ALGAL BIOMASS INDICATORS 7.4

By **Julie A. Hambrook Berkman and Michael G. Canova**

Illustrations

Tables

The citation for this section (7.4) of NFM 7 is as follows:

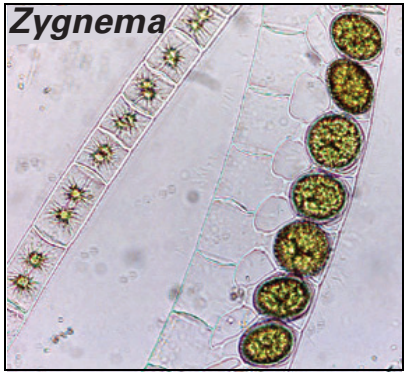
Hambrook Berkman, J.A., and Canova, M.G., 2007, Algal biomass indicators (ver. 1.0): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7, section 7.4, August, available online only from http://pubs.water.usgs.gov/twri9A/.

ALGAL BIOMASS INDICATORS 7.4

By **Julie A. Hambrook Berkman and Michael G. Canova**

Indicators of algal biomass are used to assess water quality in both moving (lotic) and stillwater (lentic) ecosystems. Algal biomass in a water body can be estimated in three ways: (1) by quantifying chlorophyll *a* (CHL *a*), (2) by measuring carbon biomass as ash-free dry mass (AFDM), or (3) by measuring the particulate organic carbon (POC) in a sample. The CHL *a* procedure measures photosynthetic pigment common to all types of algae, while AFDM and POC procedures measure the carbon in a filtered water sample.

Algae: Chlorophyll-bearing, nonvascular aquatic plants. Examples of algae include diatoms, green and red algae, and primitive photosynthetic bacteria such as Cyanobacteria (also called blue-green algae).



Note the star-shaped chloroplasts in the filament on the left and their relative size in the cell.

Figure 7.4 –1. Chlorophyll pigments in the filaments of the green alga *Zygnema*. (Photo provided by Morgan L. Vis, Ohio University, 2007.)

Chlorophyll is the green molecule in plant cells essential for energy fixation in the process of photosynthesis. Besides its importance in photosynthesis, chlorophyll is probably the most-often used estimator in North America of algal biomass in lakes and streams. Chlorophyll is used to measure algal biomass that is relatively unaffected by non-algal substances. Chlorophyll provides an estimate for measuring algal weight and volume, and acts as an empirical link between nutrient concentration and other biological phenomena in aquatic ecosystems. Nutrients and other chemicals in a watershed, together with factors such as temperature and light, affect the biomass production of algae in streams and lakes. Algal production, in turn, affects the entire biological structure of an ecosystem.

Measurement of algal biomass is common in many river and lake studies and may be especially important in studies that address nutrient enrichment or toxicity. High nutrient concentrations can affect recreational water users when the nutrients produce dense growths of algae and (or) aquatic vegetation, which are aesthetically undesirable. Use of waters for a public water supply can be affected if algal blooms result in an unpleasant taste and odor in the treated water. Fisheries, up to a point, are positively affected by increased primary algal production resulting from increased nutrient loads. However, when eutrophication begins to reduce dissolved oxygen concentrations substantially, fisheries can be adversely affected. CHL *a*, AFDM, and POC are measured because they form the direct link between the excessive nutrients and the degradation of recreational waters and ecosystem health.

The relations among algal indicators can provide additional information regarding the condition of an algal community. The amount of CHL *a* per cell changes based on the health and growth status of the cell. This means that carbon-to-CHL *a* ratios change dramatically based on the physiological status of the algal populations. The carbon-to-CHL *a* ratio can be used as a diagnostic tool, as can other elemental ratios. The AFDM/CHL *a* ratio, known as the autotrophic index, has been used to indicate organic inputs (for example, from wastewater) where the higher the ratio, the greater the amount of bacteria, and the lower the quality of water. Consistent field-sampling techniques are necessary to allow for comparisons among studies.

Currently, the United States has some regulations or guidelines for protecting human health and ecosystem viability from nuisance levels of algal biomass or from cyanobacteria algal blooms, which can be detrimental to water quality when they occur in fresh, estuarine, and marine water environments. For example, North Carolina has a 40 µg/L standard for CHL *a* in lakes, and Texas uses narrative water-quality standards to prevent nuisance levels of algae (Texas Water Conservation Association, 2005). The U.S. Environmental Protection Agency (USEPA) requires States to establish nutrient criteria levels in order to control excessive algal growth and to provide protection for the aquatic ecosystems in each State. Some States are considering use of standards for CHL *a* instead of nutrient standards. Green algae (Chlorophyta) and blue-green algae (Cyanophyta/cyanobacteria) commonly are associated with the nuisance algal blooms, but they are just 2 of 10 algal divisions (Bold and Wynne, 1985), each of which contains CHL *a* and a distinct combination of additional pigments that can be used to assess community composition and algal biomass.

Procedures for determining algal biomass include CHL *a* quantification, measurement of organic biomass as AFDM, and determination of POC.

- **Quantifying the amount of CHL *a*.** CHL *a* provides a measure of the amount of active algal biomass (as periphyton) present per area of stream bottom, or a measure of phytoplankton from a volume of water. CHL *a* is a photosynthetic pigment present in all green plants and occurs in the chloroplast of most plant cells. Figure 7.4–1 illustrates the filamentous green alga *Zygnema*, where the chlorophyll in the star-shaped chloroplast can be seen as part of the cell contents.
  - Pigments that occur in varying concentrations along with CHL *a* include CHL *b*, CHL *c*, phycocyanin, allophycocyanin, and phycoerythrin, depending on the evolutionary line of the algal division. Algae also have secondary or assessory pigments and degradation products. Phaeophytin *a* is the most common degradation product resulting from the loss of a magnesium atom.
  - Depending on the objective of a water-quality study, CHL *a* may need to be distinguished from the other primary and secondary pigments. Select the laboratory method to be used for analysis accordingly.

- **Measuring the carbon biomass associated with an algal sample as AFDM.** The AFDM analysis measures the difference in mass of a dried (dewatered) sample after organic matter in the sample has been incinerated (American Public Health Association, 1999).
  - AFDM is recommended for analysis of periphyton biomass instead of a dry mass analysis because silt can account for a substantial portion 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 (Stevenson and Bahls, 1999).
  - AFDM concentrations are near the detection level in phytoplankton samples unless the sample is collected from a highly eutrophic stream or lake. However, periphyton samples can be concentrated through filtration or centrifugation for AFDM.
- **Analysis of POC.** An alternative approach to measuring AFDM for phytoplankton samples is to obtain a measure of carbon by laboratory analysis of a subsample for POC.
  - The POC fraction is derived by subtracting the particulate inorganic carbon (PIC) from the total particulate carbon (TPC): POC = TPC - PIC (see NFM 5).[1]
  - The presence of macroalgae or aquatic plants in large amounts may necessitate accounting for this biomass. As a general rule, if aquatic plants are more than 5 centimeters (cm) long or if they cover areas of a square meter or more, use the methods described in section 7.4.4.B, "Macroalgae and macrophyte ash-free dry mass sampling methods."

[1]U.S. Geological Survey (USGS) personnel can find the correct method and parameter codes for entry into the USGS National Water Information System (NWIS) by accessing the QWDATA component of NWIS or by accessing the Office of Water Quality spreadsheet available at http://water.usgs.gov/owq/FieldManual/Chapter7/7.4.html.

PRESAMPLING CONSIDERATIONS AND PLANS 7.4.1

The purpose and objectives of a study will determine whether samples are to be collected from natural and (or) artificial substrates, and whether the sampling methods used are to yield quantitative and (or) qualitative results. For the purpose of measuring algal chlorophyll, this section (7.4) of the *National Field Manual* of the U.S. Geological Survey *(USGS)* covers sampling methods for the quantitative collection of algal samples from natural substrates and the water column. Procedures are described for the collection of periphyton from river and stream habitats; also, considerations for collecting phytoplankton from lakes, reservoirs, and rivers are discussed. Although the procedures have general applicability to other habitats such as estuaries and wetlands,[2] the specific adaptations required for sampling in such environments are beyond the scope of this section. Also not included in this section are qualitative sample-collection methods used to identify the taxonomic composition of the algal community, and collecting samples from artificial substrates such as concrete channels.[3]

The two primary habitats for sampling algal chlorophyll are (1) the water column (phytoplankton/seston), as described in sections 7.4.2 and 7.4.3, and (2) benthic substrates (periphyton), as described in section 7.4.4. Within each of these macrohabitats are numerous microhabitats to consider. For example, differences between natural lakes and manmade reservoirs, with respect to the hydrology and relative contributions from the perimeter of the basin, will influence the location for collecting phytoplankton samples. Reservoirs may receive only a small portion of their total inflow as direct runoff from the adjacent watershed, with the majority of the water, nutrient, and sediment load entering from one or two tributaries located a considerable distance upstream from the dam. Selection of sampling locations should be based on study objectives; for example, managing

[2]Methods for using algae to assess environmental conditions in wetlands can be found in U.S. Environmental Protection Agency (2002), and Danielson (2006).

[3]Qualitative sample-collection methods can be found in Moulton and others (2002). Refer to Porter and others (1993) and Stevenson and Bahls (1999) for literature references for collecting algal samples from artificial substrates.

reservoir water quality may include sampling at the headwater inflow(s) as well as at the dam or outflow. Lakes are more variable in the relative contribution of runoff from the surrounding area and sampling should take that into consideration. The depth and condition of the water column are important considerations for collecting water-column samples from streams. Upstream from riffle areas provides deeper locations for evaluating light penetration, whereas downstream from riffle areas provides well-mixed stream water for collecting an integrated water-quality sample. Larger rivers typically are sampled from a bridge.

In addition to selecting the sampling location within the water body, one must determine the appropriate depth(s) within the water column from which to collect the sample. Depending on the sampling objectives of the study, either discrete or integrated samples of the whole water column, or euphotic zone sampling, may be warranted. A measure of water transparency typically is used to estimate the euphotic depth, and the sampling depth is adjusted to collect the sample from the area where plankton will receive sufficient light to grow. Although a Secchi disk commonly is used for a depth estimate, a light meter is preferred because it can accurately measure the euphotic depth. Recent advancements in technology provide equipment that can record *in vivo* measurements of chlorophyll so that sampling can be targeted to collect from specific zones of production that would be of interest for documenting the biomass of toxic algal blooms. For example, depending on study objectives, peak algal biomass can occur in or below the thermocline at depths in lakes and reservoirs where 1 to 3 percent of photosynthetically available surface irradiance penetrates (Fee, 1976).

Euphotic depth: The depth at which 1 percent of subsurface irradiance remains (also known as the **light extinction depth).**

Before field work can begin, project personnel need to:

1. Identify the type of water body to be sampled.
2. Determine what the data collected are to represent and the intended use of the data (for example, for status, trends, or regulatory purposes).
   - Decide on the number and type of environmental and quality-control samples to be collected.
   - Decide if an in situ sensor will be used.
   - Determine the ancillary data needed, the frequency of collection, and the methods to be used.
3. Determine sampling methodology.
   - Select the laboratory method to be used or, if using an in situ sensor, follow proper calibration procedures.
   - Consider whether a benthic sample (periphyton), or water-column sample (phytoplankton, seston) or both are to be collected.

Field work requires close attention to safety practices and regulations. Field personnel should comply with U.S. Geological Survey (2005) safety guidelines. All members of sampling teams are advised to wear properly fitted personal safety devices when working in or near water and review field-safety guidance presented in this *National Field Manual*, chapter 9 (NFM 9) (Lane and Fay, 1997).

10/23/2007 UPDATE
TAKE NOTE OF THE FOLLOWING INFORMATION

After sample collection and processing:

-- Chlorophyll samples must be kept frozen until analysis and should be shipped within 1 week of sampling.

-- The holding time for the frozen chlorophyll samples is 24 days from the date of sample collection. Although the NWQL will analyze chlorophyll/pheophyton samples that arrive in excess of this holding time, the data will be qualified appropriately if samples are analyzed after 25 days from sample collection.

****

7.4.1.A SELECTING A CHLOROPHYLL EXTRACTION METHOD

Three common laboratory extraction methods can be used to measure the concentration of chlorophyll pigments and degradation compounds. Each method has advantages and disadvantages that depend on the method sensitivity and the ability of the method to distinguish between the various pigments and degradation products (table 7.4–1 and table 7.4–2). The method to use will depend on project objectives and the type of water body being sampled. If the objective includes comparing values with historical records or with other studies, then the same methodology and laboratory should be used. Common similarities and differences between analytical methods used in chlorophyll extraction are shown in table 7.4–3 and figure 7.4–2. Split samples can be analyzed between methods and between laboratories; however, a correction factor may not be possible to develop, especially with historic records, and an evaluation of laboratory quality and precision is an important consideration in these method comparisons.

- **Fluorometry** is recommended for low-concentration freshwater that predominately consists of CHL *a* greater than 1 microgram per liter (µg/L) or where pigment differentiation is not a concern.
- **Spectrometry** is recommended for freshwater that has a moderate to high concentration of CHL *a* (greater than 1 milligram per liter (mg/L)) or where precise pigment differentiation is not a concern.
- **High performance liquid chromatography** (HPLC) is the most precise (greater than 1 nanogram per liter (ng/L)) of the three methods and is recommended for marine waters, where a higher concentration of CHL *b* may bias results from other methods.

Table 7.4–1. Advantages and disadvantages of three U.S. Environmental Protection Agency laboratory extraction methods commonly used to measure the concentration of photosynthetic pigments.

[EPA, U.S. Environmental Protection Agency; HPLC, high performance liquid chromatography; CHL, chlorophyll; DMSO, dimethyl sulfoxide; USGS, U.S. Geological Survey]

Method	Advantages	Disadvantages
Fluorometric EPA 445.0[1]	• Better precision than the HPLC method • Lower associated cost than the HPLC method • Requires less sample than spectrometry • Uses fewer hazardous chemicals [2]	Cannot distinguish between the various photosynthetic pigments and may overestimate or underestimate CHL *a* concentration.
Spectrophotometric EPA 446.0	• Simple method, somewhat capable of distinguishing between CHL *a*, *b*, and *c*	The least sensitive of the three methods.
HPLC EPA 447.0[1]	• Able to distinguish between the various photosynthetic pigments • Potentially useful for determining the type of algae in blooms	Most expensive of the three methods; values are generally lower than other methods; difficult to use compared to other methods. Uses DMSO, a hazardous material.

[1]The specific method codes and parameter codes used in the USGS National Water Information System (NWIS) are available from http://water.usgs.gov/owq/FieldManual/Chapter7/7.4.html.
[2]As documented in EPA method 445.0, the fluorometric method uses fewer hazardous chemicals (acetone and hydrochloric acid) than the HPLC method (dimethyl sulfoxide, methyl alcohol, and diethyl ether). Although DMSO has been used as an extraction solvent in combination with the fluorometric method, it is not specifically mentioned as a primary or alternative solvent for method 445.0. DMSO poses a potential health hazard, so use of 90 percent acetone is recommended instead of DMSO.

Table 7.4–2. Comparison of instrumental detection limits for chlorophyll *a*, phaeophytin, and chlorophyll *b*

[EPA, U.S. Environmental Protection Agency; CHL *a*, chlorophyll pigment common to all photosynthetic organisms; Pheo *a*, phaeophytin pigment; CHL *b*, a chlorophyll pigment; µg/L, micrograms per liter; HPLC, high performance liquid chromatography; N/A, not applicable]

Method and Instrument		Instrumental Detection Limits (in µg/L)		
EPA Method[1]	Instrument Type	CHL *a*	Pheo *a*	CHL *b*
445.0	Fluorometer	0.05	0.06	N/A
446.0	Spectrophotometer	80	85	93
447.0	HPLC chromatograph	0.0007	N/A	0.0004

[1]The upper concentration limit for use of EPA method 445.0 is 250 µg/L. There is no upper concentration limit for use of EPA methods 446.0 or 447.0; however, if the concentration is greater than 250 µg/L, the chlorophyll extract must be diluted and reanalyzed.

