Maryland Biological Stream Survey Laboratory Methods for Benthic Macroinvertebrate Processing and Taxonomy

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Contents

1   Introduction

This manual describes the methods used for benthic macroinvertebrate processing, identification, and data management for the Maryland Department of Natural Resources Maryland Biological Stream Survey (MBSS). It is intended to serve as the Standard Operating Procedure for DNR staff involved in benthic processing and identification as well as a guide for other agencies, organizations, and businesses wishing to use comparable methods. Field methods for MBSS benthic sampling may be found in the MBSS Sampling Manual.

2   Sample Check-In and Inspection

Upon arrival at the DNR Field Office (see Contact Information, Appendix A) list each sample on the Benthic Macroinvertebrate Sample Chain of Custody Form (Appendix B).

Each sample container is given a unique "Log Number" by Field Office staff. The Log Number is sequential for each calendar year with the first two digits indicating the year of collection and the last four digits indicating the sequential number of each sample container in the order that it is received by the lab (e.g., 000487 is sample bucket number 487 in calendar year 2000). Log Numbers, which are kept in a separate written notebook for each calendar year, are especially useful in avoiding confusion related to duplicate samples taken at the same site and for tracking the status of sample buckets by indicating sample condition (e.g., rotten sample, dry sample).

Check each sample container for an adequate quantity of preservative (see Appendix C for a description of preservative). Ideally, there should be about twice as much preservative as there is sample material (by volume). If the preservative is low due to spillage or evaporation, add more. Check sample containers for damaged or poorly-fitting lids. Correct these problems as needed.

3   Laboratory

3.1   Preparation and Subsampling

Fill out all information on a small label to be placed inside the picked sample jar. The label should include the site name, log number, collector, date collected, sub-sampler, number of grids picked, and pertinent remarks after subsampling. Use cotton bond paper or paper that will not degrade in ethanol over time. Under a fume hood, remove the sample container lid and inspect the sample contents. Verify that sample numbers on both outside and inside labels are the same and complete. Note any instances of sample drying, dried organisms, mold, unusual color or odors, etc. in the comments section of the Bench Sheet (example provided in Appendix D).

Over a large and well-ventilated sink, pour the sample contents through a U.S. Standard #30 (600 micron mesh) sieve, catching the ethanol preservative in a clean bucket positioned beneath the sieve. Once most of the ethanol is poured through the sieve, remove it from the sink. Gently rinse the interior of the sample bucket with tap water (sides, bottom, and lid) into the sieve until the ethanol odor is undetectable. Check that all organisms are removed from the sample bucket. Save the ethanol in the original labeled sample bucket for sortate storage (only ethanol that is not degraded [i.e., without a pungent odor or without discoloration] is saved for reuse). Rinse the sample material in the sieve with tap water to remove fine sediments. Clean large objects such as stones, sticks, and large leaves with a scrub brush to remove organisms. Discard these large objects after inspection. If the sample from a site required multiple buckets, all the sample material should be placed in the sieve at one time (if possible) and gently combined to make sure the sample material is homogeneous before splitting the sample into the subsample tray.

Position the 34in X 12in subsampling tray (see Appendix C for a description of MBSS standard subsampling tray) on a flat and level surface in good light. Rinse the contents of the sieve into the subsampling tray. Ensure that all sample material is transferred to the subsampling tray. Spread the sample material evenly over the entire tray bottom. Add tap water to the tray until the sample material is completely covered. Allow the sample material to hydrate for about 10 minutes. If the quantity of sample material is more than one sample bucket (about 86 ounces), the material may be split into (approximate) halves (or split more if necessary). The subsampling steps are repeated for each half. When splitting a sample, half of the picked organisms should be picked from each tray (60 from first tray, 60 from second tray), always completely picking the last randomly chosen grid.

Using a random numbers table or a similar method of choosing numbers, randomly choose a number between 1 and 100 (there are 102 2in^2 grids in the subsampling tray in which 2 grids are blocked off; see Appendix C). After positioning a light over the grid to be picked, begin removing organisms from the randomly chosen grid with forceps and place them in a watch glass or container with 95% ethanol. Remove floating organisms first and disregard any organisms that float into the grid while the remainder of the grid is being picked.

Entry-level processors should place picked organisms into a watch glass to be viewed by the lab supervisor under a low magnification stereoscope to verify that only benthic macroinvertebrates were picked and counted as an organism. This is done until the lab supervisor is confident that the processor can separate organisms from other material. After the lab supervisor is confident with the processing, that individual can process samples.

