

U.S. Geological Survey
National Water Quality Laboratory

**Qualitative Visual Sort Method for Processing
Benthic Macroinvertebrate Samples**

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Qualitative Visual Sort Method for Processing Benthic Macroinvertebrate Samples

1. Scope, application and summary

- 1.1. Summary of Procedure—The goal of the qualitative visual sort processing method is to produce a comprehensive list of benthic macroinvertebrate (BMI) taxa present in a sample (Moulton and others, 2000). This method can be applied to any BMI sample collected in the field. Samples are visually sorted for up to 2 hours. To increase sorting effectiveness, samples are first size-fractionated to separate coarse and fine detritus. The coarse size fraction is sorted for about 0.25 hours; the fine size fraction is sorted for up to 1.75 hours. Sorting targets mature, undamaged specimens that contribute to successful genus- or species-level taxonomic resolution. Immature or damaged specimens are sorted only if they are likely to represent new taxa. The objective of sorting is to find as many distinct taxa as practical within the 2-hour time limit. Taxa are reported only as present; individual abundances of each taxon are not determined.
- 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. Lab codes supported by this method—2176
- 1.4. Reporting units and levels—Standard Taxonomic Assessment (see NWQL SOP No. BIOB0335.x)
- 1.5. Detection limits—not applicable
- 1.6. Interferences
 - 1.6.1. Sorting effectiveness varies with the type and amount of sample detritus. An excessive amount of organic detritus makes it difficult to observe and/or remove specimens (especially small, cryptic specimens) from the sample detritus.
 - 1.6.2. It is difficult to sort specimens from clumps of algal filaments; the algae must be carefully separated or delicate specimens (for example, mayfly larvae) may be damaged, losing taxonomically valuable body parts such as gills or legs.
 - 1.6.3. In samples with few BMIs or with small amounts of detritus, sorting may be terminated before the 2-hour time limit.

2. Reasons for 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.

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NWQL SOP BIOB0332.3	Analytical Method	Controlled copy
SOP title: Qualitative visual sort method for processing benthic macroinvertebrate samples		
Authors: Scott Grotheer and Robert Hood		Contact: Rob Hood
Effective date: 7 June 2011		
Approved by and date: Duane S. Wydoski, 06/07/2011		Revise or reapprove by: 06/07/2014
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- 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 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 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*

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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

4.1. Record all dates on jar/vial labels in a ‘dd-mm-yyyy’ format, using Roman numerals for the month. For example, July 12, 2004 is recorded as 12-VII-2004.

4.2. Place each portion of the sample in a ventilated metal cabinet designated by the BG Production Coordinator.

4.2.1. Unprocessed Samples

4.2.1.1. Each sample received is washed and re-preserved in 70-percent ethanol or processed within approximately 2 weeks of receipt at the NWQL (See NWQL SOP No. BIOP0331.x). Samples preserved in ethanol can be stored indefinitely.

4.2.1.2. Re-preserved samples are stored in their original container. If the original container is damaged, a suitable alternate may be used.

4.2.2. Sorted Sample Remnant

4.2.2.1. Label the outside of the sample container with the sample identification code; the first initial and last name of the individual who processed the sample, and the date sample processing was completed.

4.2.2.2. Return the sorted sample remnant to its original container. If the original container cannot be used, ensure that the proper sample identification code is on the replacement container.

4.2.2.3. Add enough 70-percent ethanol to cover the remnant sufficiently and secure the container lid.

4.2.2.4. Store the remnant in the designated cabinet.

4.2.3. Unidentified BMIs

4.2.3.1. Place a label appropriate to the taxonomic group (see Section 7.5.4) in each container along with the sample identification code and portion of sample from which it was obtained.

