CHARACTERIZING DIATOM ASSEMBLAGES

IN PONDED WETLANDS

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

Using biological assemblages for evaluating ecosystem integrity is dependent upon robust sampling techniques that adequately characterize the species composition. This thesis evaluated the performance of a wetland surface-sediment diatom collection methodology. The methodology did not result in collection of 95% of species present due to the richness of rare species in the ponded wetlands. However, because rare species added little to relative abundance, the method provided a community characterization that separated the communities based upon ponds within complexes and ponds between complexes. The mantel test indicated species distribution was dependent upon pond and complex ($p<.001$) with a model matrix of dissimilarity based upon 0, within pond, ½, within complex, and 1, between complex. The Multi-response Permutation Procedure supported the method's ability to separate complexes based upon communities ($p=.012$). The significant p-value is attributed to the very large affect size ($A=.45$) which adds further credence to the method's efficacy. Nonmetric Multidimensional Scaling ordination was used to graphically interpret the separation of ponds. The ordination shows sizeable separation between complexes, considerable separation between most ponds within a complex, and relatively little separation between replicates. Culling rare species further improved the ordination with 91% of the variance explained by 2 axes with a stress of 7.35 and a probability of obtaining that stress by a chance of $p=.004$. The sampling methodology also provided an assemblage that correlated with biotic and abiotic environmental variables. The Mantel test indicated

correlation with water chemistry variables ($r=0.564$, $p=0.028$) after controlling for vegetation variables. Vegetation variables also correlated with the diatom community ($r=0.700$, $p=0.001$) after controlling for chemical variables. Correlation increases for groups of environmental variables emphasizes the abilities of the Mantel test and BIOENV over ordination techniques in ponded wetlands when the factors influencing the community composition are working as a consortium. However, the ordination is still useful because it indicated the diatom community corresponds with our knowledge of shallow lake ecology where biotic and abiotic factors work as a consortium. The sampling methodology could provide another tool for environmental assessment and enhance our ability to deduce wetland status and promote desired wetland biota.

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CHAPTER 1

INTRODUCTION

Using biological assemblages for evaluating ecosystem integrity is dependent upon robust sampling techniques that adequately characterize the species composition. Characterizing wetlands is extremely challenging because of their internal spatial heterogeneity and the large variations in wetland types. Confounding the complexity of heterogeneous habitat, biological communities of wetlands in temperate latitudes also experience seasonal heterogeneity. The following thesis attempts to determine if the diatom assemblage can be used to characterize the communities of wetlands managed for waterfowl on the eastern shores of the Great Salt Lake, Utah, and if so, determine the strengths of those relationships between the community and both abiotic and biotic wetland characteristics.

Although wetlands occupy only 6% of the Earth's terrestrial surface (Maltby and Turner 1983) their importance in biogeochemical cycling (Reddy and DeLaune 2008) and food webs (Bott 1996; Lamberti 1996; Mitsch and Gosselink 2000) is much greater than their relative area. Wetlands are highly productive ecosystems that support internal, terrestrial and aquatic food webs (Westlake, Kvét et al. 1998).

Historically, wetlands have been viewed as detritus-based systems (Mitsch and Gosselink 2000) with most primary productivity coming from macrophyte vegetation. That view has changed recently because algae have been shown to contribute a significant proportion of wetland primary productivity and also play a large role in wetland food webs (see review in Stevenson et al., 1996). As such, the importance of algae to

wetland ecosystem functions has typically been underestimated (Batzer and Wissinger 1996)

Aquatic algae are species rich in most aquatic environments even though they are subject to extreme competition for limited resources (Hutchinson 1961). Algae are also very sensitive to environmental gradients and have been used in both riverine and lacustrine assessments of trophic status and ecosystem functions (Lowe and Pan 1996). The presence of "signature" algal communities in a system is a result of abiotic conditions and biotic interactions. One algal group, diatoms (*Bacillariophyta),* have been used extensively in various aquatic paleolimnological studies to determine the abiotic conditions historically present in the depository waters (Dixit, Smol et al. 1992). Diatoms have also been used in contemporary watershed science to determine trophic states (i.e. nutrient status) of rivers and lakes (Van Dam, Mertens et al. 1994). Diatoms, however, have not been used extensively in wetland studies. I intend to explore the possibilities of diatoms for use as environmental indicators for wetlands with this proposed research.

