Freshwater Macroinvertebrates Protocol

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 footware
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?

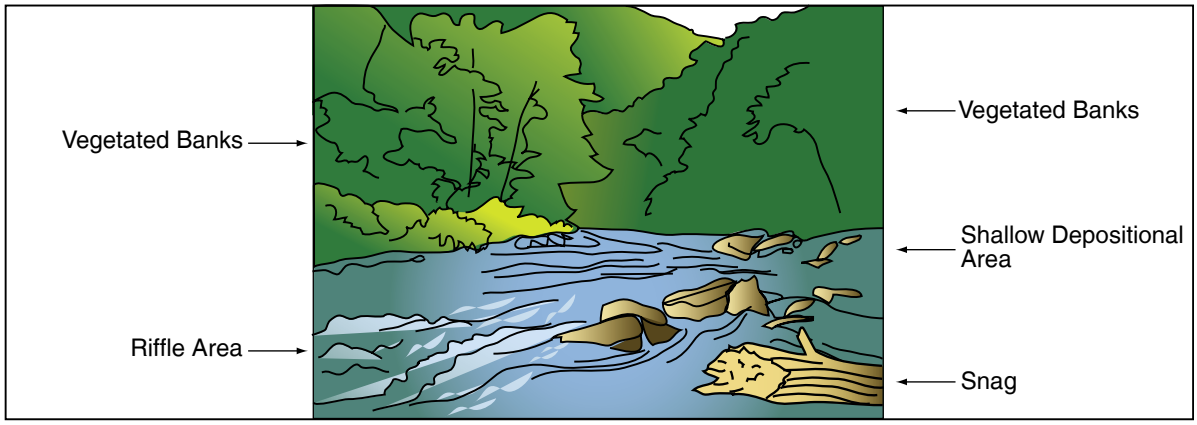
If your site has running water and stones, indicate the riffle habitats, the run habitats, the pool habitats and their substrate: boulder, cobble, or gravel. Other potential habitats in running waters or more stagnant waters and wetlands are: vegetated banks, submerged vegetation, snags, logs, roots,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

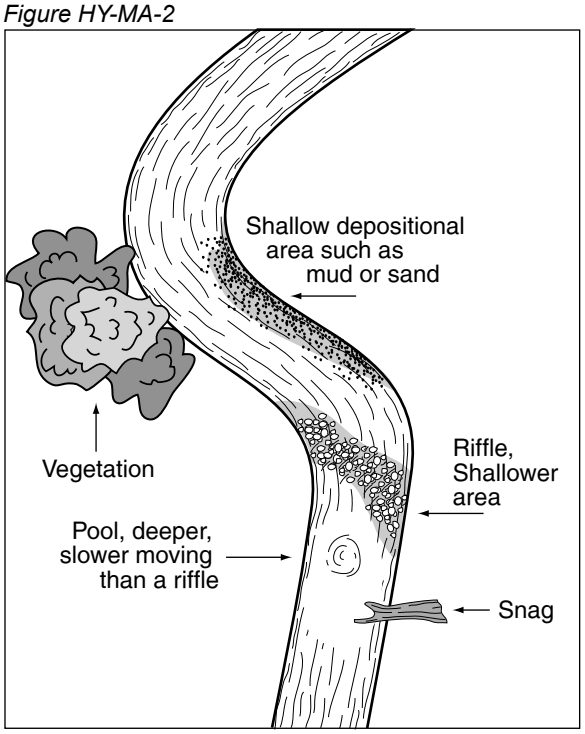
If the water is deeper than 90 cm or if many habitats are present, use the *Multi-habitat Freshwater Macroinvertebrate Protocol.* When mapping, pay special attention to identify all the aquatic habitats present and estimate the area covered by each habitat. The proportion that each accessible habitat 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 small and pass through the mesh of the net or be difficult to identify.

Figure HY-MA-2



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 classes.

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 macro-invertebrate 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?

Rocky Substrates in Running Water Macroinvertebrate Protocol

Field Guide

Task

Collect three samples of macroinvertebrates. Where you sample depends on what is available at your site.

Select sampling areas in the following order:

1. 3 different riffles
2. 2 different riffles, 1 run
3. 2 different runs, 1 riffle

If there is no combination of 3 different riffles and runs, then include a pool habitat as long as the pool contains a rocky substrate. If pools and other habitats are present, use the *Multi-habitat Freshwater Macroinvertebrate Protocol*.

What You Need

- ❑ *Freshwater Macroinvertebrate Identification Data Sheet*
- ❑ *Sorting, Identifying and Counting Freshwater Macroinvertebrate Protocol Lab Guide*
- ❑ Hydrology Site Map
- ❑ Equipment and *Hydrology Data Sheets* for collection of water chemistry measurements (optional)
- ❑ Square of white fabric (at least 110 cm by 110 cm)
- ❑ Two to six 5-L white buckets
- ❑ Forceps
- ❑ Stop Watch or watch
- ❑ Latex gloves
- ❑ Kick-net
- ❑ Sieve (0.5 mm or smaller)
- ❑ 1 x 1 meter quadrat
- ❑ One to four spray bottles (1 to 2-L)

In the Field

1. Locate the areas where you will collect your three samples on your map and in the water.
2. If collecting water chemistry measurements, do before collecting macroinvertebrates. Be careful not to disturb the areas where you will be collecting macroinvertebrates.
3. Fill a bucket with water from the site.
4. While holding the sieve over a second bucket, pour water through the sieve. Use the sieved water to fill (and refill as needed) the plastic squirt or spray bottles. Keep sieved water in the shade.
5. Rinse sieve downstream of the sampling sites.
6. Begin sampling in the area farthest downstream. Work in a team of 3 or 4. Place the 1 x 1 meter quadrat on the bottom of the stream so that two sides are perpendicular to the water flow.

