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ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM-SURFACE WATERS:

WESTERN PILOT STUDY
FIELD OPERATIONS MANUAL FOR
WADEABLE STREAMS

Edited by

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SECTION 8
PERIPHYTON

by
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Periphyton are algae, fungi, bacteria, protozoa, and associated organic matter associated with channel substrates. Periphyton are useful indicators of environmental condition because they respond rapidly and are sensitive to a number of anthropogenic disturbances, including habitat destruction, contamination by nutrients, metals, herbicides, hydrocarbons, and acidification (e.g., Hill et al., 2000).

Modifications to the periphyton sampling procedures from the published EMAP-SW field operations manual (Hill, 1998) are summarized in Table 8-1. These modifications include increasing the number of transects where samples are collected, and reducing the number of composite samples from two to one per site. Also, pre-leached and pre-weighed glass-fiber filters are no longer required. Beginning in 2001, modifications include changing the containers used for chlorophyll and biomass samples, and eliminating the collection of the acid/alkaline phosphatase activity (APA) sample.

The "biomorphs" (refer to Figure 2-1) collect periphyton samples are collected at each transect at the same time as benthic macroinvertebrate samples (Section 11). Periphyton samples are collected from the dominant habitat type (erosional or depositional) located at each of the eleven cross-section transects (transects "A" through "K") established within the sampling reach (Section 4). At each stream, a single composite "index" sample of periphyton is prepared by combining individual transect samples. At the completion of the day's sampling activities, but before leaving the stream, four types of laboratory samples are prepared from each composite index sample.

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TABLE 8-1. SUMMARY OF CHANGES IN PERIPHYTON PROCEDURES FOR THE WESTERN PILOT STUDY

Changes from Hill (1998)
1. The number of transects where periphyton samples are collected is increased from nine to eleven.
2. A single composite sample is prepared from the 11 cross-section samples, rather than preparing separating samples for erosional and depositional transect samples.
3. The same glass-fiber filters are now used for both chlorophyll and biomass samples. Previously a pre-treated and pre-weighed filter was provided to use for the biomass sample.
Changes from Year 2000 Western Pilot Study Activities
1. Filters for chlorophyll and biomass are no longer wrapped in foil, but are folded and placed in separate 50-mL centrifuge tubes, which are labeled and then placed in a black plastic bag.
2. Samples for acid/alkaline phosphatase activity (APA) will not be collected in 2001.

8.1 SAMPLE COLLECTION

The general scheme for collecting periphyton samples from the sampling reach at each stream is illustrated in Figure 8-1. The procedure for collecting periphyton samples is presented in Table 8-2. At each transect, samples are collected from an assigned sampling point (left, center, or right). Sampling points at each transect may have been assigned when the sampling reach was laid out (Figure 8-1; refer also to Section 4; Table 4-3). If not, the sampling point at Transect "A" is assigned at random using a die or other suitable means (e.g., digital watch). Once the first sampling point is determined, either an erosional or depositional sample is collected, depending on whether the dominant habitat at the sampling point is flowing water (e.g., a riffle or run) or slack water (e.g., a pool). A composite sample for the reach is prepared by combining the individual transect samples as they are collected into a single plastic bottle. The volume of the composite sample are recorded on the Sample Collection Form as shown in Figure 8-2.

8.2 PREPARATION OF LABORATORY SAMPLES

Four different types of laboratory samples are prepared from the composite index sample: an ID/enumeration sample (to determine taxonomic composition and relative