Tally the total number of organisms removed from each randomly-chosen grid that are placed in a sample jar. Also, tally the number of grids subsampled (including those with no organisms). While subsampling, any organism that extends over a line separating multiple grids is considered to be in the grid containing its head. If the processor is unsure which end is the head, then the organism is considered to be in the grid being picked if that grid contains greater than 50% of the organism.

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If the total number of organisms removed from the first grid is equal to or greater than 120, subsampling is complete for the sample. If not, repeat the above process for another randomly-chosen grid. Continue this process until at least 120 organisms have been subsampled. The last grid chosen must be picked in its entirety. For some samples, the total number of organisms may be less than 120, even after picking all grids (100) in the subsample tray. The 120-organism target is used to allow for organisms that are missing parts needed for identification, non-target organisms (e.g., mites), or non-organism material counted in the subsample. If possible, at least 100 picked organisms must be identifiable.

Once the target number of organisms is tallied, record the number of grids required for the subsample on the label. If the sample was split and subsampled twice, make a note of the number of grids needed to get the first and second groups of (approximately) 60 organisms. The total number of grids is divided by the number of trays picked and rounded up, if necessary, to a whole number. This number is entered on the bench sheet when identifying the sample. For example, if two trays are picked for one sample and the first tray contained 60 organisms in 6 grids and the second tray contained 60 organisms in 10 grids, then the number of grids to be entered on the sample's bench sheet would be 8. Once the label is completely filled out it is placed in a sample jar with the picked sample.

After subsampling, pour the remaining sample material back into the laboratory sieve, draining off the water. Place the sample material back into the original sample container(s) and add the original ethanol. Ensure that the subsample tray and laboratory sieve are rinsed well and free from remaining organisms prior to beginning another sample.

3.2   Identification

A list of taxonomic keys used for the identification of MBSS benthic macroinvertebrates can be found in Appendix E. Note that this list may be updated more often than this manual is revised.

Fill out the information at the top of a blank MBSS Benthic Macroinvertebrate Laboratory Bench Sheet (Appendix D), including Site Name, Log Number, Collector, Collection Date, Subsampler, Number of Grids, Date IDed, and Taxonomist Name.

In the event that less than 100 organisms are identified in a sample, verify its grid count. If 100 grids are reported, then identification is complete. If less than 100 grids are reported, subsample an additional grid in its entirety and add these organisms for identification. If after this step more than 100 organisms have been identified, then identification is complete. If less than 100 organisms are still present, continue subsampling individual grids until at least 100 organisms have

been identified or 100 grids have been subsampled.

3.2.1 Core and Targeted MBSS

For core (randomly-selected) and targeted MBSS samples, most organisms are identified to genus, if possible, using stereoscopes.

Refer to Appendix G for decision rules on exceptions. Those taxa not identifiable to genus (due to small size or damage) may be left at family level or higher. These are noted on the bench sheet at the higher taxonomic level. Counts at levels higher than genus are not assumed to be different taxa from those identified to genus. Likewise, counts at levels higher than family are not assumed to be different taxa from those identified to family.

When entering taxa and respective counts into the Microsoft Access program, each entry is prompted with an 'Exclude?' checkbox option (Appendix F). This Exclude checkbox should be checked when an organism should not be included in any taxa richness metric counts for IBI calculations. This box should only be checked when an organism can only be identified to family level (or above) due to immaturity, size, or damage, and the sample includes the taxon from the same family (or above) that has been identified to genus level.

3.2.1.1 Chironomid identification can follow either the subsampling method described below or identified without subsampling (all Chironomids are identified).

Chironomid Subsampling Method: Divide all Chironomid larvae within the subsample into Subfamilies (i.e., Chironominae, Orthocladiinae, Tanypodinae, Diamesinae) or Tribes (i.e., Tanytarsini, Chronomini) and count the total number in each group. If either the total number of Chironomids or the total number of individuals within a Subfamily or Tribe is ten or less, all larvae are identified (using slide mounts; see below). If there are more than 10 total Chironomids or more than 10 individual Chironomids within a Subfamily or Tribe, then approximately 20% of the individual larvae (rounded to the nearest whole number) within each Subfamily or Tribe are slide mounted and identified. For example, if you have a total of 60 Chironomid larvae in a subsample which are separated into 20 Orthocladiinae, 15 Chironomini, 16 Tanytarsini, 5 Tanypodinae and 5 Diamesinae, you would slide mount 4 Orthocladiinae, 3 Chironomini, 3 Tanytarsini, all 5 Tanypodinae, and all 5 Diamesinae.

