

U.S. Environmental Protection Agency  
Region 4, Science and Ecosystem Support Division  
Athens, Georgia

## OPERATING PROCEDURE

Title: **Multi-Habitat Macroinvertebrate Sampling in Wadeable  
Freshwater Streams**

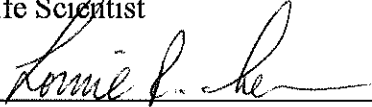
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## Revision History

This table shows changes to this controlled document over time. The most recent version is presented in the top row of the table. Previous versions of the document are maintained by the SESD Document Control Coordinator.

| History   | Effective Date           |
|---|--------------------------|
| <p>SESDPROC-508-R2, <i>Multi-Habitat Macroinvertebrate Sampling in Wadeable Freshwater Streams</i>, replaces SESDPROC-508-R1.</p> <p><b>Revision History:</b> Changed Field Quality Manager to Document Control Coordinator.</p> <p><b>Section 1.3:</b> Omitted reference to the H: drive. Changed the Field Quality Manager to the Document Control Coordinator.</p> <p><b>Section 1.5:</b> Updated references.</p> <p><b>Section 2.3.2.2:</b> Reformatted procedures for collecting sample.</p>   | <p>February 17, 2010</p> |
| <p>SESDPROC-508-R1, <i>Multi-Habitat Macroinvertebrate Sampling in Wadeable Freshwater Streams</i>, replaces SESDPROC-508-R0.</p> <p><b>General</b><br/>Corrected any typographical, grammatical, and/or editorial errors.</p> <p><b>Title Page</b><br/>Changed title for Bill Cosgrove from Acting Chief to Chief.</p> <p><b>Table of Contents</b><br/>Renamed Section 1.5, and deleted Section 3.</p> <p><b>Section 1.3</b><br/>Updated information to reflect that procedure is located on the H: drive of the LAN. In addition, text has been revised in this section.</p> <p><b>Section 1.42</b><br/>deleted</p> <p><b>Section 1.5</b><br/>Citations of SESD procedures used in this procedure added. SHEMP citation added. References in Section 3 merged with this section. Other changes made to be consistent.</p> <p><b>Section 1.6.1</b><br/>Title of Safety, Health, and Environmental Management Program Procedures and Policy Manual corrected, and citation added.</p> | <p>November 1, 2007</p>  |

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|--|--------------------------|
| <p><b>Section 1.6.2, 4<sup>th</sup> bullet</b><br/> Added references to the CFR and IATA's Dangerous Goods Regulations.</p> <p>Added bullets 8, 9, and 10.</p> <p><b>Section 2.3.2.1</b><br/> Deleted Bullets 4 through 8 and referred reader to operating procedure for <i>in situ</i> water quality monitoring.</p> <p><b>Section 2.3.2.3</b><br/> Added additional bullet for collecting GPS coordinates following SESD operating procedures.</p> <p><b>Section 2.3.3</b><br/> Deleted reference to an SOP for Macroinvertebrate Sample Handling and Receiving in Bullet 3.</p> <p><b>Section 2.4</b><br/> Deleted procedure for collecting duplicate sample.</p> <p><b>Section 3</b><br/> Moved this section to Section 1.5.</p> |                          |
| <p>SESDPROC-508-R0, <i>Multi-habitat Macro-Invertebrate Sampling in Wadeable Streams</i>, Original Issue</p>   | <p>February 05, 2007</p> |

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# **1 General Information**

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## **1.1 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).

## **1.2 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 a 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. Methods and equipment may vary, depending upon the objectives of the study and stream type.

## **1.3 Documentation/Verification**

This procedure was prepared by persons deemed technically competent by SESD management, based on their knowledge, skills and abilities and has been tested in practice and reviewed in print by a subject matter expert. The official copy of this procedure resides on the SESD Local Area Network (LAN). The Document Control Coordinator is responsible for ensuring the most recent version of the procedure is placed on the SESD LAN and for maintaining records of review conducted prior to its issuance.

## **1.4 Definitions**

### ***1.4.1 Coarse Particulate Organic Matter (CPOM)***

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

### ***1.4.2 Reach***

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

### ***1.4.3 Riffle***

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.

#### **1.4.4 Run**

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

#### **1.4.5 Sample**

The term "sample" is used to refer to the entire sample which is collected from various habitats.

