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METHODS FOR EVALUATING WETLAND CONDITION

#11 Using Algae To Assess Environmental Conditions in Wetlands

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Notice

The material in this document has been subjected to U.S. Environmental Protection Agency (EPA) technical review and has been approved for publication as an EPA document. The information contained herein is offered to the reader as a review of the "state of the science" concerning wetland bioassessment and is not intended to be prescriptive guidance or firm advice. Mention of trade names, products or services does not convey, and should not be interpreted as conveying official EPA approval, endorsement, or recommendation.

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http://www.epa.gov/ost/standards

http://www.epa.gov/owow/wetlands/bawwg

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Foreword

In 1999, the U.S. Environmental Protection Agency (EPA) began work on this series of reports entitled *Methods for Evaluating Wetland Condition*. The purpose of these reports is to help States and Tribes develop methods to evaluate (1) the overall ecological condition of wetlands using biological assessments and (2) nutrient enrichment of wetlands, which is one of the primary stressors damaging wetlands in many parts of the country. This information is intended to serve as a starting point for States and Tribes to eventually establish biological and nutrient water quality criteria specifically refined for wetland waterbodies.

This purpose was to be accomplished by providing a series of "state of the science" modules concerning wetland bioassessment as well as the nutrient enrichment of wetlands. The individual module format was used instead of one large publication to facilitate the addition of other reports as wetland science progresses and wetlands are further incorporated into water quality programs. Also, this modular approach allows EPA to revise reports without having to reprint them all. A list of the inaugural set of 20 modules can be found at the end of this section.

This series of reports is the product of a collaborative effort between EPA's Health and Ecological Criteria Division of the Office of Science and Technology (OST) and the Wetlands Division of the Office of Wetlands, Oceans and Watersheds (OWOW). The reports were initiated with the support and oversight of Thomas J. Danielson (OWOW), Amanda K. Parker and Susan K. Jackson (OST), and seen to completion by Douglas G. Hoskins (OWOW) and Ifeyinwa F. Davis (OST). EPA relied heavily on the input, recommendations, and energy of three panels of experts, which unfortunately have too many members to list individually:

- Biological Assessment of Wetlands Workgroup
- New England Biological Assessment of Wetlands Workgroup
- Wetlands Nutrient Criteria Workgroup

More information about biological and nutrient criteria is available at the following EPA website:

http://www.epa.gov/ost/standards

More information about wetland biological assessments is available at the following EPA website:

http://www.epa.gov/owow/wetlands/bawwg

List of "Methods for Evaluating Wetland Condition" Modules

Summary

Algae play important roles in wetland function and can be valuable indicators of biological integrity and ecological condition of wetlands. Sampling designs for algal assessment vary with objectives of programs and the algal characteristics that are measured. Both structural and functional attributes of algae can be measured, including diversity, biomass, chemical composition, plus productivity and other metabolic functions. Species composition of algae, particularly diatoms, is commonly used as an indicator of biological integrity of wetlands and the physical and chemical conditions in wetlands. These latter conditions can be inferred based on species environmental preferences and species composition of algae in wetlands. Sampling methods for algae on plants and sediments and floating in the water are well established, are reviewed in detail in another chapter of this book, and are used in streams and lakes as well. Laboratory methods are also well established for most algal characteristics with relatively standard protocols used in several national stream programs. Guidelines for data analysis are also reviewed in this chapter, which includes basic metric development and also the development and application of indices that infer physical and chemical conditions in wetlands. Case studies are presented on the development of algal indicators for Maine wetlands and use of algae to assess ecological conditions in the Everglades.

Purpose

The purpose of this chapter is to provide guide lines for the use of algae to assess biological integrity and ecological condition of wetlands.

Introduction

Background

Algae are an ecologically important and often conspicuous feature of both freshwater and estuarine wetlands (see reviews by Vymazal 1994, Goldsborough and Robinson 1996, Sullivan 1999). Periphyton, assemblages of algae and other microorganisms attached to submerged surfaces, occur in nearly all shallow-water habitats wherever sufficient light penetrates (Table 1). Periphyton grow attached to submerged surfaces, such as sediments, woody and herbaceous plants, and rock substrata. In many wetlands aggregations of algae, called metaphyton, grow entangled among macrophytes either at the water surface or below. Phytoplankton also can be abundant in deeper and larger wetlands where water-column nutrient levels are high, flushing rates are low, and grazing pressure by planktivores is low. Algae provide a food source for invertebrates and small fish in wetlands (Sullivan and Moncreif 1990, Murkin et al. 1992, Campeau et al. 1994, Browder et al. 1994, Mihuc and Toetz

Table 1: Major Habitats and Algal Assemblages in Wetlands

Community type		Growth form and habitat
Phytoplankton		Unicellular and colonial forms suspended in the water column
Periphyton:	Epidendron	Mats or films growing on dead wood
	Epilithon	Mats or films attached to hard surfaces
	Epipelon	Mats, flocs, or films growing on soft sediments
	Epiphyton	Mats or films attached to submerged macrophyte stems and leaves
	Metaphyton	Mats or filaments floating on the water surface or suspended in the water column, often entangled with macrophytes

1994). Algal photosynthesis and respiration can account for a significant fraction of wetland metabolism in some habitats and, therefore, can strongly influence water-column oxygen dynamics (McCormick et al. 1997). Algae are important in energy and nutrient cycling, stabilizing substrata, and serving as habitat for other organisms in wetlands (Sullivan and Moncreif 1988, Sundbäck and Granéli 1988, Browder et al. 1994, MacIntyre et al. 1996, Miller et al. 1996, Wetzel 1996). In some cases, algal mats may serve as refugia for invertebrates during periods of wetland desiccation (Harrington 1959).

Algae are among the most widely used indicators of biological integrity and physico-chemical conditions in aquatic ecosystems (see reviews in McCormick and Cairns 1994, Adamus 1996, Danielson 1998, Stoermer and Smol 1999, Stevenson and Smol in press, Stevenson in press). Algal growth and taxonomic composition respond predictably and sensitively to changes in pH, conductivity, nutrient enrichment, organic contamination, sediments, pesticides and many other contaminants (Round 1981, Stevenson et al. 1996). Algae provide some of the first indications of changes in wetlands because they respond directly to many environmental changes, they have high dispersal rates, and they have rapid growth rates. Algae provide highly precise assessments of changes in wetlands because they have high species numbers and each species is differentially sensitive to a broad range of environmental conditions. In fact, algae may provide a more precise indication of wetland nutrient status than sporadic measurements of water chemistry, particularly in wetlands that receive pulsed nutrient inputs (e.g., storm-water runoff). Effects of varying nutrients are measurable in a historical record manifested in algal assemblage characteristics (see Stevenson in press). Because of their role in ecosystem energetics and biogeochemical cycling, algae provide an integrated picture of wetland condition. The glass cell walls of diatoms that accumulate in sediments provide a historic record of ecological conditions in wetlands and are an important indicator in paleolimnological studies. The persistence of diatoms in sediments, even when wetlands are dry, may provide a year-round approach for assessing the ecological integrity of wetlands when other organisms are not present. Thus, diatoms could be used to provide a basis for developing regulatory decisions when many other organisms may not be present. In addition, the rapid growth rates of algae enable experimental manipulation of environmental conditions to determine cause-effect relationships between algal response and specific environmental stressors (McCormick and O'Dell 1996, Pan et al. 2000).

A common assumption is that algal assessments require unusually great expertise and effort to complete. Even though detailed, species-level assessments do require substantial skill in algal taxonomy, these skills can be readily developed with an introductory course in algal taxonomy, experience, a good library of taxonomic literature, and periodic consultations with other taxonomists. The taxonomy of most common algal genera and diatom species is well established and taxonomic keys are widely available. Coarser taxonomic analyses (e.g., dominant genera) require considerably less training and nontaxonomic metrics (e.g., chlorophyll *a*, nutrient content) require only a general background in analytical laboratory practices. Field sampling and laboratory processing times for algal taxonomic analyses are comparable or less than for other commonly used indicators. Thus the utility of using algae as an assessment tool should not be overly constrained by a lack of staff expertise and resource limitations.

Considerations When Formulating a Sampling Design

Objectives of the project

Sampling design is highly dependent on the objectives of specific projects. Initial projects may be designed to develop and test metrics for applica-

tion in a specific class of wetlands and in specific geographic regions. After metrics have been developed, sampling design should change (e.g., random selection of sites) to apply these metrics to assess site-specific conditions or status and trends in wetlands within a region. During development of metrics, the most efficient sampling design is to select wetlands with a wide range of adverse human influences. Metrics developed with this strategy will apply to the range of wetland conditions in a region. Developing metrics using sites that were randomly selected from the set of all sites may cause oversampling of impaired or unimpaired wetlands and may diminish development effort. If management concerns are based on specific types of adverse human influences, for example, nutrient enrichment or hydrologic alterations, then metric development should be focused on wetlands with a range of nutrient or hydrological conditions. After metrics are developed, sampling designs should be planned to test hypotheses, for example:

- Are conditions at a specific site significantly different from those found at a population of reference sites; or
- Do wetland conditions in a region have a specific mean or modal condition and variance?

The effects of project objectives on sampling design are discussed more completely in Module 4: Study Design for Monitoring Wetlands.

Wetland class

Algal attributes can differ considerably among different wetland types within the same state or ecoregion (e.g., Stewart et al. 1985). Therefore, it often will be necessary to define reference conditions separately for each wetland type. Light, pH, available nutrients, and the mineral content of the water determine the type of algal community that develops in a wetland. As a result of shading effects, algal biomass and productivity typically are lower in forested and emergent-plant wetlands than they are in sparsely vegetated systems dominated by submerged aquatic vascular plants (SAV). Rainfall-driven wetlands, which tend to have low ionic content and a neutral-acidic pH, contain a periphyton community dominated by chlorophytes and acidophilous diatoms. Wetlands fed by groundwater, which typically has a higher pH and mineral content, contain a greater abundance of cyanobacteria and alkaliphilous diatoms.

Hydrology is less important than water chemistry in determining periphyton structure and function. Most algae require saturated or flooded conditions to grow, and hydrologic parameters, such as water depth, influence light penetration to phytoplankton in the water column and to submerged surfaces where periphyton grow. However, many algae possess adaptations that allow them to either survive dry conditions or to recolonize quickly, which enables rapid recovery of antecedent algal communities following marsh reflooding. Thus, many attributes of the wetland algal community are less sensitive to hydrologic modifications as compared with macrophytes. However, species composition of algae may respond indirectly to hydrologic conditions because hydrology often regulates water chemistry.

Classification systems (discussed in Module 7: Wetlands Classification) provide a starting point for classifying wetland types for periphyton sampling purposes. However, periphyton characteristics may not follow hydrogeomorphic classification schemes exactly, as this community is influenced most strongly by water chemistry. Initial sampling in reference wetlands provides the best means of classifying wetland types according to their periphyton characteristics.

Habitats sampled

Algal characteristics also can vary considerably among habitats within a wetland. Periphyton grows on most submerged surfaces (e.g., hard and soft sediments, living and dead vegetation), with the ex-

ception of those that exude allelopathic or otherwise inhibitory compounds. A distinct phytoplankton community also may develop. Both the amount of light and the nature of the substratum affect periphyton abundance, growth, nutrient content, and taxonomic composition. For example, senescent or decomposing vegetation may release more nutrients than actively growing plant stems, thereby increasing periphyton growth and nutrient content. The sensitivity to environmental change of different algal communities within a wetland also may vary; for example, phytoplankton and floating periphyton mats (metaphyton) respond quickly to changes in water-column nutrients whereas periphyton growing on sediments (epipelon) are influenced most by nutrient fluxes from the underlying substrate. Given the potential for such variability in algal metrics among habitats, it is important that algal habitats be sampled in a consistent manner across wetlands so that changes in wetlands can be assessed precisely.

