STANDARD OPERATING PROCEDURES

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BENTHIC MACROINVERTEBRATE SAMPLING

CONTENTS

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1.0 SCOPE AND APPLICATION

The purpose of this Standard Operating Procedure (SOP) is to describe the procedures for sampling benthic macroinvertebrate populations. Analysis of benthos will be used, in conjunction with other bioassessment techniques, to assess the direct and/or indirect impact of contamination to benthic communities, composition, and functionality.

2.0 METHOD SUMMARY

Benthic sampling can be qualitative (a general assessment of the benthic taxa present, possibly with some observations of their relative abundance) or quantitative (an estimate of the numbers [total or by taxa] present so that a statistical confidence interval of the estimate can be calculated). Quantitative sampling is necessary to determine ratios of various functional feeding groups of benthic macroinvertebrates; for example, the ratio of the number of forms that skeletonize leaf litter (shredders) to the number that graze on attached algae (scrapers). All representative subhabitats of a given system should be thoroughly sampled. Different methods may be used depending on the type of habitat sampled (e.g., lentic or lotic).

2.1 Lentic Habitats

A variety of bottom dredges (grabs) are available for use in lentic, or still water habitats. Examples of some common collecting instruments are the Ekman dredge, Peterson dredge, and Ponar dredge. The Ekman dredge is the easiest to use since it is light and relatively easy to "set". However, use is limited to soft mud, silt, or finely divided sand bottoms. For sampling where the bottom material is compacted or consists of pebble, gravel, or organic litter substrate, the Peterson or Ponar dredge is preferred since these dredges are heavier than the Ekman dredge. Usually, small obstacles will not prevent the closing of these dredges and are crushed by the jaws, whereas the same materials may block the operation of the Ekman dredge.

2.2 Lotic Habitats

Benthic macroinvertebrate sampling from lotic, or running water habitats differs in both the type of the organisms collected, and the means used for collection. Because of the scouring action of the current, soft sediments are rarely found. Organisms of running waters are usually heavy bodied or have special means of attachment. Finally, because of the lack of fine sediments, the distribution of organisms with depth in the sediment is usually greater in running waters than in standing waters. An example of a commonly utilized sampling device used in running waters is the Surber stream bottom sampler. This device has a major drawback in that its use is restricted to waters of less than 30 centimeters (cm) in depth and of slow to moderate velocity.

3.0 SAMPLE PRESERVATION, CONTAINERS, HANDLING, AND STORAGE

After a representative sample has been collected, animals, vegetation, and substrate are preserved for picking, sorting, and analysis (e.g., taxonomic, statistical). The organisms from each sample are placed into a separate jar or vial and covered with a preservative such as 70 percent (%) ethyl alcohol, 40% isopropyl alcohol, or neutral formalin. Alcohol is less irritating to use than formalin but should be used only for short-term storage unless the animals are fixed first in formalin. Organisms collected for residue analysis are not chemically preserved, but are frozen on dry ice.

4.0 INTERFERENCES AND POTENTIAL PROBLEMS

There are several potential problems and interferences, most of which are physical in nature and may occur when sampling benthic communities. Temperature, recent storm events, high/low water levels and other factors can impact local benthic communities. Care must be taken to ensure that the sample locations are not impacted by these factors. If it cannot be avoided (e.g., flood event), the problem/interference should be properly documented in field logbooks as per SERAS SOP #4001, *Logbook Documentation.*

5.0 EQUIPMENT/APPARATUS

The following equipment is useful for benthic sampling:

Surber stream bottom sampler
Trowels
Forceps
Stiff bristle brush (wire or nylon)
Dredge, line, messenger weight, and pole
Plastic bucket, 5-gallon
No. 35 (0.5 millimeter) soil sieve
Wide-mouthed high density polyethylene (HDPE) bottles (500 to 1,000 milliliters)
D-frame nets
Hip or chest waders
Artificial substrates (e.g., Hester and Dendy)
Anchor for artificial substrates
White plastic trays or pans
Wooden stakes
Flagging tape
Marking pens
Shoulder length gloves
Acetate cores with caps
Surgical gloves
Tape measure

6.0 REAGENTS

Buffered neutral formalin is used to fix samples, and 70% ethyl alcohol or 40% isopropyl alcohol are used for sample preservation when samples are archived or submitted for taxonomic analysis only.

7.0 PROCEDURES

7.1 Preparation

1. Determine the extent of the sampling effort, the sampling methods to be employed, the types and amounts of equipment and supplies needed.

2. Ensure that all equipment is in working order.

3. Perform a general survey prior to site entry in accordance with the Work Plan (WP) and Health and Safety Plan (HASP).

4. Use stakes, flagging tape, buoys, or global positioning system (GPS) generated waypoints to identify and mark all sampling locations.

