6 PERIPHYTON PROTOCOLS

**By R. Jan Stevenson, University of Louisville, and
Loren L. Bahls, University of Montana**

Benthic algae (periphyton or phytobenthos) are primary producers and 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 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 numbers 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.

Periphyton indices of biotic integrity have been developed and tested in several regions (Kentucky Department of Environmental Protection 1993, Hill 1997). Since the ecological tolerances for many species are known (see section 6.1.4), changes in community composition can be used to diagnose the environmental stressors affecting ecological health, as well as to assess biotic integrity (Stevenson 1998, Stevenson and Pan 1999).

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

Presently, few states have developed protocols for periphyton assessment. Montana, Kentucky, and Oklahoma have developed periphyton bioassessment programs. Others states are exploring the possibility of developing periphyton programs. Algae have been widely used to monitor water quality in rivers of Europe, where many different approaches have been used for sampling and data analysis (see reviews in Whitton and Rott 1996, Whitton et al. 1991). The protocols presented here are a composite of the techniques used in Kentucky, Montana, and Oklahoma (Bahls 1993, Kentucky Department of Environmental Protection 1993, Oklahoma Conservation Commission 1993).

Two Rapid Bioassessment Protocols for periphyton are presented. These protocols are meant to provide examples of methods that can be used. Other methods are available and should be considered based on the objectives of the assessment program, resources available for study, numbers of streams sampled, hypothesized stressors, and the physical habitat of the streams studied. Examples of other methods are presented in textboxes throughout the chapter.

The first protocol (6.1) is a standard approach in which species composition and/or biomass of a sampled assemblage is assessed in the laboratory. The second protocol (6.2) is a field-based rapid survey of periphyton biomass and coarse-level taxonomic composition (e.g., diatoms, filamentous greens, blue-green algae) and requires little taxonomic expertise. The two protocols can be used together. The first protocol has the advantage of providing much more accuracy in assessing biotic

integrity and in diagnosing causes of impairment than the second protocol, but it requires more effort than the second protocol. Additionally, the first protocol provides the option of sampling the natural substrate of the stream or placing artificial substrates for colonization.

6.1 STANDARD LABORATORY-BASED APPROACH

6.1.1 Field Sampling Procedures: Natural Substrates

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).

Two sampling approaches are described for natural substrate sampling. Multihabitat sampling best characterizes the benthic algae in the reach, but results may not be sensitive to subtle water quality changes because of habitat variability between reaches. Species composition of assemblages from a single habitat should reflect water quality differences among streams more precisely than multi-habitat sampling, but impacts in other habitats in the reach may be missed.

The length of stream sampled depends upon the objectives of the project, budget, and expected results. Multihabitat sampling should be conducted at the reach scale (30-40 stream widths) to ensure sampling the diversity of habitats that occur in the stream. Ideally, single habitat sampling should also be conducted at the reach scale. A shorter length of stream can probably be sampled for single habitat samples than multihabitat samples because the chosen single habitat (e.g., riffles) is usually common within the study streams.

6.1.1.1 Multihabitat Sampling

The following procedures for multihabitat sampling of algae have been adapted from the Kentucky and Montana protocols (Kentucky DEP 1993, Bahls 1993). These procedures are recommended when subsequent laboratory assessments of species composition of algal assemblages will be performed.

1. Establish the reach for multihabitat sampling as per the macroinvertebrate protocols (Chapter 7). In most cases, the reach required for periphyton sampling will be the same size as the reach required for

FIELD EQUIPMENT FOR PERIPHYTON SAMPLING--NATURAL SUBSTRATES

- stainless steel teaspoon, toothbrush, or similar brushing and scraping tools
- section of PVC pipe (3" diameter or larger) fitted with a rubber collar at one end
- field notebook or field forms*; pens and pencils
- white plastic or enamel pan
- petri dish and spatula (for collecting soft sediment)
- forceps, suction bulb, and disposable pipettes
- squeeze bottle with distilled water
- sample containers (125 ml wide-mouth jars)
- sample container labels
- preservative [Lugol's solution, 4% buffered formalin, "M3" fixative, or 2% glutaraldehyde (APHA 1995)]
- first aid kit
- 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).

macroinvertebrate or fish sampling (30-40 stream widths) so that as many algal habitats can be sampled as is practical.

2. Before sampling, complete the physical/chemical field sheet (see Chapter 5; Appendix A-1, Form 1) and the periphyton field data sheet (Appendix A-2, Form 1). Visual estimates or quantitative transect-based assessments can be used to determine the percent coverage of each substrate type and the estimated relative abundance of macrophytes, macroscopic filamentous algae, diatoms and other microscopic algal accumulations (periphyton), and other biota (see section 6.2).

3. Collect algae from all available substrates and habitats. The objective is to collect a single composite sample that is representative of the periphyton assemblage present in the reach. Sample all substrates (Table 6-1) and habitats (riffles, runs, shallow pools, nearshore areas) roughly in proportion to their areal coverage in the reach. Within a stream reach, light, depth, substrate, and current velocity can affect species composition of periphyton assemblages. Changes in species composition of algae among habitats are often evident as changes in color and texture of the periphyton. Small amounts (about 5 mL or less) of subsample from each habitat are usually sufficient. Pick specimens of macroalgae by hand in proportion to their relative abundance in the reach. Combine all samples into a common container.

Table 6-1. Summary of collection techniques for periphyton from wadeable streams (adapted from Kentucky DEP 1993, Bahls 1993).

Substrate Type	Collection Technique
Removable substrates (hard): gravel, pebbles, cobble, and woody debris	Remove representative substrates from water; brush or scrape representative area of algae from surface and rinse into sample jar.
Removable substrates (soft): mosses, macroalgae, vascular plants, root masses	Place a portion of the plant in a sample container with some water. Shake it vigorously and rub it gently to remove algae. Remove plant from sample container.
Large substrates (not removable): boulders, bedrock, logs, trees, roots	Place PVC pipe with a neoprene collar at one end on the substrate so that the collar is sealed against the substrate. Dislodge algae in the pipe with a toothbrush, nail brush, or scraper. Remove algae from pipe with pipette.
Loose sediments: sand, silt, fine particulate organic matter, clay	Invert petri dish over sediments. Trap sediments in petri dish by inserting spatula under dish. Remove sediments from stream and rinse into sampling container. Algal samples from depositional habitats can also be collected with spoons, forceps, or pipette.

4. Place all samples into a single water-tight, unbreakable, wide-mouth container. A composite sample measuring four ounces (ca. 125 ml) is sufficient (Bahls 1993). Add recommended amount of Lugol's (IKI) solution, "M3" fixative, buffered 4% formalin, 2% glutaraldehyde, or other preservative (APHA 1995).

5. 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-2, Form 1). Place another label with the same information inside the sample container. (Caution! Lugol's solution and other iodine-based preservatives will turn paper labels black.)

6. After sampling, review the recorded information on all labels and forms for accuracy and completeness.

7. Examine all brushing and scraping tools for residues. Rub them clean and rinse them in distilled water before sampling the next site and before putting them away.

8. Transport 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 taxonomic evaluation is completed.

9. Log in all incoming samples (Appendix A-2, Form 2). At a minimum, record sample identification code, date, stream name, sampling location, collector's name, sampling method, and area sampled (if it was determined).

