Standard Operating Procedure for Benthic Invertebrate Laboratory Analysis

LG407

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TABLE OF CONTENTS

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1.0 SCOPE AND APPLICATION

1.1 The procedures outlined here are for the sorting, identification, and storage of benthic invertebrates in samples collected from the Great Lakes National Program Office surveys.

2.0 SUMMARY OF METHOD

2.1 This procedure explains how to 1) prepare samples for processing, 2) separate animals from sediment, 3) prepare, record, and maintain specimens for taxonomic analysis and storage, and 4) perform required QA/QC steps.

3.0 EQUIPMENT LIST

3.1 Equipment

Scintillation Vials (5 mL)
Larger Storage Jars (for larger or numerous specimens)
Preserved Field Samples (1 L)
Petri dishes, gridded
Forceps (Fine Points, 2 pair per worker)
Watchglasses
Spoon
Wash bottle
Temporary Storage Jars
Dissecting Microscope
Compound Microscope
Slides (25 x 75 mm)
Coverslips (No. 1, 18 x 18 mm or 22 x 22 mm)
Slide Storage Cases
Taxonomic Keys (section 10.0)

3.2 Reagents

70% to 80% ethanol (EtOH) containing 5% glycerin
Mounting Media (CMC-9, CMC-10, or Hoyer's)
Formalin (3.7% formaldehyde)

4.0 SORTING PROCEDURES

4.1 Label a group of scintillation vials for each replicate field sample. Labels for the laboratory sample vials must include the sample number, lake and station location number (e.g. MI 47), and station replicate (FD1, FD2). There should be a scintillation vial for each major taxonomic group present (amphipods, oligochaetes, chironomids, mollusks) for each replicate field sample.

4.2 Sorting benthic invertebrates

4.2.1 Rinse the formalin out of the sediments by placing the entire sample into a 500-µm sieve within a tray/pan. Dispose of formalin using proper procedure. Under a fume hood, run tap water over the sample until the formalin is gone. If multiple bottles are present for a single sample (check field sample log) they can be combined at this point, or they can be processed separately; be sure to label all jars properly (e.g. 1 of 3, 2 of 3, 3 of 3). However, keep the replicate samples for each station separate.

4.2.2 Rinse the entire sample from the sieve and into a 16-oz glass jar or other temporary receptacle. Label the jar exactly as the original jar is labeled.

4.2.3 Samples that contain large quantities of variously sized materials (especially zebra mussels) may be initially separated into size fractions prior to picking in order to maximize the ability to efficiently and accurately pick out organisms from the debris. The sample can be sieved through a large mesh sieve (e.g. U.S. No. 4, with a 4.75-mm opening), then a medium-sized mesh sieve (e.g. U.S. No. 8, with a 2.36-mm opening), and then finally the standard U.S. No. 35 sieve (500- µm opening). Each fraction is separately kept and processed in its entirety, with all organisms from the fractions pooled together.

4.2.4 Under a dissecting microscope, remove organisms from the sediment, and transfer them to the appropriately labeled vial (see 4.1). It will be necessary to do this by spooning a small amount of sediment into the gridded Petri dish and then viewing through the dissecting microscope to locate all appropriate organisms.

4.2.5 Place spent sediments back into the sample bottle with 70% to 80% ethanol or 10% formalin solution for further QC picking. Be sure the label on the bottle is correct. For samples that have been fractionated, spent sediments from each fraction should be separately kept until QC checks have been completed.

5.0 TAXONOMIC ANALYSIS PROCEDURES

5.1 These procedures should be used for oligochaete worms.

5.1.1 For each sample, place all worms into one or two small watchglasses for viewing through a dissecting microscope (16-40 X magnification with proper lighting). Identify, count, and remove all positively identifiable fragments of worms. [*A fragment is a piece of worm that lacks its head. If it is not clear whether a specimen possesses its head, mount the specimen for identification.*] Place the fragments in a separately labeled vial containing ethanol. Record the count on the bench sheet under oligochaete fragments.

