Region 4 U.S. Environmental Protection Agency Laboratory Services & Applied Science Division Athens, Georgia	
Operating Procedure	
Title: Multi Habitat Macroinvertebrate Sampling	ID: LSASDPROC-508-R6
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Purpose

The purpose of this document is to describe methodology, equipment, and sample handling practices applicable to the collection of macroinvertebrates from wadeable freshwater streams using a standard D-frame net. The procedure is intended to describe a workable approach to sampling, but other methods such as Standard Operating Procedures (SOP) for collecting macroinvertebrates from wadeable freshwater streams that were developed by other agencies should be used if warranted by the objectives of the study. The sampling procedure used must be documented in the study plan or Quality Assurance Project Plan (QAPP).

Scope/Application

The methodology, equipment and sample handling procedures described in this document allow the investigator to assess the health of flowing freshwater ecosystems of perennial nature. Due to their limited mobility and relatively long-life span, benthic macroinvertebrates integrate and reflect water quality effects over time, thus allowing the investigator to detect stress within a stream or between streams. Samples are collected using the multi-habitat Rapid Bioassessment Protocol (RBP) approach.

While this SOP may be informative, it is not intended for and may not be directly applicable to operations in other organizations. Mention of trade names or commercial products in this operating procedure does not constitute endorsement or recommendation for use.

Note: LSASD is currently migrating to a paperless organization. As a result, this SOP will allow for the use of electronic logbooks, checklists, signatures, SOPs, and forms as they are developed, which will also be housed on the Local Area Network (LAN) and traceable to each project.

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1. Precautions

1.1. **Safety**

1.1.1. Proper safety precautions must be observed when collecting macroinvertebrate samples. Refer to the Laboratory Services and Applied Science Division (LSASD) Safety and Health Management System (SHMS) and any pertinent site-specific Health and Safety Plans (HASP) for guidelines on safety precautions. These guidelines, however, should only be used to complement the judgment of an experienced professional. When using this procedure, minimize exposure to potential health hazards through the use of protective clothing, eye wear and gloves. Address chemicals that pose specific toxicity or safety concerns and follow any other relevant requirements, as appropriate.

1.2. Equipment Handling

1.2.1. The following precautions should be considered when collecting samples:

- Special care must be taken not to contaminate samples. This includes storing samples in a secure location to preclude conditions, such as desiccation, which could alter the properties of the sample. Samples shall be custody sealed during long-term storage or shipment.

- Collected samples are in the custody of the sampler or sample custodian until the samples are relinquished to another party.

- If samples are transported by the sampler, they will remain under his/her custody or be secured until they are relinquished.

- Shipped samples shall conform to all U.S. Department of Transportation (DOT) rules of shipment found in Title 49 of the Code of Federal Regulations (49 CFR parts 171 to 179), and/or International Air Transportation Association (IATA) hazardous materials shipping requirements found in the current edition of IATA's Dangerous Goods Regulations.

- Documentation of field sampling will be done in a bound logbook, in accordance with LSASD Operating Procedure for Logbooks (LSASDPROC-1002).

- Chain-of-custody documents will be filled out and remain with the samples until custody is relinquished.

- All shipping documents, such as bills of lading, will be retained by the project leader and stored in a secure place.

- Samples must not be collected if visibility is compromised, either by ambient light conditions or turbidity of the water.

- Samples must not be collected when heavy rain (> 2 inches) has occurred within the prior two weeks.

- Samples must not be collected when there is not sufficient flow to carry material into the net.

2. Methodology

2.1. Summary of Procedure

2.1.1. This procedure describes methods for collecting samples from flowing freshwater wadeable streams. The intent of this methodology is to collect a representative sample. Samples are collected using tools that are selected to support the objectives of the study. Selection of containers and preservatives, as well as holding times, will be addressed in this procedure. Macroinvertebrates are collected from all available habitats. The methodology consists of multiple sampling efforts in unique microhabitats in strict assignment as follows:

- Riffles - 3 “kicks” in the faster current with D-frame net (a “kick” involves disturbing the bottom substrate of a riffle using either the foot or hands to dislodge organisms).

- Run - 3 “kicks” in the slower current with the D-frame net.

- Snags/woody debris - 5 pieces washed in sieve bucket or D-frame net.