According to Gibson (Gibson, Bowman et al. 2000), environmental trophic indicators should fulfill the following five requirements: a) be related to biological integrity, b) respond monotonically to environmental stresses, c) be measurable with a low error, d) be cost effective, and e) be environmentally benign to measure. The sampling protocol outlined in the Materials and Methods section of this paper fulfills the last two requirements. The purpose of this research is to test the sampling protocol and assemblage for the first three requirements. This research should also illuminate what abiotic characteristics are the greatest contributors to shaping species assemblage and which ones may be acting as environmental stressors.

Diatoms have been used in freshwater research to assess environmental conditions in aquatic systems for over a century(Kolkwitz and Marssln 1908). Historically the focus has been on using benthic algae in riverine systems for assessing

environmental conditions associated with point source pollution (Hansmann and Phinney 1973; Lowe and McCullough 1974; Cooper and Wilhm 1975; Morgan 1987; Biggs 1989; Stevenson, Bothwell et al. 1996). Numerous autecological tables exist for lake and riverine systems and the ecological "preferences" of many diatoms are available (Van Dam, Mertens et al. 1994; Kelly and Whitton 1995). These indices are generally not correlated to specific abiotic characteristics; however, they are more frequently correlated with specific abiotic states, also known as trophic states. It would be of great value to wetland scientists and managers to correlate diatom guilds with actual concentration gradients of abiotic characteristics.

Numerous chemical and physical variables exist that promote and corrupt wetland functions and most of them can be measured directly. One could reasonably ask why monitor biology when the abiotic factors can be measured directly? The question is reasonable but measuring chemical and physical parameters has several disadvantages. First the actual cost associated with measuring the plethora of chemical and physical parameters can be prohibitive. Even if it was possible to continuously monitor for all chemical and physical parameters affecting the biological communities, impacts of different combinations of abiotic or environmental factors can be substantially different from the singular impacts of individual factors. Furthermore, the biological components of an ecosystem are the actual result of the biotic and abiotic interactions and provide the best information about the health of the system.

I prefer to ask the question; why are we monitoring the chemical and physical components of the system when it is the biology of the system providing the ecosystem goods and services? If the biology of wetland systems is what we need to understand and what governmental policies are attempting to protect, we should be developing robust biological monitoring practices and models. Only through biological assessment will we be able to determine which processes are being promoted and which ones are

being corrupted by the enormous number of potential environmental variables. We understand a considerable amount about wetland biogeochemical functions and their relationship to environmental variables. This knowledge could be used to manage and assess wetlands experiencing anthropogenic influences if we can develop adequate biological monitoring practices.

Algae play a pivotal role in all aquatic ecosystems as primary producers and basic components of the food web (Goldsborough and Robinson 1996). In aquatic systems, they are the interface between chemical and physical components and primary productivity. Algae can account for a significant portion of the primary productivity in wetlands (Burkholder and Wetzel 1989; Pinckney and Zingmzrk 1993; Cronk and Mitsch 1994) and the importance of algal assemblages to secondary producers in wetland food webs is being shown to be greater than previously assumed (Lamberti 1996). Historically, wetlands have been considered detritus-based systems (Mitsch and Gosselink 2000) and algae have been shown to decompose up to seven times faster than macrophytes (Pieczyńska 1986). Algae are significantly important in both the herbivorous and detrital food webs of wetlands (Smalley 1958; Soszka 1974; Kirby and Gosselink 1976). The biological importance of algae promotes their candidacy for use in biological monitoring.

Although sampling for algae in rivers and lakes systems is relatively uncomplicated, sampling wetlands for algae has several confounding factors. Stating hydrology is the key determinant of wetlands is somewhat of a cliché in contemporary literature but the point is that varying hydrology creates varying wetlands that have varying sampling issues. Overcoming the issues of inevitably sampling diverse conditions in wetlands is not a trivial problem. Wetlands vary in hydrologic gradients and vegetation associated with differing gradients. Wetlands span a range of inundation from waters 2 m deep to periodically dry conditions.

