

# Taxonomic Identification of Benthic Macroinvertebrates

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NWQL SOP #: BIOB0335.2	Analytical Method	Controlled copy
SOP Title: Taxonomic Identification of Benthic Macroinvertebrates		
Authors: Scott Grotheer and Robert Hood		
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It is the user's responsibility to verify that these revision numbers match.		

# U.S. GEOLOGICAL SURVEY

## Taxonomic Identification of Benthic Macroinvertebrates

### 1. Scope, Application and Summary

- 1.1. Summary of procedure—The goal of this method is to standardize procedures used for the taxonomic identification of benthic macroinvertebrates (BMIs). These procedures can be applied to any BMI sample submitted to the National Water Quality Laboratory (NWQL) for analysis. Taxonomic identification of BMIs requires experienced personnel trained in zoological taxonomic principles and possessing a broad knowledge of all aquatic macroinvertebrate groups. Typically, dichotomous keys, which are used to identify specimens, offer a formal, stepwise method for arriving at the name of a specimen based primarily on its morphological characteristics. Progression through the dichotomous key results in classification of the specimen according to a nomenclatural hierarchy (for example, order→family→genus→species) of increasing morphological similarity. Identifying BMIs can require viewing the whole specimen at the low magnification of a dissecting microscope or clearing and mounting the entire specimen (or its parts) on a microscope slide for the viewing at higher magnification of a compound microscope.
- 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 covered by this method—2172 and 2176
- 1.4. Reporting units and levels—Standard Taxonomic Assessment (STA) and Rapid Taxonomic Assessment (RTA) (as defined in this SOP and Moulton and others, 2000)
- 1.5. Detection limits—not applicable.
- 1.6. Interference
  - 1.6.1. Identification to a recommended level (for example, genus or species) may not be possible if the specimen is immature or damaged. In this case, identify the specimen to the most reliable level, usually family or order, and note why the recommended level could not be achieved (see Attachment 1).
  - 1.6.2. Dirt and debris can obscure diagnostic morphological structures (for example, setae), which are necessary for identification. Using a sable brush, forceps, or sonicator can often remove debris from specimens.

### 2. Reasons for Revision and Summary of Changes

- 2.1. This SOP is being updated to the new format as per NWQL SOP No. QUAX0001.x.
- 2.2. References to other NWQL SOPs and Technical/Policy Memoranda are being corrected.
- 2.3. Records management section is being updated to reflect current standards.
- 2.4. Additional quality control checks are being added.

### 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 protective eyewear and nitrile gloves when preparing reagents, standards and solvents.
- 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. Maintain an erect sitting posture while working at a microscope.
- 3.1.7. Follow other safety procedures described in the U. S. Geological Survey (USGS) Occupational Hazards and Safety Procedures Handbook (September 1999).
- 3.2. Waste Disposal and Environmental Compliance
  - 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 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).
  - 3.2.5. Follow the guidelines set forth in the Safety, Health, and Environmental Compliance Policy Memorandum 2006.01 (<http://wwwnwql.cr.usgs.gov/USGS/policy/policy.06-01.doc>).
- 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://wwwnwql.cr.usgs.gov/USGS/policy/policy.04-01.html>). Refer to NWQL SOP No. SHEX0355.x, Waste Disposal at the National Water Quality Laboratory, for further information.

**4. Sample Preservation, Handling, Containers, Analytical Processing/Holding Times, Cautions and Disposal**

- 4.1. Refer to NWQL SOP No. BIOB0332.x and NWQL SOP no. BIOB0333.x.

**5. Preparation of Reagents/Standards/Solvents**

- 5.1. 70-percent ethanol
  - 5.1.1. Prepared according to the procedure in NWQL SOP No. BIOP0331.x.
  - 5.1.2. Taxonomists are responsible for filling their own squirt-bottle supply as needed. All squirt bottles must be labeled as 70-percent ethanol.

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## 5.2. Glycerin

5.2.1. Stock supply kept in BG laboratory. Taxonomists may keep small quantities at their workstations in eyedropper bottles.

5.2.2. There are no hazard warnings for this chemical.

## 5.3. Aqueous potassium hydroxide (KOH)

5.3.1. A supply in pellet form is located in the chemical storage locker located beneath the fume hood in the BG laboratory.

5.3.2. Preparation of KOH solution for clearing BMI tissues:

5.3.2.1. Wear safety glasses and rubber gloves when preparing KOH solution.

5.3.2.2. Dissolve 3 to 5 pellets in about 10 mL of tap or distilled water.

5.3.3. Label container (for example, a scintillation vial) with "KOH" if clearing overnight at room temperature.

## 6. Apparatus

### 6.1. Labware

6.1.1. 2-inch wide clear tape

6.1.2. Forceps

6.1.3. Probes

6.1.4. Watch glasses or other similar glass dishes

6.1.5. 2-inch wide clear tape

6.1.6. Scissors

6.1.7. Screw-cap vials (4–6 dram preferred) with polyseal caps

6.1.8. Scintillation vials

6.1.9. Vial racks

6.1.10. Shell vials (1/4 dram)

6.1.11. Genitalia microvials

6.1.12. Cotton

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

6.1.14. Pigma pen (#01, 0.28 mm), India ink pen, or pencil.

6.1.15. Fine sable-hair brush

6.1.16. Pipette

6.1.17. 0.5 cc syringe

### 6.2. Equipment

6.2.1. Tally counter

6.2.2. Compound microscope (40 to 1000X magnification)

6.2.3. Binocular microscope (6 to 50X magnification)

6.2.4. Fiber-optic illuminator

6.2.5. Sonicator

## 7. Analysis

### 7.1. Taxonomic Information Resources

7.1.1. Confirm identifications by consulting descriptions, reviews and revisions of taxa, monographs of regional faunas, and distributional checklists.

