

Standard Operating Procedure 8: Quantitative Sampling of Macroinvertebrates, version 1.0

The purpose of quantitative sampling of macroinvertebrates is to estimate the abundance of individuals and richness of taxa occurring in the most productive microhabitat in streams (i.e. riffles). Quantitative samples are taken with a Portable Invertebrate Box Sampler (PIBS) or Surber sampler, both outfitted with a 363- μ m mesh catch net. The activities involved with quantitative sampling include: choosing the sample location, choosing the sampling device, collecting the sample, and preserving the sample. Required equipment for this task is a PIBS or Surber sampler, vegetable brush, three bags to retain the samples, three labels with proper site number and replicate, cover datasheet, and 95% ethanol.

Choosing the Sample Location

Three quantitative samples are taken at each stream site (100 meter reach). Upon arriving at a designated site, survey the reach from the bank and choose three specific locations in riffles that are suitable for sampling.

Quantitative samples are taken in shallow, wadeable riffles where the substratum consists predominantly of cobble (6.0 cm -25.0 cm) and pebble (2.0mm -6.0 cm). The sampler must be placed in a location where the depth and current are sufficient to wash dislodged organisms into the catch net but neither so deep that the sampler is completely submerged nor so swift that the sampler cannot be held in place on the bottom. In addition, the stream channel must be wider than the base of the sampler.

Choose sample locations in three different riffles if possible. If not, spread the samples out as much as possible within the same riffle.

There are some sample sites where it is sometimes impossible to get all three samples within the designated 100 m reach. This is typically due to flow volume or inappropriate substrates. In these cases, it is permissible to get the necessary samples from outside the site. Simply walk upstream or downstream and find the nearest suitable riffle. In the notes section of the data cover sheet, record the approximate distance and direction outside the site the sample is taken.

Choosing the Sampling Device

The first choice for a sampling device is always the PIBS because the foam bottom establishes a thorough seal by taking the shape of the substrate, and it is completely enclosed on all sides so organisms cannot escape or enter while sampling is in progress. However, the PIBS requires sufficient depth for the water to flow over the foam bottom into the net. In addition, the channel must be wide enough to accommodate the width of the foam bottom. The PIBS requires at least 10 cm of water depth (with new foam) to capture macroinvertebrates efficiently. As the foam ages, shallower areas may be sampled. The foam should be replaced when it degrades to a thickness of 4 to 5cm. It is advantageous to keep one PIBS with older foam to facilitate sampling in lower flows or smaller streams. Keep in mind the condition of the foam and replace if needed.

If these conditions cannot be met, the Surber sampler should be used. The Surber should not be used in less than 4cm of water. The PIBS and Surber Samplers sample slightly different size areas. Do not use two different sampling devices at a stream site on the same occasion because it may complicate interpretation of the data collected. If it is necessary to use the Surber sampler at one sampling location at a site, then take all three samples with the Surber.

Taking the Samples

Always take the first quantitative sample at the most downstream location within a site and proceed upstream for the second and third samples. This avoids walking through riffles before samples are taken, which causes mobile macroinvertebrates to swim or drift away. Always approach riffles as discretely as possible, especially in low water, because macroinvertebrates will also swim or drift away from your movement on the bank or your shadow.

Place the sampler over an area of stream bottom that contains a mix of cobbles and pebbles. Homogeneous areas of small gravel and sand should be avoided because the diversity and abundance of macroinvertebrates will be very low.

After placing the sampler on the bottom, check the inside of the sampler to make sure that there is a good seal between the sampler and the substrate. The substratum where the sampler is placed should not be so irregular that the sampler will not seal against the stream bottom because this allows macroinvertebrates to escape. If the sampler does not seal tightly with the stream bottom, ensure that the net is clean and move the sampler to another location.

When using the PIBS, the best position for the person taking the sample is to kneel behind the sampler with the knees on the edges of the device and the catch net passing between the legs. Alternatively, the person can sit on the top of box. With either position the weight of the person taking the sample pushes the PIBS tightly against the substratum. If it is difficult to establish a good seal, the second person can assist by pushing on the PIBS with a foot at the appropriate place. If the seal is not good after sitting on the PIBS, pick up the sampler, invert the net (remove debris if needed), and move to another location. The Surber can be held in place by the second person exerting pressure with their hand on the top of the frame.

Each individual rock, beginning with the largest ones first, is picked up and brushed on all sides with the vegetable brush so that the organisms are dislodged and swept into the catch net. Hold the rocks underwater while brushing to make sure that organisms are not thrown out of the sampler. Brushing underwater also reduces damage to the organisms, which makes them harder to identify. Brush all cobbles and pebbles that are large enough to be stable on the bottom of the riffle. Ensure that water flows through the net at all times. Some sites have a large amount of filamentous algae that tends to clog the net. If water stops flowing through the net, grab the net and shake it without moving the sampler. This should move the algae to the tip of the net and allow water to flow through the sampler again.