6.1.1.2 Single Habitat Sampling

Variability due to differences in habitat between streams may be reduced by collecting periphyton from a single substrate/habitat combination that characterizes the study reach (Rosen 1995). For comparability of results, the same substrate/habitat combination should be sampled in all reference and test streams. Single habitat sampling should be used when biomass of periphyton will be assessed.

1. Define the sampling reach. The area sampled for single habitat sampling can be smaller than the area used for multihabitat sampling. Valuable results have been achieved in past projects by sampling just one riffle or pool.

2. Before sampling, complete the physical/chemical field sheet (see Chapter 5; Appendix A-1, Form 1) and the periphyton field data sheet

CHLOROPHYLL *a* SUBSAMPLING (OPTIONAL)

1. Chlorophyll *a* subsamples should be taken as soon as possible (< 12 hours after sampling). Generally, if chlorophyll subsamples can not be taken in the lab on the day of collection, subsample in the field.

2. Homogenize samples. In the field, shake vigorously. In the lab, use a tissue homogenizer.

3. Record the initial volume of sample on the periphyton sample log form.

4. Stir the sample on a magnetic stirrer and subsample. When subsampling, take at least two aliquots from the sample for each chlorophyll sample (two aliquots provides a more representative subsample than one). Record the subsample volume for chlorophyll *a* on the periphyton sample log form.

5. Concentrate the chlorophyll subsample on a glass fiber filter (e.g., Whatman® GFC or equivalent).

6. Fold the filter and wrap with aluminum to exclude light.

7. Store the filter in a cold cooler (not in water) and eventually in a freezer.

(Appendix A-2, Form 1). Complete habitat assessments as in multihabitat sampling so that the relative importance of the habitats sampled can be characterized.

3. The recommended substrate/habitat combination is cobble obtained from riffles and runs with current velocities of 10-50 cm/sec. Samples from this habitat are often easier to analyze than from slow current habitats because they contain less silt. These habitats are common in many streams. In low gradient streams where riffles are rare, algae on snags or in depositional habitats can be collected. Shifting sand is not recommended as a targeted substrate because the species composition on sand is limited due to the small size and unstable nature of the substratum. Phytoplankton should be considered as an alternative to periphyton in large, low gradient streams.

4. Collect several subsamples from the same substrate/habitat combination and composite them into a single container. Three or more subsamples should be collected from each reach or study stream.

5. The area sampled should always be determined if biomass (e.g., chlorophyll) per unit area is to be measured.

6. If you plan to assay samples for chlorophyll *a*, do not preserve samples until they have been subsampled (see textbox entitled "Chlorophyll *a* Subsampling").

7. Store, transport, process, and log in samples as in steps 4-9 in section 6.1.1.1.

6.1.2 Field Sampling Procedures: Artificial Substrates

Most monitoring groups prefer sampling natural substrates whenever possible to reduce field time and improve ecological applicability of information. However, periphyton can also be sampled by collecting from artificial substrates that are placed in aquatic habitats and colonized over a period of time. This procedure is particularly useful in non-wadeable streams, rivers with no riffle areas, wetlands, or the littoral zones of lentic habitats. Both natural and artificial substrates are useful in monitoring and assessing waterbody conditions, and have corresponding advantages and disadvantages (Stevenson and Lowe 1986, Aloi 1990). The methods summarized here are a composite of those specified by Kentucky (Kentucky DEP 1993), Florida (Florida DEP 1996), and Oklahoma (Oklahoma CC 1993).
Although glass microslides are preferred, a variety of artificial substrates have been used with success (see #2 below and textbox on p 6-6).

QUALITY CONTROL (QC) IN THE FIELD

1. Sample labels must be accurately and thoroughly completed, including the sample identification code, date, stream name, sampling location, and collector's name. The outside and any inside labels of the container should contain the same information. Chain of custody and sample log forms must include the same information as the sample container labels. **Caution!** Lugol's solution and iodine-based preservatives will turn paper labels black.

2. After sampling has been completed at a given site, all brushes, suction and scraping devices that have come in contact with the sample should be rubbed clean and rinsed thoroughly in distilled water. The equipment should be examined again prior to use at the next sampling site, and rinsed again if necessary.

3. After sampling, review the recorded information on all labels and forms for accuracy and completeness.

4. Collect and analyze one replicate sample from 10% of the sites to evaluate precision or repeatability of sampling technique, collection team, sample analysis, and taxonomy.

1. Microslides should be thoroughly cleaned before placing in periphytometers (e.g., Patrick et al. 1954). Rinse slides in acetone and clean with Kimwipes®.

2. Place surface (floating) or benthic (bottom) periphytometers fitted with glass slides, glass rods, clay tiles, plexiglass plates or similar substrates in the study area. Allow 2 to 4 weeks for periphyton recruitment and colonization.
3. Replicate a minimum of 3 periphytometers at each site to account for spatial variability. The total number should depend upon the study design and hypotheses tested. Samples can either be composited or analyzed individually.

4. Attach periphytometers to rebars pounded into the stream bottom or to other stable structures. Periphytometers should be hidden from view to minimize disturbance or vandalism. Avoid the main channel of floatable, recreational streams. Each periphytometer should be oriented with the shield directed upstream.

5. If flooding or a similar scouring event occurs during incubation, allow waterbody to equilibrate and reset periphytometers with clean slides.

6. After the incubation period (2-4 weeks), collect substrates. Remove algae using rubber spatulas, toothbrushes and razor blades. You can tell when all algae have been removed from substrates by a change from smooth, mucilaginous feel (even when no visible algae are present) to a non-slimy or rough texture.

7. Store, transport, process, and log in samples as in steps 4-9 in section 6.1.1.1.

8. One advantage of using artificial substrates is that containers (e.g., whirl-pack bags or sample jars) can be purchased that will hold the substrates so that substrates need not be scraped in the field. Different substrates can be designated for microscopic analysis and chlorophyll assay. Then algae and substrates can be placed in sampling containers and preserved for later processing and microscopic analysis or placed in a cooler on ice for later chlorophyll *a* analysis. Laboratory sample processing is preferred; so if travel and holding time are less than 12 hours, it is not necessary to split samples before returning to the lab.

FIELD EQUIPMENT/SUPPLIES NEEDED FOR PERIPHYTON SAMPLING-- ARTIFICIAL SUBSTRATES

- periphytometer (frame to hold artificial substrata)
- microslides or other suitable substratum (e.g., clay tiles, sanded Plexiglass® plates, or wooden or acrylic dowels)
- sledge hammer and rebars
- toothbrush, razor blade, or other scraping tools
- water bottle with distilled water
- white plastic or enamel pan
- aluminium foil
- sample containers
- sample container labels
- field notebook (waterproof)
- preservative [Lugol's solution, 4% buffered formalin, "M[3]" fixative, or 2% glutaraldehyde (APHA 1995)]
- cooler with ice

6.1.3 Assessing Relative Abundances of Algal Taxa: Both "Soft" (Non-Diatom) Algae and Diatoms

The Methods summarized here are a modified version of those used by Kentucky (Kentucky DEP 1993), Florida (Florida DEP 1996), and Montana (Bahls 1993). For more detail or for alternative methods, see Standard Methods for the Examination of Water and Wastewater (APHA 1995).

Many algae are readily identifiable to species level by trained personnel who have a good library of

literature on algal taxonomy (see section 6.3). All algae can not be identified to species because: the growth forms of some algal species are morphologically indistinguishable with the light microscope (e.g., zoospores of many green algae); the species has not been described previously; or the species is not in the laboratory's literature. Consistency in identifications within a laboratory and program is very important, because most bioassessment are based on contrasts between reference and test sites. Accuracy of identifications becomes most important when using autecological information from other studies. Quality assurance techniques are designed to ensure "internal consistency" and also improve comparisons with information in other algal assessment and monitoring programs.

