

Freshwater Macroinvertebrates Protocol



Welcome

Introduction

Protocols

Learning Activities

Appendix

Purpose

To sample, identify and count macroinvertebrates at your Hydrology Site

Overview

Students will collect, sort, identify, and count macroinvertebrates from habitats at their site.

Student Outcomes

Students will learn to,

- identify taxa of macroinvertebrates at their site;
- understand the importance of representative sampling;
- use biodiversity and other metrics in macroinvertebrate research (advanced);
- examine reasons for changes in the macroinvertebrate community at their Hydrology Site (advanced);
- communicate project results with other GLOBE schools;
- collaborate with other GLOBE schools (within your country or other countries); and
- share observations by submitting data to the GLOBE archive.

Science Concepts

Earth and Space Sciences

Soils have properties of color, texture and composition; they support the growth of many kinds of plants.

Soils consist of weathered rocks and decomposed organic matter.

Life Sciences

Organisms have basic needs.

Organisms can only survive in environments where their needs are met.

Earth has many different kinds of environments that support different combinations of organisms.

Organisms functions relate to their environment.

Organisms change the environment in which they live.

Humans can change natural environments.

Ecosystems demonstrate the complementary nature of structure and function.

All organisms must be able to obtain and use resources while living in a constantly changing environment.

All populations living together and the physical factors with which they interact constitute an ecosystem.

Populations of organisms can be categorized by the function they serve in the ecosystem.

Living systems require a continuous input of energy to maintain their chemical and physical organizations.

The interaction of organisms have evolved together over time.

Scientific Inquiry Abilities

Identify answerable questions.

Design and conduct scientific investigations.

Use appropriate mathematics to analyze data.

Develop descriptions and explanations using evidence.

Recognize and analyze alternative explanations.

Communicate procedures and explanations.

Time

3 to 6 hours to collect samples, count, identify, and preserve specimens

Time will vary with the abundance and diversity of organisms.



Level

Middle and Secondary

Frequency

2 times a year

Materials and Tools

Macroinvertebrate Identification Data Sheet

Equipment used to collect water chemistry measurements at your Hydrology Site (optional)

Latex gloves

Many clear plastic jars (0.5 to 3 L)

Many small plastic vials.

One to four plastic squirt or spray bottles (1 to 2 L)

Many 20-mL bulb basting syringes (end should be approximately 5 mm diameter)

Several eyedroppers (end should be approximately 2 mm diameter)

Large and small plastic or metal forceps

Several magnifying glasses or loupes

Two to six 5-L white buckets

White trays

Sub-sampling tray (optional)

Two sieves: one 0.5 mm (or smaller), and one between 2-5 mm

Locally-applicable macroinvertebrate identification keys

Appropriate footwear

Specimen bottles with preservation solution (70% ethanol) and tight lids (optional)

1 x 1 m quadrat (optional)

For Rocky Substrates in Running Water Protocol:

- Kick-net (0.5 mm mesh)

- Stop watch or watch

- Square of white fabric (about 110 cm by 110 cm)

For Multi-habitat Freshwater Macroinvertebrate Protocol:

- D-net (0.5 mm mesh)

- Trowel or shovel

Preparation

Practice identifying the macroinvertebrates using local keys to macroinvertebrates.

Make or buy the appropriate net for your Hydrology Site.

Collect and make materials for sampling.

Collect pictures or books illustrating local macroinvertebrates.

Prerequisites

None

Freshwater Macroinvertebrates Protocol – Introduction

Macroinvertebrates are small animals without a backbone that can be seen without a microscope. They live around living or dead vegetation, on the surface or in the sediments of water bodies. They include many larvae of insects such as mosquitoes, dragonflies and caddis flies that begin their lives in the water before becoming land dwelling insects when they mature. Other examples of common macroinvertebrates include crustaceans (such as crayfish), snails, worms and leeches. Macroinvertebrates can populate ponds or streams in amazing numbers – some of them up to thousands in a square meter. They are an important part of the food chain.

