels of intensity, more intensive survey methods are necessary to describe the condition of the waterbody in question. In the case of these impairment characterization assessments the following procedures will apply.

II. FIELD ASSESSMENTS:

In order to evaluate the aquatic life uses of the targeted streams mentioned above, assessments will require more rigorous field data collection and observations. Physical, chemical, habitat, and biological data may be collected as prescribed below as determined by the identified

potential of specific source(s) and cause(s) for each waterbody. The minimum data collection requirements and assessment options are described below.

A) Physical – Chemical Field Data and Observations

1) Field Chemistry (required)

Detailed field observations on land use and potential sources of pollution in the study watershed are recorded on field data collection forms following a thorough reconnaissance of the watershed. Dissolved oxygen, pH, specific conductance, and temperature are measured in the field using hand-held meters calibrated according to manufacturer specifications. Total alkalinity can be measured using available field test kits or a water sample can be sent to the Bureau of Laboratories for analysis.

2) Water Chemistry (as needed)

Chemical characterization of the water body is driven by the need to identify sources and causes of impairment and/or the needs of the TMDL model.

Water samples for laboratory analyses are collected in 125 and/or 500 ml plastic bottles with appropriate fixatives added in the field (as needed) in accordance with the DEP Laboratory's prescribed Analytical Methods and the QAPP for this survey protocol. All samples are iced and returned to the DEP laboratory for analysis. If needed, separate water samples for dissolved metals and dissolved phosphorus analyses are filtered in the field through 0.45-micron filters using a portable filtration apparatus. Samples are collected throughout the watershed in such a manner to identify potential sources of impairment.

Measurement of stream discharge is required when water chemistry samples are collected and bankfull channel cross-section are measured if needed for the TMDL model, or if stormwater or nutrients are involved in the use impairment, according to the Department's Stream Flow Measurement Protocol (Appendix D). At least one discharge and bankfull channel cross-section measurement will be made at each sampling station.

Standard Analysis Codes (SACs) are lists of chemical parameter analyses required to confirm specific suspected source and cause impairments. The SACs recommended for specific impairments are indicated in pertinent source and cause sections that follow and in Appendix B. The investigator is not limited to the parameters in the SACs and may need to add additional parameters of special concern in order to identify causes of impairment.

a) Point Source

For these follow-up surveys, representative water samples are collected from the discharge pipe, from upstream (control), and downstream locations at a minimum. Sampling stations located upstream of the discharge pipe should be in a non-impacted zone to serve as a control. If

there are multiple discharges, then sample stations should be placed to bracket individual discharges in order to better characterize each source. For sampling downstream of the discharge pipe, the investigator should avoid the immediate vicinity of the discharge point and select a sample point far enough downstream to allow for mixing between the discharge and stream flow. Conductivity measurements may help determine the point of complete mix. If the point of complete mix is unclear or too far downstream for representative sampling, then multiple samples should be collected across a transect. For very large streams and rivers it may be necessary to composite samples collected along a cross channel transect to accurately characterize water quality of the sampled stream segment. At least one sample should be collected downstream of the discharge point, but multiple samples may be collected throughout the impacted reach if deemed necessary.

i) Municipal Point Source

Analysis should be conducted for BOD_5, DO, TSS, phosphorus, ammonia, nitrite, and nitrate using SAC 907 (Appendix E).

ii) Point Source Toxic Effects

Analysis should be conducted for alkalinity, hardness, magnesium, cadmium, copper, lead, nickel, zinc, and aluminum using SAC 908 (Appendix E).

b) Non-Point Source

i) Stormwater

For these follow-up surveys, a minimum of one sample is collected during low or dry weather flow to determine background conditions, and from 3 to 5 high flow (storm) events in conjunction with stream flow measurements to characterize pollutant loadings. For storm events it is important for the biologist to make collections during the first flush and/or while the hydrograph is rising. Analysis should be performed for metals (Fe, Al, Cu, Pb, Zn, Cd, Cr, Hg) oils and grease, pathogens, and for total and dissolved nutrients (Appendix E). Analysis is not limited to the above and parameters of special concern (e.g. fertilizers, pesticides and other organic chemicals) may be added as necessary.

ii) Nutrients

If deemed necessary by the investigator, nutrient sampling will occur during the growing season at least once a month from May through October. Sampling should occur during both dry and wet weather in order to adequately characterize loadings. Wet weather samples should be collected during the rising hydrograph. In

addition, stream discharge will be measured at least once. Water quality analysis should be conducted for total and dissolved nutrients using SAC 047 (Appendix E).

iii) Abandoned Mine Discharges

For acid mine discharges, samples should be collected from the points of discharge, if possible. In addition, flow from the discharge(s) should be measured to determine loading rates for TMDL development. Flow and channel cross section are measured in the field according to standard USGS stream gauging techniques.

Analysis is performed for metals, alkalinity and acidity using SAC 909 (Appendix E).

iv) Acid Precipitation Analysis

For suspected cases of impairment caused by atmospheric deposition, the Acid Precipitation Protocol will be used (Appendix F). Acid precipitation sampling should occur in late winter/early spring during heavy snowmelt and/or storm events to capture episodic acidification. Sampling should occur during peak flow conditions to characterize worst-case conditions. This protocol includes a filtering method for dissolved aluminum that differs from that prescribed for other dissolved metals. Water for the dissolved aluminum analysis is filtered through a 0.1-micron filter rather than through the standard 0.45-micron filter. The results from this alternate dissolved aluminum analysis correlates well with the occurrence of inorganic monomeric aluminum species, which causes the lethal responses in fish. Analysis is performed for metals, alkalinity and acidity using SAC 910 (Appendix E).

v) Potable Water Supply

For surface waters used as sources of drinking water, the potable water supply use can be evaluated by collecting a minimum of 8 samples over a period of one year. Samples are collected upstream of the surface water withdrawal at a minimum of one location, but multiple locations may be necessary to identify potential sources of pollution.

Analysis is performed for total nitrites, iron, manganese, chloride, fluoride, sulfate, color and dissolved solids using SAC 166 (Appendix E). Additional microbiological parameters can be added on a site-specific basis – see section B.3 below.

3) Habitat Assessment

a) Qualitative Assessment (required)

A habitat assessment is conducted on a measured 100-meter reach of stream, at a minimum. The habitat assessment process involves rating twelve parameters as excellent, good, fair, or poor by using a numeric value (ranging from 20-0), based on the criteria included in the Riffle/Run Habitat Assessment protocol. The Riffle/Run Habitat Assessment protocol and field data sheets (Appendix B) are presented in the Department's Standardized Biological Field Collection and Laboratory Methods (PaDEP "Methods"). The twelve habitat assessment parameters used for Riffle/Run prevalent streams are: instream fish cover, epifaunal substrate, embeddedness, velocity/depth regime, channel alteration, sediment deposition, riffle frequency, channel flow status, conditions of banks, bank vegetative protection, grazing or other disruptive pressures, and riparian vegetative zone widths.

b) Stormwater Impacted Habitat (as needed)

For cases of suspected stormwater runoff induced impairments a zigzag pebble count procedure developed by Bevenger and King (1995) will be used to measure increases in the percentage of fine particles in gravel and cobble bed streams. Prior to field collections, reference and study reaches should be identified and classified according to the Rosgen stream classification system using topographic quadrangles and aerial photographs. Sampling should only occur on streams that are classified as B and C with gravel or cobble beds as other Rosgen stream types may provide erroneous results.

The zigzag pebble count procedure will be applied to both reference and study stream reaches for purposes of comparison (Appendix G). The sample stream reaches must include at least 2 pool and 2 riffle habitat units, if present, or be conducted over a minimum reach of 200 meters. Particles are collected from the substrate within the active channel from bank toe to bank toe along a zigzag transect. For all reaches, a minimum total of 200 particles will be sampled. Particles are selected by placing a finger at the toe of one boot, and without looking, sliding the finger down to the streambed until touching the substrate. The first particle touched is selected and the intermediate axis is measured to the nearest millimeter and tallied according to Wentworth size class on the Pebble Count Field Form (Appendix H).

An alternative assessment method for excess sediment is the Watershed Assessment of River Stability and Sediment Supply (WARSSS) developed by the US Environmental Protection Agency (EPA). Information on the use of WARSSS can be found on the US EPA website www.epa.gov/warsss/index.htm

B) Biological Sampling Methods

At least one of the biological sampling methods listed below will be applied in each Instream Comprehensive Evaluation survey conducted. The biological method selected for use must be the most appropriate for assessing the attainment of designated use of interest. In most instances benthic macroinvertebrates will be the primary biological assessment method. To quantify the precision of the overall method 10 percent of biological samples are replicated. Replicate samples should be collected within the same reach and by the same investigator to minimize variability.