7. You and a partner hold the Kick-net vertically in the water column, perpendicular to the water flow. Press the Kick-net firmly against the bottom of the streambed lined up with the quadrat and one meter downstream of the quadrat. Water must not flow above or under the net.
8. Start working in the part of the quadrat farthest away from the net. Two other students overturn and scrape the undersides of rocks and wood found in the quadrat. The rocks and wood may be placed outside the quadrat until the sample is collected. Place large crustaceans and mollusks directly in the bucket. If large organisms escape outside the quadrat, mentally note their identity and numbers to record on the *Freshwater Macroinvertebrate Identification Data Sheet* later.
9. After scrapping rocks and wood, use your feet, hands or a stick to disturb the stream bottom within the quadrat for exactly 3 minutes. One student watches the time while one or more students kick.
10. Lift the Kick-net from the water by moving the bottom of the frame forward in a scooping motion so that nothing escapes from the net.
11. Return to shore with net.
12. Place the net over the square of white fabric.
13. Carefully remove large organisms and large debris with your hands or forceps and put them in a tray half filled with the sieved water from the site.
14. Two students lift the net while others squirt water on the net to concentrate all organisms and small debris in one corner of the net.
15. Place the corner of the net with the sample into a bucket. Tip the net and squirt water to move all of the contents into the bucket.
16. Rinse the square of white fabric into the bucket to make sure that you have all the macroinvertebrates in the sample.
17. Place the bucket in the shade until you are ready to sort, identify, and count organisms.
18. Repeat steps 6 -17 for the other two samples.
19. Use the *Sorting, Identifying and Counting Freshwater Macroinvertebrate Protocol Lab Guide* to sort, identify and count the macroinvertebrates you collected.

Multi-habitat Freshwater Macroinvertebrate Protocol

Field Guide

Task

Collect macroinvertebrate samples from one or more of following habitat types: vegetated banks, submersed vegetation, snags, logs, roots, mud, sand, and gravel. The number of samples for each habitat type is proportional to the area that habitat type covers at your hydrology site. Collect a total of 20 samples.

What You Need

- ❑ *Freshwater Macroinvertebrate Identification Data Sheet*
- ❑ Hydrology Site Map
- ❑ Equipment and *Hydrology Data Sheets* for collection of water chemistry measurements (optional)
- ❑ One to four spray bottles (1 to 2-L)
- ❑ Two to six 5-L white buckets
- ❑ 1 x 1 meter quadrat (for mud, sand and gravel habitats)
- ❑ Sieve (0.5 mm or smaller)
- ❑ Latex gloves
- ❑ Trowel or shovel
- ❑ D-net
- ❑ Calculator (optional)

In the Field

1. Locate the areas where you will collect your samples on your map and in the water.
2. Estimate the proportion of each accessible habitat type within your hydrology site.
3. Use the *Freshwater Macroinvertebrate Identification Data Sheet* to calculate the number of samples collected within each habitat type for a total of 20 samples.
4. If collecting water chemistry measurements, do before collecting macroinvertebrates. Be careful not to disturb the areas where you will be collecting macroinvertebrates.
5. Fill a bucket with water from the site
6. While holding the sieve over a second bucket, pour water through the sieve. Use the sieved water to fill (and refill as needed) the spray bottles. Keep sieved water in the shade.
7. Rinse sieve downstream of the sampling sites (or away from sites if water is not flowing).
8. Start macroinvertebrate sampling downstream and move upstream as you collect samples from different habitat types. If the water is not visibly moving, collect samples in the order that will minimize the impact of taking one sample on taking the others.

9. Use the *Field Guides* to collect samples in
   - submersed vegetation,
   - vegetated banks or around snags, logs, and roots,
   - muddy bottom, and
   - gravel and sand.
10. Record the number of samples taken in each habitat on the *Freshwater Macroinvertebrate Identification Data Sheet.* The total should be 20 samples. If the number of samples per habitat is different than what was planned, explain why in the comment section.

Freshwater Macroinvertebrate Sampling Technique

for Submersed Vegetation

Field Guide

In the Field

1. Put the D-net in the water until it almost reaches the bottom in front of the vegetation. Make sure that the net is folded out away from the opening and ready to sample.
2. Push the D-net horizontally into the vegetation bouncing the net into the sediments twice.
3. Vertically bring the D-net up through the vegetation at a constant rate until you reach the surface of the water.
4. Slowly lift the D-net out of the water. As the water flows through, make sure that no organisms escape by climbing out. This is one sample.
5. Use the sieved water in squirt bottle to concentrate all organisms and debris at the bottom of the net.
6. Grab the bottom of the net and overturn the net carefully to release all of its content into a bucket. Use the squirt bottles to make sure that all organisms and debris have been transferred to the bucket.
7. Place the bucket(s) in the shade until you are ready to sort, count and identify organisms.
8. Repeat steps 1-7 until you have collected the number of samples you need for this habitat type.

