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Chapter 15

Biotic Indices of Stream Macroinvertebrates for Fun and (Educational) Profit

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Joy Perry earned a B.S. in General Biology from Purdue University long ago, and an M.S. in Plant Pathology and Integrated Pest Management from the University of Wisconsin – Madison. Her career has included stints as Academic Staff Specialist researching diseases of vegetable and field crops at UW-Madison, being the owner-operator of Savage River Tissue Culture, a commercial micropropagation lab, and a teaching career that began at Allegany College and Frostburg State University in Maryland. Since 1994 she has been a Lecturer in Biological Sciences at UWFox, one of the 13 campuses of the University of Wisconsin Colleges. She teaches courses in general biology, environmental biology, botany, ecology, and in a learning community combining political science, art and environmental science. She has received several campus and institutional awards for excellence in teaching. Mostly, though, she likes to poke around outside and turn over rocks in streams to see who's under there.

Contents

Introduction

> "He sat on the bank, while the river still chattered on to him, a babbling procession of the best stories in the world, sent from the heart of the earth to be told at last to the insatiable sea." From "The Wind in the Willows" by Kenneth Grahame

Rivers do, indeed, tell stories, especially about human activities occurring in their watersheds. We can help our students discover and interpret those stories through field- and lab-based monitoring. Water quality monitoring activities can support student inquiry into ecological concepts and pollution issues, as well as offer insight into integrating field and lab work

This lab-based activity provides an introduction to mid-level laboratory identification of macroinvertebrates to the order or family taxon level. The identification process is made reasonable for beginning students by a pictorial dichotomous key based largely on readily visible features. Pollution tolerance values for many taxa are provided as well, and students calculate three different biotic indices for their macroinvertebrate samples. The index values can be compared and analyzed to provide an overall indication of water quality.

This article offers ideas for integrating this lab with field-based studies as well as extensions of this activity for more advanced students. A list of resources is provided.

Student Outline

You may have made measurements of physical features of a stream, and performed chemical tests, in order to determine water quality. The purpose of this exercise is to introduce you to a more sophisticated water quality analysis using the benthic macroinvertebrate community of a stream. (Benthic macroinvertebrates are those small animals that live on and just beneath the substrates of a stream bottom. They include insect larvae, arthropods and others.) After identifying the macroinvertebrates, you will calculate several numerical biotic indices by noting the abundance of macroinvertebrates and their ability to tolerate water pollution. Each biotic index will provide you with an assessment of the water quality of the stream from which the macroinvertebrates were collected.

Aquatic entomologists may spend a lifetime studying the fine points of benthic macroinvertebrate taxonomy. However, with reasonable care and good identification keys, you can identify most collected organisms to taxonomic family, a level which allows use of several informative biotic indices.

Biotic indices are routinely used in biological monitoring of stream water quality. Such monitoring can give an indication of past water quality conditions as well as current conditions, unlike physical and chemical monitoring. Benthic macroinvertebrates tend to be relatively sedentary and many are also relatively long-lived in their larval forms. They are therefore continuously exposed to the conditions of a given stream region. The pollution tolerance of many taxa has been established, making these organisms ideal indicators of water quality. In general, a larger proportion of pollution intolerant taxa, along with greater numbers of individuals of those pollution intolerant taxa, indicate higher water quality of a stream.

Macroinvertebrate Identification (aka "bug-pickin')

Depending on the number of organisms collected and the time available, your instructor will ask you to perform this procedure individually, or to pool your data as part of a larger group.

- Drain the macroinvertebrate sample through a fine sieve (no. 60) to remove any alcohol preservative. If the specimens are living and you wish to keep them healthy, rinse them with cold stream water. If the specimens are preserved, rinse with tap water.
- Transfer sample to a small bucket of water, swirl it to mix up all the macroinvertebrates, and randomly pour a sample of material into a white pan. Randomly pour or scoop a subsample into a Petri dish; guard yourself against the temptation to select only larger specimens. Our goal is to identify a *representative* sample of organisms.
- Examine your Petri dish subsample under a stereozoom microscope, keeping the specimens wet. Separate all organisms larger than about 1.5 mm from the debris. These organisms are sorted into major "look-alike" groups, identified to family level wherever possible using the attached key (Figure 1).

As you begin to sort and identify each specimen, ask yourself the following questions:

- Does it have a distinct head?
- Does it have legs? If so, how many? An organism with six legs is an insect.
- Does it have what appear to be "tails"? If so, how many?
- Is its body hard or soft? What is the body shape?

As you work, keep count of the number of individuals of each type. Record data on form Appendix A. Continue sorting and identifying specimens until 100 organisms have been identified. Your instructor may direct you to preserve the sorted, identified organisms in labeled vials of 70% ethanol as archived voucher specimens.

Biotic Indices: What Do We Do Now That We've Identified The Critters?

Coal miners used canaries as indicators of mine air quality. Unlike a belly-up canary though, the macroinvertebrate population of a stream must be evaluated in more painstaking manner to determine what it indicates about water quality. Most rating methods, called biotic indices, are based on the fact that the healthiest streams have the greatest number of different types of pollution-sensitive organisms. We will calculate and use three indices: Species richness, EPT richness, and the Hilsenhoff Biotic Index.

Species/taxa richness index (SPP): This index is simply the number of species or taxa identified in the sample. Higher species richness index values are generally associated with cleaner water conditions.

