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USE OF ALGAE IN ENVIRONMENTAL ASSESSMENTS

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I. INTRODUCTION

Algae have long been used to assess environmental conditions in aquatic habitats throughout the world. During the early part of the twentieth century, algae were exploxed as indicators of organic pollution in European streams and rivers (Kolkwitz and Marsson, 1908). Between 20 and 50 years ago, use of algal indicators of environmental conditions flourished based on the environmental sensitivities and tolerances of individual taxa and species composition of assemblages (e.g., Butcher, 1947; Fjerdingstad, 1950; Zelinka and Marvan, 1961; Slàdecek, 1973; Lowe, 1974; Lange-Bertalot, 1979). Nutrient stimulation of algal growth made algae part of the problem in the eutrophication of lakes such that trophic status of lakes was also characterized by the amount of algae (Vollenweider, 1976; Carlson, 1977). In North America, Ruth Patrick and C. Mervin Palmer were pioneers in the development of large monitoring programs to assess the ecological health of rivers and nuisance algal growths (Patrick, 1949; Patrick *et al.*, 1954; Palmer, 1969). More recently, the sensitivity of many algal taxa to pH, combined with preservation of certain algal cell wall components (e.g., diatom frustules and chrysophyte scales) in sediments, has been employed to assess problems with acid deposition and to determine if rates of lake acidification have been enhanced by human contributions to acid deposition (Smol, 1995; Battarbee *et al.*, 1999). Government agencies throughout the world now use algae to monitor and assess ecological conditions in many types of aquatic ecosystems (e.g., Weber, 1973; Dixit and Smol, 1991; Dixit *et al.*, 1992, 1999; Bahls, 1993; Kentucky Division of Water, 1993; Whitton and Rott, 1996; Biggs *et al.*, 1998; Kelly *et al.*, 1998; Stevenson and Bahls, 1999). Thus, characterization of algal assemblages has been important in environmental assessment, both in indicating changes in environmental conditions that impair or threaten ecosystem health and in determining if algae themselves are causing problems.

Algae are particularly valuable in environmental assessments. Algae are the base of most aquatic food chains, are important in biogeochemical cycling, and serve as habitat for many organisms in aquatic ecosystems (e.g., Minshall, 1978; Wetzel, 1983; Power, 1990; Carpenter and Kitchell, 1993; Vymazal, 1994; Bott, 1996; Lamberti, 1996; Mulholland, 1996; Wetzel, 1996). Thus, a natural balance of species and assemblage functions is important for ecosystem health (Angermeier and Karr, 1994). Increases in algal biomass and shifts in species composition can cause problems with many ecosystem services by causing taste and odor problems in water supplies (Sigworth, 1957; Palmer, 1962; Arruda and Fromm, 1989), toxic algal blooms (Bowling and Baker, 1996; Burkholder and Glasgow, 1997), and low dissolved oxygen levels (Lasenby, 1975).

In many aquatic habitats, algae are the most diverse assemblage of organisms that can be easily sampled and readily identified to species (particularly diatoms and desmids). The great species-specific sensitivity of algae to environmental conditions and their high diversity in habitats provide the potential for very precise and accurate assessments of the physical, chemical, and biological conditions that may be causing problems. Moreover, algae and paleolimnological techniques can be used to infer historical conditions in lakes, wetlands, and even reservoirs and rivers (Fritz, 1990; Smol, 1992). Algae occur in all aquatic habitats, so they could be very valuable for comparison among ecosystems with the same group of organisms. From a logistical perspective, algae are relatively easy to sample, and analysis is relatively inexpensive compared with bioassessment with other groups of organisms. In addition, many characteristics of algal assemblages can be measured and used as multiple lines of evidence for whether ecological integrity has been altered and the causes of those alterations. Algal bioassessment complements physical and chemical data by providing corroborative evidence for environmental change.

Both structural and functional characteristics of algae can be used to assess environmental conditions in aquatic habitats. Algal biomass (measured as chlorophyll *a*, cell numbers, and/or algal biovolume; Stevenson, 1996) can be used to indicate the presence of toxic pollutants as well as trophic status and nuisance algal growths (Carlson, 1977; Dodds *et al*., 1998). Taxonomic composition and diversity of algal assemblages are used to assess ecological health of habitats and to infer probable environmental causes of ecological impairment (e.g., Patrick *et al*., 1954; Smol, 1992; Stevenson and Pan, 1999). Ratios of chemicals in algal samples can be used to indicate algal health (phaeophytin:chlorophyll *a*) and nutrient limitation (N:P) (Weber, 1973; Hecky and Kilham, 1988; Biggs, 1995). Photosynthesis, respiration, and phosphatase activity are examples of algal metabolism that can be used to assess the amount of algae in habitats, physiological impairment, and phosphorus limitation (Blanck, 1985; Hill *et al*., 1997; Newman *et al*., 1994).

In this chapter, the abundant and diverse methods of using algae to assess environmental conditions in all aquatic habitats are organized in a risk assessment framework (U.S. Environmental Protection Agency, 1992, 1996, 1998). Many reviews of algal methods for environmental assessment have been published in recent years (Stevenson and Lowe, 1986; Round, 1991; Coste *et al*., 1991; Smol, 1992; Whitton and Kelly, 1995; Rosen, 1995; Reid *et al*., 1995; Lowe and Pan, 1996; Stevenson, 1998; McCormick and Stevenson, 1998; Wehr and Descy, 1998; Kelly and Whitton, 1998; Kelly *et al*., 1998; Ibelings *et al*., 1998; Prygiel *et al*., 1999a; Stevenson and Pan, 1999; Stevenson and Bahls, 1999; see many chapters in Whitton *et al*., 1991; Whitton and Rott, 1996; Stoermer and Smol, 1999; Prygiel *et al*., 1999b). We take this abundance of recent reviews as an indication of the growing importance of algae in environmental assessment. In our chapter, we emphasize understanding the goals of environmental programs, developing and testing hypotheses that address program goals, and selecting the simplest and most direct methods for achieving program goals. We present the characteristics of algae that can be used in environmental assessments and then elaborate on how these characteristics can be related by using them in the five steps of ecological risk assessment. Although algae have been used for such assessments in habitats throughout the world, great potential exists for developing indices that more directly meet the needs of specific environmental assessment programs. Thus, in this chapter, we present the many approaches for developing algal methods for environmental assessment, and then we describe the application of algal methods for assessment.

II. GOALS OF ENVIRONMENTAL ASSESSMENT WITH ALGAE

The goals of environmental assessment programs can be established by legislation, by government officials and policy decision makers, by scientists, or by the general public. In most cases, scientists play an important role in translating the official goals of an environmental program into hypotheses that can be tested and in developing a practical study plan that can be implemented within the budget allocated for the project. The United States Environmental Protection Agency (U.S. EPA) risk assessment and risk management framework (U.S. EPA, 1992, 1996, 1998) (Fig. 1) is valuable for translating the many goals of 'environmental problem solving' into a series of testable hypotheses and for providing a sound scientific approach for solving problems.

The overall goals of environmental assessment, with algae or other organisms, are to characterize the effects or potential effects of human activities and to implement management strategies that reduce the risk of ecological impairment and restore valued ecological conditions. In addition to the actual state of the ecosystem, factors such as economic, social, and legal issues may affect decisions about how to protect or restore valued ecological characteristics (Fig. 1). Because of the complexity of many environmental issues, clearly stated goals permit the development of testable hypotheses, sampling and statistical design, and choice of the best methods. The ecological risk assessment (ERA) framework helps to organize and relate the many issues associated with environmental problems and form these hypotheses. The ERA helps distinguish between the ecological conditions that the public wants to protect, the stressors that threaten those conditions, the human activities causing those stressors, and the many other factors involved in decisions on how to solve the problem.

In general, most environmental assessments involve one or more steps in the ERA (Fig. 1). A full risk assessment involves five steps of ERA: problem formulation, hazard (response) assessment, exposure (stressor) assessment, evaluating the stressor–response relationships, and then characterizing the risk associated with each stressor and responses of interest. Problem formulation is the identification of the ecological attributes and stressors with which the public are most concerned. We may start from the perspective that a valued ecological attribute, such as water clarity or biotic integrity (*sensu* Karr and Dudley, 1981), is threatened or impaired and that we need to determine the cause of the problem. Are aesthetics or taste and odor impaired by nuisance growths of algae? Are toxic algal blooms occurring? Alternatively, we may be concerned about how a stressor, such as acid deposition or nutrients, could be affecting ecosystems. During problem formulation, identifying and distinguishing valued ecological attributes and stressors is very important, so that cause–effect relationships can be identified and targeted in an ERA.

FIGURE 1 Elements of the risk assessment and risk management framework. Modified from U.S. Environmental Protection Agency (1996).

Our adaptation of the ERA for algal bioassessments has broad applications in the integration of the diversity of information that can be obtained in ecological assessments with algae and applying them to hazard assessment and exposure assessment. Hazard assessment is determining whether the ecological conditions in a habitat are impaired and is an assessment of the dependent variable (valued ecological attribute or an indicator of that attribute, a response variable) in the problem (Fig. 2). For example, have algae accumulated to nuisance levels or have sensitive species been lost from the habitat. Exposure assessment is an evaluation of the intensity, frequency, and duration of altered habitat conditions or contamination. For example, what is the pH, total phosphorus concentration, or organic load in the habitat, and how long does it last? Exposure assessment is a measurement of the stressor, which is the independent variable in the stressor–response relationship.

The stressor–response relationship permits determination of the stressor that is likely to be most threatening or causing impairment of ecological conditions (Fig. 2). Stressor–response relationships may be found in previously published literature or in studies that accompany the ERA. Ecological risk associated with each stressor should then be characterized by comparing assessed response (hazard assessment) and stressor levels (exposure assessment) with the stressor–response relationship (Fig. 2). One or more stressors should be identified as being the likely stressors that most threaten impairment or cause impairment (see Stevenson, 2001, for more discussion). Most stressors should be too low to cause the observed impairment. However, at least one stressor, or an interaction of multiple stressors, should be high enough to cause the observed response.

Algae can be used in ERA to determine whether a problem exists, to infer levels of specific stressors in a

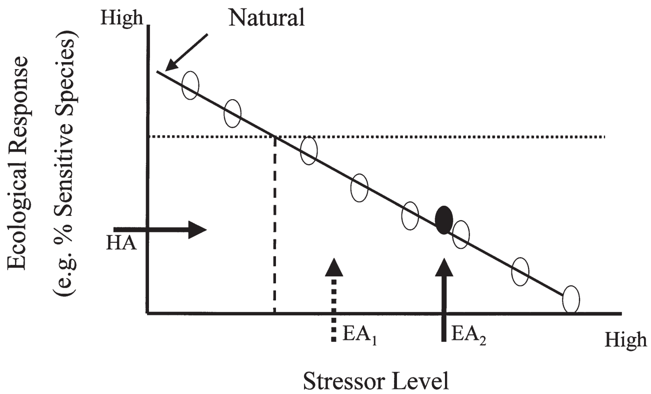

FIGURE 2 Response–stressor relationship between hypothetical ecological response and stressor with hazard assessment and exposure assessment indicated. Acceptable levels of the ecological response are indicated by the horizontal dotted line. The stressor level that produces that level of response is indicated by the vertical dashed line. The observed ecological response (indicated by horizontal solid arrow marked HA on the *y* axis) is below acceptable levels. Two stressors were measured or inferred based on algal indices (indicated by the vertical arrows marked EA (exposure assessment) on the *x* axis). One stressor (indicated by EA_1) is too low to cause the observed response, whereas the other has a high certainty of causing the observed ecological condition.

habitat, and to characterize stressor–response relationships. Algal indices are used to assess both stressors as well as valued ecological attributes. Probably more than any other group of organisms, algae have been used to infer physical and chemical conditions (potential stressors) in a habitat through the determination of species composition of assemblages and species' ecological preferences. Potential for inferring stressor conditions exists for other organisms, but algae are used much more often than fish, macroinvertebrates, or insects in terrestrial habitats to infer levels of pH, conductivity, trophic status, and sewage contamination. Therefore, distinguishing between whether algal indices are indicating valued ecological attributes or stressors is important for relating results of algal bioassessments to goals of the ERA. This opportunity to use algae to determine whether problems exist, potentially even forecast problems, and to diagnose causes of problems should be emphasized in algal bioassessments.