- Coarse Particulate Organic Matter (CPOM) - equivalent to half the D-frame net bag.

- Undercut banks - 6 “jabs” with D-frame net (a “jab” equals a one meter sweep).

- Bottom substrate - 3 one-meter sweeps in sediment (disturb sediment to 3 centimeters [cm] depth).

- Submerged macrophytes - 3 one-meter sweeps of submerged plant material.

2.1.2. If a microhabitat is not present in the sampling reach, no sample is collected from that habitat and no attempt is made to reallocate the sampling effort to another habitat.

2.2. Equipment

- Sieve bucket
- Soft brush
- Tweezers
- Wide mouth 1 liter Nalgene jars with screw-on lid
- White pans
- Labels
- Global positioning system (GPS) receiver
- Field record book
- Waterproof pens/pencils
- Field data forms printed on waterproof paper
- Chest waders
- Latex gloves
- Ethanol (90% or greater)
- Multi-probe water quality measuring instrument
- Digital camera
- D-frame biological dip net with 500 micrometer (µm) mesh opening
- Wash bucket

2.3. Procedure

2.3.1. Planning and Preparation

- A project plan and site safety plan must be prepared prior to conducting any field sampling activity.

- The project plan will include a description of the sampling method to be used. If following another agency's SOP, indicate the date the method was adopted. Document any departures from the planned methods in the field logbook.

- Determine the study objectives and the data quality objectives. These will be included in the project study plan.

- Assemble the necessary sampling equipment. Ensure all sampling gear is in working order and that replacement parts are on hand. Ensure that adequate amounts of instrument calibration standards are on hand and that the standards meet quality assurance (QA) requirements.

- Ensure that adequate amounts of preservative (ethanol 90% or greater) are on hand. Ensure that proper safety measures are in place when working with or transferring ethanol.

2.3.2. Collection of Samples

2.3.2.1. Collection of In- Situ Water Quality Data

- Establish the sampling reach. The sample reach should represent a 100- meter segment of instream habitats having no major tributaries in the assessment area, unless the sampling method specifies a different way of establishing the reach. The reach length should be recorded on the field data sheet.

- A digital photograph of the upstream and downstream reach will be taken and the photograph numbers and picture orientation will be recorded in the field logbook.

- If samples for chemical analysis are to be collected, they will be collected at this point in the procedure. Sample collection for chemical analysis should follow the SESD Operating Procedure for Surface Water Sampling (SESDPROC-201).

2.3.2.2. Collect water quality parameters in accordance with SESD Operating Procedure for In Situ Water Quality Monitoring (SESDPROC-111).

- Collection of Macroinvertebrate Sample

- Survey the entire reach and determine where samples will be collected. Do so in a manner that least disturbs the microhabitats to be sampled.

- Assemble the necessary equipment and supplies. Labels for the sample jar must be filled out and affixed before collecting the sample.

- Add ethanol to the sample jar. Fill the sample jar approximately 1/3 full with ethanol. Additional preservative can be added to ensure all sample material is covered and a nominal concentration of 70% ethanol maintained.

2.3.2.2.1. Riffle/Run

- Sampling is conducted from downstream to upstream in the riffle and run by placing the bottom of the net securely on the stream bed and perpendicular to the flow. Any large gravel, cobble or boulders should be removed downstream and from under the lip of the net to ensure an even flow through the net and to minimize flow around or under the net.

- Use the foot or hand to dislodge organisms within an area no wider than the net and 18 inches upstream of the net.

- This procedure is repeated until a total of 3 “kicks” in the riffle and 3 “kicks in the run have been collected. If the net gets clogged with debris so that the sample flows around the net, wash the contents of the net into the sieve bucket and rinse the mesh of the net with the wash bucket before collecting additional organisms.

- Material collected from the riffle/run should be placed in a white pan with water. This material shall be “coarse picked” in the field to remove large sticks, rocks and leaves. The sampler(s) must inspect each piece of debris and remove any attached organisms before the material is discarded.

- The remaining material and water in the pan are poured through the net and allowed to drain before being placed into the collection jar.

