Limestone Stream Macroinvertebrate Surveys

This document outlines the procedures for conducting macroinvertebrate field collection, sample processing, and interpretation of the results in true limestone streams. The method is excerpted from the DEP document "An Index of Biological Integrity (IBI) for 'True' Limestone Streams, March 2009, William Botts." This latter document presents the technical details of the metric selection process and scoring presented in this condensed narrative.

Stream Classification

A true limestone stream must meet the following criterion for this method to be applicable.

Limestone Streams Criteria

Parameter	Criterion	Explanation
Alkalinity	Minimum 140 mg/l	Stream must maintain high alkalinity throughout the year
Temperature	40 to 65 deg. F 4 to 18 deg. C	Constant temperatures are very important, check to see if the stream is ice free in the winter
Stream originates from limestone springs or very strongly influenced by limestone springs		
Drainage Area	Maximum 20 sq. miles	There maybe exception to this parameter as long as all other criteria are met
Designated Water Use	Cold Water Fishery (CWF)	Must be designated a CWF in Chapter 93

Field Sampling and Sample Processing Methods

Net Mesh Considerations

All invertebrate samples collected for the development of this document used net mesh in the 800-900µ range. In recent years, many state water quality programs, federal agencies (e.g. EPA, USGS), and other water quality monitoring organizations began using net sampling devices with 500µ mesh nets. Due to the natural conditions of limestone streams 500µ mesh size quickly clogs preventing macroinvertebrates and vegetation from entering the net resulting in a poor sample. In order to insure an accurate assessment 800-900µ net mesh must be used to collect samples.

D-Frame Net

The handheld D-frame sampler consists of a bag net attached to a half-circle ("D" shaped) frame that is 1 ft. wide. The net is employed by one person facing downstream and holding the net firmly on the stream bottom. One "d-frame effort" is defined as: vigorous kicks in an approximate area of 1m2 (1 x 1 m) immediately upstream of the net to a depth of 10cm (or 0 approximately 4", as the embeddedness of the substrate will allow) for approximately one minute. All benthic dislodgement and substrate scrubbing should be done by kicks only. Substrate handling should be limited to the removal of large rocks or debris (as needed) with no hand washing. Since the width of the kick area is wider than the net opening, net placement is critical to assure all kicked material flows

toward the net. Avoiding areas with crosscurrents, the substrate material from within the 1 m2 area should be kicked toward the center of the square meter area.

Semi-Quantitative Method (PaDEP-RBP):

The PaDEP-RBP is a modification of the EPA RBP III (Plafkin, J.L, M.T. Barbour, K.D. Porter, S.K. Gross, and R.M. Hughes. 1989. Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic macroinvertebrates and fish. EPA/440/4-89-001. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.); designed to be compatible with Pennsylvania's historical database. Modifications include: 1) the use of a D-frame net for the collection of the riffle/run samples, 2) different laboratory sorting procedures, 3) elimination of the CPOM (coarse particulate organic matter) sampling, and 4) metrics substitutions. Unlike the EPA's RBP III methodology, no field sorting is done. Only larger rocks, detritus, and other debris are rinsed and removed while in the field before the sample is preserved. While EPA's RBP III method was designed to compare impacted waters to reference conditions (cause/effect approach), the PaDEP-RBP modifications were designed for un-impacted waters, as well as impacted waters.

Sample Collection

The purpose of the standardized PaDEP-RBP sampling procedure is to obtain representative macroinvertebrate fauna from comparable stations. The PaDEP-RBP assumes the riffle/run to be the most productive habitat. Riffle/run habitats are sampled using the D-frame net method described above. For limestone stream surveys, two paired D-frame efforts are collected from each station - one from an area of fast current velocity and one from an area of slower current velocity within the same riffle. Limestone streams have low gradient often making it difficult to locate well developed riffles. If there are no riffles in the sample area, a run or the best rock substrate available is sampled. The resulting "D-frame efforts" (two) are composited into one sample jar (or more as necessary). Care must be taken to minimize "wear and tear" on the collected organisms when compositing the materials. It is recommended that the benthic material be placed in a bucket filled with water to facilitate gentle stirring and mixing. The sample is preserved in ethanol (95%) and returned to the lab for processing.

Sample Collection Period

Samples must be collected from January through May. All samples used to develop this IBI were collected in this time period. Limestone streams have a low number of sensitive taxa and only a few of these taxa are generally found in large numbers. One very important sensitive taxon is *Ephemerella*. A good population of *Ephemerella* generally indicates better water quality. The three species of *Ephemerella*: *invaria (rotunda)* and *dorothea* found in Pennsylvania limestone streams emerge in May and June and are normally difficult or impossible to collect after emergence and until nymphs mature. Collecting samples from January through May ensures this very important ecological indicator taxa will not be missed.

Sample Processing

Samples collected with a D-frame net are generally considered to be qualitative.
However, the preserved samples can be processed in a manner which yields data that is

"semi-quantitative" – 0 collected by qualitative methods but yielding information that is almost statistically as strong as that collected by quantitative methods.

The following procedure is adapted from EPA 1999 RBP methodology and used to process qualitative D-frame samples so that the resulting data can be analyzed using benthic macroinvertebrate biometric indices (or "metrics"). Equipment needed for the benthic sample processing includes:

- 2 large laboratory pans gridded into 28 squares (more gridded pans may be necessary depending on the size of the sample). White polyethylene pans 18"L x 12"W x 3.5"D were used, but any similarly sized pan with 28 equal grids may be used.
- Illuminated magnifying viewer. (optional)
- Slips of paper (numbered from 1 to 28) for drawing random numbers, and
- Forceps (or any tools that can be used to pick floating benthic organisms),
- Grid cutters made from tubular material that approximates an inside area of 4 in2.