Recent evidence indicates that samples from targeted habitat samples more precisely indicate wetland change than composite samples from multiple habitats. In a study of correlations between algal attributes and several indicators of human disturbance in wetlands in Maine (see case study at end of module), similar numbers of statistically significant correlations were observed with assemblages from sediments, plants, and plankton. More correlations and higher correlations were observed in targeted habitat samples than when epipelon, epiphyton, and plankton were combined in a single, multihabitat sample.

Introduced substrates (e.g., unglazed clay tiles, glass slides, acrylic rods) are commonly used to provide a standardized surface for periphyton growth. The use of introduced substrates minimizes problems associated with substrate comparability among sampling locations. While the periphyton community developing on such surfaces may differ in certain respects from that growing on natural substrates (Tuchman and Stevenson 1980), this type of sampling provides a reliable indicator of wetland nutrient status and other changes in water chemistry (McCormick et al. 1996). In fact, the use of inert surfaces for algal colonization may enhance the sensitivity of the community to changes in water-column nutrient availability because nutrients leak from plants and sediments (Moeller et al. 1988, Burkholder 1996, Wetzel 1996). However, if the assessment goal is to assess periphyton condition within the wetland and not simply to use periphyton as an indicator, then natural substrates should be sampled. Disadvantages to the use of introduced substrates include the requirement for two visits to each sampling location, once for deployment and again for retrieval. In addition, these substrata are susceptible to loss as a result of vandalism, animal damage, or fluctuating water levels. The strengths and weaknesses of using artificial substrates have been debated extensively in the literature (e.g., Stevenson and Lowe 1986, Aloi 1990).

Sampling frequency

Algal communities typically exhibit distinct seasonal patterns of standing crop and species composition, and these patterns can differ among wetland types. For example, deciduous forested wetlands may exhibit maximum algal growth during spring or following leaf fall, when light penetration is highest, whereas periphyton abundance in other wetland types may peak during the warmer months. Temperature tolerances and optima vary among species and major algal groups, with diatoms being relatively more abundant during cooler months and cyanobacteria (blue-green algae) being more common during the summer. Furthermore, although less sensitive to hydrology than macrophytes, most algal communities require some surface water to remain active. Disturbance events may be less common and less severe in most wetlands than in most streams and rivers, but still they can affect periphyton growth and abundance temporarily. For example, heavy rainfall and wind can disrupt or even

disintegrate floating algal mats and introduce sediment, nutrients, and other pollutants that can affect algal metabolism and growth.

Some familiarity with the temporal dynamics of algal communities in wetland classes of interest should be gained before initiating routine sampling. If possible, sampling should be conducted during more than one season to provide an integrated assessment of periphyton and nutrient conditions. If this frequency is not practical, then sampling should be conducted during the peak growing season or at a time when stressor impacts are most likely to occur. Wetlands to be compared should be sampled at approximately the same time of year to minimize the confounding influence of seasonal variability on algal metrics.

Spatial sampling intensity

The sampling intensity required to adequately assess algal conditions is related to the complexity and spatial variability of the algal community, which in turn is a function of habitat heterogeneity. The sampling effort will be relatively low in instances where a single algal community predominates and is distributed in a relatively homogeneous manner across the wetland. In most instances, however, multiple communities will coexist and be distributed patchily within and among vegetative habitats.

Sampling intensity also is affected by the presence of nutrient or other disturbance gradients within the wetland. For example, point-source nutrient inputs can produce localized zones of enrichment that will support an algal community quite different from that in unenriched portions of the same wetland, whereas nonpoint source inputs or those that are exceedingly high relative to the size of the wetland may enrich the entire system.

The spatial heterogeneity of the wetlands to be assessed should be evaluated in both qualitative and quantitative terms to develop a sampling protocol that optimizes the tradeoff between precision and cost. First, major algal habitats and communities should be identified. Secondly, preliminary sampling should be conducted to assess redundancy of information among different communities and, in conjunction with power analysis, to determine the optimal sampling effort from a statistical standpoint. Insufficient sampling relative to background variability will reduce the ability to discern trends and impacts. Oversampling is not cost-effective and may preclude measurement of other valuable indicators. In heterogeneous environments, there are several ways to reduce the sampling effort without introducing excessive variability into the results by (1) conducting a composite sampling, i.e., combining several field samples into a single sample for processing to account for local variation without increasing costs, (2) selecting a single "indicator" community for sampling rather than attempting to sample all algal habitats, and (3) deploying introduced substrates (as discussed above). The efficacy of these various alternatives will depend on assessment objectives and the types of wetlands being sampled.

Quantitative versus qualitative sampling

In addition to the frequency and intensity of sampling, one of the most important determinants of effort—and therefore cost—is the type of metrics to be measured. Quantitative measures of algal standing crop, nutrient content, and productivity provide valuable insight into wetland function and the relative importance of periphyton processes. Unfortunately, they also are extremely costly to obtain because they are so spatially and temporally variable. Costs of some area-specific measures can be reduced if measures are restricted to specific habitats. By contrast, qualitative sampling (e.g., collecting grab samples of the dominant periphyton community from natural substrates) offers a more cost-effective tool for assessing the nutrient status of the wetland, albeit providing somewhat less information on overall periphyton condition.

Considerations When Selecting Metrics

The types of algal metrics selected for assessment and monitoring depend on several factors as follows.

Overall objectives of the sampling program

Sampling is based on the objectives of the program. For example, is the objective to assess the periphyton condition of the wetland or simply to get an indicator of wetland condition? Assessing periphyton condition requires sampling a broad array of quantitative metrics on natural substrates whereas an assessment of wetland condition, such as trophic status, can be accomplished using a limited number of measures obtained from either natural or standardized substrata and need not be quantitative in most cases.

Wetland type

Available habitats and the relevance and utility of different metrics vary among wetland types. Some wetlands do not have herbaceous macrophytes and others seldom have standing water. Algae on sediments occur in almost all habitats. Although we have not thoroughly tested which habitat is most sensitive to changes in wetland condition, algal metrics from all three of the habitats we studied can reflect changes in wetland condition (see Maine case study). In the Everglades, the occurrence of a floating calcareous algal mat is characteristic of oligotrophic conditions, but this algal attribute is not found in oligotrophic wetlands from many other ecoregions.

Frequency of sampling

Sensitive metrics that respond quickly to changes in nutrient availability and other physicochemical conditions are useful when wetlands are to be monitored frequently. If sampling is conduct infrequently (e.g., annually) or is limited to a single assessment, emphasis should be placed on metrics that integrate conditions over longer periods of time and, therefore, are not overly susceptible to short-term fluctuations. Surface sediment diatom assemblages, for example, probably provide the greatest temporal integration of wetland conditions, because diatoms have accumulated in those sediments over the last several years. However, surface sediment diatom assemblages may not be as sensitive to nutrient conditions because of their location on sediments. Epiphytic algae may integrate environmental conditions over the last couple of months as diatom assemblages developed on the plants. Phytoplankton probably reflects the shortest historical context of all the assemblages. Taxonomic attributes (especially presence/absence of taxa versus their relative abundances and density) typically have longer histories than metabolic attributes (photosynthesis, phosphatase activity).

Existing capabilities

Metrics selected for use undoubtedly will be influenced by existing facilities and staff expertise. Thus metrics may be similar to those currently being used to monitor other types of aquatic systems. In many instances, algal metrics and protocols used to sample other benthic habitats can be adapted for use in wetlands.

Typical Algal Attributes used for Metrics

Algal attributes generally are classified as either structural or functional. Structural attributes are based on measurements of biomass, nutrient content, and taxonomic composition of samples. Functional attributes are based on measurements of growth rates and rates of metabolism, such as photosynthesis, respiration, nutrient uptake, and enzyme activity.

Algae, more than other assemblages, have been used to indicate physicochemical conditions as well as assess biological integrity in a habitat (Figure 1).

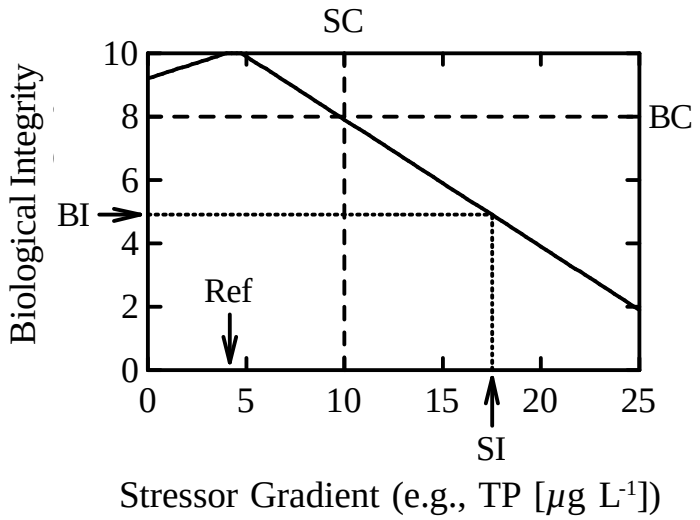

FIGURE 1: RELATIONS BETWEEN BIOLOGICAL INTEGRITY AND STRESSOR INDICATORS ALONG A STRESSOR GRADIENT (SOLID LINE)

These relations can be developed during indicator development and testing or determined from the literature. These relations often are most precise between specific assemblage attributes and specific stressors rather than between multimetric indices and specific stressors and are important for applying a risk assessment approach to environmental monitoring (adapted from Stevenson in press). Correspondence between observed measures of biological integrity (BI) and specific stressors (SI) can help diagnose causes of or threats to ecological impairment. BI - measured biological integrity; SI - measured stressor indicator; RC - Response Criteria; and SC - Stressor Criteria (dashed lines). Reference (Ref) indicated by arrow.

In other words, many attributes of algal assemblages can be used as indicators of a natural, resilient flora, but species composition of algal assemblages and environmental preferences of algae have been particularly powerful indicators for diagnosing causes of environmental impairment. Diagnostic indicators, usually referred to as stressor indicators (Paulsen et al. 1991, U.S. EPA 1998), usually are considered to be actual measures of altered physical, chemical, or biological attributes caused by human disturbance. Direct measurements of nutrient concentrations, pH, conductivity, acid-neutralizing capacity, depth and period of inundation, or densities of non-native taxa commonly are used as stressor indicators related to nutrient enrichment, hydrological alterations, and introduction of non-native taxa. Thus stressor indicators based on algal assemblages can be used to complement direct measurement of environmental conditions. In general, all attributes of algal assemblages could be used to assess biological integrity, but species composition and, to some extent, chemical composition most often are used to diagnose causes of problems.

Taxonomic composition

Taxonomic characteristics of algal assemblages provide some of the most sensitive and robust indications of wetland nutrient status (e.g., McCormick and O'Dell 1996). Species-level assessments, although requiring the greatest taxonomic expertise to perform, yield the most precise indicators of environmental conditions and biological integrity. However, considerable information can be obtained from assessments performed at the genus level or higher (e.g., Prygiel and Coste 1993, VanderBorgh 1999). Taxonomic analyses often focus on diatoms within the algal assemblage because the taxonomy and species' autecologies of this group are relatively well established and readily determined from field collections without additional effort (e.g., culturing).

Taxonomic characteristics of assemblages can be used to assess biological integrity of wetlands and diagnose causes of or threats to impairment. Biological integrity of algal assemblages can be evaluated at many taxonomic levels, for example, looking just for changes in species composition or changes in algal growth form. Whereas the latter may reflect changes in food availability and habitat structure, changes in species composition alone are important indicators that biodiversity has been altered and that the environmental factors regulating microbial processes in the wetland have changed.