III. SAMPLING AND ASSESSING ALGAL ASSEMBLAGES FOR ENVIRONMENTAL ASSESSMENT

A. Sampling Algae in Freshwater Habitats

Sampling techniques and design may vary with the objectives of the assessment, probable factors affecting algae and anticipated problems, water body type, targeted habitat within the water body, and budget. A complete discussion of the most appropriate sampling methods and design is beyond the scope of this chapter, but we will review some of the important issues related to sampling for bioassessment. In general, the same basic approaches are useful for solving most problems in any water body or habitat. Algal indicators may, however, be more precise if they are refined with regional datasets for specific water body types, but this problem will be discussed later with development of indicators of exposure assessment (weighted averaging inference models).

1. Sampling Design

Objectives of an environmental assessment should be defined as clearly as possible for the formulation of hypotheses and a sampling design (e.g., Green, 1979). Testable hypotheses should be formed so that results provide answers that address the objectives of the assessment and that have a defined error or uncertainty. Testing hypotheses requires a sampling design that includes replicate sampling or some form of assessment of error variation. Estimates of ecological health in small, local studies are usually based on replicate sampling at all sites and provide means and estimates of variation at each site to test the hypothesis that conditions at a tested site are significantly different from conditions at a reference site or from a criterion. However, replicate sampling at all sites in large surveys is often not affordable; then replicate sampling at a random subset of sites (often 10% or more) can be used to characterize variation in estimates of ecological health. The error variation associated with the random subset of sites in large surveys is a measure of the precision (standard deviation or standard error) of assessments at all sites.

Before sampling, investigators should define the extent of each sample site so that it can be repeated at all locations. The open-water region of lakes usually defines the horizontal extent of plankton habitats sampled at a lake site, but the vertical extent (depth of sampling) may vary with goals of the project. In many river programs, for example, sampling one riffle has been considered sufficient to characterize the ecological health of a stream with benthic algae (Bahls, 1993), but in other programs the extent of the habitat is defined as a stream reach with a length that is 40 times the width of the stream (Klemm and Lazorchak, 1994; Stevenson and Bahls, 1999). Similarly, a representative portion of wetland should be chosen to sample (e.g., Stevenson *et al.*, 1999).

Most algal sampling strategies focus on a specific habitat within the water body (Wehr and Sheath, Chap. 2, this volume), such as plankton, algae on rocks (epilithic) in riffles, or algae on plants (epiphytic). Alternatively, objectives of a project may call for characterizing the diversity of algae in a habitat and consequently sampling all suitable habitats within a water body (defined site) (Porter *et al.*, 1993). The presumed advantage of sampling targeted habitats is that algal indicators are more sensitive and can more precisely detect changes in environmental conditions if interhabitat variability is reduced (Rosen, 1995). In a recent review, Kelly *et al.* (1998) make strong arguments for sampling rocks or other hard substrata, if they are present. In some cases, however, sampling the same targeted habitat in all water bodies of a project is impractical. Although finding plankton in lakes is usually not a problem, finding cobble riffles in all streams or open water in all wetlands can be a problem. Multihabitat sampling is one solution to the problem of habitat diversity among sites. One advantage to multihabitat sampling is a more complete assessment of all taxa at a site, which potentially is a better characterization of biodiversity and biointegrity than assemblages from targeted habitats. Another solution is to classify streams and wetlands by size and hydrogeomorphology (Vannote *et al.*, 1980; Biggs and Close, 1989; Rosgen, 1994; Biggs, 1995; Brinson, 1993; Goldsborough and Robinson, 1996; Biggs *et al.*, 1998), so they have similar habitats, and then to develop sampling strategies and indicators for specific hydrogeomorphic classes of streams and wetlands.

Estimates of algal biomass, which are particularly important in characterizing trophic state, require quantitative sampling of habitats. Habitats are quantitatively sampled by measuring the volume of water collected or area of substratum sampled and accounting for the proportion of sample assayed. Algal attributes (e.g., biomass or productivity) can be expressed on a volume-specific or area-specific basis by correcting measurements for volume or area sampled and proportion of sample assayed (Wetzel and Likens, 1991; APHA, 1998). The main disadvantage of quantitative sampling is the time required and the practicality of precisely characterizing the area sampled. Measuring sample volume, in the field or in the laboratory, requires relatively little extra time. The benefits of quantitative sampling are also reduced when habitat conditions are spatially or temporally variable such that biomass is affected. Thus, quantitative sampling is particularly problematic in structurally diverse and hydrologically variable streams and wetlands.

Qualitative algal sampling is recommended in habitats that have great spatial and temporal variation and when sufficient time is not available to measure the substratum area. One goal of qualitative sampling could be sampling all species at a site (Porter *et al.*, 1993), which would call for sampling all habitats, water column, rocks, plants, and sediment in different physical settings (e.g., light, depth, current velocity, etc.). Another goal could be to determine the dominant algae at a site, which would require estimating the relative areas of different habitats and proportional sampling of those habitats.

Variation in quantitative or qualitative estimates of algal attributes, due to spatial variation in habitat conditions, can be reduced by composite sampling. Reducing spatial variation calls for subsampling of many areas throughout the defined extent of the study site and putting all subsamples into a composite sample. Variation in estimates of algal attributes due to temporal variation in habitats is more difficult to reduce because it requires sampling throughout a study period and return visits to a site, which may not be practical. To reduce effects of temporal variation on quantitative attributes in highly variable ecosystems like streams, it is best to sample after an extended (1–2-week) period of stable habitat conditions so that algal assemblages have reached peak or sustainable biomass and a relatively predictable state (Stevenson, 1990; Peterson and Stevenson, 1992; Biggs, 1996; Stevenson, 1996).

Semiquantitative approaches for assessing algal biomass and percentage cover of different algal groups have been used to reduce field and lab time and to increase the spatial and temporal extent of algal assessments in streams. Secchi disc transparency is a semiquantitative approach for assessing plankton biomass in lakes (Brezonik, 1978; Davies-Colley and Vant, 1988; Wetzel and Likens, 1991). In streams, visual characterization of algal type, percentage cover, filament length, and periphyton mat thickness along multiple transects have been used in many situations (Holmes and Whitton, 1981; Sheath and Burkholder, 1985; Rout and Gaur, 1990; Stevenson and Bahls, 1999). These techniques can easily be employed in all sampling programs because they require little time and can provide biomass assessments over large areas. They may be excellent quantitative tools for volunteer programs because they require little taxonomic expertise.

2. Sampling Techniques

a. Sampling Present-Day Assemblages Numerous algal habitats can be sampled within water bodies. Phytoplankton can be sampled at specific depths with Van Dorn, Kemmerer, or similar discrete-depth samplers (APHA, 1998). Depth-integrated samples can be collected with devices (e.g., peristaltic pumps) that allow water to slowly enter a sampling chamber or by compositing samples collected from specific depths.

Phytoplankton biomass is almost always expressed per unit volume (e.g., Wetzel and Likens, 1991). Qualitative samples can be collected with plankton nets; however, we recommend collecting whole water samples with known volumes whenever possible so that small algae are not missed and samples can be assayed quantitatively. Algae in whole water samples can be concentrated by filtering or settling (e.g., Wetzel and Likens, 1991; APHA, 1998).

Metaphyton are macroalgal and microalgal masses suspended in the water column and entangled among macrophytes or along shorelines, typically in slow or still water (Hillebrand, 1983; Goldsborough and Robinson, 1996). Quantitative sampling of metaphyton requires collecting algae from a vertical column through the assemblage. Coring tubes can be used to isolate and collect a column of metaphyton. Scissors are useful for cutting horizontal filaments that block the insertion of the tube through the metaphyton assemblage. The depth of the core should not extend to the substratum surface. The diameter of the core depends upon the spatial variability of the metaphyton, on necessary sample size, and on the ability to isolate the core of algae from surrounding metaphyton (Stevenson, personal observation). Metaphyton in the form of unconsolidated green clouds requires wider cores (ca. 10 cm) because filaments are difficult to isolate in narrow cores. Narrower cores (ca. 3 cm) can be used to sample consolidated microalgal mats. Metaphyton biomass should be expressed on an areal basis (e.g., m^{-2}). Qualitative samples of metaphyton can be gathered with grabs, forceps, strainers, spoons, or cooking basters.

Benthic algae are sampled by scraping hard or firm substrata, such as rocks, plants, and tree branches, usually after they have been removed from the water (Stevenson and Hashim, 1989; Aloi, 1990; Porter *et al.*, 1993). Cores of algae should be collected on soft or unconsolidated substrata, such as sediments and sand (Stevenson and Stoermer, 1981; Stevenson and Hashim, 1989). Area of substrata sampled should be recorded to quantify samples. Some substrata, such as bedrock and logs, cannot be removed from the water. In those cases, vertical tubes can be used to isolate an area of substratum. After algae are scraped from the substratum in the tube, algae and water in the tube can be removed with a suction device.

Artificial substrata are also used to assess benthic algal assemblages (Patrick *et al.*, 19547; Tuchman and Stevenson, 1980; Aloi, 1990). They are typically uniform substrata (e.g., glass or acrylic slides, clay tiles, acrylic or wooden dowels) that can be used across many water body types (streams, rivers, wetlands, lakes). If placed in similar light and current environments in all habitats, differences in assemblages among sites should be highly sensitive to water chemistry. However, placement and sampling of artificial substrata require more than one trip to sample sites. Samples are often lost because they are subject to vandalism. Finally, assemblages on artificial subtrates may not reflect historical changes in habitats or changes in physical habitat structure as well as assemblages on natural substrata (see also Section II). Most large national and state programs have chosen to sample natural substrata (Porter *et al.*, 1993; Klemm and Lazorchak, 1994), but artificial substrata can be valuable in smaller-scale programs where travel time is limited or in ecosystems with great habitat diversity.

b. Sampling Historic Assemblages Sediment sampling in lakes, streams, rivers, and wetlands with deposited sediments can include an algal assemblage that has accumulated for months, years, or centuries, depending upon the depth and disturbance of sediments. A large variety of coring apparatuses are available to retrieve sediment cores (several are illustrated in Smol and Glew, 1992), with the choice of equipment largely dependent on the type of system being studied and the temporal resolution required. The resolution is also dependent on the type of sectioning techniques and equipment one uses. Close-interval sectioning equipment and techniques (e.g., Glew, 1988) are available that can provide lake managers with a high degree of temporal resolution.

The overall paleolimnological approach is summarized in the accompanying schematic (Fig. 3). Once the study site is chosen, a sediment core is removed, usually from near the center of the lake. In general, the central, flat portion of a basin integrates indicators from across the lake, and so a more holistic record of past environmental change is archived.

Once the core is retrieved and sectioned, the "depth-time" profile must be established. This requires dating a sufficient number of sediment layers to attain a reliable chronology. For most paleolimnological studies dealing with recent environmental assessments, ^{210}Pb dating is most often used (Oldfield and Appleby, 1984), as the half-life (22.26 years) of this naturally occurring isotope enables one to date, with reasonable certainty, approximately the last century or so of sediment accumulation. In some lake systems, close to annual (and sometimes subannual) resolution is possible.

