Chapter 5 - Biological Monitoring

Benthic Macroinvertebrates

Benthic macroinvertebrates are animals that are big enough (macro) to be seen with the naked eye. They lack backbones (invertebrate) and live at least part of their lives in or on the bottom (benthos) of a body of water.

Macroinvertebrates include aquatic insects (such as mayflies, stoneflies, caddisflies, midges, beetles), snails, worms, freshwater clams, mussels, and crayfish. Some benthic macroinvertebrates, such as midges, are small and grow no larger than 1/2 inch in length. Others, like the three ridge mussel, can be over ten inches long.

Why Do We Monitor Them?

Biological monitoring focuses on the aquatic organisms that live in streams and rivers. Scientists observe changes that occur in the number of types of organisms present in a stream system to determine the richness of the biological community. They also observe the total number of organisms in an area, or the density of the community. If community richness and community density change over time, it may indicate the effects of human activity on the stream.

Biological stream monitoring is based on the fact that different species react to pollution in different ways. Pollution-sensitive organisms such as mayflies, stoneflies, and caddisflies are more susceptible to the effects of physical or chemical changes in a stream than other organisms. These organisms act as indicators of the absence of pollutants. Pollution-tolerant organisms such as midges and worms are less susceptible to changes in physical and chemical parameters in a stream. The presence or absence of such indicator organisms is an indirect measure of pollution. When a stream becomes polluted, pollution-sensitive organisms decrease in number or disappear; pollution-tolerant organisms increase in variety and number.

In addition to being sensitive to changes in the stream's overall ecological integrity, benthic macroinvertebrates offer other advantages to scientists looking for indications of stream pollution.

- Benthic macroinvertebrates are relatively easy to sample. They are abundant can be easily collected and identified by trained volunteers.
- They are relatively immobile. Fish can escape toxic spills or degraded habitats by swimming away. Migratory animals may spend only a small portion of their life cycles in a particular stream before moving to larger rivers, wetlands, or other streams. However, most macroinvertebrates spend a large part of their life cycle in the same part of a stream, clinging to objects so they are not swept away with the water's current.
- Benthic macroinvertebrates are continuous indicators of environmental quality. The composition of a macroinvertebrate community in a stream reflects that stream's physical and chemical conditions over time. Monitoring for certain water quality parameters (such as the amount of dissolved oxygen) only describes the condition of the water at the moment in time the samples were taken.
- Benthic macroinvertebrates are a critical part of the aquatic food web. They form a vital link in the food chain connecting aquatic plants, algae, and leaf litter to the fish species in streams. The condition of the benthic macroinvertebrate community reflects the stability and diversity of the larger aquatic food web.

How Do We Collect Them?

Kick Seine Sampling Method

The **kick seine method** is a simple procedure for collecting stream-dwelling macroinvertebrates. It is used in riffle areas where the majority of the organisms live. This technique can be quite effective in determining relative stream health; **however, it is only as good as the sampling technique.** Two to three people work together to perform the technique properly. Follow the procedures as closely as possible.

1. Locate a "typical riffle." Such a riffle is a more shallow, faster moving mud-free section of stream with a stream bed composed of material ranging in size from ten-inch cobbles to one-quarter inch gravel or sand. The water ranges in depth from approximately two inches to a foot, with a moderately swift flow. Avoid riffles located in an area of a stream that has been recently disturbed by anything, including construction of a pipeline crossing or roadway.

2. Once the riffle has been located, select an area measuring 3 feet by 3 feet that is typical of the riffle as a whole. Avoid disturbing the stream bed upstream from this area.

3. Examine the net closely and remove any organisms remaining from the last time it was used.

4. **Approach the sampling area from downstream!**

5. Have one person place the net at the downstream edge of the sampling area. (It may take two people to hold it in place.) The net should be held perpendicular to the flow, but at a slight downstream angle. Stretch the net approximately three feet, being certain that the bottom edge is lying firmly against the bed. If water washes beneath or over the net you will lose organisms.

6. Another person comes upstream of the net. **Stand beside, not within the sampling area.** Remove all stones and other objects two inches or more in diameter from the sampling area. Hold each one below the water as you brush all organisms from the rock into the net. You can also place rocks on the bottom of the net to help hold it down.