Freshwater Macroinvertebrate Sampling Technique for

Vegetated Banks or Around Snags, Logs, and Roots

Field Guide

In the Field

1. Hold the D-net in the air so that it unfolds and is ready to sample.
2. In a constant motion, submerge the net in the water, move it into the vegetated bank, or around the snag(s), log(s), or root(s) heading towards the bottom.
3. Bounce the net into the sediments twice.
4. Bring the net up through the water.
5. Slowly lift the D-net out of the water. As the water flows through, make sure that no organisms escape by climbing out. This is one sample.
6. Use the sieved water in squirt bottle to concentrate all organisms and debris at the bottom of the net.
7. Grab the bottom of the net and overturn the net carefully to release all of its content into a bucket. Use the squirt bottles to make sure that all organisms and debris have been transferred to the bucket.
8. Place the bucket(s) in the shade until you are ready to sort, count and identify organisms.
9. Repeat steps 1-8 until you have collected the number of samples you need for this habitat type.

Freshwater Macroinvertebrate Sampling Technique

for Muddy Bottom

Field Guide

In the Field

1. Use a quadrat or estimate a 1 x 1 m square.
2. Place the mouth of the D-net inside one side of the quadrat (downstream if moving water) and lower it 4 cm into the sediments.
3. Move the net over the 1 x 1 m square and then slowly lift the D-net partly out of the water.
4. Move the bottom of the net back and forth in the water to wash out some of the sediments.
5. Lift the net out of the water and as the water flows through, make sure no organisms escape by climbing out. One student may have to hold the net itself underneath since it may be quite heavy. This is one sample.
6. Use the sieved water in squirt bottle to concentrate all organisms and debris at the bottom of the net.
7. Grab the bottom of the net and overturn the net carefully to release all of its content into a bucket. Use the squirt bottles to make sure that all organisms and debris have been transferred to the bucket.
8. Place the bucket(s) in the shade until you are ready to sort, count and identify organisms.
9. Repeat steps 1-8 until you have collected the number of samples you need for this habitat type.

Freshwater Macroinvertebrate Sampling Technique

for Gravel and Sand

Field Guide

In the Field

1. Lay the quadrat on the sand or gravel and place the D-net downstream (if moving water) inside and along one side of the quadrat.

2. One student holds the net while another uses a trowel or shovel to lift the top 4 cm of the substrate and place it into the net. Move the net next to where the student is digging until the whole quadrat is sampled.

3. Slowly lift the D-net partly out of the water. Move the bottom of the net back and forth in the water to wash out the finer sediments.

4. Lift the net out of the water and as the water flows through, make sure no organisms escape by climbing out. One student should hold the net itself underneath to prevent the net from ripping since the sample may be heavy. This is one sample.

5. Use the sieved water in squirt bottle to concentrate all organisms and debris at the bottom of the net.

6. Grab the bottom of the net and overturn the net carefully to release all of its content into a bucket. Use the squirt bottles to make sure that all organisms and debris have been transferred to the bucket.

7. Place the bucket(s) in the shade until you are ready to sort, count and identify organisms.

8. Repeat steps 1-7 until you have collected the number of samples you need for this habitat type.

Sorting, Identifying and Counting Freshwater Macroinvertebrate Protocol

Lab Guide

Task

Sort macroinvertebrates into taxonomic groups.

Count or estimate the number of individuals in each taxon.

Preserve three voucher specimens of macroinvertebrates for each taxon (optional).

What You Need

- ❑ Several basting syringes (20 ml with end approximately 5 mm diameter)
- ❑ Large plastic forceps
- ❑ Small forceps
- ❑ Several magnifying glasses or loupes or boxes
- ❑ Several eyedroppers (3 ml with end approximately 2 mm diameter)
- ❑ Many clear plastic jars (0.5 to 3 L) labeled (as you go) with the name of a taxon
- ❑ One to four spray bottles (1 to 2-L)
- ❑ At least 2 white trays
- ❑ Two sieves (0.5 mm (or smaller), and one between 2 and 5 mm) (optional)
- ❑ Two – six buckets
- ❑ Many small plastic vials
- ❑ Small specimen bottles with labels filled with 70% ethanol with lids that are sealing or covered with paraffin
- ❑ Permanent markers
- ❑ Pencils
- ❑ Latex gloves
- ❑ Macroinvertebrates identification keys
- ❑ *Freshwater Macroinvertebrate Identification Data Sheet*

In the Lab

1. Fill out the top portion of the *Freshwater Macroinvertebrates Identification Data Sheet.*
2. Put on gloves.
3. Use a basting syringe or forceps to pick out large organisms from your buckets. Put these organisms in a tray.

   Note: You have the option to combine all samples together to sort, identify or keep the samples separated by habitat type.
4. If you have rocks in your sample, take them out of the bucket and use the spray bottle to rinse the rocks over the sample bucket before discarding the rocks.

5. If the water in your buckets is clear, free of debris, and rather a small amount, pour sample on tray to sort. Go to step 13.

6. If you have a lot of water, sediments or debris, pour the samples through the sieves. Place the sieve with the finer mesh size below the other sieve. Hold the sieves inside the top of a clean bucket.

7. Gently and slowly pour the water from the bucket containing the organisms into the sieves. If a sieve is clogged, gently tap the bottom of the clogged sieve to allow water to escape.

8. Every so often, transfer and rinse the contents of the sieves into trays using a squirt bottle. Other students can start sorting organisms in the trays.