To extrapolate the numbers of individuals within each Chironomid group genera, the subsample counts of each of the identified genera are divided by the fraction of the group that was subsampled, and the result is rounded to the nearest whole

number. Expanding on the previous example, if the subsample of Tanytarsini (3 out of 16 total) was identified as 2 Stempellina and 1 Tanytarsus, both would be divided by 3/16, resulting in extrapolations of 11 Stempellina and 5 Tanytarsus.

Clearing and mounting larvae

Remove Chironomid larvae from the ethanol and place them in water for about 10 minutes. Ensure that the larvae are totally immersed in the water and not floating. Place the larvae in 10% potassium hydroxide (KOH) in a small heat-resistant crucible. KOH serves as a clearing agent that digests the inner muscle tissues and leaves the sclerotized portions of the larval exoskeleton, including the body (Epler 2001). Heat them on low heat using a hotplate until the internal tissues are clear. If unable to use a hotplate, the larvae can be placed in room temperature KOH overnight or until internal tissues are cleared. Place the larvae in water again for about 5 minutes. Return the larvae to ethanol.

With a drop or two of ethanol on a microscope slide, place several cleared larvae in a row with all heads toward one edge of the slide and dorsum down (mouthparts upward). Do not allow the larvae to dry, as air bubbles within the integument may block essential structures from view. Add one or two drops of mounting media (CMCP 10/CMCP 9AF; see Appendix C for further details) next to the larvae. Carefully lower a cover slip (one edge down first) over the larvae. Try to prevent movement of the larvae and air bubbles from being trapped beneath the coverslip. Gently press on the coverslip with a pencil eraser to spread mouthparts and extrude air. Identify the larvae using a compound microscope. After identification, place a bead of clear nail polish around the edge of the coverslip to render the mount permanent. Detailed procedures for the mounting and identification of Chironomid larvae may be found in EPA (1990) or Epler (2001). Store all slide mounted Chironomid larvae in a slide storage box with the corresponding box of subsamples from the same sampling year.

Chironomid pupae are identified to Subfamily or Tribe without subsampling or mounting.

3.2.1.2 Mounting Oligochaeta

Place Oligochaetes in a drop of ethanol on a microscope slide. Place several drops of mounting medium (CMCP 10/CMCP 9AF) over the organisms. Carefully place a coverslip over the worms and gently press with a pencil eraser to remove

bubbles. Place the slide in a drying oven on low heat for 5 to 10 minutes or until tissues clear.

Place counts of all organisms in the subsample on the Bench Sheet. Include comments on sample conditions, etc. in the Comments section of the Bench Sheet. All identified non-Chironomid and non-Oligochaete organisms are placed into a sample jar and stored in numerical (Log Number) order.

4.0   Quality Assurance/Quality Control

4.1   Repeated Subsampling

Using sequential Log Numbers, every 20th sample (if two buckets were required at a site, they should be treated as a single unit) is randomly chosen for re-subsampling and identification according to the following procedure:

A.   Subsample and identify the subsample as usual EXCEPT - identify Chironomids to Subfamily or Tribe (do not slide mount the larvae) and Oligochaetes to Class. This serves as the QA/QC dataset.

B.   Return the once-identified organisms to the original sample bucket containing the sortate and preservative, and re-subsample.

C.   Identify the second subsample according to standard procedures (i.e., slide mount Chironomid larvae and Oligochaetes and identify them to genus and family, respectively, if possible). This serves as the finalized dataset used in IBI calculations.

D.   QC comparisons are made on the two taxa lists and benthic Index of Biotic Integrity (BIBI; see Southerland et al. 2005) values generated from the two subsamples (of the same sample). Note that the BIBI generated from the second subsample (the one with genus-identified chironomids) should be calculated with subfamily or tribe-level chironomid counts. Differences of less than 1 BIBI value are generally considered acceptable.

4.2   Taxonomy

Questionable identifications are verified by consulting other DNR benthic taxonomists, regional experts, and regional keys for certain taxonomic groups. Maryland DNR requires that MBSS benthic taxonomists pass both the EPT East (or EPT East Images), and North America Chironomidae (or North America Chironomidae Images) tests administered by the Society for Freshwater Science.

5.0   Literature Cited

EPA 1990. Macroinvertebrate field and laboratory methods for evaluating the biological integrity of surface waters. U.S. Environmental Protection Agency. EPA/600/4-90/030. Cincinnati, Ohio.