EPT richness (EPT): EPT is the total number of species or higher taxa of mayflies (Order **E**phemeroptera), stoneflies (Order **P**lecoptera), and caddisflies (Order **T**richoptera) found in a 100-organism subsample. These are considered clean-water organisms and their presence is generally correlated with good water quality. The higher the EPT index, the cleaner the stream.

Hilsenhoff Biotic Index (HBI): Hilsenhoff (1988) determined pollution tolerance values for benthic macroinvertebrate families (Table 1.) This information is combined with the identification data from the 100-organism sample to produce the family-level HBI as follows (see sample calculation, Figure 2):

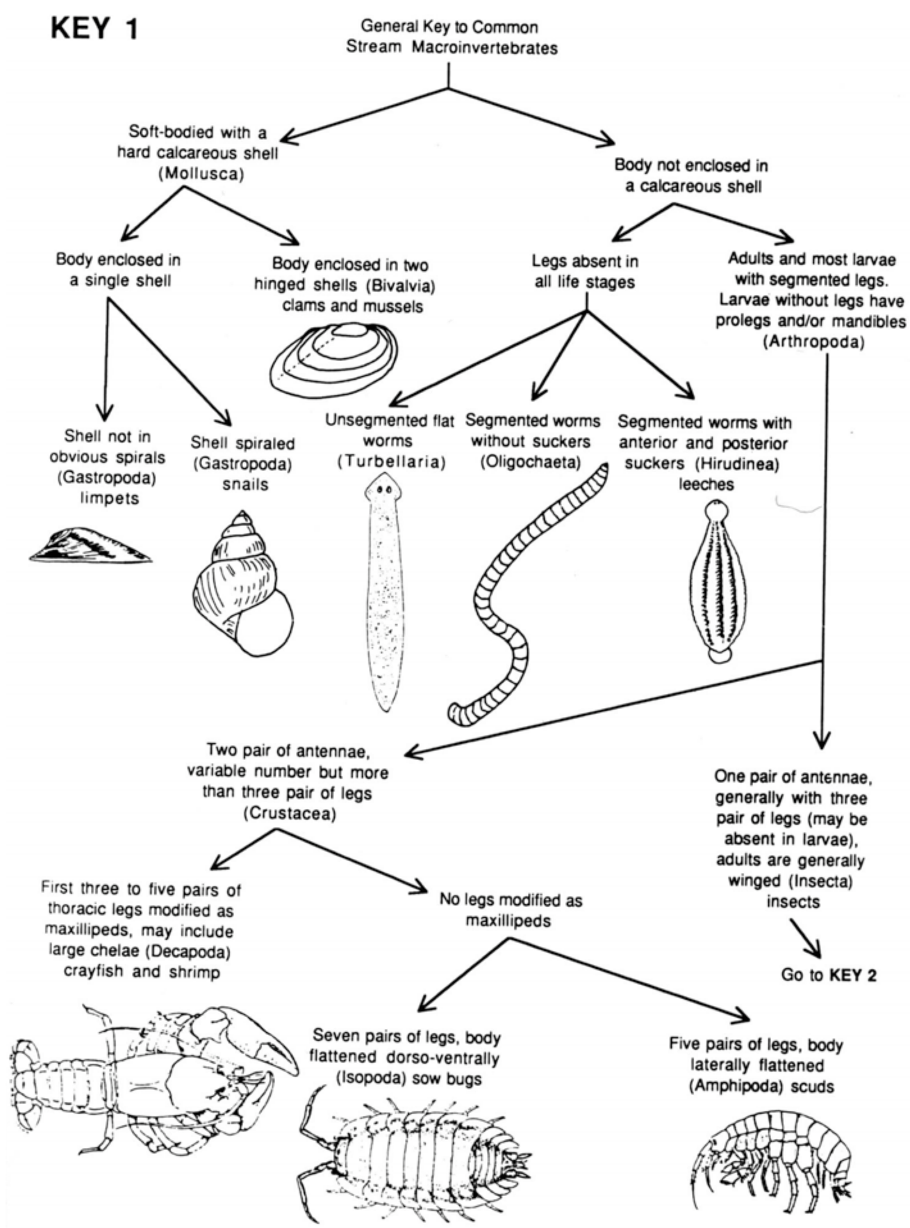
1. Determine the tolerance value for each family in the sample. Each value is an assigned number from 0 to 10, with 0 being very intolerant and 10 being very pollution and stress tolerant.
2. For each taxon, multiply the number of individuals by its tolerance value to yield a "biotic score."
3. Sum all these individual biotic scores.
4. Divide the sum of the biotic scores (from step 3) by the total number of individuals in the sample (usually 100.) This is the HBI family value.