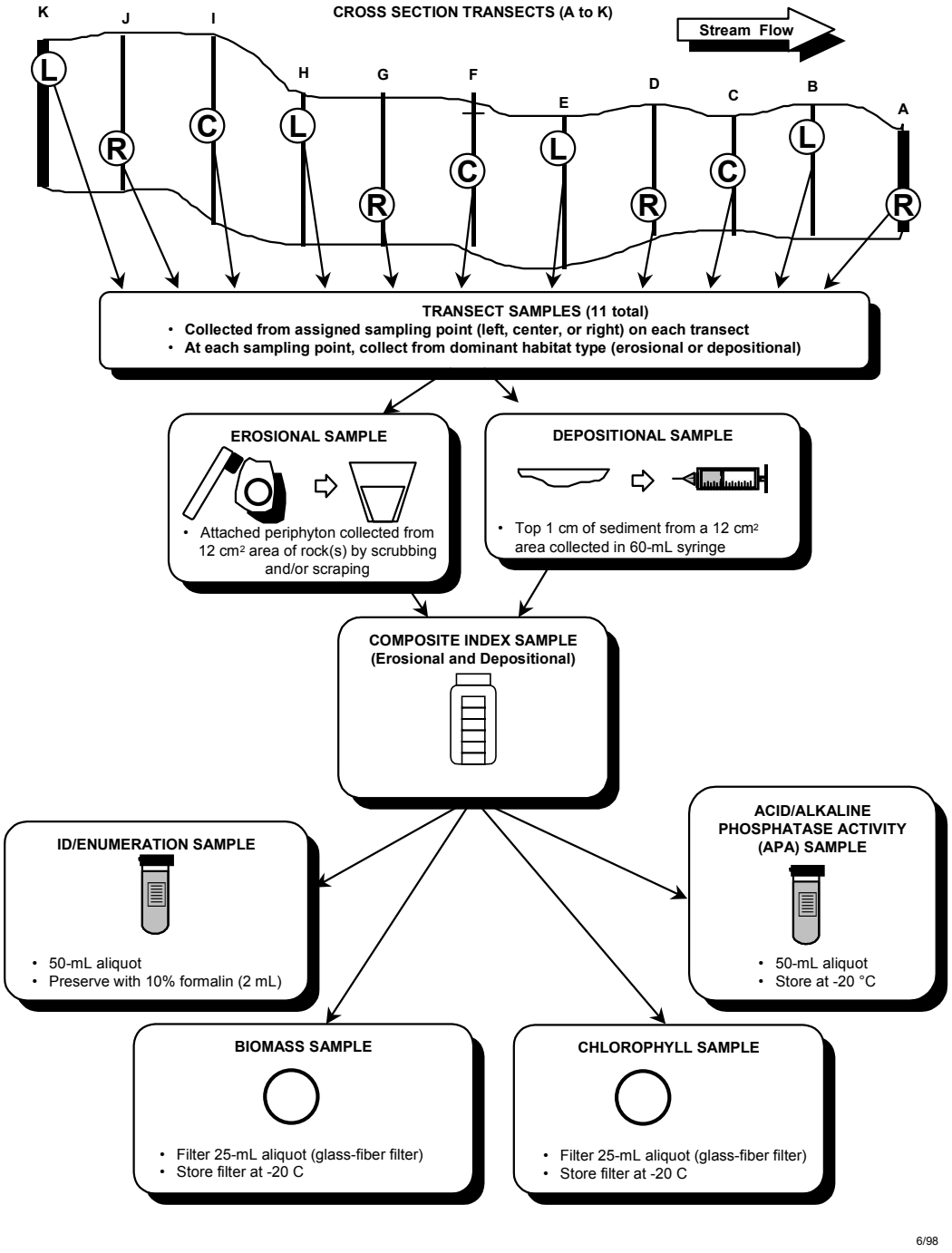

Figure 8-1. Index sampling design for periphyton.

TABLE 8-2. PROCEDURE FOR COLLECTING COMPOSITE INDEX SAMPLES OF PERIPHYTON

1. Starting with Transect "A", determine if the assigned sampling point (Left, Center, or Right) is located in an erosional (riffle) habitat or a slack water (pool) habitat. Collect a single sample at the point using the appropriate procedure in Step 2 below.

   If the sampling points were not assigned previously when laying out the sampling reach, proceed to Transect "A". Roll a die to determine if it is a left (L), center (C), or right (R) sampling point for collecting periphyton and benthic macroinvertebrate samples. A roll of 1 or 2 indicates L, 3 or 4 indicates C, and 5 or 6 indicates R (or use a digital wristwatch and glance at the last digit (1-3=L, 4-6=C, 7-9=R). Mark L, C, or R on the transect flagging. Assign sampling points at each successive transect in order as L, C, R after the first random selection.

