U.S. Geological Survey
National Water Quality Laboratory

Preparing Benthic Macroinvertebrate Samples for Processing

Contents

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1. Scope, application, and summary

1.1. General scope of the procedure—This procedure supports lab codes 2172, 2176, and U.S. Geological Survey Open-File Report 00-212 (Moulton and others, 2000). 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. The use of trade, product, or firm names in this procedure is for descriptive purposes only and does not imply endorsement by the U.S. Government.

1.3. 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.

1.4. Reporting units and levels – not applicable

1.5. Detection limits – not applicable

1.6. These procedures are used by anyone responsible for the laboratory processing of BMI samples.

1.7. Interferences

1.7.1. 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.

1.7.2. 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.

1.7.3. 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.

2. Reasons or and summary of changes:

2.1. This SOP is being updated to the new format as per NWQL SOP No. QUAX0001.x.

2.2. Links to NWQL Technical/Policy Memoranda are being deleted

2.3. References to documents that are not utilized are being deleted

2.4. A statement was added to the 'Deviations' section of this SOP to allow for deviations from this SOP at the request of a customer.

2.5. The analyst is not required to submit a new demonstration of capability based upon the revised SOP. The analyst is not required to submit a document training form for the revised SOP.

3. Health, safety, and waste disposal information

3.1. Applicable Health and Safety Issues

3.1.1. Wear long pants and closed-toed shoes at all times when working in the laboratory.

3.1.2. Wear an apron, nitrile gloves, and protective eyewear during sample processing in the hood.

3.1.3. Nitrile gloves must be changed after no more than 50 minutes of exposure to ethanol to avoid breakthrough.

3.1.4. Know the location of the nearest eyewash and shower stations.

3.1.5. Do not eat or drink in the laboratory.

3.1.6. 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. Waste Disposal and Environmental Compliance

3.2.1. 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.

3.2.2. Know the location of and be familiar with the Material Safety Data Sheets (MSDS) for each chemical used by the BG.

3.2.3. Solid waste including gloves, organic and inorganic detritus are disposed of in properly labeled solid waste containers.

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. All NWQL personnel will follow the guidance outlined in the following policy memoranda, accessible on the NWQL Home page (internal USGS access only) > Technical > Policy Memoranda. Supervisors will ensure that employees submit completed training forms, documenting that they have read and understood the information contained in these policy memoranda.

3.3.1. Safety, Health and Environmental (SHE) Compliance Section's Policy Memorandum 2006.01, *General laboratory health, safety, and waste disposal requirements*

3.3.2. NWQL Policy Memorandum 2008.01, *Conducting chemical and other potentially hazardous operations at the NWQL*

3.3.3. NWQL Policy Memorandum 04.01, *Pollution prevention and waste minimization policy*

3.3.4. NWQL Policy Memorandum 2010.02, *NWQL hours of operation*: High, Moderate, and Low Risks, pp. 3–4

3.4. All NWQL personnel will follow the guidance outlined in the NWQL SOP SHEX0355.x, *Waste disposal at the National Water Quality Laboratory*.

4. Sample preservation, handling, containers, analytical processing/holding times, and disposal

Refer to NWQL SOP No. BIOB0332.x and NWQL SOP No. BIOB0333.x.

5. Preparation of reagents, standards, and solvents

5.1. Tap water

5.2. 70-percent ethanol (see Attachments 1, 2, and 3)

6. Apparatus

6.1. Supplies

6.1.1. 2-inch wide clear tape

6.1.2. Forceps

6.1.3. Large plastic spoon

6.1.4. Plastic washbasin

6.1.5. Scoopula® or other small scoop

6.1.6. Scrub brush

6.1.7. Squirt bottle(s) for 70-percent ethanol and/or water

6.1.8. White sorting trays of various sizes (for example, 15 x 20 cm and 20 x 30 cm)

6.2. Equipment

6.2.1. Standard metal sieves with mesh size equal to or slightly smaller than the field-collection mesh size. (see Section 9.2)

7. Sample preparation, analysis, and instrument operation and shutdown

7.1. Replacement of Original Sample Preservative

7.1.1. If a sample will not be sorted within approximately two weeks of receipt by the NWQL, the original field preservative should be replaced with 70-percent ethanol in all sample containers.

7.1.2. Perform these steps in the fume hood.

7.1.3. Remove sealing tape from container.

7.1.4. Place an appropriate sieve (see Section 6.2.1) 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.

7.1.5. Remove the container's lid and pour the original preservative through the selected sieve and collect it in the washbasin.

