Section 7
Periphyton

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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.

Periphyton samples are collected at the near-shore shallows when stopped at each of the cross-section transects (transects "A" through "K") established within the sampling reach (Section 4). Periphyton samples are collected at each transect at the same time as sediment samples (Section 8) and benthic macroinvertebrate samples (Section 9). One composite "index" sample of periphyton is prepared for each river site. At the completion of the day's sampling activities, but before leaving the river, four types of laboratory samples are prepared from the composite periphyton sample.

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7.1 Sample Collection

The general scheme for collecting periphyton samples from the sampling reach at each river is illustrated in Figure 7-1. At each transect, samples are collected from the shoreline assigned during the layout of the reach (Section 4). The substrate selected for sampling should be collected from a depth no deeper than can be reached by submerging your arm to mid-bicep depth. If a sample cannot be collected because the location is too deep, skip the transect. The procedure for collecting samples and preparing a composite sample is presented in Table 7-1. One sample is collected from each of the transects and composited in one bottle. The volume of the sample is recorded on the Sample Collection Form as shown in Figure 7-2.

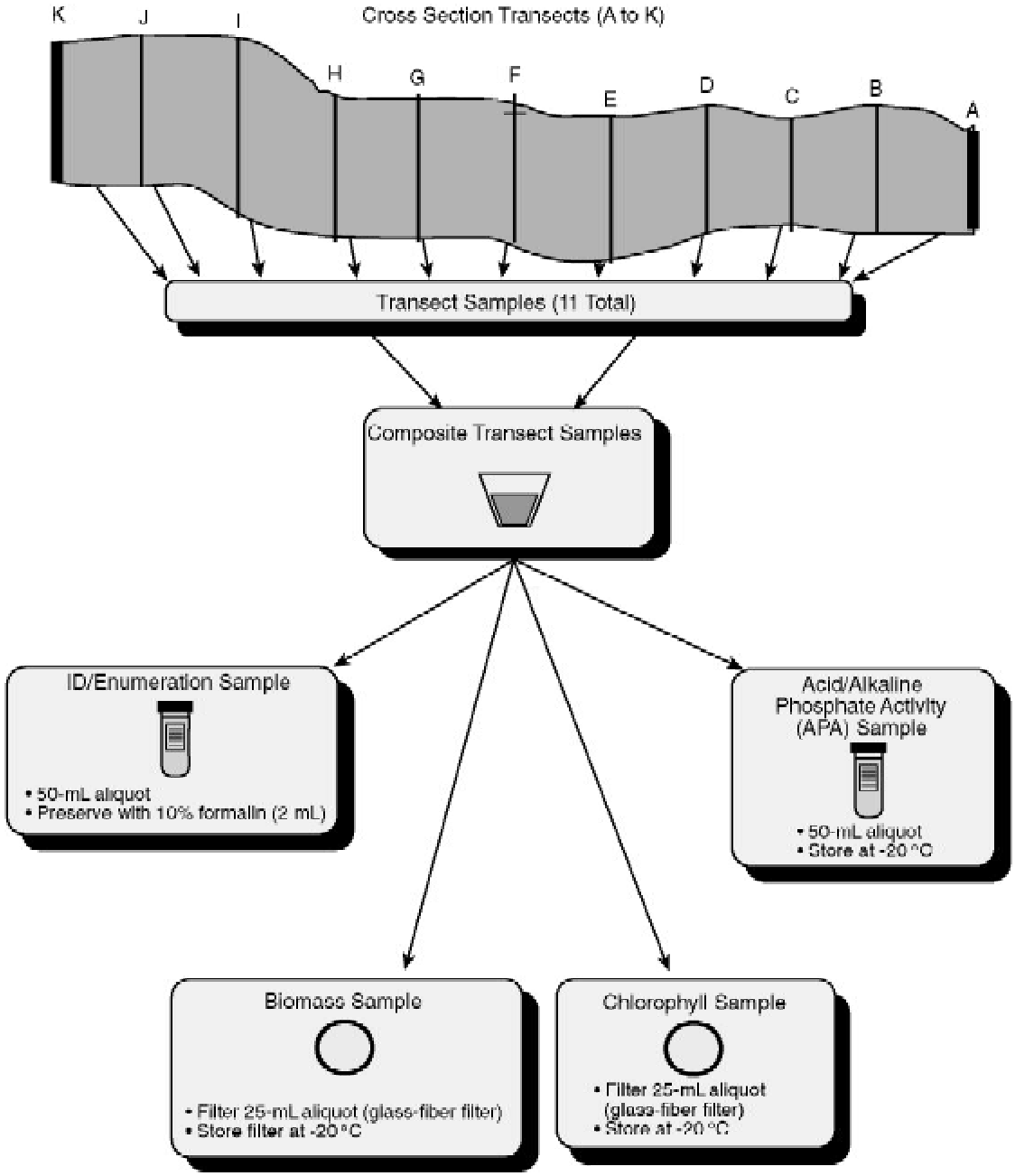

Figure 7-1. Index sampling design for periphyton.