Table 7.4–3. Similarities and differences between chlorophyll extraction analytical methods (Edward T. Furlong, U.S. Geological Survey, written commun., 2006)

[HPLC, high-performance liquid chromatography; DMSO, dimethyl sulfoxide]

Sample Preparation and Analysis	Analytical method					
	Spectro-photometric	Trichromatic	Fluorometric, acidified	Fluorometric, non-acidified	Gradient HPLC	Isocratic HPLC
Extraction with 90 percent aqueous acetone	X	X	X	X	X	
Extraction with DMSO, diethyl ether, methanol						X
HPLC separation using gradient elution profile					X	
HPLC separation using isocratic elution profile						X
Analysis by fluorescence spectroscopy			X	X	X	X
Analysis by absorbance spectroscopy	X	X				

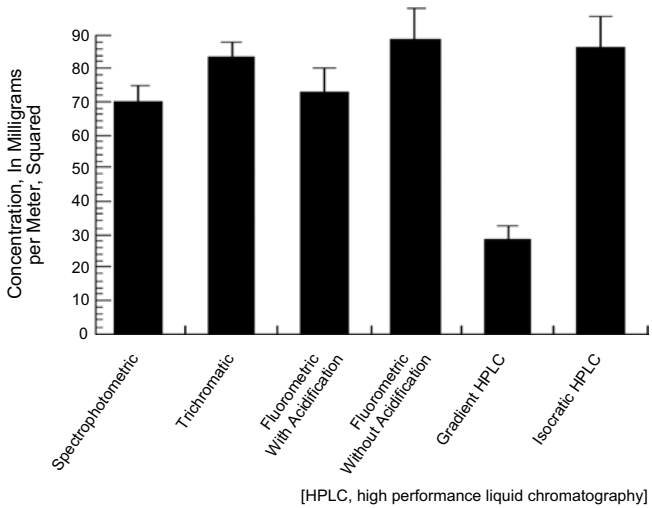


Figure 7.4–2. Example of midrange concentration results for six methods of chlorophyll *a* analysis (from Edward T. Furlong, U.S. Geological Survey, written commun., 2006).

The bar graph in figure 7.4–2 shows mean CHL *a* concentrations for several split samples analyzed by the different methods. At midrange concentrations, the gradient HPLC method determined lower values than the whole-extract spectrophotometric methods. But concentrations determined by the isocratic HPLC method determine equivalent if not higher values than whole-extract-spectrophotometric methods. A similar analysis was done for phytoplankton from seawater (U.S. Environmental Protection Agency, 1997a, table 9). The results show less variance for phytoplankton than the results for the periphyton samples illustrated in figure 7.4–2. Jeffrey and others (1997) provides a more detailed discussion of algal pigments and analysis.

COLLECTING ANCILLARY DATA 7.4.1.B

Seasonal differences in primary physicochemical factors (light, temperature, current velocity, and nutrients) influence the development, structure, and growth rates of algal communities. Collecting ancillary data therefore is recommended, as well as sampling during normal, low-flow, or stable-flow periods. Sampling should be delayed at least 2 weeks after stormwater runoff has disturbed and scoured the periphyton from the substrate in streams or increased turbidity in lakes and reservoirs.

Selecting the specific ancillary data to be collected at each site depends on study objectives and site conditions; however, basic water-quality field measurements (see NFM 6), recording of visual observations, physical habitat measurements, nutrient concentrations, and measurement of light availability are recommended and are routine within the USGS (table 7.4–4). These data can be important for the interpretation of the chlorophyll and biomass sample analysis. In general, such ancillary data take only a short time to collect in the field; however, they require prior planning, training, and preparation of appropriate field forms, as well as acquiring and maintaining field instruments.

Table 7.4–4. Suggested ancillary data for chlorophyll and biomass sampling.

[NFM, *National Field Manual for the Collection of Water-Quality Data*; USEPA, U.S. Environmental Protection Agency; CHL, chlorophyll; PAR, photosynthetically active radiation; *in vivo*, measured in the water column from living cells]

Ancillary data	Description	Reference(s)
Basic field measurements	Measure and record: • discharge • temperature, water and air • dissolved oxygen in water • specific electrical conductance • pH • alkalinity, acid neutralizing capacity • turbidity	• Rantz and others, 1982 • NFM 6.0, 6.1 • NFM 6.0, 6.2 • NFM 6.0, 6.3 • NFM 6.0, 6.4 • NFM 6.6 • NFM 6.7
Site information and visual observations	Record site information and observations on field forms: describe • algae • water color and clarity • presence of surface scum • severity of streamflow • number of days since rainfall • extent of periphyton coverage Take at least three photographs at each site from vantage points that provide the best overall view of the site and document the sampling location. Record on the field form the photo or image number and brief description; print the photos and file them with the field forms.	• NFM 4 • Barbour and others, 1999, Chapter 5
Physical habitat conditions	Describe weather and bank conditions and the type of riparian area(s). Measure and record: • wind speed and water body width and depth • percent vegetation cover; percent bank erosion • width and type of riparian areas	• Fitzpatrick and others, 1998 (for rivers and streams) • USEPA, 1998 (for lakes and reservoirs)
Nutrient concentrations	Collect samples for analysis of total and dissolved forms of nutrients, including phosphorus and nitrogen[1]. Carbon is determined by analysis of particulate organic carbon.	• NFM 4.0, 4.1 • NFM 5
Light availability	Select the appropriate measuring method: • Secchi disk measures transparency; widely used in lakes and reservoirs • Turbidimeter - portable instruments, including a turbidity sensor in multi-parameter instruments, are used routinely for USGS water-quality studies[2]. • Light meter with underwater quantum sensor - provides quantitative measurements of PAR available to algae. Use of a light meter is recommended, as it provides a direct measure of the energy available for algal growth.	• Procedures described in 7.4.1.B and at http://dipin.kent.edu/secchi.htm • NFM 6.7 • Procedures described in section 7.4.1.B, and in Moulton and others, 2002.
In vivo CHL	Fluorescence and other sensors used in situ to measure relative CHL concentration; these measurements must be supported with extractive *in vitro* laboratory analysis.	• Procedures and pros and cons are described in table 7.4–7, and USEPA (1996)

[1]Growth of algae is dependent on availability of nutrient concentrations to the plants; total nitrogen and phosphorus include the phytoplankton itself, resulting in strong correlations between phytoplankton and total phosphorus. The amount of dissolved nutrients represents what is available to the plants for growth.

[2]Turbidity tubes are an alternative method for turbidity measurement, but require care in reading and interpretation because of the tendency for particulates to fall out of suspension; the reading is subjective and its accuracy relies heavily on the experience of the analyst.

Continuous monitoring

Instruments are available for continuous monitoring of all basic field properties (Wagner and others, 2006) and for some chemical constituents and measures of physical habitat. Continuous measurements can be useful in modeling, particularly in studies of climate variability where the interaction of temperature and water are important. Continuous monitoring over several days is recommended for measuring properties such as dissolved-oxygen (DO) concentration and pH, which can be controlled by biological activity. Monitoring DO concentration over 24 hours is especially important, since the diurnal variation of DO in water bodies with high algal concentrations can be extreme. Thus, minimum, maximum, and mean daily values can be helpful in water-body assessment and data interpretation. Measures of DO over time can be used to calculate rates of oxygen production and respiration. The minimum DO concentration usually occurs in the early morning and the maximum occurs in the late afternoon or early evening, depending on the available sunlight and water temperature.

Measures of light availability: Secchi disks and light meters

Measurement of light availability through the water column is an important factor for algal studies. Once light reaches the water surface, particulate matter within the water column (sediment, plankton, and other organic matter) absorb, reflect, and scatter the wavelengths of light. The amount of light available to periphyton and phytoplankton will influence the type of taxa and the amount of chlorophyll and biomass. Another consideration for sampling in lakes is the importance of light profiles to help locate layers of algae in the lake. These layers are referred to as subsurface or deep chlorophyll maxima layers. When present, these subsurface layers of algal concentrations can represent 80 percent of the algal biomass in the water column.

Transparency: A measure of water clarity.

Water clarity can be measured using Secchi disks, turbidimeters, light meters, and sensors. Procedures for the use of Secchi disks and light meters and sensors to measure light availability are described below. Procedures for use of turbidimeters and discussion of the capabilities of various types of turbidimeters are detailed in NFM 6.7.

TECHNICAL NOTES AND TIPS–1. Transparency:

- Transparency can be affected by the color of the water, as well as by algae and suspended sediments in the water.
- Transparency decreases as color, suspended sediments, or algal abundance increases.
- Water often is stained yellow or brown by decaying plant matter. In bogs and some lakes, the brown stain can make the water the color of strong tea.
- Transparency can be affected by the amount of nutrients and suspended sediment coming into the water body from natural and anthropogenic sources; wind speed can be responsible for the resuspension of bottom sediments.

Secchi disks (fig. 7.4–3). Secchi disks are widely used in lakes and reservoirs to measure transparency, but the method, although inexpensive and easy to use, usually is not practical in streams. The water might be too clear and pools might not be deep enough to take a reading, or the maximum wadeable depth might be less than the Secchi depth. The Secchi disk is typically an 8-inch-diameter (20 cm) disk with alternating black and white quadrants. The disk is lowered into the water until the observer can no longer see it. The average of the depth of disappearance and reappearance is called the Secchi depth and is a measure of the transparency of the water (see **Technical Notes and Tips-1**).

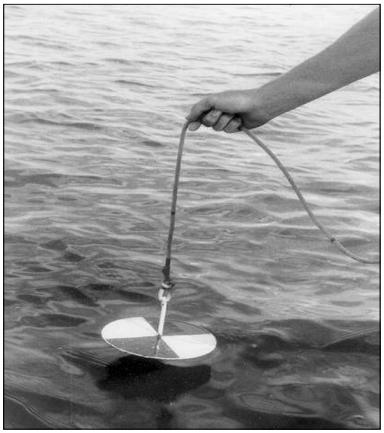


Figure 7.4–3. Lowering a Secchi disk into water. (Photo provided by Kent State University, Ohio.)

- Many State agencies routinely obtain Secchi depth measurements in lakes from the shaded side of a boat. Investigators should be aware, however, that minor differences, such as whether observations are made in full, partial, or shaded sunlight, can affect the measurements (Kent State University, 2007).
- Establish a uniform procedure for measuring Secchi depth in individual studies and water-quality monitoring programs to ensure data comparability and interpretability.
  - If transparency is measured at regular intervals throughout the year, trends in transparency may be observed.
  - If light profiles are taken at the same time as Secchi-depth transparency, a general relation can be developed between Secchi-depth transparency and percentage of incident light energy.

To make a transparency measurement using a Secchi disk:

1. Attach a measuring tape (recommended) or rope to the Secchi disk.
2. Lower the disk straight down into the water until the disk just disappears from sight.
   - The sun should be behind you whenever a Secchi depth measurement is made. An exception to the rule would be when the sun is directly overhead.
   - Sunglasses should be removed unless conditions warrant otherwise.
   - If an anchor is used to prevent drifting from a sampling site, be careful not to disturb the sediments on the bottom as this could cloud the water and interfere with the reading, especially in shallow lakes. The same is true if a Secchi depth measurement is made in a stream, especially in slow moving streams.
3. If the disk is suspended with a measuring tape, note this on the field form and record the depth. If a rope suspends the disk, mark the rope at the depth at which the disk disappears.
4. Slowly raise the disk through the water column until it becomes visible. Record the depth at which the disk reappears (or mark the rope at this depth).

5. The Secchi depth is the midpoint between the disappearance and reappearance measurements. In other words, this point is one-half the distance between the point of disappearance of the disk and the point of reappearance.
6. Measure the distance from the Secchi disk to the midpoint determined in step 5.
7. Record the Secchi depth on the field form to the nearest centimeter or tenth of a foot, and repeat the measurement a second time for quality control.

Light meters and submersible sensors. Light meters and underwater sensors, such as the LI-CORTM 250 meter with LI-CORTM 192SA (illustrated here, for estimates of light penetration in streams) or the LI-193SA (spherical quantum sensor that measures light from all directions-better for deeper water bodies) underwater quantum sensor, can be used to obtain quantitative measurements of the **photosynthetically active radiation** available to the algal community (fig. 7.4–4).

- Use of a light meter is the recommended approach because it provides a direct measure of the energy available for algal growth.
- Light profiles may be used to estimate the light attenuation coefficient as well as the compensation point.

Photosynthetically active radiation: Radiation in the 400 to 700 nanometer waveband.

Light meter and sensor disk with sensor cord running through a graduated PVC pipe. The light-sensor disk is taped so it is facing upward on the measuring rod.

Measuring light penetration using a light meter and light-sensor disk.

Figure 7.4–4. Examples of (*A*) light meter and sensor disk, and (*B*) a light-penetration measurement using a light meter and light-sensor disk. (Photographs by S. Mark Nelson, Bureau of Reclamation.)

To measure photosynthetically active radiation:

1. Assemble the light meter and underwater light sensor to read and record the measurement, in units of photon flux density (micromoles of photons per square meter per second).
   a. Attach the light sensor to a 1.5-m-long 1.3-cm-diameter PVC pipe by feeding the sensor cord through the pipe for streams (fig. 7.4–4a), or using a standard hanging frame for the sensor when used in lakes or reservoirs.
   b. Secure the sensor to the bottom of the pipe with duct tape and a plastic tie, or use a hanger frame and clamp the sensor to the PVC pipe.
   c. Mark the pipe at 10-cm intervals from where the sensor is attached.
   d. If necessary, modify the PVC pipe to ensure that it remains plumb and steady while light readings are taken. Helpful modifications include adding weight to the bottom of the pipe or a bubble level to the top of the pipe.
2. Locate a pool in the reach (or an area in a lake with steady sun or shade) from which light readings can be taken. Ideally, the pools should be shaded if complete cloud cover is not available on the day of sampling. If pools are not present in the reach, locate a pool outside the reach (for example, at a bridge scour) that can be representative.
3. When taking light readings, be aware that the amount of available light at the surface is sensitive to changes in the wind, cloud cover, and disturbance at the water surface.
4. Lower the sensor into the water and take a light reading about 1 cm below the air/water interface (fig. 7.4–4b).
5. Continue lowering the sensor and take a reading at 5- to 10-cm intervals (in streams; take readings at feet or meter intervals for lakes) until the sensor reaches the stream bottom or the instrument reads 1 percent of the first (subsurface) light reading, whichever comes first. The objective is to maximize the number of light readings at each location. During stable flow, wadeable streams rarely have sufficient depth and material to fulfill the condition of 1 percent of the surface reading.

6. Record on the field form the beginning time of the light readings, the depth (in centimeters as indicated by the depth interval on the PVC pipe), and the light readings for all measurements taken at each location. Repeat two or three times and record the measurements each time (replicate measurements). See examples of field forms in Appendix 7.4–A.

7. For data from deep pools or lakes: plot the data using a log-linear plot of readings and depths. The slope of this line approximates the light attenuation coefficient for the water body being sampled. (Section 7.4.2 below on sample collection provides more detail on light profiles.)

TECHNICAL NOTES AND TIPS–2. Calculating the portion of the water body in which light is sufficient for photosynthesis (the euphotic depth):

The light intensity or irradiance (I), at depth z, is a function of intensity at the surface (I_0) to the log base (e) of the negative extinction coefficient (n) at the depth distance, z in meters (Wetzel, 1975, p. 53):

$$I_z = I_0 e^{-nz}$$

where, I = Photosynthetically Active Radiation, in micromoles of photons per square meter per second, or other consistent measurement of irradiance or light,

z = depth, in meters,

n = extinction coefficient, in per meter

Taking the natural logarithms of both sides:

$$Ln(I_z) = Ln(I_0) - nz$$

Using a statistical package, data pairs of light intensity and depth can be used to estimate regression coefficients that correspond to $Ln(I_0)$ and n. Let $Ln(I_z)$ be the light readings (dependent variable) and z be the depths at which they were taken (independent variable), so that the statistics generated are **n** (the extinction coefficient) and $Ln\ I_0$ (Y-intercept). In a statistical package, simply set the depth as the independent variable and LOG_LIGHT as the dependent variable. The CONSTANT (the Y-intercept) and the COEFFICIENT (the slope, the extinction coefficient) are determined from X and Y (data) values. Also, the antilog of the CONSTANT (the Y-intercept) should be very close to the light reading recorded at the surface (I_0) (James F. Coles and Stephen D. Porter, U.S. Geological Survey, written commun., 2001).