7. When all materials two inches or larger have been brushed, step into the upstream edge of the sampling area and kick the stream bed vigorously until you have disturbed the entire sampling area. Kick from the upstream edge toward the net. Try to disturb the bed to a depth of at least two inches. You can also use a small shovel to disturb the bed. Kick for at least 2-3 minutes.

8. Carefully remove the net with a forward upstream scooping motion. DO NOT allow water to flow over the top of the net or you may lose organisms.

9. Carry the seine to a flat area on the stream bank. Remove leaves, rocks, and other debris. Examine them for any attached organisms. Using fingers or forceps, remove organisms from the net and place in a plastic container for later identification. If nothing appears to be on the net, leave it alone for a few minutes. The insects will begin to move around because they are out of the water.

10. Perform steps 1-9 a total of three times in the same riffle or different riffles within your site.

11. Sort all the organisms collected from the three samples according to body shape using ice cube trays or petri dishes. Record the presence of each type of organism and estimate the number of each type.

Dip Net Sampling Method

If there are no riffles at your stream site to perform the kick seine sampling method, then you should use the dip net to perform your biological monitoring. Dip nets are useful for sampling aquatic habitats other than riffles. One dip net "jab" involves forcing the dip net against the stream bottom repeatedly, starting close to your body and finishing with arms fully outstretched. However, sampling technique differs depending on specific habitat conditions. The following is a list of habitat-specific sampling hints *(modified from the Clinton River Watershed Teacher Training Manual)*:

- **Leaf Pack**: Look for leaves that are brown and slightly decomposed (only a handful of leaves is necessary). Place the bottom of the net immediately downstream from the leaf pack with handle perpendicular to stream flow. Gently shake the leaf pack in the water to release organisms, and then quickly scoop up the net, capturing both the organisms and the leaves. It is recommended to do 3 "jabs" with the net.

- **Tree Roots, Snags (accumulations of debris), and Submerged Logs:** Select an area approximately 3 by 3 feet in size. Begin working downstream, scraping the surface of roots, logs, or debris with the net. You may also disturb such surfaces with a large stick, your foot, or by removing some of the bark to expose hidden organisms. In all cases, be sure that the net is positioned downstream from the snag, root, or log, so that dislodged material floats into it. It is recommended to do 4"jabs" with the net.

- **Undercut Banks (see picture CQHEI instructions):** Place the net below the surface under the overhanging vegetation. Move the net in a bottom-up motion, jabbing at the bank several times in a row to loosen organisms. It is recommended to do 10 "jabs" with the net.

- **Sediments:** (Sampling technique useful in areas of mostly sand and/or mud). The person holding the net stands downstream of sediment area with dip net resting on the bottom. Another person begins upstream, kicking and disturbing sediments to a depth of about two inches as they approach the net. The "netter" sweeps the net upward to collect organisms as the kicker approaches. Finally, keeping the opening of the net at least an inch or two above the surface of the water, wash sediment out of the net by moving the net back and forth in the stream water. It is recommended to do 3 "jabs" with the net.

Take a total of twenty jabs in a variety of habitats. After two or three jabs are performed with one net, dump the collected materials from the net into a shallow white container or bin - a dish pan works well. The materials in the bin may be quite muddy and turbid (depending upon your stream habitat). If this is the case, once you find macroinvertebrates in your sample, you may want to place them into another clean container with a small amount of clear water for easier identification.

Combination Sampling Method

If your 200 foot sampling site has a variety of habitats, including riffles, then you may perform a combination of sampling methods. Record the types of equipment used and the types of habitats sampled on the Biological Monitoring Data Sheet.

How Do They Develop?

Most of the benthic macroinvertebrates you will encounter are aquatic insects. Aquatic insects have complex life cycles and live in the water only during certain stages of development.

Complete Metamorphosis

Aquatic insects may go through one of two kinds of development or metamorphosis. Those that go through complete metamorphosis undergo four stages of development: egg, larva, pupa, and adult. They lay their eggs in water; eggs then hatch into larvae that feed and grow in the water. (These larval insects do not resemble the adult insects; many appear worm-like.) The fully-grown larvae develop into pupae and then into adults. The fully-formed adults of some species (midges and flies, for example) emerge from the water and live in the habitat surrounding the stream. Others, such as riffle beetles, continue to live in the stream as adults. After mating, adults of all aquatic insect species lay eggs in the water, beginning the life-cycle all over again.

