Chapter 5.0 Algae

with contributions from **Lei Zheng**[1]

This chapter…

- presents methods for sampling periphyton and phytoplankton
- recommends a periphyton-based approach

Algae are…

- a basal food resource for much of the riverine food web
- important biological indicators
- the most responsive indicators for nutrients

5.1 Introduction

Algae are a highly diverse group of photosynthetic organisms with unicellular reproductive structures. They have important functions in aquatic habitats as producers of organic matter and play a vital role in inorganic nutrient retention, transfer and cycling (Stevenson 1996). Large bodies of freshwater, such as large rivers, are usually dominated by diatoms, which are generally referred to as microalgae. The degree to which components of the algal assemblage are used in bioassessment and monitoring programs across the country varies. Diatoms, for example, are widely used as indicators, whereas cyanobacteria (commonly referred to as blue-green algae) and green algae are only occasionally used. This is in part because of differences in taxonomic development, availability of tolerance values, and availability of protocols. The routine use of algae as indicators is also more limited often due to a lack of expertise within monitoring entities. Another factor limiting use is the substantial spatial and temporal variability in species composition even without changes in water quality (Wetzel 2001). The use of cyanobacteria has recently increased because of a need to monitor the occurrence and extent of harmful algae blooms.

It has long been recognized that pollution can change the structure and function of the natural algal assemblage, especially diatoms (Patrick et al. 1954, Patrick 1977), and thus have substantial utility for biological assessments. A number of algal metrics and indices (a majority of which are diatom metrics) have been developed and used to indicate various environmental changes. Most of them belong to one of three categories of methods. The first category is the saprobic system and its derivatives in which diatom assemblages are characterized by their tolerance to organic pollution (Kolkwitz and Marsson 1908, Liebmann 1962, Sladecek 1973). The second category is based on the classification of diatoms according to their sensitivity to all types of pollution (Fjerdingstad 1950, 1965, Coste 1974). Fjerdingstad (1950, 1965) classified diatom species according to their ability to withstand varying amounts of pollution and then described communities in terms of dominant and associated species. The third category of methods is based on the diversity of diatom assemblages. These methods include plotting the number of species against the number of individuals per species (Patrick 1968) and calculating diversity indices (review by Archibald 1972).

This chapter provides brief reviews of several different protocols for sampling periphyton and phytoplankton in a variety of ways (Hill and Herlihy 2000, Stevenson and Bahls 1999, Moulton et al. 2002). The LR-BP for periphyton presented here is an amalgam of methods used by these

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programs. If field sampling methods other than those recommended are more suitable to your program, they should be thoroughly tested to ensure that they return data of sufficient quality and provide the capacity to address their intended and stated purposes.

5.1.1 Benthic Algae Overview

The benthic algal assemblage in streams and rivers is increasingly being used as an indicator of environmental condition (USEPA 2002). Sampling is generally active through scraping rocks, sticks, or other substrata, or passive by use of artificial substrata. In streams where flow and substratum characteristics create efficient interactions between water and the benthic algal assemblage, benthic algae reflect recent water chemistry (Lowe and Pan 1996). However, in large rivers, suitable attachment surfaces may only occur along banks. In some cases, little suitable substrates may be present for sampling, which may limit the utility of benthic algae as indicators of water chemistry in some rivers. This is particularly relevant in impounded systems where light and flow rates are reduced.

Periphyton assemblage composition is strongly influenced by land-water interactions, and also by river size and the level of human disturbance. In relatively undisturbed rivers, primary productivity is directly correlated with stream order because the surface area of substrata available for periphyton production is increasing and light penetration is adequate. With the increase of ecosystem disturbance (e.g., deforestation and agriculture), periphyton production declines with increasing river size and turbidity (Naiman 1983). The appropriate sampling depth for periphyton in rivers, therefore, will depend heavily on turbidity. It should be noted, however, that periphyton photosynthesis can occur at relatively low light intensities (e.g., 5-25 μmol m^{-2} s^{-1}) (Wetzel 2001).

Bioassessment programs use algal surveys for two primary purposes: 1) to quantify biomass and 2) to characterize species composition. Benthic algal biomass can be generally characterized by different measures, including cell density/biovolume, chlorophyll *a*, ash-free dry mass (AFDM) and dry mass measurement. Qualitative field observation of algal status also helps to identify environmental impairment in rivers. When combined with chemistry information and other biological metrics, qualitative site ranking of the algal assemblage can help decision making. The Kentucky Division of Water (DOW) (2002) uses a 1 (lowest quality) to 5 (highest quality) scoring system and a number of criteria to describe the algal assemblage. The criteria include phytoplankton density, presence/absence of floating algal mats, diversity of several divisions (e.g., chrysophytes, chlorophytes, cyanobacteria, rhodophytes) and the thickness and color of filamentous algae.

5.1.2 Phytoplankton Overview

Phytoplankton is that portion of the plankton composed of algae and cyano bacteria. In general, phytoplankton diversity and biomass are much greater in high order rivers than in low order streams, although their productivity is also often limited by light, as is true for periphyton. The sampling depth of phytoplankton is also regulated by flow, turbidity, and light. In deep, well-mixed large rivers or shallow rivers (i.e., 2-3 m in depth), one phytoplankton sample collected at the depth of 0.5 to 1 m may be adequate. Usually, it is desirable to sample the main channel of the rivers and avoid inlets, backwater, and sloughs areas. If it is determined that phytoplankton distribution is variable or patchy in a very heterogeneous river channel, compositing samples from multiple locations in a reach is recommended. The planktonic assemblages in general (i.e., phytoplankton and zooplankton) are potentially useful indicators of environmental condition because they are important to the trophic structure of larger rivers, and they are likely sensitive to a number of anthropogenic disturbances, including flow regulation, habitat alteration, invasive species, and contamination by nutrients, metals, and herbicides (Angradi 2006).

Important issues to consider prior to launching a program using periphyton or phytoplankton as a biological indicator include:

- sampling period,
- quantitative and/or qualitative samples,
- collection method to use,
- substrata to sample,
- target indicator to use,
- whether to composite samples,
- sample locations, and
- level of taxonomic identification.

Additional issues to consider associated with phytoplankton include:

- hydrologic seasonality,
- distance from impoundments,
- presence of flushable backwaters, and
- water residence time.

5.2 Discussion on Algal Methods

The protocols in this section have largely been designed for specific applications. However, most can be adapted to meet the differing needs of researchers and resource managers, depending on specific objectives for individual programs and projects. A few questions should be addressed before selecting a field protocol, including, will the focus of the sampling be quantitative or qualitative? If the focus is quantitative, how many parameters will be measured? Is the targeted habitat a single habitat type or multihabitat? Other aspects of the protocol to consider include reach lengths, sampling points and transects, and algal count methods.