Wetlands may have emergent, submergent or hydrophytic vegetation on continually wet or periodically dry soils. Algae live on all habitats available in wetlands. There is a "free" living phytoplankton component, an epiphytic component, a metaphyton and a benthic component. Algae even colonize macroinvertebrates and vertebrates (Hart 1935; Croll and Holmes 1982; Holmes and Croll 1984; Holmes 1985). In other words, algae are ubiquitous in wetlands.

A record of the entire algal community (and sometimes the associated terrestrial community) exists in the soils of wetlands and other lentic systems (Stevenson and Smol 2003). Sediment samples can be collected at all wetland sites either from a boat, by wading, or walking. Since sedimentation rates in the wetlands I am studying along the eastern shores of the Great Salt Lake are less than .5 cm/year (Miller 2008), seasonal and temporally variable algae will be present in the surface sediments and will provide a record of at least the most recent year's flora. Paleolimnological studies often use the sediment diatom assemblage because such assemblages are believed to integrate diatoms from various habitats over several years (Dixit, Smol et al. 1992; Fritz, Cumming et al. 1999).

Dry soils generally do not contain abundant "soft" algae because desiccation destroys their cellular structure. Diatoms are also intolerant to desiccation, but their unique silica wall structure allows post mortem identification centuries or millennia later. Diatoms are the organisms most often used for environmental paleolimnological research (Dixit, Smol et al. 1992). Their very distinctive and unique silica shells are easily identifiable to species level with relatively inexpensive microscopic laboratory techniques. The preparatory and slide mounting techniques enable samples to maintain their viability for centuries allowing for reanalysis at a later date and provide a historic record. Because surface-sediment diatom assemblage can be sampled in all wetlands,

even if they have a “dry” hydrologic phase, I have chosen surface-sediment diatom assemblages as the matrix of interest for this study.

Determining the “best” location for sampling diatom assemblages can be problematic because sedimentation rates can vary spatially in wetlands (Fennessy, Brueske et al. 1994). Diatom assemblages in lakes have also been shown to vary spatially (Bradbury and Winter 1976; Earle, Duthie et al. 1988). Natural wetlands generally have spatial heterogeneity associated with water depth and algal biomass and assemblage can be related to water depth (Schalles and Shure 1989; Robinson, Gurney et al. 1997a; Robinson, Gurney et al. 1997b). Macrophyte vegetation and its associated algal epiphyte community in wetlands are also dependent on water levels (Pip and Robinson 1984; Cattaneo, Galanti et al. 1998). Given this information, one would conclude that benthic diatom and algal assemblages may not be homogenous and could be spatially variable. Weilhoefer and Pan (Weilhoefer and Pan 2006) showed spatial variation of benthic diatom assemblages within the Rooster Rock wetland of Oregon. They concluded that species richness leveled after sampling a composite of five surface-sediment samples with about 95% of the species accounted for. With the replicate composite samples in my methods, I propose to determine if five composited surface sediment samples can adequately and uniquely characterize the wetlands diatom assemblage in ponded wetlands of the east shore of the Great Salt Lake, Utah.

This thesis is designed to answer three questions related to diatom surface-sediment assemblages. First, does the sampling methodology adequately characterize the community assemblage? This question is often overlooked by most researchers. It is important since any conclusions drawn from methodologies that do not adequately characterize the diatom community and are not reproducible are prone to error. Second, which environmental gradients are most responsible for community structure? Although a large list of possible gradients have been selected and measured *a priori,* the

community assemblage itself will *a posteriori* determine which abiotic components influence the community most strongly. This is an important distinction from other research where the consequences of environmental gradients are predetermined by the researcher based upon their opinion of what should be significant. Third, which community characteristics or attributes respond appreciably to the environmental gradients? This question is also being pursued on an *a posteriori* basis.