2A. Erosional habitats:

   (1) Collect a sample of substrate (rock or wood) that is small enough (< 15 cm diameter) and can be easily removed from the stream. Place the substrate in a plastic funnel which drains into a 500-mL plastic bottle with volume graduations marked on it and labeled "PERIPHYTON."

   (2) Use the area delimiter to define a 12-cm^2 area on the **upper** surface of the substrate. Dislodge attached periphyton from the substrate within the delimiter into the funnel by brushing with a stiff-bristled toothbrush for 30 seconds. **Take care to ensure that the upper surface of the substrate is the surface that is being scrubbed, and that the entire surface within the delimiter is scrubbed.**

   (3) Fill a wash bottle with stream water. Using a minimal volume of water from this bottle, wash the dislodged periphyton from the rock, delimiter, and funnel into the 500-mL bottle.

2B. Depositional habitats:

   (1) Use the area delimiter to confine a 12-cm^2 area of soft sediments.

   (2) Vacuum the top 1 cm of sediments from within the delimited area into a 60-mL syringe.

   (3) Empty the syringe into the 500-mL 'PERIPHYTON" bottle (combining it with samples collected from erosional habitats).

3. Repeat Steps 1and 2 for transects "B" through "K" to produce the composite index sample for the stream reach. Keep the collection bottle out of direct sunlight as much as possible to minimize degradation of chlorophyll.

4. After samples have been collected from all eleven transects, mix the 500-mL bottle thoroughly. Record the total estimated volume of the composite sample in the periphyton section of the Sample Collection Form. Also record the number of transects at which you obtained a periphyton sample.

SAMPLE COLLECTION FORM - STREAMS

Reviewed by (initial): DW

SITE ID: WXXP99-9999 DATE: 07/01/2001

WATER CHEMISTRY

Sample ID	Transect	Comments
229015	X	

REACH-WIDE BENTHOS SAMPLE

Sample ID	No. of Jars	Comment
999001	2	FOR TRANSECT K OTHER

TRANSECT		A		B		C		D		E		F		G		H		I		J		K	
SUBSTRATE	CHAN.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.	Sub.	Chan.
Fine/Sand	Pool	☐ F	☐ P	☒ F	☐ P	☒ F	☒ P	☐ F	☐ P	☐ F	☐ P	☐ F	☐ P	☐ F	☐ P	☒ F	☐ P	☒ F	☒ P	☒ F	☒ P	☐ F	☒ P
Gravel	Glide	☒ G	☐ G	☐ G	☒ G	☐ G	☐ G	☒ G	☒ G	☒ G	☐ G	☐ G	☐ G	☒ G	☐ G	☐ G	☒ G	☐ G	☐ G	☐ G	☐ G	☐ G	☐ G
Coarse	Riffle	☐ C	☒ RI	☐ C	☐ RI	☐ C	☐ RI	☐ C	☐ RI	☐ C	☒ RI	☒ C	☒ RI	☐ C	☒ RI	☐ C	☐ RI	☐ C	☐ RI	☐ C	☐ RI	☐ C	☐ RI
Other: Note in Comments	Rapid	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☐ O	☐ RA	☒ O	☐ RA

TARGETED RIFFLE BENTHOS SAMPLE

Sample ID	No. of Jars	Comment
999002	1	

NEAREST TRANSECT	A	A	E	E	F	F	G	G
Dom. Substrate: Fine/Sand	☐ F/S	☐ F/S	☐ F/S	☐ F/S	☐ F/S	☐ F/S	☐ F/S	☐ F/S
Gravel	☒ G	☒ G	☐ G	☒ G	☐ G	☐ G	☒ G	☒ G
Coarse	☐ C	☐ C	☒ C	☐ C	☒ C	☒ C	☐ C	☐ C
Other: Note in Comments	☐ O	☐ O	☐ O	☐ O	☐ O	☐ O	☐ O	☐ O

SUBSTRATE SIZE CLASSES
F/S - ladybug or smaller (<2 mm)
G - ladybug to tennis ball (2 to 64 mm)
C - tennis ball to car sized (64 to 4000 mm)
O - bedrock, hardpan, wood, etc

Additional Benthos Comments

COMPOSITE PERIPHYTON SAMPLE

Sample ID	Composite Volume (mL)	Number of transects sampled (0-11):
800990	500	11

Assemblage ID (50-mL tube, preserved)		Chlorophyll (GF/F filter)		Biomass (GF/F Filter)	
Sample Vol. (mL)	Flag	Sample Vol. (mL)	Flag	Sample Vol. (mL)	Flag
50		25		25	

Flag	Comments

Flag codes: K = Sample not collected; U = Suspect sample; F1, F2, etc. = misc. flag assigned by field crew. Explain all flags in comment sections.

