

CHAPTER 7

BENTHIC ALGAE AND AQUATIC MACROPHYTES

Disclaimer

Methodologies for assessing ALU and other regulatory bioassessments 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 wadable freshwater systems. Benthic algae are an important component of the periphytic 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 (U.S. EPA 1976).

Overview of Methods for Collecting Benthic Algal Samples

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

Sampling of benthic algae and aquatic macrophytes is not part of routine monitoring; however, special studies may require qualitative analyses. Special studies require an approved quality-assurance project plan or quality-assurance plan before sampling. If a study is in progress, refer to the study's QAPP or QAP for details. If you are developing a QAPP or QAP for a special study, contact appropriate SWQM personnel for assistance.

Equipment

Equipment for collecting benthic algal samples is minimal. Visual assessment requires a transect line or quadrat, a ruler marked with centimeters and millimeters, and a field notebook for recording observations. Qualitative sample collection requires a sample collection jar, a pocketknife 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. See Appendix A for a complete list of equipment needed for benthic algal sampling.

Records

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

Field Notebook

For each algal sample event, record the following in a field logbook.

- date and time of sample collection
- location of the sample site (station ID)
- name of each collector
- method of collection
- number and type of samples collected
- number of sample containers
- preservative used

Sample-Tracking Logbook

Maintain a logbook that documents when samples arrive at the laboratory or headquarters, the steps in processing samples, and who has custody or responsibility for each sample.

Upon return to the laboratory, assign a unique sample tracking number to each jar containing the fish specimens according to the sequence in the fish sample-tracking logbook. For example, an instance of numbering may look like *BA 040 14*, where *BA* refers to 'benthic algae,' *040* refers to sample number 40, and *14* refers to the year 2014.

Record the tracking number and related information on the sample in the logbook. This information includes:

- sample tracking number
- date and time of collection
- station number and location description
- name of each collector
- collection method (for example, kicknet or snag)
- preservative used
- number of containers in sample

Laboratory Bench Sheets

Maintain laboratory bench sheets where specimen identification and enumeration occur. These bench sheets document the raw counts of individuals for each taxon and notes relevant to their identification and counting. See Appendix H for a sample algae laboratory bench sheet.

Wadable Streams and Rivers

Visual Assessment of Algae

Visual assessment gives an estimate of percent cover of both macroscopic and microscopic benthic algae. It is adapted from published methods such as those used in the *Stream Periphyton Monitoring Manual* by Biggs and Kilroy (2000). Such information is useful in determining if algal abundance is great enough to indicate nutrient enrichment. Nuisance algal growths can affect recreational uses such as swimming, fishing, and general aesthetic enjoyment of a water body.

The visual assessment method is a relatively rapid qualitative estimate of biomass. This assessment can be done by an observer trained in algal identification who at a minimum must be able to distinguish moss from filamentous algae, and be able to distinguish diatom mats from bluegreen algal mats or other microalgae. It would be helpful for the observer to be able to identify various divisions of algae for best results. In addition, it is preferable for the same observer to assess all transects to minimize variability.

Required Equipment

- tape measure
- 0.25 meter² quadrat or viewing bucket with a known area.
 - depending on the water clarity, use either a viewing bucket with a clear bottom or a square 0.25 meter quadrat constructed of perforated 1" PVC pipe for the observations; other quadrats of similar size are acceptable as long as the same size is used consistently
- small white plastic ruler marked in mm and cm
- field data sheet for visual assessment of benthic algae (Figure 7.1)
- clipboard
- pencil

Categories of Algae

Nine categories of algae are used in the visual assessment.

Long Filaments

This category includes filamentous green algae that are 30 mm in length or longer. Treat unattached filamentous algae that is entangled and not floating downstream as if it were attached to the substrate. Common filamentous green algae that may be encountered include *Cladophora*, *Stigeoclonium*, *Rhizoclonium*, *Hydrodictyon*, *Oedogonium*, *Spirogyra*, and *Mougeotia*. The yellow-green filamentous alga *Vaucheria* should be included in this category. It is not necessary to identify algae in the field. Samples may be returned to the laboratory and identified if desired.

