Standard Operating Procedure (SOP)

Aquatic Invertebrate Collection, Habitat Assessment,

and Laboratory Sample Processing

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Center for Water and the Environment
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EMPLOYEE TRAINING

This document describes the sample collection and processing procedures used by the Microscopy Laboratory at the University of Minnesota Duluth's (UMD) Natural Resources Research Institute (NRRI), and is a revised version of NRRI/TR-99/37. Prior to any work-related effort, individuals are trained in laboratory safety requirements, project and field safety, and are approved by the department safety officer. For detailed information regarding general laboratory safety and protocols, refer to The University of Minnesota Department of Health and Safety Laboratory Safety Plan (see http://www.nrri.umn.edu/safety/policies.html for documentation). All project personnel are trained in sampling techniques by experienced CWE personnel, made aware of laboratory chemical inventory (Table 1), and sample or process samples only under the supervision of personnel experienced in these sampling techniques until they demonstrate proficiency.

Table 1. Microscopy Laboratory chemical inventory and documentation. Material safety data sheets for any chemicals/products are in the general Laboratory Standard Operating Procedure manual located in NRRI Rm. 486. Rooms 486 and 488 are laboratories, and room 106 is dedicated for building chemical storage.

Microscopy Laboratory Sample Storage And Chemical Inventory		
Chemical/Product	*CAS#	NRRI Room No.
Formalin (37% formaldehyde)	50-00-0	486, 488, 106
Ethyl Alcohol (ETOH, 190 Proof)	64-17-5	486, 488, 106
Euparal (Eucalyptol)		486
Permount Solution		486
CMC-10 Mounting Media		486
Sodium Nitrate	7631-99-4	486
Kahle's Preservative (≈4% formaldehyde, 38% ETOH, 58% H_20 when mixed in sample) (≈ 7.4% formaldehyde, 76% ETOH, 16.6% H_20 when un-mixed)		106
LUDOX (Silicoidal Solution)		106
Sodium Phosphate	7558-79-4	486
FINQUEL (MS-222)	886-86-2	486

FIELD SAMPLING

The number and type of samples to be collected depends on project goals and the types of habitats under consideration. Samples obtained through petite ponar dredge, Hess, Surber, and corer capture organisms from a known area, so quantitative inferences can be made from the data. D-frame dip net samples offer only qualitative data. Rock bags are artificial substrates of relatively constant area; rock scrubs sample natural substrates of a relatively constant area. Quantitative inference can be made for both sample types. Target depth for samples which "grab" (ponar and corer), or capture dislodged invertebrates from agitation (D-net, Hess, Surber) is 0.20 m, which is the substrate depth utilized by most macroinvertebrates (Culp et al. 1983; Maridet et al. 1996). Sample types and collection methods are described below and in Table 2.

Table 2. Aquatic invertebrate sample gear types and their effective sample conditions.

Sample Type	Water Depths	Ecosystems	Effective Habitat Types
Ponar	> 1 m	Lakes, large rivers	Bottom substrates of fine sand or smaller particles
Rock bags	> 1 m	Lakes, large rivers	Hard bottoms which render ponars ineffective (bedrock, boulder, packed sand, etc.)
Dnet	< 1 m	All wadeable habitats	Any habitat that can be agitated by foot or sweeping to dislodge and capture invertebrates
Hess	15 – 35 cm	Flowing, wadeable streams	Bottom substrates of sand through large gravel
Surber	1 – 30 cm	Flowing, wadeable streams	Bottom substrates of sand through large gravel
Portable invertebrate box (PIB)	10 – 40 cm	Flowing, wadeable streams	Bottom substrates of large gravel through bedrock
Corer	< 1 m	Fluvial deposition areas, wetlands	Sediments dominated by sand or finer particles
Rock scrub	< 1 m	Lakeshores, wadeable streams	Appropriate when substrate sizes are large cobbles to small boulders, render D-netting ineffective