Using appropriate statistical techniques, the community members that respond to environmental gradients will be tested for degree of responsiveness and significance. Although essential work on metrics that respond to environmental gradients has been done on rivers and streams (USEPA 2002), wetlands are a different environment. Generally, wetlands are considered harsh environments and the fauna associated with them is tolerant of those harsh conditions (Mitsch and Gosselink 2000). Therefore, the response of those communities to environmental gradients may not vary considerably as the community is composed of tolerant individuals. The question of whether diatom communities significantly differ in response to environmental gradients could help elucidate the sensitivities of wetlands to anthropogenic influences.

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CHAPTER 2

EFFICACY OF A DIATOM SURFACE-SEDIMENT SAMPLING METHODOLGY IN PONDED WETLANDS OF THE GREAT SALT LAKE, UTAH, USA

Abstract

The performance of a wetland composite surface-sediment diatom collection methodology was evaluated with individual and replicate samples from ponded wetlands in three different wetland waterfowl management areas associated with the Great Salt Lake, Utah. The methodology did not result in the collection of 95% of species present due to the richness of rare species in these wetlands. Because the rare species added little to the total relative abundance, however, the method did provide an adequate community characterization that separated diatom communities of ponds within complexes and ponds between complexes.

The Mantel test indicated the species distribution was dependent upon pond and complex ($p<.001$) with a model matrix of perceived dissimilarity based upon 0 within ponds, ½ within complexes, and 1 between complexes. The Multiresponse Permutation Procedure (MRPP) supported the ability of the method to separate complexes based upon diatom communities ($p=.012$). Significant p-values can be difficult to obtain with this test and small samples sizes ($n=7$) and is attributed to the very large effect size ($A=0.45$). The large effect size ($A=0.45$) adds further credence to the efficacy of the method.

Nonmetric Multidimensional Scaling ordination was also used to determine the strengths of community characterization and to graphically interpret the separation in species space of within pond replicates, ponds within complexes, and ponds from different complexes. The ordination graphically demonstrates the ability of the method to characterize the diatom community. Precision is demonstrated by the close clustering of replicate samples and accuracy is demonstrated by clear separation of assemblages from different complexes.

Culling rare species further improved the ordination with 91% of the variance explained by 2 axes with a stress of 7.35 and a probability of obtaining that stress by chance of p=.004. Although the sampling method and laboratory analysis did not elucidate 95% of the diatom species richness in these ponded wetlands, the method provided reproducible results that appear to provide sufficient abundance information to unambiguously characterize the different diatom community assemblages.

Introduction

Using biological assemblages for evaluating ecosystem integrity is dependent upon robust sampling techniques that adequately characterize species composition. Characterizing wetlands is challenging because of their internal spatial heterogeneity and the large variation in wetland types. Confounding the complexity of heterogeneous habitat, biological communities of wetlands in temperate latitudes also experience seasonal heterogeneity.

Weilhoefer and Pan (2006) determined the efficacy in terms of richness for surface-sediment diatom sampling was maximized by compositing five surface-sediment cores into one sample for the Rooster Rock wetland, Oregon. The following research evaluates the reproducibility of samples collected by the surface sediment diatom collection methodology recommended by Weilhoefer and Pan (2006) and the ability of

the method to adequately distinguish between diatom communities in different water-fowl management areas on the eastern edge of the Great Salt Lake, Utah. If the method provides reproducible results and can distinguish between communities in different waterfowl management areas, it could be a useful tool for environmental assessments and wetland research.

According to Gibson (2000), metrics used to determine trophic status, i.e. environmental trophic indicators, should fulfill the following five requirements: a) be related to biological integrity, b) respond monotonically to environmental stresses, c) be measurable with a low error, d) be cost effective, and e) be environmentally benign to measure. The sampling protocol outlined in this research accomplishes the last two requirements. The purpose of this work is to evaluate the third requirement, the ability of the composite sampling protocol to discriminate between diatom communities based upon the error components of reproducibility and specificity. For this research, reproducibility and specificity will be evaluated using distance scores based upon sample species assemblage. Replicates samples should have small difference scores relative to difference scores of samples between complexes.

