4. Periphyton (Attached Algae and Aquatic Plants) as Indicators of Watershed Condition

For the purposes of this manual, “periphyton” consists of the plants attached to benthic sediment, rock, and each other at the bottom and edges of water-bodies. In many areas around the world they are considered appropriate indicators of ecological condition and pollution. In California water-bodies there are algal species and vascular plants that can be used in this way too. The method described here is for measuring the occurrence and amount of periphyton in streams and rivers, though it could possibly be adapted for use in lakes, as well. The focus and examples are primarily for algae, but the principles apply to vascular plants too.

A. Overview

Periphyton in streams and rivers are an important component of aquatic ecosystems, providing food for invertebrates, and thus fish, in local and downstream ecosystems (e.g., Finlay et al., 2002). Periphyton growth can be light-limited (Kiffney and Bull, 2000; Quinn et al., 1997a,b) or nutrient-limited (Cascallar et al. 2003; McCormick and Stevenson, 1998; Perrin and Richardson, 1997), or both, and is influenced by temperature (Francoeur et al., 1999; Morin et al., 1999; Robinson and Minshall, 1998; Weckstroem and Korhola, 2001). In addition, periphyton communities can rapidly deplete waterways of nutrients, assuming no additional inputs, and communities vary compositionally (i.e., species types) with nutrient concentrations (Marinelarena and Di Giorgi, 2001). Excessive periphyton growth can occur in rivers and lakes as a result of high water temperatures from reduced managed flows or excess nutrient production from human development on the landscape, through releases from wastewater treatment facilities, agricultural operations, deforestation, and soil disturbance, and therefore can serve as an ecological indicator for these disturbances (Bojsen and Jacobsen, 2003; Cascallar et al., 2003; Chessman et al., 1999; Delong and Brusven, 1998; Giorgi and Malacalza, 2002; Harding et al., 1999; Siva and John, 2002; Winter and Duthie, 1998). “Excessive growth” is defined here as growth that is not normal for the system and that causes local or downstream negative impacts such as changes in the particulate and dissolved organic carbon budget, nutrient cycling, biological and chemical oxygen demand, pH, and/or methylation.
and accumulation of mercury in fish. Increases in aquatic vegetation growth can change and negatively impact benthic macroinvertebrate abundance and species richness and their functional role in the ecosystem as consumers of organic material and prey to larger invertebrates and vertebrates (Collier, 2002; Nelson and Lieberman, 2002; Quinn et al., 1997; Robinson and Minshall, 1998; Suren et al., 2003).

The conceptual model below (Figure 1) shows the ecosystem processes and attributes that are important when considering periphyton measurements. Land and water management actions may contribute to periphyton growth, which may in turn impact water chemistry (dissolved oxygen and pH) and fixed carbon production (dissolved organic carbon, DOC). These may impact local aquatic habitats and wildlife (benthic macroinvertebrates, fish, etc.) and downstream aquatic habitat and drinking water quality (e.g., mercury methylation and bromine-reactive DOC compounds). Methylated mercury can enter the food chain and pose health risks to wildlife and humans; bromine-reacted DOC compounds in drinking water are also health hazards. The parameters shown here, as well as consequences of land and water management actions (elevated temperature and nutrient concentrations) can be measured in order to understand the role of periphyton growth as an environmental indicator, and potential nuisance, in your watershed.

Figure 1 Conceptual model of potential effects on periphyton growth and potential effects of periphyton growth.
Periphyton community structure, species composition, and succession respond to environmental conditions and thus can be used to classify waterways (Denicola et al. 2004; Wargo and Holt, 2004). In addition, these algal communities can and have been used as biotic indicators of ecological condition and change in condition in response to human and natural disturbance (Cascallar et al., 2003; Chessman et al., 1999; Denicola et al., 2004; Hamsher and Vis, 2003; Komulainen, 2002; McCormick and Stevenson, 1998; Stevenson, 1998). In rivers in New Zealand and in the Sacramento River, periphyton succession and total biomass may be a driving or explanatory variable in determining benthic macroinvertebrate community structure (Harding et al., 1999; Nelson and Lieberman, 2002; Suren et al., 2003). Dams and flow regulation are correlated with downstream increased periphyton biomass and decreased taxonomic richness, biomass, and density of invertebrate communities (Collier, 2002; Growns and Growns, 2001). Periphyton growth on the edge of Lake Tahoe has been suggested as a useful environmental indicator for human-induced nutrient enrichment in that system (Hackley et al., 2001).

The algal flora in California is not well described. The periphyton algae sampled in the Sierra Nevada (e.g., Figure 2) seems to be primarily of the division Chlorophyta (green algae) and the genus Cladophora, which forms branched or unbranched filaments up to several meters long and has the common name “blanket weed”. It is uncommon in waters low in calcium, nitrogen, and phosphorous. Moderate growth of this alga can occur in high quality water, though large mats and long filaments are signs of “eutrophication” (nutrient enrichment) of waters. Most freshwater algae are primarily growth-limited by the availability of phosphorous, and secondarily nitrogen. (Canter-Lund and Lund, 1995). There may also be excessive growth of aquatic vascular plants in certain creeks and rivers.

Live algal mats can dramatically alter the dissolved oxygen concentration in the benthos and water column during the day (increase) and night (decrease) (Lavoie et al., 2003). Excessive organic matter from the algae, when it dies, results in biological oxygen demand (BOD). Increasing organic carbon availability, and consequently reducing oxygen concentrations, creates conditions facilitating mercury methylation both in-stream and in downstream reservoirs. Excessive amounts of live algae can also cause wide daytime swings in pH due to the uptake of carbonic acid (a source of carbon dioxide) for photosynthesis.

B. Condition assessment using a periphyton bioindicator

Several key watershed-management issues can be addressed through assessment of the periphyton community, both in terms of its density and its composition.

1) Location and severity of nutrient pollutant inputs to a waterway. Although you may not be able to estimate the concentration of nutrients from measuring periphyton, extensive growth
of periphyton or dominance by particular pollutant-tolerant species can indicate excessive nutrients.

2) Location and severity of high temperatures in waterways that would naturally be cold. Even in the absence of high nutrient concentrations (e.g., in oligotrophic waters), it is possible to get excessive periphyton growth or dominance by particular pollutant-tolerant species because of higher-than-normal water temperatures. These high temperatures could originate from water diversion/storage that result in low in-stream flows or flows from warm reservoirs. They could also result from point-sources of warm water where the water was warmed during use or because it was exposed to warm surfaces or conditions (e.g., street surfaces or waste-water treatment plant). Finally, abnormally warm water could be caused by a loss of riparian canopy and resulting excessive sun exposure.

3) Invasion of a waterway by a periphyton species that does not normally occur, but can dominate the local waterway flora and become a debilitating nuisance to human and ecological needs. An example of this is the water Hyacinth (*Egeria densa*), which is a rapidly-growing aquatic flowering plant that grows in slow-moving waters of the Bay-Delta watershed and Southern California ([http://dbw.ca.gov/aquatic.htm](http://dbw.ca.gov/aquatic.htm)). It forms dense mats that dominate all other ecological features and influences processes in the waters beneath it.

