

CHAPTER 7

BENTHIC ALGAE AND AQUATIC MACROPHYTES

Disclaimer

Methodologies for assessing ALU based on benthic algae or macrophytes have not been developed for Texas waters. Before conducting any biological monitoring activities using benthic algae or macrophytes, it is imperative to coordinate this work with the TCEQ and the TPWD. As methodologies and metrics become established, this manual will be updated to reflect those changes.

Objective

The objective of this chapter is to describe methods recommended by the TCEQ for the collection and assessment of benthic algal assemblages in wadeable freshwater systems. Benthic algae are an important component of the periphyton community. Periphyton is best described as the community of microscopic organisms associated with submerged surfaces of any type or depth, including bacteria, algae, protozoa, and other microscopic animals (USEPA 1976).

The purpose of this sampling is to determine the kinds and total number—or relative abundances—of benthic algae present. Depending on the sample location, there may be macroscopic and microscopic benthic algae (periphyton), microscopic plankton (algae and other organisms suspended in the water column), and possibly visible surface “scums.” Record general types and abundances of these algae on a field data sheet. The type of sampling and analysis of algal assemblages performed will depend on the purpose of the study.

Overview of Benthic Algal Sample Collection Methods

The TCEQ recommends different sampling techniques for algae depending upon the habitat being sampled. This chapter outlines methods for sample collection, processing, preservation, and evaluation for qualitative and quantitative benthic algal samples. Aquatic macrophyte sample collection methods are included at the end of the chapter.

Equipment

Benthic algal sample collection equipment is minimal. Qualitative sample collection requires a sample collection jar, a pocket knife or similar device for scraping algae from hard substrates, a pipette for suctioning algae from soft substrates, and the proper preservatives. Quantitative sample collection requires a bit more equipment, as described in the section on quantitative sample collection.

Records

In addition to sample labeling requirements as specified in this chapter, maintain the following records for algal sampling.

Field logbook. For each algal sample event, record all relevant information, including the date and time of sample collection, location of the sample site (station ID), collectors name(s), method of collection, number and type of samples collected, number of sample containers, and preservative used, in a field logbook.

Sample tracking logbook. Maintain a sample tracking logbook. This logbook contains the information described in the QA chapter of this manual. This logbook documents when samples arrive at the laboratory or headquarters facility, when each sample enters each sample processing step, and who has custody or responsibility for the sample.

Laboratory bench sheets. Maintain laboratory bench sheets at the location where specimen identification and enumeration occurs. These bench sheets document the raw counts of individuals for each taxon and notes relevant to the identification and enumeration process.

Wadeable Streams and Rivers

Benthic Algal Qualitative Sample Collection Procedures

For a synoptic analysis of the benthic algal community, collect a qualitative composite sample from all available habitats. Sample those habitats in approximately the same proportion they appear in the sample reach. For example, if the reach is approximately 60 percent riffle by area, then 60 percent of the sample volume must be from riffle areas, and the other 40 percent from other habitats, such as snags, depositional areas, and aquatic vegetation. The algal sample must contain any macro-algae, green and blue-green algal mats, and diatom mats located in the sample reach.

Collect macro-algae with forceps and place in a separate sample jar. The macro-algae can be later identified and not lost during the diatom sample processing. Continue sampling until 20 to 50 mL of algal material has been collected.

Hard substrates (epilithic algae). Sample hard substrates, such as rocks, boulders, turtle shells, or mollusc shells by scraping with a knife or stiff brush and rinsing into the sample jar.

Woody debris (epidendric algae). Collect samples by brushing, scraping, or picking algae from submerged snags. If possible, move the snags from beneath the water surface before scraping to avoid losing algae.

Sand or silt (epipsammic or epipellic algae). In depositional areas with no current, sample algal mats growing on top of fine sediments by using the sharp edge of a pocket knife or micro-spatula and gently lifting the top layer into the sample jar. A pipette can also be used to suction algae from the surface of fine sediments.

Macrophytes, roots wads, mosses (epiphytic algae). Rub algae from plant material with fingertips and place it in the sample jar. Squeeze water and algae from mosses into sample jar. Place bits of plant material into jar and shake vigorously to remove attached algae. Some plant material can be left in the sample jar to be examined later for tightly adhered diatoms and other epiphytic algae.

