

Standard Operating Procedure 15: Laboratory Processing of Macroinvertebrate Samples, version 1.0

Note: The following procedures outline specific actions taken by Virginia Tech staff who processed macroinvertebrate samples for the first ~30 years of the SHEN macroinvertebrate program. Future contractor procedures should not differ substantially or in a manner that would influence results.

Laboratory processing of macroinvertebrate samples involves a series of activities that generate the desired raw data from the preserved samples that were collected in the field. Each Portable Invertebrate Box Sampler (PIBS), Surber, or D-net sample is maintained as a separate unit. Before the individual macroinvertebrates can be identified and counted, they must first be removed from the mineral substrate and organic debris that is also contained in the samples. The basic steps in laboratory processing of macroinvertebrate samples includes: washing, sorting, identification, and enumeration and recording, along with quality control. The ultimate raw data consist of the numbers of individuals in each taxon that occurs in each sample. Required equipment includes 355- μ m brass soil sieve (U. S. Series No. 45), water, 70% ethanol, shallow white enamel metal pan, forceps, teaspoon, numerous 2-oz (59-ml) bottles with screw caps, vials in unit trays, watch glasses, porcelain spot test plates, 2X lighted magnifier, stereomicroscope, taxonomic code dictionary, and bench data sheet.

Washing

The first step in analyzing a benthic macroinvertebrate sample is to wash it in order to remove any remaining fine detritus and the original preservative, which will have become darkly stained from leaf leachate. The purpose of this procedure is to make the sample clean enough for the organisms to be seen and removed.

Place the sample in a 355- μ m brass soil sieve (U. S. Series No. 45), and wash it thoroughly with tap water from a faucet in a sink. A sieve of this particular mesh size is used to correspond with the mesh size of the nets on the samplers that were used to collect the samples in the field. The original coded label that was placed in the sample during field collection should be kept with the sample throughout all steps of laboratory processing.

Gently stir and shake the sample while it is being washed. Wash the sample thoroughly, but do not damage the specimens because accurate identifications depend on having all external structures intact. If there are any large pieces of leaves or sticks in the sample, these should be removed individually, washed over the sieve, examined for adhering organisms, and then discarded. Adhering organisms are removed with forceps and placed in the sieve.

Sorting

The next step is to remove all macroinvertebrates from the debris in the washed sample. This procedure can be referred to as “sorting,” but it is more commonly called “bug picking.” Samples are sorted in their entirety. Transfer a small amount (1 to 3 teaspoons) of the washed sample to a shallow white enamel metal pan. An effective size of enamel pan is 19.5 cm wide x 31 cm long x 5.5 cm high.

If the sample contains a great deal of debris or a very high number of macroinvertebrates, a smaller portion of sample should be placed in the sorting pan at one time and vice versa for a sample that is very clean and contains few macroinvertebrates.

Cover the sample material with approximately 1 cm of water and remove the macroinvertebrates with fine tipped forceps. Examine the pan in a systematic fashion, proceeding along imaginary rows and columns. Gently stir the debris with the tips of the forceps as the sample is examined. There should be a strong light immediately above the pan, such as a desk lamp with a flexible arm. Carefully examine the entire pan, then scan the entire contents a second time using the imaginary rows and columns in a different pattern. For example, examine the pan in rows from left to right the first time and in columns from top to bottom the second time. Use the 2X lighted magnifier for the second examination. After the second examination of the pan, dispose of the material and continue sorting portions of the sample until finished.

As macroinvertebrates are sorted from the washed sample material, place them into a single container containing 70% ethanol along with the original coded label that was placed in the sample during field collection. A 2-oz (59-ml) bottle with a screw cap is a good size for holding all organisms from a single sample.

When the last washed material is removed from the sieve for sorting, special efforts are required to make sure that all macroinvertebrates are removed from the sieve. They tend to adhere to the fine wire mesh of the sieve because of hooks and hairs on their bodies and their overall small size. The last washed material should be concentrated at the bottom of the sieve by holding the sieve at an angle under a faucet. A flexible hose attached to the faucet is particularly effective for this task. Transfer the last of the washed sample to the sorting pan by gently washing it out of the sieve with a squeeze bottle containing water. The bottom area of the sieve should be back-washed with the squeeze bottle into the sorting pan to complete the removal of the sample.

When the sample has been completely sorted, fill the bottle containing the macroinvertebrates with 70% ethanol and seal tightly. Double check to make sure the bottle contains the original field label.

