Standard Operating Procedure for Benthic Invertebrate Field Sampling

LG406

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1.0 SCOPE AND APPLICATION

1.1 This standard operating procedure describes a method for collection and preservation of benthic invertebrate and sediment characterization samples from the soft sediment typical of Great Lakes deepwater benthic habitats.

2.0 SUMMARY OF METHOD

2.1 Three separate samples of benthic invertebrates are taken with a Ponar grab sampler at each designated sample site in each lake. Each sample is processed, preserved and stored separately. Samples are processed by elutriation. A fourth ponar sample is taken and the surface sediments are stored in separate containers for sediment grain size and sediment nutrient analysis.

2.2 During the spring cruise, samples are taken at five sites (Saginaw Bay, Green Bay, and the Western Basin of Lake Erie) and are analyzed specifically for the mayfly Hexagenia. Green Bay will be sampled from the Lake Guardian as part of the Lake Michigan survey. If ice cover presents sampling at the beginning of the survey, the station will be sampled on the way back to Milwaukee at the end of the survey. Saginaw Bay samples are collected by local collaborators. During the summer cruise, all stations are sampled except station HU 98, which is sampled separately by Michigan DNR, and samples analyzed for all benthic invertebrates.

3.0 EQUIPMENT

2 Full-size Ponar grab samplers
Sturdy tub to hold Ponar sample (2 to 4)
Elutriator, 500-µm mesh sleeve and deck stand

Hose/pump for water supply
Adjustable spray nozzle for hose
Funnel
1-L plastic wide-mouthed field sample bottles (2/3 of total amount)
0.5-L plastic wide-mouthed field sample bottles (1/3 of total amount)
6 oz glass jars with Teflon lids for sediment characterization (nutrients)
4 oz plastic jars for sediment characterization (grain size)
Homogenizing bowls (2-3 with minimum capacity of 2 cups)
Stainless steel spoons (2-3)
Labels and marking pens
Benthos field sheet
Ponar Grab Data Sheet (GLENDA data sheet)
Clear tape for affixing labels
Parafilm

4.0 REAGENTS

4.1 37 % Formaldehyde

4.2 Rose Bengal stain (powder)

4.3 Buffer (Borax)

4.4 Reagent Preparation

To make the stock solution, add approximately 1 g of Rose Bengal stain and 80 g of Borax per 1 gallon of 37% formaldehyde. To make one gallon of 10% formalin solution, add 380ml of stock solution (37% buffered formaldeyhyde with rose Bengal) to one gallon container with a spigot, and fill the remaining space with Deionized water (DI water). To make 10 L of 10% formalin solution, fill 10 L container with 9 L of DI water, then add 1 L of stock solution and mix well. To make a larger volume of 10% formalin solution, fill 20 L container with 18 L of DI water, then add 2 L of stock solution and mix well. This should be done on the ship, prior to arrival at the first site.

5.0 ON-STATION PONAR SAMPLER PROCEDURES

5.1 Sample Collection

5.1.1 Cock the arms of the Ponar grab sampler to open position and insert the spring loaded safety pin.

5.1.2 Lower the Ponar to the sediment surface. The grab sampler should be lowered slowly to within 5 m of the bottom, then allowed to free fall to the bottom. The jaws will close automatically as the grab sampler is raised from the sediment surface. If the Ponar grab sampler descends too quickly, it creates a "bow wave" that can push animals out from under the sampler, as well as strike the bottom at an improper angle. However, if it strikes the sediment without enough force, it will not penetrate deep enough for a good sample. Stopping the sampler during the descent may cause the trigger to release. The speed at which it is raised is not as critical as the speed of descent.

5.1.2.1 For the sediment characterization ponars, it is also best to avoid a "bow wave". The rate of ascent should be observed so that the ponar flaps remain in a closed position. This will minimize the disturbance of the surficial layer of sediment in the ponar. A successful grab is one having relatively level, intact sediment over the entire area of the grab, and lack of obvious disturbance or washout. Grabs containing little to no sediment or grossly slumped surfaces are unacceptable. Grabs completely filled to the top, where the sediment is in direct contact with the hinged top, are also unacceptable.

