Macroinvertebrate Sample Collection: Rocky Substrate

ALWAYS collect a water quality sample immediately before you collect the macroinvertebrate sample. When collecting your water sample, do not disturb the area you plan to collect your macro sample in.

Equipment for Macro Collection

Equipment provided by River Watch:

- A modified D-net (18" x 8"); the net is a 500-micron mesh net.
- Two forceps to pick organisms from net
- A 600-micron sieve (#30)
- One small brush
- Two containers with alcohol – one 500 mL and one 1000 mL bottle (you will have four bottles if you are to collect a QA/QC sample).

Additional equipment provided by you:

- Two 3-5 gallon clean sample buckets. Preferably white/light colored buckets (**do not use** the River Watch water quality sample bucket)
- A squirt bottle (can be any water bottle with a squirt nozzle)
- A timing device that can time 60 seconds (a second hand on a watch, phone, stopwatch)
- Waders
- A ruler to measure substrate sizes > or < than 12", 6" and 3"
- A measuring stick, broom, pole or pipe with inch and foot marks on it to measure depths
- A tape measure (can be marked string or twine) to measure stream widths
- Rubber gloves (optional) and magnifying glasses (optional)
- (Optional) A large **white** plastic tray – or white trash bag (easy to see bugs on)

Collection Instructions

- Macro collection is best done with at least 3-4 people. Assign each person on the team one of the following roles: holding the net, kicking, timing, and recording data.
- Select four riffles in your reach to kick, choosing two fast riffles and two in slow riffles.
- Starting at the most downstream riffle, measure the kick area by laying the net down to help make a mental map of a 5.5 ft x 3 ft area on the stream bottom, or the same area covered by length of handle and the width of the net. This is your micro habitat/kick area. Fill out boxes 1 and 2 of the datasheet.
- Prepare the net on the downstream side of the kick area facing upstream, standing with the net at your feet, no more than 1 ft. away from the kick area. **Do not place the net in the water until the 60 second timer begins.**
- Prepare the timer - **Each kick is 60 seconds**. Once the timer starts, the kicker will step into the kick area at the downstream side where the net is and begin to kick moving upstream (slowly) towards the end of the kick area. Kicker will dig into the substrate with their feet and disturb the habitat by digging with their heels and toes as much as possible (this will get tiring).
- As the kicker moves upstream in the kick area, the net holder will move upstream with the kicker until they've reached the end of the kick area. During the kick, place the net on the stream bottom, but do not allow water to flow over the top of the net. You want the water to flow **THROUGH** the net.
- Do not kick the larger substrate, such as large rocks and logs. Instead, pick up these objects and brush them off in the water upstream of the net, so any bugs will be captured by the net downstream.
- After 60 seconds of kicking, slowly raise the net out of the water, carefully holding the net steady so that no bugs fall out.
- **Do not empty the net.** Repeat these steps for each of the 4 kicks at the different selected riffles, moving upstream, while filling out the microhabitat data for each kick in your datasheets.
- **Once all four kicks have been collected in the net**, you will begin to process the sample.

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Now, it's time to begin sample processing:

- Carry the net to shore.
- Fill a large bucket ½ to ⅔ full of stream water.
- Gather the sample material into one corner of the net by tilting the net and pouring some water through the outside of the net (so you aren't introducing anything into the sample). Over a clean bucket, carefully invert the net inside out into the bucket by grabbing the clump of material from the opposite side of the net with your hand and dumping it into the bucket. Knock all bugs and debris out of the net and into the bucket. Once the net is inverted, rinse the net by pouring water from the other bucket through the net to release bugs and debris into the bucket.
- Pull the net clear of the bucket and examine the net for any leftover bugs and pluck these off the net with forceps/tweezers. Place these bugs directly into the sample jar with alcohol. Set the net aside.
- Pick larger debris out of the bucket first (larger rocks, sticks etc.), one at a time while checking to see if there's organisms on them. These larger items will not go in your sample jar. Hold each piece of debris over the sieve. Rinse the debris with a squirt bottle containing river water or DI water over the sieve (NOT over the sample jar). Place any macroinvertebrates you find into the sample jar using forceps.
- Place algal masses on the sieve, and let as much water as possible drain out of the masses. Do not smash the masses to drain them. Place completely drained algal masses into the sample jar.
- Separate the organisms from the rest of the smaller debris by swirling the sample around in the bucket. Aggressively swirl the bucket for a minimum of 15-20 swirls. Swirl until lighter organisms and debris have floated to the top, and heavier sediment has sunken to the bottom.
- Pour out the top section of the water and floating material into the sieve. Limit scraping or any movements that would smash bugs. Collect bugs from the sieve with forceps or clump organisms/algae together carefully with hands or useful devices and place into the sample jar.
- Repeat swirling until there is no more floating material remaining. Add more river water if needed. Pick up a handful of sediment and inspect for bugs. If there are no more bugs, stop swirling.
- Pour the remaining contents of the bucket onto the sieve in manageable batches. Spread out the material and look for more bugs to place into the sample jar.
- Place this material in the white tray and inspect for bugs one last time. Place all bugs from the sieve and tray into the sample jar.
- Once all debris from the net is processed, rinse the net, sieve, and pan thoroughly in the river, until no debris is visible. Let these items dry completely.
- Fill out 2 duplicate macro labels with a pencil. Place one label inside the jar and tape one label to the outside of the jar.

Quality Assurance Macroinvertebrate Collection (Duplicate Sample)

- Find four riffles in the sample collection area that are wide enough to collect two samples right next to each other.
- Collect your "normal" macro sample as described above. Two fast riffles, two slow riffles, filling out the datasheets and processing the sample as normal making sure not to disturb the second half of each riffle where you will collect your QA sample.
- Once your normal sample is complete, collect a "duplicate" sample right next to your initial sample moving downstream to upstream, like you would a normal sample at all four kicks.
- **Make sure your duplicate kicks aren't overlapping your first set of kicks, they should be next to the original kick areas, not on top of these kick areas.**
- Process your sample in a separate bottle marked "QA Macro Sample".
- Clean your net, sieve, brush and buckets.

Complete the Physical Habitat Data Sheet in addition to the macro datasheets.