Incomplete Metamorphosis

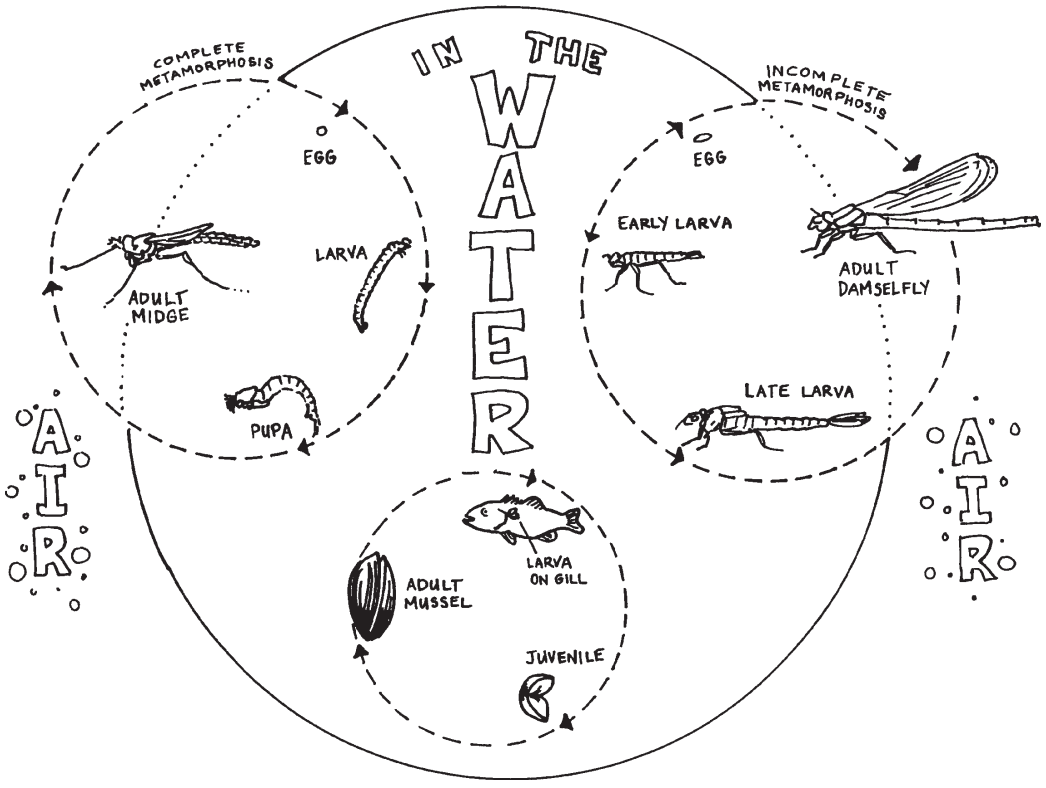
Aquatic insects that go through incomplete metamorphosis undergo only three stages of development; eggs, nymphs and adult. The eggs hatch into nymphs (also called larvae). Nymphs feed and grow in the water while they develop adult structures and organs. The life cycle begins again when adults lay eggs in the water.

Incomplete metamorphosis:
egg → nymph → adult

(mayfly, dragonfly, stonefly, true bugs)

Complete metamorphosis:
egg → larvae → pupa → adult

(true flies, beetles, caddisfly)



What and How Do They Eat?

Macroinvertebrates may be categorized by their feeding groups - the type of food they eat and the manner in which food is obtained/collected.

Shredder

Shredders feed on coarse, dead organic matter (leaves, grasses, algae, and rooted aquatic plants), breaking it into finer material that is released in their feces. Shredders include stonefly nymphs, caddisfly larvae, cranefly larvae.

Collector

Collectors feed on fine, dead organic matter, including that produced by the shredders.

F**iltering collector**: filters particles out of flowing current. Examples include blackfly larvae and net-building caddisflies.

Gathering collector: gathers matter while crawling along the river bottom. Gatherers include mayfly nymphs, adult beetles, midge larvae.