Identification

The last step of laboratory processing involves identifying the specimens to the lowest practical taxonomic level and counting the number of specimens in each taxon. Each specimen is carefully examined with a reasonably good quality stereomicroscope having magnification up to at least 40X. Identification is accomplished by using the most up to date taxonomic publications that contain descriptions and keys (Brigham et al. 1982, Pennak 1989, Stewart and Stark 1989, Wiggins 1996, Merritt et al, 2008). Macroinvertebrates will be identified to at least the following taxonomic levels:

Insecta - genus (except Chironomidae, family)

Crustacea - genus

Mollusca - family

Oligochaeta - class

Turbellaria - class

Enumeration and Recording

As specimens are identified they will be temporarily stored separately. A series of vials lined up on the bench in special unit trays is handy for this purpose. Watch glasses and porcelain spot test plates also work well for temporarily holding individual taxa while a sample is being identified. Specimens should always be kept immersed in 70% alcohol.

For the quantitative benthic samples (i.e. PIBS and surber samples), all specimens will be enumerated. Tally meters will be used for both continuous and final counting. Individuals of abundant taxa will be tallied continuously while the sample is being identified. Individuals of sparse taxa will be tallied once at the end of the sample.

For the qualitative benthic samples (i.e. D-net), taxa are only recorded as being present; there is no need to count the specimens.

All data are recorded on a Benthic Macroinvertebrate Worksheet (SOP 20). It is very important to fill in all of the information at the top of each worksheet (site code, date, sample type and replicate, person making the identifications). The column for comments is used to record any special circumstances, such as questionable identifications that need more attention or specimens having been sent to a specialist for verification.

Macroinvertebrate data are entered into the database management system by taxonomic codes, rather than scientific names. After the samples are identified and enumerated, the taxonomic codes should be written on the worksheets in the column labeled "Taxa Codes" to make data entry faster and more accurate. The taxonomic codes can be found in the dictionary for the aquatic component of the database management system (Table "W_AIzdd_taxa").

When a sample is completely identified, counted, and recorded on bench sheets, all specimens are stored collectively by replicate in a 2-oz bottle containing 70% alcohol. Make sure the bottles are filled and tightly sealed. Each bottle contains a label bearing the site code, site name, replicate number, date, taxonomic name, and name of the person making the identification. Bottles will be stored in collective lots corresponding to site, date, sample type, and replicate. The original field label will be kept with the corresponding lot.

Information on the bench sheets is then entered into an Access database provided by Park staff.

Control (QC) for Sorting and Identification

QC checks of sorting effectiveness are performed on 5% of the samples for an annual set of samples by a qualified co-worker. Samples are randomly selected for QC checks.

For each sample selected for a QC check, first sort the sample as usual, but save all material that remains in the sorting pan after the original sorter thinks all macroinvertebrates have been removed. Next, count the total number of organisms sorted from the sample. Then a qualified co-worker resorts the sample by the same procedures and counts the total number of organisms found. All organisms recovered during resorting are added to the originally sorted specimens and become a permanent part of the sample. The number of organisms found during resorting of the sample is compared to the total number of organisms found in the original sorting of the sample.

If the number of organisms from resorting the sample is less than 10% of the original total, the sample passes.

If the number of macroinvertebrates from resorting the sample is more than 10% of the original total, the sample fails. If a majority of the QC samples fail, sorters will undergo more training and another 5% of the samples will be randomly selected for additional QC checks until a majority of the QC samples pass.

QC checks of identification accuracy are performed on 5% of the samples for an annual set of samples by a qualified co-worker. Samples are randomly selected for QC checks.

Both the accuracy of taxonomic identification and enumeration of specimens belonging to each taxon are checked. All taxonomic identifications must be correct. Errors in enumeration less than 5% are considered acceptable. Any misidentifications or errors in enumeration are corrected for the sample selected for QC and become the final data for that sample. Corrective actions for future samples are discussed with the worker making the identifications and enumerations.

At the end of the contract year, preserved samples, hard copies of datasheets, and electronic data are sent to Shenandoah. Shenandoah National Park is responsible for long term storage of specimens and merging aquatic invertebrate data into the master database.

References

- Brigham, A. R., W. U. Brigham and A. Gnilka, eds. 1982. Aquatic insects and oligochaetes of North and South Carolina. Midwest Aquatic Enterprises, Mahomet, Illinois.
- Merritt, R. W., K. W. Cummins, and M. B. Berg, eds. 2008. An introduction to the aquatic insects of North America, 4th ed. Kendall/Hunt, Dubuque, Iowa.
- Pennak, R. W. 1989. Fresh-water invertebrates of the United States, 3rd ed. John Wiley and Sons, New York.
- Stewart, K. W. and B. P. Stark. 1989. Nymphs of North American stonefly genera (Plecoptera). Volume 12, Thomas Say Foundation Series, Entomological Society of America, Hyattsville, Maryland.
- Wiggins, G. B. 1996. Larvae of North American caddisfly genera (Trichoptera). 2nd ed. University of Toronto Press, Toronto, Ontario.

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 #