Although sampling for algae in rivers and lakes systems is comparatively uncomplicated, sampling wetlands for algae has several confounding factors. Saying hydrology is the key determinant of wetlands is somewhat of a cliché in contemporary literature but varying hydrology creates variable wetlands with varying sampling issues. Overcoming the issues of inevitably sampling diverse conditions in wetlands is not a trivial problem. Wetlands vary in hydrologic gradients and vegetation associated with differing gradients. Wetlands span a range of inundation from waters 2 meters deep to periodically dry conditions (Cowdin, Carter et al. 1979).

Wetlands may have emergent, submergent or hydrophytic vegetation on continually wet or periodically dry soils. Algae live in all habitats available in wetlands

including a “free” living phytoplankton component, an epiphytic component, a metaphyton and a benthic component. Algae also colonize macroinvertebrates and vertebrates (Hart 1935; Croll and Holmes 1982; Holmes and Croll 1984; Holmes 1985). In other words, algae are ubiquitous in wetlands.

A record of the entire algal community (and sometimes the associated terrestrial community) may exists in the sediments of wetlands and other lentic systems (Stevenson and Smol 2003). Sediment and soil samples can be collected at all wetland sites from a boat, by wading, or walking. Paleolimnological studies often use sediment diatom assemblages because they are believed to integrate diatoms from various habitats over several years (Dixit, Smol et al. 1992; Fritz, Cumming et al. 1999).Sedimentation rates in the wetlands studied along the eastern shores of the Great Salt Lake are less than 0.5 cm/ year(Miller 2008) and temporally and spatially variable algae should be present in the surface sediments and should provide a description of at least the most recent year’s flora.

Determining the “best” location for sampling diatom assemblages can be problematic since sedimentation rates can vary spatially in wetlands (Fennessy, Brueske et al. 1994). Diatom assemblages in lakes have also been shown to vary spatially (Bradbury and Winter 1976; Earle, Duthie et al. 1988). Natural wetlands often show spatial heterogeneity associated with water depth and algal biomass and algal assemblages can be related to water depth (Schalles and Shure 1989; Robinson, Gurney et al. 1997a;Robinson, Gurney et al. 1997b). Macrophyte vegetation and associated algal epiphyte communities in wetlands are also dependent on hydrologic conditions (Pip and Robinson 1984; Cattaneo, Galanti et al. 1998). Given this information, one would conclude that benthic diatom and algal assemblages may not be homogenous and could be spatially variable. Weilhoefer and Pan (2006) showed that indeed a spatial variation of benthic diatom assemblages did exist within the Rooster

Rock Wetland of Oregon. They concluded that sample species richness leveled off after sampling a composite of five surface-sediment samples. With replicates of composited samples in my methods, I propose to determine if five composited surface sediment samples can adequately characterize and distinguish diatom assemblages of wetlands in this study.

Materials and Methods

Study Site

Sediment samples were collected in the fall of 2008 at three ponded wetland complexes on the eastern shores of the Great Salt Lake, Utah (Figure 2.1). All three complexes contain several connected ponded wetlands and are managed to enhance waterfowl habitat. A total of seven ponds were sampled: three at the private Newstate Duck Club, two at the federal Bear River Migratory Bird Refuge and two at the State of Utah Public Shooting Grounds (Figure 2.1). Although the three complexes are managed similarly, they have different source water with differing water quality and therefore should have differing diatom community assemblages.

The three complexes have similar altitudes and are proximate enough to have similar climate. The three complexes have similar hydrology and are managed for water fowl. Managers of each facility control pond depth to cultivate *Stuckenia* and *Ruppia*. Ponds are drained in late January to control nuisance fish species (common carp) and filled during spring runoff in late February or early March. The rest of the year pond depths are generally maintained around 16 inches (40.6 cm).

Figure 2.1. Location of ponded complexes along the eastern shore of the Great Salt Lake studied in this research.

