

The following methods were adopted from the USGS's *METHODS FOR COLLECTING ALGAL SAMPLES AS PART OF THE NATIONAL WATER-QUALITY ASSESSMENT PROGRAM* <http://water.usgs.gov/nawqa/protocols/OFR-93-409/alg1.html>

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Background:

Benthic algae (periphyton) and phytoplankton communities are characterized in the U.S. Geological Survey's National Water-Quality Assessment Program as part of an integrated physical, chemical, and biological assessment of the Nation's water quality. Water quality can be characterized by evaluating the results of qualitative and quantitative measurements of the algal community. Qualitative periphyton samples are collected to develop a list of taxa present in the sampling reach. Quantitative periphyton samples are collected to measure algal community structure within selected habitats. These samples of benthic algal communities are collected from natural substrates, using the sampling methods that are most appropriate for the habitat conditions. Estimates of algal biomass (chlorophyll content and ash-free dry mass) also are optional measures that may be useful for interpreting water-quality conditions.

Periphyton Microhabitats

Periphyton microhabitats are relatively small areas of submerged surfaces in streams and rivers that support the attachment of algae or are otherwise associated with the accumulation of algal biomass. Periphyton may be collected by scraping, brushing, siphoning, or by other methods appropriate to each microhabitat.

Epilithic - periphyton attached to rocks, bedrock, or other hard surfaces. Remove rocks from water and scrape (or hand pick) algal material into a sample container using a pocket knife or brush. Bedrock may be sampled using a PVC pipe sampler or the periphyton sampling device described in the section, "Quantitative Targeted-Habitat Periphyton Samples." It is desirable to collect epilithic samples that represent all combinations of microalgal texture and pigmentation present on rocks within the sampling reach in erosional and depositional areas.

Epidendric - periphyton attached to submerged tree limbs and roots, or on other wood surfaces. Collection methods are similar to those described for epilithic microhabitat.

Epiphytic - periphyton attached to submerged aquatic plants or macroalgae. Scrape or brush algal biomass attached to roots, stems, and leaves of aquatic vascular plants into a sample container. Squeeze the liquid contents of filamentous algal mats and aquatic vascular plants into the same container.

Epipellic - periphyton associated with fine streambed sediments. Motile algal taxa, such as diatoms, euglenophytes, and blue-green algae, occur in the top 5-10 mm of the surface

sediment. Filamentous algae also can be loosely associated with, but not necessarily attached to, the streambed in depositional areas of the sampling reach. Collect epipelic algae with a disposable pasteur pipette and bulb or with a larger suction device, such as a poultry baster. The epipelon also may be collected with a spoon or scoop in wadeable streams or from the upper surface of sediment samples collected with an Ekman or Ponar dredge in nonwadeable streams and rivers. Periphyton collections should be attempted only when there is visible pigmentation, such as brownish-gold or dark green, associated with the streambed. An attempt should be made to exclude excessive amounts of inorganic silt from the periphyton sample.

Epipsammic - periphyton associated with coarse streambed sediments, such as sand. Collection methods are similar to those described for epipelic microhabitat. Only the top 510 mm layer of sand should be collected.

The collection of representative, quantitative periphyton samples from natural substrates is preferred but presents special sampling challenges that directly affect the accuracy and precision of various structural estimates of algal standing crop. Because algal colonization (immigration and reproduction) is affected by numerous factors, such as light intensity, depth, velocity, and substrate texture, the distribution of periphyton in flowing streams is typically heterogeneous, or patchy. Although the use of artificial substrates (glass slides, clay tiles, or other introduced materials) may reduce the variability associated with natural substrates, careful sampling of natural substrates is likely to yield more complete information regarding the structure of the periphyton community and relations with benthic herbivores (invertebrates and fish).

Artificial substrates can be considered for stream reaches where natural substrates cannot be sampled because of safety issues or habitat inaccessibility, or when uniformity of substrate surfaces is an important consideration for interpreting water quality. However, quantitative algal data from artificial substrates are not directly comparable to data from natural substrates. Methods for using artificial substrates are discussed later in this document.

Macroalgae

Quantitative periphyton samples of macroalgae (for example, filamentous assemblages of *Cladophora*) require sampling from relatively larger areas than suggested for microalgae in order to provide a characterization of conditions in the sampling reach. Estimates of macroalgal biomass 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 and rivers. A limitation of quantitative collection methods for macroalgae is that the microalgal community component can be severely under-represented or absent. Therefore, a quantitative microalgal sample should be collected in conjunction with the macroalgal sample to assess the autecological character of the periphyton community.