The targeted sub-sample size is 300 for Limestone surveys (± 20%), (240 to 360 organisms). Samples must be properly prepared for sub-sampling. Macroinvertebrates tend to clump so the sample should be mixed in the sample container or the sub-sample pan to make it as homogenous as possible. If necessary the sample maybe mixed in a bucket prior to being placed in the pan. In order to further reduce the effect of clumping a two-tiered sub-sampling technique is employed. A minimum of 4 grids must be selected from the first pan.

Tier 1 – Rinse the sample in a standard USGS No. 35 sieve to remove fine materials and residual preservative. During the rinse, larger rocks, sticks, and leaves maybe removed making sure to retain all the macroinvertebrates. Place the rinsed sample in a 28-square gridded pan (Pan1) and add enough water to distribute the sample evenly. Randomly select 4 grids using the 28 random number set and, using the grid cutters, remove the debris and organisms entirely from within the grid cutter and place in a second gridded pan (Pan2). Selecting a minimum of 4 grids reduces the effect of clumping. Do a visual scan of Pan2 to ensure that there are enough identifiable (this excludes pupae, extremely small instar larvae, and empty shells or cases) organisms to reach the targeted sub-sample size (300 +/- 20%). If there do not appear to be enough organisms randomly select additional grids until there appears there are a minimum of 300 +/- 20% organisms.
Note: In limestone streams, more than 4 grids has never been needed.

Tier 2 –Randomly select grids from pan2 removing all the organisms from each grid until there is a sub-sample of 300 +/- 20%. If it appears that the number of benthic organisms from the last grid will cause the sub-sample to exceed it's target size by more than 20% (>360 organisms), count them and place in a clean gridded pan (Pan3) with enough water to facilitate gentle stirring and even distribution. Randomly select grids from Pan3 and remove individuals until the count of organisms remaining in Pan3 falls within the +20% upper limit.

Comments:

1. If the sample is too large to fit in pan 1 evenly divide sample into 2 or more pans. Randomly select a minimum 4 grids from each pan and place them in a pan.
2. The benthic material remaining after the target sub-sample has been picked can be returned to its original sample jar and preserved. It must be retained in accordance with QA retention times as specified for this respective survey type.
3. Any grid chosen must be picked in its entirety.

Identification, Taxonomic Level

The level of identification for most aquatic macroinvertebrates is to genus. Some individuals collected will be immature and not exhibit the characteristics necessary for confident identification. If an individual cannot be confidently identified to the proper level it should be discarded. All pupae are discarded. Certain groups are identified to a higher taxonomic level as follows:

Flatworms (Turbellaria) - Phylum Turbellaria
Segmented worms (Annelida) aquatic earthworms & tubificids - Class Oligochaeta
Proboscis worms – Phylum Nemertea
Roundworms - Phylum Nematoda
Water mites - "Hydracarina" (an artificial taxonomic grouping of several mite superfamilies)
Midges – Family Chironimadae

Metrics

The following table describes the metrics used to evaluate the macroinvertebrate communities.

Category	**Metric**	**Definition**	**Response to Pollution**
Richness Measure	Total Taxa	Number of taxa in the subsample	Decreases
	EPT Taxa	Number of taxa in the orders Ephemeroptera, Plecoptera and Trichoptera	Decreases
Tolerance/ Intolerance Measures	Beck's Index, 4	Taxa with a HBI of 0 or 1 are given 2 points and HBI of 2, 3, or 4 are given 1 point	Decreases
	% Tolerant	Percent of organisms considered to be tolerant of pollution, HBI >6	Increases
	HBI	The biotic index and abundance of each taxa are used to find a biotic index for the sample	Increases
Composition Measures	Shannon Diversity	Uses both taxa richness and abundance to measure general diversity and composition	Decreases

The individual metrics are scored as follows using the standardization formula in the following tables. The first table scores the metrics that increase as conditions improve and the second table scores the metrics that increase as conditions degrade.

	Standard (best value)		
Metrics	X_{95}	X_{min}	Standardization Formula
Total Taxa	18.0	0	Score = (X/18.0) x 100
EPT Taxa	8.0	0	Score = (X/8.0) x 100
Beck's Index, 4	12.0	0	Score = (X/12.0) x 100
Shannon Diversity	2.13	0	Score = (X/2.13) x 100

Metrics such as % Tolerant and HBI increase with greater impairment. The lower the score for these metrics the better the ecological condition.
Metrics

	Standard (best value)		
Metrics	X_5	X_{max}	Standardization Formula
% Tolerant	1.5	100	Score = (100 - X/100 - 1.5) x 100
HBI	3.84	10	Score = (10 - X/10 - 3.84) x 100

Threshold Scoring

The final score is compared to the values in the table below and assigned to one of four categories. Sites scoring less than 60 are considered impaired and should be placed on Integrated List Category 5 of impaired streams requiring TMDLs.

IBI Scoring Thresholds

Limestone Stream IBI Scoring Thresholds

	CWF	CWF	Impaired CWF	
Classification	Reference	Attaining	Moderately	Severely
IBI Score	>72	72-60	59-30	<30

Less Than <60 is impaired