5.1.2 Procedures 5.1.2.1 to 5.1.2.7 should be followed by staff who are learning or becoming familiar with the oligochaete fauna of the Great Lakes region.

5.1.2.1 Mount ALL worms that possess their heads and any fragments that are in question.

5.1.2.2 Place worms on the slides length-wise, either all horizontal or all vertical. Place heads on same side or location (i.e., all heads on left side or on top). An attempt should be made to mount specimens of the same size on a slide. Mounting thick bodies with thinner bodies will make the latter hard to see. When possible, avoid allowing specimens to cross each other or wrap over onto themselves. This may make the mount too thick and cause excessive air bubbles, and it may obscure anatomy/morphology necessary for identification.

5.1.2.3 Mount 1-10 animals per coverslip (depending on size of specimens), with a maximum of 2 coverslips per slide.

5.1.2.4 CMC-9 (low viscosity) or CMC-10 (high viscosity) mounting media can be used, or the two can be mixed to provide the best thickness for the animals being mounted. In addition, Hoyer's mounting medium may be used. After placing the coverslip(s) on the mounted specimens, set the slide(s) aside for a few minutes; add more mounting medium along the edges if air bubbles appear. Large specimens may require additional medium if air bubbles appear after a few days. After the slides have properly dried, a sealant (eg. Cytoseal™ 60) should be placed around the edge of the coverslip to provide additional protection from the development of air pockets.

5.1.2.5 Labeling is done directly on the slide using a "superfrost" permanent marker. Label the slide with sample number, sampling period, station, number in the slide sequence for that replicate, taxonomic group, and number of individuals in each taxon.

99GB49S36
Summer 1999
HU 32 (1 of 6)
Limnodrilus hoffmeisteri – 5
Stylodrilus – 3

5.1.2.6 Appendix A in the Guide to the Freshwater Oligochaetes of North America (Kathman and Brinkhurst, 1998) should be consulted for technical guidance in mounting oligochaetes.

5.1.2.7 Identify and count the specimens, and record the information on the bench sheet and on the slide.

5.1.3 Procedures 5.1.3.1 to 5.1.3.5 can be followed by people with considerable experience and expertise with the oligochaete fauna of the Great Lakes region.

5.1.3.1 After removal of fragments in procedure 5.1.1, sort the remaining specimens into groups. Taxa that are listed in 5.1.3.2 may be identified and counted using a dissecting microscope. All other specimens must be mounted for identification and enumeration according to procedures in 5.1.2.

5.1.3.2 The following taxa of worms can be identified using a dissecting microscope without having to mount the specimens. **Specimens must be viewed under relatively high magnification (16-40 X) with proper lighting**. Any and all specimens that are even remotely different in appearance, or for which the worker is unsure of the identity, should be mounted for identification.

Lumbriculidae: *Stylodrilus heringianus* Claparède, 1862, (a common species, many have their penes protruding, often the only species present at great depths in the Great Lakes)

Tubificinae:

1) immature tubificines without hairs (no sign of tissue density or sexual maturity in the clitellar region. **Warning**: some species are small when they are mature, e.g. *Thalassodrilus hallae*);
2) immature tubificines with hairs (no sign of tissue density or sexual maturity in the clitellar region);
3) mature *Potamothrix moldaviensis* Vejdovsky and Mrazek, 1902(with no hairs and everted penes);
4) *Potamothrix vejdovskyi* (Hrabe, 1941) (with short, bent hairs. **Warning**: do not confuse with *Aulodrilus pluriseta*);
5) *Branchiura sowerbyi* Beddard, 1892 (distinctive posterior gills);

6) *Quistadrilus multisetosus* (Smith, 1900) (very bristly with distinct papillae in rings around the body);
7) *Spirosperma ferox* Eisen, 1879 (hairy, with fine whitish particulate matter on nearly the entire body [often absent in clitellar region], bifid ventral chaetae anteriorly, retractile prostomium, small papillae throughout body, often occurs in enriched areas);
8) *Spirosperma nikolskyi* (Lastochkin and Sokolskaya, 1935) (also hairy, with fine whitish particulate matter, simple chaetae anteriorly, occurs most often in deeper oligotrophic areas).

