ENVIRONMENTAL MONITORING AND ASSESSMENT PROGRAM-SURFACE WATERS:

FIELD OPERATIONS AND METHODS FOR MEASURING THE ECOLOGICAL CONDITION OF WADEABLE STREAMS

Edited by

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SECTION 8 PERIPHYTON

by Brian H. Hill¹

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.

The "biomorphs" (refer to Figure 2-1) collect periphyton samples after completing activities pertaining to water chemistry (Section 5) and discharge (Section 6). Periphyton samples are collected from erosional and depositional habitats located at each of the nine interior cross-section transects (transects "B" through "J") established within the sampling reach (Section 4). Periphyton samples are collected at each transect at the same time as sediment samples (Section 9) and benthic macroinvertebrate samples (Section 11). At each stream, composite "index" samples of periphyton are prepared for erosional and depositional habitats. 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.

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 8-1. 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,

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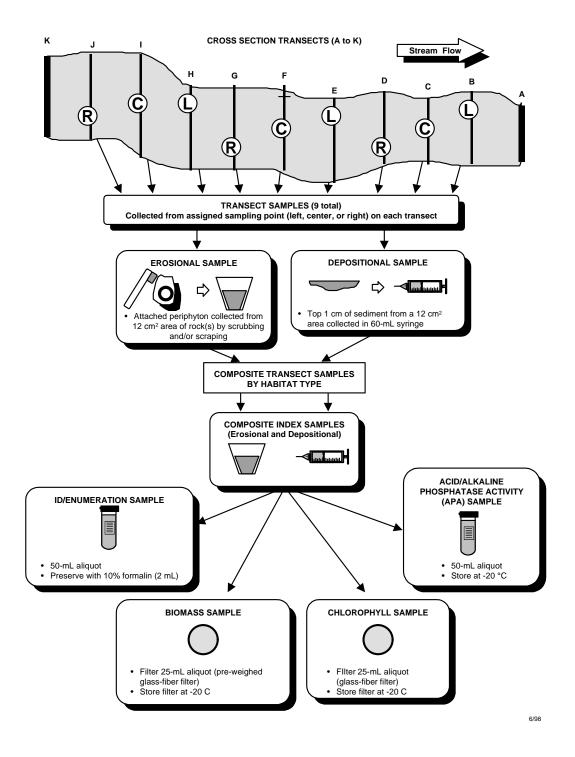


Figure 8-1. Index sampling design for periphyton.

TABLE 8-1. PROCEDURE FOR COLLECTING COMPOSITE INDEX SAMPLES OF PERIPHYTON

1. Starting with Transect "B", 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 "B". 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 "EROSIONAL."
 - (2) Use the area delimiter to define a 12-cm² 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 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 a 500-mL plastic bottle with volume graduations marked on it and labeled "DEPOSITIONAL."
- 3. Repeat Steps 1and 2 for transects "C" through "J". Place the sample collected at each sampling site into its appropriate 500-mL bottle ("EROSIONAL" or "DEPOSITIONAL") to produce the composite index sample for each habitat type.
- 4. After samples have been collected from all nine transects, mix each 500-mL bottle thoroughly. For each composite sample, place an "X" in the appropriate habitat type box ("riffle" for erosional; "pool" for depositional) and record the total estimated volume of the composite sample in the periphyton section of the Sample Collection Form.

the sampling point at Transect "B" is assigned at random using a die or other suitable means (e.g., digital watch). Once the first sampling point is determined, depositional sample, depending on whether the habitat at the site is flowing water (e.g., a riffle or run) or slack water (e.g., a pool). Composite samples for erosional and depositional habitats are prepared by combining the individual transect samples as they are collected from each habitat into separate plastic bottles. The habitat type and volume of each 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 each of the two composite index samples: an ID/enumeration sample (to determine taxonomic composition and relative abundances), a chlorophyll sample, a biomass sample (for ash-free dry mass [AFDM]), and an acid/alkaline phosphatase activity (APA) sample. 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 habitat type of the composite index sample and 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, avoid covering any volume graduations and markings on the container.