Biomass is often the primary concern when extensive algal growth and associated nutrient enrichment are present. For this type of assessment, quantitative sampling to characterize algal biomass is the best approach. However, algal species composition, especially for diatoms, is a useful tool for metrics and indicator development, and can be characterized as relative abundance of individual taxa in a sample. Table 5-1 summarizes the advantages and disadvantages of various algal measures.

Different field sampling methods for freshwater algae can yield similar results (taxonomic composition and relative abundance) providing considerable flexibility in selection of field techniques. This is likely due to the general ubiquitous distribution of algae in water bodies. As a result, field efficiency can be increased by allowing for the coordinated collection of multiple assemblages at the same collection points of a single design. For example, to facilitate the collection of periphyton sampling from a study reach without significant increases in field time, periphyton samples are regularly collected using the collection techniques discussed in Sections 5.3 and 5.4, but using the field design developed for the LR-BP for benthic macroinvertebrates (Chapter 6).

TABLE 5-1. Advantages and disadvantages of selected algal methods.

Measures	Purpose	Advantages	Disadvantages
Rapid periphyton survey	Quantifying macroalgae and periphyton cover and thickness in a stream reach.	Provides relative biomass of dominant macroalgae and periphyton without laboratory processing and counting.	Requires 3-10 transects for algal cover. Increases field time.
Chlorophyll *a*	Frequently used for indirectly estimating algal biomass.	Measures only the algal portion of the biomass.	Has a relatively short holding time (24 hours) before filtering. Samples must be kept on ice, in a freezer, or in liquid nitrogen in the field, and in the dark prior to laboratory freezer storage and later analysis.
AFDM	Direct measure of algal biomass.	Adds little additional field time. Easy to analyze in the laboratory.	Can include debris and other organic material in the sample. The proportion of algae, bacteria and debris can significantly change the AFDM/dry mass ratio in a sample.
Dry Mass	Direct measure of algal biomass.	Adds little additional field time. Easy to analyze in the laboratory.	Silt can account for a substantial proportion of dry mass in some samples. The proportion of algae, bacteria and debris can significantly change the AFDM/dry mass ratio in a sample.
Cell Density / Biovolume	Estimates the total number algal cells in a sample area.	Provides the most accurate and reliable estimates of total algal standing crop.	Costs more and requires longer processing time.

5.3 Field Sampling Methods

Although there have been efforts to develop broadly-consistent sampling protocols, some differences remain. Basic sampling approaches for periphyton and phytoplankton are provided by the USEPA RBP (Stevenson and Bahls 1999 in Barbour et al. 1999), USEPA EMAP (Hill and Herlihy 2000), USGS-NAWQA program (Moulton et al. 2002), and the USEPA EMAP-GRE (Table 5-2).

TABLE 5-2. Major large river periphyton and phytoplankton sampling methods.

Program	Protocol Summary	Citation
USEPA RBP (periphyton)	Representative samples taken from natural materials (organic and inorganic) and from artificial substrata, and are scraped, drawn, or washed into sample containers; all microhabitat types sampled, or all surfaces from artificial substrata scraped. As appropriate, composite sample preserved or frozen (analyzed for taxonomic composition, biomass, condition index).	Stevenson and Bahls 1999 (from Barbour et al. 1999)
USEPA EMAP-Surface Waters Non-wadeable Streams and Rivers (periphyton)	Individual sample units are taken at eleven transects over a 40 or 100X sampling reach length, on each bank. Use stiff-bristled brush to dislodge periphyton from defined area of rock or wood, wash into sample container as composite sample. Preserve or ice, as necessary. Syringe used to draw sample from soft sediment (analyzed for species composition, relative density, chlorophyll *a*, biomass, enzymatic activity).	Hill and Herlihy 2000
USGS NAWQA Program (periphyton)	Qualitative and quantitative samples taken from epilithic, epidendric, epiphytic, epipelic, and epipsammic habitats over a 500 to 1000 m sampling reach. Use, as appropriate, tools to scrape from rock, wood, or other plant material, and some suction device or spoon to draw soft sediment. For quantitative samples, 25 representative subsamples with controlled effort, and composited into one sample jar. Preserve on ice as necessary (analyzed for species composition, relative density, chlorophyll *a*, and biomass.	Moulton et al. 2002, Porter et al. 1993
USGS NAWQA Program (phytoplankton)	A subsurface grab or depth/width-integrating sampler is used to collect a quantitative whole-water sample. A 1-L sample is sufficient for productive, nutrient-enriched; larger volumes up to about 5 L may be necessary for unproductive, low-nutrient rivers. Subsample volumes may range from 50 mL to more than 500 mL. Subsamples are prepared for chlorophyll a, particulate organic carbon, and biomass.	Moulton et al. 2002, Porter et al. 1993
USEPA EMAP GRE (phytoplankton)	A quantitative phytoplankton sample is collected as a ~2-L composite and preserved with formalin. Samples are analyzed for assemblage structure, body size distribution, and trophic structure. Separate water samples are collected for chlorophyll *a* analysis.	Angradi 2006

Periphyton data were found to be more consistent across different field designs (Charles Lane, US Environmental Protection Agency, personal communications) than are benthic macroinvertebrate data or fish data (Blocksom and Flotemersch 2005, Flotemersch and Blocksom 2005). Collection points for periphyton are, thus, relatively flexible and can be placed according to the needs of the less-flexible designs for benthic macroinvertebrates and fish, thus increasing field efficiency. The field sampling design of the LR-BP for periphyton, as presented in this document, is configured for field compatibility with that of benthic macroinvertebrates (12 sampling zones) and fish LR-BPs (Chapters 6 and 7).

5.4 The Large River Bioassessment Protocol (LR-BP) for Periphyton

Each sampling site consists of a 500-m reach. The GPS coordinates correspond to the downstream end of the sampling reach. At each site, there are a total of six transects. Transect A is located at the downstream end of the reach (0 m) with the remaining five transects at 100 m, 200 m, 300 m, 400 m, and 500 m from the downstream end (Figure 5-1). At each transect, a 10-m sample zone (5 m on each side of transect) on each bank defines the area that will be searched for a substratum suitable for collecting a periphyton sample. The zone extends from the edge of the water to the midpoint of the river, or to a depth of 1m.