5.1.3 Once the grab sampler is on deck, check to see that the jaws are closed properly and that there is an adequate sample of sediment inside the Ponar.

5.1.4 Empty the grab sampler into a plastic tub.

5.1.5 Rinse the sediment and animals from the top screen and the interior of the Ponar.

5.1.6 If the substrate at the site is hard packed clay, sand or bedrock, the grab sampler will come up empty. Because the sample sites were originally located in depositional areas, none of these situations should occur. If these problems do occur, the station should be relocated to deeper water (moving as short a distance as possible, less than 500 feet) and the procedure re-started. If problems persist, consult with the GLNPO Chief Scientist on duty. Any decision to discontinue sampling *must be made by the GLNPO Chief Scientist*, and noted as such in the benthos field collection worksheet. If it is not possible to obtain a sample from the site, it might be necessary to revise the station location before the next cruise.

5.1.7 Benthos samples are rinsed by elutriating (section 5.2) and sediment characterization samples are processed according to section 5.4.

5.2 Elutriating the Sample

5.2.1 The elutriation method should be used for all samples. Visually examine the sediments during the elutriation process and note on the field sheet if there are large numbers of live or dead *Dreissena* spp. mussels, live *Dreissena* spp. present, and sediment type as relative percentages of the major elements (example: 50% clay, 40% mud, and 10% shells).

5.2.2 Place entire sample in the elutriator, fill it with water, and then gently stir the water and sediment together with your hand to break apart the sediment. Agitating the water too vigorously will destroy a large number of animals (soft bodied oligochaetes are most susceptible) and compromise the laboratory results.

5.2.3 Lift the handle edge of the elutriator and pour the water into the nozzle/net/field sample bottle GENTLY. Avoid squeezing the sleeve as this will damage and break fragile oligochaetes (and produce multiple oligochaete fragments) and molluscs shells. The sample will require several washings to rinse out material smaller than 500 microns. Pour the whole sample through the net and into the sample bottle. If large pieces of substrate are present (gravel, wood, coal), remove them from the elutriator, wash and then put directly to the bottle. If material is too large to fit into bottle, wash extremely well in the elutriator, ensure no specimens are attached, and then discard.

5.2.4 The efficiency of the elutriator separation step will vary somewhat with the sediment type at the site. Samples with high percentages of large detritus (e.g., samples from Lake Erie, Green Bay, or Saginaw Bay) may not separate well and two or more bottles per replicate may be needed to preserve the sample. Make sure all bottles of that replicate are labeled the same and make a note of how many bottles were used on the Benthos Field Collection Worksheet. If sandy substrate is present, multiple bottles may be needed to contain all material, this can be washed down in the biology lab with a 500µm sieve to attempt to place whole sample in one bottle, if not use multiple bottles.

5.2.5 The ENTIRE sample should be washed and placed in sample bottles. Do not overfill bottles, sediment should not take up any more than 2/3 of the space. If many dreissenids or organic matter is present, fill no more than halfway. Try to remove as much water that is possible from the bottle; the sample will preserve much better with less water.

5.3 To complete replicate sampling, repeat Steps 5.1 through 5.3 twice more, for a total of three replicates at each benthic station visited.

5.4 Sediment Characterization Samples

5.4.1 The fourth Ponar sample for sediment characterization is collected for stations in the summer survey at the direction of the Chief Scientist. Sample collection follows steps 5.1.1 through 5.1.3.

5.4.1.1 Drain water from the ponar (not allowing water into the tub).

5.4.1.2 Place the fourth sample GENTLY into a tub. It is important not to disturb the sample. Do not spray ponar with hose.

5.4.1.3 Approximately one half of the surface of the sample is needed in order to get a representative sample. Select a collection area or areas of the sample which make up approximately one half of the surface of the sample and where the top sediment is mostly intact, as evidenced by visible integrity of the surface layer and lack of obvious disturbance or washout. Using a stainless steel spoon, remove and discard large, non-living surface items such as rocks or pieces of wood, any submerged aquatic vegetation (SAV), and any visible organisms or mussel shells from the collection area.

5.4.1.4 Using a spoon collect the top 2 cm of sediment from the collection area and place in the homogenizing container. Total collected in the homogenizing container should be around 500 mL, but must be at least 300 mL (approximately 10 fluid oz).