6.1.3.1 "Soft" (Non-Diatom) Algae Relative Abundance and Taxa Richness

1. Homogenize algal samples with a tissue homogenizer or blender.

2. Thoroughly mix the homogenized sample and pipette into a Palmer counting cell (see textbox for alternative methods). Algal suspensions that produce between 10 and 20 cells in a field provide good densities for counting and identifying cells. Lower densities slow counting. Dilute samples if cells overlap too much for counting.

3. Fill in the top portion of the benchsheet for "soft" algae (Appendix A-2, Form 3) with enough information from the sample label and other sources to uniquely identify the sample.

4. Identify and count 300 algal "cell units" to the lowest possible taxonomic level at 400X magnification with the use of the references in Section 6.3.

   - **!** Distinguishing cells of coenocytic algae (e.g., *Vaucheria*) and small filaments of blue-green algae is a problem in cell counts. "Cell units" can be defined for these algae as 10mm sections of the thallus or filament.
   - **!** For diatoms, only count live diatoms and do not identify to lower taxonomic levels if a subsequent count of cleaned diatoms is to be undertaken (See section 6.1.3.2).
   - **!** Record numbers of cells or cell units observed for each taxon on a benchsheet.
   - **!** Make taxonomic notes and drawings on benchsheets of important specimens.

5. Optional - To better determine non-diatom taxa richness, continue counting until you have not observed any new taxa for 100 cell units or about three minutes of observation.

6.1.3.2 Diatom Relative Abundances and Taxa Richness

1. Subsample at least 5-10 mL of concentrated preserved sample while vigorously shaking the sample (or using magnetic stirrer). Oxidize (clean) samples for diatom analysis (APHA 1995, see textbox entitled "Oxidation Methods for Cleaning Diatoms").

2. Mount diatoms in Naphrax® or another high refractive index medium to make permanent slides. Label slides with same information as on the sample container label.

3. Fill in the top portion of the bench sheet for diatom counts (Appendix A-2, Form 4) with enough information from the sample label to uniquely identify the sample.

4. Identify and count diatom valves to the lowest possible taxonomic level, which should be species and perhaps variety level, under oil immersion at 1000X magnification with the use of the

references in Section 6.3. At minimum, count 600 valves (300 cells) and at least until 10 valves of 10 species have been observed. Be careful to distinguish and count both valves of intact frustules. The 10 valves of 10 species rule ensures relatively precise estimates of relative abundances of the dominant taxa when one or two taxa are highly dominant. Six hundred valve counts were chosen to conform with methods used in other national bioassessment programs (Porter et al. 1993). Record numbers of valves observed for each taxon on the bench sheet. Make taxonomic notes and drawings on benchsheets and record stage coordinates of important specimens.

5. Optional - To estimate total diatom taxa richness, continue counting until you have not observed any new species for 100 specimens or about three minutes of observation.

6.1.3.3 Calculating Species Relative Abundances and Taxa Richness

1. Relative abundances of "soft" algae are determined by dividing the number of cells (cell units) counted for each taxon by the total number of cells counted (e.g., 300). Enter this information on Appendix A-2, Form 3.

2. Relative abundances of diatoms have to be corrected for the number of live diatoms observed in the count of all algae. Therefore, determine the relative abundances of diatom species in the algal assemblage by dividing the number of valves counted for each species by the total number of valves counted (e.g., 600); then multiply the relative abundance of each diatom taxon in the diatom count by the relative abundance of live diatoms in the count of all algae. Enter this information on Appendix A-2, Form 4. Some analysts prefer to treat diatom and soft algal species composition separately. In this case, determine the relative abundances of diatom species in the algal assemblage by dividing the number of valves counted for each species by the total number of valves counted (e.g., 600).

3. Total taxa richness can be estimated by adding the number of "soft" algal taxa and diatom taxa.

6.1.3.4 Alternative Preparation Techniques

Palmer counting cells are excellent for identifying and counting soft-algae in most species assemblages. When samples have many very small blue-green algae or a few, relatively important large cells, other slide preparation techniques may be useful to increase magnification and sample size, respectively. Because accurate diatom identification is not possible in Palmer cells, we have recommended counting cleaned diatoms in special mounts. However, if the taxonomy of algae in samples is well known, preparation and counting time can be reduced by mounting algae in syrup. In syrup, both soft algae and diatoms can be identified, but resolution of morphological details of diatoms is not as great as in mounts of diatoms in resins (e.g., Naphrax®).

Assemblages with many small cells: We recommend a simple wet mount procedure when samples contain many small algae so samples can be observed at 1000X. A small volume of water under the coverglass prevents movement of cells when adjusting focus and using oil immersion. These preparations usually last several days if properly sealed (see below).

Wet mounts:

1. Clean coverglasses and place on flat surface.

2. Pipette 1.0 mL of algal suspension onto the coverglass.

3. Dry the algal suspension on the coverglass. For convenience, the evaporation of water can be increased on a slide-warmer or slowed by drying the sample in a vapor chamber (as simple as a cake pan or aluminum foil hood placed over samples).

4. As soon as the algal suspension dries, invert the coverglass into the 0.02 mL of distilled water on a microscope slide.

5. Seal the water under the microscope slide with fingernail polish or polyurethane varnish.

Assemblages with a few large cells: Sedgewick-Rafter counting chambers, which are large modified microscope slides with 1.0 mL wells, increase sample size. Counts in Sedgewick-Rafter counting cells should be done after counts in Palmer cells or wet mounts so that the relation between sample proportions with the two methods can be determined. While keeping track of the proportion of sample observed, identify and count large algae in transects at 200X or 100X magnification in the counting cell.

Syrup mounts:

1. Prepare Taft's syrup medium (TSM) by mixing 30 mL of clear corn syrup (e.g., Karo's® Corn Syrup) with 7 mL of formaldehyde and 63 mL of distilled water. Dilute a 10 mL proportion of this 100% TSM with 90 mL of distilled water to make 10% TSM.

2. Place 0.2 mL of 10% TSM on coverglass.

OXIDATION (CLEANING) METHODS FOR DIATOMS

Concentrated Acid Oxidation:

1. Place a 5-10 mL subsample of preserved algal sample in a beaker.

2. Under a fume hood, add enough concentrated nitric or sulfuric acid to produce a strong exothermic reaction. Usually equal parts of sample and acid will produce such a reaction.
   (Caution! With some preservatives and samples from hard water, adding concentrated acid will produce a violent exothermic reaction. Use a fume hood, safety glasses, and protective clothing. Separate the sample beakers by a few inches to prevent cross-contamination of samples in the event of overflow.)

3. Allow the sample to oxidize overnight.

4. Fill the beaker with distilled water.

5. Wait 1 hour for each centimeter of water depth in the beaker.

6. Siphon off the supernatant and refill the beaker with distilled water. Siphon from the center of the water column to avoid siphoning light algae that have adsorbed onto the sides and surface of the water column.

7. Repeat steps 4 through 6 until all color is removed and the sample becomes clear or has a circumneutral pH.

Hydrogen Peroxide/Potassium Dichromate Oxidation:

1. Prepare samples as in step 1 above, but use 50% H_2O_2 instead of concentrated acid.

2. Allow the sample to oxidize overnight, then add a microspatula of potassium dichromate.
   (Caution! This will cause a violent exothermic reaction. Use a fume hood, safety glasses, and protective clothing. Separate the sample beakers by a few inches to prevent cross-contamination in the event of overflow.)