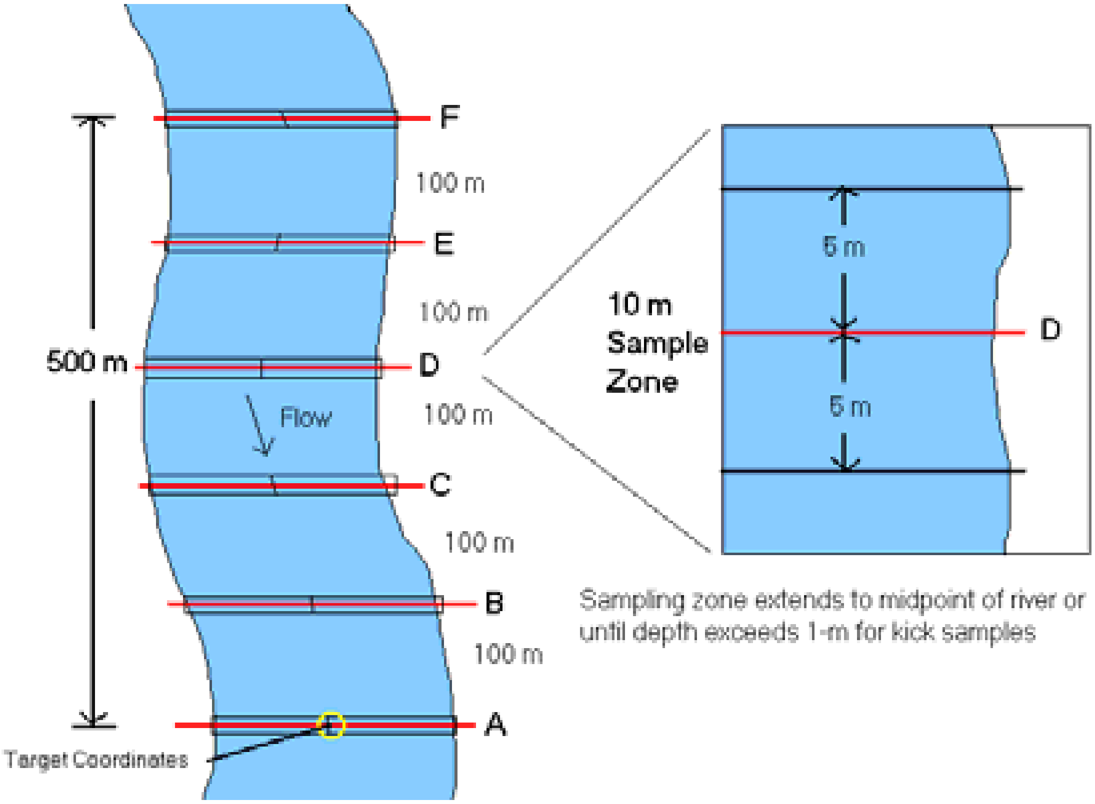

FIGURE 5-1 Example of the six transects and 12 sample zones for collection of periphyton in large rivers using the LR-BP design.

5.4.1 Substrata Selection

At each of 12 sampling zones established, a suitable substratum is selected for collection of a single sample. The substratum selected for sampling should be collected from a location where light penetration reaches the bottom such that it can support algal development. Hill and Herlihy (2000) suggested that the sample be collected from a depth no deeper than can be reached by submerging your arm to mid-bicep depth. If water at a site is >1m deep at the water's edge or the bank is steep, the substratum may be sampled by reaching out of the boat. If a suitable substratum cannot be located or safely sampled, the transect can be bypassed and the exception noted on the field forms.

Often, the selected substratum may arguably be the richest habitat, but this is not the guiding factor in the selection of a suitable substratum. The substratum selected should be one with a high likelihood of producing a quality sample, that is, one that strikes a balance between being representative of the sample station and being suitable for processing in the laboratory. Samples containing excessive sediment are less desirable because they generally take much longer to process, require less than ideal levels of dilution, and can result in poor measures of chlorophyll. The best samples are those from surfaces with a well-developed algal assemblage (e.g., biofilm, algal mat) and a minimum of non-productive sediment. As a guide, epilithic (rock), epidendric (wood) and epiphytic (plant) substrata are preferred (in that order), but other substrata can be sampled, including non-natural surfaces. An example would be when submerged rocks have been covered by a layer of sediment while a suspended piece of woody debris has not. The sample of substratum that is selected is usually small enough (<15 cm diameter) and can be easily removed from the river. At some sampling stations, this may not be the best substratum, but rather the most suitable for the protocol. If the majority of substrata present at a sampling station are so large as to prohibit removal from the river, a longer section of PVC pipe can be used (as described by Hill and Herlihy 2000) that has been fitted with a gasket to seal around the delimited area. The sample can then be removed with a long barrel syringe.

5.4.2 Sample Collection

The following sample collection procedure is a modified version of that outlined by Hill and Herlihy (2000). Once the substratum has been identified, the periphyton sample is collected by removing attached algae from a defined area. Several options exist for delineating the area. Two of the more common apparatuses used for this are a short section of PVC pipe (Hill and Herlihy 2000), and the barrel of a syringe fitted with an O-ring (Porter et al. [1993], and citations therein). The O-ring on the syringe provides for a better seal on the substratum. Two other approaches are the plastic frame of a 35 mm or medium format slide and a rubber mat with an opening. The slide frame is preferred by some because it is more flexible and form-fitting than a section of PVC pipe or the barrel of a syringe. The rubber mat is likewise flexible with the added feature of covering the area outside of that delineated and when rinsed, reduces the potential for sample contamination.

Program Highlight

USGS National Water Quality Assessment (NAWQA) Algal Assessment Protocols for Non-wadeable Streams and Rivers

The USGS NAWQA program has developed a suite of protocols for the collection of algae from non-wadeable streams and rivers (Moulton et al. 2002). These include protocols for the active collection of qualitative and quantitative periphyton samples that use artificial substrata and phytoplankton samples. The sampling reach length and location used for the collection of algal samples are determined on the basis of a combination of repeating geomorphic channel units (Meador et al. 1993). However, given the realities often faced on large river systems, minimum and maximum reach lengths of 500 and 1000 m, respectively, have been deemed acceptable (Meador et al. 1993).

In general, periphyton samples are collected from the surfaces of natural substrata in relation to the presence of microhabitats in the sampling reach by scraping, brushing, siphoning, or other methods appropriate to each microhabitat (Porter et al. 1993). Periphyton is sampled in erosional habitats by removing the designated substratum from the stream, dislodging the attached material from a predetermined area on the upper surface of the substratum with a stiff-bristled brush, and then washing the material into a sample bottle. In depositional habitats, a predetermined area of soft sediment is collected using a syringe or a spoon and transferred to the sample bottle. Sampling is conducted at locations chosen to represent combinations of natural and anthropogenic factors (Porter et al. 1993).

Qualitative Multihabitat Sampling Method

For this protocol, periphyton samples from all instream microhabitat types present in the sampling reach are composited (Porter et al. 1993).