Short Filaments

This category includes filamentous green algae that are shorter than 30 mm.

Thin Diatom Mat

The category includes mats less than 0.5 mm thick. This can range from a slimy surface to just visible cover, thickness not measurable. Color will range from dark brown to light brown to greenish brown. Mats on soft substrates such as silt and sand will be recognizable by color differences and occasionally small bubbles on the substrate, especially on sunny days.

Medium Diatom Mat

This category includes mats that are 0.5 to 3 mm thick. This will be a measurable thickness, up to the thickness of two pennies stacked together. Color will range from dark brown to light brown to greenish brown.

Thick Diatom Mat

This category includes diatom mats that are thicker than 3 mm—greater than two pennies stacked together. Color will range from dark brown to light brown to greenish brown. At times diatom mats may resemble filaments but will dissolve into individual cells if rubbed between the fingers. The nuisance alga *Didymosphenia* or “rock snot,” which has been identified in Texas, can form thick mucilaginous mats or blobs.

Thin Blue-Green Mat

This category includes blue-green algal mats that are less than 0.5 mm thick. This can range from a slimy surface to just visible cover, thickness not measurable. Their color will range from dark green to bluish green to dark brown or black.

Medium Blue-Green Mat

The category includes blue-green algal mats that are 0.5 to 3 mm thick. This will be a measurable thickness, up to the thickness of two pennies stacked together. Their color will range from dark green to bluish green to dark brown or black.

Thick Blue-Green Mat

This category includes blue-green algal mats that are thicker than 3 mm—greater than two pennies stacked together. Their color will range from dark green to bluish green to dark brown or black. Short intertwined filaments may be observed within the mat.

Other

This category includes other groups that are not classified as green or blue-green algae or diatoms and are attached to the substrate. Examples include reddish or brown filaments, *Chara*, *Nitella*, sewage fungus (*Sphaerotilus*), or moss. Identify these taxa to division or genus level if known, or provide description. Do not record attached or floating macrophytes, including duckweed or rooted emergent or submergent plants. If they are abundant, note that in the “Observations” section at the bottom of the data sheet.

Procedure

1. Establish four transects across the stream in riffle, run, or glide habitats, in that order of preference, avoiding pools if possible. Lay out transects from downstream to upstream to avoid disturbing the substrate and making the water too turbid for viewing. On the field data sheet, circle the type of habitat—“riffle,” “run,” “glide,” or “pool”—for each transect. See Chapter 9 for more information on these habitat types. Along each transect choose five equally spaced observation points—right bank (RB), mid-right (MR), center (C), mid-left (ML), and left bank (LB).

Left-Bank and Right-Bank Orientation—to be consistent and to help orient others to the location of observations, the convention *left bank–right bank* is used. “Left” and “right” refer to the banks to those sides of an observer when facing downstream.

2. Starting at the right bank (facing downstream), place the quadrat or viewing bucket one quadrat width from the wetted edge. This viewing area is “RB” on the field data sheet.
3. Estimate and record the percentage cover of each category of algae listed on a field data sheet for visual assessment of benthic algae (see Figure 7.2 for example). If a particular algae type is not present, record a zero for that category. Record percentage cover to the nearest 5 percent. If the water is too turbid to see clearly, it may be necessary to pick up a piece of substrate to make the field measurements, or carefully feel the substrate with your fingers to estimate the percent cover.
4. If filamentous algae are present, record the length (mm) of the longest filament at each observation point to the nearest 5 mm. If no filamentous algae are present, record a zero.
5. Move to the next observation point, MR, which should be midway between the first point and the center of the stream. Repeat steps 3 and 4.
6. Continue across the transect to observation points C, ML and LB, repeating steps 3 and 4 at each observation point.
7. Repeat this pattern for the next three transects, always starting at the right bank. That way, the field data sheet will serve as a “map” of algal distribution.
8. After all transects are completed, circle the length of the longest filament (max) on the bottom row of the field data sheet. Record “ $n =$ ” at the top of the last column as the total number of observation points. Normally $n = 20$ unless any number of transects other than four are used.