Naidinae:

1) *Arcteonais lomondi* (Martin, 1907) (with short proboscis and long, bunched hairs);
2) *Chaetogaster diaphanus* (Gruithuisen, 1828) (short, chunky, anteriorly truncate);
3) *Dero* sp. (posterior gills);
4) *Ophidonais serpentina* (Muller, 1773) (eyes, transverse pigment stripes anteriorly, no dorsal hairs, stout dorsal chaetae);
5) *Slavina appendiculata* (d'Udekem, 1855) (narrow, debris-laden, with long hairs on segment VI);
6) *Stylaria lacustris* (Linnaeus, 1767) (a distinctive species with a proboscis);
7) *Ripistes parasita* (Schmidt, 1874) (dorsal chaetal bundles on segments VI-VIII with very long hairs);
8) *Pristina leidyi* Smith, 1896 (very long dorsal hairs on segment III–unless broken off).

Enchytraeidae

5.1.3.3 Temporary wet mounts may be done to verify a specimen under the compound microscope (eg. *Spirosperma ferox* vs. *Spirosperma nikolskyi*, *Potamothrix vejdovskyi* vs. *Aulodrilus pluriseta*). After verification, the specimen(s) must be returned to the labeled vial.

5.1.3.4 Representative specimens from the above list must be initially mounted for verification under a compound microscope; this should be accomplished for each site. The remainder of the specimens of these species must be stored in separate, labeled vials containing ethanol for archival storage; this must be completed for each site. Voucher specimens of these taxa must be placed in the reference collection both as slide mounts and in vials.

5.1.3.5 The label (Laser printing with acid-free paper containing 25% cotton) for a vial must include at least the sample number, station number, and the sampling period. A separate label with the identification can be added, or the taxonomic name can be written on the back side of the location label (India ink or pencil).

E.g.

U.S. EPA GLNPO	Summer 2003
Ponar Benthos	
MI 48	03GA59D36FD1

5.2 These procedures should be used for chironomid midges.

5.2.1 Procedures 5.2.1.1 to 5.2.1.4 should be followed by staff who are learning or becoming familiar with the chironomid fauna of the Great Lakes region.

5.2.1.1 Mount all midges for identification. Larval chironomids should be mounted according to the publication Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina (Epler, 2001).

5.2.1.2 Mount 1-10 animals per coverslip depending on size of specimens, maximum of 2 coverslips per slide. Mount the body as well as the head capsule. If the body is too large, it may be preserved in a vial of ethanol; the vial and slide must be cross referenced.

5.2.1.3 Labeling should be the same as oligochaete slides (see 5.1.2.5).

5.2.1.4 Identify and count the specimens, and record the information on the bench sheet.

5.2.2 Procedures 5.2.2.1 to 5.2.2.2 can be followed by people with considerable experience and expertise with the chironomid fauna of the Great Lakes region.

5.2.2.1 Some chironomid midges can be identified using a dissecting microscope without having to mount the specimens. **Specimens must be viewed under relatively high magnification (16-40 X) with proper lighting**. Any and all specimens that are even remotely different in appearance, or for which the worker is unsure of the identity, should be mounted for identification.

Taxa that may be identified using a dissecting microscope include the following:

1). Chironomus 2). *Coelotanypus* 3). *Cryptochironomus*
4). *Heterotrissocladius subpilosus* group
5). *Procladius* 6). *Protanypus*
7). *Paracladopelma winnelli* (verification specimens should be mounted on a routine basis to ensure proper species identification for this taxon).

Additional taxa may be included in this list, especially once the taxa are known from a specific site, but representative specimens of the above 7 taxa, and any additional taxa, must be slide mounted and also placed in vials of ethanol for the reference collection.

5.2.2.2 Temporary wet mounts may be done to verify a specimen under the compound microscope (eg. *Paracladopelma winnelli*). After verification, the specimen(s) must be returned to the labeled vial.