Benthic Algal Sample Preservation

The preferred preservative for algal samples is 2 percent glutaraldehyde. If glutaraldehyde is not available, preserve in 3 to 5 percent formalin (three to five parts full-strength formalin and 97 to 95 parts water). Place algal samples in a cool dark container until analysis. Samples in glutaraldehyde that are kept refrigerated in the dark tend to maintain their natural pigmentation longer than samples preserved in formalin.

Safety Note: Avoid breathing **formalin** fumes! Glutaraldehyde and formalin are corrosive to the eyes, skin, and respiratory tract. Wear safety glasses and latex when working with these chemicals. **Formalin is a suspected carcinogen.** Always work in a well ventilated area or under a hood when preparing glutaraldehyde or formalin solutions.

Check the formalin solution and glutaraldehyde Material Safety Data Sheets for proper handling requirements.

Labeling the Container

Affix a label to the outside of the container, making sure the container is dry, and wrap with clear tape to ensure the label will not come off. Do not place labels for algal samples inside the sample container because the algae will discolor them and make them illegible. Label the container with the following information:

- Station number and location description
- Date and time of collection
- Collection method (for example: hard substrate, snags, or macrophytes)
- Preservative used
- Name of collector(s)
- Sample type
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

If two or more samples are collected at a site, label them accordingly; for example, one may be labeled as macro-algae and the other as composite.

Field Notes

Record the following information in a field notebook:

- Type of macro-algae present
- Percent of the substrate covered by algae
- Presence of any extensive growths of filamentous algae or surface algal blooms
- Evidence of recent scouring
- Any other pertinent observations

Laboratory Procedures for Qualitative Benthic Algal Sample Processing and Identification

Process benthic algal samples in a laboratory for microscopic examination. Process samples in two parts.

1. **Non-diatom.** Examine samples to inventory the algal community on a generic level. These include the macro- and microscopic algae, except for the diatoms.
2. **Diatom.** Clean and mount samples on slides for identification to species level. Species-level identification allows calculation of several metrics for more in-depth analysis of biotic integrity. Metric calculations are described in the “Sample Analysis” section of this chapter.

Chapter 11 provides a complete listing of required and recommended algal identification references.

Microscopic Taxonomic Analysis of Non-Diatom Samples

The purpose of microscopic examination of the non-diatom algae sample is to inventory the algal community. Examine samples within 24 hours of collection unless they have been chemically preserved in formalin or another chemical fixative. Preserved samples can be stored indefinitely; however, pigmentation that may aid in identification will fade quickly, making it preferable to analyze them as soon as possible upon return to the laboratory.

1. Thoroughly shake the sample to mix it and dislodge epiphytes from filamentous algae. Using fine-tipped forceps, pick representative macro-algae from the mixture and place them on a microscope slide with a few drops of water. Cover with a cover slip and identify the filaments under a compound microscope that is equipped with 10x, 20x, and 40x objectives. Do not attempt to examine a wet mount using oil and an oil immersion objective.
2. Mix the sample again and pipette a few drops onto a new slide to identify the non-filamentous algae. If there are many diatoms present, it may be useful to clean them as described below to aid in their identification. For non-diatom algae, examine a minimum of three slides, continuing to scan the slides until no new taxa are encountered.
3. Identify the algae to the lowest possible taxonomic level. Most taxa must be identifiable to genus. Record the observed taxa on a laboratory bench sheet along with estimated relative abundance, such as abundant, common, or rare.
4. If counting the algae in the sample to get a numerical estimate of taxa richness and relative abundance, count colonies as individual units, and filaments in 10 μm segments. For example, a *Pediastrum* colony would be counted as 1 unit, while a 100 μm filament of *Cladophora* sp. would be counted as 10 units. Count at least 300 units, continuing to scan the slide until no new taxa are encountered.

Microscopic Taxonomic Analysis of Diatom Samples

For some studies, it may be desirable to analyze only the diatoms in an algae sample. This may be especially advisable if analytical time or resources are limited. Diatoms are most easily identified if the cells are “cleaned” and mounted in a permanent mounting media as described below.

