

APPENDICES

- I. Biological Methods for Kick Sampling
- II. Macroinvertebrate Community Parameters
- III. Levels of Water Quality Impact in Streams
- IV. Biological Assessment Profile Description
- V. Water Quality Assessment Criteria
- VI. Traveling Kick Sample Illustration
- VII. Macroinvertebrate Illustrations
- VIII. Rationale for Biological Monitoring
- IX. Glossary
- X. Impact Source Determination Methods and Models
- XI. Methods for Calculating the Nutrient Biotic Index

BIOLOGICAL METHODS FOR KICK SAMPLING

A. Rationale. The use of the standardized kick sampling method provides a biological assessment technique that lends itself to rapid assessments of stream water quality.

B. Site Selection. Sampling sites are selected based on these criteria: (1) The sampling location should be a riffle with a substrate of rubble, gravel, and sand. Depth should be one meter or less, and current speed should be at least 0.4 meters per second. (2) The site should have comparable current speed, substrate type, embeddedness, and canopy cover to both upstream and downstream sites to the degree possible. (3) Sites are chosen to have a safe and convenient access.

C. Sampling. Macroinvertebrates are sampled using the standardized traveling kick method. An aquatic net is positioned in the water at arms' length downstream and the stream bottom is disturbed by foot, so that the dislodged organisms are carried into the net. Sampling is continued for a specified time and for a specified distance in the stream. Rapid assessment sampling specifies sampling five minutes for a distance of five meters. The net contents are emptied into a pan of stream water. The contents are then examined, and the major groups of organisms are recorded, usually on the ordinal level (e.g., stoneflies, mayflies, caddisflies). Larger rocks, sticks, and plants may be removed from the sample if organisms are first removed from them. The contents of the pan are poured into a U.S. No. 30 sieve and transferred to a quart jar. The sample is then preserved by adding 95% ethyl alcohol.

D. Sample Sorting and Subsampling. In the laboratory the sample is rinsed with tap water in a U.S. No. 40 standard sieve to remove any fine particles left in the residues from field sieving. The sample is transferred to an enamel pan and distributed homogeneously over the bottom of the pan. A small amount of the sample is randomly removed with a spatula, rinsed with water, and placed in a petri dish. This portion is examined under a dissecting stereo microscope and 100 organisms are randomly removed from the debris. As they are removed, they are sorted into major groups, placed in vials containing 70 percent alcohol, and counted. The total number of organisms in the sample is estimated by weighing the residue from the picked subsample and determining its proportion of the total sample weight.

E. Organism Identification. All organisms are identified to the species level whenever possible. Chironomids and oligochaetes are slide-mounted and viewed through a compound microscope; most other organisms are identified as whole specimens using a dissecting stereomicroscope. The number of individuals in each species, and the total number of individuals in the subsample is recorded on a data sheet. All organisms from the subsample are archived (either slide-mounted or preserved in alcohol). If the results of the identification process are ambiguous, suspected of being spurious, or do not yield a clear water quality assessment, additional subsampling may be required.

MACROINVERTEBRATE COMMUNITY PARAMETERS

1. Species richness is the total number of species or taxa found in the sample. For subsamples of 100-organisms each that are taken from kick samples, expected ranges in most New York State streams are: greater than 26, non-impacted; 19-26, slightly impacted; 11 - 18, moderately impacted; less than 11, severely impacted.

2. EPT Richness denotes the total number of species of mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddisflies (Trichoptera) found in an average 100-organism subsample. These are considered to be mostly clean-water organisms, and their presence generally is correlated with good water quality (Lenat, 1987). Expected ranges from most streams in New York State are: greater than 10, non-impacted; 6- 10 slightly impacted; 2-5, moderately impacted; and 0- 1, severely impacted.