Similarity in taxonomic composition between reference and test sites is the most direct approach for assessing biological integrity of algal assemblages in wetlands. The ability to distinguish impaired from reference assemblages requires precise characterization of taxonomic composition of assemblages in reference wetlands. Precision in estimates of reference assemblages can be increased by classifying wetlands and sampling multiple reference wetlands for each wetland class (see Module 7: Wetlands Classification). Similarity can be measured with many indices (e.g., % similarity, Euclidean distance; Pielou 1984, Jongman et al. 1995) and with many types of taxonomic data. Similarity based on taxonomic data can be calculated based on densities, relative biovolumes, relative abundances, or presence/absence of species, genera, or functional groups. Similarity calculated with both species and genus composition of assemblages provides sufficient data to compare assemblage similarity. Functional groups often are defined by growth form and major taxonomic divisions, such as unicellular, colonial, and filamentous forms of cyanobacteria; diatoms; green algae; euglenoids; and cryptomonads. These groups are thought to represent different food categories for herbivores (Porter 1977, Lamberti 1996). We recommend two measures of similarity in algal assemblages: similarity in relative biovolumes of functional groups and similarity in relative abundances of diatom species as two relatively standard indicators of biological integrity.

Another approach to assessing biological integrity of algal assemblages in wetlands is to compare the relative abundance of organisms and the number of taxa in genera that are common in reference conditions and rare in disturbed wetlands. For example, we often find high numbers of taxa in the diatom genera *Eunotia* and *Pinnularia* in relatively undisturbed wetlands. The % of cells and % of taxa of *Eunotia* and *Pinnularia* can be used as indicators of biological integrity of wetlands, if reference wetlands have high numbers of these diatom genera. These metrics probably are less precise than direct species-level comparisons with assemblages from reference conditions, but they may be valuable metrics if reference conditions are not defined precisely.

A successful approach used in streams is first to define the sensitivity of all taxa that are common in reference conditions and rare or absent from disturbed conditions and then to determine the % of sensitive cells or taxa in assemblages at test sites. Percents of sensitive cells or taxa are measurements of similarity between reference and impaired sites. By placing an emphasis on taxa autecologies (e.g., sensitivity to pollution), the emphasis on wetland classification can be reduced; pairing of wetland classes and identifying taxa in reference sites is not as great an issue. Sensitive taxa in any wetland class, across all classes of wetlands, should be sensitive to the same disturbances by humans.

Taxonomic composition, when combined with specific environmental tolerances of taxa, can be used to calculate stressor indicators to infer environmental conditions in wetlands (Pan and Stevenson 1996, Stevenson et al. 1999, see case studies). Such indices usually are referred to as autecological because they are based on the autecological characteristics of taxa. Different levels of accuracy in environmental tolerances can be used to distinguish two groups of autecological indices. For example, in some cases, environmental tolerance categories are known for taxa (Lowe 1974,

VanLandingham 1982, Van Dam et al. 1994). In other cases, specific pH, salinity, or total phosphorus (TP) optima for taxa are known (Pan and Stevenson 1996, Stevenson et al. 1999). Assessments of environmental conditions based on species optima may provide more accurate inferences of conditions. However, use of indices based on species optima characterized in another geographic region may be biased. Indices based on categorical characterizations of species autecologies may be more transferable among regions. Probably they are more reliable in the early stages ofimplementingalgae assessment programs until species environmental optima in the study region have been evaluated.

Conceptually, autecological indices also might be used to infer biological integrity in cases where reference conditions in wetlands are not well defined. Many environmental contaminants, such as nutrients, are not commonly found in abundance in natural aquatic ecosystems. Thus, high proportions of taxa that require high nutrients would indicate that the biological integrity of the habitat had been compromised and that nutrients were a highly probable cause of impairment. Wetland conductivity (ionic concentration) often changes with hydrologic alterations, and algae are highly sensitive to changes in conductivity. Thus, changes in algal species composition could be used to indicate hydrologic alteration of wetlands.

Diversity

Richness of taxa numbers and evenness of taxa abundances are two characteristics of many diversity indices (sensu Shannon 1948, Simpson 1949, Hurlbert 1971) that are used to describe biological assemblages. Although diversity often is used in environmental assessments, basic problems with its use exist. First, species diversity and evenness are highly correlated with standard 300-600 cell counts; in these counts many species usually have not been identified, so richness is more a function of evenness than evenness a function of richness (Patrick et al.1954, Archibald 1972, Stevenson and Lowe 1986). Using standard counting procedures, nonmonotonic (showing both positive and negative changes as the independent variable increases) responses of algal diversity can occur along environmental gradients, which introduces ambiguity into the interpretation of diversity indices. This ambiguity seems to be related to the maximum evenness of tolerant and sensitive taxa at midpoints along environmental gradients, fewer species being adapted to environmental extremes at both ends of environmental gradients, and subsidy-stress perturbation gradients (Odum et al. 1979). Despite these difficulties, species richness and evenness may respond monotonically (exhibiting only positive or negative changes, but not necessarily linear ones, as the independent variable increases). Likewise, they may respond sensitively and precisely to gradients of human disturbance in some settings and should be tested for use as metrics.

Biomass

Biomass of algae often increases with resource availability and decreases with many toxic and sediment stressors caused by humans. The relationship between algal biomass and nutrient inputs is one of the most widely used indicators of eutrophication in aquatic ecosystems (e.g., Vollenweider 1976, Schindler et al. 1978, Dodds et al. 1998). Most relationships have been established for lake phytoplankton; however, periphyton in streams also is closely related to nutrient status, as increased nutrient availability will yield more biomass (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 with season and weather (Whitton 1970, Wong et al. 1978, Lembi et al. 1988), the timing of sampling is important. Biomass is an important attribute in environmental assessments because it is related to productivity and nuisance problems.

Biomass can be estimated using several measurements, including cell density, cell biovolume, ash-free-dry-mass (AFDM), and chlorophyll (chl) *a*. Each of these measurements has strengths and weaknesses as described by Stevenson (1996, in press), and the use of multiple estimates can increase the amount of information obtained. For example, estimates of periphyton chlorophyll *a* and AFDM can be used to calculate the autotrophic index (Weber 1973), which indicates the dominance of heterotrophs (e.g., bacteria, fungi) relative to algae in the periphyton community. Ratios between nutrients and AFDM in samples provide indicators of nutrient availability and trophic status (McCormick and O'Dell 1996, Pan et al. 2000).

In practice, quantitative measures of areal periphyton biomass (e.g., McCormick et al. 1998) can be time-consuming, destructive and therefore costly to obtain. Measuring biomass can require harvesting of all periphyton and associated substrates from known areas of marsh. Sampling with this approach focuses on particular vegetative habitats or can be conducted on a habitat-weighted basis to characterize the entire wetland. Multiple plots must be sampled from each habitat to account for spatial variation in biomass. Considerable processing time is required to separate periphyton from associated macrophyte material and other substrates when sampling at this scale. A second quantitative approach is to sample at smaller scales, by algal habitats: plants, sediments, floating mats, and water column. Then, based on quantitative assessment of area of these habitats within the wetland, algal biomass can be estimated with a habitat-weighted calculation.

Qualitative (i.e., presence-absence) or semi-quantitative (e.g., percent cover) measures of periphyton abundance may provide a cost-effective alternative indicator of nutrient status and wetland condition in some instances and can be accompanied by visual assessments of algal taxonomic composition. These techniques have been employed successfully in streams (Sheath and Burkholder 1985, Stevenson and Bahls 1999). Biomass accumulation on introduced substrates (see growth rate below) also can be used to assess biomass-nutrient relationships among wetlands, although these measurements are not always a reliable predictor of periphyton abundance on natural substrata within the same wetland.

Although the relationship between algal biomass and nutrient availability is logical, it has limitations in practice. Biomass can be highly variable in space and time (e.g., episodic algal blooms, sloughing of periphyton from surfaces) and may respond differently to enrichment in different wetlands. For example, algal biomass in open-water habitats in the Florida Everglades declines with increased phosphorus availability, which differs from many other aquatic systems (McCormick and O'Dell 1996, Pan et al. 2000). Biomass also is affected by differences in the light regime and grazing pressure among wetlands as well as nutrient levels. Biomass-nutrient relationships also may be confounded by the presence of other stressors such as toxic chemicals. Thus, the accurate interpretation of biomass changes requires an understanding of ecological processes and study of cumulative impacts within a wetland.

Chemical composition

Chemical composition of algal assemblages can be used to assess trophic status or contamination of food webs by toxic compounds. Water-column nutrient concentrations, particularly those of bioavailable (i.e., dissolved inorganic) forms, can be highly variable over short periods as a result of weather events (e.g., heavy rainfall) and pulsed nutrient inputs from anthropogenic sources. In oligotrophic wetlands, water-column nutrient concentrations may be below, at, or near the minimum detection limits, thus increasing the relative uncertainty associated with analytical results. Periphyton nutrient concentrations integrate variation in nutrient bioavailability over time scales of weeks, thus providing an indication of the recent nutrient status of

the wetland that is not unduly influenced by episodic events or other short-term fluctuations. Measurements of periphyton nutrients complement soil nutrient analyses, which indicate the nutrient history of the wetland over years or decades. Many toxic contaminants, such as heavy metals and organic contaminants, are rapidly adsorbed or taken up by periphyton (e.g., Vymazal 1994). Their presence in the water column far underestimates their presence in the habitat and their potential availability to the food web.

Total phosphorus (TP) and nitrogen (TN) concentrations of water and periphyton have been used to characterize trophic status (Carlson 1975, Dodds et al. 1998, Biggs 1995). TN:TP ratios are widely used to infer which nutrient regulates algal growth (Hecky and Henzel 1980, Hecky and Kilham 1988, Biggs 1995). In many of these assessments, most of the total P and N is 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 1975), but use has also been proposed for streams, rivers, and wetlands (Vymazal and Richardson 1995, Dodds et al. 1998, McCormick and Stevenson 1998). TP and TN per unit biomass in benthic algae also have correlated positively with 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.

The chemical composition of algae is a valuable piece of information 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). The toxicity of heavy metals to algae is one reason for monitoring them. Other reasons include their bioaccumulation in waste streams, and the movement of heavy metals into the food web (Whitton and Shehata 1982, Vymazal 1984, Radwin et al. 1990).

Growth

In the absence of other environmental limitations (e.g., light availability, grazing), algal net production is positively related to nutrient bioavailability. Periphyton production is quantified most easily in the field by measuring biomass accumulation on introduced substrates over a standardized period of time. Though growth rates on these substrates may differ from those on natural wetland substrates, they do provide a reliable indicator of nutrient availability. A similar level of light should be maintained for substrates placed in all wetlands that will be compared; otherwise, the relationship between biomass accumulation and nutrient availability will be hard to interpret. Maintaining this uniformity may require clipping macrophyte material in heavily vegetated wetlands. Incubation times should be sufficiently long (e.g., 10-20 d) to allow for accumulation of measurable biomass, but not so long as to result in sloughing. Determining a standardized incubation period can be difficult when working in wetlands of widely varying trophic status. Typically, periphyton accumulate much faster at enriched sites and therefore may slough before sufficient biomass has been achieved at low-nutrient sites. We recommend placing plenty of artificial substrata at the sites and sampling frequently throughout the colonization period to ensure the best characterizations of algal growth rates and peak biomass (see Stevenson 1996). Differences in grazing pressure or levels of nonnutrient stressors among wetlands may confound the relationship between biomass accumulation and nutrient availability.

Metabolism

Metabolic activities of algal assemblages are important wetland functions and therefore may be included in assessments. Photosynthesis (gross primary productivity), respiration, net primary productivity (photosynthesis-respiration), nutrient uptake, and phosphatase activity (PA) are commonly measured in ecological studies and are sensitive to environmental change (Bott et al. 1978, Healey and Hendzel 1979, Wetzel and Likens 1991, Marzolf et al. 1994, Hill et al. 1997, Young and Huryn 1998, Whitton et al. 1998). Phosphatase activity is highly sensitive to even slight changes in trophic status of P-limited oligotrophic habitats. Phosphatases are extracellular enzymes that cleave phosphate molecules from organic compounds, thereby making the P available to algal and other cells. Algal and bacterial cells excrete these enzymes in response to P deprivation; thus, PA levels in the water and periphyton of wetlands are inversely proportional to P availability (Newman et al. in press). Nitrogen deficiency also can be assessed by measuring the level of N fixation activity in plankton and periphyton samples. Heterocystous algae and some bacteria are capable of converting dinitrogen gas into ammonia through a series of enzymatic reactions. Through this same pathway, acetylene is converted into ethylene. Thus, the level of N-fixation activity can be assessed by measuring the rate at which acetylene, injected into a sealed vessel containing the water or periphyton sample, is converted to ethylene. Because cellular N-fixation is an energy-intensive process, generally it is only induced in response to intracellular N limitation. A gas chromatograph is required to measure the quantity of ethylene produced during this assay.