Interpreting The Results

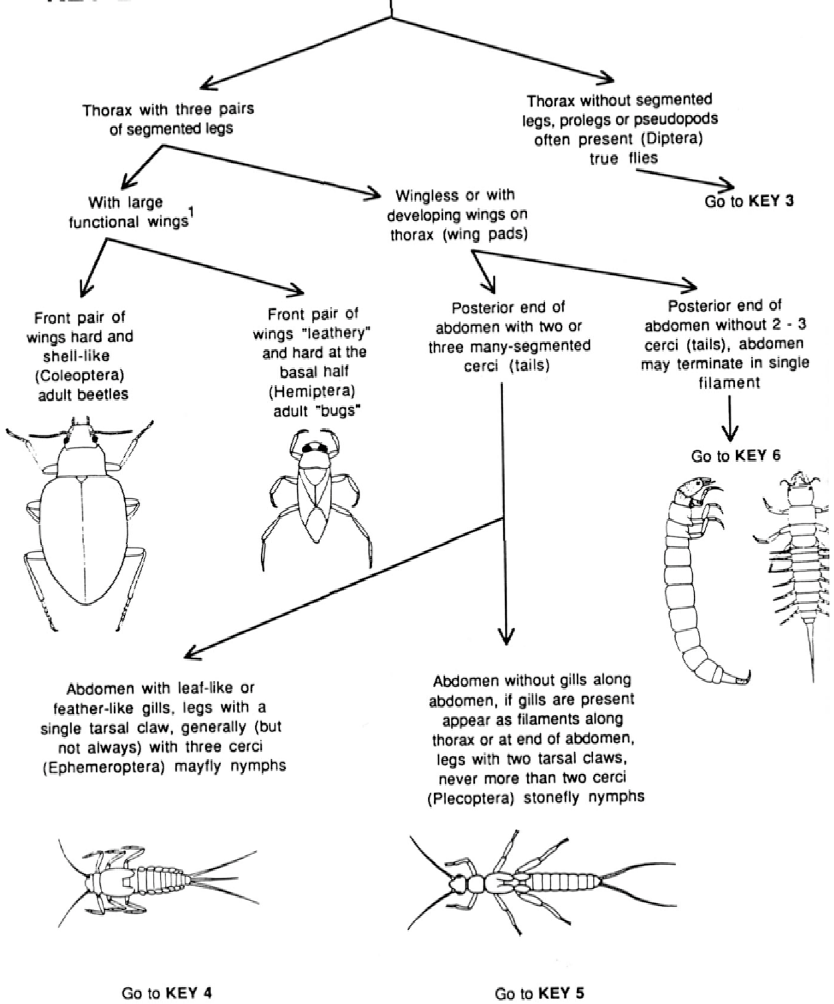
The Biological Assessment Profile (Figure 3) allows you to determine overall water quality impacts of the stream by comparing the index values of the three indices on a common scale. Mean (average) scale value for the three indices represents the overall impact at a site.

Figure 1. A Simplified Key for the Rapid Identification of the Most Commonly Occurring Stream Macroinvertebrates* (Hauer and Resh. 1996)

*Note: This key does not include the Phylum Odonata, the dragonflies and damselflies. The larvae of these insects are distinguished by their lower lip (labium) which is long, hinged, and folded back against the head (and thus most visible from the side). Wing pads are present on the thorax (center body section), as well as three pairs of legs. Each leg ends with a pair of claws. Refer to the print resources in your classroom to distinguish dragonflies from damselflies, and to identify these insects to the family level.