Quantitative Targeted-Habitat Sampling Method

The goal of quantitative periphyton sample collection is to measure relative abundance and density of taxonomically representative periphyton within: (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. Another quantitative method is the use of artificial substrata, which don't necessarily target any specific habitat. In both RTH and DTH, the protocols specify sampling from five representative substrata at five locations within the designated reach. This results in a final composite sample (for both the RTH and DTH) that is composed of, at most, 25 subsamples each (if five substrata are available).

Richest-Targeted Habitat (RTH)

Typical RTH areas include riffles in shallow, coarse-grained, high-gradient systems, or woody snag habitats in sandy-bottomed systems. At each of the five locations, samples are taken from five representative substrata (25 total samples). In order of preference, samples are taken from epilithic, epidendric, and epiphytic substrata. A simple sampling device is used to quantify the size of the sampled area (Porter et al. 1993). The device consists of a 60-cc syringe barrel fitted with a rubber O-ring on one end. The end with the rubber o-ring is placed flat on the substratum surface so that a seal is formed. A brush is then placed through the syringe barrel and used to dislodge the attached periphyton from the surface of the substratum. The sample area is then washed with a squirt bottle and the dislodged periphyton is rinsed into the sample collection container. If the substratum surface is irregular so that the rubber o-ring cannot form a seal, the periphyton can be brushed from the entire substratum and the entire substratum is then fitted with aluminum foil. The substratum is discarded and the foil is returned to the laboratory so that the surface area of the substratum can be determined. If bedrock is to be sampled, then a PVC pipe sampler is used. The periphyton from all 25 subsamples are composited into one sample jar.

An example of a DTH area is an organically-rich depositional area such as a pool. If epilithic or epidendric substrata are available in the DTH area, then periphyton should be collected in the same manner as they are collected from the RTH areas. However, if these substrata are not present, then epipelic or epipsammic microhabitats should be sampled. In order to sample epipsammic or epipelic habitats, the top half of a disposable 47-mm plastic petri dish is gently pushed into the streambed sediment. Then, a small sheet of Plexiglas or a spatula is slipped under the petri dish top so that the sediment is trapped inside. The contents are then rinsed into a sample jar. Because the volume of the petri dish top can be measured, then the sample can be quantified. Five sediment samples are taken for the entire reach. All DTH samples (sediment and any other available substratum samples) are composited into one sample jar.

Artificial Substratum Sampling Method

When natural substrata cannot be sampled because of inaccessibility of the microhabitats, cost of sample collection or safety issues, artificial substrata can be used in sampling reaches. These limitations occur in large rivers and should be considered when designing a sampling program for this type of system. Samples obtained from artificial substrata typically have reduced heterogeneity compared to those obtained from natural substrata but can be used to compare water quality among rivers with disparate periphyton microhabitats. However, data from artificial substrata cannot be compared with data from natural substrata. If artificial substrata are used 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. The advantages and limitations of artificial substrata are discussed in Porter et al. (1993).

Quantitative Phytoplankton Protocol

Phytoplankton are more reflective of conditions in the open water column, whereas periphyton represent conditions at the sediment/substratum-water interface. Quantitative phytoplankton samples are obtained by collecting representative whole-water samples. A sample volume of 1 L is sufficient for samples collected from productive, nutrient-enriched rivers as indicated by water color, but a larger sample volume is required for samples collected from unproductive, low nutrient rivers as indicated by water transparency. Phytoplankton samples, taken in conjunction with water chemistry sampling, are taken with a depth-integrating sampler. Alternatively, quantitative phytoplankton samples can be collected with a water sampling bottle or with a pump. If chlorophyll is not to be measured, the entire sample is preserved with buffered formalin. For chlorophyll measurements, an unpreserved subsample is withdrawn from the phytoplankton sample, and the aliquot is filtered onto a glass fiber filter. The filtered subsample volume should be sufficient to ensure that adequate algal biomass is retained on the filter. Filters are then wrapped in aluminum foil and immediately stored on dry ice (Porter et al. 1993).

Place the substratum in a plastic funnel which drains into a 500-ml plastic bottle with volume graduation. Use the area delimiter to define an area on the upper surface of the substratum. Dislodge attached periphyton from the substratum within the delimiter into the funnel by brushing with a stiff-bristled toothbrush for 30 seconds. Take care to ensure that the upper surface of the substratum is the surface that is being scrubbed, and that the entire surface within the delimiter is scrubbed. It may be necessary to mark the area contained in the delimiter with a tool, remove the delimiter and proceed with processing the surface.

In systems where the material within the area of the delimiter takes the form of a thick mat rather than a biofilm, the delimiter is placed on the substratum particle as previously described, but before the surface is scrubbed, a micro-spatula or spoon may be used to scoop out the material. This activity may or may not need to be followed by brushing. A minimal volume of river water from a bottle is used to wash the dislodged periphyton from the funnel into the 500-ml bottle. This process is repeated on left and right banks of all six transects and samples are composited into the same 500-ml bottle.

After samples have been collected from all 12 sample zones, the 500-ml bottle is thoroughly mixed regardless of substratum type. The sample container should be placed on ice until preservation. The total estimated volume of the composite sample is recorded. This volume will be used for subsequent preparation of samples for laboratory processing. The total area sampled for the reach will be 12X the area of the delineator minus those sample zones, if any, that were not sampled. Place samples on dry ice. Within 24 hours, place 48 mL of the samples into a separate container and preserve with 2 ml of 10% buffered formalin.

5.5 Laboratory Processing

As many as five different types of samples could be shipped to laboratories for analyses: Chlorophyll (Chl) *a*, Biomass (AFDM and dry mass), Acid/Alkaline Phosphatase Activity (APA), algal mass nutrient contents, and algal ID/enumeration samples. The Chl *a* and AFDM analyses estimate algal total biomass by area in the sampling reach. Standard methods have been developed for these measurements (APHA 1998). The APA and periphyton C:N:P ratio are optional measurements of algal nutrient limitation for determining which nutrient is limiting an aquatic system (Hill and Herlihy 2000). They are not as frequently used as algal biomass parameters. Algal ID/enumeration samples are used for algal taxonomy and algal cell density/biomass measurements.

5.5.1 Chlorophyll a and AFDM Analyses

Chl *a* analysis methods have been developed for both phytoplankton and periphyton (APHA 1998). After samples are filtered through a glass fiber filter, they are extracted using 90% aqueous acetone (APHA 1998) and stored for 24 hours at 4 °C in the dark. Three techniques for measuring Chl *a* in solution are the spectrophotometric, the fluorometric, and the high-performance liquid chromatography (HPLC) techniques. Hydrochloric acid (HCl) is used to correct phaeophytin *a* concentration. Dry mass and AFDM can be determined by weighing dry algal mass and weighing ash after incinerating organic material at 500 °C for 1 hour (APHA 1998).

