Chapter 5 - 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 more than ten inches long.

What is the ecological importance of benthic macroinvertebrates? Benthos are an important part of the food chain, especially for fish. Many feed on algae and bacteria which are on the lower end of the food chain. Some shred and eat leaves and other organic matter that enters the water. Because of their abundance and position as "middleman" in the aquatic food chain, benthos play a critical role in the balance and natural flow of energy and nutrients. As benthos die, they decay, leaving behind nutrients that are reused by aquatic plants and other animals in the food chain. *(Source: Maryland Department of Natural Resources)*

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 or diversity of the biological community. They also observe the total number of organisms in an area, or the density of the community. If diversity and density change over time, it may indicate the effects of human activity on the stream.

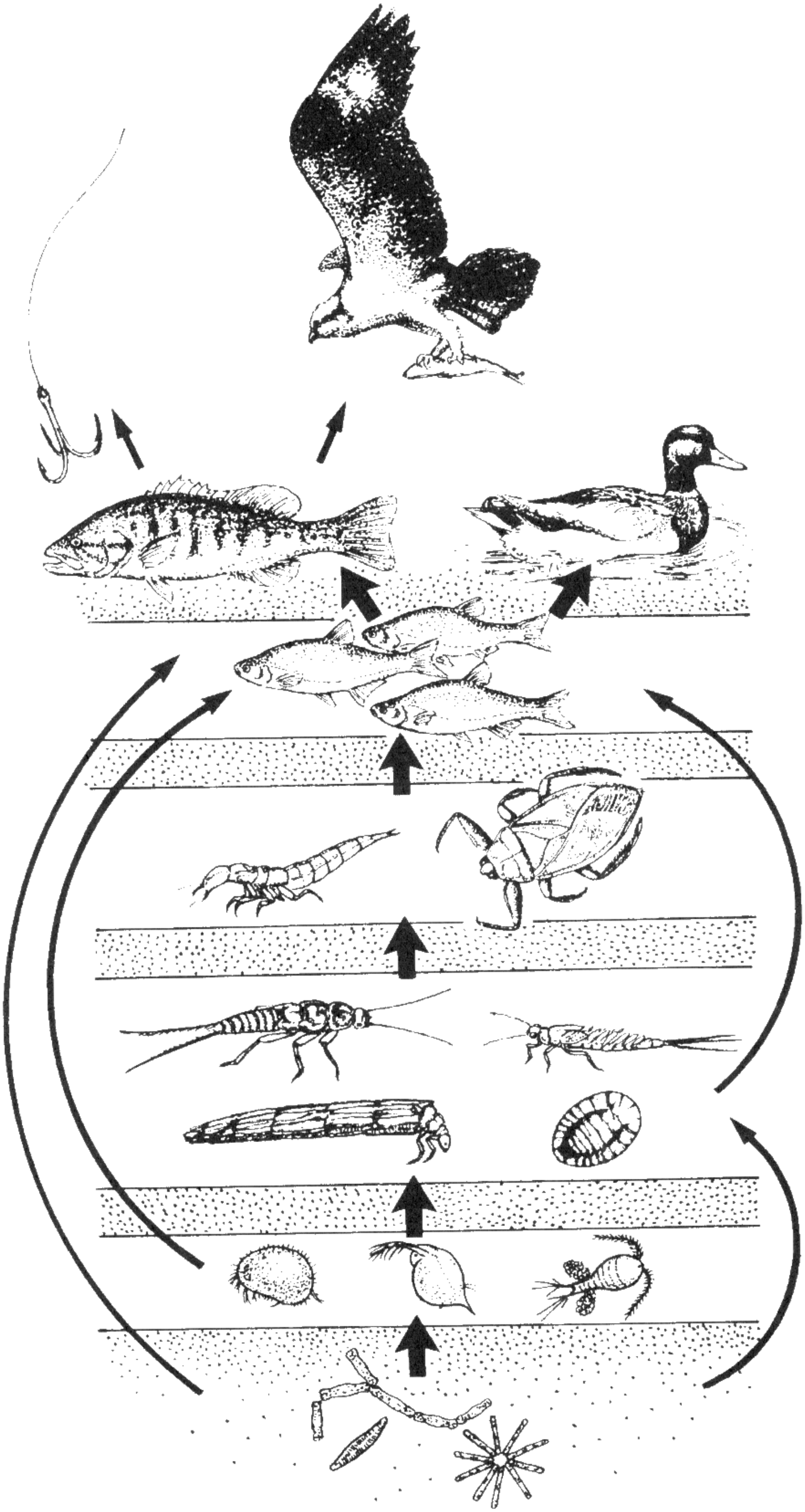
Biological stream monitoring is based on the fact that different species react to pollution in different ways. Pollution-sensitive organisms such as mayflies, stoneflies, and caddisflies are more susceptible than other organisms to the effects of physical or chemical changes in a stream. These organisms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

- Benthic macroinvertebrates are relatively easy to sample. They are abundant and 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 (Figure 26). 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.

Aquatic Food Web *(from Pond & Stream Safari, Cornell Cooperative Extension)*

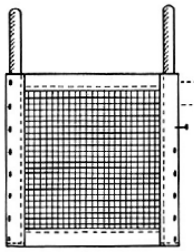
Figure 26



How Do We Collect Them?

Macroinvertebrate Collection Tips

You will want to collect macros no more than three times per year. Collecting more often can impact populations. Limit collection time to 30 minutes, and do not collect between mid-November and mid-April. The time of year in which you monitor will influence the macros you find due to their life cycles.



Aquatic Kick Seine

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 prefer to live. This method can be quite effective in determining relative stream health. Two to three people work together to perform the method. Carefully read the procedures, and follow them as closely as possible.