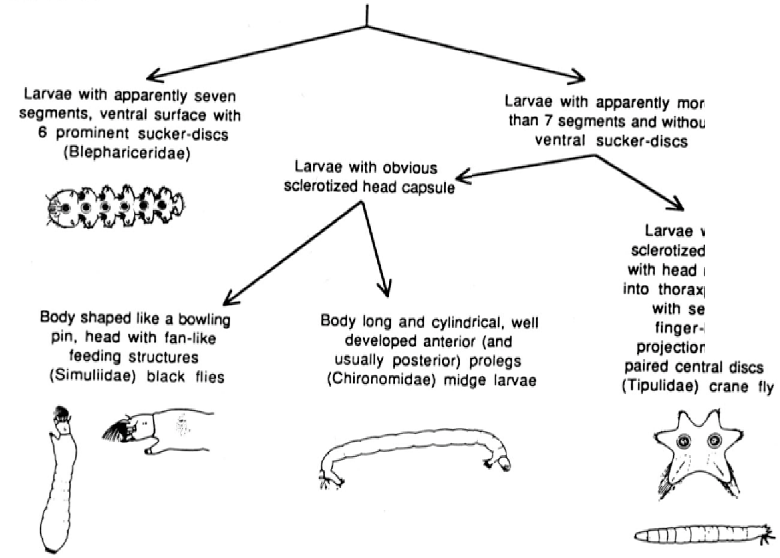


KEY 2

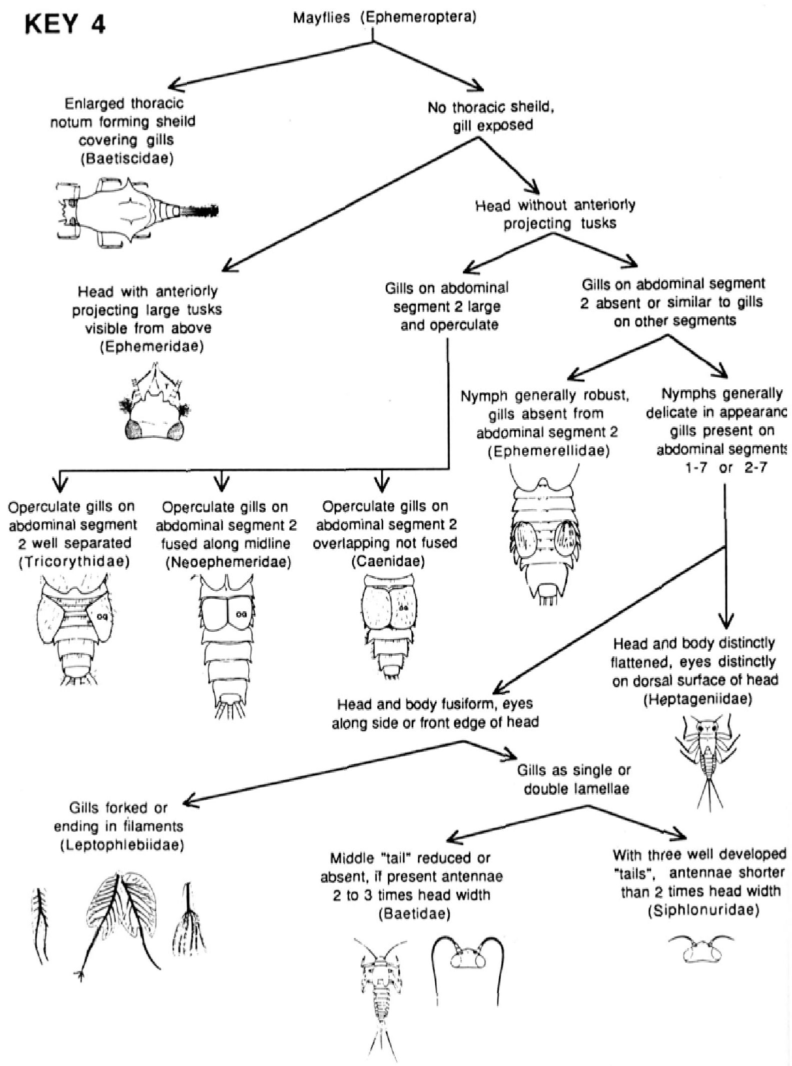


[1] NOTE: Other winged insects may be present from either terrestrial forms or the aerial adults of aquatic larvae

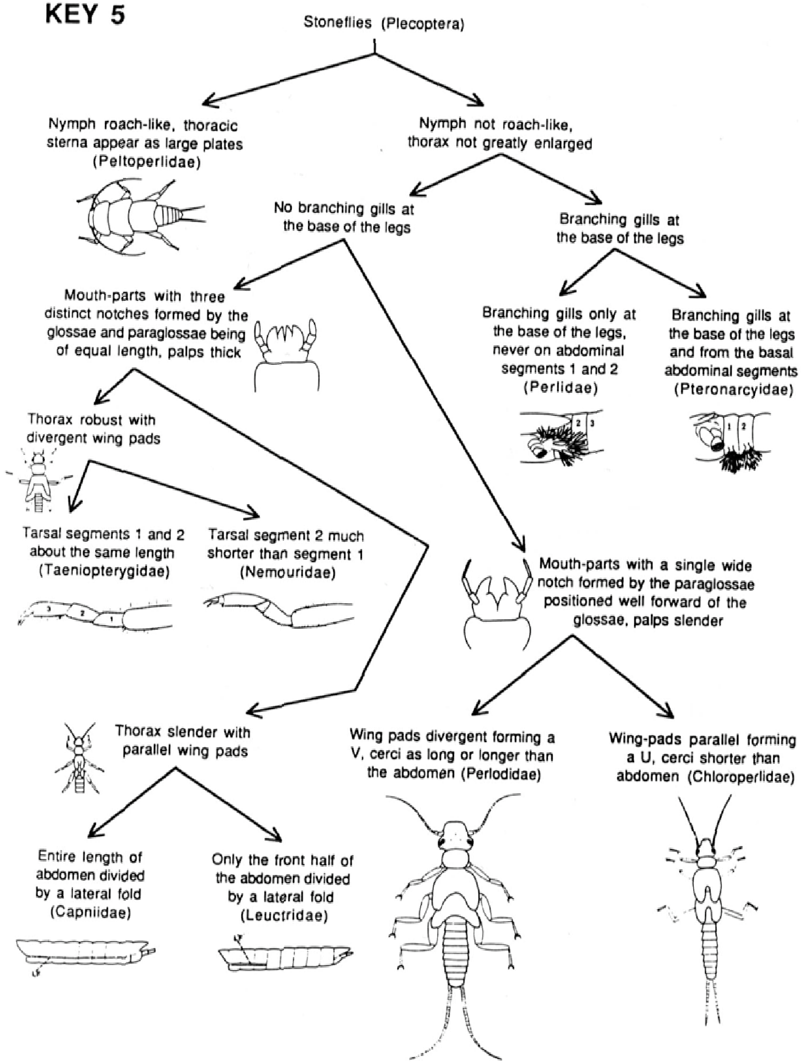
KEY 3



KEY 4


Mayflies (Ephemeroptera)
Enlarged thoracic notum forming sheild covering gills (Baetiscidae)
No thoracic sheild, gill exposed
Head without anteriorly projecting tusks
Head with anteriorly projecting large tusks visible from above (Ephemeridae)
Gills on abdominal segment 2 large and operculate
Gills on abdominal segment 2 absent or similar to gills on other segments
Nymph generally robust, gills absent from abdominal segment 2 (Ephemerellidae)
Nymphs generally delicate in appearanc gills present on abdominal segments 1-7 or 2-7
Operculate gills on abdominal segment 2 well separated (Tricorythidae)
Operculate gills on abdominal segment 2 fused along midline (Neoephemeridae)
Operculate gills on abdominal segment 2 overlapping not fused (Caenidae)
Head and body distinctly flattened, eyes distinctly on dorsal surface of head (Heptageniidae)
Head and body fusiform, eyes along side or front edge of head
Gills as single or double lamellae
Gills forked or ending in filaments (Leptophlebiidae)
Middle "tail" reduced or absent, if present antennae 2 to 3 times head width (Baetidae)
With three well developed "tails", antennae shorter than 2 times head width (Siphlonuridae)