7.1.2. Use taxonomic literature located in BG laboratory. Mouton and others (2000) provide a partial list of the major references used.

7.1.3. Consult taxonomic information (for example, checklists) on the Internet as necessary.

7.1.4. Know the limitations of the taxonomic literature being used. Apply caution when using unpublished information or literature not subjected to peer-review.

### 7.2. Reference Collection

7.2.1. A reference collection of specimens representing taxa found in BMI samples is maintained in a designated area of the BG laboratory.

7.2.2. Specimens added to the reference collection should either be verified by a second senior BG taxonomist or by a recognized specialist.

7.2.3. When necessary, confirm identifications by comparing specimens to taxa in the BG reference collection.

### 7.3. Taxonomic Specialists

7.3.1. Consult external taxonomic specialists as necessary to assist with problematic taxonomic issues or to confirm identifications. This is required for all BMI taxa classified as state or federal threatened or endangered species.

7.3.2. Copy all correspondence with external taxonomic specialists to other BG taxonomists.

7.3.3. External taxonomic specialists must be recognized experts in their area of taxonomic interest and have a demonstrated record of peer-reviewed publications in taxonomy, systematics, and/or biogeography of BMIs.

### 7.4. Levels of Taxonomic Assessment

7.4.1. The taxonomist will identify specimens in a STA to the following recommended levels listed in Attachment 1 provided the specimens are mature enough and undamaged.

7.4.2. The taxonomist will identify specimens in a RTA to the following recommended levels listed in Attachment 2 provided the specimens are mature enough and undamaged.

### 7.5. Identify the Sample

7.5.1. Transfer specimen(s) from their container into a dish containing 70-percent ethanol.

7.5.2. Identify and/or enumerate each specimen.

7.5.2.1. Identify specimens to the level of taxonomic assessment specified by the project or customer (see Section 7.4).

7.5.2.2. Place different life stages for the same taxon in separate vials.

7.5.2.3. If necessary, clear specimens in KOH.

- 7.5.2.3.1. These steps should be performed in a fume hood.
- 7.5.2.3.2. Wear safety glasses and rubber gloves.
- 7.5.2.3.3. Place the whole specimen or a dissected part of a specimen (for example, abdomen from a caddisfly pupa or adult) into about 10 mL of 10-percent KOH solution (see Section 5.3) in a 50-mL beaker.
- 7.5.2.3.4. Warm the KOH solution gently on a hot plate for several minutes. Avoid boiling the KOH solution. Clearing time will vary depending on the size of the specimen or structure being cleared (for example, a small hydroptilid caddisfly may require 2 to 3 minutes; a large limnephilid caddisfly abdomen may take 5 to 10 minutes to clear).
- 7.5.2.3.5. Alternatively, clearing can be performed overnight, by placing a specimen in a sealed vial of KOH at room temperature.
- 7.5.2.3.6. Check the specimen to determine whether additional clearing time is required.
- 7.5.2.3.7. Remove the specimen or structure from the KOH using forceps and place it in a dish of 70-percent ethanol.
- 7.5.2.3.8. Flush dissolved body tissues from dissected insect abdomens by inserting a 0.5 cc syringe filled with 70-percent ethanol. Inject ethanol into the open end of the abdomen to flush out dissolved tissues.
- 7.5.2.4. Identify and enumerate the immature stages (larvae or nymphs) of all complete aquatic BMIs.
  - 7.5.2.4.1. Do not identify and enumerate BMI fragments unless a head is present.
  - 7.5.2.4.2. In general, BMI's without heads are not identified and enumerated; however,
    - 7.5.2.4.2.1. In qualitative samples, mollusks may be identified based on an empty shell; this should only be done if the taxon is not already represented in the sample by an entire specimen.
    - 7.5.2.4.2.2. In quantitative samples, identify Bryozoa only if the zooid is present. Since these specimens are colonial do not attempt to enumerate them; record "1" to indicate presence in the count column.
    - 7.5.2.4.2.3. In qualitatively processed samples, identify Bryozoa using the entire specimen or stalk fragments (missing zooids).
- 7.5.2.5. Identify and enumerate the mature stages (adults or pupae) of all complete aquatic BMIs.
  - 7.5.2.5.1. When necessary, use morphological characters located in the terminal abdominal segments (for example, genitalia) to identify pupae and adults. In most cases, these segments must be present to achieve low-level taxonomic resolution (for example, genus or species). For this reason, insect pupae and adults can be identified and enumerated when at least the terminal abdominal segments and some portion of the thorax are present.