3. When the sample color changes from purple to yellow and boiling stops, fill the beaker with distilled water.

4. Wait 4 hours, siphon off the supernatant, and refill the beaker with distilled water. Siphon from the center of

3. Place 1.0 mL of algal suspension on coverglass. Consider using several dilutions.

4. Let dry for 24 hours. Alternatively, dry on slide warmer on low setting. Do not overdry or cells will plasmolyze.
5. Place another ≈ 1.0 mL of 10% TSM on cover glass and dry (overnight or 4 hours on a slide warmer). Apply 10% TSM quickly to avoid patchy resuspension of the original layer of TSM and algae.

6. Invert coverglass onto microscope slide; place slide on hot plate to warm the slide and syrup. Do not boil, just warm. Press coverglass gently in place with forceps, being careful to keep all syrup under the coverglass. The syrup should spread under coverglass.

7. Remove the slide from the hotplate. Cooling should partially seal the coverglass to the slide.

8. More permanently seal the syrup under slides by painting fingernail polish around the edge of the cover glass and onto the microscope slide.

Note: Preserve color of chloroplasts by keeping samples in dark.

Special Note: If slides get too warm in storage, syrup will loose viscosity and become runny. Algae and medium may then escape containment under coverglass. Store slides in a horizontal position.

6.1.4 Metrics Based on Species Composition

The periphyton metrics presented here are used by several states and environmental assessment programs throughout the US and Europe (e.g., Kentucky DEP 1993, Bahls 1993, Florida DEP 1996, Whitton et al. 1991, Whitton and Kelly 1995). Each of these metrics should be tested for response to human alterations of streams in the region in which they are used (see Chapter 9, Biological Data Analysis). In many cases, diatom and soft algal metrics have been determined separately because changes in small abundant cyanobacteria (blue-green algae) can numerically overwhelm metrics based on relative abundance and because green algae with large cells (e.g., *Cladophora*) may not have appropriate weight. However, attempts should be made to integrate diatoms and soft algae in as many metrics as possible, especially in cases such as species and generic richness when great variability in relative abundance is not an issue.

Many metrics can be calculated based on presence/absence data or on relative abundances of taxa. For example, percent Pollution Tolerant Diatoms can be calculated as the sum of relative abundances of pollution tolerant taxa in an assemblage or as the number of species that are tolerant to pollution in an assemblage. Percent community similarity can be calculated as presented below, which quantifies the percent of organisms in two assemblages that are the same. Alternatively, it can be calculated as the percent of species that are the same by making all relative abundances greater than 0 equal to 1. The following metrics can also be calculated with presence/absence data instead of species relative abundances: % sensitive taxa, % motile taxa, % acidobiontic, % alkalibiontic, % halobiontic, % saprobiontic, % eutrophic, simple autecological indices, and change in inferred ecological conditions. Although we may find that metrics based on species relative abundances are more sensitive to environmental change, metrics based on presence/absence data may be more appropriate when developing metrics with multihabitat samples and proportional sampling of habitats is difficult. In the latter case, presence/absence of species should remain the same, even if relative abundance of taxa differs with biases in multihabitat sampling.

COSTS AND BENEFITS OF SIMPLER ANALYSES

- We recommend that all algae (soft and diatom) be identified and counted. Information may be lost if soft algae are not identified and counted because some impacts may selectively affect soft algae. Most of the species (and thus information) in a sample will be diatoms. Costs of both analyses are not that great.
- Costs can be reduced by only counting diatoms or soft algae. Since diatoms are usually the most species-rich group of algae in samples and most metrics are based on differences in taxonomic composition, we recommend that diatoms be counted. In addition, permanently preserved and readily archived microslides of diatoms can serve as a historic reference of ecological conditions.
- In general, identifying algae to species is recommended for two reasons: (1) to better characterize differences between assemblages that may occur at the species level and (2) because large differences in ecological preferences do exist among algal species within the same genus.
- However, substantial information can be gained by identifying algae just to the genus level. Whereas identifying algae only to genus may loose valuable ecological information, costs of analyses can be reduced, especially for inexperienced analysts.
- If implementing a new program and only an inexperienced analyst is available for the job, identifying diatom genera in assemblages can provide valuable characterizations of biotic integrity and environmental conditions.
- As analysts get more experience counting, the taxonomic level of their analyses should improve. The cost of an experienced analyst counting and identifying algae to species is not much greater than analysis to genus.

The metrics have been divided into two groups which may be helpful in developing an Index of Biotic Integrity (IBI). Metrics in the first group are less diagnostic than the second group of metrics. Metrics in the first group (species and generic richness, Shannon diversity, etc.) generally characterize biotic integrity ("natural balance in flora and fauna…." as in Karr and Dudley 1981) without specifically diagnosing ecological conditions and causes of impairment. The second group of metrics more specifically diagnoses causes of impaired biotic integrity. Metrics from both groups could be included in an IBI to make a hierarchically diagnostic IBI. Alternatively, an IBI could be constructed from only metrics of biotic integrity so that inference of biotic integrity and diagnosis of impairment are independent (Stevenson and Pan 1999).

Autecological information about many algal species and genera has been reported in the literature. This information comes in several forms. In some cases, qualitative descriptions of the ecological conditions in which species were observed were reported in early studies of diatoms. Following the development of the saprobic index by Kolkwitz and Marsson (1908), several categorical classification systems (e.g., halobian spectrum, pH spectrum) were developed to describe the ecological preferences and tolerances of species (see Lowe 1974 for a review). Most recently, the ecological optima and tolerances of species for specific environmental conditions have been quantified by using weighted average regression approaches (see ter Braak and van Dam 1989 for a review). We have compiled a list of references for this information in Section 6.4. These references will be valuable for developing many of the metrics below.

Metrics of Biotic Integrity

1. **Species richness** is an estimate of the number of algal species (diatoms, soft algae, or both) in a sample. High species richness is assumed to indicate high biotic integrity because many species are adapted to the conditions present in the habitat. Species richness is predicted to decrease with increasing pollution because many species are stressed. However, many habitats may be naturally stressed by low nutrients, low light, or other factors. Slight increases in nutrient enrichment can increase species richness in headwater and naturally unproductive, nutrient-poor streams (Bahls et al. 1992).

2. **Total Number of Genera** (Generic richness) should be highest in reference sites and lowest in impacted sites where sensitive genera become stressed. Total number of genera (diatoms, soft algae, or both) may provide a more robust measure of diversity than species richness, because numerous closely related species are within some genera and may artificially inflate richness estimates.

3. **Total Number of Divisions** represented by all taxa should be highest in sites with good water quality and high biotic integrity.

4. **Shannon Diversity (for diatoms)**. The Shannon Index is a function of both the number of species in a sample and the distribution of individuals among those species (Klemm et al. 1990). Because species richness and evenness may vary independently and complexly with water pollution. Stevenson (1984) suggests that changes in species diversity, rather than the diversity value, may be useful indicators of changes in water quality. Species diversity, despite the controversy surrounding it, has historically been used with success as an indicator of organic (sewage) pollution (Wilhm and Dorris 1968, Weber 1973, Cooper and Wilhm 1975). Bahls et al. (1992) uses Shannon diversity because of its sensitivity to water quality changes. Under certain conditions Shannon diversity values may underestimate water quality e.g., when total number of taxa is less than 10. Assessments for low richness samples can be improved by comparing the assemblage Shannon Diversity to the Maximum Shannon Diversity value (David Beeson[1], personal communication).

