PROTOCOL 5. COLLECTING AQUATIC INVERTEBRATES

Objective

To safely collect, sort, and maintain samples of stream invertebrates prior to assessing biotic integrity.

Background

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Topic: aquatic entomology
Go to: *www.sciLINKS.org*
Code: WD14

A healthy stream is a dynamic system and a home to many living organisms. One way to assess the health of a stream is to study the organisms living in it. Aquatic invertebrates, including insects that live part of their lives in water, are commonly used as indicators of stream health. Some, such as stonefly nymphs, are usually found only in very clean water because they require lots of dissolved oxygen. Others, such as some species of aquatic worms, may be able to survive in water that has very little oxygen. We call these organisms *biotic indicators* of water quality because their presence (or absence) gives us information about the quality of the stream.

SCILINKS® THE WORLD'S A CLICK AWAY

Topic: freshwater ecosystems
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Code: WD15

Most streams—even polluted ones—contain a variety of organisms, and many invertebrate taxa (e.g., family or genus) include both pollution-tolerant and pollution-sensitive species. For example, most stoneflies are sensitive to pollution, but there are some exceptions. In other words, assessing the ecological health or *biotic integrity* of a stream is usually more involved that just looking for the stonefly order. Protocols 6 and 7 provide instructions for using aquatic invertebrates to assess the biotic integrity of streams, but before you can do that, you'll need to collect samples. This protocol guides you through the collection of aquatic invertebrate samples.

Materials (per student group)

- Waders, waterproof boots, or other non-slip footgear suitable for the conditions
- D-nets
- Collecting pans and 10–15 smaller containers
- Tweezers and plastic spoons for picking up animals
- Latex gloves (if the streamwater quality could be poor)
- Walking sticks to provide stability on slippery rocks
- Personal flotation devices (PFDs) if the stream is not uniformly shallow
- Stream Invertebrate Identification sheet (pp. 81–84) and other identification materials
- Hand lenses or plastic "bug boxes" with magnifying lids

Procedure

You will work in teams of three students to collect invertebrates. Because safety is very important while working in streams, your teacher will review precautions appropriate to your site.

Part 1. Collecting Macroinvertebrates

1. Sample an area of the stream that is shallow enough to wade but is moving fairly quickly. This type of microhabitat is called a *run*. All three of you should wade into the run: one carries a D-net, one carries a collecting pan, and one—the "shuffler"—needs both hands free.
2. Facing upstream into the current, the person with the D-net should hold the handle upright and place the net on the bottom of the stream. The shuffler should stand a foot or two upstream of the net and disturb the stream bottom by slowly shuffling his or her feet, dislodging stones. Many invertebrates will jump off into the current and be carried down to the waiting net (that's one reason you're sampling where there is a good current). If the water isn't too deep or cold, reach to the bottom and lift up and brush some rocks as well. Try not to kick up too much silt and sediment.
3. After a couple of minutes of shuffling, check the net. If there is a lot of mud in it, raise and lower it into the water a few times to rinse. Then empty the contents of the net into your pan, which should start with about an inch of water.
4. Look in the pan to see if you collected any animals. You may need to wait a minute or so for sediments to settle. Your goal is to collect about 100 macroinvertebrates, but chances are good that there are many more in the pan than you see at first.
5. Move a few feet from your original sampling site and repeat the shuffling-and-netting procedure. Unless your collecting pan is getting crowded with leaves, stones, and other debris, you will be able to add the contents of several nets to the pan before returning to shore.

Part 2. Preliminary Processing

1. Unless you are planning to preserve your sample in alcohol, do your best to keep alive the animals you collected. Two common physical problems with collected aquatic invertebrates are thermal shock (sudden temperature change) and insufficient dissolved oxygen. If it is a sunny day and the air is much warmer than the water, do your on-shore work in the shade. Periodically give the animals some fresh water, and don't crowd lots of larger animals into a small container. Cool water holds more dissolved oxygen than warm water, so keeping the water cool will reduce both types of mortality.
2. Now observe the animals you've collected. How do they move? How might they breathe? How do they appear adapted to the habitat in which you caught them? Take a look at the Stream Invertebrate Identification sheet and try to identify a few organisms from the drawings.

Part 3. Sorting

1. Put some fresh water into your smaller containers and begin sorting the invertebrates by appearance. You can pick them up gently with the forceps or use a plastic spoon or small container to scoop them. At this point, you are only estimating how many taxa and how many individuals you have, and do not need to specifically identify each one. A taxon (plural taxa) is a group of related individuals. Note that many aquatic invertebrates go through several similar-looking life stages that differ primarily in size. Individuals that look the same in every respect except size should be considered members of the same taxonomic group.