4) Natural succession of periphyton genera and species disrupted seasonally and with watershed disturbance. Aquatic plant communities, like their terrestrial counterparts, change composition in response to seasonal changes (e.g., flow and temperature) and watershed disturbance (e.g., water diversion, fire, or development activities). By analyzing composition of periphyton communities over several seasons, and in disturbed and less-disturbed conditions, you can determine which community properties can best serve as indicators for change in your region and even your watershed.

C. Description of method

Most of this section describes how to assess periphyton growth and describe the periphyton community. *Method 1* is quantitative, while *Method 2* is not quantitative, but may help you determine whether or not a problem exists and what the potential causes are. This second method can be used if resources are lacking to conduct a complete study, as described in *Method 1*. Because of the similarity in many ways between sampling benthic macroinvertebrates (BMIs) and periphyton, *Method 1* is structured like that which is presented in Chapter 5, which describes the survey approach for BMI communities.
Method 1 – Biological, Chemical, and Physical Habitat Investigation

1) Identify the type of impacts to be studied through a periphyton community assessment

Examples of types of impacts are:

- A fixed source of pollution that periodically or regularly delivers water-borne pollutants to a waterway.
- Diffuse sources of pollution affecting a given waterway.
- Range of extents and types of disturbances across watershed.

Step 1 Outline the watershed management issues you want to study using periphyton and the reasons you think periphyton are an appropriate indicator for the investigation. Draw a conceptual model and study design that shows how you think parts of the system work together and how you would study them.

2) Select a number of sites in a waterway appropriate for understanding the degree of impacts

As with BMIs, the number of sites chosen to measure periphyton depends on the complexity of the monitoring and assessment situation. In this case, “site” means sampled reach. In addition, at each site you might take several samples at independent locations (>3) in order to understand the natural variability in periphyton distribution. This will also allow you to compare different waterways or reaches along a waterway and to compare impacts from point and non-point sources. The number and distribution of sampling sites between disturbed and control conditions, and the number of these sites compared to all possible sites within the study area affect how statistical analyses are conducted and how results are interpreted. In addition, because there is natural variation in the physical nature of riffles, both within riffles and among riffles, the samples collected potentially represent different natural physical conditions, which may confound their comparison with other samples from nearby riffles. Because of this, the distribution and number of sites is an important consideration.

There are no hard and fast rules for decisions about site selection, but there are some guidelines you can use:

1) Randomly distribute your sites/reaches along the waterway. This will increase the likelihood that your measurements reflect the average condition for the waterway and that you can estimate variability in the measured parameter (e.g., mass of periphyton) for the reach.
2) Target potential point sources, but randomly distribute the above-point and below sites/reaches and the sampling locations within the sites/reaches. For point source pollution inputs you would want at least one sampling reach above and one below the point of input and preferably three, the minimum for statistical comparisons between types of sites. For non-point source pollution, the site number and distribution would depend more on the size of the area involved and the resolution desired for measuring an impact or change over space and/or time.
3) Use previous experience or preliminary measurements to determine the number of sites and samples/site needed to account for variability. Within a 100 m reach, you could have periphyton biomass range from zero to hundreds of milligrams per square meter, depending on water depth, flows, light availability and other local environmental factors. Preliminary measurements allow you to calculate variation, which you can then use to calculate the number of samples and sampling sites you should use in order to have sufficient statistical power to detect change over time or difference between locations.

4) Make sure sites cover the range of light, water depth, shading, and substrate types found in the reach.

**Step 2** Select sites (riffles) according to the types of impacts/conditions you wish to study.

3) Describe physical habitat and chemical water quality characteristics

The California Stream Bioassessment Procedure (CSBP, Harrington and Born, 2003; Aquatic Bioassessment Laboratory, 2006) provides guidance for physical habitat and water quality assessment in association with monitoring benthic macroinvertebrates (see Chapter 5 in this Volume). This guidance is suitable for periphyton studies too, with some added chemical measurements.

**Water Quality**

The primary water characteristics measured in the field at the time of periphyton sampling are: temperature, specific conductance, dissolved oxygen, pH. In many cases you would also want to take samples for measuring concentrations of nitrogen and phosphorous-containing nutrients. Standard EPA, USGS, or SWRCB protocols should be used for the field measurements. These are described elsewhere in the Manual (Volume II, Chapter 3). Nutrient concentrations can be measured as: total Kjedahl nitrogen, nitrates, nitrites, ammonia, total phosphorous, particulate phosphorous, and soluble reactive phosphorous (methods examples, Hatch et al., 2001; Hunter et al., 1993; Kampahke et al., 1967; and Murphy and Riley, 1962). These methods have been and are currently being used to measure these nutrients in Lake Tahoe and can be very sensitive.

**Physical Habitat Quality**

The methods from the 2003 & 2006 CSBP summarized below are for streams with residual or dominant natural sediment and plant cover. They can be adapted for use with developed areas where concrete channel bottoms are prevalent. In this case “riffles” can be replaced with “channel site or section”.

a) Measure study reach length and average channel site dimensions (length, width, slope, and depth). Measure exact depth of periphyton sampling locations.

b) Estimate stream water velocity by measuring the rate of movement of a floating object, or measure flow velocity using a flow meter.

c) Estimate or measure canopy cover over the sampling site/riffle by eye or using a spherical densiometer.
d) Estimate or measure benthic substrate complexity and embeddedness in the entire riffle length.

e) Estimate proportion of riffle in sediment categories ranging from fines to large boulders.

f) (2006 CSBP) Measure particle size frequencies (Wolman, 1954 technique, 5 particles) for each of the 11 major transects (n=55 particles) and 10 inter-transects (n=50 particles). Sample a single particle at each bank and at ¼, ½, and ¾ the width of the creek.

g) (2006 CSBP) Estimate percent that the sampled particles at each transect are embedded in fine sediments.

h) (2006 CSBP) Use inter-transect distances and elevation changes to calculate average reach slope.

i) (2006 CSBP) Record any of the various categories of human activities present in the riparian centered on each transect.

j) (2006 CSBP) Record size, type, and condition of riparian vegetation and bank stability.

k) (2006 CSBP) Record in-stream habitat complexity, including natural and human elements.

l) (2006 CSBP) Record particle size frequencies at the 10 inter-transects.

m) (2006 CSBP) Record flow-based habitat types at each of the 11 major transects.

n) (2006 CSBP) Measure bank-full width and multiple depths at a single representative transect.

**Step 3** Measure water quality and physical habitat attributes in the sampling riffles and reaches.

**4) Sample periphyton from benthic sediments at each site**

Within each sampling site/reach, three to five individual samples should be taken of periphyton attached to rocks within riffles. Sample locations should be chosen using a random number chart to choose the distance in meters from the downstream end of the riffle (method used by Harrington et al., California Department of Fish and Game for benthic macroinvertebrate sampling). A 1/16 m² quadrat can be used to delineate a collection area within which all cobble can be sampled. A quadrat is a square made of sturdy material (such as wood or PVC) and the area of the open square is exactly determined and constant. If you want to calculate the amount of periphyton in the reach, your quadrat should be large enough to fit at least five to ten representative cobbles within the open sampled area. If you want to calculate the distribution of algae on the surface of cobbles at finer scales (e.g., does the periphyton grow on the sides or tops of cobbles?), the sampled area can be smaller than the size of individual cobbles. An alternative to collecting rocks is to place artificial substrate on the benthos (e.g., ceramic tile) for several weeks to months and retrieve them for sampling. Because these tiles or other materials can be of known size and can be distributed randomly or with specific intention, the sampling process will be much easier. However, because the physical attributes of the tile do not exactly mimic native substrata, the periphyton community colonizing the tiles may not be representative of the flora of the reach. Use of tiles may therefore not be appropriate, depending on the goals of the assessment.