The euphotic depth is defined as the depth at which $I_z/I_0 = 0.01$, and Ln (0.01) = -4.6. By rearranging terms in the previous equation:

$$Euphotic\_Depth = 4.6 / n$$

7.4.1.C EQUIPMENT AND SUPPLIES

Equipment and supplies should be ordered and checked in advance of a field trip (tables 7.4–5 and 7.4–6). All equipment and field instruments must be in good operating condition and cleaned according to USGS recommendations and requirements. Instrument calibration or calibration checks should be executed onsite on the day of sampling.

To prevent sample contamination, sample-collection equipment (table 7.4–5) and sample-processing equipment (table 7.4–6) must be cleaned properly. This is especially important in eutrophic systems where it is best to sample from sites of lowest concentration (cleanest sites) to higher concentration sites.

- Follow the USGS cleaning procedures for inorganic-constituent equipment that are detailed in NFM 3, for pre-field and onsite equipment rinsing and cleaning.
- Thoroughly rinse the equipment for sample collection or sample processing with tap water or deionized water (DIW) between each sample collected and before the equipment has time to dry. **Do not let sample-wetted equipment dry before this preliminary rinse is done and before the actual equipment cleaning.**
- **Multiple samples at the same site.** Thoroughly rinse the processing equipment immediately after processing each sample, and before the equipment has time to dry. Rinse all surfaces of the equipment three times with tap water and three times with DIW, or six times with DIW if tap water is not available, before processing another sample.
- **Between sites.** Apply the following procedures to in-field cleaning:

  1. Rinse the equipment with tap water (or DIW) immediately after use.
  2. Soak equipment for 30 minutes in a 0.1- to 0.2-percent non-phosphate detergent solution.
  3. Scrub equipment with a brush to remove residues from the previous sample.
  4. Rinse the equipment three times with tap water, followed by three or more rinses with DIW.

- **Sites with high algal concentrations** — the equipment may require a 30-minute soak in acid solution to assist in the removal of residues (see NFM 3).

Table 7.4–5. Example checklist of basic and ancillary field supplies and sampling equipment used in the collection of algal samples

[L, liter; mL, milliliter; mm, millimeter]

Items for sample collection	✓
Phytoplankton Collection	
Secchi disk and a measuring tape	
Water sampler (Kemmerer, a weighted bottle sampler, DH 81, or other sampler)	
1-L amber high-density polyethylene sample bottles	
Periphyton Collection	
Wide-mouth sample bottles (for example, Nalgene™) (size depends on sampling method, typically between a 100 mL and 1 L bottle)	
SG-92 with brushes (see section 4.3.1 in Moulton and others, 2002)	
Ruler and tape measure	
Wax pencil (for top-rock scrape)	
Aluminum foil	
Hand brush	
Plastic petri dishes (47-mm diameter)	
Spatula (without holes)	
Gravel sampler with masonry trowel	
General	
PFD (personal flotation device)	
Forceps	
Graduated cylinder	
Squirt bottles for washing algae off sampling forceps, pipettes, and pans	
Sample labels and tape	
Field forms, printed on waterproof paper, for recording date, time, conditions, area samples and volumes filtered	
Camera	
Global positioning system unit, to document site and sample location	
100-meter tape	
Depth measuring stick	
Gravelometer or ruler to measure substrate size	
Angular densiometer to measure channel shading	
Clinometer to measure stream slope	
Light meter and quantum sensor, or Secchi disk for lakes	
Instrument(s) for measuring water pH, conductance, temperature, and dissolved-oxygen concentration	
In vivo chlorophyll or phaeophytin sensor	

Table 7.4-6. Example checklist of equipment and supplies used in the processing of algal samples

[L, liter; mm, millimeter; TNPC-POC, total nitrogen and particulate carbon and particulate organic carbon; lbs, pounds]

Items for sample processing	✓
Hand-held electric stirrer (periphyton homogenizer) or blender	
Graduated cylinders	
Pipettor and disposable pipette tips (for example, Oxford™ or Eppendorf™)	
Plastic Erlenmeyer flask (1 or 2 L) with appropriately sized one-hole stopper	
Filter funnel and base (for 47-mm-diameter filters). Note: adapt to 25 mm for TNPC-POC	
Vacuum pump with pressure gage	
Glass fiber filters, 47-mm diameter (for example, WhatmanTM). Note: 25 mm for TNPC-POC	
Plastic gloves	
Forceps	
Aluminum foil	
Dry ice, 10 lbs. per day of transit	
Cooler to hold dry ice and filtered samples	
Plastic petri dishes (47-mm diameter)	
Electrical tape to seal petri dishes	
Squirt bottles for washing algae off sampling forceps, pipettes, and pans	

7.4.1.D QUALITY CONTROL

Algal concentration may vary substantially from sample to sample. The variability can be attributed to spatiotemporal variability of the collection, as well as variability in the processing, storage, and analysis of the sample. Quality-control samples, such as blanks and replicates, should be used to determine the cause of the variability or to assess precision. One quality-control sample is typically collected for every 10 to 15 samples; however, the frequency, types, and distribution of quality-control samples must be based on the data-quality objectives of the study. Note that analyses using fewer than four blanks or replicates carry little statistical power.

- In water bodies with high algal concentrations, collect blanks to help determine the effect of carryover or positive bias.
  - Blanks should be targeted for field and laboratory methods. Field blanks are best collected at the end of the day, after the last sample.
  - Blanks intended to determine laboratory carryover should be collected and processed under the controlled conditions of the office laboratory.

- Collect replicate and split samples to determine the variance in laboratory or field procedures.
  - Consider split and replicate samples to partition the variance associated with laboratory analysis of the sample and the variance associated with collecting the sample. Chlorophyll concentrations can vary with light (riparian shading and (or) water turbidity), velocity, algal community composition (particularly when filamentous green algae are predominant in periphyton samples), community age and patchiness, and other factors. Develop a quality-control design that takes into account the results of preliminary measuring and recording of these ancillary data.
  - Special considerations for lake plankton include water column structure, euphotic depth, wind speed, and wind direction. Consider how the algae are distributed with depth, especially for stratified lakes or deep pools in streams. If the mixed layer of the lake or pool is very deep, or if light penetrates deep into the lake, consider collecting samples at multiple depths. Samplers also are available to collect depth-integrated samples over the mixed layer. In general, lake algae are not uniformly distributed with depth, and so the sampling plan should take this into account. For lakes and reservoirs, sampling at multiple depths is more important than collecting replicate samples at the same depth, because the major source of variability at any one site is the change in concentration of algae with depth. See section 7.4.3 on sample collection for more detail on integrated sampling.
  - If chlorophyll extraction from the sample will be performed at multiple laboratories or by multiple laboratories or personnel within a laboratory, use of split samples for inter- and intra-laboratory comparisons is recommended.
  - **Post sample-collection handling.** Algae samples are susceptible to degradation. Heat and light are the primary sources of sample degradation. Remember that algae are living microorganisms with relatively fast growth rates. **Samples cannot be held for more than 24 hours before significant changes begin to occur.** Sample filtration followed by freezing is the best protection from degradation; however, freezing and thawing can also cause sample degradation.

7.4.2 *IN VIVO* MEASUREMENT OF CHLOROPHYLL AND PHYCOCYANIN

Field measurements taken *in vivo* (within a living organism or a natural setting) are performed on whole living algal cells and are adequate to provide a realistic estimate of CHL *a*. Fluorescence-based sensors (*in vivo* fluorescence[4]) can be used in situ to provide a continuous record of CHL *a* concentration. These chlorophyll sensors output a relative measure of the total chlorophyll concentration; to adjust the readings to CHL *a*, the total concentration must be related to the analysis of extracted CHL *a* samples collected from the site. The fluorometer emits an excitation light at a particular wavelength (approximately 430 to 470 nanometers (nm)) that causes the CHL *a* contained within the algal cells to fluoresce at another wavelength (approximately 650 to 700 nm). The concentration of CHL *a* is proportional to the amount of CHL *a* fluorescence emitted. All algae contain CHL *a*, but there are many kinds of algae and each division has distinctly different accessory pigments that fluoresce at different wavelengths. Some accessory pigments such as CHL *b* and CHL *c* fluoresce within the same wavelength and may influence the CHL *a* determination.

Cyanobactera: A group of phytoplankton and periphyton organisms containing the photosynthetic pigments chlorophyll *a* and accessory pigments that give cyanobacteria their blue-green color.

Cyanobacteria or cyanophyta are blue-green algae found in all aquatic environments throughout the year. In addition to CHL *a*, these phytoplankton and periphyton organisms contain phycocyanin, allophycocyanin, and phycoerythrin, collectively referred to as phycobilin pigments. The abundance of cyanobacteria in phytoplankton samples is expressed as the number of cells or trichomes (filaments) per milliliter (cells/mL or trichomes/mL) or as a biovolume in cubic micrometers per milliliter (μm^3/mL). Excessive growth of cyanobacteria can cause the nuisance water-quality conditions referred to as Cyanobacterial Harmful Algal Blooms, or CyanoHABs.

[4]Throughout this section, *in vivo* fluorescence refers to the measurement of any of the photosynthetic pigments.

An estimated concentration of cyanobacteria in the plankton community can be measured *in vivo* by measuring phycocyanin fluorescence in much the same way as CHL *a* fluorescence. The primary differences are the wavelength of light used by the sensor, and the units of measure; the measured fluorescence is correlated to a cell count (cells/mL). Phycocyanin is an accessory pigment in cyanobacteria that is measured in freshwater environments (using an excitation wavelength of approximately 595 nm and an emission wavelength of approximately 670 nm). Phycoerythrin is another pigment that occurs in cyanobacteria and typically is measured in marine waters using an excitation wavelength of approximately 528 nm and an emission wavelength of approximately 573 nm. Advantages and disadvantages of using *in vivo* measurements are included in table 7.4–7.

Table 7.4–7. *In vivo* chlorophyll and phycocyanin measurement: advantages and disadvantages

Advantages	Disadvantages
The measurement is simple and laboratory equipment is not required.	The measured fluorescence is affected by cell structure, particle size, organism type, physiological state of the cell, and environmental conditions.
In vivo spot sampling can determine points of interest in real time and can indicate where to collect samples for extractive analysis.	Different phytoplankton species may show differing fluorescence intensities even with similar chlorophyll contents. It is not usually possible to differentiate between different forms of chlorophyll.
Measurements from the sensor can provide useful information in either vertical or horizontal profiling studies. Instantaneous, short-term, and long-term measurements can be made.	Interferences may occur from other fluorescent species and sunlight. Depending on the sensor type, anything that fluoresces may be detected. The time of day and the turbidity of the water may affect fluorescence intensity due to the impact of available light, resulting in fluorescence variation even though chlorophyll (phytoplankton) remains constant.
In vivo fluorescence measurements allow for the continuous monitoring of chlorophyll and the observation of trends in phytoplankton concentration.	Many environmental factors can affect fluorescence, about which little is known (including diurnal variations). For example, *in vivo* fluorescence responses exhibit photoinhibition over the daily irradiance cycle, making time of day and light intensity important sampling variables. Drifting and fouling of the sensors on continuous monitors require periodic (in some cases frequent) servicing and recalibration.
In vivo sampling can complement extractive analysis by limiting labor, expense, and (or) number of samples.	The calibration of chlorophyll sensors may present a challenge. True chlorophyll standards are an extract dissolved in acetone. This mixture is not recommended for sondes. The use of a secondary standard to check sensor performance is typical among the available sonde chlorophyll sensors.

7.4.2.A SENSOR RANGE

In vivo fluorescence must be measured and calibrated within the linear range of the sensor. Most commercially available sensors operate at ranges up to 200-400 *in vivo* fluorescence units (or 200-400 µg/L). Measures that are greater than the sensor's range will increasingly become negatively biased. Refer to the sensor user's manual to determine the maximum range of the sensor and select a sensor that meets the range requirements for the study. If environmental conditions are greater than approximately 10 times the maximum range of the sensor, sample quenching may occur. The observed effect of quenching is readings that decrease even though concentrations are increasing. Quenching is due to light-absorption losses in the sample (Turner Designs, Inc., 2004). Quenching can be a problem because high concentrations of chlorophyll will produce low fluorescence measurements that may appear to be within the linear range of the sensor.

7.4.2.B CALIBRATION

The majority of *in vivo* fluorescence sensors are not calibrated using a certified primary standard but are checked using a secondary standard in order to monitor sensor performance. A primary standard contains a known concentration of chlorophyll, usually dissolved in an organic solvent such as acetone. A secondary standard contains some other fluorescent material in place of chlorophyll. As a result, the measured values are not directly related to CHL *a* concentration in micrograms per liter, such as can be obtained when calibrating with a primary standard. Rather, readings are a relative change in measured fluorescence over time and are reported in *In Vivo* Fluorescence Units (IVFU). Even if a primary standard is used, the fluorescence produced from an extracted chlorophyll standard is unlikely to be the same as the fluorescence produced by the same concentration of chlorophyll present in a whole living cell (YSI Incorporated, 2001). Since most sensors report measurements in extrapolated units—either as microgram per liter or cells per milliliter—the user must be aware of the units being reported relative to the calibration method so that data are represented appropriately.

Calibration typically is performed using a secondary standard such as rhodamine dye or a solid block standard. Consult the equipment user's manual for the type of standard, the standard preparation instructions, and the sensor's verification procedure recommended by the manufacturer.

- Due to the temperature dependency of fluormetric measurements, the temperature of the standard should be as close as possible to that of the environmental conditions.
- Always verify sensor performance before use.

Basic Calibration Procedure

Record the results of each of the following steps and the sample temperature on the field form, as appropriate, and in the instrument logbook.

1. Rinse the sensor with DIW three times and fill with DIW to obtain an initial blank reading. This step ensures no contamination is present.
2. Use at least one secondary standard to verify sensor performance. The sensor should be checked at a minimum of two points, bracketing the expected environmental concentration. Use as many points as possible.
3. Rinse the sensor with DIW to obtain a second blank reading. This step is critical if rhodamine dye is used as a secondary standard because the dye has a tendency to persist and remain detectable at low concentrations. A minimum of six DIW rinses typically is needed to obtain a reading equivalent to the initial blank reading.
4. If the data are to be quantified, obtain a measurement and collect and process a representative sample of the native water. Depending on the environmental conditions, this may be a concurrent sample or a split sample.

7.4.2.C INTERFERENCES

The distribution and concentration of various algal species can affect the variability of the measurement.

- The **physiological state and morphology of the cell** affects the correlation between the amount of fluorescence and the actual concentration of CHL *a*, because excitation and emission occur through the algal cell wall. Unhealthy cells fluoresce more than healthy cells due to the decreased ability of the unhealthy cells to use available light for photosynthesis (Turner Designs, Inc., 2004).
- **Temperature** has an inverse relation with fluorescence: as temperature increases, fluorescence decreases. Temperature coefficients vary from instrument to instrument. Check the instrument specifications or user's manual to determine the effect of temperature on the measurement. Some sensors may automatically compensate for temperature.
- **Photoinhibition** is the inhibition of chlorophyll to fluoresce in the presence of ambient light (YSI Incorporated, 2006). This would result in an apparent diurnal cycle showing less fluorescence during the day and more at night. Photoinhibition must be taken into account to avoid introducing significant error. Some sensors are capable of ambient light rejection. Thus, the impact of photoinhibition will vary depending on the sensor being used. The use of a flowthrough cell also can help to reduce the effect of photoinhibition by providing the cells with more of a constant light history before measuring fluorescence (Turner Designs, Inc., 2004).
- **Turbidity** can introduce significant error when turbidity levels are high or variable, by increasing light scatter and reducing fluorescence measurements by absorbing light. Some sensor arrays are capable of compensating for the effects of turbidity. The effects of turbidity on *in vivo* chlorophyll fluorescence can be corrected by developing a multiple regression equation using data on *in vivo* fluorescence, turbidity, and CHL *a* concentration from extracted samples (Turner Designs, Inc., 2006).

- ***In vivo* fluorometers cannot distinguish between different algal species or any other compounds that fluoresce at the measured emission wavelengths.** The optical filter bandwidth of the sensor is an important consideration because it defines the range of the excitation and emission wavelengths. The amount of interference from fluorescing compounds, other than CHL *a*, depends on the bandwidth of the optical filters (Turner Designs Inc., 2004, p. 32). A narrow bandwidth is more specific and minimizes interference.
- **Bubbles on the optical sensor** can interfere with readings. Many instruments are equipped with a mechanical wiper to remove bubbles that may have formed on the sensor surface between readings.

