

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

OPERATING PROCEDURE

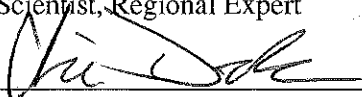
Title: Periphyton Sampling and Algae Surveys in Wadeable Streams

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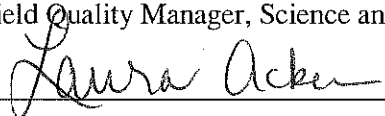
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<p>Section 2.4.1 Added referenced procedures.</p> <p>Section 2.7 Deleted the term habitat evaluation forms.</p>	
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Table of Contents

1	General Information	5
1.1	Purpose.....	5
1.2	Scope/Application	5
1.3	Documentation/Verification.....	5
1.4	Definitions.....	6
1.6	General Precautions.....	7
1.6.1	<i>Safety</i>	7
1.6.2	<i>Procedural Precautions</i>	7
2.1	Summary of Procedures	9
2.2.1	<i>Semi-Qualitative Periphyton Sampling</i>	9
2.2.2	<i>Rapid Periphyton Survey</i>	9
2.2	Equipment	9
2.3	Planning and Preparation	10
2.4	Collection of Data and Samples	10
2.4.1	<i>Site Observations and Collection of Supplementary Data</i>	10
2.4.2	<i>Semi-Qualitative Periphyton Sampling</i>	11
2.4.3	<i>Rapid Periphyton Survey</i>	12
2.5	Sample Handling, Preservation, and Transport	13
2.6	Quality Control	13
2.7	Records.....	13

List of Tables and Figures

Table 1.	Example Aliquot Apportionment Strategies	14
Table 2.	Percent Cover and Thickness Classes	14
Figure 1.	Locating Transects	12
Figure 2.	Observation Points	12
Figure 3.	Rapid Periphyton Survey Data Sheet	15

1 General Information

1.1 Purpose

The purpose of this document is to describe methodology, equipment, and sample handling practices applicable to collecting periphyton samples and conducting substrate algal coverage surveys in wadeable freshwater streams. The procedures are intended to describe a workable approach to investigating aquatic algae, but other methods such as Standard Operating Procedures (SOP) 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 generally assess the quality of flowing freshwater ecosystems. Specifically, the methods described will allow the investigator to document the extent of stream substrate algal coverage and to characterize the species composition of the stream algal community. Both methods have been shown to be necessary and effective tools for investigating aquatic nutrient dynamics. Algae have been shown to be particularly useful in nutrient investigations because:

- Algal species are sensitive to nutrients.
- Different algal species have different sensitivities to nutrients.
- Algal communities develop over long enough time periods that they can integrate the variability of nutrient conditions associated with temporal, diurnal and flow-related events.

Methods and equipment may vary, depending upon the objectives of the study and stream type. In the event that Science and Ecosystem Support Division (SESD) field personnel determine that any of the procedures described in this section are inappropriate, inadequate or impractical and that another procedure must be used, the alternate procedure will be documented in the field log book (in accordance with SESD operating Procedure SESDPROC-010, Logbooks), along with a description of the circumstances requiring its use.

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 H: drive of the SESD local area network. The Field Quality Manager (FQM) is responsible for ensuring the most recent version of the procedure is

placed on the H: drive and for maintaining records of review conducted prior to its issuance.

1.4 Definitions

1.4.1 Biofilm

Slick layer found on submerged substrate. Typically composed of diatoms, but may also include other algae, bacteria, fungi and meiofauna.

1.4.2 Filamentous Algae

Strands of green algae (Chlorophyta) composed of spherical or cylindrical cells attached end to end. The strands or filaments can be branched or un-branched.

1.4.3 Macroalgae

Filamentous algae.

1.4.3 Microalgae

Biofilms, diatoms.

1.4.4 Periphyton

Algae attached to submerged substrate in aquatic environments.

1.4.5 Reach

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

1.4.6 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.

1.4.7 Run

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

1.4.8 Sample

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

1.4.9 Site

The sampling location, or reach as defined above.

1.4.10 Snags

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

1.5 References

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

SESD Operating Procedure for Logbooks, SESDPROC-010, 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 Surface Water Sampling, SESDPROC-201, Most Recent Version.

