Standard Operating Procedure for Benthic Invertebrate Laboratory Analysis

LG407

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for Benthic Invertebrate Laboratory Analysis

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 quality analysis/quality control (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)
Bogorov Counting Chamber (90x115mm)
Petri dishes
Dissecting needles
Click Counter
Balance (desired precision ±0.0001 g)
Forceps (Fine Points, 2 pair per worker)
Spoon
Wash bottle
Temporary Storage Jars
Dissecting Microscope
Compound Microscope
Slides (25 x 75 mm)
Slide labels
Coverslips (No. 1, 18 x 18 mm, 22 x 22 mm, and 24 x 50 mm)
Slide Storage Cases
Taxonomic Keys (section 10.0)
Cellulose filter paper (suggested brand: Whatman 11.0 cm Grade 1 Qualitative)
500 µm Sieve
Goggles
Laboratory Coat
Laboratory Gloves

3.2 Reagents

70% to 80% ethanol (EtOH) containing 5% glycerin [Alcohol Glycerin Water (AWG)]
Mounting Media (CMC-9, CMC-10, CMCP-9, CMCP-10 or Hoyer's) Clear Fingernail Polish
5-10% Neutral Buffered Formalin.

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 (e.g., 12GC51S16), lake and station location number (e.g., MI 47), and station replicate (e.g., RFS, or the first replicate, FD1, the second, or FD2, the third). 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 under a fume hood. Dispose of formalin using proper procedure. 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 as long as major taxonomic groups are combined; 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 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 Bogorov counting chamber with water and then viewing through the dissecting microscope to locate all appropriate organisms. If a piece of material does not fit into the counting chamber, place it separately in a Petri dish and check for organisms.

4.2.4 Place spent sediments back into the sample bottle with 10% neutral buffered formalin solution for further QC picking (the poured off formalin from initial sample preservation can be used for this). 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.

4.2.5 Dreissenid organisms will be picked, counted, and weighed using the following procedure. All dreissenid mussels should be sorted by species, and then divided, counted, and weighed by size fractions (0-4.99 mm, 5-9.99 mm, 10-14.99 mm, 15-19.99 mm, etc.) to the nearest 0.001 g after being blotted dry on absorbent paper to remove external water (total wet weight, soft tissue plus shell). The number and weight of all dreissenids in each size category, and the total number and weight of all dreissenids should be recorded by species. If the sample contains an exceptionally large number of dreissenid mussels (>5000) then the sample can be split using a gridded tray or beaker. The sample will be placed in the beaker or gridded tray, stirred to distribute the organisms, and then split into two parts. Another way to split the sample is subsampling by weight relative to total. The splitting procedure will be repeated until approximately 100-200 organisms remain in a subsample to be counted. The subsample fraction of the original sample is to be recorded on the laboratory data sheet and used to estimate the total density of dreissenids in the sample based on number of splits. This subsample will be picked, identified, counted, weighted and the resulting organisms reported by species and size fractions. This procedure, however, should be avoided when possible due to potential bias in samples containing a mix of small and large mussels.

Shell size will be divided and reported in 5mm size fractions: 0-4.99 mm, 5-9.99 mm, 10-14.9

mm, 15-19.99 mm, etc. The remaining sample will be saved and labeled noting the split proportion of the remaining portion.

5.0 TAXONOMIC ANALYSIS AND WET WEIGHT PROCEDURES

5.1 These procedures should be used for wet weight determination.

5.1.1 Before weighing anything on the analytical balance (desired precision ±0.0001 g) make sure that it is leveled and zeroed.

5.1.2 To check the leveling on the balance, look at the leveling bubble (centered in printed circle) on the floor of the weighing chamber. If it is not centered, center it by turning the leveling screws on the bottom toward the back of the balance.

5.1.3 Once the balance is leveled, close all the chamber doors and press the control bar on the front of the balance. After a few seconds, a row of zeroes will appear. This indicates that the balance is zeroed and ready for use.

5.1.4 Separate organisms by taxonomic group or size category, count individuals, and remove foreign material.

5.1.5 Blot dry each individual, individuals of the same species, or size category on cellulose filter paper (can be reused until soiled) to remove external water until the wet spots left by animal(s) on the absorbent paper disappear. Drying time varies based on the surface area/volume ratio of the organisms (but should never cause damage to them): approximately 30 seconds for large and medium chironomids and oligochaetes, less time (10-20 seconds) for smaller worms. Be careful not to over dry the organisms. Larger organisms (like Dreissenids) may take longer to dry and are done drying when moved to a different part of blotting paper and do not let off excessive water.

5.1.6 *Dreissena* may take longer to dry and the following procedure should be followed for larger individuals:

5.1.6.1 Dry large individuals on paper towels spaced out. Do not weigh until externally adhering fluids are blotted off shells.

