METHODS FOR COLLECTING ALGAL SAMPLES AS PART OF THE NATIONAL WATER-QUALITY ASSESSMENT PROGRAM

U.S. GEOLOGICAL SURVEY

Open-File Report 93-409

COVER PHOTOGRAPH: Little Naches River at mouth near Cliffdell, Washington. Inset shows circular areas on rocks following removal of algae by scraping; periphyton brush (lower left) and pipettor also shown.

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U.S. DEPARTMENT OF THE INTERIOR
BRUCE BABBITT, Secretary

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CONVERSION FACTORS AND ABBREVIATIONS

Multiply	By	To obtain
	Length	
micron (µm)	0.00003937	inch
millimeter (mm)	0.03937	inch
centimeter (cm)	0.3937	inch
meter (m)	3.281	foot
	Area	
square centimeter (cm^2)	0.001076	square foot
	Volume	
liter (L)	0.264	gallon
milliliter (mL)	0.000264	gallon
	Mass	
gram (g)	0.03527	ounce, avoirdupois
	Pressure	
kilopascal (kPa)	0.1450	pound-force per square inch

Abbreviations used in this report in addition to those shown above:

cm/s	centimeter per second
°C	degrees Celsius
ft	foot
in.	inch

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ABSTRACT

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. This multidisciplinary approach provides multiple lines of evidence for evaluating water-quality status and trends, and for refining an understanding of the factors that affect water-quality conditions locally, regionally, and nationally. 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 of 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. Phytoplankton samples may be collected in large nonwadeable streams and rivers to meet specific program objectives. Estimates of algal biomass (chlorophyll content and ash-free dry mass) also are optional measures that may be useful for interpreting water-quality conditions. A nationally consistent approach provides guidance on site, reach, and habitat selection, as well as information on methods and equipment for qualitative and quantitative sampling. Appropriate quality-assurance and quality-control guidelines are used to maximize the ability to analyze data locally, regionally, and nationally.

INTRODUCTION

The collection of algal samples is designed to complement the collection of physical and chemical data at National Water-Quality Assessment (NAWQA) Program basic fixed sites and at stream locations chosen for synoptic surveys and case studies. The sampling methods and procedures described are intended to provide guidance to personnel collecting algal samples as part of the NAWQA Program.

Background

The U.S. Geological Survey's (USGS) NAWQA Program is designed to assess the status and trends of the Nation's water quality and to develop an understanding of the major sources and factors that affect the observed water-quality conditions and trends (Hirsch and others, 1988; Leahy and others, 1990; Gurtz, 1993). The NAWQA Program is organized into 60 study units on the basis of hydrologic systems (major river basins and large parts of aquifers). Personnel in each study unit conduct water-quality investigations for 4 to 5 years, followed by 5 years of low-level monitoring, with the cycle then repeated (Hirsch and others, 1988; Leahy and others, 1990). Activities are staggered so that approximately one-third of the study units are in an intensive data-collection phase each year.

The combination of physical, chemical, and biological data to be collected as part of NAWQA provides an integrated assessment of water quality within selected environmental settings. Natural and human factors that influence the quality of water will be addressed in the integrated-assessment approach of the NAWQA Program. These factors include ecoregion, geology, hydrology, and stream size, as well as land- and water-use activities. The objectives of NAWQA ecological surveys are to characterize benthic invertebrate (Cuffney and others, 1993), fish (Meador, Cuffney, and Gurtz, 1993), and algal communities, in addition to documenting instream and riparian habitat conditions (Meador, Hupp, and others, 1993).

The algal component of NAWQA ecological surveys is designed primarily to characterize the species distribution and community structure of benthic algae (periphyton) and their relation to water quality. Estimates of algal biomass (for example, ash-free dry mass and chlorophyll content) are optional and may be made in some study units. The collection of phytoplankton samples (or the use of artificial substrates for collecting periphyton samples) may be considered for large, nonwadeable streams and rivers. Stream locations are chosen to represent major natural and human factors that are thought to significantly influence the quality of water. The sampling reach, described by Meador, Hupp, and others (1993), represents the sampling unit for ecological assessments within NAWQA study units. Relations among biological community structure, water chemistry, and major natural and human factors among NAWQA study units form the basis for a national synthesis of water-quality conditions and trends.

Periphyton samples for NAWQA ecological surveys typically are collected in conjunction with the sampling of benthic invertebrates described by Cuffney and others (1993). Periphyton microhabitats are submerged surfaces in streams and rivers, such as rocks, logs, plants, sand, and silt, that support the attachment and growth of algae. Qualitative periphyton samples are intended to provide a list of species (taxa richness) present in the sampling reach. Samples of algae are collected from each periphyton microhabitat present in the sampling reach and composited into one sample. Quantitative periphyton samples are collected to measure the relative abundance and density (algal cells per square centimeter) of each taxon present in each of two contrasting instream habitat types in a sampling reach. Quantitative samples are collected using a variety of sampling devices; the appropriate choice of sampling equipment is dictated by the character of the dominant periphyton growth forms and microhabitats in the sampling reach.

Three sampling reaches are established at a subset of the NAWQA basic fixed sites to assess spatial and short-term temporal variability. For each of these intensive ecological assessments, one reach will be sampled every year for 3 successive years to assess short-term temporal changes. To evaluate the magnitude of reach-to-reach variability, two additional reaches will be sampled during 1 of the 3 years. Periphyton data will be related to corresponding physical, chemical, and biological data at each basic fixed site to evaluate taxon-specific responses to differences or changes in water and sediment chemistry, to assess the effects of algal communities on water quality, and to integrate physical, chemical, and biological characteristics into regional and national assessments of water quality.

Purpose and Scope

This report describes methods, procedures, and equipment for collecting algal samples at basic fixed sites as part of the U.S. Geological Survey's NAWQA Program. The sampling

design for algal communities uses an approach that provides a common spatial scale (a defined sampling reach) to assess biological communities and habitat characteristics. This design also considers seasonal and hydrologic conditions that affect the algal communities. Other collection methods and equipment for algae sampling are described by Patrick and Reimer (1966), Weber (1973), Pryfogle and Lowe (1979), Stevenson and Lowe (1986), Britton and Greeson (1988), and Aloi (1990).

A variety of sample-collection methods focuses on qualitative multihabitat and quantitative targeted-habitat periphyton samples. The methods used depend on the type of sample to be collected, physical conditions in the sampling reach, and the relevance of the measurement to study-unit and national objectives. Forms for recording sampling data are provided.

The scheme for processing algal samples stresses preservation methods, preparation of subsamples, and labeling. Samples sent to contract laboratories for processing are monitored according to established quality-assurance and quality-control criteria.

NATIONAL WATER-QUALITY ASSESSMENT PROGRAM SAMPLING DESIGN

The NAWQA sampling design emphasizes a multidisciplinary approach using physical, chemical, and biological tools to provide multiple lines of evidence with which to evaluate water-resource conditions. The NAWQA Program focuses on a broad spectrum of attributes and sampling approaches to collect data in relation to (1) benthic invertebrate, fish, and algal communities; (2) stream habitats; (3) water-column measures of inorganic constituents (major ions, trace elements, nutrients), physical characteristics (suspended sediment, conductance, temperature), radionuclides, and organic compounds; (4) trace elements and organic compounds in bed material and aquatic biota; and (5) hydrology. Study-unit investigations consist of four main components: (1) retrospective analysis and reconnaissance, (2) occurrence and distribution assessment, (3) assessment of long-term trends and changes, and (4) source, transport, fate, and effect studies.

Retrospective analyses and reconnaissance efforts provide information used to focus NAWQA issues and aid in the design of NAWQA studies. Retrospective analyses are designed to provide historical perspectives of water-quality conditions and biota within a study unit and to assist in the identification of major natural and human factors that control water quality in that study unit. Analysis of retrospective information also provides baseline information to assist with the identification of candidate sampling locations. Sampling locations are chosen following a reconnaissance and evaluation of candidate sampling locations.

A reconnaissance consists of a rapid site assessment, including evaluations of stream access, stream habitat conditions, proximity of major natural or human stream influences, and methods and equipment appropriate for conducting various types of sampling at that location. A reconnaissance is conducted to familiarize project personnel with watershed features of the study unit and to evaluate and select candidate locations for subsequent sampling of biological, chemical, and physical characteristics of streams. This subsequent, integrated sampling effort is known as an occurrence and distribution assessment.

The occurrence and distribution assessment characterizes geographic and seasonal distributions of water-quality conditions in relation to major natural and human sources of constituents. This assessment is designed to fill crucial gaps in existing data for each NAWQA study unit. The design of water-quality investigations conducted during the occurrence and distribution assessment represents a balance between study-unit flexibility, to target issues of local importance, and national consistency in relation to parameters measured, sampling approaches, and spatial and temporal resolution to allow for comparisons among study units. The occurrence and distribution assessment serves as a basis for designing field activities to evaluate long-term changes in water-quality conditions and studies of sources, transport, fate, and effects.

Occurrence and distribution sampling includes two distinct types of sampling locations: basic fixed sites and synoptic survey sites. Basic fixed sites are geographically "fixed" sites at which sampling for a broad suite of chemical constituents, along with continuous discharge measurements and ecological surveys, is conducted. Basic fixed sites form the basis for long-term trend and transport assessments, as well as integrated physical, chemical, and biological studies within and among cycles of the NAWQA Program. Synoptic surveys are conducted generally as one-time collections of a limited number of constituents, with the objective of answering questions concerning the sources, occurrence, and spatial distributions of constituents within a study unit.

Assessments of long-term trends and changes in selected water-quality characteristics are designed from the results of the retrospective analyses, reconnaissance, and occurrence and distribution assessments. In many study units, assessments of long-term trends and changes are conducted at a restricted number of fixed sites in a few basins chosen to represent selected environmental settings.

Source, transport, fate, and effect studies are conducted to test hypotheses and examine specific issues about characteristics and causes of any water-quality degradation. These studies are directed at high-priority water-quality issues for individual study units and the Nation. The results of these studies among study units enable the linkage of broad assessments of water-quality status and trends to specific causes and processes by example and inference. Source, transport, fate and effect studies are designed by project personnel in individual study units and are conducted at a wide range of spatial and temporal scales.