#### **1.4.6 Site**

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

#### **1.4.7 Snags**

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

#### **1.4.8 Submerged Macrophytes**

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

#### **1.4.9 Undercut Bank**

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

### **1.5 References**

American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. Standard Methods for the Examination of Water and Wastewater. 20<sup>th</sup> Edition. Washington, D.C.

International Air Transport Authority (IATA). Dangerous Goods Regulations, Most Recent Version

SESD Operating Procedure for Global Positioning System, SESDPROC-110, Most Recent Version.

SESD Operating Procedure for *In situ* Water Quality Monitoring, SESDPROC-111, Most Recent Version.

SESD Operating Procedure for Logbooks, SESDPROC-010, Most Recent Version

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

Title 49 Code of Federal Regulations, Pts. 171 to 179, Most Recent Version

United States Environmental Protection Agency (USEPA). 1989. Rapid Bioassessment Protocols for Use in Streams and Rivers: Benthic Macroinvertebrates and Fish. EPA 440-4-89-001. Office of Water Regulations and Standards.

USEPA. 1990. Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters. EPA-600-4-90-030. Environmental Monitoring Systems Laboratory.

USEPA. 1999. Rapid Bioassessment Protocols for use in Streams and Wadeable Rivers: Periphyton, Benthic Macroinvertebrates, and Fish. Second Edition. EPA 841-B-99-002. Office of Water.

USEPA. Most Recent Version. Safety, Health and Environmental Management Program Procedures and Policy Manual. Science and Ecosystem Support Division, Region 4, Athens, Georgia.

## **1.6 General Precautions**

### ***1.6.1 Safety***

Proper safety precautions must be observed when collecting macroinvertebrate samples. Refer to the Science and Ecosystem Support Division (SESD) Safety, Health and Environmental Management Program Procedures and Policy Manual 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.

### ***1.6.2 Procedural Precautions***

The following precautions should be considered when collecting samples:

1. 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.
2. Collected samples are in the custody of the sampler or sample custodian until the samples are relinquished to another party.
3. If samples are transported by the sampler, they will remain under his/her custody or be secured until they are relinquished.

4. 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.
5. Documentation of field sampling is done in a bound logbook, in accordance with SESD Operating Procedure for Logbooks (SESDPROC-010).
6. Chain-of-custody documents will be filled out and remain with the samples until custody is relinquished.
7. All shipping documents, such as bills of lading, will be retained by the project leader and stored in a secure place.
8. Samples must not be collected if visibility is compromised, either by ambient light conditions or turbidity of the water.
9. Samples must not be collected when heavy rain (> 2 inches) has occurred within the prior two weeks.
10. Samples must not be collected when there is not sufficient flow to carry material into the net.

## **2 Methodology**

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### **2.1 Summary of Procedure**

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:

1. 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).
2. Run - 3 "kicks" in the slower current with the D-frame net.
3. Snags/woody debris - 5 pieces washed in sieve bucket or D-frame net.
4. Coarse Particulate Organic Matter (CPOM) - equivalent to half the D-frame net bag.
5. Undercut banks - 6 "jabs" with D-frame net (a "jab" equals a one meter sweep).
6. Bottom substrate - 3 one-meter sweeps in sediment (disturb sediment to 3 centimeters [cm] depth).
7. Submerged macrophytes - 3 one-meter sweeps of submerged plant material.

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**

1. Sieve bucket
2. Soft brush
3. Tweezers
4. Wide mouth 1 liter Nalgene jars with screw-on lid
5. White pans
6. Labels
7. Global positioning system (GPS) receiver
8. Field record book
9. Waterproof pens/pencils
10. Field data forms printed on waterproof paper
11. Chest waders

12. Latex gloves
13. Ethanol (90% or greater)
14. Multi-probe water quality measuring instrument
15. Digital camera
16. D-frame biological dip net with 500 micrometer ( $\mu\text{m}$ ) mesh opening
17. Wash bucket
18. Camera

## 2.3 Procedure

### 2.3.1 *Planning and Preparation*

1. A project study plan and site safety plan must be prepared prior to conducting any field sampling activity.
2. 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.
3. Determine the study objectives and the data quality objectives. These should be included in the project study plan.
4. Assemble the necessary sampling equipment. Assure all sampling gear is in working order and that replacement parts are on hand. Assure that adequate amounts of instrument calibration standards are on hand and that the standards meet quality assurance (QA) requirements.
5. Assure that adequate amounts of preservative (ethanol 90% or greater) are on hand. Assure 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*

1. 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.
2. A digital photograph of the upstream and downstream reach should be taken and the photograph numbers and picture orientation should be recorded in the field logbook.