Epler, J. H. 2001. Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida. Raleigh, NC.

Southerland, M., Rogers, G., Kline, M., Morgan, R., Boward, D., Kazyak, P., Klauda, R., and Stranko, S., 2005. New Biological Indicators to Better Assess the Condition of Maryland Streams. Versar, Inc. Columbia, Maryland.

Stranko, S. 2009. Maryland Biological Stream Survey Sampling Manual. Maryland Department of Natural Resources. Monitoring and Non-tidal Assessment Division. Annapolis.

Contact Information

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Appendix B

MBSS Benthic Macroinvertebrate Sample Chain-of-Custody Sheet

Site ID	Collector (print)	Collection Date (DD/MM/YY)	Date Delivered to Field Office (DD/MM/YY)	Relinquished By (print)	Received by (print)	Field Office Log Number

Comments

**Guidance for MBSS Benthic Macroinvertebrate Sample Chain-of-Custody Sheet**

General

This sheet provides a means of tracking the transfer of benthic macroinvertebrate samples between field collecting crews and DNR field office personnel responsible for processing the samples. If multiple sample buckets are delivered for a single site, enter each bucket on a separate row. If entries are repeated down a row, it is not necessary to enter the information in each cell. Simply use an arrow or quote marks to indicate the information is repeated down the row. Please write as legibly as possible following the guidelines below. The entry of a printed name indicates the responsibility of the individual for relinquishing or receiving each sample.

1. **Site ID** — Enter the site ID just as it appears on the field data form.

2. **Collector (print)** — Print the name of the person who collected the benthic sample.

3. **Collection Date** — Enter the date the sample was collected (using DD/MM/YY format) just as it appears on the field data form.

4. **Date Delivered to Field Office** — Enter the date the sample was delivered to the field office using DD/MM/YY format.

5. **Relinquished By (print)** — Enter the printed name of the person relinquishing the sample to the appropriate field office staff member.

7. **Received By (print)** — Enter the printed name of the person receiving the sample at the field office.

8. **Field Office Log-In Number** — (Done by field office personnel) Enter the Benthic Sample Log-in number.

9. **Comments** — Place any pertinent comments regarding the delivered samples, including unusual circumstances, here. Examples include "label for sample from site X fell off - see label in bucket" or "some of sample for site Y spilled while in transport".

Appendix C

Supplies and Equipment
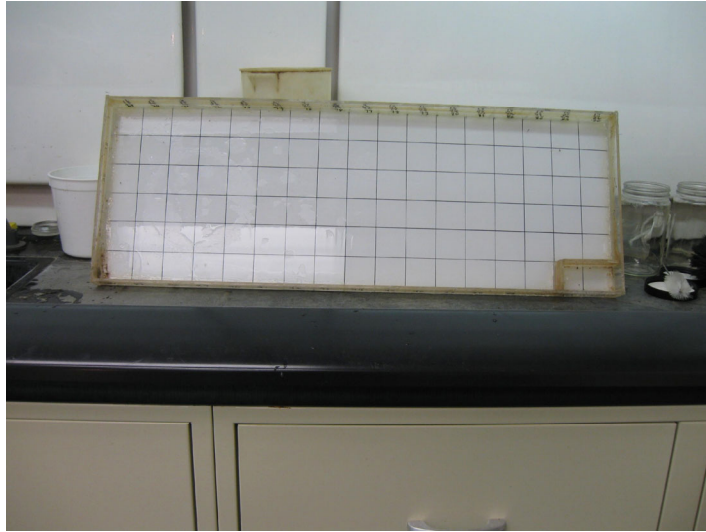
Subsampling Tray

34in X 12in plastic tray with 2" high walls; 102 2in X 2in black square grids drawn on the tray bottom. Block off 2 grids for a total of 100 available grids. Trays used by DNR were constructed by DNR staff. For detailed information on the DNR subsampling tray, contact Neal Dziepak.

Laboratory Sieve

12" diameter brass sieve; U. S. #30 (600 µm mesh)

Mounting Medium for Chironomidae and Oligochaeta

The two products used are CMCP-9AF and CMCP-10. The mixture used by DNR staff for mounting Chironomidae and Oligochaeta is 2/3 CMCP-9AF and 1/3 CMCP-10.