- 7.5.2.5.2. To avoid a potentially redundant record, head and thorax combinations from pupae and adult insects are only enumerated if at least some of the anterior abdominal segments are present as well.
- 7.5.2.5.3. Do not attempt to match fragments with the remainder of the body.
- 7.5.3. Preserve the specimens of each identified taxon in an appropriately labeled container (refer to NWQL SOP No. BIOB0332.x or NWQL SOP No. BIOB0333.x).
- 7.5.4. Place specimen parts that are cleared, dissected or inadvertently fragmented during identification in a ¼ dram shell vial or genitalia microvial containing 70-percent ethanol and plug with cotton. Place the shell vial or microvial inside the primary taxon vial. Place larval sclerites from pupal metamorphotypes in either the puparium or a microvial.
- 7.5.5. When appropriate, use a standardized conditional or provisional designation listed in Attachment 3 to convey as much taxonomic information as possible.
- 7.5.6. When appropriate, use a standardized note list in Attachment 4 to justify a determination when the requested level of identification is not achieved.
- 7.5.7. When appropriate, use a standardized note listed in Attachment 5 to convey additional information about the identification
- 7.5.8. When appropriate, designate identified specimens as reference taxa for inclusion in the BG reference collection.
  - 7.5.8.1. Select specimens that are mature, intact, and, when possible, are available in a series (several specimens of a taxon) from a particular sample. Specimens may be selected despite their condition if they represent the only or first verifiable record of a particular taxon.
  - 7.5.8.2. When possible, place the specimen(s) in a patent lip vial and seal with an Ethylene Propylene Diene Monomer (EPDM) stopper. Have a second senior taxonomist verify the identification. The second taxonomist should also place a determination label in the vial; this label should include the identification, the taxonomist's first initial and last name, and the date (for example, October 8, 2004 is recorded as 8-X-2004).
  - 7.5.8.3. If a patent lip vial and EPDM stopper cannot be used, select an appropriate container and label as in Section 7.5.8.2.
  - 7.5.8.4. If mounted specimens are selected, record the determination and position(s) on the slide label(s). Have a second senior taxonomist initial on the label(s) to indicate agreement with the determination.
- 7.5.9. Compare the taxonomic names on the completed bench data sheet to those recorded on the vial labels for the sample to ensure that a corresponding vial exists for each entry.
- 7.5.10. Confirm that the BMI Identification and Enumeration Bench Data Sheet (see Attachments 6, 7, and 8) and other associated paperwork from the sample are complete.
- 7.6. Special procedures used prior to mounting Chironomidae larvae from samples sorted qualitatively using NWQL SOP No. BIOB0332.x.
  - 7.6.1. All chironomid larvae are slide mounted when 50 or fewer larvae were sorted.
  - 7.6.2. If more than 50 chironomid larvae were sorted from the sample, separate 50 larvae from the group.

7.6.2.1. This step is performed using a dissecting microscope

7.6.2.2. Try to maximize the number of taxa by selecting 50 larvae with as many different combinations of diagnostic characters as possible. For example, see Table 1.

Table 1. Gross morphological characteristics for the major Chironomidae (Diptera) subfamilies.

Subfamily	Morphological character			
	Antennae	Ligula	Ventromental plates	Shape of head capsule
Chironominae	non-retractile	absent	well developed/ striated	round
Diamesinae	non-retractile/ annulated	absent	reduced	round/square
Orthocladiinae	non-retractile	absent	reduced	round/square
Tanypodinae	retractile	present	absent	square

7.7. Special Procedures when identifying specimens mounted on microscope slides.

7.7.1. Obtain paperwork for each sample from the bin designated by the BG Production Coordinator.

7.7.2. Obtain the slide mounted material and the corresponding Slide Preparations — Identification and Enumeration Worksheet (see Attachment 9) for each sample to be identified.

7.7.3. Verify that sample identification codes for the slides and the worksheet match.

7.7.4. Locate the first specimen under the first cover glass using scanning magnification.

7.7.5. Identify specimens, in order, as they appear under the cover glass(es).

7.7.5.1. For midges mounted following NWQL SOP No. BIOP0334.x, when the label can be read right side up, the first specimen is the left-most specimen under the left-most cover glass.

7.7.5.2. For oligochaeta mounted following NWQL SOP No. BIOP0334.x, when the label can be read right side up, the first specimen is the top-most specimen under the left-most cover glass.

## 8. Data Acquisition, Calculations and Data Evaluation/Reduction

8.1. Quantitative and semi-quantitative data are electronically corrected for laboratory subsampling according to methods described in NWQL SOP No. BIOB0333.x.

## 9. Quality Control and Quality Assurance

9.1. General

9.1.1. Record dates on 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.

9.1.2. Clean microscopes, objective lenses, and light sources as necessary.

9.1.3. Return all literature to the proper filing cabinet or shelf.

9.1.4. Work on only one sample at a time.

- 9.1.5. Do not mix taxa from different samples in the same petri dish except for making side-by-side comparisons.
- 9.1.6. Use extreme care when handling reference specimens to prevent damage.
- 9.1.7. Consider all lines of morphological and distributional evidence available when identifying a taxon for the first time; use the BG reference collection and consult with other taxonomists in the laboratory as needed.

9.2. Enumeration

- 9.2.1. The enumeration criteria should be evaluated prior to mounting any BMIs on slide, so that the sample can be completely recombined and reprocessed if necessary.
- 9.2.2. There will be differences between the total number of BMIs reported by the technician that sorted the sample and the taxonomist that identified the sample. Documenting all of this variation is not the intent of these checks. The intent of these checks is to discover significant enumeration differences between the technician and the taxonomist that might have resulted in different processing decisions during the sorting phase. This is a minimum count method, so unless it is determined that significant savings in time can be gained, gross errors have been made, or the significant differences persist, the recommendations will be limited to future modifications of the technician's procedures.
- 9.2.3. If 1-stage subsampling was performed and the following is true, consult with an individual designated by the BG production coordinator to determine if the sample should be reprocessed. See Table 2 for general guidance

$$T < Q - \left[ \frac{Q}{X} \right]$$

where,  $Q$  = the total specimens identified by the taxonomist from the stage 1 grids

