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ASSESSING WETLANDS AND THEIR RESTORATION USING ALGAE

By

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ABSTRACT

ASSESSING WETLANDS AND THEIR RESTORATION USING ALGAE

By

Lei Zheng

This dissertation develops a better understanding of relationship among a variety of ecological factors within wetlands, evaluates a number of valuable ecological attributes to develop indicators for wetland bioassessment and wetland restoration, and establishes biological and environmental criteria to protect wetland ecosystems. Ecological assessment in wetlands has to develop habitat or class specific attributes to reduce the spatial and temporal variability. I first evaluated spatial and temporal variation of environmental and algal attributes among habitats within wetlands and between restored and extant wetlands in Michigan. The results indicated non-diatom algal assemblages differed among habitats, but sediment and epiphytic diatom assemblages were not significantly different. Epiphytic assemblages in the restored and extant wetlands were relatively distinct, while both sediment and phytoplankton assemblages were not as different between restored and extant wetlands. The differences in algal assemblages among habitats within wetlands and between restored and extant wetlands were related to a number of environmental characteristics, but particularly nutrients and sediment organic matter. In my study of salt marsh restoration, organic matter in sediments and the similarity of diatom species composition between paired restored and reference salt marshes increased with age of restored marshes during spring and summer. Primary production by epiphytic and sediment algae in summer showed site-specific changes and did not change consistently with marsh age. In this study, pairwise comparison of a

restored marsh and a nearby reference marsh reduced regional variation among wetlands and helped identify the successional pattern of ecological development after restoration. Because nutrients were one of the most important factors regulating periphyton growth and species composition in wetlands, experimental and field surveys were conducted to determine the limiting factors in wetlands. Experiments indicated that algae were nitrogen limited and limitation was directly related to nutrient concentrations and nutrient ratios. Although individual diatom taxa responded to nutrient additions differently, the responses of indicator taxa in the nutrient bioassays were positively correlated with natural nutrient gradients among natural wetlands indicating variation in species composition among restored wetlands was related to N-limitation. Based on the establishment of a cause-effect and stressor-response relationships between nutrient stressors and algal attributes, I evaluated the response of a number of valued ecological attributes to nutrient stressors. The similarity in species composition to reference conditions and the number of native species decreased and trophic index calculated with species composition increased with increasing nutrient concentrations. Diatoms as indicators of wetland nutrient pollution and human disturbance were developed using paleoecological diatom assemblages to characterize reference conditions. Biological criterion was then established based on the historic trophic status indicated by diatom species composition, and a nutrient criterion to protect or restore the biological integrity of wetlands was then established from the relationship between trophic status index and nutrients and using the predicted nutrient concentration. My studies indicated that algal assemblages were valuable indicators for assessing biological condition of wetlands, monitoring restoration, and diagnosing stressors.

This thesis is dedicated to

My wife

Jingfang Chen

And

My parents

For their infinite love and constant inspiration

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I would like to thank my wife Jenny Chen for her support and love during the dissertation writing. Her encouragement made this dissertation possible. My parents receive my deepest gratitude and love for their dedication and many years of support.

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Chapter 1

Algae as indicators of biological integrity in restored and extant wetland ecosystems

1.1 Wetland bioassessment

Wetlands have suffered serious impairment and loss since European settlement in North America. Approximately 53% of the wetlands in the United States have been lost since the late 1700s (Mitsch and Gosselink 1999), and a large proportion of wetlands have been identified as impaired by various pollutants such as nutrients and other environmental stressors. The U.S. Congress passed the Clean Water Act (CWA) in 1972 to preserve and improve the quality of the nation's water resources, including wetlands. Over the past 30 years, wetland mitigation has been increasingly used to counteract loss of wetland habitat and the associated ecosystem functions. As a result, thousands of wetlands have been restored and created to fulfill the goal of the "no-net-loss" policy (Section 307 of the Water Resources Development Act). However, even for the elementary purpose of maintaining water quality, the success rates of wetland mitigation have been very low (USEPA 2000).

To attain the stated goal of the CWA to " restore and maintain physical, chemical, and biological integrity" of wetlands, a set of structural and functional criteria for extant and restored wetlands needs to be developed and evaluated. Traditional wetland assessment techniques evaluate physical and chemical conditions, especially chemical influences from point-source discharges. Increased human activities have contributed to nonpoint source runoff, introduced species, and other anthropogenic impacts that have imposed increasing damage to wetland ecosystems. New approaches based on structural and

functional assessment of biological communities have been developed during the last two decades. Two of the most common approaches for assessing wetlands are the Hydrogeomorphic (HGM) approach, which focuses on functional assessment (Hauer & Smith 1998), and the Index of Biotic Integrity (IBI) approach of bioassessment (Danielson 2001). The HGM functional assessment approach is based on the hydorgeomorphic concepts for classifying wetlands and was specifically developed for rapid wetland assessments in response to the requirements of "no overall net-loss" of wetland area and functions (Conservation Foundation, 1988). In contrast, the IBI approach of bioassessment was largely developed to meet water quality and biocriteria requirements and is based on the conventional Cowardin et al. classification systems (Cowardin et al. 1978). These two approaches both contrast and complement each other and both contribute to assessment of wetland structure and function (Stevenson and Hauer 2002).

In the past 20 years, biological assessment has been developed to evaluate the integrity of aquatic systems. Biological integrity is a comprehensive reflection of physical and chemical factors and is defined as "the ability of an aquatic ecosystem to support and maintain a balanced, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of natural habitats within a region" (Karr and Dudley 1981). By evaluating the condition of one taxonomic community relative to physical and chemical conditions across a range of sites, biological assessment can identify stressors that impair ecological health and also diagnose the cause of environmental impairment. Bioassessment can also be used to evaluate wetland restoration and changes in ecosystem functions over time.

Evaluation of wetland restoration and wetland impairment involves assessment of nutrient enrichment at multiple spatial and temporal scales and how they affect biological communities. Numerous studies have documented wetland structural and functional changes after restoration (Middleton 1999; Streever 1999). Most of these studies have focused on changes in biogeochemistry, macrophyte community structure, and invertebrate species composition. It is the general expectation that restored wetlands should increase in species diversity, nutrient retention, systems sustainability, and productivity over time. The direct impact of nutrient accrual and recycling during wetland development is a major regulator of wetland structure and function.

1.2 Algal indicators

As pointed out by Landis and McLaughlin (2000), indicators have three basic purposes: to measure current status, to predict future changes in the system, and to predict progress toward the restoration of a system. The most common use of indicators is to establish the current status of a system.

Algae are one of the most sensitive indicators for biological assessment of aquatic systems. The importance of algal assemblages and function has been summarized in a numbers of reviews (Dixit et al. 1992, Stevenson and Pan 1999, Stevenson 2001). Algal characteristics that contribute to their effectiveness in assessment, include great diversity, presence in almost all aquatic ecosystems, sensitivity to chemical and physical changes, and taxon-specific responses to environmental changes (Shubert 1984, Steneck & Dethier 1994, Stoermer 1990). Environmental stressors could lead to degradation of valued ecological attributes and thus the sensitive response of algal assemblages could be useful indicators of environmental degradation (Figure 1). Over the past several decades,

evaluation of streams and lakes using algal attributes has proved successful (Lowe & Pan 1996). Although the biological communities and the underlying biogeochemical processes of wetlands are more complex than streams and lakes, the general approaches and methods for streams and lakes assessment can also be applied to wetland systems (McCormick and Stevenson 1998, Danielson 2001). Attempts to evaluate environmental gradients using algal attributes have been successful in Kentucky wetlands and the Everglades (Pan et al. 1996, McCormick 1998, Pan et al. 2000) and have proved to be successful.

1.3 Wetland biogeochemistry and primary producers

Wetland systems have complicated biogeochemical characteristics that vary both within and among different types of wetlands (Odum 1988, Mitsch and Gosselink 2000). Salt marshes differ from freshwater marshes because they receive chemicals from both oceanic and freshwater input. Anaerobic decomposition is predominated by sulfur reduction and fermentation in salt marshes. Large amounts of sulfur gases and CO_2, low amounts of methane, and unknown amounts of nitrous oxides are the main biogenic gases. Nitrogen is the major limiting factor for primary production in salt marsh ecosystems, while phosphorus is accumulated. Salt marshes have been shown to be both sources and sinks of nutrients, particularly nitrogen. The general model of salt marsh nutrient flux suggests that salt marshes act primarily as transformers of nutrients by importing dissolved oxidized inorganic forms and exporting dissolved and particulate reduced forms (ammonium, organic nitrogen, and phosphorous compounds). Salt marshes tend to be a net importer of nutrients during spring and summer and a net exporter in the autumn and winter (Odum 1988).

The water and soil chemistry of freshwater marshes is dominated by a combination of factors associated with the underlying mineral soils, overlain with autochthonous inputs of organic matter from vegetation production (Mitsch and Gosselink 2000). The inflowing water has high amounts of dissolved materials and nutrients, derived from stream, groundwater, and surface water inflow. Organic matter in freshwater marshes can vary widely, but is generally higher compared to salt marshes (Mitsch and Gosselink 2000). Dissolved inorganic nitrogen and phosphorus vary seasonally from very low concentration in the summer to high concentration in the winter. Thus, nutrient limitation varies seasonally. Sulfur reduction is an important anaerobic decomposition process, but is of less significance in freshwater marshes than in salt marshes. Anaerobic decomposition is predominated by methanogenesis and fermentation in freshwater marshes. Large amounts of methane and CO_2, small amounts of sulfur gases, and unknown magnitude of nitrous oxides are the main biogenic gases. Most studies show that freshwater wetlands are nutrient sinks and transformers. Nitrogen and phosphorus are generally limiting factors and are probably related to each other (Mitsch and Gosselink 2000).

Both salt marshes and inland freshwater wetlands are among the most productive ecosystems in the world. Marsh grasses, periphyton, and phytoplankton compose the three major autotrophic units in salt marshes (Odum 1988). Periphyton production can be as high as or higher than that of the macrophyte community's production. Vascular plant communities are always dominated by a few species, whereas algal species attain high species diversity and production. Freshwater marshes, on the contrary, have a much higher diversity of vascular plants but a relatively lower diversity of algae than salt

marshes (Odum 1988). The plant productivity of inland marshes can be as high as salt marshes (Mitsch and Gosselink 2000).

1.4 Objectives of the dissertation

The central goal of this dissertation was to apply algal biological assessment approaches that are broadly used in lakes and streams to evaluate ecological health of wetlands and to assess wetland restoration. Based on this objective, my research focused on how algal assemblages in both coastal salt marshes and inland freshwater wetlands respond to environmental changes and human disturbance. I conducted both field observations and experiments to delineate the pattern of algal responses along human disturbance gradients, especially nutrient gradients. I evaluated algal indicators for wetland nutrient enrichment and wetland restoration in both coastal salt marshes and freshwater wetlands. Both simple statistics and multivariate approaches were used to identify, develop, evaluate, and integrate a suite of algal indicators of ecological condition. Assessment of ecological conditions in least disturbed conditions and stressor-response relationships were used for development of biological criteria and corresponding stressor criteria for environmental monitoring and management (Fig. 1).

This dissertation includes the following major components as separate chapters:

Part I (chapter 2): Freshwater wetland restoration was evaluated by: examining the difference of environmental variables and algal assemblages between restored and extant wetlands; identifying what environmental variables causing the difference in algal assemblages between restored and extant wetlands; and comparing algal assemblages among different habitats and how similarities among habitats change with change of environmental conditions.

Part II (chapter 3): The success of salt marsh restoration was assessed by: using the reference approach and pairing a restored wetland with a nearby extant wetland to minimize regional difference among wetlands; evaluating a suite of algal attributes and finding the best indicators for salt marsh restoration; and evaluating the structural and functional development of wetland ecosystem development over time.

Part III (chapter 4): Nutrient enrichment experiments were conducted in a set of restored freshwater wetlands to determine: nutrient limitation, how algae respond to nutrient enrichment in artificial habitats, and how these responses are different or similar in experimental examination compared to natural environmental gradients, and thus establish indicators based on cause-effect relationship for wetland bioassessment.

Part IV (chapter 5): A set of wetlands across Michigan were used to: determine stresses and stressor gradients through diatom ecological response in wetland ecosystems; investigate how these stressors are related to human disturbance gradients based on diatom ecological responses; and set biological and stressor criteria to protect restored wetlands.

The ultimate goal of this research was to provide new tools for diagnosing and quantifying risks from pollution that threaten wetland ecosystems. The results of my research should support ecologically sensitive decision-making in land planning and wetland restoration. Specifically, I was able to define ecological thresholds based on stressor-response relationships and reference approaches. These results can be applied for watershed management by providing targets for decreasing anthropogenic pollution and thereby increasing the rate of successful restoration projects. I also developed easy and rapid biotic and abiotic indicators that serve as qualitative and quantitative criteria to

assess water quality and ecosystem health. These studies help to identify ecosystems at risk due to excess nutrients in the environment.

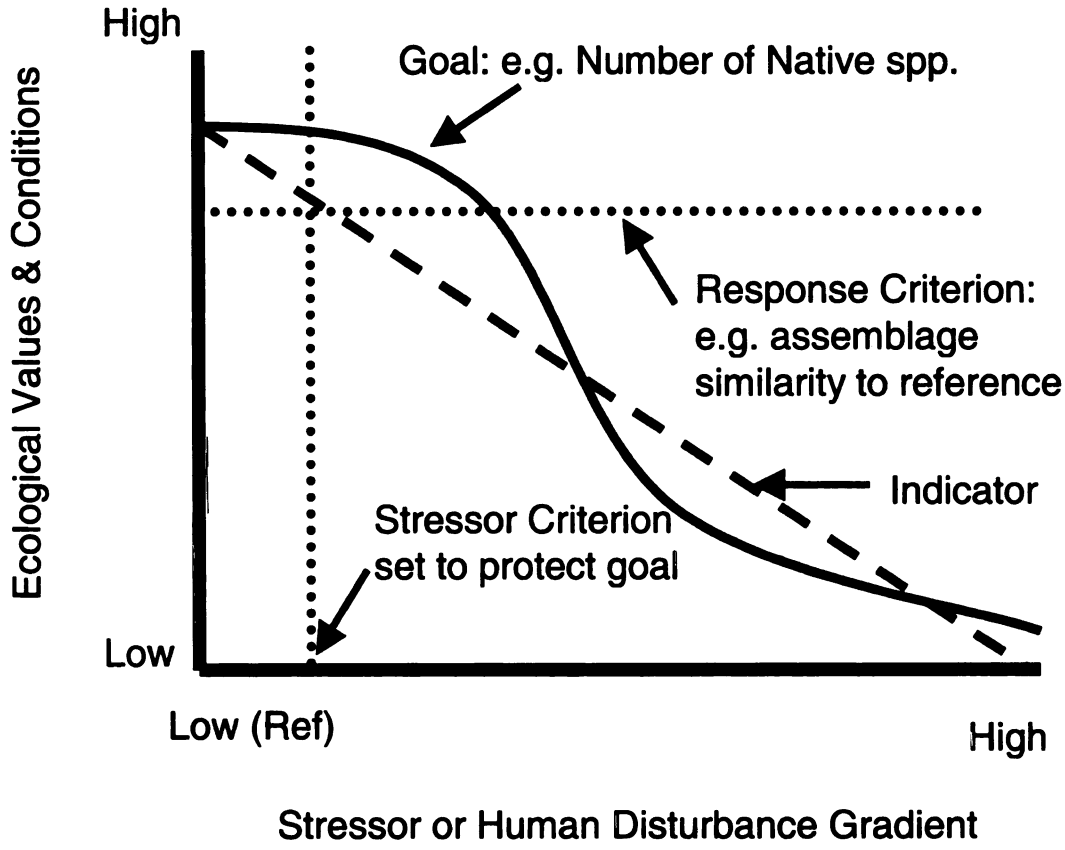

Figure 1.1 Stressors-response relationship between human disturbance and response of ecological valuable attributes

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CHAPTER 2

ALGAL ASSEMBLAGES IN MULTIPLE HABITATS OF RESTORED AND EXTANT WETLANDS

2.1 Abstract The functional and structural attributes of algal assemblages were studied in 25 restored and 20 extant depressional, palustrine wetlands in southern central Michigan. Environmental conditions and algal assemblages were compared between restored and extant wetlands and among habitats within wetlands. Restored marshes generally had lower shading by macrophytes, nutrient concentrations, and organic matter in sediments. Relative biovolume of non-diatom algae was significantly different among habitats in restored wetlands, but did not differ between macrophytes and sediments in extant wetlands. Species composition of diatom assemblages was not significantly different between epiphytes and sediment in both restored and extant wetlands. The discovered differences in non-diatom algae could not be attributed to any environmental variables measured; however, diatom assemblage differences between habitats increased with light irradiance. Differences in sediment diatom assemblages were observed between restored and extant wetlands and were related to differences in nutrients, pH, and canopy cover. Differences were also observed between epiphytic diatom assemblages in restored and extant wetlands and they were related to light and dissolved oxygen.

Key index words: diatoms; non-diatom algae; epiphyton; epipelon; phytoplankton; habitats; restored wetlands; extant wetlands; periphyton

Abbreviations: DIN, dissolved inorganic nitrogen; DO, dissolved oxygen; LEC, light extinction coefficients; MANOVA, multivariate analysis of variances; MRPP, multi-

response permutation procedure; NMDS, nonmetric multi-dimensional scaling; OMP, organic matter proportion; PCA, principle component analyses; SRP, soluble reactive phosphorus; TN, total nitrogen; TP, total phosphorus

2.2 INTRODUCTION

Wetland restoration has been increasingly used to mitigate loss and anthropogenic impact of wetland systems (McDonnell and Pickett 1990, Holland et al. 1995, Detenbeck et al. 1999, Zedler 2000). Beginning in the early 1990's, a large effort was initiated in Michigan to compensate wetland losses by establishing wetland restoration programs (MDEQ 2001a). Hundreds of wetland restoration projects have been carried out since then. Most of the wetland restoration projects involve simple techniques, such as plugging agricultural ditches or breaking field tiles to restore the hydrology of an area, and then restore the biological community. Restored wetlands support aquatic and terrestrial habitats and sustain many plant, invertebrate, and wildlife species (NRC 1995, USEPA 2000).

Ecological development after wetland restoration has been studied extensively (Whigham et al. 1990, Patten 1994, Middle 1999, Keddy 1999), and many environmental variables change during transition from one successional state to another (Gosz 1992). Nutrient accumulation is one of the most noticeable changes in biogeochemical process affecting the biological community in wetlands. In human impacted landscapes, nutrient enrichment from human activities affects the wetland restoration process, and its detrimental effects on wetland systems have been frequently reported (Koerselman and Beltman 1988, Blair 1996, Magee et al. 1999). Nutrient accumulation and storage from agricultural and urban runoff affect wetland productivity, species diversity, water quality, as well as the restoration rate of wetlands (Rader and Richardson 1992, Craft and Casey 2000).

Algae are one of the most important groups of primary producers in wetlands, and they play crucial roles in wetland biogeochemical cycles (Vymazal 1995). Algae, especially diatoms, are effective indicators of ecological conditions (Zedler 1993, Adamus 1996, Cottingham and Carpenter 1998, Detenbeck 2001, Stevenson et al. 2001) and have been used as indicators of ecological conditions in wetlands (Pan and Stevenson 1996, Stevenson et al. 1999, Pan et al. 2000, Stevenson 2001). Algae are also sensitive to nutrient concentration change (Tilman 1982). Soil organic C, N and P accumulation during wetland restoration can affect the biomass and species composition of the algal community (McCormick et al. 1996, Pan et al. 2000). Therefore, algal species assemblages could be indicators of restoration success because algal species composition and diversity would differ in low- and high-nutrient wetlands (Mayer and Galatowitsch 1999).

Habitat differences, along with a number of other environmental factors, contribute to spatial and temporal variability of algal assemblages in aquatic ecosystems (Leland 1995, Stevenson 1996, Stevenson 1997). The extent and conditions in four major algal habitats, phytoplankton, epiphyton, epipelon, and metaphyton, vary temporally at different stages in wetland cycles (Goldsborough and Robinson 1996). A number of studies have reported that different habitats support different algal assemblages in aquatic systems (Stevenson and Hashim 1989, Pringle 1990, Burkholder 1996, McCormick et al. 1998, Hillebrand and Kahlert 2001, Lim et al. 2001). Thus the similarity of algal assemblages between different habitats should increase with the increase of wetland age and increasing similarity of habitat characteristics.

Environmental factors such as, substratatum, substratratum stability, and nutrient availability have been suspected as major determinants of species composition differences among habitats (Stevenson 1996, Burkholder 1996). Changes in environmental conditions of algal habitats during wetland development flowing creation or restoration may regulate heterogeneity in species composition among habitatis. The lack of substratum should distinguish the plankton from the two benthic habitats of all stages of wetland development, but heterogeneity between phytoplankton and benthic habitats could decrease if more benthic algae were suspended in the water during either early or late stages of development. Algae on plants and sediments get some nutrients direcetly from the substratum. Nutrient supply from sediments directly to epipelon may be available earlier during wetland development than the indirect supply from sediments, through plants, to epiphyton. Differences between epiphyton and epipelon probably decrease with wetland development as nutrient availabitliyt increases and differences between habitats decrease.

To evaluate the success of wetland restoration and investigate the impact of human disturbance to both natural and restored wetland systems, I selected 45 (25 restored, 20 extant) freshwater depressional wetlands in the Maple and Upper Grand watersheds in southern Michigan. The environmental characteristics and algal assemblages of phytoplankton, epiphyton, and epipelon on different habitats in these wetlands were examined. I hypothesized that: environmental variables would differ between restored and extant wetlands; algal assemblages would differ among habitats and between restored and extant wetlands; differences in algal assemblages among habitats and between restored and extant wetlands would decrease with increasing age of restored wetlands;

and that decrease would be due to increasing similarity of environmental characteristics. I also expected to identify environmental factors that determine the distribution of algal assemblages and biodiversity among habitats and between restored and extant wetlands.

2.3 METHODS

Site description. Forty-five (25 restored, 20 extant wetlands) depressional, palustrine wetlands (Cowardin et al. 1979) were selected in the Maple and Upper Grand River watersheds of Southern Michigan for this study (Figure 2.1). All restored wetlands were reestablished within the past 15 years as part of wetland mitigation program. All wetland sites were located in landscapes dominated by agricultural and urban land. The sampled wetlands were mostly *Typha* or *Nuphar* dominated marshes, but also included several swamps. Watershed land use data were obtained from the Michigan DNR spatial data library to determine land use around these wetlands. Wetland boundaries for each site were determined based on shifts from wetland to upland vegetation and changes in slope between the wetlands and the

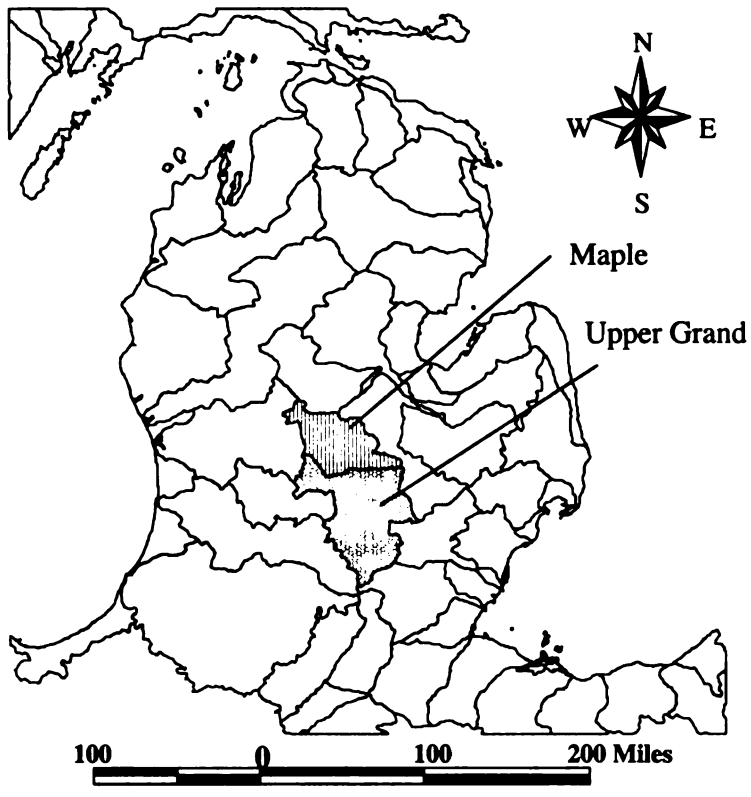

Figure 2.1. Wetland sampling locations in southern Michigan. 45 sites were selected in Maple and Upper Grand River watersheds.

adjacent upland. Duckweed covered at least some proportion of all wetlands during the sampling period.

2.3.1 Sampling. The selected wetlands were sampled and assessed during mornings of July 2000. Water temperature and dissolved oxygen (DO) were measured with a YSI® DO meter (YSI Inc., Yellow Springs, Ohio, USA) at the beginning and at the end of the sampling period, allowing at least one hour between measurements. Wetland net production was calculated based on DO change over one hour period times the estimated average depth of the wetland. Conductivity and pH were measured using a YSI® model 33 SCT meter with a conductivity and pH electrode (Denver Instrument Company). Canopy cover by emergent vegetation was estimated with a spherical canopy densiometer held at water level and by averaging canopy cover from 4 directions. Duckweed coverage was estimated based on the proportional coverage of the wetland surface. Irradiance at the water surface (I_0) and 30 cm below the water surface (I_z) were recorded with a LICOR light meter. Light extinction coefficients (LEC) were calculated as $\ln(I_0)$-$\ln(I_z)$/0.3 m (Wetzel 2001). Water samples from open water area in each wetland were collected in 2 125-ml acid-washed polyethylene bottles, stored in ice, transported to the laboratory, and frozen until chemical analysis. Phytoplankton samples from 10 cm below the water surface were collected in 2 1-L polyethylene bottles for chlorophyll *a* and algal cell counts. Sediment samples were collected randomly from eight different locations in each wetland using hard plastic tubes (internal diameter = 3.8 cm, length = 20 cm). Only the top 1 cm of sediment was collected. All 8 sediment samples from a wetland were composited in a Whirl-pak® bag. Epiphytic algae were collected by cutting 4-5 macrophyte stems, brushing epiphytes from each macrophyte surface, and putting them

in a Whirl-pak® bag. Metaphyton were collected for qualitative identification. Algae were preserved with M3 (APHA 1998) after subsampling for chl *a* analysis.

2.3.2 Sample analysis. One 250-ml water sample from each wetland was analyzed for total nitrogen (TN) and total phosphorus (TP). Another bottle was filtered through Coleman® glass fiber filters (0.45um diameter) and the filtrate was analyzed for nitrate-nitrite (NO_3^--N + NO_2^--N) and soluble reactive phosphorus (SRP) using a Spectronic® Genesys™ 2 Spectrophotometer. TP and SRP were measured using the ascorbic acid method, while TN and nitrate and nitrite (NOx-N) were measured using cadium reduction methods (APHA 1998). Ammonia (NH_4-N) was analyzed using a Wuick-Chem 8000 autoanalyzer at University of Michigan Biological Station (UMBS). Dissolved inorganic nitrogen (DIN) was calculated as the sum of ammonia and nitrate-nitrite concentrations. Sediment samples were rinsed with 200 ml of deionized water and homogenized using a Biospec® homogenizer. Appropriate dilution was made for sediment samples to measure sediment TN and TP concentration following the same methods as the water samples.

Chl *a* in water sample was assayed by extracting in 90% buffered acetone overnight at 4°C, reading absorbance on a Spectronic® Genesys™ 2 Spectrophotometer, and calculating pheophytin-corrected chl a concentration (APHA 1998). Dry mass (DM) and ash free dry mass (AFDM) of periphyton were determined according to standard methods (APHA 1998). Organic matter proportion in sediment (OMP) was the AFDM/DM ratio of surface sediments.

Phytoplankton, epiphytic algae, and sediment algae were diluted or condensed as necessary before counting. Algal densities and non-diatom species composition were determined using a Leica® microscope and a Palmer-Maloney (0.1 ml) counting chamber

under 400X and by counting 300 natural units. Algal taxa were identified to the lowest possible taxonomic level. Biovolume was estimated for at least 15 cells of each taxon by assigning them a geometric shape and measuring appropriate cell dimensions (Hillebrand et al. 1999, Charles et al. 2002). Diatom taxa and relative abundance were determined from permanent Naphax mounts of acid-cleaned diatoms (Stosch and Reimann 1970). At least 500 diatom valves were counted. Alpha diversity (species richness per locality), gamma diversity (total species diversity within landscape, all wetland sampled), and beta diversity (variation in species composition between 2 communities or habitats) were calculated for non-diatom algae and diatoms (Beilman 2001).

2.3.3 Statistics. To compare environmental variables between restored and extant wetlands, both individual variables and scores of principle component analyses (PCA) were used. Environmental variables were log-transformed to evenly distribute the variance before analysis, if necessary. The Student t-test was used to compare individual variables between extant and restored wetlands. PCA summarized major environmental patterns in this region. Multiple analysis of variance (MANOVA) was also used the test overall differences between restored and extant wetlands. The scores of the first three PCA axes were used as composite variables for MANOVA. All statistics were performed using SYSTAT® v. 10.

Attributes of non-diatom algae and diatoms were analyzed separately. The relative biovolume of non-diatom algae and relative abundance of diatom species were calculated and used for comparisons. Only diatom species with a relative abundance $\geq 1\%$ in a minimum of three sites or $\geq 5\%$ in at least one site were included in the analysis.

Nonparametric multidimensional scaling (NMDS), a multivariate ordination technique (Clarke 1993), was used to summarize and illustrate patterns of non-diatom and diatom distribution among different habitats and between restored and extant wetlands. Multi-response permutation procedure (MRPP), a non-parametric procedure for testing the hypothesis of no difference between two or more groups (Biondini et al. 1985), was used to assess differences in taxonomic composition of non-diatom and diatom assemblages among different habitats and between restored and extant wetlands. A p-value was provided by the MRPP to characterize the significance of between group differences. An A value (0-1) characterizes within-group homogeneity compared to the random expectation. When all items are identical within groups, then A = 1; if heterogeneity within groups equals the expectation by chance, then A = 0. Both MRPP and NMDS were performed using PC-ORD ® v.4.0 (McCune and Mefford 1997).

To relate differences in taxa composition to environmental variables, Bray-Curtis similarity matrics (smilarity in relative abundances or biovolumes) were calculated for species composition of non-diatom and diatom assemblages among different habitats and between restored and extant wetlands. Stepwise automatic linear regressions were used to explore the relationship between non-diatom or diatom assemblages and environmental variables. This was done by using similarity of non-diatom or diatom assemblages between habitats as separate dependent variables and a set of 19 uncorrelated variables as independent variables. Using these same procedures, similarities of species composition between restored and extant wetlands were calculated and expressed as a function of differences in environmental variables between wetlands. The dependent variables were the average Bray-Curtis similarities in species composition of a restored wetland with all

the extant wetlands. The Euclidean distance matrix for each environmental variable among different wetlands was calculated to indicate environmental differences between wetlands and the average Euclidean distances of individual environmental variables between a restored wetland with all extant wetlands were used as independent variables. Those variables that had the highest correlations with other variables were screened out, so only the most uncorrelated environmental variables were included for the regression analyses. For example, the distance of conductivity between restored and extant wetlands was positively correlated with the distance of sediment TP (r=0. 424, P=0.035), negatively correlated with pH distance (r=0.430, p=0.032), and related to several other variables. Therefore, it was excluded from the model. All these analyses were performed using Systat® v10.

2.4 RESULTS

2.4.1 Environmental variables in restored and extant wetlands.

Land cover in the region was dominated by agriculture (78%) with smaller portions of urban (9%), forest (6%), and other land types. Most of the restored wetlands were *Typha*-dominated marshes. In contrast, extant marshes in this area were seldom dominated by one macrophyte species. *Phragmites, Typha, Nuphar, Ceratophyllum demersum, Myriophyllum*, and *Scirpus* were co-dominant in many of the extant marshes.

Three PCA scores accounted for 42% of environmental variance in the data set (Table 2.1). The first axis was positively correlated with major nutrients, such as TP (r=0.621), ammonium (r=0.784), and DIN (r=0.675) in water column, as well as light supplies (duckweed cover and light extinction coefficient). The second axis was negatively

correlated with N, P content in sediments and shading by vegetation, and positively correlated with conductivity. The third axis was positively correlated with SRP and DO. Overall, the scores of the first 3 axes were significantly different between restored and extant wetlands (MANOVA, Wilks's lamba F3, 42= 5.938, P=0.002). Univariate F test indicated that PCA axis 1 score was significantly different between restored and extant wetlands (P=0.012), while the scores of PCA axis 2 and axis 3 were not different (P>0.1). Many variables did not differ between the two types of wetlands (P>0.1). Water temperature varied from 18.1 to 32.2°C among wetlands during the sampling period. Conductivity was highly variable (from 149 to 1003, 499±34 μmhos/cm). pH varied greatly among sites (from 6.8 to 9.6, 7.92±0.9 (Average ±S.E.)), but was not significantly different between restored and extant wetlands (P=0.110). DO in the morning could be as high as 11 mg/L and as low as 0.7 mg/L in some of the wetlands (4.567±0.381mg/L). Wetland net production based on DO change ranged from –1.0 to 3.17 $g \cdot m^{-2} \cdot h^{-1}$ representing heterotrophic to autotrophic wetland types.