7.2 Surber Stream Bottom Sampler

1. Select a sampling site representative of the area desired with a depth no greater than the height of the net frame, or a depth easily reached by the sampler. Shallow riffle areas of relatively fast moving streams with cobble, gravel, and sand substrates are often best sampled using this method. The velocity of the stream must not be so great as to cause a "pressure head" of water to flow around the mouth of the sampler.

2. Wade from downstream, and place the sampler with the mouth of the net facing upstream in an undisturbed area. Care must be taken that there is no disturbance of the substrate upstream from the net, since organisms may be dislodged and washed into the net.

3. Lower the square foot frame onto the substrate anchoring it in place by setting the sampler between the feet of the person sampling. Pick up the larger rocks or bits of substrate within the frame, and while holding them in the mouth of the net, brush them free of all organisms, allowing the current to carry them into the net. Discard these rocks outside the frame.

4. After all the organisms have been brushed off the larger rocks and debris, use hands or a trowel to stir up or agitate the substrate within the square foot frame so that dislodged organisms can be carried into the mouth of the net and not around it. The substrate within the frame should be disturbed to a uniform depth.

5. After a sample has been collected, empty the contents of the net into a white pan, and remove rocks and large debris after inspection for clinging organisms. When completed, transfer the contents of the white pan into a wide-mouthed jar. Turn the net "wrong side out", and using forceps, pick off any organisms that have attached to the net fabric and place them into the jar. Depending on field conditions, number of organisms, etc., organisms may be partially

6. or fully picked in the field. This may save time compared to laboratory processing, since the organisms are alive and easier to spot. If required, target organisms being submitted for residue analysis should be removed, placed into an appropriate container, and stored on dry ice.

7. Depending on the number of replicates desired, move the sampler to other sites, and repeat steps 1 through 5.

8. After sampling is complete, add a sufficient amount of fixative or preservative to cover the substrate and organisms in the bottle. Each jar should be labeled indicating the date, site name, sample location and replicate number. Samples should be shipped to the appropriate laboratory for further processing as per SERAS SOP #2004, *Sample Packaging and Shipment*.

7.3 Hess Sampler

1. Select a sampling site representative of the area desired with a depth no greater than the height of the sampler, or a depth easily reached by the sampler. Due to the design of the sampler, deeper riffle areas (too deep for use with a Surber Sampler) of relatively fast moving streams with cobble, gravel, and sand substrates are often best sampled using this method.

2. Wade from downstream, and embed the base of the sampler into the stream substrate with the sampler body mesh facing upstream and the sample collection net facing downstream in an undisturbed area.

3. Set the sampler between the feet of the person sampling. Pick up the larger rocks or bits of substrate within the sampler, and while holding them in the mouth of the net, brush them free of all organisms, allowing the current to carry them into the net. Discard these rocks outside the frame.

4. After all the organisms have been brushed off the larger rocks and debris, use hands or a trowel to stir up or agitate the substrate within the sampler so that dislodged organisms can be carried into the mouth of the net. The substrate within the frame should be disturbed to a uniform depth.

5. After a sample has been collected, empty the contents of the net into a white pan, and remove any large debris after inspection for clinging organisms. When completed, transfer the contents of the white pan into a wide-mouthed jar. Turn the net "wrong side out", and using forceps, pick off any organisms that have attached to the net fabric and place them into the jar. Depending on field conditions, number of organisms, etc., organisms may be partially or fully picked in the field. This may save time compared to laboratory processing, since the organisms are alive and easier to spot. If required, target organisms being submitted for residue analysis should be removed, placed into an appropriate container, and stored on dry ice

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7.4 Dredges

1. Select sampling sites representative of the area to be sampled, and determine the number of replications desired. Dredges are particularly useful in areas of deep water, or soft bottom sediments.

2. Secure the dredge to a length of heavy rope. Ekman dredges can be operated by securing the dredge and a weighted messenger to a length of rope, or by bolting it to the end of the pole. After the selected dredge is secured, set the trip mechanism.

3. Lower the dredge slowly, particularly through the final 0.5 meters (m) of water above the substrate surface. If the depth of the water is unknown, lower the dredge slowly to the bottom and then raise it, move 1 or 2 meters laterally, and re-lower gently. Trip the dredge by dropping the weighted messenger, or depressing the tripping mechanism on the pole for the Ekman, or by allowing the line to slacken for the Peterson dredge or Ponar.

4. Retrieving and emptying the contents of a dredge requires two people due to the weight and bulkiness of a filled dredge. Lift the filled dredge to the surface with a smooth, even motion to avoid jarring out contents. It is recommended that the dredge be held over a pail while it is brought over the side of a boat or recovered. Care must be taken when retrieving and handling a dredge since they are especially heavy when loaded.