Sample Collection Types

1. **Petite ponar dredge.** The majority of benthic samples in water depths >1 m are collected via petite ponar dredge (sample area = 0.023 m^2). A visual estimate of ponar fullness is recorded to quantify the effectiveness of the ponar grab sample. Fullness estimates less than 25% of ponar capacity will be discarded as ineffective sample attempts. Ponar samples over 70% full will be retained as successful samples. Up to 3 attempts will be made to get a > 70% full sample at each point. If unsuccessful, the fullest ponar sample will be kept and its percent fullness recorded on the data sheet as a range of 25-70%. Extra or "alternate" points are typically assigned in project design to accommodate some sample attempt failures. All petite ponar samples are washed through a 250 µm mesh net or sieve to remove silt and fine sand, and preserved in a labeled sample container.

2. **D-frame dip net.** D-frame dip nets (a.k.a. D-nets) are used to acquire samples from a multitude of habitats in shallow water (<1 m depth), and are often taken to collect macroinvertebrates representative of emergent vegetation. The openings on our dip nets are approximately 0.30 m wide by 0.14 m high (area = 0.0355 m^2). D-nets are outfitted with 500 µm mesh. Samples of invertebrates are taken by bouncing the net off of the bottom and then moving the net quickly up through the water column, brushing and "sweeping" the net through any vegetation in the process. In wetlands each sweep covers approximately 1 m; 10 sweeps constitute one sample. In streams D-nets are used to sample various habitats not sampleable by Hess, Surber, or corer (i.e., root wads, overhanging vegetation, SAV, woody debris). Stream D-net samples do not necessarily have a sweep number associated with them, but the types of habitats sampled are recorded. Each sample is washed into a labeled sample container and filled with preservative.

   D-frame dip nets are most often used in the "sweep" method described above. However, they can also be used to "scoop" material not easily sampled by sweeping, or that cannot be sampled by a preferred method (e.g., wood chips unsampleable by petite ponar dredge). If the water depths are not greater than the length of the net handle, a "scoop" can be taken of bottom substrates to simulate the amount of material which would be collected in a petite ponar. The scooped material is transferred immediately from the dip net to a 250 µm mesh net or sieve to wash down.

   D-frame dip nets can also be used to capture invertebrates dislodged while disturbing larger substrates by foot upstream from the net. This technique is useful in flowing water (riverine) habitats where the rocks are too large to manipulate with the end of the net. Similarly, along lakeshores or in riverine habitats with little noticeable flow, a backwards shuffle can be used to collect invertebrates. While performing this technique the net is held with the net opening facing the operator, and rocks are dislodged by foot while shuffling backwards and sweeping the net through the cloud of dislodged sediments and organisms. Collected material is washed down within the net and material placed in a labeled sample container with preservative.

3. **Modified Hess sampler.** Our modified Hess sampler is a 0.30 m diameter (area = 0.086 m^2) steel cylinder outfitted with 250 µm mesh. The Hess is used in wadeable riverine habitats with unidirectional flow, and where benthic sediments are loose enough to press the lower 0.20 m into the bottom substrate. Water depths should be between approximately 0.15 and 0.35 m for correct operation of the Hess. The bottom substrate encompassed within the Hess is agitated by hand to dislodge invertebrates that are then carried by the current into a collection chamber. Larger rocks are scrubbed by hand within the Hess. After thorough rinsing the collection chamber is removed and the material placed in a labeled sample container with preservative.

4. **Surber sampler.** The Surber is a 0.305 x 0.305 m (area = 0.093 m^2) box with an open bottom and collapsible sides. The Surber has a 250 µm mesh expansion "bag" for dislodged invertebrates to be captured. A Surber is used where flow is unidirectional (streams) and when bottom substrates are impenetrable or water depths are too shallow to accommodate a Hess sampler. The Surber sampler is effective in sampling water 0.01 to 0.30 m. The substrate materials encompassed within the bottom frame of the Surber are agitated by hand to dislodge invertebrates into the net. Larger rocks are scrubbed by hand. After thorough rinsing the material collected is placed into a labeled sample container with preservative.