1. Locate a "typical riffle." Such a riffle is a shallow, fast moving mud-free section of stream with a stream bed composed of material ranging in size from one-quarter inch gravel or sand to ten-inch cobbles. 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 is held perpendicular to the flow, but at a slight (45 degree) downstream angle. Stretch the net approximately three feet, being certain that the bottom edge is lying firmly against the bed. You can hold the poles closer at the top to create a pocket for catching macros. 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 edge of the net to help hold it in place against the stream bottom.
7. When all materials two inches or larger have been brushed, step into the upstream edge of the sampling area 3 feet from the net 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 four to six inches. You can also use a small shovel to disturb the bed. Kick for approximately 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. Place it on a large white sheet, plastic table cloth, garbage bag, or shower curtain. Remove leaves, rocks, and other debris; examining them for any attached organisms. Using fingers or forceps, remove organisms from the net and place in another container with water for later identification. If nothing appears to be on the net, leave it alone for a few minutes, and the organisms will begin to move around because they are out of the water. Be sure to check your white ground cover for any creatures attempting to escape. If you happen to collect live mussels (native or exotic) in your net, **please see page 73 for further instruction**.
10. Perform steps 1-9 a total of three times at different locations within your 200' site. Your goal is to collect at least 200 organisms.
11. Sort all the organisms collected from the three samples according to body shape using ice cube trays or petri dishes. Record the number of each type of organism (if more than 100; record >100).

D-Net Sampling Method

D-net

If there are no riffles at your stream site to perform the kick seine sampling method, then you should use the D-net to perform your biological monitoring. Take a total of twenty jabs in a variety of habitats (Figures 27 and 28). 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 habitat conditions. *(Modified from the Clinton River Watershed Teacher Training Manual)*

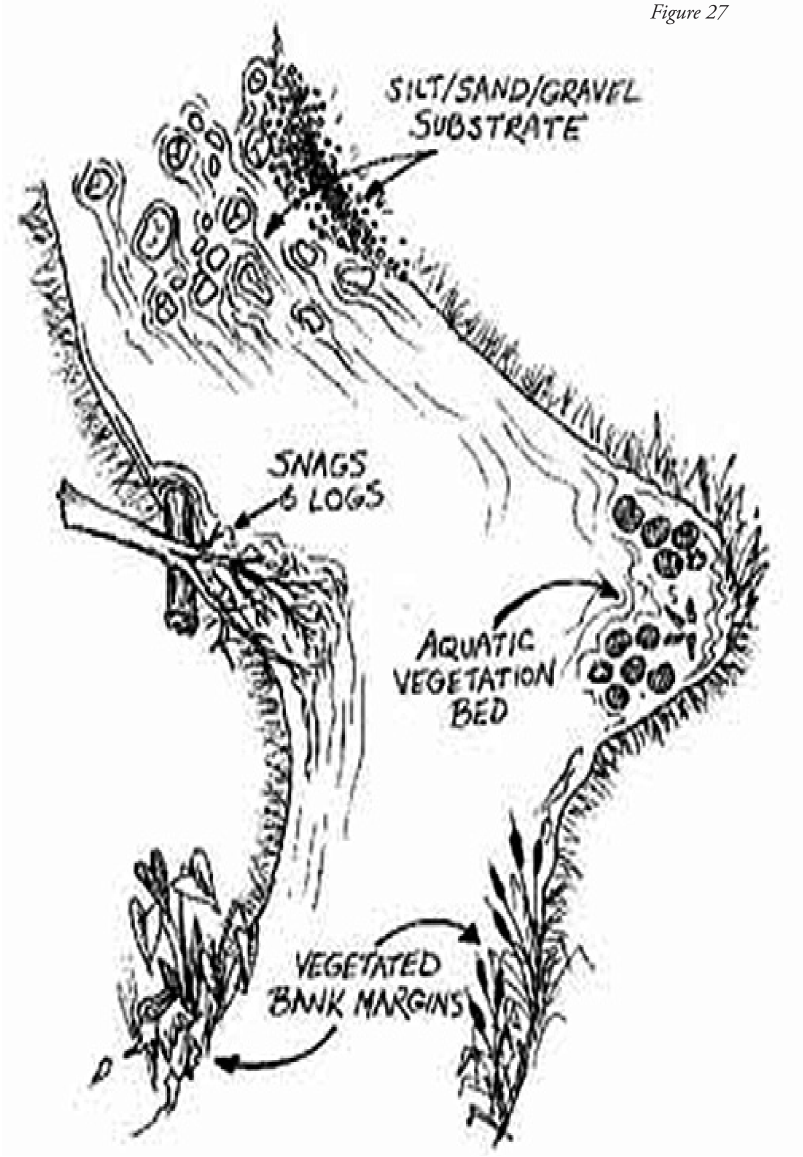
- Leaf Pack: Shake the leaf pack in the water to release organisms, and then quickly scoop up the net, capturing both the organisms and the leaves. (See information on the next page for experiments using leaf packs.)
- Tree Roots, Snags (accumulations of debris), and Submerged Logs: Select an area approximately 3 x 3 feet in size. Scrape the surface of roots, logs, or debris with the net, a large stick, or your hand or foot. Be sure the net is positioned downstream so that dislodged material floats into it.
- Undercut Banks (page 25 - Figure 12): Place the net below the overhanging vegetation. Move the net in a bottom-up motion, jabbing at the bank several times to loosen organisms.
- Sediments (sand/mud): If there is not much flow, jab the net into the bottom with a sweeping motion. If flow is good, stand upstream of the net holding it against the bottom of the stream and kick in front of the net so that the flow washes organisms into the net. To rinse, keep the opening of the net at least 1-2 inches above the surface of the water, and move it back and forth to wash small particles out of the net.

After two or three jabs with one net, dump the collected materials into a shallow white container (a dishpan works well). The materials in the bin may be quite muddy and turbid (depending upon your stream habitat). When you find macroinvertebrates, place them into another container (white pan, petri dish, bug board, ice cube tray) with clear water for easier identification.