Quantitative samples are collected to determine the biomass of macroalgae that is attached to a defined area of the streambed. Sample-collection methods described for macroalgae are also applied to quantitative sampling of aquatic mosses. A qualitative sample of the macroalgal or aquatic moss assemblage should also be collected for species identification if the taxon is not recognizable in the field. Periphyton biomass can be measured as dry mass (DM), ash-free dry mass (AFDM), or photosynthetic-pigment content (for example, chlorophyll *a* and *b* (CHL) concentrations).

Quantitative samples of macroalgae can be collected with benthic invertebrate sampling gear, such as a Surber sampler, Hess sampler, or box sampler (Cuffney and others, 1993), or with other devices, such as a cylindrical coring device or template, that defines a measured area of stream bottom. The sampling device is placed over a representative macroalgal assemblage, and algae within the template of the sampling device are removed by hand or with the use of a brush or scraper. Quantitative macroalgal samples also can be collected by scraping or brushing algae from the surface of representative rocks and estimating surface area by the foil-template method.

Sample processing methods for macroalgae differ with respect to the nature of the biomass measurement. If macroalgal samples are to be analyzed for AFDM, pour off any residual stream water from the sample, place the sample in a plastic bag with a sample label, chill the sample, and transport the sample to the laboratory. Record the area of the macroalgal sample on the field data sheet (fig. 5) and on the sample label. If weather conditions permit, the macroalgal sample can be air dried during the site visit; the dried sample is placed in a plastic bag or other container with a sample label. Air-dried samples of macroalgae do not require chilling. Determinations of AFDM can provide an inexpensive estimate of algal biomass in a stream reach, indicating relative differences in loads of nutrients and other water-chemistry constituents among streams in a basin. If project personnel have access to an analytical balance, drying oven, and muffle furnace, AFDM can be determined by laboratory methods described in Britton and Greeson (1988) or Clesceri and others (1989).

The biomass of macroalgae also can be estimated by determining the CHL content of the periphyton assemblage. This determination is particularly appropriate for studies designed to address the effects of benthic algal processes on water quality, such as relations with instream concentrations of dissolved oxygen, alkalinity, and pH. Processing of a macroalgal sample for CHL analysis is accomplished by (1) obtaining a representative subsample volume from the total volume of the macroalgal sample, (2) collecting the subsample volume on a glass-fiber filter (Whatman GF/F or equivalent) using a filtration apparatus and hand vacuum pump, and (3) wrapping the filter in aluminum foil, placing the foil into a pre-labeled container, and transporting the container to the laboratory on dry ice. Specific details of the filtration procedure are discussed in the collection procedures for microalgae.

Obtaining representative chlorophyll subsamples from samples of macroalgae can be a challenge, particularly for filamentous taxa such as *Cladophora glomerata*. The recommended sample-processing method used will depend in part on the capabilities of

the analytical laboratory and on recommendations from the regional biologist. Several sample-processing methods are suggested below. The analytical laboratory should be contacted prior to the collection of quantitative macroalgal samples for CHL determinations, particularly if sample-processing methods (2) or (3) are selected.

- 1. Cut algal filaments into smaller lengths with scissors; add sufficient stream water to constitute a known volume (for example, 1 L) of algae-water suspension; pour the suspension into a churn splitter (Ward and Harr, 1990), and withdraw a subsample volume (for example, 50 mL) for filtration. The specific subsample volume withdrawn from the churn splitter is related to the volume of algal biomass in the algae-water suspension. Sufficient subsample volume should be withdrawn to ensure that adequate algal biomass (green or brown color) is retained on the surface of the glass-fiber filter after the filtration process. Include the following information on the field data sheet (fig. 5) and on the sample label (fig. 3): area of the macroalgal sample, volume of algae-water suspension, and volume of subsample filtered.
- 2. Collect and process a quantitative macroalgal sample for DM and AFDM. Collect a smaller representative amount of macroalgal biomass from the same general stream location; place the biomass into an externally labeled sample container, and transport the sample to the laboratory on dry ice. Request that the laboratory report the CHL concentration in relation to the biomass of the sample, for example, milligrams of CHL per gram of DM. Estimate the CHL concentration per unit area by multiplying the laboratory datum by the result of the DM determination.
- 3. Collect a quantitative macroalgal sample and submit the entire sample to the laboratory for CHL analysis. All samples for CHL analysis must be placed into containers that prevent exposure to sunlight and must be shipped to the laboratory on dry ice. Record the area of the macroalgal sample on the field data sheet and on the sample label.