3. Hilsenhoff Biotic index is a measure of the tolerance of the organisms in the sample to organic pollution (sewage effluent, animal wastes) and low dissolved oxygen levels. It is calculated by multiplying the number of individuals of each species by its assigned tolerance value, summing these products, and dividing by the total number of individuals. On a 0-10 scale, tolerance values range from intolerant (0) to tolerant (10). For purposes of characterizing species' tolerance, intolerant = 0-4, facultative = 5-7, and tolerant = 8-10. Values are listed in Hilsenhoff (1987); additional values are assigned by the NYS Stream Biomonitoring Unit. The most recent values for each species are listed in the Quality Assurance document (Bode et al., 1996). Ranges for the levels of impact are: 0-4.50, non-impacted; 4.5 1-6.50, slightly impacted; 6.5 1-8.50, moderately impacted; and 8.51 - 10.00, severely impacted.

4. Percent Model Affinity is a measure of similarity to a model non-impacted community based on percent abundance in seven major macroinvertebrate groups (Novak and Bode, 1992). Percent abundances in the model community are 40% Ephemeroptera, 5% Plecoptera, 10% Trichoptera, 10% Coleoptera, 20% Chironomidae, 5% Oligochaeta, and 10% Other. Impact ranges are: greater than 64, non-impacted; 50-64, slightly impacted; 35-49, moderately impacted; and less than 35, severely impacted.

Bode, R.W., M.A. Novak, and L.E. Abele. 1996. Quality assurance work plan for biological stream monitoring in New York State. NY S DEC technical report, 89 pp.

Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. *The Great Lakes Entomologist* 20(1): 31-39.

Lenat, D. R. 1987. Water quality assessment using a new qualitative collection method for freshwater benthic macroinvertebrates. North Carolina DEM Tech. Report. 12 pp.

Novak, M.A., and R. W. Bode. 1992. Percent model affinity: a new measure of macroinvertebrate community composition. *J. N. Am. Benthol. Soc.* 11(1):80-85.

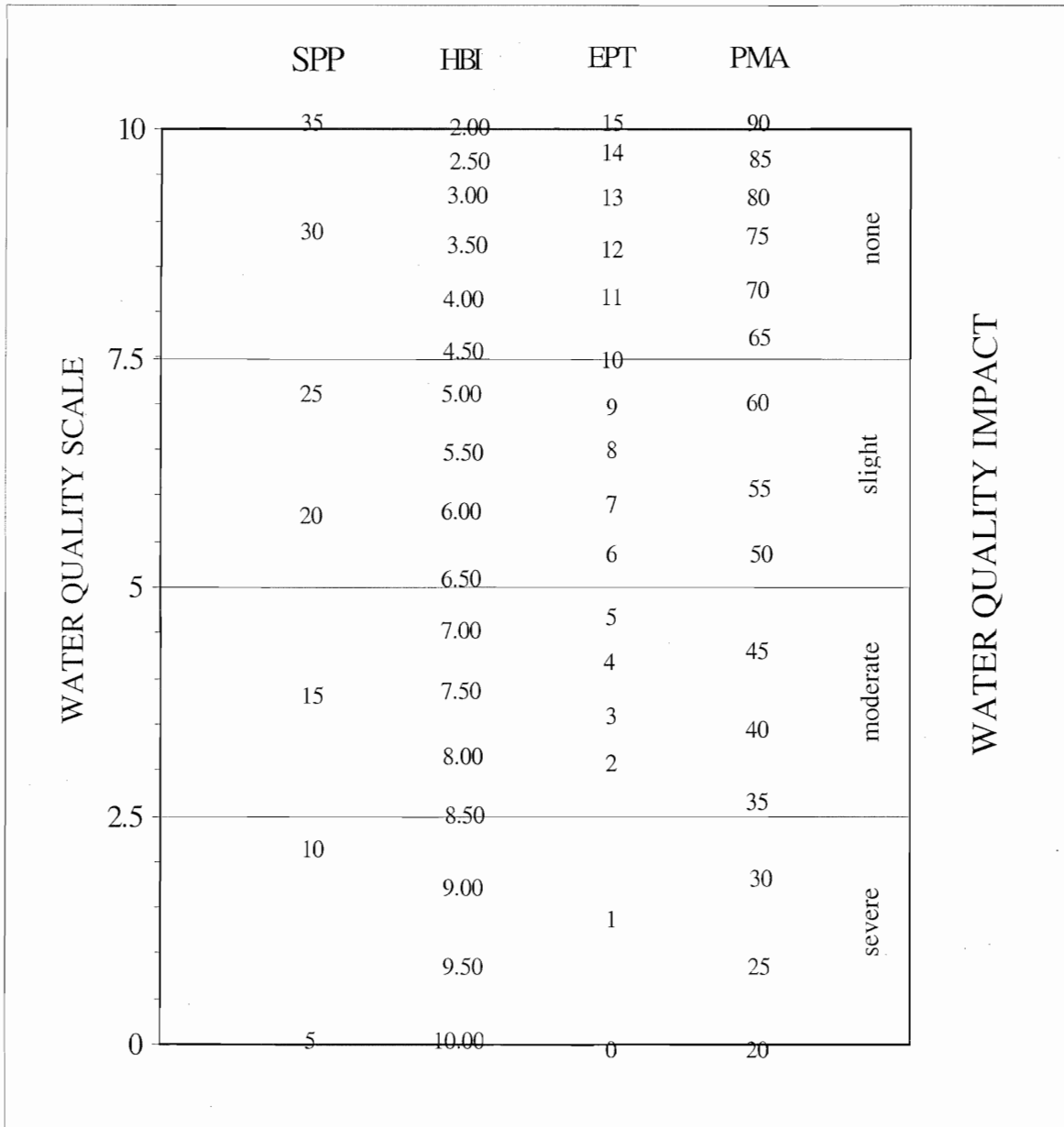
LEVELS OF WATER QUALITY IMPACT IN STREAMS