Clearing Agent for Mounting Chironomidae

10% Potassium Hydroxide (KOH)

Appendix D

Portion of the MBSS Benthic Laboratory Bench Sheet

MBSS Benthic Macroinvertebrate Laboratory Bench Sheet

Site: _____-_____-_____-_____   Collection Date _____   Date IDed _____
Log No. _____   Subsampler _____   Taxonomist _____
Collector _____   No. of Grids _____

GORDIIDAE		**GAMMARIDAE**		**SIPHLONURIDAE**		**PERLODIDAE**	
		Gammarus sp.		Siphlonurus sp.		Clioperla sp.	
DUGESIIDAE						Cultus sp.	
Cura sp.		**TALITRIDAE**		**ODONATA**		Diploperla sp.	
Girardia sp.		Hyalella sp.		**AESHNIDAE**		Isoperla sp.	
				Boyeria sp.			
PLANARIIDAE		**PALAEMONIDAE**				**PTERONARCYIDAE**	
Phagocata sp.		Palaemonetes sp.		**CALOPTERYGIDAE**		Pteronarcys sp.	
				Calopteryx sp.			
OLIGOCHAETA		**ISOPODA**				**TAENIOPTERYGIDAE**	
ENCHYTRAEIDAE		**ASELLIDAE**		**COENAGRIONIDAE**		Oemopteryx sp.	
LUMBRICULIDAE		Caecidotea sp.		Argia sp.		Strophopteryx sp.	
NAIDIDAE		Lirceus sp.		Enallagma sp.		Taeniopteryx sp.	
TUBIFICIDAE				Ischnura sp.		Taenionema sp.	
Limnodrilus sp.		**CAMBARIDAE**				**HEMIPTERA**	
Spirosperma sp.		Cambarus sp.		**CORDULEGASTRIDAE**		**BELOSTOMATIDAE**	
		Orconectes sp.		Cordulegaster sp.		Belostoma sp.	
HIRUDINEA		Procambarus sp.					
BACTRACOBDELLA				**CORDULIIDAE**		**CORIXIDAE**	
ERPOBDELLIDAE		**INSECTA**		Somatochlora sp.		Palmacorixa sp.	
		COLLEMBOLA				Trichocorixa sp.	
GLOSSIPHONIIDAE		**ISOTOMIDAE**		**GOMPHIDAE**			
Placobdella sp.		Isotomurus sp.		Arigomphus sp.		**GERRIDAE**	
				Dromogomphus sp.		Gerris sp.	
PISCICOLIDAE		**EPHEMEROPTERA**		Gomphus sp.		Trepobates sp.	
Piscicola sp.		**AMELETIDAE**		Hagenius sp.		Limnoporus sp.	
		Ameletus sp.		Lanthus sp.		Metrobates sp.	
GASTROPODA				Progomphus sp.			
ANCYLIDAE		**LEPTOPHLEBIIDAE**		Stylogomphus sp.		**NOTONECTIDAE**	
Ferrissia sp.		Habrophlebia sp.				Buenoa sp.	
		Leptophlebia sp.		**LESTIDAE** Lestes sp.		Notonecta sp.	

Appendix E

List of Commonly-Used Taxonomic Keys

Epler, J. H. 2001. Identification Manual for the Larval Chironomidae (Diptera) of North and South Carolina. A guide to the taxonomy of the midges of the southeastern United States, including Florida. Raleigh, NC.

Merritt, R. W., K. W. Cummins, and M. B. Berg. 2019. An Introduction to the Aquatic Insects of North America. 5th Edition, Kendall Hunt Publishers. Dubuque, Iowa.

Morse, J. C., W. P. McCaferty, B. P. Stark and L. M. Jacobus (Editors), 2017: Larvae of the Southeastern USA Mayfly, Stonefly and Caddisfly Species (Ephemeroptera, Plecoptera and Trichoptera). Clemson University. Clemson, South Carolina.

Peckarsky, B. L., P. R. Fraissinet, M. A. Penton, and D. J. Conklin, Jr. 1990. Freshwater Macroinvertebrates of Northeastern North America. Comstock Publishing Association. Ithaca, New York.

Pennak, R. W. 1989. Freshwater Invertebrates of the United States. Third Edition. John Wiley and Sons, Inc. New York, New York.

Stewart, K.W. and Stark, B.P. 2002: Nymphs of North American stonefly genera (Plecoptera), 2nd edition. The Caddis Press, Columbus, Ohio, XII + 510 pp.

Wiggins, G. B. 1996. Larvae of the North American Caddisfly Genera (Trichoptera). Second Edition. University of Toronto Press. Toronto, Canada.