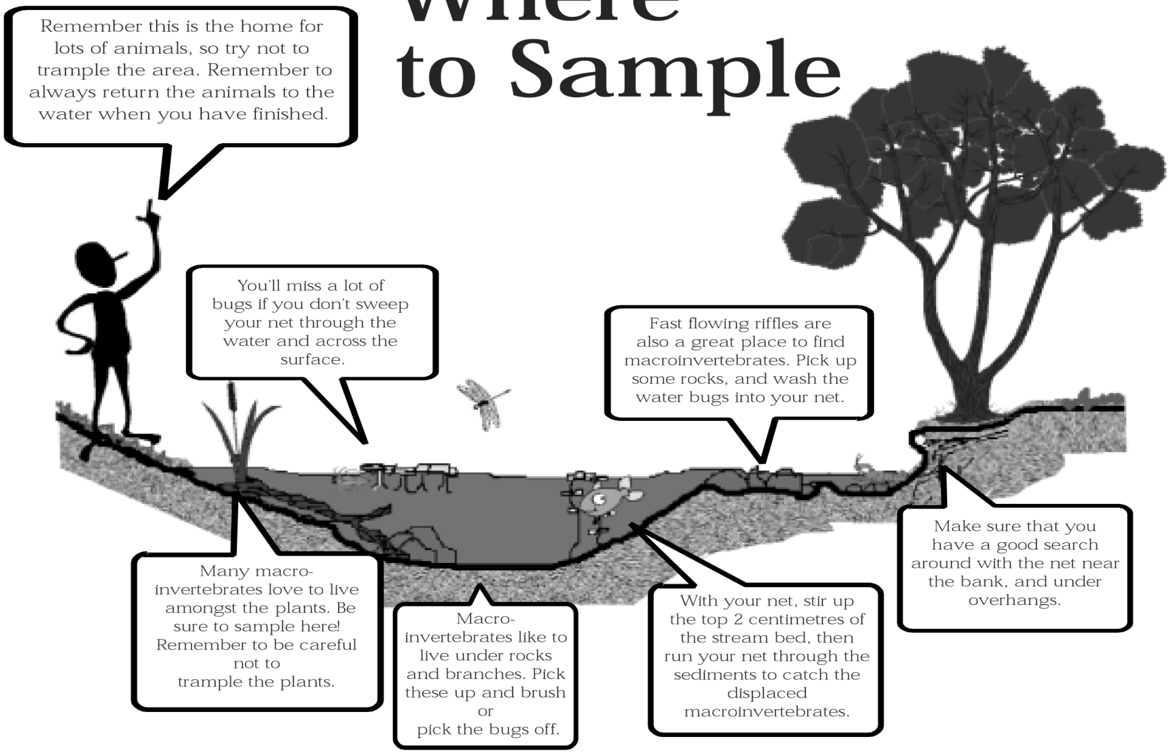
Combination Sampling Method

If your 200' site has a variety of habitats, including riffles, then you may perform a combination of sampling methods. Record the equipment used and the types of habitats sampled on the Biological Monitoring Data Sheet (page 75).

Figure 27



Where to Sample



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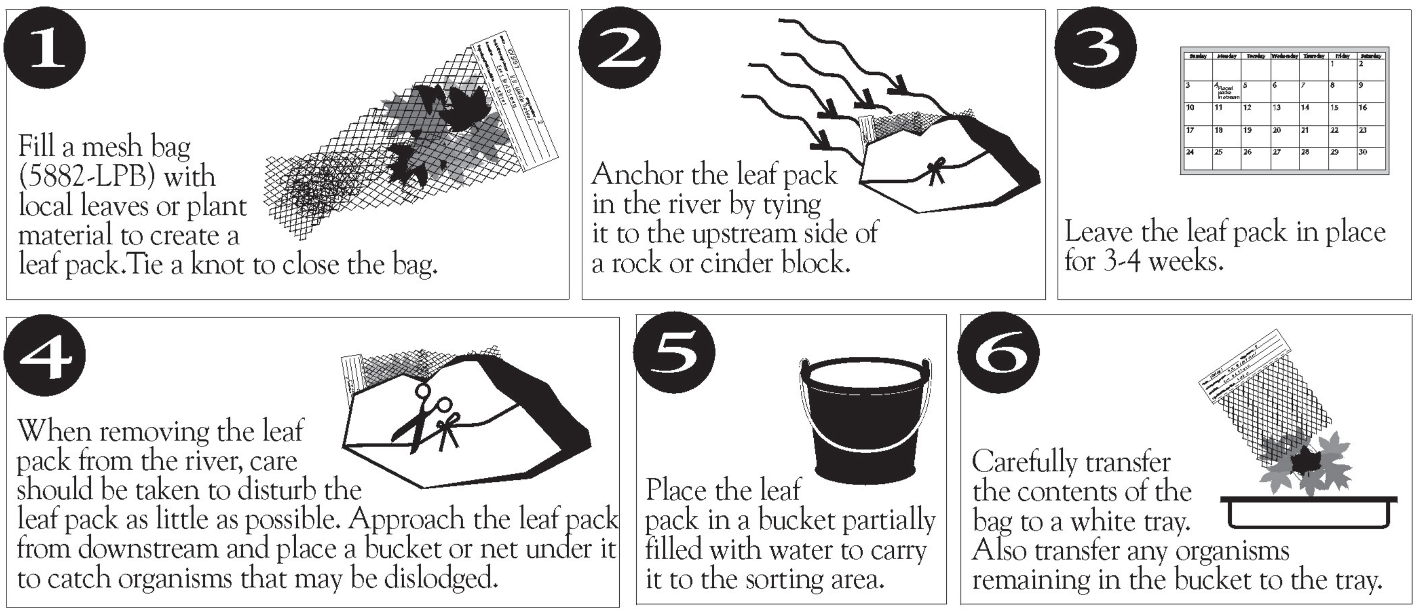
Leaf Pack Experiments or Hester-Dendy Samplers

Another type of sampling involves placing an artificial substrate in the stream for a number of weeks, then collecting it after has been colonized by macroinvertebrates. This method is useful if you are sampling deeper water and use of a net is difficult, or if you do not have sufficient time at the stream to perform the proper kick seine or D-net sampling procedures. Instructions for making your own Hester-Dendy are provided in Appendix A. Leaf pack experiments (below) are flexible. However, each experiment will: 1) provide an understanding of the structure and function of macroinvertebrates within a stream community and 2) relate the abundance and variety of macroinvertebrates colonizing artificial leaf packs to: habitat quality, water quality, and the influence of the forested riparian area.