These techniques to measure metabolism rarely are incorporated into routine monitoring and survey work because they require more field time than typical water, phytoplankton, and periphyton sampling. In addition, they are related closely to biomass, which can vary substantially on a seasonal basis and in relation to weather-related disturbances. Thus, although metabolic attributes of algal assemblages are important attributes of wetlands, algal metabolism usually is not incorporated into environmental assessments until the later stages of more in-depth investigations.

Bioassay

For purposes of discussion such as this one, bioassays usually are defined as in-lab cultures 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, United States Environmental Protection Agency 1978, Trainor and Shubert 1973, Greene et al. 1976, Ghosh and Gaur 1990, McCormick et al. 1996). Samples are inoculated with known amounts of the test alga and incubated under controlled conditions to determine the biomass yield after 14 days. This yield, known as the algal growth potential (AGP), indicates the bioavailability of nutrients in the water sample. In a second assay, the Limiting Nutrient Algal Assay (LNAA), replicate water samples are spiked with different nutrients, either alone or in combination. The nutrient yielding the greatest amount of biomass is identified as being most limiting to the growth of this test population. The test alga used in these assays is by no means ubiquitous among wetlands, and certainly the response of this species to nutrient enrichment differs from that of many other algae. Furthermore, populations of algae within a wetland community may be limited by different nutrients. However, results from these simple tests often provide reliable predictions of wetland nutrient status and limitation (McCormick et al. 1996). Another advantage of these tests is that they can be standardized easily for use among wetlands that may contain different algal communities. Disadvantages include the effort required to establish and maintain testing and culture facilities.

Alternatively, bioassays can assess planktonic or benthic assemblages from reference or test sites

(rather than a standard test organism) and can be conducted under highly controlled laboratory conditions using waters from the studied habitats or else conducted in the field. Different dilution levels of effluents entering the regions under study or specific stressors (like phosphate) can be added to lab cultures or field mesocosms. Twist et al. (1997) introduced the novel approach of embedding test organisms in alginate (agarous substance) and culturing them in situ. Nutrient-diffusing substrata, microcosms, and mesocosms can be deployed at many scales in the field (e.g., Cotê 1983, Fairchild et al. 1985, Gensemer 1991, Hoagland et al. 1993, Lamberti and Steinman 1993, McCormick and O'Dell 1996, Pan et al. 2000). Most investigators tend to think that larger scale (bigger space and longer time) manipulations are more likely to reflect changes that occur in natural systems.

The response of organisms to bioassays can provide another valuable line of evidence for identifying causes of environmental stress. The results of bioassays where specific chemicals or effluents were 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). If changes in multiple algal assemblage attributes, particularly shifts in species composition, are similar among in situ, laboratory bioassays and along environmental gradients in the field, then strong evidence has been found to link causes of change in biological integrity in the field to the factors that were manipulated in the bioassays.

Field Methods

Sampling Present-Day Assemblages

Many algal habitats can be sampled within wetlands using standard methods for other habitats (Goldsborough and Robinson 1996, Goldsborough in press). Composite sampling is recommended to reduce variability in assessments resulting from spatial variability within wetlands. Composite sampling means gathering multiple collections (usually about five) from areas around the wetland and putting them into the same container. Composite samples all may be from the same habitat (plankton, plants, sediments) and thus are referred to as targeted habitat samples; or they may include subsamples from many habitats and thus are referred to as multihabitat samples.

In deep, open water wetlands, phytoplankton can be sampled with Van Dorn, Kemmerer, or similar discrete-depth samplers (APHA 1998). However, in most cases, whole-water phytoplankton samples can be collected by immersing a container. To avoid sampling particulate material suspended while an investigator enters the wetland, a small plastic container can be attached to the end of a pole (ca. 2 m long). 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 (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 to cut horizontal filaments that block the insertion of the tube through the metaphyton assemblage. Depth of the core should not extend to the substratum. Diameter of the core depends upon spatial variability of the metaphyton, on necessary sample size, and on ability to isolate the core of algae from surrounding metaphyton (Stevenson personal observation). Metaphyton in the form of unconsolidated

green clouds requires use of wider (ca. 10 cm) cores, because filaments are difficult to isolate in narrow cores and related edge-effect error is reduced with wider cores. Narrower cores (ca. 3 cm) can be used to sample consolidated microalgal mats. Metaphyton biomass should be expressed on an areal basis. Qualitative samples of metaphyton can be gathered with grabs, forceps, strainers, spoons, pipettes, 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). Epiphytes can be collected by cutting plants close to the sediment with scissors and placing them in a plastic bag with water. 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 with a brush, algae and water in the tube can be removed with suction pumps.

Artificial substrata, such as microscope slides or dowels made from glass, acrylic plastic, or wood, can be used to assess benthic algal assemblages in wetlands (McCormick et al. 1996). Dowels are particularly easy to use in wetlands because they only require sticking into sediments. If placed in the same light and depths, assemblages should be highly sensitive to water chemistry. Multiple samplings of substrata during colonization can be used to determine dispersal, growth rates, and peak biomasses of assemblages (Stevenson et al. 1991, Stevenson 1996).

Samples should be preserved for later processing as soon as possible. If chlorophyll *a* will be analyzed, however, samples cannot be preserved until subsamples have been extracted in the lab. During large survey projects when immediate sample processing is not practical, samples can be frozen to preserve them. Freezing disrupts only large cells with large-cell vacuoles, like *Spirogyra* or *Vaucheria*. Small cells like diatoms and cyanobacteria are not affected. Samples for AFDM and cell count assays can be preserved with many different preservatives. In most situations, M3 (a combination of formaldehyde, glacial acetic acid, and iodine; APHA 1998) is effective and reduces formaldehyde concentrations in samples and in the lab. If flagella of phytoplankton are important for identification, Lugol's preservative (APHA 1998) is recommended. Some caution should be exercised when fixing microorganisms. Double concentrations of preservative are recommended for phytoplankton when treating dense periphyton assemblages. Many artifacts can surface in the course of sampling in wetlands, which are home to a great diversity of organisms, not all of which will be equally well preserved (Klein Breteler 1985, Sherr and Sherr 1993). Artifacts can be detected by examining some samples immediately after sampling and before preservation. Artifacts can be standardized by using the same preservative.

Sampling Historic Assemblages

In wetlands with relatively undisturbed sediments, a chronological record of environmental conditions may have been stored by algae that settled to the bottom (Slate 1998, Slate and Stevenson 2000). Newer assemblages lie close to the surface, and older assemblages lie deep within the sediments. Algal assemblages in sediments may have accumulated for months, years, or centuries, depending on the depth and disturbance of sediments. A large variety of coring apparatuses have been used to retrieve sediment cores (Smol and Glew 1992). Telling the time of historic changes depends on the type of sectioning techniques and equipment used. Close-interval sectioning equipment and techniques (e.g., Glew 1988, 1991) can provide a high degree of temporal resolution.

A sediment core usually is removed from near the center of the wetland. In general, the central, flat portion of a basin collects cells from the broadest region and retains deposits best, and so a more holistic and complete record of past environmental change is archived there.

Sediments and diatom assemblages in them can be dated. ^{210}Pb often is used to date sediments (Oldfield and Appleby 1984), because it has a half-life of 22.26 years and occurs naturally. Dating with ^{210}Pb is reasonably accurate for about a century. Alternatively, paleolimnologists have sometimes used a "top/bottom" approach by removing surface sediment cores as they would in a detailed paleoenvironmental assessment. Instead of sectioning and analyzing the entire core, however, they simply analyze the top 1 cm of sediment (= present-day conditions) and a sediment level known to have been deposited before anthropogenic impact (i.e., the bottom sediment section). This approach has been used effectively to infer the amount of acidification occurring in lakes (Cumming et al. 1992, Dixit et al. 1999) and eutrophication (e.g., Dixit and Smol 1994, Hall and Smol 1992). The employment of diatom community composition, diatom autecological information, and paleoenvironmental approaches can imply if not indicate the background conditions of a system and provide mitigation targets for environmental remediation (Smol 1992).

Laboratory Methods

Laboratory procedures for most routine algal measures (e.g., chlorophyll, AFDM, cell identification and counts) have been standardized (e.g., APHA 1998, Stevenson and Bahls 1999). These methods should be followed whenever feasible so that comparability among studies is as great as possible. In this section, we set out the steps to determine algal attributes, and we provide references to standard methods.

Upon return of samples to the lab, fill out all sample chain of custody (COC) forms and check sample labels to ensure their adhesion. If samples need to be subsampled for multiple assays, homogenize them with a biohomogenizer (tissue homogenizer) in a beaker before subsampling. Subsampling can introduce error. Therefore, put homogenized samples on a magnetic stirrer and remove two or more aliquots of sample for each subsample to reduce measurement error.

Structural Attributes

Taxonomic composition

Taxonomic composition of algal assemblages requires microscopic examination of samples. The methods used for microscopic identification and counting of algae depend on the objectives of data analysis and type of sample. A two-step algal counting process is being used in many environmental programs. The first step is designed to characterize species composition of nondiatom algae in samples. This step is eliminated from projects in which only diatoms are analyzed. In this first step, count all algae and identify only nondiatom algae in a wet mount at 400X (e.g., Palmer cell). If many small algae occur in samples, count algae at 1000X with an inverted microscope (Lund et al. 1958) or with a regular microscope by drying samples onto a cover glass, inverting the sample onto a microscope slide in 0.020 mL of water, and sealing the sample by ringing the cover glass with fingernail polish or varnish (Stevenson and Bahls 1999). The second step is to count diatoms after oxidation of organic material out of diatoms and mounting them in a highly refractive mounting medium (e.g., Stevenson and Bahls 1999). This two-step technique provides the most complete taxonomic assessment of an algal assemblage. Counts of 300 algal cells, colonies, or filaments and about 500 diatom valves are the standard approach used in some national programs (Porter et al. 1993, Pan et al. 1996). Counting these numbers of cells usually provides relatively precise estimates of the relative

abundances of the dominant taxa in sample (with observation of 10 or more cells, colonies, or filaments of each taxon). Alternatively, counting rules can be 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 of 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). The numbers of species in diatom assemblages usually are sufficient to show a response, provide an indicator of algal biological integrity, and provide indicators of environmental stressors.

Taxonomic composition can be recorded as presence/absence, percent or proportional relative abundances, percent 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 levels of taxonomic scale and probably reflect a gradient "from least variable to most variable" on a temporal scale. Presence/absence records of species should be based on observations of thousands of cells and should reflect long-term changes in habitat conditions, especially if immigration and colonization of habitats regulates species membership in a sample. Relative abundance and biovolumes of taxa probably reflect recent habitat conditions more than long-term conditions because of recent species responses to their environment. Densities and biovolumes of taxa change daily, so absolute densities and biovolumes may be too sensitive to detect more long-term environmental changes. The relative abundance of cells more commonly is used as a metric than are relative biovolumes, but the latter are particularly valuable when cell sizes vary greatly among taxa within samples.

Biomass

Biomass of algal assemblages can be estimated with measurements of chl *a*, dry mass, ash-free dry mass, algal cell density, biovolume, or chemical mass of samples. All these measurements have pros and cons (see Stevenson 1996 for review), because none directly measures all constituents of algal biomass or only algal biomass. Chl *a* is first extracted from cells in acetone or methanol before measurement 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 fluorometric 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 counting cells 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).