7.1.6. Rinse any detritus that accumulated on the sieve back into the original jar using 70-percent ethanol.

7.1.7. Completely refill the original container with 70-percent ethanol and tightly reseal it using the original lid.

7.1.8. 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).

7.1.9. 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.

7.2. Sample Washing and Sieving Prior to Processing

7.2.1. If large-rare sample components (for example, Moulton and others, 2002) exist, process them first (see Section 7.3).

7.2.2. If possible, limit preparation time to 30 minutes or less.

7.2.3. Perform these steps in the fume hood.

7.2.4. 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.

7.2.5. Place an appropriate sieve (see Section 6.2.1) within a washbasin. The washbasin will allow the sample to be recovered if spilled.

7.2.6. Open the sample container.

7.2.7. 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.

7.2.8. If the original preservative has not previously been replaced (see Section 7.1), transfer the liquid from the washbasin into the waste drum with the vacuum waste disposal system before adding additional water.

7.2.9. 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.

7.2.10. Transfer liquid waste from the washbasin into the disposal drum using the vacuum waste disposal system.

7.2.11. Repeat steps 7.2.9 and 7.2.10 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.

7.3. Large-rare sample component (for example, Moulton and others, 2002)

7.3.1. Process multiple large-rares separately (see Section 7.1 and 7.2), maintaining the integrity of the component as it was sent to the BG.

7.3.2. Transfer the specimen(s) from each container into a separate white-bottomed tray.

7.3.3. Separate specimens that are entangled with larger specimens,

7.3.4. If the large-rare contains only a minimal number of large or mature specimens (Moulton and other, 2002), label, preserve, and store them as unidentified specimens (see NWQL SOP No. BIOB0332.x or NWQL SOP No. BIOB0333.x).

7.3.5. If the large-rare either contains a significant amount of detritus or contains significantly greater than 20 large or mature specimens as defined in Moulton and others, 2002:

7.3.5.1. 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.

7.3.5.2. As a general rule, although multiple specimens from a single container may be sorted, redundant taxa from multiple containers should not be sorted.

7.3.5.3. If the sample was NOT SPLIT in the field (for example, Moulton and others, 2002):

7.3.5.3.1. Sort out no more than 20 unique specimens (see Section 7.3.5.2).

7.3.5.3.2. Label, preserve, and store the unidentified specimens (see NWQL SOP No. BIOB0332.x or NWQL SOP No. BIOB0333.x).

7.3.5.3.3. After obtaining the approval and initials of a taxonomist, recombine the remaining specimens and/or detritus with the rest of the sample.

7.3.5.3.4. Label and store the empty original container as a sorted sample remnant (see NWQL SOP No. BIOB0332.x or NWQL SOP No. BIOB0333.x).

7.3.5.4. If the sample was SPLIT in the field (for example, Moulton and others, 2002):

7.3.5.4.1. Sort out no more than 20 unique specimens (see Section 7.3.5.2).

7.3.5.4.2. Label, preserve, and store the unidentified specimens (see NWQL SOP No. BIOB0332.x or NWQL SOP No. BIOB0333.x).

7.3.5.4.3. 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. BIOB0332.x or NWQL SOP No. BIOB0333.x).

7.3.5.4.4. 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. BIOB0332.x or NWQL SOP No. BIOB0333.x).

7.4. Supplemental Swirl and Pour Sample Flotation

7.4.1. If the sample contains substantial amounts of inorganic detritus (for example, sand or gravel) then perform a swirl and pour flotation.

7.4.2. Swirl and pour flotation can be performed outside of the fume hood, if the sample has been washed (see Section 7.2).

7.4.3. Place the sample in a washbasin.

7.4.4. Fill the basin with enough water to cover the sample with at least 2.5 cm of water.

7.4.5. Carefully mix the sample with a spoon or by gently swirling the basin to suspend the organic detritus.

7.4.6. Place an appropriate sieve (see Section 6.2.1) 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.

7.4.7. Repeat steps 7.4.4 to 7.4.6 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.

7.4.8. 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.

7.5. Remove large detritus (for example, sticks, large leaves, or rocks) from the sample.

7.5.1. 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.

7.5.2. Return any specimens that are found to the sample.

7.5.3. Discard the inspected detritus in the solid waste container.

7.6. If further processing of a prepared sample cannot continue within 48 hours, re-preserve the sample in 70-percent ethanol.

7.7. Clean-Up

7.7.1. Inspect all sieves and trays for retained specimens. Return any specimens found back to the sample.

7.7.2. Rinse and clean all sieves, sorting trays, and washbasins used to process a sample.

7.7.3. Scrub sieves with a brush and rinse from both sides to remove entrained sample detritus.

7.7.4. Wipe up water and clean workstation.

7.7.5. Put away all supplies and equipment.

7.8. 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. BIOB0332.x or BIOB0333.x. If a custom method is requested then follow the procedures described in the customer's proposal.