5. **Percent Community Similarity (PS_c) of Diatoms**. The percent community similarity (PS_c) index, discussed by Whittaker (1952), was used by Whittaker and Fairbanks (1958) to compare planktonic copepod communities. It was chosen for use in algal bioassessment because it shows community similarities based on relative abundances, and in doing so, gives

[1]David Beeson is a phycologist with Schafer & Associates, Inc.

more weight to dominant taxa than rare ones. Percent similarity can be used to compare control and test sites, or average community of a group of control or reference sites with a test site. Percent community similarity values range from 0 (no similarity) to 100%.

The formula for calculating percent community similarity is:

$$PS_c = 100 - .5\Sigma_{i=1}^{s}|a_i - b_i| = \Sigma_{i=1}^{s}\min(a_i, b_i)$$

where:

a_i = percentage of species i in sample A
b_i = percentage of species i in sample B

6. **Pollution Tolerance Index for Diatoms.** The pollution tolerance index (PTI) for algae resembles the Hilsenhoff biotic index for macroinvertebrates (Hilsenhoff 1987). Lange-Bertalot (1979) distinguishes three categories of diatoms according to their tolerance to increased pollution, with species assigned a value of 1 for most tolerant taxa (e.g., *Nitzschia palea* or *Gomphonema parvulum*) to 3 for relatively sensitive species. Relative tolerance for taxa can be found in Lange-Bertalot (1979) and in many of the references listed in section 6.4. Thus, Lange-Bertalot's PTI varies from 1 for most polluted to 3 for least polluted waters when using the following equation:

$$PTI = \frac{\Sigma n_i t_i}{N}$$

where:

n_i = number of cells counted for species i
t_i = tolerance value of species i
N = total number of cells counted

In some cases, the range of values for tolerances has been increased, thereby producing a corresponding increase in the range of PTI values.

7. **Percent Sensitive Diatoms.** The percent sensitive diatoms metric is the sum of the relative abundances of all intolerant species. This metric is especially important in smaller-order streams where primary productivity may be naturally low, causing many other metrics to underestimate water quality.

8. **Percent *Achnanthes minutissima*.** This species is a cosmopolitan diatom that has a very broad ecological amplitude. It is an attached diatom and often the first species to pioneer a recently scoured site, sometimes to the exclusion of all other algae. *A. minutissima* is also frequently dominant in streams subjected to acid mine drainage (e.g., Silver Bow Creek, Montana) and to other chemical insults. The percent abundance of *A. minutissima* has been found to be directly proportional to the time that has elapsed since the last scouring flow or episode of toxic pollution. For use in bioassessment, the quartiles of this metric from a

population of sites has been used to establish judgment criteria, e.g., 0-25% = no disturbance, 25-50% = minor disturbance, 50-75% = moderate disturbance, and 75-100% = severe disturbance. Least-impaired streams in Montana may contain up to 50% *A. minutissima* (Bahls, unpublished data).

9. **Percent live diatoms** was proposed by Hill (1997) as a metric to indicate the health of the diatom assemblage. Low percent live diatoms could be due to heavy sedimentation and/or relatively old algal assemblages with high algal biomass on substrates.

Diagnostic Metrics that Infer Ecological Conditions

The ecological preferences of many diatoms and other algae have been recorded in the literature. Using relative abundances of algal species in the sample and their preferences for specific habitat conditions, metrics can be calculated to indicate the environment stressors in a habitat. These metrics can more specifically infer environmental stressors than the general pollution tolerance index.

10. **Percent Aberrant Diatoms** is the percent of diatoms in a sample that have anomalies in striae patterns or frustule shape (e.g, long cells that are bent or cells with indentations). This metric has been positively correlated to heavy metal contamination in streams (McFarland et al. 1997).

11. **Percent Motile Diatoms.** The percent motile diatoms is a siltation index, expressed as the relative abundance of *Navicula* + *Nitzschia* + *Surirella*. It has shown promise in Montana (Bahls et al. 1992). The three genera are able to crawl towards the surface if they are covered by silt; their abundance is thought to reflect the amount and frequency of siltation. Relative abundances of Gyrosigma, Cylindrotheca, and other motile diatoms may also be added to this metric.

12. **Simple Diagnostic Metrics** can infer the environmental stressor based on the autecology of individual species in the habitats. For example, if acid mine drainage was impairing stream conditions, then we would expect to find more acidobiontic taxa in samples. Calculate a simple diagnostic metric as the sum of the percent relative abundances (range 0-100%) of species that have environmental optima in extreme environmental conditions. For example (see Table 6-2):

% acidobiontic + % acidophilic
% alkalibiontic + % alkaliphilic
% halophilic
% mesosaprobic + % oligosaprobic + % saprophilic
% eutrophic

13. **Inferred Ecological Conditions with Simple Autecological Indices (SAI)** - The ecological preferences for diatoms are commonly recorded in the literature. Using the standard ecological categories compiled by Lowe (1974, Table 6-2), the ecological preferences for different diatom species can be characterized along an environmental (stressor) gradient. For example, pH preferences for many taxa are known. These preferences (Θ_i) can be ranked from 1-5 (e.g., acidobiontic, acidophilic, indifferent, alkaliphilic, alkalibiontic, Table 6-2) and can be used in the following equation to infer environmental conditions (EC) and effect on the periphyton assemblage.

$$SAI_{EC} = \Sigma \Theta_i p_i$$

14. **Inferred Ecological Conditions with Weighted Average Indices** are based on the specific ecological optima (β_i) for algae, which are being reported more and more commonly in recent publications (see Pan and Stevenson 1996). Caution should be exercised, because we do not know how transferable these optima are among regions and habitats. Using the following equation, the ecological conditions (EC) in a habitat can be inferred more accurately by using the optimum environmental conditions (β_i) and relative abundances (ρ_i) for taxa in the habitat (ter Braak and van Dam 1989, Pan et al., 1996) than if only the ecological categorization were used (as above for the SAI). Optimum environmental conditions are those in which the highest relative abundances of a taxon are observed. These can be determined from the literature or from past surveys of taxa and environmental conditions in the study area (see ter Braak and van Dam 1989). In a pH example, the specific pH in a habitat can be inferred if we know the pH optima (H_i) of taxa in the habitat, and use the following general equation:

$$WAI_{EC} = \Sigma \beta_i p_i$$

and modify for inferring pH:

$$WAI_{pH} = \Sigma H_i p_i$$

15. **Impairment of Ecological Conditions** can be inferred with algal assemblages by calculating the deviation (Δ_{EC}) between inferred environmental conditions at a test site and at a reference site.

Compare inferred ecological conditions at the test site to the expected ecological conditions (EC_{ex}) of regional reference sites by using either simple autecological indices (SAI_{EC}) or weighted average indices (WAI_{EC}):

$$\Delta_{EC} = |SAI_{EC} - EC_{ex}|$$

$$\Delta_{EC} = |WAI_{EC} - EC_{ex}|$$

Table 6-2. Environmental definitions of autecological classification systems for algae (as modified or referenced by Lowe 1974). Definitions for classes are given if no subclass is indicated.