9. Rinse twigs over the sieves.

10. Put twigs in a tray with water. Examine twigs for macroinvertebrates.

11. Rinse the bucket several times with your spray bottles and pour the water down the sieves.

12. Invert each sieve over a tray and squirt water on the back of the sieve to remove contents.

13. Work in teams. Use identification keys to identify individuals to the most detailed level possible (Phylum, Class, or Order required and Family, Genus, or Species if possible). Keep in mind that appendages like legs and antennae may be missing because they may have broken in the net or the sieves.

14. Use the vials to sort organisms into different taxa. If you do not know the taxon of an organism, place in a separate vial to examine later under a dissecting scope or with the help of an expert.

15. If organisms are large and clinging to debris, use forceps to gently pull them free. If they are floating or swimming, use a basting syringe or an eyedropper to catch them.

16. If different teams are sorting and identifying organisms, combine the vials of the same taxon. Do this for all the taxa.

17. To count the number of individuals in each taxon, isolate organisms a few at a time using forceps, an eye dropper, or a basting syringe and transfer them into another jar as you go. Keep a tally on paper.

18. Count macroinvertebrates in each taxon up to 100 individuals. If you have more than 100 individuals in a taxon, you can do three things:

    1. report >100,

    2. continue counting,

    3. use the *Freshwater Macroinvertebrate Sub-sampling Field Guide* to estimate the total number of organisms of this taxon.

    Note: If possible, count all individuals since it is more accurate than sub-sampling, but sub-sampling is more informative than reporting >100.

19. As you count, look closely at the individuals to make sure that there are no mistakes in identification. If you find an individual that belongs to a different taxon, notify the student who is doing the count for that taxon and transfer the organism.

20. Report the total number of organisms found for each taxon on the *Macroinvertebrates Identification Data Sheet*. Include organisms that were counted at the site but could not be collected because they escaped.

21. Optional: For each taxon you identify, preserve three individuals as voucher specimens for future reference. Place the three organisms in a specimen bottle containing 70% ethanol solution.

22. Label the bottle with:

Name of Sample Site
Date
Phylum, Class, Order (family, genus and species, if known)
70% ethanol

23. Return remaining live macroinvertebrates to the water.

Freshwater Macroinvertebrate Sub-sampling

Field Guide

Task

To collect 20% of original sample for each taxon

What You Need

- ❑ Sub-sampling grid with level
- ❑ Pieces of paper with grid labels
- ❑ Hat or bag
- ❑ 500-ml beaker

In the Field

1. Record grid volume on *Data Sheet.*
2. Record total number of squares on grid on *Data Sheet.*
3. Multiply total number of squares by 0.2 to calculate the number of grids you need to sample.
4. Write grid numbers on pieces of paper and put in bag or hat. Pick enough for the 20%. Macroinvertebrates will be taken from those squares on the grid.
5. Place all the organisms from the taxon to sub-sample in beaker. The volume of water plus the organisms must equal the grid.
6. Adjust the sub-sampling grid so that it is perfectly leveled.
7. Mix the contents of the jar and pour onto the grid, spreading the sample evenly over the grid. If the grid is leveled and the volume is right, the organisms will be contained in their own 'pools' made from the raised lines on the grid.
8. If the grid is very stable and the number of organisms per square is small, the organisms in the randomly selected squares can be counted on the grid. Otherwise, use a basting syringe to remove the organisms from the randomly selected squares and transfer then to a jar and then count them.
9. Calculate the total number of individuals for this taxon. If you counted 20% of your squares, multiply the number of organisms you counted by 5 to estimate the total number of individuals for this taxon.
10. Report the percent of squares sub-sampled and the estimated total number of individuals that you sampled for this taxon on the *Macroinvertebrate Identification Data Sheet.*

Frequently Asked Questions

1. Do I have to use a 0.5 mm-mesh net?

Yes. If too large of a mesh is used, small macroinvertebrates will be lost from your sample. Everyone needs to use the same mesh size for the nets so that data are comparable among sites.

2. Why do we need to sample from as many habitats as possible?

To get as many different organisms as are present. The variability in organisms found can be greater between habitats than between years. By sampling many habitats, we get a better idea of biodiversity and health of the ecosystem.

3. What if we want to identify macro-invertebrates at the family and genus species levels?

You are encouraged to do so using local books, keys, field guides, and experts to help you. You can write the information on a *Macroinvertebrate Identification Data Sheet*, and additional sheets if you need more space. You can report these data on the data entry page on the GLOBE Web site.

4. Why aren't we counting protists and other groups such as Gastrotrichs?

These organisms also play a very important role in aquatic ecosystems. However, most of the species are very small. Only a few are slightly above 0.5 mm, they are not considered macroinvertebrates.

5. Why are there different levels of identification for different groups of animals?

Classification is very helpful for us to organize objects, thoughts and the world. However, not all organisms fit neatly into groups. You are identifying many organisms to the Order level. For some groups, that level of identification would require extensive knowledge of obscure external or internal features, or using high power microscopy to look at the shape of features such as tiny hairs. The taxonomic level of identification that we suggest is more easily accessible with low magnifying powers. If you enjoy taxonomy and want to identify organisms to the family, genus, or species levels, please do so and report your data on the web.

6. What should we do if the quadrat sinks in the mud and cannot be seen?

You can attach floaters to the quadrat or just estimate the 1 x 1 m area.