$T$  = 5-percent beyond the fixed count minimum for the sample as defined in NWQL SOP No. BIOB0333.x

$X$  = total number of grids sorted from the stage-1 subsampling frame as in NWQL SOP No. BIOB0333.x

- 9.2.4. If 2-stage subsampling was performed and the following is true, consult with an individual designated by the BG production coordinator to determine if the sample should be reprocessed. See Table 2 for general guidance.

$$T < Q - \left[ \frac{Q}{Z} \right]$$

where,  $Q$  = the total specimens identified by the taxonomist from the stage 1 grids

$T$  = 5-percent beyond the fixed count minimum for the sample as defined in NWQL SOP No. BIOB0333.x

$Z$  = total number of grids sorted from the stage-2 subsampling frame as in NWQL SOP No. BIOB0333.x

Table 2. Minimum total number of specimens indicating action might be warranted for common final grid counts.

<sup>1</sup>action level differs from the formula due to the fact that it is assumed that either 150 or 300 organisms would have been sorted from either the 1<sup>st</sup> or 2<sup>nd</sup> grid.

Final Grids Sorted	Action Level
3	450 <sup>1</sup>
4	420
5	394
6	378
7	368

9.2.5. If either of the previous is true it is important to evaluate and attempt to correct any issues that resulted in the error(s).

9.2.6. If the total specimens identified by the taxonomist from either the stage 1 grids or stage 2 grids, is greater than 5-percent below the method's fixed-count minimum (for example, less than 285 for the 300 fixed-count method),

9.2.6.1. Attempt to obtain additional organisms from the sorted sample remnant.

9.2.6.2. If this cannot be achieved, the sample must be recombined and reprocessed.

### 9.3. Taxonomic Identifications

9.3.1. A second taxonomist verifies identifications that are deposited in the BG reference collection or that are selected during the weekly internal QC process.

9.3.2. A block of samples, for the purposes of internal QC, is defined as all samples for which the non-midge determinations have been completed by the close of business on Friday of each week.

9.3.3. When identifications are complete for the block of samples, enter the data into the BG database as needed.

9.3.4. Taxonomic names in the data file are compared with a master taxa list maintained by the BG to ensure that all taxonomic names are consistently recorded. Discrepancies between the master list and the data file are resolved.

9.3.5. Taxa are selected for internal QC checks.

9.3.5.1. Taxa new to the BG master taxa list are selected.

9.3.5.2. Taxa/life-stage combinations that are new from an individual BG taxonomist are selected.

9.3.5.3. Taxa/life-stage combinations generated by a BG taxonomist that have not been verified in the last 3 years from that taxonomist are selected.

9.3.5.4. Monotypic genera or genera with only one known North American species that are only identified to genus are selected.

9.3.5.5. Ten percent of the taxa remaining in the block are randomly selected.

9.3.6. Check selected taxa to ensure that they were properly identified. All specimens in the vial and all slide-mounted specimens representing the selected taxa are checked. In

quantitatively processed samples, specimens are also re-enumerated. Incorrect determinations and/or enumerations are recorded on the Random Taxonomic QC Worksheet.

- 9.3.7. Place a QC verification label in each vial reviewed as part of the internal QC process; these labels contain the first initial and last name of the taxonomist who verified the determination(s) and the date of the verification(s) (see Section 9.1.1).
- 9.3.8. The initials of the second taxonomist are placed in the cell of the Random Taxonomic QC Worksheet associated with each selected determination and/or enumeration.
- 9.3.9. Communicate the results of the internal taxonomic QC evaluation to each taxonomist and summarize to the BG staff as needed.
- 9.3.10. Consult with the original taxonomist when problems are discovered and correct them by updating both the original paperwork and electronic file(s). Problems corrected after data release might only be documented electronically and/or on the labels for the impacted specimen(s); the original paperwork might not be corrected.

## 10. Data Management and Records Management

- 10.1. Record identification results legibly on the BMI Identification and Enumeration Bench Data Sheet (see Attachments 6, 7, and 8). If the data are entered electronically print out a copy of the data and write: "see attached" in the appropriate section(s) of the BMI Identification and Enumeration Bench Data Sheet (see Attachments 6, 7, and 8). Regardless of the mode of entry, the following information is to be recorded and available in hardcopy when done identifying the sample:
  - 10.1.1. The correction factor entered on the Fixed-Count Processing—Subsampling and Preliminary Enumeration Worksheet (see NWQL SOP No. BIOB0333.x and Attachment 10 of this SOP) should be confirmed and also entered on this data sheet to facilitate data entry.
  - 10.1.2. All BMI identifications in the 'Taxon' column. If the specimens are identified to species, provide the authority (for example, *Hydropsyche simulans* Ross). Record the species epithet for a monotypic genus or one for which there is only one known North American species. When recording conditional or provisional designations follow the conventions presented in Attachment 3.
  - 10.1.3. The life stage of each taxon, if applicable, in the 'LS' column. Standard life-stage notations are as follows: **L** is larva(e); **P** is pupa(e); or **A** is adult(s). Use **L** to designate nymphal life stages as well. Record multiple life stages for the same taxon on separate lines.
  - 10.1.4. Supporting taxonomic note(s) (see Attachment 4 and Attachment 5) where applicable. Use a semi-colon to separate multiple notes for a single taxon (for example, imm.; dam.)
  - 10.1.5. Specimen counts; recorded in the appropriate column.
    - 10.1.5.1. In qualitatively processed samples (for example, NWQL SOP No. BIOB0332.x), even when multiple specimens are present, record a "1" in the pre-labeled 1:1 correction factor column.
    - 10.1.5.2. In quantitatively processed samples (for example, NWQL SOP No. BIOB0333.x), record the actual number of specimen identified for each taxon. Specimen counts from the large-rare scan are recorded in the pre-labeled 1:1

column. Specimen counts from the grid subsamples are recorded in the column that corresponds to the appropriate subsampling correction factor.