Grazer

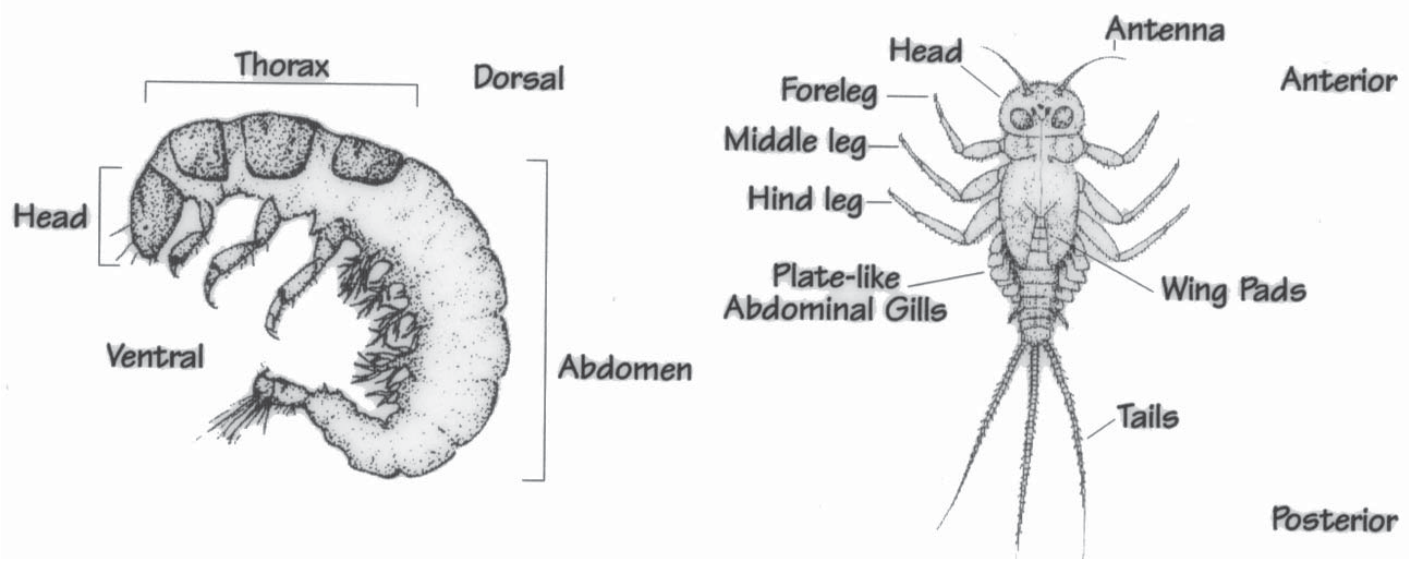
These organisms graze on algae growing on rocks in the substrate or on vegetation. Grazers include snails and water pennies.

Predator

Predators feed on other invertebrates or small fish. Mouth parts are specially adapted to feed on prey. Dragonflies and damselflies have scoop-like lower jaws, the jaws of hellgrammites (dobsonflies) are pincer-like, and water strider's mouth parts are spear-like. Also includes beetle adults and larvae.

What Do They Look Like?

A simple key to bethic macroinvertebrates is provided on the following pages. The organisms are grouped according to pollution tolerance, starting with the most intolerant families. The figure below may help you identify the distinguishing features of many of the organisms.



Figures from GREEN Standard Water Monitoring Kit

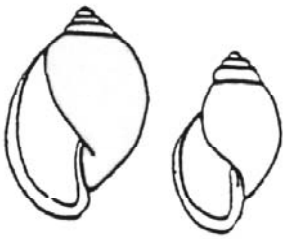
Taxonomic Key to Benthic Macroinvertebrates

The purpose of this taxonomic key is to assist volunteer monitors, who are not trained in taxonomy, with the identification of benthic macroinvertebrates found in the Great Miami River Watershed. This key is a simplified version of more complex keys. The taxonomic level of this key is intended for use by citizen monitoring groups. When using this key please note that each couplet offers two or three options. Each couplet is numbered and the numbers in bold refer to the next couplet (the next set of numbers that you proceed to).

Please be aware that some macroinvertebrates may have missing body parts - so you should look at more than one organism!