5.2.2.3 Representative specimens from the above list must be initially mounted for verification under a compound microscope. The remainder of the specimens of these species must be stored in separate, labeled vials containing ethanol for archival storage. This must be accomplished for each site. Voucher specimens of these taxa must be placed in the reference collection both as slide mounts and in vials.

5.3 These procedures should be used for amphipods and all other non-slide mounted animals.

5.3.1 Identify and count the specimens, and record the information on the appropriate bench sheet. Only specimens that possess their heads should be included for identification and enumeration, even if only the head is the piece that is present; all other parts, pieces, exuviae, and empty shells should be ignored. **EXCEPTION**: if enough of the body of a specimen (excluding exuviae and empty shells) is present to make a valid identification, and it is assured that other body parts of the specimen are not present in the sample (i.e. the specimen is in pieces and is being counted more than once), then the piece may be included in the taxonomic analysis and enumeration. For example, if there are two whole amphipods and one amphipod with the head missing (total of only three specimens), but the technician can identify the headless animal, the specimen should be identified and counted. However, this is not a common situation.

5.3.2 Return all the specimens for each taxon to their individual, labeled vial for archived sample storage.

5.4 Taxa that do not appear on a current species list for this project are periodically encountered. Specimens of new taxa should be identified to the lowest possible taxonomic level and properly preserved in ethanol, or on a slide, or both. These individuals will serve as voucher specimens and should be sent to an expert for external confirmation. A notice of confirmation by the outside expert should be submitted to the WAM. The taxon can be added to the species list after written notification of acceptance by the WAM. The specimen(s) should then be added to the reference collection.

6.0 SAMPLE PRESERVATION AND STORAGE

6.1 Slide-Mounted Organisms: Mounted chironomid midges and oligochaete worms will be stored as archived samples.

6.2 Organisms in Vials: All invertebrates can be stored in their properly labeled scintillation vials with 70-80% ethanol that contains 5% glycerin. The glycerin provides a lubricant to the sample in the event that the alcohol evaporates from the vial, preventing the specimens from desiccating. The samples should be stored together according to site, lake, season, and year, and placed into sealed containers. For long-term, archival storage at the GLNPO facility, the sealed containers with vials should be placed into larger storage receptacles that contain ethanol.

6.3 Spent Sediment: The remainder of the benthic sample, which may contain sediment, zebra mussels, nematodes, and other non-target organisms should remain preserved in the original sample bottle with 70-80% ethanol or formalin until proper QC checks are completed. After completion of QC checks, the sample may be properly discarded by the laboratory personnel.

6.4 After analysis is complete and data have been submitted and approved by the WAM, all archived samples will be shipped to, and stored at, the GLNPO storage facility at 536 S. Clark St. Chicago IL

7.0 DATA HANDLING AND CALCULATIONS

7.1 The data must be converted from estimated raw sample counts to abundance per m2 for each species by multiplying the totals by the number: 19.12. This conversion factor is derived from dimensions of the Ponar grab sampler. If a different sampler is used the factor should be recalculated.

7.2 Calculate a mean abundance for each species and report the mean of each species (or lowest taxonomic level) by Station Number.

8.0 SAFETY AND WASTE HANDLING

8.1 Personal protection equipment (safety glasses, gloves and lab coat) should be worn in the laboratory while preparing and handling samples for analyses.

8.2 All samples preserved with formalin should be handled under the hood prior to being rinsed for sorting (section 4.2.1.).

8.3 Follow laboratory waste disposal guidelines regarding formalin solution waste. Sample waste should be emptied into a waste container.

9.0 TRAINING AND QUALITY CONTROL

9.1 New analysts are required to receive formal training in the areas of terminology, anatomy, morphology, and taxonomy of Great Lakes benthic invertebrates. This can be accomplished in one of two ways: instruction from a senior benthic analyst in the laboratory, or by attending an external course taught by benthic specialists. Acceptable training courses can be found by contacting either the North American Benthological Society or the International Association of Great Lakes Research.

9.2 Quality Control Checks on the processing of benthic macroinvertebrates.

9.2.1 Picking Samples

The senior taxonomist or the lead assistant will "second pick" at least 10% of the samples picked by each assistant. One sample from a block of 10 consecutively picked samples by each person will be randomly chosen for second picking. Error percentages in picking should be less than 10%, preferably less than 5%.