SAMPLING DESIGN FOR ALGAL COMMUNITIES

Ecological surveys characterize biological communities (algae, benthic invertebrates, and fish) and stream habitats at locations chosen to represent combinations of major natural and human factors thought to significantly influence water quality nationally and within the study unit. The communities and habitat conditions associated with a site are characterized within a defined length of the stream referred to as the "sampling reach." This approach provides a common spatial scale upon which to assess biological communities and habitat characteristics. Each sampling reach is characterized using a combination of qualitative and quantitative algal samples. The character of periphyton microhabitats present in the sampling reach determines the types of sampling devices and collection methods used for collecting representative algal samples.

Establishing Sampling Reaches

The location and length of the sampling reach are determined on the basis of a combination of repeated geomorphic channel units (Meador, Hupp, and others, 1993) and fish sampling considerations (Meador, Cuffney, and Gurtz, 1993). Composite qualitative and quantitative samples are collected within each sampling reach to characterize the algal community. Typically, a single sampling reach is established at each site; however, three sampling reaches are established at a subset of sites in order to assess variability among sampling reaches.

The primary determinant of the length of the sampling reach is the presence of repetitions of two geomorphic channel units, such as a sequence of pool, riffle, pool, riffle. Only those geomorphic channel units (riffle, run, and pool) that cover greater than 50 percent of the active channel width are considered when determining the length of the reach. If repetitions of geomorphic channel units are not present or are present at intervals of greater than 1,000 m (for example, in large rivers), then the length of the reach is determined to be 20 channel widths based on the width of the channel at the boundary of the reach. Theoretically, this length represents at least one complete meander wavelength (Leopold and Wolman, 1957). Regardless of the method used to establish the length of the sampling reach, the minimum and maximum acceptable ranges are 150 to 500 m for wadeable sites and 500 to 1,000 m for nonwadeable sites (Meador, Hupp, and others, 1993).

The location of each sampling reach is related to a durable reference point, such as a stream gage or bridge pier (Meador, Hupp, and others, 1993), that is used to permanently define the location of the sampling reach. Sampling reaches are located where instream and riparian-habitat conditions are representative of the local area and support NAWQA study-unit objectives (for example, representative of a specific land use, agricultural practice, or reference condition). In order to meet these objectives, the sampling reach may be located upstream, downstream, or adjacent to the site location as long as the water chemistry and hydrologic data collected at the site accurately reflect conditions within the sampling reach or reaches.

A hypothetical intensive ecological assessment site (basic fixed site with multiple sample reaches) is shown in figure 1. Each sampling reach is composed of repeating geomorphic units, two pools (shaded areas) and two riffles (unshaded areas). In this example, sampling reach "A" is located upstream of the basic fixed site; sampling reach "B" is located at the basic fixed site; and sampling reach "C" is located downstream from the basic fixed site. Alternatively, the study-unit biologist might decide to locate all three sampling reaches upstream or downstream from the basic fixed site as long as there are no significant intervening changes in water chemistry, hydrology, or habitat conditions among sampling reaches. Where possible, multiple sampling reaches are separated by a minimum of 150 m.

Types of Samples Collected

Qualitative periphyton samples are collected to document the occurrence of algal taxa in as many available periphyton microhabitats within the sampling reach as possible. The purpose of qualitative sampling is to develop a detailed list of the taxa present in the reach at the time of collection. This type of sample, referred to as a qualitative multihabitat (QMH) periphyton sample, is prepared by compositing collections of periphyton from microhabitats present in the sampling reach.

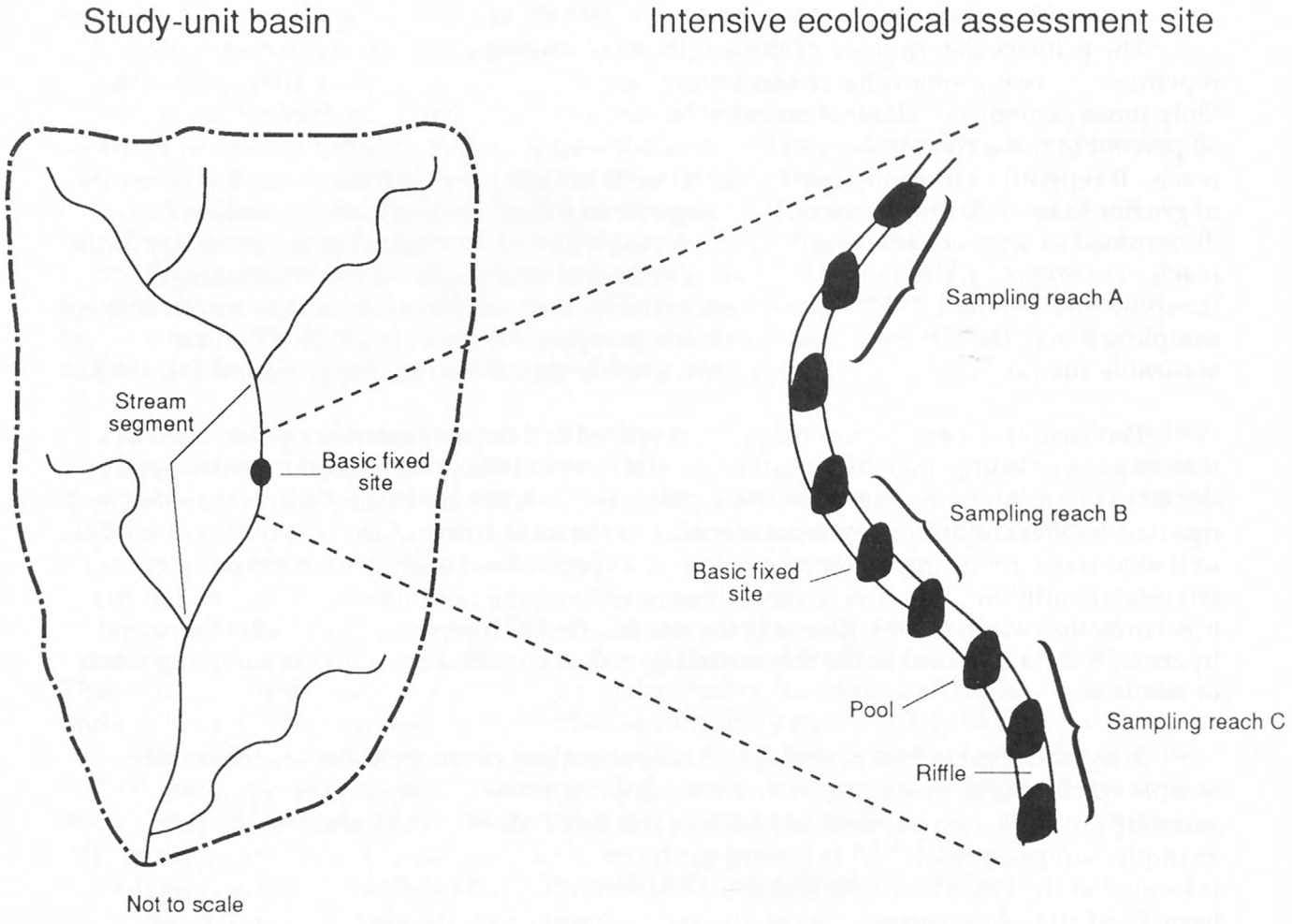

Figure 1.--Hypothetical location of a basic fixed site and three associated sampling reaches used for intensive ecological assessments.

Quantitative periphyton samples are collected to measure algal community structure and, optionally, chlorophyll content and ash-free dry mass within two contrasting instream habitat types: a taxonomically "richest" habitat (richest-targeted habitat or RTH) and a depositional-targeted habitat (DTH). These samples, along with the corresponding chemical and physical data, are used to (1) characterize the community within the sampling reach, (2) compare reaches among environmental settings, (3) compare changes in communities over time, and (4) couple physical and chemical water-quality characteristics with biological characteristics. Specific locations of the RTH and DTH habitats within a stream reach are consistent with those selected for invertebrate sampling (Cuffney and others, 1993).

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.

Epipelic - 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 5-10 mm layer of sand should be collected.

Appropriate Season and Hydrologic Conditions for Sampling

Periphyton samples collected during NAWQA ecological surveys are generally collected concurrently with benthic invertebrate samples. Although not implicitly stated as an objective of NAWQA ecological surveys, the relation of benthic algal biomass and community composition with the abundance and distribution of herbivorous invertebrates and fish likely will benefit water-quality interpretations of the floral and faunal components of aquatic biological communities. The invertebrate protocol recommends sampling to be conducted during normal, low- or stable-flow periods and delayed approximately 4 weeks following a flood with a recurrence interval greater than 5 years (Cuffney and others, 1993). This recommendation is based partly on logistical considerations because it is physically easier to sample a stream at a low, steady stage. Although algae are generally in all aquatic habitats, algal community structure and biomass can vary seasonally and in relation to antecedent hydrologic conditions. Light, temperature, current velocity, and nutrients are primary factors that influence the development, structure, and growth rates of periphyton and phytoplankton communities. Seasonal differences in these factors account for successional changes in the relative dominance of algal taxa in streams and rivers.

The intensity, duration, and quality of light influence the dominance of algal species, the structure of algal communities, and the relative amount of photosynthetic pigments produced by algal cells (Antoine and Benson-Evans, 1983; Shortreed and Stockner, 1983). For example, algal cells subjected to shaded conditions synthesize larger amounts of chlorophyll *a*, relative to algal biomass, than those exposed to high light intensity (Darley, 1982; Rosen and Lowe, 1984). Thus, the ratio of chlorophyll *a* to ash-free dry mass (CHL/AFDM) of algal communities can be relatively larger during certain seasons of the year (for example, following leaf-out of deciduous, riparian trees during spring) and in shaded stream reaches. The effects of light quantity and quality should be considered in the selection of an appropriate sampling time and location.

Water temperature influences the metabolic and reproductive rates of algae, benthic invertebrates, fish, and other aquatic organisms. Although algal growth rates can be relatively lower during periods of cold water temperature, the standing crop or biomass of periphyton communities can be comparatively large because of the absence or inactivity of grazing organisms. Temperature also can influence periphyton species composition and community structure to a greater extent than it affects the total biomass or photosynthetic pigment concentration of the algal community.

Discharge and velocity conditions at the time of sampling, as well as conditions prior to the date of sampling, must be considered when determining the appropriate time to collect algal samples. Access to designated sampling reaches can be limited during seasonal high-flow conditions. Periodic high flows, or spates, result in scouring of benthic microhabitats and washout of periphyton and phytoplankton communities. However, modest increases of current velocity following storms may actually enhance rates of algal accumulation (Stevenson, 1990; Humphrey and Stevenson, 1992). Because rates of nutrient uptake and boundary-layer diffusion between the water and benthic algal mats increase with current velocity (Whitford, 1960; Horner and Welch, 1981), moderate increases in velocity can result in increased algal biomass. Differences or changes in current velocity also are likely to affect the ratio of chlorophyll *a* to ash-free dry mass and the composition and structure of algal communities (McIntire, 1968; Belanger and others, 1985).