Biomass also can be estimated rapidly with field assays, such as Secchi depth in the water column and percent cover and thickness of algal assemblages on substrata (Wetzel and Likens 1991). Assessments of algal biomass with Secchi depth can be confounded by suspended inorganic material and other factors (Preisendorfer 1986). The advantage of assessing benthic algal biomass with percent cover and thickness of algal assemblages is that biomass over a large area can be characterized readily (Holmes and Whitton 1981, Sheath and Burkholder 1985, Stevenson and Bahls 1999).

Chemical Composition

Periphyton chemical concentrations can be determined by collecting several (e.g., five) represen-

tative grab samples of the dominant community from different locations to account for spatial variation within a wetland. If an environmental gradient is known or suspected to exist within the wetland as a result of, for example, point-source discharges, then sites along this gradient should be sampled separately. Comparisons among wetlands or locations within a wetland should be done on a habitat-specific basis (e.g., metaphyton, epiphyton, epipelon), as habitat can affect periphyton chemical concentration. For example, in reference areas of the Everglades, epipelon phosphorus concentrations typically are twice those in the metaphyton (McCormick et al. 1998). Grab samples can be combined into a single composite sample to reduce analytical costs and then are frozen until processing. Samples are processed in the same manner as for macrophyte material to determine N and P content. N is determined by CHN and P is determined after digestion and oxidation to PO_4. Heavy metals are determined by atomic absorption after digestion or by other instruments (e.g., inductively couple mass spectrometry). Many other chemical contaminants also can be assayed in algal samples, such as toxic organics. Chemical contaminants usually are expressed on a dry-mass basis, which reflects the presence of contaminants in the organic (e.g., algal cells, detritus) and inorganic (e.g., precipitated Ca or sediment) fractions of samples. See Module 10: Using Vegetation to Assess Environmental Conditions in Wetlands, to compare with vegetation-based indicators.

Functional Attributes

Measuring gross and net productivity and respiration can be done in the field with light and dark chambers and changes in oxygen concentration if the water column is mixed, as is common in shallow wetlands (Bott et al. 1978, Wetzel and Likens 1991). Alternatively, productivity can be estimated with changes in oxygen concentration in the water during a diel sampling period or at two locations in a stream, if diffusion of oxygen from the water column is accounted for properly (Kelly et al. 1974, Marzolf et al. 1994, Young and Huryn 1998). Nutrient uptake can be measured as depletion of nutrients in closed chambers. Phosphatase is measured with water samples in the laboratory (Healey and Hendzel 1979).

QA/QC

Proper quality assurance/quality control (QA/QC) is essential, both to ensure that results are accurate and defensible and to quantify the degree of uncertainty associated with each measurement. In this regard, requirements for algal sampling protocols are no different than for any other assessment. Standard operating procedures (SOPs) that detail each sampling and processing procedure must be distributed to all personnel participating in the assessment process. One or more individuals within the assessment group must be designated as the QA/QC officer and be responsible for conducting routine audits of field and laboratory personnel to ensure compliance with SOPs. Deviations from SOPs resulting from unusual field events or conditions should be documented in writing, using a standard form. Chain of custody sheets should follow samples through the processing train; such documentation is important particularly if samples are to be transferred among laboratories. Written documentation, as just described, often is needed to recreate the sampling and analysis process, to account for missing data, and perhaps to explain anomalous results. This reconstruction process can be critical at the regulatory and litigation stage, which may occur several years after the samples were collected and key personnel have left the organization.

Collection of duplicate samples at a subset of all wetlands studied (e.g., 5-10% of samples) provides information on the amount of variation associated with field sampling procedures. Similarly, processing duplicate subsamples allows for laboratory varia-

tion to be quantified, as is done routinely for water chemistry samples. The amount of acceptable variability depends on the degree of resolution required. If variability is excessive, a search should be made for the causes (e.g., habitat heterogeneity, sample preparation, or taxonomic inconsistencies).

Taxonomic QA/QC requires the greatest effort and should include both photographic documentation and archived specimens of all identified taxa. The first step in taxonomic QA/QC is having a good taxonomic library. A table of taxonomic references can be found in Stevenson and Bahls (1999). Photographs provide a convenient means for comparing identifications among laboratory staff. However, microscopic examination of archived material often is necessary to confirm identifications. Diatom samples can be archived on microscope slides when mounted in most resin media, like NaprhaxÒ. Long-term archiving of preserved algal samples and cleaned diatom samples is possible by sealing containers with tape and wax to prevent evaporation. Channels of communication within and among laboratories (e.g., regularly scheduled lab meetings, Web sites that are updated regularly) must be formalized to ensure consistency in taxonomic identifications and nomenclature. These channels of communication should be developed early in the project and be maintained. Often, taxonomic workshops are held at regional and national meetings. Periodically, quantitative QA/QC determinations (e.g., laboratory staff counting the same fields of the same mount) should be performed to determine the degree of variability among counters.

Data Analysis

Objectives

The objectives of programs and specific steps in data analysis should be defined clearly. First, attributes of algal assemblages should be calculated, which may include the following: areal density of cells, pigments, or mass; relative abundances of taxa; diversity attributes; % of organisms or taxa within taxonomic, autecological, or functional group categories; species environmental optima and tolerances; and inferred environmental conditions based on species relative abundances. Early exploration of the data will involve multivariate analyses to assess correlation among environmental variables and major changes in algal attributes (see Lowe and Pan 1996 for discussion). Early metric development may include testing algal attributes for merit as metrics (see Module 6: Developing Metrics and Indexes of Biological Integrity) and delineating wetland classes to increase precision of metrics and ability to distinguish impairment (see Module 7: Wetlands Classification).

Data analysis during early stages of metric development can be simple. The main goal is to find wetland attributes that reliably change along with human disturbance (e.g., box plot analysis). During later stages of metric development, studies should be designed to describe stressor-response relationships more accurately so that criteria can be established for protecting valued ecological attributes (linear and nonlinear regression, change point analysis). Indicators and stressor-response relationships may vary among classes of wetlands; such variability should be evaluated.

Finally, projects should be established to monitor status and trends in wetlands, and data analysis should be used to determine whether sites or populations of sites meet specific criteria and how wetland condition changes over time. Each of these stages can be approached with a variety of data analyses to provide multiple lines of evidence on which management decisions can be based and benefits of corrective actions monitored. Although a complete review of these analyses is beyond the scope of this module, the following paragraphs detail the calculation of environmental optima and of autecological indicators of environmental conditions.

An overview of data analysis used in environmental programs is described briefly. Case studies following this section provide examples of how these steps of data analysis are integrated into environmental programs.

Environmental Optima and Autecological Indices

Environmental optima for taxa can be calculated with a very straightforward approach, if algal assemblages are collected from a range of environmental conditions and if taxa respond to those conditions (Zelinka and Marvin 1961, ter Braak and van Dam 1989). Computer programs have been developed especially to develop and test autecological indices of environmental conditions (Line et al. 1994, Juggins and ter Braak 1992). Often these calculations are preceded by a multivariate assessment of variability in species composition among assemblages and correlations between patterns in species composition and environmental factors (see review in Lowe and Pan 1996). Identifying environmental factors that are highly correlated with changes in species composition helps to narrow the selection of environmental factors that should be used to characterize species preferences. Environmental optima (Θ_{ik}) for each species are calculated as a weighted average of the relative abundance of species *i* in different habitats *j* (n_{ij}) and the k^{th} environmental factor (e_{jk}) in habitat *j*:

$$\Theta_{ik} = \Sigma_{i=1,S} n_{ij} e_{jk} / \Sigma_{i=1,S} n_{ij}$$

Environmental conditions in a habitat (EC_k) can then be inferred based on species autecologies (Θ_{ik}) and on relative abundances of species for which autecologies are known (n_{ij}):

$$EC_k = \Sigma_{i=1,S} n_{ij} \Theta_{ik} / \Sigma_{i=1,S} n_{ij}$$

Table 2: Calculation of total phosphorus optima for 4 diatom species with the total phosphorus concentrations and relative abundances (N_{IJ}) of 4 taxa at 10 sites

Site (J)	TP(µG/L)	Frsynegr		Masmithi		Nacryten		Niamphib	
		n_{ij}	TP* n_{ij}	n_{ij}	TP* n_{ij}	n_{ij}	TP* n_{ij}	n_{ij}	TP* n_{ij}
1	24.8	0.052	1.30	0.348	8.64	0.003	0.07	0.004	0.11
2	79.3	0.028	2.21	0.032	2.56	0.029	2.32	0.526	41.72
3	12.4	0.089	1.11	0.340	4.22	0.000	0.00	0.000	0.00
4	14.5	0.085	1.24	0.277	4.02	0.000	0.00	0.000	0.00
5	110.3	0.012	1.37	0.123	13.57	0.023	2.58	0.329	36.23
6	31.7	0.111	3.52	0.075	2.39	0.033	1.04	0.027	0.86
7	57.5	0.066	3.82	0.043	2.49	0.037	2.13	0.326	18.74
8	46.9	0.024	1.12	0.093	4.34	0.115	5.39	0.287	13.44
9	9.9	0.308	3.05	0.223	2.21	0.000	0.00	0.000	0.00
10	10.0	0.090	0.90	0.531	5.31	0.000	0.00	0.000	0.00
col. Sums (Σ)		0.867	19.63	2.087	49.74	0.240	13.53	1.499	111.09
Optima			22.65		23.84		56.32		74.14

Frsynegr = Fragilaria synegrotesca; Masmithi = Mastogloia smithii; Nacryten = Navicula cryptotenella; and Niamphib = Nitzschia amphibia.

The following example shows how these indices are calculated. In Table 2, the total phosphorus concentrations in which 4 taxa (indicated by 8 letter species codes) were found were recorded for 10 sites. The relative abundances (n_{ij}) were the proportions of diatom assemblages that these taxa represented at the 10 sites. The products of the phosphorus concentrations and relative abundances ($TP_* n_{ij}$) were calculated; for example, that product is 1.30 for *Fragilaria synegrotesca* at site 1 and 1.04 for *Navicula cryptotenella* at site 6. The sum of the relative abundances of each taxon at all sites (Σn_{ij}) and the sum of the P concentration/relative abundance products ($\Sigma TP_* n_{ij}$) were calculated and represent column sums (col. sums), for example, 0.867 and 19.63, respectively, for *F. synegrotesca*. The environmental optima then were calculated by dividing the sum of the P concentration/relative abundance products by the sum of relative abundances of each taxon, for example, 19.63/0.867=22.65. Based on these calculations, *F. synegrotesca* and *Mastogloia smithii* have lower total phosphorus optima than *Navicula cryptotenella* and *Nitzschia amphibia*.

In Table 3, inferred environmental conditions (i.e., total phosphorus concentration) were calculated with relative abundances (n_{ij}) and environmental optima (Θ_{ik}) for diatom taxa in samples from two sites. Products of species-relative abundances and environmental optima ($\Theta_* n_{ij}$) were calculated (e.g., 6.80 for *F. synegrotesca* at site 1) and summed for each sample, as were the relative abundances of species for which environmental optima are not known (n_{ij}*). If environmental optima are known for all taxa, $n_{ij} = n_{ij}$*. Inferred environmental conditions, (i.e., the TP concentrations in this example) at sites 1 and 2 were calculated as the sum of products of species-relative abundances and environmental optima ($\Sigma Q_* n_{ij}$) divided by the sum of species-relative abundances (Σn_{ij}*) for which environmental optima were known (e.g., 29.24=26.90/0.92 at site 1).

Developing and Testing Metrics

Developing and testing metrics for algae involve the same procedure as for other kinds of organisms (see Module 6: Developing Metrics and Indexes of

Table 3: Calculation of inferred total phosphorus concentration based on the relative abundances (N_{IJ}) of five taxa at two sites and known total phosphorus optima for four of the five taxa

Taxa	TP optima (µg/L)	Site 1				Site 2	
		n_{ij}	$Q^* n_{ij}$	n_{ij*}	n_{ij}	$Q^* n_{ij}$	n_{ij*}
Frsynegr	22.65	0.30	6.80	0.30	0.05	1.13	0.05
Masmithi	23.84	0.50	11.92	0.50	0.10	2.38	0.10
Nacryten	56.32	0.04	2.25	0.04	0.40	22.53	0.40
Niamphib	74.14	0.08	5.93	0.08	0.20	14.83	0.20
Syulna	na	0.08	na		0.25	na	
col. sums (Σ)			26.90	0.92		40.87	0.75
Inferred TP conc.				29.24			54.50

Frsynegr = Fragilaria synegrotesca; Masmithi = Mastogloia smithii; Nacryten = Navicula cryptotenella; and Niamphib = Nitzschia amphibia; and Sulna = Synedra ulna. na = not available. nij* indicates the relative abundances of taxa for which autecological optima are known.