Classification System/ Ecological Parameter	Class	Subclass	Conditions of Highest Relative Abundances
pH Spectrum	Acidobiontic		Below 5.5 pH
	Acidophilic		Above 5.5 and below 7 pH
	Indifferent		Around 7 pH
	Alakaliphilic		Above 7 and below 8.5 pH
	Alkalibiontic		Above 8.5 pH
Nutrient Spectrum - based on P and N concentrations	Eutrophic		High nutrient conditions

Classification System/ Ecological Parameter	Class	Subclass	Conditions of Highest Relative Abundances
	Mesotrophic		Moderate nutrient conditions
	Oligotrophic		Low nutrient conditions
	Dystrophic		High humic (DOC) conditions
Halobion Spectrum - based on chloride concentrations or conductivity	Polyhalobous		Salt concentrations > 40,000 mg/L
	Euhalobous		Marine forms: 30,000-40,000 mg/L
	Mesohalobous	Alpha range	Brackish water forms: 10,000-30,000 mg/L
	Mesohalobous	Beta range	Brackish water forms: 500-10,000 mg/L
	Oligohalobous	Halophilous	Freshwater - stimulated by some salt
	Oligohalobous	Indifferent	Freshwater - tolerates some salt
	Oligohalobous	Halophobic	Freshwater - does not tolerate small amounts of salt
Saprobien System - based on organic pollution	Polysaprobic		Characteristic of zone of degradation and putrefication, oxygen usually absent or low in concentration
	Mesosaprobic	Alpha range	Zone of organic load oxidation — N as amino acids
		Beta range	Zone of organic load oxidation — N as ammonia
	Oligosaprobic		Zone in which oxidation of organics complete, but high nutrient concentrations persist
	Saprophilic		Usually in polluted waters, but also in clean waters
	Saproxenous		Usually in clean waters, but also found in polluted waters
	Saprophobic		Only found in unpolluted waters

6.1.5 Determining Periphyton Biomass

Measurement of periphyton biomass is common in many studies and may be especially important in studies that address nutrient enrichment or toxicity. In many cases, however, sampling benthic algae misses peak biomass, which may best indicate nutrient problems and potential for nuisance algal growths (Biggs 1996, Stevenson 1996).

Biomass measurements can be made with samples collected from natural or artificial substrates. To quantify algal biomass (chl *a*, ash-free dry mass, cell density, biovolume cm^{-2}), the area of the substrate sampled must be determined. Two national stream assessment programs sample and assess area-specific cell density and biovolume (USGS-NAWQA, Porter et al. 1993; and EMAP, Klemm and Lazorchak 1994). These programs estimate algal biomass in habitats and reaches by collecting composite samples separately from riffle and pool habitats.

Periphyton biomass can be estimated with chl *a*, ash-free dry mass (AFDM), cell densities, and biovolume, usually per cm^2 (Stevenson 1996). Each of these measures estimates a different component of periphyton biomass (see Stevenson 1996 for discussion).

LABORATORY EQUIPMENT FOR PERIPHYTON ANALYSIS

- compound microscope with 10X or 15X oculars and 20X, 40X and 100X (oil) objectives
- tally counter (for species proportional count)
- microscope slides and coverglasses
- immersion oil, lens paper and absorbent tissues
- tissue homogenizer or blender
- magnetic stirrer and stir bar
- forceps
- hot plate
- fume hood
- squeeze bottle with distilled water
- oxidation reagents (HNO_3, H_2SO_4, $K_2Cr_2O_7$, H_2O_2)
- 200-500 ml beakers
- safety glasses and protective clothing
- drying oven for AFDM
- muffle furnace for AFDM
- aluminum weighing pans for AFDM
- spectrophotometer or fluorometer for chl *a*
- centrifuge for chl *a*
- graduated test tubes for chl *a*
- acetone for chl *a*
- $MgCO_3$ for chl *a*

6.1.5.1 Chlorophyll *a*

Chlorophyll *a* ranges from 0.5 to 2% of total algal biomass (APHA 1995), and this ratio varies with taxonomy, light, and nutrients. A detailed description of chlorophyll *a* analysis is beyond the scope of this chapter. Standard methods (APHA 1995, USEPA 1992) are readily available. The analysis is relatively simple and involves:

1. extracting chlorophyll *a* in acetone;

2. measuring chlorophyll concentration in the extract with a spectrophotometer or fluorometer; and

3. calculating chlorophyll density on substrates by determining the proportion of original sample that was assessed for chlorophyll.

6.1.5.2 Ash-Free Dry Mass

Ash-free dry mass is a measurement of the organic matter in samples, and includes biomass of bacteria, fungi, small fauna, and detritus in samples. A detailed description of analysis is beyond the scope of this chapter, but standard methods (APHA 1995, USEPA 1995) are readily available. The analysis is relatively simple and measures the difference in mass of a sample after drying and after incinerating organic matter in the sample. We recommend using AFDM versus dry mass to measure periphyton biomass because silt can account for a substantial proportion of dry mass in some samples. Ash mass in samples can be used to infer the amount of silt or other inorganic matter in samples.

6.1.5.3 Area-Specific Cell Densities and Biovolumes

Cell densities (cells cm^{-2}) are determined by dividing the numbers of cells counted by the proportion of sample counted and the area from which samples were collected. Cell biovolumes (mm^3 biovolume cm^{-2}) are determined by summing the products of cell density and biovolume of each species counted (see Lowe and Pan 1996) and dividing that sum by the proportion of sample counted and the area from which samples were collected.

6.1.5.4 Biomass Metrics

High algal biomass can indicate eutrophication, but high algal biomass can also accumulate in less productive habitats after long periods of stable flow. Low algal biomass may be due to toxic conditions, but could be due to a recent storm event and spate or naturally heavy grazing. Thus, interpretation of biomass results is ambiguous and is the reason that major emphasis has not been placed on quantifying algal biomass for RBP. However, nuisance levels of algal biomass (e.g., > 10 µg chl *a* cm^{-2}, > 5 mg AFDM cm^{-2}, > 40% cover by macroalgae; see review by Biggs 1996) do indicate nutrient or organic enrichment. If repeated measurements of biomass can be made, then the mean and maximum benthic chl *a* could be used to define trophic status of streams. Dodds et al. (1998) have proposed guidelines in which the oligotrophic-mesotrophic boundary is a mean benthic chl *a* of 2 µg cm^{-2} or a maximum benthic chl *a* of 7 µg cm^{-2} and the mesotrophic-eutrophic boundary is a mean of 6 µg chl *a* cm^{-2} and a maximum of 20 µg chl *a* cm^{-2}.

QUALITY CONTROL IN THE LABORATORY

1. Upon delivery of samples to the laboratory, complete entries on periphyton sample log-in forms (Appendix 2, Form 2).

2. Maintain a voucher collection of all samples and diatom slides. They should be accurately and completely labeled, preserved, and stored in the laboratory for future reference. Specimens on diatom slides should be clearly circled with a diamond or ink marker to facilitate location. A record of the voucher specimens should be maintained. Photographs of specimens improve "in-house" QA.

3. For every QA/QC sample (replicate sample in every 10th stream), assess relative abundances and taxa richness in replicate wet mounts and a replicate diatom slide to assess variation in metrics due to variability in sampling within reaches (habitats), sample preparation, and analytical variability.

4. QA/QC samples should be counted by another taxonomist to assess taxonomic precision and bias, if possible.

5. Common algal taxa should be the same for the two wet mount replicates. The percent community similarity index (Whittaker 1952) (see Section 6.5.1) calculated from proportional counts of the two replicate diatom slides should exceed 75%.