4.2.3.2. Place BMIs in the appropriate container, preserve (for example, fill with 70-percent ethanol), and seal.

4.2.3.3. Place all the vials in a rack labeled with the sample identification code.

4.2.3.4. Place the rack of vials and other associated containers in the designated cabinet.

4.2.4. Identified BMIs

4.2.4.1. Place a label in each container with the determination, first initial and last name of the taxonomist, date, sample identification code, and portion of sample from which it was obtained.

- 4.2.4.2. Place BMIs in the appropriate container, preserve (for example, fill with 70-percent ethanol), and seal.
 - 4.2.4.3. After non-chironomid BMIs have been identified from a sample, put the labeled vials in a rack and place it on a shelf in the designated cabinet with other samples completed that week.
 - 4.2.4.4. Chironomidae identified to family that are not to be mounted should have the added designation “retained” recorded on the label and be placed in the rack with the other identified non-chironomid BMIs.
 - 4.2.4.5. Chironomidae identified to family and designated for mounting should have the added designation “TO MOUNT” recorded on the vial label and be placed in the rack designated for chironomids to be mounted from samples completed that week.
 - 4.2.4.6. Slide mounted chironomids should be stored in slide boxes (see NWQL SOP No. BIOP0334.x) and placed on the shelf following the vial racks for samples completed during the corresponding week.
 - 4.2.4.7. Following QC, a BG representative will add selected vials and/or slides of identified BMI taxa to the reference collection. All non-referenced vials/slides are stored in designated cabinets.
- 4.3. Sample remnants and/or BMIs can be disposed of according to NWQL Policy Memorandum 00.03.

5. Preparation of reagents, standards, and solvents

- 5.1. Tap water
- 5.2. 70-percent ethanol (see NWQL SOP No. BIOP0331.x)

6. Apparatus

- 6.1. Labware
 - 6.1.1. 2-inch wide clear tape
 - 6.1.2. Forceps
 - 6.1.3. Labels for vials, racks, and remnants
 - 6.1.4. Large plastic spoon
 - 6.1.5. Plastic washbasins
 - 6.1.6. Scissors
 - 6.1.7. Screw-cap vials (4–6 dram preferred) with polyseal caps
 - 6.1.8. Scrub brush
 - 6.1.9. Squirt bottle(s) for 70-percent ethanol and/or water
 - 6.1.10. Various sizes/types of containers as needed
 - 6.1.11. Vial racks

- 6.1.12. Watch glasses or other similar glass dishes
- 6.1.13. White sorting trays of various sizes (for example, 15 x 20 cm and 20 x 30 cm)
- 6.2. Equipment
 - 6.2.1. Tally counter
 - 6.2.2. Light source (fiber-optic illuminator or portable incandescent lamp)
 - 6.2.3. Standard metal sieve(s) with mesh size equal to or slightly smaller than the field-collection mesh size (see Section 9.3.1).
 - 6.2.4. Standard 4.75-mm metal mesh sieve

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

- 7.1. Work order/worksheet handling—not applicable
- 7.2. Obtain the Subsampling and Preliminary Enumeration Worksheet (see Attachment 6 in NWQL SOP No. BIOB0335.x), the BMI Identification and Enumeration Bench Data Sheet (see Attachments 8 or 9 in NWQL SOP No. BIOB0335.x), the Slide Preparations – Identification and Enumeration Worksheet (see Attachment 10 in NWQL SOP No. BIOB0335.x), the preprinted vial labels and original sample container(s) for the sample from the BG Production Coordinator or from its designated location(s).
- 7.3. Prepare the sample according to NWQL SOP No. BIOP0331.x.
- 7.4. Size-fractionate the sample. (NOTE: If sample does not contain coarse sample detritus, bypass these steps)
 - 7.4.1. Working over the washbasin, place the sample in the 4.75-mm mesh sieve. If the sample volume is excessive, smaller amounts can be washed incrementally in the sieve (for example, one-fourth of the sample at a time).
 - 7.4.2. Fill a plastic washbasin with enough water to completely cover the sample.
 - 7.4.3. With the sample on the 4.75-mm sieve, gently agitate the sieve within the washbasin to allow fine sample detritus to pass through.
 - 7.4.4. Set the 4.75-mm sieve aside on a tray.
 - 7.4.5. Place a second sieve with mesh size equal to or slightly smaller than the field-collection mesh size in a second washbasin.
 - 7.4.6. Pour the sample detritus from the first washbasin that passed through the 4.75-mm sieve through the second finer mesh sieve into the second washbasin.
 - 7.4.7. Dispose of the water collected in the second washbasin.
 - 7.4.8. If necessary, repeat steps 7.4.1 to 7.4.7 until the entire sample has been size-fractionated. A properly size-fractionated sample consists of two portions: (1) fine sample detritus (detritus passing through the 4.75-mm sieve) and (2) coarse sample detritus (detritus retained by the 4.75-mm sieve).
- 7.5. Sort the sample.