How do they develop?

Many 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 (Figure 29).

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 wormlike.) 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.

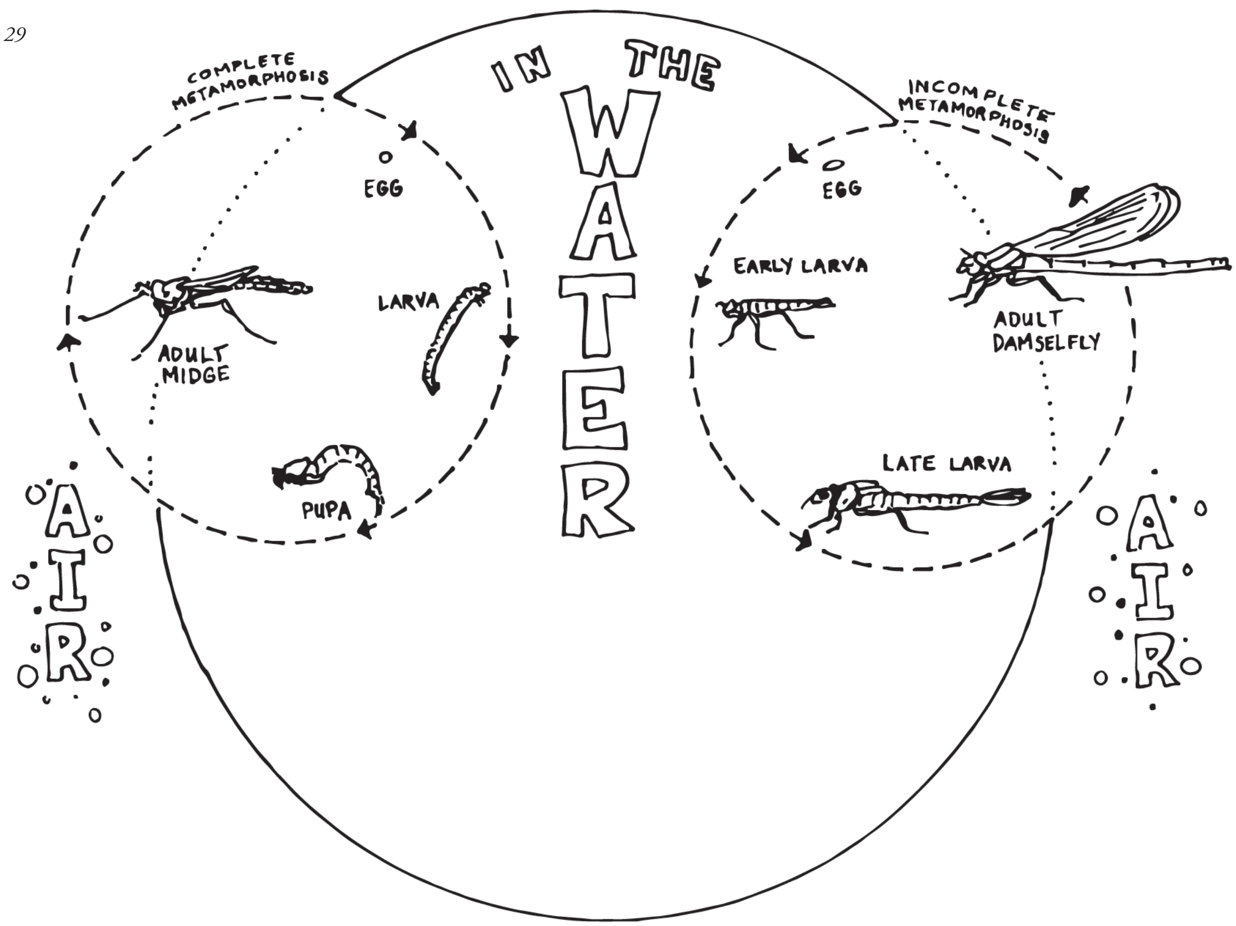
Complete metamorphosis: **egg > larvae > pupa > adult** (true flies, beetles, caddisflies)

Incomplete Metamorphosis

Aquatic insects that go through incomplete metamorphosis undergo only three stages of development: eggs, nymphs and adult. The eggs hatch into nymphs which feed and grow in the water while they develop adult structures and organs. Nymphs often look similar in body shape to the adults. The life cycle begins again when adults lay eggs in the water.

Incomplete metamorphosis: **egg > nymph > adult** (mayflies, dragonflies, stoneflies, true bugs)

Figure 29



What and How Do They Eat?

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

Shredder: Feeds 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: Feeds on fine, dead organic matter, including that produced by the shredders.

> **Filtering collector:** Filters particles out of flowing water.
> Examples include blackfly larvae and net-building caddisflies.
>
> **Gathering collector:** Gathers matter while crawling along the river bottom.
> Gatherers include mayfly nymphs, adult beetles, and midge larvae.