5.5.2 Taxonomy and Enumeration: Soft-bodied Algae

The methods summarized here are a combination of the protocols provided by Barbour et al. (1999) and Moulton et al. (2002). Direct laboratory analysis results in density and abundance values for both soft-bodied algae (non-diatom) and diatoms. However, in many cases, data from diatoms only are sufficient for indicators of river condition.

Specification of laboratory procedures (counting, subsampling, and taxonomy) is extremely important for developing datasets for algae-based indicators. Subsamples of soft algae are used to determine density or biovolume of major taxa. After appropriate dilution, a Palmer-Maloney counting cell at 400X magnification can be used for both identification and enumeration. A common procedure for counting soft algae is to count 300 natural units (i.e., each individual filament, colony, or isolated cell). This procedure prevents a colonial or filamentous cluster from dominating a count and allows the assemblage structure to be assessed. All algae should be identified to the lowest possible taxonomic level, recording its name and density. Many of the non-diatom taxa, however, are not easily identified to the species level without culturing of the taxa. For most purposes of biological assessment, genus level identification is usually adequate. References for soft algae taxonomy are *Freshwater Algae of North America* (Wehr and Sheath 2003), and *Algae of the Western Great Lakes Area* (Prescott 1962). Although cell density of each taxon is recorded during the soft algae count, it is also recommended to convert cell density to biovolume (Hillebrand et al. 1999) or bio-surface area (APHA 1998) to account for the actual biomass of algae. Biovolumes and biosurface areas of all common taxa (relative abundance >5%) in any sample are determined by measuring at least 15 cells of each taxon present. Density and live:dead ratios of diatoms are recorded in the Palmer-Maloney soft algae count. Identification of diatoms is not necessary in the soft algae count.

Another algal counting method is a simple wet mount procedure used to identify small algal cells under high magnification (1000X). Sedgewick-Rafter counting chambers (large modified slides with 1-ml wells) are also often used for counting large filamentous algae and larger cells under lower magnification (100X).

5.5.3 Taxonomy and Enumeration: Diatoms

Diatom subsamples can be digested with hydrogen peroxide or nitric acid to remove organic matter (Patrick and Reimer 1967). Permanent slides are prepared using Naphrax, a high refractive index mounting medium, following APHA (1998). Approximately 300 diatom cells (600 valves) are counted at random or fixed transects and identified to the lowest possible taxonomic level (usually species or subspecies).

Both soft algae and diatom counts require highly trained taxonomists to perform consistent identification. The four-volume series on the diatoms of Middle Europe (Krammer and Lange-Bertalot 1986, 1988, 1991a, 1991b) is the primary diatom reference used by many taxonomists. This series updates diatom structure with detailed micrographs of each taxon. In *The Diatoms of the US,* Patrick and Reimer (1967, 1975) include the major algal taxa found in the USA. The recent treatments by Round et al. (1990) provide details on diatom ultrastructure. Terminology necessary to work through the keys and descriptions of genera is presented in the glossary.

Other taxonomic references include the diatom naming conventions adopted by the Academy of Natural Sciences of Philadelphia (Morales and Potapova 2000).

5.6 Data Entry

Taxonomic nomenclature and counts are frequently entered into the data management system directly from handwritten bench or field sheets. Depending upon the system used, there may be an autocomplete function that helps prevent misspellings. There are two methods for assuring accuracy in data entry. One is the double entry of all data by two separate individuals, and then the performance of a direct match between databases. Where there are differences, it is determined which database is in error, and corrections are made. The second approach is to perform a 100% comparison of all data entered to handwritten data sheets. Comparisons should be performed by someone other than the primary data enterer. When errors are found, they are hand-edited for documentation, and corrections are made electronically. The rates of data entry errors are recorded, and then, in the overall database are segregated by data type (e.g., fish, benthic macroinvertebrates, periphyton, header information, latitude longitude, physical habitat and water chemistry).

5.7 Data Reduction (Metric Calculation)

The current literature identifies various diatom metrics that indicate responses to different environmental stressors (Van Dam et al. 1994). Metrics summarized by Stevenson and Bahls (1999) are used by several States (Bahls 1992, Kentucky DOW 2002). Of the metrics discussed in the RBP manual, nine represent metrics of biotic condition while six others are diagnostic metrics. Hill et al. (2000, 2003) and Kentucky DOW (2002) developed periphyton indices of biotic condition using multiple metric approaches. Van Dam (1994) and Kelly (1998) also provided valuable information on diatom autecological indices that have been widely used in Europe. Table 5-3 lists the most commonly used metrics.

5.7.1 Diversity Metrics

Two periphyton metrics are measurements of taxa richness (i.e., total taxa and Shannon diversity) and are estimated from the count of taxa found in a target number of cells (e.g., 300 cells). Diversity metrics are less persuasive in indicating nutrient enrichment because of the confounding effect of algal diversity increasing with increased nutrient levels in oligotrophic systems as a consequence of adding homogenizing species.

5.7.2 The Pollution Tolerance Index (PTI) of Diatoms

An example of a water quality assessment method based on the pollution tolerance of diatom assemblages is the pollution tolerance index (PTI), which is used by the Kentucky Department of Environmental Protection (Kentucky DOW 2002). The PTI is similar to that used by Lange-Bertalot (1979) and resembles the Hilsenhoff biotic index (HBI) for macroinvertebrates (Hilsenhoff 1987). There are three categories of diatoms according to documented pollution tolerance, with the most tolerant taxa assigned a value of 1 and the most sensitive taxa assigned a value of 3. For the PTI, the categories are expanded to four with the resulting values ranging from 1 to 4. Similarly, percent sensitive and percent tolerant taxa can be derived from this method. The formula used to calculate PTI is:

$$PTI = \sum \frac{n_i t_i}{N},$$

where n_i is the number of cells counted for species i, t_i is the tolerance value of species i (1-4), and N is the total number of cells counted. Tolerance values have been generated from several sources, including Patrick and Reimer (1966, 1975), Lowe (1974), Patrick (1977), Descy (1979), Lange-Bertalot (1979), Kelly (1988), Sabater et al. (1988), and Bahls (1992).

TABLE 5-3. Diatom and non-diatom metrics summarized from various sources.