Analyzing a sediment core at close intervals (e.g., every cm or every 0.5 cm) is time consuming and may not be practical for some large-scale, regional environmental assessments. For these cases, paleolimnologists have sometimes used a "snapshot" approach, which

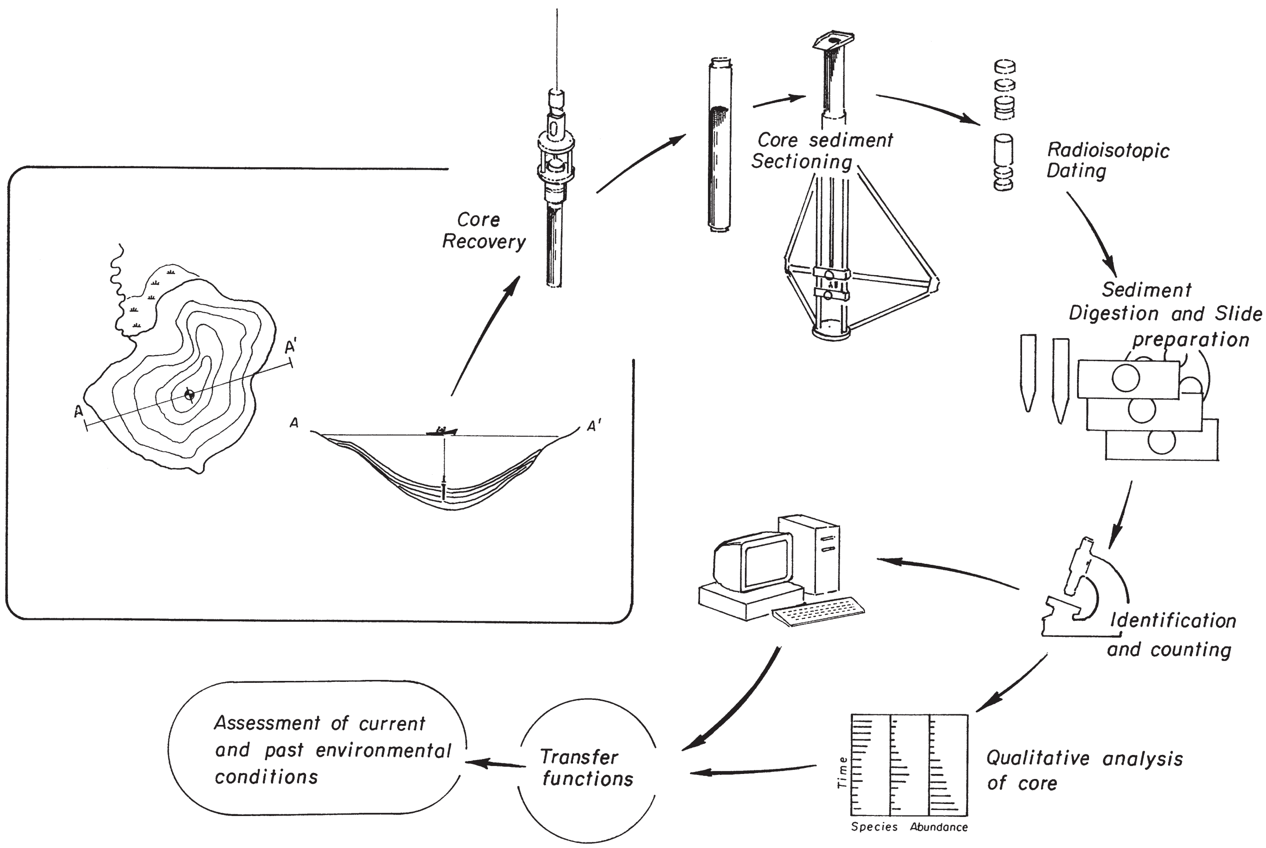

FIGURE 3 Schematic diagram showing the major steps involved in a paleolimnological assessment. Modified from Dixit *et al*., (1992a).

attempts to estimate what conditions were like before anthropogenic impacts and how much degradation has occurred. This so-called top/bottom approach is a very simple but effective tool for obtaining regional assessments of environmental change. Paleolimnologists remove surface sediment cores as they would in a detailed paleoenvironmental assessment; but instead of sectioning and analyzing the entire core, they simply analyze, for example, diatom valves and/or chrysophyte scales in the top 1 cm of sediment (= present-day conditions) and from a sediment level known to have been deposited before anthropogenic impact (i.e., the bottom sediment section). This before-and-after approach has been effectively used to infer environmental change, such as acidification (Cumming *et al*., 1992a; Battarbee *et al*., 1999; Dixit *et al*., 1999) and eutrophication (e.g., Dixit and Smol, 1994; Hall and Smol, 1999) that has occurred on regional scales. In addition to providing some estimate of degradation, these paleoenvironmental data also provide important information on the natural background conditions of a system and therefore provide important mitigation targets for environmental remediation efforts (Smol, 1992). To determine the rates and trajectories of past changes, more detailed paleoenvironmental assessments are required.

The next step is to recover any paleoenvironmental information archived in dated sediment cores. Our focus here is on the paleophycological data, but many other types of proxy data are available. For example, past changes in terrestrial vegetation can be inferred from the analyses of fossil pollen grains (the field of palynology); paleomagnetic measurements and other techniques can be used to estimate past erosion rates (e.g., Dearing *et al*., 1987); and isotope and geochemical analyses of metals and other contaminants (e.g., PCBs, DDT, etc.) can be analyzed from the sedimentary profiles (Autenrieth *et al*., 1991). Despite these other powerful approaches, the mainstay of many paleolimnological assessments is algal data. Virtually every algal group leaves some sort of morphological or chemical fossil in the sedimentary record, but the indicators that are most often used are diatom valves (Dixit *et al*., 1992), chrysophyte scales and cysts (Smol, 1995), and fossil pigments (Leavitt, 1996). As shown in examples given later in this chapter, these indicators can be used to reconstruct past limnological characteristics (such as pH, eutrophication variables, and salinity). Paleolimnological approaches have been subjected to a large amount of quality assurance and quality control considerations; if undertaken carefully and correctly, the paleolimnological approach is robust, reproducible, and powerful.

B. Attributes of Algal Assemblages for Environmental Assessment

Many attributes of algal assemblages can be used to assess environmental conditions in a habitat or site (Table I). Structural attributes (e.g., species composition) and functional attributes (e.g., productivity) can be measured in the field or the laboratory. The diversity of these attributes and the pros and cons of their uses have been discussed in recent studies (Stevenson, 1996; Stoermer and Smol, 1999; Stevenson and Pan, 1999; Stevenson and Bahls, 1999). In this treatment, we will focus more on the value of these attributes in detecting effects of humans on ecological systems.

For this review, we will use a set of terms recommended by Karr and Chu (1999) to clarify discussions of using biological data for bioassessment. An *attribute* is any characteristic of an assemblage that can be measured, such as chlorophyll *a* (chl *a*), number of species, or net primary productivity. A *metric* is an attribute that responds to human disturbances of habitats. Some attributes, such as the number of diatom genera in a 200-valve count, may not reliably respond to human impacts. Karr and Chu (1999) also recommend the use of the term *index* for statistical and other mathematical summaries of many metrics and indices. Multimetric indices of biotic integrity, such as the diatom bioassessment index (Kentucky Division of Water, 1993), are examples of multiple metrics being averaged or summed to compose a single, summary index. Many of the water quality indices that are commonly used in Europe (e.g., pollution tolerance index (Lange-Bertlalot 1979), generic diatom index (Coste and Ayphassorho, 1991), and trophic diatom index (Kelly and Whitton, 1995)) should probably be characterized as metrics, according to the method of Karr and Chu (1999), but we do not recommend renaming these indicators and being encumbered by semantics.

TABLE I Basic Attributes of Algal Assemblages That Can Be Measured and Potentially Be Used to Assess Environmental Conditions

Structural attributes
Biomass
chl *a*
Ash free dry mass
Cell density
Cell biovolume
Taxonomic composition
Species relative abundances
Species relative biovolume
Functional group biovolume
Diversity
Species richness
Genus richness
Evenness
Chemical composition
chl *a*:(Phaeophytin+Chl *a*) ratio
chl *a*:ash free dry mass ratio
P or N/ash free dry mass
N:P ratio of algal assemblages
Functional attributes
Photosynthesis rates
Respiration rates
Net primary productivity
Growth rates
Nutrient uptake rates

For a more detailed list with literature citations, see McCormick and Cairns (1994).

Algal assemblages can be characterized through the use of two basic kinds of attributes, structural and functional (Table I). Structural attributes are instantaneous characterizations of assemblages, such as biomass per unit area or volume of habitat, taxonomic and chemical characterizations of community composition, and diversity of community taxa (e.g., species richness). Functional attributes are measures or indicators of assemblage metabolism, such as photosynthetic rate (gross primary production), respiration rate, net primary productivity, nutrient cycling, phosphatase activity, and population growth rates. Functional assessments usually require more time in the field or multiple trips, so are used less in routine environmental assessments. However, they can be important for understanding impairment of algal and microbial activity.

1. Biomass

Biomass of algae usually increases with resource availability and decreases with many stressors caused by humans (Vollenweider, 1976; Dodds *et al.*, 1998). Removal of riparian (stream-side) canopies along streams and nutrient loading in all water bodies increase light, temperature, and nutrient availability, which can limit algal growth rates and biomass accrual (see reviews in Biggs, 1996; Hill, 1996; DeNicola, 1996; Borchardt, 1996). Sediments, toxic substances, and removal of benthic habitat can limit algal growth and accrual (Genter, 1996; Hoagland *et al.*, 1996). Because biomass and the potential for nuisance algal growths vary temporally with season and weather (Whitton, 1970; Wong *et al.*, 1978; Lembi *et al.*, 1988), timing of sampling is important. In most habitats, peak biomass occurs after periods of undisturbed habitat conditions (e.g., post-flood) when algal biomass has had an opportunity to accrue. Spring and summer blooms of phytoplankton are common in lakes with spring turnover and warm summer temperatures (Wetzel, 1983; Harper, 1992). Nuisance algal growths may occur, with filamentous benthic algal accrual during seasonal optima (Whitton, 1970; Biggs and Price, 1987; Dodds and Gudder, 1992; Lembi *et al.*, Chap. 24, this volume), or in the water column during summer low flow, when water residence time is sufficient for algal accrual in the water column (Bowling and Baker, 1996). Biomass is an important attribute in environmental assessments because it is related to productivity and nuisance problems.

Biomass of algal assemblages can be estimated with laboratory assays of chl *a*, dry mass, ash-free dry mass, algal cell density, biovolume, or chemical mass of samples. All of these measurements have pros and cons (see Stevenson, 1996, for a review), because none directly measure all constituents of algal biomass or only algal biomass. However, all are reasonable estimates of algal biomass in different situations. Chl *a* must be extracted from cells in organic solvents, such as acetone or methanol, and then assayed by spectrophotometry, fluorometry, or high-performance liquid chromatography (HPLC) (Lorenzen, 1967; Mantoura and Llewellyn, 1983; Wetzel and Likens, 1991; APHA, 1998; Van Heukelem *et al.*, 1992; Millie *et al.*, 1993). Spectrophotometric and flourometric chl *a* assays should be corrected for phaeophytin. Dry mass and ash-free dry mass are measured by drying and combusting samples (APHA, 1998). Cell density is measured after cells are counted microscopically (Lund *et al.*, 1958; APHA, 1998; Stevenson and Bahls, 1999). Algal biovolume can be measured by distinguishing sizes of cells during microscopic counts, multiplying biovolume by cell size for all size categories, and finally summing biovolumes for all size categories in the sample (Stevenson *et al.*, 1985; Wetzel and Likens, 1991; APHA, 1998; Hillebrand *et al.*, 1999). Because of variation in vacuole size and cell walls (e.g., Sicko-Goad *et al.*, 1977), cell surface area may be a valuable indicator of biomass because much cytoplasm is within 1–2 μm of the cell membrane.

Biomass can also be estimated rapidly with field assays, such as secchi depth in the water column and percentage cover and thickness of algal assemblages on substrata (Wetzel and Likens, 1991). Secchi depth characterizes light attenuation in the water column; assessments of algal biomass by this method are confounded by suspended inorganic materials, dissolved substances, and other factors (Preisendorfer, 1986). The strengths of assessing benthic algal biomass from percentage cover and thickness of algal assemblages is that biomass throughout a stream reach can be readily characterized (Holmes and Whitton, 1981; Sheath and Burkholder, 1985; Stevenson and Bahls, 1999). Remote sensing of algal biomass also shows promise for assessing spatially and temporally variable growths (Cullen *et al.*, 1997). These rapid assessment techniques may permit more thorough spatial and temporal assessments, which may improve the notoriously variable relationships between biomass and nutrient concentrations or loading.

2. Taxonomic Composition

Taxonomic composition of algae is a powerful tool for assessing biotic integrity and diagnosing the direct and indirect causes of environmental problems (Stevenson, 1998). Differences in taxonomic composition of assemblages between an assessed site and a reference (desired) site can indicate impairment of biotic integrity and environmental conditions, if natural variation in assemblage composition is well documented (e.g., McCormick and O'Dell, 1996; McCormick and Stevenson, 1998). When natural seasonal or interhabitat variation in composition is not well known, changes in taxonomic composition can be related to human activities by comparing shifts in taxonomic composition to environmental change with autecological characteristics of species and relating inferred environmental changes to human activities (e.g., Kwandrans *et al.*, 1998). Shifts in functional groups of algae (defined as different growth forms and divisions of algae; e.g., Pan *et al.*, 2000) can also indicate an important change in food quality and in habitat structure for benthic invertebrates. For example, the food quality and accessibility of diatoms are usually greater than cyanobacteria and filamentous green algae for many herbivores (Porter, 1977; Lamberti, 1996). In addition, the habitat structure for benthic invertebrates differs greatly with changes from microalgae (e.g., diatoms) to macroalgae (e.g., *Cladophora*) (Holomuzki and Short, 1988; Power, 1990).