Methods:

Our general approach came from the USGS methods and Mike Deas' discussion with S. Porter and consisted of:

- a) a fixed area sample (a 1x3" microscope) slide area of substrate was sampled.
- b) Two samples per location are collected for (a) chlor a (c) species identification and enumeration
- c) To identify sites that had consistent characteristics we used the following criteria
 - a. Depth: 1 to 2 feet (used current meter staff)
 - b. Velocity: 1 to 2 feet per second (current meter)
 - c. Exposure: clear sky (i.e., no serious topographic shading, no riparian shading)
- d) Thus the sites were not "random" – instead the community that was probably most prevalent in the river (i.e., not the very-near shore assemblage, not the deep water assemblage) was selected

- e) Contact the specific lab/analyst to identify preservative and handling methods. Currently, YTEP is using Aquatic Analysts to process our benthic periphyton samples. We've been shipping both samples on wet ice in an insulated cooler and have had good luck – as per analyst request.

At each sampling location, a representative area was identified that has the above depth and velocity characteristics and 5 cobbles are selected that could readily be sampled care is taken to avoid collecting rocks in extremes of algal cover and physical site conditions (see 1x3sample.jpg). At each sampling location, five rocks (five rocks are sampled for the chl.a sample and five rocks are sampled for the speciation and enumeration sample) are placed in a plastic tub below the water surface to reduce loss of periphyton. The rocks are transported in the tub to a convenient sample-processing area.

Record the stream velocity, water depth, distance from the shore and the stream width for the location in which rocks will be removed for sampling on the datasheet (see grid datasheet031308.doc). Also, record any general observations that may be useful such as weather conditions and/or any drastic change in stream flow that could influence the periphyton community (i.e., recent rain event that caused increase in flow or scheduled flow releases or reductions).

Rocks were sampled by selecting five rocks that were large enough to place a 1 inch X 3inch microscope slide, firmly hold microscope slide to rock (pinning down the algae), then with a brush clean off that face of the rock. This allows you to wash away all the excess material around the microscope slide, then brush your 1x3 sample into a small plastic tray or directly into the sample jar other tools that are available at the hardware store to aid in the brushing process includes: toothbrushes, scrapers, razor blades and spatulas. Then carefully pour the contents of the tray into the sample bottle. Using distilled water is recommended to help wash all of the trays contents into the sample jar.
POUR DI WATER INTO THE CHL. A PERIPHYTON SAMPLE JAR ONLY.

Place the chl.a sample jar on ice immediately. The algae speciation and enumeration sample jar does not need to be stored on ice before it is delivered to the lab. The periphyton chl.a sample jar has a preservative of saturated solution of $MgCO_3$ already placed in the sample jar for this test and the sample jars can be requested in advance from the lab performing the analysis. The periphyton speciation and enumeration sample jar has Lugol's solution that preserves the sample for the cell ID and counts by the lab. The chl.a sample must be mailed overnight in a sealed cooler packed with wet or dry ice so the lab can perform the analysis within the 48 hour hold time. Normally, both sample jars are mailed off to the lab so they receive both samples in a timely manner.

Percent cover is measured by using a grid made out of a mesh that is approximately 1.5 square feet that is laid on the river bed to determine approximate percentage of cover (see grid.jpg). This grid data is recorded on a separate datasheet (see grid datasheet031308.doc). The information that is collected here helps measure the percent of the gravel covered by periphyton and can help characterize how dense the algae is from month to month. Place the mesh on the stream bed in an area adjacent to the area where the sampler removed the

rocks that were scraped. Effort is made to select an area that has not been disturbed by the sampling crew but still meets the same depths and velocities of location where the rocks were collected for scraping. Place your feet on the edges of the grid so that it does not float away in the river current. With the view finder visually inspect the amount of periphyton or macrophyte in each quadrant and record the amount that is covering the stream substrate and record this information on the datasheet. The datasheet contains room for two locations to place the mesh grid to record percent cover. If the sampling location has a homogenous benthic periphyton distribution then only one location is necessary. If the benthic algae community is distributed in a more patchy heterogeneous nature record percent covers in two locations to reflect the representative nature of the sampling site.

Record water quality parameters onto the datasheet (see [algaedatasheet031008.doc](#)) with a freshly calibrated multi-parameter water quality probe and record the type of sample collected, date and time and any other pertinent observations that may be useful when reporting this data.