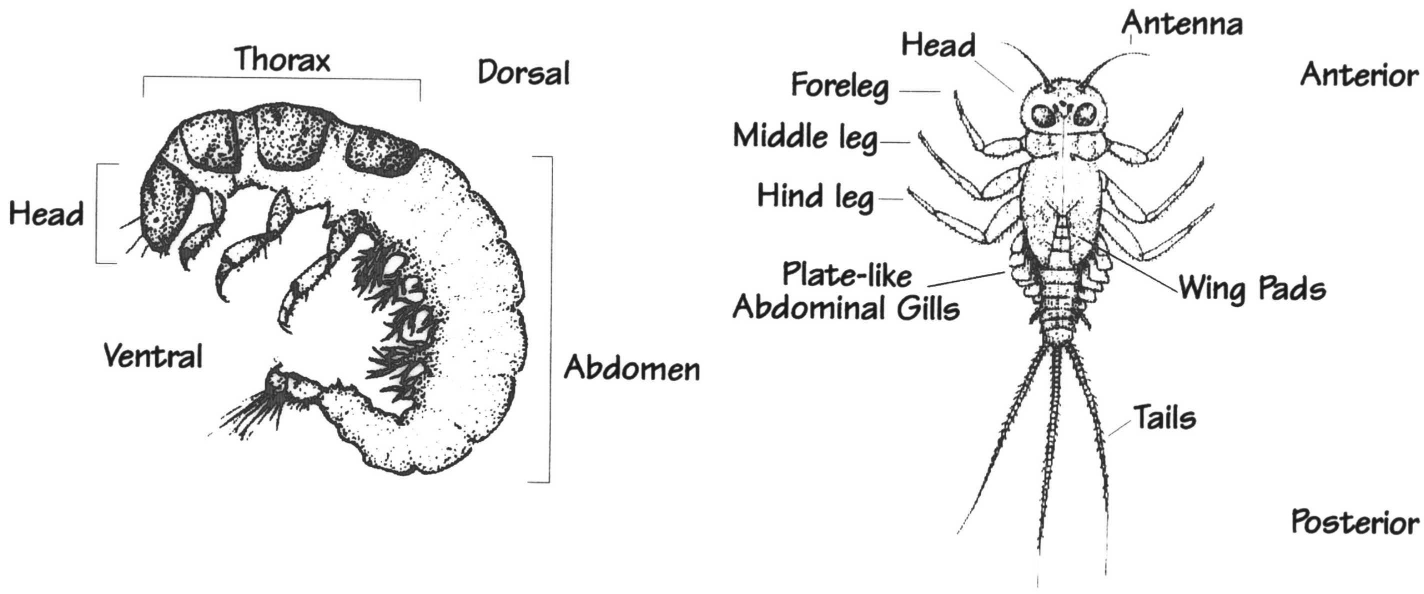
Grazer: Grazes on algae growing on rocks in the substrate or on vegetation. Grazers include snails and water pennies (a type of beetle larvae).

Predator: Feeds 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 (larval dobsonflies) are pincher-like, and a water strider's mouth parts are spear-like. Also includes beetle adults and larvae.

What Do They Look Like?

A simple key to benthic macroinvertebrates is provided in Appendix B. The organisms are grouped according to pollution tolerance, starting with the most intolerant families. Figure 30 below (*from the GREEN Standard Water Monitoring Kit*) may help you identify the distinguishing features of many of the organisms.

Figure 30



What If You Find Freshwater Mussels?

Freshwater mussels are the most endangered group of animals in Indiana! Of the 77 species that once inhabited Hoosier lakes, rivers and streams, 10 are now extinct, 17 are endangered, and 7 are of special concern. Since the presence and diversity of freshwater mussels serve as an indicator of river and stream health, we must minimize our impact on the stream substrate to protect these important species.

Follow these guidelines:

- **AVOID** sampling (especially kick seining) where you observe live mussels or a bed of mussel shells (open or closed).
- If you happen to collect mussels when sampling for macroinvertebrates, **you MUST replace ALL mussels** in the stream in the exact location and orientation in which you found them. Observe any live mussel's shell for clues to its original orientation. If part of the mussel is covered in algae and part in mud, the algae side was sticking up toward the sun while the other side was buried in the substrate. Also, the hinge (closure) should face downstream, with the opening toward the streamflow. If you are unsure how to put a mussel that you kicked up back into the substrate, then just lay it gently on the bottom of the stream in the area from which it came and allow it to reorient and re-bury itself.
- Be careful not to spread exotic species. **Volunteers sampling in zebra mussel infested waters should allow their equipment to dry completely** before using it in another water body. Zebra mussel veligers (planktonic larvae) can live for a while out of water. If the equipment must be used in a different waterbody soon after sampling in infested waters, you must rinse the equipment thoroughly with hot water!

Identifying mussel species is not an easy task; only specially-trained biologists are able to differentiate species. It is unlikely we, as volunteer stream monitors, will be able to distinguish an endangered mussel from a nonendangered species. Therefore, **ALL mussels should be treated as though they are endangered**!

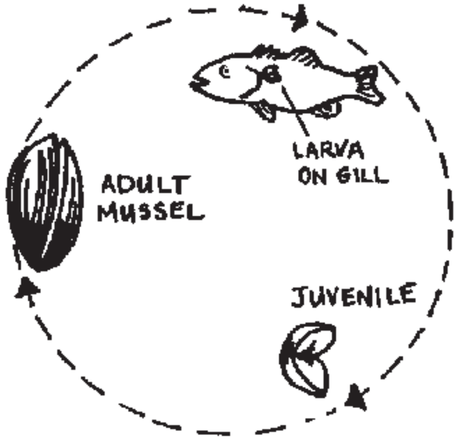
Freshwater Mussels Regulations

In an effort to reverse statewide declines in their populations, the removal of freshwater mussels, both live specimens and dead shells, from Indiana waters became illegal in 1991. It is illegal to have live or dead mussel shells in your possession. (Live shells are closed and are held together by the living mussel inside.) Leaving the mussels in the streams not only protects them, but you as well.

Why are Freshwater Mussels in Danger?

Mussels have a very complicated life cycle (Figure 31), which may make it difficult for some species to persist. Male mussels release their sperm into the water column, and the sperm must then be lucky enough to be siphoned in by a female mussel downstream of the male *(which is why it is VERY important that you replace a mussel exactly where and how you found it)*. After a time, the female will release mussel larvae or glochidia into the water where they will die unless they attach to a host fish. The fish serves as a source of food, shelter and locomotion for the developing larvae. Without the proper fish to serve as host, many mussel species could not expand their range or survive!

Figure 31



Many aquatic populations, including fish and mussels, have suffered because of habitat disturbances (such as dam construction, channelization, dredging) and watershed activities (such as construction and agriculture) which can lead to increased siltation and polluted runoff to rivers, streams, and lakes.

Commercial demands for freshwater mussel shells have also contributed to their decline. Mussel shells were used to make pearl buttons from the late 19th and early 20th centuries until the 1940s when plastic became the material of choice. Current commercial use involves grinding freshwater mussel shells to insert into oysters and stimulate the production of cultured pearls. Poaching remains a threat to mussel populations. If you suspect poaching of mussels, report it to the Indiana Department of Natural Resources immediately through their toll-free number **1 (800) TIP-IDNR.**

Other Biological Indicators of Stream Health

If any of these indicators are present, please check the appropriate box on the bottom of the biological Monitoring Data Sheet.