1) Benthic Macroinvertebrates (required)

Because aquatic organisms are excellent indicators of water quality, and are routinely sampled as part of Pennsylvania's ongoing water quality management program, benthic macroinvertebrates will be collected in most instances to assess the attainment of aquatic life uses. The primary method used to collect these organisms will be the semi-quantitative method described below.

a) Semi-Quantitative (PaDEP-RBP) Method

For this method, benthic macroinvertebrate samples are collected with a handheld D-frame net employing the semi-quantitative "kick" method in shallow, fast and slow riffle areas. Sample collection consists of 6 D-frame sample efforts from each station, composited and returned to the lab for further processing and identification (Pa DEP "Methods", Section V.C.). This 6 D-frame sample collection method applies year round (Pa DEP "Methods", Section V.C.).

b) Quantitative Method

In some instances, such as establishing baseline conditions, it may be necessary to collect quantitative benthic samples from wadeable streams. In these cases, the traditional quantitative sampling methods (PaDEP "Methods", Section V.D.) should be used in place of the D-frame net. Recommended gear includes Surber-type samplers, artificial substrate (multi-plate) samplers, and grab sample devices. Sample processing will follow procedures set forth in PaDEP "Methods", Section V.C.

c) Sample Preservation

Samples collected using any of the above benthic methods are placed in labeled containers, preserved with 70-80 percent ethanol and returned to the laboratory for identification. In the laboratory, the organisms are sorted from debris and are identified using standard taxonomic references (PaDEP "Methods", Section IX).

2) Fish Survey Protocol (as needed)

In cases of large (4th order or larger) wadeable warm water streams and rivers or streams and rivers impacted by abandoned mine drainage, use of benthic macroinvertebrates to assess aquatic life uses may not be practical or appropriate. For these wadeable streams and rivers, fish sampling methods can be employed to assess the attainment of aquatic life uses. Pennsylvania DEP is developing a Fish Index of Biotic Integrity (PaFIBI) protocol (See Section a) below). In the interim, the Qualitative Fish Sampling Protocol described below in Section b) will be used.

a) Pennsylvania Fish Index of Biotic Integrity

For large wadeable warm water streams, fishes are collected by electrofishing using a backpack or boat-mounted electrofisher. The sample reach is 10 times the mean stream width, or a minimum of 100 meters. A sample reach should not: include major tributaries; be close to the mouth; or be immediately downstream of impoundments. Every effort is made to collect and identify as many individual fish as possible. Individuals are enumerated and recorded. Specimens that cannot be field identified are preserved in a 10 percent formalin solution for laboratory identification. A detailed description of the Pennsylvania Fish Index of Biotic Integrity ("Methods" Section VI.C.3) will be included in DEP's "Methods" when completed and verified with an independent data set.

b) Qualitative Fish Sampling Protocol

Fish sampling is conducted over a representative 100-meter minimum stream reach. Sampling of the reach is continued until no new species of fish are found ("Methods", Section VI.B.). When possible, the fish are identified in the field and released. Specimens which cannot be field identified are preserved in a 10 percent formalin solution for laboratory identification. Presence of each species and enumeration of individuals are reported on appropriate field forms (Attachment F).

3) Bacteria (as needed)

Bacteriological samples are collected at the discretion of the field investigator, and are used to assess potable water supply or recreational use impairment.

Samples for bacteriological analysis may be collected at each station using a 125 ml sterile bottle. At a minimum, a total of 10 samples are to be collected, one sample each on five different days, during two (2) 30-day periods, from May 1 to September 30. This supports the calculation of a geometric mean comparable to criteria specified in Chapter 93. The samples are iced and returned to the DEP laboratory where analysis is conducted following Standard Methods.

4) Aquatic Plants and Periphyton (as needed)

In cases of noxious plant or algal growth, or when deemed appropriate by the field investigator, aquatic vascular plants, bryophytes, algae, and periphyton are noted in the field where they occurred. Those which cannot be field identified may be preserved for laboratory analysis. Specimens returned to the laboratory are identified using standard taxonomic keys (PaDEP 2003, Methods Section IX).

III. DATA ANALYSIS:

A) Field Chemistry

Field chemistry, while important for general characterization of water quality conditions, has limitations as a basis for making aquatic life use attainment decisions. In all instances, results of physical/chemical field measurements clarify and support use attainment decisions that are primarily based on water chemistry and biological data.

B) Water Chemistry

Water chemistry is analyzed to determine if chronic Chapter 93 criteria violations are occurring. These data will be used in conjunction with field chemistry and biological data to determine aquatic life use impairment and aid in identification of sources and causes of the impairment.

C) Habitat

1) Qualitative Habitat

After all parameters in the matrix are evaluated, the scores are summed to derive a total habitat score for that station. The habitat parameters of "instream cover", "epifaunal substrate", "embeddedness", "sediment deposition", and "condition of banks" are more critical because they evaluate the instream habitat components that have the most affect on the benthic macroinvertebrate community. Scores in the "marginal" (6-10) or "poor" (0-5) categories for these parameters are of greater concern than for those of the other parameters due to their ability to influence instream benthic macroinvertebrate habitat. Total scores in the "optimal" category range from 240-192; "suboptimal" 180-132, "marginal" 120-72, and "poor" is 60 or less. The decision gaps between these categories are left to the discretion of the field investigator.

2) Stormwater Impacted Habitat

For stormwater-impacted sites where a pebble count analysis was conducted, data analysis procedures are presented in the Pebble Count Procedure For Assessing Stormwater Impacts (Appendix G). Briefly summarized here, the cumulative particle size distribution of reference and study reaches are plotted on graph paper or electronically to generate a graph or spreadsheet for data interpretation (Example in Appendix G). Reference reaches are those streams that have less than 15 percent of total particles finer than 8 mm, and stable study reaches are

those streams with less than 30% of particles finer than 8 mm. If total fine particles are greater than 35 percent (estimated) the study reach is very likely unstable and may be impaired. These percentage fines are to be used as a general guideline and will vary from stream to stream with some streams being unstable at lower percentage fines while others will be stable at higher percentage fines.

If the WARSSS method was used to assess excess sediment, then analysis is in accordance with the WARSSS methodology.

D) Benthic Macroinvertebrates

Biological metrics are calculated, compiled and compared to a composite benchmark threshold score. These metrics were developed through the PA Tiered Aquatic Life Uses IBI workshop and include: EPT taxa richness, total taxa richness, Shannon Diversity Index, Beck's Index, Hilsenhoff Biotic Index and %Intolerant Individuals and will discriminate between impaired and unimpaired waters and are based on data collected to date. The metric scoring categories and decision matrix is presented in Appendix H along with a more detailed discussion.

E) Fishes

1) Pennsylvania Fish Index of Biotic Integrity

In the absence of quantitative fish IBI protocols (currently under development), fish data collected from small or large wadeable streams will be analyzed as required by the Qualitative Fish Sampling Protocol (PaDEP "Methods", Section VI.C.3.k). Fish communities characterized by unbalanced populations of predator species vs. prey species or the absence of predatory species indicate impairment. (Once PA fish IBI protocols are implemented, this section will be superseded by data analysis requirements of these new protocols.)

2) Qualitative Fish Sampling Protocol

For fish data collected from small or large wadeable streams in the Susquehanna or Delaware River basins, data will be analyzed as required by the Qualitative Fish Sampling Protocol (PaDEP "Methods", Section VI.B).

IV. REFERENCES:

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APPENDIX B

HABITAT ASSESSMENT FORMS

3800-FM-WSFR0402 Rev. 10/2008

pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF WATER STANDARDS AND FACILITY REGULATION

WATER QUALITY NETWORK
HABITAT ASSESSMENT

WATERBODY NAME _____ STR CODE/RMI _____

STATION NUMBER _____ LOCATION _____

DATE _____ TIME _____

AQUATIC ECOREGION _____ COUNTY _____

INVESTIGATORS _____

FORM COMPLETED BY _____ ***RIFFLE/RUN PREVALENCE***

Habitat Parameter	Category			
	Optimal	**Suboptimal**	**Marginal**	**Poor**
1. Instream Cover (Fish)	Greater than 50% mix of boulder, cobble, sub-merged logs, undercut banks, or other stable habitat.	30-50% mix of boulder, cobble, or other stable habitat; adequate habitat.	10-30% mix of boulder, cobble, or other stable habitat; habitat avail-ability less than desirable.	Less than 10% mix of boulder, cobble, or other stable habitat; lack of habitat is obvious.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
2. Epifaunal Substrate	Well developed riffle and run, riffle is as wide as stream and length extends two times the width of stream; abundance of cobble.	Riffle is as wide as stream but length is less than two times width; abundance of cobble; boulders and gravel common.	Run area may be lack-ing; riffle not as wide as stream and its length is less than two times the stream width; gravel or large boulders and bed-rock prevalent; some cobble present.	Riffles or run virtually nonexistent; large boulders and bedrock prevalent; cobble lacking.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
3. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
4. Velocity/Depth Regimes	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow).	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score lower than if missing other regimes).	Dominated by 1 velocity/depth regime (usually slow-deep).
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
5. Channel Alteration	No channelization or dredging present.	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.	New embankments present on both banks; and 40-80% of stream reach channelized and disrupted.	Banks shored gabion or cement; over 80% of the stream reach channelized and disrupted.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Total Side 1 _____				

RIFFLE/RUN PREVALENCE

Habitat Parameter	Category			
	Optimal	Suboptimal	Marginal	Poor
6. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from coarse gravel; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, coarse sand on old and new bars; 30-50% of the bottom affected; sediment deposits at obstruction, constriction, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% 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**
7. Frequency of Riffles	Occurrence of riffles relatively frequent; distance between riffles divided by the width of the stream equals 5 to 7; variety of habitat.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream equals 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is between ratio >25.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
8. 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**
9. Condition of Banks	Banks stable; no evidence of erosion or bank failure.	Moderately stable; infrequent, small areas of erosion mostly healed over.	Moderately unstable; up to 60% of banks in reach have areas of erosion.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; on side slopes, 60-100% of bank has erosional scars.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
10. Bank Vegetative Protection	More than 90% of the streambank surface covered by vegetation.	70-90% of the stream-bank surface covered by vegetation.	50-70% of the stream-bank surfaces covered by vegetation.	Less than 50% of the streambank surface covered by vegetation.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
11. Grazing or Other Disruptive Pressure	Vegetative disruption, through grazing or mowing, minimal or not evident; almost all plants allowed to grow naturally.	Disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	Disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Disruption of vegetation is very high; vegetation has been removed to 2 inches or less in average stubble height.
SCORE _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
12. Riparian Vegetative Zone Width	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 _____	**20 19 18 17 16**	**15 14 13 12 11**	**10 9 8 7 6**	**5 4 3 2 1**
Total Side 2 _____				
Total Score _____				

APPENDIX C

FLOWING WATERBODY FIELD FORMS

3800-FM-WSFR0086 Rev. 12/2008

pennsylvania
DEPARTMENT OF ENVIRONMENTAL PROTECTION

COMMONWEALTH OF PENNSYLVANIA
DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF WATER STANDARDS AND FACILITY REGULATION

FLOWING WATERBODY FIELD DATA FORM

(Information and comments for fields boxed in double lines are required database entries. Other fields are optional for personal use.)