Observations

Record any pertinent information, such as type of taxa observed, abundant macrophyte types, or unusual observations at the bottom of the field data sheet.

Note: If samples are returned to the laboratory for identification, label and preserve as described in the “Benthic Algal Qualitative Sample Collection Procedures” section of this chapter. Useful identification resources include Biggs and Kilroy (2000) and Prescott (1978).

Figure 7.1. Example field data sheet for visual assessment of benthic algae—percent cover.

Station ID: _____ Site Name: _____ Date: _____ Sampler: _____

Record percent cover to nearest 5%	Transect 1 riffle run glide pool					Transect 2 riffle run glide pool					Transect 3 riffle run glide pool					Transect 4 riffle run glide pool					\bar{X}
	RB	MR	C	ML	LB	RB	MR	C	ML	LB	RB	MR	C	ML	LB	RB	MR	C	ML	LB	$n =$
Long filaments (> 20 mm)																					
Short filaments (< 20 mm)																					
Thin diatom mat (< 0.5 mm)																					
Medium diatom mat (0.5–3 mm)																					
Thick diatom mat (> 3 mm)																					
Thin blue-green mat (< 0.5 mm)																					
Medium blue-green mat (0.5–3 mm)																					
Thick blue-green mat (> 3 mm)																					
Other (describe):																					
Total % cover (Σ all categories)																					
Longest filament (mm)—Circle Max																					
Observations:																					

Data Evaluation

Calculate and record the mean percentage cover for each algal category and total mean percentage in the last column of the field data sheet. Include zeros in the calculation.

Visual assessment data can be used alone as an estimate of the stream or river bed that is covered with algae and the relative lengths and thickness of the algae present. It can also be used in conjunction with quantitative analyses of the algal community. Data that are reported include

1. mean percent cover of each category of algae
2. mean total length of longest filament
3. mean percent cover of the stream or river bed

Mean percent cover, longest filament length, percent cover of long filaments, and percent cover of thick diatom or thick blue-green mats may be useful metrics in assessing nutrient enrichment.

Collecting Qualitative Benthic Algal Samples

For a synoptic analysis of the benthic algal community, collect a qualitative composite sample from each available habitat. 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 macroalgae, green and bluegreen algal mats, and diatom mats in the sample reach.

Collect macroalgae with forceps and place them in a separate sample jar for later identification. This also keeps macroalgae from being lost during diatom sample processing. Continue sampling until 20 to 50 mL of algal material has been collected.

Habitat Types

Hard Substrates

Algae living on hard substrates are called *epilithic* algae. Sample hard substrates, such as rocks, boulders, turtle shells, or mollusk shells by scraping with a knife or stiff brush and rinsing into the sample jar.

Woody Debris

Algae living on hard substrates are called *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

Algae living on hard substrates are called *epipsammic* or *epipellic* algae. In depositional areas with no current, sample algal mats growing on top of fine sediments using the sharp edge of a pocketknife or microspatula and gently lifting the top layer into the sample jar. You can also use a pipette to suction algae from the surface of fine sediments.

Macrophytes, Root Wads, Mosses

Algae living on hard substrates are called *epiphytic* algae. Rub algae from plant material with fingertips and place it in the sample jar. Squeeze water and algae from mosses into the sample jar. Place bits of plant material into the 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.

Preserving Benthic Algal Samples

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

Safety

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 material-safety data sheets for formalin solution and glutaraldehyde for proper handling requirements.

Labeling the Container

Attach a label to the outside of the container, making sure the container is dry, and wrap with clear tape. 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 each collector
- sample type
- container replicate number if needed (for example, *1 of 2* or *2 of 2*)

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

Field Notes

Record the following information in a field notebook.