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

USEPA. 2007. 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 periphyton samples. Refer to the SESD Safety, Health and Environmental Management Program Procedures and Policy Manual (USEPA 2007) and any pertinent site-specific Health and Safety Plans (HASPs) 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:

- 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 is done in a bound logbook.
- 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.

- Global positioning system (GPS) receiver
- Waterproof pens
- Permanent markers (sharpies)
- Densimeter
- Multi-meter datasonde

2.3 Planning and Preparation

- A project study 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 should be included in the project study plan.
- 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.
- Assure that adequate amounts of preservative (glutaraldehyde 5% or greater) and rinse water are on hand. Assure that proper safety measures are in place when working with or transferring preservatives.

2.4 Collection of Data and Samples

2.4.1 Site Observations and Collection of Supplementary 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. If an alternate sampling methodology is being used, the reach should be established accordingly. The reach length should be recorded on the field data sheet.
- A digital photograph of the upstream and downstream reach should be taken.
- 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 SESD Operating Procedure Surface Water Sampling (SESDPROC-201).
- If *in situ* water quality monitoring data are required, the appropriate monitoring devices should be deployed and operated at this point in the procedure, according to the SESD Operating Procedure for *In situ* Water Quality Monitoring, SESDPROC-111.
- Global positioning system coordinates should be recorded at the beginning point of the sample reach, following the SESD Operating Procedure for Global Positioning System, SESDPROC-110.

2.4.2 *Semi-Qualitative Periphyton Sampling*

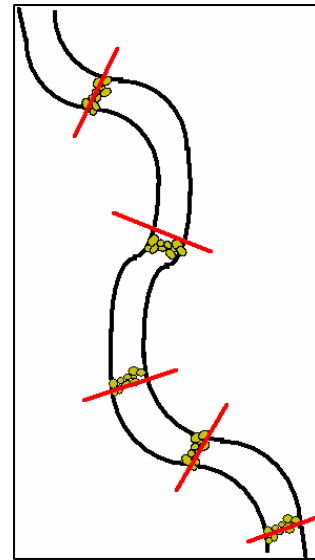
1. Conduct assessment of productive substrates within the defined sample reach and determine sample strategy.
2. Identify target substrates where algae can be collected within 0.5 meters (about an arm's length) from the water surface:
 - Typical substrates include removable portions of vascular plants or mosses, snags, roots, leaf packs or mats, and rock.
 - **DO NOT CONSIDER SEDIMENTS AS A TARGET SUBSTRATE.**
 - Substrate should be “seasoned” (not recent or “new fall”).
 - Strategy: “equally” apportion substrates into 10 aliquots.
 - See Table 1 for examples of aliquot apportionments.
3. Fill wide mouth sample jar with site water to 100 mL fill mark.
4. Remove a target substrate from water.
5. Select an amount of the removed substrate with surface area of approximately 9 cm diameter:
 - Use mouth of sample jar to estimate the 9 cm diameter.
 - Remove excess substrate by breaking or cutting.
 - For substrate that is unbreakable, disregard the excess area.
6. Remove algae from substrate:
 - Using fingers, rub substrate surface into the 100 mL of site water.
 - For any substrate that was not reduced to a 9 cm diameter area in Step 5, ignore excess substrate area and concentrate on removing algae from an area matching the mouth of the sample jar.
 - Rub the entire substrate surface three times to ensure algae removal.
 - Rinse fingers in the jar with the site water.
7. Take 2 sub-samples and composite into centrifuge tube:
 - Stir well to homogenize periphyton in the 100 mL slurry.
 - Remove first 2 mL subsample with pipette and transfer into centrifuge tube.
 - Repeat stirring procedure in 100 mL slurry.
 - Remove second 2 mL subsample with pipette and transfer into centrifuge tube.
8. Repeat steps 3 through 6 for the 9 additional aliquots:
 - Final sample volume in the centrifuge tube should equal 40 mL.
 - $2 \times 2 \text{ mL subsample} = 4 \text{ mL aliquot}$; $4 \text{ mL} \times 10 \text{ aliquots} = 40 \text{ mL}$.
9. Label centrifuge tube with identifying information:
 - Site number, date, collector initials.
10. Add 2 mL of a 3-5% glutaraldehyde solution to the 40 mL of periphyton sample in the centrifuge tube.