5. **Portable invertebrate box (PIB).** The PIB is a 0.32 x 0.32 m (area = 0.10 m^2) box with an open bottom rimmed by a 5 cm lip onto which is affixed 6 cm of compressible foam. The PIB has a 250 µm mesh net and collection chamber in which dislodged invertebrates are captured. The PIB is used where flow is unidirectional (streams), when bottom substrates are impenetrable, and when depth and flow are too great to use a Surber sampler. The PIB is effective in sampling water 0.1 to 0.40 m. The operator kneels on the sampler lip, compressing the foam to the bottom substrate and forming a tight seal with the substrate. Substrate materials encompassed within the bottom frame are scrubbed by hand and the current carries the invertebrates into the collection net. After thorough rinsing the material collected is placed into a labeled sample container with preservative.

6. **Sediment corer.** A core tube is useful when sampling fine sediments (silt, fine sand, fine particular organics) in < 1 m water depth. A plexiglas tube of 0.07 m diameter (area = 0.00385 m^2) is inserted approximately 0.20 m into soft sediments. Placing an open hand or cork over the open end of the tube creates suction, allowing the tube to be withdrawn containing the sediment core. The collected material is washed through a 250 µm mesh net or sieve and placed in a labeled sample container with preservative.

7. **Rock scrubs**. When other sample methods fail, coarse, large substrates can be scrubbed for invertebrates in streams or along rocky lakeshores. Rocks are collected by hand from water < 1 m depth in a size and quantity appropriate to evenly fill an enamel tray of approximately 0.23 by 0.39 m (area = 0.09 m^2). The rocks are scrubbed with hand-held brushes and all material is rinsed through a 250 µm mesh net or sieve and placed in a labeled sample container with preservative. Scrubbed rocks are returned to the environment.

8. **Deployable rock bags**. Rock bags can be deployed as artificial substrates to sample benthic invertebrates from deep (> 1 m) habitats with hard bottoms that render a dredge ineffective. Bags should be deployed at depths that avoid heavy wave action or floods, and that are not in routine boating or shipping areas. Rock bags are constructed from approximately 2.5 cm netting (1") and the bag bottoms are lined with 250 µm mesh. The bags are filled with fist-sized washed river rock (cobble) that is smooth and has few cracks and crannies where invertebrates could hide. Once filled with rock a drawstring is pulled tight to close the opening and secure the rocks within. Nylon ropes (1/4") are attached to each rock bag to match the water depth and fitted with a brightly colored cork float to aid retrieval. Rock bags should be deployed in groups of 10 individual bags placed in close proximity. The entire group, called a "pod", is marked with a 4' high colored buoy per Minnesota Lake Superior management regulation. Pods should remain in place and undisturbed for 30 days to allow colonization. Upon retrieval, the rock bags are raised steadily to the surface at an even speed, and a 250 µm mesh hand net is quickly placed under each rock bag as it breaks the water surface to minimize loss of invertebrates. The rocks are removed from each bag, scrubbed, and retained for re-use. The material from each rock bag is rinsed through a 250 µm mesh net and placed in an individual labeled sample container with preservative (i.e. each rock bag is treated as an individual sample).

Sample Preservation and Inventory

Invertebrate samples are preserved in Kahle's solution, which is a combination of 95% ethyl alcohol (ETOH) and 37% formaldehyde (Formalin) in a ratio of 4:1, respectively, then mixed with equal parts sample water. Samples with high organic content are mixed with less water to ensure adequate preservation. One of the benefits of Kahle's solution is that it can be mixed at a higher concentration than needed as a preservative and diluted with equal parts sample water in the field. This reduces the volume of preservative to be transported, but increases the concentration of formaldehyde and alcohol per unit volume. At transportation and storage concentration, Kahle's solution consists of approximately 7.4% formaldehyde, 76% ETOH, and 16.6% H_20. The composition of Kahle's solution when mixed into a sample, and therefore equal part (50%) sample water, is approximately 3.7% formaldehyde, 38% ETOH, 58.3% H_20. Samples which contain no or little organic material may be preserved in 95% ETOH to reduce human exposure to formaldehyde. While an excellent preserving agent, formaldehyde is a known carcinogen.