5.4.1.5 Using the stainless steel spoon, stir the sample to thoroughly homogenize it, then fill each of the two containers to 2/3 full.

6.0 SAMPLE PRESERVATION AND LABELING

6.1 Sample Labeling

6.1.1 Field sample bottles should be labeled with lake, cruise, sample site, and parameter name (benthos, organic matter or sediment type). Duplicate and triplicate benthos samples are designated by a D and T, respectively, in the 7th field of the GLNPO number; all other samples have an S in that field. Sample labels are to be provided by GLNPO personnel aboard the Lake Guardian for each cruise. A list of the stations and sample ID's are to be provided by GLNPO personnel one month prior to the start of each cruise.

6.2 Preservation of Biological Samples

6.2.1 Once the sample is elutriated, return the bottles to the shipboard lab for preservation.

6.2.2 Fill remaining space in bottle with 10% buffered formalin solution made on the ship.

6.2.3 Wrap the top of the jar in parafilm to prevent leakage and store the sample in a designated cooler in the walk-in refrigerator or another designated area.

6.3 Preservation of Sediment Characterization Samples

6.3.1 Samples for sediment nutrient analyses in the 6 oz. glass jars should be stored in the shipboard freezer.

6.3.2 Samples for grain size analysis in the 4 oz. plastic jars should be stored in the shipboard refrigerator at 4º C. It is critical the grain size samples are not frozen.

6.4 Benthos Field Documentation

6.4.1 Notes should be made in the field log book to indicate any changes to the normal sampling procedure (e.g., more than one bottle used; sample skipped by authority of GLNPO Chief Scientist; unusual substrate encountered, etc.).

6.4.2 The field technician should also complete the Ponar Grab Data sheet and enter the data into the onboard computer GLENDA database.

7.0 QUALITY CONTROL

7.1 Precision of the sampling process is obtained by having all crew members follow the same steps in the same order for the entire cruise.

7.2 New crew members or those who have not previously performed the procedures must read a copy of this SOP before boarding the boat. A copy will also be available on the boat.

7.3 The more experienced benthic sampler must closely supervise the new sampler for at least two stations after which the new sampler is expected to perform sampling unsupervised.

7.4 Field duplicates for the sediment characterization samples will be collected at approximately five percent of the stations sampled to determine the precision associated with sample collection, preservation and storage.

8.0 SAFETY AND WASTE HANDLING

8.1 Refer to GLNPO's *Health, Safety and Environmental Compliance Manual* (May 1997, or as amended) and individual instrument procedural operations manuals for specific details on applicable 1) personal health and safety issues; 2) instrumental, chemical, and waste handling procedures; and 3) accident prevention. This applies to all EPA personnel, EPA contractors or federal, state, or local government agencies, and persons who operate or are passengers onboard US EPA GLNPO vessels during all activities and surveys.

8.2 All applicable safety and waste handling rules are to be followed. These include proper labeling and disposal of chemical wastes. Over-board discharges of chemical wastes are forbidden.

8.3 During sampling, caution, common sense, and good judgment should dictate appropriate safety gear to be worn in any given situation on deck. Hard hats, gloves, and steel-toed shoes must be worn in working conditions where there is a possibility of injury to the head, hands, or feet; however, if in doubt, please ask the Chemical Hygiene Officer.

8.4 Collecting samples in cold weather, especially around cold waterbodies, carries the risk of hypothermia and frostbite. Sampling team members should wear adequate clothing for protection in cold weather. For specific information regarding sampling during cold conditions, please refer to the US EPA GLNPO *Standard Operating Procedures for Winter Operations* (December 1994, or as amended).

8.5 Collecting samples in extremely hot and humid weather carries the risk of dehydration and heat stroke. Sampling team members should carry an adequate supply of water or other liquids for protection against dehydration in hot weather.

8.6 Work vests must be worn while working on the fantail and Rosette deck.

8.7 Safety glasses, hard hat and gloves are to be worn as directed by the ship-board chemical hygiene officer. The 37% formaldehyde solution and stock solution will be stored in the hazardous chemical storage locker (enough 10% formalin for a shift's stations can be kept in the hood in the biology lab) and used under a chemical hood.