- 10.1.6. The individual that identified the non-midge taxa should initial in the appropriate space after inventorying the vials (see Section 7.5.9)
  - 10.1.7. The individual that identified the non-midge taxa should record their first initial, last name, and the date (mm/dd/yyyy) this step was completed.
  - 10.1.8. Block Code for the week that the non-midge identifications are completed. For example, if the sample is completed during the week ending Friday, October 1, 2004, record 20041001.
  - 10.1.9. Circle either 'YES' or 'NO', depending on whether data is continued on the back of the sheet as appropriate.
  - 10.1.10. Problems or errors associated with the sample (for example, sample was spilled or wrong sieve was used). Attach additional sheets as needed.
- 10.2. After completion of identifications of the unmounted material, place the completed packet of datasheets and worksheets in the bin designated by the BG Production Coordinator.
- 10.3. Record identification results legibly on the Slide Preparations — Identification and Enumeration Worksheet (see Attachment 9). The following information is recorded:
- 10.3.1. Record the determination for the first taxon (see Section 10.1.2) on the line numbered "1" in the right-hand section of the worksheet. Record its life stage (see Section 10.1.3) and appropriate notes (see Sections 10.1.4) on the line as well. Enter a corresponding circled "1" in the first cell of the left-hand section of the worksheet. Each additional specimen that receives the same determination as that recorded on line #1 should have an uncircled "1" recorded in the cell that corresponds to its position on the slide. The second unique determination should be recorded on line #2 and a circled "2" should be recorded in the cell that corresponds to its position on the slide; subsequent 2's should not be circled.
  - 10.3.2. Continue by recording each unique determination on a new line, indicating in which position(s) on which slide(s) it is found by recording its corresponding line number in the appropriate cell(s), until all specimens have been identified. The first occurrence of each number in the left-hand section should always be circled.
  - 10.3.3. To enumerate (see Section 10.1.5), start with each circled number and count the number of occurrences of that number. Record the total occurrences of each number on the corresponding line in the right-hand section of the worksheet.
  - 10.3.4. First initial and last name of the individual that identified the chironomids and the date (mm/dd/yyyy) that the chironomid identification was completed.
  - 10.3.5. Initials of the individual that identifies the chironomids and the date (mm/dd/yyyy) should be entered in the appropriate space at the bottom of the sheet regarding the acceptability of the mounted material. Note any problems on the back of the form or in the notes section.
  - 10.3.6. Circle 'YES' or 'NO' as appropriate if the taxa list continues on the back of the form.
- 10.4. After completing identification of the mounted material, place the completed packet of datasheets in the bin designated by the BG Production Coordinator so that data entry can be completed.

- 10.5. After completing data entry, the packet(s) are placed in a bin designated by the BG Production Coordinator awaiting internal QC checks.
- 10.6. Following QC and prior to data release, the original paperwork packet(s) for each sample or block of samples is filed, along with samples completed during the corresponding week, in a location designated by the BG Production Coordinator until all samples for a project are completed.
- 10.7. Following data release, the original paperwork packet for each sample is stored by project and labeled with the name of the project and the applicable year(s) during which the sample(s) were collected.
- 10.8. Maintain all paperwork on file in the BG for a period at least as long as the voucher specimens for the sample(s) are stored at the BG.
- 10.9. Back up electronic BMI data routinely.

## 11. Definitions

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

## 12. Deviations

- 12.1. When performing either RTA or STA, the recommended level of identification for water mites (Hydrachnidia) was changed from 'Order' to 'Subclass' (Acari) (see Attachment 1).
- 12.2. When performing STA, the recommended level for Capniidae (Plecoptera) was changed from 'Genus' to 'Family/Genus' (see Attachment 1).
- 12.3. When performing STA, the recommended level for Athericidae (Diptera) was changed from 'Genus' to 'Genus/Species' (see Attachment 1).
- 12.4. When performing STA, the recommended level for Sciomyzidae (Diptera) was changed from 'Genus' to 'Family/Genus' (see Attachment 1).
- 12.5. Internal taxonomic QC protocol updated (see Section 9.3).

## 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. National Research Council, 1995, Prudent practices in the laboratory—Handling and disposal of chemicals: Washington, D.C., National Academy Press, 427 p.
- 13.3. 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>

## 14. Key Words

benthic macroinvertebrate, taxonomic identification, enumeration, determination, reference collection, standard taxonomic assessment, rapid taxonomic assessment

## 15. Attachments

NWQL SOP #: BIOB0335.2	Analytical Method	Controlled copy
SOP Title: Taxonomic Identification of Benthic Macroinvertebrates		
Authors: Scott Grotheer and Robert Hood		
Effective Date: 07/30/2008		
Approved by and date of approval: David Reppert, 07/30/2008. Revision / reapproval due by 07/30/2011		
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It is the user's responsibility to verify that these revision numbers match.		

