

Standard Operating Procedure #8:

Collection of Periphyton

Version 1.0

In Monitoring Protocol for Wadeable Streams of the Great Lakes Network

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Revision History Log

The following table lists all edits and amendments to this document since the original publication date. Information entered in the log must be complete and concise. Users of this standard operating procedure will promptly notify the project manager and/or the Great Lakes Network (GLKN) data manager about recommended and required changes. The project manager must review and incorporate all changes, complete the revision history log, and change the date and version number on the title page and in the header of the document file. For complete instructions, please refer to Procedure for Revising the Protocol, SOP #16.

Previous Version #	Revision Date	Author (with title and affiliation)	Location in Document and Concise Description of Revision	Reason for Change	New Version #
Add rows as needed for each change or set of changes tied to an updated version number					

Acknowledgements

This standard operating procedure is drawn directly from the periphyton protocols by Stevenson and Bahls (1999) within the EPA Rapid Bioassessment of Wadeable Streams manual (Barbour et al. 1999). Portions of the protocol are copied here *verbatim*.

8.0 Introduction

Benthic algae (periphyton or phytobenthos) are primary producers and are an important foundation of many stream food webs. These organisms also stabilize substrata and serve as habitat for many other organisms. Because benthic algal assemblages are attached to substrate, their characteristics are affected by physical, chemical, and biological disturbances that occur in the stream reach during the time in which the assemblage developed.

Diatoms, a major group of algae that are among the most common types of phytoplankton, in particular are useful ecological indicators because they are found in abundance in most lotic ecosystems. Diatoms and many other algae can be identified to species by experienced algologists. The great number of species provide multiple, sensitive indicators of environmental change and the specific conditions of their habitat. Diatom species are differentially adapted to a wide range of ecological conditions.

Algae have been widely used to monitor water quality in rivers of the United States, Europe, and New Zealand, where many different approaches have been used for sampling and data analysis (Limbeck and Smith 2007, Raunio and Soininen 2007, Moulton et al. 2002, Biggs and Kilroy 2000, Porter et al. 1993; also see reviews in Whitton and Rott 1996, Whitton et al. 1991). Periphyton indices of biotic integrity have also been developed and tested in several regions (Kentucky Department of Environmental Protection 1993; Pan et al. 1996; Hill 1997; Hill et al. 2000, 2003; Hamsher and Vis 2003), and several states have developed protocols for periphyton assessment (e.g., Bahls 1993, Kentucky Department of Environmental Protection 1993, Oklahoma Conservation Commission 1993). Because the ecological tolerances for many species are known (Lange-Berlatot 1979, Renberg 1990, Fritz et al. 1999, Batterbee et al. 2001), changes in community composition can be used to diagnose the environmental stressors affecting ecological health, as well as to assess biotic integrity (Stevenson 1998, Chessman et al. 1999, Stevenson and Pan 1999, Komulainen 2002, Cascallar et al. 2003, Hamsher, and Vis 2003, Ramstack et al. 2003, Denicola et al. 2004).

Periphyton assessments may be used by themselves, but they are most effective when used with habitat data or data from one or more of the other taxonomic assemblages. Periphyton assessments should be used with habitat and benthic macroinvertebrate assessments, particularly, because of the close relation between periphyton and these elements of stream ecosystems.

Periphyton samples will be collected from riffles (where present) for analysis of, chlorophyll-a and species composition. The main objectives for measuring these parameters are to assess the condition and quality of the stream reach through the use of algal indicators and to analyze for trends in trophic condition. Determination of species composition allows an accurate assessment of stream biotic integrity and facilitates diagnosis of causes of impairment. Chlorophyll-a serves as an indicator of stream productivity and trophic condition.

This SOP describes the methods for collecting and preserving periphyton samples from wadeable streams. It is closely linked to SOP #7, Collection of Benthic Macroinvertebrates.

8.1 General Overview

Periphyton samples should be collected during periods of stable stream flow. High flows can scour the stream bed, flushing the periphyton downstream. Recolonization of substrates will be faster after less severe floods and in streams with nutrient enrichment. Peterson and Stevenson (1990) recommend a three-week delay following high, bottom-scouring stream flows to allow for recolonization and succession to a mature periphyton community. However, recovery after high discharge can be as rapid as 7 days if severe scouring of substrata did not occur (Stevenson 1990).

All stream sampling will be conducted during a single site visit, so the visit must be scheduled at the appropriate time for macroinvertebrates (see SOP #7, Collection of Macroinvertebrates) and at a time when high flows have not scoured the stream bed. If large rain events have occurred within three weeks of a scheduled sampling event, a reconnaissance visit may be needed to determine whether the stream bed has been scoured. If evidence of scouring exists, reschedule the sampling visit.