Most restored wetlands had relatively low shading by vegetation (26.2±3.8%, mean ± standard error). Extant wetlands had higher shading than restored sites (t-test, P=0.031) (Figure 2.2). Duckweed and other floating plants appeared in at least 2/3 of the sampled sites with an average coverage of 38.2% (±31.5%) of water surface during sampling period. Duckweed coverage was much higher in extant wetlands than restored ones (P=0.003). LEC at 0.3m depth in open water (if available) of restored wetlands (1.207 ±0.121 μmol $m^{-3}s^{-1}$) was much lower than in extant wetlands (3.173±0.777 μmol $m^{-3}s^{-1}$) (P=0.01) indicating that extant wetlands had much lower light avalaibility in the water column and at the bottom.

Table 2.1. Correlation matrix of environmental variables measured and principal component analysis (PCA) loadings of environmental variables in the 45 wetlands. When a correlation coefficient r >0.30, Dunn-Sidak P<0.05; when r>0.39, P<0.01. Correlation between age and environmental variables had a degree of freedom 24 and none of the correlation was significant.

	Shading	LEC	Duckweed	DO	Prod.	Cond.	pH	TN	TP	DIN	NH_3	SRP	DIN:SRP	TN:TP	Chl a	OMP	Sed. N	Sed. P	Sed. N:P	Age
Shading	1.00	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
LEC	0.05	1.00	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Duckweed	0.16	0.65	1.00	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
DO	-0.21	-0.37	-0.54	1.00	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Prod.	0.11	-0.17	0.07	0.18	1.00	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Cond.	-0.21	-0.13	-0.14	-0.10	0.12	1.00	.	.	.	.	.	.	.	.	.	.	.	.	.	
pH	0.32	-0.06	0.01	0.11	0.05	-0.29	1.00	.	.	.	.	.	.	.	.	.	.	.	.	
TN	0.05	0.00	0.07	-0.01	-0.09	-0.01	-0.17	1.00	.	.	.	.	.	.	.	.	.	.	.	
TP	0.18	0.48	0.27	-0.14	0.07	-0.03	0.17	0.08	1.00	.	.	.	.	.	.	.	.	.	.	
DIN	0.40	0.43	0.28	-0.29	-0.03	-0.01	0.03	0.24	0.25	1.00	.	.	.	.	.	.	.	.	.	
NH_3	0.44	0.51	0.37	-0.31	-0.10	-0.07	0.04	0.31	0.35	0.96	1.00	.	.	.	.	.	.	.	.	
SRP	0.10	0.21	0.11	0.19	-0.01	-0.12	-0.22	0.37	0.46	0.23	0.30	1.00	.	.	.	.	.	.	.	
DIN:SRP	-0.20	-0.10	-0.16	0.09	0.00	0.16	-0.02	0.11	-0.25	0.29	0.20	-0.23	1.00	.	.	.	.	.	.	
TN:TP	-0.18	-0.14	0.00	-0.01	0.06	0.01	-0.15	0.13	-0.51	0.01	-0.06	-0.31	0.30	1.00	.	.	.	.	.	
Chl a	0.03	0.38	0.38	0.02	0.23	-0.17	0.09	0.11	0.35	0.19	0.26	0.29	-0.12	-0.13	1.00	.	.	.	.	
OMP	0.17	0.29	0.31	-0.15	-0.06	-0.17	-0.01	0.04	0.25	0.32	0.39	0.28	-0.12	-0.09	0.49	1.00	.	.	.	
Sed. N	0.28	-0.03	0.16	-0.08	-0.06	-0.29	0.13	-0.13	-0.07	0.01	-0.05	0.00	-0.02	0.06	-0.05	0.29	1.00	.	.	
Sed. P	0.26	-0.02	0.16	-0.07	-0.06	-0.17	-0.10	-0.04	0.04	0.16	0.11	0.11	0.09	-0.05	-0.04	0.12	0.76	1.00	.	
Sed. N:P	-0.05	0.10	0.34	-0.21	0.24	0.08	0.07	0.03	-0.14	-0.11	-0.09	-0.09	0.09	0.44	0.02	0.09	0.01	-0.22	1.00	
Age	0.22	0.12	0.07	-0.07	0.16	-0.02	0.15	-0.19	0.03	-0.14	-0.21	0.02	0.02	0.32	0.02	0.02	0.00	0.04	0.07	1.00
PC1	0.24	0.69	0.63	-0.33	0.073	-0.17	-0.05	0.38	0.62	0.68	0.78	0.45	-0.22	-0.25	0.65	0.66	0.14	0.08	0.02	-0.22
PC2	-0.61	0.17	-0.16	0.14	-0.08	0.48	-0.42	0.24	0.01	0.14	0.18	0.13	0.12	0.12	0.18	0.29	-0.79	-0.69	0.32	-0.24
PC3	0.04	-0.21	-0.36	0.75	0.10	-0.42	0.17	0.26	0.38	-0.12	-0.22	0.64	-0.23	-0.39	0.03	-0.13	-0.44	-0.08	-0.22	0.37

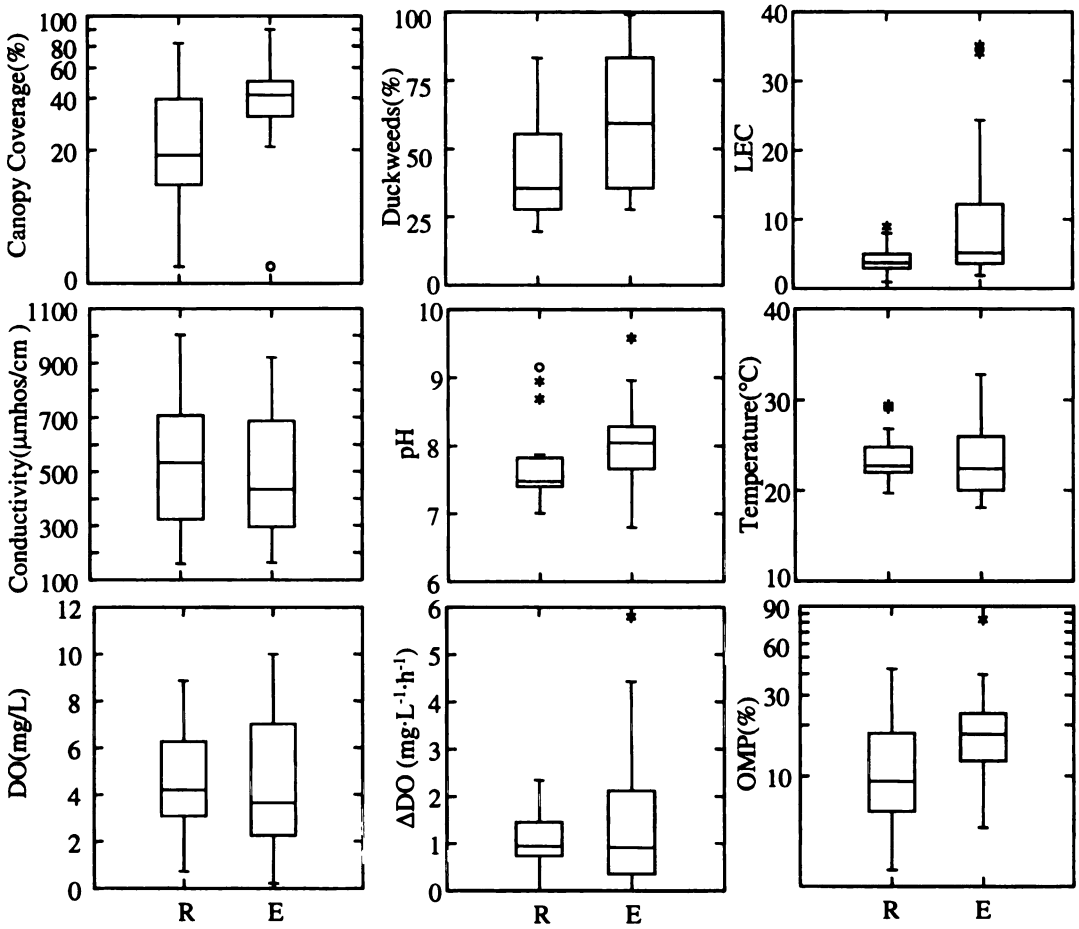

Figure 2.2. Comparisons of physical and chemical characteristics between restored and reference wetlands.

Nutrient concentrations in these two types of wetlands were different (Figure 2.3). TP (1.858±0.314 mg/L) and SRP (0.406±0.102 mg/L) were high in most of the wetlands, and had significantly higher concentrations in extant wetlands than in restored wetlands (P=0.007 and 0.027 respectively). TN (0.779±0.069 mg/L overall) in extant wetlands was not different from restored wetlands (P>0.1); the major form of inorganic N was NH_4^+, and NO_3^- was almost depleted in most of the wetlands (<0.010mg/L). DIN (0.054±0.007) was significantly higher in extant wetlands (P=0.023) than restored wetlands. N:P and

DIN: SRP ratios were generally lower than 17. N: P ratio was higher in restored wetlands than in extant wetlands (P=0.002) while DIN:SRP ratio was not significantly different between these two types of wetlands (P=0.210). Most of wetlands had mineral soils with an average OMP 17.4 (±2.3)%, which was higher in extant wetlands than in restored sites (P=0.035). TN (3.911±1.106 mg/L) and TP content (0.757±0.186 mg/g) in soil surface was higher in extant wetlands than in restored wetlands (P=0.025 and 0.058 respectively), but N: P ratio (3.78±1.86) in sediment were not different (P>0.128).

Many of the environmental variables related to trophic condition correlated with each other (Table 2.1). LEC at 30 cm depth positively correlated with duckweed cover, chl *a*, DIN, ammonia, TP concentration in the water column, and sediment organic matter, and negatively correlated with DO. DO in the water column negatively correlated with duckweed cover, LEC, and ammonia concentration. Most nutrient factors were also correlated with each other. TN in water column positively correlated with SRP, DIN, NH_4^+, and sediment TN. TP in water column positively correlated with SRP, ammonia, chl a. Sediment TN and TP content were positively correlated with each other. LEC, Duckweeds, SRP, and TP positively correlated with age of wetlands.

Age of restored marshes was not a significant factor in differentiating the first 3 PCA axes. Environmental conditions between marshes less than 5-year-old and 5-10 year old were not significantly different (MANOVA, Wilks's lamba $F_{3,25}$=0.833, P=0.48) based on the three PCA axes. None of the environmental variables correlated with age of wetlands.

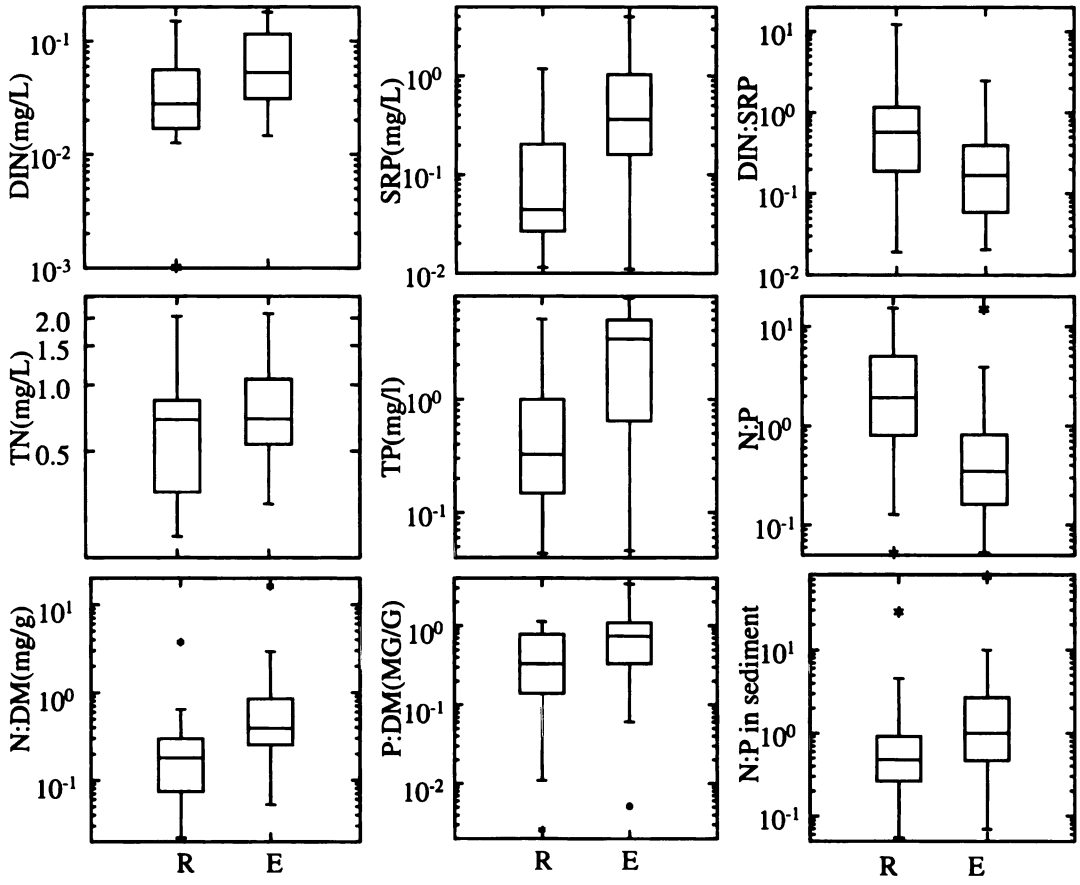

Figure 2.3. Wetland nutrient concentration and comparison between restored and extant wetlands.

2.4.2 Non-diatom algal assemblage and diatom assemblages in restored and extant wetlands

Of the 311 epiphytic algal taxa identified, diatoms had the highest taxa richness (157), followed by Chlorophyta (99 taxa) and cyanobacteria (34). Other groups (Euglenophyta, Dinophyta, and Chrysophyta) appeared but with less abundance. Chlorophyta were the most abundant, with an average of 48.2% (±6.9% SE) of the total cell density and 46.1% ±5.0% of total biovolume. *Stigeoclonium, Mougeotia,* and

Oedogonium were the most abundant genera. The next abundant group in epiphytes was diatoms with 30.3% (±4.5%) of total density and 26.8% (± 4.3%) of total biovolume. *Navicula minimum, Cocconeis placentula, Achnanthidium minutissimum,* and *Navicula cryptocephala* were the most dominant diatom taxa. Cyanobacteria, Euglenophyta, Dinophyta, and Chrysophyta appeared but were low in density in epiphytic samples. Total number of algal taxa (gamma diversity) and average taxa richness (alpha diversity) were higher in restored wetlands (250 and 30.0 respectively) than in extant wetlands (189 and 25.8, respectively), mostly because of higher diatom gamma diversity in restored wetlands (Table 2.2). Beta diversity of diatoms was also higher in restored wetlands than in extant wetlands.

Of the 235 epipelon taxa identified, diatoms had both the highest taxa richness (199) and abundance with an average of 54.4 % (±6.6% SE) of total cell density and 48.8% (± 6.5%) of total biovolume. *Navicula minima, A. minutissimum, N. cryptocephala,* and *Nitzschia palea* dominated diatom communities. Chlorophyta was the next dominant group with 19 taxa and 22.3 (±4.3%) of total density and 12.9% (±4.1%) of total biovolume because of dominance of *Scenedesmus* in some of the wetlands. Only 8 cyanobacteria taxa were found, but they remained in low density (13±2.3%) and biovolume (16.4±4.7%). Other groups (Euglenophyta, Dinophyta and Chrysophyta) appeared but with relatively low abundance. Although the alpha diversity of non-diatom algae and diatoms were pretty similar in restored (3.2 and 28.2 respectively) and extant wetlands (2.9 and 29.7 respectively), the gamma diversity of diatom taxa in restored wetlands (165) was much higher than in extant wetlands (129) and caused higher beta diversity in restored wetlands (Table 2.2).

Phytoplankton biomass was low in most sampled wetlands (0.030±0.009 mg chl a/L) and was not significantly different between restored and extant wetlands (P=0.113). The direct correlations of phytoplankton biomass with major nutrient gradients were weak (r=0.368 with TP, r=0.126 with TN), but were significantly related to OMP in sediments (r=0.499, P=0.025).

Phytoplankton taxa richness was relatively low compared to other habitats. A total of 153 non-diatom taxa were found in phytoplankton samples, but diatom density was very low in most wetlands. Most of the phytoplankton were Chlorophyta (42.3%) and cyanobacteria (33.1%) on the basis of cell density, only 15.2(±3.3%) of total algal abundance was diatom. The average relative biovolume was dominated by dinoflagellates (37.9± 4.7%), Englenophyta (26.5±3.7%), chrysophyta (10.5±4.4%) and green algae (9.0±2.9%). Cyanobacteria (3.5±1.6%) and diatom biovolume (2.3±0.7%) were very low. Restored wetlands had higher alpha, beta, and gamma diversity than extant wetlands (Table 2.2). Planktonic diatoms were not used for quantitative analysis because of their extremely low density in half of the wetland sites.

Metaphyton appeared in the majority of the wetlands and completely covered the basin of at least ¼ of wetlands. The most commonly found macroalgae were *Stigeoclonium, Oedogonium*, and *Mougeotia*. Because of the difficulty of quantifying their biomass, metaphyton were not used for further analysis.

Non-diatom and diatom diversity among different habitats were quite different. Alpha, beta, and gamma diversity of non-diatom epiphyton and phytoplankton were

Table 2.2. Mean alpha diversity (Number of species per site), Gamma (landscape) diversity, and beta diversity between habitats within a wetland and between restored and extant wetlands

		Alpha (average + s.e.)			Gamma			Beta		
		Epiphytic	Sediment	planktonic	Epiphytic	Sediment	planktonic	epiphytic	sediment	planktonic
Restored	Diatoms	21.6±1.3	28.2±1.8		132	165		6.1	5.9	
	Non-diatom	8.7±1.2	3.2±0.3	9.3±0.76	118	26	111	13.6	8.1	12.0
	All algae	30.0±1.9	31.3±1.9		250	191		8.3	6.1	
Extant	Diatom	17.6±1.9	29.7±2.4		100	129		5.7	4.3	
	Non-diatom	9.7±1.4	2.9±2.0	7.9±0.65	89	28	90	9.2	9.6	11.4
	Algae	25.8±2.7	32.6±2.4		189	157		7.3	4.8	
All site	Diatom	20.5±1.1	29.5±1.3		157	199		7.7	6.7	
	Non-diatom	9.1±0.9	3.0±0.2	8.7±0.52	154	38	153	16.9	12.7	17.6
	Algae	28.7±1.5	31.2±1.6		311	235		10.8	7.5	

higher than epipelon in all wetlands. Sediment diatoms had higher alpha diversity, higher gamma diversity, but lower beta diversity than epiphytic diatoms (Table 2.2).

2.4.3 Difference of taxonomic composition among habitats and between restored and extant wetlands

Differences in algal assemblages were commonly observed among habitats within wetlands. Most comparisons of non-diatom algae among habitats were different in both restored and extant wetlands, except that the difference between epiphyton and epipelon assemblages was not significantly different in extant wetlands (P=0.113) (Table 2.3, Figure 2.4). Similarly, epiphytic diatom assemblages and sediment diatom assemblages were not significantly different in restored or extant wetlands (Table 2.3). The sample ellipse of epiphytic diatom assemblages (Figure 2.4) was larger than that of sediment diatoms, indicating a larger beta diversity for epiphytic diatom assemblages than sediment diatoms.

Differences in species composition of algal assemblages between restored and extant wetlands revealed habitat specific patterns (Table 2.4, Fig. 2.5). Both epiphytic non-diatom algae and diatom assemblages in restored wetlands were significantly different from those in extant wetlands (Table 2.3, Fig. 2.5). However, sediment algae and phytoplankton were not different between these two types of wetlands.

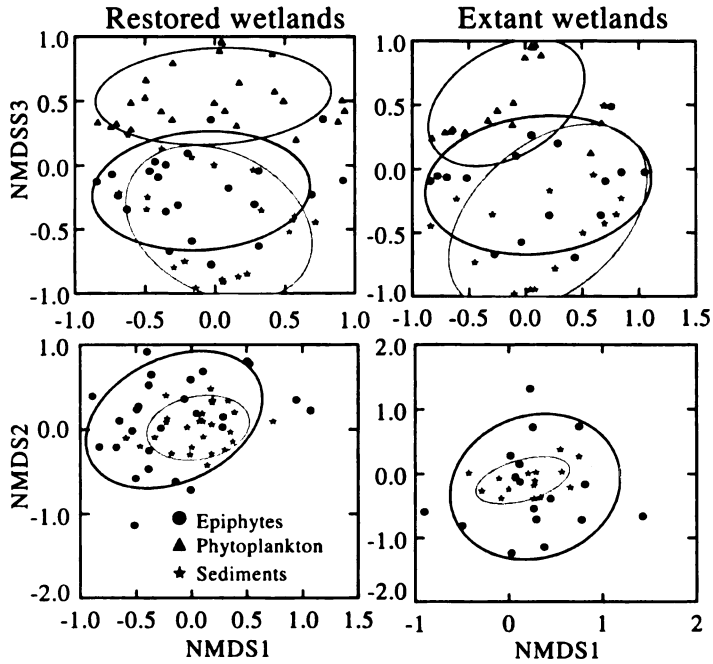

Figure 2.4. Non-metric multidimentional scaling (NMDS) of the restored and extant wetlands based on non-diatom and diatom assemblages in different habitats. The circles are the sample ellipse which is centered on the sample means of the x and y variables. The size of the ellipse is specified at a probability value of 0.6827.

Table 2.3. MRPP comparisons of species composition in non-diatom algae and diatom between different habitats in restored and extant wetlands

Comparisons	Wetland type	T statistic	A	P
Based on non-diatom algal biovolume				
Epiphytes *vs.* Sediments	Restored	-3.940	0.0145	0.002
	Extant	-1.241	0.0051	0.113
Phytoplankton *vs.* Epiphytes	Restored	-5.333	0.0174	0.000
	Extant	-4.876	0.0276	0.001
Phytoplankton *vs.* Sediments	Restored	-8.660	0.0371	0.000
	Extant	-6.944	0.0442	0.000
Based on diatom relative abundance				
Epiphytes *vs.* Sediments	Restored	-0.138	0.0006	0.389
	Extant	-1.118	0.0057	0.133

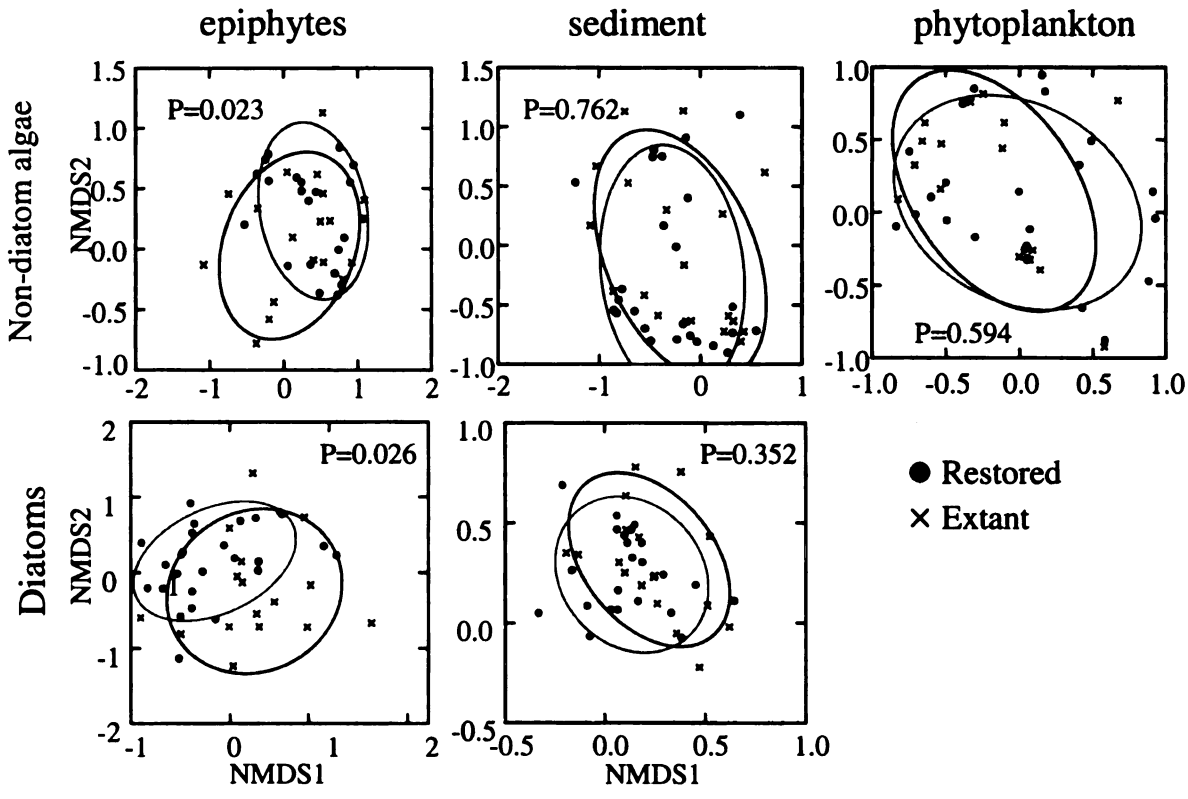

Figure 2.5. Non-metric multidimentional scaling (NMDS) of the restored and extant wetlands based on non-diatom and diatom assemblages in different habitats. The circles are the sample ellipse which is centered on the sample means of the x and y variables. The size of the ellipse is specified at a probability value of 0.6827.

Table 2.4. MRPP comparisons of species composition of non-diatom algae and diatoms between restored and extant wetlands

	habitats	T statistic	A	P
Non-diatom algae	Epiphytes	-2.219	0.0070	0.023
	Sediments	0.760	-0.0042	0.762
	Phytoplankton	0.466	-0.0033	0.594
Diatom	Epiphytes	-2.322	0.0100	0.026
	Sediment	-0.279	0.0010	0.352

Table 2.5. Pearson correlation matrix of dissimilarities in species composition of non-diatom algae and diatoms between restored and extant wetlands in different habitats. A * indicates a P value <0.05.

	Epiphytic diatom	Sediment Diatom	Epiphytic non-diatom algae	Planktonic non-diatom	Sediment non-diatom algae
Epiphytic diatom	1.000				
Sediment Diatom	0.613*	1.000			
Epiphytic Non-diatom	0.560*	0.440*	1.000		
Planktonic non-diatom	0.691*	0.377	0.656*	1.000	
Sediment non-diatom	0.394	0.163	0.418*	0.477*	1.000

Differences in species composition of algal assemblages between restored and extant wetlands consistently showed the same pattern in different habitats (Table 2.5). The dissimilarities of non-diatom algae between restored and extant wetlands and the dissimilarity of diatoms between these two types of wetlands were correlated ($P<0.1$) (Table 2.5). The only correlation not statistically significant was between the dis-similarities of sediment diatom and non-diatom algae.

2.4.4 Algal assemblages and environmental variables.

Multiple linear regression of non-diatom algal similarities among habitats indicated that little variance could be explained by environmental variables (Table 2.6). P and sediment TP had very weak effect on algal assemblages among habitats. However, none of the regression models was significant.

Bray-Curtis similarity between epiphytic and sediment diatoms, however, revealed significant regression model with shading by vegetation, LEC, and DO as predictors

(R^2=0.346, P=0.001). With the increase of plant shading, LEC, and DO, similarity in diatom species composition between epiphytes and epipelon increased.

Similar to inter-habitat comparison, similarity between restored vs. extant wetlands using non-diatom algae could not be as easily explained by environmental differences as diatom assemblages (Table 2.8). Only similarity of planktonic non-diatom algae between restored and extant wetlands slightly responded to canopy coverage (P=0.132). Non-diatom algae in other habitats did not respond to environmental variables.

Similarity in diatom species composition between restored and extant wetlands in different habitats were related to different environmental factors (Table 2.8). Similarity of epiphytic diatom species composition between restored and extant wetlands could be negatively affected by LEC difference and positively to differences in plant shading. Similarity in sediment diatom species composition related to a number of factors, such as differences in pH, plant shading, and nutrient differences between restored and extant wetlands (R^2=0.540, P=0.007). With the decrease of nutrient difference between restored and extant wetlands, similarity in diatom species composition increased.

Table 2.6. Regression by forward stepwise selection of independent variables. Dependent variable is similarity among epiphytic, phytoplankton, and sediment non-diatom algae, and dissimilarity between epiphytic and sediment diatom assemblages, independent variables are uncorrelated environmental variables.

Species composition	Habitats	Variables	N	Coefficient	T value	R^2	P(2 Tail)
Non-diatom algae	Epiphytes vs. Phytoplankton	SRP	45	-0.954	88.464	0.073	0.073
	Epiphytes vs. Sediments	P in sediment	45	0.254	-1.619	0.065	0.114
	Phytoplankton vs. Sediments	pH	45	-0.007	0.540	0.037	0.637
		conductivity		-0.015	1.206		
		ΔDO		-0.001	0.089		
Diatoms	Epiphytes vs. sediments	Constant	45	0.475	39.308	0.374	0.000
		DIN:SRP		-0.016	1.327		0.192
		DO		0.027	-2.030		0.049
		Canopy coverage		0.050	-3.955		0.000
		LEC		0.034	-2.567		0.014
		Regression					0.001

Table 2.7. Linear regression by forward stepwise selection of independent variables. Dependent variable is average Bray-Curtis similarity of algal species compositions in a restored wetland with extant wetlands. Independent variables are average distances of a variable between a restored wetland with all extant wetlands.

Species composition	Habitats	Variables	N	coefficient	t-value	R square	P
	Epiphytes		25			0	
Non-diatom algae	plankton	Canopy Coverage	22	0.021	-1.569	0.110	0.132
	Sediments		25			0	
	Sediments	Constant		0.239	9.944		0.000
		pH		0.015	-1.894		0.074
		TN		-0.011	1.681		0.109
		DIN		-0.024	2.559		0.019
		Canopy Coverage		0.021	-2.515		0.021
		Sediment TP		-0.108	3.211		0.005
Diatoms		Regression	25			0.540	0.007
	Epiphytes	Constant		0.038	12.821		0.000
		LEC		-0.086	-1.637		0.054
		Canopy Coverage		0.020	2.037		0.114
		Regression	25			0.196	0.091

2.5 DISCUSSION

Distinct ecological differences were evident between the restored and extant wetlands in southern-central Michigan. In addition to difference in shading by vegetation and duckweeds on water surface, nutrient concentrations and soil organic matter proportion also differed between these two types of wetlands. Differences in algal species composition between two types of wetlands depended on habitat. Epiphytic assemblages were more different between restored and extant wetlands than sediment and phytoplankton assemblages. Algal assemblages also differed among habitats. Abiotic environmental variables were related to many of the changes in algal assemblages among habitats in wetlands and between restored and extant wetlands. Age of a restored site was not a determinant factor for attributes in these wetlands.

2.5.1 Wetland restoration and environmental change in restored wetlands

Althought the initial goal of restoration is usually to restore the original physical and chemical characteristics and biological communities of ecosystems, the actual consequences of wetland restoration and construction are variable (Mitsch and Wilson 1996, Kaiser 2001). Wetland restoration can recover some aspects of the lost function and structure of pristine wetlands (USEPA 2001), but most evidence (van der Valk 1981, Galatowitsch and van der Valk 1996, Magee et al 1999) indicates that restored wetlands differ from extant wetlands in a number of ways. Restored wetlands generally have different hydrogeomorphology, more flood area with expanse of open water (Gwin et al. 1999), narrow borders of vegetation (Kentula et al. 1992, Bedford 1996), low floristic diversity, and different floristic composition (Galatowitsch and van der Valk 1996,

Magee et al. 1999) than natural wetlands. In this study, the extant wetlands in southern Michigan generally had complete emergent, submergent, and floating zones. Vegetation in these wetlands was more diverse, and vegetation shading was higher than restored wetlands. Restored wetlands were mainly composed of submergent zone with fewer sedge meadows. Restored vegetation was composed of one or fewer species, and canopy cover was lower. The higher canopy cover and duckweeds cover in extant wetlands indicated light limitation for algal growth.