During relatively stable hydrologic conditions, mature periphyton communities can develop in streams and rivers after a short period (several weeks to months) of colonization and reproduction. However, algal biomass and community composition can vary considerably within and among sampling reaches in relation to differences of current velocity, light intensity, and water-chemistry factors. Field measurements of current velocity, temperature, and riparian canopy should be made in conjunction with all periphyton collections. Measurements of the relative availability of light to the periphyton community (for example, photosynthetically active radiation (PAR) or Secchi depth) should be measured when possible so that reach-specific factors may be distinguished from the effects of larger-scale, human and natural factors that influence the quality of water.

METHODS FOR COLLECTING ALGAL SAMPLES

Many types of sampling equipment and various techniques have been developed for the collection of algal samples. The proper choice of sampling equipment and technique depends on physical conditions in the sampling reach (for example, water depth, current velocity, and habitat conditions) and whether the sample is intended to provide qualitative or quantitative data. The following sections establish guidelines for collecting qualitative and quantitative samples of periphyton from natural substrates, using artificial substrates to collect periphyton, and collecting quantitative samples of phytoplankton.

Qualitative Multihabitat Periphyton Samples

Qualitative periphyton samples are composite samples collected from all periphyton microhabitats present within the sampling reach. These samples are designed to give a thorough representation of the number of taxa (taxa richness) and identity of algal taxa present in the reach, but not their abundance. A subsample of the qualitative periphyton sample also can be used to assist with the identification or verification of diatom species and varieties in quantitative periphyton samples from the same location. Water quality can be assessed by interpreting autecological information, the taxon-specific physiological requirements or tolerance for defined ranges of water-quality conditions, which is known for over 3,000 algal species (Kolkwitz and Marsson, 1908; Kolbe, 1927; Cholnoky, 1968; Palmer, 1969; Lowe, 1974; VanLandingham, 1976; Descy, 1979a and 1979b).

The objective of qualitative multihabitat (QMH) sampling is to obtain as complete a list as possible of periphyton taxa present in the sampling reach in the sampling time available, usually about 30 minutes. Qualitative algal samples should represent all possible texture and pigmentation combinations of three major periphyton growth-forms present on submerged surfaces in the sampling reach. The three major growth-forms of freshwater periphyton are operationally defined as macroalgae (morphology is visible to the eye), microalgae (morphology is microscopic, appearing as pigmented accumulations or films attached to submerged surfaces), and aquatic mosses (nonalgal periphyton, Division Bryophyta). Examples of macroalgae include filamentous growth-forms (*Cladophora*, *Spirogyra*, *Hydrodictyon*, and *Batrachospermum*), plant-like algae with leaf-like structures (*Chara* and *Nitella*), round or flattened colonies (*Nostoc*), gelatinous masses (*Chaetophora* and *Tetraspora*), and short, tubular strands (*Lemanea*). It is important to collect periphyton from a variety of locations in the sampling reach, representing the range of conditions of current velocity, water depth, and riparian shading present at the time of sampling. If all periphyton growth-forms are present in the sampling reach, the QMH periphyton sample would consist of three sample containers: (A) a sample of macroalgae, (B) a composite sample of microalgae, and (C) a sample of aquatic mosses. In contrast, if neither macroalgae nor

aquatic mosses were present in the sampling reach, the QMH periphyton sample would be represented by only one sample container containing microalgae.

Samples of macroalgae (A) are collected with forceps or by hand and placed into a sample container. Microalgae are collected by scraping, brushing, or suctioning material from all periphyton microhabitats present in the sampling reach and placing algal collections from each microhabitat into a separate container. A composite sample of microalgae (B) is prepared by compositing subsamples from the microhabitat collections. When present in the sampling reach, a sample of aquatic mosses (C) is collected with forceps or by hand and placed into a separate sample container.

The composite sample of microalgae (B) is prepared by the equally weighted composite (EWC) method. With this method, equivalent volumes of algal biomass from each microhabitat subsample are composited into a single sample container. For example, if five periphyton microhabitats are present in the sampling reach, equal volumes of biomass from each microhabitat collection are composited into a single sample of microalgae; the EWC sample of microalgae would contain approximately 20 percent of the periphyton biomass from each microhabitat subsample.

Qualitative periphyton samples are preserved with formalin and are identified with a sample label and with information recorded on the field data sheet (fig. 2). Samples are transported and stored in boxes or containers that protect the samples from exposure to light. Preserve samples with a volume of concentrated, buffered formalin sufficient to constitute a 3- to 5-percent concentration of preservative in the periphyton sample. A sample label (fig. 3) containing the following information is affixed to the sample container: site name, site identification number, sampling reach, date, time, name of the person who collected the sample, type of sample, area sampled (for quantitative periphyton samples), subsample information, type and amount of preservative, and sample identification number. The sample identification number is a unique 16-character identification code, described later in this document (refer to "Sample Processing and Labeling").

Information concerning the ecological site and sampling procedure is entered on a field data sheet (fig. 2) at the time qualitative periphyton samples are collected. The date, site name and identification number, sampling reach, and sampling team members are recorded in the "Site Information" section. The section on "Related Sampling Activities" provides space to enter benthic invertebrate, fish, water chemistry, discharge measurement, or other sampling activities that co-occur with or immediately precede algal sampling. The "Physical Site Conditions" section is for recording weather condition, water temperature, and stream stage. Data on local weather conditions that might affect sampling, such as percent cloud cover, estimated wind direction and speed, and the type, relative intensity, and duration of precipitation, are entered in the appropriate spaces. Other relevant weather-related conditions are entered in the space provided for "Other." Water temperature and time of determination are entered for the start and finish of the algal sampling effort. Stream stage is recorded if the stream reach is located at or near a USGS gaging station. All times are entered in a 24-hour (h) format. Observations on water clarity and riparian shading are recorded by circling the most appropriate descriptor.

QUALITATIVE MULTIHABITAT (QMH) PERIPHYTON SAMPLE

SITE INFORMATION		
Site name:		Date: (MM/DD/YY) _____/_____/_____
Site identification number:	Reach ID:	Time:_____ h
Sampling team (leader):		

RELATED SAMPLING ACTIVITIES

PHYSICAL SITE CONDITIONS			
Clouds: %	Wind:	Precipitation:	
Other:			
Water temp. -- Start: °C @ time h		Finish: °C @ time h	
Stream stage: ft @ time h		Velocity (range) cm/s	
Water clarity (circle): Very turbid	Turbid	Slightly turbid	Clear
Riparian shading (circle): Exposed	Partially shaded	Shaded	
Remarks:			

SAMPLING INFORMATION			
Number of containers for this sample:		Preservative: mL	
Periphyton abundance (circle one): Dense	Moderate	Sparse	None Not rated
Recognizable algal taxa (circle or indicate): *Cladophora*	*Nostoc*	*Spirogyra*	_____
_____	_____	_____	_____
Periphyton microhabitats:	Epilithic %	Epidendric %	Epiphytic %
	Epipsammic %	Epipelic %	Other %
Sample type:	Macroalgae	Microalgae	Aquatic Mosses
Sample identification numbers:	A	B	C

Figure 2.--Example of a field data sheet to record sampling information during collection of qualitative multihabitat periphyton samples.

NAWQA SAMPLE

Site name: Yakima R bl Toppenish Cr

Site ID no. 12507525 Reach: A

Date 10 / 22 / 93 Time: 1015 h

Collected by: Di A. Tom, Al G. Bloom, S.C. Umgetter

Type of sample (check one):

PERIPHYTON-QUALITATIVE (QMH):

Macroalgae (A) _____ Microalgae (B) ✓ Aquatic mosses (C) _____

PERIPHYTON-QUANTITATIVE: RTH _____ DTH _____

PHYTOPLANKTON-QUANTITATIVE: _____

SA diameter: NA cm No. SA's: NA Total area sampled NA cm^2

Subsample type (circle one):
(ID) CHL AFDM

Subsample volume: 20 mL Total sample volume: 40 mL

Preservative: Formalin ; 1 mL

ID no. YAKI1093AQE0142B

EXPLANATION

NAWQA	National water-quality assessment
ID no.	Identification number
QMH	Qualitative multihabitat
RTH	Richest-targeted habitat
DTH	Depositional-targeted habitat
SA	Sampling area
CHL	Chlorophyll *a* and *b*
AFDM	Ash-free dry mass
QA	Quality assurance
YAKI	Yakima River Basin (table 1, p. 29)

Figure 3.--Example of a completed sample label.

The "Sampling Information" section of the field data sheet contains entries for the total number of containers included in the sample, the type and volume of algal preservative used, and qualitative observations of periphyton abundance and recognizable algal taxa. Periphyton microhabitats present in the sampling reach are circled; if desired, the relative percentage of each microhabitat is estimated and recorded. Periphyton growth-forms present in the reach (macroalgae, microalgae, and aquatic mosses) are indicated by circling the sample types collected and recording the sample identification number for each one.

Quantitative Targeted-Habitat Periphyton Samples

Quantitative periphyton samples provide data on the occurrence and abundance of benthic algal taxa in two contrasting instream response habitats: (1) a richest-targeted habitat (RTH), which supports the taxonomically richest assemblage of organisms within a sampling reach, and (2) a depositional-targeted habitat (DTH), where organisms are likely to be exposed to sediment-borne contaminants for extended periods of time. Examples of RTH habitats include a riffle in a shallow, coarse-grained, high-gradient stream (epilithic periphyton microhabitat) or snag habitats in a sandy-bottom, Coastal Plain stream (epidendric periphyton microhabitat). The DTH habitat is typically an organically rich, depositional area such as a pool (epipelic or epipsammic microhabitats). The selection of an appropriate RTH is based on information derived during the NAWQA retrospective data analysis, input from the study-unit liaison committee, consultation with the USGS regional biologist, and results from reconnaissance sampling.