- type of macroalgae present
- percentage of the substrate covered by algae
- any extensive growths of filamentous algae or surface algal blooms
- evidence of recent scouring
- any other pertinent observations

Processing and Identification of Qualitative Benthic Algal Samples in the Laboratory

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 macroscopic 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 “Multimetric Analysis” section of this chapter.

Chapter 11 contains a complete list of required and recommended references on algal identification.

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 dislodge epiphytes from filamentous algae. Using fine-tipped forceps, pick representative macroalgae filaments from the mixture and place them on a microscope slide with a few drops of water. Place a cover slip over the filaments and identify them under a compound microscope equipped with 10×, 20×, and 40× objectives. Do not attempt to examine a wet mount using oil and an oil-immersion objective.
2. Shake the sample again and pipette a few drops onto a new slide with a cover slip to identify 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 at least 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 identifying and counting algae 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 a sample of algae. 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 medium 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 Erlenmeyer 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 hot plate 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 a 2000 mL flask or larger to prevent acid from boiling over.

4. After oxidation overnight, or after the sample is cooled after boiling, fill the flask with distilled water. Allow the sample to settle overnight.
5. Decant or siphon off the supernatant, and refill the flask. Allow it to settle overnight again.
6. Siphon off the supernatant and pour the cleaned sample into a 1000 mL glass cylinder. Fill it with distilled water and allow it to settle overnight, or at least four hours, until all the diatom frustules have settled to the bottom of the cylinder. 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 the diatom sample for at least 60 seconds.
2. Pipette two to three drops of sample onto a cover slip on a cool hot plate 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 hot plate next to it. Put a drop of Naphrax or another highly refractive index-mounting medium onto the slide and invert the cover slip onto it. Turn the hot plate on low and heat it until the slide begins to bubble.
4. Remove the hot slide from the hot plate with flat-bladed forceps and set it to cool on a heat-resistant surface (a piece of corrugated cardboard is suitable).
5. After the slide is cool and hardened, scrape any excess mounting medium from it.
6. Permanently label the slide. Slides with a frosted end are preferable, 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 100× 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. This method of identifying diatoms will speed up counting.
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. Identify and 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 in Chapter 11.

Evaluation of Benthic Algae from Qualitative Samples

Non-Diatom Benthic Algal Samples

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 Assemblages

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 studies of river-water monitoring. 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.
- The taxa and individuals found at any given site are usually sufficiently numerous 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 rapidly respond and recover 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).

Multimetric Analysis

The diatom community lends itself to multimetric 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 collected, 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). The following list of example metrics is not inclusive; other metrics can and should be calculated and evaluated.

1. **Richness of taxa.** High species richness is assumed in an 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). While higher values for this metric have historically been assumed to indicate higher water quality, this interpretation can be misleading if richness of taxa 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). Use caution in comparing diversity-index values to those published in the literature unless you are confident they are calculated using the same formula you have used. Different formulas exist.
3. **Percent dominance.** Recently, the diversity index has been replaced by indices that more directly measure the two components of the original index, richness of taxa (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).**

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

—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 benthic macroinvertebrates (Hilsenhoff 1987), with the exception that tolerance values range from 1 to 4, and increasing numbers signify increased sensitivity. While tolerance values for Texas have not been published, values have been generated for a Kentucky database 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 (1977–93) and data collections by the Kentucky Nature Preserves Commission (1979–86) 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. **Richness of *Cymbella* group taxa.** 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. Higher percent similarity to the reference site may indicate higher water quality, assuming the reference site is of high quality.

Collecting Quantitative Benthic Algal Samples

Sampling methods for quantitative analysis depend on the type of study and should follow general guidelines described in this section. Modifications are acceptable as long as they are detailed in the QAPP or QAP.

