7.18

STANDARD OPERATING PROCEDURES FOR LABORATORY PROCESSING OF MACROINVERTEBRATE SAMPLES

Summary

Macroinvertebrate samples collected in the field by either the single or multi-habitat method are best processed in the laboratory under controlled conditions. Aspects of laboratory sample processing include washing, rinsing, sub-sampling, sorting, identification, and enumeration of organisms.

The following protocol describes a method to sub-sample macroinvertebrates collected from a site. In cases where the sample contains large numbers of organisms, sub-sampling reduces the effort required for sorting and identification. The following protocol is based on a 500 organism sub-sample, but it can be used for any size sub-sample (100, 200, 300, etc.).

Equipment list

√	Item
	Laboratory sample log in forms (Figure 7.20.1)
	Laboratory bench sheets for sorting and identification (Figure7.20.1)
	Sorting Pans (surface area of pan should be divided into grids of equal size for picking)
	Forceps (both fine tipped, medium tipped and curved)
	Dissecting Probes and Needles
	Watch Glasses
	Dissecting Scope (9X to 110X for final IDs)
	Dissecting Scope (7X to 30X to aid in sorting)
	Compound Microscope (4X, l0X, 40X, and 100X oil objectives and phase contrast optics)
	Specimen Vials (assorted sizes of 1, 2, and 4 drams and larger with screw cap vials for voucher specimens)
	Squeeze bottles (1 liter for 70% ethanol)
	Eyedroppers
	Tally counter
	Hot plate
	Microscopes slides
	Microscope cover slips 1 oz. Round
	Magnifying lens with light source for picking samples
	Taxonomic keys
	70% Ethanol
	Euparol and/or CMC 10 mounting media
	Potassium Hydroxide (KOH) 10% by volume
	Illuminator compatible with dissecting scope
	Deck of numbered cards

Procedures

1. Sample Login In

Upon receipt by laboratory personnel, record all samples on the laboratory sample log in form (Figure 7.18.1). Include the date received and all information from the sample container label. If more than one container was used, record the number of containers per sample. All samples should be sorted in the same laboratory to enhance quality control.

2. Washing and Preparing the Sample for Sorting

Thoroughly rinse the sample in a 500 µm-mesh sieve to remove preservative and fine sediment. Large organic material (whole leaves, twigs, algae, or macrophyte mats, etc.) not removed in the field should be rinsed, visually inspected, and discarded. If the samples have been preserved in alcohol, it will be necessary to soak the sample contents in water for about 15 minutes to hydrate the benthic organisms. This will prevent them from floating on the water surface during sorting. If the sample was stored in more than one container, the contents of all containers for a given sample should be combined at this time. Gently mix the sample by hand while rinsing to make the entire sample homogeneous.

After washing, spread the sample evenly across a pan marked with numbered grids approximately 6 cm × 6 cm. Along the sides and top of the gridded pan, line up numbered specimen vials, which will hold the sorted organisms. Start with vials 1-15 set up and have vials 16-30 available, if needed. If the sample is to be identified that day, these jars can contain water. If it is towards the end of the day and they will not be identified in the next twelve hours the jars should contain 70 percent ethanol.

3. Sample Sorting and Counting

Using a deck of cards that contains numbers corresponding to the numbered grids in the pan, draw a card to select a grid within the gridded pan. This is done to make sure a random sample is carried out. Begin picking organisms from that square and placing them in the numbered vials. Any organism that is lying over a line separating two grids is considered to be on the grid containing its head. In those instances where it may not be possible to determine the location of the head (worms for instance), the organism is considered to be in the grid containing most of its body. Each numbered vial should contain one taxon of organisms. Use a tally counter to keep track of the total number of organisms. The tally counters can also be used to keep track of specific taxa (i.e., scuds or corixids) that may be in high abundance. When all organisms have been removed from the selected grid, draw another card and remove all the organisms from that grid in the same manner. If new taxa are found, place them in the next empty vial. Continue this

process of drawing cards and picking grids. After 10 grids have been picked, determine the average number of organisms per grid and determine approximately how many total grids will be picked to reach 500 organisms. When approaching that number of grids, monitor the total count of organisms. A sample should not be stopped in the middle of picking a grid, so stop on a grid that will give a number of 500 organisms or more. This is done to eliminate any bias as to which organisms would be picked in the last grid. Rarely will the final count be exactly 500 organisms. Note on the bench data sheet how many grids were picked to get the final count. Save the remaining unsorted sample debris residue in a separate container labeled "sample residue"; this container should include the original sample label.

