

# U.S. GEOLOGICAL SURVEY

## Preparing Benthic Macroinvertebrate Samples for Processing

### 1. Scope, Application and Summary

- 1.1. General scope of the procedure—This procedure supports lab codes 2172 and 2176. Preparation procedures described herein can also be applied to benthic macroinvertebrate (BMI) samples collected in the field using other quantitative, semi-quantitative, or qualitative sampling methods. These procedures do not include some method-specific preparation steps (for example, size-fractionation in qualitatively processed samples).
- 1.2. Short summary of the procedure—A sample must be prepared in the laboratory to remove field preservatives (for example, formalin or ethanol) and fine detritus before methods can be applied to sort and identify specimens. Each sample is washed in a fume hood. Once the preservative waste and obvious odors are minimized, each sample is further processed at a sink. Sieving and flotation techniques are used to separate portions of the detritus.
- 1.3. These procedures are used by anyone responsible for the laboratory processing of BMI samples.

### 2. Reasons For Revision and Summary of Changes:

- 2.1. This SOP is being updated to the new format.
- 2.2. Detail is being added to sections as appropriate to better define the methods.
- 2.3. An attachment is being added to detail the preparation and use of ethanol.

### 3. Health, Safety, and Waste Disposal Information

- 3.1. Personal Safety
  - 3.1.1. Wear long pants and closed-toed shoes at all times when working in the laboratory.
  - 3.1.2. Wear an apron, gloves, and protective eyewear during sample processing in the hood.
  - 3.1.3. Know the location of the nearest eyewash and shower stations.
  - 3.1.4. Do not eat or drink in the laboratory.
  - 3.1.5. Follow other safety procedures described in the U.S. Geological Survey (USGS) Occupational Hazards and Safety Procedures Handbook (September 1999).
- 3.2. Chemical Safety
  - 3.2.1. Only work in the laboratory when the room ventilation system and fume hoods are working properly. Leave the laboratory and contact the Biological Group (BG) supervisor or appropriate Support Services section representative if the ventilation systems are not working properly.
  - 3.2.2. Use the vacuum waste disposal system to transfer preservative waste from the fume hood to the storage drum. Contact the BG supervisor or appropriate Support Services section representative if the system is not functioning properly. Contact a representative from the National Water Quality Laboratory (NWQL) Safety, Health, and Environmental Compliance (SHE) section when the waste drum is full and needs to be replaced.

- 3.2.3. Know the location of and be familiar with the Material Safety Data Sheets (MSDS) for each chemical used by the BG.
- 3.2.4. Know how to report and handle chemical and sample spills using procedures described in the NWQL Chemical Hygiene Plan (available from the NWQL SHE section).
- 3.3. Follow other standard safety guidelines provided by the National Research Council (1995).
- 3.4. All NWQL personnel will follow the guidance outlined in the NWQL Pollution Prevention and Waste Minimization Policy (see NWQL Policy Memorandum 04.01 at <http://www.nwql.cr.usgs.gov/USGS/policy/policy.04-01.html>). Refer to NWQL SOP No. TX0035.x, Waste Disposal at the National Water Quality Laboratory, for further information.

#### 4. Procedure and Responsibilities

- 4.1. Obtain the following supplies, chemicals, and equipment before preparing a sample.
  - 4.1.1. Supplies
    - 4.1.1.1. 2-inch wide clear tape
    - 4.1.1.2. Forceps
    - 4.1.1.3. Large plastic spoon
    - 4.1.1.4. Plastic washbasin
    - 4.1.1.5. Scoopula<sup>®</sup> or other small scoop
    - 4.1.1.6. Scrub brush
    - 4.1.1.7. Squirt bottle(s) for 70-percent ethanol and/or water
    - 4.1.1.8. White sorting trays of various sizes (for example, 15 x 20 cm and 20 x 30 cm)
  - 4.1.2. Chemicals
    - 4.1.2.1. Tap water
    - 4.1.2.2. 70-percent ethanol (see Attachment 1)
  - 4.1.3. Equipment
    - 4.1.3.1. Standard metal sieves with mesh size equal to or slightly smaller than the field-collection mesh size. (see Section 5.2)
- 4.2. Interferences
  - 4.2.1. Residual specimens on processing supplies and/or equipment can cause cross contamination between samples. Inspect and clean all equipment and supplies prior to preparing a sample to minimize the possibility of cross contamination.
  - 4.2.2. Sample washing is ineffective when too much material is washed at one time, especially if the material is fine detritus or silt. In this case, wash smaller portions of the sample separately and then recombine the sample in a washbasin or another sieve.
  - 4.2.3. If field preservation is obviously inadequate from the odor of the sample and/or condition of the specimens, reseal the container and contact the BG Production Coordinator immediately.
- 4.3. Replacement of Original Sample Preservative