Taxonomic composition of algal assemblages can provide a highly precise and accurate characterization of biotic integrity and environmental conditions (Stoermer and Smol, 1999). Taxonomic composition of assemblages develops over periods of time, ranging from weeks to years, and should reflect environmental changes during that period. Even though taxonomic composition varies spatially and temporally in a water body, autecological characterizations of environmental conditions based on taxonomic composition should consistently reflect the physical and chemical changes caused by humans. For example, if trophic status of a habitat is being assessed, only low-nutrient indicator taxa should occur in low-nutrient habitats, even though temperature and shading and stage of community development may change with time and local habitat structure. Species presence and success in assemblages are fundamentally constrained by environmental conditions and interactions (e.g., competition) with other species in the habitat (Stevenson, 1997). Thus, attributes of assemblages based on percentage taxonomic similarity of assemblages at a test site and a reference site (Raschke, 1993; Stevenson, 1984) and percentage sensitive species should be good metrics because typically they sensitively, precisely, and monotonically change along gradients of human disturbance.

Taxonomic composition of algal assemblages usually requires microscopic assessments of samples, but some basic information about growth form and class can be obtained with rapid field assessments (e.g., Sheath and Burkholder, 1985; Stevenson and Bahls, 1999). The methods used for microscopic identification and counting of algae depend upon the objectives of data analysis and type of sample. A two-step process has been adopted by the national stream assessment programs in the United States. The first step is to count all algae and identify only nondiatom algae in a wet mount at either 400× (e.g., Palmer cell) or 1000×, if many small algae occur in samples. Algae can be counted in wet mounts at 1000× with an inverted microscope (Lund *et al.*, 1958) or with a regular microscope by drying samples onto a coverglass, inverting the sample onto a microscope slide in 20 μL of water, and sealing the sample by ringing the coverglass with fingernail polish or varnish (Stevenson, unpublished method). The second step is to count diatoms after oxidizing organic material out of diatoms and mounting them in a highly refractive mounting medium (Stevenson and Bahls, 1999). This technique provides the most complete taxonomic assessment of an algal assemblage. Using counts of 300 algal cells, colonies, or filaments and about 500 diatom valves is a standard approach of some U.S. national programs (Porter *et al.*, 1993; Pan *et al.*, 1996) and usually provides relatively precise estimates of the relative abundances of the dominant taxa in a sample. Alternatively, counting rules have been defined so that cells of all algae are identified and counted until at least 10 cells (or natural counting units = cells, colonies, or filaments) of the 10 dominant taxa are counted (Stevenson, unpublished data). This type rule, rather than a fixed total number of cells, ensures precision in estimates of a specified number of taxa. Some assessment programs primarily use diatoms (Bahls, 1993; Kentucky Division of Water, 1993; Kelly *et al.*, 1998; Kwandrans *et al.*, 1998), because the number of species in diatom assemblages is usually sufficient to show a response.

Taxonomic composition can be recorded as presence/absence, percentage or proportional relative abundances, percentage or proportional relative biovolumes, or absolute densities and biovolumes of taxa (cells or μm^3 cm^{-2} or mL^{-1}). Although there is no published comparison of these forms of data, they represent scales of biological resolution and probably reflect a gradient from least sensitive to most sensitive and least variable to most variable. Presence/absence records of species should be based on observations of thousands of cells and conceptually should reflect long-term changes in habitat conditions if immigration and colonization of habitats are a selective barrier. Relative abundances and biovolumes of taxa probably reflect recent habitat conditions more than long-term conditions because of recent species responses to environment. Densities and biovolumes of taxa change daily, so absolute densities and biovolumes may be too sensitive to detect more long-term environmental changes. Relative abundance of cells is more commonly used than relative biovolumes (because of ease of use), but the latter is particularly valuable when cell sizes vary greatly among taxa within samples.

3. Diversity

Richness and evenness of taxa abundances are two basic elements of diversity (Shannon, 1948; Simpson, 1949; Hurlbert, 1971) of biological assemblages. Richness and evenness are hypothesized to decrease with increasing human disturbance of habitats; however, evenness of species abundances may increase if toxic stresses retard the growth of dominant taxa more than rare taxa (e.g., Patrick, 1973). Two problems develop with use of diversity measures in environmental assessment: standard counting procedures may not accurately assess diversity (Patrick *et al.*, 1954; Stevenson and Lowe, 1986), and diversity may not change monotonically across the gradient of human disturbance (Stevenson, 1984; Jüttner *et al.*, 1996; Stevenson and Pan, 1999). Species diversity and evenness are highly correlated with standard 300–600 cell counts (Archibald, 1972). In these counts many species have usually not been identified, so richness is more a function of evenness than evenness is a function of richness (Patrick *et al.*, 1954; Stevenson and Lowe, 1986). As shown by standard counting procedures, nonmonotonic (showing both positive and negative changes as the independent variable increases) responses of algal diversity to some environmental gradients seem to be related to maximum evenness of tolerant and sensitive taxa at midpoints along environmental optima, to fewer species being adapted to environmental extremes at both ends of environmental gradients, and to subsidy-stress perturbation gradients

(Odum *et al.*, 1979). Despite these difficulties, species richness and evenness may respond monotonically (having only positive or negative changes, but not necessarily linear changes, as the independent variable increases), sensitively, and precisely to gradients of human disturbance in some settings and should be tested for use as metrics.

4. Chemical Composition

The chemical composition of algal assemblages can be used to assess the trophic status of water bodies (e.g., Carlson, 1977), such as total phosphorus (TP) and nitrogen (TN) concentrations of water and periphyton (Dodds *et al.*, 1998; Biggs, 1995). TN:TP ratios are widely used to infer which nutrient regulates algal growth (Healey and Hendzel, 1980; Hecky and Kilham, 1988; Biggs, 1995). In many of these assessments, most of the total P and N are particulate, and much of the particulate matter is algae. Thus, measurements of TP or TN per unit volume or area of habitat largely reflect the amount of algae in the habitat. Of course, the most widespread use of trophic assessments with TP and TN is phytoplankton in lakes (Carlson, 1977), but use has also been proposed for streams, rivers, and wetlands (Dodds *et al.*, 1998; McCormick and Stevenson, 1998). TP and TN per unit biomass in benthic algae have also been positively correlated to benthic algal biomass in streams; however, negative density-dependent effects may reduce biomass-specific concentrations of benthic algal TP and TN and confound estimates of P and N availability to cells (Humphrey and Stevenson, 1992). Volume-specific, area-specific, and biomass-specific estimates of TP and TN do increase monotonically with most gradients of human disturbance and may be good metrics for trophic status in streams, rivers, and wetlands as well as lakes.

Chemical assessments are also valuable for monitoring heavy metal contamination in rivers, lakes, and estuaries (Briand *et al.*, 1978; Whitton *et al.*, 1989; Say *et al.*, 1990). Many algae accumulate heavy metals when exposed to them in natural environments (Whitton, 1984). While toxicity of heavy metals to algae is one reason for monitoring heavy metals in algae, other reasons include bioaccumulation and metal removal from waste streams and movement of heavy metals into the food web (Whitton and Shehata, 1982; Vymazal, 1984; Radwin *et al.*, 1990).

5. Functional Attributes

Metabolism of algal assemblages is highly sensitive to environmental conditions and is important to the assessment of ecosystem function and many ecosystem services. Estimates of photosynthesis (gross primary productivity), respiration, net primary productivity, nutrient uptake and cycling, and phosphatase activity are common functions measured in ecological studies (Bott *et al.*, 1978; Healey and Hendzel, 1979; Wetzel and Likens, 1991; Marzolf *et al.*, 1994; Hill *et al.*, 1997; Whitton *et al.*, 1998). These techniques are rarely incorporated into routine monitoring and survey work because they require more field time than typical water, phytoplankton, and periphyton sampling. However, they can be valuable additions to bioassessment projects. Biggs (1990) describes using algal growth rates to assess stream enrichment. Metabolism can be based on an area-specific, volume-specific, or biomass-specific basis. The most direct measurement of cellular performance is biomass-specific rates of metabolism; however area-specific and volume-specific measurements directly relate to community performance and ecosystem services. Caution in the accurate use of these attributes must be exercised. Area- and volume-specific measurements of productivity and respiration increase with biomass in the habitat, irrespective of human influence of biomass-specific rates. However, for periphyton, biomass-specific rates of productivity and nutrient uptake decrease substantially with increasing biomass in the habitat (e.g., Hill and Boston, 1989), presumably because of shading and impairment of nutrient mixing through the microbial matrix (Stevenson and Glover, 1993).

Gross and net productivity and respiration can be measured in the field with light and dark chambers and changes in oxygen concentration (Bott *et al.*, 1978; Wetzel and Likens, 1991). Alternatively, productivity can be estimated with changes in oxygen concentration in the water during the sampling period or at two locations in a stream, if diffusion of oxygen from the water column is properly accounted for (Kelly *et al.*, 1974; Marzolf *et al.*, 1994). Furthermore, algae secrete an enzyme called phosphatase when in low-P environments. The phosphatase enzyme cleaves PO_4 from organic molecules and makes it biologically available. Phosphatase is measured with water samples in the laboratory (Healey and Hendzel, 1979).

6. Bioassay

In this discussion we define bioassays as in-lab culture of organisms in waters from the study site. A valuable, field-based use of this technique is the *Selenastrum* bottle assay, in which known quantities of this highly culturable green alga are added to water from the study site and growth is monitored over a predefined period (Cain and Trainor, 1973; U.S. Environmental Protection Agency, 1971; Trainor and Shubert, 1973; Greene *et al.*, 1976; Ghosh and Gaur, 1990; McCormick *et al.*, 1996). Alternatively, planktonic or benthic assemblages from reference or test sites could be cultured in bioassays with waters from those habitats or different dilution levels of effluents entering those regions. Twist *et al*. (1997) introduced the novel approach of embedding test organisms in alginate and culturing them *in situ*. Nutrient-diffusing substrata, microcosms, and mesocosms are also valuable bioassay techniques in field settings (e.g., Cotê, 1983; Fairchild *et al*., 1985; Gensemer, 1991; Hoagland *et al*., 1993; Lamberti and Steinman, 1993; Thorp *et al*., 1996). Response of organisms to bioassays can provide another valuable line of evidence for identifying causes of environmental stress. Bioassay results with specific chemicals or effluents added can be used to confirm cause–effect relations between parameters for which only observational correlations can be obtained in field surveys (e.g., McCormick and O'Dell, 1996; Pan *et al*., 2000).

IV. DEVELOPING METRICS FOR HAZARD ASSESSMENT

A. Relating Goals to Ecological Attributes

Hazard assessments are the determination of the intensity, spatial extent, frequency, and duration of environmental problems or the threat of environmental problems in which ecological conditions do not meet designated use (U.S. EPA, 1996). Designated use describes the goals for environmental protection in U.S. management plans, such as preserving biotic integrity and biodiversity, maintaining fishable and swimmable conditions, minimizing taste and odor problems or risks to human health in water supplies, optimizing sustainable fisheries production, and protecting human health. Designated use emphasizes valued ecological attributes that the public wants to protect. Many algal attributes can be related to these designated uses. We recommend selecting as many metrics as possible to develop multiple lines of evidence to help assess ecological conditions, which can be altered in many ways. Thus, hazard assessment requires identifying the goals of environmental assessment, selecting algal metrics that represent qualities of ecosystems that are related to designated uses, and then measuring those algal metrics to determine if goals are being met.