DATA QUANTIFICATION 7.4.2.D

The relative measured fluorescence for total CHL *a* and phycocyanin is proportional to the actual concentrations of CHL *a* and the density of cyanobacteria (respectively) in the sample and may be correlated to an extractive analysis. If fluorescence measurements are to be quantified, they must be supported with extractive laboratory analysis. To relate measured fluorescence to CHL *a* concentration, collect multiple CHL *a* samples over the range of fluorescence measurements taken in the field. A correlation rating can then be developed between fluorescence and CHL *a*. The type of extractive analysis must be taken into consideration and should be consistent throughout a study.

Extraction is the primary difference between the *in vivo* field measurement and the in vitro (an artificial environment outside of a living organism) laboratory measurement. The purpose of the extraction step used in laboratory analysis is to disrupt the cell wall and dissolve the photosynthetic pigments into an organic solvent, resulting in a filtered homogeneous sample that effectively removes many of the interferences mentioned above for the *in vivo* measurement. Although the physiological interferences have been removed, other interferences, such as from CHL *b* and *c*, may still be present.

***In vivo* chlorophyll:** Active "live" chlorophyll.
Extracted chlorophyll: "Dead" chlorophyll that is no longer part of an active cell function.

To correlate the relative measured fluorescence to an actual concentration, wholewater samples for laboratory analysis are collected and processed as described in section 7.4.3. For estimates of cyanobacteria, cell counts are used rather than an extract, because of difficulty in extracting the phycobilin pigments. The correlation is calculated the same way as chlorophyll. The number of samples to be collected will depend on the length of deployment, the variability of environmental conditions, and data-quality objectives. Since chlorophyll samples degrade rapidly once they are filtered, samples should be processed, frozen, and shipped to the laboratory by overnight express delivery before Friday. The samples for laboratory analysis should be collected throughout the range of fluorescence measured in the field.

- For long-term deployments, collect a sample for laboratory analysis during each site visit, typically 26 samples per year.
- Samples should be collected throughout the entire range of fluorescence measured in the field.
- The sample collected must be representative of the *in vivo* measurement. The greatest accuracy is achieved if the collection method:
  - Uses split or concurrent samples to compare a fluorescence reading to a laboratory analysis, or
  - Collects a point sample as close to the deployed sensor as possible and compares the in situ reading to the laboratory analysis.

Quantified data can be misinterpreted and must be presented with caution.

DATA INTERPRETATION 7.4.2.E

Several methods are used to calculate a concentration from a relative measured value. The methods range from a simple ratio, or scatter plot, to multiple-regression equations. The appropriate calculation method will depend on the environmental conditions and the number of interferences present. The number and type of interferences that may be present in various environmental settings may cause the data to be highly variable. Depending on the amount of variability and the types of interferences affecting the raw measured values, the development of a useful relation between *in vivo* fluorescence and extractive analysis may be unfeasible.

Understanding the raw fluorescence values may be more beneficial than applying an extrapolated concentration.

- Diurnal fluctuations in fluorescence are indicative of a healthy algal population.
- Increasing fluorescence values over a period of weeks may be indicative of increased algal abundance.
- An increase in the ratio of phycocyanin fluorescence to chlorophyll fluorescence may indicate increased dominance by cyanobacteria.

QUALITY CONTROL 7.4.2.F

The measurement of *in vivo* fluorescence can be highly variable. This method currently is in development. Documenting the methods used in the field and maintaining a continuous record of adaptations for specific sites is crucial to the development of a method. Use of the following steps is recommended to minimize the uncertainty of the measurement and improve the quality of the data.

- Periodically analyze a "true blank" using filtered native water and a glass fiber filter or capsule filter. The purpose is to remove algal cells from the water being measured in order to check for background interferences of dissolved constituents.

- If there is an issue with possible quenching (a decreasing instrument reading for a sample with increasing and extreme algal biomass), consider using diluted replicates to test for linearity and quenching. That is, in addition to an environmental sample and a true blank, consider analyzing a 1:1 or 1:2 mixture of environmental sample and filtered (blank) environmental water.
- If standards are prepared inhouse, then careful quality control of laboratory practices is essential. The field measurements are only as good as the standards being used. Use clean, Class A glassware to prepare standards.
- If samples are being collected to quantify data, all of the quality-control procedures described in 7.4.1.D apply.
- Sensor performance must be verified at the beginning and at the end of deployment and periodically during extended sampling.

7.4.3 PHYTOPLANKTON SAMPLING PROCEDURES FOR CHLOROPHYLL *a* AND PARTICULATE ORGANIC CARBON

The procedures for collecting phytoplankton/seston samples are described below for wadeable streams (section 7.4.3.A) and from lakes, reservoirs, and large rivers (section 7.4.3.B). Collection procedures for POC are similar to those for CHL *a*. Refer to POC methods in NFM 5.2.2.C and compare when to use each set. For POC, take an additional aliquot of sample with the same volume as the CHL *a*, and process the sample through a 25-mm glass fiber filter. Similar samples may be useful for other photopigments in some studies.

Phytoplankton (PHY): Floating or weakly swimming microalgae. Algae floating in streams **(algal seston)** include periphyton that have been lifted or scoured off the stream bottom.

COLLECTING SAMPLES FROM WADEABLE STREAMS 7.4.3.A

Quantitative phytoplankton samples are typically collected along with water-chemistry samples at streamgage locations or at ecological sites of interest. The water sample is poured into a churn splitter and the phytoplankton sample and chemical-quality samples are taken directly from the churn splitter (NFM 4.1 and NFM 5; Ward and Harr, 1990).

To collect phytoplankton samples from wadeable streams:

1. Select a sampling site/location to sample upstream from turbulent areas; for example, in a run or pool above the riffle.
2. Obtain a representative wholewater sample of sufficient volume to ensure adequate phytoplankton biomass for analysis. Use a depth-integrating sampler, such as a DH-81 sampler, or use grab samples at shallow ecological sites. Refer to NFM 4 for isokinetic and nonisokinetic sampling methods. Advantages and disadvantages of sampler types are described in table 7.4–8.
   - A 1-L sample is sufficient for productive, nutrient-enriched rivers, as indicated by a noticeable color to the water.
   - In contrast, a large sample volume, such as 5 L, may be required for phytoplankton samples collected from clear, ground-water fed streams or unproductive, low-nutrient rivers (indicated by water transparency).
3. Withdraw unpreserved subsamples from the churn splitter into an amber high-density polyethylene (HDPE) bottle. Adequate subsample volumes range from 50 mL to more than 5 L for clear, ground-water fed streams. When withdrawing a churn sample for CHL *a* analysis, filter and freeze the samples as soon as possible.
4. Label the sample bottle and store it on ice in the dark until the sample is processed.
5. Record the total sample volume and subsample volume on the field form and sample labels. The water depth at which samples are collected, along with a measure of light availability, water transparency, or the light extinction depth also should be recorded at the sample location.
6. The recommended amount of time from the time of collection until when the live samples are processed by filtering and freezing (section 7.4.5) varies, depending on study or program requirements. **Once samples are filtered, they must be frozen immediately and kept frozen until extraction in the laboratory.**

Table 7.4–8. Phytoplankton sampler types: advantages and disadvantages

[ft, feet; ft/sec, feet per second; L, liter; <, less than; lbs/in^2, pounds per square inch]

Sampler Type	Advantages	Disadvantages
Isokinetic samplers	• Common sampler and already in use. • Ideal for flowing water. • Capable of collecting a representative sample from stratified or poorly mixed streams. • Other constituents can be collected at the same time.	• Algal cells can be stressed by the swirling of the sample and the use of a churn splitter. • A minimum stream velocity is required, otherwise the sampling method is equivalent to a multiple vertical grab using an open-mouth bottle. • Maximum depth limit for 3/16-inch nozzle intake: – DH 81, 15 ft – U-DH-2, 35 ft (2 to 6 ft/sec) – D-96, 110 ft (Refer to NFM 4 and Davis (2005) for sampler selection.)
Kemmerer or Van Dorn samplers	• Ideal for slow-moving to still water. • Capable of collecting an undisturbed point sample at a specific depth; not limited in the depth capability. • Most widely accepted method to collect an algal sample.	• Can be difficult to clean between sites. • Cannot collect integrated samples. • Moving parts in the trigger mechanism may increase the likelihood of equipment failure. • May drift in flowing water.
Open-top bailer	• Can be used to collect an integrated water-column sample. • Sample volume depends on the length and diameter of the bailer selected.	• Can only collect a mixed sample. • Starts at the surface and is limited in depth by the length of the bailer and the rate of filling.
Weighted bottle or open-mouth bottle grab samplers	• Works well for surface or near-surface samples. • Sampler is easy to use and equipment is inexpensive. • The sample is collected in the actual sample container, or in a collapsible 3- to 6-L bag. • May be the only method possible in shallow, narrow streams.	• Sampling begins as soon as the sampler is submersed. • Unacceptable for deep samples in stratified waters. • Limited in the maximum depth that can be sampled. • The sampler will cause some mixing as it is lowered to the appropriate depth and as it fills.
Pump samplers	• The sampling pump may already be in use, such as on a boat. • Sample can be pumped through a glass fiber filter if the pressure is controlled to < 5 lbs/in^2. • Also capable of sampling at a specific location and depth.	• Depending on the ability to regulate pumping rate and flow, certain pumps may deform or destroy the algal cells. • May bias results, depending on the analysis to be performed. • **This sampling method is not acceptable for algal speciation.**

COLLECTING SAMPLES FROM LAKES, RESERVOIRS, AND LARGE RIVERS 7.4.3.B

Quantitative phytoplankton samples collected from lakes, reservoirs, and large rivers are typically collected in the euphotic zone (Britton and Greeson, 1987).

Euphotic zone: The part of the aquatic environment in which the light is sufficient for photosynthesis; commonly considered to be that part of a water body in which the intensity of underwater light equals or exceeds 1 percent of the intensity of surface light.

To collect samples from the euphotic zone:

1. Determine the euphotic zone using secchi depth and (or) a light meter, and the location to be sampled. (Note: The calculated euphotic depth is comparable to a Secchi-disk depth in lakes).
2. Collect samples in the euphotic zone. This depth commonly is approximated by measuring the Secchi depth and collecting the sample at half of the Secchi depth.
   - If the water body being sampled is too shallow to collect at half of the Secchi depth, then collect the sample at half the depth of the water column.
   - These methods may vary depending on study objectives.
3. Samples should be collected in a manner that does not rupture the cells. The common sampler types for lakes and reservoirs include the Kemmerer sampler, Van Dorn sampler, weighted-bottle sampler, and peristaltic pump, listed from most preferred to least. For illustrations and guidance regarding sampler types, see Britton and Greeson (1987).
4. Collect enough sample to fill a 1-L amber HDPE bottle. A 1-L sample is sufficient for productive, nutrient-enriched rivers and reservoirs, as indicated by a noticeable color to the water. A large sample volume (for example 5 L) may be required for phytoplankton samples at some locations.

5. **Store the sample bottle on ice in a dark cooler immediately after collection and until the sample is processed.** Do not exceed the holding time from sample collection to sample processing, as required by the study or program.

6. Record total sample volume and subsample volume on the field form and on the sample labels. The water depth during sample collection, along with a measure of light availability, water transparency, or the light extinction depth also should be recorded for the sample location.

TECHNICAL NOTES AND TIPS–3. Calibrating a sampling pump for accurate representation samples:

When collecting chlorophyll samples using a pump, the time required to completely fill the pump tubing must be determined before sampling. The pump should run for three times the determined length of time before sample collection begins, to allow for three volume rinses of the tubing. If field properties are being monitored, they must stabilize according to stability criteria (NFM 6).

- It is important that the same equipment is used to determine the pump time and to collect the sample. Variations in pumps, tubing, power supplies, and head can affect the pump time.

- If possible perform the following steps in the field immediately before sampling. It is assumed a peristaltic pump is being used.

To determine pump time:

1. Load the tubing into the pump head.
2. Place the end of the pump tubing in deionized water.
3. Note the speed the pump is set at. All samples must be collected at that speed or higher.
4. Turn on the pump and measure the amount of time it takes the deionized water to travel the length of the tubing.
5. Multiply the amount of time by three. This is how long the pump must run before a sample can be collected.

PERIPHYTON SAMPLING PROCEDURES FOR CHLOROPHYLL AND ASH-FREE DRY MASS IN STREAMS, LAKES, RESERVOIRS, AND LARGE RIVERS 7.4.4

The sampling procedures for periphyton vary by the substrate to be sampled. Operationally, periphyton growth forms are classified as either microalgae or macroalgae. Microalgae are microscopic, typically single-celled, and appear as pigmented accumulations or films attached to submerged surfaces. Macroalgae are visible without magnification and typically are filamentous. Macrophytes are vascular plants that typically have roots and are not periphyton. Macrophytes may account for important nutrient uptake and photosynthetic biomass in aquatic ecosystems, and for those reasons they are included with macroalgae in section 7.4.4.B.

Periphyton can be collected from a variety of locations in the sampling reach, representing the range of current velocity, water depth, and riparian shading at the time of sampling. Sampling reaches in rivers and streams typically will have all of the microhabitats listed in table 7.4–9, while the littoral zones of lakes and reservoirs more often are dominated by soft substrates. Depending on the focus of the study and the need to be able to compare results between sites over a range of physiographic regions, it may be important to choose the most representative substrate to sample among the sites.

Periphyton (benthic algae):
Algae attached to an aquatic substrate.

Recommended methods for collecting quantitative periphyton samples from the different in-stream microhabitats (table 7.4–9) and from lakes and reservoirs are described with the intention of reducing the variance in algal collection techniques and increasing data comparability. Where possible, a single habitat is selected for all sites within each study area. Seasonal differences in primary physicochemical factors (light, temperature, current velocity, and nutrients) influence the development, structure, and growth rates of algal communities; therefore, **sampling is recommended during normal, low- or stable-flow periods and delayed at least 2 weeks after spates that have disturbed and removed the periphyton.**

Table 7.4-9. Recommended quantitative periphyton sampling devices or methods for common microhabitat and substrate types

Periphyton Habitat Types		Recommended Sampling Device or Method
Microhabitat	**Substrate**	
Epilithic	Cobble	SG-92 sampler (for smooth surfaces) Top-rock scrape (for irregular surfaces)
	Gravel	Gravel sampler
	Bedrock, boulders, concrete	SG-92 sampler (or a larger adaptation using PVC pipe) Artificial substrate methods (fig. 7.4–5e; Porter and others, 1993; Britton and Greeson, 1987)
Epidendric	Woody snags	Cylinder scrape
Episammic	Sand	Inverted petri dish with spatula
Epipelic	Silt, fine-particle organic matter	Inverted petri dish with spatula

Typical examples of riverine habitats with hard substrates include a riffle in a shallow, coarse-grained, high-gradient stream (epilithic periphyton microhabitat) and a woody snag in a sandy-bottom, coastal plain stream (epidendric periphyton microhabitat). Soft substrate habitats include organically rich depositional areas, such as backwater areas or pools (epipelic or episammic microhabitats). Although these usually are not sampled for chlorophyll, soft substrates that are present in the main channel may be considered representative of the reach for those streams that lack a hard substrate.

To minimize disturbing the stream bottom, stream sampling begins at the downstream boundary of the sampling reach and progresses upstream. Stream depth, velocity, and light should be measured at locations where quantitative periphyton samples are collected, and these site characteristics are recorded on the field form. The area sampled, total sample volume, and subsample volume should also be recorded on the field data form and **on the sample labels.**

Lake edges may have abundant growth of periphyton (as well as macroalgae and macrophytes), and therefore may require a quantitative measure of chlorophyll for documenting near-shore plant biomass. Macroalgal growth such as *Cladophora* spp. can be problematic in lakes that are relatively clear but have cold, nutrient-rich inflows. Collection methods presented here may be used in lakes and reservoirs in wadeable areas near shore (especially the soft substrate), using macroalgal sampling methods. Ancillary data and photographs documenting the coverage of *Cladophora* and the macrophyte Stargrass by distance from shore, depth, and breadth along the shoreline, as well as knowledge of bathymetry and euphotic depth, will help determine the extent to which to apply the sample results (see section 7.4.4.B).

MICROALGAE CHLOROPHYLL AND ASH-FREE DRY MASS SAMPLING METHODS 7.4.4.A

Microalgal sampling procedures for periphyton should be used up to the point at which visible macroalgae become either too long or too dense for practical use of these procedures; at that point use the macrophyte methods described in section 7.4.4.B. It is important to recognize that periphyton represent a dynamic community made of microscopic forms that can accumulate into films, mats, and filaments that become visible macroalgae.