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03/26/2001 2001 Sample Collection

Figure 8-2. Sample Collection Form, showing data recorded for periphyton samples.

abundances), an acid/alkaline phosphatase activity (APA) sample, a chlorophyll sample, and a biomass sample (for ash-free dry mass). All the sample containers required for an individual stream should be sealed in plastic bags until use (see Section 3) to avoid external sources of contamination (e.g., dust, dirt, or mud) that are present at streamside.

A set of completed periphyton sample labels is shown in Figure 8-3. All labels in a set have the same sample ID number. Circle the appropriate type of sample (chlorophyll, biomass, etc.) on each label. Attach completed labels to the appropriate containers and cover with clear tape. When attaching the completed labels, do not cover any volume graduations and markings on the container.

8.2.1 ID/Enumeration Sample

Prepare the ID/Enumeration sample as a 50-mL aliquot from the composite index sample, following the procedure presented in Table 8-3. Preserve each sample with 2 mL of 10% formalin., observing all safety precautions associated with handling formalin solution. Record the ID number (barcode) from the sample container label and the total volume of the sample (50 mL) in the appropriate fields on the Sample Collection Form as shown in Figure 8-2. Explain any deviations from the 50 mL target volume in the comments field of the collection form. Store the preserved samples upright in a container containing absorbent material, according to the guidelines provided for handling formalin-preserved samples.

8.2.2 Acid/Alkaline Phosphatase Activity Sample

NOTE: The Acid/Alkaline Phosphatase Activity Sample will not be prepared in 2001.

The Acid/alkaline phosphatase activity (APA) sample is prepared as a 50-mL subsample of the composite index in the same manner as the ID/enumeration sample (Table 8-3). No field treatment (i.e., filtration, preservation) of the APA sample is necessary. Complete a label for each sample as shown in Figure 8-3 and affix it to a 50-mL centrifuge tube. Record the ID number (barcode), and the volume of the subsample on the Sample Collection Form (Figure 8-2). Check to ensure that the information recorded on the Sample Collection Form matches the corresponding information recorded on the sample label. Store APA samples frozen until shipment to the laboratory (Section 3).

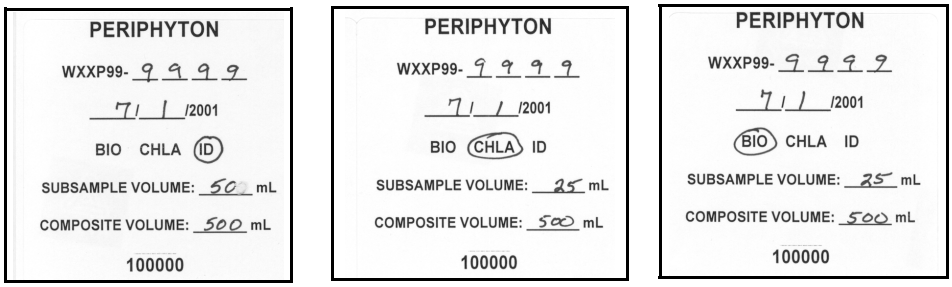

Figure 8-3. Completed set of periphyton sample labels.

8.2.3 Chlorophyll Sample

Prepare a chlorophyll sample by filtering a 25-mL aliquot of the composite index sample through a glass fiber filter (Whatman GF/F or equivalent). The procedure for preparing chlorophyll samples is presented in Table 8-4. Chlorophyll can degrade rapidly when exposed to bright light. If possible, prepare the samples in subdued light (or shade), filtering as quickly as possible after collection to minimize degradation. The filtration apparatus is illustrated in Figure 8-4. Rinse the filtration chamber with deionized water each day before use at the base site and then seal in a plastic bag until use at the stream (see Section 3). Keep the glass fiber filters in a dispenser inside a sealed plastic bag until use.