Date-Time-Initials* Example 20040212-0312-XYZ	- - Date Time Initials	**Watershed Code (HUC)**	**Stream Code**	**Ch. 93 Use**
Secondary Station ID		Surveyed by:		

*Date as YYYYMMDD, time as military time, and your initials uniquely identify the stream reach.

SWP Watershed	

Survey Type

(1) Basin Survey, (2) Cause / Effect, (3) Fish Tissue, (4) Instream Comprehensive Evaluation [ICE], (5) Point-of-First-Use, (6) SERA, (7) Antidegradation [Special Protection], (8) Toxics, (10) Use Attainability, (11) WQN, (12) Limestone, (13) Low-gradient [Multihabitat]	

Location

County:		**Municipality:**		**Topo Quad:**	

Location Description:

Land Use

Residential:	%	Commercial:	%	Industrial:	%	Cropland:	%	Pasture:	%
Abd. Mining:	%	Old Fields:	%	Forest:	%	Other:	%		

Land Use Comments:

Canopy cover: open partly shaded mostly shaded fully shaded

Water Quality

	Collector-sequence #	**Field Meter Readings:**					**Bottle Notes (N-normal, MNF-metals non-filtered, MF-metals filtered, B-bac't, Others: indicate)**
		Temp (^{0}C)	**DO (mg/L)**	**pH**	**Cond. (umhos)**	**Alkalinity mg/l**	
1.							
2.							
3.							

Water Appearance/Odor Comments: (* see bottom of back for common descriptors)

Findings

Not Impaired:	☐	Impaired biology?	☐	Impaired habitat?	☐	Is impact localized?	☐	Reevaluate designated use?	☐

Decision comments. Describe the rationale for your "Not Impaired" or "Impaired" decision; reach locations for use designation reevaluations; special condition comments; etc.:

IBI Score:		**Total Habitat Score:**	

Macroinvertebrate sampling
Sampling method: Std. kick screen: ☐ D-frame: ☐ Surber: ☐ Other: ☐ method?: _____
Comments/Abundance Notes:

Habitat Impairment Thresholds	Metric Score
#3 Riff/Run: embeddedness *or* #3 Glide/Pool: substrate character + #6 Sediment Deposition = 24 or less *(20 or less for warm water, low gradient streams)*	
#9 Condition of Banks + #10 Bank Vegetation = 24 or less *(20 or less for warm water, low gradient streams*	
Total habitat score 140 or less for forested, cold water, high gradient streams *(120 or less for warm water, low gradient streams)*	
Habitat Comments:	

Special Condition

Use this block to describe conditions that justify attainment/impairment of stations with IBI score <63 and >53.

***Common descriptors: Water Odors -** none normal sewage petroleum chemical other; **Water Surface Oils -** none slick sheen globs flecks; **Turbidity -** clear slight turbid opaque; **NPS Pollution** - no evidence some potential obvious; **Sediment Odors -** none normal sewage petroleum chemical anaerobic; **Sediment Oils -** absent slight moderate profuse; **Deposits –** none sludge sawdust paper fiber sand relict shells other. **Are the undersides of stones deeply embedded black?**

APPENDIX D

STREAM FLOW MEASUREMENT PROTOCOL

STREAM FLOW MEASUREMENT PROTOCOL FOR INSTREAM DISCHARGE (Q) CALCULATION

The estimate of stream discharge (Q) requires careful field measurements during variable flow conditions. Since stream discharge is a volume estimate, three dimensions must be measured. Stream width (W) and stream depth (D) are simple measurements equivalent to the cubical width and height. Since streams are flowing, the cubical length equivalent becomes a distance/time dimension (velocity, or V).

The following protocol provides guidelines outlining procedures designed to assure that W, D, and V are measured as accurately and consistently as possible. This protocol follows a "6/10th" depth method similar to that described in USGS field methodology manuals and other sources.

1. Equipment needs:

(a) Flow meter (This protocol is written for "electromagnetic probe" type flow meters similar to Marsh-McBirney models.)

(b) Standard wading rod

(c) 100' cloth tape measure (English/metric in 1/10ths)

(d) two rods/stakes for anchoring measuring tape

(e) clip board & data entry form or field data book

(f) pencils and spare meter batteries

(g) flow calculation program

(h) proper wading gear (hip or chest waders (preferred) with felt soles (a must!)

2. Stream reach selection and site conditions

(a) Select stream reach location that properly reflects the cumulative flow from upstream study area.

(i) Avoid sampling immediately downstream from road crossings, road drainage ditches, tributary "plumes" (in the mixing zone - before the "zone of complete mix").

(ii) Be sure to sample or place the transect far enough downstream to reflect upstream discharges: point sources, nonpoint sources, and tributaries.

(b) Be sure flow conditions are measurable (water is moving) and wadeable (<1 meter deep & <1m/sec).

3. Transect Placement - Open channel/flow considerations

(a) Strive for the "ideal transect" - stretch your tape across the stream; perpendicular to the direction of mid-channel flow, where you find the best combination of the following "ideal" conditions:

(i) Straight channel - try to find a stream section with a straight distance that is 2X the stream width. For stream widths > 10', straight distances <2X width can be considered IF there are no (or very few) obstacles, large vortices, or mid-channel flow diversions.

(ii) Laminar flow - the channel bottom should be as smooth as possible.

(iii) No obstacles - avoid sections where there are protruding boulders, sandbars, deflecting structures (logs, brush, debris, etc.).

(iv) Uniform depth -"U-shaped" channel with steady, gradual, tapering depths. Avoid abrupt, almost vertical changes in depth.

(v) No backwater flow

(b) In many cases, in-stream conditions may be altered to reduce the overall inaccuracy by moving some submerged materials and obstacles that deflect flow or cause associated turbulence.

4. Meter and wading rod preparation

(a) Check batteries.

(b) Calibrate meter according to manufacturer's specifications.

(c) Attach meter probe to wading rod so that the signal wire exits from the top and is parallel to the wading rod's vertical shaft.

5. Velocity measurements

Once the tape transect has been positioned, flow measurements may begin following these guidelines:

(a) Meter operation - (This protocol is written for "electromagnetic probe" type flow meters similar to Marsh-McBirney models. If other models are used, follow the manufacturer's instructions to render a velocity reading.)

(i) meter is "readied" (turn on and set scale to "ft/sec").

(ii) Meter is set for any "time constant."

(iii) Velocity is read once it has stabilized.

(b) Wading rod placement and operation ("6/10th depth" method)

(i) With the operator standing downstream from the tape, the wading rod is held behind the tape at straight-arm length, aligned at the first width increment, and rested on the stream bottom in a perpendicular position.

(ii) Measure depth and adjust meter probe to proper depth setting by depressing the sliding rod lock and sliding it up to align with the "tenth scale" depth. The sliding rod is calibrated with single lines in 1.0 foot increments. The appropriate foot marker on the sliding rod is aligned with its corresponding "1/10th foot reading. For example, the depth was measured to be 2.3 feet. The "2" foot marker on the sliding rod is aligned with the "3" line on the "tenth scale". Because of the wading rod's construction, the meter's probe depth is now properly positioned at "6/10ths of the total depth" from the surface.

(iii) After each velocity reading, move the rod to the next width increment, reset the meter probe depth and measure the velocity.

(iv) Repeat until all required width increments have been measured.

6. Cross-section measurements ("Mid-section" Method)

Cross-section measurements are taken to provide the "W" and "D" dimensions for Q calculations. Since the stream depth and velocities vary widely across any given transect, the cross-section will be divided into many smaller sub-sections (at least 20); each with its own W, D, and V measurements. This is to assure that no more than 5 percent of the total transect Q flows through any one sub-section and that inaccuracies introduced by widely variable depths and velocities are minimized.

(a) Anchor tape to both stream banks and measure width.

(b) Record W, D, and V entries on a flow data sheet for each width increment. It is more convenient for data recording to measure width increments in ascending order across the transect. The first depth and velocity entries should begin at the shoreline and be recorded as "0" and "0" respectively.