One of the most fundamental goals of environmental assessment is to determine if the natural balance of flora and fauna has been altered in a habitat. The concept of biotic integrity and natural balance of flora and fauna is a legislated goal in the United States' Clean Water Act (Karr and Dudley, 1981; Adler, 1995), is fundamental to ecosystem protection and sustaining biodiversity (Angermeier and Karr, 1994), and is broadly applied in U.S. monitoring programs in which indices of biotic integrity are used to identify ecological problems (Plafkin *et al*., 1989; Barbour *et al*., 1999; Karr and Chu, 1999). Biotic integrity or ecosystem health can be defined as the similarity between assemblages in an evaluated habitat and assemblages in a set of reference habitats (*sensu* Hughes, 1995). That assessment of similarity can be based on structural and functional characteristics. In most cases, assessment of biotic integrity has been based on changes in diversity, species composition, functional groups (such as proportions of diatoms versus green algae and cyanobacteria), and changes in ecological conditions inferred by species composition and species autecological characteristics (Karr, 1981; Smol, 1992; Kerans and Karr, 1994; Stevenson and Bahls, 1999).

Alternatively, more specific assessments of algal nuisance can be the goals of projects. Such nuisances may cause reduced water clarity, hypolimnetic deoxygenation, taste and odor problems, habitat alteration, or toxic effects on other organisms, including humans (Carmichael, 1994, Chap. 24). In these cases, algal biomass or specific problem taxa may be important attributes for assessment.

Attributes that respond to gradients of human disturbance are classified as metrics. A good metric for hazard assessment is unambiguous, sensitive, precise, reliable, and transferable among regions and perhaps water body types (Murtaugh, 1996; Karr and Chu, 1999). McCormick and Cairns (1994) list other ideal qualities of indicators that should also be considered, such as relevance, redundancy, and cost-effectiveness. An unambiguous attribute responds monotonically to increasing levels of human disturbance (Fig. 4a and b). An attribute that can be equal at low and high levels of human disturbance (Fig. 4c) is ambiguous and should not be used as a metric. A good attribute may respond nonlinearly to human disturbance, but most change substantially (i.e., be sensitive) over the range of human disturbance being assessed. Precision (low variability in repeated measures) of metrics is important for detecting responses. In addition, metrics will be much more effective if they respond to human disturbance at many times of year and if they respond in many regions.

Two categories of attributes can be identified that characterize or indicate valued ecological attributes, and these categories are distinguished by the degree to which reference conditions need to be characterized. All attributes in the first category can be directly related to gradients of human disturbance by regression analysis to determine their use as metrics without rigorous identification and characterization of reference conditions and assemblages at reference sites. The first category includes attributes such as biomass,

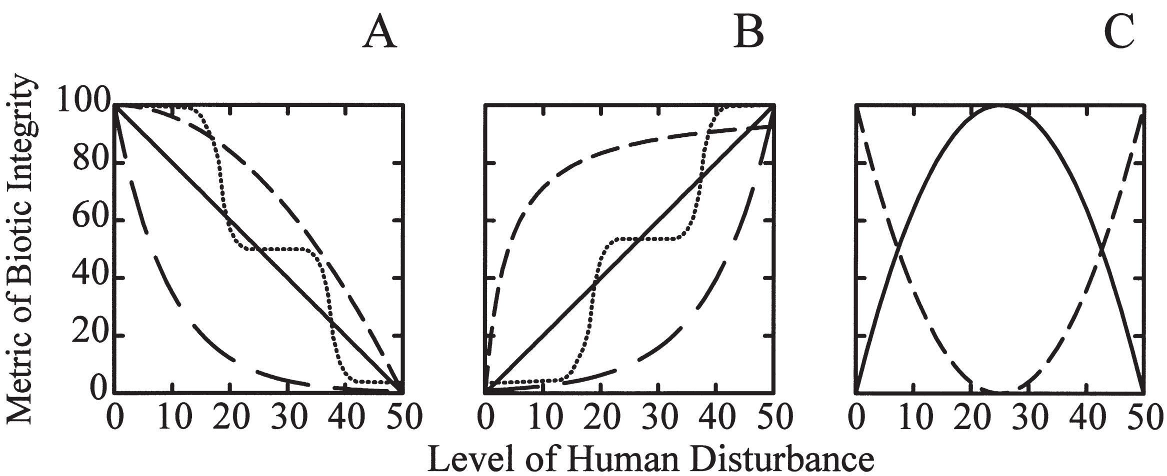

FIGURE 4 Examples of attribute responses that provide useful metrics. (A) Negative attribute responses along a gradient of human disturbance. (B) Positive responses along a gradient of human disturbance. (C) Ambiguous responses along a gradient of human disturbance. The different lines in each figure (A–C) represent different patterns that would fit the categories represented in the figures (negative, positive, and ambiguous, respectively).

species richness, Shannon (1948) diversity, relative abundances of specific nuisance taxa or functional groups of taxa, relative abundances of pollution-sensitive and pollution-tolerant taxa, and function of assemblages (Tables I and II; Stevenson and Bahls, 1999).

A second category of metrics requires comparison of differences between reference and test sites as the response variable to the gradient of human disturbance (Table II). Similarity of species composition between test sites and reference sites is one potential metric. Similarity of species composition between reference and test sites should decrease with increasing human disturbance at test sites. Many different formula can be used to calculate percentage similarity. One compares

TABLE II Examples of Metrics That Are Based on Taxonomic Composition of Assemblages and Autecological Characteristics of Species

Class		*Stressor*	*Representative references*
SC	BI	Pollution in general (based on species)	Lange-Bertalot (1979), Descy (1979), Coste (1982), Bahls (1993)
SC	BI	Pollution in general (based on genera)	Rumeau and Coste (1988), Coste and Ayphassorho (1991)
SC	D	pH	Whitmore (1989)
SC	D	Trophic status	Whitmore (1989), Kelly and Whitton (1995)
SC	D	Organic wastes	Zelinka and Marvan (1961), Palmer (1969), Sládeček (1973, 1986), Watanabe *et al.* (1986)
SC	D	Salinity	Zeimann (1991)
WA	D	pH	Charles and Smol (1988), ter Braak and van Dam (1989), Sweets (1992), Cumming *et al.* (1992a, b)
WA	D	Salinity	Fritz (1990), Cumming and Smol (1992)
WA	D	TP	Anderson *et al.* (1993), Reavie *et al.* (1995), Pan *et al.* (1996), Pan and Stevenson (1996)
WA	D	Fish presence	Kingston *et al.* (1992)

Metrics are classified based on whether they are based on simple categorical autecological characterizations (SC) or accurate weighted-average (WA) autecological characterizations and on whether they could be used to infer biotic integrity (BI) of sites or are highly diagnostic (D) of stressors that may threaten or impair biotic integrity.

relative abundances of species in two samples and bases similarity upon the sum of the lower relative abundances of each taxon in the two communities. The percentage community similarity (PS_c; Whittaker, 1952) is a straightforward example, where

$$PS_C = \Sigma_{i=1,S} \min(a_i, b_i)$$

Here a_i is the percentage of the ith species in sample a, and b_i is the percentage of the same ith species in sample b.

A second kind of similarity measurement is based on a distance measurement, which is a dissimilarity measurement rather than a similarity measurement, because the index increases with the greater dissimilarity (Pielou, 1984; Stevenson, 1984). Euclidean distance (ED) is a standard, where

$$ED = \sqrt{(\Sigma_{i=1,S}(a_i - b_i)^2)}$$

When using these dissimilarity indices, we recommend log-transforming relative abundances to reduce the importance and variability of common taxa.

A third category of similarity indices only compares the observed taxa at an assessed site with the taxa that are expected at that site to determine the proportion of species that have been lost from assessed sites. This technique has been used widely with macroinvertebrates (Moss *et al.*, 1999) and was recently employed with diatom genera (Chessman *et al.*, 1999). This approach has the potential for increasing the precision of algal metrics by distinguishing species in three categories: species that should be there and are still there, species that have been lost, and species that have invaded. First, loss of species is an important impairment of biodiversity. Second, diagnosis of causes of impairment may be substantially enhanced by linking autecological information to whether species have resisted disturbance, not resisted disturbance, or invaded to exploit disturbance.

In our application of the ERA framework, we emphasize the distinction between algal indicators that characterize designated use and ecological values in which the public are most interested and algal indicators that diagnose the stressors that may threaten or cause impairment of designated use. Some protocols recommend that metrics indicating the status of designated use be called "response" or "condition" indicators, and metrics that diagnose the physical, chemical, or biological factors that could be impairing designated use be called "stressor" or "causal" indicators (Paulsen *et al.*, 1991; U.S. Environmental Protection Agency, 1998). This distinction of types of metrics emphasizes the diversity of information that can be obtained with algal assessments and how to apply that information in environmental problem solving. Therefore, algal indicators that use environmental preferences of species, such as weighted average indices of pH or TP, will be described later under exposure assessment, rather that here under hazard assessment.

B. Testing Metrics

Metrics can be tested with measurements of attributes at multiple sites with varying levels of human disturbance and either parametric or nonparametric statistical methods (see Sokal and Rohlf, 1998). Sites with different levels of human impact should be chosen and sampled to assess the ecological response to human disturbance. The level of human disturbance at sites can be characterized with multiple lines of evidence. When point sources of pollution occur, environmental gradients are relatively simple to establish with a reference condition upstream from the point source and a decreasing gradient of disturbance at increasing distances downstream from the point source. When non-point-source pollution is a concern, human disturbance can be estimated by land use type, intensity, and proximity to a habitat or by concentrations of contaminants. The multivariate nature of complex non-point-source contamination can be simplified with the use of ordination techniques and axis scores as a ranking scale of human disturbance.

Reference sites help define expected conditions in a habitat if it had not been affected by human activities (Hughes, 1995); these are typically the least impacted ecosystems in the region. Reference sites can be sites upstream from a point source of pollution in a stream, whereas test sites can be downstream. Alternatively, reference sites for a specific climatic and hydrogeomorphic class of habitats can be defined as a set of sites with lowest human disturbance or greatest riparian buffer within their watersheds. Reference sites may have the lowest level of a specific stressor in them, such as low phosphorus and other specific indicators of human disturbance.

Algae are particularly useful in establishing reference conditions in lakes and wetlands, where sediments are continuously deposited because algal remains in sediments from times of low human disturbance can be used to infer historical conditions in those habitats. Paleolimnological approaches provide direct measurements of long-term environmental trends at a specific site, which increases certainty about how fast and the extent to which a system is deteriorating. To propose realistic mitigation procedures, paleolimnological reconstruction of past conditions can provide a realistic target for restoration. Long-term data can also show critical loads of pollutants or stressors that a system can handle before negative effects are manifested

(Smol, 1990, 1992, 1995; Anderson and Battarbee, 1994; and papers in Stoermer and Smol, 1999).

Paleolimnological approaches are based on fairly straightforward principles. Under ideal conditions, sediments slowly accumulate at the bottom of lakes, without disruptions. Certainly, in some cases, problems may occur (e.g., excessive bioturbation), but these problems can usually be recognized and assessed. Over time, therefore, the history of the lake and its watershed is archived in the depth/time profile of the sediments. Incorporated in these sediments is a surprisingly large library of information on the conditions present in the lake (from autochthonous indicators), as well as environmental conditions that existed outside the lake (from allochthonous indicators). Physical, chemical, and biological information is archived in sediments; however, for the purpose of this chapter, we will primarily focus on algal data. Paleolimnology is now widely recognized as a robust environmental management tool. We mainly discuss lake paleoenvironmental studies in this chapter, as most of the research has centered on these systems. However, many paleo approaches can easily be transferred to other aquatic systems such as ponds (Douglas *et al.*, 1994), rivers (e.g., Reavie and Smol, 1987, 1998; Amoros and Van Urk, 1989; Reavie *et al.*, 1998), wetlands (Bunting *et al.*, 1997), estuaries (Cooper, 1999), and marine systems (Anderson and Vos, 1992).

After algal attributes from habitats with different levels of human disturbance have been assessed, their response to human disturbance can be characterized. Log-transformation of biomass-related variables (such as chl *a*, cell density, and biovolume) is recommended to meet the equal variances assumption of regression analysis and sometimes to make patterns more linear. Other data transformations may be necessary to meet assumptions of statistical tests (e.g., Green, 1979; Sokal and Rohlf, 1998) or to manage the sensitivity of metrics. For example, arc-sine transformations of proportional data and log or square-root transformations of relative abundances should increase the normality of the data (Sokal and Rohlf, 1998) and can reduce the importance of highly variable abundant taxa, which increases the precision of some metrics.

Both nonparametric and parametric statistical techniques can be used to determine whether algal attributes respond to gradients of human disturbance. The simplest and most direct method is to compare attributes to the gradient of human disturbance by regression or correlation (e.g., Hill *et al.*, 2000), if human disturbance can be quantified on a continuous scale. Alternatively, human disturbance can be categorized as low and high, and ANOVA or Mann–Whitney U tests can be used to test for differences in metrics with differences in human disturbance (Green, 1979; Barbour *et al.*, 1992; Sokal and Rohlf, 1998; Barbour *et al.*, 1999).