Selection of a hard substrate versus a soft substrate depends on study objectives. In general, hard substrates are preferred because, as erosional areas, they represent conditions at the time of sample collection. Soft substrates, on the other hand, often consist of a mixture of local community biomass as well as deposition from upstream; however, study objectives might dictate selection of soft substrates for this reason. Of the hard substrates, epilithic is the preferred sampling substrate (when it is available throughout the study area) instead of sampling epidendric substrates; this is because the woody branches often are suspended in the water column and located near the edge of the water, and thus are not as representative of the main stream reach.

Epilithic habitat sampling methods

Epilithic sampling locations are randomly selected from five areas in the reach. At each of the areas, a specified minimum number of cobble-sized rocks (5 to 25 cm (~2 to 10 inches)) or gravel samples are carefully lifted from the water and placed in a white plastic dishpan to minimize the loss of periphyton (fig. 7.4–5a). The rocks or gravel are transported in the tub to a convenient onsite sample-processing area. An ideal processing location is shady and flat enough to accommodate a seat (such as an upside-down bucket) for the processor to remove periphyton from the substrate. A composite sample is prepared by combining the algae scraped from the rocks collected in the five areas of the reach.

Epilithic: Benthic habitat consisting of natural, coarse-grained substrates (for example, gravels, cobble-sized rocks, or boulders) or bedrocks, or artificial hard substrates such as submerged concrete on which organisms are attached or loosely associated.

Epilithic microhabitats are sampled using one of three methods, depending on the substrate being sampled (table 7.4–9). When the algae are present in mats or as long filaments, extra effort will be needed to reduce the clumps though mechanical blending to improve reproducibility of results. Long filaments and clumps will need to be reduced to 2 millimeters (mm) or less to improve subsample reproducibility.

- The SG-92 sampler is a modified-syringe sampling device (fig. 7.4–5b) described in Moulton and others (2002) and works best on relatively smooth rock surfaces with moderate to dense assemblages of diatoms or blue-green microalgae. Large filamentous algae can be a problem when trying to prevent leakage between the SG-92 and the sampling area.
- A "top-rock scrape" sampling method is recommended for irregular rock surfaces or when rock surfaces have either sparse periphyton coverage or substantial growths of filamentous algae (fig. 7.4–5c).
- The gravel sampler (fig. 7.4–5d) is recommended for sampling periphyton attached to gravel substrates.

a. Collecting cobble-sized rocks from a stream into a dishpan for processing on the stream bank.

b. Preparing to scrape a rock using the SG-92 sampler (showing o-ring) and periphyton brush.

c. Foil covering the scraped area of a rock following the top-rock scrape method.

d. The gravel sampler. Note beveled edge on bottom of sampler to improve coring.

e. Example of an artificial substrate sampler using clay tiles placed on concrete pavers.

f. A soft substrate, epipelic sample being collected with the inverted petri dish method. Note how a spatula is placed beneath the petri dish to keep the sample intact.

Figure 7.4–5. Photographs of sampling methods for collecting periphyton. (Photographs are by U.S. Geological Survey employees: *a*–*c* and *f* by Richard Frehs, *d* by Stephen Porter, *e* by Carmen Burton.)

To use the SG-92 sampling method:

1. Assemble the SG-92 sampler and periphyton brushes, following the instructions in Moulton and others (2002).
2. Collect five cobble-sized rocks at a time from each of five sampling locations (a total of 25 cobbles per site) into a white plastic dishpan (fig. 7.4–5a) and transport the rocks to an onsite processing station to remove the periphyton.
3. Place the SG-92 barrel with the O-ring on a smooth part of the rock where the algal density is typical. Press down and rotate slightly to create a good seal.
4. Using a pipettor, dispense 5 mL of water into the SG-92 barrel on the cobble. If the water leaks out the side, select another place on the cobble and try again. If the water does not leak, insert the brush into the barrel and scrub to remove the periphyton from the enclosed area on the cobble.
5. Remove periphyton and water mixture with the pipette and dispense the sample into a 100-mL graduated cylinder.
   - Use additional volumes of water as needed.
   - Repeat the process several times until all of the visible periphyton is removed.
   - Pour the contents of the graduated cylinder into a 500-mL sample bottle.

   Note: Dispensing into a graduated cylinder instead of a 500-mL sample bottle is recommended in case the SG-92 seal fails while collecting the sample, thereby causing the collector to start over. If the seal fails, then only the contents of the graduated cylinder are discarded.
6. Repeat the sampling procedures for a single area on each of the rocks selected (the composite sample is composed of 25 discrete collections taken from 25 rocks). Place the sample bottle on ice in a cooler and keep dark until the sample is processed.
7. Measure the diameter of the area scraped by the SG-92 at the beginning and end of sampling. Record these diameters on the Periphyton Field Form (Appendix 7.4–A, fig. 2) to establish an average scrape diameter from which the sampling area can be calculated.

8. Calculate the total area of the composite sample using the average scrape diameter.

$$\text{Total area sampled } (cm^2) = n\pi\,(d/2)^2$$

where, n = number of sample replicates

$\pi = 3.1416$

d = average scrape diameter in centimeters (cm)

Note: If using the inside diameter of a 30-mL syringe, then the total surface area sampled for 25 rocks will be about 75 cm^2.

TECHNICAL NOTES AND TIPS–4. Helpful suggestions for improving the performance of the SG-92 sampler:

- To improve the seal of the SG-92: Replace the rubber O-ring with a neoprene O-ring. The added thickness provides more flexibility to seal the SG-92 against rough rock surfaces.
- If the SG-92 seal fails: Using a pen point, scribe the inside circumference of the SG-92. Set the SG-92 aside. Use a knife, brush, or single-side razor blade to remove the algae from inside the scribed area. Rinse the sample from the brush or blade into the sample bottle.
- Reverse the procedure by scraping away all the algae on the rock around the SG-92; then remove the SG-92 and measure the diameter of your sample and scrape it off into the collection bottle.

To use the top-rock scrape sampling method:

1. Select one to five representative rocks (5 to 25 cm in size) from each of five representative locations in the reach and place them in a white plastic dishpan. Avoid nearshore and shaded areas by selecting rocks from the main part of the riffle or run, partly because edge areas fluctuate with regard to water covering the substrate. The number of rocks to sample depends on periphyton density.

   - Where periphyton density is low (bare rocks), scraping up to 25 rocks may be necessary.
   - If the rocks have a thick biofilm or filamentous growth, scraping five representative rocks should be sufficient.
   - If the coverage of periphyton is variable, scraping 10 rocks is recommended.

2. Identify the area on the top of the rock where periphyton are growing and use a red wax pencil to draw a line around the middle (side) of the rock, outlining the area of periphyton growth on the upper-side of the rock (exposed to some level of light) to be sampled. In some cases, where the steambed is unstable or when excessive scouring has occurred, it can be difficult to assess the area to sample; often the bottom of the rock that has been in contact with the surrounding substrate is darker, especially in streams embedded with silt and organic matter. If it is impossible to determine the top of the rock then pick another rock, or consider sampling the whole rock and determining the area of the entire rock.

3. Using a small brush (nailbrush or toothbrush) or a pocketknife, scrape the periphyton from the sampling area on each rock (typically the top and sides) down to the red line.

4. Rinse the periphyton from each rock into the dishpan using a poultry baster and filtered stream water. If filaments are present, measure the longest one and record the length on a Periphyton Field Form, then cut all filaments into smaller pieces (approximately 2 mm) and include them in the sample to improve homogenizing and splitting the sample.

5. Pour the contents of the dishpan through a funnel into a labeled 500-mL sample bottle. **Place the bottle on ice in a cooler and keep in the dark until the sample is processed** (see "Sample Processing and Preservation," section 7.4.5).

6. The total volume of the composite periphyton sample (including dishpan rinse water) should be less than 475 mL (if mixing in a 500-mL bottle).

7. Trace the outline of each rock on waterproof paper. More than one rock can be outlined per page; number each rock outline sequentially. Label each page with site name, date, number of rocks collected from each site, and initials of the person making the outline.

8. Wrap aluminum foil around the surface of each rock, covering the area that was scraped to remove periphyton (down to the red line on the side). Mold the foil tightly (fig. 7.4–5c) and trim the excess from the bottom edge of the scraped area.

9. Remove the foil from the rock. Make radial cuts in the foil to allow it to be flattened. Place the foil templates into a labeled resealable plastic bag for later determination of the area of each template.

10. In the laboratory, determine the area of each scrape as soon as practical, using a clear plastic grid, digitizer, or mass-area relations.
    - Note that determining a mass area requires an analytical balance; any excess foil not accounting for sample area must be removed.
    - For the digitized foil areas use the hand-drawn outlines of the rocks as a reference.
    - Sum the area for all the rocks sampled in the reach and record the total area on sample labels and on the Periphyton Field Form (Appendix 7.4–A, fig. 2) (modify the section for measured areas sampled by device and microalgal cover).

To use the gravel-sampler sampling method:

1. Assemble the gravel sampler from a plumbing "clean-out" pipe (7.6 cm diameter) (fig. 7.4–5d). Attach the threaded cap; bevel the bottom edge of the clean-out by using a grinding wheel to improve the coring capability of the sampler. Obtain a large masonry trowel wide enough to enclose completely the bottom of the samples.
2. Select 5 to 10 sampling areas throughout the reach.
3. Press the beveled end of the sampler into the gravel substrate to a depth that will exceed the height of the sampler cap. After the sampler is in place, carefully remove the gravel surrounding the outside of the sampler and insert the masonry trowel.
4. Slide the sampler onto the trowel and carefully lift it out of the water.
5. Quickly invert the sampler to contain the gravel and water in the sampler cap.
6. Unscrew the cap and let the excess gravel fall back into the stream, retaining all the surficial gravel that supports periphyton and discarding excess water and granules that have no periphyton.
7. Pour each discrete collection into a dishpan and rinse the sampler before collecting another discrete sample.
8. Repeat these steps to complete 5 to 10 discrete sample collections, which form the composite sample.
9. Extract macroalgal filaments (if present) from the gravel with forceps and then cut them into fine pieces about 2 mm long or less.

10. Brush and rinse the gravel with dishpan water to remove the algae. Recycle the processing water to limit the total sample volume.
11. Rinse the gravel with clear stream water or tap water and pour the rinsate into the sample bottle until there is no sign of periphyton on the gravel in the pan.
12. Pour the sample from the dishpan through a funnel into a 500-mL sampling bottle. **Place the bottle on ice in a cooler and keep in the dark until the sample is processed. Do not exceed holding times** (see "Sample Processing and Preservation," section 7.4.5).
13. Calculate the total sampling area by using the formula presented for the SG-92 sampling method, and record the total area on the field form and sample label.

Epidendric habitat sampling: Cylinder scrape (snag) method

Collecting quantitative epidendric samples (woody snags) from natural substrates presents a challenge because they have an irregular surface and are difficult to remove from the water without loss of algal biomass.

Epidendric: Benthic habitat consisting of woody substrates on which organisms are attached or loosely associated.

Since roots and branches tend to be near the water's edge where streamflow and light often are reduced, selection of epidendric microhabitats with streamflow and exposure to sunlight should be considered before sampling. In cases where the only epidendric surface is too large to be removed, an artificial sampling substrate made from wood can be used. If the woody snag has a smooth surface, it can be sampled in a similar manner to epilithic habitats by using the SG-92 sampler. Otherwise, periphyton is collected from woody snags by using the cylinder scrape method.

To use the cylinder scrape (snag) sampling method:

1. Look for woody snags, roots, and branches that are cylindrical in shape, and select one woody snag in each of five locations throughout the reach.
2. Record the current velocity, water depth, and light-extinction measurements for each sampling site on the Periphyton Field Form.
3. Identify the part of the branch that will be sampled for periphyton. Carefully cut off a 10- to 20-cm-long piece with pruning shears so the attached algal community is not disturbed, and place the snag in a white plastic dishpan.
4. Scrub the entire surface of each woody snag section in the dishpan with a stiff brush. Rinse the brush and each section into the dishpan. Recycle rinse water to minimize the sample volume.
5. Pour the sample from the dishpan through a funnel into a plastic graduated cylinder to measure the volume, and then pour the sample into the 500-mL sampling bottle.
6. **Place the bottle on ice in a cooler and keep in the dark until the sample is processed. Do not exceed the sample holding times** (see sections 7.4.5.A and 7.4.6).
7. Measure the length and diameter of each cleaned woody snag section and calculate the total sampling area by using the following formula (assumes a cylinder):

$$\text{Total sampling area } (cm^2) = \Sigma_{i}^{n} \pi \, d_i \, l_i$$

where,

$\pi = 3.1416$

d_i = diameter of a snag, in centimeters

h_i = height (length) of a snag, in centimeters

Alternatively, a foil template can be used (see the top-rock scrape method) for irregularly shaped woody snag sections.

Episammic and epipelic habitat sampling: inverted petri dish method

Soft substrates such as silt, clay, and sand are frequently sampled to represent depositional habitats. Quantitative periphyton samples are collected from the upper 5- to 7-mm layer of sand (episammic) or fine silt (epipelic) microhabitats in depositional areas of the sampling reach. Depositional habitats where sediment and algae accumulate are: on the downstream side of an object that protrudes into the flow, such as a log or large boulder; at the water's edge in slow-flow areas; and on the bottom of pools.

Episammic: Benthic habitat consisting of sand-sized (>0.064 to 2 mm) particles on which organisms are attached or loosely associated.

Epipelic: Benthic habitat consisting of silt-sized (<0.064 mm) streambed sediments on which organisms are loosely associated. This habitat is commonly found in areas of low velocities, such as pools and side-channel areas, where silt can deposit.

To compare the data from different sites, it is best to target one type of habitat. There are some difficulties collecting samples from the bottom of pools: the sample collector often must swim to get to the bottom of the pool; deep pools often have low visibility; and soft substrates are easily disturbed, so any movement in the pool can disrupt the sample area. For these reasons, collecting from the first two areas mentioned above are recommended. Soft substrates are sampled with the inverted petri dish method.

TECHNICAL NOTES AND TIPS–5. Use only the lid of the petri dish and keep only the lid with sampling equipment and supplies to prevent use of the petri dish bottom, which has a different area.

To use the inverted petri dish method:

1. Select five locations in the reach that have a depositional zone consisting of either sand or silt substrates. All five locations must be either sand or silt.
2. At each location, hold the lid of a small plastic petri dish (about 47 mm, or approximately 17 cm^2) upside down in the water; rub the inside of the lid to remove air bubbles.
3. Turn the inside of the lid toward the substrate that will be sampled without disturbing the sediments.
4. Carefully and slowly press (in cookie-cutter fashion) the petri dish lid into the streambed sediment.
5. Slide the lid onto a spatula (fig.7.4–5f) or trowel to enclose the discrete collection. Holding the petri dish tight against the spatula, carefully wash extraneous sediment from the spatula and then lift out of the water.
6. Invert the lid and remove the spatula.
7. Rinse the sediment from the lid with filtered stream water into a 500-mL sample bottle.
8. Repeat this collection procedure at each additional sampling location in the reach.
9. Combine the five discrete collections in a 500-mL sample bottle (the total area will be about 85 cm^2).
10. Rinse the silt and sand from the threads of the sample bottle, tighten the lid, and **keep the bottle on ice in the cooler until the sample is processed.**
11. Measure and record the depth, current velocity, and light conditions at each location. Include these records along with the total sampling area on the sample label and Periphyton Field Form.

7.4.4.B MACROALGAE AND MACROPHYTE ASH-FREE DRY MASS SAMPLING METHODS

To adequately characterize biomass from quantitative periphyton samples of macroalgae, for example, *Cladophora* (fig. 7.4–6a), or of macrophyte/vascular plants such as stargrass (fig. 7.4–6b), requires sampling from relatively larger areas than suggested for microalgae. Estimates of macroalgal biomass based on analysis of AFDM can be valuable for water-quality modeling and eutrophication-process studies, such as the effect of benthic macroalgae on diel cycles of dissolved-oxygen concentrations, pH, and alkalinity in the water of nutrient-enriched streams.

Macrophytes: Aquatic plants growing in or near water that are either emergent, submergent, or floating. **Aquatic plants** are also called **hydrophytic plants** or **hydrophytes.** Aquatic vascular plants can be ferns or angiosperms (from both monocot and dicot families).