(c) Repeat, measuring at least 20 subsections. The final W, D, V readings recorded should be measured at the water's edge on the opposite bank and, again be entered as "0" and "0", respectively.

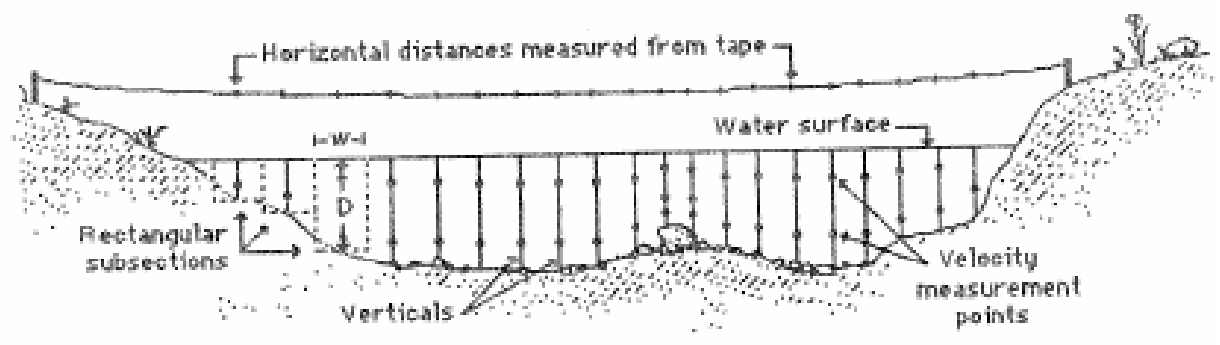
(d) Special conditions or situations to consider:

(i) For meter operation, probe must be completely submerged (approx. 3" depth.

(ii) Sub-section increments must be shortened significantly whenever velocities or depths change dramatically. Measuring smaller width increments may increase the number of sub-sections in any given transect.

(iii) Avoid placing transects in areas where backflow occurs.

Figure 1


Horizontal distances measured from tape
Water surface
W
D
Rectangular subsections
Verticals
Velocity measurement points

3800-FM-WSFR0401 10/2005

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DEPARTMENT OF ENVIRONMENTAL PROTECTION
BUREAU OF WATER STANDARDS AND FACILITY REGULATION

WATER QUALITY INSTREAM FLOW MEASUREMENTS

STREAM _____ DATE _____

STATION _____ SEGMENT _____

STREAM WIDTH _____ RMI _____ EST. FLOW _____

COLLECTORS _____

DIST (ft)	DEPTH (ft)	VEL (ft/s)	DIST (ft)	DEPTH (ft)	VEL (ft/s)

APPENDIX E

PARAMETERS FOR INSTREAM COMPREHENSIVE EVALUATION SURVEY ANALYSES

PARAMETERS FOR INSTREAM
COMPREHENSIVE EVALUATION SURVEY ANALYSES

		Standard Analysis Code						
Parameter	**Method**	**047**	**166**	**907**	**908**	**909**	**910**	**Storm water**
pH	00403			X	X	X	X	X
Alkalinity, Total as $CACO_3$ (Titrimetric)	00410	X			X	X	X	X
Hardness, Total (Calculated)	00900				X			X
Acidity, Total hot as $CACO_3$ (Titrimetric)	70508					X	X	
Biochemical Oxygen Demand 5 Day	00310			X				
Residue, Dissolved at 105° C	00515		X	X				
Total Suspended Solids	00530			X				
Nitrogen, T	00600A	X						
Ammonia, Total as Nitrogen	00610A			X			X	X
Nitrite Nitrogen, Total	00615A			X				X
Nitrate as Nitrogen	00620A			X			X	X
Nitrite + Nitrate, Total	00630A		X					
Phosphorus, Total as P	00665A	X		X			X	X
Phosphorus, Dissolved as P	00666A							X
Phosphorus, Ortho Dissolved	00671A							X
Phosphorus, Total, Orthophosphate as P	70507A							X
Calcium, Total by Trace Elements	00916A				X		X	
Magnesium, Total by Trace Elements	00927A				X		X	
Cadmium, Total by Trace Elements	01027H				X			X
Copper, Total by Trace Elements	01042A				X			X
Lead, Total by Trace Elements	01051H				X			X
Nickel, Total by Trace Elements	01067H				X			
Zinc, Total by Trace Elements	01092H				X	X		X
Aluminum, Total by Trace Elements	01105H				X		X	X
Aluminum, Dissolved 0.1 micron filter	01106D						X	
Sulfate by Ion Chromatography	00945		X				X	
Iron, Total by Trace Elements	01045A		X			X	X	X
Manganese, Total by Trace Elements	01055A		X			X	X	
Chloride by Ion Chromatography	00940		X				X	
Chromium, Total by Trace Elements	01034A							X
Mercury, Dissolved	718901							X
Fluoride by Ion Chromatography	00951		X					
Color	00080		X					

Required Bottles								
	Fixative	**Number of Bottles**						
		Standard Analysis Code						
		047	**166**	**907**	**908**	**909**	**910**	**Storm water**
500 ml, inorganics	None	1	1	1	1	1	1	1
500 ml, NH_3-N, Kjeldahl-N, Tot P	1:1 H_2SO_4							1
125 ml, fixed N/P	1:1 H_2SO_4	1						
125 ml, fixed metals	1:1 HNO_3		1		1	1		1
125 ml, filtered 0.45µ, Dissolved P	1:1 H_2SO_4							1
125 ml, filtered 0.45µ, Ortho-P	None							1
500 ml, filtered 0.1µ, Dissolved Aluminum	1:1 HNO_3						1	

APPENDIX F

ACID PRECIPITATION PROTOCOL

ACID PRECIPITATION PROTOCOL

I. PURPOSE:

Acid precipitation impairment is difficult to detect using the standard SSWAP biological screening protocol, particularly when the impairment is due to episodic acidification. Small, forested, headwater streams with low alkalinity are generally unproductive. Low numbers of benthic macroinvertebrates with relatively low diversity are frequently observed in these types of streams. The collected organisms are also generally sensitive to organic pollution, so the benthic community will normally be dominated by taxa with low Hilsenhoff scores. Depending on the season and recent precipitation history, field water chemistry measurements will document the low alkalinity, but may fail to detect a low pH event. Assuming that no major component of the benthic community is missing (e.g. mayflies), the standard SSWAP biological screening protocol may lead to the potentially erroneous conclusion of no biological impairment.

The SWWAP biological screening methodology may fail to identify acid precipitation impacts because it typically does not assess the fish community. A fish community may slowly decline as year classes are lost to episodic acidification and sensitive species are eliminated from a given reach, but this trend may go unnoticed if the benthos alone is used to detect biological impairment. Macroinvertebrates are better able to recolonize stream reaches than fish due to the shorter time between successive generations, and may not exhibit the same symptoms as fish communities when challenged by episodic acidification. Thus, a relatively healthy macroinvertebrate community may not infer that a healthy fish community is present, and therefore may not give a complete indication of the stream's biological impairment due to acid precipitation.

Macroinvertebrate metrics provide only an indirect indication of potential acid precipitation impairment. When abundance and diversity are obviously low, community composition is abnormal (e.g. no mayflies), and field alkalinity and pH are both low (alkalinity < 5 ppm; pH < 5.0), the standard SSWAP biological screening protocol can support a decision of biological impairment due to acidification. When these conditions are not observed and acid impairment is suspected, a more detailed investigation may be warranted to conclusively identify an acid precipitation problem. Other evidence that may also trigger a detailed follow-up survey would include anecdotal information indicating a decline in a fishery; cessation of trout stocking by PFBC due to poor survival; and fisheries data documenting population changes and species loss over time.

The best way to document acid precipitation impairment is to collect water samples during spring snowmelt or storm events that document conditions known to be lethal to fish. The most critical measurements are pH and dissolved aluminum. Low pH and high concentrations of dissolved aluminum have been linked to high fish mortality in studies of episodic acidification. Dissolved inorganic monomeric aluminum is the aluminum species most strongly correlated to fish mortality, but analysis for this form of aluminum is more complicated than for the more traditional "total dissolved aluminum" concentration. Total dissolved aluminum concentrations obtained via the standard method of field filtration through a 0.45 μ filter are only weakly correlated with lethal response in fish, and are of limited value for identifying impairment due to acidification. An alternate dissolved aluminum analysis that correlates well with inorganic monomeric aluminum concentrations and is useful for identifying acid impairment is one conducted on water samples filtered through a 0.1 μ filter.

II. FIELD COLLECTION:

Follow-up sampling to detect acid impairment should be concentrated during storm events and periods of heavy snowmelt. Ideally, water samples should be collected during peak flows to characterize worst-case conditions. Grab samples collected during high flow events should be adequate for most follow-up surveys. A low flow sample may be collected for comparison, but is not necessary; if the high flow sample documents stressful conditions (i.e. low pH and high dissolved aluminum levels), then some degree of biological impairment is likely. Prior to shipping the sample to the lab, a 500 ml aliquot must be filtered through a 0.1 µ filter.

