

Chapter 6

The Use of the Biotic Index as an Indication of Water Quality

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Introduction

This exercise has six objectives:

1. Learn about the food web, functional feeding groups, and diversity of a typical freshwater stream ecosystem.
2. Understand how the biological and chemical traits of the stream change with pollution.
3. Develop the concept of organisms as indicators of organic pollution and calculate the Biotic Index.
4. Examine community concepts of species diversity.
5. Use a dichotomous key to classify organisms.
6. Finally, extend our investigative skills to combine field and laboratory methodology, data collection, and analysis into an interpretation of ecosystem events.

The write-up of this laboratory is intended to serve not only as a basis for a laboratory in a general biology course for majors, but also provide enough information for an independent project or a general ecology laboratory. The construction of the stream food web and Biotic Index has served as: (1) investigative independent exercises for general biology students at Cornell University and, (2) a formal laboratory in the general biology course at Lycoming College.

The complete study (food web construction, measurement of BOD, coliforms, and chemical/physical parameters, and the determination of the Biotic Index and species Diversity Indexes) has served as a basis for a 2-week investigative laboratory in general ecology at Lycoming College. When the complete laboratory is done, the first week is used by the class to collect all field samples and finish water chemical tests in the laboratory. The second week is used to classify organisms and begin data analysis. Students, as a class, finishing the entire study are then given 2 weeks to complete data analysis and summarize results in a paper written independently in the format of a scientific journal.

When the laboratory is used as a 1-week exercise in general biology, field collected samples are brought into the laboratory and students classify organisms, construct a food web, calculate the Biotic Index, and discuss their results. In conjunction with this laboratory, I assign either one of the following two articles by Cummins (1974, 1975a): Structure and Function of Stream Ecosystems or The Ecology of Running Waters: Theory and Practice. If students are writing papers, additional articles are put on reserve in the library.

Student Outline

Background

As human populations have grown, more and more categories of pollution of our surface waters have occurred. One of the most common pollution categories is organic pollution caused by oxygen-demanding wastes as domestic sewage, wood fiber from pulp and paper mills, effluent from food processing plants, and run-off from agricultural areas (especially hay, dairy, and cattle farms). Dissolved oxygen is consumed either through chemical oxidation of these substances or through the respiratory processes of biological decomposition. Decomposition of materials is a normal process in all aquatic ecosystems and is a function of decomposers such as bacteria and fungi. These organisms metabolize the organic matter as an energy and nutrient source and utilize dissolved oxygen in the process. However, serious consequences can result if these natural mechanisms are overloaded by large influxes of organic matter. Severe oxygen depletion can result in the loss of desirable aquatic life and may produce an odorous anaerobic system. Although sewage may contain a variety of pollutants (i.e. heavy metals, pesticides), only the general impact of organic loading is considered in this exercise.

The effects of oxygen-demanding wastes on a stream are depicted in Figure 6.1. The severity and duration of the pollution episode depend upon many factors including amount of waste, size of stream, and temperature. Biochemical Oxygen Demand (BOD) is a common measure of the strength of an effluent containing biodegradable organic matter. BOD is defined as the total amount of oxygen required by microbes to decompose a given amount of waste. BOD levels are high at the source of the effluent and gradually decrease over time and downstream as the waste is stabilized. As dissolved oxygen (DO) is depleted, the macroinvertebrates (those invertebrates retained in a No. 30 sieve) and fish which require high concentrations may be eliminated and replaced by pollution-tolerant forms. Algae may be eliminated at the outfall by high turbidity levels but also stimulated downstream by the release of nutrients from microbial activities. Eventually the waste is metabolized and microbial populations are reduced by organisms feeding on them. The final step in the process of recovery is the reappearance of pollution-sensitive fish and invertebrate life.

BOD values are routinely determined in laboratory tests of many types of waste-water. However, there is no short or simple way to measure BOD. The standard test is a bioassay which extends over 5 days and is carried out within carefully defined conditions. In addition, the BOD test and many other chemical/physical determinations of water quality, evaluates specific characteristics of the water only at the time of sampling and do not measure past short-term pollution stresses (perturbations). This is why organisms, especially slow sessile aquatic invertebrates which cannot swim away from intermittent perturbations, can be used as biological indicators of water pollution because their presence or absence may reflect conditions not otherwise evident when the researcher checks the site. Furthermore, they are probably best suited because they are numerous in almost every stream, are readily collected and identified, and can be classified as pollution sensitive (or in the case of organic pollution to be “tolerant”, “facultative” or “intolerant” of low dissolved oxygen conditions).