7.2 Preparation of Laboratory Samples

Four different types of laboratory samples are prepared from the 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

Table 7-1. Procedure for Collecting Composite Index Samples of Periphyton.

1. Starting with Transect "A", collect a single sample from the assigned shoreline using the procedure below.
   (a) Collect a sample of substrate (rock or wood) that is small enough (< 15 cm diameter) and can be easily removed from the river. Place the substrate in a plastic funnel which drains into a 500-mL plastic bottle with volume graduations marked on it.
   (b) 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.
   (c) Fill a wash bottle with river water. Using a minimal volume of water from this bottle, wash the dislodged periphyton from the funnel into the 500-mL bottle.

   If no coarse sediment (cobbles or larger) are present:
   (d) Use the area delimiter to confine a 12-cm2 area of soft sediments.
   (e) Vacuum the top 1 cm of sediments from within the delimited area into a de-tipped 60-mL syringe.
   (f) Empty the syringe into the same 500-mL plastic bottle as above.
2. Repeat Step 1 for transects "B" through "K". Place the sample collected at each sampling site into the single 500-mL bottle to produce the composite index sample.
3. After samples have been collected from all 11 transects, thoroughly mix the 500-mL bottle regardless of substrate type. Record the total estimated volume of the composite sample in the periphyton section of the Sample Collection Form.

(APA) sample. All the sample containers required for an individual river 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 river shorelines.

A set of completed periphyton sample labels is shown in Figure 7-3. 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.

7.2.1 ID/Enumeration Sample

Prepare the ID/Enumeration sample as a 50-mL aliquot from the composite index sample, following the procedure presented in Table 7-2. Preserve each sample with 2 mL of 10% formalin. Record the ID number (barcode) from the container label and the total volume of the sample in the appropriate fields on the Sample Collection Form as shown in Figure 7-2. Store the preserved samples upright in a container containing absorbent material, according to the guidelines provided for handling formalin-preserved samples.

7.2.2 Chlorophyll Sample

Prepare the chlorophyll sample by filtering a 25-mL aliquot of the composite index sample through a glass fiber filter (0.45 m nominal pore size). The procedure for preparing chlorophyll samples is presented in Table 7-3. 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 collec-

Reviewed by (initial) DKA

SAMPLE COLLECTION FORM - RIVERS

SITE NAME: BEAVER RIVER DATE: 8 / 5 / 98 VISIT ☐0 ☒1 ☐2 ☐3

SITE ID: ORRV 98-999 TEAM ID (X): ☒1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8

COMPOSITE BENTHOS SAMPLES

BANK SAMPLED (LOOKING DOWNSTREAM): ☒LEFT ☐RIGHT

S = SHORE M = MID-CHANNEL D = DRIFT

SAMPLED (BARCODE)	HABITAT (CIRCLE ONE)	NO. OF JARS	FLAG	COMMENTS
243612	S M (D)	1		RIGHT BANK - SAMPLED AT LAUNCH
243611	(S) M D	1		NO SHORE KICK AT TRANSECTS
	S M D			A,G,K => TOO DEEP

STATION (CIRCLE IF DONE)	A	B	C	D	E	F	G	H	I	J
SHORE KICK	S	(S)	(S)	(S)	(S)	(S)	S	(S)	(S)	(S)
MID-CHANNEL	M	M	M	M	M	M	M	M	M	M

DRIFT NET	TRANSECT	TIME (24-HR CLOCK) START	TIME (24-HR CLOCK) END	VELOCITY (FT/SEC) XX.XX
	☒A ☐B ☐C ☐D ☐E ☐F ☐G ☐H ☐I ☐J ☐	09 : 40	19 : 45	3.0

COMPOSITE PERIPHYTON SAMPLES

SAMPLE ID (BARCODE) ➤ 244013 COMPOSITE VOLUME xxxx mL ➤ 325

ASSEMBLAGE ID (50-mL TUBE)	CHLOROPHYLL (GF/F FILTER	BIOMASS (TARED FILTER)		APA SAMPLE (50-mL TUBE)
SUB. SAMPLE VOL. xx mL	VOL. FILTERED xxxx mL	FILTER NO.	VOL. FILTERED xxxx mL	SUB. SAMPLE VOL. xxxx mL
50	25		25	50

COMMENTS:

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

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SAMPLE COLLECTION FORM - RIVERS - 1

Figure 7-2. Sample Collection Form (page1) showing data recorded for periphyton samples.