- Native Mussels
- Zebra Mussels – Invasive species
- Rusty Crayfish – Invasive species
- Aquatic Plants – Indicators of clear water and stable substrate. They provide habitat and stabilize the stream bed during high flow conditions. They also produce oxygen and take contaminants out the sediment via root absorption (From IOWATER Program Handbook). However, exotic invasive plant can cause serious damage to an ecosystem.
- % Algae Cover - Excess algae can be caused by too many nutrients in the stream. Too much algae can lead to oxygen depletion. Estimate the amount of the stream bottom (or the rocks) within your 200' stream section covered with algae - in increments of 25%, 50%, 75%, or 100%.

Biological Monitoring Data Sheet

The biological monitoring data sheet can be taken into the field to record the results of your biological sampling. The sheet includes information about the day, equipment used to collect samples (kick net or D-net), habitats sampled, and organisms collected. The bottom of the sheet includes other biological indicators. Although these do not factor into scoring, they can help you document what is going on in your stream.

The macroinvertebrate index is divided into Pollution Tolerance Groups 1, 2, 3, and 4. These groups represent the different levels of pollution tolerance; the higher the number, the higher the pollution tolerance level. Record the number of macroinvertebrates you find in this space.

An example completed data sheet can be found on page 76. After counting and recording numbers of organisms found in the appropriate space, you count the number of taxa present and record that information in the # of Taxa box. (Do not make the mistake of adding the numbers of total individuals together). You will then multiply the # of Taxa by the appropriate weighting factor and total these to get a Pollution Tolerance Index (PTI) score.

Note: The volunteer stream monitoring database (www.HoosierRiverwatch.com) will perform the calculations for you when you submit data.

Once you have identified the macroinvertebrates in your river or stream samples and noted the number of each taxa, the data can be easily applied to more than one index. These include *Virginia Save our Streams Multimetric Index* and the *Macroinvertebrate Diversity Index*. You can use these indices for education and further reference about your stream.

Hoosier Riverwatch
Biological Monitoring Data Sheet

Date: _____ / _____ / _____ Volunteer ID: ___________ Site ID: ___________

Stream Name: ________________________________ Latitude: ________ Longitude: _______

Time ______:_____ AM / PM Time Sampling: _______ hrs Air Temp.: ____________ °C

Current Weather: ☐ Clear/Sunny ☐ Overcast ☐ Showers ☐ Rain (steady) ☐ Storm (Heavy)

Worst Weather (past 48 hours): ☐ Clear/Sunny ☐ Overcast ☐ Showers ☐ Rain (steady) ☐ Storm (Heavy)

Check Methods Used: ☐ Kick Seine Net (3 times) ☐ Dip Net (20 jabs or scoops)

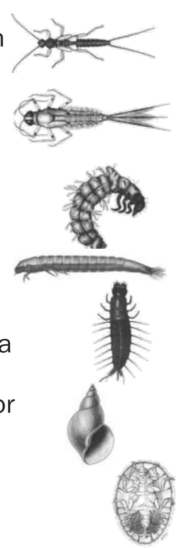
Check Habitats Sampled: ☐ Undercut Banks ☐ Riffles ☐ Leaf Packs ☐ Snags/Vegetation ☐ Sediment

Pollution Tolerance Index (PTI)

Record the taxa (group) represented in your sampling by either entering the number of organisms you counted or a ✓

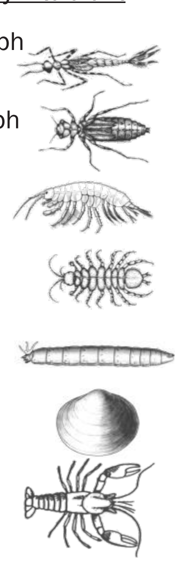
Group 1 - Intolerant

☐	Stonefly nymph
☐	Mayfly nymph
☐	Caddisfly larva
☐	Riffle Beetle
☐	Dobsonfly Larva
☐	Right-Handed or Gilled snail
☐	Water Penny
☐	**# of TAXA represented**
☐	Weighting Factor (x4)



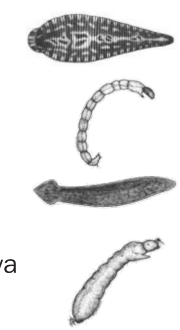
Group 2 - Moderately Intolerant

☐	Damselfly nymph
☐	Dragonfly nymph
☐	Scud
☐	Sowbug
☐	Cranefly larva
☐	Clam/Mussel
☐	Crayfish
☐	**# of TAXA represented**
☐	Weighting Factor (x3)



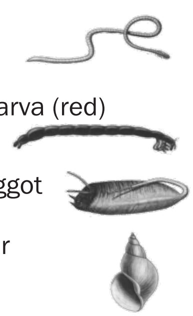
Group 3 - Fairly Tolerant

☐	Leech
☐	Midge larva
☐	Planaria/ Flatworm
☐	Black fly larva
☐	**# of TAXA represented**
☐	Weighting Factor (x2)



Groups 4 - Very Tolerant

☐	Aquatic worm
☐	Blood midge larva (red)
☐	Rat-tailed Maggot
☐	Left-Handed or Pouch snail
☐	**# of TAXA represented**
☐	Weighting Factor (x1)



Pollution Tolerance Index Rating

(Add the final index values for each group)

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

Please check other Biological Indicators you observed:

☐ Native Mussels ☐ Zebra Mussels ☐ Rusty Crayfish ☐ Aquatic Plants ________ % Algae Cover ________ Diversity Index

Hoosier Riverwatch
Biological Monitoring Data Sheet

Date: 10/04/2009 | Volunteer ID: 1000 | Site ID: 1000

Stream Name: Example Stream Indiana | Latitude: 40.76054 | Longitude: - 85.76369

Time: 12:15 AM / PM | Time Sampling: 2.5 hrs | Air Temp.: 29.5 °C

Current Weather: ☒ Clear/Sunny ☐ Overcast ☐ Showers ☐ Rain (steady) ☐ Storm (Heavy)