It is a well known fact (see Figure 6.1) that pollution of a stream reduces the number of species of the system (i.e., Species Diversity), while frequently creating an environment that is favorable to a few species (i.e., pollution-tolerant forms). Thus, in a polluted stream, there are usually large numbers of a few species, while in a clean stream there are moderate numbers of many species. For instance, many gill-breathing mayfly, stonefly, and caddisfly larvae can survive only where

there is abundant oxygen in the water. Other invertebrates can tolerate low oxygen water because they breathe atmospheric oxygen via snorkel-like breathing tubes (i.e., the rat-tailed maggot) or have some other special adaptations like respiratory pigments which enable them to more efficiently obtain oxygen that is in low concentration (i.e., Tubifax worms and chironomid midge larvae).

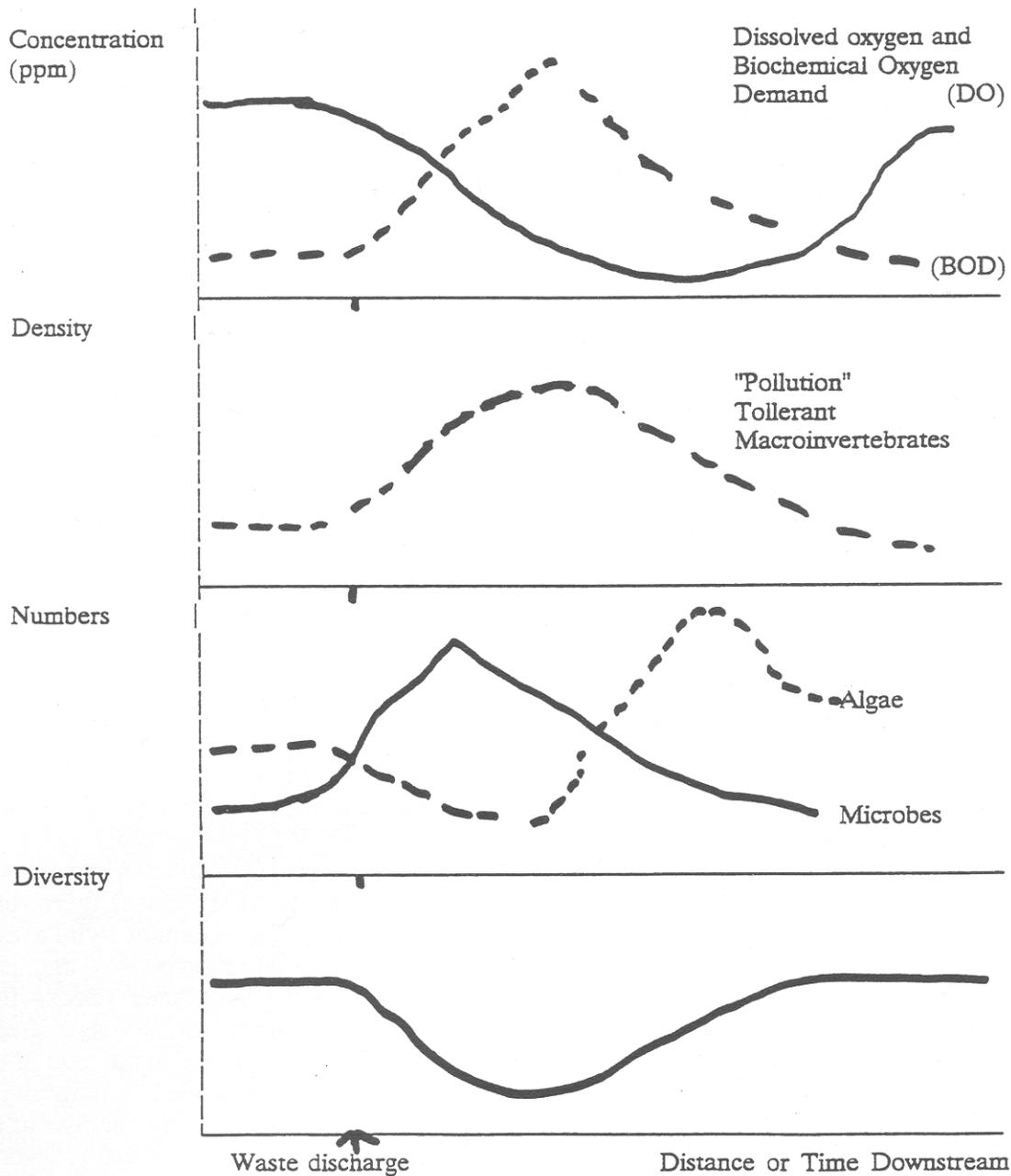


Figure 6.1. Effects of organic pollution (i.e., raw sewage) on a stream ecosystem. Adapted from Bartsch and Ingram (1975).

Because both pollution sensitive and tolerant forms are present in “clean” waters, it is the absence of the former coupled with the presence of the latter which may indicate damage. This is the basis of the *Biotic Index*. The Biotic Index (BI) is based on categorizing macroinvertebrates into categories depending on their response to organic pollution (i.e., the tolerance of various levels of dissolved oxygen; see Figure 6.2). One of the most comprehensive of these indexes is the one proposed by the Hilsenhoff (1977) formula:

$$BI = \frac{\sum n_i a_i}{N}$$

Where n_i is the number of specimens in each taxonomic group, a_i is the pollution tolerance score for that taxonomic group, and N is the total number of organisms in sample. Macroinvertebrates are given a numerical pollution tolerance score (a_i) ranging from 0 to 5.