The objective of quantitative habitat-based sampling of periphyton is to obtain representative algal samples from the predominant periphyton microhabitat in each of two instream habitats (RTH and DTH). These objectives parallel those described for benthic invertebrates (Cuffney and others, 1993). When periphyton sampling is conducted in association with benthic invertebrate sampling (for example, at NAWQA basic fixed sites), representative microhabitats are sampled from the same general location in the sampling reach as the benthic invertebrate samples. The quantitative periphyton samples should be obtained prior to collecting qualitative algae and benthic invertebrate samples unless there are sufficient personnel and space within the sampling reach to ensure that the two sampling activities do not interfere with one another. Sampling should begin at the downstream end of the sampling reach and progress upstream.

Richest-Targeted Habitats

The purpose of collecting quantitative samples of microalgae is to measure algal abundance, species composition, and community structure in the predominant periphyton microhabitat of the RTH selected for sampling benthic invertebrates. Periphyton microhabitats in the RTH generally can be classified as epilithic, epidendric, or epiphytic; however, the RTH in some sampling reaches may be characterized by epipsammic or epipelic microhabitats. In addition to relative abundance information, which is required for NAWQA ecological surveys at basic fixed sites, optional information may include density and biovolume of algal species, chlorophyll *a* and *b*, ash-free dry mass, and other properties of the periphyton community. The appropriate periphyton sampling methods, as well as the components to be measured, should be chosen in consultation with the regional biologist and dictated by microhabitat conditions of the instream habitats selected in the sampling reach.

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.

Epilithic periphyton microhabitats

Quantitative periphyton samples of microalgae are collected from rocks or other solid, flat surfaces with the NAWQA periphyton sampling device (SG-92) (fig. 4). The SG-92 is a modified syringe sampling device similar to those described by Cushing and others (1983), Britton and Greeson (1988), and Aloi (1990). The SG-92 is constructed by cementing (cyanoacrylate adhesive) an O-ring (inside diameter, 2.06 cm [13/16 in.]; outside diameter, 2.70 cm [1 1/16 in.]) to the flanged end of the barrel of a 30-mL syringe. Periphyton brushes (fig. 4) are constructed by affixing (epoxy adhesive) small, circular sections of bristles from a stiff-bristled toothbrush to the ends of 0.64-cm [1/4-in.] diameter plastic rods. The length of the bristles is trimmed to approximately 4 mm, and the periphyton brushes are discarded when the bristle length decreases to 1 mm.

When quantitative periphyton samples are collected in conjunction with benthic invertebrate sampling, rocks with algal cover representative of the invertebrate sampling locations are chosen without intentional bias; extreme conditions, such as extremely dense or sparse algal cover, are avoided. If benthic invertebrate samples are not collected, rocks are collected from five locations representative of the epilithic microhabitat distributed throughout the sampling reach, again avoiding extremes of algal cover and physical site conditions. Stream depth and velocity are measured at the location where quantitative periphyton samples are collected, and the measurements are recorded on the field data sheet (fig. 5). At each sampling location, a minimum of five rocks 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.

Quantitative RTH periphyton samples are collected by removing attached algae contained within the circular sampling area (SA) of the SG-92 sampler with a periphyton brush. The SG-92 is firmly sealed to the substrate by pressing the O-ring of the SG-92 assembly against the rock and rotating the assembly approximately one quarter turn before dislodging periphyton with the brush. The algae-water mixture is withdrawn from the SG-92 with a 5-mL hand pipettor and placed into a sample container. Filtered stream water is then added to the SG-92, and the procedure is repeated until all algal biomass has been removed from the SA.

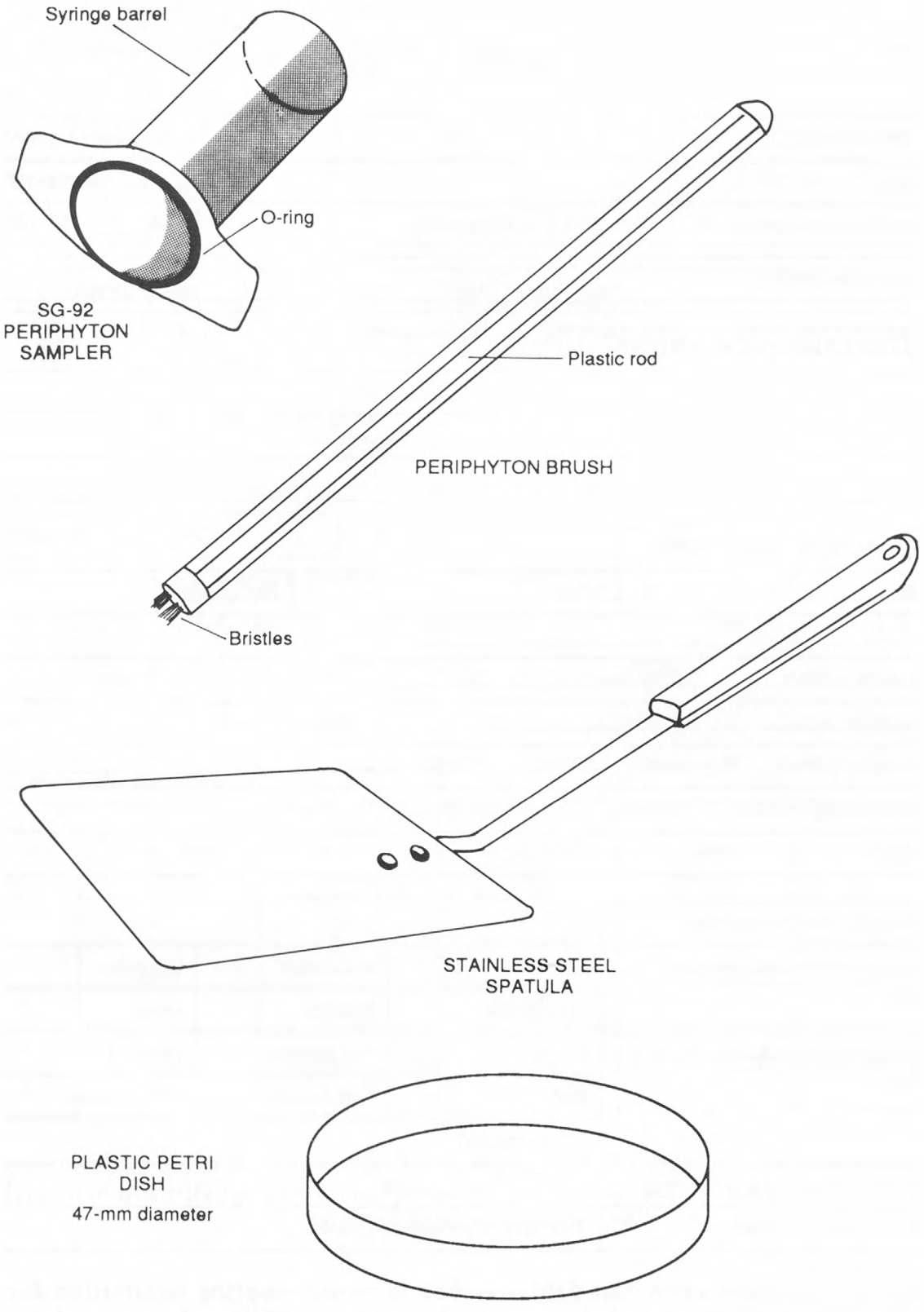

Figure 4.--Diagrams of quantitative periphyton-sampling equipment.

QUANTITATIVE TARGETED-HABITAT PERIPHYTON SAMPLES

RTH DTH (circle one)

SITE INFORMATION		
Site name:		Date: (MM/DD/YY) _____/_____/_____
Site identification number:	Reach ID:	Time:_____ h
Sampling team (leader):		

RELATED SAMPLING ACTIVITIES

PHYSICAL SITE CONDITIONS		
Clouds: %	Wind:	Precipitation:
Other:		
Water temp. -- Start: °C @ time h	Finish: °C @ time h	
Stream stage: ft @ time h	Velocity (range) cm/s	
Water clarity (circle): Very turbid Turbid Slightly turbid Clear		
Riparian shading (circle): Exposed Partially shaded Shaded		
Remarks:		

SAMPLING INFORMATION			
Periphyton microhabitat sampled (check):	Epipelic	Epidendric	Epiphytic
	Epipsammic	Epilithic	Other
Sampling method or device (check or specify):	SG-92	Foil template	Petri dish
	Surber	Pipe sampler	PVC cylinder
	Other (specify)		
Area sampled by device (SA):	Begin: cm^2	End: cm^2	
Number of areas sampled:	Total area of periphyton sample: cm^2		

Figure 5.--Example of a two-page field data sheet to record sampling information during collection of quantitative periphyton samples from richest- (RTH) or depositional- (DTH) targeted habitats.

SAMPLING INFORMATION (continued)			
Periphyton subsamples (check):	ID/enumeration ____	Chlorophyll ____	Ash-free dry mass ____
Subsample ID no.:			
Subsample volume:	mL	mL	mL
Preservative:	mL	Dry ice	Dry ice
Total volume of periphyton sample: mL		Surface area:	cm^2

Quality assurance sample? Y N	Type: Split Replicate		
Subsample type:	ID/enumeration ____	Chlorophyll ____	Ash-free dry mass ____
Subsample ID no.:		Subsample volume: mL	
Preservative (if required): mL			
Remarks:			

Artificial substrates used? Y N	Type:
Beginning of colonization period (MM/DD/YY): / /	Time: h
End of colonization period (MM/DD/YY): / /	Time: h
Total length of substrate exposure: days	
Remarks:	

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

Figure 5.--Example of a two-page field data sheet to record sampling information during collection of quantitative periphyton samples from richest- (RTH) or depositional- (DTH) targeted habitats--Continued.

For the purpose of NAWQA ecological surveys, a composite RTH periphyton sample is prepared by combining periphyton collections from five locations within the sampling reach into a single sample. At each location, periphyton is brushed and withdrawn from a minimum of one SA from each of five representative (relative to size and periphyton abundance, texture, and color) rocks. Periphyton collections from a total of five SA's, representing a total sampling area of approximately 15 cm^2 are made at each location. Periphyton collections from the five locations are composited to form the RTH periphyton sample, representing a total of 25 SA's and a total area sampled of approximately 75 cm^2. However, if periphyton abundance is very sparse, algal biomass from additional rocks or SA's must be collected in order to provide sufficient material for laboratory analysis. The diameter of the SA (typically 1.8 to 2.0 cm) is measured from rocks that have been sampled at the beginning and at the end of sampling activities at a sampling reach to ensure there has been no change during the sampling period. The area sampled (typically 2.5 to 3.1 cm^2) is calculated and recorded on the field data sheet (fig. 5). The area sampled and the total number of SA's collected for the quantitative periphyton sample are recorded on a sample label (fig. 3) and on the associated field data sheet.