Quantitative samples of macroalgae and macrophytes can be collected with invertebrate sampling equipment such as a Slack sampler (Cuffney and others, 1993; Moulton and others, 2002; Peterson and Porter, 2002), or with other devices that define a measured area of stream bottom (Britton and Greeson, 1987). **The recommended sampling method for lotic (flowing water) habitats (described here) uses the Slack sampler with a sampling area template (Moulton and others, 2002, pages 38, 39, and 75) that is placed over a representative macroalgal assemblage.** A separate Quantitative Macroalgae Field Form should be used to record the wet and dry weights along with the supporting information regarding the locations where they were collected. See example in Appendix 7.4–A, figure 3.

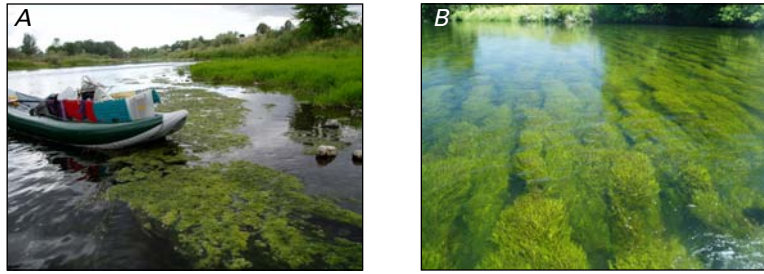


Figure 7.4–6. Examples of (*A*) filamentous green algae (*Cladophora*), and (*B*) Water Stargrass (*Heteranthera dubia*) a rooted macrophyte in the Yakima River, Washington. (Photographs by Kurt Carpenter, U.S. Geological Survey.)

To use the slack-sampler sampling method:

1. Place the Slack sampler and frame on the stream bottom, facing upstream into the flow.

2. Remove the large filaments by hand or by using a pocketknife, scissors, or wire brush, and allow filaments to flow into the net. If filaments extend beyond the sampling frame, scissors can be used to cut and remove the extended filaments so that only the algae covering the designated area of the sampling frame are included in the collection.

3. After collecting the macroalgae from within the frame, remove the sample from the net and rinse it in a dishpan with stream water to remove most of the invertebrate biomass.

4. Place the macroalgae in a labeled, resealable plastic bag. Keep the bag on ice in a cooler and do not expose to light until it is time to measure wet and dry weights.

5. Repeat these steps at four other locations for a total of five separate samples.

6. Record the depth and current velocity at each sample location on the Quantitative Macroalgae Field Form (Appendix 7.4A, fig. 3).

To use the top-rock scrape sampling method:

Quantitative macroalgal samples also can be collected with the top-rock scrape method previously described for sampling epilithic microhabitats, as follows.

1. Instead of using a frame, select and arrange representative rocks to cover the bottom of a dishpan, and measure the area of the dishpan bottom. (Measuring the area of the dishpan bottom using a foil template is not necessary if the tops of the rocks are flat.)

2. To harvest the biomass of macroalgae such as *Cladophora*: remove extraneous invertebrates and silt, pat the macroalgal/macrophyte biomass to remove excess water, and obtain a wet weight.

3. Take subsamples of the wet weight that can be processed for CHL *a*, ADFM and/or POC from the fresh (wet) subsamples of the clipped material.

4. Dry the subsamples of the clipped biomass and weigh the dried sample to obtain the dry mass of macroalgae per square meter. The subsample analysis, of CHL *a* for example, can then be estimated per meter square of biomass.

To use the clip-plot sampling method:

1. In lentic (stillwater) habitats, select representative sample points, use a frame/template to define the area, and remove all biomass above the sediment.

2. If there is sufficient velocity in the water, collect the clippings in a Slack sampler; otherwise, collect the clippings in a dishpan, rinse them with native water to remove most invertebrates, drain through a fine mesh to reduce water volume, and store in a ziplock bag.

3. Record on field forms: depth, velocity, and area. Repeat as needed to represent the habitat unit.

To use the habitat survey method:

In order to estimate macroalgal percent cover in wadeable streams, a visual survey of the stream can be made using a viewbox approach, as described by Stevenson and Bahls (1999) or a modification using a Periphyton Abundance Survey Field Form (Appendix 7.4–A, fig. 4) that incorporates the Wolman pebble count (Wolman, 1954). For example, the results from the form can be summarized based on 100 particles for each geomorphic unit (riffle, pool, or run); or based on 100 particles throughout the targeted sampling area, where

n = Total number of locations assessed,

Microalgal percent cover = (number of checks in columns 0 to 5/n) × 100, and

Macroalgae percent cover = (number of checks in macroalgae column/n) × 100.

Using the Periphyton Abundance Survey to account for macroalgae abundance in the reach, it is also possible to summarize the particle size in the reach and relate the abundance of periphyton to particle size.

SAMPLE PROCESSING AND PRESERVATION 7.4.5

Sampling and processing methods are similar, whether algal biomass will be determined from CHL *a*, AFDM, or POC. There are many items to consider between the field collection and processing of samples.

- Laboratory analyses for CHL *a*, AFDM, and POC each utilize the entire portion of sample that is submitted. Therefore,
  - — Process and submit a separate filter or subsample for each analysis being requested.
  - — Check laboratory requirements in case more than one filter is needed for a given analytical method.
- For POC follow the procedures described in NFM 5.2.2.C "Procedures for Processing Samples for Carbon Analysis."
  - — The maximum pressure of the filtering apparatus must not exceed 15 pounds per square inch (lb/in^2) for POC samples. Greater pressure may cause loss of material through the filter and will rupture algal cells, transferring carbon and (or) chlorophyll pigment from the suspended to the dissolved portion of the sample.
  - — The amount of water to be filtered to obtain a sufficient quantity of material for the analysis depends on the suspended-sediment concentration and (or) on the concentration of humic and other substances (such as organic and inorganic colloids).
  - — A graph of the historical stream stage plotted against suspended materials concentration can aid in estimating loads of suspended materials. Suspended-material concentrations can be used to help select the volume of sample to be filtered for a POC determination.
  - — Particulate analytes (TPC, PIC, POC, SOC) are reported in units of mass per volume (milligrams per liter), and therefore the volume of sample passed through each filter must be measured accurately and recorded on the field form and on the Analytical Services Request form. Record the filtrate volume passed through each filter used for particulate analysis. This is critical for calculation of POC concentrations.
- If samples must be filtered in the field, care should be taken to avoid sample exposure to direct sunlight and to be as consistent as possible with subsampling techniques.

7.4.5.A FILTERING SAMPLES FOR CHLOROPHYLL *a*, ASH-FREE DRY MASS, AND PARTICULATE ORGANIC CARBON

Use this procedure to isolate a representative fraction of any algal sample on a filter for later analysis of CHL *a*, AFDM, or POC. A similar procedure for POC is described in NFM 5.2.2.C. This procedure may also be useful for other photopigments or algal chemicals. Filter enough of the sample to retain a mass within the laboratory calibration limits.

- When filtering, apply a pressure of about 10 lbs/in^2. The maximum pressure of the filtering apparatus must not exceed 15 lb/in^2 for POC and dissolved organic carbon (DOC) samples. Greater pressure may cause loss of material through the filter and will rupture algal cells, transferring carbon and (or) chlorophyll pigment, from the suspended to the dissolved portion of the sample.
- Filter large samples to obtain a more representative sample. The volume filtered will depend on the amount of suspended material in the water, the amount of sample being collected, and the filtration time. A moderate amount of sediment or color is more than enough.
- **Do not overload the filter.** More than a 1-mm thickness of matter accumulation on the filter is too much material. If the sample is too dense to filter at 15 lb/in^2, discard it and begin with a smaller volume of water.
- The maximum/minimum calibration range ratio for chlorophyll is greater than for many other analytical methods. For example, the calibration range of the chlorophyll is 5 to 800 µg/L with reference to the 20-mL final solution extracted from the filter(s) provided by the field personnel (Bruce Anderson, U.S. Geological Survey, written commun., 2007). This calibration range corresponds to 0.1 to 16 µg CHL a on the filter.
- Visible green or brown coloration on a single filter usually indicates sufficient CHL *a* for the analysis (fig. 7.4–7f). Nondetects of CHL *a* are rare when determined using EPA Method 445.0 (U.S. Environmental Protection Agency, 1997a).

When filtering the sample:

1. Record the original water volume or habitat area from which the sample was collected, the volume or mass of the entire sample, and the volume or mass of the filtered subsample. Record these values in sampling notes, on sample containers sent to the laboratory, and in the remarks section of the Analytical Services Request form.

2. Assemble the filtration unit and vacuum flask, and connect to the vacuum pump (fig 7.4–7a).

3. Using clean forceps to place a 47-mm glass fiber filter (Whatman™ GF/F or equivalent) on the base of the filter unit (fig 7.4–7b), clamp the funnel onto the filter unit, wet the filter with DIW (fig 7.4–7c), and use the hand pump to draw a vacuum of 3 lb/in^2. **If the vacuum does not hold, check for leaks, re-wet the filter, and try again.**

4. Homogenize the full sample to create a uniform suspension of algae from which CHL *a*, AFDM, or POC subsamples are taken. Repeat the homogenization before further processing each subsample.

   - For phytoplankton, invert the sample bottle 10 times, mix in a churn splitter, or shake vigorously for 30 seconds.
   - For periphyton, invert the sample bottle 10 times and use a battery powered stirrer to break up the clumps.
   - For macroalgae or macrophytes, remove excess moisture, as in measuring wet weight as part of the dry mass procedure (section 7.4.4.B). Repeat the homogenization before further processing of each subsample.

5. Withdraw a measured subsample volume or mass, record the amount, and pour or wash the subsamples through the filter using additional DIW as necessary (fig 7.4–7d). The volumes indicated in the bullets that follow pertain to chlorophyll and AFDM; a greater volume is recommended for POC, unless a large amount of suspended material in the water causes clogging of the filter.

   - **Phytoplankton** — Pour the subsample into a graduated cylinder, working in 50- to 100-mL increments. Record the volume increment and pour into the filter funnel. Depending on plankton density, filter a total of 50 to 200 mL, although as much as 500 mL may be needed for clear oligotrophic habitats.

- **Periphyton** — Withdraw 5.0-mL aliquots using a pipette and dispense onto the glass-fiber filter. A total of 10 mL is typically sufficient for the analysis.
- **Macroalgae and macrophytes** — Cut or tear 0.5 to 2 g wet weight of representative material from the full sample and transfer it to the filter (not to exceed 1 mm height of biomass on the filter), using a small amount of DIW if needed.

6. Apply the vacuum. Do not exceed 15 lbs/in^2, depending on the units printed on the vacuum gage. Pressures above 15 lbs/in^2 will physically break algal cell walls and carbon will transfer from the suspended phase to the dissolved phase (James Kammer, U.S. Geological Survey, written commun., 2007).
7. Do not filter a particular sample for more than 10 minutes.
8. Do not allow the filter to be sucked dry. Slowly decrease the vacuum as the water level in the filter funnel gets close to the filter.
9. Rinse any subsample containers into the filter funnel and rinse the sides of the funnel with DIW. Allow water to be vacuumed completely from the sample, then release the vacuum from the system.
10. Examine the filter (fig. 7.4–7f). Sample volumes should be withdrawn to ensure that adequate algal biomass, **indicated by a green or brown color,** is retained on the surface of the glass-fiber filter after the filtration process. If algal coverage is sparse, repeat the previous steps 4 through 10 to add an additional subsample aliquot.
11. Using clean forceps, remove the filter from the filter unit, fold it in quarters (pie shape), wrap the folded filter in aluminum foil to protect from light, and place it in a clean petri dish (fig. 7.4–7g).
12. Seal the petri dish and label the sample (figs. 7.4–7h and 7.4–7i).
13. Immediately store the filtered sample in a freezer or on dry ice. **Filter replicate samples and keep them frozen so that they can be sent to the laboratory later in case of loss, damage, or unusual results.**

a. Assemble the filtration unit and vacuum flask, and connect to the vacuum pump.

b. Use clean forceps to place a 47-millimeter glass fiber filter (Whatman™ GF/F or equivalent) on the base of the filter unit.

c. Clamp the funnel onto the filter unit, wet the filter with deionized water, and use the hand pump to draw a vacuum of 10 pounds per square inch.

Figure 7.4–7. Steps for processing algal samples.

d. Withdraw a measured volume, making sure the solution is well-mixed.

e. Pour measured amounts through the filter and record the volume.

f. Examine the filter. Sample volumes should be withdrawn to ensure that adequate algal biomass, indicated by a green or brown color, is retained on the surface of the glass-fiber filter after the filtration process. If algal coverage is sparse, repeat the previous step. This filter shows an ideal amount of algal biomass from a water source with little suspended mineral sediment.

Figure 7.4–7. Steps for processing algal samples — *continued.*

g. Using clean forceps, remove the filter from the filter unit, fold it in quarters (pie shape), wrap the folded filter in aluminum foil to protect from light, and place it in a clean petri dish.

h. Seal the petri dish with waterproof tape.

i. Label the sample, put it in a sealed plastic bag, and store in a freezer or on dry ice.

Figure 7.4–7. Steps for processing algal samples — *continued.*

TECHNICAL NOTES AND TIPS–6. Pipetting accurate volumes:

Measure and adjust, if necessary, the volume delivered by the pipettor—generally 5.0 mL—into a 10-mL graduated cylinder. The precise amount delivered by the pipettor may or may not be indicated by the graduations on the unit. Loosen the knob on the pipettor to adjust the volume up or down, depending on whether more or less volume delivery is indicated by the measurement in the graduated cylinder. Once adjusted, the volume delivered remains constant (± 0.1 mL), at least for that day.

7.4.5.B MEASURING MACROALGAL AND MACROPHYTE DRY MASS

Subsamples are processed for dry mass using Standard Methods (American Public Health Association, 1999, section 10400.D.3).

Use the following procedure when preparing a subsample for macroalgal and macrophyte dry-mass determination:

1. Measure the sample wet (fresh) weight
   a. Wash the sample to remove invertebrates, sediment, and organic debris.
   b. Remove excess moisture from the sample to obtain good consistency among samples and subsamples.
   c. Blot moisture from the sample using absorbent material such as paper towels.
   d. Supporting the sample on a nonabsorbent screen, centrifuge the sample by hand, or low-speed mechanism, or use another consistent method.
   e. Weigh the sample to the nearest 0.1 g.
2. Extract a representative subsample (at least 10 percent) from the wet-weight sample. Weigh the subsample and the record wet weight of the full sample and the subsample on field forms and the Analytical Services Request form.
3. Seal the subsample in aluminum foil, then label, freeze, and send to the laboratory.

SAMPLE HOLDING TIMES 7.4.5.C

The recommended length of time from the point of collection to the point in time when live samples are processed by filtering and freezing varies, depending on study or program requirements. The amount of time that a sample can be kept in the dark on ice without significant degradation of chlorophyll concentration ranges from hours for the most sensitive phytoplankton (oligotrophic lakes to less sensitive massive blooms of cyanobacteria), to days for periphyton (often with clumps and filaments) and larger, tougher macroalgal/macrophyte plants. Holding time is an important consideration because time-sensitive samples may need to be filtered in the field and placed on dry ice.

- The more biomass that needs to be processed, the greater the importance of doing the work under controlled conditions in the laboratory. Biggs (1987) found that mechanical blending to break up clumps of periphyton improved subsampling reproducibility. Studies by USEPA using methods 445.0 and 446.0 on phytoplankton processing from seawater suggest that the sample should be filtered as soon as possible after sampling since algal populations (thus, CHL *a* concentrations) can change in relatively short periods of time.
  - Strickland and Parsons (1972) state that phytoplankton may be stored in a cool dark place for up to 8 hours or for more prolonged periods at -20^{o}C after being filtered.
  - Storage experiments on periphyton samples (Biggs, 1987) found that it is possible to obtain reliable estimates of periphyton CHL *a* concentrations from 2 to 3 days after sample collection, as long as the samples are on ice and in the dark.
- **Once samples are filtered they must be frozen immediately and kept frozen until extraction in the laboratory.** The filters can be held in a freezer at the Water Science Center over the weekend. Holding time of filters for analysis is 24 days from the date of sample collection, so it is best to ship within a week of sampling.