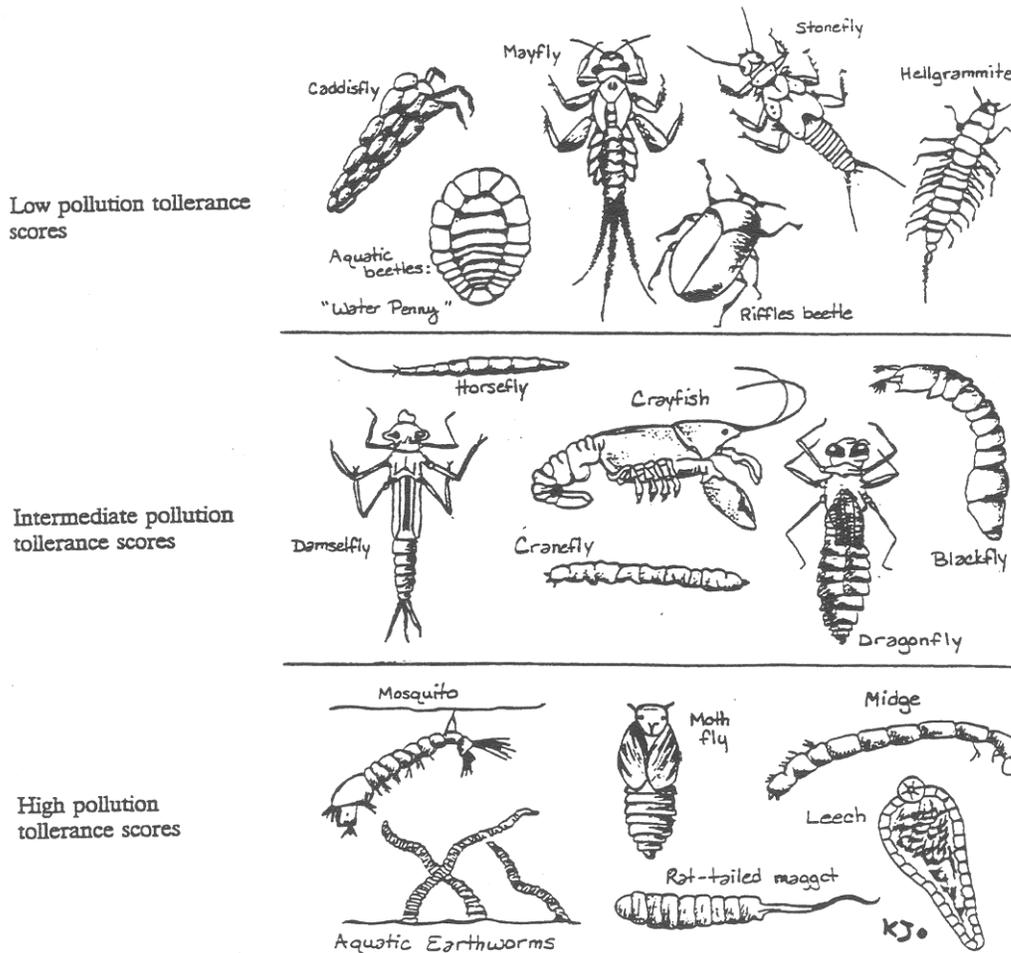


Figure 6.2. General pollution tolerance for common aquatic organisms.

In 1987, Hilsenhoff reevaluated the pollution tolerance scores and expanded the range from 0 to 10. The value is based on field and laboratory responses of these organisms toward organic pollution. Zero taxa are extremely intolerant of low dissolved oxygen; taxa with scores of 2 through 9 are tolerant to varying degrees; taxa which can survive great amounts of pollution are scored 10. A problem with the application of this form of the biotic index (BI) for general use is the requirement to identify the organisms to genus and/or species.

In 1988, Hilsenhoff proposed a family-level biotic index (FBI). The purpose of the FBI is to provide a rapid, but less critical, evaluation of streams and is not intended as a substitute for the BI when detailed taxonomic information is available. The FBI uses the same formula as the BI but substitutes average pollution tolerance scores (ai) for a family instead of differences in species. FBI uses the same formula as the BI but substitutes average pollution tolerance scores (ai) for a family instead of differences in species. Table 6.1 summarizes the family pollution tolerance scores (ai) for a variety of stream arthropods. Table 6.2 summarizes ranges of FBI scores for a stream site water quality interpretation as proposed by Hilsenhoff (1988a, 1988b).