6. If it is not possible to get another taxonomist in the lab to QA/QC samples, an outside taxonomist should be consulted on a periodic basis to spot-check and verify taxonomic identifications in wet mounts and diatom slides. All common genera in the wet mount and all major species on the diatom slide (>3% relative abundance) should be identified similarly by both analysts (synonyms are acceptable). Any differences in identification should be reconciled and bench sheets should be corrected.

7. A library of basic taxonomic literature is an essential aid in the identification of algae and should be maintained and updated as needed in the laboratory (see taxonomic references for periphyton in Section 6.5). Taxonomists should participate in periodic training to ensure accurate identifications

6.2 FIELD-BASED RAPID PERIPHYTON SURVEY

Semi-quantitative assessments of benthic algal biomass and taxonomic composition can be made rapidly with a viewing bucket marked with a grid and a biomass scoring system. The advantage of using this technique is that it enables rapid assessment of algal biomass over larger spatial scales than substrate sampling and laboratory analysis. Coarse-level taxonomic characterization of communities is also possible with this technique. This technique is a survey of the

natural substrate and requires no laboratory proc essing, but hand picked samples can be returned to the laboratory to quickly verify identification. It is a technique developed by Stevenson and Rier[2].

1. Fill in top of Rapid Periphyton Survey (RPS) Field Sheet, Appendix A-2, Form 5.

2. Establish at least 3 transects across the habitat being sampled (preferably riffles or runs in the reach in which benthic algal accumulation is readily observed and characterized).

3. Select 3 locations along each transect (e.g., stratified random locations on right, middle, and left bank).

4. Characterize algae in each selected location by immersing the bucket with 50-dot grid (7 x 7 + 1) in the water.
   - **!** First, characterize macroalgal biomass.
     - Observe the bottom of the stream through the bottom of the viewing bucket and count the number of dots that occur over macroalgae (e.g., Cladophora or Spirogyra) under which substrates cannot be seen. Record that number and the kind of macroalgae under the dots on RPS field sheet.
     - Measure and record the maximum length of the macroalgae.
     - If two or more types of macroalgae are present, count the dots, measure, and record information for each type of macroalgae separately.
   - **!** Second, characterize microalgal cover.
     - While viewing the same area, record the number of dots under which substrata occur that are suitable size for microalgal accumulation (gravel > 2 cm in size).
     - Determine the kind (usually diatoms and blue-green algae) and estimate the thickness (density) of microalgae under each dot using the following thickness scale:
       0 - substrate rough with no visual evidence of microalgae
       0.5 - substrate slimy, but no visual accumulation of microalgae is evident
       1 - a thin layer of microalgae is visually evident
       2 - accumulation of microalgal layer from 0.5-1 mm thick is evident
       3 - accumulation of microalgae layer from 1 mm to 5 mm thick is evident
       4 - accumulation of microalgal layer from 5 mm to 2 cm thick is evident
       5 - accumulation of microalgal layer greater than 2 cm thick is evident
       Mat thickness can be measured with a ruler.
     - Record the number of dots that are over each of the specific thickness ranks separately for diatoms, blue-green algae, or other microalgae.

FIELD EQUIPMENT FOR RAPID PERIPHYTON SURVEY

- viewing bucket with 50-dot grid [Make the viewing bucket by cutting a hole in bottom of large (≥0.5 m diameter) plastic bucket, but leave a small ridge around the edge. Attach a piece of clear acrylic sheet to the bottom of the bucket with small screws and silicon caulk. The latter makes water tight seal so that no water enters the bucket when it is partially submerged. Periphyton can be clearly viewed by looking down through the bucket when it is partially submerged in the stream. Mark 50 dots in a 7 x 7 grid on the top surface of the acrylic sheet with a waterproof black marker. Add another dot outside the 7 x 7 grid to make the 50 dot grid.]
- meter stick
- pencil
- Rapid Periphyton Survey Field Sheet

[2]S.T. Rier is a graduate student at the University of Louisville.

5. Statistically characterize density of algae on substrate by determining:
   - total number of grid points (dots) evaluated at the site (D_t);
   - number of grid points (dots) over macroalgae (D_m)
   - total number of grid points (dots) over suitable substrate for microalgae at the site (d_t);
   - number of grid points over microalga of different thickness ranks for each type of microalga (d_i);
   - average percent cover of the habitat by each type of macroalgae (i.e., $100X\ D_m/D_t$);
   - maximum length of each type of macroalgae;
   - mean density (i.e., thickness rank) of each type of macroalgae on suitable substrate (i.e., $\Sigma d_i r_i/d_t$); maximum density of each type of microalgae on suitable substrate.

6. QA/QC between observers and calibration between algal biomass (chl *a*, AFDM, cell density and biovolume cm^{-2} and taxonomic composition) can be developed by collecting samples that have specific microalgal rankings and assaying the periphyton.

6.3 TAXONOMIC REFERENCES FOR PERIPHYTON

A great wealth of taxonomic literature is available for algae. Below is a subset of that literature. It is a list of taxonomic references that are useful for most of the United States and are either in English, are important because no English treatment of the group is adequate, or are valuable for the good illustrations.

Camburn, K.E., R.L. Lowe, and D.L. Stoneburner. 1978. The haptobenthic diatom flora of Long Branch Creek, South Carolina. *Nova Hedwigia* 30:149-279.

Collins, G.B. and R.G. Kalinsky. 1977. Studies on Ohio diatoms: I. Diatoms of the Scioto River Basin. *Bull. Ohio Biological Survey*. 5(3):1-45.

Cox, E. J. 1996. *Identification of freshwater diatoms from live material*. Chapman & Hall, London.

Czarnecki, D.B. and D.W. Blinn. 1978. *Diatoms of the Colorado River in Grand Canyon National Park and vicinity*. (Diatoms of Southwestern USA II). Bibliotheca Phycologia 38. J. Cramer. 181 pp.

Dawes, C. J. 1974. *Marine Algae of the West Coast of Florida*. University of Miami Press.

Dillard, G.E. 1989a. Freshwater algae of the Southeastern United States. Part 1. Chlorophyceae: Volvocales, Testrasporales, and Chlorococcales. *Bibliotheca*, 81.

Dillard, G.E. 1989b. Freshwater algae of the Southeastern United States. Part 2. Chlorophyceae: Ulotrichales, Microsporales, Cylindrocapsales, Sphaeropleales, Chaetophorales, Cladophorales, Schizogoniales, Siphonales, and Oedogoniales. *Bibliotheca Phycologica*, 83.

Dillard, G.E. 1990. Freshwater algae of the Southeastern United States. Part 3. Chlorophyceae: Zygnematales: Zygenmataceae, Mesotaeniaceae, and Desmidaceae (Section 1). *Bibliotheca Phycologica*, 85.

Dillard, G.E. 1991. Freshwater algae of the Southeastern United States. Part 4. Chlorophyceae: Zygnemateles: Desmidaceae (Section 2). *Bibliotheca Phycologica*, 89.

Drouet, F. 1968. *Revision of the classification of the oscillatoriaceae*. Monograph 15. Academy of Natural Sciences, Philadelphia. Fulton Press, Lancaster, Pennsylvania.

Hohn, M.H. and J. Hellerman. 1963. The taxonomy and structure of diatom populations from three North American rivers using three sampling methods. *Transaction of the American Microscopal Society* 82:250-329.