Sampling Methodology and Study Design

Diatom samples were collected by wading transects parallel to impoundment dikes about 50 meters from the pond's controlling culvert. Each sample from a pond was a composite of five core grab samples. Five random numbers between 1 and 100 were generated for sampling along 100 meter transects. Core samples were collected by pushing a 2" ID clear plastic tube into the sediment approximately 30 cm. The top 1 cm of the core was placed in an appropriately labeled polyethylene container. The process was repeated at the next four random points along the transect and the composite of five surface sediment samples was blended with a putty knife, capped and frozen until analysis. To obtain three samples (replicates) at one pond, 15 cores were collected along one transect by generating three random sets of five numbers. The three composite samples of five cores were collected during one pass along the transect to avoid collecting at a disturbed location. A total of 13 samples each containing 5 cores was collected.

Laboratory Analysis

Strewn mounts were prepared by following the methods of Sgro and Johansen (1995). Microscopy was performed using an Olympus BX51 research microscope equipped with differential interference phase contrast optics. Permanent strewn mounts were examined along linear, horizontal transects of the slide. Each species with at least 50% of a single valve present was counted until a minimum total of 600 valves were enumerated. Identifications were performed using standard taxonomic works and personal reference slide collections.

Six hundred valves were counted in each sample. The 13 samples contained a total of 130 species. Forty-one species appeared in only one sample, and each sample contained a large number of species with only one individual identified. These

singletons provide a large number of the total species present in the samples, but account for a small proportion of the total relative abundance.

Data Analysis

Diatom counts were log base 2 transformed for a graphical interpretation of species abundance curves. For other analyses, the diatom counts were first relativized by dividing the individual counts by the total number of valves counted for each sample and then transformed by taking the natural log of the relative abundance plus 1 (ln(RA +1)). This is a common transformation for community data sets that reduces distribution skew (Gotelli and Ellison 2004). Replicate samples were used to generate species accumulation curves and estimate species richness with PCORD 5.0 software jackknife estimators (Palmer 1990; Palmer 1991).

Bray-Curtis difference scores were calculated with PC-ORD 5.0 on the full data set to compare dissimilarity. The Bray-Curtis difference calculation is used throughout this research because it does not overemphasize large differences in community data sets that are characterized by a few abundant species and numerous rare species. Furthermore, the Bray-Curtis difference measurement is amenable to Nonmetric Multidimensional Scaling which was used for an ordination analysis (McCune and Grace 2002).

The Mantel test with asymptotic approximation was used to determine if ponds contained significant differences in diatom communities based on this data set (Urban, Goslee et al. 2002). The Mantel procedure tests the null hypothesis that two distance matrices of equal dimension are not related. In this context, it is used to test the sample distance matrix against a perceived distance matrix where replicates are very similar, differences between ponds from different complexes are large, and differences between ponds within a complex are intermediate. The first matrix was the Bray-Curtis difference

scores and the second matrix was populated with perceived distance relationships of 0 for differences of replicate samples in a single pond, 0.5 for differences between samples from the same complex, and 1 for samples from different complexes.

Multiresponse Permutation Procedures (MRPP) were performed to test the ability of the sampling method to distinguish between complexes. MRPP is a nonparametric test and does not require assumptions of normality and equal variance that are often absent in community data sets. The software for the analysis was PC-ORD 5.0 which uses methods described in Mielke and Berry (2001). As in previous analyses, Bray-Curtis difference scores were used. MRPP tests the null hypothesis that there is no difference between grouped communities. Groups were defined by the complexes, Public Shooting Grounds, Bear River Bird Refuge, and Newstate Duck Club. Since the test was between ponds in the complexes, replicate samples were combined to preclude pseudo-replication enhancement of the test statistic.

Ordination of the entire data set was performed with Nonmetric Multidimensional Scaling (NMS) to graphically analyze and determine the strength of the method to separate ponds between complexes and ponds within complexes. The ordination also allows graphical analysis of the method's reproducibility with replicates within ponds. The PC-ORD software uses an algorithm developed by Mather (1976) and Kruskal (1964). Several random starting positions were used and although there were small differences in axis orientation, they all provided an equivalent ordination. Two hundred and fifty runs were requested with real data and a 2-dimensional model was determined sufficient since adding a third dimension did not decrease the final stress by greater than 5.