Cleaning Method for Diatoms

1. Shake the sample jar thoroughly to homogenize the sample. Pour a small subsample, about 5 to 10 mL, into a 2000 mL Ehrlenmeyer flask.
2. *Working under a fume hood*, pour approximately 50 mL of concentrated nitric acid into the flask.

Safety Note: This will produce an exothermic reaction and fumes. Make sure to wear eye protection and gloves that are resistant to acid. Avoid breathing fumes. Always add acid to water. Do not attempt this procedure without use of a fume hood.

3. Allow the sample to oxidize overnight. To reduce the oxidation time, gently boil the sample for a few minutes on a hotplate under the fume hood; delicately silicified diatom frustules may be damaged by this procedure, however.

Safety Note: Use extreme caution if you boil the sample, as additional fumes will be produced. Use insulated gloves to handle the hot flask. Always use at least a 2000 mL flask to prevent acid from boiling over.

4. After oxidation overnight, or after sample is cooled if the boiling method is used, fill the flask with distilled water. Allow the sample to settle overnight.
5. Decant or siphon off the supernatant, and refill the flask. Allow to settle overnight again.
6. Siphon off the supernatant and pour the cleaned sample into a 1000 mL glass cylinder. Fill with distilled water and allow to settle overnight again, or at least four hours, until all the diatom frustules have settled to the bottom of the cylinder.

7. Siphon off the supernatant and pour the diatom sample into a small vial. Scintillation vials with polyethylene cap liners work well for storing cleaned diatom samples. Add one drop of preservative (formalin or glutaraldehyde) to prevent bacterial growth in the stored sample.

Slide Preparation Method for Diatoms

1. Shake diatom sample for at least 60 seconds.
2. Pipette 2 to 3 drops of sample onto a cover slip placed on a cool hotplate under a fume hood. Immediately pipette enough distilled water (approximately 1 mL) onto the cover slip to dilute the diatom solution without breaking the surface tension over the cover slip. This may take practice to learn but will aid in making slide mounts with evenly distributed diatom frustules for identification and counting.
3. Let the cover slip dry, then place a microscope slide on the hotplate next to it. Place a drop of Naphrax or another high refractive index mounting medium onto the slide and invert the cover slip onto it. Turn the hotplate on low and heat until the slide begins to bubble.
4. Remove the hot slide from the hotplate with flat bladed forceps and set on a heat resistant surface (a piece of corrugated cardboard is suitable) to cool.
5. After the slide is cool and hardened, scrape any excess mounting medium from the slide.
6. Permanently label the slide. Slides with a frosted end are preferred, as information can be written directly on the slide; however, adhesive labels are acceptable.

Taxonomic Analysis of the Diatom Sample

Examine the diatom slide on a compound microscope equipped with a 100x oil immersion objective. *Quality optics and lighting are critical for identification of diatoms to species.*

1. Before counting, scan the slide and record the taxa encountered until no new species is observed for at least three transects across the slide. Identification of the diatoms in the sample in this way will speed the counting process.
2. To begin counting, select a random spot on the slide and scan across the slide in transects. Be careful not to scan the same area of the slide twice.
3. Once counting has begun, count the first 500 diatom frustules encountered. A tally counter will help to keep track of the most numerous taxa. Record any new taxa encountered. Identify diatoms to species, if possible, using the references at the end of this chapter.

Benthic Algal Data Evaluation for Qualitative Samples

Non-diatom Benthic Algal Evaluation

The following metrics may be useful in evaluating the non-diatom algal community.

Number of algal divisions present. The number will be higher in sites with good water quality and high biotic integrity. Dominance by filamentous green algae (for example: *Cladophora*) may indicate nutrient enrichment.

Generic taxa richness. This is generally higher in reference sites and lower in impaired sites. Total number of genera, diatoms, soft algae, or both, provides a robust measure of diversity (Barbour et al. 1999).

Indicator taxa. Certain genera of non-diatom algae can be used as indicators of different levels and causes of pollution (Bahls 1992; Palmer 1969, 1977).

Diatom Assemblage Evaluation

Diatom assemblages are especially well suited as biological indicators of environmental impacts in streams and have been used extensively for this purpose. Round (1991) has published a

thorough review of the use of diatoms in river water monitoring studies. Diatoms have historically been used as environmental indicators because of the following qualities.