7.4.6 FIELD-DATA DOCUMENTATION, LABELING, AND SAMPLE PACKAGING AND SHIPPING

Following the correct procedures for documentation, labeling, packaging, and shipping of samples is as neccessary for preserving sample integrity as is using the correct sampling and sample-processing methods and technique.

7.4.6.A Review of field forms and sample labels

The following guidance pertains to USGS protocols and procedures.

- Before departing from the sampling site, the field team should review all of the information recorded on the field forms, sample bottles, and petri dish labels for accuracy and completeness. Sample labels must include the station name and station number, date, time, type of sample, area or substrates sampled, total sample volume, subsample volume, and collector's name.
- Check the calculations of total volume and other sample information that were recorded on the field forms. Place clear tape over the completed sample label to protect it against deterioration in ice-filled or dry-ice filled shipping coolers. Place all field forms (blank and completed) in separate resealable bags for additional protection.

To complete field data documentation:

- To complete field data documentation:

  1. Record the following parameter codes on the field form and enter them into NWIS-QWDATA.
     - 00078 Secchi Depth
     - 00003 Sampling Depth
     - 84164 Sampler Type
     - 82398 Sampling Method
  2. Use the Data Qualifier Codes and the Field Result Comment to record any deviations from the sampling protocols, such as holding times, filtration times, and storage temperatures.

When labeling samples:

1. Label the petri dish with the following information:
   - Site identifier (ID)
   - Date
   - Time
   - Volume processed for CHL *a*, AFDM, or POC:
     – Record volume filtered in milliliters for phytoplankton.
     – Record total area (in square centimeters sampled, total sample volume (in milliliters), and subsample volume (in milliliters) filtered for periphyton.
   - Container type (for analysis of CHL *a*, AFDM, or POC)
2. Recommendation: On the foil surrounding the filter, write the date and a shortened form of the site and sample identifiers, in the event that the filter gets separated from its petri dish in the laboratory.

Packaging and shipping microalgal samples 7.4.6.B

Special procedures must be followed when packaging samples for microalgal analysis in order to prevent sample degradation. In addition, Federal regulations apply to all samples that are shipped on dry ice, including how to fill out the shipping label and airbill.

Sample packaging

▶ Package each filter separately by folding the filter twice with the sample folded inside, wrapping it in a small piece of foil, and placing it in a plastic petri dish (47 mm).

— Each sample that is sent to the USGS National Water Quality Laboratory (NWQL) will require a separate petri dish and label. However, if sample splits are being analyzed at a local laboratory, several filters of the same filtered volume may be placed in a single labeled petri dish.

— Although some laboratory schedules may include analysis for both CHL *a* and AFDM, these analytes have different holding times and the filters need to be packaged separately.

- The filter for POC analysis should be placed in aluminum foil envelope(s), then into two Whirl-Pak® bags (6-ounce and then a larger 18-ounce bag), and then maintained in an ice-filled cooler at or below 4°C during storage and shipment to the laboratory.
  - — **Samples can be shipped on ice or on dry ice.** Samples are frozen once they reach the NWQL.
  - — Ship POC samples and CHL *a* samples in two separate coolers.
- Seal the edge of the petri dish with plastic tape. Ensure that the necessary information is recorded on the sample label on the petri dish (site, date, total sample area, total volume, subsample volume, and sample identifier) and on the appropriate field form.
- Place the labeled petri dishes in resealable plastic bags in a cooler containing dry ice.
- Attach the necessary Analytical Services Request (ASR) form and data information to the inside lid of the cooler that contains the sample(s) and dry ice. These **ASR forms must be in the same cooler as the samples** so they do not become separated, especially if a cooler is placed within a cooler.

Shipping samples on dry ice by Priority Overnight

The U.S. Department of Transportation regulates the shipment of dry ice because it is a hazardous material. Atmospheric concentrations of carbon dioxide greater than 5 percent (60 g/m^3) are immediately dangerous to life or health. Dry ice is classified as a class 9 miscellaneous hazard and there are fines and penalties that the shipper may be subject to if improper procedures are followed. **The improper packaging of dry ice may result in explosion, suffocation, and (or) severe burns.**

- Use a minimum of 10 lbs of dry ice per day to keep samples frozen.
- The packaging must be able to withstand the extremely cold temperature of the dry ice without becoming brittle in order to prevent exposure to humans. Usually a small cooler or ice chest is used.

- The packaging must allow for the escape of carbon dioxide gas in order to prevent explosion. Punch a small hole in any plastic bags that may contain the dry ice. When taping the cooler lid shut leave a little slack in the packing tape so that the lid can move slightly.
- Samples must be secured so that they will remain in contact with the dry ice during shipping, taking into account that the dry ice will shrink as it sublimates.
- All free space must be occupied with packing material to minimize the volume of air. Good examples of packing materials are cardboard, newspaper, and styrofoam. **Do not use bubble wrap,** as the extreme cold of the dry ice causes the air to contract, turning the bubble wrap into a sheet of plastic.
- Do not ship samples unless they can be received by the laboratory the next day. Ship Monday through Thursday overnight if possible. Notify the analytical laboratory that samples are on the way.

Shipping label and airbill

- Dry ice labels can be obtained directly from the selected transportation carrier. Fill out the label completely. A dry ice sticker selection can be found at: http://images.google.com/images?svnum=10&hl=en&lr=&q=dry+ice+label (Accessed August 28, 2007.)
- On the an airbill, section 6 Special Handling, under the question "Does this shipment contain dangerous goods?" you must check "Yes Shipper's Declaration not required." Just to the right of that box is another checkbox, number 6, for Dry Ice. Check the box and enter the number of packages and their weight in kilograms.
- Note for USGS personnel when submitting frozen samples to NWQL:
  - **Call the NWQL** and make arrangements to ship CHL *a*/AFDM filters to NWQL with a shipper that can handle the dry-ice component of the samples. Prepare the NWQL ASR forms and shipping labels in advance.
  - **Do not ship samples on Fridays.** The filters can be held in a freezer at the Water Science Center over the weekend. The holding time of filters for analysis is 24 days; **ship samples within 1 week of sampling.**

— **Samples that must be submitted frozen, such as chlorophyll, will be qualified as "Sample warm when received" if they arrive at the laboratory (NWQL) at a temperature greater than 2°C.** The collector and project chief listed on the ASR will receive an e-mail indicating that an exception was created due to samples arriving at the improper temperature.

— Retain duplicate CHL *a* filters frozen, as backup samples, for analysis in case of shipping problems.

TECHNICAL NOTES AND TIPS–7. Maintaining dry ice:

To prevent dry ice from sublimating, keep the dry ice well insulated. Use a small cooler in the field for freezing the filtered samples. Package the cooler with newspaper or brown paper bags, and layer the top with a plastic bag to create an air-tight seal. Place samples at the bottom of the cooler, because warm air rises. Only open the cooler for brief periods when necessary to add or remove samples. Following these tips will decrease the amount of dry ice required (10 lbs for small 'pony' coolers) and extend the viability of the dry ice. For shipping to the NWQL, 10 lbs of dry ice per day of travel is recommended (a minimum of 20 lbs to ensure that samples arrive safely).

— Example of log-in codes for CHL *a*, AFDM, and POC, for submitting samples to the NWQL are provided in table 7.4–10. Use a consistent scheme for recording times based on the kind of sample. For example, the blank is recorded 1 minute before the environmental sample. The environmental sample is set for the nearest 10 minutes (such as 0900, 0910, 0920). The first replicate recorded as 1 minute after the environmental sample and the second replicate is recorded as 2 minutes after the environmental sample. **Note that medium codes are different for chlorophyll samples collected from phytoplankton/seston in the water column where the medium code is '9' for surface water, or from periphyton where the medium code is 'D' for plant tissue.**

Table 7.4–10. Example of log-in codes for submitting samples of chlorophyll *a,* ash-free dry mass, and particulate organic carbon to the USGS National Water Quality Laboratory.

[POC, particulate organic carbon; AFDM, ash-free dry mass; lab, laboratory; IND, Indiana Water Science Center laboratory; Q, quality-assurance sample-artificial; 2, blank sample; NWQL, National Water Quality Laboratory; 9, surface water; 7, replicate; D, plant tissue; R, quality-assurance sample-surface water; Y, quality-assurance sample-plant tissue; OEPA, Ohio Environmental Protection Agency; NA, not collected; envi, environmental; rep, replicate; --, not applicable]

	Phytoplankton and POC					Periphyton and AFDM				
Time	**Kind**	**Lab**	**Medium Code**	**Sample type**	**POC lab**	**Kind**	**Lab**	**Medium Code**	**Sample type**	**AFDM lab**
0959	blank	IND	Q	2	NA	blank	IND	Q	2	NA
1000	envi	IND	9	7	NWQL	envi	IND	D	7	IND
1001	rep	IND	R	7	NA	rep	IND	Y	7	IND
1002	rep	IND	R	7	NA	rep	IND	Y	7	IND
1003	rep	NWQL	R	7	NA	rep	NWQL	Y	7	NWQL
1004	rep	OEPA	R	7	NA	rep	OEPA	Y	7	OEPA
Total filters per site	6	--	--	--	3	6	--	--	--	5

NOTE: Only one sample is collected per site for POC (total particulate carbon and nitrogen, NWQL schedule 2631), but the sample requires three filters per sample. For the phytoplankton samples, only one filter is needed (NWQL schedule 1637:CHL *a)* because POC is collected, but for the periphyton sample the schedule calls for two filters per sample (NWQL schedule 1632: 1 for CHL *a* and one for AFDM).

7.4.7 SELECTED REFERENCES FOR ALGAL BIOMASS INDICATORS

American Public Health Association, 1999, Standard methods for the examination of water and wastewater: American Public Health Association, American Water Works Association, and Water Pollution Control Federation, 20th ed., Washington, D.C.

Barbour, M.T., Gerrtisen, J., Snyder, B.D., and Stribling, J.B., 1999, Rapid bioassessment protocols for use in streams and wadeable rivers — Periphyton, benthic macroinvertebrates and fish (2d ed.): EPA 841-B-99-002, chap. 5, p. 5-1 to 5-30.

Biggs, B.J.F., 1987, Effects of sample storage and mechanical blending on the quantitative analysis of river periphyton: Freshwater Biology, v. 18, p. 197-203.

Bold, H.C., and Wynne, M.J., 1985, Introduction to the algae structure and reproduction (2d ed.): Englewood Cliffs, N.J., Prentice-Hall, Inc., 720 p.

Britton, L.J., and Greeson, P.E., 1987, Methods for collection and analysis of aquatic biological and microbiological samples: U.S. Geological Survey Techniques of Water-Resources Investigations, book 5, chap. A4, 363 p.

Carlson, R.E., and Simpson, J., 1996, A coordinator's guide to volunteer lake monitoring methods: North American Lake Management Society, 96 p.

Cuffney, T.F., Meador, M.R., and Gurtz, M.E., 1993, Methods for collecting benthic invertebrate samples as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 93-406, 66 p.

Danielson, T., 2006, Standard operating procedure methods for sampling stream and wetland algae: Maine Department of Environmental Protection, Bureau of Land and Water Quality, Division of Environmental Assessment Biomonitoring Program DEPLW0634, 18 p.

Davis, B.E., 2005, A guide to the proper selection and use of federally approved sediment and water-quality samplers: U.S. Geological Survey Open-File Report 2005-1087, 20 p.

Fee, E.J., 1976, The vertical and seasonal distribution of chlorophyll in lakes of the Experimental Lakes Area, northwestern Ontario — Implications for primary production estimates: Limnology and Oceanography, v. 21, no. 6, p. 767-783.

Fitzpatrick, F.A., Waite, I.R., Arconte, P.J.D., Meador, M.R., Maupin, M.A., and Gurtz, M.E., 1998, Revised methods for characterizing stream habitat in the National Water-Quality Assessment Program: U.S. Geological Survey Water-Resources Investigations Report 98-4052, 77 p.

Jeffrey, S.W., Mantoura, R.F.C., and Wright, S.W., 1997, Phytoplankton pigments in oceanography — Guidelines to modern methods: Paris, UNESCO Publishing, 661 p.

Kent State University, 2007, The great North American Secchi dip-in: accessed April 10, 2007, at http://dipin.kent.edu/index.htm.

Lane, S.L., and Fay, R.G., 1997, Safety in field activities: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. 9, accessed January 25, 2007, at http://pubs.water.usgs.gov/twri9A9/.

Mantoura, R.F.C., Jeffrey, S.W., Llewellyn, C.A., Claustre, H., and Morales, C.E., 1997, Comparison between spectrophotometric, fluorometric and HPLC methods for chlorophyll analysis, Chapter 14 *in* Mantoura, J.S.W., and Wright, S.W., eds., Phytoplankton pigments in oceanography: Paris, UNESCO Publishing, p. 361-380.

Moulton II, S.R., Kennen, J.G., Goldstein, R.M., and Hambrook, J.A., 2002, Revised protocols for sampling algal, invertebrate, and fish communities as part of the National Water-Quality Assessment Program: U.S. Geological Survey Open-File Report 02-150, 75 p.

Peterson, D.A., and Porter, S.D., 2002, Biological and chemical indications of eutrophication in the Yellowstone River and major tributaries during August 2000, *in* Proceedings of the National Monitoring Conference 2002, National Water-Quality Monitoring Council, 13 p.

Porter, S.D., Cuffney, T.F., Gurtz, M.E., and Meador, M.R., 1993, Methods for collecting algal samples as part of the National Water-Quality Assessment program: U.S. Geological Survey Open-File Report 93-409, 39 p.

Rantz, S.E., and others, 1982, Measurement and computation of streamflow — Volume 1, Measurement of stage and discharge: U.S. Geological Survey Water-Supply Paper 2175, 284 p.

Stevenson, R.J., and Bahls, L.L., 1999, Chapter 6, Periphyton protocols, *in* Barbour, M.T., Gerrtisen, J., Snyder, B.D., and Stribling, J.B., Rapid bioassessment protocols for use in streams and wadeable rivers — Periphyton, benthic macroinvertebrates and fish (2d ed.): EPA 841-B-99-002, p. 6-1 to 6-22.

Strickland, J.D.H., and Parsons, T.R., 1972, A practical handbook of seawater analysis (2d ed.): Fisheries Research Board of Canada, Bulletin 167, p. 187.

Texas Water Conservation Association, 2005, Development of use-based chlorophyll criteria for recreational uses of reservoirs: 127 p., accessed October 5, 2006, at http://www.amsa-cleanwater.org/getfile.cfm?fn=2005-06TXNutrientReport.pdf. (Accessed August 28, 2007.)

Turner Designs, Inc., 2004, SCUFA self-contained underwater fluorescence appartatus-User's Manual (rev. 2.3): Sunnyvale, Calif., 43 p., accessed August 28, 2007 at http://www.turnerdesigns.com/t2/doc/manuals/scufa_manual.pdf

Turner Designs, Inc., 2006, Effects of turbidity on *in vivo* chlorophyll fluorescence: Sunnyvale, Calif., 43 p., accessed September 2007 at http://www.turnerdesigns.com/t2/esci/turbidity_effects.html.

U.S. Environmental Protection Agency, 1996, EPA information collection rule (ICR) microbial laboratory manual: EPA/600/R-95/178, variously paged.

U.S. Environmental Protection Agency, 1997, EPA 440.0 Determination of carbon and nitrogen in sediments and particulates of estuarine/coastal waters using elemental analysis, Revision 1.4, September 1997: U.S. Environmental Protection Agency, National Exposure Research Laboratory, Office of Research and Development, 10 p., accessed August 28, 2007, at http://www.epa.gov/microbes/m440_0.pdf.

U.S. Environmental Protection Agency, 1997a, EPA 445.0 *In vitro* determination of chlorophyll *a*, and pheophytin *a* in marine and freshwater algae by fluorescence, Revision 1.2, September 1997: U.S. Environmental Protection Agency, National Exposure Research Laboratory, Office of Research and Development, 22 p., accessed March 14, 2007, at http://www.epa.gov/nerlcwww/m445_0.pdf.

U.S. Environmental Protection Agency, 1997b, EPA 446.0 *In vitro* determination of chlorophylls *a*, *b*, $c_1 + c_2$ and pheopigments in marine and freshwater algae by visible spectrophotometry, Revision 1.2, September 1997: U.S. Environmental Protection Agency, National Exposure Research Laboratory, Office of Research and Development, 26 p., accessed March 14, 2007, at http://synectics.net/resources/sac-app-01/public/marine/m446_0.pdf.