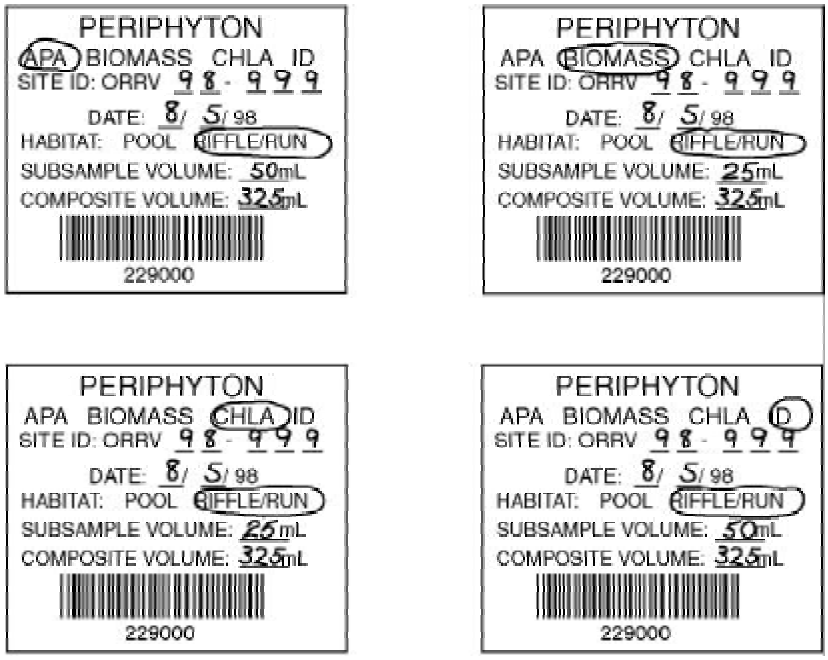

Figure 7-3. Completed set of periphyton sample labels.

Table 7-2. Preparation of ID/Enumeration Samples for Periphyton.

1. Prepare a barcoded sample label and circle the sample type ("ID") 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.

2. Record the sample ID number (barcode) of the label and the total volume of the composite index sample on the form.

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

4. Thoroughly mix the bottle containing the composite sample.

5. Withdraw 50 mL of the mixed sample into the syringe. Right after mixing, place the contents of the syringe sample into the labeled 50-mL centrifuge tube.

6. 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.

Table 7-3. Procedure for Preparing Chlorophyll Samples for Periphyton.

1. 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.
2. Rinse the sides of the filter funnel and the filter with a small volume of deionized water.
3. Rinse a 25-mL or 50-mL graduated cylinder three times with small volumes of deionized water.
4. Mix the composite sample bottle thoroughly.
5. Measure 25 mL (±1 mL) of sample into the graduated cylinder.
   - NOTE: For a composite sample containing fine sediment, (e.g., the "DEPOSITIONAL" sample), allow grit to settle for 10 - 20 seconds before pouring the sample into the graduated cylinder.
6. Pour the 25-mL aliquot into the filter funnel, replace the cap, and pull the sample through the filter using the hand pump. NOTE: Vacuum pressure from the pump should not exceed 15 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 sample (filtrate) side folded in on itself. Wrap the folded filter in a small piece of aluminum foil.
8. Complete a periphyton sample label for chlorophyll, including 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.
9. 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.
10. Place the plastic bag containing the filter into a portable freezer or between two sealed plastic bags of ice or frozen gel packs in a cooler.
11. Rinse the filter funnel, filter holder, filter chamber, and graduated cylinder thoroughly with deionized water.

tion to minimize degradation. The filtration apparatus is illustrated in Figure 7-4. 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 15 pounds per square inch (psi) to avoid rupturing cells. If the vacuum pressure exceeds 15 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 each sample, wrap the filter in aluminum foil. Complete a sample label (Figure 7-3) 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 7-2). Make