- Since they are attached to the substrate, they are subjected to immediate, intermittent, or prolonged disturbances.
- Diatoms are ubiquitous, with at least a few species found under almost any aquatic environmental condition.
- Total number of taxa and individuals found at any given site is usually high enough for use in metric calculation.
- Most diatoms can be identified to species level by trained phycologists.
- Tolerance of, or sensitivity to, pollutants is understood for many species or assemblages of diatoms.
- Diatom populations have rapid response and recovery times because of their relatively short life cycle (compared to fish or macroinvertebrates) and their ability to quickly recolonize formerly disturbed sites (Dixit et al. 1992).

Verification of diatoms performed by outside experts will be circled on the slide with a diamond pencil and labeled appropriately with the taxon name.

Multiple Metric Analysis

The diatom community lends itself to multiple metric analysis due to its historical use as a water quality indicator, the many species found in the benthic algae, and the known ecological tolerances of many species. At the time of this publication, a diatom IBI has not been developed for Texas. However, the following metrics can be calculated and, as data are gathered, regional scoring criteria could be developed to aid in assessment of the algal community. Other potential diatom metrics and IBIs are described in the *USEPA Rapid Bioassessment Protocols for Periphyton* (Barbour et al. 1999), *Methods for Assessing Biological Integrity of Surface Waters in Kentucky* (Kentucky Division of Water 2002), and *Montana Water Quality Monitoring Standard Operating Procedures* (Bahls 1992).

1. **Taxa richness.** High species richness is assumed to be the case in a unimpaired site and species richness is expected to decrease with increasing perturbation. Slight levels of nutrient enrichment may increase species richness in naturally unproductive, nutrient-poor streams. In general, however, higher values for this metric indicate higher water quality.
2. **Diversity.** The diversity index has been used in water pollution surveys extensively in the past as an indicator of organic pollution (Weber 1973; Weitzel 1979). The Shannon index is calculated as:

$${}_s H' = - \sum_{i=1} P_i \log P_i$$

where s is the number of species and P_i is the proportion of individuals in the i^{th} species.

While higher values for this metric have historically been assumed to indicate higher water quality, this interpretation can be misleading if taxa richness is extremely low due to toxicity and the few individuals present are evenly distributed among a few tolerant taxa (Stevenson 1984). Compare values to those from a reference stream (Pontasch and Brusven 1988).

3. **Percent dominance.** Recently, the diversity index has been replaced by indices that more directly measure the two components of the original index, taxa richness (above) and evenness of distribution. Since biological assemblages are naturally not evenly distributed, a “better” metric measures the amount of unevenness. Percent dominance of one or a few taxa indicates an unbalanced community. The relative abundance of the three most common taxa can be a useful replacement for the Shannon index. Higher values indicate lower water quality.

4. **Pollution tolerance index (PTI).** Calculated using the formula,

$$PTI = \sum n_i \times t_i / N$$

where n_i is the number of individuals of a particular species, t_i is the tolerance value of that species, and N is the number of organisms in the sample.

This diatom index is modeled after the HBI for macroinvertebrates (Hilsenhoff 1987), with the exception that tolerance values range from 1 to 4, and increasing numbers signify increased sensitivity. Tolerance values were generated from a literature review including Lowe (1974), Patrick and Reimer (1966, 1975), Patrick (1977), Lange-Bertalot (1979), Descy (1979), Sabater et al. (1988), and Bahls (1992). An extensive Kentucky Division of Water database (1977-1993) and data collections by the Kentucky Nature Preserves Commission (1979-1986) were also instrumental in assigning tolerance values. General tolerances of the most common species are fairly well understood. If no information is available for a given species, do not include individuals of that species in the PTI calculation. Higher values for this metric would indicate higher water quality.