U.S. Environmental Protection Agency, 2006, Habitat measurement *in* chap. 5, Lake and reservoir bioassessment and biocriteria - Technical guidance document, accessed August 28, 2007, at http://www.epa.gov/owow/monitoring/tech/chap05.html.

U.S. Environmental Protection Agency, 2002, Methods for evaluating wetland condition — Using algae to assess environmental conditions in wetlands: EPA-822-R-02-021, accessed March 14, 2007, from http://www.epa.gov/ost/standards.

U.S. Geological Survey, 2005, Environmental compliance and protection handbook: U.S. Geological Survey Manual 445-1-H, accessed April 18, 2007, at http://www.usgs.gov/usgs-manual/handbook/hb/445-1-h/toc.html.

Vis, Morgan, 2007, Directory of algal images — Chlorophyta - Zygnema sp.: accessed April 16, 2007, at http://vis-pc.plantbio.ohiou.edu/algaeimage/pages/Zygnema.html.

Wagner, R.J., Boulger, R.W., Jr., Oblinger, C.J., and Smith, B.A., 2006, Guidelines and standard procedures for continuous water-quality monitors — Station operation, record computation, and data reporting: U.S. Geological Survey Techniques and Methods 1-D3, 51 p.

Ward, J.R., and Harr, C.A., 1990, Methods for collection and processing of surface-water and bed-sediment samples for physical and chemical analyses: U.S. Geological Survey Open-File Report 90-140, 71 p.

Wetzel, R.G., 1975, Limnology: Philadelphia, W.B. Saunders Company, p. 52.

Wolman, M.G., 1954, A method of sampling coarse riverbed material: Transactions, American Geophysical Union, v. 35, no. 6, p. 951-956.

YSI Incorporated, 2006, *In Vivo* Measurement of Chlorophyll and the YSI 6025 Wiped Chlorophyll Sensor: Yellow Springs, Ohio, p. 4, accessed September 2007 at https://www.ysi.com/DocumentServer/DocumentServer?docID=YSI_WP_E44.

7.4.8 ACKNOWLEDGMENTS

The information included in this section of the National Field Manual relies on a spectrum of expertise within the USGS and the wider scientific community, including that gained from the publications that have been referenced. The protocols that were developed for the USGS National Water-Quality Assessment (NAWQA) Program (Moulton and others, 2002) provided a foundation from which to develop this guidance document. In addition, the authors are indebted to Morgan L. Vis of Ohio University, and thank the following colleagues for their time and effort in helping to enhance the utility and technical content of this report: Kurt D. Carpenter, James H. Eychaner, Jeffrey W. Frey, Edward T. Furlong, Stephen R. Glodt, Jennifer L. Graham, James A. Kammer, Richard L. Kiesling, Carolyn J. Oblinger, Stephen D. Porter, Iona P. Williams, and Andrew C. Ziegler.

Our appreciation goes, also, to Iris M. Collies and Loretta J. Ulibarri for editorial and production assistance; to Franceska D. Wilde for her contributions as managing editor; and to Timothy L. Miller, Chief of the USGS Office of Water Quality, for his continued support of the *National Field Manual for the Collection of Water-Quality Data*.

APPENDIX 7.4–A.
Examples of Field Forms

(Modified from Moulton and others, 2002)

Page

STATION INFORMATION

Station name:		Date: (MM/DD/YY) _____/_____/_____
Station identification number:	Reach ID:	Time:_____ h
Collectors (leader):		

RELATED SAMPLING ACTIVITIES (check)

Water chemistry	Discharge	Habitat Survey	Tissues
Invertebrate survey	Fish Survey	Bed Sediment	Other:
Sample and Photographic Notes:			
Remarks:			

PHYSICAL SITE CONDITIONS

Clouds: %	Wind (circle): Calm Light Moderate Gusty	Precipitation (circle): None Rain Sleet Snow
Other:		
Stream stage: ft @ time h	Discharge: ft^3/s	Velocity (range) cm/s
Start time:____ h ; Water Temp. ___ C ; Dissolved oxygen (DO) _____ mg/L; ___pH; Specific cond.: ___ uS/cm		
Finish time: h; Temp. C; DO mg/L; pH; Specific conductance: uS/cm		
Water clarity (circle): Very turbid Turbid Slightly turbid Clear		Turbidity: NTUs
Water color (circle): Clear Black Brown Silty-tan Stained Golden Reddish Light green Dark green		
Riparian shading (circle): Exposed Partially shaded Shaded		

SAMPLING INFORMATION — Phytoplankton

Sampling method or device (check or specify):	DH 81 sampler _____	Kemmerer _____	Van Dorn _____
	Grab sample_____	Other (specify):	
	Subsurface depth where sample is collected: _____cm		
Location where sample is collected in the reach:			

Phytoplankton subsample type: (check)	Subsample volume:	Preservative volume in mL:	Sample identification numbers:
ID/enumeration:	_____mL	mL	
Chlorophyll:	___filters x ____mL=____mL	Dry ice	
Ash-free dry mass:	___filters x ____mL=____mL	Dry ice	
Residual volume:	___mL	NA	Spilled or decanted volume, no ID
Total volume of sample before subsampling: mL			
Type of preservative for ID sample, if included (circle one): Conc. Formalin Formalin (10%) Lugol's other_____			

Figure 1. Quantitative Phytoplankton Field Form

PHYTOPLANKTON SAMPLING INFORMATION (continued)			
Quality assurance sample? Y N	Type and number of QA samples: Split _____		Replicate _____
Subsample type: (check)	Subsample volume:	Preservative	Subsample ID no.:
ID/enumeration:	mL	mL	
Chlorophyll:	mL	Dry ice	
Ash-free dry mass:	mL	Dry ice	
Residual volume:	mL	NA	NA
Total	mL	NA	NA
Type of preservative (circle one): Conc. Formalin Formalin (10%); Lugol's; other _____			
Remarks:			

SUPPORTING INFORMATION					
Sample location number	Water depth (cm)	Velocity (cm/s)	Secchi depth (cm) or Turbidity NTUs	Light intensity	Other
1					
2					
3					
4					
5					

LIGHT MEASUREMENTS					
Distance from water surface	Water depth (cm)	Light intensity Reading Location #1	Light intensity Reading Location #2	Light intensity Reading Location #3	Average Light intensity Reading
Reference above water surface	-10				
1.Below water surface	1		0		
2.At equal depth intervals down to the euphotic depth, where possible					
3.					
4.					
5.					
6.					
7.					
8.					
9.					
10.					
11.					

Figure 1. Quantitative Phytoplankton Field Form — *continued*

STATION INFORMATION

Station name:		Date: (MM/DD/YY) _____/_____/_____
Site identification number:	Reach ID:	Time:_____ h
Collectors (leader):		

RELATED SAMPLING ACTIVITIES (check)

Water chemistry	Discharge	Habitat Survey	Tissues
Invertebrate survey	Fish Survey	Bed Sediment	Data sonde_____ Hobo Temp. ___Other:
Sample and Photographic Notes:			
Remarks:			

PHYSICAL SITE CONDITIONS

Clouds: %	Wind (circle): Calm Light Moderate Gusty	Precipitation (circle): None Rain Sleet Snow
Other:		
Stream stage: ft @ time h Discharge ft^3/s Velocity (range) cm/s		
Start time: h; Water temp. ____ C; Dissolved oxygen (DO) ___ mg/L; ___ pH; Specific cond.: ___uS/cm		
Finish time: h; Water Temp. C; DO mg/L; pH; Specific cond.: uS/cm		
Water clarity (circle): Very turbid Turbid Slightly turbid Clear : Turbidity _____NTU		
Water color (circle): Clear Black Brown Silty-tan Stained Golden Reddish Light green Dark green		
Riparian shading (circle): Exposed Partially shaded Shaded		

SAMPLING INFORMATION — MICROALGAE

Periphyton microhabitat sampled (check):	Epipelic	Epidendric	Epiphytic
	Episammic	Epilithic	Other
Sampling method or device (check or specify):	SG-92	Top-rock scrape	Cylinder scrape
	Slack sampler	Gravel sampler	Petri dish
	Other (specify)		
Measured areas sampled by device and microalgal cover (0-5) see scale below:	Beginning diameter: cm/	Ending diameter: cm/	

cm/	cm/	cm/	cm/	cm/	cm/	cm/	cm/
cm/	cm/	cm/	cm/	cm/	cm/	cm/	cm/
cm/	cm/	cm/	cm/	cm/	cm/	cm/	cm/

Average area sampled by device (SA): cm^2	
Number of areas sampled:	Total area of microalgal periphyton sample: cm^2
	Total volume of sample before subsampling: mL

Microalgal cover	Definition of periphyton abundance survey form categories
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 microalgae layer from 0.5 mm to 1 mm thick is evident
3	Accumulation of microalgae layer from 1 mm to 5 mm thick is evident
4	Accumulation of microalgae layer from 5 mm to 2 cm thick is evident
5	Accumulation of microalgae layer greater than 2 cm thick is evident

Figure 2. Quantitative Targeted-Habitat Periphyton Field Form

SAMPLING INFORMATION – MICROALGAE (continued)			
Subsample type:(check)	Subsample volume amounts:	Preservative volume:	Sample identification numbers:
ID/enumeration:	____mL	mL	
Chlorophyll:	___filters x____mL=____mL	Dry ice	
Ash-free dry mass:	___filters x____mL=____mL	Dry ice	
Residual volume:	_____mL	NA	Spilled or decanted volume, no ID #
Total volume of sample before subsampling: mL			
Type of preservative (circle one): Formaldehyde; Formalin (10%); Lugol's; other_____			

Quality assurance sample? Y N		Type: Split Replicate	
Subsample type: (check)	Subsample volume:	Preservative:	Sample identification numbers:
Chlorophyll:	#___filters x____mL=____mL	Dry ice	
Ash-free dry mass:	#___filters x____mL=____mL	Dry ice	
Total volume of sample before subsampling: mL			

SUPPORTING INFORMATION					
Sample location number	Water depth (cm)	Velocity (cm/s)	Secchi depth (cm) or Turbidity NTUs	Riparian shading (shaded, partial or full sun)	Macroalgae present:Y N Type of algae or color; Other:
1					
2					
3					
4					
5					

LIGHT MEASUREMENTS							
Distance from water surface	Water depth (cm)	Light intensity reading location 1	Light intensity reading location 2	Light intensity reading location 3	Average light intensity (units)	Difference between subsurface reading and available light	Percent reduction of available light with depth
Reference above water surface	-10						
1.Below water surface	1					0	0
2.At 5 to10 cm below water surface, and equal depth intervals down to streambed							
3.							
4.							
5.							
6.							
7.							
8.							
9.							
10.							
11.							

Figure 2. Quantitative Targeted-Habitat Periphyton Field Form — *continued.*

STATION INFORMATION

Station name:		Date: (MM/DD/YY) _____/_____/_____
Site identification number:	Reach ID:	Time:_____ h
Collectors (leader):		

RELATED SAMPLING ACTIVITIES (check)

Water chemistry	Discharge	Habitat Survey	Tissues
Invertebrate survey	Fish Survey	Bed Sediment	Data sonde_____ Hobo Temp. ___Other:
Sample and Photographic Notes:			
Remarks:			

PHYSICAL SITE CONDITIONS

Clouds: %	Wind (circle): Calm Light Moderate Gusty	Precipitation (circle): None Rain Sleet Snow
Other:		
Stream stage: ft @ time h Discharge ft^3/s Velocity (range) cm/s		
Start time:_____h; Water temp. ____ C; Dissolved oxygen (DO)____ mg/L; ____pH; Specific cond.: ____uS/cm		
Finish time: h; Water Temp. C; DO mg/L; pH; Specific cond.: uS/cm		
Water clarity (circle): Very turbid Turbid Slightly turbid Clear : Turbidity _____NTU		
Water color (circle): Clear Black Brown Silty-tan Stained Golden Reddish Light green Dark green		
Riparian shading (circle): Exposed Partially shaded Shaded		

SAMPLING INFORMATION — MACROALGAE (RTH component A)

Periphyton microhabitat sampled (check):		Epipelic	Epidendric		Epiphytic	
		Episammic	Epilithic		Other	
Sampling method or device (check or specify):		Slack sampler	Top-rock scrape		Gravel core	
Other (specify)		Square frame				
Measured areas sampled by device:	Beginning area:		cm^2	Ending area:		cm^2
Average area sampled by device (SA):						
Number of areas sampled:	Total area of macroalgal periphyton sample:					m^2

Wet weight per sampling area:	g	g	g	g	g	Total g/m2
Dry weight per sampling area:	g	g	g	g	g	Total g/m2

Sample identification number:			
Subsample type (check):	Subsample volume:	Preservative voulme:	Sample identification number:
ID/enumeration:	____mL	mL	
Chlorophyll:	filters x mL= mL	Dry ice	
Ash-free dry mass:	filters x mL= mL	Dry ice	
Residual volume:	____mL		NA
Total sample volume:	____mL		NA
Type of preservative for ID sample, if included (circle one):	Conc. Formalin	Formalin (10%)	Lugol's other_____

Figure 3. Quantitative Macroalgae Field Form

Page No. [ ]

STATION INFORMATION		
Station name:		**Date: (MM/DD/YY)** _____/_____/_____ **Time:**_____ **h**
Site identification number:	Reach ID:	
Collectors (leader):		

RELATED SAMPLING ACTIVITIES (check)			
Water chemistry	Discharge	Habitat Survey	Tissues
Invertebrate survey	Fish Survey	Bed Sediment	Other:
Sampling conditions and Photographic Notes:			

Particle size diameter mm or Si = silt, Sa = sand, O = organic matter	Periphyton abundance									Comments: Periphyton color; name of dominant algae; maximum length of macroalgae
	Microalgae (check)							Macroalgae (Check)		
	0	0.5	1	2	3	4	5	Macroalgae (M or check)	Macroalgal cover: nearest 10 percent (0 to 100 percent)	
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
Subtotal										

Figure 4. Periphyton Abundance Survey Field Form

Page No. [ ]

Particle size diameter mm or Si = silt, Sa = sand, O = organic matter	Periphyton abundance: Site name and date.									Comments: Periphyton color; name of dominant algae; maximum length of macroalgae
	Microalgae (check)							Macroalgae (Check)		
	0	0.5	1	2	3	4	5	Macroalgae	Macroalgal cover: nearest 10 percent (0 to 100 percent)	
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
10										
31										
32										
33										
34										
35										
36										
37										
38										
39										
40										
41										
42										
43										
44										
45										
46										
47										
48										
49										
50										
Subtotal										

Figure 4. Periphyton Abundance Survey Field Form — *continued*

Page No. [ ]

Particle size diameter mm or Si = silt, Sa = sand, O = organic matter	Periphyton abundance, Site name and Date.									Comments: Periphyton color; name of dominant algae; maximum length of macroalgae
	Microalgae (check)							Macroalgae (Check)		
	0	0.5	1	2	3	4	5	Macroalgae	Macroalgal cover: nearest 10 percent (0 to 100 percent)	
51										
52										
53										
54										
55										
56										
57										
58										
59										
60										
61										
62										
63										
64										
65										
66										
67										
68										
69										
70										
71										
72										
73										
74										
75										
76										
77										
78										
79										
80										
81										
82										
83										
84										
85										
Subtotal										

Figure 4. Periphyton Abundance Survey Field Form — *continued*

Page No. [ ]

Particle size diameter mm or Si = silt, Sa = sand, O = organic matter	Periphyton abundance: Site name and date									Comments: Periphyton color; name of dominant algae; maximum length of macroalgae
	Microalgae (check)							Macroalgae (Check)		
	0	0.5	1	2	3	4	5	Macroalgae (M or check)	Macroalgal cover: nearest 10 percent (0 to 100 percent)	
86										
87										
88										
89										
90										
91										
92										
93										
94										
95										
96										
97										
98										
99										
100										
Totals										

Microalgal cover	1. Definition of periphyton abundance survey form categories
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 microalgae layer from 0.5 mm to 1 mm thick is evident
3	Accumulation of microalgae layer from 1 mm to 5 mm thick is evident
4	Accumulation of microalgae layer from 5 mm to 2 cm thick is evident
5	Accumulation of microalgae layer greater than 2 cm thick is evident
Macroalgae percent cover	Record M if macroalgae are present and estimate the percent to the nearest 10 percent

Figure 4. Periphyton Abundance Survey Field Form — *continued*