In this laboratory/field exercise, you are asked to determine the water quality of stream sites based primarily on the family biotic index. Additional support for your interpretation may come from analyses of species diversity, density, and/or analysis of chemical parameters. You may also wish to examine the difference in functional feeding categories of the stream food web proposed by Cummins (1974, 1975a, 1975b) between sites of varying water quality.

Materials (Students work in pairs)

1. For collection of macroinvertebrates:

- Surber stream-bottom sampler or D-frame aquatic nets (No. 30 sieve)
- Small stiff "floor" brush and trowel
- Bucket
- Wide-mouth quart jar with lids
- Grease pencils or labels
- Blunt forceps
- Formalin (neutral formalin made by saturating 37% formalin with calcium carbonate; make up final solution as 10%)
- Hipboots (and trappers gloves if collection made during cold weather)

2. For collection of stream depth, width and velocity data:

- Metric tape
- Meter stick
- Float or cork
- Stopwatch or watch with second hand or stopwatch and Gurley Pygmy Current Meter (Model 625)

3. For collection of water temperature and dissolved oxygen (DO):

- Thermometer (°C)
- Yellow Springs Instrument Co. (YSI) Oxygen Meter and Thermistor (Model 57) or similar meter kit or chemicals to determine DO by Winkler Method

Table 6.1. Family-level pollution tolerance scores for the Hilsenhoff Biotic Index. Adapted from Bode (1988), Hilsenhoff (1988a, 1988b), and Lehmkuhl (1979).