Standard Analysis Code 910 (SAC 910) has been established for use by the SSWAP biologists when investigating potential acid precipitation problems. The analyses conducted as part of SAC 910 are listed in Table 1. The most important parameters for identifying acid precipitation impairment are pH and dissolved aluminum concentrations (with 0.1 micron filtration). Elevated dissolved aluminum concentrations (>150 µg/l) and low pH (<5.8) can be lethal to brook trout, depending on duration of exposure. When a stream survey documents pH depression and dissolved aluminum levels above 150 µg/l (after 0.1 micron filtration), it is probably appropriate to consider the stream to be biologically impaired due to acid precipitation. For 303d list reporting purposes, acid precipitation is the source and pH is the cause of impairment.

Table 1. Analyses included under the Standard Analysis Code for acid precipitation samples (SAC 910).

Test Description	Reporting units
Specific conductivity	umhos/cm
pH	pH units
Alkalinity total as $CaCO_3$	mg/l
Acidity, mineral as $CaCO_3$	mg/l
Calcium, total	mg/l
Magnesium, total	mg/l
Chloride	mg/l
Sulfate	mg/l
Iron, total	µg/l
Manganese, total by trace elements	µg/l
Aluminum, total by trace elements	µg/l
Aluminum, dissolved 0.1 micron filter	µg/l

Table 2. Sample handling requirements and holding times required for SAC 910.

Analysis	Container	Containers Per Sample	Preservation
Metals	125 ml Plastic (HDPE)	1	1 ml 1:1 HNO_3pH < 2, ship on ice
General Chemistry	500 ml Plastic (HDPE)	1	Must be shipped to lab on ice within 24 hours.
Dissolved Aluminum	500 ml Plastic (HDPE)	1	Filtered (0.1 µ) Fixed 5 ml HNO_3, ship on ice

APPENDIX G

PEBBLE COUNT PROCEDURE FOR ASSESSING STORMWATER IMPACTS

PEBBLE COUNT PROCEDURE FOR ASSESSING STORMWATER IMPACTS

I. PURPOSE:

This survey protocol is to be applied to riffle/run dominated, gravel or cobble bed stream segments identified as being at risk of impairment, or impaired by stormwater runoff as determined by the Statewide Surface Water Assessment Program (SSWAP) screening protocol or other assessment methods.

Flow regime alteration (change in volume and/or timing of discharge) is a major cause of stream instability and habitat alteration. One aspect of concern is the delivery of fine sediments to streams and their effects on aquatic habitat. One method of monitoring these sediment effects is "A Pebble Count Procedure for Assessing Watershed Cumulative Effects" by Bevenger and King (1995). This procedure utilizes a reference stream approach in evaluating the stability of study or candidate streams. The procedure characterizes particle size distributions of reference and study streams, where reference streams are defined as "natural" or "least impacted" and study streams as "disturbed" or "impacted". These particle size distributions can be used for comparative purposes to determine, with statistical reliability, if there has been a shift toward finer size materials in the study stream. This protocol employs a modification of the Wolman (1954) pebble count procedure to a zigzag pattern through a continuum along a longitudinal reach of the stream. This allows for numerous meander bends and associated habitat features to be sampled as an integrated unit.

II. FIELD COLLECTION:

Wadeable reference and study streams should be selected from the same ecoregion, and the streams should be classified according to the Rosgen stream classification system (Rosgen, 1994, 1996) prior to conducting the field collection. Streams classification can be accomplished in the office using topographic quadrangles and aerial photographs, and the classification should be confirmed when the sample site is visited. This protocol should only be applied to those streams that are classified as B and C types with cobble (B3 or C3) or gravel beds (B4 or C4). If the classification results in stream types G, F, or D, then field collection may not be necessary since, in most cases, these stream types are the result of channel instability. If the instability were a result of natural conditions the stream would not be classified as impaired. Also, if the classification results in stream types A and E, which are ordinarily stable, then field collection is not necessary. In addition, this procedure should not be conducted on "natural" sand or silt/clay bottom streams, as fine particles will be the predominate substrate type, thus resulting in potentially misleading indications of instability.

A) <u>Particle Count Procedures</u>

Once reference and study streams have been identified, the sample stream reach should include at least two riffle and two pool habitat units if present, or a minimum of 200 meters. The chosen sample reach habitat units should be representative of the streams. Study and reference streams must have a minimum mean width of 3 meters. If mean stream width is greater than 20 meters, then sample reach must be extended 100 meters for each 10-meter increment increase in width. Sampling of reference streams should occur within a few days of the sampling of study streams when possible and should always occur within the same year and season. In order to confirm stream

classification, at least two stream cross-sections (one riffle and one pool) should be measured from bankfull elevation to bankfull elevation within the study reach, prior to conducting the pebble count.

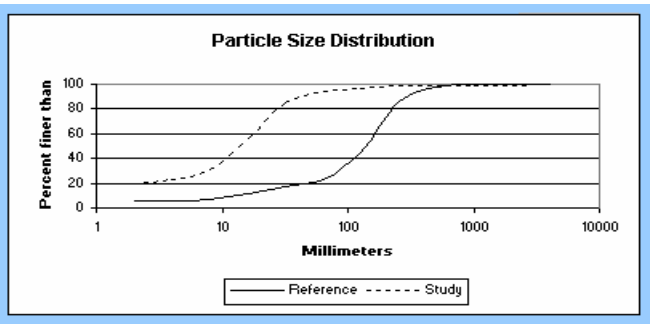
Pebble counts are conducted on the selected reach beginning at the head of a riffle and continuing through 4 habitat units (2 riffle, 2 pools if present), or for a minimum of 200 meters. At least 200 particles are to be sampled for the stream reach. Pebble counts are conducted along a zigzag transect from bank toe to bank toe in the active channel (Figure 1). The angle of the transect from the bank should be maintained as best as possible and can be aided by identifying a location to walk to on the opposite bank. Particles are selected beginning at the start point by placing a finger at the toe of one boot, and without looking, sliding your finger down to the stream bottom until it comes into contact with a particle (Figure 1). Each particle selected is measured along the intermediate axis (Figure 1) and the measurement is recorded on the Pebble Count field form attached to this document. Alternatively, each particle measurement may be tallied according to Wentworth size classes (<2 mm, 2-4 mm, >4-8 mm, >8-16 mm, etc.) on the Alternative Pebble Count Field form attached to this document. The investigator then paces off a chosen distance to the next point and samples another particle in the same manner as the first. The distance to the next sample point should be no less than 2.1 meters to avoid correlation between particles sampled.

III. DATA ANALYSIS:

Collected data are plotted on graph paper, or entered into Excel spreadsheets (Size-Class Pebble Count Analyzer V1 2001.xls by John Potyondy and Kristin Bunte or zig-zag Pebble Count Analyzer V1 2001.xls by Gregory S. Bevenger and Rudy M. King) and plotted electronically, as cumulative percentages for both reference and study streams. Particles 8 mm or smaller are of primary concern since they should have the most biological significance and are most likely to smother macroinvertebrate and fish spawning habitat. Reference streams should have no more than 15 percent of particles smaller than 8 mm. Impaired reaches are study streams with ≥35 percent (subject to change, and will vary by stream type) of particles smaller than 8 mm.

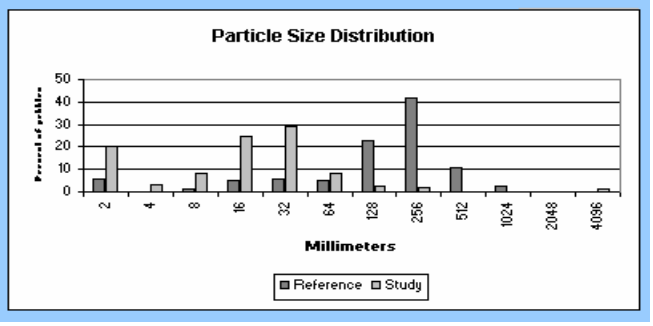
Cumulative Distribution

Class	Reference	Study
2	6	20
4	6	23
8	7	32
16	12	57
32	18	86
64	23	94
128	45	97
256	87	99
512	98	99
1024	100	99
2048	100	99
4096	100	100



Histogram

Class	Reference	Study
2	6	20
4	0	3
8	1	9
16	5	25
32	6	29
64	5	9
128	23	3
256	42	2
512	11	0
1024	3	0
2048	0	0
4096	0	2