- Attachment 1 — Recommended levels of identification for STA
- Attachment 2 — Recommended levels of identification for RTA
- Attachment 3 — Standardized conditional or provisional designations
- Attachment 4 — Standardized notes used to justify a determination
- Attachment 5 — Standardized notes used to convey additional information
- Attachment 6 — Example Invertebrate Identification and Enumeration Bench Data Sheet (quantitative samples)
- Attachment 7 — Example Invertebrate Identification and Enumeration Bench Data Sheet (qualitative samples)
- Attachment 8 — Example Invertebrate Identification and Enumeration Bench Data Sheet (field large-rare)
- Attachment 9 — Example Slide Preparations – Identification and Enumeration Worksheet
- Attachment 10 — Example Invertebrate Processing -- Subsampling and Preliminary Enumeration Worksheet

**Attachment 1**  
**Recommended levels of identification for STA**

Taxon	Level of identification
Porifera	Family
Cnidaria	Family
Platyhelminthes	Class
Nematoda	Phylum
Nemertea	Genus
Nematomorpha	Phylum
Bryozoa	Phylum
Gastropoda	Genus
Bivalvia	Genus
Polychaeta	Family
Aphanoneura	Family
Oligochaeta	Family
Hirudinea	Family
Acari	Subclass
Amphipoda	Genus
Isopoda	Genus
Decapoda	Genus
Collembola	Order
<b>Ephemeroptera</b>	
Acanthametropodidae	Genus/Species
Ameletidae	Genus
Ametropodidae	Genus/Species
Arthropleidae	Genus/Species
Baetidae	Genus/Species
Baetiscidae	Genus/Species
Behningiidae	Genus/Species
Caenidae	Genus/Species
Ephemeridae	Genus/Species
Ephemerellidae	Genus/Species
Heptageniidae	Genus/Species
Isonychiidae	Genus
Leptohyphidae	Genus/Species
Leptophlebiidae	Genus/Species
Metretopodidae	Genus/Species
Neophemeridae	Genus/Species
Oligoneuriidae	Genus/Species
Polymitarcyidae	Genus/Species
Potamanthidae	Genus/Species
Pseudironidae	Genus/Species
Siphonuridae	Genus/Species
<b>Odonata</b>	
Calopterygidae	Genus/Species
Coenagrionidae	Genus/Species

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<b>Taxon</b>	<b>Level of identification</b>
Lestidae	Genus/Species
Protoneuridae	Genus/Species
Aeshnidae	Genus/Species
Cordulegastridae	Genus
Corduliidae	Genus/Species
Gomphidae	Genus/Species
Libellulidae	Genus/Species
Macromiidae	Genus/Species
Petaluridae	Genus/Species
<b>Plecoptera</b>	
Capniidae	Family/Genus
Chloroperlidae	Genus
Leuctridae	Genus
Nemouridae	Genus
Peltoperlidae	Genus
Perlidae	Genus/Species
Perlodidae	Genus/Species
Pteronarcyidae	Genus/Species
Taeniopterygidae	Genus
<b>Heteroptera</b>	
Belostomatidae	Genus/Species
Corixidae	Genus
Gelastocoridae	Genus
Gerridae	Genus/Species
Hebridae	Genus
Hydrometridae	Genus
Macroveliidae	Species
Mesoveliidae	Genus
Naucoridae	Genus
Nepidae	Genus/Species
Notonectidae	Genus
Ochteridae	Genus
Pleidae	Genus
Saldidae	Genus
Veliidae	Genus
<b>Megaloptera</b>	
Corydalidae	Genus/Species
Sialidae	Genus
<b>Neuroptera</b>	
Sisyridae	Genus
<b>Trichoptera</b>	
Apataniidae	Genus/Species
Beraeidae	Genus
Brachycentridae	Genus/Species
Calamoceratidae	Genus/Species
Dipseudopsidae	Genus

<b>Taxon</b>	<b>Level of identification</b>
Ecnomidae	Genus/Species
Glossosomatidae	Genus/Species
Goeridae	Genus/Species
Helicopsychidae	Genus/Species
Hydrobiosidae	Genus/Species
Hydropsychidae	Genus/Species
Hydroptilidae	Genus/Species
Lepidostomatidae	Genus
Leptoceridae	Genus/Species
Limnephilidae	Genus/Species
Molannidae	Genus/Species
Odontoceridae	Genus/Species
Philopotamidae	Genus
Phryganeidae	Genus/Species
Polycentropodidae	Genus/Species
Psychomyiidae	Genus/Species
Rhyacophilidae	Genus/Species
Rossianidae	Genus/Species
Sericostomatidae	Genus/Species
Uenoidae	Genus/Species
Xiphocentronidae	Genus/Species
<b>Lepidoptera</b>	
Arctiidae	Genus
Cosmopterigidae	Genus
Nepticulidae	Genus
Noctuidae	Genus
Pyralidae	Genus
Tortricidae	Genus
<b>Coleoptera</b>	
Amphizoidae	Genus
Anthicidae	Family
Carabidae	Family
Chrysomelidae	Family
Curculionidae	Family
Dryopidae	Genus/Species
Dytiscidae	Subfamily/Tribe/Genus
Elmidae	Genus/Species
Epimetopidae	Genus
Georyssidae	Genus
Gyrinidae	Genus/Species
Halplidae	Genus
Helophoridae	Genus
Heteroceridae	Family
Histeridae	Family
Hydraenidae	Genus
Hydrochidae	Genus
Hydrophilidae	Genus
Hydroscaphidae	Species
Lampyridae	Family