5.1.6.2 If there are many *Dreissena*, a petri dish may be used to place on the scale. Place the empty petri dish on the balance pan and close the doors. Tare the container by briefly pressing the control bar. The readout will read zero with the container sitting on the pan. This allows the mass of your sample to be read directly. Place the sample in the petri dish. Be sure not to press the control bar again as the readout will be incorrect. With the sample and the petri dish sitting on the balance, close the chamber doors and read the display to find the mass of your sample after the readout stabilizes or after one minute.

5.1.7 After blotting dry, weigh all individuals of each species (or size category, e.g., as for Oligochaeta and *Dreissena*) and record the total number and weight for the group.

5.1.8 To calculate the total weight for each species that was mounted by size groups for identification (e.g., Oligochaeta), multiply the number of individuals of the species found in each size category by the average weight of worms in that category. If a species was found in

more than one size categories, sum the number and weight of the species across all categories and report as a total number, total weight (g), density (number per m-2) and biomass (weight per m-2) per replicate.

5.2 These procedures should be used for oligochaete worms.

5.2.1 For each sample, place all worms into one or two small watch-glasses for viewing through a dissecting microscope (16-40 X magnification with proper lighting), or a benthic sorting paddle. Identify, count, weigh (§5.1) and remove all positively identifiable fragments of worms. *[A fragment is a piece of worm that does not have a head (for example a tail-end or mid-section piece). If it is not clear if a specimen possesses a head, mount the specimen for identification.]* All fragments should be weighed together after being counted. Place the fragments in a separately labeled vial containing preservative (70-80% ethanol that contains 5% glycerin [Alcohol Glycerin Water (AWG)]. Record the count and weight on the bench sheet under "Oligochaeta fragments".

5.2.2 Procedures 5.2.2.1 to 5.2.2.7 should be followed by staff who are learning or becoming familiar with the oligochaete fauna of the Great Lakes region.

Count all Oligochaeta species that can be identified without mounting. Weigh Oligochaeta in groups by species and record the count and weight on bench sheets.

5.2.2.1 Divide all other worms by size categories (if many; for example: extra-large, large, medium, small and extra small). Size categories are relative to the size of Oligochaetes in each sample, there is no set size requirement for each size category. Count, weigh (§5.1, by each size category) and record the number and weight of worms in each size category. Make sure to indicate on the bench sheet the number of slide(s) each size category was mounted on. Mount ALL worms that possess their heads and any fragments that are in question. All Oligochaetes with heads should be mounted, despite being a complete specimen or a broken (head-end) worm with a head.

5.2.2.2 Mount worms by size category. Place worms on the slides lengthwise, either all horizontal (preferred) or all vertical. Place heads on same side or location (i.e., all heads pointing right or pointing up). An attempt should be made to mount specimens of the same size on a slide. Mounting thick bodied worms with thinner bodied worms will make the latter hard to identify. When possible, avoid allowing specimens to cross each other or wrap over onto themselves, as this may make the mount too thick and cause excessive air bubbles, as well as obscure anatomy/morphology necessary for identification.

5.2.2.3 Mount 1-10 animals per coverslip (depending on size of specimens), with a maximum of 2 small (22 x 22 mm) coverslips, or one large (24 x 50mm) coverslip per slide, for a maximum of 20 animals per slide.

5.2.2.4 CMC-9 (low viscosity) or CMC-10 (high viscosity) mounting media can be used. In addition, Hoyer's mounting medium may be used. After placing the coverslip(s) on the mounted specimens, gently tap down the coverslip into place, then 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 (e.g., Cytoseal™ 60 or clear fingernail polish) should be placed around the edge of the coverslip to provide additional protection from the development of air pockets.

5.2.2.5 Labeling is done directly on the slide using a "superfrost" permanent marker. Label the slide with sample number, sampling period, station, and number in the slide sequence for that replicate. Sticker labels with station information may also be used in addition to permanent markers to prolong the life of the label.

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5.2.2.6 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.

5.2.2.7 Identify and count the specimens, and record the information on the bench sheets.

5.2.3 Procedures 5.2.3.1 to 5.2.3.4 can be followed by people with considerable experience and expertise with the oligochaete fauna of the Great Lakes region.

5.2.3.1 After removal of fragments in procedure 5.2.1, sort the remaining specimens into groups. Taxa that are listed in 5.2.3.2 may be identified, counted using a dissecting microscope, and weighed (§5.1). All other specimens must be mounted for identification and enumeration according to procedures in 5.2.2.

5.2.3.2 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. 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:

1) *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) *Branchiura sowerbyi* Beddard, 1892 (a relatively large Oligochaeta with distinctive posterior gills. Body often appears squared off in cross-section).

2) *Quistadrilus multisetosus* (Smith, 1900) (very bristly with distinct papillae in rings around the body).

3) *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). Can be identified using a dissecting microscope only from Lake Erie samples to avoid misidentification with S. *nikolskyi*.

5.2.3.3 Representative specimens from the above list must be initially mounted for verification under a compound microscope; this should be accomplished when found for the first time for each lake. 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.

5.2.3.4 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	
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5.3 These procedures should be used for chironomid midges.