KEY 5


Stoneflies (Plecoptera)
Nymph roach-like, thoracic sterna appear as large plates (Peltoperlidae)
Nymph not roach-like, thorax not greatly enlarged
No branching gills at the base of the legs
Branching gills at the base of the legs
Mouth-parts with three distinct notches formed by the glossae and paraglossae being of equal length, palps thick
Branching gills only at the base of the legs, never on abdominal segments 1 and 2 (Perlidae)
Branching gills at the base of the legs and from the basal abdominal segments (Pteronarcyidae)
Thorax robust with divergent wing pads
Tarsal segments 1 and 2 about the same length (Taeniopterygidae)
Tarsal segment 2 much shorter than segment 1 (Nemouridae)
Mouth-parts with a single wide notch formed by the paraglossae positioned well forward of the glossae, palps slender
Thorax slender with parallel wing pads
Wing pads divergent forming a V, cerci as long or longer than the abdomen (Perlodidae)
Wing-pads parallel forming a U, cerci shorter than abdomen (Chloroperlidae)
Entire length of abdomen divided by a lateral fold (Capniidae)
Only the front half of the abdomen divided by a lateral fold (Leuctridae)

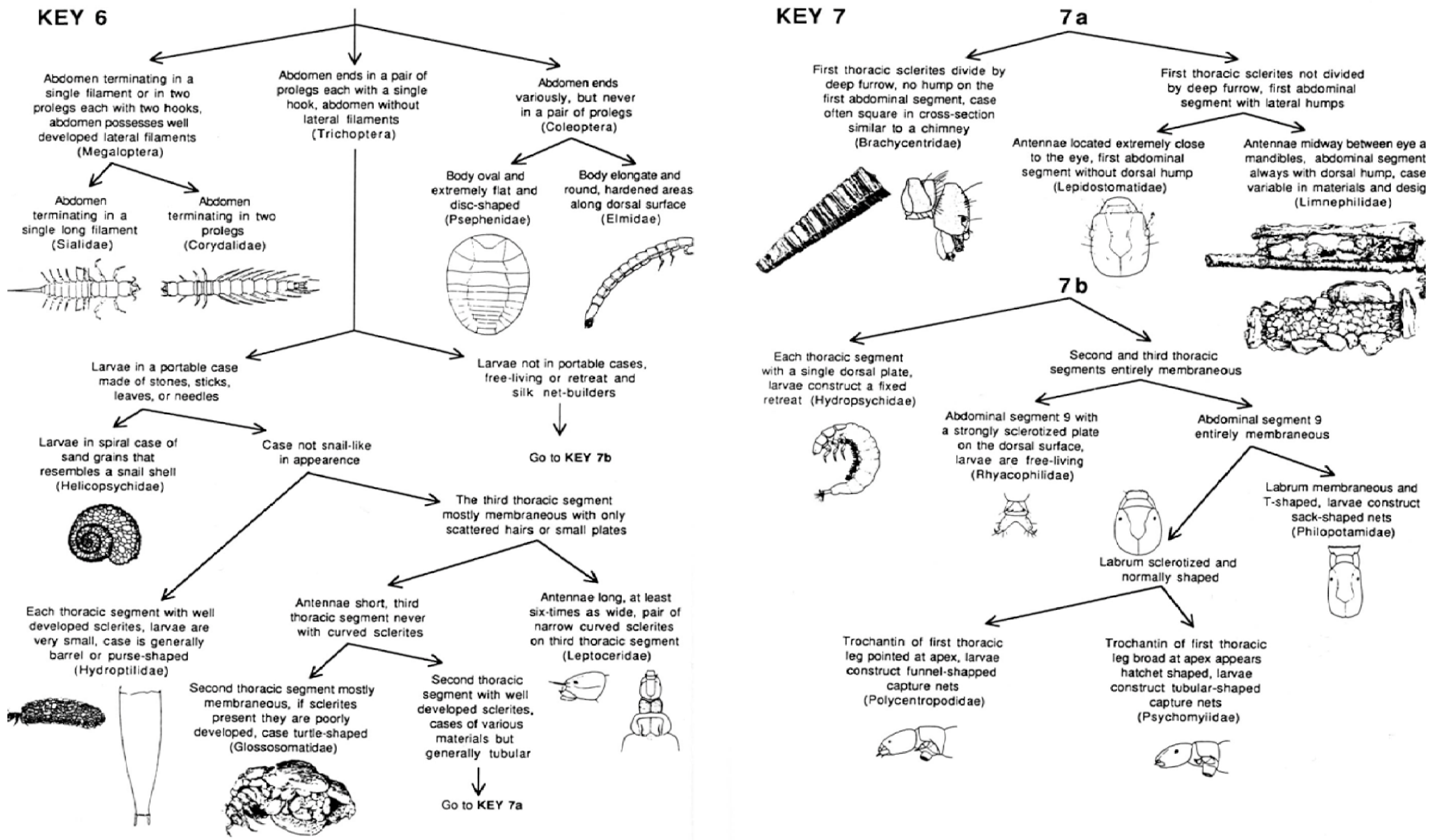

KEY 6
Abdomen terminating in a single filament or in two prolegs each with two hooks, abdomen possesses well developed lateral filaments (Megaloptera)
Abdomen ends in a pair of prolegs each with a single hook, abdomen without lateral filaments (Trichoptera)
Abdomen ends variously, but never in a pair of prolegs (Coleoptera)
Abdomen terminating in a single long filament (Sialidae)
Abdomen terminating in two prolegs (Corydalidae)
Body oval and extremely flat and disc-shaped (Psephenidae)
Body elongate and round, hardened areas along dorsal surface (Elmidae)
Larvae in a portable case made of stones, sticks, leaves, or needles
Larvae not in portable cases, free-living or retreat and silk net-builders
Go to KEY 7b
Larvae in spiral case of sand grains that resembles a snail shell (Helicopsychidae)
Case not snail-like in appearence
The third thoracic segment mostly membraneous with only scattered hairs or small plates
Each thoracic segment with well developed sclerites, larvae are very small, case is generally barrel or purse-shaped (Hydroptilidae)
Antennae short, third thoracic segment never with curved sclerites
Antennae long, at least six-times as wide, pair of narrow curved sclerites on third thoracic segment (Leptoceridae)
Second thoracic segment mostly membraneous, if sclerites present they are poorly developed, case turtle-shaped (Glossosomatidae)
Second thoracic segment with well developed sclerites, cases of various materials but generally tubular