- 4.3.1. If a sample will not be sorted within two weeks of receipt by the NWQL, the original field preservative should be replaced with 70-percent ethanol in all sample containers.
  - 4.3.2. Remove sealing tape from container.
  - 4.3.3. Place an appropriate sieve (see Section 4.1.3.1) within a washbasin in the fume hood. If a 210-micrometer sieve is not too coarse, it works well for this step, as the detritus is easier to rinse back into the original jar.
  - 4.3.4. Remove the container's lid and pour the original preservative through the selected sieve and collect it in the washbasin.
  - 4.3.5. Rinse any detritus that accumulated on the sieve back into the original jar using 70-percent ethanol.
  - 4.3.6. Completely refill the original container with 70-percent ethanol and tightly reseal it using the original lid.
  - 4.3.7. Place a label on the outside of the container indicating that the original preservative has been replaced with 70-percent ethanol and the date (dd-mm-yyyy); use roman numerals for the month (for example, July 15, 2004 would be recorded as 15-VII-2004).
  - 4.3.8. Rinse all sieves and/or trays with water. Use the vacuum waste disposal system to transfer all of the rinse water and original preservative into the waste drum.
- 4.4. Sample Washing and Sieving Prior to Processing
- 4.4.1. If large-rare sample components (for example, Moulton and others, 2002) exist, process them first (see Section 4.5).
  - 4.4.2. If possible, limit preparation time to 30 minutes or less.
  - 4.4.3. Perform these steps in the fume hood.
  - 4.4.4. Transfer all waste and wash water generated during washing and sieving within the fume hood to a disposal drum by using the vacuum waste disposal system.
  - 4.4.5. Place an appropriate sieve (see Section 4.1.3.1) within a washbasin. The washbasin will allow the sample to be recovered if spilled.
  - 4.4.6. Open the sample container.
  - 4.4.7. Pour the sample and preservative into the sieve within the washbasin. If the sample volume is substantial, incrementally pour smaller portions of the sample into the sieve.
  - 4.4.8. If the original preservative has not previously been replaced (see Section 4.3), transfer the liquid from the washbasin into the waste drum with the vacuum waste disposal system before adding additional water.
  - 4.4.9. Carefully wash the sample in the sieve with tap water. Add enough water to the basin to completely cover the sample and carefully agitate it. Samples with minimal detritus may not need to be rinsed with as much water.
  - 4.4.10. Transfer liquid waste from the washbasin into the disposal drum using the vacuum waste disposal system.
  - 4.4.11. Repeat steps 4.4.9 and 4.4.10 at least two more times. If large amounts of sand and/or silt are still visible in the washbasin during the third rinse or an obvious preservative odor is still present when the sample is removed from the hood, additional washing may be needed.
- 4.5. Large-rare sample component (for example, Moulton and others, 2002)