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- 7.5.1. Evenly distribute the coarse sample detritus into one or more trays so that the white bottom of the tray can easily be seen and fill the trays with enough water to cover the sample detritus.
- 7.5.2. Evenly distribute small portions of the fine sample detritus into one or more white bottom sorting trays and fill the trays with enough water to cover the sample detritus. As a general guide, about 50 percent of the white bottom should be visible once the portion is evenly distributed.
 - 7.5.2.1. If distributing the fine sample detritus requires more than the available number of trays, leave the undistributed detritus in the sieve within a washbasin, immerse it in water, and cover it to prevent drying.
 - 7.5.2.2. Obtain small portions of the sample as needed from that sieve.
- 7.5.3. Develop a time budget that allows at least 0.25 hours to examine the coarse sample detritus and evenly apportion the remaining time (up to 1.75 hours) for the fine sample detritus and any elutriated inorganic detritus (see NWQL SOP No. BIOP0331.x)
 - 7.5.3.1. Total sorting time is limited to 2 hours and an effort should be made to use the entire allotted time on all samples.
- 7.5.4. Prepare a labeled rack of vials filled with 70-percent ethanol corresponding to the following taxonomic groupings as needed: Gastropoda, Bivalvia, Oligochaeta, Hirudinea, Acari, Decapoda, Amphipoda/Isopoda, Ephemeroptera, Odonata, Plecoptera, Heteroptera, Megaloptera, Trichoptera, Lepidoptera, Coleoptera, Diptera (excluding Chironomidae), Chironomidae, Other, or Miscellaneous.
- 7.5.5. Systematically visually sort each tray of sample detritus. After sorting a tray, gently rock the tray to redistribute the sample detritus and then quickly re-scan; remove additional specimens if necessary.
 - 7.5.5.1. Place specimens sorted from the sample detritus into vials according to their taxonomic grouping.
 - 7.5.5.2. Select mature, undamaged specimens whenever possible.
 - 7.5.5.3. Sort sufficient numbers of specimens from groups that are difficult to identify to genus or species visually (for example, hydrosychid caddisflies and elmids beetles).
 - 7.5.5.4. Sort empty mollusk shells only if other similar looking shells do not contain soft body parts.
 - 7.5.5.5. Do not sort the following specimens or life stages: vertebrates, arthropod exuvia, branchiobdellids (worm-like crayfish parasites), eggs, microcrustaceans, and terrestrial specimens.
 - 7.5.5.6. Consider life history information for taxa when sorting a sample that contains a range of instars and life stages. This may result in the addition of taxa to the sample data.
 - 7.5.5.7. If possible, sort at least 50 Chironomidae larvae from the entire sample.

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- 7.5.5.7.1. Try to maximize the number of taxa by selecting larvae with as many different combinations of diagnostic characters as possible.
- 7.5.5.7.2. If the sample occupies multiple trays, select a few specimens from each tray to obtain the number required; don't sort all the specimens from just a few of the trays.
- 7.5.5.7.3. Use a tally counter to keep a running total.
- 7.5.5.7.4. Pupal and/or adult chironomidae may be sorted if fewer than 50 larvae are found.