Macroinvertebrates can tell us a lot about the conditions within a water body. Many macroinvertebrates are sensitive to changes in pH, dissolved oxygen, temperature, salinity, turbidity and other changes in their habitat. Habitat is a place that includes everything that an animal needs to live and grow. It includes food resources, the physical characteristics of the environment, as well as places and materials to build nests, raise young and keep them safe from predators. Habitats include rocks, sticks, dead and decaying vegetation and other living organisms such as plants.

For the *Freshwater Macroinvertebrates Protocols* we want to estimate biodiversity, examine the ecology of the water body and explore relationships among water chemistry measurements and organisms at your Hydrology Site. Most often it is impossible to count all individuals of every species present in a habitat. So, we take samples of organisms in habitats, and calculate the diversity found in these samples to estimate true biodiversity in the habitats. Biodiversity is the number of different kinds of organisms in an ecosystem and the number of individuals of each kind. Often biodiversity is estimated from species data, but it can also be the number in broader categories like the number of different kinds of arthropods.

Scientists often use metrics to learn about the ecology of the water body. Metrics are derived from counts of organisms in samples at your and other sites. A simple metric is the number of organisms. Organisms can also be put into groups such as the percentages of feeding strategies (grazers, filter feeders, and predators), or percentages of long-lived and short-lived taxa.

Taking chemical measurements in a water body is like looking at a picture of what is going on in the water at that time. Taking biological measurements is like watching a movie of things that happened over time in the water in a single visit. Macroinvertebrates record the history of a water body because many are sessile or stay within a small area and live one or more years while the water flows by. Changes in the habitats (including water chemistry) most likely will cause changes in the macroinvertebrate assemblage.



Teacher Support

Advance Preparation

Many teachers and students have little background in the study and identification of freshwater macroinvertebrates, and may be reluctant to begin such a class project. This is not a problem, since students find the critters so fascinating they will be teaching themselves and each other.

There are many local experts to call on. Often, local water quality monitoring groups are willing to work with students. These people can, for example, help with family level identification (which is encouraged but optional) and with discussing important indicator species, as well as endemic and introduced organisms present in your area. Macroinvertebrate identification keys are available on the Internet or in printed manuals and books. Select an identification key that is applicable to your locality.

Contact local experts in the area to make sure that you are not sampling at a site where other people are conducting research or where there are endangered species. You do not want to inadvertently hurt a long-term monitoring site or harm endangered species.

To have the students become familiar with macroinvertebrates before you go to the field, students can bring in macroinvertebrates from their neighborhoods to identify in class.

Site Definition and Mapping

Select a 50-meter section of your stream, pond, or lake where you will sample freshwater macroinvertebrates. Select sites that can be accessed and sampled safely.

It is important to create a map of the 50-meter section that includes all the important features surrounding and within your water body, in particular, the types of habitats where macroinvertebrate sampling will be done (see *Hydrology Site Definition and Mapping Protocol*). Represent all the habitats on your map even if certain habitats cannot be reached. Habitat description and mapping are important for understanding and interpreting your data.

Each time you visit your site and collect macroinvertebrates, describe the habitats at the site at the time of sampling. Over time, habitats may change at your site and this could then affect which macroinvertebrates are found. In addition, if you are using the *Multi-habitat Freshwater Macroinvertebrate Protocol*, the amount and types of habitats at your site will determine your macroinvertebrate sampling strategy. An up-to-date map will allow you to calculate how many samples to collect in each habitat in proportion to the new coverage of all accessible habitats.

Here are some questions to ask yourself to help identify different habitats where macroinvertebrates live.

1. Is the water flowing or stagnant? If both, identify where.
2. If flowing, where would you consider it fast-flowing or slow-flowing (at least relative to the other places within your site)?
3. What and where are the substrates – boulders, cobbles, pebbles, sand or mud?
4. Are plants growing in the water body?
5. Are the banks vegetated?
6. Which areas are being eroded?
7. Where are snags, logs and roots?
8. Does the surrounding vegetation provide shade to the water?