5. Empty the contents of the dredge into a pail. Repeat the sampling procedure until all of the desired replicates (each replicate is considered one discrete sample) have been collected at the sampling location.

6. Gently pour the contents of the pail into a No. 35 soil sieve. Repeat this step until all of the

contents of the pail have been poured through the sieve. A gentle spray of water can be used to break up compacted particles, and to facilitate passing the sediment through the sieve.

7. The material remaining on the sieve is the collected sample. Place the sieve into a pail of water, or surface water being sampled and agitate, taking care not to let the water run over the top of the sieve. This will wash smaller material through the sieve, and will concentrate the remaining material at one edge if the sieve is held at an angle.

8. When all materials and organisms are concentrated on one side of the sieve, remove the rocks and large debris that are free of clinging organisms and scrape them into a wide-mouthed jar. If necessary, wash the sieve again, and place any additional organisms/materials left in the sieve into the bottle, picking out the remains with forceps if necessary.

9. After sampling is complete, add a sufficient amount of fixative or preservative to cover the substrate and organisms in the bottle. Each jar should be labeled indicating the date, site name, sample location and replicate number. Samples should be shipped to the appropriate laboratory for further processing as per SERAS SOP #2004, *Sample Packaging and Shipment*.

7.5 Kick Nets

1. Select sampling sites representative of the area to be sampled, and determine the number of replicates desired. Riffle areas of relatively fast moving streams with cobble, gravel, and sand substrates are often best sampled using this method. This sampling method requires at least two people. The name “kick net” describes its functionality where, a field team member kicks up the substrate upstream, and allows the material to flow into the net.

2. The first field team member wades in from downstream, and places the net with the mouth facing upstream in an undisturbed area. The net is gently placed on the stream bottom in a relatively flat area and not on large rocks or debris.

3. While one field team member holds the net in place, another carefully wades into the stream perpendicular to the current, approximately 1 to 2 meters (m) upstream of the net. This team member begins kicking up the stream bottom while moving steadily downstream toward the net. This method can be “area” specific, or “timed”. Once the area has been disturbed thoroughly, the person holding the net lifts the net out of the water with a gentle scooping motion arcing the net forward and upward.

4. After a sample has been collected, empty the contents of the net into a white pan, and remove any debris after inspection for clinging organisms. When completed, transfer the contents of the white pan into a wide-mouthed jar. Turn the net "wrong side out", and using forceps, pick off any organisms that have attached to the net fabric and place them into the jar. Depending on field conditions, number of organisms, etc., organisms present may be partially or fully picked in the field. This may save time compared to laboratory processing since the organisms are alive and easier to spot. If required, target organisms being submitted for residue analysis should be picked out and placed into the appropriate container and stored on dry ice.

5. Depending on the number of replicates desired, move the sampler to other sites, and repeat steps 1 through 4.

6. After sampling is complete, add a sufficient amount of fixative or preservative to cover the substrate and organisms in the bottle. Each jar should be labeled indicating the date, site name, sample location and replicate number. Samples should be shipped to the appropriate laboratory for further processing as per SERAS SOP #2004, *Sample Packaging and Shipment*.

7.6 Artificial Substrates

1. Select sampling sites representative of the area to be sampled, and determine the number of replicates desired. Artificial substrates are particularly useful in areas of deep water, swift currents, or soft bottom sediments.

2. Anchor the substrates to a weighted object. The anchor and the means of attachment will be dependent on existing conditions at the sampling location. For example, the threaded "eye" bolt used to assemble a Hester and Dendy may be threaded onto a steel frame.

3. Place the substrates in the area to be sampled, and allow them to remain in place for an appropriate period of time (typically one month) in order for macroinvertebrates to colonize.

4. After the pre-determined time, retrieve the substrates and place into a jar to be preserved, or scrape off the colonists using a soft rubber spatula and place into a wide-mouth jar. If required, target organisms being submitted for residue analysis should be picked out and placed into the appropriate container and stored on dry ice.

5. After sampling is complete, add a sufficient amount of fixative or preservative to cover the substrate and organisms in the bottle. Each jar should be labeled indicating the date, site name, sample location and replicate number. Samples should be shipped to the appropriate laboratory for further processing as per SERAS SOP #2004, *Sample Packaging and Shipment*.

7.7 Artificial Sediment Cores

1. Select sampling sites representative of the area to be sampled, and determine the number of replicates desired. Sediment coring is particularly useful in estuarine environments such as tidal flats or salt marshes, and is often conducted during low tide. It is suggested that the cores be no less than 8.8 centimeters (cm) in diameter, and 10 cm in length.