A number of studies have shown that extant wetlands in human impacted landscape had much higher nutrient concentration and sediment organic matter than restored wetlands, which has been related to long-term accumulation and wetland's function in nutrient retention (Craft et al 1991, Holland et al. 1995, Craft and Richardson 1997). Studies (Bishel-Machung et al. 1996, Craft and Casey 2000) also found reference wetlands had higher C and N content in sediments than in constructed wetlands. Continuous saturation near the soil surface should limit decomposition of organic matter and retain N in natural wetlands (Stolt et al. 2000). My results also showed that nutrient concentration in the extant wetlands were well above average level (MDEQ 2001b) in southern Michigan, while restored marshes were at the beginning of succession and had relatively low nutrient concentration and organic accumulation. I also found significant differences in organic matter and nutrient concentrations between restored and extant wetlands, which may cause different biological communities.

2.5.2 Difference of algal species composition between restored and extant

Presumably, algal assemblages in restored wetlands would increase its similarity with extant wetlands and eventually would have the same assemblages as the extant wetlands

due to an increasing similarity of environmental characteristics. However, the starting point of a restored wetland may be different from its original state. The physical, chemical, and biological conditions during succession could also differ from natural wetland succession. Human perturbation during succession could also significantly change the path of succession (Detenbeck et al. 1999). Thus, a restored wetland may not necessarily develop to a state similar to mature natural wetlands (Mitsch and Wilson 1996, Lockwood and Pimm 1999, Kaiser 2001).

Although high variability in community composition among extant wetlands suggested a high degree of environmental heterogeneity in this study, both environmental conditions and epiphyton in restored wetlands still were significantly different from those in extant wetlands. None of the environmental variables or algal assemblage changes correlated with age of restored wetlands in this study. No linear relationship between age of wetland and organic carbon content in constructed wetlands was found. Previous studies (Bishel-Machung et al. 1996) also indicated that many environmental variables did not reach restoration goals within 10 years of initiating restoration. However, some authors have suggested that wetland nutrient content might recover after more than 15 years of restoration (Craft and Richardson 1998). According to MDEQ (2001a), only 29% of the mitigation projects in Michigan were considered biologically successful, and 26% of the mitigation sites in southern Michigan were observed to have poor water clarity. Mitsch and Wilson (1996) suggested that mitigation projects should be given as much as 15-20 years rather than 5 years before judging their success. The apparent difference of biogeochemical conditions between restored and extant wetlands make diatom assemblages great indicators of wetland conditions.

Generally, restored wetlands had higher diatom gamma diversity, beta diversity, and alpha diversity on epiphytes than observed in extant wetlands. Other studies have also observed that restored wetlands did not necessarily have less biodiversity of plant and invertebrates than reference sites (Scatolini and Zedler 1996, Bedford et al. 1999, Magee et al. 1999, Knutson 1999, Williams and Zedler 1999), and no evidence indicated a lower diatom species diversity in restored wetlands (Mayor et al. 1999). Two reasons might have caused low diversity in extant wetlands. First, eutrophication decreases algal species diversity and evenness (Detenbeck et al. 1999, Hillebrand and Sommer 2000). The significantly higher nutrient levels in extant wetlands may be the cause of lower alpha diversity. The other possible explanation was that higher spatial heterogeneity of restored wetlands at different stages of succession provided greater habitat variety for different algal species. The lower beta diversity of diatoms in extant wetlands suggested that diatom species dispersed evenly in extant wetlands, while the higher beta diversity suggested heterogeneous distribution of diatom assemblages among habitats in restored wetlands.

The patterns in similarity of diatom species composition in restored wetlands with the extant wetlands were related to environmental factors that may regulate biological differences during the ecological succession. Differences in algal species composition on macrophytes between these two types of wetlands were complexly related to light variables, but the model only explained 20% of the variance of diatom similarity among wetlands. On the other hand, the similarity in sediment diatom species composition between extant and restored wetlands could be explained by difference in pH, nutrients, and light effects. This model was much stronger (explaining 54% of the variance in

diatom similarity) and indicated the importance of nutrient accumulation and light regime, particularly for regulating algal assemblages during wetland succession.

2.5.3 Algal species composition among different habitats within wetlands

Phytoplankton communities were very different from benthic algal assemblages. As expected, phytoplankton and benthic algal communities had different growth forms which were adapted to planktonic and benthic habitats (Stevenson 1996). Phytoplankton communities contained few diatoms, despite their abundance in the benthos. The majority of southern Michigan wetlands in this study were in open and shelter states (Goldsbough and Robinson 1996), which should be dominated either by epiphyton or metaphyton. Phytoplankton density was generally very low. Duckweeds and macrophytes shading had not only caused light limitation for phytoplankton, but may also have competed with phytoplankton for nutrients as suggested in other studies (Herbst and Hartman 1981). Phytoplankton had high beta diversity, and the structural features of algal assemblages lacked a common pattern among sites. Grazing, allelopathy, light limitation, and alternating self-organization processes in these wetlands make phytoplankton dynamics more complex than in lakes due to the large number of interacting factors (Rojo 2000a, b).

The composition of non-diatom algae differed much more between benthic habitats than diatom species composition. The difference of non-diatom algae between macrophytes and sediments in restored wetlands was probably due to the very complicated interactions of algae and their substrata, involving both physical and chemical processes (Burkholder 1996). The physical and chemical habitats for benthic

algae on organic substrata is very different than on firm substrata of macrophytes. Lower light due to denser macrophytes and duckweed cover may have constrained the development of differences between non-diatom epipelon and epiphyton growth in extant wetlands, the only comparison of non-diatom algae that was not significantly different among habitats.

Diatom assemblages on macrophytes and in sediments, however, were not different in restored and extant wetlands. Lim et al. (2001) claimed that sediment diatom communities reflect changes in the diatom communities on epiphytic and rock substrates in response to limnological conditions. This study also demonstrated that sediment diatom assemblages contained the majority of epiphytic diatom taxa within a wetland, but included a wider range of species than epiphyton. Although epiphytic and sediment diatom did not differ in restored and extant wetlands, the regression model indicated that differences between these two habitats were a function of several different factors including light, DO, and nutrient ratio.

Light and DO are inter-correlated and may present different perspectives to diatom assemblages. The effect of light on diatom assemblages and productivity has been reviewed by Hill (1996). In addition to the demonstrated effects of conductance and pH on diatom assemblages, % open canopy was another important variable that determined patterns of algal taxa among sites in streams (Carpenter and Waite 2000). The direct effect of increased macrophyte shading and duckweed cover in Michigan wetlands could be providing light limited environment for epiphytes, thereby increasing the similarity of epiphytic algae to those in the limited-light environment of sediments. The indirect effect of decreasing light was to decrease DO concentrations. DO is a comprehensive variable

and could be determined by algal productivity and plant and bacteria respiration (Kemp and Dodds 2002). DO also related to many other environmental variables. The negative relation between DO and NH_4^+ concentration may be related to high decomposition, animal excretion, nitrification of ammonia, and decomposition of high N organic matter. Duckweed cover might cause light limitation for algal productivity and low DO in the water column, but algal biomass was not eliminated, which was indicated by the positive relation between duckweed cover and chl a concentration in the water column. Relatively higher DO environment for epiphytic diatoms and lower DO for sediment diatoms may be another regulating factor for autotrophic and heterotrophic diatom taxa and thus affect diatom species composition.

In summary, the physical, chemical, and biological features of restored wetlands differed from extant wetlands in a number of ways. In particular, light reduction and nutrient concentrations, were distinctly greater in extant wetlands, and probably contributed to differences in algal communities among habitats within wetlands and between restored and extant wetlands. I found no direct evidence for increasing similarity in algal assemblages between restored and extant wetlands with increasing age of restored wetlands. However, response of algal assemblages to nutrient concentrations and light, two factors distinguishing restored and extant wetlands, indicated indirectly that successional processes in restored wetlands were regulating algal assemblages. Restored wetlands < 10 years old may need more time to develop mature qualities.

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Chapter 3

CHANGES IN BENTHIC ALGAL ATTRIBUTES DURING SALT MARSH RESTORATION AND USE IN BIOASSESSMENT

3.1 Abstract: Although salt marsh restoration has been used to mitigate loss of habitat and ecosystem function and hundreds of restored marshes have been built along the US coast, few effective indicators have been developed to determine success of ecological restoration in salt marshes. To assess attributes of algal assemblages as indicators of salt marsh restoration, I chose 8 pairs of salt marshes in North Carolina, each pair with one restored marsh (from 1 to 28 year old) and a nearby reference salt marsh. Algae on *Spartina alterniflora* and sediments (sediment algae) were collected in each marsh during spring and summer 1998 for assaying biomass (dry mass (DM), ash free dry mass (AFDM), chl a content, algal biovolume), species composition and diversity, and gross primary production. An attribute restoration ratio was calculated by dividing attribute values from each restored marsh by values from the paired reference marsh. Controlling for regional variation in reference marshes substantially increased precision in relations between attributes and age of restored marshes. Organic matter restoration ratios of sediments increased with age of restored marshes in spring and summer. Algal biomass restoration ratios of epiphytes, calculated with algal biovolume and chl a, increased with restored marsh age in summer, but not during spring. In contrast, sediment algal biomass was not related to marsh age. The species diversity of sediment algae in summer showed an asymptotic relationship with sediment nutrient concentration. The similarity of diatom species composition between paired restored and reference sites increased with age of

restored marshes during both spring and summer. Primary production by epiphytic and sediment algae in summer showed site-specific changes and did not vary consistently with marsh age. Algal biomass, algal diversity, and diatom species assemblages during summer were positively correlated with sediment nitrogen and phosphorus concentration. Overall structural and functional development of restored wetlands, especially nutrient storage in sediments, regulates algal community structure and function, which can be used to evaluate marsh restoration success.

Key Words: salt marsh, restoration, algal attributes, succession, algal diversity, diatoms, biomass, nutrients, environmental variation

3.2 INTRODUCTION

Ecosystem restoration is one of the most pressing problems for applied ecologists (NRC 1992). With expanding urbanization and other human activities along coasts, many salt marshes have been degraded. A large effort has been mounted to stabilize these coastal ecosystems (Adam 1990). Along the US coast, hundreds of degraded or lost salt marshes have been restored in the past decades. Currently, few criteria exist to guide assessment of wetland restoration, so their evaluation is often superficial and subjective. Furthermore, very few reference data sets exist that can be used to compare restored habitats with natural habitats (Lockwood and Pimm 1999, Keer and Zedler 2002).

The most commonly used criterion for judging the success of ecosystem restoration is whether or not the restored community resembles the original (Adamus 1988). Developing criteria to evaluate progress in restoration can be done by characterizing development of a salt marsh ecosystem following initial restoration and identifying attributes in that marsh that increase in similarity relative to mature, existing marshes. Alternatively, multiple restored marshes with different ages could be compared to reference marshes to relate successional processes in restored ecosystems to a reference system (Lockwood and Pimm 1999). Living and nonliving organic matter, species diversity, and nutrient retention increase during ecosystem development (Odum 1969). Previous studies of restored salt marshes suggest that some of these attributes develop very slowly (e.g., 15 years for soil nutrient pools, Craft et al. 1988), while others recover within 1-5 years (Craft et al. 1991). Slow changes in biogeochemistry, water chemistry and plant and invertebrate species composition demonstrate the importance of long-term

research to characterize ecosystem succession (Hook 1988, Craft et al. 1991, Sacco et al. 1994, Scatolini and Zedler 1996).

Algae are important primary producers in salt marsh habitats and affect secondary production, nutrient retention, and biogeochemical cycling (Pomeroy 1959, Leach 1970, Gallagher and Daiber 1974, Van Raalte 1976, Zedler 1980, Sullivan and Moncreiff 1988, 1990, Pinckney and Zingmark 1993a, b). Algal photosynthesis can account for a large proportion of primary production in salt marshes, ranging from 25% to as much as 70% in some coastal marshes (Adamus 1988). Teal (1962) estimated that 47 percent of the total net primary productivity in salt marshes was lost through respiration by microbes, however, algal debris still contributed to more than one third of soil organic matter. Algae are also a major energy resource and provide the primary diet for metazoans and other invertebrate grazers (Kreeger and Newell 2000). Finally, nitrogen fixed by cyanobacteria is a very important nutrient resource in nitrogen limited salt marsh ecosystems (Jones 1974, Raalte et al. 1976, Sage and Sullivan 1978).

Algal species diversity, species composition, biomass, chemical composition, and productivity are most important attributes for assessing ecosystem structure and function in aquatic systems (Stevenson and Pan 1999). Algae, especially diatoms, have been widely used as indicators of ecological conditions in freshwater, marine, and terrestrial ecosystems (Shubert 1984, Steneck and Dethier 1994, Stevenson and Pan 1999, Sullivan and Currin 2000, Stevenson et al. 2001), but they have never been used to assess salt marsh condition. Because of their species-specific response to biogeochemistry (Shubert 1984), changes in diatom community structure and function could be used to assess restoration of wetland structure and function.

In this project, algal attributes in restored salt marshes of different ages were compared with reference marshes to answer the following key questions: 1) what timescales are required to achieve full restoration of algal-related attributes of salt marsh structure and function; 2) which indicators can be used to determine whether full restoration of salt marsh structure and function has been achieved; and 3) can ecological succession theory apply to salt marshes restoration? I hypothesized that 1) algae would rapidly colonize restored salt marshes and establish high primary production; 2) algal species diversity and organic matter on the surface of sediments would increase slowly over time and became more similar to reference sites with greater age of restored marshes; and 3) diatom species assemblages and organic matter in sediments would be important indicators of successful restoration.

3.3 METHODS

3.3.1 Site Locations

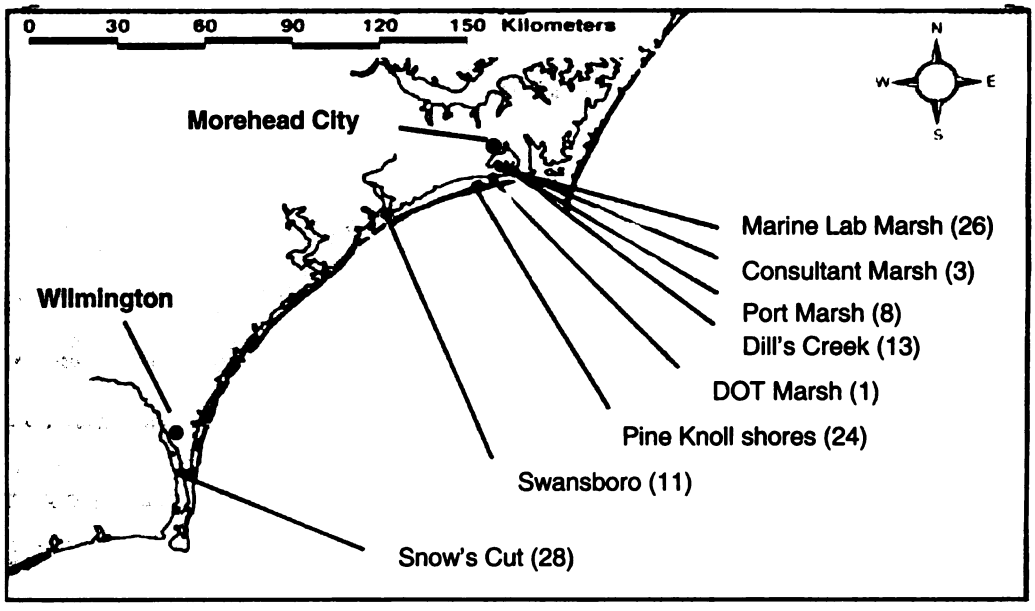

Figure 3.1. Map of sampling sites between Morehead City and Wilmington along the North Carolina coast. Age (yr) of restored marshes is reported in parentheses.

Salt marshes were sampled in the urbanizing area between Morehead City and Wilmington along the North Carolina coast (Fig. 3.1). The restored marshes examined were established for shoreline stabilization, mitigation of wetland loss, or research. The reference marshes were natural marshes near the restored marshes so that differences in water chemistry factors that affect algal assemblages, such as salinity and nutrient concentrations, would not vary due to regional factors. Each pair of wetlands received water from the same source. Eight pairs of salt marshes were selected and sampled in March 1998 (Figure 3.1): Long Beach (LB), Consultant Marsh (CS), Port Marsh (PT), Swansboro Marsh (SB), Dill Creek (DC), Pine Knoll (PN), Marine Lab (ML), and Snow's Cut (SC). All restored marshes were planted with *Spartina alterniflora* Loisel at the time of restoration. Long Beach and Pine Knoll marshes were dropped from the study in July 1998 because of the poor macrophyte growth and an additional pair of salt marshes (Department of Transportation (DOT)) was added.

To determine whether changes in developing salt marshes of North Carolina were similar to those in other regions, samples were collected from 3 pairs of restored and reference salt marshes in Chesapeake Bay at Virginia Beach, VA during summer 1999. The restored VA marshes were 2, 10, and 20 years old. These marshes represent very different habitats than those in North Carolina because they were far from the ocean, had salinity below 10 ppt, and were brackish to oligohaline.

3.3.2 Field Sampling

An area referred to as "the front marsh" was sampled. This area was approximately 1-2 meters from the levee away from the water and relatively low and flat. Sampling transects 30 to 50 m long were established parallel to the beach in this zone. Because some of these marshes did not have well-developed levee zones and several of them didn't have *Spartina* in back marshes, only the front marsh area was included in this study. *Spartina* stem density was measured in 0.25 m^2 plots (10 per marsh) in October 1998. Plant stem density in the front marsh zone of reference sites ranged from 201 to 316 stems/m^2 except in Pine Knoll marsh, which had much higher stem density (855/m^2) than any other reference sites. Restored sites had stem density ranging from 240 to 498 stems/m^2 (Table 3.1). Salinity was measured in the field using a refractometer. Surface soils (0-10 cm, n=10 per marsh) were collected in June 1998 and analyzed for total N and P as described in Craft et al. (1999). N and P concentrations in younger restored marshes were generally lower than its nearby reference marshes (Table 3.1).

Table 3.1. Sampling sites and their properties during the summer sampling season (from Robert Freese and Christopher Craft, unpublished data).

		Salinity(ppt)	Plant Stem density (/m^2)		Nitrogen (mg/g) in surface sediment		Phosphorus (mg/g) in surface sediment	
Age	Site		Reference	Restored	Reference	Restored	Reference	Restored
1	DOT	20-30	266	498	2.1	0.2	0.264	0.211
3	CS	17-32	208	304	2	0.2	0.368	0.143
8	PT	18-30	255	342	1.2	0.3	0.955	0.263
11	SB	20-30	316	398	4.8	0.4	0.545	0.409
13	DC	14-33	280	240	3	1	0.358	0.236
24	PN	20-30	855	512	0.3	0.6	0.453	0.370
26	ML	20-30	201	423	2.1	3	0.369	0.408
28	SC	5-20	271	297	4.4	3	0.492	0.371

Algal productivity on sediments and plants was measured during summer 1998. Eight sediment samples were collected from each transect by using plastic tubes (internal diameter = 3.8 cm, length = 20 cm) along each restored and reference site. Samples were taken early in the morning and brought back to the shore immediately. The sediments in the corers were covered with 30-ml of estuary water and then sealed air-tight with a thin, transparent plastic sheet and rubber bands. The cores from each transect were incubated in a light or dark chamber for 4 hours (4 cores in each treatment). Subsamples of 15 ml seawater were taken from each core to measure dissolved oxygen concentration (DO) using Winkler methods (APHA, 1998). The top 1-cm of sediment from each core was sampled and stored on dry ice for later analysis of algal biomass.

Epiphytic samples were collected from front marshes by cutting 1-3 randomly selected stems of *Spartina* from just above the ground and placing them into a zip-lock bag. Eight bags of stems were randomly collected in each zone of each marsh. All stems in a bag were washed using 200 ml of estuary water and removed from the bag. The epiphytes were left in the bag. All bags were then sealed underwater to avoid trapping air bubbles. The surface area of macrophytes from where the samples were taken was measured using a ruler. Four samples from each site were put in a light chamber, four were put in a dark chamber, and all were incubated for 4 hours. The experiments were done under light irradiance ranging from 800 to 2,200 μmole quanta$\cdot m^{-2}s^{-1}$, 32 ppt salinity, and a water temperature of 30±1°C. DO was measured using 60 ml subsamples before and after incubations. The remaining portions of samples were frozen on dry ice for later analysis of algal biomass.

3.3.3 Laboratory Analysis

In the laboratory, the frozen algae sample was homogenized and split into different volumes for assay of organic mass (30 ml), chlorophyll (15 ml), and separate microscopic assays for all algae (10 ml) and diatoms (10 ml). 200 ml of filtered seawater were added to sediment samples to enable subsampling. Dry mass (DM) and ash free dry mass (AFDM) were analyzed using standard methods (APHA 1998) to estimate sediment and epiphytic mass and organic matter. The organic matter proportion of sediments (OMP) was then calculated based on the organic proportion of total sediment (AFDM/DM). Chlorophyll a (chl a) contents of sediment algae and epiphytes in summer were measured using a Spectronic Genesys2® spectrophotometer (Spectronic Instruments) following standard methods (APHA 1998). Chl a and phaeophytin were related to area of ground and plant surface sampled.

Algal biomass and species composition of all algae and diatoms were determined by microscopically identifying and counting algae using two preparation techniques. After suitable dilution and settling, 300 natural units of algae in the chamber were identified and counted within a known portion of the samples. Non-diatom algae were recorded and identified to genus; diatoms were divided into 5 groups according to their length and width (less than 10, 11-20, 21-40, 41-60, and >60 μm) and counted. Soft algal volume was determined by measuring at least 10 individuals or colonies. Average volumes of diatom size groups were estimated by measuring 30 cells for each group. Total algal biovolume was calculated to provide a second measure of algal biomass with chl a. Diatom samples were mounted in Naphrax® after digestion using HNO_3 and $K_2Cr_2O_7$

(Stosch and Reimann 1970). Diatoms were identified to species and at least 500 valves were counted for each sample.

Among all pairs of restored and nearby salt marshes, I determined that Snow's Cut (28 year old) was a poor reference site for the nearby restored marsh, which was located in the middle of the Cape Fear River channel. Differences in algal assemblages between these marshes were probably related to large differences in salinity, nutrient input, and sedimentation. Therefore I decided to exclude this site from analyses that involved diatom species composition.

3.3.4 Statistical analyses

I used SYSTAT® 10 to calculate summary statistics, t-test for means, F statistics for variance, and Analysis of variance (ANOVA) to compare attribute characteristics between restored and reference marshes. Analysis of Covariance (ANCOVA) was used to compare slopes of two different regression lines.

Attribute restoration ratios were calculated to indicate relative condition in a restored site compared to the nearby mature reference marsh. A ratio equal to one meant that the attribute of the restored marsh was the same as in reference marsh. Thus, the organic matter restoration ratio (OMRR) was defined as (OMP in restored marsh)/(OMP in reference marsh). An algal chlorophyll restoration ratio (ACRR) was similarly calculated to relate chlorophyll concentrations in restored and reference marshes. The algal biovolume restoration ratio (AVRR) was defined as the ratio of total algal biovolume per unit surface area in a restored marsh vs. biovolume in the respective reference marsh and was calculated for both epiphytes and sediment algae. A primary production restoration

ratio (PRR) was defined as the ratio of gross primary production per unit surface area in a restored marsh vs. that in its reference marsh for both epiphytes and sediment algae.

A percentage similarity index (PSI) was used to indicate the similarity of diatom species composition in restored and nearby reference marshes (Gauch 1982). The index was calculated as

$$PSI = \Sigma \text{ minimum } (p_{1i}, p_{2i}),$$

where *PSI*= percentage similarity between samples 1 and 2, p_{1i} = percentage of the i^{th} species in the restored marsh (i = 1,....., k species), and p_{2i} = percentage of species i corresponding reference marsh.

PSI of algal functional groups was also calculated by comparing the biovolumes of functional groups in restored and reference marshes. Functional groups were defined by algal division and growth form (Steneck and Dethier 1994). All algae were divided into 9 functional groups: 5 diatom size groups, cyanobacteria, green algae, brown algae and red algae.

To evaluate successional patterns of change of algal attributes, linear regression analyses (SYSTAT ®10) were used to relate algal attributes, restoration ratios, and diatom community similarities to age of restored marshes in both spring and summer. Nonlinear regression analyses were also performed to relate changes in attributes to environmental variables (SYSTAT® 10). Canonical correspondence analysis (CCA) was performed using the computer program CANOCO (ter Braak, 1987) v4.02 to relate diatom species data to environmental variables. Sediment and epiphytic diatom taxa with relative abundances (taxa proportions of assemblages) greater than 1% in one or more sites during summer were included in our CCA with respect to the available

environmental variables: sediment total nitrogen, total phosphorus, salinity, stem density, OMP, and chl a. A forward-selected CCA was then used to determine which environmental variables accounted for the greatest amount of variance in the distribution of the diatom taxa. The significance of each variable added in this fashion was tested using a Monte Carlo permutation test with 99 unrestricted permutations ($P \leq 0.05$) (ter Braak 1990).

To determine the importance of regional factors, three pairs of restored and reference salt marshes from the Virginia coast were compared to the North Carolina summer sample set to determine how similar restoration patterns were between different regions.

3.4 RESULTS

3.4.1 Biomass Development

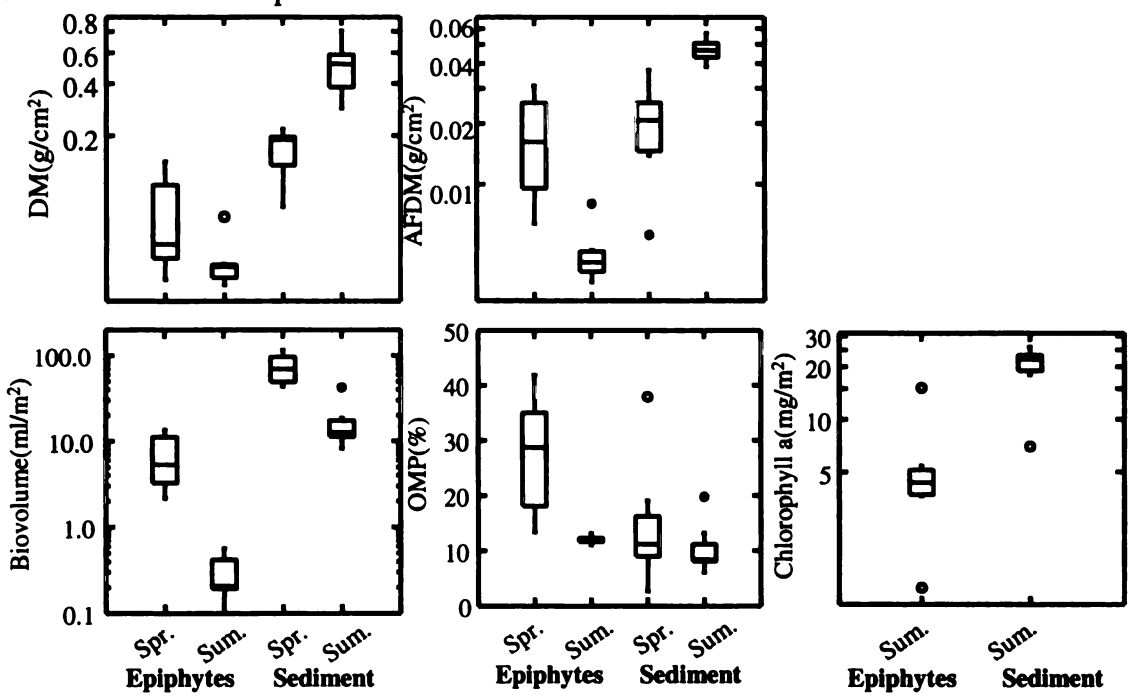

Figure 3.2. Box-plot of dry mass (DM), ash free dry mass (AFDM), the organic matter proportion of sediments (OMP), algal biovolume, and chl a samples from epiphytes and sediments in reference marshes during spring (8 samples) and summer 1998 (7 samples).

Characteristics of sediments (DM, AFDM, and OMP) and algal biomass (chl a and biovolume) in reference marshes differed between macrophytes and surface sediments (Figure 3.2). During spring, DM and algal biovolume on macrophytes were much lower than in surface sediments (t-test, $P<0.05$), AFDM on macrophytes was not different from sediment ($P>0.05$), but the OMP on macrophytes (27.3±2.8 %) was higher than in sediment (14.2±3.7 %)($P<0.05$). During summer, DM, AFDM, algal biovolume, and chl a on macrophytes were all significantly lower than in surface sediments ($P<0.05$), but OMP on macrophytes did not differ from sediment ($P>0.05$).

Sediment mass (DM and AFDM), algal biovolume, OMP on macrophytes, and algal biomass in surface sediment were higher in spring than summer ($P<0.05$). Measures of sediment mass (DM and AFDM) were less in spring than summer on surface sediment ($P<0.05$) (Figure 3.2).

DM, AFDM, and OMP in restored marshes were not related well to restored marsh age ($P>0.05$). However, the organic matter restoration ratio (OMRR) on macrophytes and in sediment during spring was linearly related to age of restored marshes (Figure 3.3). During summer, the OMRR on macrophytes was not related to age of restored marshes and the OMRR in sediments again significantly increased with age of restored marshes in a manner not statistically different from that observed in spring (ANCOVA, $P=0.450$).

Algal biomass in young restored marshes was about half that observed in mature reference marshes. In general, algal biomass restoration ratios increased with age of restored marshes. AVRR was not related to restored marsh age during spring either on macrophytes and in sediments ($P>0.05$) (Figure 3.3e-f). During summer, sediment AVRR and sediment ACRR were not related to restored marsh age, but epiphytic AVRR and ACRR were significantly related to age of restored marshes (Figure 3.3g-j). The AVRR

patterns were statistically similar to the ACRR pattern for epiphytic and sediment algae in summer (ANCOVA, P=0.375 and P=0.453 respectively).

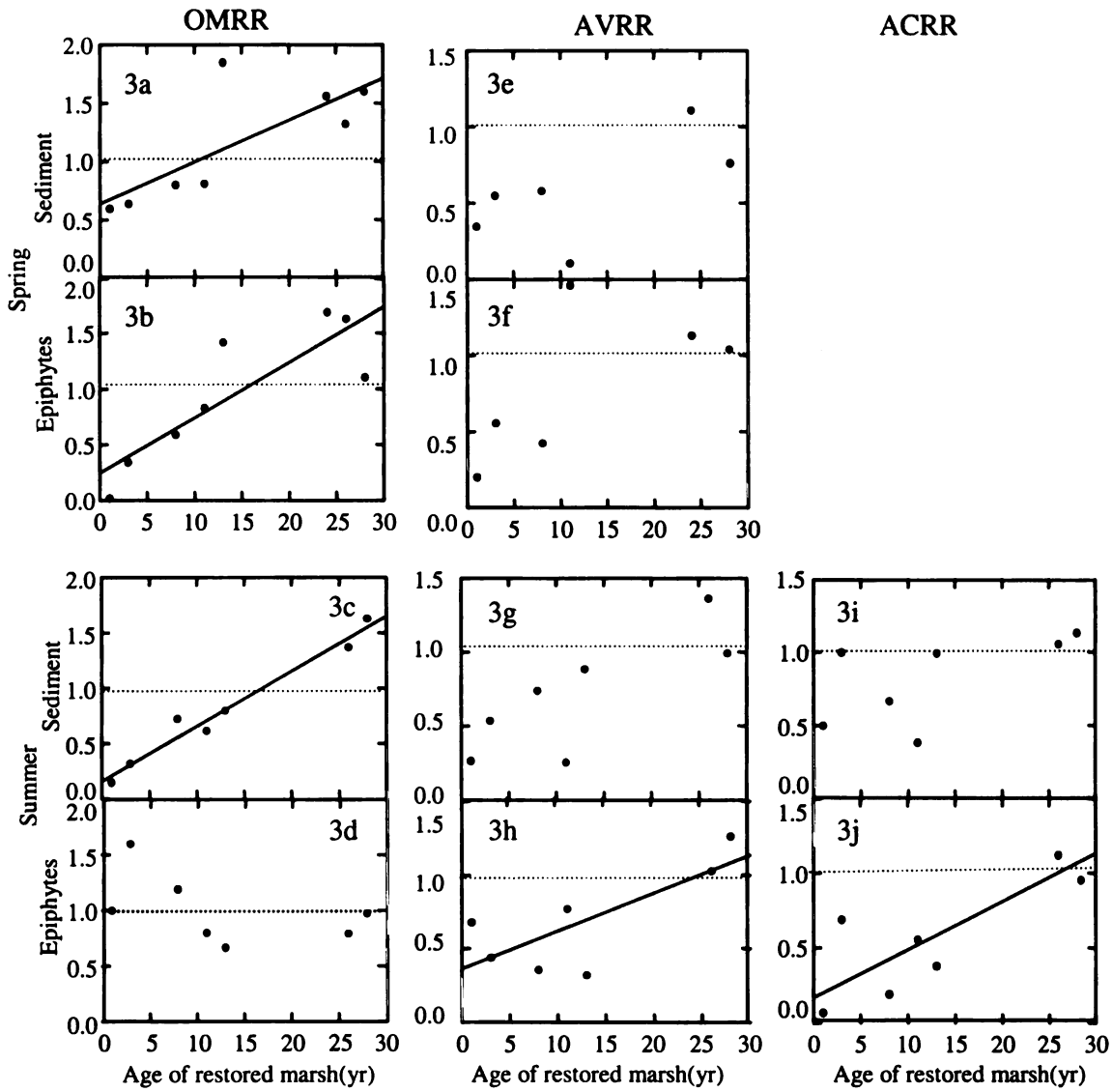

Figure 3.3. Relationship between the attribute restoration ratio and age of restored marsh in surface sediment and on macrophytes during spring and summer 1998. The OMRR is the comparison of OMP of sediments in a restored site with that of its reference marsh. The ACRR is the comparison of algal chlorophyll a concentration in a restored site with that of its reference marsh. The AVRR is the comparison of algal biovolume in a restored site with that of its reference marsh. The line represents a significant relationship (linear regression $P<0.05$). The correlation coefficients (R^2) for each relationship are: 3a: 0.727; 3b, 0.931; 3c, 0.969; 3d, 0.235; 3e, 0.304; 3f, 0.647; 3g, 0.577; 3h, 0.749; 3i, 0.442; 3j, 0.741.