For example, quantitative sampling of benthic algae may be necessary to determine if nuisance levels of periphyton are present. While no screening criteria are yet established, periphyton chlorophyll *a* biomass of > 200 mg/m² 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 estimates of chlorophyll *a* and other biomass as well as qualitative counts of algal abundance and distribution. It may be desirable to collect quantitative samples for algal biomass in conjunction with the qualitative visual assessment described earlier in this chapter.

Collecting Quantitative Samples 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. The number of transects will depend on such factors as the objectives of the study, the size of the stream, the size of the sampling device, and the patchiness of the algae within the stream. Refer to the study's QAPP for specific guidance on setting up transects.

Use the following method to collect replicate samples from a riffle or run along each transect.

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 a predesignated number of intervals. Start sampling at the midpoint of the first interval.
5. Move to the first midpoint and, without looking, reach down and select the first rock you touch for a cobble sample, or sample other substrate types using appropriate collection methods as outlined below.
6. Move to the midpoint of the next interval and collect the second replicate. Repeat until all replicates are collected.
7. Move to the next transect and continue until all replicates from all transects are collected.
8. Label and preserve samples as outlined below in “Sample Preservation,” later in this chapter. **Do not** preserve samples collected for biomass (chlorophyll *a*, ash-free dry mass, etc.). Wrap those samples in aluminum foil to exclude light and keep them on ice until transport to the laboratory.

Sample Collection

Use the sample-collection method that is most appropriate for the habitat. In all cases, accurately measure the surface area of the bedrock or cobble sampled and attempt to collect all the algal material within that selected area. For detailed instructions on how to measure the area

of the rock surface, see USGS (2002). If you use the syringe–PVC pipe method to collect the sample, accurately measure the area collected by the sample device. 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 collecting samples from a pool or depositional area.

Bedrock, Boulder, or Other Large-Substrate Habitats

After setting up the transect use the following method to sample areas of rock, bedrock, or other large substrates (such as logs in low-gradient streams without rocky substrates) with a brush and suction device. See Figure 7.2 for images of the following procedures.

1. Press a 60 mL syringe with the end cut off against the substrate tightly enough that water and dislodged algae does not leak out of the enclosed area. If a sampling area with a larger diameter is desired, it is possible to use a 3" to 4" section of PVC pipe with a rubber gasket glued to the end that is pressed against the substrate.
2. Scrape and remove as much filamentous algae as possible from within the enclosed area and place it in a sample jar. If the algal filaments are particularly long, it helps to cut around the outside of the syringe with small scissors.
3. Brush the remaining algal material off the substrate with a stiff brush. A toothbrush bent at the head at a 90-degree angle or a stiff artist's paint brush are suitable.
4. Keeping the syringe or PVC 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 hand-operated vacuum pump.
5. Repeat this process until at least five replicates are collected from each transect.
6. Keep replicate samples separate.

Cobble Habitats

After setting up the transect, use the following method to sample cobble riffle habitat.

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 should 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 the surface area and sample volume for later biomass calculations.
5. Repeat this process along the transect until at least five replicates are collected from each transect.
6. Keep replicate samples separate.



Figure 7.2. How to remove algae from rock surface and how to measure rock surface area. (USGS 2002.)

Collecting Quantitative Samples in Streams with Clay, Silt, or Sandy Substrates, or Non-Wadable Streams

If the stream has a clay, silt, or sandy substrate, and does not contain any large substrates that can be sampled, the above methods are not appropriate. In this case, the following collection methods may apply.

Macrophytes and Snags

Cut sections of submerged plant material or woody snags and wash the algal material 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 submerged in the stream long enough to have developed a natural algal community and not material 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 should have observable attached filamentous algae or diatom growth.

Artificial Substrates

Use artificial substrates if there is no other way to collect a sample. While the benthic algal community that colonizes artificial substrates is usually not representative of the community that colonizes a natural substrate, artificial substrates 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 vandalism, redeploy fresh ones.

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. See the “Laboratory Procedures for Quantitative Benthic Algal Sample Processing and Identification” section for details on processing chlorophyll *a* samples.