However, samples that yield very low numbers of individuals can lead to high percentages of error (eg. missing 1 specimen from a total of 4 specimens equals a 25% "picking error"). High error percentages from these types of samples will be taken into consideration when determining if the sample passes or fails the Quality Control Check. The main criteria in this determination will be deciding whether the error affects the ecological interpretation of the data.

Samples that do not pass the QC Check will lead to the repicking of another sample within the assistant's block of 10 samples. If the second sample fails the QC Check, all of the samples within the block of 10 will be repicked by the same assistant. Another round of QC Checks will be repeated on the block of 10 samples.

During the initial training period of an assistant, additional samples will be 2nd picked until it is assured that the assistant is effectively removing 90-100% of the total number of organisms.

Samples containing fingernail clams (*Sphaeriidae)* will be given special attention during the training process and the random QA/QC checks. Their small size (some appear as large sand grains) and resistance to stain (some appear translucent) cause them to sometimes be overlooked.

9.2.2 Taxonomy

Most, if not all, of the taxonomic identifications will be done by the senior or lead taxonomist(s). Identifications may also be accomplished by a lead assistant(s) provided they have the training and experience required to identify taxa from the Great Lakes.

Unusual or difficult oligochaetes, chironomids, or other taxa may be sent to acceptable outside taxonomic experts for identification.

The taxonomy and enumeration for 10% of the samples must be checked for Quality Control. The QC sample will be randomly chosen from batches of 10 samples. Specimens from the QC samples will be analyzed by another trained and experienced individual within the same laboratory, or by someone in an outside laboratory. Percent similarity of the two samples must exceed 90%. Samples that fail the QC check must be examined to determine the type of problem encountered. The original lab should re-examine all of the samples in the batch. Further training may be required for the lab personnel depending on the type of errors encountered. This aspect should be worked out between the original lab, the EPA's WAM, and the personnel that conducted the QC check.

Oligochaete fragments will not be included in the QC check because they have the propensity to multiply every time they are handled. However, they should be checked to ensure no identifiable specimens are present.

10.0 TAXONOMIC REFERENCES

10.1 Primary Taxonomic Sources for Identification and Nomenclature

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Pennak, R.W. 1989. Fresh-water Invertebrates of the United States. Porifera to Mollusca. 3rd Edition. John Wiley & Sons, Inc. New York, NY.

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Thorp, J.H. and A.P. Covich. 2001. Ecology and Classification of North American Freshwater Invertebrates. 2nd Edition. Academic Press. San Diego, CA.

10.2 Additional Taxonomic Sources for Species Identification

10.2.1 Insecta: Diptera: Chironomidae

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Epler, J.H. 1995. Identification Manual for the Larval Chironomidae (Diptera) of Florida. Final Report for DEP Contract Number WM579. Florida Department of Environmental Protection, Tallahassee.

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10.2.2 Insecta: Ephemeroptera

McCafferty, W.P. 1975. The burrowing mayflies of the United States (Ephemeroptera: Ephemeroidea). Transactions of the American Entomological Society 101:447-504.

10.2.3 Insecta: Trichoptera

Wiggins, G.B. 1996. Larvae of the North American Caddisfly Genera (Trichoptera). 2nd Edition. University of Toronto Press, Inc. Toronto, Ontario.

10.2.4 Insecta: Heteroptera

Hilsenhoff, W.L. 1984. Aquatic Hemiptera of Wisconsin. The Great Lakes Entomologist 17: 29-50.

Hungerford, H.B. 1948. The Corixidae of the Western Hemisphere (Hemiptera). University of Kansas Science Bulletin 32: 1-288, 408-827.

10.2.5 Annelida

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Klemm, D.J. 1985. A Guide to the Freshwater Annelida (Polychaeta, Naidid and Tubificid Oligochaeta, and Hirudinea) of North America. Kendall/Hunt Publishing Co. Dubuque, Iowa.

10.2.6 Mollusca

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