8.2.1 ID/Enumeration Sample

Prepare the ID/Enumeration samples as 50-mL aliquots from each composite index sample, following the procedure presented in Table 8-2. Preserve each sample with 2 mL of 10% formalin. For each habitat type (riffle and pool), record the ID number (barcode) from each sample container label and the total volume of the sample in the appropriate fields on the Sample Collection Form as shown in Figure 8-2. Store the preserved samples upright in a container containing absorbent material, according to the guidelines provided for handling formalin-preserved samples.

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COMPOSITE PERIPHYTON SAMPLES					HABITAT TYPE (X) → 🔀 Riff								
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SUB. SAMPLE VOL.		VOL. FILTERED				FILTER NO. VOL. FILTERED							
<u>50 ml</u>		<u>25</u> ML			╇	<u>999 25 ML</u>			<u>S</u> ML	<u> </u>			
COMPOSITE PERIPHYTON SAMPLES					HABITAT TYPE (X) → □ RI								
SAMPLE ID (BARCODE) →		229003				Composite Volume →				<u>300</u> ML			
Assemblage ID (50-mL tube)		CHLOROPHYLL (GF/F FILTER)				BIOMASS (TARED FILTER)				APA SAMPLE (50-ML TUBE)			
SUB. SAMPLE VOL.		VOL. FILTERED				FILTER NO.		VOL. FILTERED		SUB. SAMPLE VOL.			
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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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SAMPLE COLLECTION FORM - STREAMS - 1

Figure 8-2. Sample Collection Form (page1) showing data recorded for periphyton samples.

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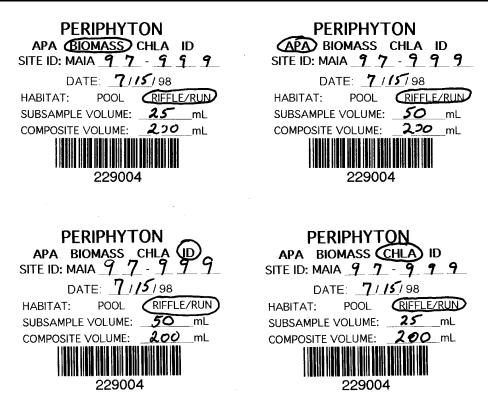


Figure 8-3. Completed set of periphyton sample labels.

8.2.2 Chlorophyll Sample

Prepare chlorophyll samples by filtering a 25-mL aliquot of each composite index sample through a glass fiber filter (0.4 to 0.6 : m nominal pore size). The procedure for preparing chlorophyll samples is presented in Table 8-3. 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

TABLE 8-2. PREPARATION OF ID/ENUMERATION SAMPLES FOR PERIPHYTON

- 1. Thoroughly mix the bottle containing the "EROSIONAL" composite index sample.
- 2. Prepare a barcoded sample label. Circle the sample type ("ID") and habitat type ("RIFFLE/RUN" for EROSIONAL. "POOL" for DEPOSITIONAL) on the label. Record the volume of the subample (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. Place and "X" in the appropriate "HABITAT TYPE" box (riffle or pool) in the first "COMPOSITE PERIPHYTON SAMPLE" section of the Sample Collection Form. Record the sample ID number (barcode) of the label and the total volume of the composite index sample on 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.
- Wearing gloves and safety glasses, use a syringe or bulb pipette to add 2 mL of 10% formalin solution to the tube. Cap the tube tightly and seal with plastic electrical tape. Shake gently to distribute preservative.
- 7. Record the volume of the sample in the centrifuge tube (excluding the volume of preservative) in the "ASSEMBLAGE ID SUBSAMPLE VOL." field of the Sample Collection Form.
- 8. Repeat Steps 1 through 7 above for the DEPOSITIONAL composite index sample. Record information in the second "COMPOSITE PERIPHYTON SAMPLE" section of the Sample Collection Form.