The description of overall stream water quality based on biological parameters uses a four-tiered system of classification. Level of impact is assessed for each individual parameter, and then combined for all parameters to form a consensus determination. Four parameters are used: species richness, EPT richness, biotic index, and percent model affinity (*see Macroinvertebrate Community Parameters Appendix*). The consensus is based on the determination of the majority of the parameters. Since parameters measure different aspects of the macroinvertebrate community, they cannot be expected to always form unanimous assessments. The assessment ranges given for each parameter are based on subsamples of 100-organisms each that are taken from macroinvertebrate riffle kick samples. These assessments also apply to most multiplate samples, with the exception of percent model affinity.

1. *Non-impacted* Indices reflect very good water quality. The macroinvertebrate community is diverse, usually with at least 27 species in riffle habitats. Mayflies, stoneflies, and caddisflies are well-represented; EPT richness is greater than 10. The biotic index value is 4.50 or less. Percent model affinity is greater than 64. Water quality should not be limiting to fish survival or propagation. This level of water quality includes both pristine habitats and those receiving discharges which minimally alter the biota.
2. *Slightly impacted* Indices reflect good water quality. The macroinvertebrate community is slightly but significantly altered from the pristine state. Species richness usually is 19-26. Mayflies and stoneflies may be restricted, with EPT richness values of 6-10. The biotic index value is 4.51-6.50. Percent model affinity is 50-64. Water quality is usually not limiting to fish survival, but may be limiting to fish propagation.
3. *Moderately impacted* Indices reflect poor water quality. The macroinvertebrate community is altered to a large degree from the pristine state. Species richness usually is 11-18 species. Mayflies and stoneflies are rare or absent, and caddisflies are often restricted; the EPT richness is 2-5. The biotic index value is 6.51- 8.50. The percent model affinity value is 35-49. Water quality often is limiting to fish propagation, but usually not to fish survival.
4. *Severely impacted* Indices reflect very poor water quality. The macroinvertebrate community is limited to a few tolerant species. Species richness is 10 or less. Mayflies, stoneflies, and caddisflies are rare or absent; EPT richness is 0-1. The biotic index value is greater than 8.50. Percent model affinity is less than 35. The dominant species are almost all tolerant, and are usually midges and worms. Often 1-2 species are very abundant. Water quality is often limiting to both fish propagation and fish survival.

Biological Assessment Profile: Conversion of Index values to Common 10-Scale

The Biological Assessment Profile of index values, developed by Phil O'Brien, Division of Water, NYSDEC, is a method of plotting biological index values on a common scale of water-quality impact. Values from the four indices, defined in the Macroinvertebrate Community Parameter Appendix, are converted to a common 0-10 scale using the formulae in the Quality Assurance document (Bode, et al., 2002) and as shown in the figure below.