- Inspect both the inside of the net and the sieve bucket to make sure no organisms adhered to these surfaces and were not transferred to the sample container. This must be done to ensure that a representative sample is collected and to make sure there is no cross contamination between sampling sites. Inspection of the net and sieve bucket must be done after sampling each microhabitat and when sampling at a site is completed.

2.3.2.2.2. Woody Debris

- Five pieces of woody debris are selected for sampling. Samplers should target woody debris that has begun to decay and is not transient. Collection from a variety of wood types is preferable.

- Organisms should be dislodged from the wood into the net using the hands, tweezers, soft brush and/or water.

- Woody debris that can be broken apart should be “crumbled” into the net.

- Material collected from the woody debris should be placed in a white pan with water. This material shall be “coarse picked” in the field to remove large sticks, rocks and leaves. The sampler(s) must inspect each piece of debris and remove any attached organisms before the material is discarded.

- The remaining material and water in the pan is poured through the net and allowed to drain before being placed into the collection jar.

2.3.2.2.3. CPOM

- Collection of CPOM is collected by hand and transferred into the dip net.

- The collected CPOM should be placed in a white pan with water. This material shall be “coarse picked” in the field to remove large sticks, rocks and leaves. The sampler(s) must inspect each piece of debris and remove any attached organisms before the material is discarded.

- The remaining CPOM fragments and water in the pan are poured through the net and allowed to drain before being placed into the collection jar.

2.3.2.2.4. Undercut Banks

- Undercut banks are sampled from downstream to upstream so that stream flow helps move organisms and material into the net. Samplers should optimize available habitat by collecting from a variety of undercut bank habitats, if present.

- Material collected from the undercut bank should be placed in a white pan with water. This material shall be “coarse picked” in the field to remove large sticks, rocks and leaves. The sampler(s) must inspect each piece of debris and remove any attached organisms before the material is discarded.

- The remaining material and water in the pan are poured through the net and allowed to drain before being placed into the collection jar.

2.3.2.2.5. Bottom Substrate

- The bottom substrate is sampled from downstream to upstream to facilitate collection of macroinvertebrates. The net should be bumped along the bottom to reduce the amount of debris in the sample.

- The collected sediment and organisms are placed in the pan and can be "coarse picked" by carefully examining small (1 tablespoon) aliquots of sediment. Organisms are removed and put directly into the sample jar.

- The remaining sediment and water can be carefully swirled to form a slurry which can be poured through the net. Any organisms retained by the net should be added directly to the sample jar.

2.3.2.2.6. Submerged Macrophytes

- Submerged macrophytes are sampled by drawing the net through the vegetation from the bottom to the surface of the water. Shallow habitats are sampled by bumping the net along the rooted plant material. Care should be taken to avoid collecting sediments.

- The collected plant material and organisms should be placed in a white pan and "coarse" picked. The sampler(s) must inspect each piece of debris and remove any attached organisms before the material is discarded.

- The remaining material and water in the pan is poured through the net and allowed to drain before being placed into the collection jar.

- Additional preservative can be added to the jar if there is enough space remaining. After the sample container is topped off with preservative, the lid can be screwed on. Samplers should check that the lid is secure before transporting the sample.

2.3.2.3. Collection of Habitat and Physical data

- Assemble the necessary forms. All field data sheets, as well as the field logbook should be made of waterproof paper. Indelible pens should be used to record data and observations.

- Complete the physical habitat characterization data sheet.

- Complete the appropriate RBP habitat evaluation form. There are two habitat evaluation form options. One form pertains to high gradient streams and the other to low gradient streams. Ensure that the correct form is used.

- Global positioning system coordinates should be recorded in the general area of the sampling reach, following the LSASD Operating Procedure for Global Positioning System, LSASDPROC-110. Another set of GPS coordinates should be collected at duplicate stations.

2.3.3. Sample Handling, Preservation, and Transport

- Macroinvertebrate samples should be carefully inverted a few times before transportation to ensure the sample has adequate contact with the preservative. Care should be taken not to damage organisms during transfer from the net or during transportation.

- Sample jars should be transported in a crate or similar device that keeps the samples secure while being transported.

- Samples collected by field personnel are transferred to the LSASD sample custody room and logged into the laboratory LIMS system by the sample custodian. A sample receipt record will be generated, and the receipt will reside in the project file. The unprocessed samples are placed in the storage cabinet located in the benthic laboratory.