TABLE 8-3. PROCEDURE FOR PREPARING CHLOROPHYLL SAMPLES FOR PERIPHYTON

- 1. Mix the "EROSIONAL" 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, (e.g., the "DEPOSITIONAL" sample), 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. Wrap the folded filter in a small piece of aluminum foil.
- 9. Complete a periphyton sample label for chlorophyll, including the type of composite index sample and the volume filtered, and attach it to the foil. Cover the label completely with a strip of clear tape. Place the foil packet into a self-sealing plastic bag.
- 10. Place and "X" in the appropriate "HABITAT TYPE" box (riffle or pool) in the first "COMPOSITE PERIPHYTON SAMPLE section of the Sample Collection Form. Record the sample ID number (barcode) of the label and the total volume of the composite index sample on the 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 filter 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 for the "DEPOSITIONAL" composite index sample. Record information in the second "COMPOSITE PERIPHYTON SAMPLE" section on the Sample Collection Form.

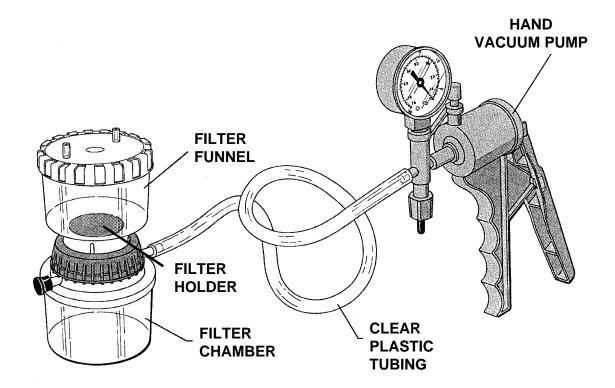


Figure 8-4. Filtration apparatus for preparing chlorophyll and biomass subsamples for periphyton. Modified from Chaloud et al. (1989).

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. Rinse the filtration unit and the graduated cylinder thoroughly with deionized water between the two composite index samples.

After filtering each sample, wrap the filter in aluminum foil. 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 foil packet and cover it completely with a strip of clear tape. Record the barcode assigned to the sample 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-

tion Form if there are any problems in collecting the sample or if conditions occur that may affect sample integrity. Store each foil packet in a self-sealing plastic bag. Store the sample frozen until shipment to the laboratory (Section 3).

8.2.3 Biomass Sample

Prepare the biomass samples from 25-mL aliquots of each composite index sample. The filters for the biomass samples (the same type as is used for chlorophyll) may be provided in a sealed, numbered container. These filters have been prepared by combusting (30 min at 525 /C), desiccating, re-hydrating, drying (60 /C for 24 hours), then weighed to the nearest 0.01 mg. Prepare each sample according to the procedure presented in Table 8-4. Take extra care in handling the filters, as they may be very fragile as a result of their preparation. 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 two composite index samples with deionized water.

After filtering each sample, do not fold the filter (as was done for the chlorophyll sample). Place the unfolded filter back into its numbered container. Complete a sample label as shown in Figure 8-3. Check each sample label to ensure that all written information is complete and legible. Affix the label to the filter container and cover it completely with clear tape. Record the bar code assigned to the sample, the container number, and the volume filtered on the Sample Collection Form as shown in Figure 8-2. Make sure the information recorded on each sample label and filters container 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 filter container frozen until shipment to the laboratory (Section 3).