When periphyton cannot be sampled with the SG-92 sampler (for example, because the rock surface is irregular and the sampler cannot be sealed to the rock without leakage), quantitative periphyton samples can be collected from the sampling reach by scraping or brushing the entire surface of representative rocks and determining the surface area by the foil template method. Individual rocks are placed into a small plastic tub with a small amount of native stream water; periphyton are scraped or brushed from the rock surface, and the algae-water suspension is poured into a sample container. The rock surface is covered with a sheet of aluminum foil, and the foil is trimmed to match the area where algae were attached. The trimmed foil template is placed into a labeled collection envelope and submitted with the quantitative periphyton sample. The surface area of the foil template can be estimated with a planimeter or by mass. Alternatively, graph paper can be used to estimate surface area colonized by periphyton. The surface area is determined by summing the number of graph squares contained in the replicate and multiplying by the area of one graph square. Additional methods for estimating the surface area of rocks are discussed by Graham and others (1988).

Collecting representative periphyton samples from gravel-sized epilithic microhabitat can be difficult because the rocks are smaller than the diameter of the SG-92 periphyton sampler, and the foil-replicate method would likely introduce substantial error into estimates of the surface area colonized by periphyton. The suggested quantitative method for sampling periphyton attached to gravel substrates involves the use of a 15.24-cm (6-in.) diameter PVC cylinder, or a similar device that serves as a template to outline a measured area of streambed (for example, a Surber sampler). The sampling device is placed in a representative stream location, and individual gravel-sized rocks are retrieved from inside the sampling device and placed in a small plastic container. A small volume of native stream water is added to the container, and periphyton are removed from all rock surfaces with a stiff-bristled toothbrush. The algae-water mixture is poured into a sample container, and the area enclosed by the sampling device (approximately 182 cm^2 for the PVC cylinder) is recorded on the sample label and on the field data sheet.

Quantitative periphyton samples may be collected from bedrock microhabitats with a PVC pipe sampler functionally similar to the SG-92 periphyton sampler. The pipe sampler is constructed from a 61-cm (2-ft length of thick-wall PVC pipe, with an inside diameter of

10.16 cm (4 in.) or more. A rubber seal is affixed to one end of the pipe. The pipe sampler is used in the same manner as described for the SG-92 sampler; a long-handled bottle brush is used to brush periphyton contained within the sampling area, and the algae-water mixture is removed by aspiration (for example, with a poultry baster). The diameter of the sampling area and the total number of areas sampled are included on the sample label and on the field data sheet.

Epidendric periphyton microhabitats

Collections of quantitative epidendric samples from natural substrates represent a special challenge because the surfaces of submerged tree limbs, trunks, and roots are generally irregular and can rarely be removed from the water without significant loss of algal biomass. In cases where the epidendric surface is relatively flat, the SG-92 periphyton sampler may be used as described for sampling of epilithic periphyton. Alternatively, a section of submerged tree root or limb can be wrapped with aluminum foil and the section severed with pruning shears or by sawing. The foil-covered section is then placed into a plastic bag and removed from the water. Periphyton biomass is brushed or scraped from the surface of the section into a sample container, and periphyton remaining on the foil or plastic bag is rinsed into the container. The surface area of algal colonization is determined by the foil-template method, as described previously. Sampling information is recorded on the field data sheet (fig. 5).

Artificial substrates may be considered for obtaining quantitative periphyton samples in reaches where neither the SG-92 nor the foil-replicate method can be used. Although artificial substrates, such as glass slides or clay tiles, may be used for this purpose, the use of weathered wood-substrates more accurately represents the availability and abundance of epidendric algal food resources for aquatic herbivores in the sampling reach.

Epiphytic periphyton microhabitats

Quantitative periphyton samples are collected from submerged leaves and stems of aquatic vascular plants (macrophytes) by gently excising submerged sections of macrophytes (leaves, leaf sections, or stems) with scissors, placing the sections into a submerged plastic bag, and removing the bag from the water. Periphyton is brushed or scraped from the macrophyte surface, and the area of colonization is determined by the foil-template method or by direct measurement of leaf dimensions. To obtain an epiphytic sample from linear-shaped macrophyte leaves (for example, *Vallisneria*), submerged sections may be gently cut with scissors, placed into submerged plastic bags, and processed as described previously. The surface area is determined by measuring the dimensions of the leaf sections and calculating the total area of periphyton colonization represented in the sample. Sampling information is recorded on the field data sheet (fig. 5).

Epiphytic samples from macrophytes with small or finely dissected leaves (for example, *Elodea* or *Ceratophyllum*) are difficult to quantify because the surface area of periphyton colonization cannot be reliably determined in the field. Although quantitative samples should be collected from these macrophytes if the epiphytic periphyton microhabitat is predominant in the RTH of the sampling reach, the regional biologist should be consulted in relation to site-specific methods of sample collection, processing, and preservation.

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 sub-sample 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.

Depositional-Targeted Habitats

Although depositional habitats may support fewer algal species than erosional habitats, algal species diversity, biomass, and primary production can be large in epipelic and epipsammic periphyton microhabitats (Hickman and Round, 1970). Photosynthesis by benthic algae in depositional habitats can contribute to oxygenation of streambed sediments (Antoine and Benson-Evans, 1985; Baillie, 1986), potentially influencing trace-element partitioning between the sediment and water (Horowitz, 1991). Benthic algal communities in depositional habitats also are likely to be exposed to sediment-borne contaminants for extended periods of time.

DTH's are typically dominated by epipelic or epipsammic periphyton microhabitats. Although epipelic and epipsammic microhabitats are relatively easy to sample

quantitatively in shallow, wadeable streams, collecting benthic algal samples from these microhabitats can be difficult in nonwadeable streams and rivers, generally requiring the use of a dredge or divers. If the DTH selected for NAWQA benthic invertebrate sampling is dominated by rocks or aquatic macrophytes, then epilithic or epiphytic periphyton microhabitats should be sampled rather than epipelic or epipsammic microhabitats.

Epipsammic or epipelic periphyton microhabitats can be prevalent throughout entire sampling reaches of streams in certain regions of the United States (notably Coastal Plain streams), as well as in streams that receive drainage from unstable soils or land-disturbance activities. Most benthic primary production occurs near the margins of these streams, where there is sufficient light penetration to support the growth of algae. Periphyton are visible as thin, pigmented (green, gold, or brown) organic accumulations on the surface of coarse or fine sediments. These periphyton communities are inherently unstable; the algal community structure reflects water-quality conditions over a period of days to weeks, depending on antecedent hydrologic conditions. The periphyton community structure also is influenced by streambed-sediment chemistry conditions at the time the sample is collected. Quantitative periphyton samples should represent conditions in the stream margins where algae are actively growing, rather than conditions in the stream thalweg where streambed scouring and lack of light penetration limit algal growth to a greater extent than do water-chemistry conditions.

Quantitative periphyton samples are collected from the upper 5- to 7-mm layer of coarse (epipsammic microhabitats) or fine (epipelic microhabitats) streambed sediments in depositional areas of a sampling reach. The top half of a disposable 47-mm plastic petri dish is gently pushed into the streambed sediment, and a small Plexiglas sheet or stainless-steel spatula (fig. 4) is slipped under the petri-dish half, sealing the sample inside the petri dish. The sealed petri-dish half is gently lifted from the stream bottom, briefly agitated (below the water surface) to remove material trapped around the edge of the petri dish, and the sample is rinsed into a sample container. The petri-dish half encloses a sampling area of approximately 17 cm^2. The DTH periphyton sample is prepared by compositing five replicate petri-dish samples collected in the sampling reach, representing a total sampling area of approximately 85 cm^2. The total sampling area is included on the sample label and on the field data sheet (fig. 5).

Using Artificial Substrates to Collect Periphyton

Artificial substrates can be considered for sampling reaches when natural substrates cannot be sampled because of inaccessibility of the microhabitat, cost of sample collection, or safety issues associated with the collection of representative samples (for example, the use of divers in large, fast-flowing rivers). Artificial substrates can also reduce the heterogeneity (or patchiness) of algae occurring on natural substrates and can be used to compare water quality among streams with disparate periphyton microhabitats. However, data from artificial substrates cannot be compared with data from natural substrates because of differences in factors that contribute to algal processes and differences in the age of the periphyton community. If artificial substrates are considered for one or more stream reaches in a basin, it is recommended that they be used at all sites so that meaningful water-quality interpretations can be made.

Artificial substrates for periphyton include benthic substrates (for example, rocks, bricks, clay tiles, glass or plastic rods, and wood dowels) and suspended substrates (for

example, styrofoam and "periphytometers" that hold glass or Plexiglas slides or coverslips). Plastic rods, flagging tape, and artificial aquarium plants also have been used to simulate epiphytic microhabitats (Aloi, 1990). In addition, nutrient-diffusing substrates can be used to assess nitrogen or phosphorus limitation in streams and offer the potential for assessing the effects of herbicides and other water-quality constituents. Examples of nutrient-diffusing substrates include clay pots or saucers (Fairchild and Lowe, 1984; Fairchild and others, 1985) and nutrient-enriched sand substrates (Pringle and Bowers, 1984; Pringle, 1987).

Artificial substrates have a number of limitations that should be considered in relation to the primary objectives of NAWQA ecological surveys, or secondary objectives within a NAWQA study unit. For example, (1) artificial substrates require a minimum of two trips to the sampling reach (installation and retrieval), separated by an interval of colonization time that can vary with season, discharge, temperature, and other environmental variables; (2) they can be susceptible to loss from vandalism or from washout during periods of storm-water discharge; (3) periphyton communities that develop on introduced substrates can be biased toward algal taxa that were actively immigrating or colonizing at the time of substrate placement and might not reflect the types or the relative abundance of algal taxa present on natural substrates (Lamberti and Resh, 1985); and (4) artificial substrates can provide a less sensitive indication of changes or differences in water quality associated with land disturbance, including increases in soil erosion and sediment transport or relative changes in the distribution of periphyton microhabitats within a sampling reach.

However, artificial substrates can be considered for obtaining quantitative periphyton samples (1) from large nonwadeable rivers, or sampling reaches where quantitative samples cannot be collected from natural substrates, (2) when uniformity of substrate surface is an important consideration for water-quality interpretations, or (3) when time-series information, such as determination of algal species-growth rates, is a secondary objective of the ecological survey (for example, a case study to investigate eutrophication processes or the effects of herbicides or other toxicants on primary production).