In summer, sample OMP increased significantly with increasing nitrogen and phosphorus concentration of surface sediments (Table 3.2). Sediment chl a concentration was asymptotically related to sediment nitrogen concentration and was barely correlated with surface phosphorus concentration (Table 3.2). Sediment algal biovolumes showed the same correlation with N and P as chl *a*. All three attributes were not significantly related with macrophyte stem density (Table 3.2).

Table 3.2. Summary of regression analyses between algal attributes and environmental variables. Each marsh (both restored and reference marshes) was treated as one site.

Attributes	Environmental variables	Adj R^2	n	Equation	P(2 tail)	Model
		0.372	14	5.409+0.192X	0.012	linear
OMP	TP	0.531	13	-0.870+30.306X	0.003	linear
	Stem density	0.464	14		0.465	none
	TN	0.459	14	23.278 + (X /(0.311+ X))	0.039	asymptotic
Chlorophyll	TP	0.232	13	5.501+33.551X	0.055	linear
	Stem density	0.119	14	8.558+0.029 X	0.227	linear
	TN	0.505	14	4.841+4.502X	0.003	linear
Biovolume	TP	0.564	13	-8.015+15.990X	0.002	linear
	Stem density	0.039	14	2.317+0.037X	0.241	linear
	TN	0.540	14	3.546 * (X / (X+0.024))		asymptotic
Biodiversity	TP	0.419	13	3.714 * (X / (X + 0.026))		asymptotic
	Stem density		14			none

3.4.2 Algal Species Diversity and Composition

Table 3.3. Number of species found in all sites, habitats and seasons.

	Habitat	No. of algal species				No. of diatom taxa			
Season	type	Res.	Ref.	Total	Common	Res.	Ref.	Total	Common
Spring	Sediment	204	218	292	137(46.9%)	212	197	277	132(47.7%)
	Epiphyte	167	170	225	104(46.2%)	155	149	205	98(47.8%)
Summer	Sediment	164	159	205	117(57.1%)	154	156	198	114(57.6%)
	Epiphyte	131	118	161	85(52.8%)	112	123	150	81(54%)

A total of 420 algal taxa, including 400 diatom taxa, were found on all substrates in the spring and summer samples. Of them, 362 algal taxa were found during spring and the majority (342 and 94.5%) were diatom taxa. Generally, more algal species were observed in sediment samples (292 taxa) than on plants (225 taxa) (Table 3.3). Fewer algal species were found in summer than in spring. Two hundred fifty-two algal taxa with 241 diatom taxa were found in all sampling sites in summer with more algal species (205 algal taxa) in sediments than on plants (161 species) (Table 3.3).

Restored and reference marshes shared fewer algal taxa (about 47%) and fewer diatom taxa (about 48%) in spring than in summer (>52.8%)(Table 3.3). Shannon diversity indices of epiphytes varied from 2.02 to 3.10 in restored marshes and 2.59 to 3.00 in reference marshes in summer, Shannon indices of sediment algae ranged from 2.93 to 3.55 in restored marshes and from 3.36 to 3.58 in reference marshes. Generally, the index values for restored sites in summer were more variable than for reference marshes (F test, P=0.002 for epiphytes and P=0.016 for sediment algae). However, neither the epiphytic nor sediment diversity indices differed between restored sites and reference sites (P>0.05). Asymptotic relationships were observed between sediment algal diversity (*H'*) and sediment nitrogen and phosphorus concentrations (Table 3.2) in summer.

Algal species were mostly composed of diatoms, filamentous green algae, and cyanobacteria. The most frequently observed soft algal genera were *Enteromorpha* spp., *Rhizoclonium* spp., *Oscillatoria* spp., and *Schizothrix* spp. *Enteromorpha* was distributed patchily in most marshes; however, because of the difficulty of quantifying the occurrence of macroalgal species such as *Enteromorpha* spp. and relative few numbers of

macroalgae taxa, they were not used to compare species composition of algal assemblages. The most common epiphytic algal group was diatoms with an average of 75% (±13% SE) of total algal biovolume in spring and 89% (±4.8%) in summer. Diatoms also were dominant in sediment assemblages, with an average of 73% (±12%) of total biovolume in spring and 93% (±4%) in summer. Epiphytic cyanobacteria algae were 0.3% to 35.2% of total algal biovolume in restored marshes and from 0.04 to 22.3% in reference marshes. Sediment cyanobacteria were less than 10% of algal biovolume in both restored and reference marshes in spring.

PSI of functional group biovolume between restored and its respective reference marshes did not change with restored marsh age in spring or summer (Figure 3.4a, b). Most of the paired sites had high similarity of functional group composition (larger than 50%) on both macrophytes and sediments.

Diatom composition on macrophytes and in sediment shifted seasonally. The most dominant epiphytic species on macrophytes in spring were *Melosira nummuloides* Agardh, *Denticula subtilis* Grunow, *Synedra fasiculata* Grunow, and *Navicula perminuta* Grunow. During summer, *M. nummuloides* became much less abundant, while *Nitzschia nana* Grunow, *Amphora coffeaeformis* (Agardh) Kutzing, and *Nitzschia frustulum* Grunow became the most dominant epiphytic species. Dominance of sediment diatom assemblages changed little from spring to summer. With *M. nummloides*, *A. coffeaeformis*, and *Rhaphoneis surirella* (Ehrenberg) Grunow being most common (Table 3.4).

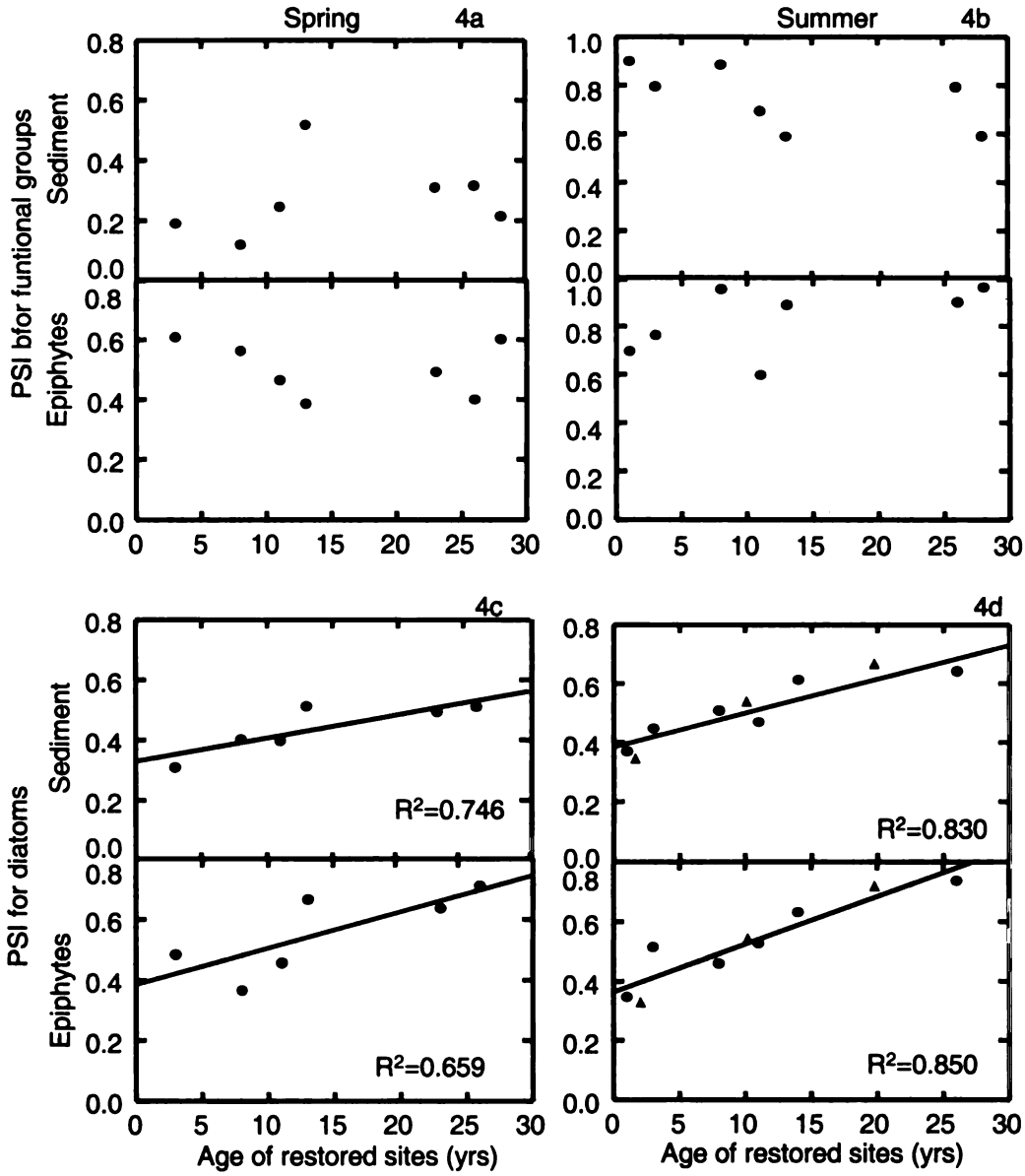

Figure 3.4. Relationship between the percentage similarity index (PSI) of algal functional groups (4a, 4b) and diatom assemblages (4c, 4d) and the age of restored marshes in surface sediments and on macrophytes during spring and summer 1998. PSI was calculated for algal functional groups or diatom assemblages between restored and reference marshes. The line represents a significant relationship (linear regression $P<0.05$). The triangles represent sites in Virginia, and dots represent sites in North Carolina.

PSI of diatom species composition between restored and reference marshes increased with age of restored salt marshes both on macrophytes and in sediments in the spring

(Figure 3.4 c). The rate of change in PSI between restored and reference marshes with age of restored marshes did not differ between epiphytic and sediment algae (ANCOVA, P=0.602). The PSI changed from 37% to 70% on epiphytes and from 30% to 50% in sediment.

Similarity in epiphytic and sediment diatom communities between restored and reference marshes during summer also increased with increasing age of restored salt marshes (Figure 3.4d). The rate of change in PSI between restored and reference marshes with age of restored marshes did not differ between epiphytic and sediment algae (ANCOVA, P=0.160). The similarity changed from 30% to 73% on macrophytes and from 36% to 65% in sediment.

Canonical correspondence analysis of epiphytic diatom assemblages showed that 26.8 of the total variance in the species data was explained by the first two axes. The first axis was correlated with OMP (r=0.769), nitrogen (r=0.495), chl a (r=0.524), and salinity (r = -0.467); the second axis was correlated with stem density (r=0.493), salinity (r = -0.474) and OMP(r=0.394). Similarly, CCA of sediment diatom assemblages and environmental variables showed that 26.2% of the species variance was explained by the first two axes. The first axis was negatively correlated with OMP (r=0.617), nitrogen (r=0.503), and chl a (r=0.490); the second axis was negatively correlated with salinity (r = -0.737).
In epiphytic samples, *Achnanthes exigua var. elliptica* Hustedt, *Actynoptychus splendens* Ralfs, *and Skeletonema costatum* (Greville) Cleve dominated in high N concentration sites, while *Navicula pygmaea* Kutz., *Nitzschia clausii* Hantzsch, and *Amphora proteus* Gregory dominated in low N environments. In sediment samples, *Plagiogramma pygmaeum* Grev., *Actinoptychus splendens* (Shadbolt) Ralfs in Pritchard, *Gyrosigma balticum* (Ehrenberg) Rabenhorst, *Diploneis papula* (A. Schmidt) Cleve, *Amphora gigantean* Grunow ex A.

Schmidt were negatively correlated with nitrogen concentration. With an increase of nitrogen concentration, diatom species composition shifted to *Nitzschia debilis* (Arnott) Grunow.

3.4.3 Productivity

Gross primary production (GPP) of algae had a wide range during summer. The GPP of epiphytes ranged from 3.832 to 75.338 mg C m^{-2} h^{-1} in reference marshes, and from 6.503 to 137.068 mg C m^{-2} h^{-1} in restored marshes. The sediment algal GPP showed less variation, ranging from 4.56 to 16.56 mg C m^{-2} h^{-1} in reference marshes and from 4.08 to 46.981 mg C m^{-2} h^{-1} in restored marshes. PRR did not change significantly with age of restored marshes either for epiphytes or sediment algae (Figure 3.5). PRR was generally higher in younger marshes. Great variation among different pairs of marshes was observed, but age of restored marshes was not a determinant of algal productivity in this study.

3.4.4 Algal Attribute Development In Virginia Salt Marshes

DM, AFDM, OMP, and algal biovolume in Virginia salt marshes varied among different marshes and did not change with age of restored marshes. Sediment DM (0.182 ± 0.043 g/cm^2) of Virginia reference marshes was lower than DM in North Carolina reference marshes ($P<0.05$); AFDM (0.033 ± 0.010 g/cm^2) and OMP in sediment (18.2 ± 3.5%) were similar to North Carolina marshes. DM on plants (0.43 ± 0.12mg/cm^2),

Table 3.4. The 10 most common diatom species in all sites (sorted by relative abundance rank, habitat type, marsh type, and season).

	Edaphic diatoms		Epiphytic diatoms	
	Reference marshes	Restored marshes	Reference marshes	Restored marshes
Spring	*Melosira nummuloides* Agardh	*Melosira nummuloides* Agardh	*Melosira nummuloides* Agardh	*Melosira nummuloides* Agardh
	Navicula phyllepta Kutzing	*Achnanthes haukiana* Grunow	*Denticula subtilis* Grunow	*Denticula subtilis* Grunow
	Achnanthes haukiana Grunow	*Navicula halophila* (Grunow)	*Synedra fasciculata*(Agardh)	*Nitzschia brevissima* W. Smith
	Rhaphoneis surirella	Cleve	Kutzing	*Synedra fasciculata* (Agardh)
	(Ehrenberg)Grunow	*Nitzschia dissipata (*Kutzing)	*Navicula. sp*	Kutzing
	*Nitzschia dissipata (*Kutzing)	Grunow	*Navicula perminuta* Grunow	*Navicula perminuta* Grunow
	Grunow	*Nitzschia thermaloides* Hustedt	*Nitzschia laevis* Hustedt	*Navicula sp.*
	Amphora coffeaeformis (Agardh)	*Rhaphoneis surirella*	*Rhaphoneis surirella* (Ehrenberg)	*Nitzschia laevis* Hustedt
	Kutzing	(Ehrenberg)Grunow	Grunow	*Berkeleya fennica* Juhlin-Dannfe
	Berkeleya fennica Juhlin-Dannfelt	*Amphora coffeaeformis* (Agardh)	*Achnanthes haukiana* Grunow	*Nitzschia dissipata (*Kutzing)
	Cymatosira belgica Grunow	Kutzing	*Nitzschia frustulum* Grunow	Grunow
	Navicula perminuta Grunow	*Navicula phyllepta* Kutzing	*Navicula halophila* Grunow	*Amphora coffeaeformis* (Agardh)
	Eunotogramma marinum	*Nitzschia aequorea* Hustedt		Kutzing
	(W.Smith) H. et M.	*Denticula subtilis* Grunow		
Summer	*Melosira nummloides* Agardh	*Melosira nummloides* Agardh	*Nitzschia nana* Grunow	*Denticula subtilis* Grunow
	Amphora coffeaeformis (Agardh)	*Amphora coffeaeformis* (Agardh)	*Denticula subtilis* Grunow	*Nitzschia frustulum* Grunow
	Kutzing	Kutzing	*Navicula sp.*	*Amphora coffeaeformis* (Agardh)
	Denticula subtilis Grunow	*Denticula subtilis* Grunow	*Amphora granulata* Greg.	Kutzing
	Paralia sulcata (Ehrenberg)	*Achnanthes exigua* Grunow	*Amphora coffeaeformis* (Agardh)	*Navicula sp.*
	Cleve	*Cymatosira belgica* Grunow	Kutzing	*Nitzschia nana* Kutzing
	Rhaphoneis surirella (Ehrenberg)	*Paralia sulcata* (Ehrenberg) Cleve	*Nitzschia frustulum* Grunow	*Nitzschia inconspicua* Grunow
	Grunow	*Nitzschia inconspicua* Grunow	*Navicula cryptocephala* Kutzing	*Achnanthes haukiana* Grunow
	Cymatosira belgica Grunow	*Opephora pacifica* (Grunow) Petit	*Melosira nummuloides* Agardh	*Cymatosira belgica* Grunow
	Achnanthes haukiana Grunow	*Nitzschia frustulum* Grunow	*Nitzschia dissipata (*Kutzing)	*Navicula cryptocephala* Kutzing
	Achnanthes exigua Grunow	*Rhaphoneis surirella* (Ehrenberg)	Grunow	*Nitzschia brittoni* Hagelst
	Nitzschia frustulum Grunow	Grunow	*Nitzschia inconspicua* Grunow	
	Nitzschia inconspicua Grunow			

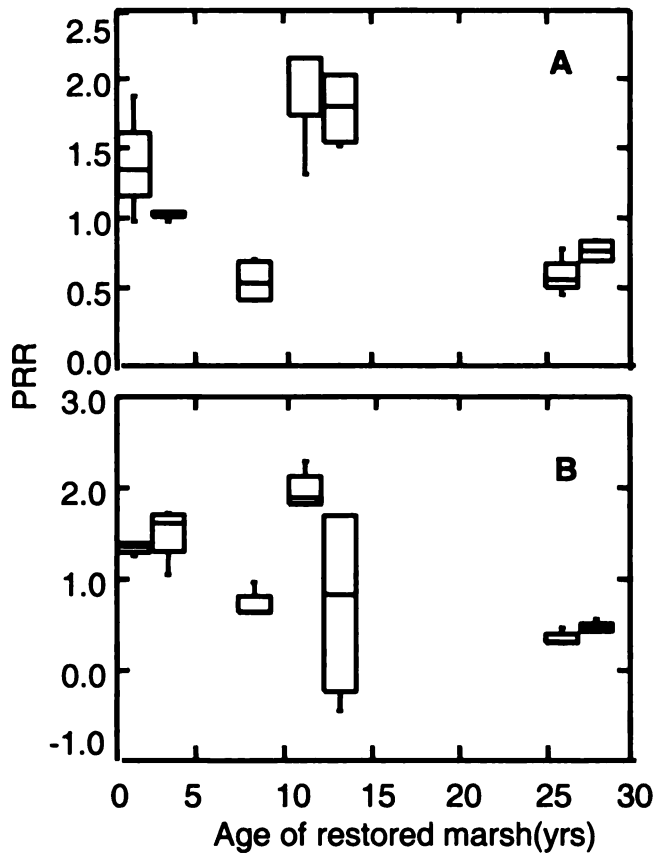

Figure 3.5. Relationship between algal productivity ratio and age of restored marshes in summer 1998. Productivity ratio compared between algal GPP between restored and reference sites in surface sediments (A) and on macrophytes (A).

AFDM ($0.03 \pm 0.01 mg/cm^2$), and OMP (<20%) were lower than in North Carolina marshes. OMRR in Virginia did not fit well in the OMRR pattern found in North Carolina.

Shannon's species diversity indices in reference marshes varied from 1.72 to 2.64 on macrophytes and from 2.86 to 3.43 in sediments, which were similar to that in North Carolina marshes. The diversity index in a restored marsh was most similar to its reference marsh but did not vary with age of restored marshes. PSI of diatoms in Virginia marshes fit well into the PSI pattern with restored marsh age for North Carolina (Figure 3.4d).

3.5 DISCUSSION

The species composition of algae in salt marshes is determined by multiple environmental variables and clearly responds to age of restored marshes. Previous studies (Pinckney and Zingmark 1993b, Mayer and Galatowitsch 1999) questioned the use of diatom assemblages as indicators of ecological restoration in wetlands because of the high degree of environmental heterogeneity in reference wetlands. Controlling for regional and habitat variation by comparing restored marshes to nearby reference marshes enabled us to increase the precision in relations between algal attributes and the age of restored marshes. Algal biomass, algal species composition, and organic matter responded, like invertebrate, soil, and plant attributes (Craft 1988, 1991, Sacco et al. 1994, Scatolini and Zedler 1996), to age of restored wetlands. Observed relations between algal attributes and other environmental factors provided the base for hypotheses as to how changing wetland processes that regulated algal attribute restoration.

A number of spatial and temporal factors are account for variation of organic matter in soil sediment and sediment on plants. Organic matter in Mississippi salt marshes ranged from 14.3 to 23.3% by weight of the soil surface sediments (Sullivan and Moncreiff 1988). OMP in our reference marshes in North Carolina was more variable among different marshes than in Mississippi but remained constant with depth in soil cores. OMP decreased with depth in restored marshes indicating gradual accumulation of organic matter over time (Craft et al. 1999). Sediments on macrophytes had higher OMP than soil surface sediments during spring, probably because of a larger algal proportion of total mass on plants and less inorganic matter (silt) than in sediment. So I presumed that

the OMP on plants could be a different indicator of algal biomass change over time than sediment OMP. Seasonal variation of OMP in NC salt marshes showed higher OMP during spring than summer, which was probably caused by high organic matter input from runoff and input from rivers and high tides during spring (Fong and Zedler 1993).

Algal biomass in North Carolina salt marshes, represented by algal biovolume and chl a concentration, was higher in spring than summer on both macrophytes and in surface sediments. Seasonal succession of the algal community from large green macroalgae to mainly diatom-dominated microalgal communities may contribute to that variation (Mitsch and Gosslink 2000). In North Carolina salt marshes, the diatom proportion of biovolume was much higher during summer than in spring. In addition to changes in algal growth form, low nutrient concentrations in summer in the water column of North Carolina salt marshes and the importance of sediment nutrients in regulating algal growth (Larned 1998, Fong et al. 2001) also indicated that nutrients were important in regulating algal biomass and caused the seasonal variation. Relatively high nutrient availability and low grazing pressure by meiofauna, junvenile macrofauna, and fiddler crabs during spring are commonly related to seasonal variability in algal biomass (Sullivan and Daiber 1975, Darley et al. 1981, Montagna et al. 1983, Wear et al. 1999).

Algal biomass also varied among habitats in salt marshes (Pinckney and Zingmark 1991, 1993b). Sediment algal biomass was much higher than epiphytic algal biomass in the North Carolina salt marshes. Vertical migration of algae in sediments enables occupation of a 3-dimensional habitat that can extend to at least the upper 5 mm. Thus a much thicker algal accumulation in surface sediments (Leach 1974) can support a much higher areal algal biomass than on macrophytes. The increase of *Spartina* canopy density,

water column turbidity, and different species of marsh plant (Pip and Robinson 1984) may also affect the light availability for algal growth on macrophytes (Colijn 1982, Zedler 1993) and lead to varied distribution of algal biomass in different salt marshes.

Algal diversity and species composition were highly related to N concentrations and sediment organic matter in North Carolina salt marshes as in other marshes (McCormick and O'Dell 1996, Stevenson et al. 1999, Pan et al. 2000). Shannon diversity of sediment algae was positively correlated to sediment nutrient concentration, especially TN, indicating a release of constraints on species membership in assemblages by N limitation. Algal species diversity was higher during spring than summer, which may have been due to higher N runoff to upstream watersheds. Shifts in diatom species composition on plants and sediments were also related to OMP and TN concentration in sediments.

The relationships between algal biomass and species composition with sediment N and OMP in NC salt marshes are likely due to organic matter sources of N and release of algae from N limitation. Algae as well as macrophytes in many estuarine habitats can be N limited (Cargill and Jefferies 1984, Pedersen and Borum. 1996, Kiehl et al. 1997, van Wijnen 1999). Increases in sediment organic matter increase N supply to macrophytes such as *Spartina* (Kiehl et al 1997, Craft 2001). Benthic algae can acquire nutrients that leak from plants and sediments as well as from the water column (Moeller et al 1988, Burkholder and Wetzel 1990, Fong and Desmond 1997, Fong et al. 2001). Sediment algae probably get higher proportions from their substratum because of proximity to the two nutrient supplies. If nutrient concentrations in the water column are lower during summer than spring due to runoff patterns and if nutrient fluxes from sediments stay relatively constant, then seasonal effects of nutrient supply should be greater for

epiphytic algae than sediment algae, as I observed. In addition, the relationships between algal biomass and nutrients were stronger for epiphytic than sediment algae and during summer than spring. Thus N limitation was probably related to organic matter in sediments and was greater during summer than spring and for epiphytic than sediment algae in NC marshes.

Despite variability in salt marsh algal assemblages related to season, habitat, OMP and N, I was still be able to detect relations between OMRR in sediment, AVRR and ACRR on macrophytes during summer, and PSI of diatoms on plants and sediments increased with age of restored marshes. Despite regional differences, the similarities of diatom assemblages between 3 pairs of restored and reference marshes in Virginia fit well into the similarity-age relationship found in North Carolina. Most processes of benthic algal community development probably occur on much shorter time scales than the 10-15 years required for the algal community development observed in restored NC salt marshes. Algal immigration, growth, and community development occur during times scales ranging from weeks to months, not years (Fisher et al. 1982, Stevenson 1990, McCormick and Stevenson 1991). Therefore, exogenous processes at scales greater than the periphyton community, perhaps at the wetland scale, probably regulate succession of algal assemblages after the first year of wetland restoration (Figure 3.6). Relations between organic matter, algal biomass on macrophytes during summer, and algal species composition indicated a linkage between algal community development in microhabitats of marshes and the larger-scale successional process of organic matter accrual in wetlands. Organic matter accrual in restored NC salt marshes requires at least 15 years before it reaches the level of reference marshes (Craft et al. 1999, Craft 2000).

The increase in organic matter in salt marshes probably regulated benthic algal succession indirectly during marsh restoration through control of N supply. CCA of species-environment relationships showed that the difference in nutrient concentration between restored and reference marshes was one of the main causes of species assemblage differentiation. The increased nutrient concentration in restored marshes with age of marsh correlated with similarity of diatom assemblage to reference marshes. Epiphytic algal biomass was constrained by nutrient limitation during summers, probably due to N limitation. Thus, development of algal species composition on macrophytes and sediments and algal biomass on plants during summer was probably regulated by biogeochemical processes in wetlands and may be valuable indicators of those processes (Figure 3.6)(Sullivan 1975).

Due to the high spatial and temporal variation of algal biomass and productivity, no relations were found between algal primary productivity or respiration and age of restored marshes. A number of studies have been done to accurately measure the biomass and production in estuarine habitats (Sullivan and Moncreiff 1990, Pinckey and Zingmark 1993a, b). Sediment grain size, sediment moisture, ambient irradiance, salinity, H_2S concentration, temperature, pH, and nutrients all affected algal production and biomass (Frey and Basan 1985, Sullivan 2000). Estimates of annual sediment primary production varied from 4 to 267 g C m^{-2} yr^{-1} depending on different marsh types and nutrient conditions (Leach 1976, Zedler et al. 1978, Zedler 1980, Pinckney and Zingmark 1993b). Although algal productivity is a very important attribute of algal community function, it is also a complicated attribute and can easily be affected by microbial activity, macrophyte shading, irradiance, and algal biomass (Sullivan 2000). Algal primary

production on macrophytes and in sediments in my studies were also subject to a wide range of variation, but all were within the range of previous results. Spatial and temporal variability and difficulties in measuring primary production in field settings probably contributed to variation in this study and the lack of strong relations between algal productivity, biomass, and wetland development.

I hypothesize that marsh-wide algal productivity should increase relatively rapidly during restoration. Algal productivity on macrophytes during summer should have increased with algal biomass on plants and restored marsh age due to release from N limitation. However, algal biomass in sediments was much higher than on plants and was not as sensitive to age of restored marshes as algae on macrophytes. Sediment algae should be exposed to higher nutrient concentrations than algae on macrophytes. In addition, irradiance and photosynthetic rates should be higher during early stages of restoration because plant density is lower. Thus, limitation of algal biomass and productivity on a marsh-wide scale is probably not as sensitively related to organic matter build-up and development of biogeochemical cycling of nutrients as algal species composition and epiphytic algae biomass (Figure 3.6).

In summary, diatom species composition, epiphytic algal biomass in summer, and organic matter proportions in sediments are good indicators for success of salt marsh restoration when regional variables are controlled by comparing a restored marsh to a nearby mature, reference marsh. Historic problems with use of algal assemblages as indicators of wetland restoration were related to variability among wetlands (Pinckney and Zingmark 1993b, Mayer and Galatowitsch 1999), which is a common problem

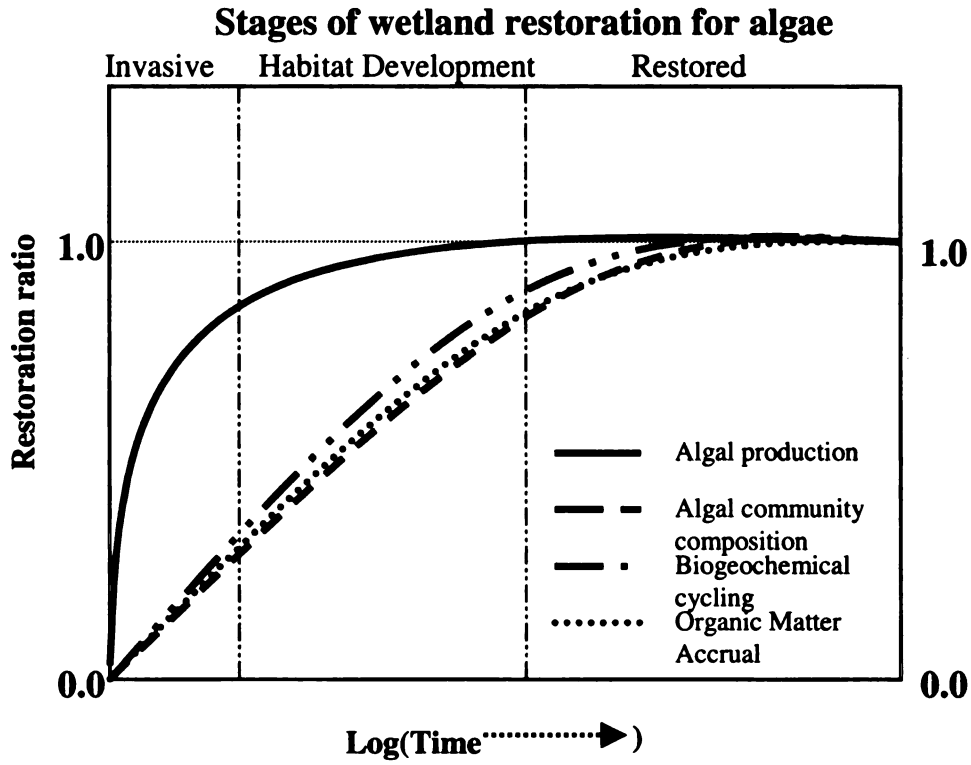

Figure 3.6. Conceptual model of algal structural and functional development during marsh restoration.

in ecological assessment. Precise characterization of expected reference conditions calls for classifying wetlands based on the natural regulatory factors that affect biota, such as climate, hydrology, geochemistry, and salinity in coastal environments (Hawkins et al. 2000, USEPA 2002a, Stevenson et al. in review). Thus, precision of algal assessments of wetland restoration, as any wetland assessments, should be greatly enhanced by identifying the class of restored wetland and comparing observed conditions to conditions found in relatively natural, reference wetlands of the same class (Stevenson 2001, USEPA 2002b). With increasing understanding of algal ecology in wetlands, algal sensitivity to environmental conditions, and new developments in assessment protocols,

great potential exists for diagnosing factors that retard wetland restoration, establishing criteria for wetland restoration, and improving assessments.