IV. REFERENCES:

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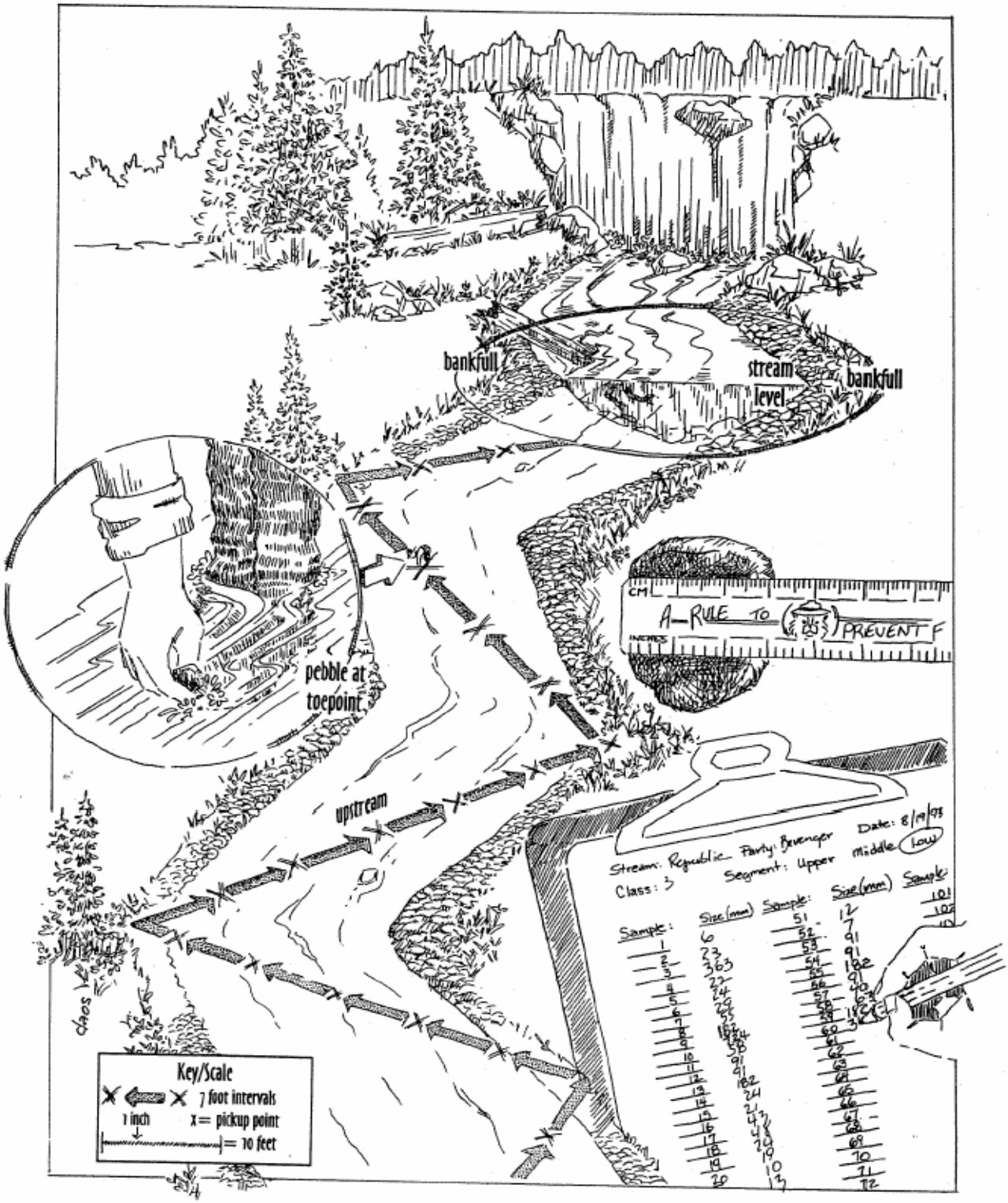

Figure 1. Zig-zag pebble count procedure from Bevenger and King, 1995.

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Pebble Count Form

GIS Key: _____ **Survey Crew:** _____

Stream: _____ **County:** _____ **SWP:** _____

Mean Width: _____ **Sample Interval:** _____ **Reach Length:** _____

Station Description: _____

_____

1		35		69		102		135		168	
2		36		70		103		136		169	
3		37		71		104		137		170	
4		38		72		105		138		171	
5		39		73		106		139		172	
6		40		74		107		140		173	
7		41		75		108		141		174	
8		42		76		109		142		175	
9		43		77		110		143		176	
10		44		78		111		144		177	
11		45		79		112		145		178	
12		46		80		113		146		179	
13		47		81		114		147		180	
14		48		82		115		148		181	
15		49		83		116		149		182	
16		50		84		117		150		183	
17		51		85		118		151		184	
18		52		86		119		152		185	
19		53		87		120		153		186	
20		54		88		121		154		187	
21		55		89		122		155		188	
22		56		90		123		156		189	
23		57		91		124		157		190	
24		58		92		125		158		191	
25		59		93		126		159		192	
26		60		94		127		160		193	
27		61		95		128		161		194	
28		62		96		129		162		195	
29		63		97		130		163		196	
30		64		98		131		164		197	
31		65		99		132		165		198	
32		66		100		133		166		199	
33		67		101		134		167		200	
34		68									

Comments: _____

_____

_____

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Alternative Pebble Count Field Form

Station GIS Key:	**Station Description:**
Survey Crew:	
Reach Length (meters):	
Sample Interval (meters):	**Mean Steam Width (meters):**

Particle Description	Intermediate Axis of Particle (mm)	Substrate Type	Particle Count Tally	Particle Count Results		
				Total#	Item %	Cumulative %
Silt/Clay	<.062	Silt/Clay				
Very Fine	.062-.125	Sand				
Fine	>.125-.25					
Medium	>.25-.5					
Coarse	>.5-1.					
Very Coarse	>1-2					
Very Fine	>2-4	Gravel				
Fine	>4-6					
Fine	>6-8					
Medium	>8-11					
Medium	>11-16					
Coarse	>16-23					
Coarse	>23-32					
Very Coarse	>32-45					
Very Coarse	>45-64					
Small	>64-90	Cobble				
Small	>90-128					
Large	>128-180					
Large	>180-256					
Small	>256-362	Boulder				
Small	>362-512					
Medium	>512-1024					
Large-Very Large	>1024					
Bedrock		Bedrock				
Sample Size:		**Totals:**				

APPENDIX H

PA-DEP RBP METRICS TABLE AND SUPPORT MATERIALS

INDEX OF BIOLOGICAL INTEGRITY FOR WADEABLE, FREESTONE, RIFFLE-RUN STREAMS IN PENNSYLVANIA

Introduction

The Pennsylvania Department of Environmental Protection (DEP) developed an index of biotic integrity (IBI) for benthic macroinvertebrate communities in Pennsylvania's wadeable, freestone, riffle-run type streams as a scientifically credible biological assessment tool. This indicator assists in guiding and evaluating legislation, policy and management strategies as well as setting goals for aquatic resources by enabling direct quantification of important ecological attributes along a gradient of biological conditions and ecosystem stressors (Davis and Simon 1995; Davies and Jackson 2006; Hawkins 2006). This indicator serves as a measure of the extent to which anthropogenic stressors impair the capability of a stream to support a healthy aquatic community (Davis and Simon 1995).

Biological Sampling Methods

This IBI applies to benthic macroinvertebrate samples collected any time of the year from wadeable, freestone, riffle-run streams in Pennsylvania using a D-frame net with 500-micron mesh. Sampling biologists composite six kicks from riffle areas distributed throughout a 100-meter stream reach, working progressively upstream, with each kick disturbing approximately one square meter immediately upstream of the net for approximately one minute to an approximate depth of 10 cm, as substrate allows. Composited samples are preserved with 95% ethanol in the field and transported back to the laboratory for processing. In the lab, each composited sample is placed into an 18 x 12 x 3.5 inch pan marked with 28 four-square inch grids. The contents of four grids are randomly selected from the pan, extracted using a four-square inch circular "cookie cutter," and placed into another identical empty pan. From this second pan, organisms are picked from randomly selected grids until a 200-organism sub-sample (+/- 40 organisms) is obtained. Organisms in the sub-sample are identified and counted. Midges are identified to the family level of Chironomidae. Roundworms and proboscis worms are identified to the phylum levels of Nematoda and Nemertea, respectively. Flatworms are identified to the class level of Turbellaria. Segmented worms, aquatic earthworms, and tubificids are identified to the class level of Oligochaeta. All water mites are identified as Hydracarina, an artificial taxonomic grouping of several mite superfamilies. All other macroinvertebrates are identified to genus level. More detailed information on field sampling and laboratory methods is available upon request.

Most of the samples used to develop the IBI were taken from relatively small, mostly first through third order riffle-run type streams draining less than 25 square miles, so this IBI should be applied with discretion to other stream types (e.g., limestone type streams) and larger stream/river systems. Currently, DEP does not apply any regionally-based classification to wadeable, freestone, riffle-run streams in the Commonwealth for purposes of applying this IBI.

The Metrics

A number of different metric combinations were evaluated during index development and the following six metrics were selected for inclusion as core metrics in the IBI based on various performance characteristics. These six metrics all exhibited a strong ability to distinguish between reference and stressed conditions. In addition, these six metrics measure different aspects of the biological communities represented by the sub-samples, and when used together in a multimetric index, they provide a solid foundation for assessing the biological condition of benthic macroinvertebrate assemblages in Pennsylvania's wadeable freestone riffle-run stream ecosystems.

Total Taxa Richness

This taxonomic richness metric is a count of the total number of taxa in a sub-sample. Generally, this metric is expected to decrease with increasing anthropogenic stress to a stream ecosystem, reflecting loss of taxa and increasing dominance of a few pollution-tolerant taxa. Other benefits of including this metric include its common use in many biological monitoring and assessment programs in other parts of the world as well as its ease of explanation and calculation.

Ephemeroptera + Plecoptera + Trichoptera Taxa Richness
(Pollution Tolerance Value 0 – 4 only)

This taxonomic richness metric is a count of the number of taxa belonging to the orders Ephemeroptera, Plecoptera, and Trichoptera (EPT) in a sub-sample – common names for these orders are mayflies, stoneflies, and caddisflies, respectively. The aquatic life stages of these three insect orders are generally considered sensitive to, or intolerant of, pollution (Lenat and Penrose 1996); in fact, this metric only counts EPT taxa with pollution tolerance values (PTVs) of 0 to 4, excluding a few of the most tolerant mayfly and caddisfly taxa. This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting the loss of taxa from these largely pollution-sensitive orders. This metric has a history of use across the world and is relatively easy to use, explain and calculate (Lenat and Penrose 1996).