Biological Assessment Profile: Plotting Values

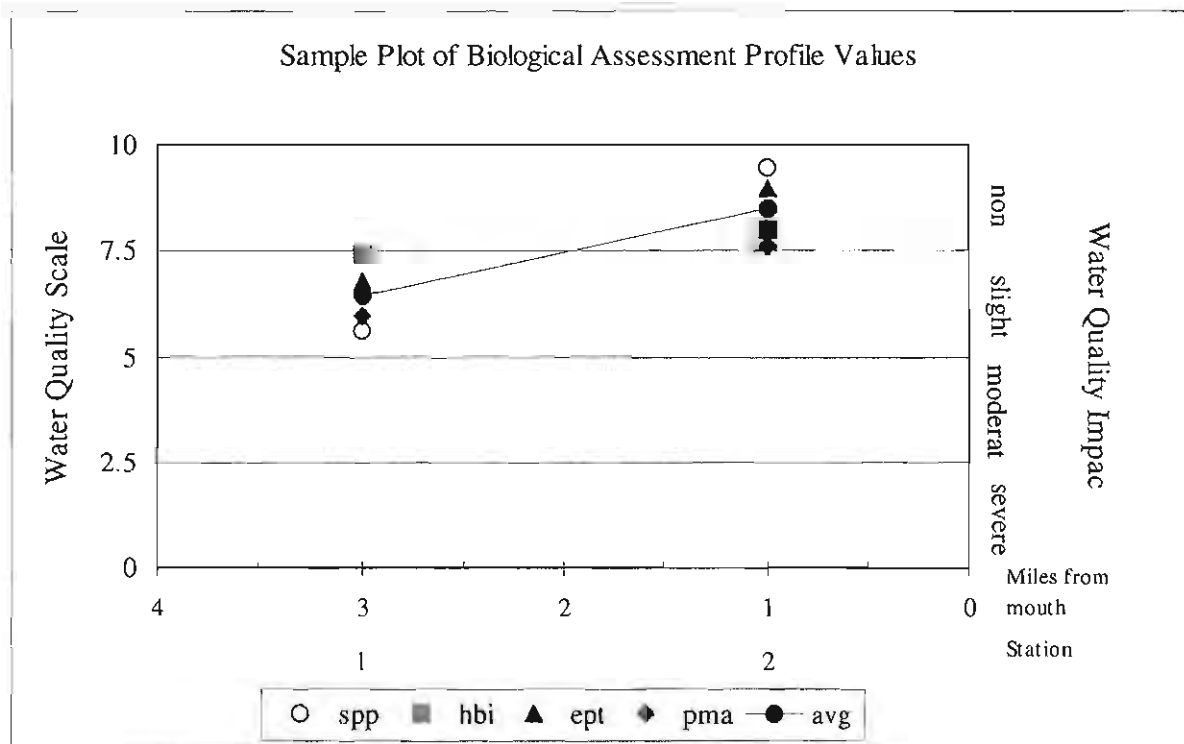
To plot survey data:

1. Position each site on the x-axis according to miles or tenths of a mile upstream of the mouth.
2. Plot the values of the four indices for each site as indicated by the common scale.
3. Calculate the mean of the four values and plot the result. This represents the assessed impact for each site.

Example data:

	Station 1		Station 2	
	metric value	10-scale value	metric value	10-scale value
Species richness	20	5.59	33	9.44
Hilsenhoff biotic index	5.00	7.40	4.00	8.00
EPT richness	9	6.80	13	9.00
Percent model affinity	55	5.97	65	7.60
Average		6.44 (slight)		8.51 (non-)

Table IV-B. Sample Plot of Biological Assessment Profile values



Water Quality Assessment Criteria

Water Quality Assessment Criteria for Non-Navigable Flowing Waters

	Species Richness	Hilsenhoff Biotic Index	EPT Richness	Percent Model Affinity#	Species Diversity*
Non-Impacted	>26	0.00-4.50	>10	>64	>4
Slightly Impacted	19-26	4.51-6.50	6-10	50-64	3.01-4.00
Moderately Impacted	11-18	6.51-8.50	2-5	35-49	2.01-3.00
Severely Impacted	0-10	8.51-10.00	0-1	<35	0.00-2.00

Percent model affinity criteria are used for traveling kick samples but not for multiplate samples.