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 dark container and kept cool 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

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 material-safety data sheets for formalin solution and glutaraldehyde for proper handling requirements.

Labeling the Sample Container

Attach a label to the outside of the container making sure the container is dry, and wrap it with clear tape to ensure the label stays on 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 each collector
- sample type
- container replicate number if needed (for example, *1 of 2* or *2 of 2*)

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

Field Notes

Field notes comprising 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.

Processing and Identification of Quantitative Benthic Algal Samples in the Laboratory

Estimating Biomass

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 2012). Samples for chlorophyll *a* analysis must be processed and filtered within 24 hours of collection. Filters can then be kept frozen in a dark container for 28 days before extraction. While the *Standard Methods* are written for samples from artificial substrates, they can be easily adapted for qualitative samples from natural substrates. Content-analysis for ash-free weight and chlorophyll is described in methods 10030.C.5 and 10030.C.6. If fluorometric analysis of chlorophyll is to be performed, use EPA method 445.0 (U.S. EPA 1997).

Taxonomic Analysis

Chapter 11 gives a complete list of required and recommended references on algal identification.

The person initially performing the taxonomic identification of diatoms will seek verification by outside experts of those diatoms for which identification is unsure. The person seeking the verification will circle those diatoms that need verification on the slide with a diamond pencil and will number each specimen circled with an accompanying reference to the taxon name.

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 are needed to report the data as cells per mm². Using a laboratory bench sheet record 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 and then proceed to count strips or fields. See *Standard Methods* (APHA 2012) for details on using counting chambers.

Identify algae to genus whenever possible. Count 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 (APHA 2012).

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

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) (mm^2)
 V_s = sample volume used in chamber (mL)
 A_s = surface area of substrate (mm^2)

Evaluating Benthic Algal Data for Quantitative Samples

Evaluating Periphyton Biomass

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 $> 200 \text{ mg/m}^2$ 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 (algae, cyanobacteria, and moss) and heterotrophs (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).

Evaluating Benthic Algal Assemblages

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

Macrophyte sampling is not a routine SWQM activity; however, special studies of aquatic macrophytes in specific areas may be desirable. The purpose of macrophyte sampling may be to illustrate short- and long-term changes in the environment, or simply to inventory the types of macrophytes present in a water body.

Contact the SWQM central-office staff for assistance when developing a macrophyte sampling project. Know the specific objective of the monitoring so that a QAPP can be written to address its purpose and how the data will be used.

Data may be used to describe presence or absence of nuisance growths of aquatic plants, can be expressed as percent cover or abundance of individual plants or taxa, or can be an estimate of total biomass of macrophytes, depending on the study objectives.

Seagrass

Seagrass communities serve as critical nursery habitat for estuarine fisheries and wildlife. Additionally, seagrasses serve as food for fish, waterfowl and sea turtles; contribute organic material to estuarine and marine food webs; cycle nutrients; and stabilize sediments. They are economically important, based on their function in maintaining Gulf fisheries, and were identified as a critical area by the Coastal Coordination Act in 1977.

Three state agencies with primary responsibility for conserving coastal natural resources—the Texas General Land Office, the TCEQ, and the Texas Parks and Wildlife Department—signed the Seagrass Conservation Plan for Texas in 1999. One component of the Seagrass Conservation Plan, the Texas Seagrass Monitoring Plan, provided for the formation of a stakeholder work group. Since that time members of the seagrass-monitoring work group have been developing a monitoring plan for seagrass on the Texas coast; the work group proposed this plan in 2010 (Dunton, Pulich and Mutcher, 2010).

At this time, methodologies for assessing ALU and other regulatory bioassessments based on seagrass have not been established for Texas waters. Currently, the SWQM program is developing seagrass monitoring procedures to be used by agency personnel when sampling seagrass. Before conducting any biological monitoring involving seagrass, with the intention of submitting data for inclusion in the TCEQ SWQMIS database, it is important 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.