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Chapter 4

Relations between nutrient conditions, algal growth, and species composition in the restored wetlands of southern Michigan

4.1 Abstract: Nutrient limitation is one of the main factors regulating periphyton growth and species composition in wetlands. To study nutrient limitation of algae and their response to nutrient addition in restored wetlands, I placed nutrient diffusing substrata in 22 restored wetlands for 3 weeks in southern Michigan during the summer of 2000. Physical and chemical variables, as well as epiphytic, sediment, and planktonic algal assemblages were evaluated during the experimental period. Nitrogen (N) was the main limiting nutrient for periphyton growth in restored Michigan wetlands. Algal N limitation was positively correlated with phosphorus (P) concentration and negatively correlated with N:P ratios in wetland water and sediment. Canonical correspondence analysis (CCA) indicated that changes in diatom assemblages in natural habitats and control artificial habitats were correlated mainly to pH and secondarily to nutrient, primarily total phosphorus (TP) and dissolved inorganic nitrogen (DIN). Individual diatom taxa responded to nutrient addition differently. Overall, the responses of indicator species in nutrient bioassays were positively correlated with natural nutrient gradients among natural wetlands indicating a good match of diatom indicators between natural and experimental systems.

4.2 Introduction

Wetlands have increasingly been impacted by nutrient input from point and non-point sources (Carpenter et al. 1998). A considerable body of evidence indicates nutrient pollution not only alters the structural composition of a biological community in aquatic systems, but also changes decomposition rates, productivity, and other ecosystem functions (Craft et al 1995, Newman et al 1996, Bedford et al. 1999, Craft and Richardson 1997, Qualls and Richardson 2000, Svengsouk and Mitsch 2001). In natural communities, nutrient enrichment and shifts in resource ratios will lead to changes in algal production and shifts in species composition (Tilman 1981,Tilman et al. 1986, Sommer 1993). Thus, identifying the limiting nutrients in wetlands could help us understand factors regulating community structure and function and better manage these valuable ecosystems.

The primary limiting nutrients in aquatic systems are usually either nitrogen (N), phosphorus (P), or both, depending on different systems and geological regions (Downing et al. 1999, Bedford et al. 1999). Early studies of nutrient limitation in freshwater systems used multiple approaches to show P limitation of phytoplankton ranging from algal cultures in incubators to phytoplankton response to whole lake fertilizations (Emery et al. 1973, Hecky and Kilham 1988, Elser 1990, Sommer 1993, Wurtsbaugh et al 2001). Periphyton bioassays have also been used to identify nutrient limitation in lotic systems (Bothwell and Stockner 1980, Elwood et al. 1981, Peterson et al. 1983, Grimm and Fisher 1986, Mulholland et al. 1991, Francoeur et al. 1999, Chambers et al. 2000, Wurtsbaugh et al. 2001) and have shown relations among region, geology, and N or P limitation. Boreal and temperate eastern and central streams are

generally P-limited (Peterson et al. 1983), while temperate western and southwestern streams in landscapes of volcanic or tectonic origin are generally N-limited (Grimm & Fisher 1986).

Few studies have examined nutrient limitation and enrichment in wetlands (Hooper-Reid and Robinson 1978, Bayley et al. 1985, Rader and Richardson 1992, McDogal et al. 1994, McCormick et al. 1996, 2001, Pan et al. 2000), where plants and algae can be N or P limited depending on wetland type. It is widely assumed that most freshwater wetlands are P-limited in north temperate zone of North America, but this conclusion is based on little data (Goldsborough and Robinson 1996). A meta-analysis on nutrient limitation and plant diversity suggested (Bedford et al. 1999) a large proportion of North American wetlands, especially bogs and fens, were either P limited or co-limited by N and P. They also used N: P ratios in plant tissues and surface soils to predict that marshes and the majority of swamps were N limited. Aquatic systems in Southern Michigan were found to be mostly P-limited (Hart and Robinson 1990, Fahnenstiel et al. 2000, Wetzel 2001) or not limited (Hamilton 2000) by nutrients. But N: P ratios in ground water and precipitation were generally very high, which also indicates P-limitation (Hamilton, Personal communication).

Experimental determination of algal nutrient limitation at a regional scale has never been conducted in wetlands. Nutrient diffusing substrata have been an effective way to determine nutrient limitation in stream and lake systems at regional scales (Grimm et al. 1981, Lowe et al. 1986, Pringle et al. 1986, Genter et al. 1987, Hecky and Kilham 1988, Carrick et al. 1993, Wurtsbaugh et al. 2001). They have also been used to determine

nutrient limitation and algal production in individual wetlands (McCormick et al. 1996, 2001, Pan et al. 2000).

The main purpose of this research was to examine the effects of nutrient enrichment on periphyton production and algal species composition in restored wetlands. Field experiments were conducted with nutrient diffusing substrata to determine the effects of N and P availability on periphyton accrual in 22 restored wetlands in southern Michigan. While previous studies indicate both N- and P-limitation were limited in aquatic ecosystems of temperate Northern America, I hypothesized that P would be the primary limiting factor for algal growth in restored wetlands of southern Michigan because of high groundwater N:P ratios. I also tested the hypotheses that 1) algal nutrient limitation are correlated with nutrient concentrations and the N:P ratio in the wetlands, and 2) nutrient conditions regulate the natural variability in algal species among wetlands. The latter hypothsis was tested by experimental nutrient addition to establish cause-effect relations and with variation in species composition and nutrient conditions among wetlands.

4.3 Methods

4.31 Sample collection

Twenty-two restored depressional wetlands were selected in the Maple and Upper Grand watersheds of southern Michigan for this study (Figure 4.1). All these mitigated wetlands were restored within the past 15 year and were located in an agricultural and urban dominated landscape. Previous research showed that nutrient concentration and

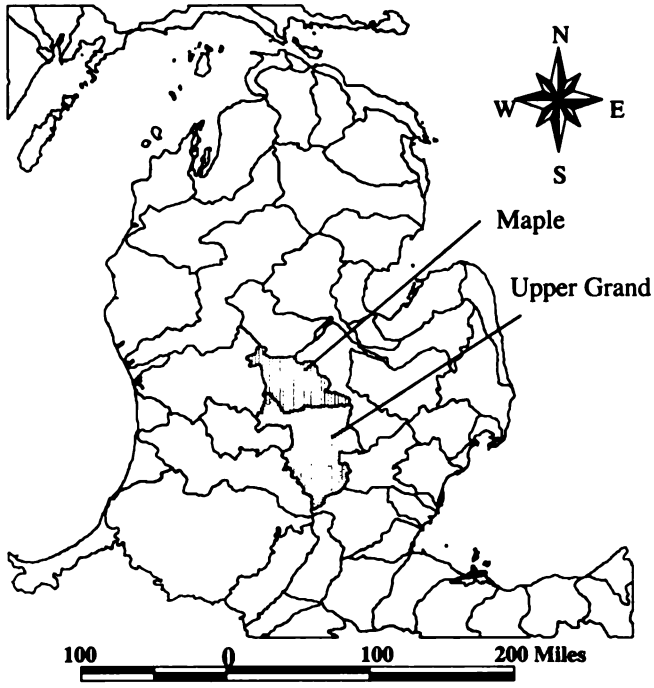

Figure 4.4.1. Map of Lower Peninsula of Michigan, showing the Upper Grand River and Maple River watersheds where sampling sites were located.

algal assemblages were not related to age of these wetlands (Zheng and Stevenson, unpublished data). The sampling sites were mainly *Typha*- or *Nuphar*- dominated marshes intermingled with several swamps. Duckweed covered at least some proportion of all wetlands during the experimental period. Physical, chemical, and biological variables were assayed or collected during the mornings of July 2000. Water temperature and dissolved oxygen (DO) was measured with a YSI® DO meter (YSI Inc., Yellow Springs, Ohio, USA) at the beginning and at the end of the sampling period, allowing at least one hour between measurements. Conductivity and pH were measured using a YSI® model 33 SCT meter with conductivity and pH electrodes (Denver Instrument Company). Canopy coverage by emergent vegetation was estimated with a spherical canopy densiometer held at water level and by averaging canopy cover from 4 directions. Duckweed coverage was estimated by averaging the coverage of the wetland surface before and after the nutrient-enrichment experiments. Irradiance at the water surface (I_0)

and 30 cm below the water surface (I_z) were recorded with a LICOR light meter. Light extinction coefficients were calculated ($\ln(I_0)-\ln(I_z)/Z$, Wetzel 2001). Water samples were collected in 2 125-ml acid-washed polyethylene bottles, stored in ice, transported to the laboratory, and frozen until chemical analysis. Phytoplankton samples were collected in 2 1-L polyethylene bottles for chlorophyll *a* and algal cell counts. The top 1 cm of sediment was collected randomly from eight different locations in each wetland using plastic tubes (internal diameter = 3.8 cm, length = 20 cm). All 8 sediment samples from a wetland were combined in a Whirl-pak® bag. Epiphytic algae were collected by cutting 4-5 macrophyte stems, brushing epiphytes from each macrophyte surface, and putting them in a Whirl-pak® bag. Metaphyton were collected for qualitative identification. Algae were preserved with M3(APHA 1998) after subsampling for chl *a* analysis.

4.3.2 Nutrient diffusion experiment

Nutrient enrichment experiments were conducted in each wetland to determine whether nutrients limited periphyton growth. Although a variety of experimental approaches can be used to study the effects of nutrient enrichment, nutrient diffusing substrata are an effective and economical way to determine nutrient limitation of periphyton (Fairchild et al. 1985, Hecky and Kilham 1988, Wurtsbaugh et al. 2001). Nutrient-diffusing bioassays were condicted with clay flowerpots (upper diameter = 4.1cm, lower diameter =5.8 cm, height = 4.1 cm) as described in Fairchild et al. (1985). Eight pots for each wetland were sealed with plastic plates on the bottom and filled with 2% Bacto® agar and one of four different nutrient treatments: an untreated control, N-enriched (0.5M $NaNO_3$,), P-enriched (0.5 M Na_2HPO_4), and NP-enriched (0.5M $NaNO_3$

and Na_2HPO_4) treatments. Pots were capped with acid-washed rubber caps. Eight pots were attached to the corners of two 25 X 25 cm tiles to ensure stability during the experimental period.

Nutrient diffusion rates were monitored over a 3-week period in distilled water in the laboratory. Water samples in the containers for all treatments were sampled and analyzed every two days. Nutrient diffusion rates were constant over the 3-week period.

The pots were placed at a 20-40 cm depth in each wetland. After three weeks, the pots were collected and immediately transported in a cooler to the laboratory. The pot surface was cleaned with a razor blade and a hard-bristled toothbrush. Periphyton from the two pots in the same treatment were combined and rinsed into a glass beaker with deionized water. The periphyton samples were shaken well and then subsampled for assays of total nitrogen (TN) and total phosphorus (TP), chlorophyll *a* (chl *a*), dry mass (DM), ash free dry mass (AFDM), and species composition, cell density, and biovolume of non-diatom algae and diatoms.

4.3.3 Laboratory analyses

One 250-ml water sample from each wetland was analyzed for TN and TP. Another bottle was filtered through Coleman® glass fiber filters (0.45um diameter) and the filtrate was analyzed for nitrate (NO_3^--N) and soluble reactive phosphorus (SRP) in a Spectronic® Genesys™ 2 Spectrophotometer. TP and TN were oxidized to SRP and NO_3^- (APHA 1998). TP and SRP were measured using the ascorbic acid method, while TN and nitrate and nitrite (NOx-N) were measured using cadium reduction methods (APHA 1998). Ammonium (NH_4^+-N) was analyzed using a Wuick-Chem 8000 autoanalyzer in University of Michigan Biological Station (UMBS). Dissolved inorganic

nitrogen (DIN) was thus calculated as the sum of ammonia, nitrate, and nitrite concentrations. Sediment samples were rinsed with deionized water to 200 ml and homogenized using a homogenizer. Appropriate dilution was made for sediment samples to measure sediment TN and TP concentration following the same methods as the water samples. Periphyton samples from clay pots were filtered on GF/C filters for the analysis of algal cell nutrient contents.

Chl *a* samples were assayed by extraction in 90% acetone overnight at 4°C, reading absorbance with a Spectronic® Genesys™ 2 Spectrophotometer and calculating pheophytin-corrected chl a concentration (APHA 1998). DM and AFDM of periphyton were determined according to standard methods (APHA 1998).

Phytoplankton, epiphytic algae, sediment algae, and clay pot algae were diluted or condensed as if necessary before counting. Algal densities and non-diatom species composition were determined using a Leica® microscope and a Palmer-Maloney (0.1 ml) counting chamber under 400X and by counting 300 natural units. Algal taxa were identified to the lowest possible taxonomic level. Diatom taxa and relative abundance were determined from permanent Naphrax® mounts of acid-cleaned diatoms (Stosch and Reimann 1970). At least 500 diatom valves were counted. Biovolume was estimated for at least 15 cells of each taxon by assigning geometric shapes and measuring appropriate cell dimensions (Stevenson et al. 1985, Hillebrand et al. 1999)

4.3.4 Data analysis

To evaluate the magnitude of N and P limitation, I defined primary nutrient limitation in the i^{th} wetland as algal biomass of either N or P treatment (N_i or P_i) minus biomass in

the control treatment (C_i) then divided by C_i. For example, primary N limitation=(N_i - C_i)/C_i and primary P limitation =(P_i -C_i)/C_i. Algal secondary nutrient limitation in a wetland was defined as algal biomass in the NP treatment (NP_i) minus either P_i or N_i, then divided by biomass in that nutrient treatment. Therefore, secondary N limitation=(NP_i -P_i)/P_i and secondary P limitation =(NP_i -P_i)/N_i.

To calculate cellular nutrient content in periphyton mass, particulate N and P contents of algal biomass on artificial substrata were divided by algal DM. Mass N:P ratios were also calculated based on mass N and P contents.

To determine which treatment led to biomass accrual, algal cellular N and P contents, chl *a*, algal biovolume, and algal density were compared among treatments. Because of the data distribution and site-specific dependence, a non-metric paired comparison was conducted using a Wilcoxon Signed Ranks Test in Systat® 10.

To determine if the type and magnitude of nutrient limitation was correlated with nutrient concentrations or nutrient ratios, correlations were calculated using Systat ®10. I related nutrient limitation to nutrient concentrations and the following molar ratios: TN/TP, DIN/SRP, and DIN/TP to determine the relative importance of dissolved and total nutrient in regulating nutrient limitation.

Algal species composition of phytoplankton, and diatom species composition on macrophytes, in sediments, and on control treatments of artificial substrata were related to environmental factors using canonical correspondence analysis (CCA) (CANOCO 4.0, ter Braak and Smilauer 1997). Diatom taxa with a relative abundance greater than 1% and appearing in at least 3 wetlands, or with relative abundance greater than 5% and appearing in at least 1 wetland were selected for CCA. Relative abundance values were

square-root transformed to reduce the importance of dominant taxa and attain normal data distribution. Algal relative biovolume of phytoplankton genera were also selected and transformed as the same rules as relative abundance of diatom assemblages. A forward-selected CCA ordination was then used to determine which environmental variables accounted for the greatest amount of variance in the distribution of the algal or diatom taxa. The significance of each variable added in this fashion was tested using a Monte Carlo permutation test with 99 unrestricted permutations ($P \leq 0.05$) (ter Braak and Smilauer 1997).

To compare the relationships between diatom species responses to nutrients among wetlands and their responses to experimental N, P, and NP enrichment, I identified indicator species that specifically responded to N or P additions on aritificial substrata. Diatom species whose relative abundance in N treatments were significantly higher than control treatments, or in NP treatment significantly higher than P treatments were defined as N indicator species (Wilcoxon Signed Ranks Test $P<0.1$). Diatom species whose relative abundance in P treatments were higher than in control treatments and higher in NP treatments than N treatments were defined as P indicator species. The sum of the relative abundances of indicator species for either N or P in each wetland was then related to nutrient concentrations in wetlands.

4.4 Results

4.4.1 Wetland habitats

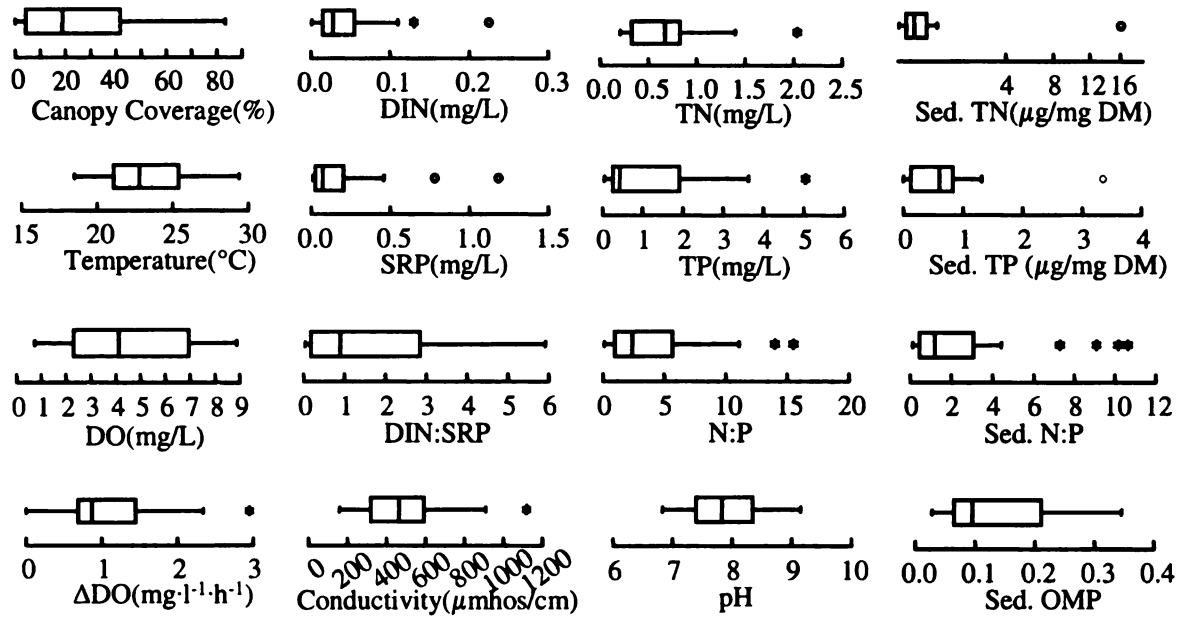

Figure 4.2. Box plot of environmental variables in the 22 wetlands.

Although most of the 22 wetlands were not forested, the canopy 27(±25%) and duckweed cover (and other floating plants) (45±12%) on the surface of most wetlands reduced irradiance to extremely low levels. Light available for use by underwater algae (30cm below water surface) averaged 21 (± 18) % of irradiance at the water surface, and the light extinction coefficients (LEC) averaged 1.20 (±0.12). Water of these wetlands had a wide range of conductivity (490±251 μmol/cm). pH was generally neutral (7.86±0.66). Average DO was 4.58 (± 2.59) mg/L before 10 a.m. and ranged from complete depletion (0.7 mg/L) to oversaturation (11mg/L) in different wetlands. The productivities based on DO change rates were 0.76 (±0.15) $g \cdot m^{-2} \cdot h^{-1}$ (Figure 4.2).

Table 4.1. Correlation matrix of ambient nutrients in 22 wetlands. A * indicates a significant correlation (Dunn-Sidak P<0.05).

	NOx	NH_4^+	DIN	SRP	TN	TP	Sediment TN
NOx	1.000						
NH_4^+	0.432	1.000					
DIN	0.771*	0.758*	1.000				
SRP	-0.126	0.259	0.135	1.000			
TN	0.070	0.517*	0.515*	0.009	1.000		
TP	-0.128	-0.146	-0.109	0.532*	-0.137	1.000	
Sediment TN	0.112	-0.058	0.065	-0.099	-0.141	-0.161	1.000
Sediment TP	0.119	0.139	0.207	0.151	0.017	0.027	0.852*

DIN (0.047±0.050 mg/L) and TN (0.680±0.448 mg/L) concentrations were generally low in the water column compared with high SRP (0.195±0.286 mg/L) and TP (1.143 ±1.353 mg/L) concentrations (Figure 4.2). NH_4^+ was the main form of DIN while NO_3^- was completely depleted (<0.010 mg/L) in 17 of the 22 wetlands. Both DIN: SRP ratios and TN: TP ratios were less than 17 indicating potential N limitation in the water column. The predominant substratum was generally sand overlain by very fine organic material and silt. Sediments were mineral soils with organic matter (OMP) composing less than 35%. Sediment N:P ratios showed extremely low values (<2), except in one site, which also indicated an N-limited habitat.

Nutrient concentrations in the water column were not correlated with nutrient concentrations or ratios in sediments (Table 4.1). TN in the water column was correlated with inorganic nitrogen concentration (r=0.517 with DIN, r=0.515 with NH_4^+), and TP in the water column was correlated with SRP (r=0.532). TN and TP in sediment (mg/kg DM) were highly correlated with each other (r=0.852).

4.4.2 Effects of nutrient enrichment and nutrient concentrations

Nutrient enrichment through clay pots significantly changed algal cellular N content, but had little effect on P content (Figure 4.3). Nitrogen content (TN: DM) in both the N-enriched and the NP-enriched treatments was much higher than in the control treatments (P=0.003 and 0.014) and the P enriched treatments (P=0.001 and 0.004). P content of periphyton (TP: DM) in the nutrient enrichment treatments was not significantly different from that in the control treatment (P > 0.10). P content in the P enriched treatments was marginally higher than in the N-enriched treatments (P=0.062).

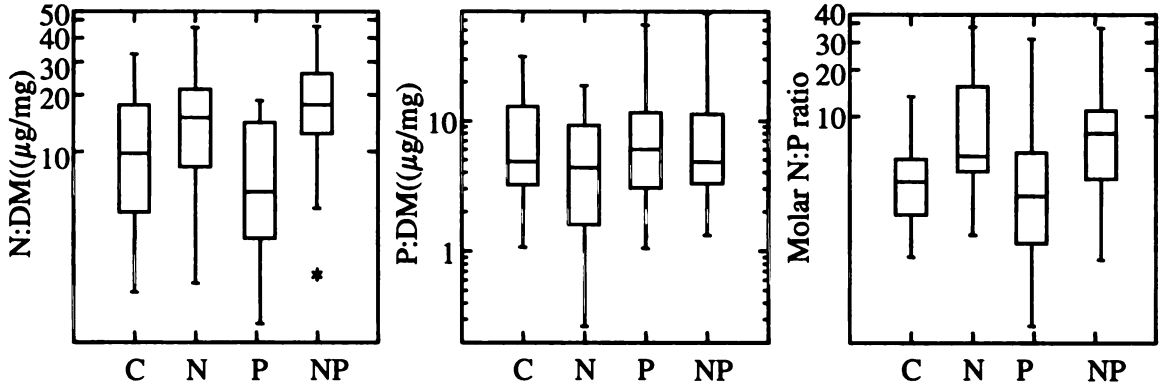

Figure 4.3. Algal cellular nutrient contents and molar N:P ratios in control, N-enriched, P-enriched, and NP-enriched treatments on artificial substrata.

Low cellular N:P ratios on clay pots indicated that algal growth would be N limited (Figure 4.3). Molar cellular N:P ratio averaged 4.490 (±0.686) in the control treatment, 11.148(±2.163) in the N-enriched treatment, 5.584(±1.614) in P-enriched treatment, and 9.971(±2.163) in NP treatment. Cellular N:P ratio in N- and NP-enriched treatments were significantly higher than control (P=0.004 and 0.016) and P-enriched (P=0.042 and 0.059) treatments. N:P ratios were not less in P-enriched than control treatments.

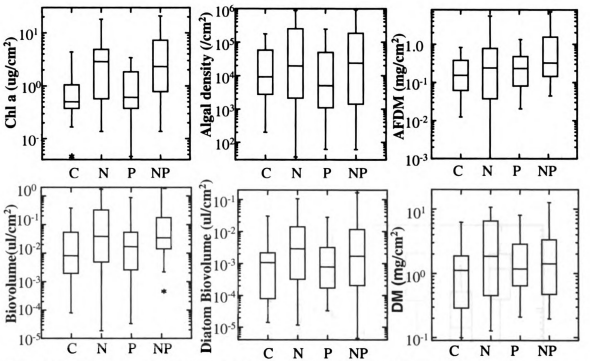

Figure 4.4. Algal biomass/production in artificial substrata of control, N-enriched, P-enriched, and NP-enriched treatments. They are represented by different attributes.

Differences in algal biomass on nutrient diffusing substrata also indicated that nitrogen was overall the most important limiting nutrient in the 22 restored Michigan wetlands (Figure 4.4). Chl *a*, algal cell density, and algal biovolume were all significantly higher on N- and NP-enriched substrata than on control and P-enriched substrata ($P<0.05$). DM and AFDM responses to N enrichment were not as great as chl a, cell density, and biovolume. DM with N and NP additions was significantly greater over the control treatment ($P=0.001$ and 0.026 respectively) but not among other treatments. AFDM in the NP enriched treatments was higher than in control, P-enriched, and N-enriched treatments ($P<0.01$). No differences between the P-enriched treatments and control treatments or between NP- and N-enriched treatments were found (Figure 4.4). Little evidence of secondary P limitation was found.

Chl *a*: DM and Chl *a*: AFDM ratios were different among different treatments (Figure 4.5). N- and NP- enriched treatments had significantly higher Chl *a* :DM ratios than control and P-enriched treatments ($P<0.05$). N-enriched treatment also had higher Chl *a*: AFDM ratio than the control, P-enriched, and NP-enriched treatments. chl *a* : AFDM in the NP-enriched treatment did not differ from control treatment, but was higher than the P-enriched treatment.

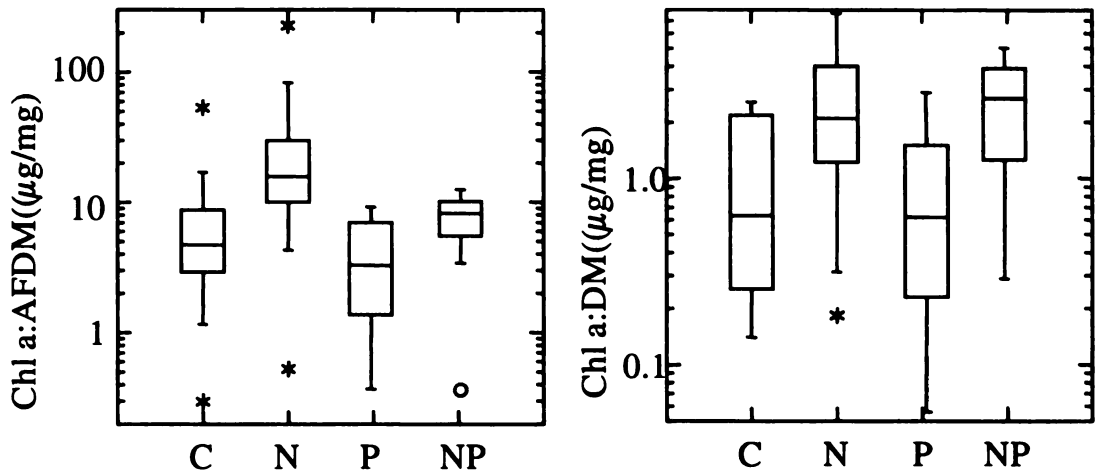

Figure 4.5. Chl a: AFDM and Chl a:DM ratios in control, N-enriched, P-enriched, and NP-enriched treatments on artificial substrata.

Table 4.2. Algal nutrient limitation and corresponded water nutrients and sediment nutrients. Value in the parentheses are correlations between log-transformed value and algal nutrient limitation. A * indicates significant correlation (Dunn-Sidak $P<0.05$)

		Observed Correlation						
		water nutrients				sediment nutrients		
		N	P	DIN:TP	N:P	N	P	N:P
Primary N limitation	(N-C)/C	-0.034 (-0.122)	**0.612*** **(0.592*)**	-0.437 **(-0.546*)**	-0.297 **(-0.601*)**	-0.199 (-0.082)	**0.634*** (0.150)	-0.378 (-0.430)
Primary P Limitation	(P-C)/C	-0.121 (-0.069)	0.239 (0.253)	-0.203 (-0.237)	-0.300 (-0.282)	-0.141 (-0.138)	-0.089 (-0.043)	-0.137 (-0.089)
Secondary N Limitation	(NP-P)/P	0.143 (0.028)	0.062 (0.131)	-0.139 (0.049)	-0.014 (-0.115)	-0.181 (-0019)	-0.153 (-0.106)	-0.007 (-0.019)
Secondary P Limitation	(NP-N)/N	-0.116 (-0.141)	-0.049 (0.049)	-0.168 (0.011)	-0.180 (-0.115)	-0.064 (-0.206)	-0.239 **(-0.567*)**	0.309 (0.382)

The magnitude of N- and P-limitation in individual wetlands also showed that N limitation was consistently greater than any evidence of P limitation (Figure 4.6). Several wetlands showed some signs of P-limitation. However, the magnitude of N limitation, ranging from 0-13.5 units was much greater than the 0-2.5 units range in P-limitation.

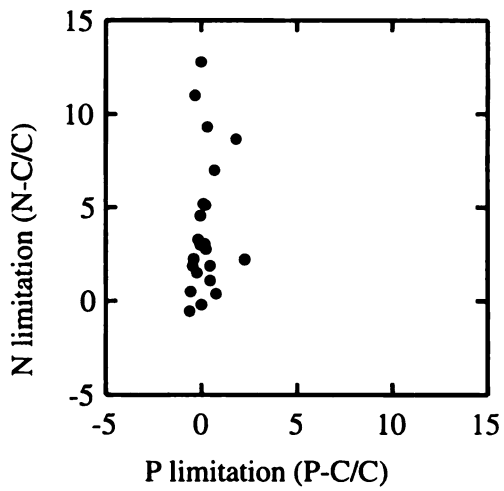

Figure 4.6. Comparisons of algal N and P limitation in all 22 wetlands. Primary N or P limitation is defined as algal biomass of that nutrient treatment minus control treatment then weighted by control treatment. Primary nutrient limitation is defined as algal biomass of that nutrient treatment minus control treatment then weighted by control treatment.

Primary limitation of algal accrual by N supply was related to nutrient concentrations and ratios both in the water column and sediments (Table 4.2, Figure 4.7). Direct correlation between N-limitation and DIN concentration was very weak. However, the magnitude of N-limitation in a wetland was positively correlated with TP both in the water and in the sediments, negatively correlated with DIN:TP and N: P ratios in the water column, and negatively correlated with N:P ratio in the sediments (Table 4.2, Figure 4.7). Secondary P limitation was negatively correlated with TP concentration in the sediments (Table 4.2).

4.4.3 Algal species composition in natural and artificial substrata

Changes in species composition among wetlands indicated nutrients were an important factor regulating algae in epiphytic habitats. A total of 115 diatom taxa out of 213 algal taxa were found in epiphytic samples. Epiphytic non-diatom algae were

predominated by *Oedogonium, Mougeotia, Scenedesmus, Pediastrum*, and *Trachemonas* species, while *Navicula minima, Achnanthes minutissima, and Navicula cryptocephala* were the dominant diatom species. CCA based on epiphytic diatom assemblages and environmental variables showed that 31.4 % of the total variance of diatom assemblages were explained by the first three CCA axes (Table 4.3). Epiphytic diatom assemblages were closely correlated with pH (P=0.005). Conductivity (P=0.07), DIN (P=0.075), and TP (P=0.015) were the other important environmental factors with high eigenvalues explaining diatom distribution (Table 4.3, Figure 4.8).