The general sequence of events should be as follows:

1. Prepare sample bottles and labels in advance.
2. Review checklist of field gear.
3. Prepare new data sheets for each monitoring station, printed on waterproof paper. Separate data sheets are needed for a) mapping and measuring habitat features; b) habitat sampling along transects; c) station summary, including water quality; d) flow; e) macroinvertebrate collection; and f) periphyton collection.
4. Conduct daily calibration of appropriate meters and probes.
5. Inspect motorized vehicles/boats at the beginning of every field day, including all safety and directional lights, oil, gasoline, and air pressure levels of tires.
6. Travel to monitoring site. Refer to description of monitoring station location, directions, and photos to verify correct location. Verify coordinates on GPS unit.
7. Measure water quality variables with a multiprobe and transparency with a transparency tube downstream of first transect per SOP #6; record measurements on data sheet.
8. Obtain mean stream width to calculate $40 \times \text{MSW}$ to find sample reach Length, as needed.
9. Moving from downstream to up, conduct physical habitat assessment instream and in the riparian area along transects, Map and measure habitat features as you go. Also as you move upstream, select three riffles or other appropriate habitats for collecting macroinvertebrate and periphyton samples (per SOPs #7 and #8).
10. Collect and preserve macroinvertebrate and periphyton samples, per SOPs #7 and #8, respectively.

11. Measure water quality variables with a multiprobe and transparency with a transparency tube upstream of last transect per SOP #6; record measurements on data sheet.
12. Measure flow along a cross section of the stream (per SOP #10) at an appropriate place within the sampling reach; record measurements on data sheet.
13. Download data from continuous logging devices, replace batteries or logging devices if necessary, and re-deploy.
14. Record stage reading.
15. Verify that all data sheets are completely filled out, and initial them.
16. If sampling more than one monitoring station in a day, go back to step 6, ensuring that equipment has first been decontaminated for invasive species per SOP #5.
17. Return to office or park laboratory.
18. Clean and decontaminate sampling equipment (SOP #5) and perform calibration checks, as detailed in SOP #6.
19. Download field data and check against hard copy field data sheets as soon as feasible.
20. Enter all data into NPSTORET at the end of the season.
21. This SOP contains details on mapping and measuring habitat features and conducting physical habitat assessments instream and in the riparian area.

8.2 Equipment and Supplies

Refer to the checklist of supplies and equipment needed for collecting periphyton samples (Table 1) prior to each sampling trip. Plan ahead to be sure all equipment is available, clean, and ready for use on the day it is needed. Keep on hand all necessary forms, field logbooks, field data sheets, procedural manuals, and equipment instructional manuals. Copy field data sheets on waterproof paper. See Appendix A for a blank data sheet. Specific sections of the protocol narrative and this SOP that might be important to have in the field should be copied onto waterproof paper and remain in the field kit.

Table 1. Checklist of equipment needed for periphyton sampling.

	scalpel, toothbrush, or similar brushing and scraping tools
	plastic funnel
	suction bulb, and disposable pipettes
	filtering apparatus, hand pump, Whatman GF/C filters, forceps
	wash bottle for filtered stream water
	O-ring area delimiter
	60 mL syringe
	sample composite container (500 mL wide mouth Nalgene)
	plastic graduated cylinder (500 mL)
	sample containers for chlorophyll and taxonomy
	sample container labels
	field notebook or field forms*; pens and pencils
	preservative [2% glutaraldehyde (APHA 1998)]
	first aid kit
	GPS and extra batteries
	Camera
	clipboard, data sheets, pencils, etc.
	wrist watch
	waders, hat, bug head-net
	cooler with ice

*During wet weather conditions, waterproof paper is useful or copies of field forms can be stored in a metal storage box (attached to a clip-board).

8.3 Sampling

Prior to sampling periphyton, filter approximately 500 mL of stream water through a Whatman GF/C filter and transfer water to a wash bottle (for rinsing periphyton into sample bottle). Select three riffles that are similar to one another in bottom substrate size and composition and indicate their locations on the site map (see SOP #7 for more details on site mapping and selecting riffles). Periphyton and macroinvertebrate samples will be collected in these three riffles. If the sampling reach does not contain three riffles, periphyton should be collected from large coarse woody debris, if available.

8.3.1 Riffle Habitats

Three random periphyton subsamples will be collected from each of three riffles. The subsamples from each individual riffle will be composited into a single sample for each riffle, resulting in three samples for each reach.

After mapping the sampling reach (SOP #7) and selecting the riffles for sampling, macroinvertebrate sampling sites are randomly-chosen (see SOP #7). Each periphyton sampling site will be to the left of each macroinvertebrate sampling site. For example, if the first macroinvertebrate site is in the right third of the riffle, then the first periphyton sampling site will be in the center third; if the macroinvertebrate sample is collected from the center third, the periphyton sample will be collected from the left third; and if the macroinvertebrate sample is collected in the left third of the riffle, the periphyton sample will be collected from the right third (Figure 1). The process is repeated for the next two riffles.