Suggested Readings and Web sites:

A Guide to Common Freshwater Invertebrates of North America. J. Reese Voshell, Jr. The McDonald & Woodward Publishing Company. Blacksburg, Virginia. 2002

An Introduction to the Aquatic Insects of North America. R. M. Merritt and K. W. Cummins (eds). Kendall/Hunt Publishing Company. Dubuque, Iowa 1996.

Aquatic Entomology: The Fishermen's and Ecologists' Illustrated Guide to Insects and Their Relatives. W. P. McCafferty. Jones and Bartlett Publishers. Sudbury, Massachusetts. 1998.

Fresh-Water Invertebrates of the United States: Protozoa to Mollusca. R. W. Pennak. John Wiley & Sons, Inc. New York. 1989

Save Our Stream (SOS). http://www.sosva.com/download_the_field_sheets_for_th.htm

ECOSTRIMED protocol: Bioassessment to define river's ecological status. http://geographyfieldwork.com/ECOSTRIMED%20Protocol%20Procedure.htm

Two good macroinvertebrate keys for North America can be obtained from the University of Wisconsin's Extension/Wisconsin Department of Natural Resources, and may be reproduce for education non-profit purposes. One is the "Key to Macroinvertebrate Life in the River" and the other is "Key to Life in the Pond".

http://clean-water.uwex.edu/wav/otherwav/

http://clean-water.uwex.edu/wav/otherwav/riverkey.pdf

http://clean-water.uwex.edu/wav/otherwav/pondkey.pdf (contains a few vertebrates)

Freshwater Macroinvertebrates Protocol – Looking at the Data

Are the data reasonable?

When you look at the types of taxa you recorded, make sure that these taxa are found in your region. For example if you live in higher latitudes where water temperatures are relatively cold and recorded a taxon that only lives in warm waters, you might question whether you identified that taxon correctly. Check your voucher specimen to verify your identification.

Check to see if the macroinvertebrate taxa you collected are found in the types of substrate you sampled. If you sampled in a lake with a muddy bottom and found mainly stoneflies that typically live on rocky substrates, you would want to check your voucher specimen to make sure.

Also, if you find a large abundance of a rare taxon, again check your voucher specimen. If you are positive that you identified the taxon correctly, you might want to contact a local expert from a government agency or university because that may be very valuable information.

What do people look for in these data?

Scientists look at macroinvertebrate data for the distinct types of organisms present and the variety of organisms (biodiversity). There are many different types of macroinvertebrates! Certain types of macroinvertebrates are more commonly found in one type of habitat than another. For instance *Oligochaeta* (segmented worms) may be much more abundant in a muddy pond environment than in a gravelly stream whereas the abundance of *Plecoptera* (stoneflies) may be much less.

Scientists can compare water chemistry data and the macroinvertebrate data to see what types of patterns can be found and relate these to habitat conditions such as the water properties measured in GLOBE. Scientists compare different sites to see patterns among these sites, and look at the same site to see what changes happen through the seasons and over the years.

Biodiversity Estimates

To estimate biodiversity, scientists look at both the number of organisms and the number of different taxa. The number of different taxa is called *richness*. The number of organisms is called the abundance. Scientists also look at the relative abundances of the taxa; this is called *evenness*. High richness and high evenness are generally considered by scientists to indicate high biodiversity. The example below illustrates why both the number of different taxa and the number of individuals for each taxon are needed to estimate biodiversity. Students collected data from three streams:

Stream 1	**Stream 2**	**Stream 3**
50 worms	25 worms	45 worms
50 leeches	25 leeches	50 leeches
100 total	25 dragonfly larvae	2 dragonfly larvae
	15 caddisfly larvae	2 caddisfly larvae
	10 beetle larvae	1 beetle larvae
	100 total	100 total

All three streams have a total of 100 organisms, but their diversities are different. The biodiversities are greater in Stream 2 and Stream 3 because there are five kinds of organisms (taxa), while there are only two taxa in Stream 1. However, Stream 3 has many worms and leeches and only a few dragonfly, caddisfly and beetle larvae. Stream 2 has a more even distribution of amounts found for each taxon. In this example, Stream 2 has the highest biodiversity since it has a higher evenness than Stream 3.

The only way we can know the exact biodiversity of a stream, lake or pond is to count all the organisms. Generally, this is impossible! So scientists take samples, identify and count the different taxa within the sample, as you have done, and use mathematical equations to calculate the biodiversity in the sample. The biodiversity value of the sample is used as an estimate of the biodiversity in the water body overall.

There are many different mathematical formulas used to estimate biodiversity from a sample of organisms. The Shannon-Weiner Index (shown below) is a commonly used formula. It combines evenness and richness and reaches its maximum value when all species are evenly distributed. Values of the Shannon-Weiner Index, as well as other biodiversity indices, can be compared among different water bodies to evaluate which has the greatest diversity of organisms. In general, greater diversity indicates a more robust ecosystem when you are comparing similar sites, for example, a comparison of two small streams in the same watershed.

Shannon-Weiner Biodiversity Index:

$$BI = -\sum_{i=1}^{k} x_i \log_2 x_i$$

where:

k = number of taxa you found, and

x_i = the percentage of taxa i

$\log_2$ = logarithm in base 2

So, let's compare the biodiversities of the three streams.