Plecoptera	Capniidae 1, Chloroperlidae 1, Leuctridae 0, Nemouridae 2, Perlidae 1, Perlodidae 2, Pteronarcyidae 0, Taeniopterygidae 2
Ephemeroptera	Baetidae 4, Baetiscidae 3, Caenidae 7, Ephemerellidae 1, Ephemeridae 4, Heptageniidae 4, Leptophlebiidae 2, Metretopodidae 2, Oligoneuriidae 2, Polymitarcyidae 2, Potomanthidae 4, Siphonuridae 7
Tricorythidae	4
Odonta	Aeshnidae 3, Calopterygidae 5, Coenagrionidae 9, Cordulegastridae 3, Corduliidae 5, Gomphidae 1, Lestidae 9, Libellulidae 9, Macromiidae 3
Trichoptera	Brachycentridae 1, Glososomatidae 0, Helicopsychoidea 3, Hydropsychidae 4, Hydroptilidae 4, Lepidostomatidae 1, Leptoceridae 4, Limnephilidae 4, Molannidae 6, Odontoceridae 0, Philopotamidae 3, Phryganeidae 4, Polycentropodidae 6, Psychomyiidae 2, Rhyacophilidae 0, Sericostomatidae 3
Megaloptera	Corydalidae 0, Sialidae 4
Lepidoptera	Pyralidae 5
Coleoptera	Dryopidae 5, Elmidae 4, Psephenidae 4
Diptera	Athericidae 2, Blephariceridae 0, Ceratopogonidae 6, Blood-red Chironomidae (Chironomini) 8, Other (including pink) Chironomidae 6, Dolichopodidae 4, Empididae 6, Ephydriidae 6, Psychodidae 10, Simuliidae 6, Muscidae 6, Syrphidae 10, Tabanidae 6, Tipulidae 3
Amphipoda	Gammaridae 4, Talitridae 8
Isopoda	Asellidae 8
Acariformes	4
Decapoda	6
Gastropoda	Amnicola 8, Bithynia 8, Ferrissia 6, Gyraulus 8, Helisoma 6, Lymnaea 6, Physa 8, Sphaeriidae 8
Oligochaeta	Chaetogaster 6, Dero 10, Nais barbata 8, Nais behningi 6, Nais bretscheri 6, Nais communis 8, Nais elinguis 10, Nais pardalis 8, Nais simplex 6, Nais variabilis 10, Pristina 8, Stylaria 8, Tubificidae: Aulodrilus 8, Limnodrilus 10
Hirudinea	Helobdella 10
Turbellaria	4

Table 6.2. Water quality based on Family Biotic Index (adapted from Hilsenhoff, 1977).

Biotic Index	Water quality	Degree of organic pollution
0.00–3.50	Excellent	No apparent organic pollution
3.51–4.50	Very good	Possible slight organic pollution
4.51–5.50	Good	Some organic pollution
5.51–6.50	Fair	Fairly significant organic pollution
6.51–7.50	Fairly poor	Significant organic pollution
7.51–8.50	Poor	Very significant organic pollution
8.51–10.0	Very poor	Severe organic pollution

4. For collection/determination of other chemical/physical properties of water:

The HACH Portable Engineers' Water Test Laboratory (there are several models, which use either spectrophotometers or colorimeters, to choose from) or similar portable kit is used for determination of: pH, total phosphate, turbidity, nitrate nitrogen, conductivity, nitrite nitrogen, and ortho-phosphate.

5. For determination of BOD and coliforms:

HACH BOD Kit or *Standard Methods* analysis
Millipore Coli-count or HACH Coliform tube assembly

6. For macroinvertebrate identification:

Enamel trays for sorting
Dissecting microscopes
Blunt and fine forceps
Dissecting pins
Medicine droppers
Small jars or vials
“Watch” glass
Manuals for identification of aquatic macroinvertebrates (suggested are Lehmkuhl, 1975, 1979)

Procedure

For easier direct comparison and for purposes of statistical analysis, two riffle sections of the same stream (a “clean” water upstream site and a downstream polluted site) should be examined. Ideally, each site should be of the same stream order (see Cummins, 1975), and similar in width and depth. If a Surber sampler is used, depth must be less than 1 foot. For purposes of statistical analysis (non-parametric tests are most appropriate), at least six samples of each of the biological, chemical, and physical parameters described below should be collected from each site.

At each site:

1. Make some *general observations* about each site (i.e., type of surrounding vegetation, type of stream substrate).

2. At each of the riffle sites where *benthic samples* are to be collected with the Surber, wade into the water from downstream and place the net with its mouth facing upstream. Lower the square-foot frame of the sampler onto the substrate and hold in place. Pick up all rocks, and while holding them in the mouth of the net, brush them free of all organisms (allow the current to carry them into the net). When done, discard each rock outside the frame. When all rocks have been brushed, use a garden trowel to stir up the substrate within the square-foot frame. Be sure that the stirring motion is toward the center of the frame so that organisms that are dislodged will be carried into the mouth of the net and not around it. Attempt to stir this area thoroughly and to a uniform depth. Empty contents of the Surber net into a labelled collecting jar. To empty the Surber, the net must be turned "inside out" and organisms attached to the fabric must be picked from the net with forceps and placed into the jar. Add sufficient 10% formalin or 70% ethanol to just cover substrate and organisms in the jar.