C. Multimetric Indices

Summarizing data in the form of multimetric indices has been a valuable method for communicating results of complex analyses that often involve multiple lines of evidence. This method has been used commonly with fish and invertebrate assemblages as multimetric indices of biotic integrity (IBI) (Karr, 1981; Kerans and Karr, 1992), but it has also been used with periphyton (Kentucky Division of Water, 1993; Hill *et al.*, 2000). Development of a multimetric index calls for selecting 6–10 metrics that describe a diversity of responses of assemblages that will be sensitive to all probable environmental stressors. For example, species richness, percentage of diatoms, percentage similarity to reference assemblages, number of taxa sensitive to pollution, percentage motile diatoms, percentage aberrant diatoms, inferred trophic status, inferred salinity (conductivity), inferred saprobity, and inferred pH could be used in a multimetric index, if they all responded to gradients of human disturbance (i.e., performed as good metrics). The range of each metric should be normalized, for example, to a range of 0–10, so that each metric has equal weight (see Hill *et al.*, 2000). Then values of each metric for a sample can be summed. In an example with 10 metrics and each ranging from 0 to 10 in scale, the multimetric index would then range from 0 to 100.

The value of multimetric indices is that they provide a single number as a summary of multiple lines of evidence. Such a summary statistic is highly valuable for communicating information to a lay audience, especially when compared with interpretations of ordination analyses (Karr and Chu, 1999). The disadvantage is that they may mask effects on one or two metrics; however, they can provide a hierarchically decomposable system of metrics for assessing ecological risk and even diagnosing causes or threats to impairment (Stevenson and Pan, 1999).

D. Multivariate Statistics and Hazard Assessment

Multivariate statistics are powerful and informative statistical tools for determining the major patterns of change in species composition and relating them to physical, chemical, or other biological characteristics of the habitats studied. We regard a multimetric index of biotic integrity (IBI) and multivariate statistics as complementary tools.

Cluster analysis and ordination provide multivariate methods for grouping stations by similarity in assemblage structure, exploring patterns in data, and illustrating those patterns (Hill, 1979; Pielou, 1984; Jongman *et al*., 1995). Cluster analyses can be bottom-up, such as UPGMA, or top-down, like TWINSPAN. A recent evaluation comparing these approaches showed that UPGMA, compared with TWINSPAN, grouped artificial assemblages better (developed based on a selected set of assumptions and assignment of species' relative abundances, based on a probabilistic distribution) (Belbin and McDonald, 1993). However, many researchers use TWINSPAN and find results with actual data to be highly interpretable (e.g., Pan *et al*., 2000).

Ordination (e.g., correspondence analysis and principal components analysis) condenses patterns in assemblage characteristics to axes that explain the covariation in assemblage characteristics among samples. Similarity in species composition among sites and species responses to environmental conditions at sites can be compared by plotting sample scores and species loadings in ordination space (Fig. 5). Environmental factors can then be incorporated into correspondence analyses (CAs) to relate variation in species distributions and sampled sites to variation in environmental conditions among sites. Many canonical ordination techniques can be applied to relating species and environmental variance among sites. If great differences occur in species composition among sites, a U-shaped pattern in ordination scores of sites often occurs; this artifact can be reduced by using detrending techniques (e.g., DCA) (Jongman *et al*., 1995).

Multivariate statistics are valuable for the early stages of environmental programs when initial relationships between changes in assemblages and environmental conditions are being explored to develop metrics and multimetric IBI (e.g., Pan *et al*., 1996). They can be an important part of any program when it is necessary to reduce the complexity of multivariate data. Even though cluster analysis and ordination group assemblages in classes that are often biologically interpretable, they do not test hypotheses that are directly related to questions of whether a site or a group of test sites is impaired or not. Testing these hypotheses calls for multivariate analysis of variance or discriminate function analysis (Pan *et al*., 2000). These latter approaches may be valuable for finding thresholds along gradients and being able to establish a probability that sites with specific characteristics were exposed to unacceptable levels of human disturbance and were impaired. Another weakness in multivariate statistics as an endpoint in ERA is that results are often not easily and repeatedly interpretable by audiences that are not trained in the use of multivariate statistics. Reviews of multivariate statistics and their use can be found in Green (1979), Pielou (1984), Jongman *et al.* (1995), and Birks (1995, 1998).

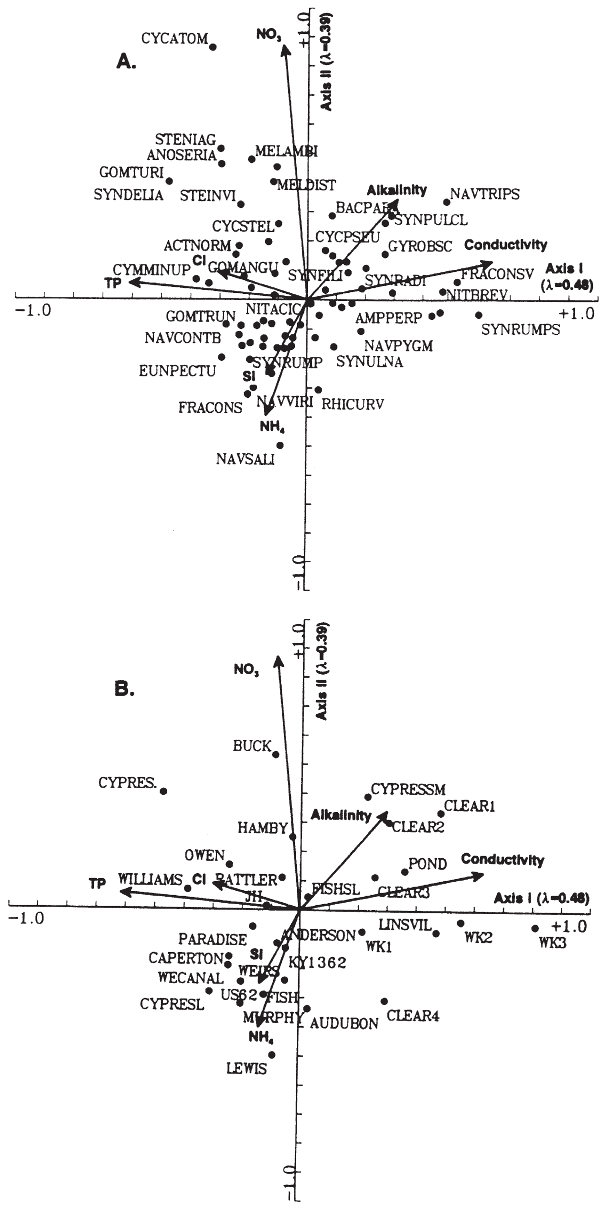

FIGURE 5 Plots of sample sites and species along ordination axes to indicate the relationship between similarity in species composition among sites and the species that are most important in defining that similarity (from Pan and Stevenson, 1996). (A) Plot of species and environmental variables (arrows) along ordination axes to show which species are most important in defining similarity among assemblages. (B) Plot of sample sites that shows sites with similar species composition located in similar locations along ordination axes with related environmental variables (arrows).

V. EXPOSURE ASSESSMENT: WHAT ARE ENVIRONMENTAL CONDITIONS?

Exposure assessment may be as simple as measuring stressors, such as pH or phosphorus concentration, directly, or it may call for using biological indicators of exposure. Exposure assessment is important for precisely characterizing the level or intensity of environmental conditions that may be affecting valued ecosystem components or services. Often, environmental conditions in a habitat cannot be measured accurately or precisely, particularly in shallow-water habitats like streams and wetlands, where environmental conditions change diurnally and seasonally with biological activity and from day to day with weather.

Many algal taxa have long been recognized to be, with varying degrees of specificity, restricted to certain aquatic environments (Kolkwitz and Marsson, 1908); therefore, they can potentially be used as bioindicators of environmental conditions. The simplest of the quantitative stressor or causal indicators are simply the sum of relative abundances of organisms that are either tolerant or sensitive to a specific environmental stressor, such as the relative abundance of motile diatoms or aberrant diatoms, which indicate silt and heavy metal pollution, respectively (Bahls, 1993; McFarland *et al*., 1997). Alternatively, the relative abundance of organisms adapted to environmental extremes could be used to diagnose stressors, such as high organic contamination, high salinity, low dissolved oxygen, or low pH (Stevenson and Bahls, 1999). More complex quantitative approaches use species composition of algal assemblages and categorical rankings of species environmental preferences, with either weighted average equations (Zelinka and Marvan, 1961) or regression equations (Renberg and Hellberg, 1982) to infer the stressor level. Recently, new accessibilities to personal computers and new statistical techniques (weighted average assessment of species preferences) have enabled the development of more accurate characterizations of species environmental preferences and more accurate and precise biological indicators of stressors in ecosystems (see Birks, 1995, 1998, for reviews). Thus, stressor levels in a habitat can be inferred with weighted average equations with species autecologies that were developed based on a categorical ranking of species environmental preferences or with autecologies determined with weighted average techniques.

Simple autecological ranks have been assigned to characterize environmental preferences for many taxa and many environmental characteristics (see van Dam *et al*., 1994, for a review). These autecological characteristics of taxa have been compiled in several reviews: Lowe (1974), Beaver (1981), Denys (1991), Hofmann (1994), and van Dam *et al.* (1994). Using a weighted average formula, stressor levels in habitats can be inferred based on the categorical autecological ranks of taxa (often eight or fewer categories) and relative abundances of taxa in samples. For example, a simple autecological index (SAI) for trophic status can be developed based on autecological ranks (Θ_i) of 0–7 for taxa observed to be most abundant in waters classified as ultraoligotrophic, oligotrophic, oligo- to mesotrophic, mesotrophic, meso- to eutrophic, eutrophic, and hypereutrophic, respectively (see van Dam *et al*., 1994). Then the trophic index can be calculated as

$$\mathrm{SAI}_{\mathrm{TI}} = \Sigma_{i=1,S} p_i \Theta_i$$

where p_i is the proportion of the ith species and Θ_i is the ecological condition in which the highest relative abundances of the ith species are collected. If autecological information is not known for all taxa, valuable information can still be obtained by correcting the index for the proportion of taxa with autecological characterizations. Next, one can redefine the community as the subset of taxa for which autecological characteristics are known by dividing the SAI by the sum of the proportional abundances of taxa with known autecological information.

This weighted average approach with simple autecological characterizations of taxa has been used extensively in stream assessments with algae, particularly in Europe (Table II) (see reviews in Whitton *et al*., 1991; Whitton and Rott, 1996; Prygiel *et al*., 1999). Software and databases of diatom autecological characteristics (e.g., OMNIDIA; Lecointe *et al*., 1993) have been developed that can be used to calculate these indices. In tests of these indices, some perform better than others when used in regions other than those for which they were originally developed (e.g., Kwandrans *et al*., 1998). Regional calibration of these indices may be required to improve performance by reassessment of algal autecological characteristics.

Stressor indicators based on accurate weighted-average assessments of species' environmental optima are more precise than indicators based on categorical characterizations of species' autecologies (e.g., ter Braak and van Dam, 1989; Agbeti, 1992). However, acquiring accurate descriptions of species' autecologies may be more difficult than using categorical characterizations. Weighted-average inference (WAI) models have been widely used to precisely infer environmental characteristics (Birks, 1998). The general principle for characterizing species autecological preferences is that, under most circumstances, the distributions of most algal taxa will exhibit a unimodal, Gaussian response curve (Fig. 6), if the gradient is long enough. The optimum (m) is estimated by the position along the environmental gradient where the taxon is most common, and the tolerance (t) can be estimated by the standard deviation of the curve. Because different taxa will have different optima and tolerances to environmental variables, these data can be used to make quantitative inferences of these variables (Fig. 7).

