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 Macroinvertebrates Protocol Lab Guide
- Hydrosphere Investigation Site Map
- Equipment and Hydrosphere 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 scraping 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.