5.3.1 Procedures 5.3.1.1 to 5.3.1.5 should be followed by staff that are learning or becoming familiar with the chironomid fauna of the Great Lakes region.

5.3.1.1 Divide all chironomids into size categories (for example: large, medium, small). Size categories are relative to the chironomids in each sample, there are no set lengths for each category. Count, weigh (§5.1, by size category), and record information on bench sheets. Be sure to indicate what slide(s) that size category was mounted on.

5.3.1.2 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; Canada Balsam/Euparal Method (Epler, 2001).

5.3.1.3 Using 22x22 mm coverslips, mount 1-10 animals per coverslip depending on size of specimens, with a maximum of 2 coverslips per slide. Do not remove the heads of specimens, 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.

5.3.1.4 Labeling should be the same as oligochaete slides (see 5.2.2.5).

5.3.1.5 Identify and count the specimens, and record the information on the bench sheet, reporting counts according to size category.

5.3.2 Procedures 5.3.2.1 to 5.3.2.2 can be followed by people with considerable experience and expertise with the chironomid fauna of the Great Lakes region.

5.3.2.1 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**. 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 accurately using a dissecting microscope, specifically *Chironomus*, do not have to be mounted.

5.3.2.2 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 AGW for archival storage. This must be accomplished for each year and lake. Voucher specimens of these taxa must be placed in the reference collection both as slide mounts and in vials. Specimens that can be identified without mounting should be counted, weighed, and recorded by taxa on the appropriate bench sheet. Specimens should be kept in vials filled with ethanol and labeled as described above.

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

5.4.1 Identify, count, and weigh (§5.1, in groups by species) 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 a third amphipod with the head missing (total of only three specimens), and the technician can identify the headless animal, the specimen should be identified and counted. Please note that this is not a common situation.

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

5.5 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, counted, weighed (§5.1) 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 request for a taxonomic addition should include taxon name, naming authority, citation for primary taxonomic source used in making the determination, written description of distinguishing characteristics of new taxon, citation(s) of previous records of occurrence in the Great Lakes, written confirmation of identification by a qualified outside expert, drawings and/or photographs of new taxon and reference to voucher specimen location, and relation to any taxa currently in the GLNPO species list. This documentation should be submitted to the Data Submission System (DSS) for review and approval by the GLNPO Technical Lead. When approved, the taxon will be added to the GLNPO Great Lakes taxa list, and 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 (AGW). The glycerin provides a lubricant to the sample if 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.

6.3 Dreissenids in Jars and Vials: All specimens should be stored in 10% Neutral Buffered Formalin. After data submission and approval, dreissenids can be combined to station level to save space and preservative. All labels should be kept and stored in the sample jar to easily show that they had been combined to station level (i.e., all replicates from a single station combined into one jar). It is recommended that small dreissenids be kept in separate 20ml scintillation vials deposited into larger jars with large mussels. The samples should be stored together according to site, lake, season, and year.

6.4 Spent Sediment: The remainder of the benthic sample, which may contain sediment, nematodes, and other non- target organisms should remain preserved in the original sample bottle with formalin until proper QC checks are completed. After data have been approved by the GLBMP Technical Lead, the sample may be properly discarded by the laboratory personnel.

6.5 After the grant ends and all data have been submitted and approved by the GLNPO, all archived samples will be shipped to GLNPO for long-term sample storage.

7.0 DATA HANDLING AND CALCULATIONS

7.1 The data must be converted from estimated raw sample counts and weight to abundance and biomass per m^2 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 total abundance and biomass for each species and report the total of each species (or lowest taxonomic level) per replicate.

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 Society for Freshwater Science (SFS) or the International Association of Great Lakes Research (IAGLR).

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. If the assistant is newly hired or has had difficulty in the past, the senior taxonomist may enforce a probationary period where 20% of the samples picked will undergo a second picking until the assistant is considered proficient. 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 (e.g., 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. It is recommended that the assistant be placed on probationary period if their failing of QC results in complete repicking of 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. The samples selected should 1) contain taxa and 2) be analyzed for all taxa, not a select group. Additional QC should be completed on samples that contain only selected groups (e.g., *Dreissena* spp. and/or Diporeia). 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%. Similarity of the two samples can be measured by Whittaker's percent similarity of community (PSc) index. This index compares relative abundances of taxa in two samples according to the following formula:

$$PSc = 100 - 0.5\sum_{i=1}^{k}|a - b|$$

where a and b are, for a given species, percentages of the total samples A and B which that species represents. The absolute value of their difference is then summed over all species. The resulting scores range between 1 (or 100%) if all species are represented in the same proportions in the two samples, and 0 (or 0%) if the samples have no species in common. 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. Actions required because of a failed QC standard will be decided on a case basis following conversations between the original lab, the EPA's GLNPO, and the personnel that conducted the QC check.

Oligochaete fragment counts 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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10.2 Additional Taxonomic Sources for Species Identification

10.2.1 Insecta: Diptera: Chironomidae

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10.2.3 Insecta: Trichoptera

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10.2.4 Insecta: Heteroptera

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