It is important to measure the volume of the sample being filtered accurately (±1 mL) with a graduated cylinder. During filtration, do no exceed 7 pounds per square inch (psi) to avoid rupturing cells. If the vacuum pressure exceeds 7 psi, prepare a new sample. If the filter clogs completely before all the sample in the chamber has been filtered, discard the sample and filter, and prepare a new sample using a smaller volume of sample.

After filtering the sample, fold the filter paper in half and place it in a 50-mL centrifuge tube. Complete a sample label (Figure 8-3) and check it to ensure that all written information is complete and legible. Affix the label to the centrifuge tube and cover it completely with a strip of clear tape. Record the sample ID number printed on the label on the Sample Collection Form (Figure 8-2). Make sure the volume recorded on each sample label matches the corresponding volume recorded on the Sample Collection Form. Record a flag and provide comments on the Sample Collection Form if there are any problems in collecting the sample or if conditions occur that may affect sample integrity. Store each

TABLE 8-3. PREPARATION OF ID/ENUMERATION AND ACID/ALKALINE PHOSPHATASE ACTIVITY SAMPLES FOR PERIPHYTON

NOTE: THE APA sample is not prepared in 2001.

1. Thoroughly mix the bottle containing the composite index sample.

2. Prepare a barcoded sample label. Circle the sample type ("ID" or "APA") on the label. Record the volume of the subsample (typically 50 mL) and the volume of the composite index sample on the label. Attach the completed label to a 50-mL centrifuge tube; avoid covering the volume graduations and markings. Cover the label completely with a clear tape strip.

3. Record the sample ID number (barcode) of the label and the total volume of the composite index sample on the Sample Collection Form. Explain any deviations from the target volume in the comments section of the form.

4. Rinse a 60-mL syringe with deionized water.

5. Withdraw 50 mL of the composite index sample into the syringe. Place the contents of the syringe sample into the labeled 50-mL centrifuge tube.

6. Repeat Steps 1 through 5 for the acid/alkaline phosphatase activity (APA) sample. **Note that in 2001, the APA sample is not collected.**

7. A. For the ID sample (wearing gloves and safety glasses), use a syringe or bulb pipette to add 2 mL of 10% formalin solution to the ID sample tube. Cap the tube tightly and seal with plastic electrical tape. Shake gently to distribute the preservative.

   B. Do NOT add preservative to the APA sample. Cap the tube tightly and seal with plastic electrical tape. Place in a cooler

8. Record the volume of each sample (typically 50 mL; exclude the volume of preservative added to the ID sample) on the Sample Collection Form. Double check that the volumes recorded on the collection form matches the total volume recorded on the corresponding sample labels.

TABLE 8-4. PROCEDURE FOR PREPARING CHLOROPHYLL AND BIOMASS SAMPLES FOR PERIPHYTON

1. Mix the composite index sample bottle thoroughly.

2. Using clean forceps, place a glass fiber filter on the filter holder. Use a small amount of deionized water from a wash bottle to help settle the filter properly. Attach the filter funnel to the filter holder and filter chamber, then attach the hand vacuum pump to the chamber.

4. Rinse the sides of the filter funnel and the filter with a small volume of deionized water.

5. Rinse a 25-mL or 50-mL graduated cylinder three times with small volumes of deionized water. Measure 25 mL (±1 mL) of sample into the graduated cylinder.

   - NOTE: For composite samples containing fine sediment, allow grit to settle before pouring the sample into the graduated cylinder.

6. Pour the 25-mL aliquot into the filter funnel, replace the cap, and pump the sample through the filter using the hand pump. **NOTE: Vacuum pressure from the pump should not exceed 7 psi to avoid rupture of fragile algal cells.**

   - If 25 mL of sample will not pass through the filter, discard the filter and rinse the chamber thoroughly with deionized water. Collect a new sample using a smaller volume of sample, measured to ±1 mL. Be sure to record the actual volume sampled on the sample label and the Sample Collection Form.