Biological Integrity). Box plots and regression analysis can be used to relate algal attributes to human disturbance. Like other metrics, algal metrics, should be evaluated for their capability to distinguish impaired condition from reference condition (power) within a region and their applicability to different regions.

Indexes of biological integrity (IBIs) provide a means of summarizing complex multimetric results. These indices combine the responses of several metrics (e.g., taxonomic information, biomass, and growth measures) to derive a single number describing wetland condition. These indices provide a useful and objective means of summarizing complex ecological data and have been adopted for use by a number of states (USEPA 1996). As for any summary, these indices are most informative when presented in conjunction with the response of individual metrics. The development and application of IBIs to assessing aquatic ecosystem condition are discussed by Karr (1981) and Barbour et al. (1999) and described in Module 6. Algal IBIs currently are used and more are under development for assessing streams and rivers (Kentucky Division of Water 1993, Hill et al. 2000). No IBIs currently exist, however, for assessing algal condition in wetlands. Development of such is discussed by Stevenson (in press).

Developing Criteria

Assessing biological impacts requires some sort of threshold or criterion as to what is considered to be an unacceptable condition. Defining a wetland's condition in terms of biological integrity provides a response variable for assessment. Changes in habitat structure, productivity, and the functional groups within wetlands are gross changes in biological integrity that affect other organisms in wetlands. More subtle changes in algal species composition are a concern because they indicate a change in biological integrity of wetlands. Moreover, subtle changes in species composition also may indicate alteration of the fundamental environmental constraints that regulate microbial processes in wetlands.

Algal characteristics indicative of undisturbed and altered conditions have been identified for lakes and rivers, and, in many cases, they apply as indicators of conditions in wetlands as well. However, the point where integrity is impaired can be difficult to determine when metrics change gradually in response to enrichment. Abrupt changes in algal metrics along a gradient of human disturbance within or among wetlands provide relatively clear evidence of impairment. These abrupt changes often are most precisely indicated by changes in algal species composition. Significant changes along these gradients can be detected using statistical procedures such as change-point analysis (see case studies). Nonlinear responses along disturbance gradients, therefore, can provide a basis for establishing criteria if they are supported by an ecological basis for labeling a change as an impact. Indicators that change more linearly along disturbance gradients actually may be most valuable for assessing status and trends and detecting changes in ecosystems at low levels of human disturbance as well as at high levels.

Limitations of Current Knowledge– Research Needs

The basic tools exist to use algae in wetland assessments. However, greater precision and statistical power for detecting impairment can be achieved. Several factors could improve that effort. First, a more precise characterization of expected condition in wetlands would allow a more sensitive detection of impairment. That precise characterization depends on better classification of wet-

lands and selection of metrics. Still poorly understood is the scale of biological resolution involved, such as genus versus species level metrics or relative abundances versus presence/absence data.

Other limitations of current knowledge are related to predicting stressors, effects of changes in algal biological integrity on other organisms, and results of different wetland protection and remediation approaches. Although first principles of ecology will enable development of good predictions, little research has been done in wetlands relating algal assemblages and production to elements of wetland ecology. More basic research in the understanding of the role of algae in wetlands will enable more effective use of algal assessments and more successful management of wetlands.

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Case Study 1: Developing Algal Indicators of the Ecological Integrity of Maine Wetlands

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Project Objectives

This project was designed to:

- Develop sampling methods for algae and macroinvertebrates
- Develop biological criteria for Maine wetlands
- Diagnose stressors degrading wetlands

Project History

Maine DEP initiated the Casco Bay Watershed biological assessment project in 1998 and has completed 2 years of sampling. The Casco Bay study is a cooperative effort between Jeanne Difranco of Maine DEP and Jan Stevenson of Michigan State University. As of the January 2000 BAWWG conference, Jeanne Difranco and the Maine DEP staff have analyzed 1998 macroinvertebrate data and are processing the data from the summer 1999 season. Jan Stevenson also has completed 2 years of sampling for algae and is now developing algal protocols and metrics for Maine wetlands. In this presentation of the Maine case study, some of the results from the algal part of the project will be presented from the first sampling season.

Study Design

In 1998, the first sampling season, 20 wetlands were selected in the Casco Bay Watershed. All the wetlands are semi-permanent or permanent depressional wetlands and were selected based on six criteria: hydrologic regime, distribution of sites, landscape position, disturbance gradient, management significance, and accessibility. Six of the 30 wetlands were selected as minimally disturbed reference sites and the others range in condition from good to poor quality.

Sampling Methods: Algae

Quantitative and qualitative algae samples were gathered from the same 20 wetland sites as used for macroinvertebrate sampling. Algae from plants, sediments, and the water column were sampled from multiple sites within each wetland and composited into one sample for each habitat. In addition, a multihabitat sample was collected from each site in which algae on plants, on sediments, and suspended in the water were placed in the same container.

Four algal sample types were collected to determine which produced the best indicators. Samples were collected from the water column, plants, and sediments, and across the wetland as a multihabitat sample. Samples were examined microscopically to determine species numbers and relative abundances of different species in samples. Chlorophyll *a* in the water column was assessed as an indicator of algal biomass.

Among the tools used for sampling were scissors to clip plants off of the bottom substrate, a turkey baster to collect sediment samples, and a bottle on a short pole or a hand-held cup to collect water samples. For the multihabitat sample, doses from each sample were combined into one container.

Analytical Methods: Algae

Three disturbance indicators were used to evaluate responses of algal attributes to human alteration of wetlands: a land use indicator developed by Maine DEP, trophic status indicators (total Nitrogen, total Phosphorus, and chlorophyll *a*), and hydrologic and sewage chemicals (Ca, Na, Cl). A suite of algal attributes was compared to the disturbance indicators to determine which types of indicators responded. The algae indicators included biological integrity measures such as genus and species richness, Shannon diversity, and a number of taxa in different genera. European autoecological information (Van Dam et al. 1994) was used to determine environmental preferences for the taxa. Weighted-average autecological indices were calculated with species-relative abundances and their autecological characterizations to infer environmental stress as a consequence of low moisture, organic N, low oxygen, pH, salt, and nutrients. A preliminary analysis of the data is presented in which only a few of the many algal attributes are compared with human disturbance indicators of the 20 wetlands.

Lessons Learned

Diatoms from plants, sediments, and the water column provided similar numbers of metrics of biological integrity and indices of stressors. Attributes of diatom assemblages in multihabitat samples were not as well correlated with indicators of environmental gradients of human disturbance as were attributes in assemblages in samples from single habitats (Table CS-1). Only 55 of the possible 400 algal attributes of multihabitat samples were correlated with $r>0.30$, where as between 86 and 90 of the possible attributes of algae on plants, on sediments, and in the water column were correlated to the indicators of human disturbance.

Table CS-1. Number of times algal attributes were correlated (r>0.30) to 20 possible disturbance indicators for assemblages from each habitat

Habitat	Genera	S	N	S/N	H	Even	Rich	Poss
Water	1	1	2	2	2	5	1	n=20
Sediments	1	4	0	4	2	2	2	
Plants	5	4	4	4	3	2	4	
Multihabitat	1	1	5	2	2	4	1	
Sum	8	10	11	12	9	13	8	n=80

Habitat	Achn	Cymb	Euno	Navi	Nitz	Pinn		
Water	4	6	4	5	8	7		
Sediment	3	3	8	7	6	5		
Plants	1	6	5	2	6	5		
Multihabitat	4	3	2	2	5	2		
Sum	12	18	19	16	25	19		

Habitat	Moist	Org. N	O_2 Tol	pH	Salt	Org Tol	Trophic	Sum
Water	1	8	6	6	7	5	8	89
Sediment	1	7	3	6	7	7	8	86
Plants	0	8	7	4	6	7	7	90
Multihabitat	10	6	0	1	2	1	1	55
Sum	12	29	16	17	22	20	24	n=400

The table is broken into three parts, each with a heading and the sum of times over all habitats that each attribute was correlated to the 20 possible disturbance indicators (maximum = 80). The three panels are organized into: diversity metrics (Genera = number of genera, S = number of species, N = number of organisms in count, S/N = number of organisms, H = Shannon diversity, Even = Hurlburt's Evenness, Rich = theoretical species richness based on S and Even); species-based metrics (number of taxa of the diatom genera Achnanthes (Achn), Cymbella (Cymb), Eunotia (Euno), Navicula (Nav), Nitzschia (Nitz), and Pinnularia (Pinn); and autecological indices (moisture index, organic N index, low O_2 tolerance index, pH, salinity, organic pollution index, and trophic status index).

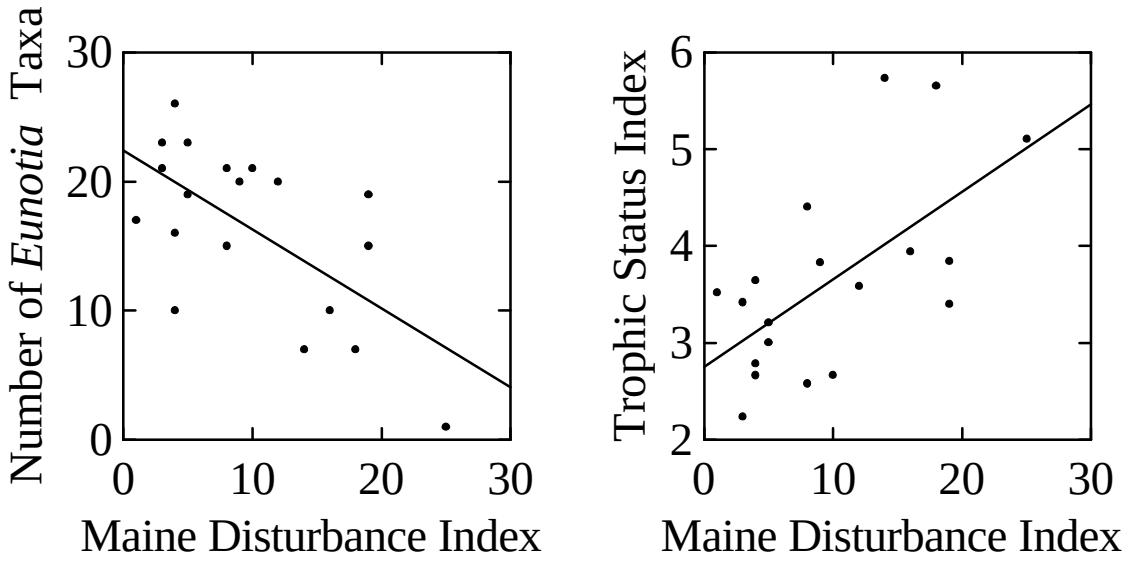

FIGURE CS-1. CHANGE IN NUMBER OF SPECIES IN THE DIATOM GENUS EUNOTIA AND THE TROPHIC STATUS AUTECOLOGICAL INDEX WITH INCREASING LEVELS OF THE MAINE DEP DISTURBANCE INDEX.

Metrics based on the number of taxa in common genera and on autecological characteristics of species were more highly correlated with indicators of human disturbance than diversity characteristics of diatom assemblages. Indices based on autecological characteristics of diatom species were slightly more reliable than genus- based metrics (Figure CS-1), even though those characteristics were based on autecological characterizations for European populations of the same species. We expect that these autecological indices will improve when environmental preferences are based on distributions of regional populations.