Stream 1

Taxa	Amount	x = Percentage (Amount/total)	$\log_2 x$	$x\log_2 x$
Worms	50	50/100 = 0.5	-1	-0.5
Leeches	50	50/100 = 0.5	-1	-0.5

$$DI = -\sum_{i=1}^{k} x_i \log_2 x_i$$

$$= -(-0.5 + -0.5) = 1$$

Stream 2

Taxa	Amount	x = Percentage (Amount/total)	$\log_2$ **x**	$x\log_2 x$
Worms	25	0.25	-2	-0.5
Leeches	25	0.25	-2	-0.5
Dragonfly larvae	25	0.25	-2	-0.5
Caddisfly larvae	15	0.15	-2.74	-0.41
Beetle larvae	10	0.1	-3.32	-0.33

$$BI = -\sum_{i=1}^{k} x_i \log_2 x_i$$

$$= -(-0.5 + -0.5 + -0.5 + -0.41 + -0.33) = 2.24$$

Stream 3

Taxa	Amount	x = Percentage (Amount/total)	$\log_2 x$	$x\log_2 x$
Worms	45	0.45	-1.15	-0.52
Leeches	50	0.5	-1.00	-0.50
Dragonfly larvae	2	0.02	-5.64	-0.11
Caddisfly larvae	2	0.02	-5.64	-0.11
Beetle larvae	1	0.01	-6.64	-0.07

$$BI = -\sum_{i=1}^{k} x_i \log_2 x_i$$

$$= -(-0.52 + -0.53 + -0.11 + -0.11 + -0.07) = 1.31$$

So, the biodiversity index for Stream 2 is the highest, 2.24, followed by Stream 3 with a value of 1.31 and then Stream 1 with a value of 1, which confirms what we initially thought.

Using macroinvertebrates to indicate how stressed the water body is:

Scientists studying ecological systems often are interested in what happens to organisms when exposed to different types of stresses. Stresses can be caused by natural events or human activities. An example of a natural stress in an aquatic system is a major storm that causes extensive flooding. Many macroinvertebrates may die or be washed away. The flooding may cause mud to be deposited in areas that were mainly gravel. This will cause a change in the types of macroinvertebrates that can live there.

Macroinvertebrate metrics are often used to examine the types of stresses affecting water bodies. Metrics are defined as easily calculable characteristics of the macroinvertebrate data that respond to stress in some predictable way. The metrics are designed to evaluate responses in the macroinvertebrate community to things affecting their habitats. By combining data on abundance of different taxa with characteristics such as ecological roles of these taxa in the ecosystem and tolerance to stress, one can learn much about the aquatic ecosystem.

To describe the water body and see the extent to which the macroinvertebrates may be living in a habitat that has undergone some sort of stress, scientists analyze macroinvertebrate data to obtain metrics in different categories. These include:

- richness measures,
- composition measures,
- stress tolerance or intolerance measures,
- feeding measures,
- habit measures, and
- life-cycle measures.

Below are explanations of some these used metrics. There are many more that can be found in books and journals.

Richness Measures

A commonly used richness measure for rivers or streams is the number of *Ephemeroptera* (mayflies), *Trichoptera* (caddisflies) or *Plecoptera* (stoneflies) found at a site. For wetlands, scientists often look at the number of *Hemiptera* (water bugs), *Coleoptera* (water beetles), and *Odonata* (damselflies and dragonflies). The abundance of these taxa is expected to decrease with increased stress.

Composition Measures

In rivers and streams, the percentage of macroinvertebrates present in the samples that are *Ephemeroptera +Trichoptera +Plecoptera* (%EPT) is used. In wetlands, scientists look at the percentage of *Ephemeroptera*, *Trichoptera*, *Sphaeriidae* (fingernail clams), and *Odonata* (%ETSD). Lower percentages may indicate a stressed environment. It would be interesting to see what happens to these percentages during and after a dry year.

Scientists also measure the % *Diptera* (mosquitoes, midges, flies) or % *Chironomidae* (midges). Studies have shown that both tend to increase with increased stress, for example, increased deposits of mud or decreased dissolved oxygen content.

The % Dominant Taxon (%DT) is the number of organisms in the most abundant taxon relative to the total number of organisms in the sample. Higher values may indicate a more stressed environment where only one taxon can flourish.

Tolerance/intolerance Measures

One can also compare the percentage of taxa that are considered tolerant to perturbation with the percentage of taxa that are intolerant. A high ratio of %tolerant/%intolerant indicates a more stressed environment.

Feeding Measures

We can learn a lot about the ecosystem by looking at how the organisms eat. In fast moving waters, the percentages of collectors, filterers, omnivores and scavengers often increase with stress such as a drought that results in slower-moving waters and decreased dissolved oxygen levels, but can represent quite a diverse community in wetlands. A shift from herbivores and filter feeders to scavengers such as worms may indicate that sedimentation is occurring.

Habit Measures

A habit measure often used is the percentage of clingers. These taxa have retreats or attachments that allow them to stay in place in flowing water. Their numbers decrease with stress.

Life-cycle Measures

Life-cycle measures refer to organisms that develop rapidly and live a short time or ones that are long-lived. Many short-lived taxa increase when stress increases while long-lived taxa decrease. Some short-lived taxa are highly seasonal.

As you can see, your data allow you and scientists to explore and learn a great deal about specific aquatic environments!

An Example of Student Research Investigation

Two schools in the same watershed decided to do a collaborative project. They wanted to learn about the types of macroinvertebrates in nearby streams and how the types and abundance of macroinvertebrates varied between two sites in the same watershed. They predicted that the macroinvertebrate data would be similar between the sites. The students were also curious to see if differences would be found between the autumn and spring samples at the same site. They predicted that the types of macroinvertebrate taxa would be different between the autumn and spring samples, but that the biodiversity values would be similar.

