**U.S. GEOLOGICAL SURVEY**
**NATIONAL WATER QUALITY LABORATORY**
**SOP — Laboratory Analytical Method or Procedure**

| SOP # | EFFECTIVE DATE: | PREPARING BENTHIC MACROINVERTEBRATE SAMPLES FOR PROCESSING |
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| BS0331.0 | April 7, 2000 | |
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| WRITTEN BY: S.R. Moulton, II and M. Siebenmann | APPROVED BY: Merle W. Shockey | |

## 1. Scope, Application and Summary

1.1. General Scope of Procedures—Laboratory preparation of a benthic macroinvertebrate (BMI) sample involves washing and sieving procedures to clean the sample prior to sorting organisms from the sample matrix. Preparation procedures described herein can be applied to BMI samples collected in the field using quantitative, semiquantitative, or qualitative sampling methods. These procedures do not include some method-specific preparation steps (for example, size-fractionation in qualitatively processed samples).

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

1.3. Summary of Procedures—A sample must be prepared in the laboratory to remove field preservatives (for example, formalin and ethanol) and fine debris (for example, sand and silt) before methods can be applied to sort and identify organisms. 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 sample matrix.

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

This is a new SOP.

## 3. Health and Safety Warnings

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, rubber gloves, and protective eyewear during sample preparation.

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 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 BG supervisor if the ventilation systems are not working properly.

3.2.2. Use the preservative waste pump system to transfer preservative waste from the fume hood to the storage barrel. Contact the BG Supervisor if the system is not functioning properly. Contact the BG Safety Committee representative when the storage barrel 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 in the laboratory.

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 Safety Program).

3.3. Follow other standard safety guidelines as describe in National Research Council (1995).

**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. Forceps

4.1.1.2. Scoopula®

4.1.1.3. Large plastic spoon

4.1.1.4. Scrub brush

4.1.1.5. Bucket (approximately 2.5 gallons)

4.1.1.6. Plastic wash basins

4.1.1.7. Wash bottle

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

4.1.3. Equipment

4.1.3.1. Standard metal sieves (mesh size equal to field-collection mesh size)

4.2. Interferences

4.2.1. Sample washing is ineffective when too much material is washed at one time, especially if the sample matrix consists of fine detritus or silt. Wash smaller portions of the sample separately and then recombine in a wash basin or another sieve.

4.3. Sample Washing and Sieving

4.3.1. Obtain a standard metal sieve having a mesh size equal to or slightly less than the field-collection mesh size. If the mesh size is unknown, stop work immediately and contact the Production Coordinator.

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

4.3.3. Perform all initial washing in the fume hood over a wash basin. This will allow the sample to be recovered if spilled.

4.3.4. Open the sample container.

4.3.5. Pour the sample into the selected sieve held in the wash basin. If the sample volume is substantial, pour only a small portion of the sample into the sieve. (Note: Verify that the correct mesh-size sieve is being used.)

4.3.6. Transfer waste from the wash basin into the disposal barrel using the pump system.

4.3.7. Carefully wash the sample in the sieve with tap water.

4.3.8. Remove large organic debris (for example, sticks and large leaves) from the sample by rinsing each piece with water and then inspecting it for attached organisms.

4.3.9. Slightly immerse the sieve in a wash basin of water and carefully agitate the sample.

4.3.10. Repeat these procedures until only trace amounts of sand or silt are visible in the wash water.

4.3.11. Transfer all waste and wash water to a disposal barrel by using the pump system.

4.4. Supplemental Swirl and Pour Sample Flotation

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

4.4.2. Perform at sink outside the fume hood after the sample has been washed.

4.4.3. Place the sample in a bucket.

4.4.4. Fill the bucket one-third full of water.

4.4.5. Carefully mix the sample with a spoon to suspend the organic detritus.

4.4.6. Swirl the bucket to keep the organic detritus in suspension.

4.4.7. Slowly pour the suspended organic detritus into the selected sieve held in a wash basin. Stop pouring when the inorganic debris reaches the edge of the bucket.

4.4.8. Repeat these steps until the inorganic debris remaining in the bucket appears free of organic detritus and most organisms; some organisms (for example, mollusks or caddisfly larvae in stone or heavy cases) may remain in the inorganic portion.

4.4.9. Place the inorganic debris in a white sorting tray and process further according the selected method.

4.5. If further processing of a prepared sample cannot continue immediately, preserve the sample in 70-percent ethanol and place the sample in the project cabinet.

4.6. Preparation of 70-percent ethanol from a 55-gallon supply of 95-percent ethyl alcohol.

4.6.1. Pump out 14.5-gallons of 95 percent ethyl alcohol from a new 55-gallon drum of ethyl alcohol. Place in alcohol the overflow drum.

4.6.2. Add 14.5 gallons of distilled water to the new 55-gallon drum of ethyl alcohol.

4.6.3. Place the 14.5-gallons of ethyl alcohol in a reserve storage container.

4.7. Clean-Up

4.7.1. Rinse and clean all sieves, buckets, wash basins, and sorting trays used to prepare the sample.

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4.7.2. Scrub sieves with a brush and rinse from both sides to remove entrained sample debris. Inspect each sieve prior to preparing another sample to ensure that the sieve mesh is free of organisms or debris.

4.7.3. Wipe up water and clean workstation. Put away all supplies and equipment.

4.8. Process the prepared sample according to the method prescribed by the customer. If a standard BG method is used then process according to either SOP No. BS0332.0 or BS0333.0. If a custom method is requested then follow the procedure describe in the customer proposal.

**5. Quality Control and Quality Assurance**

5.1. Inspect all sieves, wash basins, and sorting trays before preparing a sample to ensure tno organisms or sample debris remain from a previously processed sample.

5.2. Verify the mesh-size sieve to be used.

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

5.4. Contact the Production Coordinator immediately if there are any problems with the sample.

**6. Data and Records Management**

6.1. Record the following information in the laboratory record book for each sample prepared.

6.1.1. Sample identification code

6.1.2. Total time to prepare the sample

6.1.3. Problems or errors associated with the sample (for example, sample was spilled or wrong mesh-size sieve was used)

**7. Definitions**

7.1. Flotation—The separation of organic (for example, detritus, woody debris, plant material) from inorganic (for example, sand, gravel) debris in a sample. Typically performed when substantial amounts of inorganic debris is present in the sample. Flotation promotes effective subsampling and removal of organisms from the remaining organic sample matrix.

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

8.2. National Research Council, 1995, Prudent practices in the laboratory—Handling and disposal of chemicals: Washington, D.C., National Academy Press, 427 p.

8.3. 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**

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benthic macroinvertebrate, sample preparation, washing, sieving, flotation