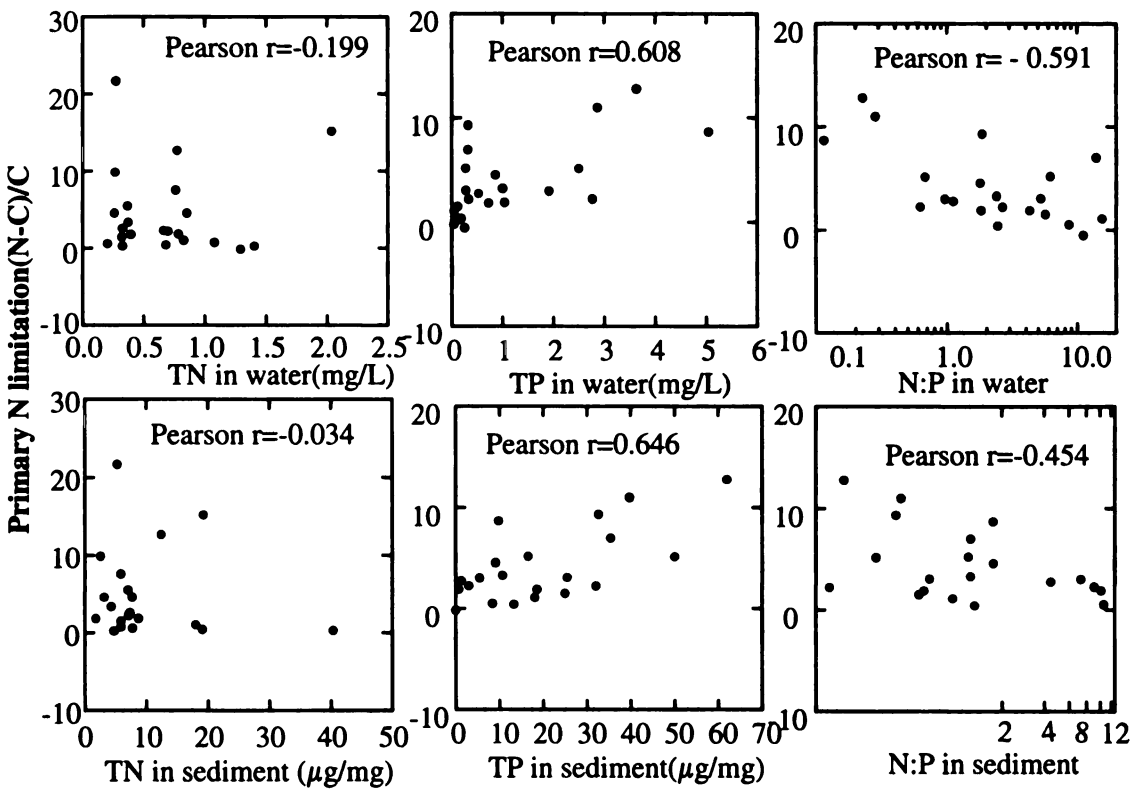

Figure 4.7. Comparisons of algal N and P limitation in all 22 wetlands. Primary N or P limitation is defined as algal biomass of that nutrient treatment minus control treatment then weighted by control treatment.

Changes in algal assemblages of sediment also revealed that nutrients regulated species composition. The majority of algal taxa (173) found in sediment samples were

diatom taxa (148). Non-diatom algae were mainly composed of *Oscillatoria* and *Scenedesmus*, while diatom communities were dominated by *N. minima, A. minutissima, N. cryptocephala,* and *Achnanthes lanceolata* var. *frequentissima.* CCA related changes in diatom assemblages to pH and N gradients (Figure 4.8). The first three ordination axes of CCA explained 27.4% of diatom taxa data. The first axis was correlated with pH (P=0.01), and the second axis was positively correlated with DIN (P=0.04)(Table 4.3).

Table 4.3. Weighted correlation matrix from Canonical Correspondent Analysis (CCA).

	Epiphytes			Sediment			phytoplankton			Artificial substrata		
	Axis1	Axis2	Axis3	Axis1	Axis 2	Axis 3	Axis1	Axis 2	Axis 3	Axis1	Axis2	Axis 3
Variance	14.3	9.2	7.9	11.5	8.2	7.7	9	8.5	7.9	14.3	9.0	8.0
pH	**0.596**	-0.453	0.128	**-0.655**	-0.026	-0.330	0.433	0.090	0.040	**-0.509**	-0.245	**0.609**
Conductivity	-0.036	**0.497**	-0.208	-0.248	0.031	-0.482	-0.099	-0.076	-0.385	-0.244	**0.746**	0.261
Canopy Cov.	-0.259	0.338	0.052	0.088	0.325	0.148	-0.075	0.338	-0.045	-0.073	-0.286	0.456
DIN	-0.308	0.363	**-0.520**	-0.314	**0.577**	-0.511	-0.189	-0.044	0.084	-0.261	0.390	0.378
SRP	**-0.454**	-0.094	0.135	0.123	-0.331	-0.156	-0.460	0.223	-0.250	-0.230	0.277	0.160
TN	-0228	-0.201	**-0.502**	0.193	-0.250	-.0118	-0.353	-0.040	0.304	-0.230	0.277	0.160
TP	-0.331	-0.008	**-0.654**	0.216	-0.034	0.296	-0.415	**0.660**	0.051	-0.115	0.104	**0.492**
TN:TP	0.289	0.374	-0.052	-0.066	-0.144	-0.115	-0.022	0.022	0.233	-0.125	**-0.620**	-0.421
Sed. N	0.138	-0.062	-0.117	0.013	-0.202	0.283	-0.456	0.005	0.304	-0.172	-0.132	0.250
Sed. P	0.218	0.335	0.152	-0.407	-0.148	-0.416	-0.293	0.045	0.304	0.268	0.167	0.175

Phytoplankton assemblages were also affected by nutrient factors. Phytoplankton samples were very low in algal species and cell density in most wetlands. Only 99 non-diatom taxa were found. Swimming or floating genera, such as *Scenedesmus, Pediastrum, Euglena, Trachelomonas,* and *Oscillatoria* appeared in some of these sites, but none of them dominated in all wetlands. Diatom density was extremely low so they were not used for further analysis. CCA of non-diatom assemblages related changes in non-diatom species composition to TP (Figure 4.8). The first three ordination axes of CCA explained 25.4% of diatom taxa data. The only significant environmental variable was TP (P=0.03), which correlated with the second CCA axis (Table 4.3).

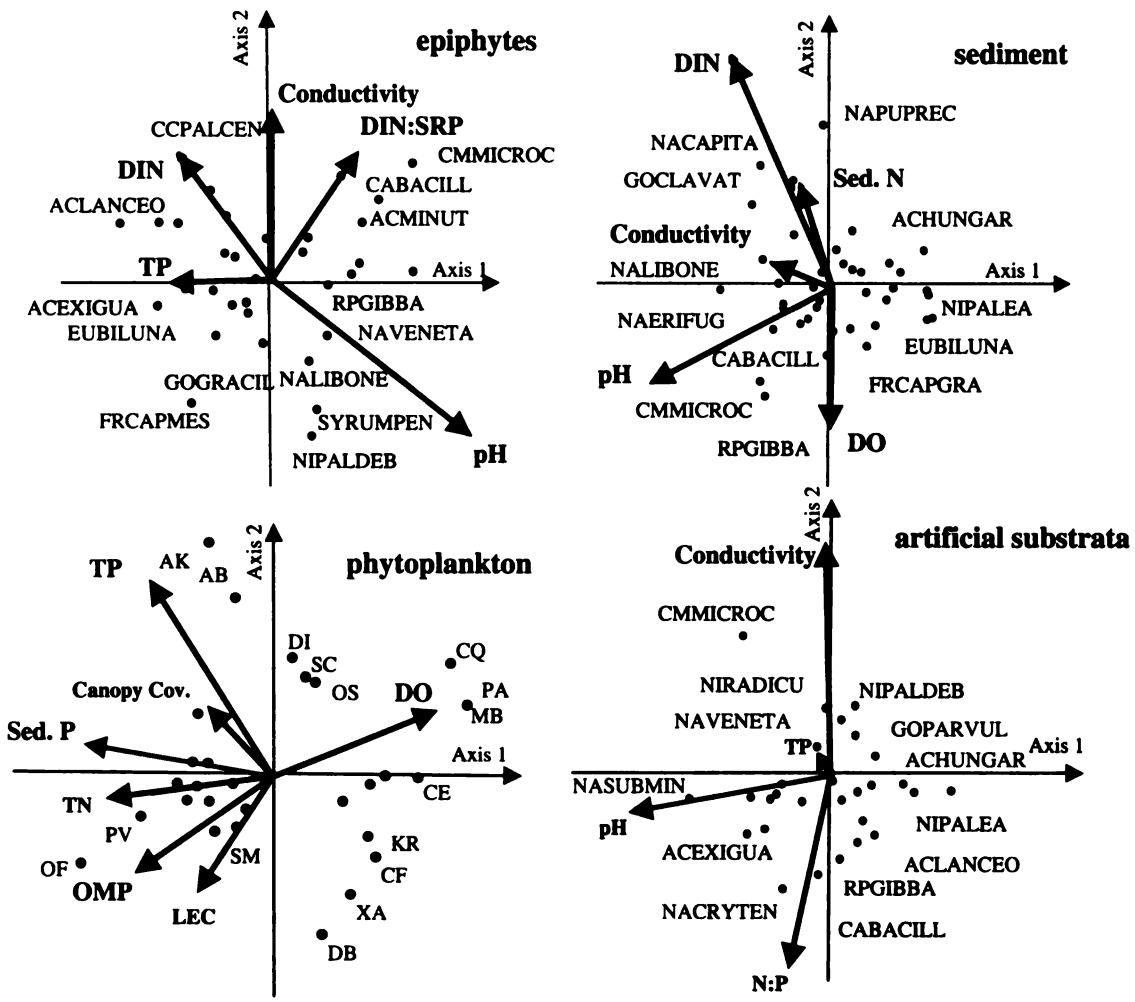

Figure 4.8. CCA biplots of algal assemblages and environmental variables. Diatom taxa on macrophytes, in sediments, and on artificial substrata in control treatments, and algal genera of phytoplankton were used for the CCA. Codes for diatoms: ACEXIGUA-*Achnanthes exigua, ACHUNGAR- Achnanthes hungrarica, ACLANCEO- Achnanthes lanceolata, ACMINUTI- Achnanthese minutissima, CABACILL- Caloneis bacillum, CCPLALIN- Coconeis placentula* var. *linearis, CMMICROC-Cymbella microcephala, EUBILUNA- Eunotia bilunaris, FRCAPGRA- Fragilaria capucina* var. *gracilis, FRCAPMES-Fragilaria capucina* var. *mesolopta, GOCLAVAT-Gomphonema clavata, GOPARVUL-Gomphonema parvulum, GOGRACIL-Gomphonema gracilis, NACAPITA-Navicula capitata, NACRYPTO- Navicula cryptocephala, NACRYTEN- Navicula cryptotenella, NAERIFUG- Navicula erifuga, NALIBONE- Navicula libonensis, NAMINMA- Navicula minima, NAPUPREC-Navicula pupula* var. *rectangularis, NAVENETA- Navicula veneta, NIFRUSTU-Nitzschia frustulum, NIPALEA-Nitzschia palea, NIPALDEB-Nitzschia palea* var. *debilis, NIRADICU- Nitzschia radicula, RPGIBBA-Ropalodia gibba, SYRUMPEN-Synedra rumpens.* Code for phytoplankton genus: AB-*Anabaena*, AK- *Ankiostrodesmus*, DI-diatoms, CE-*Coelastrum*, CF-*Cladophora*, CQ-*Coelosphaerium*, DB- *Dinobryn*, GL-*Glenodinium*, KR- *Ceratium*, MB -*Micrasterias*, OF- *Ophiocytium*, OS-*Oscillatoria*, PA- *Pediastrum*, PV- *Pandorina*, SC-*Scenedesmus,* XA- unknown algae.

Only 137 non-diatom taxa and 113 diatom taxa were found on the control artificial substrata. About half of the wetlands were dominated by *Stigeoclonium, Oedgonium,* and *Mougeotia* green macroalgae. The others were mostly diatom species, predominantly *N. minima, A. minutissima,* and *N. cryptocephala.* CCA indicated that 31.3% of variance in the diatom taxa data was explained by the first three CCA axes. Diatom assemblages in control substrata were correlated with pH (P=0.005), conductivity (P=0.015), N: P (P=0.06), and TP (P=0.065) (Table 4.3).

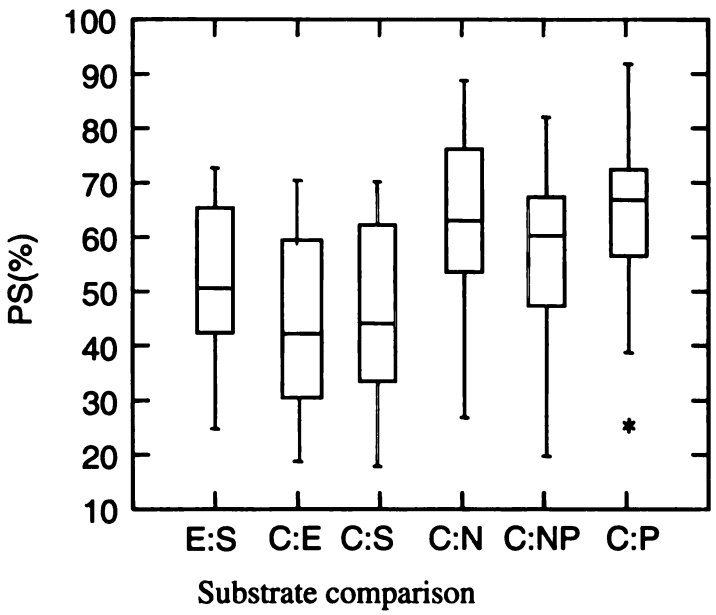

Figure 4.9. Box-plots of similarities of diatom assemblages between different habitats and treatments. E-epiphytes, S-sediment, C-control, N-N treatment, P-P treatment, NP- NP treatment. X-axis shows the comparison of two paired habitats. Y-axis shows the Percentage similarity between the two habitats.

Different substrata might have different effects on diatom species assemblages, however, the similarities in species composition on natural habitats, epiphyton (E) and sediments (S), was not significantly different than between natural habitats and control treatments (Figure 4.9). The average similarities between epiphytic and sediment diatoms, and epiphytic and control treatment diatoms, and sediment and control treatment diatoms (0.444(±0.053), 0.442 (±0.038), and 0.474 (±0.034) respectively) were not

significantly different (P>0.05). Diatom species assemblages of all treatments on artificial substrata were more similar (P<0.05) to each other than with those on natural substrata (Figure 4.9).

4.4.4 Algal assemblage response to nutrient additions

Both diatom and non-diatom algae increased in density and biovolume in N-enriched treatments and NP-enriched treatments (P<0.01). The most dominant algal taxonomic group in control treatments was Chlorophyta (45.2 ± 0.077%), followed by diatoms (21.9± 6.2%), Euglenophyta (15.7± 5.9%). Only a small proportion of the biovolume was cyanobacteria (5.6 ± 2.7%). N-enrichment did not change the relative biovolume of diatoms (29.6 ± 6%) and filamentous green algae (48.5± 7.6%) overall, but the relative biovolume of cyanobacteria (0.9± 0.6%) was significantly lower in the N-enriched treatment than in the control treatment (P<0.05). P-enrichment did not change the relative biovolume of any of the algal groups. NP-enrichment also decreased the relative biovolume of cyanobacteria (1.9± 0.9%). The most dominant algal genera, *Oedogonium, Mougeotia*, and *Stigeoclonium,* did not respond to nutrient addition based on relative biovolume (P>0.1), although they all increased in total biovolume and density with nitrate addition.

The most abundant diatom taxa responded to nutrient additions in a variety of ways on artificial substrata (Table 4.4). The relative abundance of the most abundant taxa, such as *N. minima, A. minutissima, A. exigua, C. placentula* var. *lineata,* and *N. frustulum* did not change with nutrient addition. The relative abundance of *G. parvulum, and N. cryptocephala* increased with nitrogen addition (P<0.1), while the relative abundance of

N. palea and *N. palea* var. *debilis* decreased. The relative abundance of *A. lanceolata* and *A. lanceolata* v. *frequentissima* increased with P enrichment.

Table 4.4 Twenty-six most dominant taxa and their responses to nutrient additions. The 4-digit number represents comparisons of the relative abundance of a diatom taxa in a treatment with control, N-enrichment, P-enrichment, and NP-enrichment treatments. A significant change ($P<0.1$) of relative abundance between treatments was presented by 1, and insignificant effect was 0. * is empty when compared with itself.

Diatom taxa	N-enriched	P-enriched	NP-enriched	Indicator
Achnanthes exigua Grun.	*0*00*	*00*0*	*000**	-
Achnanthes hungarica (Grun.) Grun	*1*00*	*00*0*	*000**	*N*
Achnanthes lanceolata (Breb.) Grun.	*0*00*	*10*0*	*100**	*P*
Achnanthes lanceolata v. *frequentissima* Lange-Bert.	*0*00*	*10*0*	*000**	*P*
Achnanthes minutissima Kütz.	*0*00*	*00*0*	*000**	-
Amphora veneta Kutz.	*0*00*	*00*0*	*000**	-
Cocconeis placentula v. *lineate* (Ehr.) V. H	*0*00*	*00*0*	*000**	-
Cymbella microcephala Grun.	*0*00*	*00*1*	*101**	*N*
Epithemia sorex Kutz.	0*00	00*0	000*	-
Eunotia billularia (Ehr.) Mills	*0*00*	*00*0*	*000**	-
Fragillaria construens(Ehr.) Grun.	*0*00*	*00*1*	*001**	*N*
Fragillaria capucina v. *gracilis* (Østr.) Hust.	*0*00*	*00*0*	*000**	-
Gomphonema clavatum Ehr.	*1*01*	*11*1*	*001**	*N and P*
Gomphonema gracile Ehr. Emend. V.H.	*0*00*	*00*0*	*000**	-
Gomphonema parvulum Kutz.	1*00	00*1	001*	*N*
Navicula capitata Kutz	*0*00*	*00*0*	*000**	-
Navicula cryptocephala Kutz	*1*00*	*00*0*	*000**	*N*
Navicula minima Grun.	*0*00*	*00*0*	*000**	-
Navicula semilunum Grun	*0*10*	*11*1*	*001**	*N*
Navicula trivialis Kutz	*0*00*	*00*0*	*000**	-
Navicula veneta Kutz	*0*00*	*00*0*	*000**	-
Nitzschia amphibia Grun.	*1*00*	*10*0*	*000**	*N*
Nitzschia frustulum (Kutz) Grun.	*0*00*	*00*0*	*000**	-
Nitzschia palea v. *debilis* (Kütz.) Grun.	*1*00*	*01*0*	*000**	*P**
Nitzschia palea (Kuetz.) Grun.	*1*00*	*00*0*	*000**	*P**
Nitzschia perminata (Grun.) Peragallo	*0*00*	*00*1*	*001**	*P*

A number of diatom species distributed along the nutrient gradients in CCA ordinations on artificial substrata, macrophytes, and sediments (Figure 4.8). On macrophytes, *Cocconeis placentula* dominated in high DIN environments and *Nitzschia palea* var. *debilis*, *Synedra rumpens*, and *Navicula libonensis* dominated in low DIN environments. *Cymbella microcephala* preferred high DIN: SRP environments but *Fragilaria capucina* var. *mesolopta* dominated in low DIN:SRP environments. *Achnanthes exigua* and *Eunotia bilunaria* were abundant in high TP environments. In sediment habitats, *Navicula capitata, Gomphonema clavatum, Navicula pupula* var. *rectangularis* appeared in high DIN environments while *Fragilaria capucina* var. *gracilis* and *E. bilunaria* dominated in low N conditions. On artificial substrata, *Navicula cryptonella* and *Caloneis bacillum* showed preference for high N: P environments while *N. palea var. debilis* for low N: P environments.

Table 4.5. Correlations between relative abundance of nutrient indicator species and nutrient concentration in different habitats. * indicates significant correlation (P<0.05).

	DIN	SRP	TN	TP	Sed. N	Sed. P
N indicator on artificial substrata	0.310	0.236	0.034	-0.105	0.044	0.123
N indicator on macrophytes	-0.004	0.041	0.339	-0.137	-0.122	-0.350
N indicator in sediment	0.168	0.006	0.299	-0.068	-0.208	-0.079
P indicator on artificial substrata	-0.165	0.373	-0.137	**0.562***	-0.045	0.032
P indicator on macrophytes	-0.241	0.330	-0.220	**0.470***	-0.163	-0.115
P indicator in sediments	-0.019	0.282	-0.111	**0.437***	-0.040	-0.076

Although individual taxa responded to a nutrient gradient differently, the indicator species (Table 4.4) delineated from experimental nutrient additions correlated with natural nutrient concentrations of wetlands (Table 4.5). Indicator diatom species for N and P were listed in Table 4.4. Relative abundance of N indicator species on artificial substrata weakly correlated with DIN concentration (r=0.331) in water column (Table

4.5). N indicator species on macrophytes and sediments weakly correlated with TN concentrations in water column. P indicators on all substrata were consistently correlated with TP concentrations in water column (Table 4.5).

4.5 Discussion

4.5.1 Algal nutrient limitation

Four lines of evidence indicated that N was the primary limiting nutrient for periphyton growth in restored wetlands in southern Michigan. First, ambient NO_3-N concentration and N:P ratios were low. Second, N and NP additions caused increased periphyton cellular N content and cellular N:P ratios in the wetlands, which indicated a physiological N limitation for algal cells. Third, the highest algal biomass on N and NP enriched substrata revealed that N could stimulate algal biomass accrual in these wetlands. Fourth, this N limitation was 5 times stronger than any indication of P limitation in these systems.

The low DIN concentration, especially depleted NO_3^--N concentration (<10μg NO_3^--N/L), in most of the wetlands indicated strong N-limitation. Previous studies have found that periphyton growth could be limited when ambient levels were 50-90 μg NO_3-N/L (Grimm and Fisher 1986, Lohman et al. 1992, Stelzer and Lamberti 2001), while Maberly et al. (2002) found that nitrogen limitation was more likely than phosphorus limitation where the DIN was <6.5 μmol /L (71μg N/L) . The restored wetlands in southern Michigan were generally depleted in NO_3^--N, but not NH_4^+. Several possible biogeochemical processes might have led to the low NO_3^--N concentrations in these wetlands. One possible cause was sequestering of nitrogen by duckweed, which can compete with phytoplankton for nutrients and deplete DIN supply (Pokorny and

Rejmankova 1983, Oscarson et al. 1989). Denitrification also can be a significant path of nitrogen loss, especially NO_3^-. In duckweed covered wetlands, low light irradiance and high decomposition rate led to low DO concentration. The low dissolved O_2 could have limited nitrification rates and caused a relatively high NH_4^+: NO_3 ratio.

Although nutrient limitation is presumably related to the concentration of that limiting nutrient, the magnitude of nutrient limitation was not negatively correlated with DIN or TN concentrations. It was negatively correlated with TP concentrations in southern Michigan wetlands. Studies of nutrient addition experiments in New Zealand streams (Francoeur et al. 1999) indicated that the degree of nutrient limitation (mostly N limitation or NP co-limitation) was negatively correlated with NO_3-N concentration when NO_3^- ranged from 3.2 –367μg/L. NO_3^--N concentrations in our wetlands were extremely low or depleted. The absence of a gradient of N concentrations could have caused the lack of a possible relationship between N and the magnitude of nutrient limitation. The increased TP concentration increased the difficulty for periphyton to absorb NO_3-N from the water column and led to greater N-limitation with increased P gradient. In high ammonium wetlands, duckweeds covered the water surface and light may have been a limiting factor that prevented NH_4^+ utilization. Algal biomass accrual was barely detectable in these wetlands.

N:P ratios in the water column and in sediments were much lower compared with the Redfield ratio, the optimal N:P ratio for periphyton growth (Redfield 1958, Tilman 1977, Fairchild 1985, Sommer 1993, Vymaza 1995, Hillebrand and Sommer 1999, Guildford and Hecky 2000, Stelzer and Lamberti 2001). In a review study on nutrient limitation of phytoplankton in lakes and oceans, Guildfold and Hecky (2000) concluded that the

absolute value of TN might not be a strong predictor of N limitation, the TN:TP ratio might be more useful determining the potentially limiting nutrient. However, Stelzer and Lamberti (2001) found that the overall abundance of lotic periphyton responded positively to increased total nitrogen concentration, but not to the N:P ratio. Apparently, nutrient concentration and nutrient ratios could both play important roles in different systems.

Increased cellular N content and N:P ratio after N addition is a strong evidence for N limitation of algal accrual. Algal cellular N:P ratios in our control treatment were much lower than the Redfield Ratio. Increased N availability significantly increased cellular N content, but P addition did not change the P content which indicated that the ambient P concentration in the water column was saturated for algal growth. Previous studies showed that N and P enrichment would change N:P ratios and N and P contents in algal tissues (Vymazal et al. 1994, Vymazal and Richardson 1995, Hillibrand and Sommer 1999, McCormick et al. 2001, Hillibrand and Kahlert 2001, Stelzer and Lamberti 2001). In the P-limited Everglades, algal N:P ratio was higher than 100:1 (McCormick et al. 2001) and increased P loading increased P content in the algal periphyton. Experimental manipulation of stream periphyton growth under different nutrient concentrations and N:P ratios revealed that periphyton N and P content increased with the N and P concentration of stream water (Stelzer and Lamberti 2001), and the cellular N: P ratio significantly followed the N:P ratio in the stream water.

Algal chl *a* concentration, biovolume, and cell density increased with N and NP enrichments in restored Michigan wetlands, but DM and AFDM showed different response. This difference can be found by examining the chl *a* to AFDM and DM ratios.

My results showed that N-enriched periphyton was characterized by higher chl *a* to AFDM ratios than N-limited periphyton, and NP-enriched periphyton had lower chl *a* : AFDM than N enriched treatment. Previous studies also found the same results (Grimm and Fisher 1986). Triska et al. (1983) found no difference in AFDM between N enriched and control treatments despite large differences in chl *a* because of higher chl *a* to AFDM ratios of N-enriched periphyton. Healey and Hendzel (1979) proposed that chl *a* to biomass ratio was a reasonably good indicator of N deficiency for several algal species because chl *a* synthesis often was reduced or ceased in N-deficient algal cells. Such cells tend to accumulate carbon storage compounds (Healey 1973). Grimm and Fisher (1986) attributed low chl *a* :AFDM ratio to earlier development of a thick layer of organic material (detrital accumulation) on the substratum surface in N-limited substrata. Weber (1973) proposed that the autotrophic index should be calculated based on estimates of periphyton chl *a* and AFDM. Apparently, N enrichment could stimulate high algal accumulation rather than high organic matter accumulation in P additions. Competition for nutrients in N-limited substrata may have stimulated heterotrophic microbial processes (Tank and Webster 1998).

Nutrient limitation is a complicated process and could be easily confounded by other factors. Algal nutrient limitation could be affected by many biotic factors (herbivory, resource competition) and abiotic factors (light, hydrology, and substrata) (Hill and Knight 1988, Munn et al. 1989, Hansson 1992, Cronk and Mitsch 1994, Toetz 1999, Larned and Santos 2000, Rosemond et al. 2000). These factors sometimes confound change in periphyton growth with nutrient addition (Lowe et al. 1986, Hill and Knight 1988, Millard et al. 1996). The wetland systems in this study had high duckweed cover

and low phytoplankton density. Low algal accrual on control and nutrient enriched treatments indicated that light limitation and grazing were probably important in these wetlands. Despite the possibility that these confounding factors might have reduced the significance of algal nutrient limitation, nutrient diffusing bioassays still showed strong responses of algal biomass and algal assemblages with N additions.

4.5.2 Algal species composition and nutrient concentrations

Although different habitats within wetlands and pH variation among wetlands affected algal species composition, the response of algal species to nutrient and nutrient ratios were consistently observed in all three CCA in our study. Previous studies in lakes and streams have indicated that substrata type (Pringle 1990, Cronk and Mitsch 1994, Tank and Webster 1998), pH (Dixit et al. 1992), and nutrients (Schindler 1987, Elser et al. 1990, Stevenson 1998, Leland et al. 2001) are three important factors affecting algal species composition. Algal communities on different substrata had different algal assemblages. However, the similarities of diatom communities between artificial substrata with sediment and epiphytic habitats were not different than the similarities of algal assemblages between natural (sediment and plant) habitats in this study. Although algal species composition in different habitats can respond to nutrients in different ways (Pringle 1990), pH was the most important factor regulating diatom assemblages on all three substrata in my study, and in a number of studies (Dixit et al. 1992, Lindstrom 1996, Stevenson and Pan 1999). Although algal response to nutrient gradients in wetlands was not as strong as the response to pH and conductivity (Pan et al. 1996, Stevenson et al. 1999), TP and N:P ratio are also important factors regulating algal assemblages in both regional surveys and experimental manipulation of nutrient additions (McCormick et al.

1996, 2001, Pan et al. 2000). Similarly, this studies indicated nutrient concentrations and ratios could both be important in determining diatom species composition in restored wetlands.

One of the most noticeable changes of algal groups on artificial substrata was that cyanobacteria decreased in relative biovolume with N addition. The property of cyanobacteria fixing N and dominating in N deficient ecosystems have been noted by many studies (Rader and Richardson 1992, McCormick et al. 1996, 2001). Most studies indicated that N-enrichment could decrease bluegreen biomass, and low N environment stimulated cyanobacteria dominance. Marinho and Huszar (2002) reported that cyanobacteria abundance was negatively associated with NO_3^- and N/P ratio, and positively associated with temperature and light in a reservoir. My N- and NP-enriched treatments did decrease the abundance of cyanobacteria. However, our wetland system did not have high cyanobacteria biovolume. Green algae (primarily *Stigeoclonium* and *Oedogonium*) and diatoms dominated in the wetlands. Even though cyanobacteria were supposed to dominate to compensate N-limitation in N-limited systems (Smith 1986, Vitousek et al. 2002), low light levels and low temperatures in northern (high latitude) wetlands could have limited cyanobacteria dominance (Watermann et al. 1999, Sekar et al. 2002). Cyanobacteria abundance and N fixation could also have been constrained by trace-element limitation and/or grazing even when N:P ratios were low (Howarth et al. 1999).

Individual diatom taxa responded to N and P additions on artificial substrata in a similar or different manner to their response along natural gradients. Some species showed exactly the same response to a nutrient addition as I observed on natural substrata

and controlled artificial substrata. For example, *N. palea* and *N. palea* var. *debilis* were found in low N and high P conditions on natural substrata as well as previous studies (Fairchild et al. 1985). This study proved that N enrichment decreased the relative abundance of these taxa and P addition did not change their abundance in P-enriched environments. *G. clavatum* and *C. microcephala* were also found in high DIN environments in natural habitats, and N addition also increased their relative abundance on artificial substrata. On the other hand, several species that appeared in the high or low end of nutrient gradients did not change significantly with nutrient addition. For example, *Caloneis bacillum* appeared in high DIN: SRP environments but N addition did not significantly increase its relative abundance. *A. minutissima*, which was reported to positively respond to N addition (Fairchild et al. 1985), did not respond to nutrient addition in this study. Pringle (1990) reported that *A. minutissima* was significantly less for all nutrient treatments relative to the control on sand-agar. A lack of pristine P environments may have caused insensitive response from species responding both to N and P additions and thus may have confounded the effect of N addition. Complicated wetland environments such as different pH among wetlands and grazing may have confounded individual species response to nutrient additions (John 1993, Rosemond et al. 1993).

Little evidence has quantitatively related experimental response of diatom taxa to their response in natural habitats. Diatom species responses to natural nutrient gradients have been determined and indicator values have been assigned for many species (van Dam 1994, EPA 2002). Multispecies indicators of trophic status based on specis nutrients preferences proved to be applicable across geological regions (Kelly 1998). A few studies

have related natural distribution of diatom assemblages along nutrient gradients to experimental nutrient gradients (McCormick et al. 1996, 2001, Underwood et al. 1998, Pan et al. 2000). My studies indicated that despite variability among taxa, the overall response of the sensitive diatom taxa was similar for natural and experimental nutrient gradients. However, the response of N indicator species were not as strong as P indicator species. This may have been due to the lack of immediate N supply and N gradients in these wetlands resulting N indicator species not responding to N addition in a gradual way. Although the correlations between experimental results and natural gradients were weak, they provided a pioneering attempt to identify indicator species experimentally that could be used for wetlands.

4.6 Conclusions

Algal growth in the restored wetlands of southern Michigan was limited by N supply. Cellular nutrient contents and N:P ratios, algal biomass accrual response to a nutrient addition, and the strong N limitation vs. weak P limitation proved that N was the primary limiting nutrient. Although the magnitude of limitation was not directly correlated with N concentration in the water sediments, its strong correlation with P concentration and N: P ratio in both the water column and the sediments indicate a strong limitation caused by N depletion. Algal species composition in natural habitats also responded to nutrient gradients. Experimental enrichment changed relative abundance of many dominant species. Overall, the relative abundance of species that increased with N or P enrichment on experimental substrata also increased as N or P concentrations increased in wetlands.

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Chapter 5

Assessing wetland response to land use using diatoms and paleoecological characterization of reference condition

5.1 Abstract. Diatom-based biological indicators were used to establish biological and stressor criteria for wetland protection and restoration and to indicate wetland ecosystem health. Physico-chemical characteristics and surface sediment diatom assemblages were studied in 35 palustrine wetlands in the lower peninsula of Michigan across a wide range of human disturbance gradients. Paleoecological diatom assemblages from cores and pre-settlement sediments were used to represent natural, reference conditions in wetlands. Stressor-response relations were used to delineate stressor criteria and the land use causing problems when biological conditions deviated from the "normal" range of natural conditions and when deviations were associated with stressors and land use. Canonical correspondence analysis (CCA) revealed that diatom species assemblages responded mainly to conductivity and total phosphorus (TP). Weighted average predictive models for these variables were generally successful. The similarity in species composition and number of native species in current wetlands decreased with increasing nutrient concentrations. van Dam's diatom trophic index was linearly correlated with the TP gradient. Both the weighted average TP model and van Dam's trophic index positively responded to agricultural land use around wetlands. These indices deviated from the "normal" natural condition when TP exceeded 46.6 μg/L. A threshold change in TP and biotic indices was detected when agricultural land exceeded 60% total land use.