All Algae Metrics	Diatom Metrics
Taxa richness of non-diatoms or all algae	Total number of diatom taxa (TNDT)
Species dominance	Shannon diversity index
% cyanobacteria	Percent community similarity (PSc)
Number of Divisions represented by all taxa	Pollution tolerance index
Chlorophyll *a*	Percent sensitive diatoms
Ash-free dry mass (AFDM)	Percent live diatoms
Phosphatase activity	Van Dam’s diagnostic metrics
Autotrophic index	Simple autecological indices
	Percent Epithemiaceae
	Percent motile diatoms
	Percent *Achnanthidium minutissimum*

5.7.3 Percent Community Similarity (PSc)

Percent community similarity (PSc) by Whittaker (1952) is an example of a water-quality assessment method based on the diversity of diatom assemblages. The PSc was chosen for use in diatom bioassessments because it shows assemblage similarities based on relative abundances and gives more weight to dominant taxa than to rare taxa. The PSc should only be used when comparing a study site to a control site, or when conducting multivariate cluster analysis. If the emphasis is comparing a study site to a regional reference condition (i.e., a composite of sites), the PSc should not be used. The PSc values range from 0% (no similarity) to 100% (identical).

The formula for calculating PSc is:

$$PS_c = 100 - 0.5\sum_{i=1}^{s} |a_i - b_i|,$$

where a_i is the percentage of species i in sample A, and b_i is the percentage of species i in sample B.

5.7.4 *The Autotrophic Index*

Because periphyton is found on or in close proximity to the substratum, dry mass (DM) and ash-free dry mass (AFDM) values are used as assessment tools. The AFDM is an estimate of total organic material accumulated on the substratum. This organic material includes all living organisms (e.g., algae, fungi, bacteria, and macroinvertebrates) as well as non-living detritus. The DM values are used in conjunction with chlorophyll *a* as a means of determining the trophic status of rivers through the use of the autotrophic index (AI). The formula used to calculate AI is:

$$\text{AI} = \text{DM (mg/m}^2\text{)/Chlorophyll } a \text{ (mg/m}^2\text{)}.$$

High AI values (i.e., >200) indicate that the assemblage is dominated by heterotrophic organisms and can indicate poor water quality (Weber 1973, Weitzel 1979, Matthews et al. 1980). This index should be used with discretion because non-living organic detritus can artificially inflate the AFDM value. One option is to modify the AI to include AFDM and invert:

$$\text{AI} = \text{Chlorophyll } a \text{ (mg/m}^2\text{)/ AFDM (mg/m}^2\text{)}$$

In this form, the index is positively related to the autotrophic proportion of the assemblage instead of the heterotrophic proportion. Also, since chlorophyll *a* / AFDM values normally are about 0.1%, the modified index would have better statistical properties than the original index.

5.7.5 *Diagnostic Diatom Metrics*

Diatom species have different sensitivities to different types of pollution (e.g., nutrients, metals, pH, salinity). Thus, stressor-specific metrics may help to diagnose environmental pollution in aquatic systems. A number of diatom metrics have been developed to assess environmental impairment (Table 5-2). For example, % *Eunotia* species has been used to assess acidic condition (e.g., in association with acid mine drainage), and % Epithemiaceae taxa has been used to indicate nitrogen limitation (Kentucky DOW 2002). The diatom indices in Van Dam et al. (1994) are among the most complete diatom autecological references for diagnosing various environmental conditions. These indices are:

- *Trophic state index.* Eutrophic and hypereutrophic diatoms indicate elevated concentrations of nutrients that are important for diatom growth: nitrogen, phosphorus, inorganic carbon and silica. Diatom species are assigned nutrient tolerance values ranging from 1 – 6. As nutrient concentrations increase, the mean tolerance value of diatoms present increases from 1 to 6, and the proportion of eutrophenic diatoms (indicator values from 5 to 6) will increase. Therefore, the index or % eutrophenic taxa will also increase.

- *Index of nitrogen uptake metabolism*. Indicator values of nitrogen uptake from autotrophic to heterotrophic taxa range from 1 to 4. When nitrogen concentrations increase, the percentage of obligate nitrogen autotrophs will decrease, but obligate nitrogen heterotrophs will increase. Therefore, the index value will increase with organic enrichment.
- *Saprobity index*. The index characterizes waters with light to heavy loads of organic matter and with low or no oxygen. The index value will increase from 1 to 5 as organic loads (e.g., from agricultural and wastewater discharges) increase.
- *pH index*. Diatoms are extremely sensitive to pH. The index value ranges from 1 to 5, inferring acidic to alkaline conditions. The index indicates pH value from below 5.5 to above 8.5.
- *Oxygen demand index*. Oxygen demand is also classified for many diatoms, ranging from 1 to 5, indicating very low (i.e., <10% saturation) to very high (i.e., 100% saturation) dissolved oxygen. It is also an indication of organic degradation.
- *Salinity index*. A diatom-based salinity index that was formulated from statistical relationships between salinity data and diatom assemblages. The index value ranges from 1 to 4, indicating salinity from <0.2 to 9.0%.

5.8 Site Assessment and Interpretation

Although the use of diatoms for assessing stream condition is well established, their use for determining biological impairment (such as for purposes of water quality standards programs) is not as widespread and has not been extensively used in large river systems. Taken collectively, diatoms span a very wide autecological spectrum (e.g., ranging from ultraoligotrophic to hypertrophic conditions); but within this broad range, individual species display relatively consistent environmental tolerances (or autecological values), even across wide geographic distributions. These characteristics make diatom assemblages potentially useful indicators of biological impairment. The diatom diagnostic metrics discussed in Section 5.5.5 quantifies algal status and environmental characteristics in a sampling station for particular stressors. Other algal metrics (e.g., algal biomass, AFDM, Chl *a*), while not stressor-specific, can also indicate human disturbance. However, for resource management, a simple multimetric biotic index is much more practical for decision making and for implementing protection goals.

The reference approaches based on an IBI or other integrated variables (e.g., observed/expected ([O/E] ratio) could be used for periphyton assessment. Hill et al. (2000, 2003) developed a periphyton IBI that included eight metrics (i.e., species richness; species dominance; relative abundances of acidobiotic, eutraphentic, and motile diatoms; standing crops from Chl *a* and biomass; and APA). The Kentucky DOW (2002) also developed a diatom bioassessment index (DBI) comprising six metrics (i.e., diatom richness, Shannon index, PTI, Siltation index, *Fragilaria* richness, and *Cymbella* richness). A well-designed index should respond to both specific and multiple environmental stressors in a predictable manner and should not be affected by river size or watershed area. To determine an impairment threshold, a reference approach can be used. Hill et al. (2003) adopted 75th, 25th, and 5th percentile scores of the reference site distribution to set thresholds for excellent, good, fair or poor conditions, respectively, and thus set protective goals for algal status. Another approach that has been widely used in macroinvertebrate assessment is the O/E approach. Mid-Atlantic streams were assessed using diatoms by comparing the O/E approach to other metrics and multimetric approaches (R. J. Stevenson, personal communication). The results indicated that the approach to quantifying loss of native diatom taxa with increasing nutrients was not successful, but diatom autecologies are likely correlated with nutrient concentrations.