- 4.5.1. Process multiple large-rares separately (see Section 4.3 and 4.4), maintaining the integrity of the component as it was sent to the BG.
- 4.5.2. Transfer the specimen(s) from each container into a separate white-bottomed tray.
- 4.5.3. Separate specimens that are entangled with larger specimens,
- 4.5.4. If the large-rare contains only a minimal number of large or mature specimens (sensu Moulton and other, 2002), label, preserve, and store them as unidentified specimens (see NWQL SOP No. BS0332.x or NWQL SOP No. BS0333.x).
- 4.5.5. If the large-rare either contains a significant amount of detritus or contains significantly greater than 20 large or mature specimens:
  - 4.5.5.1. Consult with the BG Production Coordinator or taxonomist for guidance on the number and types of specimens that should be sorted from the large-rare.
  - 4.5.5.2. As a general rule, although multiple specimens from a single container may be sorted, redundant taxa from multiple containers should not be sorted.
  - 4.5.5.3. If the sample was NOT SPLIT in the field (for example, Moulton and others, 2002):
    - 4.5.5.3.1. Sort out no more than 20 unique specimens (see Section 4.5.5.2).
    - 4.5.5.3.2. Label, preserve, and store the unidentified specimens (see NWQL SOP No. BS0332.x or NWQL SOP No. BS0333.x).
    - 4.5.5.3.3. After obtaining the approval and initials of a taxonomist, recombine the remaining specimens and/or detritus with the rest of the sample.
    - 4.5.5.3.4. Label and store the empty original container as a sorted sample remnant (see NWQL SOP No. BS0332.x or NWQL SOP No. BS0333.x).
  - 4.5.5.4. If the sample was SPLIT in the field (for example, Moulton and others, 2002):
    - 4.5.5.4.1. Sort out no more than 20 unique specimens (see Section 4.5.5.2).
    - 4.5.5.4.2. Label, preserve, and store the unidentified specimens (see NWQL SOP No. BS0332.x or NWQL SOP No. BS0333.x).
    - 4.5.5.4.3. Unidentified specimens that are not sorted from the component should be labeled, preserved, and stored as a sorted sample remnant in the original sample container (see NWQL SOP No. BS0332.x or NWQL SOP No. BS0333.x).
    - 4.5.5.4.4. Detritus, even if it is free of specimens, should also be labeled, preserved, and stored as a sorted sample remnant in the original sample container (see NWQL SOP No. BS0332.x or NWQL SOP No. BS0333.x).
- 4.6. Supplemental Swirl and Pour Sample Flotation
  - 4.6.1. If the sample contains substantial amounts of inorganic detritus (for example, sand or gravel) then perform a swirl and pour flotation.
  - 4.6.2. Swirl and pour flotation can be performed outside of the fume hood, if the sample has been washed (see Section 4.4).
  - 4.6.3. Place the sample in a washbasin.
  - 4.6.4. Fill the basin with enough water to cover the sample with at least 2.5 cm of water.

- 4.6.5. Carefully mix the sample with a spoon or by gently swirling the basin to suspend the organic detritus.
- 4.6.6. Place an appropriate sieve (see Section 4.1.3.1) into a second washbasin and slowly pour the suspended organic detritus onto the sieve. Stop pouring when the inorganic detritus reaches the edge of the first washbasin. Dispose of the rinse water that passes through the sieve and is retained in the second washbasin.
- 4.6.7. Repeat steps 4.6.4 to 4.6.6 until the inorganic detritus remaining in the washbasin appears free of organic detritus and most specimens; some specimens (for example, mollusks or cased caddisfly larvae) may remain in the inorganic portion.
- 4.6.8. Place the inorganic detritus in a white sorting tray and process further according to the selected method. If there are numerous specimens present in this inorganic detritus, consult with the BG Production Coordinator for advice.
- 4.7. Remove large detritus (for example, sticks, large leaves, or rocks) from the sample.
  - 4.7.1. Rinse each piece of detritus with water and inspect it for attached specimens using magnification appropriate for the method that will be used to further process the sample.
  - 4.7.2. Return any specimens that are found to the sample.
  - 4.7.3. Discard the inspected detritus in the solid waste container.
- 4.8. If further processing of a prepared sample cannot continue within 48 hours, re-preserve the sample in 70-percent ethanol.
- 4.9. Clean-Up
  - 4.9.1. Inspect all sieves and trays for retained specimens. Return any specimens found back to the sample.
  - 4.9.2. Rinse and clean all sieves, sorting trays, and washbasins used to process a sample.
  - 4.9.3. Scrub sieves with a brush and rinse from both sides to remove entrained sample detritus.
  - 4.9.4. Wipe up water and clean workstation.
  - 4.9.5. Put away all supplies and equipment.
- 4.10. Process the prepared sample according to the method prescribed by the customer. If a standard NWQL method is used then process according to either the current SOP No. BS0332.x or BS0333.x. If a custom method is requested then follow the procedures described in the customer's proposal.

## 5. Quality Control and Quality Assurance

- 5.1. Individual containers from a multi-container sample should be processed on separate sieves until a second member of the BG staff approves recombination.
- 5.2. If the field-collection mesh size is unknown, stop work immediately and contact the BG Production Coordinator.
- 5.3. Inspect all sieves, washbasins, and sorting trays before preparing a sample to ensure no specimens or sample detritus remain from a previously processed sample.
- 5.4. If applicable, compare the information on the internal and external sample labels; report any discrepancies to the BG Production Coordinator.

- 5.5. Contact the BG Production Coordinator immediately if there are any problems with the sample or if this SOP does not provide guidance as to a solution for those problems.