* Diversity criteria are used for multiplate samples but not for traveling kick samples.

Water Quality Assessment Criteria for Navigable Flowing Waters

	Species Richness	Hilsenhoff Biotic Index	EPT Richness	Species Diversity
Non-Impacted	>21	0.00-7.00	>5	>3.00
Slightly Impacted	17-21	7.01-8.00	4-5	2.51-3.00
Moderately Impacted	12-16	8.01-9.00	2-3	2.01-2.50
Severely Impacted	0-11	9.01-10.00	0-1	0.00-2.00

THE TRAVELING KICK SAMPLE



← CURRENT →

Rocks and sediment in the stream riffle are dislodged by foot upstream of a net; dislodged organisms are carried by the current in the net. Sampling is continued for a specified time, gradually moving downstream to cover a specified distance.

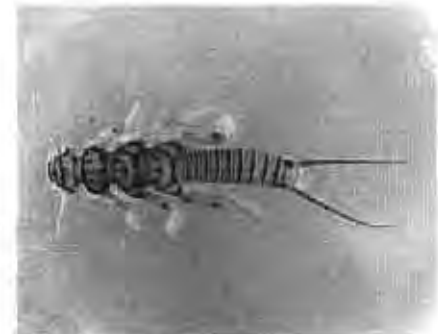
AQUATIC MACROINVERTEBRATES THAT USUALLY INDICATE GOOD WATER QUALITY

Mayfly nymphs are often the most numerous organisms found in clean streams. They are sensitive to most types of pollution, including low dissolved oxygen (less than 5 ppm), chlorine, ammonia, metals, pesticides, and acidity. Most mayflies are found clinging to the undersides of rocks.



MAYFLIES

Stonefly nymphs are mostly limited to cool, well-oxygenated streams. They are sensitive to most of the same pollutants as mayflies, except acidity. They are usually much less numerous than mayflies. The presence of even a few stoneflies in a stream suggests that good water quality has been maintained for several months.



STONEFLIES

Caddisfly larvae often build a portable case of sand, stones, sticks, or other debris. Many caddisfly larvae are sensitive to pollution, although a few are tolerant. One family spins nets to catch drifting plankton, and is often numerous in nutrient-enriched stream segments.



CADDISFLIES

The most common beetles in streams are riffle beetles and water pennies. Most of these require a swift current and an adequate supply of oxygen, and are generally considered clean-water indicators.



BEEPLES



AQUATIC MACROINVERTEBRATES THAT USUALLY INDICATE POOR WATER QUALITY

Midges are the most common aquatic flies. The larvae occur in almost any aquatic situation. Many species are very tolerant to pollution. Large, red midge larvae called "bloodworms" indicate organic enrichment. Other midge larvae filter plankton, indicating nutrient enrichment when numerous.



MIDGES

Black fly larvae have specialized structures for filtering plankton and bacteria from the water, and require a strong current. Some species are tolerant of organic enrichment and toxic contaminants, while others are intolerant of pollutants.



BLACK FLIES



The segmented worms include the leeches and the small aquatic earthworms. The latter are more common, though usually unnoticed. They burrow in the substrate and feed on bacteria in the sediment. They can thrive under conditions of severe pollution and very low oxygen levels, and are thus valuable pollution indicators. Many leeches are also tolerant of poor water quality.



WORMS



Aquatic sowbugs are crustaceans that are often numerous in situations of high organic content and low oxygen levels. They are classic indicators of sewage pollution, and can also thrive in toxic situations.

Digital images by Larry Abele, New York State Department of Environmental Conservation, Stream Biomonitoring Unit.



SOWBUGS

THE RATIONALE OF BIOLOGICAL MONITORING

Biological monitoring refers to the use of resident benthic macroinvertebrate communities as indicators of water quality. Macroinvertebrates are larger than-microscopic invertebrate animals that inhabit aquatic habitats; freshwater forms are primarily aquatic insects, worms, clams, snails, and crustaceans.