On the laboratory bench data sheet (Figure 7.18.1) write down the tentative identifications and total numbers of organisms for each vial. Examine vials under a 10X dissecting scope to count organisms and ensure that all organisms are of the same taxon. Do not try and separate taxa that are hard to differentiate, this will be done under higher power during the final identification. Once all vials have been recorded on the bench sheet, place screw tops on the vials, place the vials and bench sheet into a designated tray and bring it to the final identification station.

After laboratory processing is complete for a given sample, all sieves, pans, trays, etc., that have come in contact with the sample will be rinsed thoroughly, examined carefully, and picked free of organisms or debris; organisms found will be added to the sample residue.

4. **Sample Identification**

Final organism identifications should be done to the lowest taxonomic level practicable (genus/species preferred). In order to provide accurate taxonomic identification, midge (Chironomidae) larvae and pupae will be mounted on slides in an appropriate medium (e.g., Euparal, CMC-10); slides will be labeled with the site identifier, date collected, and the first initial and last name of the collector. As with midges, worms (Oligochaeta) must also be mounted on slides and should be appropriately labeled. All slides should be archived so further levels of identification can be done at a later date. Each taxon found in a sample is recorded and enumerated on the laboratory bench sheet (Figure 7.18.1). Any difficulties encountered during identification (e.g., missing gills) are noted on these sheets.

Record the identity and number of organisms in each taxonomic group on the laboratory bench sheet. Also, record the life stage of the organisms and the taxonomist's initials. After each taxon is identified, the organisms will be placed in a container. A label with the site number, location, date of the sample, and taxonomic identification should also be placed in the container.

5. Sample Vouchers and Storage

In order to ensure accuracy and precision it is recommended that a voucher collection be established for each set of samples, which are enumerated and identified by a specific laboratory. A voucher collection is established by extracting individual specimens of each taxon from the sample collection. These individuals will be placed in specimen vials and tightly capped. A label that includes site, date, taxon, and identifying taxonomist will be placed inside the vial. Slides that are to be included in the voucher collection must be initialed by the identifying taxonomist. A separate label may be added to slides to include the taxon (taxa) name(s) for use in a voucher or reference collection.

For archiving samples, specimen vials (grouped by voucher collection station and date) are placed in jars with a small amount of denatured 70 percent ethanol and tightly capped. The ethanol level in these jars must be examined periodically and replenished as needed, before ethanol loss from the specimen vial takes place. A stick-on label is placed on the outside of the jar indicating sample identifier, date, and preservative (denatured 70 percent ethanol). Voucher collections will be cataloged and placed in the North Dakota River and Stream Macroinvertebrate Collection located at Valley City State University by Dr. Andre DeLorme, Ph.D.

Macroinvertebrate Laboratory Bench Data Sheet

North Dakota Department of Health
Division of Water Quality- SWQMP
Telephone: 701.328.5210
Fax: 701.328.5200

Site:	Sample #:	Date sampled:
No. of Squares picked:	Date ID:	Picker(s):

Vial #	Phylum/ Order	Family	Genus Species	Final Count	Life Stage	Notes
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						
22						
23						

Figure 7.18.1. Macroinvertebrate Laboratory Bench Data Sheet.

Field Recording Form for Biological Monitoring
North Dakota Department of Health
Division of Water Quality-SWQMP
Telephone: 701.328.5210
Fax: 701.328.5200

SITE ID:_____ **DATE:** _____/ _____/ _____

FIELD NUMBER:_____ **SAMPLERS:** _____

STATION DESCRIPTION:_____

_____

LATITUDE: _____ **LONGITUDE:** _____

ECOREGION (circle one)**:** 43 42 46 48

INVERTEBRATE COLLECTION METHOD (circle one): D-NET OTHER _____

REACH LENGTH:_____ M

STREAM HABITAT TYPE (%):	RIFFLE:_____	POOL:_____	SNAG:_____	UNDERCUT BANK:_____
	AQUATIC VEG: _____	OVERHANG VEG:_____	OTHER:_____	

FIELD WATER CHEMISTRY	**SITE PHOTOS**
TEMP:	UPSTREAM:
DO:	DOWNSTREAM:
pH:	
COND:	

WEATHER CONDITIONS (Temp., Wind, etc.): _____
_____

COMMENTS: _____
_____

Figure 7.18.2. Macroinvertebrate Field Collection Data Recording Form.

SITE DRAWING (Show direction of water flow and north)

COMMENTS:

Figure 7.18.2 ctd. Macroinvertebrate Field Collection Data Recording Form (reverse).