Note: Accuracy of the biotic index depends on at least 100 organisms being processed from each sample. A D-frame kick net can be used to collect a kick sample from each site to provide enough arthropods for analysis.

3. Take measurements of stream *temperature* and DO at each site where benthic samples were collected.
4. Determine *current velocity* at each site where benthic samples were collected. If a current meter is not available, velocity can be found by dropping a fisherman's float and recording, with a stopwatch, the time required to travel 3 *meters*. Record an average of three determinations as meters per second (m/sec). If a current meter is available, determine both surface and bottom velocity (this value is closer to the organisms' habitat).

Note: It is a well known fact that stream velocity influences macroinvertebrate diversity and density. Therefore, if differences between sites are to be attributed to "pollution" it is important that no statistical difference in velocity exists between sites.

An estimate of *the volume of flow* at each site can be determined by the method outlined in Robins and Crawford (1954). Choose a cross-section of the stream where current and depth are most uniform and measure the width. Then divide this width into three equal segments. Next record the midpoint of each segment and determine the velocity of the surface current (as described above). Determine the volume of flow (R) for each segment of the cross-section by the following formula:

$$R = W D a V$$

Where *a* is a bottom factor constant (0.8 for rocks and coarse gravel; 0.9 for mud, sand, hardpan, or bedrock), *W* refers to the width of segment, *D* is the depth of the segment, and *V* equals the surface current velocity taken at the midpoint of the segment. Total volume of flow is determined by adding the R values for the three segments.

5. Collect water samples (approximately 500 ml) at each site where benthic organisms were collected and transport back to the laboratory for chemical/physical analysis. Follow directions in the HACH kit for tests. Determine average values for each site.
6. If coliform and BOD samples are to be collected, follow directions outlined in specific kits used.
7. In the laboratory, sort and identify the invertebrates in each sample to the family level of classification. To do this, place the contents of a jar in a large flat pan marked with a grid (a 30-cm by 45-cm pan with a 5-cm grid is satisfactory). Number the square in the grid and select a starting square for each sample by picking a number from a table of random numbers. Remove

all arthropods from the starting square and then remove arthropods from each successively higher numbered square until a total count of 100 organisms are collected.

8. Count all arthropods in a sample and calculate the density (organisms/m²) of organisms for each sample as well as an average for both sites. The Surber samples an area of 0.09 m².
9. Calculate both the Shannon-Wiener and Simpson Diversity Indexes. If this analysis is to be done, and you do not have the time or expertise to identify each organism to species, you can use the operational taxonomic unit (OTU) approach. Continue to sort organisms of the same family, separated in step 7 above, into individuals that look the same (i.e., seem to be members of the same species). These groups of “look-alikes” are called OTU species and can be used for calculation of species diversity. The Shannon-Wiener Index (H'), adopted from information theory, is currently one of the most widely used diversity measures. The basic formula is:

$$H' = - \sum_{i=1}^s \left(\frac{n_i}{N} \right) \log \left(\frac{n_i}{N} \right)$$

Where n_i is the number of individuals in the i th species, N equals the total number of individuals in the sample, and s equals the total number of species in the sample. This index, which usually varies from 0 to 5, shows how successful one would be at guessing the next bit of information (i.e., species) after knowing the first. The Simpson Index (C), with values ranging from 0 to 1, is the probability that if two selections are made randomly from a collection of organisms, they will be individuals of the same species. This index is calculated as follows:

$$C = 1 - \sum_{i=1}^s \left(\frac{n_i}{N} \right)^2$$

Determine the average species diversity indexes for each site and compare.

10. Summarize results of the water quality of the two stream sites in a paper written in the format of a scientific journal.
11. (Optional) Compare the functional feeding groups between the two sites (percent shredders, collectors, scrapers, predators; see Cummins, 1974, 1975a, 1975b; Merrit and Cummins, 1984). The paper by Cummins and Wilzbach (1985) provides an excellent, and easy, key to determine functional feeding groups. Has the water quality affected them?