As soon as samples are collected they are carefully labeled with interior and exterior labels which identify: 1) project name, 2) site name or number, 3) sample number and number of containers (i.e., large samples that are placed in multiple containers, A-D), 4) gear type (dnet, ponar, etc.), and 5) sample date. Interior labels are written in pencil lead or laser-printed on waterproof paper. Exterior labels are written in permanent marker.

A list of samples, including all label information, is copied into field notebooks or noted on field data sheets as samples are collected. A sample list accompanies all samples returning to the laboratory. Chain

of custody forms are completed and verified with the field sample list as incoming samples are returned to NRRI. Chain of custody forms and a field sample list are duplicated, with copies to the outside agency (where applicable), a copy filed with field notes or data sheets, and one copy placed in the project log book. Samples are clearly labeled, logged in, and stored in room 126, NRRI. Archived samples and remaining sample preservatives are deposited in Chemical Storage (Rm. 106, NRRI).

SUPPLEMENTARY HABITAT DATA COLLECTION

When invertebrates are collected from a location it is beneficial to concurrently record data on the surrounding area and water quality which represent the habitat where the invertebrates are living. Some of the habitat measurements NRRI collects as standard protocol are listed below; separated by wetland/open water or stream environments.

Wetland or open water samples

This category includes vegetated wetlands, large rivers, inland lakes, and the Great Lakes. Other measurements may be deemed necessary, but those listed below should be collected as standard protocol.

Water quality: water quality parameters (temperature (°C), specific conductivity (µS/cm), dissolved oxygen (%), dissolved oxygen (mg/L), and pH) should be recorded at each sample location using a water quality probe (Sonde). In deep water the probe should be lowered to within 1 m of the bottom. NRRI presently uses a Hydrolab MS5 manufactured by Hach Environmental. Maximum immersion depth of this model is 225 m.
Water depth (m): record water depth at sample location using a meter stick or depth finder.
Vegetation quadrat: record visual estimate of percentage of emergent, floating leaved, submergent, and open water in a 1 x 1 m quadrat. Indicate the dominant species for each vegetation morphotype.
Substrate texture: indicate whether the bottom sediments are sand, silt, clay, detritus (clearly visible pieces of decaying vegetation), or others as they occur. Record the top two dominant types.

Stream samples

Invertebrates collected in small flowing streams are often correlated with bottom substrate type, and are also subjected to a range of flow rates. Discharge and water quality probe readings need only to be taken once per stream reach unless there are contributing tributaries between sample locations. Other measurements may be deemed necessary, but those listed below should be collected as standard protocol.

Discharge (m^3/sec): may be calculated once per stream reach where invertebrates are sampled. See Stream Survey Standard Operating Procedures for calculating discharge.
Water quality: water quality parameters (temperature (°C), specific conductivity (µS/cm), dissolved oxygen (%), dissolved oxygen (mg/L), and pH) should be recorded at one location representative of the

stream reach sampled using a water quality probe. NRRI presently uses a Hydrolab MS5 manufactured by Hach Environmental.
Flow velocity (m/sec): recorded at 60% water depth from surface using a flow meter. We are currently using a Marsh-McBirney Flow Mate 2000.
Water depth (m): record water depth with a meter stick at sample location.
Bottom substrate composition: record percentages of substrate categories bedrock, boulder (>256 mm), cobble (64-256 mm), large gravel (32-64 mm), small gravel (2-32 mm), sand (0.062-2 mm), silt (0.004-0.062 mm), and clay (<0.004 mm) in a 0.5 x 0.5 m quadrat of the stream bottom.
Embeddedness: visual estimate of percent that large substrates (>32 mm) are embedded by finer particles. Can be supplemented with a meterstick measurement of depth of fine sediments.
Presence of woody debris or aquatic vegetation: note in sample log whether the location sampled had woody debris or vegetation within 1 m.