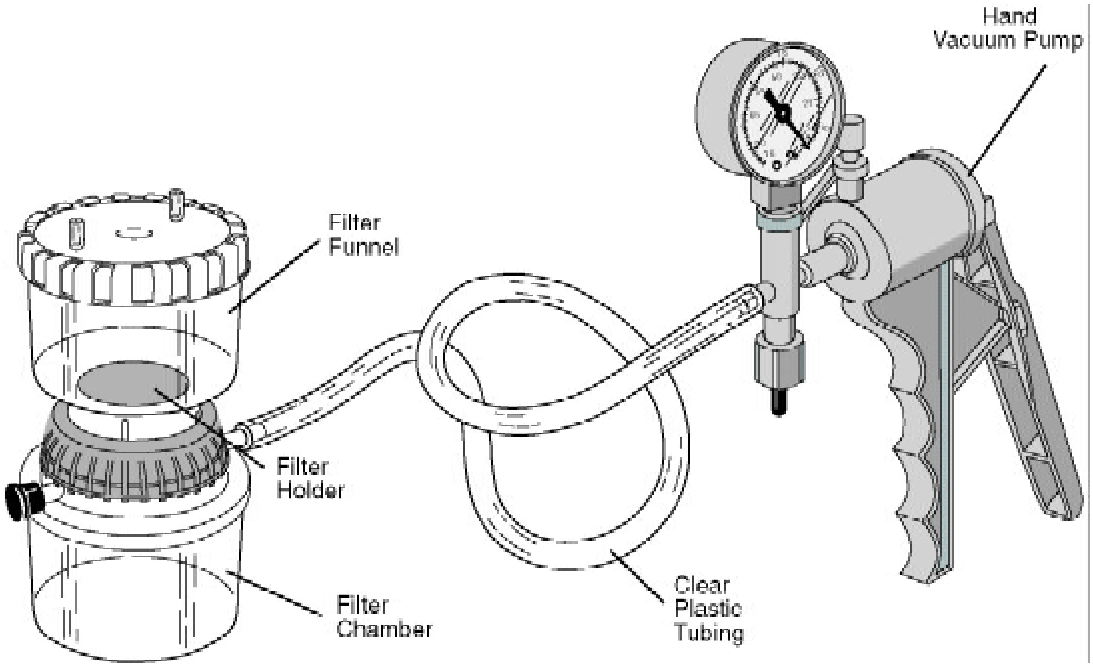

Figure 7-4. Filtration apparatus for preparing chlorophyll and biomass subsamples for periphyton. Modified from Chaloud et al. (1989).

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

7.2.3 Biomass Sample

Prepare the biomass sample from a 25-mL aliquot of the composite index sample. As with the chlorophyll sample, it is important to measure the volume to be filtered accurately (±1 mL).

After filtering each sample, complete a sample label as shown in Figure 7-3. 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 7-2. 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 con-

Table 7-4. Procedure For Preparing Biomass Samples For Periphyton.

1. Using clean forceps, remove a glass-fiber filter 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.
2. Rinse the filter chamber and filter with a small volume of deionized water.
3. Rinse a 25-mL or 50-mL graduated cylinder three times with small volumes of deionized water.
4. Mix the composite sample bottle thoroughly.
5. Measure 25 mL (±1 mL) of composite sample into the graduated cylinder.
   - NOTE: For a composite sample containing fine sediment, allow grit to settle for 10 - 20 seconds before pouring the sample into the graduated cylinder.
6. Pour the 25-mL aliquot into filter funnel, replace the cap, and pull the sample through the filter using the hand pump. NOTE: Filtration pressure should not exceed 15 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.
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 sample portion of the filter.
8. Complete a periphyton sample label for biomass, including 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.
9. Record the sample ID number (barcode) of the label and the total volume of the composite sample on the form. Record 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 cooler containing two sealed plastic bags of ice.
11. Rinse the filter funnel, filter holder, filter chamber, and graduated cylinder thoroughly with deionized water.

tainer frozen until shipment to the laboratory (Section 3).

7.2.4 Acid/Alkaline Phosphatase Activity Sample

The Acid/Alkaline phosphatase activity (APA) sample is prepared from a 50-mL subsample of the composite index sample. Table 7-5 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 7-3 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 7-2). 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).

7.3 Equipment and Supplies

Figure 7-5 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

Table 7-5. Procedure for Preparing Acid Alkaline Phosphatase Activity Samples for Periphyton.

1. Prepare a barcoded sample label. Circle the sample type ("APA") and the habitat type ("Riffle/ Run" or "Pool") 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.
2. Rinse a 60-mL syringe with deionized water.
3. Thoroughly mix the bottle containing the composite sample.
4. Withdraw 50 mL of the mixed 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 electrical tape.
5. Record the sample ID number (barcode) of the label and the total volume of the composite index sample on the form.
6. Record the volume of the sample in the centrifuge tube in the "APA Sample" field of the Sample Collection Form.

Equipment and Supplies for Periphyton		
Qty.	Item	
1	Large funnel (15-20 cm diameter)	
1	12-cm2 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	
1	500-mL plastic bottles for the composite sample.	
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 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	
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	
1 set	Sample labels (4 per set) with the same barcode ID number	
1	Sample Collection Form for river	
	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	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 periphyton	

Figure 7-5. Checklist of equipment and supplies for periphyton

the required equipment is brought to the river. Use this checklist to ensure that equipment and supplies are organized and available at the river site in order to conduct the activities efficiently.

7.4 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.