Concept

Nearly all streams are inhabited by a community of benthic macroinvertebrates. The species comprising the community each occupy a distinct niche defined and limited by a set of environmental requirements. The composition of the macroinvertebrate community is thus determined by many factors, including habitat, food source, flow regime, temperature, and water quality. The community is presumed to be controlled primarily by water quality if the other factors are determined to be constant or optimal. Community components which can change with water quality include species richness, diversity, balance, abundance, and presence/absence of tolerant or intolerant species. Various indices or metrics are used to measure these community changes. Assessments of water quality are based on metric values of the community, compared to expected metric values.

Advantages

The primary advantages to using macroinvertebrates as water quality indicators are:

- 1) they are sensitive to environmental impacts
- 2) they are less mobile than fish, and thus cannot avoid discharges
- 3) they can indicate effects of spills, intermittent discharges, and lapses in treatment
- 4) they are indicators of overall, integrated water quality, including synergistic effects and substances lower than detectable limits
- 5) they are abundant in most streams and are relatively easy and inexpensive to sample
- 6) they are able to detect non-chemical impacts to the habitat, e.g. siltation or thermal changes
- 7) they are vital components of the aquatic ecosystem and important as a food source for fish
- 8) they are more readily perceived by the public as tangible indicators of water quality
- 9) they can often provide an on-site estimate of water quality
- 10) they can often be used to identify specific stresses or sources of impairment
- 11) they can be preserved and archived for decades, allowing for direct comparison of specimens
- 12) they bioaccumulate many contaminants, so that analysis of their tissues is a good monitor of toxic substances in the aquatic food chain

Limitations

Biological monitoring is not intended to replace chemical sampling, toxicity testing, or fish surveys. Each of these measurements provides information not contained in the others. Similarly, assessments based on biological sampling should not be taken as being representative of chemical sampling. Some substances may be present in levels exceeding ambient water quality criteria, yet have no apparent adverse community impact.

Anthropogenic: caused by human actions

Assessment: a diagnosis or evaluation of water quality

Benthos: organisms occurring on or in the bottom substrate of a waterbody

Bioaccumulate: accumulate contaminants in the tissues of an organism

Biomonitoring: the use of biological indicators to measure water quality

Community: a group of populations of organisms interacting in a habitat

Drainage basin: an area in which all water drains to a particular waterbody; watershed

EPT richness: the number of species of mayflies (**E**phemeroptera), stoneflies (**P**lecoptera), and caddisflies (**T**richoptera) in a sample or subsample

Facultative: occurring over a wide range of water quality; neither tolerant nor intolerant of poor water quality

Fauna: the animal life of a particular habitat

Impact: a change in the physical, chemical, or biological condition of a waterbody

Impairment: a detrimental effect caused by an impact

Index: a number, metric, or parameter derived from sample data used as a measure of water quality

Intolerant: unable to survive poor water quality

Longitudinal trends: upstream-downstream changes in water quality in a river or stream

Macroinvertebrate: a larger-than-microscopic invertebrate animal that lives at least part of its life in aquatic habitats

Multiplate: multiple-plate sampler, a type of artificial substrate sampler of aquatic macroinvertebrates

Organism: a living individual

PAHs: Polycyclic Aromatic Hydrocarbons, a class of organic compounds that are often toxic or carcinogenic

Rapid bioassessment: a biological diagnosis of water quality using field and laboratory analysis designed to allow assessment of water quality in a short time; usually involves kick sampling and laboratory subsampling of the sample

Riffle: wadeable stretch of stream usually having a rubble bottom and sufficient current to break the water surface; rapids

Species richness: the number of macroinvertebrate species in a sample or subsample

Station: a sampling site on a waterbody

Survey: a set of samplings conducted in succession along a stretch of stream

Synergistic effect: an effect produced by the combination of two factors that is greater than the sum of the two factors

Tolerant: able to survive poor water quality

Impact Source Determination Methods and Community Models

Definition: Impact Source Determination (ISD) is the procedure for identifying types of impacts that exert deleterious effects on a waterbody. While the analysis of benthic macroinvertebrate communities has been shown to be an effective means of determining severity of water quality impacts, it has been less effective in determining what kind of pollution is causing the impact. ISD uses community types or models to ascertain the primary factor influencing the fauna.