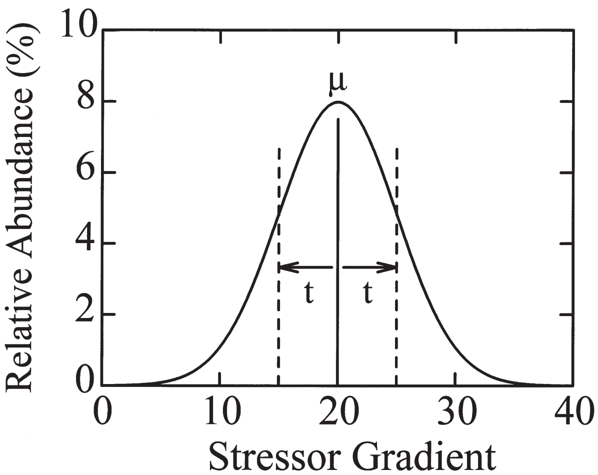

FIGURE 6 Idealized pattern in relative abundances of a single algal species along an environmental gradient showing a unimodal curve with optima (μ) and tolerance (*t*) to environmental gradients.

These approaches, which have been used extensively in both paleo- and neo-environmental assessments, are statistically robust and ecologically sound (reviewed in Charles and Smol, 1994; Birks, 1995, 1998). In general, these quantitative inferences are based on transfer functions that have been derived from paleolimnological studies, typically with the use of surface sediment calibration sets (reviewed by Charles and Smol, 1994) or with the use of present-day algal assemblages from a large suite of sites (e.g., Siver, 1995; Reavie and Smol, 1997, 1998b; Stevenson *et al.*, 1999; Pan *et al.*, 1996).

Some of the largest and most robust ecological calibration sets, or training sets, have been developed by paleolimnologists using diatoms and chrysophytes preserved in the surface (recent) sediments from a set of calibration lakes. Briefly, a calibration set is constructed by choosing a suite of sites that have been well studied (i.e., limnological characteristics are well defined) and span the gradient of interest (e.g., pH, trophic status, etc.). For example, one of the most pressing environmental questions in North America in the 1980s was, "Have lakes acidified because of acid precipitation?" Very little long-term monitoring data were available, so paleolimnological approaches were used to infer past pH and related limnological variables.

A major research effort was focused on the lakes in Adirondack Park (New York) (Charles *et al.*, 1990). To develop transfer functions to infer past lakewater acidity levels in a suite of Adirondack lakes, a calibration set of 71 lakes was chosen that ranged in present-day pH from 4.4 to 7.8 (Dixit *et al.*, 1993). From each of these lakes, the surface sediments (e.g., top 1 cm of sediment accumulation, representing the last few years of sediment deposition) were removed with a gravity corer (Glew, 1991). The indicators preserved in these sediments, in this case diatom valves (Dixit *et al.*, 1993) and chrysophyte scales (Cumming *et al.*, 1992a), were analyzed (identified to the species level, counted, and expressed as relative frequencies) from the surface sediments of the calibration lakes. This provides one of the matrices (i.e., the 71 lakes and the percentages of the taxa found in the recent sediments of lakes)

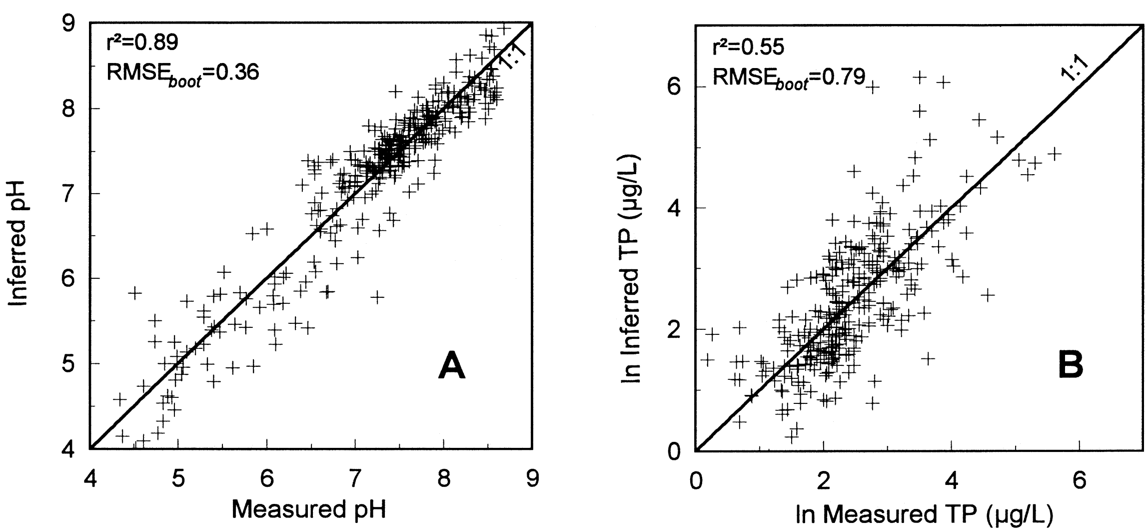

FIGURE 7 Weighted averaging calibration models for inferring lakewater pH (A) and lakewater total phosphorus (B) from diatom assemblages preserved in the surface sediments of 309 lakes in the northeastern United States. Modified from Dixit *et al.*, (1999). The error is estimated by the bootstrapped root mean squared error.

required for the calibration (i.e., the species matrix). The second matrix was composed of the present-day environmental data collected for the 71 calibration lakes (in the above example, 21 limnological variables, such as lakewater pH, monomeric aluminum, dissolved organic carbon, nutrient levels, and depth were recorded). Once these two matrices are constructed, a variety of direct gradient analysis techniques (see reviews in Charles and Smol, 1994; Birks, 1995, 1998), such as Canonical Correspondence Analysis (CCA), can then be used to determine which environmental variables are most closely related to species distributions. Thereafter, weighted averaging calibration and regression (e.g., WACALIB; Line *et al.*, 1994) were used to construct robust inference equations to infer lakewater characteristics (with known errors) from the diatoms or chrysophyte assemblages recorded in the sediments (e.g., Cumming *et al.*, 1992b, 1994). Such approaches (e.g., Fig. 8) have been used in a variety of management issues (see Smol, 1992, 1995; Anderson and Battarbee, 1994).

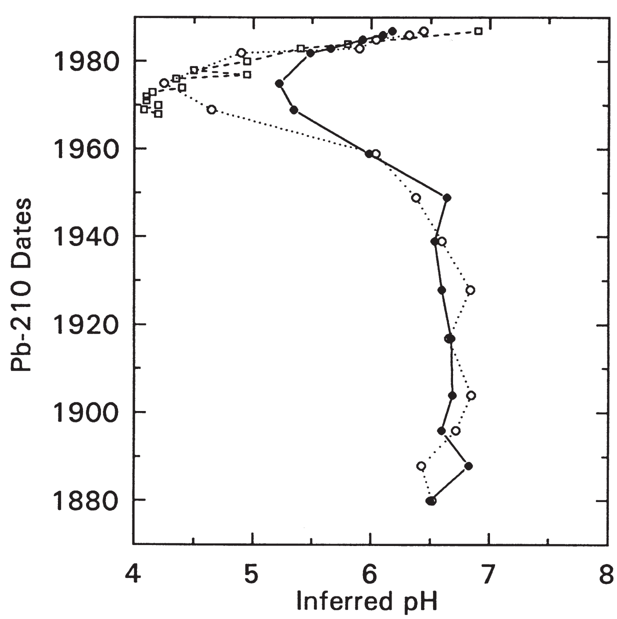

FIGURE 8 Paleolimnological assessment of acidification and recovery in Baby Lake, Sudbury, Ontario (modified from Dixit *et al.*, 1992b). The core has been dated with ^{210}Pb chronology. Closed circles represent inferred pH values from diatom assemblages, open circles represent values from scaled chrysophyte assemblages, and squares represent measured pH data (from Hutchinson and Havas, 1986) collected for the lake from various sources over the last three decades. These paleolimnological data clearly show a marked acidification of the lake in the middle part of this century, as a result of the emissions from the Sudbury smelters. Following the closure of the Coniston smelter in 1972, the fossil algal assemblages track a recovery pattern, which is matched by the measured pH data for this period. As is often the case, chrysophytes typically record more extreme acidification sequences, perhaps because they primarily bloom during spring, when the effects of acidification may be most severe.

The above calibration approaches can also be applied, in slightly modified forms, in settings where sediment accrual is not as regular as in lakes (e.g., some rivers and wetlands). For example, Reavie and Smol (1997, 1998) developed inference models from diatom assemblages attached to a variety of substrata in the St. Lawrence River and then used these transfer functions to infer past river conditions from sediment cores taken from fluvial lakes in the river system (Reavie *et al.*, 1998).

In situations where environmental conditions are highly variable, weighted averaging inference models have been shown to be better indicators of environmental conditions than one-time sampling and measurement of physical and chemical conditions (Stevenson, 1998). Field travel and sampling is an expensive part of program budgets, so habitats are often only sampled once. Water chemistry (TP concentration, for example) could be estimated based on a single water chemistry sample from a habitat or inferred based on algal species composition and autecological characteristics of those algae in a habitat. The precision of the estimate of mean TP concentration in a stream with a single water sample is the standard deviation of that assessment. The precision of an estimate of mean TP concentration in a stream with a single algal sample is the root mean square error of a weighted average regression model. Hence, 66% of estimates of mean TP concentration in a stream should be within one standard deviation of the measured TP concentration in a water sample and within one root mean square error of an inferred TP concentration from a weighted averaging inference model. Based on estimates of temporal variability in measured concentrations of TP along a wetland P gradient and among streams in a regional assessment, the standard deviation of the measured TP concentration was greater than the root mean square error of inferred TP with a weighted average model (Fig. 9; Stevenson, unpublished data).

Although intuitively one might think that it would be imperative to use ecological calibration data taken only from the region of study, and that autecological data are not readily transferable from region to region, experience sugggests that this is not strictly the case. What is most critical in ecological calibration is to capture the range of environmental conditions that one will need to infer from the biological indicators. Although regional calibration data would be more likely to contain analogues, several studies have shown that data from geographically distant regions can also be used effectively. For example, as part of the SWAP acidification program in Europe, diatom calibration

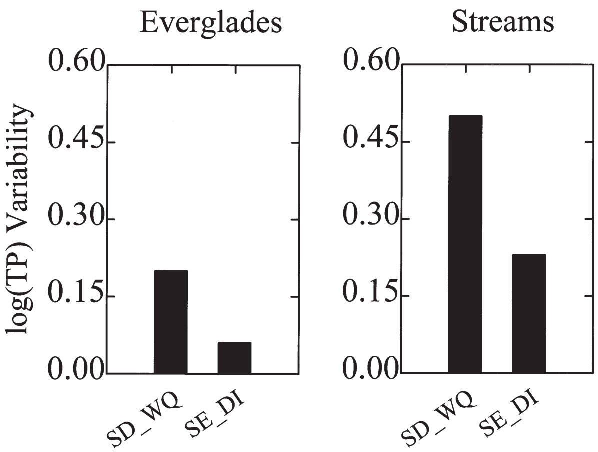

FIGURE 9 A comparison of total phosphorus (TP) variability estimated by one-time sampling and assay of water chemistry (SD_WQ) and by one-time sampling of diatom assemblages and inference with weighted average models (SE_DI). Variation in TP assessed in water chemistry (SD_WQ) is based on the standard deviation in assessed TP in the Everglades at one location over 2.5 years (provided by Paul McCormick, South Florida Water Management District, West Palm Beach, FL) and in a stream near Louisville, KY (Stevenson, unpublished data). The TP variability is based on the RMSE of diatom inference models from Everglades data (Slate, 1998) and mid-Atlantic Highlands streams (Pan *et al*., 1996).

data were effectively pooled from England, Norway, Scotland, Sweden, and Wales (Birks *et al*., 1990). Bennion *et al*. (1996) have combined regional diatom calibration sets from England, Wales, Northern Ireland, Denmark, and Sweden to develop robust transfer functions to infer lakewater epilimnetic phosphorus concentrations. As part of the CASPIA project, diatom calibration sets from North America, Africa, Australia, and Europe were pooled (Juggins *et al*., 1994). Ongoing work in our labs is also showing that calibration data are not as regional as may have once been thought, but that careful attention must be paid to taxonomic consistency and to the development of calibration data sets that effectively capture the necessary range of environmental variables.