Taxon	Level of identification
Limnichidae	Genus
Lutrochidae	Genus/Species
Melyridae	Family
Microsporidae	Genus
Noteridae	Genus
Ptilidae	Family
Psephenidae	Genus
Ptilodactylidae	Species
Salpingidae	Family
Scirtidae	Family
Staphylinidae	Family
Tenebrionidae	Family
<b>Diptera</b>	(pupae to Suborder/Family)
Athericidae	Genus
Blephariceridae	Genus
Canacidae	Genus
Ceratopogonidae	Genus (pupae to Family)
Chaoboridae	Genus
Chironomidae	Subfamily/Tribe/Genus (pupae to Subfamily; adults to Family)
Corethrellidae	Genus
Culicidae	Genus
Deuterophlebiidae	Genus
Dixidae	Genus
Dolichopodidae	Family
Dryomyzidae	Genus
Empididae	Genus
Ephydriidae	Family
Muscidae	Family
Nymphomyiidae	Genus
Pelecorhynchidae	Genus
Phoridae	Family
Psychodidae	Genus
Ptychopteridae	Genus
Sarcophagidae	Family
Scathophagidae	Family
Sciomyzidae	Family/Genus
Simuliidae	Genus
Stratiomyidae	Genus
Syrphidae	Family
Tabanidae	Genus
Tanyderidae	Family
Thaumaleidae	Family
Tipulidae	Family/Genus

**Attachment 2**  
**Recommended levels of identification for RTA**

Taxon	Level of Identification
Porifera	Family
Cnidaria	Family
Platyhelminthes	Class
Nematoda	Phylum
Nemertea	Genus
Nematomorpha	Phylum
Bryozoa	Phylum
Gastropoda	Family
Bivalvia	Family
Polychaeta	Family
Aphanoneura	Family
Oligochaeta	Family
Hirudinea	Family
Acari	Subclass
Amphipoda	Family
Isopoda	Family
Decapoda	Family
Insecta (except Collembola)	Family
Collembola	Order

### Attachment 3

#### Standardized conditional or provisional designations

Designation	Description
sp.	<ul style="list-style-type: none"> <li>Species place holder for identifications to Genus-level only</li> <li>Denotes both singular and plural forms of species</li> <li>Example: <i>Hydropsyche</i> sp.</li> </ul>
sp. nr.	<ul style="list-style-type: none"> <li>Means "species near"</li> <li>Refers to a potentially undescribed species nearest to the species/authority following the designation</li> <li>Example: <i>Hydropsyche</i> sp. nr. <i>simulans</i> Ross</li> </ul>
cf.	<ul style="list-style-type: none"> <li>Means "confer"</li> <li>Refers to a species that closely matches the species/authority following the designation but differs morphologically in some minor ways or the description in the literature is too vague or incomplete to be certain</li> <li>Example: <i>Hydropsyche</i> cf. <i>simulans</i> Ross</li> </ul>
/ "slash"	<ul style="list-style-type: none"> <li>Used to denote two or more taxa that are unresolvable or where only two species are known in a monophyletic group</li> <li>Placed between the taxa in question</li> <li>Taxa are ordered alphabetically</li> <li>If Species, authorities are included</li> <li>Example: <i>Hydropsyche rossi</i> Flint, Voshell, and Parker/<i>simulans</i> Ross</li> </ul>
sp. 1 or sp. A genus A	<ul style="list-style-type: none"> <li>Refers to provisional taxa reported in the literature where their specific identity remains unknown; also known as "operational taxonomic units" or "OTUs"</li> <li>Provisional designation is reported exactly as it appears in the literature</li> <li>Provisional designation is followed parenthetically by the author(s) and year of the publication</li> <li>Example: <i>Oecetis</i> sp. A (Floyd, 1995)</li> </ul>
group	<ul style="list-style-type: none"> <li>Denotes a group of more than two closely related species that cannot be separated or specimens that can be reliably placed in a species group where determination to species is unsupported</li> <li>If only two species in the group, then use "/" or slash designation</li> <li>Is formally recognized in the literature</li> <li>Example: <i>Hydropsyche scalaris</i> group</li> </ul>
complex	<ul style="list-style-type: none"> <li>Denotes a species for which there may be considerable variation suggesting two or more cryptic species</li> <li>Is formally recognized in the literature</li> <li>Example: <i>Oecetis inconspicua</i> complex</li> </ul>
n. sp.	<ul style="list-style-type: none"> <li>Means "new species"</li> <li>Represents a species new to science that has been verified by a recognized authority or one that appears in the literature as such</li> <li>If the designation appears in the literature, the designation must be followed parenthetically by the authors and year of the publication</li> <li>Example: <i>Hydroptila</i> n. sp. (Moulton and Stewart, 1997)</li> </ul>
Other conditional or provisional designations	<ul style="list-style-type: none"> <li>Reported exactly as they appear in the reference from which they were obtained</li> <li>The designation is followed parenthetically by the author(s) and year of the publication</li> <li>Example: <i>Stilocladius?</i> sp. (Epler, 1995)</li> </ul>