- If processing of the samples will be delayed for more than a week, the ethanol should carefully be poured through a number 35 sieve into the flammable waste container. Any organisms retained in the sieve should be placed back into the sample container.

- Fresh preservative should then be added to the sample jar so that there is very little head space left in the jar. This can be done just before samples are placed in the storage cabinet. Invert the sample jar a few times to ensure the sample has adequate contact with the preservative. If after three months the samples have not been processed, the procedure in step four

above will be repeated and the date the preservative was changed will be noted on each container, along with the initials of the person who changed the preservative. The procedure in step four will be repeated annually (before the date of the second preservation at three months) until the samples are either processed or discarded.

2.4. Quality Control

2.4.1. Ensure that sample labels are properly completed, including the station identification code, date, collectors' initials and number of containers comprising the sample. The interior label must be filled out in pencil (not pen) with pertinent sample information such as date of collection, station or sample identification, project number, and samplers' initials. Make sure the same information is recorded correctly on any field data sheets as well.

2.4.2. Any equipment that has come in contact with the sample must be examined for organisms and then thoroughly rinsed with water to remove debris. Any organisms found will be placed in the appropriate collection jar.

2.5. Records

2.5.1. Records generated will include field notes, recorded in a bound waterproof logbook, field data sheets for physical characterizations, habitat evaluation forms, digital photographs custody tags, completed chain-of-custody forms, and if needed, completed receipt for sample forms.

2.6. Coarse Particulate Organic Matter (CPOM)

2.6.1. Submerged packs of leaves, needles, twigs, bark or fragments of these that have begun to decompose.

2.7. Reach

2.7.1. The length of stream representing the site or the station. This length is generally 100 meters for RBP collections but may be different based on other methods and purposes.

2.8. Riffle

2.8.1. Riffles are shallow parts of the stream where water flows swiftly over completely- or partially-submerged pebble- to boulder-sized rocks to produce surface agitation.

2.9. Run

2.9.1. Refers to the slower current located at the tail of the riffle where less surface agitation is occurring.

2.10. Sample

2.10.1. The term “sample” is used to refer to the entire sample which is collected from various habitats.

2.11. Site

2.11.1. Site and station are synonymous terms. They refer to the sampling location or reach as defined above.

2.12. Snags

2.12.1. Refers to woody debris that has been submerged for a relatively long period of time.

2.13. Submerged Macrophytes

2.13.1. Aquatic plants that are rooted to the bottom of the stream.

2.14. Undercut Bank

2.14.1. Refers to the lower submerged portion of the stream bank where roots protrude into the water.

References

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SESD Operating Procedure for Surface Water Sampling, SESDPROC-201, Most Recent Version.

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Revision History

History	Effective Date
Changed Chief with Supervisor; General formatting revisions.	April 22, 2023
SESDPROC-508-R5, Multi-Habitat Macroinvertebrate Sampling in Wadeable Freshwater Streams, replaces SESDPROC-508-R4. General: The document was edited to reflect new Document Control Processes along with their references. Put the SOP in the new SOP format.	February 18, 2022
SESDPROC-508-R4, Multi-Habitat Macroinvertebrate Sampling in Wadeable Freshwater Streams, replaces SESDPROC-508-R3. General: Corrected any typographical, grammatical, and/or editorial errors. Additionally, the document was edited to reflect new Document Control Processes. Section 2.3.2.2: Bullet 3 was edited to dictate the target preservative for the final sample is 70% ethanol. Section 2.3.3: Edited to reflect current sample login procedure and preservation processes.	March 14, 2018
SESDPROC-508-R3, Multi-Habitat Macroinvertebrate Sampling in Wadeable Freshwater Streams, replaces SESDPROC-508-R2.	December 4, 2013
SESDPROC-508-R2, Multi-Habitat Macroinvertebrate Sampling in Wadeable Freshwater Streams, replaces SESDPROC-508-R1.	February 17, 2010
SESDPROC-508-R1, Multi-Habitat Macroinvertebrate Sampling in Wadeable Freshwater Streams, replaces SESDPROC-508-R0.	November 1, 2007
SESDPROC-508-R0, Multi-habitat Macro-Invertebrate Sampling in Wadeable Streams, Original Issue	February 05, 2007