Sites were chosen within the watershed that could be safely reached by students at each school. The students coordinated their data collection so that both schools collected macroinvertebrates on the same day and at about the same time of day. Samples were collected in both the autumn and spring, and students shared their data. Each school analyzed the data separately and compared their results. Here is what students did at School 1.

At School 1, students collected 270 organisms from 13 different taxa in the autumn and 225 organisms from 10 different taxa in the spring (Table 1). In the autumn, the sample contained many *Tricoptera*, *Chironomidae* and *Oligochaeta*, and many other taxa that had only 1 or 2 individuals in each taxon. The spring sample, however, contained a large quantity of *Chironomidae* and many *Plecoptera* (stoneflies), *Ephemeroptera* and *Tricoptera*. The autumn sample contained more organisms overall.

Table HY-MA-1: Macroinvertebrate Abundance Data, Total Number of Taxa and Total Number of Organisms Students Collected in the Autumn and Spring for the Two Schools.

	School 1		School 2	
	Autumn	**Spring**	**Autumn**	**Spring**
Plecoptera (stoneflies)	4	37		
Odonata (dragonflies, damselflies)	0	1		
Ephemeroptera (mayflies)	2	36		
Psephenidae (water pennies)	2	0		
Tricoptera (caddis flies)	51	31		
Chronomidae (midges)	126	96	29	100
Oligochaeta (worms)	80	20	80	74
Turbellaria (planarian)	1	1		
Hirudinea (leeches)	1	0		
Gastropoda (snails)	1	1	200	356
Pelecypoda (clams)	1	1		
Nematomorpha (horsehair worms)	1	0		
Amphipod (scuds)	0	1		
Total # organisms	270	225	309	530
# taxa	13	10	3	3

When the students at School 1 looked at the data from School 2, they quickly noticed large differences with their data. Although the total number of organisms collected in the autumn and spring are much larger at School 2, the sample had only 3 taxa. Furthermore, the same taxa, Oligochaeta, Chironomidae and Gastropoda, are found in both the autumn and spring samples. So, they decided to compare the biodiversities.

Using the Shannon-Weiner biodiversity equation, the students calculated an estimate of biodiversity of 1.83 in the autumn and a biodiversity of 2.25 in the spring for School 1. See Table HY-MA-2. They put the data in a spreadsheet and performed the calculations. The values of –1.83 and –2.25 are the totals within those columns. Multiplying these values by –1 gives the biodiversity values.

They were very surprised to have collected a few more individuals from more taxa in the autumn compared to what they sampled in the spring, and yet obtain a higher estimate of biodiversity in the spring. They rechecked their calculations to make sure that they made no mistakes.

Table HY-MA-2: Calculations of Biodiversity for the Data Collected at School 1

School 1	Autumn				Spring			
Taxa	Amount	Percentage	$Log_2(\%)$	$\%log_2(\%)$	Amount	Percentage	$Log_2(\%)$	$\%log_2(\%)$
Plecoptera (stoneflies)	4	0.01	-6.08	-0.09	37	0.16	-2.60	-0.42
Odonata (dragonflies, damselflies)					1	0.004	-7.81	-0.03
Ephemeroptera (mayflies)	2	0.01	-7.08	-0.05	36	0.16	-2.64	-0.42
Psephenidae (water pennies)	2	0.01	-7.08	-0.05				
Tricoptera (caddis flies)	51	0.19	-2.40	-0.45	31	0.14	-2.86	-0.39
Chronomidae (midges)	126	0.47	-1.10	-0.51	96	0.43	-1.23	-0.52
Oligochaeta (worms)	80	0.30	-1.75	-0.52	20	0.09	-3.49	-0.31
Turbellaria (planarian)	1	0.004	-8.08	-0.03	1	0.004	-7.81	-0.03
Hirudinea (leeches)	1	0.004	-8.08	-0.03				
Gastropoda (snails)	1	0.004	-8.08	-0.03	1	0.004	-7.81	-0.03
Bivalve (clams)	1	0.004	-8.08	-0.03	1	0.004	-7.81	-0.03
Nematomorpha (horsehair worms)	1	0.004	-8.08	-0.03				
Amphipod (scuds)					1	0.004	-7.81	-0.03
Total				-1.83				-2.25

The students looked at the data from School 2 to see if there is a similar pattern. They were surprised to see more individuals collected in spring than autumn (the opposite trend of what they found), and yet only 3 different taxa were found at both times (Table HY-MA-3). The biodiversity values for School 2 do not change significantly between spring and autumn (1.23 and 1.24) and the biodiversity value of 1.24 is much lower than either 1.83 or 2.01, the biodiversities calculated for School 1. These results made them curious to find out why there were such large differences between two sites in the same watershed.

Table HY-MA-3: Calculations of Biodiversity for the Data Collected at School 2

School 2	Autumn				Spring			
Taxa	Amount	Percentage	$Log_2(\%)$	$\%log_2(\%)$	Amount	Percentage	$Log_2(\%)$	$\%log_2(\%)$
Chronomidae (midges)	29	0.09	-3.41	-0.32	100	0.19	-2.41	-0.45
Oligochaeta (worms)	80	0.26	-1.95	-0.50	74	0.14	-2.84	-0.40
Bivalve (clams)	200	0.65	-0.63	-0.41	356	0.67	-0.57	-0.39
Total				-1.23				-1.24

To see what factors might account for the differences between the sites and seasons, the students looked at the water chemistry measurements taken at the times of macroinvertebrate sampling. Table HY-MA-4 shows the pH, temperature and dissolved oxygen data.