To better understand how to characterize gradients of human disturbance, the number of algal attributes that correlated with r>0.30 to each indicator of human disturbance was determined. In general, conservative ions such as Cl, Na, and Ca correlated more highly to changes in diatom assemblages (Figure CS-2) than did nutrient concentrations and field-based indicators of disturbance (e.g., Maine DEP Disturbance Index). Of the field-based indicators of human disturbance, algae related most to the percent of impervious surface and nonpoint source pollution, with 39 out of 80 algal attributes in the 4 habitats being related for the latter disturbance indicators (Table CS-2). These numbers actually were similar to the 31–45 range of correlations between disturbance and algal attributes for the chemical, general-disturbance indicators. Note that algae responded more to direct indicators of individual stressors rather than to a cumulative summary index of human disturbance based on field assessments. Total phosphorus correlated more to algal attributes than did total nitrogen or chlorophyll *a* in the water column.

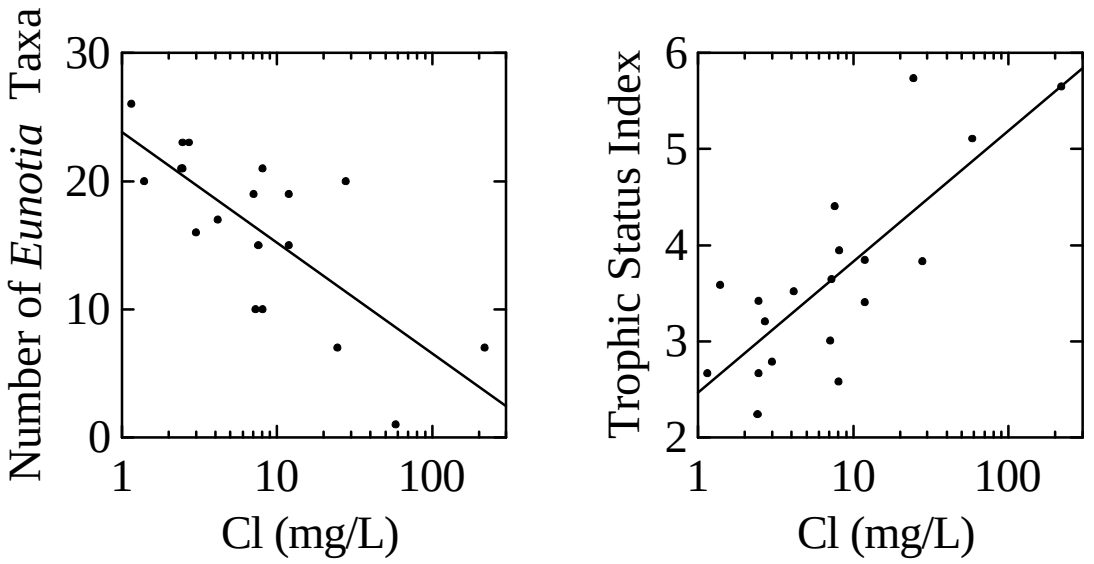

FIGURE CS-2: CHANGE IN NUMBER OF SPECIES IN THE DIATOM GENUS EUNOTIA AND THE TROPHIC STATUS AUTECOLOGICAL INDEX WITH INCREASING LEVELS OF CHLORIDE CONCENTRATION.

Table CS-2. Number of times attributes of human disturbance correlated (r>0.30) to 20 possible algal attributes for algal assemblages from each habitat

Habitat	Hydro	Veg	Imperv	NPS	Cum
Water	5	2	14	10	10
Sediment	5	1	12	9	8
Plants	1	2	3	14	9
Multihabitat	4	6	10	6	4
Sum	**15**	**11**	**39**	**39**	**31**

Habitat	TN	TP	Chl a
Water	4	9	2
Sediment	2	11	2
Plants	5	5	3
Multihabitat	5	1	3
Sum	**16**	**26**	**10**

Habitat	Ca	Na	Cl
Water	10	12	10
Sediment	13	13	10
Plants	5	15	13
Multihabitat	3	5	5
Sum	**31**	**45**	**38**

The table is broken into three parts, each with a heading and the sum of times over all habitats that each attribute of human disturbance correlated to the 20 possible algal indicators (maximum = 80). The three panels are organized into land use indicators based on field assessments by Maine DEP (hydrologic disturbance, vegetative disturbance, percent impervious surface, nonpoint source pollution, and cumulative index of all four disturbance types); trophic status indicators, total phosphorus, total nitrogen, and chlorophyll *a*; and general human disturbance indicators as concentrations of calcium, sodium, and chloride.

Case Study 2: Florida Everglades

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Purpose of Project

This project was initiated to monitor biological assemblages across a nutrient gradient in the Florida Everglades in support of regulatory efforts to define a numeric water quality criterion for phosphorus. The goal is protection of natural populations of aquatic flora and fauna in the Everglades Protection Area.

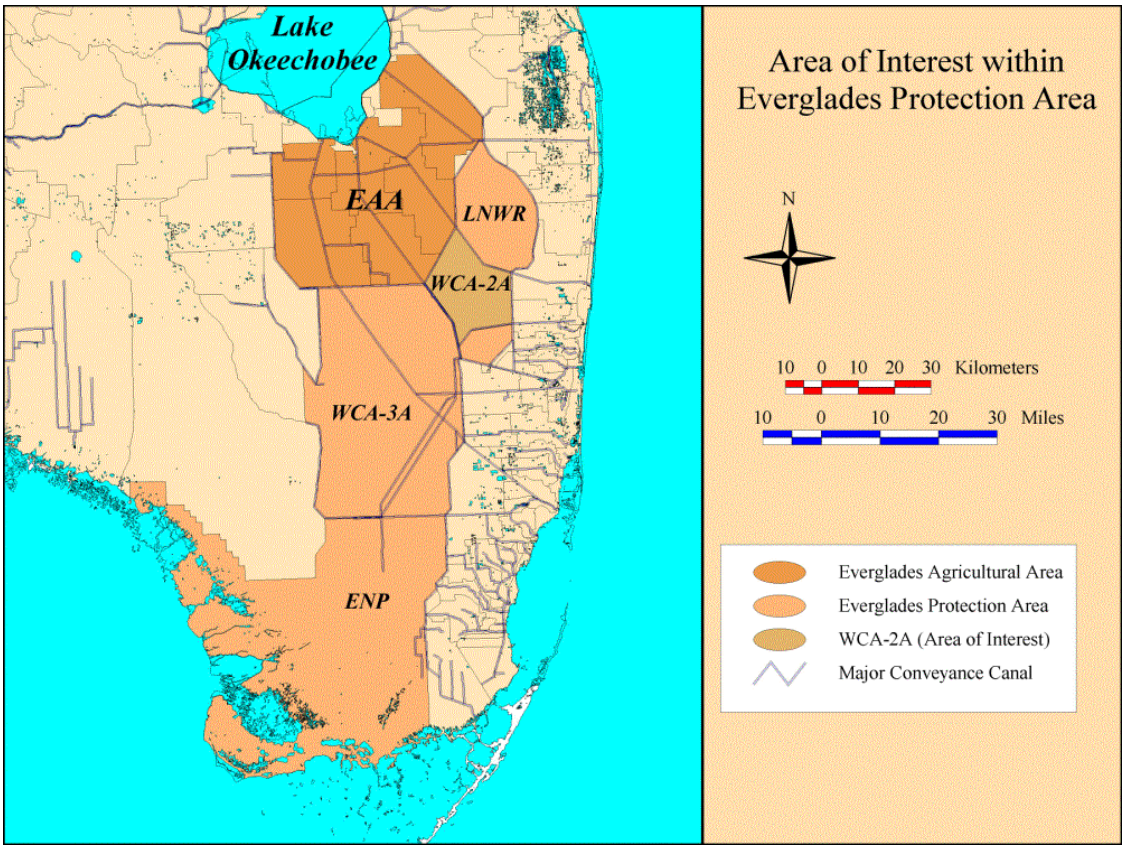
Project History

The historic Florida Everglades consisted of approximately 4 million acres of shallow sawgrass marsh, with wet prairies and aquatic sloughs interspersed with tree islands. Today, only 50 percent of the original Everglades ecosystem remains, primarily as a result of drainage and conversion of large portions of the northern and eastern Everglades to agricultural or urban land use. The remaining portions of the historic Everglades are located in the Water Conservation Areas (WCAs) and Everglades National Park (ENP).

The Everglades ecosystem evolved under extremely low phosphorus concentrations and is considered an oligotrophic ecosystem. A large body of evidence indicates that phosphorus is the primary limiting nutrient throughout the remaining Everglades. The introduction of excess phosphorus into the Everglades has resulted in ecological changes over large areas of the marsh. The Everglades Forever Act (EFA; Section 373.4592, Florida Statutes), passed by the Florida Legislature in 1994, stated that waters flowing into the part of the remnant Everglades known as the Everglades Protection Area (defined as Water Conservation Areas 1, 2A, 2B, 3A, 3B and ENP) contain excessive levels of phosphorus and that a reduction in levels of phosphorus will benefit the ecology of the Everglades Protection Area. The EFA requires the Florida Department of Environmental Protection (FDEP) and the South Florida Water Management District (SFWMD) to complete research necessary to establish a numeric phosphorus criterion for the Everglades Protection Area.

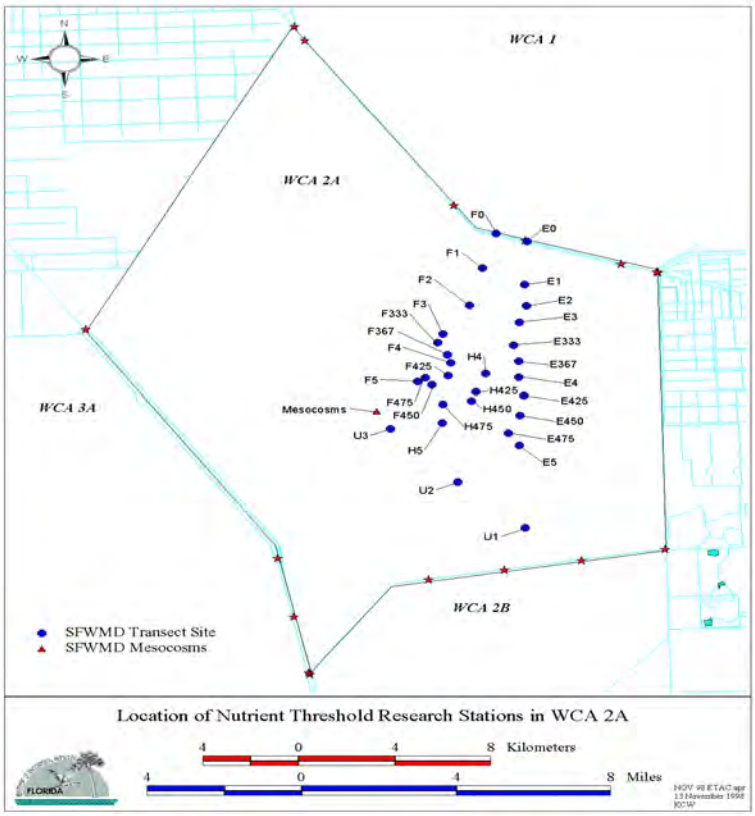
The SFWMD Everglades System Research Division (ESRD) initiated a succession of studies, beginning in 1993 and continuing to the present, as part of the research and monitoring being conducted in the

Everglades for the purposes of phosphorus criterion development. Biological monitoring for the ESRD studies was initiated in early 1994 in WCA 2A. Data from this and other studies are being used by FDEP in the development of a numeric phosphorus criterion for the Everglades Protection Area.



Study Design

SFWMD ESRD initially selected 13 sites along two transects located downstream of canals discharging into WCA 2A and extending down the phosphorus gradient into least affected areas of the marsh. Sampling sites ranged from the canal inflows (discharge structures on the north-eastern margin of WCA 2A) to a site nearly 15 km downstream from the canal inflows. Three of the 13 main sites specifically were chosen to represent the least affected area of WCA 2A with respect to anthropogenic disturbance (sites U1-U3). A series of 15 additional "intermediate" sites were added to the study later to obtain better spatial coverage of the lower portion of the transects. The sites have been monitored for water, sediment, and biological quality.



