Protocol 12: Macroinvertebrate collecting for abundance and diversity

Materials:

- Field book or field sheets and pencil or waterproof pen
- Clipboard
- D-net (500 um, handle marked with increments to measure depth)
- Collecting pan or tub (white dishwashing tubs work well)—with lid, if returning to classroom with samples
- Ice cube trays and yogurt containers (for sorting)
- Forceps
- Plastic spoons
- Sieve bucket, with 500 opening mesh
- Squirt bottles with 95% ethanol[1]
- Sample containers, sample container labels
- Sample submission jars: one for upstream, one for downstream—labeled!
- First aid kit (put this in the general field day material)
- Rubber boots, old sneakers or waders (chest-high or hip boots)
- Rinse cup or bucket
- Gloves
- 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)

Method:

Note: This protocol has three parts and is for the collecting, sorting and preserving macroinvertebrates in the field.

GENERALLY

You will sample along one transect below and one transect above the culvert. Using the D-net you will sample from one bank to the other sampling three to five quadrats of about 0.5 m^2 each.

Look at the stream above and below your culvert (about 10m above and 10m below the culvert). How would you describe what the stream looks like? You will be collecting macroinvertebrates in areas that best represent what you have just described. Imagine throwing a line from one side of the stream to the other—once below the culvert and once above the culvert. These lines will be the guides you use to sample along.

If your stream is less than a meter wide then plan on collecting samples at about five meters, 10 meters and 20 meters upstream and downstream from the culvert.

Prior to sampling

Set up the area on the side of the stream where you will be sorting your macroinvertebrates. If it is a hot day plan to do the sorting in the shade. From downstream

[1] For sample preservation. Adult use only.

of your sampling sites fill your squirt bottles with stream water. You will use this water to wash down your nets when you are sampling.
Note: we are only collecting macroinvertebrates. If you collect any fish or amphibians feel free to share them and take pictures of them, but they must be returned to the stream.

Taking your sample

1. Approach your sample site from downstream.
2. Starting on one bank, three of you should wade into the stream: one carries a D-net, one carries a collecting tub and squirt bottle, and one—the "shuffler"—needs both hands free.
3. 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.
4. Jab the net upstream for 0.5 m (jabbing means you are firmly bouncing the net along the bottom to dislodge organisms, rocks, branches).
5. Slowly lift the D-net out of the water. As the water flows through, make sure that no organisms escape by climbing out.
6. Use the squirt bottle to rinse down the sides of the net 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 bottle to rinse the net to make sure that all organisms and debris have been transferred to the collecting tub. If there are many rocks in the sample carefully rinse them, and inspect them for small or tight clinging organisms. Remove the organisms from the rocks, place the organisms in the bucket, and put the rocks back into the stream. Try to put as little material as possible into the sample bucket (remove rocks, sticks, and leaves).
8. Put the net back into the water (handle upright, bottom of the net on the bottom of the stream) the shuffler should now stand 0.5 m upstream of the net and disturb the stream bottom by slowly shuffling his or her feet, dislodging stones. Disturb the substrate with your feet in an area the width of the net and, at most, two net widths upstream. Rub by hand any large sticks and/or stones from the disturbed area to dislodge any tightly clinging organisms. **CAUTION: When disturbing and removing substrates, be aware of the possibility of sharp objects such as broken glass.** Many invertebrates will jump off into the current and be carried down to the waiting net. If the water isn't too deep or cold, reach to the bottom and lift up and brush the rocks as well. Try not to kick up too much silt and sediment.
9. After a couple of minutes of shuffling, slowly lift the D-net out of the water. If there is a lot of mud in it, raise and lower it into the water a few times to rinse. Repeat steps 6+7 (rinse and empty net into bucket).
10. Repeat steps 3-9 along your transect two to four more times
11. When the tub gets heavy return it to shore, place it in the shade, and get another tub.
12. Repeat steps 1-11 along the transect above the culvert.
13. **Remember to keep all the above-culvert and below-culvert samples separate!**

Field Sorting

1. **Remember to keep all the above-culvert and below-culvert samples separate!**
2. If your stream is less than a meter wide then do not attempt to collect samples from one side of the stream of the other. Instead, collect samples at about five meters, 10 meters and 20 meters upstream and downstream from the culvert, then keep each one of those locations separate from the other samples (in other words you will have three separate

upstream samples and three separate downstream samples).

3. Put some stream water into your ice cube trays and yogurt containers and begin sorting the invertebrates by appearance (using the Macroinvertebrate Key). Put different types of organisms in different compartments. You can pick the organisms up gently with the forceps or syringe or use a plastic spoon to scoop them. You will sort the organisms first and then tally them when everything is sorted. It is all right to put large and small individuals that look alike in the same compartment.
4. 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.
5. Use the yogurt containers for larger organisms or for organisms that you have a lot of.

Note: 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.

Checking in

After doing a quick sorting check in with your teacher and with the other groups. Ask them what they've identified and make sure that what they're calling, for example, a "caddisfly" is what your group is calling a caddisfly.

Tallying

1. Continue picking over your sample until you are certain you have found most of the organisms (have another group check your tub to make sure). Look hard for smaller and slower-moving creatures, they can be easy to miss. If there are still stones in your bucket look them over for organisms.
2. Once your sample is completely sorted double-check the identity of the organisms and tally them using the tally sheet.

Preserving

1. Once tallied place the organisms from each ice cube tray compartment in the sample jar filled about ½ full with stream water.
2. Label the jar.
3. Labels filled in with pencil can be slipped into the jar.
4. Labels must have the following information:
   School
   Name of Sample Site
   Above Culvert or Below Culvert designation
   Sample Date
   Type of organism
   "70% alcohol" (it is about 70% alcohol when you add the 95% alcohol to your jar that already has stream water in it, and you need to let other people know that it is not just water in these jars)
5. Cap the jar and bring it to an adult. The adult will fill the rest of the jar with 95% alcohol.
6. Place the sample in a cooler for safe transport.

If you are sorting and preserving in the classroom then fill a lidded bucket with one gallon of stream water. If there are sticks, rocks and leaves in your sample tub then inspect them for small macroinvertebrates and return the rocks, sticks, leaves to the stream. Strain sample through sieve, empty the sieved sample (the macroinvertebrates in the sieve) into the lidded bucket and bring the sample back to classroom. Store in a refrigerator and sort and preserve within 24 hours.

Use Protocol 13: Macroinvertebrate identification and preserving in the classroom.