5.9 Performance Characteristics for Biological Assessments Using Algae

5.9.1 Field Sampling

Quantitative (QN) performance characteristics for field sampling are *precision* and *completeness* (Table 5-4). Repeat samples for purposes of calculating precision of field sampling are obtained by sampling from two adjacent 500-m reaches (Figure 5-2). For algae, samples from the adjacent reaches (also called quality control [QC] or duplicate samples) must be laboratory-processed prior to data being available for precision calculations. These precision values are statements of the consistency with which the sampling protocols:

- characterized the algal biota of the river and
- were applied by the field team,

and thus, reflect a combination of natural variability, laboratory error, and systematic error (see Chapter 3).

The number of reaches for which repeat samples are taken varies, but a rule-of-thumb is 10% of the total number of sampling reaches constituting a sampling effort (whether a programmatic routine or an individual project), and they would be randomly selected from that list. Values for calculation of precision are dry mass (DM), ash free dry mass (AFDM), chlorophyll *a*, biovolume/density and some measure of species composition. In effect, whatever the indicator values that are to be used for site assessment, are also used to calculate relative percent difference (RPD), root-mean square error (RMSE) and coefficient of variability (CV) (Table 3-2). Acceptance criteria for each of these would be established based on programmatic capabilities demonstrated via pilot studies, or through analysis of existing datasets produced using the same protocols. These criteria are not data quality thresholds beyond which data points should be considered for discarding. Rather, they are flags for potential problems (errors) in sample collection or processing, are used to help determine the sources of the problems, and can be used to help develop recommendations for corrective actions.

Percent completeness (Tables 3-2, 5-4) is calculated to allow communication of the number of valid samples (however validity is judged) that were collected as a proportion of those that were originally planned. This value serves as one summary of overall data quality for a sampling effort, and it demonstrates confidence in the final results.

TABLE 5-4. Error partitioning framework for biological assessments and biological assessment protocols for algae. There may be additional activities or performance characteristics, and they may be quantitative (QN), qualitative (QL), or not applicable (na).

Component Method or Activity	Performance Characteristics				
	Precision	Accuracy	Bias	Representativeness	Completeness
1. Field sampling	QN	na	QL	QL	QN
2. Laboratory subsampling	QN	na	QL	QL	na
3. Taxonomy	QN	QL	QL	na	QN
4. Data entry	na	QN	na	na	QN
5. Data reduction (e.g., metric calculation)	na	na	QN	na	na
6. Site assessment and interpretation	QN	QN	QL	QL	QN

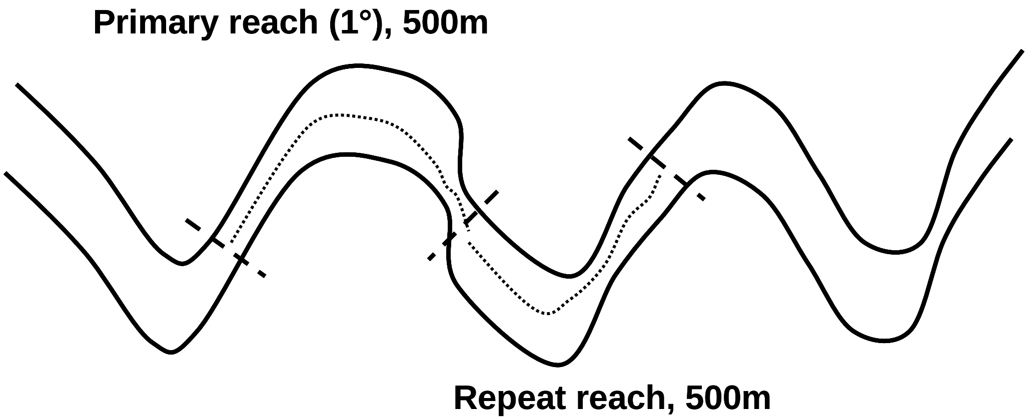

Figure 5-2. Adjacent reaches (primary and secondary) on a fluvial channel.

Qualitative (QL) performance characteristics for field sampling are *bias* and *representativeness* (Table 5-4). Attempts to minimize the bias associated with the LR-BP for algae include the fact that sample portions are taken are from areas with hard surfaces (rock, wood) that are distributed among 12 sample zones (Figure 5-1) and composited; sampling is not restricted to small, limited areas. The LR-BP field sampling method is intended to depict the algal assemblage present in the shore-zone area (out to a 1-m depth) that the large river has the conditions to support.

Accuracy is considered "not applicable" to field sampling (Table 5-4) because efforts to define analytical truth would necessitate a sampling effort excessive beyond any practicality. That is, the analytical truth would be all algae or algal taxa that exist in the river (shore zone to 1-m depth). There is no sampling approach that would collect all individual algal cells or filaments.

5.9.2 Laboratory Sorting/Subsampling

Precision is a QN characteristic of performance for laboratory subsampling of algae (Table 5-4). Subsampling of algae (specifically, for diatoms and phytoplankton) occurs as pipettes are used to draw liquid from a sample to prepare slide mounts. Comparison of the results from multiple slides prepared from the same sample provides information on the precision of subsampling, which is calculated using RPD and CV (Table 3-2) with measures of species composition as the input variables. Precision is an indication of how well the sample is mixed; it is not necessary to do this for every sample. Serial subsampling and precision estimates should be done on approximately 10% of all samples collected as part of a project and on two timeframes. First, they should be done and the results documented and reported, to demonstrate what the laboratory is capable of in application of the subsampling method. Second, they should be done periodically to demonstrate that the program routinely continues to meet that level of precision. Representativeness of the sorting/subsampling process is addressed as part of the standard operating procedure (SOP). Considered as “not applicable”, estimates of *accuracy* are not necessary for characterizing laboratory sorting performance.