Rocks are collected from within the quadrat, scrubbed free of attached algal/plant material, and returned to the riffle. The entire sample of collected periphyton is captured in a 1-liter container and stored on ice until processed. If something other than mass is going to be determined (e.g., identification of plant species present) then the sample can be crudely homogenized (e.g.,
through a large-bore 50 cc syringe) without causing cell wall disruption to allow accurate sub-
sampling of a small proportion of the sample. Algal sample dry and organic masses can be
measured by filtering the complete sample (minus sub-samples) on pre-weighed glass-fiber
filters. This can be done in the field, or within 24 hours in a laboratory. The filters containing the
plant material are dried and weighed (Dry mass = weight of dry filter & material – dry weight of
filter). They are then heated at 450°C in a muffle furnace, and re-weighed (Dry biomass = dry
filter & material – ash filter & material).

If you have large amounts of periphyton growing, which is probably the most important time to
sample it, then the sampling, concentrating, and sub-sampling will become more challenging. If
there are long strands of algae originating from your cobbles, you will need to make sure you
don’t break them off when setting down the quadrat. When retrieving the cobbles, try to make
sure that the attached long filaments are saved. Removing the material may need to involve
scraping first, then scrubbing, so that the scrubbing brush does not become fouled. You can
collect the larger material in a coarse filtering device (e.g., a sieve) and capture the smaller
material that makes it through the pores for filtering or adding back to the large material once it
is concentrated. With more material, you will need larger aluminum weighing boats. For
example, a 47 mm weighing boat will hold the amount of material coming from 1/16 m² sampled
area that looks like the cobbles in Figure 2, above.

Some known proportion of the suspended algal material can be sub-sampled for taxonomy
and/or chlorophyll-a measurement. Identification of the plant species present (remember algae
are plants) and the changes in the periphyton communities over space and time can tell you
about influences of environmental conditions on those communities.

**Take a sample for Chlorophyll-a measurement**

Chlorophyll-a is an important photosynthesis pigment and can be used to estimate relative
amounts of healthy plant material in a sample. An aliquot of suspended algae of known volume
can be taken for chlorophyll-a measurement. The algae can be suspended by passing the algal
sample through a large bore syringe to break strands. The method is after that of Parsons et al.
(1984) and is briefly described here.

- The aliquot of suspended algae is filtered onto glass-fiber filters and pigments are extracted
  with 90% acetone within hours (not days) of sampling the algae.
- The filter is shaken in 90% acetone and the resulting aqueous sample centrifuged to remove
  particulate material.
- The absorption of the supernatant is measured at 630, 647, and 664 nm, from which
  chlorophyll-a amounts and concentrations can be calculated.
- The amount of chlorophyll-a per square meter is then calculated based on the known sub-
sample volumes. These values are useful for comparing with past studies and other
geographic areas.

**Step 4 Collect periphyton at sampling locations, sub-sample periphyton for organic mass,
taxonomy, and chlorophyll-a.**
5) Identify the periphyton to genus or species

Periphyton samples collected in the field can be used to identify and count vascular plants species and soft-bodied (e.g., *Cladophora* spp.) and diatom algae. As is the case with benthic macroinvertebrates, the greater taxonomic detail (e.g., genus vs. family level identification) you can obtain about the periphyton community, the greater range of assessment questions you will be able to address. Of course, greater detail usually means greater cost and/or intensity of effort, so there is some balance you will need to reach.

The taxonomy samples are preserved in Lugol’s Iodine Solution (KI/I in 10% Acetic Acid, 1% Lugol’s in final sample) at the time of sub-sampling in the field. All taxonomy and counting must be carried out by trained taxonomists. One sub-sample, each, for vascular plants, soft-bodied algae, and diatom algae is taken from the field samples. The methods used are adapted from two main protocols used for wadeable streams. The websites below describe the protocols and each has several references for taxonomy: a) EPA rapid bioassessment protocol (Barbour et al., 1999): [http://www.epa.gov/owow/monitoring/rbp/ch06main.html](http://www.epa.gov/owow/monitoring/rbp/ch06main.html) b) USGS NAWQA: [http://water.usgs.gov/nawqa/protocols/OFR02-150/index.html](http://water.usgs.gov/nawqa/protocols/OFR02-150/index.html).

**Soft-bodied algae:**

The following is one way to count and identify soft-bodied algae (non-diatoms). Algae samples are sub-sampled and the relative abundance of various macroalgae determined. The remainder of the sample is agitated to dislodge epiphytic algae and to randomly distribute individual cells and colonies. Exactly 0.1mL of the homogenized sample is placed in a Palmer-Maloney counting chamber using a micropipette. Algae in the Palmer-Maloney counting chamber are identified and counted at 400X magnification using a light microscope. Filaments and colonies are counted as one unit.

**Diatom ID/Enumeration:**

The following is one way to count and identify diatoms. The diatom ID/enumeration samples are homogenized and a 10mL sub-sample placed in a small glass beaker. The diatom sample is treated with a 1:1 ratio of concentrated nitric acid and 10 mg of potassium dichromate (to digest all organic matter). The sample is then rinsed with de-ionized water, through repeated cycles of settling and/or centrifugation of the sample pellet, until the pH of the sample is neutral. The clean diatoms are mounted on duplicate slides in a high-resolution resin (Naphrax®) for identification under a 1000X magnification light microscope. Relative concentration of diatom species for each sample are determined by choosing an area of the slide with heterogeneous distribution of cells and then identifying diatoms, one field of view at a time, until at least 600 diatom valves are counted and identified.

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**Step 5** Identify and count periphyton to the genus/species level, depending on need.

6) Calculate periphyton metrics

Several metrics relating to periphyton growth and community composition can be calculated from the data you gather. The significance of the actual values for these metrics will depend on the location and type of waterway in which they are measured. We provide guidance here about
what the metrics mean, but the values you find will have to be compared to local or regional standards or reference sites in order to put them in context and make them meaningful.

**Biomass**

The organic mass, or “biomass”, of the sampled periphyton is that weight of material that can be burned off at 450°C in a furnace. This material consists of all of carbon-based compounds that compose part of living, and formerly-living, material. The remaining material is the “ash”, or inorganic, portion of living material. The biomass of periphyton is important to determine, because it is the best and easiest measure of periphyton growth in response to environmental conditions. However, it is not always immediately obvious what factors are controlling periphyton biomass in your study system. Excessive growth of periphyton can be due to one or more of the following: 1) high nutrient concentrations, 2) high temperatures, or 3) long periods of stable flow without scouring (high-flow events). Biggs (1996) defined nuisance levels of algal biomass, resulting from nutrient enrichment, as those exceeding 5 mg cm⁻² (cited in Barbour *et al.*, 1999). This value may be useful for you in interpreting your data and identifying potential nutrient-enrichment problems. However, only previous studies, or comparative studies among waterways with different environmental conditions will give you a sense of what is normal and excessive periphyton growth.