5. ***Cymbella* group taxa richness.** The *Cymbella* group of diatoms contains many intolerant species. This metric is calculated as the number of *Cymbella* group taxa identified in the sample. This metric can be especially important in headwater streams, where diversity and richness may be naturally lower, causing the other metrics to underestimate water quality. Higher values for this metric indicate higher water quality.
6. **Percent motile diatoms.** The combined relative abundance of motile diatoms able to glide to the surface of sediments (*Nitzschia*, *Navicula*, and *Surirella*) has been used as a siltation indicator (Bahls 1992). Other genera may be added as their silt tolerances become known. Higher values of this index indicate decreased habitat quality or increased siltation.
7. **Percent community similarity.** The percent community similarity index (PCSI) discussed by Whittaker (1952) and Whittaker and Fairbanks (1958) can be used to compare the diatom community of a reference site and one or more test sites. It can be used with relative abundance data, therefore giving more weight to dominant taxa than rare ones without disregarding the rare taxa altogether. The formula for calculating PCSI is:

$$PCSI (AB) = 100 - 0.5 \sum |a-b| \text{ where } a \text{ is the percentage of species } a \text{ in sample A and } b \text{ is the percentage of species } a \text{ in sample B}$$

Higher percent similarity to the reference site may indicate higher water quality, assuming the reference site is of high quality.

Benthic Algal Quantitative Sample Collection Procedures

Quantitative sampling of the benthic algae are not part of a routine monitoring event; however, special studies may require quantitative analyses. Sampling methods for quantitative analysis depend on the type of study and must follow general guidelines described in this section.

For example, quantitative sampling of the benthic algae may be necessary to determine if nuisance periphyton levels are present. While there are no screening criteria established yet, current information indicates that periphyton chlorophyll *a* biomass of $> 200 \text{ mg/m}^2$ is at or above nuisance levels (Dodds and Welch 2000). Future studies on nutrient enrichment and algal biomass may require quantitative sampling of the benthic algal community. These samples may include chlorophyll *a* and/or other biomass estimates as well as qualitative counts of algal abundance and distribution.

Quantitative Sample Collection Procedure in Streams with Bedrock or Cobble Substrate

Setting up the Transect

Collect and analyze a minimum of five replicate samples separately for chlorophyll *a* biomass estimates or other quantitative analyses. Collect replicate samples from a riffle or run along a transect across the stream as follows:

1. Select an undisturbed spot in the middle of the site (one that has not been walked over during sample collection procedures).
2. Drive a stake into the ground on one bank.
3. Attach a tape measure to the stake and stretch it across the stream. Secure it with another stake.
4. Divide the width of the stream into five equal increments. Start sampling at the midpoint of the first increment.
5. Move to the first increment, and without looking, reach down and select the first rock touched for a cobble sample, or sample that area of bedrock using appropriate sample collection methods as outlined below.
6. Move to the midpoint of the next increment and collect the second replicate. Repeat until the required number of replicates is collected.

Note: If distribution of periphyton is extremely patchy, more than one transect of five replicate samples may be necessary to accurately represent the sample site.

Sample Collection Methods

Use the sample collection method that is most appropriate for the habitat, making modifications as professional judgment deems necessary. In all cases, measure the surface area of the bedrock or cobble sampled as accurately as possible and attempt to collect all the algal material within that selected area. Collect all replicates from the same habitat type, as biomass will vary greatly between habitat types. Collect samples from riffles or runs if possible. These methods do not apply to pool or depositional area sample collection.

Bedrock or Boulder. After setting up the transect (see above), sample known areas of rock or bedrock with a brush and suction device as follows:

1. Press a 4 in to 6 in length of 4-in-diameter PVC pipe fitted with a neoprene gasket against the substrate. Seal the gasket tightly against the substrate so that water and dislodged algae does not leak out of the enclosed area.
2. Scrape and remove as much filamentous algae as possible from within the enclosed area and place it into the sample jar. If the algal filaments are particularly long, it helps to cut them with small scissors.
3. Brush the remaining algal material from the substrate with a stiff brush. A toothbrush bent at the head at a 90 degree angle or a stiff artist's paint brush is suitable.
4. Keeping the pipe section firmly pressed against the substrate, suction the algal material and associated stream water into the sample jar using a syringe, turkey baster, or a hand-operated vacuum pump.
5. Repeat this process along the transect across the riffle or run until five separate replicates are collected.

Cobble. After setting up the transect (see above), sample cobble riffle habitat as follows:

1. From each transect interval, carefully remove a rock from the stream, disturbing as little algae as possible, and place it in a white pan.
2. Using a combination of scraping, brushing, and rinsing with stream water, collect all the algal material from the top surface of the rock. Use as little rinse water as possible. The sample size must not exceed 500 mL.
3. Pour the sample from the pan into a 500 mL wide-mouth sample jar.
4. Measure the sampled surface area of the rock as accurately as possible, using appropriate formulas. Record surface area and sample volume for later biomass calculations.
5. Keep replicate samples separate.