	CHOOSE ONE:	GO BELOW TO:
(1)a	Has a shell(s)	2
(1)b	Has no shell	5
(2)a	Has a hinged double shell	3
(2)b	Has a single shell	4
(3)a	Adult under 2 inches long	19
(3)b	About 2-4 inches long	MUSSEL
(4)a	Right-handed opening	RIGHT-HANDED SNAIL
(4)b	Left-hand opening	LEFT-HANDED SNAIL

Right-Handed Snail



Left-Handed Snail

CHOOSE ONE:		GO BELOW TO:
(5)a	Has a segmented body or looks like a tiny tick	6
(5)b	Has an unsegmented body and has an "arrow shaped" head; 2 pigment spots (eyes)	PLANARIA


Planaria

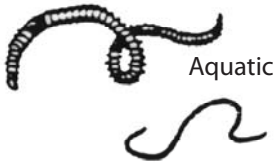
(6)a	No obvious legs	7
(6)b	Obvious legs	12
(7)a	Has no obvious appendages (long, tubular body)	8
(7)b	Has some appendages (small tubes, tiny bumps, or feathery structures)	9
(8)a	Has a smooth body and suckers	LEECH


Leech

(8)b	Has a round body and a rat tail	RAT-TAILED MAGGOT


Rat-Tailed Maggot

(8)c	Has a rounded body	AQUATIC WORMS

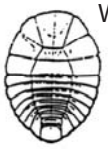
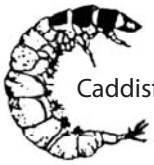

Aquatic Worms

(9)a	Body black or brown; more than 1/3 inch long; plump and catepillar-like	CRANE FLY LARVA

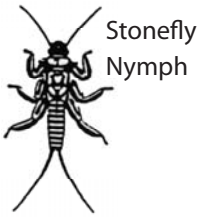

Crane Fly Larva

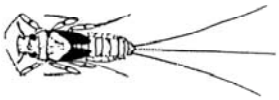
(9)b	Has a distinct head	10
(10)a	One end of body wider than other end; two tiny feather structures on smaller end	BLACK FLY LARVA

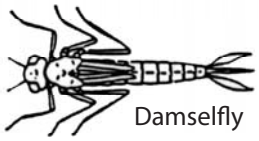

Black Fly Larva

CHOOSE ONE:		GO BELOW TO:
(10)b	No difference in diameter along body	11
(11)a	Bright red body  Chironomid	BLOOD WORM MIDGES
(11)b	Grey Body	OTHER MIDGES
(12)a	Has four pairs of legs  Water Mite	WATER MITE
(12)b	Has three pairs of legs	13
(12)c	Has many pairs of legs	26
(13)a	Has no wings or short wing pads on back	14
(13)b	Has two pairs of wings that cover the abdomen	23
(14)a	Has a flat, round body with legs underneath (wings are not obvious)  Water Penny Beetle	WATER PENNY BEETLE
(14)b	Not flat, has long body with legs	15
(15)a	Lives in a tube or a case or has two hooks in its last segment and is green with 3 plates on back behind head. (The "green caddis" builds a net & tube, but will be washed into the kick net as "free living")  Caddisfly Larva	CADDISLY LARVA
(15)b	Free-living	16

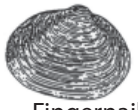
CHOOSE ONE:		GO BELOW TO:
(16)a	Abdomen possesses lateral filaments similar in size to legs	21
(16)b	Abdomen does not have "leg-like" filaments (may have feathery "gills")	17
(17)a	Always with only two tail appendages and no	STONEFLY NYMPH
(17)b	Usually has three tail appendages, and with no lateral gills on abdominal segments	18
(17)c	Tail has no appendages	25
(18)a	Has long, bristle-like tail appendages, sometimes 2 or 3	MAYFLY NYMPH
(18)b	Lower lip formed into extensible scoop-like structure and has leaf-like tail appendages	DAMSELFLY NYMPH
(19)a	Small rounded shell	20
(19)b	Small triangular shell with alternating cream and dark brown bands	ZEBRA MUSSEL (EXOTIC)
(20)a	Numerous very fine concentric rows of elevated lines, white or cream colored, with smooth lateral teeth (ridge lines on inside near point)	FINGERNAIL CLAM
(20)b	Numerous concentric elevated ridges, yellowish brown to black shell with serrated lateral teeth	ASIATIC CLAM (EXOTIC)