**Chlorophyll-a**

The amount of chlorophyll-a (chl-a) in a sample should be proportional to the amount of biomass. However, light conditions can influence the concentration of this photosynthesizing pigment in plant cells, so if you are going to use chl-a to represent biomass, you should calibrate chl-a concentrations with actual biomass. The EPA (Barbour *et al.*, 1999) suggests that trophic status of waterways can be defined in part by benthic chl-a concentration, where streams are considered oligotrophic when they have mean benthic concentrations < 2 μg chl-a cm⁻², and eutrophic, when they have mean benthic concentrations > 6 μg chl-a cm⁻² (Welch *et al.*, 1988; Dodds and Welch, 2000). As with biomass, values for chl-a concentration are most meaningful when put in the context for local and regional conditions and norms. There are high-elevation streams in California that have very little plant material naturally growing on benthic cobbles, and some growth could be considered excessive without exceeding the standard for oligotrophic streams.

**Periphphyton community composition**

There are over a dozen “metrics of biotic integrity” that the EPA associates with periphyton taxonomy (Barbour *et al.*, 1999). These are listed and described below.

*Species richness* The number of periphyton species in a sample. High richness may indicate biotic integrity, or it may indicate nutrient enrichment in a nutrient-limited system. Low richness may be a natural condition in naturally nutrient-limited systems (e.g., cold, nutrient-poor, shaded streams), or in polluted conditions where few species survive or out-grow others.

*Genus richness* The total number of genera (the plural of “genus”) may be a more robust measure of integrity than species richness, with high richness indicating higher integrity and low richness indicating stress/pollution. However, in very unproductive waters low levels of plant growth), other metrics may be more relevant.
Division richness  The total number of divisions for all taxa should be highest in waterways with good water quality.

Shannon Diversity Index (diatoms)  The Shannon Index is a combination of the number of species and the evenness of distribution of individuals among taxa (Klemm et al., 1990). It may function as a sensitive indicator for pollution when the total number of taxa is high (> 10). When taxa richness is low, interpretation of the Shannon Index may be facilitated by a comparison with the theoretical maximum Shannon Diversity value for that number of taxa.

Percent Community Similarity of Diatoms  This index allows community similarity to be assessed between sites based on relative abundance of diatom species within communities. This gives more weight to numerically dominant taxa than others. Test sites can be compared with reference sites, or all study sites can be compared pair-wise with each other.

Pollution Tolerance Index for Diatoms  Many diatom taxa (species) have been assigned tolerance ratings, from 1 to 3, based on knowledge of their tolerance of pollution. Tolerant taxa get a value of 1, and intolerant a value of 3. The index score is calculated with the following formula:

\[
PTI = \frac{\sum (n_i \cdot t_i)}{N}
\]

where \( n_i \) = number of cells counted for species \( i \)

\( t_i \) = tolerance value of species \( i \)

\( N \) = total number of cells counted

Percent Sensitive Diatoms  This is the sum of relative abundances of all species intolerant to pollution. This metric is useful in low-productivity streams where other metrics may underestimate pollution impacts to water quality.

Percent abundance Achnanthes minutissima  This species is a commonly found attached diatom that pioneers and can dominate in recently scoured or polluted sites, and it is frequently dominant in streams affected by acid mine drainage. Disturbance is crudely indicated by percent abundance of this species with the following numeric standards: 0-25% = no disturbance, 25-50% = minor disturbance, 50-75% = moderate disturbance, 75-100% = severe disturbance.

Percent live diatoms  This simple metric has been proposed as an indicator of sediment deposition on algae, or of older assemblages.

Percent aberrant diatoms  Aberrant diatoms in this case are ones that have abnormal patterns and shapes in their frustules (e.g., bending or indentation). Diatom aberrance has been associated with heavy metal contamination in streams (McFarland et al., 1997, cited in Barbour et al., 1999).

Percent motile diatoms  This is an index of siltation composed of the relative abundance of the motile genera Navicula + Nitzschia + Surirella. Individuals of these genera are capable of crawling above silt when it is deposited on algal communities and increasing percentages are thought to indicate frequent or excessive siltation. Other motile genera may be included if present (e.g., Gyrosigma and Cylindrotheca).

Simple diagnostic metrics  These are calculated as relative abundance (% species of genus X) and are related to the ecological requirements or tolerance of of the taxa. They include: % acidobiontic + % acidophilic, % alkalibiontic + % alkaliphilic, % halophilic, % mesosaprobic + % oligosaprobic + % saprophilic, and % eutrophic.
Simple Autecological Indices (SAI)  Diatoms, like all living things, have environmental/habitat preferences and relative abundances of the various taxa in a given sample can indicate the prevailing environmental conditions (e.g., acidity, salt concentration).

Inferred ecological conditions with Weighted Average Indices (WAI)  Ecological condition of the site is indicated by the relative abundances of diatom taxa. These values are compared against the maximum abundances, for each, that would be expected under optimal growth conditions, based on information from the literature.

Impairment of ecological conditions  This can be calculated by measuring the deviation from inferred environmental conditions at a test site relative to a reference site. Either the SAI or WAI from above can be used as the index of condition at a site.

**Step 6** Calculate periphyton community metrics depending on the assessment question.

7) Analyze basic statistical properties of the metrics (e.g., mean and variance) and compare among reference and impacted waterways or reaches, and/or among waterways and expected values (e.g., correlation analysis).

There are several types of metrics you can obtain from the methods described here. One is the community composition at each site, using a combination of vascular plant, diatom algae and non-diatom algae taxonomic information. The second is the dry mass and organic mass of the sampled periphyton. The third (related to the second) is the amount of chlorophyll-a per sampled area. The calculation of the mean and variance for quantitative periphyton data is the same as for any quantitative data. The California Stream Bioassessment Protocol describes the application of the Student t-test and Analysis of Variance (ANOVA) as ways to compare two sets of data (e.g., upstream vs. downstream of a point source of pollution).

One of the most important steps in your investigations will be to determine differences among sites, among sampling times, or between reference and impacted locations. To do this, scientists employ statistical tests for differences. You should carefully use these tests when using periphyton metric data because of the nature of the data (most metrics are proportions or percentages) and the impact of sampling design and natural variation on the distribution of values obtained. The values and metrics calculated in Step 6 include absolute numbers, proportions (percentages), and weighted proportions. Three types of questions, data, and tests are the following:

1) You may want to know if changes in water management that results in un-seasonally high or low flows and wide temperature swings affect the periphyton community structure. Periphyton community metrics such as abundance of particular genera may be useful for understanding these changes. For periphyton community metrics that are numbers within categories (e.g., # of a particular taxa or group), the Chi-square test (large N) and the Fisher’s exact test (smaller N) are appropriate. The Chi-square test and other analytical tools are described in Volume I, Chapter 5 and in this Volume, Chapter 1.