If your site has running water and stones, indicate the riffle habitats, the run habitats, the pool habitats and their substrate: boulder, cobble, or gravel. Other potential habitats in running waters or more stagnant waters and wetlands are: vegetated banks, submerged vegetation, snags, logs, roots, mud, sand, and gravel.

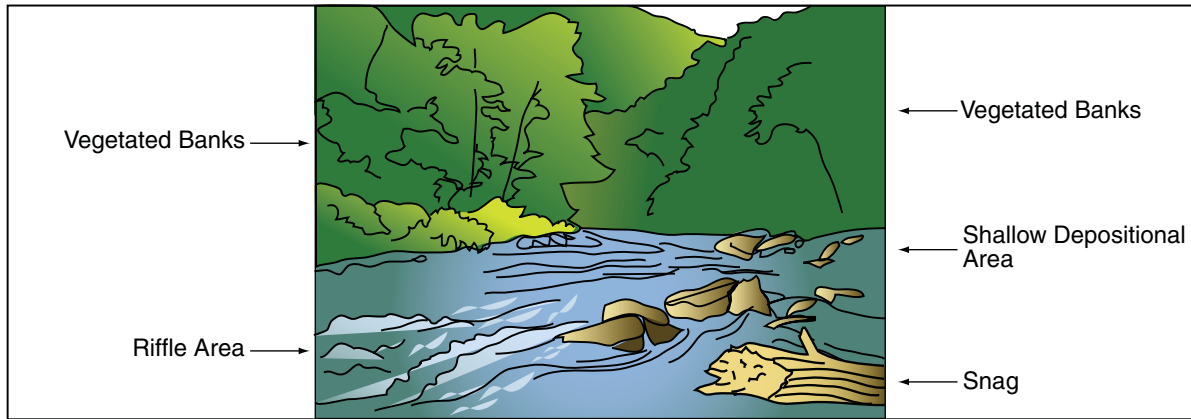
Pool: a deeper region with slower-moving water and smaller sediments.

Riffle: a shallower area with faster-flowing water and larger sediments.

Run: an intermediate category between pool and riffle. Water in a run does not have the turbulence of a riffle, but moves faster than in a pool.



Figure HY-MA-1



Snag: a tree or branch embedded in the bed of the water body.

Which Protocol to Use: Rocky-Substrates in Running Water or Multi-habitat

If your hydrology site is a body of visibly running water shallower than 90 cm with a rocky substrate, use the *Rocky Substrate in Running Water Freshwater Macroinvertebrate Protocol*.

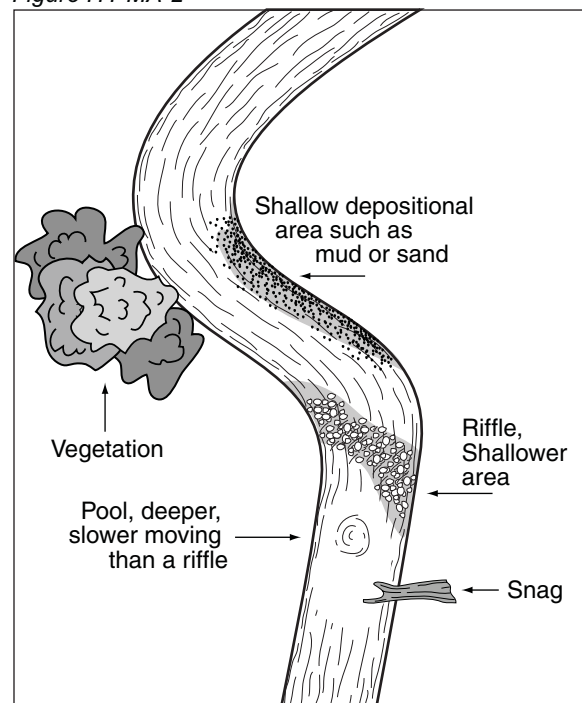
If the water is deeper than 90 cm or if many habitats are present, use the *Multi-habitat Freshwater Macroinvertebrate Protocol*. When mapping, pay special attention to identify all the aquatic habitats present and estimate the area covered by each habitat. The proportion that each accessible habitat covers will determine the number of samples taken in each habitat in the *Multi-habitat Freshwater Macroinvertebrate Protocol*.