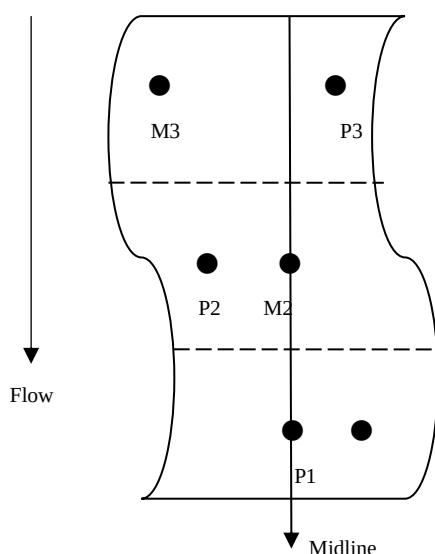


Figure 1. Placement of macroinvertebrate and periphyton samples in a riffle, with the first macroinvertebrate sample (M1) on the right and the first periphyton sample (P1) in the center, and samples 2 and 3 moving to the left as one proceeds upstream (figure from Heartland Network).

Always begin at the downstream end of the reach and proceed upstream to avoid disturbing the substrate in areas that have not yet been sampled.

At the determined sampling location (left, center, or right), haphazardly select a fist-sized cobble (ideal size) and place it in the plastic funnel, which in turn is in the composite bottle (500 mL wide-mouth Nalgene). Use the 12 cm² O-ring size delimiter to define the sampling area on the upper side of the rock. Scrape the area with a scalpel blade to dislodge large periphyton, and follow-up by scrubbing the area with a stiff toothbrush until the periphyton has been removed. Use the wash bottle with filtered stream water to wash the scraped periphyton into the funnel and composite bottle. **Use minimal water.** Return the cobble to the stream.

Repeat the process with two additional pieces of cobble at this sub-sampling site then move to the next site upstream within the same riffle. The entire periphyton sample will be a composite of three pieces of cobble per site, three sites per riffle, and three riffles (or 9 sites and 27 pieces of cobble).

If the appropriate sized cobble is not present, smaller or larger stones can be used, provided that the O-ring delimiter can be used on the stones. Use more rocks if they are too small to use the O-ring sampler to reach the desired sample area. To determine the surface area sampled of rocks too small to use the delimiter, use the rock weight or one of the displacement equations in Cooper and Testa (2001), noting which one was used on the field sheet. Record the total area of stones sampled in each riffle on the data sheet, which should be 108 cm² per riffle (27 samples from stones of 12 cm² = 108 cm²).

8.3.2 When Riffles are not Present – Woody Debris

If the sampling reach does not contain three riffles, but one or two riffles are present, sample from the riffles and use the following procedures for the remainder of the samples.

If woody debris is present, select multiple pieces of wood throughout portion of the sampling reach that does not have riffle habitat. Use the O-ring size delimiter to define the area to be sampled on each piece of wood. Scrape and brush the wood as above for cobble. Record the total area sampled on the datasheet.

The total periphyton sample from a sampling reach may be a composite of 3 riffles, 1 or 2 riffles plus wood, or all wood. In all cases, record the area sampled by substrate type on the data sheet. Composite all subsamples collected from LWD into a single sample.

8.4 Sample Processing

The composited algae sample will be processed in the field, and subsamples will be sent to a contract laboratory for analysis of chlorophyll *a* and taxonomic composition. Process the chlorophyll-*a* sample as follows:

1. Transfer the composited sample to a graduated cylinder and record the total volume of the composite sample.
2. Pour back into the bottle and mix thoroughly. If algal filaments are present, cut the filaments into pieces about 2 mm in length. Shake the sample vigorously for about 30 seconds to ensure it is well mixed. Work quickly, as direct sunlight can degrade chlorophyll-*a*.
3. Assemble the filter apparatus and place a glass fiber filter (Whatman GF/C) on the filter holder. You may want to wet the filter a little with deionized water to help keep the filter in place.
4. Using a 5 mL syringe, take two 5-mL aliquots from the mixed composite sample.
5. Filter the 10 mL through the filter using firm, steady pressure not exceeding 10 psi (69 kPa). If too much pressure is used, the algal cells can rupture, allowing chlorophyll to pass through the filter.
6. Examine the filter. If it is not green, filter another 5 mL aliquot. Repeat until the filter shows a green color.
7. Record the number of 5 mL aliquots on the data sheet.
8. Rinse the sides of the filter funnel with deionized water to include any algae that may have adhered to the sides. Pull this through the filter, but do not add this volume to the total amount of sample filtered.
9. Remove the filter with forceps and fold in quarters, with the algae on the inside. Wrap the folded filter in a small piece of aluminum foil and place in a labeled sealable plastic baggie. The label on the baggie should include the waterbody name, location, station number, date, name of collector, and volume of sample filtered.
10. Place the sample in a dark, cool place and place in a freezer as soon as possible.