Assemblages Monitored

- Algae (phytoplankton and periphyton)
- Macroinvertebrates
- Macrophytes

Sampling Methods: Algae

- Water Bottles—phytoplankton samples initially were collected monthly and later were collected quarterly using water bottles. Samples were preserved in the field and sent to the FDEP Central Biology Laboratory for taxonomic identification.

- Diatometers—racks each containing six glass diatometer slides were deployed quarterly at each site. It was determined that an 8-week period of deployment was necessary to allow for sufficient periphyton growth. Diatometers were collected and preserved and sent to the FDEP Central Biology Laboratory for processing and taxonomic identification.

- Natural Substrate (benthic)—samples of benthic periphyton were collected from surficial sediment cores at the main transect sites on several occasions. Samples were retained by SFWMD ESRD for processing and taxonomic identification.

Sampling Methods: Macroinvertebrates

- Dipnet—SFWMD staff conducted quarterly macroinvertebrate sampling using a standard D-frame dipnet with a 30-mesh bag from September 1994, through November 1995. The sampling method consisted of the collection of twenty 0.5-meter (in length) discrete dipnet sweeps from representative habitats in the area of each site on a given sampling date. The 20 dipnet sweeps for a given site were combined and sent to the FDEP Central Biology Laboratory for processing and taxonomic identification.

- Quan Net—Beginning in May 1996, SFWMD staff conducted quarterly macroinvertebrate sampling using the Quan Net method. The sampling method consisted of the deployment of a 1 m^2 frame at the site, and the collection of net samples and all vegetation within the area of the frame. Frames were deployed in each of several representative habitats, where present, in the vicinity of each site. Samples from each site/habitat were kept separate. Representative habitats were labeled as cattail, sawgrass, or slough, depending on the predominant vegetation type. The collected material from each site/habitat was subsampled, preserved, and sent to the FDEP Central Biology Laboratory for processing and taxonomic identification.

- Hester-Dendy—SFWMD staff deployed Hester-Dendy artificial substrate samplers at each of the main transect sites on a quarterly basis. The samplers were deployed for a 1-month period, after which they were collected, preserved, and sent to the FDEP Central Biology Laboratory for processing and taxonomic identification.

Sampling Methods: Macrophytes

- Macrophyte Stem Density and Frequency—In April 1997, SFWMD staff conducted a study of macrophytes at the WCA 2A transect sites. A 50-meter tape was laid out at each transect site. A 1-meter square frame was used every 2 meters along the tape to delineate the sample area for calculation of macrophyte stem densities (stems/m^2) and frequencies (# plots where a species was found/total # of plots) by species.

- Macrophyte Harvesting—On the other side of the 50-meter tape used for establishing stem densities and frequencies, SFWMD staff harvested macrophytes for biomass measurements, using the 1-meter square frame at five predetermined locations to mark the sample area for harvesting.

Analytical Methods: Algae

- Water Bottles—Samples were processed and enumerated by FDEP Central Biology Laboratory staff according to FDEP SOPs (e.g., AB-04 and AB-05; available at *http://www.dep.state.fl.us/labs/sops.htm*). Analyses from this and other studies have indicated that Everglades phytoplankton are largely periphyton that has sloughed off into the water column. Thus, algal data analysis was focused on the periphyton data.

- Diatometers—Samples were processed and enumerated by FDEP Central Biology Laboratory staff according to FDEP SOPs (*e.g.*, AB-02, AB-02.1, AB-02.2, and AB-03; available at *http://www.dep.state.fl.us/labs/sops.htm*).

- Natural Substrate (benthic)— SFWMD processed and enumerated natural substrate samples.

Analytical Methods: Macroinvertebrates

- Dipnet and Quan Net—FDEP Central Biology Laboratory staff subsampled the dipnet and quan net samples from each site and analyzed them according to FDEP SOPs (e.g., IZ-02 and IZ-06; available at *http://www.dep.state.fl.us/labs/sops.htm*).

- Hester Dendy—FDEP Central Biology Laboratory staff processed and analyzed the Hester-Dendy samples from each site according to FDEP SOPs (e.g., IZ-03 and IZ-06; available at *http://www.dep.state.fl.us/labs/sops.htm).*

Analytical Methods: Macrophytes

- Macrophyte Stem Density and Frequency—Stem densities (stems/m^2) and frequencies (# plots where a species was found/total # of plots) by species were counted at each site.

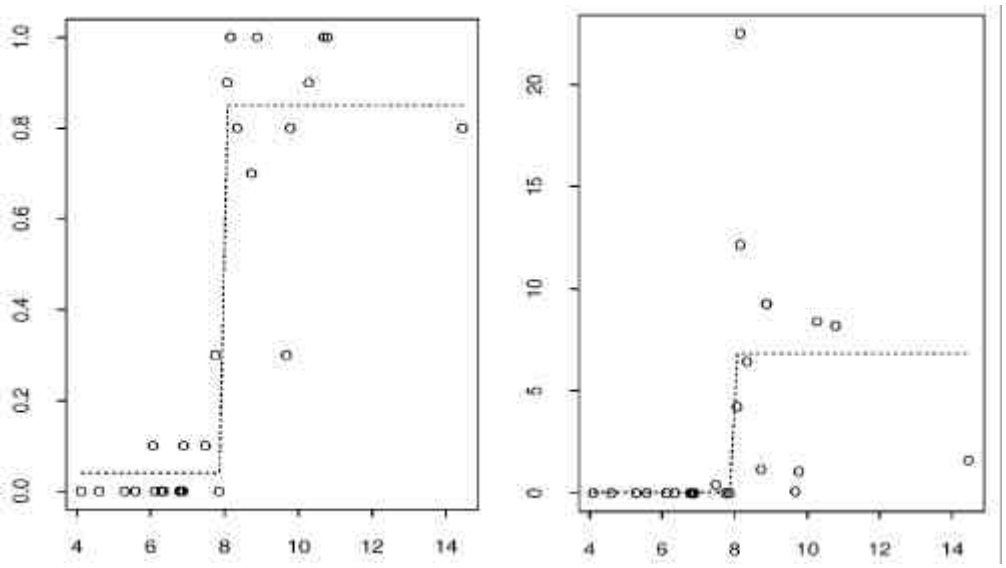
- Macrophyte Harvesting—SFWMD staff conducted biomass analysis of the harvested macrophytes for comparison of the relative biomass of several species present at each of the WCA 2A transect sites (e.g., *Eleocharis*, *Nymphaea*, *Typha*).

Lessons Learned

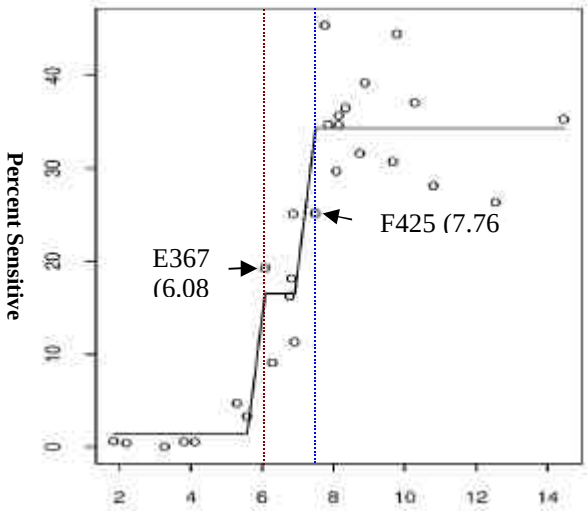
Periphyton, macroinvertebrate, and macrophyte communities in WCA 2A change substantially from reference conditions at approximately 7 to 8 km downstream of canal discharges into WCA 2A (see graphs below). Data analysis has shown that biological populations at the two stations (E5 and F5) nearest to the three initial reference sites (U1-U3) are very similar in terms of biological community structure. This analysis suggests that these areas, despite slight phosphorus enrichment, still support reference condition biota. The somewhat higher phosphorus regime at the next stations (E4 and F4 and beyond) are associated with greater biological changes. Experimental field dosing studies (mesocosms) have been conducted by SFWMD ESRD, which show that the addition of phosphorus causes changes in periphyton assemblages consistent with those observed in the transect study.

The WCA 2A transect periphyton data for each site/date have been analyzed using the entire taxonomic assemblage encountered and using lists of pollution- sensitive and tolerant species based on available literature and based on experimental phosphorus addition studies (the mesocosms) in WCA 2A. Macroinvertebrate data have been analyzed using the Florida Index and the macroinvertebrate component of the Lake Condition Index (LCI), measures of the numbers of pollution-sensitive taxa in a sample that

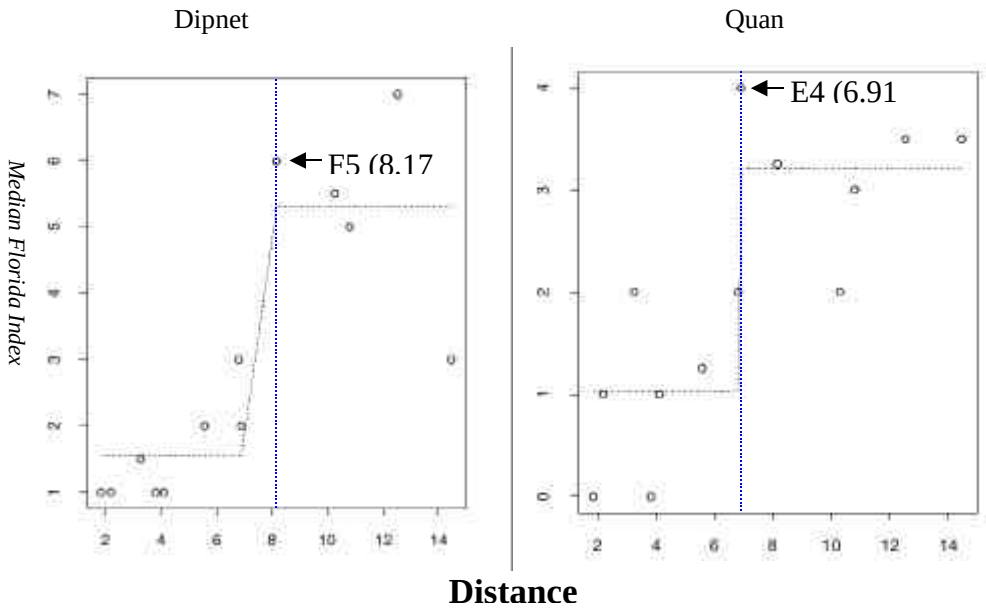
are routinely used by FDEP in bioassessments of streams and lakes. The use of these methods with the WCA 2A transect data has demonstrated a clear signal of biological disturbance along the nutrient gradient in WCA 2A. FDEP is using this information as well as information from other studies conducted in the Florida Everglades to develop a numeric phosphorus criterion for the Everglades Protection Area.



CHANGE POINT ANALYSES OF ELEOCHARIS FREQUENCY OF OCCURRENCE AND BIOMASS DATA ALONG THE SFWMD TRANSECTS. COLLECTED APRIL 1997.



RESULTS OF CHANGE POINT ANALYSES PERFORMED ON MEDIAN TOTAL PERCENTAGE OF POLLUTION-SENSITIVE (LITERATURE DETERMINED) PERIPHYTON TAXA.



RESULTS OF CHANGE POINT ANALYSES ON MEDIAN FLORIDA INDEX (MACROINVERTEBRATE) VALUES

ADDITIONAL COMMENTS

The information provided here is based solely on the transect study by SFWMD ESRD in WCA 2A. Research and monitoring of Florida Everglades water, sediment, and biological quality are being conducted by several research groups in WCA 2A, WCA 1 (Arthur R. Marshall Loxahatchee National Wildlife Refuge), Everglades National Park (ENP), and WCA 3A, including studies by SFWMD ESRD similar to the WCA 2A transect study.