## 6. Data and Records Management

- 6.1. Record the following information in a laboratory record book for each sample prepared. Information that is redundant need not be recorded. For example, the sample may be completely prepared on the same date that it is started.
  - 6.1.1. Sample identification code
  - 6.1.2. Start date (mm/dd/yyyy) and time (hh:mm).
  - 6.1.3. End date (mm/dd/yyyy) and time (hh:mm).
  - 6.1.4. If only the original preservative is replaced in a group of samples, record the start date (mm/dd/yyyy), start time (hh:mm), and the end time (hh:mm) for the group of samples. List only the projects and respective number of samples for which the preservative was replaced; individual sample identification codes need not be recorded.
  - 6.1.5. Problems or errors associated with the preparation step(s) by sample identification code (for example, sample was spilled or the wrong mesh-size sieve was used).
- 6.2. If a large-rare sample component is present (for example, Moulton and others, 2002), record the following on the BMI Identification and Enumeration Bench Data Sheet.
  - 6.2.1. The individual that sorted the component should record their initials in the appropriate space.
  - 6.2.2. If any specimens and/or detritus from the component were re-combined with the sample, the taxonomist that approved this action should record their initials in the appropriate space, otherwise enter 'N/A' (not applicable) in this space.

## 7. Definitions

- 7.1. Decant—Pour off the original preservative without additional rinsing and replace it with 70-percent ethanol.
- 7.2. Detritus—organic and/or inorganic matter of any size present in a BMI sample, excluding the specimens.
- 7.3. Flotation—The separation of organic detritus (for example, fine detritus or pieces of wood) from inorganic detritus (for example, sand or gravel) in a sample. Typically performed when a substantial amount of inorganic detritus is present in the sample. Flotation promotes effective subsampling and removal of specimens from the remaining organic detritus. This is also referred to as elutriation.
- 7.4. Large-rare—a sample component that typically consists of no more than 20 specimens such as large crayfish, hellgrammites, or mussels (Moulton and others, 2002).
- 7.5. Taxonomist—an individual trained in and hired by the BG for identification of BMIs.

## 8. References

- 8.1. Moulton, S.R., II, Carter, J.L., Grotheer, S.A., Cuffney, T.F., and Short, T.M., 2000, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory — processing,

NWQL SOP #: BIOP0331.1	General Procedure	Controlled copy
SOP Title: Preparing Benthic Macroinvertebrate Samples for Processing		
Authors: Scott Grotheer, Robert Hood, and Marcia Siebenmann		
Effective Date: 6/3/2005		
Approved by and date of approval: David Reppert, 6/3/2005. Revision / reapproval due by 06/03/08		
A paper copy of this document is only valid if its revision number matches that of the controlled copy as posted on the intranet.		
It is the user's responsibility to verify that these revision numbers match. (note: revised SOP footer added 04/30/07 by M. Cast)		

taxonomy, and quality control of benthic macroinvertebrate samples. U.S. Geological Survey Open-File Report 00-212.

- 8.2. Moulton, S.R., II, Kennen, J.G., Goldstein, R.M., and Hambrook, J.A., 2002, Revised protocols for sampling algae, invertebrate, and fish communities as part of the National Water Quality Assessment Program. U.S. Geological Survey Open-File Report 02-150.
- 8.3. National Research Council, 1995, Prudent practices in the laboratory—Handling and disposal of chemicals: Washington, D.C., National Academy Press, 427 p.
- 8.4. U.S. Geological Survey, 1999, Occupational Hazards and Safety Procedures Handbook: Manual No. 445-2-h, available at <http://www.usgs.gov/usgs-manual/handbook/hb/445-2-h.html>

## 9. Key Words

benthic macroinvertebrate, sample preparation, washing, sieving, flotation, ethanol