Hustedt, F. 1927-1966. Die kieselalgen In Rabenhorst's Kryptogamen-flora von Deutschland Osterreich und der Schweiz VII. Leipzig, West Germany.

Hustedt, F. 1930. *Bacillariophyta (Diatomae)*. In Pascher, A. (ed). Die suswasser Flora Mitteleuropas. (The freshwater flora of middle Europe). Gustav Fischer Verlag, Jena, Germany.

Jarrett, G.L. and J.M. King. 1989. The diatom flora (Bacillariphyceae) of Lake Barkley. U.S. Army Corps of Engineers, Nashville Dist. #DACW62-84-C-0085.

Krammer, K. and H. Lange-Bertalot. 1986-1991. Susswasserflora von Mitteleuropa. Band 2. Parts 1-4. Bacillariophyceae. Gustav Fischer Verlag. Stuttgart. New York.

Lange-Bertalot, H. and R. Simonsen. 1978. A taxonomic revision of the Nitzschia lanceolatae Grunow: 2. European and related extra-European freshwater and brackish water taxa. *Bacillaria* 1:11-111.

Lange-Bertalot, H. 1980. New species, combinations and synonyms in the genus Nitzschia. *Bacillaria* 3:41-77.

Patrick, R. and C.W. Reimer. 1966. *The diatoms of the United States, exclusive of Alaska and Hawaii*. Monograph No. 13. Academy of Natural Sciences, Philadelphia, Pennsylvania.

Patrick, R. and C.W. Reimer. 1975. *The Diatoms of the United States*. Vol. 2, Part 1. Monograph No. 13. Academy of Natural Sciences, Philadelphia, Pennsylvania.

Prescott, G.W. 1962. *The algae of the Western Great Lakes area*. Wm. C. Brown Co., Dubuque, Iowa.

Prescott, G.W., H.T. Croasdale, and W.C. Vinyard. 1975. *A Synopsis of North American desmids. Part II. Desmidaceae: Placodermae*. Section 1. Univ. Nebraska Press, Lincoln, Nebraska.

Prescott, G.W., H.T. Croasdale, and W.C. Vinyard. 1977. *A synopsis of North American desmids. Part II. Desmidaceae: Placodermae*. Section 2. Univ. Nebraska Press, Lincoln, Nebraska.

Prescott, G.W., H.T. Croasdale, and W.C. Vinyard. 1981. *A synopsis of North American desmids. Part II. Desmidaceae: Placodermae*. Section 3. Univ. Nebraska Press, Lincoln, Nebraska.

Prescott, G.W. 1978. *How to know the freshwater algae*. 3rd Edition. Wm. C. Brown Co., Dubuque, Iowa.

Simonsen, R. 1987. *Atlas and catalogue of the diatom types of Friedrich Hustedt*. Vol. 1-3. J. Cramer. Berlin, Germany.

Smith, M. 1950. *The Freshwater Algae of the United States*. McGraw-Hill, New York, New York.

Taylor, W. R. 1960. *Marine algae of the eastern tropical and subtropical coasts of the Americas*. University of Michigan Press, Ann Arbor, Michigan.

VanLandingham, S. L. 1982. *Guide to the identification, environmental requirements and pollution tolerance of freshwater blue-green algae (Cyanophyta)*. EPA-600/3-82-073.

Whitford, L.A. and G.J. Schumacher. 1973. *A manual of freshwater algae*. Sparks Press, Raleigh, North Carolina.

Wujek, D.E. and R.F. Rupp. 1980. Diatoms of the Tittabawassee River, Michigan. *Bibliotheca Phycologia* 50:1-100.

6.4 AUTECOLOGICAL REFERENCES FOR PERIPHYTON

Beaver, J. 1981. *Apparent ecological characteristics of some common freshwater diatoms*. Ontario Ministry of the Environment. Rexdale, Ontario, Canada.

Cholnoky, B. J. 1968. Ökologie der Diatomeen in Binnegewässern. Cramer, Lehre.

Fabri, R. and L. Leclercq. 1984. Etude écologique des riviéres du nord du massif Ardennais (Belgique): flore et végétation de diatomeées et physico-chimie des eaux. 1. Station scientifique des Hautes Fagnes, Robertville. 379 pp.

Fjerdingstad, E. 1950. The microflora of the River Molleaa with special reference to the relation of benthic algae to pollution. *Folia Limnologica Scandanavica* 5, 1-123.

Hustedt, F. 1938-39. Systamatische und ökologische Untersuchungen über die Diatomeen-Flora von Java, Bali und Sumatra nach dem Material deter Deutschen Limnologischen Sunda-Expedition. Allgemeiner Teil. I. Ubersicht über das Untersuchungsmaterial und Charakterisktik der Diatomeenflora der einzelnen Gebiete. II. Die Diatomeen flora der untersuchten Gesässertypen. III. Die ökologische Faktoren und ihr Einfluss auf die Diatomeenflora. Archiv für Hydrobiologie, Supplement Band, 15:638-790 (1938); 16:1-155 (1938); 16:274-394 (1939).

Hustedt, F. 1957. Die Diatomeenflora des Flusssystems der Weser im Gebiet der Hansestadt Bremen. Abhandlungen naturwissenschaftlichen. Verein zu Bremen, Bd. 34, Heft 3, S. 181-440, 1 Taf.

Lange-Bertalot, H. 1978. Diatomeen-Differentialarten anstelle von Leitformen: ein geeigneteres Kriterium der Gewässerbelastung. *Archiv für Hydrobiologie Supplement 51*, 393-427.

Lange-Bertalot, H. 1979. Pollution tolerance of diatoms as a criterion for water quality estimation. *Nova Hedwigia* 64, 285-304.

LeCointe C., M. Coste, and J. Prygiel. 1993. "OMNIDIA" software for taxonomy, calculation of diatom indices and inventories management. *Hydrobiologia* 269/270: 509-513.

Lowe, R. L. 1974. *Environmental Requirements and Pollution Tolerance of Freshwater Diatoms*. US Environmental Protection Agency, EPA-670/4-74-005. Cincinnati, Ohio, USA.

Palmer, C. M. 1969. A composite rating of algae tolerating organic pollution. *Journal of Phycology* 5, 78-82.

Rott, E., G. Hofmann, K. Pall, P. Pfister, and E. Pipp. 1997. Indikationslisten für Aufwuchsalgen in österreichischen Fliessgewässern. Teil 1: Saprobielle Indikation. Wasserwirtschaftskataster. Bundesminsterium für Land- und Forstwirtschaft. Stubenring 1, 1010 Wein, Austria.

Slàdecek, V. 1973. System of water quality from the biological point of view. *Archiv für Hydrobiologie und Ergebnisse Limnologie* 7, 1-218.

Van Dam, H., Mertenes, A., and Sinkeldam, J. 1994. A coded checklist and ecological indicator values of freshwater diatoms from the Netherlands. *Netherlands Journal of Aquatic Ecology* 28, 117-33.

Vanlandingham, S. L. 1982. *Guide to the identification, environmental requirement and pollution tolerance of freshwater blue-green algae (Cyanophyta)*. U. S. Environmental Protection Agency. EPA-600/3-82-073.

Watanabe, T., Asai, K., Houki, A. Tanaka, S., and Hizuka, T. 1986. Saprophilous and eurysaprobic diatom taxa to organic water pollution and diatom assemblage index (DAIpo). *Diatom* 2:23-73.