2) If you are investigating potential impacts of discharges into a waterway, you might want to compare the proportion of the periphyton community that is sensitive to discharges above and below the discharge. To compare metrics that are proportions and percentages (e.g., % sensitive diatoms), a t-test or similar test for similarity is suitable for comparing among
samples and sites. However, you must first transform your data using arcsin or logarithmic transformations before using tests like the t-test.

3) Nutrient inputs, reduction in riparian shade, and high temperatures from reduced flows can all cause periphyton blooms (high rates of growth). Measuring change of biomass or chlorophyll a per unit area of benthos can help to identify places where point source or non-point sources of impact may be causing periphyton blooms. Values for metrics that are continuous or quantification data (such as periphyton biomass) for one sample or site can be compared to those for another sample or site using the Student t-test, or comparisons can be made among multiple sites/samples using ANOVA.

As described in the CWAM, Volume I, Chapter 5, there are many resources available for conducting statistical analyses. If you use MS Excel, the Help menu in this program can guide you through conducting simple comparisons of samples, correlation/covariance analysis, and regression analysis. The most critical aspect of conducting statistical analysis with your periphyton metrics is that you are confident that the question you are asking will be addressed by the statistical test, the quality of the data, and the amount of data available.

Once you have accrued sufficient periphyton data over time, it will be tempting to conduct “trends analysis” literally meaning the trend in something over time. Trends analysis of natural systems is a very involved process, requiring knowledge of the system and very good knowledge of statistics. Most environmental processes have some periodicity or cycling associated with them, which is often due to short and long-term climatic changes. Therefore, as with any ecological indicator, trends analysis for periphyton must go beyond linear regression or similar analysis and include the potential effects of cycling changes in the environment. In addition, human land and water use and management can have cycles that are different from natural cycles. In the case of managed waterways, flows may be much higher than natural in the early summer to provide for irrigation water, or power generation. This will impact water temperatures and other in-stream processes.

**Step 7** Calculate statistical differences among/between sites and times for periphyton community metrics and amounts.

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**Method 2 – Narrative and Photo Description and Monitoring**

Unlike Method 1, the following method is not quantitative. It is provided as an alternative assessment approach for instances in which resources are lacking to conduct a complete study. It can be used to detect potential problems relating to nutrients and nuisance algal growth, and to simply assess waterway conditions over large areas.

1) **Conduct Steps 1 and 2 from Method 1.**

This will provide you with basic information about potential influences on periphyton growth and a selection of sites at which to study occurrence and growth of periphyton.

2) **At selected sites, describe channel and up-stream and surrounding landscape in narrative form.**
You may not have the time, money, or specific expertise to describe the channel and watershed conditions around your sites in a detailed or technical way. However, it is important to record these conditions so that you can later determine possible causes of any abnormal periphyton growth observed. Characteristics such as land-use (roads, houses, logging activity), adjacent riparian vegetation type, channel substrate (gravel, sand, large rocks), and watershed steepness can all help inform your assessment of periphyton communities. If you have a thermometer, water temperature is a good and easy environmental parameter to record. The state of Montana has a set of forms that you can use to track stream and associated watershed conditions (http://deq.mt.gov/wqinfo/monitoring/SOP/sop.asp).

3) At each of your selected sites, from early spring until late fall, take photographs of the channel bottom and surrounding watershed.

Photo-monitoring is an accepted method for assessing change in landscapes and vegetation. The State Water Resources Control Board provides guidance for photo-monitoring in general (http://www.waterboards.ca.gov/nps/docs/cwtguidance/4214sop.doc) that you can tailor to your needs. There are three aspects of this process that are important for preliminary periphyton investigations using photo-monitoring. One is that the picture should be taken in such a way as to minimize glare and maximizing the area recorded. The second is that a series of pictures should be taken that represent the range of sites, some of which should be “reference” sites that are similar to the study sites, but are free of the disturbance or condition under investigation. Third, photo stations should be used. These are fixed locations (several per site) from which the photographs are successively taken over time, and always with the same bearing. This provides continuity, and facilitates the ability to track changes in specific sites, as viewed from specific perspectives. Finally, for each photograph or set of photographs, it will be necessary to collect various “metadata”, or information about what is in and around the photo.

4) Compare apparent periphyton growth and differences/similarities among sites.

These observational data are qualitative, but still useful. There are two main ways to compare your photographic and descriptive characterization of periphyton growth. One is to compare conditions over time at each site: Things that you look for will be 1) when the periphyton started to appear on rocks, 2) at what water temperature (if recorded) this occurred, 3) when the periphyton growth appeared to reach its peak, 4) when the periphyton seemed to have either died in place, or disappeared due to increased fall/winter flows, and 5) if there were any changes in the appearance of the periphyton community itself, including growth or disappearance of one form (e.g., filamentous green algae) vs. another (e.g., non-filamentous brown algae). Another way to compare conditions is among sites within your watershed or within other watersheds. You will use the same kinds of variables listed for analysis over time. This can consist of comparing sites at the same time point, or if you have recorded environmental variables like water temperature, comparing sites at the same temperature. In addition, you could compare sites at the same time point, within the same elevational class in your watershed (e.g., 1,000 to 2,000 meters above sea-level). This will help you to avoid comparing upper watershed sites with valley-floor/coastal plain sites.
D. Minimum requirements to ensure data quality

There are several minimum requirements for the quality of periphyton data included in a watershed assessment. Meeting these benchmarks will maximize the robustness of decisions made using these data.

1) Number of sites

The term sites, as used here, is analogous to the riffles that are sampled using the 2003 California Stream Bioassessment Protocol (CSBP) approach for BMIs. As described above, “riffle” can be substituted with some other type of sampling stretch if the waterway does not have riffles. For each riffle, there are 3 transects containing at least 3 sampling locations. Samples within each transect are combined. The absolute minimum number of sites you would use for sampling periphyton, using approaches like the 2003 CSBP, is 2 within the point-source investigation framework. Even then, it would be better to have >1 upstream sites for reference and >1 downstream sites, in order to understand the extent of impacts. A reasonable standard would be ≥3 sites above and ≥3 sites below the point of disturbance, which will allow you to measure and to control for natural variability among your control or reference sites.

For non-point source investigations, the choice of number of sites is less straightforward and may be just as dependent on available funding and expertise as the question you are trying to answer. Under one approach in the 2003 CSBP, random stratified sampling (Step 2 above), you would first group all reaches in the study area (watershed) into categories of “likes”. Once you have grouped the reaches, you would randomly select a sub-set within each group to represent the entire group. This method may be more appropriate where there are many sub-watersheds and reaches to sample and choosing a representative sample is key. There are also non-random approaches to choosing sampling reaches, which are also based on physical and biological attributes of the reaches, and often logistical considerations, such as access to the reaches. This approach requires a field-intensive pre-survey, and may be more appropriate for smaller watersheds with fewer possible sampling reaches. For either random or non-random site selection approaches, the number of sites is hard to predict.

The best approach to take would be to estimate the variability in periphyton sampled (mass and community structure) and use that estimate and the number of individual periphyton organisms to be sampled per site to estimate the number of samples needed per condition (group of reaches), using statistical power analysis and sample size analysis (Volume I, Chapter 5).