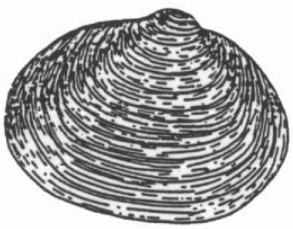

Stonefly Nymph

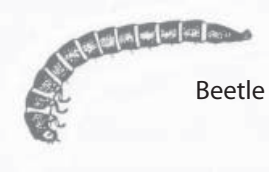
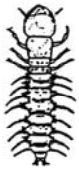
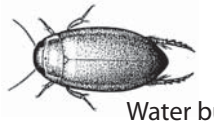
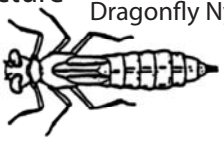
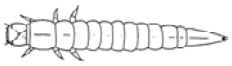
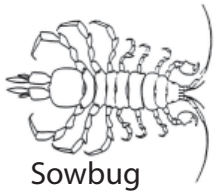

Mayfly


Damselfly


Zebra Mussel


Fingernail Clam



CHOOSE ONE:		GO BELOW TO:
(21)a	Head narrower than widest body segments  Beetle larva	BEETLE LARVAE
(21)b	Head as wide or wider than other body segments	22
(22)a	Abdomen with single long filament at end  Alderfly	ALDERFLY
(22)b	Abdomen ending with a pair of tiny hooked legs, large head with pincer-like jaws  Dobsonfly Larvae	DOBSONFLY OR FISHFLY
(23)a	Oval shaped body, legs with feathery swimming hairs  Water bug	ADULT WATER BUGS AND WATER BEETLES
(23)b	All legs smooth, without hairs, crawling  Riffle Beetle Adult	RIFFLE BEETLE ADULT
(25)a	Lower lip formed into scoop like structure  Dragonfly Nymph	DRAGONFLY LARVAE
(25)b	Looks like a tiny millipede  Riffle Beetle Larvae	RIFFLE BEETLE LARVAE
(26)a	Flattened top to bottom, crawling looks like "roly-poly" or a "pill bug"  Sowbug	SOWBUG
(26)b	Flattened side to side, swimming looks like tiny shrimp  Scud or Side-swimmer	SCUD

How to Complete the Biological Monitoring Data Sheet

Sampling Procedures

Equipment: Check one or both of the nets used to collect macroinvertebrate sample.
Habitat: Check each type of habitat sampled during this survey.

Pollution Tolerance Index

The macroinvertebrate index is divided into Pollution Tolerance Groups (PT Group) 1, 2, 3, and 4. These PT groups represent the different levels of pollution tolerance. The higher the group number, the higher the pollution tolerance level. Record the number of macroinvertebrates you find here.

PT GROUP 1 Intolerant	PT GROUP 2 Moderately Intolerant	PT GROUP 3 Fairly Tolerant	PT GROUP 4 Very Tolerant
Stonefly Nymph _____	Damselfly Nymph _____	Midge Larvae _____	Left-Handed Snail _____
Mayfly Nymph _____	Dragonfly Nymph _____	Black Fly Larvae _____	Aquatic Worms _____
Caddis Fly Larvae _____	Sowbug _____	Planaria _____	Blood Midge _____
Dobsonfly Larvae _____	Scud _____	Leech _____	Rat-tailed Maggot _____
Riffle Beetle _____	Crane Fly Larvae _____		
Water Penny _____	Clams/Mussels _____		
Right-Handed Snail _____			

The next row is the # of Taxa. Insects that have the same body shape all belong to the same taxa (see the back of your PTI macroinvertebrate data sheet for general body shape/taxa). To find the total number of taxa for each PT Group you need to add the number of types of organisms. It is possible to have a particular PT group without any numbers, therefore it will score a zero.

Do not make the mistake of adding the numbers of organisms together.

# of TAXA _____	# of TAXA _____	# of TAXA _____	# of TAXA _____

The next row is the group scores. Multiply each # of taxa by its weighting factor.

# of TAXA _____	# of TAXA _____	# of TAXA _____	# of TAXA _____
Weighting Factors: (x 4) _____	(x 3) _____	(x 2) _____	(x 1) _____

Then total all of the group scores to get the POLLUTION TOLERANCE INDEX RATING.