2. Examine your collected macroinvertebrates with a hand lens and look for differences in head and body shape, the appearance and placement of feathery external gills, coloration, and the number of tails. Put different types in different containers. If you run out of containers, you can combine the less common creatures in one container—but keep an eye on it to make sure they don't eat each other before you have a chance to record your results!

Part 4. Checking in

1. After doing a quick sorting and counting, check in with your teacher and report how many taxa and how many individuals you have. Bear in mind that you'll find more as you continue to pick over your sample. Your teacher may direct you to return to the water to do some more collecting, or may decide to combine the collections of more than one group of students. If you'll be doing Protocol 7 later, you'll probably need at least 100 individual invertebrates.
2. While you're on your feet, visit with some of the other student groups and see what they found. They may have some interesting and uncommon species that didn't show up in your sample. Ask them what they've identified and make sure that what they're calling, for example, a "mayfly" is what your group is calling a mayfly.

Part 5. Final Processing

1. Continue picking over your sample until you're pretty confident that you've found most individuals. Look hard for smaller and slower-moving creatures: they "count" as part of the biota, but are easy to miss. Also look on the surface of any stones in your sample, where some animals, such as water pennies, may be hanging on.
2. Once your sample is completely sorted, you are ready to process your samples. Processing your samples includes identifying and counting them, and is part of the separate biotic integrity protocol. When you're through with that protocol, you may want to return your animals to their native habitat. In the meantime, keep an eye on the containers and give your animals some fresh water if they appear distressed.

Analysis

Now that you have collected and identified invertebrates, you are ready to analyze the water quality of your stream section. You can use either Protocol 6 or 7 for this purpose, but there are important differences between them.

Protocol 6

The simplest method for assessing water quality is to look for invertebrates that are especially sensitive to pollution ("indicator species"). If these organisms are present in your stream, then the water is probably not very polluted. In contrast, if these organisms are missing, your stream section might be influenced by pollution. Protocol 6 allows you to assess the water quality of your stream by looking for specific types of invertebrates.

Protocol 7

Like Protocol 6, this protocol also considers what indicator species are present in your stream. However, Protocol 7 also considers how many species are present in the stream ("species richness") as well as how many individuals of each species are present ("dominance"). While Protocol 7 may take longer to complete, it will yield a more precise analysis of your stream.

STREAM INVERTEBRATE IDENTIFICATION SHEET

—Require high dissolved oxygen levels

Stonefly nymphs (order Plecoptera)

Description: 1–4 cm; six legs, each ending in double hooks; visible antennae; two tails (never three). No gills on abdomen.

Feeding: Most species gather and eat decaying plants or animals, but some eat bacteria and others are predators.

Habitat: Swiftly moving streams with high oxygen levels.

Mayfly nymphs (order Ephemeroptera)

Description: 0.5–3 cm; six legs, each ending in a single hook; visible antennae; three long tails (sometimes two). Plate-like or feathery gills along sides of abdomen.

Feeding: Grazers or gatherers; eat algae and organic matter.

Habitat: Some cling to rocks, some burrow in mud, and others are free swimmers. Diversity of mayfly species decreases with stream degradation.

Caddisfly larvae (order Trichoptera)

Description: <2.5 cm; six legs with hooked claws; two hooks at tail end. Some species build cases of small stones or sticks, and others are free-living or spin nets attached to rocks.

Net-spinner

Feeding: Some graze algae, others filter-feed detritus, and a few free-living species are predators.

Habitat: High-quality streams; some are tolerant of mild pollution.

Case-builder

Dobsonfly larvae (order Megaloptera)—also known as "Hellgrammites"

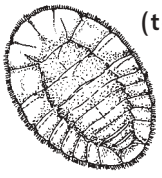
Description: 2–10 cm; six legs; large pinching jaws; pointed feelers with feathery gills along abdomen; two tail projections, each with two hooks.

Feeding: Predators with powerful chewing mouthparts. *Caution:* If they pinch you, it hurts!

Habitat: High-quality streams.

Beetle larvae and adults (order Coleoptera)

Water penny larvae



(top view)

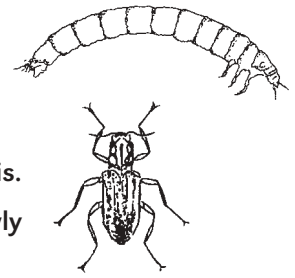
Description: 0.5–1.25 cm; broad flat saucer-shaped body; six small legs underneath.

Feeding: Graze algae and other material attached to rocks.

Habitat: Cling to rocks in cold, fast-running, high-quality streams.

(side view)

Riffle beetle larvae and adults



Description: Larvae are <1.25 cm; worm-like but hard body; six legs and small tuft of white filaments at tail end. Adults are 1–2 cm, black, and look similar to many terrestrial beetles.