Beck's Index, version 3

This taxonomic richness and tolerance metric is a weighted count of taxa with PTVs of 0, 1, or 2. The name and conceptual basis of this metric are derived from the water quality work of William H. Beck in Florida (Beck 1955). This metric is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting the loss of pollution-sensitive taxa. It should be noted that the version of the Beck's Index metric used for this project, although similar in name and concept, differs slightly in its calculation from the Beck's Index used in DEP's multihabitat protocol for assessing biological condition of low gradient pool-glide type streams.

Shannon Diversity

This community composition metric measures taxonomic richness and evenness of individuals across taxa of a sub-sample. This metric is expected to decrease in values with increasing anthropogenic stress to a stream ecosystem, reflecting loss of pollution-sensitive taxa and increasing dominance of a few pollution-tolerant taxa. The name and conceptual basis for this metric are derived from the information theory work of Claude Elwood Shannon (Shannon 1968).

Hilsenhoff Biotic Index

This community composition and tolerance metric is calculated as an average of the number of individuals in a sub-sample, weighted by PTVs. Developed by William Hilsenhoff, the Hilsenhoff Biotic Index (Hilsenhoff 1977, 1987, 1988; Klemm et al. 1990) generally increases with increasing ecosystem stress, reflecting increasing dominance of pollution-tolerant organisms.

Percent Sensitive Individuals (PTV 0 – 3)

This community composition and tolerance metric is the percentage of individuals with PTVs of 0 to 3 in a sub-sample and is expected to decrease in value with increasing anthropogenic stress to a stream ecosystem, reflecting loss of pollution-sensitive organisms.

Example calculations for each metric are provided below for a sample from Lycoming Creek.

Benthic macroinvertebrate sample from Lycoming Creek in Lycoming County taken on November 19, 2001		
Taxa Name	**Number of Individuals**	**Pollution Tolerance Value**
Acentrella	1	4
Isonychia	4	3
Epeorus	6	0
Leucrocuta	1	1
Rhithrogena	9	0
Stenonema	8	3
Ephemerella	32	1
Serratella	1	2
Paraleptophlebia	4	1
Pteronarcys	1	0
Taeniopteryx	1	2
Leuctra	2	0
Agnetina	1	2
Paragnetina	1	1
Chimarra	1	4
Dolophilodes	1	0
Cheumatopsyche	25	6
Hydropsyche	22	5
Rhyacophila	16	1
Glossosoma	2	0
Brachycentrus	3	1
Micrasema	1	2
Apatania	2	3
Psilotreta	1	0
Psephenus	3	4
Optioservus	7	4
Atherix	1	2
Antocha	2	3
Hexatoma	5	2
Prosimulium	1	2
Chironomidae	49	6
Ancylidae	2	7

Total Taxa Richness

There are ***33 taxa*** in this sub-sample, so

Total Taxa Richness = 33

EPT Taxa Richness (PTV 0 – 4 only)

There are ***9 Ephemeroptera taxa*** (Acentrella, Isonychia, Epeorus, Leucrocuta, Rhithrogena, Stenonema, Ephemerella, Serratella, Paraleptophlebia), ***5 Plecoptera taxa*** (Pteronarcys, Taeniopteryx, Leuctra, Agnetina, Paragnetina) and ***8 Trichoptera taxa*** (Chimarra, Dolophilodes, Rhyacophila, Glossosoma, Brachycentrus, Micrasema, Apatania, Psilotreta) in this sub-sample ***with PTVs ≤ 4***, so

EPT Taxa Richness (PTV 0 – 4) = 9 + 5 + 8

EPT Taxa Richness (PTV 0 – 4) = 22

Beck's Index, version 3

Beck's Index, version 3 =
(3 x (number of taxa with PTV = 0)) +
(2 x (number of taxa with PTV = 1)) +
(1 x (number of taxa with PTV = 2))

There are ***7 taxa in this sub-sample with PTV = 0***.
There are ***6 taxa in this sub-sample with PTV = 1***.
There are ***7 taxa in this sub-sample with PTV = 2***, so

Beck's Index, version 3 = 3(7) + 2(6) + 1(7)

Beck's Index, version 3 = 21 + 12 + 7

Beck's Index, version 3 = 40

Hilsenhoff Biotic Index

$$\text{Hilsenhoff Biotic Index} = \sum_{i=0}^{10} [(i * n_{indvPTVi})] / N$$

where $n_{indvPTVi}$ = the number of individuals in a sub-sample with PTV of i and N = the total number of individuals in a sub-sample

There are 22 individuals with PTV = 0
There are 57 individuals with PTV = 1
There are 11 individuals with PTV = 2
There are 16 individuals with PTV = 3
There are 12 individuals with PTV = 4
There are 22 individuals with PTV = 5
There are 74 individuals with PTV = 6
There are 2 individuals with PTV = 7
There are 0 individuals with PTV = 8 or 9
There is 1 individual with PTV = 10.

There are a total of 217 individuals in the sub-sample, so

$$\text{Hilsenhoff Biotic Index} = [(0 * 22) + (1 * 57) + (2 * 11) + (3 * 16) + (4 * 12) + (5 * 22) + (6 * 74) + (7 * 2) + (8 * 0) + (9 * 0) + (10 * 1)] / 217$$

Hilsenhoff Biotic Index = 3.47

Shannon Diversity Index

$$\text{Shannon Diversity Index} = [-\sum_{i=1}^{Rich} (n_i / N) \ln (n_i / N)]$$

where n_i = the number of individuals in each taxa (relative abundance); N = the total number of individuals in a sub-sample; and Rich = the total number of taxa in a sub-sample (total taxa richness)

There are 33 taxa in this sub-sample. The numbers of individuals in each taxa are shown in the table above. There are a total of 217 individuals in the sub-sample, so

$$\text{Shannon Diversity Index} = -(1 / 217) \ln (1 / 217) + (4 / 217) \ln (4 / 217) + (6 / 217) \ln (6 / 217) + (1 / 217) \ln (1 / 217) + (9 / 217) \ln (9 / 217) + (8 / 217) \ln (8 / 217) + (32 / 217) \ln (32 / 217) + (1 / 217) \ln (1 / 217) +$$

… (do this for all 33 taxa)

$$\ldots (1 / 217) \ln (1 / 217)$$

Shannon Diversity Index = 2.67

Percent Sensitive (PTV 0 – 3) Individuals

$$\text{Percent Sensitive (PTV 0 – 3) Individuals} = \left(\sum_{i=0}^{3} n_{indvPTVi}\right) / N * 100$$

where $n_{indvPTVi}$ = the number of individuals in a sub-sample with PTV of i and N = the total number of individuals in a sub-sample

There are 22 individuals with PTV = 0
There are 57 individuals with PTV = 1
There are 11 individuals with PTV = 2
There are 16 individuals with PTV = 3

There are a total of 217 individuals in the sub-sample, so

Percent Sensitive (PTV 0 – 3) Individuals = (22 + 57 + 11 + 16) / 217 *100

Percent Sensitive (PTV 0 – 3) Individuals = 106 / 217 * 100

Percent Sensitive (PTV 0 – 3) Individuals = 48.8%

The Index

An index is simply a means to integrate information from various measures of biological integrity, or various metrics (Barbour et al. 1999). In order to compare and combine sundry measures (e.g., percentage of individuals, counts of taxa, unitless numbers) of biological condition in a meaningful manner, it is necessary to standardize metrics with some mathematical transformation that results in a logical progression of values (Barbour et al. 1995).

The one selected core metric that increases in value with increasing anthropogenic stress (i.e., the Hilsenhoff Biotic Index) was standardized to the 5th percentile of metric scores for all samples in the IBI development dataset. Core metrics that decrease in value with increasing stress (i.e., total taxa richness, EPT taxa richness, % sensitive individuals, Shannon diversity, Beck's Index) were standardized to the 95th percentile of metrics scores for all samples in the IBI development dataset. The following table presents the standardization values used for each core metric.