Appendix F

Overview of Data Entry, Management, and Analysis

Use Microsoft Access (Office 2016 Version) to enter data from the Bench Sheet. Look-up Tables for sites and taxonomic names are provided in the program and these are updated at least annually by DNR staff as needed. The data entry screen is shown below. All benthic data (including taxon, counts, and site information) are double entered by two individuals with results compared visually following the second entry.

Once taxonomic counts are entered into Access, total organisms per subsample counts are evaluated, along with supplemental environmental (e.g., habitat, water chemistry, land use) data. Those samples with fewer than 60 organisms are evaluated carefully. For these sites, Best Professional Judgement is used to determine if low counts are likely due to sampling error or impairment at the site.

For details on MBSS data management and storage, contact a staff person listed in Appendix A.

Data Entry Screen from Access Program

Appendix G

MBSS Decision Rules for Benthic Macroinvertebrate Taxonomy

Taxon	Phylum	Class	Order	Family
IGNORED/NOT PICKED				
ACARI	Arthropoda	Arachnida		
CARABIDAE	Arthropoda	Insecta	Coleoptera	Carabidae
LAMPYRIDAE	Arthropoda	Insecta	Coleoptera	Lampyridae
COPEPODA	Arthropoda	Crustacea	Copepoda	
OSTRACODA	Arthropoda	Crustacea	Ostracoda	
CLADOCERA	Arthropoda	Crustacea	Cladocera	
HYDRA	Cnidaria	Hydrozoa		Hydridae
PORIFERA	Porifera			
BRYOZOA	Bryozoa			
URNATELLA	Entoprocta		Urnatellidae	Barentsiidae
ORTHOPTERA	Arthropoda	Insecta	Orthoptera	
TARDIGRADA	Tardigrada			
NOT ID'ED LOWER THAN PHYLUM				
NEMATODA	Nematoda			
NOT ID'ED LOWER THAN ORDER				
BRANCHIOBDELLIDA	Annelida	Oligochaeta		
NOT ID'ED LOWER THAN FAMILY				
BRACONIDAE	Arthropoda	Insecta	Hymenoptera	Braconidae
CHRYSOMELIDAE	Arthropoda	Insecta	Coleoptera	Chrysomelidae
DOLICHOPODIDAE	Arthropoda	Insecta	Diptera	Dolichopodidae
ENCHYTRAEIDAE	Annelida	Oligochaeta	Haplotaxida	Enchytraeidae
EPHYDRIDAE	Arthropoda	Insecta	Diptera	Ephydridae
GORDIIDAE	Nematomorpha	Nematomorpha	Gordioidea	Gordiidae
HAPLOTAXIDAE	Annelida	Oligochaeta	Tubificida	Haplotaxidae
ISOTOMIDAE	Arthropoda	Insecta	Collembola	Isotomidae
NAIDIDAE	Annelida	Oligochaeta	Haplotaxida	Naididae
NOCTUIDAE	Arthropoda	Insecta	Lepidoptera	Noctuidae
PELECORHYNCHIDAE	Arthropoda	Insecta	Diptera	Pelechorhynchidae
SALDIDAE	Arthropoda	Insecta	Hemiptera	Saldidae
SARCOPHAGIDAE	Arthropoda	Insecta	Diptera	Sarcophagidae
SCIOMYZIDAE	Arthropoda	Insecta	Diptera	Sciomyzidae
TORTRICIDAE	Arthropoda	Insecta	Lepidoptera	Tortricidae
ID'ED TO FAMILY BUT SHOULD NOT BE SAMPLED				
UNIONIDAE	Mollusca	Bivalvia	Unionoida	Unionidae

APPENDIX H

Glossary of Terms

Bench sheet – Paper document for handwritten (pencil) entry of benthic macroinvertebrate taxon, counts, site and sampling information, and lab processing and identification information.

Grid – A 2 in X 2 in square drawn on the bottom (outside) of the MBSS subsampling tray. The tray includes 102 such squares.

Processing – The entire series of steps from checking in an MBSS sample, washing the sample, placing the sample material in the subsampling tray and subsampling.

Picking – The process of removing individual specimens from the MBSS subsampling tray and placing them in the appropriate container during the subsampling process.

Sample – Benthic material (living and nonliving) removed from a stream, placed in one or more sample buckets, field preserved and delivered to the lab for processing and identification.

Sortate – Sample material remaining after the subsampling process is complete.

Subsample – Benthic macroinvertebrates removed (picked) from the subsampling tray for subsequent identification. The target number of individual specimens is 120.