Notes for the Instructor

Scheduling

If the distance to sampling sites is short, and with division of labor, the field collection of biological, chemical, and physical samples can be done in a 3-hour lab period. I often send part of the class back to the laboratory to run chemical tests with the HACH kit. I use a second laboratory period to identify organisms and begin data analysis. A reference collection of “typical” macroinvertebrates speeds up identification. Furthermore, additional time is saved if students have the opportunity to practice tests with the HACH kit. I generally give students 2 weeks to write up their papers.

Equipment

1. Information on HACH equipment can be obtained from HACH Chemical Co., P.O. Box 389, Loveland, CO 80537, or P.O. Box 907, Ames, IA 50010.
2. Surber samplers and nets are available from Wildlife Supply Co., 301 Cass St., Saginaw, MI 48602.
3. Millipore “coli-count” water tester is available from Millipore Corp., Bedford, MA 01730.
4. Dissolved oxygen meter is available from Yellow Springs Instrument Co., Yellow Springs, OH 45387.
5. Current meter is available from Teledyne Gurley, 514 Fulton St., Troy, NY 12181.

Biotic Index

Additional background information and data interpretation of the Hilsenhoff Biotic Index can be obtained in Hilsenhoff (1977) and Lehmkuhl (1979). Other types of biotic indexes, which use aquatic insects, are described in Beck (1954), Chutter (1972), Denoncourt (1975), Heister (1972), Rolan (1973), and Scott (1969).

Species Diversity Indexes and Water Pollution

Additional background information and interpretation can be obtained from Allan (1975), Bradt (1977), Dennis and Patil (1977), Godfrey (1978), Olive and Dambach (1973), and Ransom and Prophet (1974). In general, species diversity should be reduced by organic pollution. A simple diversity index is described by Cairns et al. (1968).

Interpretation of Chemical/Physical Data

Additional background information and interpretation of chemical/physical data can be obtained from Standard Methods (American Public Health Association, 1990) or from Bartsch and Ingram (1967), Hynes (1960, 1972), Sawyer and McCarthy (1967), or Welch (1980).

Identification Keys and Functional Feeding Groups

In addition to Lehmkuhl (1975, 1979), there are good keys in Merritt and Cummins (1984), Pennak (1978), Pekarsky et al. (1990) and Thorp and Covich (1991). Functional feeding groups are described in Cummins (1974, 1975a, 1975b), Cummins and Wilzbach (1985), and Merritt and Cummins (1978). Students can look up family (and/or genera) of each organism and determine if it is a predator, collector, scraper, or shredder.

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APPENDIX A
Form for Enumeration/identification of Aquatic Organisms

Sample type: Periphyton or Benthos
Water Temperature: _____
Slide or Container No: _____

Date: _____
Velocity: _____

Phylum (Division) -
Class -
Order -
Family -
Genus -
Number in sp. 1 -
sp. 2 -
sp. 3 -
sp. 4 -

Phylum (Division) -
Class -
Order -
Family -
Genus -
Number in sp. 1 -
sp. 2 -
sp. 3 -
sp. 4 -

Phylum (Division) -
Class -
Order -
Family -
Genus -
Number in sp. 1 -
sp. 2 -
sp. 3 -
sp. 4 -

Phylum (Division) -
Class -
Order -
Family -
Genus -
Number in sp. 1 -
sp. 2 -
sp. 3 -
sp. 4 -

-
Phylum (Division) -
Class -
Order -
Family -
Genus -
Number in sp. 1 -
sp. 2 -
sp. 3 -
sp. 4 -

Phylum (Division) -
Class -
Order -
Family -
Genus -
Number in sp. 1 -
sp. 2 -
sp. 3 -
sp. 4 -

Phylum (Division) -
Class -
Order -
Family -
Genus -
Number in sp. 1 -
sp. 2 -
sp. 3 -
sp. 4 -

Phylum (Division) -
Class -
Order -
Family -
Genus -
Number in sp. 1 -
sp. 2 -
sp. 3 -
sp. 4 -