7.6. Clean-Up

- 7.6.1. Inspect all sieves and trays for retained specimens. Return any specimens found to the sample.
- 7.6.2. Rinse and clean all sieves, sorting trays, and washbasins used to process a sample.
- 7.6.3. Scrub sieves with a brush and rinse from both sides to remove entrained sample detritus.
- 7.6.4. Wipe up water and clean workstation.
- 7.6.5. Put away all supplies and equipment.

7.7. Identify the sample

- 7.7.1. Identify all unmounted taxa referring to NWQL SOP No. BIOB0335.x
- 7.7.2. Mount taxa as per NWQL SOP No. BIOP0334.x.
- 7.7.3. Identify all mounted taxa as per NWQL SOP No. BIOB0335.x

8. Data acquisition, processing, and evaluation

Not applicable

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

- 9.1. Work with only one sample at a time.
- 9.2. Individuals sorting qualitative samples need to possess the following training and/or experience
 - 9.2.1. Ability to differentiate, without the aid of magnification, the majority of genera and families of BMI taxa. Special emphasis should be placed on groups with large diversity (for example, Ephemeroptera, Plecoptera, and Trichoptera).
 - 9.2.2. The ability to effectively communicate a summary of the taxa sorted from the sample to the individual that checks their sorting effort.
- 9.3. Before starting method
 - 9.3.1. If the field-collection mesh size is unknown, stop work immediately and contact the BG Production Coordinator.
 - 9.3.2. Inspect all sieves, washbasins, and sorting trays to make sure there are no specimens or sample detritus remaining from a previously processed sample.

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- 9.3.3. If applicable, compare the information on the internal and external sample labels; report any discrepancies to the BG Production Coordinator.
- 9.3.4. Contact the BG Production Coordinator immediately with any problems related to the processing of the sample.
- 9.4. Determine sorting effectiveness for each sample
 - 9.4.1. After sorting at least 25-percent of the sample, a second qualified individual scans the sample for missed or under-represented taxa.
 - 9.4.2. The sample is scanned for about 15 minutes.
 - 9.4.3. Additional sorted specimens are added to the original series of vials.
 - 9.4.4. Suggestions may be made for ways to improve sorting specimens from the remainder of the sample or from future samples.
 - 9.4.5. Corrective Actions
 - 9.4.5.1. Understand and implement recommendation(s) made by the second individual checking the sorting effort.
- 9.5. Taxonomic QC—as described in NWQL SOP No. BIOB0335.x.

10. Data management and records

- 10.1. BMI Identification and Enumeration Bench Data Sheet (see Attachment 8 in NWQL SOP No. BIOB0335.x):
 - 10.1.1. Circle “Qualitative”
 - 10.1.2. The individual that sorted the sample should record their first initial, last name and the date (mm/dd/yyyy) that the sorting was completed.
 - 10.1.3. The second BG staff member that checked the sorting effort (see Section 9.4) should initial in the appropriate space. The date that this check is performed is the same as the date that sorting was completed (see Section 10.1.2), so the date of the check is not necessary.
 - 10.1.4. Problems or errors associated with the sample (for example, sample was spilled or wrong sieve was used). Attach additional sheets as needed.

11. Definitions, abbreviation and acronyms

- 11.1. Detritus—organic and/or inorganic matter of any size present in a BMI sample, excluding the specimens.
- 11.2. Taxonomist—an individual trained in and hired by the BG for identification of BMIs.
- 11.3. Visual sort—Sorting specimens from a sample without the aid of magnification.

12. Deviations

- 12.1. The requirement that a ‘taxonomist’ sort the samples was removed and redefined as a ‘qualified individual’ (see Section 9.2).

12.2. 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

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.

14. Key words

benthic macroinvertebrate, qualitative sample processing, visual-sort method

15. Attachments

None