Mix the remaining sample again thoroughly. Use a 125 mL syringe and withdraw 125 mL. Place the volume into a 125 mL sample bottle. Add recommended amount of glutaraldehyde to yield a final concentration of 1-2% (APHA 1998). For a 125 mL sample add 5–10 mL of 25% glutaraldehyde solution to equal a 1%–2% final concentration.

Place a permanent label on the outside of the sample container with the following information: waterbody name, location, station number, date, name of collector, and type of preservative. Record

this information and relevant ecological information in a field notebook or on the periphyton field data sheet (Appendix A).

Before moving on to the next task, review the recorded information on all labels and forms for accuracy and completeness. Examine all brushing and scraping tools for residues. Rub them clean, rinse them in distilled water, and decontaminate them according to SOP #5 before sampling the next site and before putting them away.

Transport the samples back to the laboratory in a cooler with ice (keep them cold and dark) and store preserved samples in the dark until they are processed. Be sure to stow samples in a way so that transport and shifting does not allow samples to leak. When preserved, check preservative every few weeks and replenish as necessary until sent to a contract laboratory for taxonomic evaluation.

8.5 Packing, Storing, and Sending Samples

Samples will be sent to a contracting laboratory for analysis of chlorophyll *a* and taxonomic identification.

- Pack the chlorophyll-*a* samples together in a larger sealable plastic bag. Line a cooler with a plastic garbage bag. Surround the samples with bags of ice. Tie garbage bag closed. Complete a chain of custody (COC) form, keeping the ‘client copy’ for the project files. Seal the contractor’s copies in a one-gallon plastic sealable bag and include in the cooler with the samples.
- Carefully pack the taxonomic sample containers in a box lined with a plastic garbage bag; be sure to use enough packing material to cushion the samples. Complete a chain of custody (COC) form, keeping the ‘client copy’ for the project files. Seal the contractor’s copies in a one-gallon plastic sealable bag and include in the box with the samples.
- Prepare separate COC forms for each box and cooler. An example COC form is provided in Appendix C.
- Call an appropriate courier service ahead of time to arrange pick-up. Check to be sure the courier will accept shipments containing glutaraldehyde.
- Alert the contractor when samples have been shipped and provide them with the tracking number.

8.6 Quality Assurance/Quality Control

Avoid disturbing the substrate prior to sampling.

Whether the sample is collected from cobble, or coarse woody debris, ensure the sampling is conducted according to procedure.

Prior to departure from the sampling site, check to be sure all data forms have been filled out completely. Samples must be labeled on the inside and outside with the same information including the project, date, stream name, site code, collector’s name, and preservative. If chain-of-custody forms are used for sending samples to a contract lab, they should also contain the same information as the sample container labels.

After sampling is completed at a site, rinse all equipment and gear, especially brushes and scraping tools. Inspect to be sure no organisms or debris remains. Follow decontamination procedures described in SOP #5 before using the equipment at a new site.

Replicate samples for chlorophyll-*a* and species identification, will be taken from the same composited sample once for every approximately 10 samples, across network parks. The program manager will determine which site will serve as the replicate before sampling begins for the season.

As part of the contract to identify and enumerate benthic algal cells, the contractor will recount approximately 10% of the samples.

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Appendix A. Macroinvertebrate and Periphyton Data Sheet

Park Code _____

Stream Name _____ Site Code _____

Date (DDMMYYYY) _____ Site Name _____

Personnel _____

Macroinvertebrates

Gear Used ☐ Serber Sampler ☐ Kick Net ☐ Other

How were the samples collected ☐ From bank ☐ Wading ☐ From boat

kicks/jabs/samples taken in each habitat type

Riffles _____ Cobble (hard substrate) _____ Snags (CWD) _____

Vegetated banks _____ Submerged macrophytes _____ Sand/Silt/Mud/Muck _____

Macroinvertebrate Sample Notes:

Periphyton

Gear Used ☐ Pipette ☐ Scraping tool ☐ Toothbrush ☐ Other

How were samples collected? ☐ Wading ☐ From bank ☐ From boat ☐ Other

samples taken in each habitat type

Riffles _____ Woody debris _____ Depositional areas _____

Other _____ Estimated total area sampled (cm²) _____ Should be approx.. 108 cm²

samples taken from each substrate type

Cobble _____ Gravel/small stones _____ Woody debris _____

Sand/Silt/Mud/Muck _____ Other _____

General Comments

Appendix B. Example of Laboratory Periphyton Sample Log-In Form

Date Collected	Collected By	Number of Containers	Preservative	Station #	Park and Stream Name	Date Sent to Lab