2.4.3 Rapid Periphyton Survey

1. Establish five or ten transects across each stream reach, depending on stream width as described below (See Figure 1):

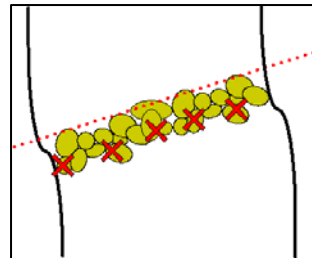
- Place transects in areas of existing algal growth or in areas of likely algal growth. Target riffles and runs, if available. The basic idea is to bias the survey towards finding algae.
- Transects can be visually established (“eyeballed”). Bank secured tape or line is not required.
- Establish 10 transects for narrow streams (less than or equal to 5 meters).
- Establish 5 transects for wider streams (greater than 5 meters).
- Target locations of likely algal growth (riffles and runs) if available.
- Attempt to place transects so that the full length of the reach is included.

Figure 1. Locating Transects



2. Visually identify 5 or 10 evenly spaced points along each transect, depending on stream width (See Figure 2):
 - Identify 5 points along transects for narrow streams (less than or equal to 5 meters).
 - Identify 10 points along transects for wide streams (greater than 5 meters).
 - 50 total observation points per reach.

Figure 2. Observation Points



3. Record observations for a 15 cm x 15 cm area around each point:
 - The observed area around each point can be delineated using the periphyton survey board or may be estimated.
 - At each point, estimate moss cover, macroalgal cover and microalgal biofilm thickness on a scale of 1 to 5. See Table 2 for details on the classification system.
 - At each point, determine if substrate is greater than 2 cm in the longest dimension (suitable size for stability).
 - All data should be recorded on the Rapid Periphyton Survey sheets (See Figure 3).

Table 1. Example Aliquot Apportionment Strategies

Number of Stable Substrates	Aliquot Apportion Strategy
1	10 aliquots from single substrate
2	5 aliquots from each substrate
3	4 aliquots from most abundant substrate 3 aliquots from remaining two substrates
4	3 aliquots from two most abundant substrates 2 aliquots from remaining two substrates
5	2 aliquots from each substrate
6	2 aliquots from four most abundant substrates 1 aliquot from two remaining substrates

Table 2. Percent Cover and Thickness Classes

<i>Coverage Classes for Moss/Macroalgae Observations</i>						
Class Number	0	1	2	3	4	
Coverage	0%	<5%	5% to 25%	25% to 50%	>50%	
<i>Thickness Classes for Biofilm Observations</i>						
Class Number	0	1	2	3	4	5
Thickness	0 mm*	<0.5 mm	0.5 to 1 mm	1 to 5 mm	5 to 20 mm	>20 mm
Characteristics	rough	Slimy, no visible biofilm	Biofilm visible			

* mm = millimeter

Figure 3. Rapid Periphyton Survey Data Sheet

Rapid Periphyton Survey Data Sheet

Site Number

Stream Name

Sample Team Members

Date

GPS Coordinates

N

W

Logged? Y/N

Trans	Point	Moss	Macro	Micro	Suitable?
	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
	11				
	12				
	13				
	14				
	15				
	16				
	17				
	18				
	19				
	20				
	21				
	22				
	23				
	24				
	25				

Trans	Point	Moss	Macro	Micro	Suitable?
	26				
	27				
	28				
	29				
	30				
	31				
	32				
	33				
	34				
	35				
	36				
	37				
	38				
	39				
	40				
	41				
	42				
	43				
	44				
	45				
	46				
	47				
	48				
	49				
	50				

Densimeter readings of canopy cover (if available)	1	
	2	
	3	
	4	

Notes:

MOSS / MACROALGAE Coverage Class					
0	1	2	3	4	
0%	<5%	5 to 25%	25 to 50%	>50%	

MICROALGAE biofilm thickness					
0	1	2	3	4	5
0 mm	<0.5 mm	0.5 to 1 mm	1 to 5 mm	5 to 20 mm	> 20 mm
<i>rough</i>	<i>slimy, not visible</i>	<i>biofilm is visible</i>			

Suitable Substrate is greater than 2 cm in longest dimension