9.13 PROTOCOL FOR INVERTEBRATE SAMPLE PROCESSING

Overview

Samples should be sieved in the net in the field. At this time, rocks, wood, leaves, and other large items found in the sample may be discarded after removing all attached benthos. Also remove and release any non-benthos animals collected (e.g., fish). If samples are to be picked live, they should be kept cool and should be processed within 48 hours. For transportation to the laboratory, decant bucket contents into a wide-mouth plastic jar to avoid spills during transportation and to conserve refrigerator space (a consideration when samples will be picked live). Label samples with lake, stream, or wetland name and/or code, date, and sample number. Insert labels in the vessel containing the sample in case labels get washed off the container; regular paper and pencil work fine for this purpose.

Samples may be preserved with 10% buffered formalin (a good fixative) as long as safety precautions are observed. Alcohol (e.g., ethanol, methanol, isopropanol) can also be used to preserve samples. If buffered formalin is used for initial fixation, replace it with alcohol after a couple of days to prevent hard body parts (e.g., clam and snail shells) from dissolving. When using alcohol for preservation in the field, a good method is to first sieve the sample to remove much of the water, transfer to a suitable container, and then add a generous amount of alcohol.

Sources

Ontario Ministry of the Environment (2005)

At a glance

remove fines

Sieving the sample

1 Remove fine particulate matter and preservative from the sample prior to picking benthos. Fines cloud the water in sorting trays, making the task of finding animals much more difficult.

2 Transfer the sample to a 500 µm sieve (500 µm D-net can be used as a sieve) and rinse well with water to remove preservative (if used) and fine suspended particles.

3 Thoroughly rinse and discard large items such as pieces of wood, rocks, and leaves.

4 Rinsate from preserved samples will be sufficiently dilute and of low enough volume to permit disposal via a septic system or municipal sewage system. When disposing sample preservative to a septic system, keep daily 10% formalin discharge to 10 L or less.

Obtaining benthos sub-samples

1 Sub-sampling is a method of removing manageable portions of the sample so that invertebrates can be more easily separated

from debris in the sample and to obtain fixed count samples.

Bucket method

1. Wash the sample from the sieve back into a large container (a bucket works well). Gently swirl the bucket contents to indiscriminately distribute the sample. Randomly remove a small quantity of the sample (using a spoon, ladle or similar gadget) and transfer it to a suitable container.

Marchant Box method

1. Wash the sample from the sieve into the Marchant Box and fill with water to a depth just below the height of the walls dividing the cells. Water depth is important. In the case of live samples, water deeper than the dividing walls will allow animals to swim between the cells once the contents have been randomized. Less water will make it difficult to distribute the sample among the 100 cells.

live samples

2. Close and fasten the lid. Invert and gently mix the sample with side-to-side rocking motions. Right the box quickly and set on a level surface to let contents settle into cells. Using random numbers for the 10 columns and 10 rows, arbitrarily select one or more cells and transfer contents to a suitable container or Petri dish using a pipette (or turkey baster), vacuum pump, or aspirator and suction flask, or similar method.
3. The cell-extraction method used for Marchant sub-sampling strongly influences sample-processing time. Consider the costs of more sophisticated equipment such as aspirators, pumps, suction flasks, and tubing in relation to the improved efficiency resulting from their use. Using an aspirator and suction flask may be the best balance between minimal cost and extraction efficiency.

Picking, identifying, enumerating and preserving benthos

1. Sub-samples should be sequentially removed and picked until at least 100 animals are retained from each sample. 100-animal fixed counts yield reliable estimates of relative abundance and allow samples to be processed relatively quickly (as opposed to full enumerations). In sparse samples (i.e., containing fewer than 100 animals), the entire sample is processed. If fewer than 80 animals are collected, re-sample.

at least 100 animals

2. To be counted, a specimen must have enough intact body parts to permit its identification to the targeted level, and it must have a head (this prevents double counting). Larval exuviate and empty shells (e.g., snails and clams) and cases (e.g., of caddis flies) are not counted.
3. To identify, tally and preserve benthos as they are picked from the sample, transfer a sub-sample into a suitable picking

container such as a Petri dish or a white tray. Add additional water to the tray to aid sorting. Sort through the sample, removing all benthos. Identify and tally animals as these are removed; however, when picking live, speed is important so identify animals after all samples are picked. Set aside specimens that require detailed observation to identify for later identification. The minimum detail for identification is a coarse 27 group mix of Phyla, Orders, Classes and Families.

when picking live, speed is important

4 Place animals into a labeled container with alcohol preservative after they are identified and tallied. Glass jars with lids that give a good seal are commonly used. Animals that cannot be identified should be archived with the rest of the sample; their presence should be recorded on the tally sheet as unknown, but their count is not considered part of the 100-animal sub-sample.

5 Continue picking the sub-sample until all benthos have been removed (no more animals are found during a reasonable period of searching).

6 Continue to sort and identify animals until at least 100 invertebrates have been tallied. The entire sub-sample that contains the 100th animal must be picked in its entirety to allow abundance estimation.