3. If samples for chemical analysis are to be collected, they should 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).
4. Collect water quality parameters in accordance with SESD Operating Procedure for *in situ* Water Quality Monitoring (SESDPROC-111).

#### 2.3.2.2 *Collection of Macroinvertebrate Sample*

1. Survey the entire reach and determine where samples will be collected.
2. Assemble the necessary equipment and supplies. Labels for the sample jar must be filled out and affixed before collecting the sample.
3. Add ethanol to the sample jar. Fill the jar approximately 1/3 full. Additional preservative can be added if needed once the sample is collected.

#### 4. **Riffle/Run**

- a. 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.
- b. Use the foot or hand to dislodge organisms within an area no wider than the net and 18 inches upstream of the net.
- c. 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.
- d. Material collected from the riffle/run should be placed in a white enamel 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.
- e. The remaining material and water in the enamel pan are poured through the net and allowed to drain before being placed into the collection jar which is partially-filled (~1/3) with ethanol.

- f. 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.

## **5. Woody Debris**

- a. 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.
- b. Organisms should be dislodged from the wood into the net using the hands, tweezers, soft brush, and/or water.
- c. Woody debris that can be broken apart should be “crumbled” into the net.
- d. Material collected from the woody debris should be placed in a white enamel 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.
- e. The remaining material and water in the enamel pan is poured through the net and allowed to drain before being placed into the collection jar.

## **6. CPOM**

- a. Collection of CPOM is collected by hand and transferred into the dip net.
- b. The collected CPOM should be placed in a white enamel 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.
- c. The remaining CPOM fragments and water in the enamel pan are poured through the net and allowed to drain before being placed into the collection jar.

## **7. Undercut Banks**

- a. 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.
- b. Material collected from the undercut bank should be placed in a white enamel 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.
- c. The remaining material and water in the enamel pan are poured through the net and allowed to drain before being placed into the collection jar.

## **8. Bottom Substrate**

- a. 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.
- b. The collected sediment and organisms are placed in the enamel 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.
- c. 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.

## **9. Submerged Macrophytes**

- a. 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.
- b. The collected plant material and organisms should be placed in an enamel pan and "coarse" picked. The sampler(s) must inspect each piece of debris and remove any attached organisms before the material is discarded.
- c. The remaining material and water in the enamel pan are poured through the net and allowed to drain before being placed into the collection jar.

10. 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**

1. 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.
2. Complete the physical habitat characterization data sheet.
3. Complete the habitat evaluation form. There are two habitat evaluation form options. One form pertains to high gradient streams and the other to low gradient streams. Assure that the correct form is used.
4. Global positioning system coordinates should be recorded in the general area of the sampling reach, following the SESD Operating Procedure for Global Positioning System, SESDPROC-110. Another set of GPS coordinates should be collected at duplicate stations.

### **2.3.3 Sample Handling, Preservation, and Transport**

1. Macroinvertebrate samples should be carefully inverted a few times before transportation to insure the sample has adequate contact with the preservative. Care should be taken not to damage organisms during transfer from the net or during transportation.
2. Sample jars should be transported in a wooden crate or similar device that keeps the samples secure while being transported.
3. Samples collected by SESD biologists are transferred to the Benthic Macroinvertebrate Laboratory. The samples are logged into the laboratory's logbook. This logbook covers the required chain-of-custody. The unprocessed samples are stored in a flammable storage cabinet in the laboratory.
4. Samples collected by other scientists must have the proper chain-of-custody documents in place before the samples can be logged into the SESD Benthic Macroinvertebrate Laboratory.
5. 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.

6. Fresh preservative should then be added to the sample jar so that there is very little head space left in the jar. Carefully invert the sample jar a few times to insure the sample has adequate contact with the preservative.

## **2.4 Quality Control**

Assure 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, number of containers comprising the sample (i.e. 1 of 1, 1 of 2, etc.), and samplers' initials. Make sure the same information is recorded correctly on any field data sheets as well.

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

## **2.5 Records**

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.