7. Remove both plugs from the filtration chamber and pour out the filtered water in the chamber. Remove the filter funnel from the filter holder. Remove the filter from the holder with clean forceps. Avoid touching the colored portion of the filter. Fold the filter in half, with the colored side folded in on itself. Place the folded filter paper into a 50-mL centrifuge tube.

9. Complete a periphyton sample label for chlorophyll, including the volume filtered, and attach it to the centrifuge tube. Cover the label completely with a strip of clear tape. Place the centrifuge tube into a self-sealing plastic bag and store in darkness.

10. Record the sample ID number (barcode) of the label and the total volume of the composite index sample on the Sample Collection Form. Record the volume filtered in the "CHLOROPHYLL" field on the Sample Collection Form. Double check that the volume recorded on the collection form matches the total volume recorded on the sample label.

11. Place the plastic bag containing the centrifuge tube into a portable freezer, a cooler containing dry ice, or between two sealed plastic bags of ice in a cooler.

12. Rinse the filter funnel, filter holder, filter chamber, and graduated cylinder thoroughly with deionized water.

13. Repeat Steps 1 through 12 to prepare the biomass sample, completing a periphyton sample label for biomass and recording sample information in the biomass section of the Sample Collection Form.

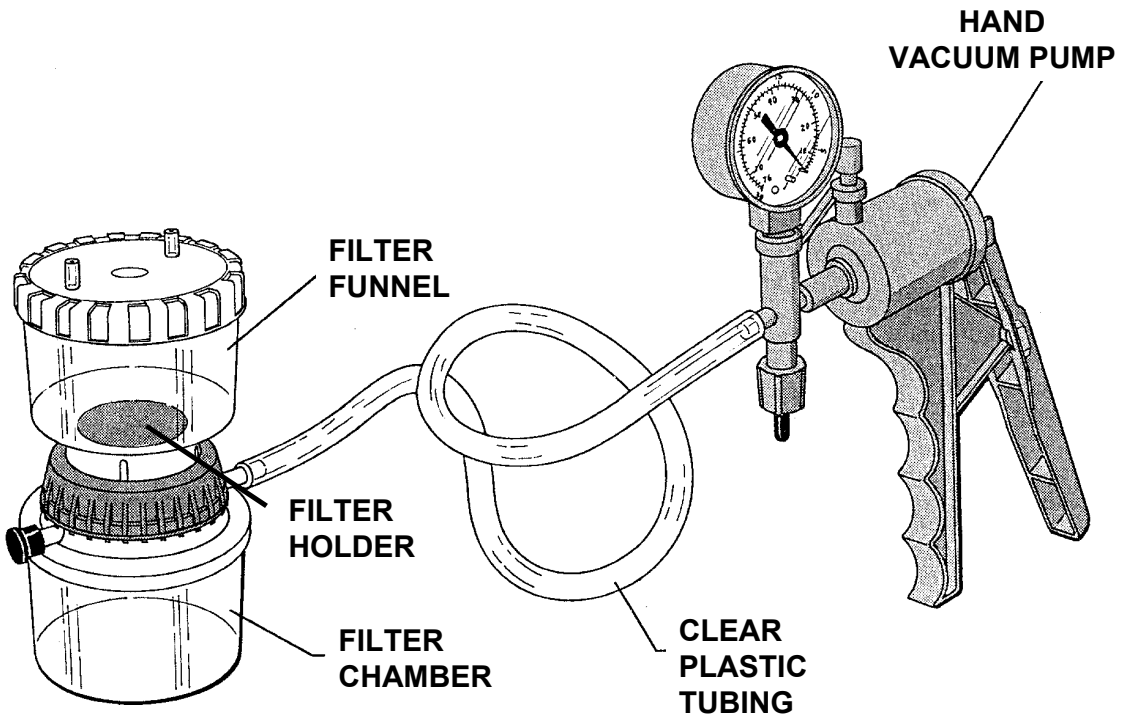

Figure 8-4. Filtration apparatus for preparing chlorophyll and biomass subsamples for periphyton. Modified from Chaloud et al. (1989).

centrifuge tube in a self-sealing plastic bag in darkness. Store the sample frozen until shipment to the laboratory (Section 3).