Go to KEY 7a
KEY 7
7a
First thoracic sclerites divide by deep furrow, no hump on the first abdominal segment, case often square in cross-section similar to a chimney (Brachycentridae)
First thoracic sclerites not divided by deep furrow, first abdominal segment with lateral humps
Antennae located extremely close to the eye, first abdominal segment without dorsal hump (Lepidostomatidae)
Antennae midway between eye a mandibles, abdominal segment always with dorsal hump, case variable in materials and desig (Limnephilidae)
7b
Each thoracic segment with a single dorsal plate, larvae construct a fixed retreat (Hydropsychidae)
Second and third thoracic segments entirely membraneous
Abdominal segment 9 with a strongly sclerotized plate on the dorsal surface, larvae are free-living (Rhyacophilidae)
Abdominal segment 9 entirely membraneous
Labrum membraneous and T-shaped, larvae construct sack-shaped nets (Philopotamidae)
Labrum sclerotized and normally shaped
Trochantin of first thoracic leg pointed at apex, larvae construct funnel-shapped capture nets (Polycentropodidae)
Trochantin of first thoracic leg broad at apex appears hatchet shaped, larvae construct tubular-shaped capture nets (Psychomyiidae)

Table 1. Tolerance Values for Macroinvertebrates
(Resh *et al*, 1996, using data from Hilsenhoff, 1988)

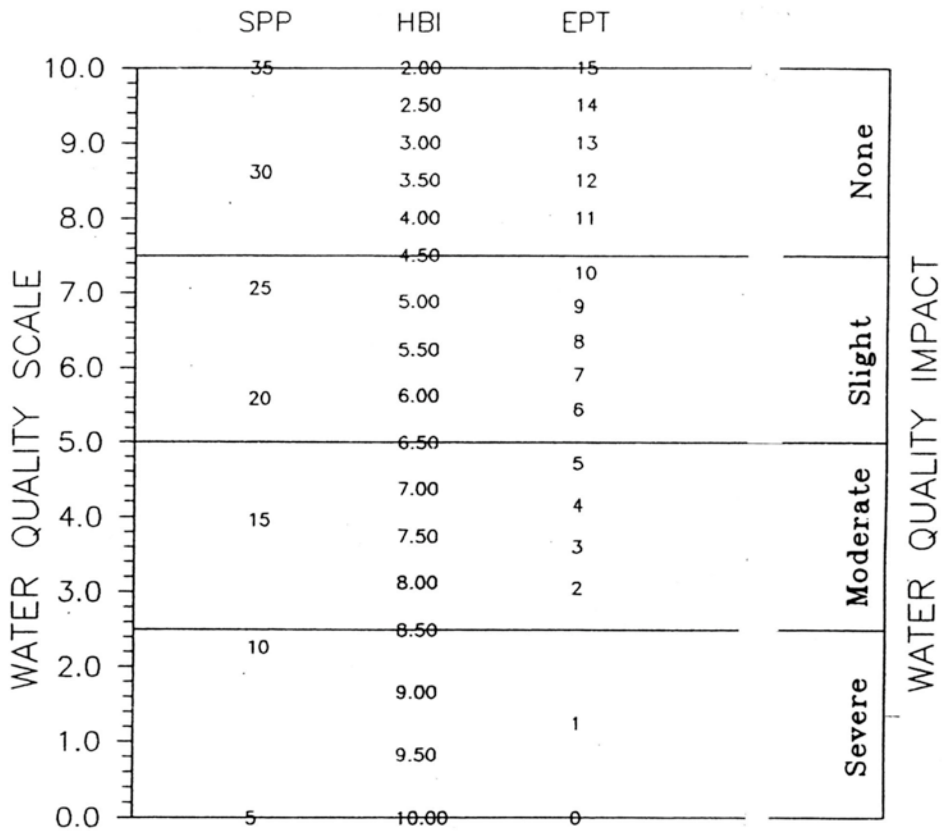
Plecoptera	
Capniidae	1
Chloroperlidae	1
Leuctridae	0
Nemouridae	2
Perlidae	1
Perlodidae	2
Pteronarcyidae	0
Taeniopterygidae	2
Ephemeroptera	
Baetidae	4
Baetiscidae	3
Caenidae	7
Ephemerellidae	1
Ephemeridae	4
Heptageniidae	4
Leptophlebiidae	2
Metretopodidae	2
Oligoneuridae	2
Polymitarcyidae	2
Potomanthidae	4
Siphlonuridae	7
Trichorythidae	4
Odonata	
Aeshnidae	3
Calopterygidae	5
Coenagrionidae	9
Cordulegastridae	3
Corduliidae	5
Gomphidae	1
Lestidae	9
Libellulidae	9
Macromiidae	3
Trichoptera	
Brachycentridae	1
Calamoceratidae*	3
Glossosomatidae	0
Helicopsychidae	3
Hydropsychidae	4
Hydroptilidae	4
Lepidostomatidae	1
Leptoceridae	4
Limnephilidae	4
Molannidae	6
Odontoceridae	0
Philpotamidae	3
Phryganeidae	4
Polycentropodidae	6
Psychomyiidae	2
Rhyacophilidae	0
Sericostomatidae	3
Uenoidae	3
Megaloptera	
Corydalidae	0
Sialidae	4
Lepidoptera	
Pyralidae	5
Coleoptera	
Dryopidae	5
Elmidae	4
Psephenidae	4
Diptera	
Athericidae	2
Blepharoceridae	0
Ceratopogonidae	6
Blood-red Chironomidae	8
Other Chironomidae	6
Dolochopodidae	4
Empididae	6
Ephydridae	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
Mollusca**	
Lymnaeidae	6
Physidae	8
Sphaeridae	8
Oligochaeta**	8
Hirudinea**	
Bdellidae	10
Turbellaria**	
Platyhelminthidae	4