8. Data acquisition, processing and evaluation

None

9. Quality control (QC) and quality assurance (QA)

9.1. Individual containers from a multi-container sample should be processed on separate sieves until a second member of the BG staff approves recombination.

9.2. If the field-collection mesh size is unknown, stop work immediately and contact the BG Production Coordinator.

9.3. Inspect all sieves, washbasins, and sorting trays before preparing a sample to ensure no specimens or sample detritus remain from a previously processed sample.

9.4. If applicable, compare the information on the internal and external sample labels; report any discrepancies to the BG Production Coordinator.

9.5. 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.

10. Data management and records

10.1. When replacing the preservative in a sample, affix a label to the outside of the container indicating that the original preservative has been replaced with 70-percent ethanol. Record the date that the preservative was replaced on that label and use roman numerals for the month (for example, July 15, 2004 would be recorded as 15-VII-2004).

10.2. Problems or errors associated with the preparation step(s) (for example, sample was spilled or the wrong mesh-size sieve was used) should either be noted on or included with the original paperwork that is associated with the sample.

10.3. 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 (see Attachment 9 in NWQL SOP No. BIOB0335.x).

10.3.1. The individual that sorted the component should record their initials in the appropriate space.

10.3.2. 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.

11. Definitions, abbreviations, and acronyms

11.1. Decant—Pour off the original preservative without additional rinsing and replace it with 70-percent ethanol.

11.2. Detritus—organic and/or inorganic matter of any size present in a BMI sample, excluding the specimens.

11.3. 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.

11.4. 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).

11.5. Taxonomist—an individual trained in and hired by the BG for identification of BMIs.

12. Deviations

NAWQA is asking the Biological Group (BG) to evaluate modifications to this procedure to gain efficiencies that may reduce the amount of time and/or supplies required to process samples. Certain sections of OFR 00-212 (http://nwql.usgs.gov/Public/pubs/OFR00-212.pdf) require that specific procedures, equipment, and/or supplies be used. Since the BG does not have access to samples that can be used for evaluation, actual field samples must be used to evaluate process and/or equipment modifications. As a result, the BG may deviate from the requirements specified in this SOP to evaluate potential procedural modifications. Deviations that are isolated to a single sample will be documented on the appropriate sample paperwork. Documentation of deviations that are more global in nature will be kept in a laboratory notebook dedicated for that purpose. All samples to which this statement applies will have a sticker affixed to them referring to this section of the appropriate SOP. In any case, the BG staff will use its best professional judgement to ensure that deviations do not violate the intent of OFR 00-212. If there is concern regarding whether or not the deviation will violate the intent of the OFR, BG staff will consult with the customer and the supervisor of the BG.

13. References

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

13.2. 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.

14. Key Words

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

15. Attachments

Attachment 1 – Preparation, Tracking, and Use of Ethanol

Attachment 2 – 70-percent ethanol mixing and use log

Attachment 3 – 95-percent ethanol OVERFLOW DRUM mixing and use log

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® 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 (Attachment 2).

1.4. Either newly designate or use the existing overflow drum. If a new overflow drum is designated, start a new 95-percent ethanol OVERFLOW DRUM mixing and use log (Attachment 3) 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 95-percent ethanol OVERFLOW DRUM mixing and use log (Attachment 3) 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 95-percent ethanol OVERFLOW DRUM mixing and use log (Attachment 3) for the new overflow drum.

2.5. If the overflow drum contains more that 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 (Attachment 2 or 3).

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 (Attachment 2 or 3).

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

Attachment 2

70-percent ethanol mixing and use log

Date (mm/dd/yyyy)	Initials	Initial concentration of ethanol (%)	Initial volume (gallons)	Ethanol withdrawn (gallons)	Distilled water added (gallons)	Final volume (gallons) (transcribe to next line under initial volume)
		95	55	13	13	55
		70	55			
		70				
		70				
		70				
		70				
		70				
		70				
		70				
		70				
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		70				
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		70				
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		70				
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		70				
		70				
		70				
		70				

Attachment 3

95-percent ethanol OVERFLOW DRUM mixing and use log

Date (mm/dd/yyyy)	Initials	Initial concentration of ethanol (%)	Initial volume (gallons)	95-percent ethanol added (gallons)	Ethanol withdrawn (gallons)	Water added (gallons)	Final volume (gallons) (transcribe to next line under initial volume)
		95	0				