2) Site distribution

The distribution of sites throughout your area of interest is important because it allows you to associate conditions in the watershed with periphyton metrics at particular sites on particular waterways. The distribution of sites for point source pollution investigation is straightforward and is defined here as being at least immediately above the point source and as near below the point source as is feasible. You may also choose other sites further upstream and further downstream to replicate your sampling at the immediate upstream and downstream sites.
For non-point source disturbance investigations, the best distribution of sites may be less intuitively obvious. In the most complex circumstances, that is, watersheds with many natural and human-induced conditions, you will need to employ a strategy such as the stratified random sampling approach described in the 2003 CSBP (see #1 above and Step 2 above).

Timing periphyton collection consistently (for a given waterway), according to season and/or according to influential environmental conditions, will increase the among-year comparability of the data obtained and improve signal-to-noise ratios for statistical analysis.

3) Timing of sampling

Periphyton communities may change in terms of relative abundance of component taxa in a particular location over seasons or in response to human actions. This can complicate sampling intensity and also can make the timing of sampling important. If the succession and growth of individual periphyton species and the community as a whole is unknown, then you may want to investigate this first in order to find out the best time for limited sampling. Once you have found out when growth and dominance of individual taxa occurs, then you will be in a position to select places and times to sample. Similarly, if you also determine what may be causing excessive growth if/when it occurs, then you can also time sampling of periphyton to follow environmental changes that may trigger growth.

The goal for appropriate site distribution is to achieve sufficient sampling sites to represent the population of conditions that you want to assess using the periphyton as indicators of watershed and waterway condition. This is best determined using statistical analysis of sample size, which can be related to the number of sites sampled.

4) Periphyton identification

Chapter 6 of the EPA Rapid Bioassessment Protocol (Barbour et al., 1999) describes, in general terms, the identification of periphyton collected during a monitoring/assessment program. As with BMI identification, you will need a trained and locally/regionally-experienced taxonomist to do most of the identification and potentially train local lay-taxonomists. If you rely on trained volunteer taxonomists, you should ensure that your identification process is yielding consistently reliable and accurate results by hiring a professional/expert taxonomist to identify periphyton in 10% of the samples, or 100% of a subset of samples. This is a way to conduct quality control on your identification process, especially if you are using trained volunteers. Another way to improve accuracy of taxonomic identification is to have all of the identification of periphyton conducted by an expert taxonomist with verifiable credentials. Both ways can be expensive, with the cost dependent upon the number of periphyton taxa and the fees of the expert.
E. Spatial and temporal scale
[This section is identical to the corresponding section in Volume II, Chapter 5]

Just as with BMIs, there are a variety of scales over which periphyton data can be used to assess waterway and watershed conditions. Obviously bioassessment accuracy increases with a higher intensity of sampling, more specific taxonomy, and the knowledge of influencing factors that can be considered the disturbance under investigation, or the environmental variables for which you would want to control, or use for explanatory purposes during data analysis. The case that may appear simplest on the surface (point-source investigation) still includes potential upstream impacts and influences, climatic variability, and dependence on sampling intensity. Here are some things to consider in relation to scale:

1) Single-reach investigations

You may be investigating a point source of pollution or the recovery of a reach after restoration through engineering/horticulture or management/ownership change. Having several sites above (control) and below (treatment) the reach of interest will help control for variation outside the scope of the point source or restoration. Things you can control for in this way include macroclimatic variation, other land and water use practices above the reference and treatment sites, and natural disturbance above the sites.

2) Multiple reaches or watershed non-point source investigations

Space
Investigations of multiple sources and types of impacts to waterways involve integrating information from several spatial scales, e.g., from points to rivers and sub-watersheds. A watershed-wide investigation will include many possible scales at which you can make conclusions, depending on sampling intensity (number of sites and longevity of program). If you have more than 3 sites for a reach or creek, you may be able to draw conclusions about an average condition compared to a reference or previous condition. Average condition can be determined for any spatial scale and will be most accurate when it includes all of the data available at that scale. You could compare the average condition of one creek that has some kind of impact with another that is relatively un-impacted. If your impacts are extensive, then averaging condition using an aggregation of data from multiple sites or reaches can improve both the sensitivity of your statistical comparison and the accuracy of your condition determination. If the impact(s) you are investigating is/are not extensive, then averaging conditions over a large area may only dilute your condition assessment. Averaging any condition assessment over any spatial scale depends on the similarity of sites and the metric values within the scale. This similarity can be determined using ANOVA to determine if there are differences among sites long a a particular creek (for example).

Because there will be at least 3 sampling transects per site, you may also be able to draw conclusions about changes from upstream to downstream, with increasing sample numbers yielding increasingly accurate estimates of condition at whatever scale is of interest. Generating an “average condition” can range from a simple task, if you have only a few sites, to complex, if you have many sites. Two possible ways to develop this average are to i) use all data to calculate the average or ii) use a random selection of data from all sites to calculate the average. This can be done at the scale of individual waterways or sub-watersheds.
In Figure 3 is a model watershed showing the different scales and potential scales for which periphyton data and metrics could be useful. In this model, boxes 3 and 4 are sub-watersheds of watershed II and boxes A and B are sub-watersheds of watershed III. For Sub-watershed I, you may have metrics for the mainstem of I and for creeks 1 and 2. The metrics for 1 and 2 could be evaluated separately and aggregated, if they are statistically similar, to contribute to a metric for the whole sub-watershed. For sub-watershed II, you could similarly compare A and B and if similar, they could be aggregated to contribute to a metric for 3, or maintained as distinct metrics. You could also compare A, B, and C and, if similar, aggregate them as metrics for a certain stream order or type. Among all 3 sub-watersheds (I, II, and III), you could compare the stream watersheds of a similar type or order (e.g., 1 to 6) and either identify one of them as a reference for the others, or develop a combined metric for all of them or a sub-group of them. Finally, you could develop a tiered or hierarchical metric system where you attribute condition scores based on an aggregation of periphyton metric scores from the top of the sub-watershed down. For example, if scores for A and B are “high” and for C “low”, for 3 “high” and for 4 “medium”, then for II, the contribution of these subsidiary creek watersheds to condition could be “medium to high”.

**Time**

In some ways, the temporal scale is more difficult to manage for bioassessment indicators than for some chemical or physical indicators, primarily because it can take many years to develop a statistically meaningful indication of change. For example, you might have many sites that satisfy statistical requirements for the spatial scale of your study, but it may still take decades to measure recovery of a system from the impacts to human activity. Fortunately (or unfortunately), it may take considerably less time to measure the evolving degradation of a waterway’s biota, or the existing degraded condition. This contrast points out one of the most critical things to consider when designing your use of periphyton data and metrics: What is the question you are trying to address and therefore what time-scale is relevant?

**F. Analysis of periphyton data for environmental assessments**

Measuring periphyton in a waterway in the context of watershed assessment is usually done in order to study the impacts of land and water management on aquatic life and community structure. Land management practices that can change (i.e., increase) periphyton growth include: 1) runoff from logging and road-building, grazing, agricultural operations, housing development and 2) discharge from commercial and industrial centers and wastewater-treatment facilities. Water-management practices that can change periphyton growth include: flood protection, water storage for consumptive use, water conveyance for irrigation and
drinking water, and hydropower generation. These changes could come about from changes in temperature, flow rates, or nutrient inputs. If, and when, you find “excessive” periphyton growth (i.e., above reference conditions or sufficient to cause negative environmental impacts) the characteristics associated with the increased growth will help determine potential causes of the excessive growth and management changes that can be made for remediating the observed impacts.