Worst Weather (past 48 hours): ☐ Clear/Sunny ☐ Overcast ☒ Showers ☐ Rain (steady) ☐ Storm (Heavy)

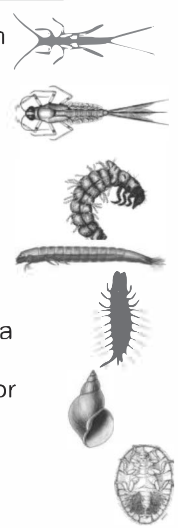
Check Methods Used: ☒ Kick Seine Net (3 times) ☒ Dip Net (20 jabs or scoops)

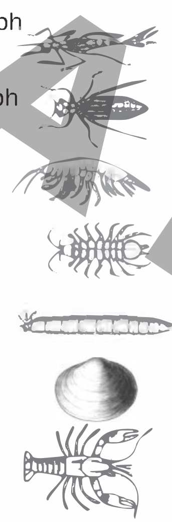
Check Habitats Sampled: ☒ Undercut Banks ☒ Riffles ☐ Leaf Packs ☒ Snags/Vegetation ☐ Sediment

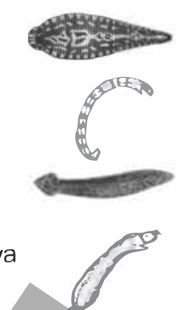
Pollution Tolerance Index (PTI)

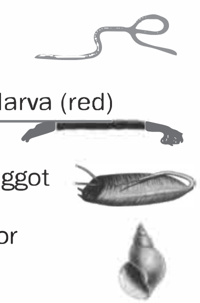
Record the taxa (group) represented in your sampling by either entering the number of organisms you counted or a ✓

Group 1 - Intolerant		Group 2 - Moderately Intolerant		Group 3 - Fairly Tolerant		Groups 4 - Very Tolerant	
6	Stonefly nymph		Damselfly nymph		Leech	25	Aquatic worm
5	Mayfly nymph	15	Dragonfly nymph	>100	Midge larva		Blood midge larva (red)
10	Caddisfly larva		Scud	16	Planaria/ Flatworm		Rat-tailed Maggot
	Riffle Beetle		Sowbug		Black fly larva	1	Left-Handed or Pouch snail
	Dobsonfly Larva		Cranefly larva				
	Right-Handed or Gilled snail		Clam/Mussel				
30	Water Penny	2	Crayfish				
4	# of TAXA represented	2	# of TAXA represented	2	# of TAXA represented	2	# of TAXA represented
16	Weighting Factor (x4)	6	Weighting Factor (x3)	4	Weighting Factor (x2)	2	Weighting Factor (x1)









Pollution Tolerance Index Rating 28

(Add the final index values for each group)

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

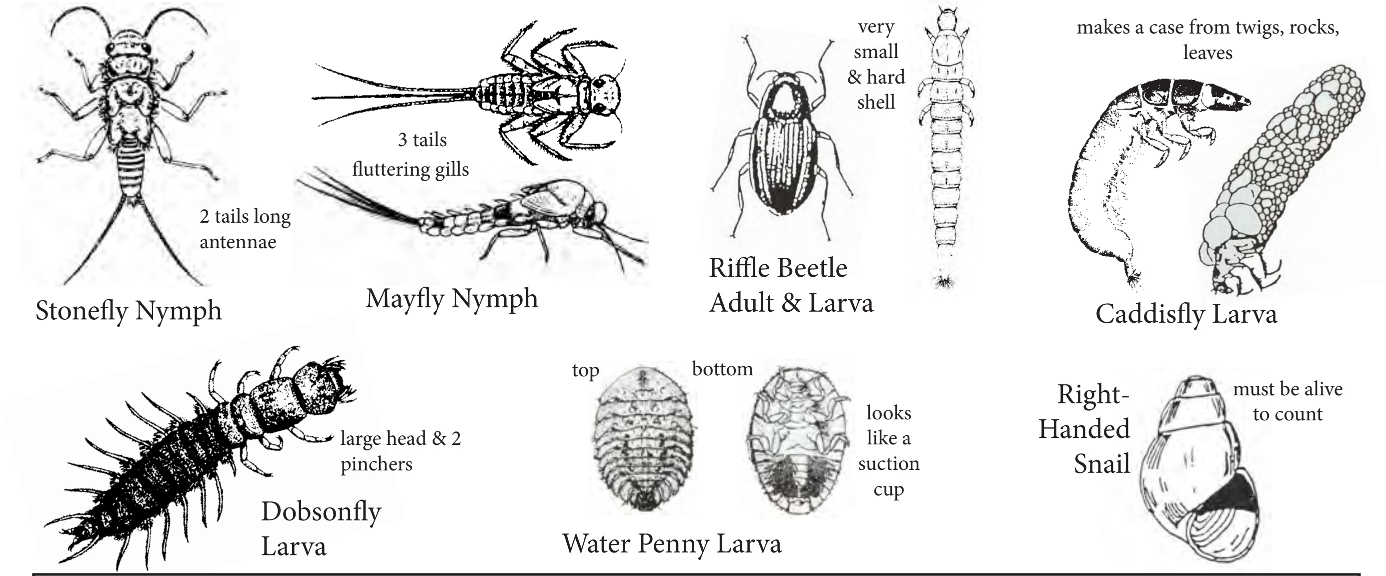
Please check other Biological Indicators you observed:

☐ Native Mussels ☐ Zebra Mussels ☐ Rusty Crayfish ☐ Aquatic Plants ______ % Algae Cover ______ Diversity Index