Optional data collection components:

Sediment particle size fractioning and organic matter content*:* A core tube of 0.07 m diameter (0.00385 m^2) is used to extract a 0.20 m deep core of sediment, which is bagged and labeled in the field. Samples are frozen until they can be processed. Upon processing, samples are thawed, dried at 105 °C for 24 hours, weighed (g), ashed at 500 °C for 1 hour, wetted down and re-dried, re-weighed, and finally put through a vertical sieve series of graded mesh sizes: 4.0, 2.0, 1.0, 0.5, 0.25, 0.063 mm, with a bottom plate capturing the smallest particles. This process can be completed for individual samples, or sample composites. This provides the percent composition of organic material and substrate sizes from coarse sand to combined silt/clay.

LABORATORY MACROINVERTEBRATE SAMPLE PROCESSING

Samples ready for processing are signed out of the project log book by lab personnel. Samples may contain multiple containers, so all containers for that sample are concurrently processed. All sample information contained inside the sample container is verified with outside labels and records from the project log book. Before processing begins the samples are drained through a 250 um sieve under a ventilation hood to remove preservative. Waste preservative is stored under the ventilation hood (Rm 485, NRRI) in containers labeled with appropriate hazardous waste information, and transferred to Hazardous Chemical Storage (Rm 138a) before reaching a 90 day maximum holding period.

After draining off preservative samples are rinsed under running tap water to remove any lingering preservative, and then washed through a sieve series in preparation for removing invertebrates from sediment and detritus. Two standard sieve sizes are used for invertebrate processing: 4 mm and 0.25 mm (250 µm). The sieves are stacked with the 4 mm on top, the 0.25 mm in the middle, and a 0.125 mm sieve at the bottom captures any material passing through the 0.25 mm sieve. The material which passes through the 0.25 mm sieve and into the 0 .125 mm sieve is not processed, and is deposited in a waste receptacle (waste material is not put down the drain).

All materials captured by the 4 mm sieve are transferred to a tray, glass pan, or sorting dish. Sample material is spread evenly throughout the pan (or multiple pans if lots of material) and invertebrates are removed under a 2X magnification lens. Typically, whole portions of the 4 mm sieve size are processed without sub-sampling. However, it may be necessary to sub-sample or elutriate the 0.25 mm portion of various samples depending on the quantity of material collected. For large amounts of sand or other heavy substrate types, elutriation is effective at separating light organic materials from heavier mineral particles. Such procedures may involve use of colloidal silica or using a saturated sucrose solution. Currently, NRRI does not use elutriation as a sample separation procedure, and is instead subsampling by splitting the sample volume into smaller amounts using a specially-designed sample splitter.

Generally, the 0.25 mm portion of samples should take a maximum of 3-4 hours to process (remove the invertebrates from the sediment, vegetation, or detritus). Samples are split when the quantity of material to sort through is too great to allow the entire sample to be processed within the 3-4 hour processing goal. If one-quarter of a sample requires less than 1 hour to process, then the entire sample will be processed (e.g., total processing time of 4 hrs). Similarly, if one-quarter of a sample requires 2 hours to complete, then a second quarter can be processed within the 4 hr time limit, resulting in one-half of the sample being processed. Samples that contain very large amounts of small detritus may take 4 hrs to process only one-quarter of the sample. Sub-sampled portions not being processed are returned to a container with 70% ethanol and labeled with appropriate sample information, and also indicate the portion of the sample remaining (e.g., “unpicked 1/2”).