The preferred sampling method under these circumstances would be to use benthic artificial substrates that serve as a model for natural substrates. For example, rocks with a mineralogic composition similar to that of natural substrates can be introduced as artificial substrates into sampling reaches where rocks do not occur or where the predominant substrate is coarse sand or gravel. Alternative sampling methods include the use of (1) unglazed clay tiles, flagstone, or frosted glass (or acrylic) as an artificial substrate for rocks (Lowe and Gale, 1980; Tuchman and Stevenson, 1980); (2) Plexiglas rods (Goldsborough and others, 1986) or plastic flagging tape (Lethbridge and others, 1988) as artificial substitutes for the stems or leaves of aquatic macrophytes; or (3) wooden dowels (Millie and Lowe, 1983) or pre-weathered wood stakes to simulate snag or epidendric microhabitats.

Suspended artificial substrates can be useful for certain water-quality studies because they (1) provide a uniform substrate surface for algal colonization, (2) normalize among-site differences relative to stream depth and light availability, (3) can reduce variance among replicate samples from a reach, and (4) are less subject to relative changes in stream stage during the colonization period. However, algal biomass and community structure on artificial substrates are often very different from that observed on natural substrates in the sampling reach. Examples of suspended artificial substrates include glass slides held in a buoyant holder (Patrick and others, 1954; Britton and Greeson, 1988), nylon lines or cords

(Lang and Austin, 1984), styrofoam floats (Flint and others, 1977), and polyurethane foam substrates (Cairns and others, 1983).

The choice of an appropriate exposure period for artificial substrates is dependent on water temperature, intensity and duration of light, nutrient status of the stream, current velocity, and the objectives of the study. If artificial substrates are used as a substitute for sampling of natural substrates, the exposure period must be long enough for the algal community to develop fully, generally 1 to 2 months. Therefore, it is necessary to place artificial substrates in identified sampling reaches well before the anticipated date of the ecological survey. The length of the exposure period, hydrologic conditions during the exposure period, and sampling season can affect the extent and taxonomic composition of periphyton growth on artificial substrates (Biggs, 1988). However, if sampling is conducted in nutrient-rich streams during the warm weather season, and if the study objective is solely to compare water-quality responses of the periphyton community among reaches, an exposure period from 3 to 4 weeks is generally sufficient. Regardless of the length of time selected as the exposure period, artificial substrates should be exposed for the same time period in all sampling reaches of an ecological study. The date of substrate placement and retrieval, as well as the total time of exposure, is indicated on the field data sheet (fig. 5) and on the sample label (fig. 3).

Quantitative Phytoplankton Samples

Phytoplankton are algae that are buoyantly suspended in the water column of streams, rivers, reservoirs, and lakes. Phytoplankton are passively transported by currents and turbulent mixing, and they respond to physical and chemical conditions present at the time of sample collection; that is, they reflect water-quality conditions of the water mass in which they occur (Clesceri and others, 1989). Although certain phytoplankton taxa are useful for assessing taste and odor problems in domestic water supplies or for determining the origin or recent history of a water mass, phytoplankton are somewhat less useful than periphyton for indicating and integrating water-quality changes relative to a fixed sampling location, particularly in wadeable streams and rivers. The plankton of many streams consist of benthic algal species that have been dislodged from periphyton microhabitats as a result of biological processes (McCormick and Stevenson, 1989; Barnese and Lowe, 1992) or physical disturbance, such as scouring. Benthic diatoms also may occur in the plankton of streams as a result of taxon-specific immigration, reproduction, and emigration processes (Korte and Blinn, 1983; Stevenson, 1983). However, potamoplankton communities (those that resemble phytoplankton communities of lakes) may develop in larger streams and rivers during periods of relatively stable hydrologic conditions, particularly in large, impounded rivers (Lowe, 1980; Round, 1981).

Phytoplankton species frequently have been used as indicators of eutrophication and other water-quality conditions in streams and lakes (Williams, 1964; Palmer, 1969; Reynolds, 1984; Charles, 1985). Physiological processes of planktonic algae also affect the quality of water, particularly with respect to water transparency (or color), alkalinity, pH, dissolved nutrients and organic carbon, and dissolved concentrations of oxygen and carbon dioxide. Certain algal taxa are toxic when ingested by fish or other animals (Palmer, 1977), and microbial decomposition of massive phytoplankton blooms often can result in the depletion of dissolved oxygen, leading to fish kills from asphyxiation.

Quantitative phytoplankton samples are obtained by collecting a representative whole-water sample of sufficient volume to ensure adequate phytoplankton biomass for analysis.

A sample volume of 1 L is sufficient for samples collected from productive, nutrient-enriched rivers as indicated by water color. In contrast, a larger sample volume is required for phytoplankton samples collected from unproductive, low-nutrient rivers as indicated by water transparency. Phytoplankton samples collected in conjunction with water-chemistry sampling at basic fixed sites are taken with a depth-integrating sampler, such as a D-77; the phytoplankton sample is taken directly from the churn splitter (Ward and Harr, 1990). Alternatively, quantitative phytoplankton samples can be collected with a water-sampling bottle (Kemmerer, Van Dorn, or another type) or with a pump (Sournia, 1978; Britton and Greeson, 1988; Clesceri and others, 1989).

Phytoplankton samples can be collected at basic fixed sites over time, similar to the approach for collecting water samples for chemical and physical determinations. Seasonal changes in the abundance and composition of phytoplankton occur on temporal scales measured in weeks or months (Reynolds, 1984). Although samples collected at 4- to 6-week intervals will generally represent seasonal patterns of algal species succession, it is desirable to sample more frequently during critical water-quality periods, such as during substantial algal blooms, seasonal taste and odor episodes, and periods when diel changes of dissolved-oxygen content may adversely affect aquatic life and other water-quality issues.

The determination of chlorophyll *a* and *b* concentrations in phytoplankton communities is a study-unit option; however, such analyses are warranted for the purposes of (1) understanding the relation between algal biomass and short-term changes in dissolved-oxygen content, pH, and alkalinity of the water, (2) use in water-quality modeling efforts, and (3) improving an understanding of the sources and loads of carbon in river systems.

If chlorophyll is not to be determined, the entire sample is preserved with buffered formalin for identification and enumeration. Information concerning the site and sampling procedures is entered onto a field data sheet (fig. 6) analogous to that used for periphyton samples. Information recorded on the sample label (fig. 3) includes total sample volume.

For chlorophyll determinations, an unpreserved subsample is withdrawn from the phytoplankton sample, and the aliquot is filtered onto a glass-fiber filter, similar to the procedure described for periphyton samples. A subsample volume of 50 mL is adequate for most phytoplankton samples; however, a larger subsample may be required for samples with exceptional water clarity. Sufficient subsample volume should be filtered to ensure that adequate algal biomass is retained on the filter. Filters are wrapped in aluminum foil, placed into a sample bottle or container, and immediately placed on dry ice. The subsample volume is recorded on the sample label (fig. 3) and on the field data sheet (fig. 6), and the filters are sent to the analytical laboratory for analysis.

SAMPLE PROCESSING AND LABELING

Processing methods for quantitative algal samples vary with the type of sample and the number of algal measurements required for the ecological study. Processing includes preservation and affixing an appropriate sample label. Some samples require decanting to remove excess water. Optional samples for determinations of chlorophyll and ash-free dry mass require filtration.

QUANTITATIVE PHYTOPLANKTON SAMPLES

SITE INFORMATION		
Site name:		Date: (MM/DD/YY) _____/_____/_____
Site identification number:	Reach ID:	Time:_____ h
Sampling team (leader):		

RELATED SAMPLING ACTIVITIES

PHYSICAL SITE CONDITIONS					
Clouds: %		Wind:		Precipitation:	
Other:					
Water temp. -- Start:	°C @ time	h	Finish:	°C @ time	h
Stream stage:	ft @ time	h	Velocity (range)		cm/s
Water clarity (circle):	Very turbid	Turbid	Slightly turbid	Clear	
Riparian shading (circle):	Exposed	Partially shaded	Shaded		
Remarks:					

SAMPLING INFORMATION			
Sampling method or device (check or specify):	D-77 sampler _____	Kemmerer _____	Van Dorn _____
	Grab sample: Subsurface depth cm		
	Other (specify):		
Phytoplankton subsamples (check):	ID/enumeration ____	Chlorophyll ____	Ash-free dry mass ____
Subsample ID no.:			
Subsample volume:	mL	mL	mL
Preservative:	mL	Dry ice	Dry ice
Total volume of phytoplankton sample: mL			

Figure 6.--Example of a two-page field data sheet to record sampling information during collection of quantitative phytoplankton samples.

SAMPLING INFORMATION (continued)			
Quality Assurance Sample? Y N	Type: Split Replicate		
Subsample type:	ID/enumeration____	Chlorophyll____	Ash-free dry mass____
Subsample ID no.:		Subsample volume	mL
Preservative (if required):		mL	
Remarks:			

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

Figure 6.--Example of a two-page field data sheet to record sampling information during collection of quantitative phytoplankton samples--Continued.

Preservative for Algal Samples

Samples for identification and enumeration of algae (ID samples) should be preserved by adding full strength buffered formalin to constitute a 3- to 5-percent concentration of preservative in the periphyton sample. Buffered formalin is prepared by dissolving 20 g sodium borate in 1 L of formalin (37-percent formaldehyde solution). The volume of preservative used should be indicated on the sample container and on the field data sheet. Following preservation, a label is affixed to the sample container; the samples are transported and stored in boxes or containers that prevent exposure to sunlight.

Formalin is an excellent preservative; however, caution should be exercised in its use because it is a known carcinogen (U.S. Environmental Protection Agency, 1981). Formalin is poisonous if ingested, and exposure to small amounts can create such symptoms as a burning sensation in the eyes and nose, watering eyes, headaches, and a sore throat. Working with formalin should be done only in well-ventilated areas. Skin can become sensitized to formalin exposure, resulting in skin rashes. Any direct skin contact with formalin should be avoided by wearing rubber gloves. If formalin does accidentally contact the skin or eyes, the affected area should be washed immediately with water. Buffered formalin is properly discarded only according to Federal, State, and local guidelines.