## 10. Attachments

Attachment 1 – Preparation, Tracking, and Use of Ethanol

## Attachment 1

### Preparation, Tracking, and Use of Ethanol

#### 1. Preparation of 70-percent ethanol from a new 55-gallon drum.

- 1.1. This procedure assumes to start with a new 55-gallon drum of 95% Ethyl Alcohol USP – 190 Proof from AAPER Alcohol and Chemical. The MSDS provided by AAPER Alcohol and Chemical states that the actual concentration of ethanol in the drum is 92.42-percent. The ethanol in these new drums will be referred to as 95-percent ethanol in this procedure.
- 1.2. Obtain the pre-marked 25 L and 20 L Nalgene<sup>®</sup> carboys that are used for ethanol mixing. If the carboys are drained on a level surface approximately 0.25 gallons will remain in the bottom of the carboy. This procedure takes that into account.
- 1.3. Obtain a new 70-percent Ethanol Mixing and Use Log.
- 1.4. Either newly designate or use the existing overflow drum. If a new overflow drum is designated, start a new Ethanol Mixing and Use Log for it.
- 1.5. Ensure that the new drum and the overflow drum are properly grounded.
- 1.6. Open both drums and install a siphon pump in the 95-percent ethanol drum.
- 1.7. Transfer 13-gallons of 95-percent ethanol from the new drum into the overflow drum
  - 1.7.1. Add 95-percent ethanol into the 25 L carboy to the top mark and drain into the overflow drum until the level in the carboy reaches the bottom mark. **ONLY drain to the bottom mark.** Approximately 5 of the 13 gallons have now been drawn off the new 95-percent drum and transferred into the overflow drum.
  - 1.7.2. Add 95-percent ethanol into the 20 L carboy to the top mark and drain the entire contents into the overflow drum. Approximately 10.25 of the 13 gallons have now been drawn off the new drum and transferred to the overflow drum.
  - 1.7.3. Add 95-percent ethanol into the same 25 L carboy used in Section 1.7.1 to the middle mark. **There should still be approximately 1.25 gallons of 95-percent ethanol in the bottom of this carboy.** Drain the entire contents into the overflow drum. Approximately 13 gallons have now been drawn off the new drum and transferred to the overflow drum.
- 1.8. Transfer 13 gallons of distilled water to the new 95-percent drum used in Section 1.7.
  - 1.8.1. Add distilled water into the 25 L carboy to the top mark and drain into the new drum until the level in the carboy reaches the bottom mark. **ONLY drain to the bottom mark.** Approximately 5 of the 13 gallons have now been added to the new drum.
  - 1.8.2. Add distilled water into the 20 L carboy to the top mark and drain the entire contents into the new drum. Approximately 10.25 of the 13 gallons have now been added to new drum.
  - 1.8.3. Add distilled water into the same 25 L carboy used in 1.8.1 to the middle mark. **There should still be approximately 1.25 gallons of distilled water in the bottom of this carboy.** Drain the entire contents into the new drum. Approximately 13 gallons have now been added to the new drum.
- 1.9. The new drum should now contain approximately 70-percent ethanol.

## 2. Preparation of 70-percent ethanol using the 95-percent ethanol in the overflow drum.

- 2.1. Once the overflow drum contains at least 42 gallons, it can be used to prepare a drum of 70-percent ethanol.
- 2.2. Continue to record information on the same Ethanol Mixing and Use Log that was used while the drum was designated as overflow.
- 2.3. Designate a new overflow drum (usually the now empty 70-percent ethanol drum).
- 2.4. Start a new Ethanol Mixing and Use Log for the new overflow drum.
- 2.5. If the overflow drum contains more than 42 gallons of 95-percent ethanol,
  - 2.5.1. Draw off an appropriate volume of 95-percent ethanol into the marked 20 L carboy referred to in Section 1.7.2 to reduce the volume to 42 gallons. **Remember, when the carboy is filled to the 2 gallon mark, it actually contains about 2.25 gallons.**
  - 2.5.2. Ensure that the new overflow drum is grounded and transfer the excess from Section 2.5.1 into the new overflow drum
  - 2.5.3. Add 13 gallons distilled water to the overflow drum (see Section 1.8).
- 2.6. If the drum contains exactly 42 gallons of 95 percent ethanol,
  - 2.6.1. Add 13 gallons distilled water to the overflow drum (see Section 1.8).

## 3. Tracking the mixing and use of alcohol

- 3.1. Each drum should have its own Ethanol Mixing and Use Log until it is empty.
- 3.2. Record volumes added or withdrawn from a drum in US gallons.
- 3.3. Track the following, if applicable, in the appropriate space on the Ethanol Mixing and Use Log.
  - 3.3.1. Initials of the person mixing/using the ethanol
  - 3.3.2. The date (mm/dd/yyyy)
  - 3.3.3. Initial concentration of ethanol in the drum
  - 3.3.4. Initial volume in the drum (from the previous line or zero)
  - 3.3.5. 95-percent ethanol added
  - 3.3.6. Ethanol withdrawn
  - 3.3.7. Distilled water added
  - 3.3.8. Final volume in the drum