2. Push the core into the sediment to the desired depth. Remove the core from the sediment by capping the top of the core and by gently rotating it as it is slowly withdrawn from the sediment. Once removed, cap the bottom end of the core for later processing. Repeat until all replicates have been taken.

3. Gently pour the contents of the core into a No. 35 soil sieve. The core may be rinsed with water to remove any sediment adhering to the insides of the core. After the core is emptied a gentle spray of water can be used to break up compacted particles, and to facilitate passing the sediment through the sieve.

4. The material remaining on the sieve is the collected sample. Place the sieve into a pail of water, or surface water being sampled and agitate, taking care not to let the water run over the top of the sieve. This will wash smaller materials through the sieve, and will concentrate the materials at one edge if the sieve is held at an angle.

5. When all of the materials and organisms are concentrated on one side of the sieve, scrape them into a wide-mouthed jar. If necessary, wash the screen again, and place any additional organisms/materials left in the sieve into the bottle, picking out any remains with forceps if necessary.

6. After sampling is complete, add a sufficient amount of fixative or preservative to cover the substrate and organisms in the bottle. Each jar should be labeled indicating the date, site name, sample location and replicate number. Samples should be shipped to the appropriate laboratory for further processing as per SERAS SOP #2004, *Sample Packaging and Shipment.*

7.8 Post Operation

7.8.1 Field

Following all sampling events any equipment used should be cleaned, and if necessary decontaminated as per Environmental Response Team (ERT)/SERAS SOP #2006, *Sample Equipment Decontamination.*

7.8.2 Laboratory Processing

The preserving fluid may be decanted off and the sample rinsed with water to prevent exposure of preservative fumes to the processor. Empty the contents of a sample container into a No. 35 sieve and rinse with water to remove the fixative or preservative. The rinsate should be collected and disposed of properly. Empty the contents of the sieve onto a white plastic tray. Samples collected may contain a mixture of mud, rocks, sand, and debris, in addition to the desired organisms. Remove the organisms from the unwanted material and separate them into similar taxonomic groupings (e.g., Order; Family) for identification and enumeration. It is recommended that the processor use illuminated dissecting lamps to aid in this task.

One benthos sample may have an excessively high density of organisms which could require several person-hours for processing. In such a case, a sub-sample may be collected from that sample. For example, the bottom of the white tray is separated into a grid consisting of 100 numbered squares. Empty the contents of the rinsed sieve, and evenly spread out the sample to cover the grid. Using a random number generator, ten squares are selected and the organisms within the squares are removed. This is the representative sample for that particular location/replicate. A variety of laboratory techniques, each specifically suited for a particular type of sample, are used by different investigators. Techniques should be modified where appropriate. The processor should be aware of whatever procedure is employed so that the final count of organisms is representative of those found in the habitat sampled.

7.8.3 Office

All field notes and/or other logging information should be included in the final report following the appropriate format outlined in SERAS SOP #4021, *Preparation of Final Reports*.

8.0 CALCULATIONS

Various indices may be calculated using the results of the final count of organisms. Calculations utilized may be project specific and may include, but are not limited to: total number of organisms, total number of taxa, ratio of pollution sensitive organisms to pollution tolerant organisms (e.g., Ephemeroptera, Plecoptera, Trichoptera [EPT}:chironomid ratio), percent dominant taxa, diversity indices, and statistical analysis.

9.0 QUALITY ASSURANCE/QUALITY CONTROL

The following Quality Assurance/Quality Control (QA/QC) procedures apply:

1. All samples must be documented on chain of custody forms, field data sheets or in site logbooks as per SERAS SOPs #4005 *Chain of Custody Procedures*, #4001 *Logbook Documentation*, and #2002 *Sample Documentation*.

2. Prior to sampling, the number and size of samples will be outlined in the site-specific WP.

3. All deliverables will receive a peer review prior to release as per SERAS Administrative Procedure (AP) #22, *Peer Review of SERAS Deliverables.*

10.0 DATA VALIDATION

Taxonomic information will be confirmed by an experienced biologist familiar with benthic organisms. Thoroughness of the sorting will be verified by having an additional person re-process ten percent of the samples. If it is determined that a large number of organisms were overlooked ,then all the samples will be re-processed.

11.0 HEALTH AND SAFETY

When working with potentially hazardous materials, follow U.S. EPA, Occupational, Safety, and Health Administration (OSHA), and corporate health and safety procedures. More specifically, refer to SERAS SOP #3001, *SERAS Health and Safety Program Policy and Implementation*.

12.0 REFERENCES

Although not cited in this document, the following documents have been useful, and are recommended references to the methods discussed in this SOP.

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13.0 APPENDIX

This section is not applicable to this SOP.