Sample Identification Codes and Labeling

Algal samples are assigned a unique 16-character identification code that is entered on sample labels (fig. 3) and on field data sheets (figs. 5 and 6). This sample ID number provides information for the (1) name of the study unit, (2) month and year of sample collection, (3) sample type and subtype, (4) sample number, and (5) sample element code as follows (fig. 7):

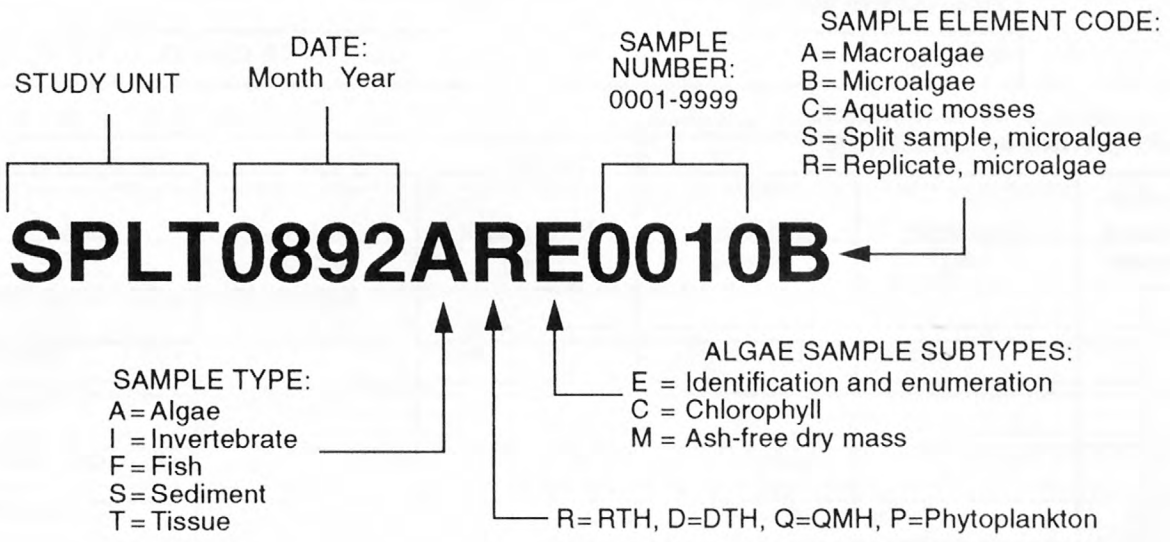

Figure 7.--Example of the 16-character sample identification code used by the National Water-Quality Assessment Program.

Characters 1-4: Study-unit abbreviation (SPLT = South Platte River Basin) (table 1)

Characters 5-8: Date of collection--MMYY (0892 = August 1992)

Character 9: Sample type, referring to the type of biological sample (A = Algae)

Character 10: First algae sample subtype, representing one of the following:

R = Quantitative richest-targeted habitat sample
D = Quantitative depositional-targeted habitat sample
Q = Qualitative multihabitat sample
P = Phytoplankton sample

Character 11: Second algae sample subtype, representing one of the following:

E = Identification and enumeration subsample
C = Chlorophyll subsample
M= Ash-free dry mass subsample

Characters 12-15: Sample number--a 4-digit sequential number for study-unit samples. Three samples, two quantitative and one qualitative, are obtained from each sampling reach and recorded sequentially. Elements of each sample are distinguished with a letter designation (character 16).

Table 1.--*Abbreviations of study-unit names used in the 16-character sample identification codes*

Study Unit	Abbreviation	Study Unit	Abbreviation
Albemarle-Pamlico Drainage	ALBE	Northern Rockies Intermontaine Basins	NROK
Allegheny and Monongahela Basins	ALGH		
Apalachicola-Chattahoochee-Flint River Basin	ACFB	Oahu	OAHU
		Ozark Plateaus	OZRK
Central Columbia Plateau	CCPT	Potomac River Basin	POTO
Central High Plains	CHPL	Puget Sound Drainages	PUGT
Central Nebraska Basins	CNBR	Red River of the North	REDN
Central Oklahoma	COKL	Rio Grande Valley	RIOG
Cheyenne and Belle	CHEY	Sacramento Basin	SACR
Chicot-Evangeline	CHEV	San Joaquin-Tulare Basins	SANJ
Connecticut, Housatonic, and Thames River Basins	CONN	Santa Ana Basin	SANA
Cook Inlet	COOK	Santee Basin and Coastal Drainage	SANT
Delaware River Basin	DELR	South Central Texas	SCTX
Delmarva Peninsula	DLMV	South Platte River Basin	SPLT
Eastern Iowa Basins	EIWA	Southeastern New England	SENE
Georgia-Florida Coastal Plain	GAFL	Southern Arizona	SOAZ
Great and Little Miami River Basin	MIAM	Southern Florida	SOFL
Great Salt Lake Basin	GRSL	Southern High Plains	SHPL
Hudson River Basin	HDSN	Southern Illinois	SILL
Kanawha-New River Basin	KANA	Trinity River Basin	TRIN
Kansas River Basin	KANS	Upper Arkansas River	UARK
Kentucky River Basin	KNTY	Upper Colorado River Basin	UCOL
Lake Erie-Saint Clair Drainage	LERI	Upper Illinois River Basin	UIRB
Long Island-New Jersey Coastal Plain	LINJ	Upper Mississippi River Basin	UMIS
		Upper Snake River Basin	USNK
Lower Susquehanna River Basin	LSUS	Upper Tennessee River Basin	UTEN
Lower Tennessee River Basin	LTEN		
Mississippi Embayment	MISE	Western Lake Michigan Drainages	WMIC
Mobile River	MOBL	White River Basin	WHIT
Nevada Basin and Range	NVBR	Willamette Basin	WILL
New Hampshire and Southern Maine Basins	NHME	Yakima River Basin	YAKI
		Yellowstone River Basin	YELL
North Platte Basin	NPLT		

Character 16: Sample element code--a single-letter code to indicate (A) the macroalgae container, (B) the microalgae container, and (C) the aquatic-mosses container. Microalgae samples that are collected for QA/QC purposes are identified by sample element codes S for split sample and R for replicate sample.

An sample label containing the following information is affixed to the sample container: site name, site ID number, sampling reach, sample ID number, date, time, type of sample, type of microhabitat, surface area of collection, and the name of the person who collected the sample. After labeling, all preserved periphyton samples are stored and transported in boxes or containers that prevent exposure of the samples to sunlight.

Preparation of Subsamples

If other periphyton measurements are required in addition to the ID sample, it will be necessary to divide the quantitative periphyton sample into three representative subsamples, preserve the ID subsample, and process the remaining subsamples for (1) CHL and (2) DM and AFDM determinations. In some circumstances, the quantitative periphyton sample will need to be homogenized (or subdivided by other means) to ensure representative subsamples for each type of periphyton measurement. Periphyton are subdivided in the field by cutting algal filaments into smaller pieces with scissors, or by using a battery-powered tissue homogenizer. The periphyton sample is shaken vigorously (approximately 40 to 50 times) prior to withdrawing subsamples for ID, CHL, and AFDM determinations. Because subsampling of filamentous periphyton assemblages can produce considerable error among the resulting replicate subsamples, it is desirable to collect separate quantitative samples for each periphyton measurement when the algal community contains appreciable amounts of macroalgae.

If all periphyton measurements are required for an ecological study, first prepare the ID subsample by withdrawing 15 mL of algae-water suspension from the quantitative periphyton sample into a 20-mL scintillation vial, using a volumetric hand pipettor. Preserve the ID subsample with 0.5 to 0.75 mL (5 to 8 drops) of concentrated, buffered formalin solution, attach a sample label to the vial, and enter the appropriate information on the field data sheet. If the ID subsample contains only small amounts of periphyton (less than a 2-mm accumulation of biomass at the bottom of the scintillation vial), prepare one or more additional 15-mL ID subsamples until sufficient biomass has been obtained. Indicate the number of containers submitted for the ID sample on the sample labels and on the field data sheet.

After the ID subsample has been withdrawn from the quantitative periphyton sample, vigorously shake the remainder of the sample and pour approximately one half of the sample volume into an unused sample container. Designate one of the two subsample containers as the CHL subsample and the other container as the AFDM subsample. Record the volume of each subsample on the field data sheet. Periphyton biomass from each subsample is filtered onto a glass-fiber filter, and the filter is submitted to the National Water-Quality Laboratory (NWQL), Arvada, Colorado, for determination of CHL and AFDM.

Procedure for Decanting Quantitative Periphyton Samples

Quantitative periphyton samples collected by certain sampling methods, such as the foil-replicate or PVC cylinder methods, usually contain a large volume of stream water relative to the biomass of algae in the sample. If CHL and AFDM are not to be determined, the sample is preserved immediately and labeled; record the volume of the sample on the field data sheet and on the sample label. If CHL and AFDM determinations are required, shake the original periphyton sample vigorously and pour a measured volume of algae-water mixture into a separate container for filtration. Record the volume of the remaining periphyton sample (the ID subsample) on the sample label and on the field data sheet; add preservative before transporting the sample to the District laboratory. Record the total volume (the original sample volume) of the periphyton sample on the field data sheet.

When field activities have been completed, excess stream water is decanted from the ID subsample to reduce the size of the container needed for subsequent transport and storage of the sample. Allow periphyton in the ID subsample to settle to the bottom of the container for a sufficient length of time (a minimum of 1 hour for each vertical centimeter of sample in the container). Water may be decanted from the sample by using a filtering flask, to which are attached (1) a vacuum pump and (2) a length of tubing with a pasteur pipette tip attached to the distal end of the tubing. A vacuum is drawn on the filtering flask and the pipette tip is inserted into the sample. Decant the samples until approximately 2 cm of water remains above the periphyton-water interface (proper disposal of preservative solution is essential). Reconstitute the sample and pour it into a (smaller) ID sample container. Place a sample label onto the container, and record the initial and final (decanted) volumes of the ID sample, as well as the name of the person who processed the sample, on the sample label and field data sheet.

Filtration Procedure for Chlorophyll and Ash-Free Dry Mass Determinations

Filtration for the optional determination of CHL and AFDM involves a stepwise procedure using equipment listed below.