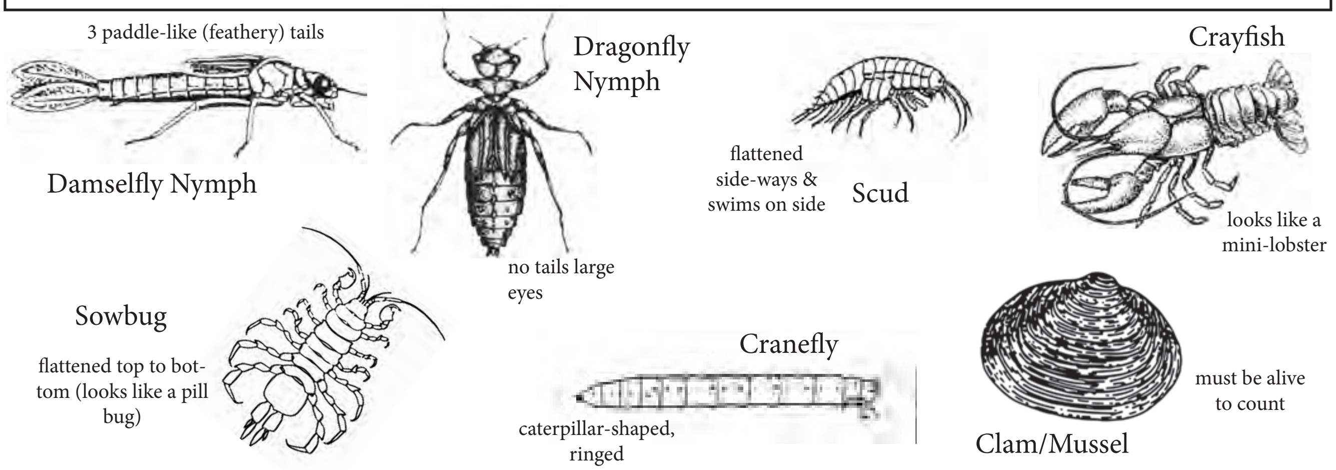
EXAMPLE

Macroinvertebrate Identification Key

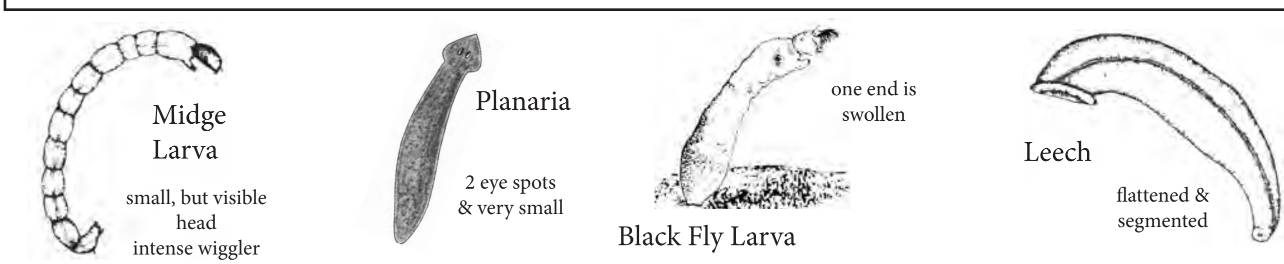
GROUP 1 – Very Intolerant of Pollution



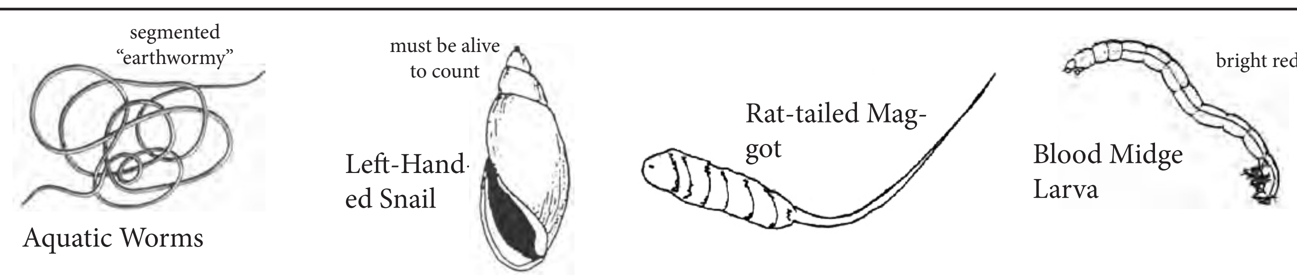
GROUP 2 – Moderately Intolerant of Pollution



GROUP 3 – Fairly Tolerant of Pollution



GROUP 4 – Very Tolerant of Pollution

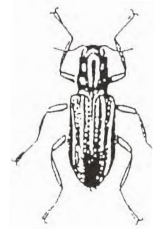


Macroinvertebrate Adults Key

GROUP 1 – Young are Very Intolerant of Pollution


Stonefly Adult


Mayfly Adult


Riffle Beetle Adult


Caddisfly Adult


Dobsonfly Adult

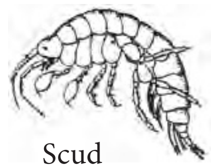

Water Penny Adult

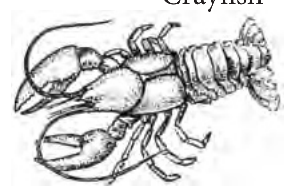

Right-Handed Snail

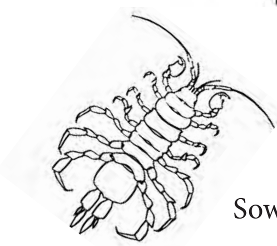
GROUP 2 – Young are Moderately Intolerant of Pollution


Damselfly Adult

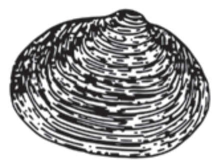

Dragonfly Adult


Scud


Crayfish


Sowbug


Cranefly Adult

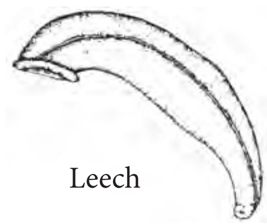

Clam/Mussel

GROUP 3 – Young are Fairly Tolerant of Pollution


Midge Adult


Planaria


Black Fly Adult


Leech

GROUP 4 – Young are Very Tolerant of Pollution


Aquatic Worms


Left-Hand-ed Snail


Hoverfly
(Rat-tailed maggot adult)


Blood Midge Adult