Understanding whether or not periphyton growth is excessive, what is causing the excessive growth, and what impact it may have had is not trivial. The possible approaches we present here span the range of types and complexity. The first approach is observation-based, the second is quantitative.

**Observation-Based**

Some degree of periphyton growth is a natural occurrence to be expected in healthy systems. However, the amount of growth and the general composition of the periphyton community may be indicative of disturbance at a site or along a waterway. One strength of the observational approach is that you can fairly rapidly and cheaply determine what reference or natural conditions are (if you have waterways that can serve as your reference), what disturbed conditions may exist, and potential sources of disturbance. The ability to do these things depends on your understanding of the underlying processes that can affect periphyton growth, the number of sites you have surveyed, whether or not you have reference conditions, and the time-frame over which you conduct your study.

You may find very obvious variation in growth of periphyton that is not explained by a corresponding variation in watershed conditions. For example, you may discover large mats of algae growing just downstream of your town, but not in similar waterways nearby that have no urban development. What is more likely is that you will find variable levels of growth corresponding to natural and human land-use conditions. For periphyton to function as an indicator in a typical watershed, relying only on observational data, you will need to have a reference waterway or reach that is similar to your waterway or reach of concern, a large difference in periphyton growth or community composition between the reference site and your waterway of concern, and a good mechanistic explanation for how watershed or waterway disturbance could lead to the excessive growth or modified community composition observed.

**Quantitative**

There are a variety of statistical approaches that have been and can be used to find a connection between water and land management activities and periphyton growth. Two are described here. The first approach (1) assumes the periphyton communities are homogeneous, and that species composition does not influence growth. The second approach (2) is based on grouping sites by periphyton species, and is thus sensitive to taxonomy. Understanding both descriptions written below requires a prior understanding of statistics.

1) An important question in measuring a biological component of streams is: "How and why did it respond to watershed processes?" The main way this is done is by conducting exploratory analyses to look for correlations between potential causes and potential effects. These correlations can be found using ordination techniques. These techniques, basic statistics, and available resources are introduced in Volume I, Chapter 5.
One commonly used ordination technique to find correlations is Principal Components Analysis, which has been used to compare watershed geographic characteristics and periphyton, macroinvertebrate, and fish assemblages (Heino et al., 2002; Ford and Rose, 2000). Another, more powerful, technique is Non-parametric Multidimensional Scaling (NMS; available in PC-ORD software; see Kruskal, 1964), which can be used as follows: 

a) To compare the distribution of watershed or waterway management practices to the biotic indices of aquatic community health. The practices serve as the “explanatory variables” (e.g., parcel densities, human population densities, number and output of wastewater treatment plants, number and volume of upstream reservoirs, and volume and proportion of upstream water diversion.) The indices of community health are the “response variables” (e.g., algal mass/unit area, benthic dissolved oxygen (DO), dissolved organic carbon (DOC) export, and benthic macroinvertebrate (BMI) distribution.)

b) To compare one response variable (e.g., algal mass/density) with the other response variables (DO, DOC, BMI). The basis for the NMS technique is the ranking of dissimilarities (in terms of Euclidean distances) among “objects” (e.g., algal mass at temperature X at site 1 in June) on dimensional scales set by the analyst, and the presentation of the objects/points in ordination space in such a way that the distance between points represents the degree of dissimilarity. Successful application of NMS preserves similarities among objects and shows dissimilarities. Advantages of NMS are that no assumptions are made about the modality of data distribution or correlations among variables and that information can be extracted effectively for non-linear relationships between variables. Several iterations should be run, using varying optimization criteria in order to maximize distance and minimize “stress”, which is the deviation of the distance metric from expected values (stress values between 0 and 0.20 are usually considered acceptable). It should be noted, however, that high stress values may indicate that more dimensions are needed for the analysis. Each iteration can be evaluated with a Shepard diagram that plots proximities of the original objects against the NMS distance metric. This process should be repeated until as much variance as possible (high r²) has been explained. NMS maps of the objects can then be evaluated for clusters of objects and “dimensions” or directionality in the occurrence of objects in the NMS map.

If NMS does not appear to be working (i.e., adequately explaining the responses measured), then Detrended Correspondence Analysis (DCA) can be used instead (Gauch, 1982; ter Braak and Šmilauer 1998). This method recognizes the possibility that distributions of periphyton species may be dependent on an environmental gradient, in contrast to NMS, which is preferable if periphyton species distribution is determined by something other than an environmental gradient (e.g., invasion in one waterway and not another; De’ath, 1999).

If there are correlations found between algal mass and the other response variables/parameters through the use of NMS or DCA, then Multiple Analysis of Variance (MANOVA) can be used to determine which of the individual watershed characteristics best explains the distribution of biotic (e.g., algal mass), chemical (e.g., nitrogenous nutrients), and physical (e.g., water temperature) conditions.

2) A combination of TWINSPAN and MANOVA can also be used to determine correspondence between periphyton growth and potential impacts (e.g., BMI community structure, benthic dissolved oxygen, DOC output). The ordination and classification method “TWINSPAN” (Two-Way Indicator Species Analysis; Hill, 1979) can be used to sort sampling sites into groups based on periphyton species occurrence. This method carries on repeated splitting of samples into daughter groups based on a combination of presence/absence of species and relative abundance of the species in the sample. The product of TWINSPAN grouping of sites is then
compared to the occurrence of environmental variables that are potentially responsible for the presence and abundance of these species (i.e., temperature, nutrient concentration, light) using MANOVA.

**Summary**

An important note is that the statistical approaches above represent the minimum research effort needed, as there are other techniques that may also be employed (e.g., pre-NMS ordination of data), if necessary. The results of these approaches should be an understanding of the immediate environmental conditions that correlate with excessive periphyton growth. Because certain of these conditions often correlate with land and water management (e.g., nutrients and urban development; water temperature and water diversion), you may find a correlation between excessive periphyton growth and landscape and water management conditions. Excessive periphyton growth may correlate with immediate environmental impacts, such as changes in the distribution of BMI taxa, and downstream impacts important to water users, such as the export of dissolved organic carbon.

**G. Reporting on the results of periphyton bioassessment**

For reporting purposes, you will want to express certain of your information about periphyton as raw data (e.g., periphyton organic mass was 80 g m\(^{-2}\) in the South Yuba River in July) and other information as a calculated metric, such as the community-based metrics. The variety of metrics available from periphyton investigations and what they mean are shown in general terms in Step 6, above (“Calculate periphyton metrics”). A caution, or caveat, for the use of all of these metrics is that local and regional conditions can affect the actual values of each metric and their significance. So for an evaluation of condition where you may conclude that a site/reach is “impaired” or polluted, you will want to have a local reference site or condition. For an evaluation of conditions among several or many waterways where you may conclude that certain are more or less polluted than others, a reference is not essential, since by design the least impacted can serve as the references.