**Attachment 4**  
**Standardized notes used to justify a determination**

Note	Description
imm.	<ul style="list-style-type: none"> <li>• Means “immature” and includes all synonyms thereof</li> <li>• Identificaton to prescribed level not supported because the specimen(s) is/are too immature</li> <li>• May be applied to larvae or pupae</li> </ul>
dam.	<ul style="list-style-type: none"> <li>• Means “damaged” and includes all synonyms thereof</li> <li>• Identification to prescribed level not supported because the specimen(s) is/are damaged</li> </ul>
mount	<ul style="list-style-type: none"> <li>• Means “poor mount” and includes all synonyms thereof</li> <li>• Identification to targeted level not supported because slide mounted specimen(s) is/are poorly oriented on slide</li> </ul>
indet.	<ul style="list-style-type: none"> <li>• Means “indeterminate” and includes all synonyms thereof</li> <li>• Identification to targeted level not supported for recently molted specimens, mayfly subimagos, mature and intact specimens because of undocumented variation or indistinct characters, required case is missing/damaged, or required habitat/ecological information is missing/unavailable</li> <li>• Unlikely that taxon is new to science</li> </ul>
gender	<ul style="list-style-type: none"> <li>• Includes males and females</li> <li>• Identification to targeted level not supported because of gender</li> </ul>
retained	<ul style="list-style-type: none"> <li>• Denotes unmounted/unidentified specimens retained in separate vial</li> <li>• For qualitatively processed samples only</li> </ul>

## Attachment 5

### Standardized notes used to convey additional information

Note	Description
new state record	<ul style="list-style-type: none"><li>Refers to a potential new state record for a taxon based on known distributional information in the published literature or other reliable source</li></ul>
new U.S. record	<ul style="list-style-type: none"><li>Refers to a potential new United States record for a taxon based on known distributional information in the published literature or other reliable source</li></ul>
new species ?	<ul style="list-style-type: none"><li>Represents a potentially undescribed species that cannot be linked to any closely related species</li><li>Used with Genus-level identification only</li></ul>
no. lost	<ul style="list-style-type: none"><li>Refers to the number of specimens accidentally lost in handling</li><li>The number of specimens lost is indicated before "lost"</li><li>Example: 2 lost, ? lost, all lost</li></ul>
artifact	<ul style="list-style-type: none"><li>Identification of a bryozoan fragment (missing zooids) or empty mollusk shell</li><li>Only used in qualitatively processed samples, when taxon is not represented by a complete specimen</li></ul>
ref.	<ul style="list-style-type: none"><li>Denotes a specimen(s) suitable for placement in a reference collection</li></ul>







## Attachment 9

**PODL0607IRM0001A**

Slide Preparations – Identification and Enumeration Worksheet

Slide	LS	Slip #1				Slip #2			
		Position #1	Position #2	Position #3	Position #4	Position #1	Position #2	Position #3	Position #4
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11									
12									
13									
14									
15									
16									
17									
18									
19									
20									
21									
22									
23									
24									
25									
26									
27									
28									
29									
30									

Printed June 2, 2008      Mounting Acceptable (ini.) \_\_\_\_\_ / \_\_\_\_\_ / \_\_\_\_\_

Block Code: \_\_\_\_\_

Mount by: \_\_\_\_\_ Date/Time: \_\_\_\_/\_\_\_\_/\_\_\_\_ : \_\_\_\_

Identified by: \_\_\_\_\_ Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

Taxon	LS	Notes	Count
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			

Data Entry: \_\_\_\_\_ (ini.)

Taxa List Continued on back ( YES | NO )

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# Attachment 10

## Invertebrate Processing -- Subsampling and Preliminary Enumeration Worksheet

Sample ID: **PODL0607IRM0001A** Collection Date: 06/12/2007 Reach: A Site ID: 01654000 Field Subsample: 100

Processed by (ini.): \_\_\_\_\_ Subsampling, Documentation, and Processing checked (ini.): \_\_\_\_\_ and Date: \_\_\_\_/\_\_\_\_/\_\_\_\_  
 Sorting Effectiveness (SE) count: \_\_\_\_\_ SE check (ini.): \_\_\_\_\_ SE Date: \_\_\_\_/\_\_\_\_/\_\_\_\_ ( PASS / FAIL ) (circle one)

Stage 1 subsampling frame		Stage 2 subsampling frame		Estimation tray		Correction factor	
Subsampler size (e.g. 6 x 7):		Sub-sampler size (e.g. 4 x 6):		Estimation tray size (e.g. 7 x 7):		$(X \times Z) : (W \times Y)$	
Total grids in stage 1 subsampler (W):		Total grids in stage 2 subsampler (Y):		Total cells in estimation tray (e):			
Grids used from stage 1 subsampler (X):		Grids used from stage 2 subsampler (Z):		Total cells counted from tray:			

### Stage 1 grid density estimation

Subsampling frame coordinates			Estimation tray coordinates/counts					Total count	MGC	EGC	Time	
Grid No.	Row (R)	Column (C)	R/C	C1	R/C	C2	R/C	C3	$(C1+C2+C3)$	$(Total\ Count/3)$		$(MGC \times e)$
1												
2												
3												
4												
5												
Recommended processing scheme:										Total ( $\Sigma$ )		
										Average ( $\Sigma/5$ )		

### Sorting data and preliminary counts from individual stage 1 subsample/stage 2 subsample grids

Grid	Row	Col	Start	Stop	Cnt	Time	Grid	Row	Col	Start	Stop	Cnt	Time	Grid	Row	Col	Start	Stop	Cnt	Time	
1							11							21							
2							12							22							
3							13							23							
4							14							24							
5							15							25							
6							16							26							
7							17							27							
8							18							28							
9							19							29							
10							20							30							
																			$\Sigma$		

Printed: June 2, 2008

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