Feeding: Collect and gather algae, diatoms, and organic debris.

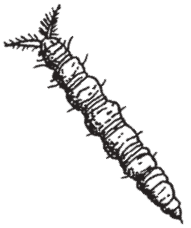
Habitat: Larvae cling to rocks in stream riffles. Adults walk slowly along stream bottom.

Notes: Unique in that larval and adult stages both are aquatic.

—Can survive with moderate oxygen levels

Fly larvae (order Diptera)

Watersnipe fly larvae



Description: <3 cm; cylindrical, slightly flattened; cone-shaped abdomen; many legs with suction tips; pale to green.

Feeding: Predators.

Habitat: Moderate quality streams and rivers.

Cranefly larvae

Description: 6 cm; large, fleshy, segmented, and worm-like, with four finger-like lobes at hind end; light brown, green, or milky color.

Feeding: Most graze on algae or are gatherers, but a few are predators.

Habitat: Can be found burrowing in mud.

Blackfly larvae

Description: <0.5 cm; head has feathery gills, tail end has suction pad. Shaped like a bowling pin.

Feeding: Filter small particles of organic matter from the current.

Habitat: Live attached to submerged rocks; require swiftly flowing water.

Damselfly and Dragonfly nymphs (order Odonata)

Damselfly nymphs

Description: 0.25–5 cm; large protruding eyes; six thin legs; long, thin, abdomen with no gills; three broad "tails" that actually are gills.

Feeding: Predators.

Habitat: Typically found in medium-quality, slowly moving water.

Dragonfly nymphs

Description: 1.25–5 cm; large protruding eyes; round to oval abdomen; six hooked legs.

Feeding: Predators; eat aquatic insects, tadpoles, and small fish.

Habitat: Slowly moving water.

Alderfly larvae (order Megaloptera)

Description: <3 cm; six legs and six to eight filaments on each side of abdomen; distinguished from Dobsonfly larvae by single-tail projection with hairs but no hooks.

Feeding: Aggressive predators.

Habitat: High- or medium-quality water.

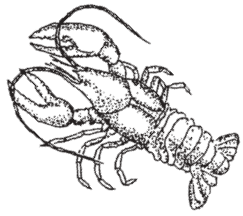
Scuds (order Amphipoda)

Description: <1.25 cm; swim rapidly on their sides and resemble shrimp; flat sides, hump-shaped back, and several pairs of legs; white, gray, or pink.

Feeding: Gather dead and decaying matter.

Habitat: Some highly sensitive to pollution; others found in moderately polluted water.

Crayfish (order Decapoda)



Description: <15 cm; look like small lobsters, with two large claws and eight smaller legs.

Feeding: Predators; use large claws to tear plant and animal prey into small chunks.

Habitat: Slow-moving streams, rivers, and ponds.

—Can live in streams with low dissolved oxygen levels

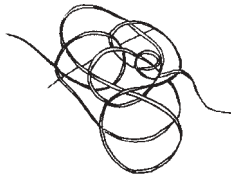
Midge larvae (order Diptera)

Description: <1.25 cm; have a worm-like body and distinct head; often C-shaped; sometimes bright red. 2 leg-like "prolegs" near the head

Feeding: Most filter-feed or gather detritus; a few prey on other insect larvae.

Habitat: Can survive in water with low oxygen concentrations.

Aquatic worms (class Oligochaeta)

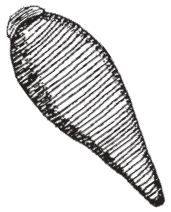


Description: Usually <7.5 cm; long, thin, segmented worms with no legs.

Feeding: Ingest mud and filter out organic material.

Habitat: Tolerant of pollution; high numbers indicate poor water quality.

Leeches (order Hirudinea)



Description: <5–8 cm; worm-like, brown, and slimy; flattened, with sucker at each end.

Feeding: Some attach suckers to prey and drink blood; others gather detritus.

Habitat: Indicators of low dissolved oxygen.

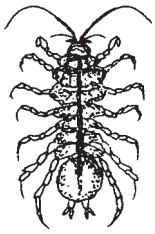
Snails (class Gastropoda)

Description: 0.5–2 cm; flat or cone-shaped shell surrounding soft body.

Feeding: Scrape algae and bacteria from surfaces of submerged rocks.

Habitat: Some species have lungs and can live in waters with low oxygen levels, and others breathe with gills and require high oxygen concentrations.

Aquatic sow bugs (order Isopoda)



Description: 0.5–2 cm; relatively flat; have long antennae and seven pairs of legs.

Feeding: Scavenge both dead and live plants and animals.

Habitat: Can tolerate high levels of decaying organic matter; typically found in muddy, slow-moving water.

Notes: Sow bugs are crustaceans, not bugs as their name suggests.