Each rock should also be visually examined at close range, because many aquatic insects have special means of attaching themselves very tightly to rock surfaces. Use forceps or a fingernail to remove any organisms found clinging after brushing and place them in the water so that they

wash into the net. Place the rocks back in the stream outside of the sampler after they are examined.

After all of the cobbles and pebbles have been brushed, examined, and removed, disturb the remaining substrate slowly with the brush handle to stir up the sediment inhabiting organisms and allow them to wash into the catch net. Disturb the substrate for a minute or more down to a depth of about 10 cm unless bedrock is reached before that depth. It is important to move the sediments slowly so that organisms will float out but sand will not be transported into the net. Sand damages the organisms and greatly increases the amount of time required to process samples in the laboratory

After disturbing the substrate for a minute or more, remove the catch net from the PIBS, or pick up the entire sampler in the case of the Surber. Any sticks or stones should be washed in the net and discarded after examining them for adhering macroinvertebrates. These will puncture the plastic bags used to hold the samples and damage the organisms contained in the sample. Also remove any salamanders or fish from the sample and return them to the stream. They are vertebrates and not included in the macroinvertebrate monitoring program, so there is no reason to include them in the sample. Crayfish, snails, limpets, clams, and all other invertebrates are included in the sample.

Wash the catch net to concentrate the contents into the end. This is best accomplished by taking the net to a nearby pool area or the riffle you are in. The mouth of the net is briefly submersed and then rapidly raised while shaking. Do not hit the bottom of the stream with your dip and do not dip downstream of your feet or other disturbance. Repeat this process for all four sides of the catch net.

Place the contents of the net into the plastic bag that will serve as the sample container (see below) by inverting the net, pushing an arm into the net, and gently tapping the apex from the inside.

Reverse the net to its original position and wash all four sides of the net a second time. Repeat the same process of emptying the net into the sample container. Examine the net carefully for any organisms that might be clinging to the mesh or stuck in seams. If there are many organisms, it is quicker to rewash the net a couple of times rather than pick each individual. Transfer these organisms with forceps or fingertips into the sample container.

Place the appropriate label into the sample container and tie the bag loosely.

1. Tags for the PIBS or Surber consist of the site number followed by QT1, QT2, and QT3 representing each of the Quantitative samples. An example using site 1F002 would be “1F002QT1”, “1F002QT2”, and “1F002QT3”. One tag will go in each sample bag starting with QT1 at the lower end of the site and progressing to QT3 at the top of the site (as quantitative samples are collected from a downstream to upstream direction).
2. The tag for the D-net sample is the site number followed by QL for qualitative. An example using site 1F002 would be “1F002QL”. This tag will go in the bag for the D-net sample.

3. A fifth tag is made with just the site number and attached to the outside of the main bag.

Invert the catch net and backwash it before proceeding to take the next sample. Lastly, record the Label IDs, Sampler (i.e. “PIBS” or “Surber”) and the collector’s initials on the site form.

Preserving the Samples and Storage

Place the samples into 1-gallon twist tie bags (as described above) with the appropriate label. These measure approximately 25 x 35 cm (10 x 14 inches). Quart and Gallon Freezer Ziplock Brand bags can also be used. Generic brands do not seal as well. When the samples are first collected at the study site, tie the open ends loosely to contain the material but to allow for easy untying at the vehicle or field pack. Upon returning to the packs or vehicle, open the bags and add undiluted 95% ethanol as a preservative. Add enough alcohol to cover the entire sample. Check to see if the bag is leaking. If so, add extra ethanol and place the original bag into a second bag. After tying each sample bag tightly, place all four sample bags into one bag and affix a site label to the outside of that bag. Place the samples in a small cooler or 5-gallon bucket for transport to the office. Try to not stack them on top of each other to prevent rupturing the sample bags. If your hike is more than 15 minutes from the vehicle, carry 0.5 gallons of 95% ethanol to the site and preserve the samples at the site.

Upon returning to headquarters, samples are stored in large plastic totes lined with two trash bags and labeled as aquatic macroinvertebrate specimens. These totes will ultimately be provided to the contractor for macroinvertebrate identification. Place the samples collected that day in a single layer in the trash bags. As noted above, stacking the sample bags can cause them to rupture. Twist the large trash bags closed and place the cover on the box. Samples from 8 to 12 sites can usually be stored in each tote box.

PIBS Notes: PIB samplers are no longer manufactured. Nets can be custom ordered from www.wildco.com. In the near future foam bases will need to be made in park. A new backup PIBS should be built out of old aluminum sign material.

SOP Revision History

All protocols, regardless of how sound they are originally, require modification as new and different information becomes available. SOP version numbers increase incrementally by tenths (e.g. version 1.1, version 1.2...etc.) for minor changes. Major revisions should be designated with the next whole number (e.g., version 2.0, 3.0, 4.0 ...). Small changes or additions to existing methods will be reviewed in-house by Shenandoah or Mid-Atlantic Network staff. However, if a complete change in methods is sought, an outside review is required.

Revision History Log:

Prev. Version #	Revision Date	Author	Changes Made	Reason for Change	New Version #