Organisms are removed from detritus using forceps under 2X to 7X magnification, depending on the size fraction being processed (higher magnification for smaller size fractions). Invertebrates are placed in vials of 70% ETOH labeled with the same information as on the container labels, as well as with the amount of sample processed (i.e., 1/4, 1/2, or whole), a vial number, the total number of vials for that particular sample (e.g., 1 of 3), and initials of lab personnel. The number of vials accompanying each sample will depend on the abundance of organisms, but one vial should be designated solely for midge larvae (Diptera: Chironomidae).

Samples that have been processed are subject to quality assurance/ quality control (QA/QC) guidelines, which specify that 10% of samples are checked for accurate removal of organisms. Accurate sample processing is defined as removal of at least 95% of all organisms. If a sample fails QC, it is repicked and other samples processed by that technician are checked until all pass QC. Each completed sample and accompanying vials are designated “complete” by placing an additional label on the container indicating its status (i.e., picked) and date completed. Sample remnants that pass QA/QC inspection may be discarded unless the sample is to be used to obtain organic content estimates. Those samples should be handled according to project protocol or standardized procedures (APHA 1985, ATSM 1985). Vials containing sorted organisms preserved in 70% ETOH are logged into a vial chain of custody form. No samples will remain outside of the appropriate sample jar when the technician responsible for a particular sample has left the laboratory.

Sample Identification

Macroinvertebrate vials are signed out for identification using the vial chain of custody form. Organisms are identified to the lowest taxonomic level using appropriate and up-to-date keys (e.g., Merritt et al. 2008, Thorp and Covich 2010, Wiggins 1996, Brinkhurst 1986), enumerated, and recorded on an identification data sheet. A reference collection can be made for each taxon identified for the project, if requested. Quality control (QC) of macroinvertebrate identifications will consist of 10% sample re-identification by another qualified NRRI taxonomist. Difficult or rare identifications will be sent out for verification to Dr. Kurt Schmude, University of Wisconsin Superior Lake Superior Research Institute, who is a regional expert on aquatic macroinvertebrates. Based on the QC results, taxa will be re-identified as necessary to correct identification data.

Identification of Chironomidae to genus

Our standard for processing midge larvae (Diptera in the family Chironomidae) is to identify to sub-family and/or tribe, but if specified in project goals, midge larvae can be identified to genus.

When identifying midge larvae to genus, the head capsules are permanently mounted on microscope slides to allow for higher magnification. Identification to genus requires head capsule removal to ensure ventral viewing of individual mouth parts. Organisms are soaked in 95% ETOH, preserved in CMC-10 mounting medium (Masters Company, Inc., Wood Dale, IL), and placed ventral side up under a cover slip. Generally, Chironomidae are sub-sampled in a watch glass, and 8 randomly-selected individuals are placed on a slide. Three slides are created per sample, yielding 24 Chironomidae specimens to represent the sample.

Permanent slide mounts are identified under a compound microscope to the lowest taxonomic level using standard identification guides (e.g., Merritt et al. 2008, Wiederholm 1983). Each individual organism is assigned a particular slide number, position, and side, so a separate reference collection for these organisms is not necessary because the location of individual organisms can be easily determined. Chironomidae identifications are usually subject to a 10% QC by Dr. Kurt Schmude, UWS.

DATA ENTRY

Data from field and laboratory sheets will be entered into an Excel spreadsheet in a format that allows for easy importation into a database. The formatting will be adjusted to match requests of the project funder. Counts will be corrected for subsampling and slide mounting. Data entry is subjected to 100% QC to ensure that all data are correct before being provided to the funder.

Raw QC'd data are merged with a taxonomic database to check for errors and provide higher taxonomic categories for all individuals identified. Individuals not listed in the database are either re-identified or the current information and taxonomic name are compared to the Integrated Taxonomic Information System (ITIS) database for confirmation (see http://www.itis.usda.gov).

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