5.2 Introduction

Assessment of wetland systems has been increasingly considered important with the growing impact of human disturbance. About 53% wetlands have disappeared or been impaired by increasing human activities since European settlement, especially due to agricultural and urban development (Mitsch and Wilson 1996, Carpenter et al. 1998, Detenbeck et al. 1999, Freeland et al. 1999, USEPA 2000). Under most circumstances, wetlands are sinks and transformers of chemicals and nutrients. They buffer the impact of terrestrial nutrient pollution on aquatic systems (Richardson 2000) and maintain biodiversity. With the better understanding of large river, stream, and lake systems in the past decades, the importance of wetland systems has received increasing attention. Many states and environmental agencies have started to incorporate wetlands into their water quality program. The U.S. Environmental Protection Agency (USEPA) recently published a series of methods for evaluating the biological integrity of wetlands (e.g., van der Valk 2001). These methods recommend using a series of ecological parameters including landscape characterization, nutrient load estimates, hydrology, algae, vegetation, and soil and water chemistry.

Ecological assessment should characterize condition of valued ecological attributes (VEA) by comparison to pre-defined criteria and diagnosis of stressors and human activities that threaten or impair valued attributes. Appropriate classification and definition of reference conditions could help to define the normal range of natural conditions and reduce natural variability among different systems. Stressor-response relation help to identify causes of impairment to VEA, and relate the stressors to human disturbance (land use). Combining the reference approach with stressor-response

relationships, appropriate biocriteria can be defined and related to stressors causing problems. Although both reference approaches and stressor-response relationships have been used for assessment of ecological conditions (Somlyody 1998, Stevenson et al. 2002), no studies have combined both reference and stressor-response relationship. Establishment of stressor criteria based on these approaches has never been reported.

Reference conditions are usually defined by characterizing chemical and biological condition at least impacted sites, which are often selected based on surrounding land use. Unfortunatedly, most aquatic systems have suffered from eutrophication by different sources, such as agriculture fertilizer and urban runoff, and finding pristine sites is unlikely (Hughes 2000). Historical records of aquatic ecosystems are well preserved in the paleoecological sediments. Shifts in species composition between historical and extant assemblages could reflect the change due to anthropogenic stresses (Wilson and Muhler 1983, Wilcox 1995). Models based on weighted average calibrations have been used by paleolimnologists to reconstruct the limnological history of lakes (Dixit et al. 1992, Cumming and Smol 1993, Hughes et al. 1993, Dixit et al. 1996, Smol et al. 1998). Thus, paleoecological records from species assemblages before European settlement could be used to establish reference conditions to indicate environmental changes before and after human disturbance. Comparison between assemblages in appropriate reference condition and impaired wetlands can help identify impaired conditions and degree of impairment in these systems.

Stressor-response relationships are the foundation for evaluating ecosystems at risk and directing ecological management (Gordon and Majumder 2000). This model is based on an understanding of relationships between human disturbance, stressors, and VEAs of

biological communities. Assessment of aquatic communities identifies degradation of VEAs. By exploring the relationships between aquatic community and possible causes of degradation based on ecological response from indicator organisms, I can relate ecological responses of VEAs can be related to stressor gradients, and used to further refine human disturbance gradients (Lopez and Fennessy 2002). A variety of ecological attributes and indices could be used to define biological criteria when comparing with reference conditions (Hill et al. 2003). Based on pairing "stressor" variables with "response" variables, ecologically meaningful criteria can be established to protect and restore VEAs.

Diatoms have been used as indicators of ecological conditions in streams and lakes (Charles 1985, Hall and Smol 1992, Dixie et al. 1996, Leland and Porter 2000), but relatively few studies have used diatoms as indicators of wetland conditions (Pan and Stevenson 1996, McCormick et al. 1996, Stevenson et al. 1999, Pan et al. 2000, Stevenson et al. 2001). Because of their great diversity and species-specific responses to varied chemical and physical conditions, diatoms should serve as valuable indicators of the heterogeneous environments in wetlands (Stevenson 1998, Stevenson et al. 2001). Diatom valves are usually well preserved in sediments because their cell walls are composed of resistant opaline silica. Therefore, diatom paleoecological assemblages represent past environmental conditions (Dixit et al. 1992). In recent years, diatom-based bioassessment has been improved and numerous indices have been developed in stream and lake habitat assessment (van Dam et al. 1994, Whitton and Kelly 1995, Hill et al. 2000, 2003). van Dam's trophic index based on diatom autoecology (van Dam et al. 1994) is one of the most successful indices proven to be valid in inferring conditions of

aquatic systems in Europe. Although the diatom paleolimnological approach has rarely been used in wetlands (Gasse 1987), diatom-based inference models by weighted average calibration have been developed for conductivity and total phosphorus in Kentucky wetlands (Pan and Stevenson 1996). Diatoms thus have all the potentials for indicating wetland health.

The goals of this research were to use diatoms to establish biocriteria and related nutrient criteria for wetland management. I used paleoecological information from pre-European settlement sediments to represent reference conditions in wetlands, and thus define biological criteria. Stressors were delineated by relating water chemistry to land use around wetlands. Stressor-response relations were used to identify nutrient criteria to protect the "normal" range of natural biological conditions.

5.3 Methods

5.3.1 Materials and methods

Preliminary analysis (Stevenson et al. 1999) of diatom assemblages in Michigan wetlands indicated low species-environmental correlation due to the great variability among wetlands. I selected 35 palustrine wetlands (as defined by Cowardin et al 1979, Detenbeck 2001) in the lower peninsula of Michigan along a north-south and an east-west transect (Figure 5.1). Although there was seasonal and annual variation, water depth of all wetlands was less than 2 m, and all but one site had water throughout the year.

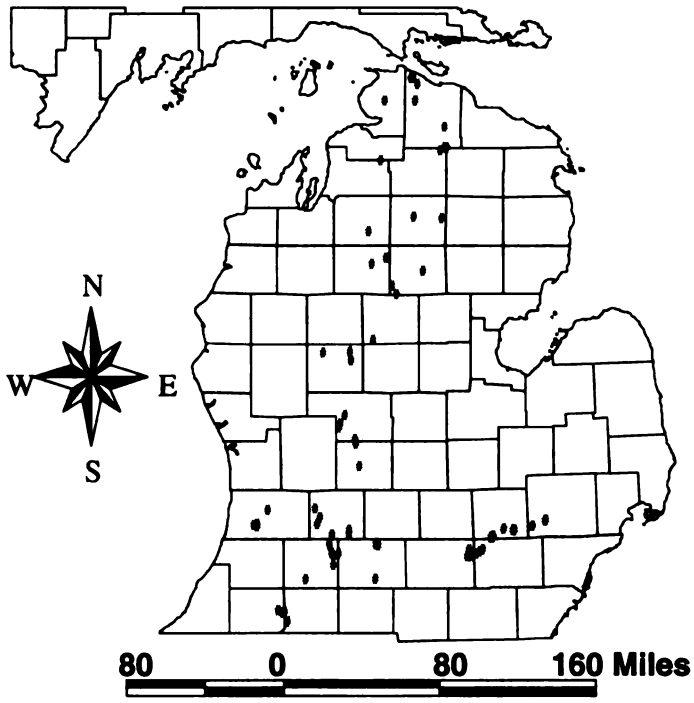

Figure 5.1. Distribution of the 35 wetlands sampled in fall 1994 and summer 1995 in the lower peninsula of Michigan along a north-south and an east-west transect.

All wetlands were visited once in fall 1994 and again in summer 1995. A sediment core was taken from a central location in each wetland. The coring methods used depended on water depth: a modified piston corer was used in waters deeper than 70 cm, and push corers (Dixit et al. 1996) were used in waters less than 70 cm. The cores, ranging in length from 20-50 cm, were cut into variable segments up to 4 cm in length. All the segments were preserved with M3 solution (APHA 1998). The top 1 cm of sediment depth was later used for analysis of surface diatom, and only fall sediment samples were included. One segment from a sediment core aged older than 200 years were used as presettlement wetland assemblages. Core samples from 14 wetlands were analyzed, and all taxa appearing in these samples were considered as native taxa.

The dissected sediment samples from fall 1994 were digested for diatom enumeration using concentrated sulfuric acid and KCrO3 (Stosch and Reimann 1970). All

surface sediments and 14 core samples from fall 1994 were selected for analysis. Diatom samples were washed with distilled water and then mounted in Naphrax®. A minimum of 500 diatom valves were identified at 1000x using a Nikon® Labophot-2 microscope. Diatom taxa were identified using the taxonomic monographs of Hustedt (1927-66), Krammer and Lange-Bertalot (1986-91), and Patrick and Reimer (1975).

All water samples were collected from the middle of the water column. A number of water chemistry parameters were monitored in the field, including pH, specific conductance, total dissolved solids (TDS), apparent and true color, and temperature. Aerated pH was measured within 24 hours after sample collection. Water samples were preserved and analyzed on a Skalar® Sans-Plus automated water chemistry system according to APHA methods (1998) for total phosphorus (TP) and total nitrogen (TN), soluble reactive phosphorus (SRP), nitrate, ammonium, and chloride. In addition, a known volume of water was filtered through a 0.45-μm Millipore® membrane filter for chlorophyll a (chl a) analysis on a Turner Designs® TOC-5050A fluorometer. Another water sample was also filtered for dissolved organic carbon (DOC) analysis on a Shimadzu® TOC-5050A.

5.3.2 Land Use coverage

Land use coverage and digital soil data were obtained from the Spatial Data Library maintained by the Michigan Department of Natural Resource (MIDNR). These data are stored as Georef projections and as 1:250,000 scale maps from the US Geological Survey (USGS). Land use was grouped as urban, agriculture, rangeland, forest, water, wetland, and barren land. Michigan major land resource and soil types were categorized according to Michigan resource inventory as Indiana and Ohio Till Plain (I), Northern Michigan and

Wisconsin Sandy Drift (II), Southern Michigan and Northern Indiana Drift Plain (III), Southern Michigan Fruit and Truck Belt (IV), and Western Michigan and Northeastern Wisconsin Fruit belt (V) (1982). They were categorized into three regions as low erosive, medium erosive, and high erosive regions. The Lower Pennisula of Michigan was classified into two sub-ecoregion according to ecoregion map. Arcview 3.2 with the Spatial Analyst 1.1 extension, along with various extensions including Michigan projection extension and MIDNR Geometry Calculator Extension, were used to perform all spatial analyses. The watershed boundaries of wetlands were determined by selecting 1 to 2 km buffer areas around palustrine wetlands (according to size of wetlands) to determine surrounding land use (Berven and Grudzien 1990, Findlay and Lenton 2001).

5.3.3 Statistical analyses

Diatom species relative abundances, species richness, and Shannon species diversity (*H'*) were calculated from the species composition and enumeration data. van Dam diatom indices (van Dam et al. 1994) were calculated as the sum of products of species indicator values and proportional relative abundance (converted for proportion of species with indicator values) and used to indicate diatoms sensitive to nutrient enrichment change. All variables were standardized before analysis. Descriptive statistics, Pearson correlations, and regression analyses were conducted in Systat® v10. The Dunn-Sidak test was used to determine the significance of the correlation.

Principal components analysis (PCA) was used to reduce and summarize variation of environmental variables in the region. The PCA (eigenvalue>1, 6 axes) sample scores were used as composite variables of chemistry to assess the differences among ecoregions or soil types.

Only diatom species with a relative abundance ≥ 1% in a minimum of three sites or ≥ 5% in at least one site were included for comparison and gradient analysis.

Nonmetric multidimentional scaling (NMDS) was used to identify diatom distribution patterns with relation to presettlement and current conditions. Euclidean distances were calculated for all samples based on diatom species composition, and then projected to 2-dimensional plots using NMDS (PCORD 4.0). Multiple response permutation procedures (MRPP), a nonmetric comparison method to discriminate differences among groups of assemblages, was used to determine the difference between reference and current diatom assemblages.

Canonical correspondence analysis (CCA) was conducted to explore the relationship between diatom species distribution and environmental conditions (ter Braak 1990). All analyses were performed with the computer program CANOCO v4.02 and PCORD v4.10 (McCune and Mefford 1999). Relative abundance data were square-root transformed and environmental variables were log transformed as necessary to obtain normal distributions. Whenever a variance inflation factor (VIF) was greater than 5, the least significant, collinear variables were dropped from the analyses .The environmental variables included all measured variables and the TN:TP ratio, DIN:SRP ratio, and wetland coordinates. Forward selection and Monte Carlo permutation (199 random permutations) were performed to determine statistical significance of a subset of environmental variables. The program CALIBRATE v0.6 (Juggins and ter Braak 1992) was used to perform weighted average regression and calibration. All models were based on square-root transformed diatom relative abundance data. Jackknifing was used to test the accuracy of the predictive models. Regression tree analysis (De'ath and Fabricius

2000) was performed using Systat® v10 to detect appropriate threshold values of environmental variables and community compositions with increasing agriculture land use.

The Bray-Curtis similarity index (SI) was calculated as similarity of the relative abundance of surface diatom assemblage at one wetland with centroid of paeleological diatom assemblages to represent similarity with reference conditions.

5.4 Results:

5.4.1 Environmental variables

The annual average of environmental variables varied among wetlands (Figure 5.2). Conductivity of these wetland waters ranged from 32.2 to 495.5 μmol/cm (average ± standard error (SE), 189.8±16.2 μmol/cm). The pH was generally neutral (6.45±0.75). DOC averaged 17.9 mg/L (±1.2 mg/L), DIN (137.2±25.9 μg/L), and TN (1456.6±95.5 μg/L) concentrations were much higher than SRP (22.0±5.8μg/L) and TP (82.8± 16.3 μg/L) concentrations. Si and Cl averaged 3.9(±0.7) mg/L and 5.2(±1.2) mg/L respectively. N:P (36.0± 3.2) and DIN:SRP (26.0± 9.0) ratios were higher than 17 indicating potential P limitation.

Environmental variables, especially nutrient variables were closely related to each other (Table 5.1). Cl correlated with conductivity($r=0.668$, $P<0.001$) and TDS (0.593, $P<0.01$). Si also correlated with conductivity and TDS ($r=0.737$, $P<0.001$), as well as NH_4 ($r=0.618$,$P<0.05$), DIN ($r=0.551$, $P<0.05$), and SRP ($r=0.547$, $P<0.05$). Nutrient concentrations were tightly correlated with each other, and related to chl a concentration in the water column. TP correlated with TN($r=0.711$, $P<0.05$), SRP($r=0.717$, $P<0.05$), and NH_4 concentrations ($r=0.753$, $P<0.001$). Chl a concentration in water column was

highly significantly (P<0.001) correlated with TP (r=0.793), TN (r=0.805), and NH_4 (r=0.850).

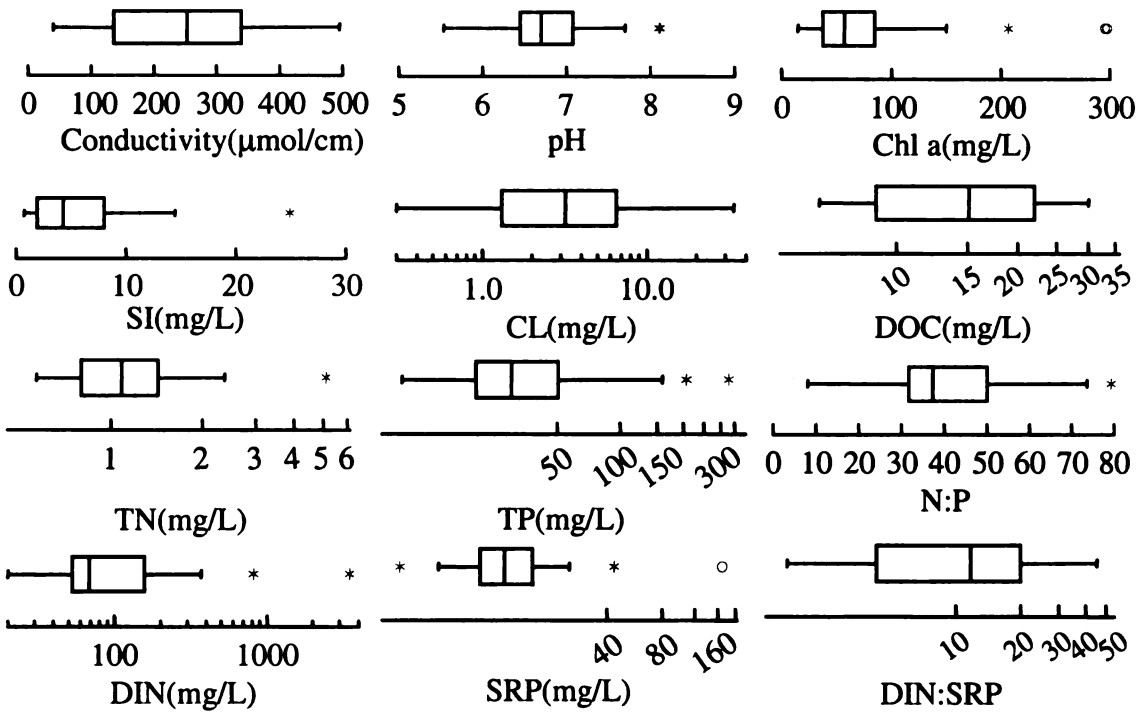

Figure 5.2. Box plot of environmental variables in 1994-1995 in the Michigan wetlands.

The first three PCA axes accounted for 61.4% of variance in the environmental data set. The first axis was correlated with ionic concentrations (conductivity and pH) and the second axis correlated with total nutrients (TN and TP). Multiviate test of the first 3 axes revealed that environmental variables in wetlands were not different among sub-ecoregions (MANOVA, Wilks's λ $F_{6,27}$=1.784, P = 0.140) and among soil types (MANOVA, Wilks's λ $F_{12,54}$=1.054, P = 0.386).

Agricultural land use around wetlands correlated with average TP concentration in 1994 to 1995 (Table 5.3, Figure 5.3). Regression tree analysis was used to explore the relationship between agricultural land use and TP concentration. A threshold effect

occurred when agricultural land use exceed 58% and average TP increased from 46.7 to 207.3 μg/L. The regression tree model explained significantly more variance than a linear model (F test, $P<0.05$).

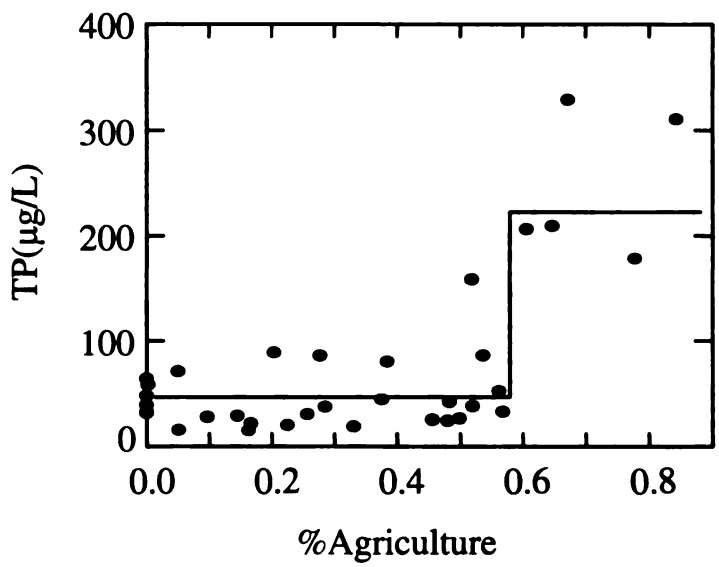

Figure 5.3. Relationship between average TP and % agricultural land use in sampled wetlands. Regression tree analysis was used to explore the relationship between TP and land use. A threshold effect occurred when agriculture occupied 58% land use around the wetland watershed.

Table 5.1. Pearson correlation matrix of environmental variables of 35 wetlands across Lower Peninsula of Michigan in 1994-995. A significant correlation (Dunn-Sidak Probabilities P<0.05) is indicated by *.

	Temp.	pH	Conductivity	TColor	DOC	Si	Cl	TN	TP	NOx	NH_4	SRP	Chl a	N:P	DIN:SRP
Temp.	1.000														
pH	-0.172	1.000													
Conductivity	-0.179	*0.616	1.000												
True Color	0.003	-0.277	0.006	1.000											
DOC	0.337	-0.339	-0.060	*0.558	1.000										
Si	0.066	0.392	*0.589	-0.053	0.280	1.000									
Cl	-0.129	0.345	*0.608	0.378	0.111	0.118	1.000								
TN	0.213	0.324	0.214	0.283	0.262	0.487	0.111	1.000							
TP	-0.125	0.085	0.293	0.296	0.009	-0.059	0.232	0.399	1.000						
NO_x	-0.129	0.271	0.230	-0.428	0.086	0.142	0.179	-0.021	-0.061	1.000					
NH_4	0.232	0.399	0.244	0.067	0.377	*0.659	0.074	*0.881	0.038	0.017	1.000				
SRP	0.070	0.019	0.203	0.169	-0.027	0.141	0.044	0.440	*0.799	-0.040	0.170	1.000			
Chl a	-0.389	0.137	0.236	-0.036	0.088	0.181	0.349	-0.062	-0.108	0.430	0.018	-0.050	1.000		
TN:TP	0.164	0.014	-0.074	-0.096	0.157	0.400	-0.203	0.109	*-0.620	0.075	0.353	-0.297	0.254	1.000	
DIN:SRP	-0.159	0.083	-0.110	-0.224	0.019	0.049	-0.157	-0.006	-0.082	-0.101	0.034	-0.183	0.075	-0.081	1.000
Agriculture	-0.275	0.099	0.376	0.108	-0.265	0.111	0.221	0.420	*0.558	0.222	0.354	*0.543	0.354	-0.465	-0.324

5.4.2 Current diatom assemblages and environmental conditions

A total of 269 diatom taxa were found in the surface sediment in the 35 palustrine wetlands during fall. Seventy-one diatom taxa (abundance > 1%, Table 5.2) from palustrine wetlands from surface sediment in fall were used in the CCA to explore the relationship between diatom assemblage and environmental variables. The first two axes explained 28.7% of cumulative variance in the taxonomical data. Stepwise permutation tests indicated that, of the 24 environmental variables, conductivity, TP, pH, and DIN: SRP ratio had significant canonical coefficients ($P<0.05$) with CCA axes and thus were selected in the final CCA. The four environmental variables alone in the first two axes captured 19.1% cumulative percentage variance of taxonomical data (Figure 5.4a, 4b). Conductivity and TP were the most significant environmental variables ($P<0.005$). The position of each taxon in the bioplot (Figure 5.4b) indicated its weighted-average optimum relative to other taxa and environmental variables. *Epithemia adnata, Cyclotella nana*, and *Gomphonema mexicanum,* among other taxa, indicated high TP concentration in the ordination space; while *Cymebella microcephala*, and *Navicula leptostriata* appeared most in low TP concentration.

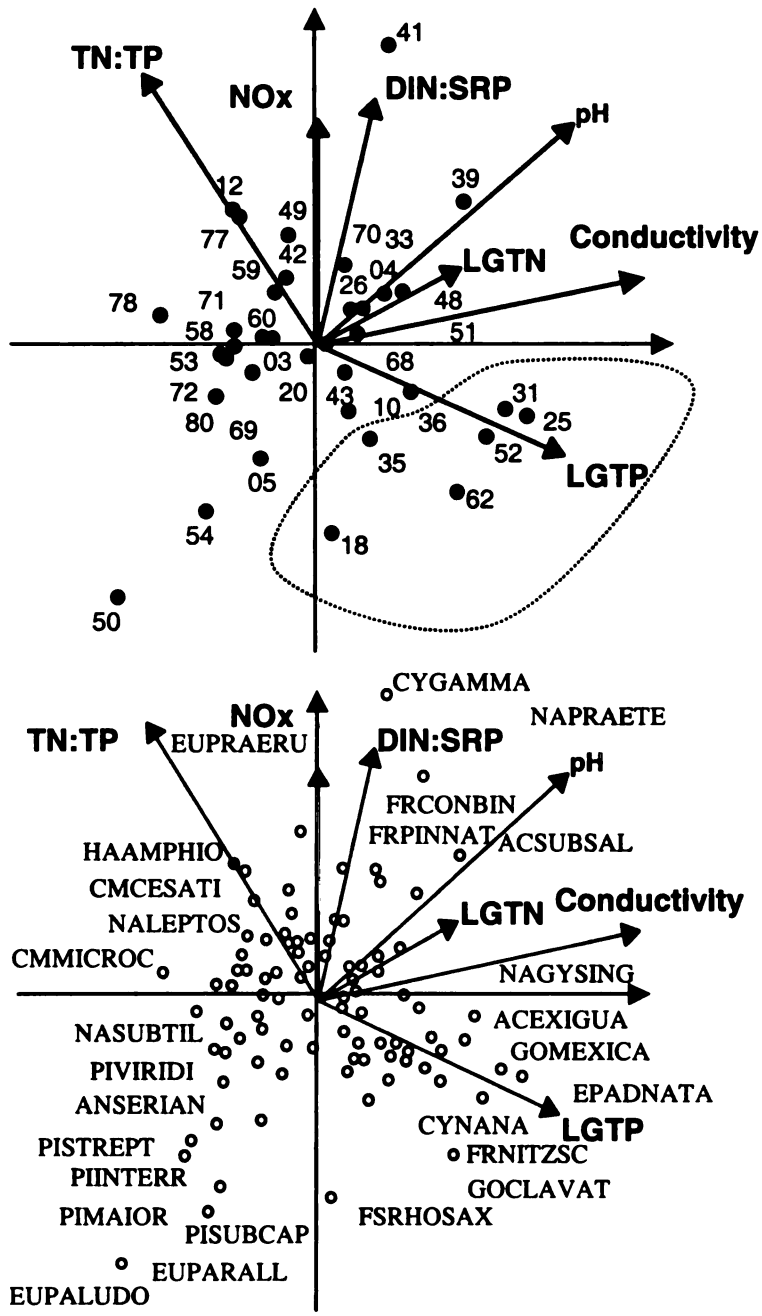

Figure 5.4. CCA biplot of sites and fall environmental variables from 35 palustrine wetlands in Michigan. (a) Dots show site scores in ordination space, (b) dots shows species scores in ordination space, and arrows indicate the strength and correlation between environmental factors and the ordination axes, The dotted circle shows sites with above 58% agricultural land cover. The dotted circle shows sites with above 58% agricultural land cover. Diatom species are indicated by eight-digits code (see Table 5.2 for complete taxon names).

Table 5.2. The most dominant diatom taxa used in CCA and the correspondent taxa code

Code	Taxa name	Code	*Taxa name*
ACCONSPI	*Achnanthes conspicua* A. Mayer	GOGRACIL	*Gomphonema gracile* Ehr. emend. V. H.
ACEXIGUA	*Achnanthes exigua* Grun.	GOPARVUL	*Gomphonema parvulum* (Kütz.) Kütz.
ACHUNGAR	*Achnanthes hungarica* (Grun.) Grun	GOTRUNCA	*Gomphonema truncatum* Ehr.
ACLANCEO	*Achnanthes lanceolata* (Breb.) Grun.	NACRYPTC	*Navicula cryptocephaloides* Hust.

ACMINUTI	*Achnanthes minutissima* Kütz.	NACRYPTO	*Navicula cryptocephala* Kütz.
ALPELLUC	*Amphipleura pellucida* (Kütz.) Kütz.	NACRYTEN	*Navicula cryptotenella* Lange-Bert.
ANSERBRA	*Anomoeoneis serians var. brachysira* (Bréb. ex Kütz.) Hust.	NALEPTOS	*Navicula leptostriata* Jorgensen
ANVITREA	*Anomoeoneis vitrea* (Grun.) Ross	NAHARDII	*Navicula hardii* Hust.
AUCRENUL	*Aulacoseira crenulata* (Ehr.) Thwaites	NAMEDIOC	*Navicula mediocris* Krasske
CCPLACEN	*Cocconeis placentula* Ehr.	NAMINIMA	*Navicula minima* Grun.
CCPLALIN	*Cocconeis placentula var. lineata* (Ehr.) V. H.	NAPUPREC	*Navicula pupula var. rectangularis* (Greg.) Grun.
CMLUNATA	*Cymbella lunata* W. Sm.	NARADIOS	*Navicula radiosa* Kütz.
CMMICROC	*Cymbella microcephala* Grun.	NASEMLUM	*Navicula seminulum* Grun.
CMMINUTA	*Cymbella minuta* Hilse ex Rabh	NASUBTIL	*Navicula subtilissima Cl.*
CMSILESI	*Cymbella silesiaca* Bleisch	NATANTUL	*Navicula tantula* Hust.
DNKUETZI	*Denticula kuetzingii* Grun.	NEIRIDIS	*Neidium iridis* (Ehr.) Cl.
EPTURGID	*Epithemia turgida* (Ehr.) Kütz.	NIAMPHIB	*Nitzschia amphibia* Grun.
EUARCUS	*Eunotia arcus* Ehr.	NIGRACIL	*Nitzschia gracilis* Hantz. ex Rabh.
EUBILMUC	*Eunotia bilunaris* var. *mucophila* Lange-Bert. & Nörpel	NIPALEA	*Nitzschia palea* (Kütz.) W. Sm.
EUCURVAT	*Eunotia curvata* (Kütz.) Lagerst.	NIPERMIN	*Nitzschia perminuta* (Grun.) Peragallo
EUEXIGUA	*Eunotia exigua* (Bréb. ex Kütz.) Rabh	NITROPIC	*Nitzschia tropica* Hust.
EUFLEXUO	*Eunotia flexuosa* Bréb. ex Kütz.	PIGIBBA	*Pinnularia gibba* Ehr.
EUNAEGEL	*Eunotia naegelii* Migula	PIINTERR	*Pinnularia interrupta* W. Sm.
EUPRAERU	*Eunotia praerupta* Ehr.	PIMAIOR	*Pinnularia maior* (Kütz.) Rabh.
EURHOMBO	*Eunotia rhomboidea* Hust.	PIMICROS	*Pinnularia microstauron* (Ehr.) Cl.
FRBREVIS	*Fragilaria brevistriata* Grun.	PISUBCAP	*Pinnularia subcapitata* Greg.
FRCAPGRA	*Fragilaria capucina var. gracilis* (Østr.) Hust.	PIVIRIDI	*Pinnularia viridis* (Nitz.) Ehr.
FRCAPMES	*Fragilaria capucinavar. mesolepta* Rabh.	SSANCEPS	*Stauroneis anceps* Ehr.
FRCONSTU	*Fragilaria construens* (Ehr.) Grun.	SSPHOENI	*Stauroneis phoenicenteron* (Nitz.) Ehr.
FRCONVEN	*Fragilaria construens var. venter* (Ehr). Grun.	SYNANA	*Synedra nana* Meist.
FRCROTON	*Fragilaria crotonensis* Kitton	SYRUMPEN	*Synedra rumpens* Kütz.
FRPINNAT	*Fragilaria pinnata* Ehr.	SYTENERA	*Synedra tenera* W. Sm.
FSRHOCRA	*Frustulia rhomboides var. crassinervia* (Bréb. ex W. Sm.) Ross	SYULNA	*Synedra ulna* (Nitz.) Ehr.
FSRHOMBO	*Frustulia rhomboides* (Ehr.) DeT.	TAFLOCCU	*Tabellaria flocculosa* (Roth) Kütz
GOACUMIN	*Gomphonema acuminatum* Ehr.	TAQUADRI	*Tabellaria quadriseptata* Knud.
GOANGUST	*Gomphonema angustatum* (Kütz.) Rabh.		

Table 5.3 Regression of observed environmental variables against predicted values based on diatom species composition. Simple regression and jackknifing cross-validation indicators based on weighted average (WA) and tolerance down-weighted WA used to compare the precision. MSE represents mean square error and RMSE represent residual of MSE.

		Simple			Cross-Validation		
		MSE	RMSE	R^2	MSE	RMSE	R^2
Conductivity	WA	4585	67.713	0.741	7132	86.693	0.578
	Tol d/w WA	3672	60.598	0.792	1.23E+04	110.879	0.425
LGTP	WA	0.0492	0.222	0.704	0.0958	0.310	0.435
	Tol d/w WA	0.0496	0.223	0.702	0.123	0.351	0.334
pH	WA	0.407	0.638	0.679	0.807	0.898	0.381
	Tol d/w WA	0.299	0.547	0.764	0.757	0.870	0.446
DIN:SRP	WA	133.8	11.568	0.634	282.1	16.795	0.255
	Tol d/w WA	131	11.447	0.641	291	17.060	0.250

Diatom-based WA inference models for conductivity, TP, pH, and DIN: SRP were developed to predict environmental conditions for palustrine wetlands (Table 5.3). The WA inference model for conductivity produced a lower R^2 (0.741) than the tolerance down-weighted WA model (WA(tol)); however, the simple WA model produced a much better model (R^2=0.578, Figure 5.5a) after cross-validation with jackknifing than the tolerance down- weighted WA model (R^2=0.425). The WA TP inference models also showed better regression (R^2=0.435) after cross-validation than the WA(tol) model (R^2=0.334). The analysis of pH data indicated that tolerance down-weighted WA models were much better than simple WA models both before and after cross-validation. R^2 values for WA models for DIN: SRP were low, especially after cross-validation jackknifing (R^2=0.250).

