

Standard Operating Procedure for Benthic Invertebrate Laboratory Analysis

LG407

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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)
Bogorov Counting Chamber (90x115mm)
Petri dishes
Dissecting needles
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)
Coverslips (No. 1, 18 x 18 mm or 22 x 22 mm)
Slide Storage Cases
Taxonomic Keys (section 10.0)
Cellulose filter paper (suggested brand: Whatman 11.0 cm Grade 1 Qualitative)
- 3.2 Reagents
70% to 80% ethanol (EtOH) containing 5% glycerin
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, FD1, or 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 μ m 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 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.
- 4.2.6 After removing all benthic organisms except for Dreissenidae from the sample sediment using procedures described in 4.2.1-4.2.5, 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 (0-5mm, 5-10mm, 10-15mm, 15-20mm, 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 a large number of Dreissenid mussels (>200) 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. Shell size will be divided and reported in 5mm size fractions: 0-5mm, 5-10mm, 10-15mm, 15-20mm, 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 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. Time drying vary based on the surface area/volume ratio of the organisms (but should never cause damage to them): approximately one minute for large and medium chironomids and oligochaetes, less time (0.6 min) 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 is a large number of *Dreissena*, a petri dish may be used to place on the scale. Place the 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 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 watchglasses for viewing through a dissecting microscope (16-40 X magnification with proper lighting). Identify, count, weigh (§5.1) 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.*] All fragments after counting should be weighed together. Place the fragments in a separately labeled vial containing ethanol. Record the count and weight on the bench sheet under oligochaete fragments.
 - 5.2.2 Procedures 5.2.2.1 to 5.2.2.8 should be followed by staff who are learning or becoming familiar with the oligochaete fauna of the Great Lakes region.
 - 5.2.2.1 Count all Oligochaeta species that can be identified without mounting. Weigh Oligochaeta in groups by species and record the count and weight in bench sheets.
 - 5.2.2.2 Divide all other worms on size categories (if many; for example: extra large, large, medium, small and extra small), count, weigh (§5.1, by each size category) and record the number and weight of worms in each category. Make sure to indicate on the bench sheet the number of slide(s) where the worms from that size category were mounted

- on. Mount ALL worms that possess their heads and any fragments that are in question.
- 5.2.2.3 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.
- 5.2.2.4 Mount 1-10 animals per coverslip (depending on size of specimens), with a maximum of 2 coverslips per slide or 20 animals per slide.
- 5.2.2.5 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 or clear fingernail polish) should be placed around the edge of the coverslip to provide additional protection from the development of air pockets.
- 5.2.2.6 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. Sticker labels with station information may also be used in addition to permanent markers to prolong the life of the label.
- 99GB49S36
Summer 1999
HU 32 (1 of 6)
Limnodrilus hoffmeisteri – 5
Stylodrilus – 3
- 5.2.2.7 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.
- 5.2.2.8 Identify and count the specimens, and record the information on the bench sheets.
- 5.2.3 Procedures 5.2.3.1 to 5.2.3.5 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. Oligochaetes that are too young to identify to family (e.g. still in egg cocoons, embryos without chaetae) should be counted as "Oligochaeta immature" (OLIIMMA). Note that their absence in previous datasets (1997-2011) is due to them not being counted, rather than them not being found.
- 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.** 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:

- 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) 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*);
- 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 plurisetus*);
- 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

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

5.2.3.4 Representative specimens from the above list must be initially mounted for verification under a compound microscope; this should be accomplished when found for 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.

5.2.3.5 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	
MI48	03GA59D36FD1

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), 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 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.

5.3.1.4 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.

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.** 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.

5.3.2.3 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.

5.3.2.3 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 year and lake. Voucher specimens of these taxa must be placed in the reference collection both as slide mounts and in vials.

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 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.

5.4.2 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 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, 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 WAM, the sample may be properly discarded by the laboratory personnel.

6.4 After the grant ends and all data have been submitted and approved by the WAM, all archived samples will be shipped to, and stored at, the GLNPO.

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 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 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 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

Merritt, R.W. and K.W. Cummins (eds.). 1996. *An Introduction to the Aquatic Insects of North America*. 3rd Edition. Kendall/Hunt Publishing Co., Dubuque IA.

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

Smith, D.G. 2001. *Pennak's Freshwater Invertebrates of the United States. Porifera to Crustacea*. 4th Edition. John Wiley & Sons, Inc. New York, NY.

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

Epler, J.H. 1987. Revision of the Nearctic *Dicrotendipes* Kieffer, 1913 (Diptera: Chironomidae). *Evolutionary Monographs* 9. 102 pp + 37 plates.

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.

Epler, J.H. 2001. *Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina*. North Carolina Department of Environment and Natural Resources.

Jackson, G.A. 1977. Nearctic and Palaearctic *Paracladopelma* Harnisch and *Saetheria* n.gen. (Diptera: Chironomidae). *Journal of the Fisheries Research Board of Canada* 34: 1321-1359.

Maschwitz, D.E. and E.F. Cook. 2000. Revision of the Nearctic species of the genus

Polypedilum Kieffer (Diptera: Chironomidae) in the subgenera *P.* (*Polypedilum*) Kieffer and *P.* (*Uresipedilum*) Oyewo and Saether. *Bulletin of the Ohio Biological Survey, New Series*. Vol. 12, No. 3.

Oliver, D.R, M.E. Dillon. 1994. Corrections and additions to "A Catalog of Nearctic Chironomidae." *Proceedings of the Entomological Society of Washington* 96:8-10.

Oliver, D.R, M.E. Dillon , and P.S. Cranston. 1990. A Catalog of Nearctic Chironomidae. Research Branch, Agriculture Canada Publication No. 1857/B.

Roback, S.S. 1985. The immature chironomids of the eastern United State VI. *Pentaneurini*— genus *Ablabesmyia*. *Proceedings of the Academy of Natural Sciences of Philadelphia* 137: 153-212.

Saether, O.A. 1973. Taxonomy and ecology of three new species of *Monodiamesa* Kieffer, with keys to Nearctic and Palaearctic species of the genus (Diptera: Chironomidae). *Journal of the Fisheries Research Board of Canada* 30: 665-679.

Walker, I.R., D.R. Oliver, and M.E. Dillon. 1992. The larva and habitat of *Parakiefferiella nigra* Brundin (Diptera: Chironomidae). *Netherlands Journal of Aquatic Ecology* 26: 527-531.

Wiederholm, T. (ed.). 1983. Chironomidae of the Holarctic region -- keys and diagnoses. Part 1. Larvae. *Entomologica Scandinavica Supplement* No.19.

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

Kathman, R.D. and Brinkhurst, R.O. 1998 (Revised 1999). *Guide to the Freshwater Oligochaetes of North America*. Aquatic Resources Center, College Grove, Tennessee.

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.

Krieger, K. A., and A. M. Stearns. 2010. *Atlas of the Aquatic Oligochaete Worms (Phylum Annelida:*

Class Clitellata: Superorder Microdrili) Recorded at the Old Woman Creek National Estuarine Research Reserve and State Nature Preserve, Ohio. National Center for Water Quality Research, Heidelberg University, Tiffin, Ohio. Available at:
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10.2.6 Mollusca

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