Metric	Standardization value
Total Taxa Richness	33
EPT Taxa Richness (PTV 0 – 4)	19
Beck's Index, version 3	38
Hilsenhoff Biotic Index	1.89
Shannon Diversity	2.86
Percent Sensitive Individuals (PTV 0 – 3)	84.5

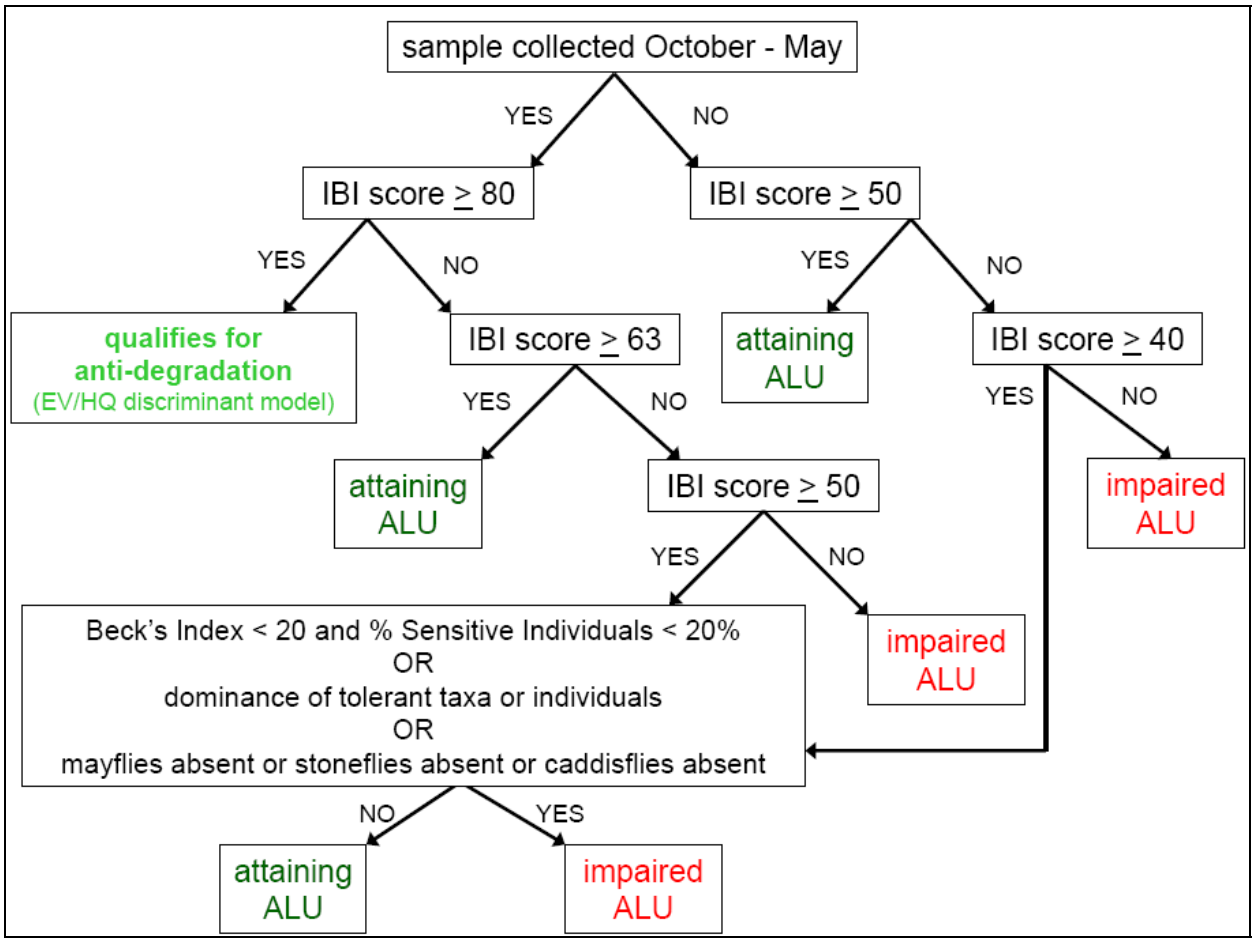
The values for standardized core metric values were set to a maximum value of 1.00, with values closer to zero corresponding to increasing deviation from the expected reference condition and progressively higher values corresponding more closely to the biological reference condition (Barbour et al. 1995). The adjusted standardized metric values for the six core metrics were averaged and multiplied by 100 to produce an index score ranging from 0 to 100. This number represents the multimetric index of biological integrity (IBI) score for a sample. The following table shows the standardized metric and index scoring calculations for the Lycoming Creek sample discussed above.

Metric	Standardization Equation	Observed Metric Value	Standardized Metric Score	Adjusted Standardized Metric Score Maximum = 1.000
Total Taxa Richness	observed value / 33	33	1.000	1.000
EPT Taxa Richness	observed value / 19	22	1.158	1.000
Modified Beck's Index	observed value / 38	40	1.053	1.000
Hilsenhoff Biotic Index	(10 – observed value) / (10 – 1.89)	3.47	0.805	0.805
Shannon Diversity	observed value / 2.86	2.67	0.934	0.934
Percent Sensitive Individuals	observed value / 84.5	48.8	0.578	0.578
Average of adjusted standardized core metric scores * 100 = **IBI Score =**				**88.6**

Aquatic Life Use Attainment Benchmarks

Based on the results of classification analyses (details available upon request), DEP decided not to establish separate reference conditions and thresholds for wadeable freestone, riffle-run type streams in separate regions of the Commonwealth. However, due to the influences of annual seasons and drainage area seen in the IBI development dataset, DEP recognizes different use attainment thresholds are appropriate for samples collected during different times of the year and from different size stream systems.

Based on the results of the analyses presented above, the results of workshops and feedback from DEP biologists and policy considerations, DEP implements a multi-tiered benchmark decision process for smaller wadeable freestone riffle-run streams in Pennsylvania that incorporates sampling season as a factor for determining aquatic life use (ALU) attainment and impairment for the cold water fishes (CWF), warm water fishes (WWF) and trout stocking (TSF) protected uses; this process is outlined in the diagram below.



The first step in the ALU assessment process for smaller wadeable freestone riffle-run streams in Pennsylvania considers sampling season (i.e. June through September versus October through May). These seasonal index periods are intended as general guidelines and may vary slightly year-to-year depending on climatological conditions; for example, a sample collected during the last week of May in a particularly hot, dry year may be more properly evaluated using procedures set forth for the summer months.

The IBI anti-degradation benchmark for smaller streams (i.e., IBI score ≥ 80) is only applicable from October through May; samples for anti-degradation surveys in wadeable freestone streams should not be collected during the summer months (i.e., June to September). Samples that qualify for anti-degradation consideration are subject to a discriminant model that then determines their status as either exceptional value (EV) or high quality (HQ) anti-degradation tiers (Pennsylvania Department of Environmental Protection 2008). The antidegradation candidacy benchmark scores will be implemented as qualifiers for special protection status only (i.e., a sample with an IBI score at or above the applicable benchmark will be afforded special protection status); they will not be used as impairment thresholds for special protection waters. The majority of the original EV and HQ designations were not based on biological sampling. As a result, we do not know what IBI score these streams may have had when and since they were designated special protection. Due to this uncertainty, we will protect a special protection stream at the IBI measured during a current survey minus the temporal precision estimated for the IBI development dataset (11.0 IBI points). For example, if Pine Run is designated HQ with no available historic metric values or IBI scores and the new method results in an IBI score of 75.0, the stream will retain its HQ status and IBI scores from future surveys will not be allowed to fall below 64.0 (75.0 minus 11.0) without listing the stream as impaired. Special protection sites that generate relatively low

IBI scores will be protected at the 63.0 impairment benchmark. For example, if Mud Run scores 68.0, the IBI will not be allowed to fall below the 63.0 impairment threshold; the impairment threshold would not be lowered to 57.0 (68.0 minus 11.0). There will be exceptional circumstances when the above scoring thresholds do not apply (e.g., when there are obvious sources of impairment or the stream never deserved special protection status); however, for the majority of cases, the IBI scoring and use attainment thresholds described in the previous paragraph will apply.

For samples collected from smaller streams between October and May, an IBI score $\geq$ 63 results in ALU attainment and an IBI score < 50 results in ALU impairment; an IBI score between 50 and 63 requires further evaluation to determine ALU impairment – three guidelines may be used: (1) if the Beck's Index score is < 20 and the % Sensitive Individuals in the sub-sample is < 20%, the ALU should be impaired without compelling reason otherwise; (2) if the sample is dominated by tolerant taxa or individuals, the ALU should be impaired without compelling reason otherwise; or (3) if mayflies, stoneflies or caddisflies are absent from the sub-sample the ALU should be impaired without compelling reason otherwise.

For samples collected between June and September from smaller streams, an IBI score $\geq$ 50 results in ALU attainment and an IBI score < 40 results in ALU impairment; an IBI score between 40 and 50 requires further evaluation to determine ALU impairment, guided by the same three guidelines outlined above for October to May samples scoring between 50 and 63 (although the absence of mayflies in samples collected immediately after spring hatches may be relaxed in some cases).

For larger wadeable freestone riffle-run type streams, DEP believes more samples are necessary to accurately establish ALU attainment and impairment benchmarks. Given the nature of flowing water bodies as gradually changing continuums, it is difficult to define a specific numeric cutoff to separate larger streams from smaller streams. However, the present dataset suggest that scores for some index metrics begin to decline for reference-quality streams drainage areas reach the 25 to 50 square mile range. Workshops conducted by DEP confirm that biological expectations or potential for most of the relatively pristine larger freestone streams in Pennsylvania are less than the biological expectations or potential for the relatively pristine smaller freestone streams.

The use assessment decision process and accompanying attainment/impairment benchmarks set forth above are intended as general guidelines, not as hard-and-fast rules. While the above guidelines will provide an accurate assessment of benthic macroinvertebrate community condition for the vast majority of samples collected from wadeable, freestone, riffle-run streams in Pennsylvania, there will be instances where a biologist's local knowledge of conditions may warrant a decision not arrived at using these guidelines. For instance, if a sample is heavily dominated by Simuliidae or Chironomidae larvae, often times this will make the metric and IBI scores difficult to interpret and the investigating biologist must rely on a more qualitative analysis of the metric scores and sample composition to arrive at an assessment decision. Similarly, samples from streams in areas receiving a substantial amount of flow from groundwater attributable to limestone geology are naturally expected to have less diversity than "true freestone" streams, so use attainment benchmarks may be justifiably relaxed for samples from these types of streams.

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