5.9.3 Taxonomy

Precision and *completeness* are QN performance characteristics used for taxonomy (Table 5-4). Precision of taxonomic identifications is calculated using percent taxonomic disagreement (PTD) and percent difference in enumeration (PDE) (Table 3-2), both of which rely on the raw data (list of taxa and number of individuals) from whole-sample re-identifications. The primary taxonomy is completed by the project taxonomist (T1); re-identifications are performed by a secondary or QC taxonomist (T2) as blind samples. The number of identifications in agreement between the two sets of results, as an inverse proportion of the total number of individuals in the sample ((1-[number of agreements])/N), is precision of the taxonomic identifications. The percent difference in sample counts by each of the taxonomists is percent difference in enumeration (PDE). These two values are evaluated individually and can be used to indicate the overall quality of the taxonomic data; and if there is a problem, they can help identify what is causing the problem. The number of samples for which this analysis is performed will vary, but 10% of the total sample lot (project, program, or year, or other) is an acceptable rule-of-thumb. Exceptions are that large programs (>~500 samples) may not need to do >50 samples; small programs (<~30 samples) will likely still need to do at least 3 samples. In actuality, it will be program-specific and the number of samples re-identified will be influenced by multiple factors, such as how many taxonomists are doing the primary identification (there may be an interest in having 10% of the samples from each taxonomist re-identified), and how confident the ultimate data user is with the results. Mean PTD and PDE across all re-identified samples is an estimate of taxonomic precision (consistency) for a dataset or a program. Percent taxonomic completeness (PTC; [Table 3-2]) quantifies the proportion of individuals in a sample that are identified to the specified target taxonomic level (lowest practical taxonomic level, species, genus, family or other, including mixed levels).

Accuracy and *bias* are QL performance characteristics for taxonomy (Table 5-4). Accuracy requires specification of an analytical truth. For taxonomy, it is a) the museum-based type specimen (holotype, or other form of type specimen), b) specimens verified by recognized expert in that particular taxon or c) unique morphological characteristics specified in dichotomous identification keys. Determination of accuracy is considered "not applicable (na)" for production taxonomy (most often used in routine monitoring programs) because that kind of taxonomy is focused on characterizing the sample; taxonomic accuracy, almost by definition, would be focused on individual specimens. Bias in taxonomy can result from use of obsolete nomenclature and keys, imperfect understanding of morphological characteristics, inadequate optical equipment, and poor training. Neither of these performance characteristics is considered necessary for production taxonomy, in that they are largely covered by the estimates of precision and completeness. For example, although it is possible that two taxonomists would put an incorrect name on an organism, it is considered low probability that they would put the same incorrect name on that organism.

5.9.4 Data Entry

Efforts to understand the quality of data entry activities may seem trivial. However, the impact of errors can be substantial, and, if undiscovered and uncorrected, can become amplified through the assessment process. This QN performance characteristic (*accuracy*) simply quantifies the number of correctly-entered data values as a proportion of the total number of data values entered. The process involves having a QC person, distinct from the staff doing the primary data entry, check all data values (100%) against the original handwritten datasheets. With the datasheets as the analytical truth, the rate of errors is the accuracy of the data entry (Table 5-4). As errors are found, they are corrected electronically. For their wadeable streams program, Mississippi Department of Environmental Quality (MDEQ) found that the two data types with the highest error rates were the datasheet header information (e.g., stream name, latitude/longitude, date of site visit, and name of field staff) and streambed particle size data (MDEQ 2006). This allowed corrective actions to be focused where needed. All other performance characteristics are considered not applicable.

5.9.5 Data Reduction (Metric Calculation)

For most biological assessment programs, raw data are the list of taxa found at a site (in a sample) and the number of individuals recorded for each taxon. Preparation of those data for analysis requires conversion to metrics or other terms; metric calculation is a form of data reduction. When electronic spreadsheets or other data manipulation techniques are used, queries are often built to perform both complex and simple calculations. If queries are not performing as intended, or links to the raw data are incorrect, errors in metric values can occur. Precision of data reduction is a QN performance characteristic (Table 5-4) that helps ensure database/ computer calculation routines are performing as intended. A subset of metric values is hand-calculated using only the taxonomic and enumeration data, which are then compared to those that result from the computer queries. A recommended approach involves calculating one metric for multiple samples (e.g., systematic, every third sample), as well as all metrics for at least one sample. If differences are found, each value should be checked for errors in the calculation process (hand calculator vs computer algorithm), and corrections made.

5.9.6 Site Assessment and Interpretation

QN performance characteristics for site assessment and interpretation are *precision*, *accuracy*, and *completeness* (Table 5-4). Site assessment precision is based on the narrative assessments from the associated index scores (e.g., good-fair-poor) from reach duplicates. It quantifies the percentage of duplicate samples that receive the same narrative assessments as the original. These comparisons are done for a randomly selected 10% of the total sample lot. Table 5-5 shows that, for this dataset, 79% of the replicates returned assessments of the same category (23 out of 29); 17% were 1 category different (5 of 29); and 3% were 2 categories different (1 of 29). Accuracy is the proportion of samples for which the biological index correctly identifies sites as impaired; the calculation is discrimination efficiency (DE) (Table 3-3). DE is a value that is developed during the index development and calibration process. Percent completeness (%C) is the proportion of sites (of the total planned) for which valid final assessments were obtained; a site assessment is considered valid when data of sufficient quality and quantity are available for that assessment.

QL performance characteristics for site assessment and interpretation are bias and representativeness (Table 5-4). The final assessment of a site can be biased if a small number of reference or stressor sites are used during the calibration process; low numbers of stressor sites can potentially result in high discrimination efficiencies that are spurious. If interpretation of assessment results fails to take into consideration abnormal or extreme hydrologic or climatic events, or other non-natural catastrophic and localized events, results could be considered non-representative of ambient conditions.

TABLE 5-5. Assessment results shown for sample pairs taken from 29 sites, each pair representing two adjacent reaches (back to back). Assessment categories are 1-good, 2-fair, 3-poor and 4-very poor.

Site	Replicate 1		Replicate 2		Categorical Difference
	Narrative	Assessment Category	Narrative	Assessment Category	
A	Poor	3	Poor	3	0
B	Poor	3	Poor	3	0
C	Good	1	Good	1	0
D	Poor	3	Very Poor	4	1
E	Fair	2	Fair	2	0
F	Poor	3	Fair	2	1
G	Poor	3	Poor	3	0
H	Very Poor	4	Very Poor	4	0
I	Very Poor	4	Very Poor	4	0
J	Poor	3	Poor	3	0
K	Poor	3	Poor	3	0
L	Very Poor	4	Very Poor	4	0
M	Very Poor	4	Very Poor	4	0
N	Poor	3	Fair	2	1
O	Poor	3	Poor	3	0
P	Poor	3	Poor	3	0
Q	Poor	3	Very Poor	4	1
R	Poor	3	Poor	3	0
S	Fair	2	Very Poor	4	2
T	Fair	2	Fair	2	0
U	Good	1	Good	1	0
V	Poor	3	Fair	2	1
W	Fair	2	Fair	2	0
X	Poor	3	Poor	3	0
Y	Poor	3	Poor	3	0
Z	Very Poor	4	Very Poor	4	0
AA	Poor	3	Poor	3	0
BB	Fair	2	Fair	2	0
CC	Poor	1	Poor	1	0