Table HY-MA-4: pH, DO, and Temperature Data Collected When the Macroinvertebrate Samples Were Taken

	School 1		School 2	
	Autumn	Spring	Autumn	Spring
pH	6.8	7.1	8.7	9.6
Temperature	11° C	14° C	10° C	16° C
Dissolved Oxygen	8.5 ppm	8 ppm	7 ppm	5.7 ppm

The pH values at School 2 were higher than at School 1. The pH values at School 1 were near the neutral value of 7 whereas the values at School 2 were basic. The autumn and spring temperature values were similar at both sites. However, one student observed that the temperature difference between the autumn and spring was greater at school 2. The temperature range at school 1 was 3° C and at School 2, it was 6° C. Another student wondered if the temperature difference was because the samples were collected on different days or at different times of the day, but then he remembered that the two schools were careful to sample at the same time. For the dissolved oxygen, the students noticed that the DO content was lower at school 2 for both autumn and spring.

To help with the interpretation of the chemical data, they looked at the ranges of pH, temperature, and dissolved oxygen necessary for selected macroinvertebrates to live (see Tables HY-MA-5, 6 and 7). They also decided to look at two metrics, %Ephemeroptera, Plecoptera and Tricoptera (EPT) and % dominant taxa (DT).

Table HY-MA-5: Required pH ranges for Selected Macroinvertebrates*

TAXA	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Mayfly				XXXXX										
Stonefly				XXXXX										
Caddisfly				XXXXX										
Snails				XXXXXXXXXX										
Clams				XXXXXXXXXX										
Mussels				XXXXXXXXXX										

* pH ranges 1-6 and 10-14 are unsuitable for most macroinvertebrates

Table HY-MA-6: Required Temperature Ranges for Selected Macroinvertebrates

TAXA	Cold Range < 12.8˚ C	Middle Range 12.8 - 20˚C	Warm Range > 20˚ C
Caddisfly	x	x	x
Stonefly	x	x	
Mayfly	x	x	
Water pennies	x		
Water beetles		x	
Water striders		x	
Dragonfly		x	x

Table HY-MA-7: Required Dissolved Oxygen Ranges for Selected Macroinvertebrates

TAXA	High Range 8 - 10 ppm	Medium Range 4 - 8 ppm	Low Range 0 - 4 ppm
Stonefly	X		
Water penny	X		
Caddisfly	X	X	
Some mayflies	X	X	
Dragonfly		X	
True bugs		X	
Damselfly		X	
Mosquito			X
Midge			X
Tubifex worm			X
Pouch/lung snails			X
Rat-tailed maggot			X

They compared the pH values for the macroinvertebrates shown in Table HY-MA-4 with the data collected by the two schools. The pH is higher than the pH required for mayflies, caddisflies and stoneflies in the stream that School 2 sampled. In addition they noticed that the pH for the stream that School 1 sampled is on the low end needed for clams to live and they wondered if that could be why there are few clams there. In contrast, the other stream has lots of clams and a high pH.

When comparing the temperature data with the required ranges for certain macroinvertebrates, the students couldn't find many reasons to help explain why there is so much difference in the macroinvertebrate assemblages. Perhaps the cool temperatures in streams explained why there was only 1 dragonfly found by the students at School 1 in the spring.

The students then examined the dissolved oxygen content. They noticed that the lower DO values found at School 2 could explain why no stoneflies and water pennies were found. These two taxa require DO concentrations of 8 ppm or greater and the DO values in the stream were 7 ppm and 5.7 ppm.

Lastly, the students looked at two metrics, % dominant taxon (%DT) and % Ephemeroptera + Plecoptera + Tricoptera (%EPT). Table 7 shows the results of their calculations. The stream that School 2 sampled had 0% EPT and higher %DT of 65% and 67%. For the sample collected during spring, School 1 had a value of 47% DT and School 2 had a value of 43%.

Table HY-MA-8: Calculations for % DT and % EPT.

	School 1		School 2	
	Autumn	**Spring**	**Autumn**	**Spring**
Dominant taxon	Chironomidae	Chironomidae	Gastropoda	Gastropoda
# dominant taxon	126	96	200	356
Total Number	270	225	309	530
% DT	(126/270) x 100 = 47%	(96/225) x 100 = 43%	(200/309) x 100 = 65%	(356/530) x 100 = 67%
E + P + T	56	74	0	0
% EPT	(56/270) x 100 = 21%	(74/225) x 100 = 32%	0	0

Based on what a local expert told them, low values of %EPT and high values of %DT indicate habitats undergoing some sort of stress, so they wondered if the stream that School 2 sampled is being stressed. This is also supported by the low diversity found there. From the water chemistry data, they thought that the high pH, in particular, was the main reason that only a few were found there. They were curious why the pH values were so basic and if the large difference in pH values between the streams was due to natural causes or human activities. They were eager to question the students at School 2.

They decided to examine the water chemistry data collected throughout the year to examine any patterns or trends. As well, they were curious to see what, if any, pattern would emerge with samples collected next autumn and spring.