Table 2. Sample calculation of Hilsenhoff Biotic Index
From a sample collected at Barr Creek, Iron County, Wisconsin, September 27, 2003

Order	**Family**	**Number of organisms** (Column A)	**Tolerance Value** (Column B)	**Column A x Column B**
Coleoptera	Psephenidae	5	4	20
	Other	1	4	4
	Elmidae	4	4	16
Diptera	Tipulidae	2	3	6
	Chironomidae	1	6	6
	Rhagionidae (Athericidae)	3	4	12
Ephemeroptera	Heptageniidae	69	2	138
	Baetidae	25	4	100
	Leptophlebiidae	49	2	98
	Siphlonuridae	14	7	98
	Neoephemeridae	16	6	96
	Ephemeridae	4	2	8
Plecoptera	Pteronarcyidae	3	0	0
	Nemouridae	1	2	2
	Leuctridae	1	0	1
	Chloroperlidae	2	0	0
	Perlidae	1	1	1
	Perlodidae	1	2	2
	Capniidae	1	3	3
Megaloptera	Sialidae	1	4	4
	Corydalidae	1	4	4
Trichoptera	Hydropsychidae	3	3	9
		$\sum = 208$		$\sum = 608$

Hilsenhoff Biotic Index = 608 / 208 = 2.92

Figure 3. Biological Assessment Profile of Index Values for Stream Riffles (Bode *et al*., 1996)



The Biological Assessment Profile of index values is a method of plotting biological index values on a common scale of water quality impact. For riffle habitats, these indices are used: SPP (species richness), HBI (Hilsenhoff Biotic Index), EPT (EPT richness). Values from the indices are converted to a common 0-10 scale as shown in this figure. The mean scale value of the indices represents the assessed impact for each site.

Literature Cited

Bode, R. W., M. A. Novak, and L. E. Abele. 1996. Quality Assurance Work Plan for Biological Stream Monitoring in New York State. Stream Biomonitoring Unit, Bureau of Monitoring and Assessment, Division of Water, NYS Department of Environmental Conservation, 50 Wolf Road, Albany NY 12233-3502

Grahame, Kenneth. 1917. The Wind in the Willows. Charles Scribner's Sons, New York, New York.

Hauer, F. R. and V. H. Resh. 1996. Benthic Macroinvertebrates, pp. 339-369 *in* F. R. Hauer and G. A Lamberti (eds), Methods in Stream Ecology. Academic Press, New York.

Hilsenhoff, W.L. 1988. Rapid Field Assessment of Organic Pollution with a Family Level Biotic Index. Journal of the North American Benthological Society 7:65-68.

Resh, V. H., M. J. Myers, and M. J. Hannaford. 1996. Macroinvertebrates as Biotic Indicators of Environmental Quality, p. 665 *in* F. R. Hauer and G. A. Lamberti (eds.) Methods in Stream Ecology. Academic Press, New York.

Materials

General

- preserved or living macroinvertebrates from stream
- no. 60 or other fine sieve
- small bucket
- several white enamel pans or shallow trays
- identification keys and references

Per student

- petri dish
- forceps
- dissecting needle
- large-bore plastic dropping pipet
- small plastic metric ruler
- squirt bottle or beaker of water
- stereozoom microscope
- calculator
- vials of 70% ethanol for voucher specimens (optional)

Notes for Instructor

This exercise introduces students to more sophisticated water quality analysis by having them assess the biodiversity and water pollution tolerance of the macroinvertebrate community of a stream, and summarize these traits in a numerical biotic index.

The rapid field identification employed in "The Kankapot Creek Coast Guard: Public Service Through Monitoring Water Quality of a Stressed Stream" (Perry 2004) is a "quick pick" method that can offer a picture of a stream's ecological integrity. However, it requires minimal macroinvertebrate identification skills and may not take into account a variety of benthic organisms present in your stream. Furthermore, it is not adequate to support many kinds of further investigations into stream ecology.

One could spend several lifetimes studying the fine points of benthic macroinvertebrate taxonomy. However, with reasonable care and good identification keys, novice students can identify most collected organisms to taxonomic family, a level that allows use of several informative biotic indices.