When To Go Sampling

You should sample twice a year in different seasons.

Warm/cold seasons: If you have warm/cold seasons, sample in the spring and autumn. Sampling in the spring should be around the time of budburst. Autumn sampling should be done around the start of green-down and before frost. Green-up and green-down are explained in the *Phenology Investigation*. If you wait until you see many insects flying in the Spring, many of the insects will have grown past their aquatic stages and left the water. You will not have them in your sample. If you sample too early, the organisms may be too

Figure HY-MA-2



small and pass through the mesh of the net or be difficult to identify.

Wet/dry seasons: If your seasons alternate between wet and dry, choose a date in the second half of the wet season and one date in the dry season six months from the first sampling if possible (or before water body becomes completely dry).

If you have no marked cyclic changes, ask local experts to find out when you should sample to find the peak abundance and diversity of macroinvertebrates in the water. Sample at that time and sample again six months later.



Sampling more than twice a year is not recommended for it may disturb and harm the habitats for the macroinvertebrates and other organisms living in the water.

Supporting Protocols



Hydrology: Students can explore relationships between the water measurements and the types of macroinvertebrates found at their Hydrology Site.

Land Cover/Biology: Students could examine relationships between the types of macroinvertebrates they find and the types of land cover surrounding their Hydrology Site and in the watershed.



Preparing for the Field

There are two sampling methods. It would be a good idea to select a site before the day of sampling and determine which sampling method will be used. The sampling method will determine which type of net you use.



Some or all of the students will be in the water. Those that walk in the water need to be appropriately dressed, in particular the footwear. Students may need waders. If using sneakers or something like sneakers, bring another pair of shoes to wear after sampling. Students may also need a change of clothes.



If available, you can take folding tables or seat desks for the students to handle and count their samples in the field.

Managing Students in the Field

If you have a large class, have students work in multiple teams. Students in a team can be responsible for different tasks. For example, two students can handle the net, one student can handle the bucket, one student can read the instructions aloud, etc.



The most time-consuming tasks are sorting and identifying the organisms. To save time, have one team of students collect a sample and start to sort and identify the organisms using the *Sorting, Identifying and Counting Freshwater Macroinvertebrate Protocol Lab Guide*. While this team is sorting and identifying, another team

can be collecting a second sample. A third team can collect a third sample. If you are collecting in riffle/run habitats, then you only need three samples. For multi-habitat environments, more samples will be collected. The more teams you have, the more buckets and other equipment you will need.

As the students work, look at the jars of sorted organisms to verify that all the students identify organisms in the same way. If not, gather the students and have them discuss the differences and determine the correct taxa.

After all the organisms are sorted and combined from the teams in separate jars for each taxon, have a committee of students and yourself look at the organisms to make sure that you all agree on identifications. Then, proceed to count organisms in each taxon and report the data on one set of data sheets. Collect voucher specimens of three individuals from each taxon, and return the rest of the organisms to the water.

Measurement Procedures

Do not sample habitats that cannot be reached safely. If your students are doing the multi-habitat sampling method, determine which habitats can be sampled safely and evaluate the percentage of coverage of each accessible habitat. Record in metadata which habitats could not be sampled.

When pouring water with macroinvertebrates through sieves or into other buckets, pour slowly and gently so that the macroinvertebrates do not get injured or die. Handle gently with forceps, fingers or syringes.

Students should only sort and count macroinvertebrates. Small fish, tadpoles, and other organisms should be removed from the samples and returned to the water.

Only count macroinvertebrates that are alive. To find out if bivalves and gastropods are alive, look for soft body tissues or for tightly closed shells (a sign that the animal is there and protecting itself). If you see many shells of dead animals, report it on the comment section and on the web site. Do not count arthropods exoskeletons. If there are many of them and it looks like the animals have just emerged out of the water,



or many are dead, report this finding on the comment section and on the Web site.