Note: A smaller suction device, such as a 30 mL syringe with the end cut off, may be appropriate for sampling cobble (USGS 2002). However, more effort is required for a representative sample. If using the USGS sampling device, samples from five separate cobbles from each transect interval must be composited for each replicate. This will total 25 separate syringe samples—five cobbles per replicate times five replicates. Label and analyze replicates separately, do not composite them.

Quantitative Sample Collection in Streams with Clay, Silt, or Sandy Substrates, or Non-wadeable Streams

If the stream has a clay, silt, or sand substrate, the above methods are not appropriate. In this case, the following collection methods may apply.

Macrophytes and snags. Cut sections of submersed plant material or woody snags and wash the algal material from them into a sample jar. Measure the surface area of the plant material sampled. This may be difficult if the plant material is highly dissected. Make sure the plant material sampled has been submersed in the stream long enough to have developed a natural algal community; it should not be material that has recently washed into the stream. In the case of woody snags, look for evidence of biological colonization, such as filamentous algae, macroinvertebrate cases, or aquatic insect larvae burrowing into the wood. Plant material must be colonized by filamentous algae or diatom mats.

Artificial substrates. Use artificial substrates if there is no feasible or safe way to collect a sample otherwise. While the benthic algal community that colonizes artificial substrates is usually not representative of the community that colonizes a naturally occurring substrate, a sample using an artificial substrate can be used to assess water quality (Patrick 1973; Stevenson and Lowe 1986). Artificial substrates include rocks, clay tiles, glass slides mounted in commercially available trays, and nutrient-diffusing substrates. Deploy artificial substrates for three to four weeks to allow sufficient time for algal colonization (Aloi 1990). If substrates are disturbed, either by natural causes (flood, drought) or vandalization, redeploy fresh ones.

Phytoplankton. See Chapter 8 for phytoplankton sample collection methods.

Sample Preservation

*Samples for chlorophyll *a* analysis must not be treated with chemical preservatives.* They must be wrapped in aluminum foil to exclude light, placed on ice, and transported to the laboratory for immediate subsampling and analysis. Samples for chlorophyll *a* analysis must be processed and filtered within 24 hours of collection. If frozen and kept in dark containers, filters can be retained for 28 days before extraction.

If both chlorophyll *a* and algal identification analyses are to be performed from the same samples, they can be subsampled in the laboratory before preservation and processing. Samples for identification and counting must be preserved in 2 percent glutaraldehyde or 3 to 5 percent formalin (three to five parts full-strength formalin and 97 to 95 parts water). Preserved algal samples must be placed in a cool dark container until analysis. Keep samples in glutaraldehyde

refrigerated in the dark to maintain their natural pigmentation longer than samples preserved in formalin. This may aid in identification.

Safety Note: Avoid breathing **formalin** fumes! Glutaraldehyde and formalin are corrosive to the eyes, skin, and respiratory tract. Wear safety glasses and latex when working with these chemicals. **Formalin is a suspected carcinogen.** Always work in a well ventilated area or under a hood when preparing glutaraldehyde or formalin solutions.

Check the formalin solution and glutaraldehyde Material Safety Data Sheets for proper handling requirements.

Labeling the Sample Container

Affix the label to the outside of the container making sure the container is dry. Wrap with clear tape to make sure the label stays fixed to the container. Do not place labels for algal samples in the sample container because the algae will discolor them and make them illegible. Labels must contain the following information.

- Station number and location description
- Date and time of collection
- Collection method (for example: hard substrate, snags, or macrophytes)
- Preservative used
- Name of collector(s)
- Sample type
- Container replicate number (for example: 1 of 2 or 2 of 2), if needed

If two or more replicate samples or sample types are collected at a site, label them accordingly.

Field Notes

Field notes comprised of at least the following information must be recorded in a field notebook.

- Type of macro-algae present
- Percent of the substrate covered by algae
- Presence of any extensive growths of filamentous algae or surface algal blooms
- Evidence of recent scouring
- Any other pertinent observations

For quantitative samples from natural substrates, record method of sampling, number of replicates, and area sampled. For artificial substrate samples, record time of deployment and substrate type (for example: periphytometer, clay tile) in the field data log.