Development of methods: The method found to be most useful in differentiating impacts in New York State streams was the use of community types based on composition by family and genus. It may be seen as an elaboration of Percent Model Affinity (Novak and Bode, 1992), which is based on class and order. A large database of macroinvertebrate data was required to develop ISD methods. The database included several sites known or presumed to be impacted by specific impact types. The impact types were mostly known by chemical data or land use. These sites were grouped into the following general categories: agricultural nonpoint, toxic-stressed, sewage (domestic municipal), sewage/toxic, siltation, impoundment, and natural. Each group initially contained 20 sites. Cluster analysis was then performed within each group, using percent similarity at the family or genus level. Within each group, four clusters were identified. Each cluster was usually composed of 4-5 sites with high biological similarity. From each cluster, a hypothetical model was then formed to represent a model cluster community type; sites within the cluster had at least 50 percent similarity to this model. These community type models formed the basis for ISD (see tables following). The method was tested by calculating percent similarity to all the models and determining which model was the most similar to the test site. Some models were initially adjusted to achieve maximum representation of the impact type. New models are developed when similar communities are recognized from several streams.

Use of the ISD methods: Impact Source Determination is based on similarity to existing models of community types (see tables following). The model that exhibits the highest similarity to the test data denotes the likely impact source type, or may indicate "natural," lacking an impact. In the graphic representation of ISD, only the highest similarity of each source type is identified. If no model exhibits a similarity to the test data of greater than 50 percent, the determination is inconclusive. The determination of impact source type is used in conjunction with assessment of severity of water quality impact to provide an overall assessment of water quality.

Limitations: These methods were developed for data derived from subsamples of 100-organisms each that are taken from traveling kick samples of New York State streams. Application of these methods for data derived from other sampling methods, habitats, or geographical areas would likely require modification of the models.

METHODS FOR CALCULATION OF THE NUTRIENT BIOTIC INDEX

Definition: The Nutrient Biotic Index (Smith, 2005) is a diagnostic measure of stream nutrient enrichment identified by macroinvertebrate taxa. The frequency of occurrences of taxa at varying nutrient concentrations allowed the identification of taxon-specific nutrient optima using a method of weighted averaging. The establishment of nutrient optima is possible based on the observation that most species exhibit unimodal response curves in relation to environmental variables (Jongman et al. 1987). The assignment of tolerance values to taxa based on their nutrient optimum provided the ability to reduce macroinvertebrate community data to a linear scale of eutrophication from oligotrophic to eutrophic. Two tolerance values were assigned to each taxon, one for total phosphorus, and one for nitrate (listed in Smith, 2005). This provides the ability to calculate two different nutrient biotic indices, one for total phosphorus (NBI-P) and one for nitrate (NBI-N). Study of the indices indicate better performance by the NBI-P, with strong correlations to stream nutrient status assessment based on diatom information.

Calculation of the NBI-P and NBI-N: Calculation of the indices [2] follows the approach of Hilsenhoff (1987).

$$NBI\ Score_{(TP\ or\ NO_3^-)} = \sum (a \times b) / c$$

Where *a* is equal to the number of individuals for each taxon, *b* is the taxon's tolerance value, and *c* is the total number of individuals in the sample (for which tolerance values have been assigned).

Classification of NBI Scores NBI scores have been placed on a scale of eutrophication with provisional boundaries between stream trophic status.

Index	Oligotrophic	Mesotrophic	Eutrophic
NBI-P	< 5.0	> 5.0 – 6.5	> 6.0
NBI-N	< 4.5	> 4.5 – 6.0	> 6.0

References:

- Hilsenhoff, W. L. 1987. An improved biotic index of organic stream pollution. *The Great Lakes Entomologist* 20(1): 31-39.
- Jongman, R. H. G., C. J. F. ter Braak, and O. F. R. van Tongeren. 1987. *Data analysis in community and landscape ecology*. Pudoc Wageningen, Netherlands 299pp.
- Smith, A.J. 2005. Development of a Nutrient Biotic Index for use with benthic macroinvertebrates. Masters Thesis, SUNY Albany. 70 pages.