Organisms may break while you process them. Count all the whole organisms first. Discard organisms that look partially decomposed. With the remaining fresh pieces, match halves of worms or count only the heads of insects for example. If you are very careful with the sieves remove heavy substrates as you go and squirt water gently, you should find most organisms intact.

For all taxa, use the *Freshwater Macroinvertebrate Identification Data Sheet* to report the number of individuals from zero to 100. In cases where you have too many animals to count in the time that you have, you can report >100 or you can take a sub-sample to count. Sub-sampling is described in the *Protocols* section. If you have enough time, count all individuals in your sample. A more accurate count of the number of individuals in each taxon allows better estimates of biodiversity and other analyses by students and scientists.

In the *Multi-habitat Freshwater Macroinvertebrate Protocol*, students can combine the samples collected from all the habitats and record total counts for each taxon, or students can examine the macroinvertebrates within each habitat type separately. By examining the habitat types separately, students can compare the macroinvertebrate assemblages among the habitat types. You can enter the data on the GLOBE Web site as either total counts for each taxon for all habitats combined, or total counts for each taxon for each habitat type.

Voucher specimens are not required, but may help with teaching the students how to properly identify the macrovertebrates before going into the field. As well, by collecting voucher specimens each time, the specimens can be compared to make sure that identifications are being done correctly each time. Specimens are preserved in a 70% ethanol solution.

Equipment Use and Maintenance

All of the sampling materials are available commercially, but students can also enjoy making

them using the instructions provided in the *Instrument Construction* section. You can also buy some parts and make others. For example, one can buy a 0.5 mm-mesh replacement net for a D-net and make the pole. This is less expensive than buying the whole device.

Sieves are very useful to remove debris and clean organisms to concentrate organisms from a large amount of water (in the bucket) to a small amount of water. These organisms can then be transferred to a tray or jar for sorting and identifying. Sieves are available commercially, but you can make your own easily (see *Instrument Construction* section). If you cannot find a small quantity of 0.5 mm-mesh netting for the sieves, you can use a piece of fabric that has a mesh visibly smaller than your sampling net (which is 0.5 mm). The smaller mesh size may cause more clogging, so you will have to pour water slowly and check more often to make sure that water does not overflow the sieves. Clogging will also occur more often if the sample has silt or sand.

The quadrat is not necessary to use and can be made out of materials other than PVC pipe. Instructions for making the quadrat are given in the *Instrument Construction* section. The quadrat makes sure that students collect samples within a 1 x 1 meter area.

After each use, rinse and dry the nets and sieves in the air. Make sure that all debris is removed and no organisms remained trapped. It is very important to check the nets and sieves before each use to make sure that the mesh is intact. Tighten pieces that come loose. Repair or replace any piece of equipment that is broken or out of place.

Do NOT use bleach to clean the nets, buckets, sieves, or anything the macroinvertebrates may contact. The bleach, even in small amounts, may harm or kill the macroinvertebrates.

Helpful Hints

As scientists do, have students keep field notes of your procedures to report what you did and if there were any deviations from your plans. Make a photo journal of your trip, and bring parents or



older GLOBE students to mentor. Enjoy learning about the diversity of animals in the world around you!



Having the students work in teams will make sample collection, sorting and identifying quicker. To work in groups, though, requires more equipment such as buckets, spray bottles, trays and magnifying glasses.

Ice cube trays can be used for sorting macroinvertebrates instead of vials.

Students can use sticks to mark boundaries of the 1-meter square area when sampling in muddy substrates. Bring a meter stick to measure the 1-meter distances.



Questions for Further Investigation

Could the surrounding plants affect which macroinvertebrates are found at your Hydrology Site?



Are there any relationships among macroinvertebrate samples and your hydrology measurements?

How could the surrounding soils affect macroinvertebrate habitats in the water?

Are there seasonal variations to the abundance and diversity of macroinvertebrates at your site? If so, suggest reasons why.



At what temperature, dissolved oxygen, and pH ranges are greater percentages of insect taxa found?

Are there types of water bodies that have a greater macroinvertebrate diversity than others?