VI. STRESSOR–RESPONSE RELATIONS

Effects of specific environmental changes on assemblage characteristics can be determined at many temporal and spatial scales and with both observational and experimental approaches. The largest scale employs surveys of large regions (e.g., ecoregions) and correlates changes in environmental conditions and assemblages, which provides observations of potential stressor–response relationships. Correlations between environmental factors and assemblage responses from surveys may show great changes and precision, and cause–effect relations may be biologically reasonable. However, experimental manipulation of environmental factors and measurement of assemblage response is important for more reliable confirmation of cause–effect relations. Experimental confirmation of stressor–response relations is particularly valuable in large-scale projects where expensive restoration efforts are planned and identification of the principal stressor is critical (e.g., McCormick and O'Dell, 1996; McCormick and Stevenson, 1998; Pan *et al*., 2000). Experimental approaches are also valuable in small-scale projects, where surveys of a large number of habitats are not practical because of budget or availability of habitats, and in toxicological studies of chemicals when chemicals are not yet widespread in the environment (Hoagland *et al*., 1993; Belanger *et al*., 1994). Observation of stressor–response correlations in large-scale surveys is useful because the relative importance of multiple environmental factors can be compared. In addition, observation of stressor–response relations in surveys shows that responses occur in the natural setting and that the relation can be expected to hold over the range of conditions studied in the survey.

Distinction between stressors and human activities that cause stressors is important in assessing stressor–response relations and in developing management strategies; this is the distinction between direct and indirect relations (U.S. Environmental Protection Agency, 1993; Yoder and Rankin, 1995; Kentucky Natural Resources and Environmental Protection Cabinet, 1997). Ecological responses to human disturbance may be caused directly by changes in physical, chemical, or biological conditions in a habitat (stressors) and indirectly by the human activities that cause those stressors. Distinguishing which stressors are most responsible for undesirable ecological responses and which human activities can be regulated to control those stressors is important for developing a plan to protect or restore environmental conditions. Many human activities (e.g., farming, logging, urbanization, sewage treatment plants) may be the source of a specific stressor (e.g., P enrichment). Many stressors (e.g., N and P enrichment, siltation, organic enrichment, and flow and light regimes) may be altered by a single human activity (e.g., farming).

The magnitude and linearity of stressor–response relationships may vary with the attribute tested. Many results indicate that metrics based on higher levels of biological organization (ecosystem/community level: e.g., biomass and productivity) are less sensitive to environmental change than metrics based on lower levels of biological organization (community/population: e.g., species composition) (Schindler, 1990; Leland, 1995). Because of high dispersal rates and high species numbers in microbial assemblages, species adapted to altered environmental conditions are probably able to invade and populate a habitat relatively quickly. Thus, impaired populations may be replaced by populations that are adapted to the altered conditions and thereby maintain ecosystem function (Stevenson, 1997). Therefore, biomass and many functional attributes of algal assemblages are often less sensitive to environmental change than changes in species composition (Schindler, 1990).

Relating the stressor–response relations between algal species composition and environmental factors to stressor–response relations for ecosystem attributes may help in the understanding of ecosystem dynamics and establishing criteria to protect ecological integrity. Recently, results from the Everglades show punctuated (sudden, discrete) changes in algal species composition along a phosphorus gradient (Pan *et al.*, 2000) (Fig. 10). Along the same gradient, higher-level biological attributes, such as biomass and productivity, change linearly or asymptotically. Thus, punctuated changes in species composition may result in multiple stable states (May, 1974) along an environmental gradient, which result from changes in the factor or factors that are the most important constraints on species composition. Punctuated changes in biomass and productivity may be blurred by species replacement along the phosphorus gradient, and spatial and temporal variability in other factors that have more short-term effects on biomass. Criteria for protecting the ecological integrity of a habitat may be established at thresholds along gradients where punctuated changes in species composition occur that correspond to undesirable changes in more than one ecological attribute (Fig. 11).

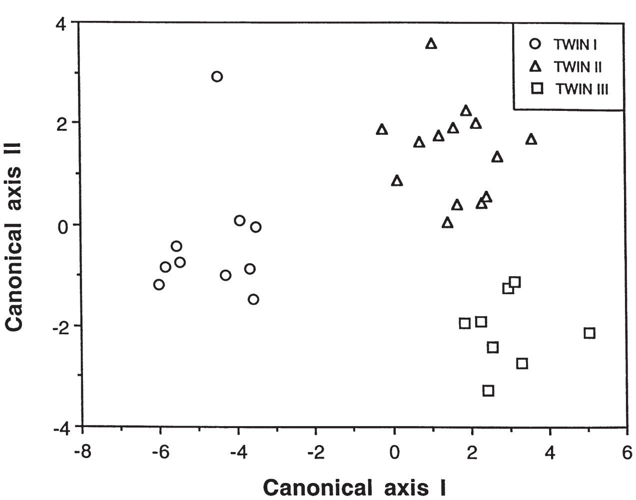

FIGURE 10 Ordination of sites based on species composition of algal assemblages at sites along a phosphorus gradient in the Everglades (Pan *et al.*, 2000).

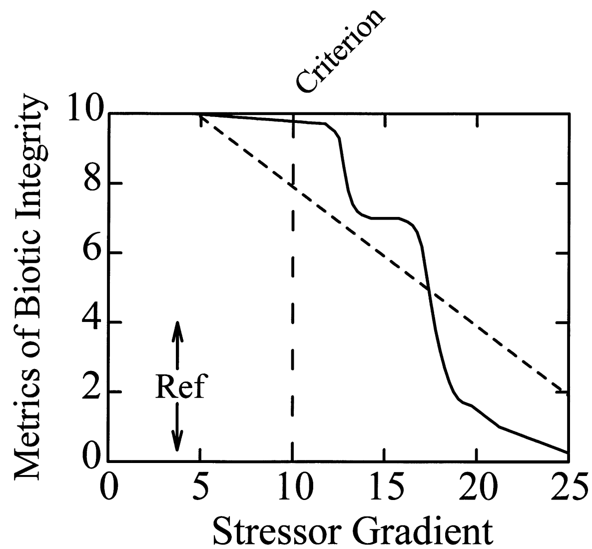

FIGURE 11 Linear (diagonal dashed line) and nonlinear (solid line) ecological responses along an environmental gradient (e.g., μg TP/L). Setting criteria for protection and remediation of ecological integrity may be facilitated by nonlinear ecological responses. Reference conditions (Ref) are indicated by the vertical arrow. The vertical line indicates a criterion.

VII. RISK CHARACTERIZATION AND MANAGEMENT DECISIONS

Risk characterization relates assessments of exposures to stressor–response relationships to evaluate the level of threat to an unimpaired system or the probable stressors and intensity of stress of impaired systems (Fig. 2) (U.S. Environmental Protection Agency, 1992). Thus, based on a set of metrics and stressor–response relationships, we can predict ecological effects of exposures to specific stressors, assess the likelihood that effects will occur, or assess the likelihood that effects were caused by specific stressors or interactions among multiple stressors. Standard risk characterizations also increase risk ratings with increasing uncertainty in information. Therefore, we are concerned with both underprotecting as well as overprotecting our resources.

Quantitatively, risk characterization on a metric-by-metric and stressor-by-stressor basis can be conducted to assess the sustainability of ecological conditions and the level of impairment or restorability of impaired conditions (Stevenson, 1998) (Fig. 12). Sustainability can be defined quantitatively as the difference between

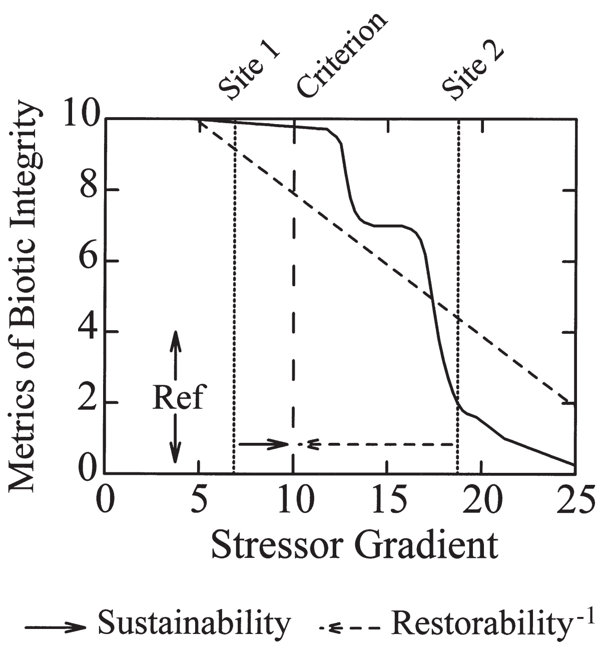

FIGURE 12 Relating ecological response to the sustainability of ecological conditions in unimpaired ecosystems and to restorability of conditions in impaired ecosystems. Reference conditions (Ref) are indicated by the vertical arrow. The vertical lines indicate a criterion and assessed conditions at two sites. Assessed conditions at Sites 1 and 2 are unimpaired and impaired, respectively. Site 1 illustrates that the greater the difference between assessed conditions and the criterion for unimpaired sites, the greater is the sustainability of valued ecological attributes at a site. Site 2 indicates that the greater the difference between assessed conditions and the criterion for impaired sites, the less restorable a habitat is.

measured exposure (stressor) levels and the exposure criteria for protecting ecological integrity (or designated use of the ecosystem). Restorability is assessed for impaired ecosystems and is equal to 1/(difference between measured exposure levels and the criteria for protecting ecological integrity). These two measures of risk characterization can provide quantitative assessments of probable effects on ecological systems—the greater the sustainability, the lower the probability of stressor effects on ecological integrity, and the lower the restorability, the greater the probability of stressor effects on ecological integrity.

Management decisions can be linked to risk assessment through risk characterizations and the stressor linkage to human activities and management options (Fig. 1). After risk characterizations are completed, management options for protecting or remediating environmental stressors will be considered with the many other factors that affect risk management decisions (e.g., political, economic, and regulatory factors). Management options depend upon the human activities that produce stressors that are causing or threatening environmental impairment. Biological assessments can actually be used to infer the human activities that cause the main ecological stressors (Yoder and Rankin, 1995; Stevenson, 1998), but detailing that approach is beyond the scope of this chapter. Thus, management options for controlling nutrients and deoxygenation in streams include reducing point sources and nonpoint sources of nutrients and BOD to streams. Point sources (e.g., municipal waste treatment plants) are easier to regulate and significantly reduce nutrient loading and related problems in many lakes and streams. However, further reduction in nutrient problems will require addressing nonpoint sources of nutrients. Thus, distinguishing the direct and indirect effects of stressors and human activities provides for a more focused approach to evaluating management options and developing a risk management decision.

VIII. CONCLUSIONS

Algae have been used successfully for environmental assessment of many streams, larger rivers, lakes, and wetlands around the world. Many different approaches can be used, which vary in the level of technical expertise required and in the amount of time required per sample. In this chapter we have tried not to make specific recommendations for which methods should be used, because different programs may call for different methods. In addition, we would not want to constrain the great promise for further development and linkage of algal methods for environmental assessment by making specific recommendations of methods. However, we have started to develop a framework for relating the different methods of environmental assessment and to explore the specific situations in which different methods should be used. We have linked the algal framework for environmental assessment to a standardized risk assessment framework so that assessments with algae can be better related to assessments with other organisms and other approaches (such as laboratory-based toxicology).

Future developments in algal methods for environmental assessment should be directed to the ultimate goal of solving environmental problems. More rigorous hypothesis testing of the precision and sensitivity of algal indicators will be important in documenting the performance of algal indicators and encouraging their application. Transferability of algal indicators among regions should be rigorously evaluated because this allows development of consistent approaches across regions (and perhaps habitat type) and saves costs of developing regional indicators. Current efforts to develop web-based access to autecological characteristics of taxa will be valuable for making this information available to a broader audience, but this approach also requires cautious evaluation of the quality of information on the web. Great challenges also exist for our more consistent application and integration of multiple lines of evidence, which may be facilitated by using a framework, such as the risk assessment framework. In addition, we must learn to communicate the results of our research more effectively and clearly to a broad audience, some of whom have little experience with interpreting mathematically complex information.

Algae can cause ecological problems, but can also perform valuable ecological services. In a recent report by the National Research Council of the US (CEIMATE, 2000), total and native species diversity, productivity, trophic status, and nutrient-use efficiency were listed as fundamental ecological indicators for the United States. Algal attributes related to these indicators, as well as other valued ecological attributes of algae, should provide one part of a rationale for assessing algal properties of ecosystems. The second part of that rationale is the extraordinary sensitivity and diagnostic power of algae to detect environmental problems and identify their causes.

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