Equipment used in filtration for chlorophyll and ash-free dry mass determinations

Filtration assembly

- Filter funnel and base to hold 47-mm diameter filters
- Filtering flask, with side tabulation, 1 L, polypropylene
- Rubber stopper to join filter funnel with filtering flask
- Hand-operated vacuum pump with vacuum gage

Filters, glass-fiber, 47-mm diameter disks, 0.7 µm pore size (Whatman GF/F or equivalent)

Graduated cylinders: 50 mL, 100 mL, and 250 mL, plastic

Vials, scintillation, 20 mL capacity

Sample cooler and dry ice

Remove the funnel from the base of the filtration assembly, place a glass-fiber filter on the base of the assembly, and replace the funnel. Wet the surface of the glass-fiber filter with de-ionized water (or filtered stream water) and apply a partial vacuum of approximately 69 kPa (10 pounds per square inch) to the assembly. Record the volume of the subsample on the field data sheet, pour the subsample into the filter funnel, and maintain a vacuum not greater than 69 kPa until all periphyton biomass in the subsample is retained on the filter, periodically rinsing down the sides of the funnel. Gently remove the funnel from the filtration assembly, remove the glass-fiber filter from the assembly base with forceps, fold the filter twice, and wrap the filter with aluminum foil. Insert the foil-wrapped filter into a pre-labeled sample container, such as a scintillation vial, and place the sample containers into a plastic bag with a label that identifies the samples and the sampling reach. Place the plastic bag in a cooler, and place a small block of dry ice on top of the plastic bags that hold the sample containers. The procedure is identical for CHL and AFDM subsamples. Do not filter the samples in direct sunlight, and protect the filters from exposure to light. Ship the filters to the laboratory for analyses as soon as possible.

CONTRACT LABORATORIES AND THE BIOLOGICAL QUALITY-ASSURANCE UNIT

Processing, enumeration, and taxonomic identifications of algal samples are performed by contract laboratories under the direction of the USGS Quality Management Group's Biological Quality-Assurance Unit (BQAU) located at the National Water-Quality Laboratory, Arvada, Colorado. The BQAU oversees and coordinates all contracts for the processing and identification of algal samples according to standardized qualification, processing, and quality-assurance/quality-control criteria analogous to those used for processing invertebrate samples (Cuffney and others, 1993). The BQAU is responsible for overseeing the quality of sample processing by contract laboratories, in terms of the accuracy of enumeration and taxonomic identifications, and for resolving taxonomic issues within and among study units. The BQAU also oversees the entry of contractor data into the National Water Information System-II (NWIS-II) data base, maintains reference collections, and deposits voucher specimens in outside museums.

Study-unit personnel send sample components to the contract laboratory and the BQAU (split-sample components) as soon as possible, preferably directly from the field. This procedure helps to minimize storage of formalin-containing samples and reduces the damage or loss of specimens and samples during shipment and storage. The study-unit biologist contacts the BQAU prior to collection of the samples to determine which contract laboratory will receive the samples. The contract laboratory is not apprised of the existence or identity of quality-assurance samples.

The buffered formalin used as a preservative for algal samples is a hazardous material; therefore, specific Federal guidelines govern the shipment of these samples. In addition, individual shipping companies could have more stringent requirements for the packaging and labeling of preserved samples. It is important to consult the shipping company regarding its requirements prior to collecting any samples. Make sure that the shipping company understands that the samples contain a solution of 3- to 5-percent formalin (not 3- to 5-percent formaldehyde) and be prepared to provide information on the maximum amount of preservative in each container and the total in each package. Packaging and labeling standards require special boxes, packing materials, and labels that need to be

ordered well in advance of their use. Therefore, the necessary shipping materials and instructions should be on hand prior to leaving for the field so that samples can be shipped directly from the field to the appropriate contract laboratory.

A complete list of the contents of each package, including appropriate information from the sample identification code, is placed in the package as a packing list. Copies of the packing list are sent to the contractor and the BQAU, and one copy is retained by the study unit. The field sample log (fig. 8) serves as the basis for the packing list by indicating information on each container returned from the field. Entries listed under the "Sample description" heading include a description of the type of sample (QMH, RTH, or DTH) and the sample element code (A, macroalgae; B, microalgae; or C, aquatic mosses). The "Disposition" column indicates the date that the containers were shipped and their destination: BQAU or the name of the contract laboratory (XYZ Laboratory). A copy of the field sample log should be sent to the BQAU to aid in inventorying and tracking samples.

Data are returned by the contractor directly to the BQAU, where data are reviewed for quality and accuracy. Provisional data are released to the study units by way of NWIS-II and are available only to the local study unit. Once appropriate quality-assurance/quality-control checks have been completed, the BQAU, in consultation with the study-unit chief, releases taxonomic data for general access.

ALGAE FIELD SAMPLE LOG

Study Unit: Lower Susquehanna River Basin Sampling Dates: Start 09/25/92

Finish 09/26/92

Sample identification code	Site code	Collection date (MM/DD/YY)	Sample description		Disposition
			Type	Compo-nent	
LSUS0992AQE0001A	12510500	09/25/92	QMH	A	XYZ Labs, 10/01/92
LSUS0992AQE0001B	12510500	09/25/92	QMH	B	XYZ Labs, 10/01/92
LSUS0992AQE0001C	12510500	09/25/92	QMH	C	XYZ Labs, 10/01/92
LSUS0992AQE0001S	12510500	09/25/92	QMH	S	NWQL, 10/01/92
LSUS0992ARE0002B	12510500	09/25/92	RTH	B	XYZ Labs, 10/01/92
LSUS0992ARC0002B	12510500	09/25/92	RTH	B	NWQL, 10/01/92
LSUS0992ARM0002B	12510500	09/25/92	RTH	B	NWQL, 10/01/92
LSUS0992ARE0002S	12510500	09/25/92	RTH	S	NWQL, 10/01/92
LSUS0992ADE0003B	12510500	09/25/92	DTH	B	XYZ Labs, 10/01/92
LSUS0992ADC0003B	12510500	09/25/92	DTH	B	NWQL, 10/01/92
LSUS0992ADM0003B	12510500	09/25/92	DTH	B	NWQL, 10/01/92
LSUS0992ADE0003S	12510500	09/25/92	DTH	S	NWQL, 10/01/92
LSUS0992AQE0004B	12510600	09/26/92	QMH	B	XYZ Labs, 10/01/92
LSUS0992APE0004B	12510600	09/26/92	P	B	XYZ Labs, 10/01/92
LSUS0992APC0004B	12510600	09/26/92	P	B	NWQL, 10/01/92
LSUS0992APM0004B	12510600	09/26/92	P	B	NWQL, 10/01/92

EXPLANATION

QMH	Qualitative multihabitat sample
RTH	Richest-targeted habitat sample
DTH	Depositional-targeted habitat sample
A	Macroalgae
B	Microalgae
C	Aquatic mosses
S	Split-sample component
P	Phytoplankton
XYZ Labs	Name of contract laboratory
NWQL	National Water-Quality Laboratory

Figure 8.--Example of a field sample log that lists collection and disposition data for samples collected.

SUMMARY

Benthic algae (periphyton) and phytoplankton communities are characterized in the U.S. Geological Survey's National Water-Quality Assessment (NAWQA) Program as part of an integrated physical, chemical, and biological assessment of the Nation's water quality. This multidisciplinary approach provides multiple lines of evidence for evaluating water-quality status and trends, and for refining an understanding of the factors that control water quality. This is accomplished by integrated sampling at locations chosen to represent combinations of natural and human factors that are important in influencing the water quality at local, regional, and national scales.

The sampling unit for algal community characterization is the sampling reach, a length of stream that contains multiple examples of the predominant geomorphic features (for example, two riffle-pool sequences) that characterize a stream or river segment. Each sampling reach is characterized using a combination of qualitative and quantitative algal samples. For the purpose of NAWQA ecological surveys, algal samples typically are collected in conjunction with benthic invertebrate samples. Periphyton samples are collected from the surfaces of natural substrates (periphyton microhabitats) in relation to the presence of microhabitats in the sampling reach and the selection of habitats for benthic invertebrate sampling. To address specific study-unit issues, phytoplankton samples can be collected at large river locations during periods of stable hydrologic conditions. The choice of sampling locations and the occurrence of different instream habitat types in the sampling reach determine the types of qualitative and quantitative algal samples that are collected. The character of periphyton microhabitats in the sampling reach determines the types of sampling devices and methods used for collecting representative algal samples.

Water quality can be characterized by evaluating the results of qualitative and quantitative measurements of the algal community. The species composition and community structure of algae provide evidence of physical and chemical conditions present in a stream, over time scales ranging from weeks to months. Qualitative periphyton samples are collected to document the occurrence of algal taxa in all available periphyton microhabitats present in the sampling reach. The purpose of qualitative sampling is to develop a detailed list of the taxa present in the reach at the time of collection. The qualitative multihabitat periphyton sample is prepared by compositing collections of periphyton from instream habitat types present in the sampling reach. Quantitative periphyton samples are collected to measure algal community structure within targeted instream habitat types--a taxonomically "richest" habitat and a depositional-targeted habitat. The specific locations of the richest-targeted and depositional-targeted habitats within a stream reach are consistent with those selected for invertebrate sampling. Relevant site information, sampling information, and microhabitat characteristics are recorded on algae field data sheets. Estimates of algal biomass (chlorophyll content and ash-free dry mass) are optional measures that are useful for interpreting water-quality conditions at some study units.

Benthic algal communities are collected from natural substrates, using the sampling method that is most appropriate for the predominant periphyton microhabitat in the designated habitat. Qualitative samples are collected from all instream habitat types in the sampling reach. Quantitative targeted habitats (richest and depositional) are sampled by collecting and compositing a minimum of five periphyton samples from each designated instream habitat type. The use of artificial substrates can be considered for nonwadeable stream locations, or when uniformity of substrate is an important consideration for detecting

differences or changes in water quality. Phytoplankton samples may be collected in large nonwadeable streams and rivers to meet specific study-unit objectives.

Algal samples are labeled with a unique 16-character code that identifies the study unit (stream location), sampling date, type of sample, subtypes, sample number, and sample element code. Optional algal samples for the determination of chlorophyll concentrations or ash-free dry mass are processed in the field, placed on dry ice, and submitted to the National Water-Quality Laboratory in Arvada, Colorado. Samples for the identification and enumeration of algal taxa are preserved with buffered formalin and shipped to a contract laboratory for analysis. The Biological Quality-Assurance Unit has responsibility for laboratory quality assurance and quality control, entry of contractor data into the national data base, and storage and maintenance of taxonomic collections. The Biological Quality-Assurance Unit also monitors the accuracy of taxonomic identifications and precision of enumeration by analyzing split samples from 10 percent of the study-unit sampling reaches.

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