8.2.4 Acid/Alkaline Phosphatase Activity Sample

The Acid/Alkaline phosphatase activity (APA) samples are prepared from 50-mL subsamples of each composite index sample. Table 8-5 presents the procedure for preparing APA samples. 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

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

- 1. Mix the "EROSIONAL" composite index sample bottle thoroughly.
- 2. Using clean forceps, remove a pre-leached, pre-weighed glass-fiber filter from its numbered container and place it 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.
- 3. Rinse the filter chamber and filter with a small volume of deionized water.
- 4. Rinse a 25-mL or 50-mL graduated cylinder three times with small volumes of deionized water. Measure 25 mL (\pm 1 mL) of composite index sample into the graduated cylinder.
 - NOTE: For composite samples containing fine sediment, (e.g., the "DEPOSITIONAL" sample), allow grit to settle before pouring the sample into the graduated cylinder.
- 5. Pour the 25-mL aliquot into filter funnel, replace the cap, and pump the sample through the filter using the hand pump. **NOTE: Filtration pressure 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 filtered on the sample label and the Sample Collection Form.
- 6. 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.
- 8. Place the **unfolded** filter back into its numbered container. Complete a periphyton sample label for biomass, including the type of index sample, the container number, and the volume filtered. Affix the label to the filter container and cover the label completely with a strip of clear tape.
- 9. Place and "X" in the appropriate "HABITAT TYPE" box (riffle or pool) in the first "COMPOSITE PERIPHYTON SAMPLE" section of the Sample Collection Form. Record the sample ID number (barcode) of the label and the total volume of the composite index sample on the form. Record the number from the filter container ("FILTER NO.") and the volume filtered in the "BIOMASS" portion on the Sample Collection Form. Double check that the volume recorded on the collection form matches the total volume recorded on the sample label.
- 10. Place the labeled filter container into a portable freezer, a cooler containing dry ice, or between two sealed plastic bags of ice in a cooler.
- 11. Rinse the filter funnel, filter holder, filter chamber, and graduated cylinder thoroughly with deionized water.
- 12. Repeat Steps 1 through 11 for the "DEPOSITIONAL" composite index sample. Record information in the second "COMPOSITE PERIPHYTON SAMPLE" section on the Sample Collection Form.

TABLE 8-5. PROCEDURE FOR PREPARING ACID/ALKALINE PHOSPHATASE ACTIVITY SAMPLES FOR PERIPHYTON

- 1. Thoroughly mix the bottle containing the "EROSIONAL" composite index sample.
- 2. Prepare a barcoded sample label. Circle the sample type ("ID") and habitat type ("RIFFLE/RUN" for the "EROSIONAL" sample; "POOL" for the "DEPOSITIONAL" sample) on the label. Record the volume of the sample (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. Rinse a 60-mL syringe with deionized water.
- 4. 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. Cap the tube tightly and seal with plastic tape.
- 5. Place and "X" in the appropriate "HABITAT TYPE" box (riffle or pool) in the first "COMPOSITE PERIPHYTON SAMPLE" section of the Sample Collection Form. Record the sample ID number (barcode) of the label and the total volume of the composite index sample on the form.
- 7. Record the volume of the sample in the centrifuge tube in the "APA SAMPLE" field of the Sample Collection Form.
- 8. Repeat Steps 1 through 7 above for the "DEPOSITIONAL" composite index sample. Record information in the second "COMPOSITE PERIPHYTON SAMPLE" section of the Sample Collection Form.

Sample Collection Form matches the corresponding information recorded on the sample label. Store APA samples 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.

QTY.	ltem	
1	Large funnel (15-20 cm diameter)	
1	12-cm ² area delimiter (3.8 cm diameter pipe, 3 cm tall)	
1	Stiff-bristle toothbrush with handle bent at 90° angle	
1	1-L wash bottle for stream water	
1	1-L wash bottle containing deionized water	
2	500-mL plastic bottles for composite index samples, labeled "EROSIONAL" and "DEPOSITIONAL"	
1	60 mL plastic syringe with 3/8" hole bored into the end	
4	50-mL screw-top centrifuge tubes (or similar sample vials)	
1 box	Glass-fiber filters for chlorophyll 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	
2	Pre-leached, pre-ashed, weighed glass-fiber filters in numbered containers for biomass sample	
2	Aluminum foil squares (3" x 6")	
2	Self-sealing plastic bags for chlorophyll 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 сору	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.