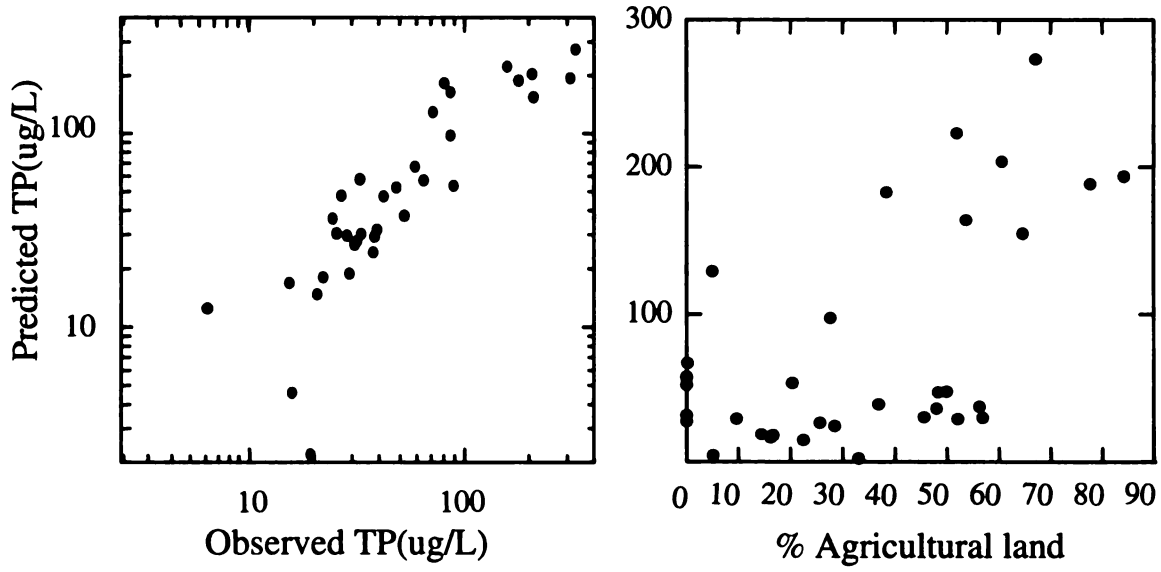

Figure 5.5. Regression models of diatom inferred environmental variables against observed environmental variables. 5b shows the relationship between diatom inferred TP and agricultural land use.

Based on diatom autecological data, predicted TP concentrations of wetlands along an agricultural land use were used to infer wetland trophic level changes (Figure 5.5a). In this model, diatom inferred TP showed a linear increase with increase of agricultural land use reached (R^2=0.246, P=0.003) (Figure 5.5b).

5.4.3 Comparison between reference and current conditions

A total of 169 diatom taxa were identified in 14 sediment core paleoecological samples, while 189 taxa were found in the corresponding surface sediment samples. An average of 55.4% (±2.4%) of the taxa in the surface samples were not found in the core samples in the same wetland, while 50% (± 4%) of the core taxa were not present in surface samples in the same site. Forty-one diatom taxa in paleoecological samples were not found in all 35 surface sediment. Diatom species compositions were significantly different between current and paleoecological samples. (MRPP, P<0.001, Figure 5.6).

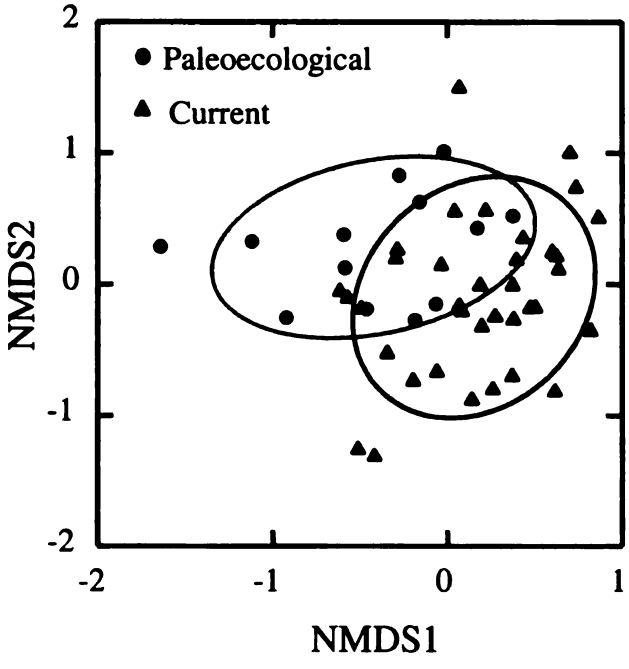

Figure 5.6. Nonmetric multidimensional scaling ordination diagrams of paleological diatom assembalges and current diatom assemblages. Reference(paleological diatom assemblages) and current (surface diatom assemblages) were indicated by different symbols. The centroids with 68.5% confident ellipse were plotted around each region.

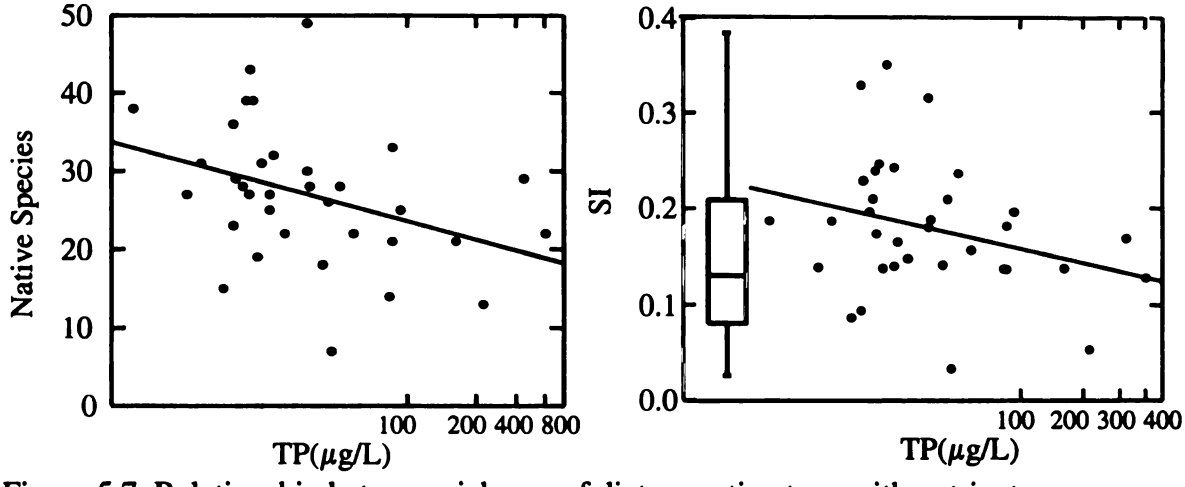

Figure 5.7. Relationship between richness of diatom native taxa with nutrient concentration (TP) (a), and relationship between similarity index (SI) of current diatom assemblage with pressettlement assemblages with nutrient concentration (TP) (b) in wetlands. Native taxa were defined as taxa found in the paleoecological samples. SI is the Bray-Curtis similarity of diatom relative abundance in surface sediment and the centroid of paeloecological samples.

Table 5.4. Correlation among community characteristics and environmental variables

	LGTN	LGTP	DIN	SRP	Chl a	% agricultural land
Species Richness	-0.471	-0.513	-0.434	-0.427	0.359	0.130
Shannon *H'*	-0.534	-0.420	-0.514	-0.357	0.265	0.093
No. of Native Species	-0.291	-0.452	-0.439	-0.302	0.195	0.015
Similarity Index	-0.453	-0.357	-0.395	-0.258	0.297	-0.062

Both the number of native taxa in surface sediments and similarity of diatom species composition with reference conditions decreased with increasing nutrient concentrations (Table 5.4, Figure 5.7). The number of native species in surface sediments was negatively correlated with increased nutrient concentrations, especially TP in wetlands. Diatom species composition in reference conditions showed large variation among different sites. The similarities ranged from 0.03 to 0.38 with a median similarity of 0.13. The similarities of current diatom relative abundances with paleoecological diatom species composition were also negatively correlated with TN and TP concentrations (r= -0.453 and –0.357 respectively). These similarities were generally low and varied within the range of similarities among reference conditions. Thus a definition of biological criterion based on similarity change was unlikely.

van Dam's trophic indices defines the importance of trophic sensitive diatom species and trophic conditions in wetlands. I defined the 75th percentile of paleoecological diatom trophic indices in wetlands as the biological criterion of pristine conditions. van Dam's trophic indices in reference conditions varied from 1.7 to 4.5 with a median value of 3.4. van Dam trophic indices in current diatom species composition was more viriable, especially at the higher value which represented higher relative abundance of trophic species in some wetlands now than in historic times. The values of indices showed a

linear increase with increase of TP concentration (R^2=0.409, P=0.001, Figure 5.8a). The nutrient concentration at the intercept of the biological criterion with regression lines represented the nutrient criterion (stressor criterion=45.6 μm/L). Change of van Dam trophic indices was also linearly correlated with agricultural gradient (R^2=0.517, Figure 5.8b). However, a regression tree analysis also showed a changepoint at 56% agricultural land use where the van Dam trophic index increased from 3.46 to 4.85.

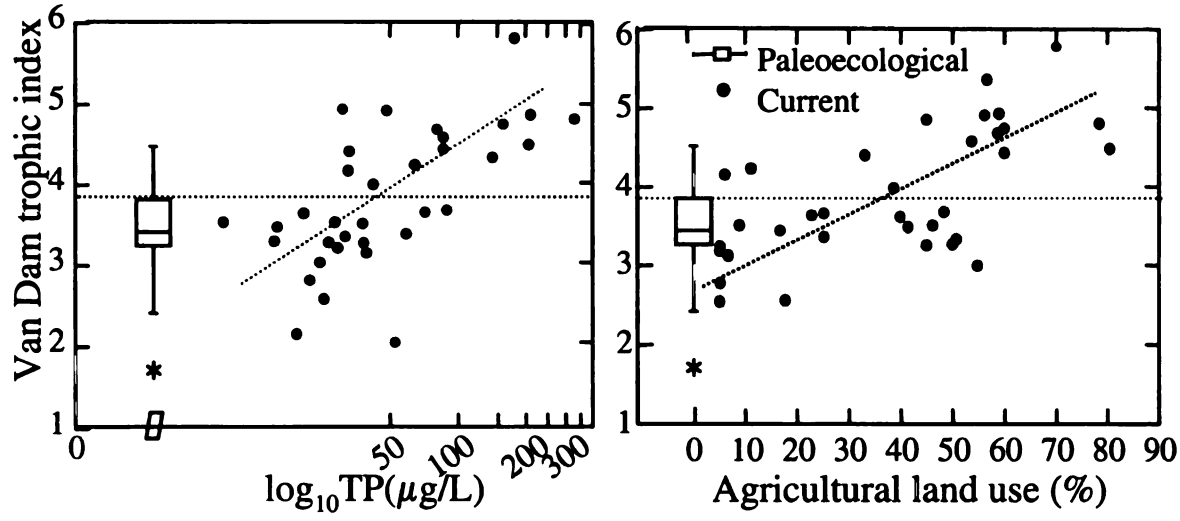

Figure 5.8a. Relationship between van Dam trophic index and palustrine wetland nutrient concentration (TP). Box-plot shows paleological diatom trophic index from 14 palustrine wetlands to represent reference wetland condition. Surface diatom trophic index (dots) respond linearly to TP concentration in wetlands (R^2=0.407, P=0.001). The intercept of biological criteria and regression line represented nutrient criteria (stressor criteria=46 ug/L). 8b. Relationship between van Dam trophic index and palustrine wetland land use. Box-plot shows paleological diatom trophic index from 14 palustrine wetlands representing reference wetland condition; dots represent change of trophic index with agricultural land use; a threshold effect was observed when agriculture exceeds 56% total land use, with van Dam trophic index increasing from 2.46 to 4.85.

5.5 Discussion

The goal of ecological assessment is to assess condition of ecosystems, often relative to an expected or desired condition, to determine the stressors that threaten or impair valued ecological attributes, and to determine the human activities producing those stressors. This has been challenging in wetlands due to spatial and temporal variability. I found that changes in algal species composition in wetlands were related to human activities and then developed biological and nutrient criteria that would protect the natural diatom assemblages of wetlands. I found that development of assessment criteria required classifying wetlands by type and restricting biological metrics to those most sensitive and directly related to stressors. Characterizing reference condition with paleoecological assemblages reduced doubt of the biological potential of the wetlands.

Nutrient concentrations, especially total phosphorus concentration in the Michigan wetlands studied, were directly related to human activities, especially agricultural land use. Human activities are well-known causes of nutrient enrichment of aquatic systems (Vitousek et al. 1997, Carpenter et al. 1998). Land cover/land use indicators of human activities and related water quality models are commonly correlated with nutrient concentrations in surface waters (Soranno et al. 1996, Tufford et al. 1998, Freeland et al. 1999). As the receiver of storm runoff, overland flow, and ground water influx, palustrine wetlands received high nutrient input from agriculture (Thompson and Polet 2000). My study not only found that high phosphorus concentration in Michigan wetlands were correlated with agricultural land use surrounding wetlands, but also discovered a threshold effect when agricultural land use exceeded 57.9% total land cover. Similarly, Waltman et al. (unpublished) also found a threshold effect on nutrient concentrations

when the % agriculture in Kentucky stream basin exceeded 60%. This effect may represent saturation of the assimilative capacity of soils within watersheds.

I found that changes in diatom species composition, number and relative abundance of individuals belonging to native species, and other valued ecological attributes changed with nutrient enrichment associated with human activities. TP, as a common cause of change in species composition, was an environmental stressor directly related to land use in Michigan wetlands. Examination of diatom taxa found in wetlands with 57.9 % agricultural land use showed that those wetlands were dominated by taxa requiring high nutrients, such as *Epithemia adnata and Cyclotella nana,* and had the lowest number of native taxa. TP enrichment was the possible cause of loss of native taxa. Both increased species diversity and decreased species diversity have been found in aquatic systems with increased nutrient concentrations (Jutter et al. 1996, Hillebrand and Sommer 2000, Pan et al. 2000). I expected a decreasing native taxa richness because native taxa were less competitive in nutrient enriched environments. Competitive exclusion for other limiting resources, increased predation, or alteration of other abiotic factors through indirect effects of nutrients (e.g., dissolved oxygen concentration, microbial interaction, or habitat structure) may all decrease native taxa richness.

The similarity of species composition to reference condition should be more sensitive than loss of native taxa (presence/absence of taxa), because relative abundance of taxa should change before they are lost from a habitat. However, my results only found a weak linear decrease in the similarity index (SI) with increasing nutrient concentration. The weak response of SI is possibly because SI was affected by multiple natural variables, such as pH and conductivity, as well as nutrient enrichment. Therefore, SI

responses are complexly relate to a stressor and its interaction with other stressors (Lansid and McLaughlin 2000). Alternatively, van Dam's trophic index represents sensitive response of diatom relative abundance specific to one environmental stressor, in this case, nutrients. Although it was developed in Europe, the strong relationship between diatom trophic indices and TP in Michigan wetlands suggests that this index could have broader geographic application.

Reference condition is ideally based on the central tendency and variability in conditions in habitats with the least human disturbance. Paleoecological assessments derived from sediments deposited before European alterations of landscapes provide the ideal characterization of reference condition. When using SI to describe reference condition, little difference was observed between reference condition and present condition in wetlands. SI was very low among paleoecological conditions in MI wetlands (usually 0.1-0.2) compared to present day variability among reference sites in the Everglades (0.7, Stevenson et al. 2002). This low similarity among reference wetlands was probably related to the great natural variability in pH, conductivity, and even development stage among wetlands. Greater differences between reference condition and present day condition of wetlands was observed when diatom assemblages were characterized using van Dam's trophic indices, probably because van Dam's trophic index based on diatom autoecological information of individual taxa characterizes biological accumulation of trophic status in the system. Nutrient conditions were probably more consistently low before European alteration of landscapes than pH, conductivity, and other naturally varying factors that affect diatoms. Compared with SI, autoecological indices are usually more precise for assessment and diagnosis of aquatic

ecosystem problems (Stevenson et al. 2001). Thus, biocriteria for wetlands were based on van Dam's trophic index indicating the relative abundance of diatoms that were sensitive to a single stressor, nutrient enrichment.

The biocriterion based on Van Dam's trophic index was established at the 75th percentile of its frequency distribution, which represents an upper bound of nutrient tolerant diatoms in the natural biological assemblages. The 75th percentile of reference conditions has been defined to indicate deterministic variation of algal assemblages and it has been used in other studies (Hill et al. 2003). A variety of percentiles in reference conditions have been used by different agencies for different purposes. An ideal approach for defining biological criteria would be based on a threshold response along a stressor gradient.

With the determination of a criterion for a valued ecological attribute, stressor criteria can be determined based on the VEA criterion and a stressor-response relationship. A cause-effect relationship has been established between nutrient enrichment and algal response in Michigan wetlands (Chapter 4). Stressor criteria should be no higher than the stressor level predicted to cause VEAs to be at the level of the biocriterion (Wang and Stevenson 2002, Stevenson et al. accepted). An alternative approach is to observe thresholds in VEA response along the stressor gradient. The TP criterion based on stressor-response relations and the biological criterion (van Dam trophic index) was 46 μg/L. This value was similar to the TP levels below the threshold response in TP with increasing agricultural land use. These complementary lines of evidence provide sound conceptual foundations for defining nutrient criterion.

The ultimate goal of assessing and managing aquatic ecosystems is to restore these systems to natural conditions. Thus, I first should estimate the natural condition should be estimated by direct assessment of reference condition with minimum human activity, contamination, and alteration. Second, cause-effect relationship should be determined between algal indicators and stressors by manipulated experimental approaches. Third, stressor criteria could be determined by integrating results from physical and chemical analyses, experimental and observational relation of stressor- response gradients, and bio-criteria based on reference approach. A suite of VEAs, such as native taxa richness, shifts in relative abundance, or sensitive taxa should be measured in relation to stressor gradients. Future research should explore ecological response of algae to nutrient concentrations in wetlands, and a nationwide database of individual diatom autoecological indices should be established. An understanding of benthic algal ecology is a prerequisite to implementing a biological approach to ecosystem management using algae.

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Chapter 6

Conclusions

This dissertation develops a better understanding of relationship among a variety of ecological factors within wetlands, evaluates a number of valuable ecological attributes to develop indicators for wetland bioassessment and wetland restoration, and establishes biological and environmental criteria to protect wetland ecosystems. Because of the spatial and temporal variability within and among wetlands, ecological assessment in wetlands has to include development of habitat or class specific attributes for effective evaluation. My studies in Michigan wetlands indicated that the similarity of algal assemblages among habitats within wetlands and between restored and extant wetlands were related to a number of environmental characteristics. Local pairwise comparison based on reference conditions could reduce regional variation among wetlands and identify the successional pattern of ecological development after restoration with restored wetland age. Algal attributes in both freshwater and salt marshes were closely related to nutrients. Experimental determination indicated that algal biomass and algal assemblages were directly related to nutrient concentrations and nutrient ratio, and a cause-effect relationship was established between algal assemblages and nutrient gradients. Based on the stressor-response relationship between nutrient stressors and algal attributes, and by choosing paleoecological diatom assemblages as reference condition, I developed bio-criteria and nutrient criteria for wetland protection.

I first evaluated spatial and temporal variation of environmental and algal attributes among habitats within wetlands and between restored and extant wetlands. Restored wetlands differed from extant wetlands in a number of ways in Southern Michigan.

Physical and chemical variables, especially plant shading and nutrient concentrations, were distinctly greater in extant wetlands and contributed to differences in algal communities among habitats within wetlands and between restored and extant wetlands. Whether algal assemblages in these two types of wetlands were similar or distinct depended on different habitats. Epiphytic assemblages in the restored and extant wetlands were relatively distinct, while neither sediment nor phytoplankton assemblages were as different between restored and extant wetlands. On the other hand, non-diatom algal assemblages from different habitats were mostly different, but sediment and epiphytic diatom assemblages were not significantly different. Ecological restoration is a long-term process and does not occur in a short period of time. I found no direct evidence for increasing similarity in algal assemblages between restored and extant wetlands with increasing age of restored wetlands. However, response of algal assemblages to nutrient concentration and light, two factors distinguishing restored and extant wetlands, indicated indirectly that succession processes in restored wetlands were regulating algal assemblages and restored wetlands < 10 years old may need more time to develop mature qualities.

In the next chapter, I evaluated a suite of algal attributes of restored salt marshes based on paired reference and restored marsh approach and developed indicators for wetland development. I assessed algal biomass (dry mass (DM), ash free dry mass (AFDM), chl *a* content, algal biovolume), algal species composition and diversity, and gross primary production on both *Spartina alterniflora* and sediments (sediment algae). Controlling for regional variation in reference marshes substantially increased precision in relations between attributes and the increase in age of restored marshes. The organic

matter restoration ratio of sediments increased with age of restored marshes in both spring and summer. The algal biomass restoration ratios of epiphytes, calculated with algal biovolume and chl *a*, increased with restored marsh age in summer but not during spring. Biomass of sediment algae was not related to marsh age. The similarity of diatom species composition between paired restored and reference sites increased with age of restored marshes during spring and summer. Primary production by epiphytic and sediment algae in summer showed site-specific changes and did not change consistently with marsh age. Algal biomass, algal diversity, and diatom species assemblages during summer were positively correlated with sediment nitrogen and phosphorus concentration. I concluded that overall structural and functional development of restored wetlands, especially nutrient storage in sediments, regulated algal community structure and function, which can be used to evaluate marsh restoration.

I then determined the limiting nutrient in wetlands and established the cause-effect relationship between nutrient and algal assemblages because both the previous two chapters indicated that nutrients were one of the most important factors regulating periphyton growth and species composition in wetlands. Algal bioassays using nutrient diffusing substrata indicated that nitrogen (N) was the main limiting nutrient for periphyton growth in restored wetlands in southern Michigan. Algal N limitation positively correlated with phosphorus (P) concentration and negatively correlated with N:P ratios in wetland water and sediment. Canonical correspondence analysis (CCA) indicated that diatom species composition in natural habitats and control artificial habitats changed mainly along a pH gradient and secondarily along nutrient gradients indicated by total phosphorus (TP) and dissolved inorganic nitrogen (DIN). Individual diatom taxa

responded to nutrient addition differently. Overall, the responses of indicator species in nutrient bioassays were positively correlated with natural nutrient gradients among natural wetlands indicating a good match of diatom indicators between natural and experimental systems.

Chapter 5 illustrated how the stressor-response relationship and reference approach could be used to develop stressor and biological criteria to protect wetland health. Diatom-based biological indicators were used to establish biological and stressor criteria for wetland protection and restoration and to indicate wetland ecosystem health. Paleoecological diatom assemblages from pre-settlement sediments were used to represent natural, reference conditions in wetlands. Stressor-response relations were used to delineate stressor criteria and human disturbance. Canonical correspondence analysis (CCA) revealed that diatom species assemblages responded mainly to conductivity and total phosphorus (TP). Similarity in species composition to reference condition and number of native species in current wetlands decreased with increase of nutrient concentrations in these wetlands. van Dam's diatom trophic index was imported to characterize wetland trophic status in North American wetlands and was linearly correlated with the TP gradient. Both the weighted average TP model and van Dam's trophic index positively responded to agricultural land use around wetlands. These indices deviated from the "normal" natural condition when TP exceeded 46 μg/L. A threshold change in TP and biotic indices was detected when agricultural land exceeded 57.9 % total land use.

In summary, most extant wetlands have suffered a certain degree of nutrient impact from human disturbance. Wetland restoration has significantly decreased nutrient

concentrations and compensated for wetland loss. However, wetland restoration is a long-term process and it takes a long time for different attributes to develop to the status of reference condition. Continuous monitoring and assessment would lead to better understanding of ecosystem processes. My studies developed algal assemblages as indicators for diagnosing and quantifying nutrient pollution in wetlands and they identified ecosystems at risk due to excess nutrients in the environment. Based on the establishment of cause-effect relationship and stressor-response relationship between nutrient and algal attributes, ecological thresholds, algal biological criteria, and stressor criteria were developed for watershed management. The results of this research could be used to support ecologically sensitive decision-making in land planning and wetland restoration by providing targets for decreasing anthropogenic pollutions and thereby increasing the rate of successful restoration projects.

Appendices

Appendix A.

Wetland sites sampled in Southern Michigan. Land use around wetland 100m radius were defined based on observation. RES: resident; AG: agricultural; CIT: constantly influenced by road; UND: undisturbed.

Site code	Code	Latitude	Longitude	Land use	County	Sample Date	Artificial substrata	Age of Res.	Site property
1	Dans13	42.56604	-84.3968	RES	INGHAM	8-18-2000	YES	6	Res.
2	Jolly18	42.68161	-84.4154	RES	INGHAM	8-24-2000	YES	11	Res.
3	Jolly19	42.682	-84.4182	RES	INGHAM	8-24-2000	NO	N/A	Ext.
4	Green20	42.90778	-84.5202	RES	CLINTON	8-23-2000	NO	N/A	Ext.
5	Green25	42.81349	-84.4527	RES	CLINTON	8-23-2000	NO	8	Res.
6	Herbison2	42.8341	-84.3962	AG	CLINTON	8-1-2000	NO	7	Res.
7	27N	42.931	-84.515	CIT	CLINTON	8-23-2000	NO	8	Res.
8	27S	42.9262	-84.5159	CIT	CLINTON	8-23-2000	NO	8	Res.
9	Dans31	42.55581	-84.3642	RES	INGHAM	8-18-2000	YES	6	Res.
10	Airport542	42.7905	-84.6067	RES	CLINTON	8-21-2000	YES	2	Res.
11	Airport539	42.79212	-84.6027	RES	CLINTON	8-21-2000	NO	N/A	Ext.
12	Airport615	42.79212	-84.6026	RES	CLINTON	8-21-2000	NO	N/A	Ext.
13	Herbison6	42.8342	-84.4007	AG	CLINTON	8-1-2000	NO	N/A	Ext.
14	Alward	42.89237	-84.4581	RES	CLINTON	8-25-2000	NO	N/A	Ext.
15	Barnes	42.52287	-84.3594	AG	INGHAM	8-16-2000	NO	N/A	Ext.
16	CampusB	42.72074	-84.4776	CIT	INGHAM	8-25-2000	YES	9	Res.
17	CampusS	42.72079	-84.4766	CIT	INGHAM	8-25-2000	YES	11	Res.
18	Centerline	42.90436	-84.4738	RES	CLINTON	8-25-2000	NO	N/A	Ext.
19	CenterRd	42.82354	-84.4178	UND	CLINTON	9-1-2000	NO	N/A	Ext.
20	CentralPk	42.72789	-84.4218	CIT	INGHAM	8-22-2000	YES	2	Res.
21	Clark55	42.81374	-84.4528	RES	CLINTON	8-25-2000	NO	N/A	Ext.
22	Clark81	42.81422	-84.4013	RES	CLINTON	8-25-2000	NO	N/A	Ext.
23	Close	42.7564	-84.5242	CIT	INGHAM	8-21-2000	YES	11	Res.
24	College	42.63054	-84.4852	CIT	INGHAM	8-24-2000	YES	5	Res.
25	Cutler	42.8625	-84.6893	UND	CLINTON	8-19-2000	NO	N/A	Ext.
26	FarmL	42.70518	-84.4804	CIT	INGHAM	8-28-2000	YES	9	Res.
27	Forest	42.8621	-84.6759	RES	CLINTON	8-19-2000	NO	N/A	Ext.
28	Hawley	42.5187	-84.3824	UND	INGHAM	8-16-2000	NO	N/A	Ext.
29	Howe	42.84207	-84.4137	RES	CLINTON	8-19-2000	NO	N/A	Ext.
30	Krepps	42.9124	-84.5073	AG	CLINTON	8-23-2000	NO	N/A	Ext.
31	Loren	42.5207	-84.3541	AG	INGHAM	8-16-2000	YES	10	Res.
32	Marsh	42.7741	-84.4124	CIT	CLINTON	9-1-2000	YES	5	Res.
33	MasonPk	42.57729	-84.4316	RES	INGHAM	8-18-2000	NO	N/A	Ext.
34	Meijer	42.5674	-84.3443	CIT	INGHAM	8-18-2000	YES	12	Res.
35	Open	42.7564	-84.5242	CIT	INGHAM	8-21-2000	YES	7	Res.
36	Orchart	42.5838	-84.4587	UND	INGHAM	8-18-2000	YES	3	Res.
37	Pratt	42.90436	-84.524	AG	CLINTON	8-23-2000	YES	10	Res.
38	Price	42.9286	-84.4418	RES	CLINTON	8-25-2000	YES	5	Res.
39	Railway	42.72044	-84.4788	CIT	INGHAM	9-1-2000	NO	N/A	Ext.
40	Rosendom	42.77521	-84.405	RES	Clinton	9-1-2000	YES	2	Res.
41	Stoll	42.79659	-84.6052	RES	CLINTON	8-21-2000	NO	N/A	Ext.
42	Tom	42.8543	-84.7094	RES	CLINTON	8-19-2000	YES	3	Res.
43	Upton	42.81598	-84.4024	RES	CLINTON	8-1-2000	NO	N/A	Ext.
44	VaughnE	42.5003	-84.363	AG	INGHAM	8-16-2000	YES	2	Res.

45	VaughnW	42.5003	-84.363	AG	INGHAM	8-16-2000	YES	2	Res.

Appendix B.

EMAP sampling location and their site property. Michigan subecoregion were classified into 6 types. Soil type are classified as: 1, Northern Michigan and Wisconsin Sandy Drift. 2, Western Michigan and Northeastern Wisconsin Fruit belt; 3, Southern Michigan and Northern Indiana Drift Plain; 4, Indiana and Ohio Till Plain; 5, Southern Michigan Fruit and Truck Belt. Agricultural land use around wetlands are based on 1 km radius around

Site Code	latitude	longitude	Ecoregion	Soil Region	% AGR
MIWET03	44.39583	-85.0242	2	1	0.166347
MIWET04	44.43513	-84.9115	2	2	0
MIWET05	44.625	-85.0552	2	1	0
MIWET10	45.36889	-84.4081	1	1	0.049657
MIWET12	44.72083	-84.4283	2	3	0.810269
MIWET18	43.26359	-85.2996	3	3	0.776972
MIWET20	42.65217	-85.5165	4	3	0
MIWET25	42.31386	-84.1737	5	4	0.842661
MIWET26	42.36821	-84.2022	5	3	0.498948
MIWET31	42.57201	-83.5506	5	4	0.518641
MIWET33	42.53601	-83.6563	5	4	0.568018
MIWET35	42.33016	-84.1287	5	3	0.646123
MIWET36	42.44701	-83.9982	5	4	0.53634
MIWET39	42.31658	-85.3603	4	3	0.276599
MIWET41	42.25543	-85.3603	4	3	0.330213
MIWET42	41.92935	-85.8401	4	3	0.256192
MIWET43	41.85054	-85.75	4	3	0.383817
MIWET48	42.40217	-85.3971	4	5	0.284899
MIWET49	41.91848	-85.7813	4	3	0.162268
MIWET50	42.14946	-85.6011	4	3	0.556
MIWET51	42.46603	-85.3695	4	3	0.482928
MIWET52	42.49796	-85.2206	4	3	0.605755
MIWET53	42.53533	-86.0404	6	3	0.455534
MIWET54	42.64674	-85.9228	6	5	0.56166
MIWET58	42.52446	-86.0055	6	5	0.479886
MIWET59	42.54755	-86.0037	6	5	0.338
MIWET60	42.46603	-85.2243	4	3	0.144218
MIWET62	42.3913	-84.977	4	3	0.671064
MIWET68	42.45788	-83.9982	5	4	0.203087
MIWET69	42.45245	-84.0055	5	4	0.002193
MIWET70	42.44973	-84.0147	5	4	0.096715
MIWET71	43.77446	-85.2132	2	3	0.224564
MIWET72	44.18278	-84.8183	2	3	0
MIWET77	45.22147	-84.3824	1	3	0.051222
MIWET78	45.55707	-84.6531	1	3	0
MIWET80	45.71603	-84.6725	1	2	0.519926