8.2.4 Biomass Sample

Prepare the biomass sample from a 25-mL aliquot of the composite index sample. Prepare the sample according to the procedure presented in Table 8-4. As with the chlorophyll sample, it is important to measure the volume to be filtered accurately (±1 mL). Rinse the filter chamber components (Figure 8-4) and the graduated cylinder thoroughly between the chlorophyll and biomass samples with deionized water.

After filtering the sample, complete a biomass sample label as shown in Figure 8-3. Check the sample label to ensure that all written information is complete and legible. Affix the label to the 50-mL centrifuge tube and cover it completely with clear tape. Record the sample ID number printed on the label and the volume filtered on the Sample Collection Form as shown in Figure 8-2. Make sure the information recorded on each sample label matches the corresponding values recorded on the Sample Collection Form. Record a flag and provide comments on the Sample Collection Form if there are any problems in collecting the sample or if conditions occur that may affect sample integrity. Store each labeled sample container frozen until shipment to the laboratory (Section 3).

8.3 EQUIPMENT AND SUPPLIES

Figure 8-5 is a checklist of equipment and supplies required to conduct periphyton sample collection and processing activities. This checklist is similar to the checklist presented in Appendix A, which is used at the base location (Section 3) to ensure that all of the required equipment is brought to the stream. Use this checklist to ensure that equipment and supplies are organized and available at the stream site in order to conduct the activities efficiently.

8.4 LITERATURE CITED

Chaloud, D.J., J.M. Nicholson, B.P. Baldigo, C.A. Hagley, and D.W. Sutton. 1989. *Handbook of Methods for Acid Deposition Studies: Field Methods for Surface Water Chemistry*. EPA 600/4-89-020. U.S. Environmental Protection Agency, Washington, D.C.

Hill, B.H. 1998. Periphyton. Pp. 199-132 IN: J.M. Lazorchak, D.J. Klemm, and D.V. Peck (eds.). *Environmental Monitoring and Assessment Program-Surface Waters: Field Operations and Methods for Measuring the Ecological Condition of Wadeable Streams.* EPA/620/R-94/004F. U.S. Environmental Protection Agency, Washington, DC.

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EQUIPMENT AND SUPPLIES FOR PERIPHYTON

QTY.	Item	
1	Large funnel (15-20 cm diameter)	
1	12-cm^2 area delimiter (3.8 cm diameter PVC pipe, 3 cm tall)	
1	Stiff-bristle toothbrush with handle bent at 90^o angle	
1	1-L wash bottle labeled “STREAM WATER”	
1	1-L wash bottle labeled for and containing deionized water	
1	500-mL plastic bottle (with volume markings) for composite index samples, labeled “PERIPHYTON COMPOSITE SAMPLE”	
1	35-60 mL catheter-tipped plastic syringe	
4	50-mL screw-top centrifuge tubes	
1 box	Glass-fiber filters for chlorophyll and biomass samples	
1 pair	Forceps for filter handling.	
1	25-mL or 50-mL graduated cylinder	
1	Filtration unit, including filter funnel, cap, filter holder, and receiving chamber	
1	Hand-operated vacuum pump and clear plastic tubing	
1	Small lightproof plastic bags for storing chlorophyll and biomass samples	
2	Self-sealing plastic bags for chlorophyll and biomass samples	
4 mL	10% formalin solution for ID/Enumeration samples	
1	Small syringe or bulb pipette for dispensing formalin	
1 pair	Chemical-resistant gloves for handling formalin	
1 pair	Safety glasses for use when handling formalin	
2 sets	Sample labels (4 per set) with the same barcode ID number	
1	Sample Collection Form for stream	
	Soft (#2) lead pencils for recording data on field forms	
	Fine-tipped indelible markers for filling out sample labels	
1 pkg.	Clear tape strips for covering labels	
1	Portable freezer, cooler with dry ice, or cooler with bags of ice to store frozen samples	
1 copy	Field operations and method manual	
1 set	Laminated sheets of procedure tables and/or quick reference guides for peri-phyton	

Figure 8-5. Checklist of equipment and supplies for periphyton.

NOTES

NOTES