# of TAXA _____	# of TAXA _____	# of TAXA _____	# of TAXA _____
(x 4) _____	(x 3) _____	(x 2) _____	(x 1) _____

23 +	Excellent
17 - 22	Good
11 - 16	Fair
10 or Less	Poor

POLLUTION TOLERANCE INDEX RATING

(Add the final index values for each group.)

Other Biological Indicators

Check the appropriate box if you find native mussels, zebra mussels, or submerged aquatic plants at your site. Estimate the percentage of algae covering rocks or the stream bottom at your site. Write your Diversity Index score if you perform the procedure.

Other Biological Indicators

☐ Native Mussels ☐ Zebra Mussels ☐ Submerged Aquatic Plants _____ % Algae Cover _____ Diversity Index

Example of a complete Pollution Tolerance Index:

POLLUTION TOLERANCE INDEX (PTI)

PT GROUP 1 Intolerant		PT GROUP 2 Moderately Intolerant		PT GROUP 3 Fairly Tolerant		PT GROUP 4 Very Tolerant	
Stonefly Nymph	__X_____	Damselfly Nymph	_____	Midges	_____	Left-Handed Snail	_____
Mayfly Nymph	__X_____	Dragonfly Nymph	_____	Black Fly Larvae	___X____	Aquatic Worms	_____
Caddis Fly Larvae	_____	Sowbug	____X___	Planaria	_____	Blood Midge	_____
Dobsonfly Larvae	_____	Scud	_____	Leech	_____	Rat-tailed Maggot	_____
Riffle Beetle	___X____	Crane Fly Larvae	___X_____				
Water Penny	_____	Clams/Mussels	___X_____				
Right-Handed Snail	__X____						
# Of TAXA	___4_____	# Of TAXA	____3____	# Of TAXA	____1____	# Of TAXA	_____
Weighting Factors: (x 4)	___16_____	(x 3)	___9_____	(x 2)	___2_____	(x 1)	___0_____

23 or More	Excellent
17 - 22	Good
11 - 16	Fair
10 or Less	Poor

POLLUTION TOLERANCE INDEX RATING 27

(Add the final index values for each group.)

BIOLOGICAL MONITORING DATA SHEET

Date ____/____/____ (MM DD YY) Begin Time _____:_____ (am/pm) # Adults _____

End Time _____:_____ (am/pm) # Students_____

Certified Monitors' Names_____

Organization Name _____

Watershed Name _____ Watershed # __ __ __ _ __ __ __ _ __

Stream/River Name _____ Site Location:

(Please do not abbreviate.)

Check Methods Used

- ☐ Kick Seine Net (3 times)
- ☐ D-Net (20 jabs or scoops)

Check Habitats Sampled

- ☐ Riffles
- ☐ Undercut Banks
- ☐ Sediment
- ☐ Leaf Packs
- ☐ Snags/Vegetation
- ☐ Other

POLLUTION TOLERANCE INDEX (PTI)

PT GROUP 1 Intolerant		PT GROUP 2 Moderately Intolerant		PT GROUP 3 Fairly Tolerant		PT GROUP 4 Very Tolerant	
Stonefly Nymph	_____	Damselfly Nymph	_____	Midges	_____	Left-Handed Snail	_____
Mayfly Nymph	_____	Dragonfly Nymph	_____	Black Fly Larvae	_____	Aquatic Worms	_____
Caddis Fly Larvae	_____	Sowbug	_____	Planaria	_____	Blood Midge	_____
Dobsonfly Larvae	_____	Scud	_____	Leech	_____	Rat-tailed Maggot	_____
Riffle Beetle	_____	Crane Fly Larvae	_____				
Water Penny	_____	Clams/Mussels	_____				
Right-Handed Snail	_____						
# Of TAXA	_____	# Of TAXA	_____	# Of TAXA	_____	# Of TAXA	_____
Weighting Factors: (x 4)	_____	(x 3)	_____	(x 2)	_____	(x 1)	_____

23 or More	Excellent
17 - 22	Good
11 - 16	Fair
10 or Less	Poor

POLLUTION TOLERANCE INDEX RATING _____

(Add the final index values for each group.)

Other Biological Indicators

☐ Native Mussels ☐ Zebra Mussels ☐ Submerged Aquatic Plants _____ % Algae Cover _____ Diversity Index