When including data and conclusions about periphyton growth and community composition, it is important to describe the significance of both the measurement and the result. Why did you measure periphyton biomass and what is the significance of the values obtained? What conclusions can you draw for what you measured in terms of periphyton and other environmental conditions (e.g., nutrient concentrations). Because high temperatures, high nutrient concentrations, impacts from animal grazers (e.g., snails), and metal/pH/salt pollution are all possible sources of variation in growth and community composition, discussing results for these other parameters is important as context for your periphyton measurements.

Because the interpretation of periphyton metrics is not an established field in California, it is worth referring to the scientific literature for other areas to get an idea for how metrics are used and what might be significant values for the metrics. This can usually be accomplished at nearby Universities with scientific journals available and searchable in a library. In general, the scientific literature supports the following general interpretations of periphyton metrics:

- Excessive chlorophyll-a (>6 μg chl-a cm\(^{-2}\)) and/or biomass (> local reference or >50 mg biomass m\(^{-2}\)) of periphyton in cold water indicates excessive nutrient inputs.
• Excessive chlorophyll-a (>6 μg chl-a cm\(^{-2}\)) and/or biomass of periphyton (> local reference or >50 mg m\(^{-2}\)) in nutrient-poor water indicates higher-than-natural temperatures and/or light conditions.
• Dominance by a single genus or species (low taxa richness) indicates a breakdown of biodiversity and dominance by one, potentially pollution-tolerant, or invasive species. An exception to this is in cold oligotrophic waters where few species may be able to survive.
• Very little periphyton growth in waterways with appropriate conditions (e.g., warm and/or non-oligotrophic water) can indicate toxic conditions to periphyton (e.g., acid mine drainage or low dissolved oxygen), or could be due to a recent scour.

H. Appropriate use and limitations of data

As with any environmental monitoring protocol, periphyton metrics are most robust when calibrated against known conditions from another watershed, or against a reference or control condition. If you have obtained certain species/genera richness values for a waterway, these values are best interpreted in the context of what your expected richness is. These expected values could come from scientific investigations conducted in a similar or nearby waterway, or from a thorough understanding of the richness you would expect, given the land cover, physiography, and in-stream habitat conditions. The more limited your knowledge of what the metric values should be, the more limited you will be in inferring impacts from the values. However, the information you have collected may become useful in future or more extensive investigations and should be reported in the watershed assessment, so that they are not lost.

Each type of data collected will have limitations on use. Many of these have been discussed. Probably one of the biggest limitations on the use of periphyton data is one that is true for all environmental studies – over or under-interpretation of the significance of results based on statistical analysis or lack of such analysis. Correlation between “excessive” periphyton growth and an environmental variable (e.g., temperature) does not mean that this variable is the causative agent for the excessive growth. Another agent (e.g., nitrogen input) could be the reason for excessive periphyton growth, which may or may not be exacerbated by high temperatures depending on the habitat requirements of the species growing. The use of particular statistical analyses should be tied to the type of data in question (e.g., biomass of periphyton vs. proportion of periphyton in one taxon) and the ecological question that drove the periphyton investigation in the first place.

I. Additional resources

The websites listed here provide additional resources for your work with periphyton. The listing of the sites is not an endorsement of them per se and is intended to show what others are doing with periphyton around the country.

Update from the Moro Bay Volunteer Monitoring Program – article about photomonitoring of algae in creeks feeding into the Bay.

Update from the Makawai Stream Alliance regarding their monitoring of water quality and stream biota (algae) in the Waiahole Stream.
http://www.pixi.com/~isd/MakawaiWQ.html
http://www.tristatecouncil.org/pages/monitoring.htm

Montana Department of Environmental Quality’s monitoring protocols. Sections 5, 9, 10, & 12 are particularly relevant here. 
Specific section in Montana DEQ protocols on studying periphyton 

J. References

Aquatic Bioassessment Laboratory (2006). Standard operating procedures for collecting benthic macroinvertebrate samples and associated physical and chemical data for ambient bioassessments in California. California Department of Fish and Game Water Pollution Control Laboratory. Rancho Cordova, CA.


Harding, JS; Young, RG; Hayes, JW; Shearer, KA; Stark, JD. 1999. Changes in agricultural intensity and river health along a river continuum. Freshwater Biology. Vol. 42, no. 2, pp. 345-357.


Quinn, JM; Cooper, AB; Davies-Colley, RJ; Rutherford, JC; Williamson, RB. 1997a. Land use effects on habitat, water quality, periphyton, and benthic invertebrates in Waikato, New Zealand, hill-country streams. New Zealand Journal of Marine and Freshwater Research. Vol. 31, no. 5, pp. 579-597.


K. Glossary of Terms

Autecological: The natural habitat conditions and preferences of a species

Benthic: Referring to the benthos, the bottom of a water-body

Biological oxygen demand: The amount of oxygen required by biological components (e.g., bacteria in benthic sediment) of a natural system (e.g., a stream or lake)

Biomass: The same as organic mass – the carbon-based material that can be ignited and burned off at 450°C in a furnace

Dissolved organic carbon: The organic carbon containing compounds, materials, and chemicals (e.g., carbohydrates or lignins) that are dissolved in natural waters

Embeddedness: The degree to which sediments are embedded in the benthic substrate. Loosely-stacked cobbles have low embeddedness, the same cobbles sunk into mud or sand have high embeddedness

Eutrophic: Waters or systems with high primary productivity

Eutrophication: Nutrient enrichment of water-bodies, often from human activities and discharges
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Frustule</td>
<td>The mineral outer shell of a diatom</td>
</tr>
<tr>
<td>Macroinvertebrates</td>
<td>Invertebrates larger than ~1 mm living primarily in the benthos of streams and the</td>
</tr>
<tr>
<td>Methylation</td>
<td>The addition of the chemical “methyl” group to reactive mercury, usually by bacteria in low-oxygen conditions</td>
</tr>
<tr>
<td>Metrics</td>
<td>Things that you can measure about a system (e.g., number of species)</td>
</tr>
<tr>
<td>Nutrient cycling</td>
<td>The natural cycle of nutrient formation and utilization, including the turnover from one form into another (e.g., from nitrate to ammonia)</td>
</tr>
<tr>
<td>Oligotrophic</td>
<td>Waters or systems with low primary productivity</td>
</tr>
<tr>
<td>Organic mass</td>
<td>The same as biomass – the carbon-based material that can be ignited and burned off at 450oC in a furnace</td>
</tr>
<tr>
<td>Periphyton</td>
<td>The vascular plants and algae that are attached to the benthic sediments and each other</td>
</tr>
<tr>
<td>Physiography</td>
<td>The physical geographic conditions (e.g., topography)</td>
</tr>
<tr>
<td>Succession</td>
<td>The replacement of one plant dominant species or community type with another</td>
</tr>
<tr>
<td>Trophic status</td>
<td>The position a species or taxonomic group has in the food chain. Primary producers, such as plants, have a low trophic status. The relative productivity of a system (e.g., eutrophic)</td>
</tr>
<tr>
<td>Vascular plants</td>
<td>Plants that have a vascular system through the stem</td>
</tr>
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