Biotic indices are routinely used in biological monitoring of stream water quality. Such monitoring can give an indication of past water quality conditions as well as current conditions, unlike physical and chemical monitoring. Benthic macroinvertebrates (those that live on or within the upper levels of bottom substrates) tend to be relatively sedentary and many are relatively long-lived in their larval forms. They are therefore continuously exposed to the conditions of a given stream reach. The pollution tolerance of many taxa has been established, making these organisms ideal indicators of water quality. In general, a larger proportion of pollution intolerant taxa, and greater numbers of individuals of the pollution intolerant taxa, indicate higher water quality of a stream.

Collecting and preserving the organisms

Benthic macroinvertebrates can be collected following the methods outlined in "The Kankapot Creek Coast Guard" (Perry 2004). Organisms that will be identified within a week can be held alive in a large volume of water in a refrigerator, with periodic aeration. Organisms can also be preserved in 95% ethanol for future identification.

Resources for identifying the macroinvertebrates

Many students are initially intimidated at the prospect of identifying dozens of macroinvertebrates. While the pictorial dichotomous key provided is relatively easy to use, plan to supplement that key with a diversity of printed resources in the lab. Following is a list of very useful resources:

McCafferty, W. Patrick. 1998. "Aquatic Entomology: The Fishermen's and Ecologists' Illustrated Guide to Insects and Their Relatives." Jones and Bartlett Publishers, Inc. Sudbury, MA 01776. This is an excellent reference with line drawings of taxa at family level; does not use highly technical language.

Merritt, R. W. and K. W. Cummins, eds. 1996. "An Introduction to the Aquatic Insects of North America," 3rd edition. Kendall/Hunt Publishing Company, Dubuque, IA 52002. May be out of print, though used copies are available from Amazon.com. As the title suggests, restricted to insects. Comprehensive, highly technical but with some line drawings; provides dichotomous keys to genera.

Pennak, R. W. 2001. "Freshwater Invertebrates of the United States," 4th ed. John Wiley & Sons, Inc., New York, NY. Textbook covering all freshwater invertebrates.

Voshell, J. R., Jr. 2002. "A Guide to Common Freshwater Invertebrates of North America." The McDonald & Woodward Publishing Company, Blacksburg, Virginia. Outstanding reference with color plates that point out distinguishing features of families. Technical language minimized.

Allen, J.D. 1995. "Stream Ecology: Structure and function of running waters." Kluwer Academic Publishers, Norwell, MA 02061

Hauer, F. R. and G.A. Lamberti, eds. 1996. Methods in Stream Ecology. Academic Press, New York.

Tips and tricks

Students will have best success separating macroinvertebrates from organic debris when the collected material is poured into white pans. White enamel pans are very expensive, though. We've found that white burner covers for electric stoves make excellent inexpensive substitutes.

Students will transfer specimens with forceps, dissecting needles or wide-bore pipettes as appropriate. Plastic dropping pipettes are usually too small in diameter to be useful; cut back the tip with scissors or razor blade to provide a wide-diameter, useful pipette.

Swirl a subsample of the collected specimens to mix before pouring out aliquots. This will suspend all the specimens in the water and better ensure that both large and small specimens will be counted. Caution students to counter their natural bias to preferentially select and identify the largest specimens.

The biotic indices in this activity are based on identification of 100 specimens. Some polluted streams will have a macroinvertebrate community that has little diversity or abundance and so is relatively quick to identify to family level, while high quality coldwater streams may have dozens of taxa represented, with large populations. I recommend that you pre-sample a new stream to gain a quick assessment of the diversity and abundance of the macroinvertebrates. You will then be better able to judge whether to ask each student, each group of students, or an entire lab section, to collectively identify 100 individuals.

What else can we do with this method?
Some possibilities:

- Test the hypothesis that a potential impact source (such as a wastewater treatment plant outfall, area of construction, farm lot, or golf course) affects water quality by comparing water quality in riffles above and below the source.
- Test the hypothesis that a trout stream will have higher water quality than a warmer water urban stream.
- Test the hypothesis that stream headwaters have less biotic diversity than mid or lower reaches.
- Compare index values from a single site but at different seasons and/or flow.
- Compare index values with results of habitat analysis, chemical analyses, etc.

Assessment of Student Learning
Lab reports are an obvious method of assessment for the types of investigations possible using these methods. Essay questions may also be useful. Possibilities:

- Single measurements of water quality are often taken at one site. Do you think water quality measures such as pH, temperature, and dissolved oxygen are more or less variable than biological measures such as the HBI or EPT indices? Why?
- How do you think collections made in different seasons might influence the HBI, EPT, and total number of individuals collected? Why?

Literature Cited

Perry, Joy. 2004. The Kankapot Creek Coast Guard: Public Service through monitoring water quality of a stressed stream. Pages 255-280, in Tested Studies for Laboratory Teaching, Volume 26 (M.A. O'Donnell, Editor). Proceedings of the 26th Workshop/Conferenceof the Association for Biology Laboratory Education (ABLE).

Appendix A. Form for Recording Macroinvertebrate Identification Data

Name ______________________________ Collection Date ______________________

Site ________________________________ Identification Date _____________________

A **Order/Family**	**B** **No. organisms in taxon**		**C** **Tolerance Value**		**D** **Biotic Score**
		X		=	
		X		=	
		X		=	
		X		=	
		X		=	
		X		=	
		X		=	
		X		=	
		X		=	
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		X		=	
		X		=	
		X		=	
		X		=	
		X		=	
		X		=	
		X		=	
	Total no. =			Col D _ =	

Family HBI = Col D _ divided by Total no. in Col. B = ________

EPT index = total number of taxa of Ephemeroptera + Plecoptera + Trichoptera = ________

SPP index = total number different taxa overall = ________