Laboratory Procedures for Quantitative Benthic Algal Sample Processing and Identification

Biomass Estimation

Quantitative samples collected for biomass estimation must be processed by the laboratory as outlined in the latest version of *Standard Methods for the Analysis of Water and Wastewater* (APHA 1999). Samples for chlorophyll *a* analysis must be processed and filtered within 24 hours of collection. Filters can then be retained, frozen in a dark container—such as a 35 mm film canister—for 28 days before extraction. While the methods described in *Standard Methods* are written for samples from artificial substrates, they can be easily adapted for qualitative samples from natural substrates. Ash-free weight and chlorophyll content analysis methods are described in methods 10030.C.5 and 10030.C.6. If fluorometric analysis of chlorophyll is to be performed, use USEPA method 445.0 (USEPA 1997).

Taxonomic Analysis

Chapter 11 provides a complete listing of required and recommended algal identification references.

If a special study requires quantitative analysis of the algal community, samples must be processed in the same way as qualitative samples, except that calculations will have to be made to report the data as cells per mm². Record on a laboratory bench sheet the original sample volume, sample area, subsample volume, and, if algal density is high, any serial dilutions.

When counting, use a Sedgewick Rafter or Palmer counting chamber filled with exactly 1 mL of sample. Use of an inverted microscope and volumetric counting chambers is acceptable, as well. Allow a short period of time for algal cells to settle to the bottom of the chamber, then proceed to count strips or fields. See *Standard Methods* (APHA 1999) for details on using counting chambers.

Identify algae to genus whenever possible, counting unicellular algae and colonies as individual units, and filaments in 10 µm segments (one 10 µm segment = one unit). Calculate and report benthic algal data as cells/mm², using the following formula from *Standard Methods*.

$$\text{Organisms/mm}^2 = \frac{N \times A_t \times V_t}{A_c \times V_s \times A_s}$$

Where: N = number of organisms counted
A_t = total area of chamber bottom
V_t = total volume of original sample suspension (mL)
A_c = area counted (strips or fields) (mm²)
V_s = sample volume used in chamber (mL)
A_s = surface area of substrate (mm²)

Benthic Algal Data Evaluation for Quantitative Samples

Periphyton Biomass Evaluation

Two common measurements of biomass are chlorophyll *a* and ash-free dry mass (AFDM). A ratio of these measurements can be used to calculate an autotrophic index (AI) (Weber 1973).

Chlorophyll *a* gives an estimate of the autotrophic component (photosynthetic) of the periphyton sample. While there are no screening criteria established yet, current information indicates that periphyton chlorophyll *a* biomass of > 200 mg/m² is at or above nuisance levels (Dodds and Welch 2000).

AFDM gives an estimate of the entire amount of organic material in the sample, including autotrophs (for example: algae, cyanobacteria, and moss) and heterotrophs (for example: bacteria, fungi, and living microinvertebrates, as well as dead algae, other organisms, and organic litter).

The AI is calculated as the ratio of the AFDM to chlorophyll *a*. This index is indicative of the relative proportions of autotrophic to heterotrophic components of the benthic periphyton community. Values of 50 to 100 are characteristic of non-polluted conditions with little organic detritus (Biggs and Kilroy 2000); whereas, values greater than 400 may indicate assemblages affected by organic pollution (Collins and Weber 1978).

Benthic Algal Assemblage Evaluation

Quantitative samples can be analyzed for density and biovolume. Conversion of algal density information into biovolume enables a more accurate analysis of the biomass dominance of different taxa. By calculating representative biovolumes for a sample of each of the main taxa, the data can be corrected for the contribution of each taxon to the total amount of organic matter at the site (Biggs and Kilroy 2000).

Aquatic Macrophytes

The purpose of macrophyte sampling is to illustrate short- and long-term changes in water quality. Select a sample site that is representative of the attached vegetation found at a monitoring station and which is readily accessible for future sampling. Collect samples by hand, or using a rake or grapple. Report data as number of the individuals per unit area. Data can also be used to describe presence or absence of nuisance growths of aquatic plants.