The data set was then culled by eliminating all species that were not present in more than two samples (15.4% of the samples), or did not have a relative abundance of greater than 1% in any sample. This was done in an attempt to improve the ordination

and separation between complexes. The same NMS parameters used to ordinate the full data set were used to ordinate the culled data set.

Results

One hundred and thirty one taxa were identified in the thirteen composite samples. The species richness of the samples was large in all samples from all complexes with a median of 46 and a range of 29 to 52. The samples had an average Shannon-Wiener diversity index of 2.99 and ranged from 2.09 to 3.34. Sample evenness averaged .79 and ranged from .60 to .86. The relative abundance in the samples has the characteristic distribution typically found in ecological community surveys. A few species in each sample represent the majority of the relative abundance and a large number of species are represented by few individuals.

Logarithmic transformation of species abundance curves can normalize their distribution (Preston 1962). Figure 2.2 is a histogram of transformed abundance data from a representative sample. The curve appears to be only half of the theorized normal curve. Replicate samples were used to generate species accumulation curves. The average number of species found in one sample through permutation techniques was 63% of the total number of species found in the three replicates. The average number of species found in one sample was 49% of the average jack knife estimates of total species richness. Species accumulation curves for the three replicate samples are shown in Figure 2.3.

The Bray-Curtis difference score matrix for samples and the hypothesized distance matrix are shown in Table 2.1. The replicates, ponds within a complex, and ponds between complexes had average distance scores of .360, .425 and .563, respectively. The results of the Mantel test were highly significant ($p<.001$) with a

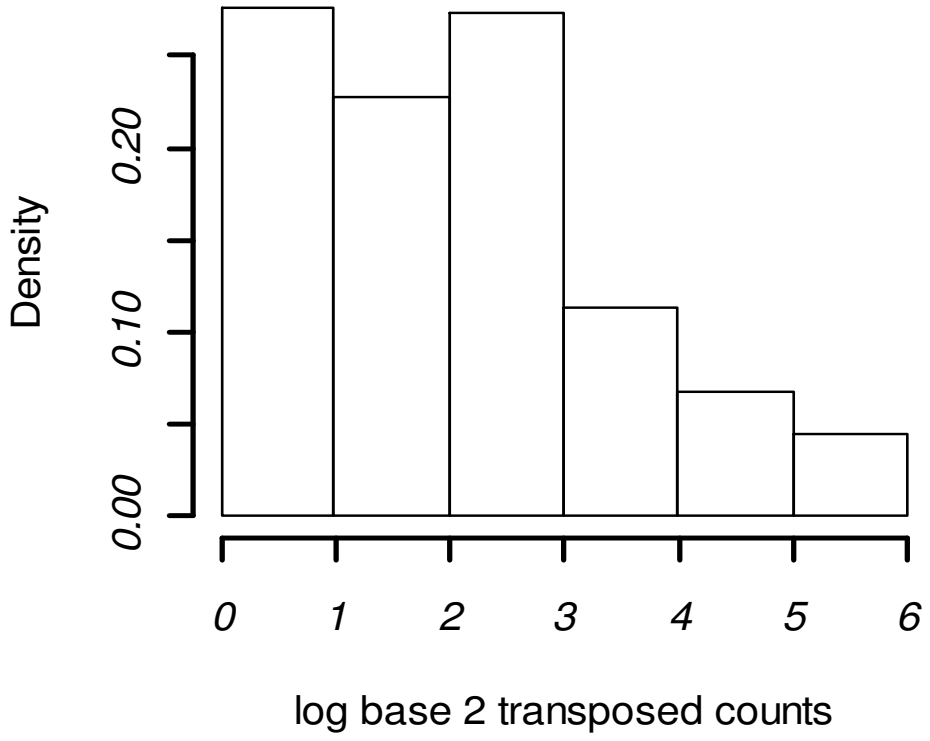


Figure 2.2. Density of species by counts transformed by log base 2 from one sample at the Widgeon Pond (PSGWI) in the Public Shooting Grounds.

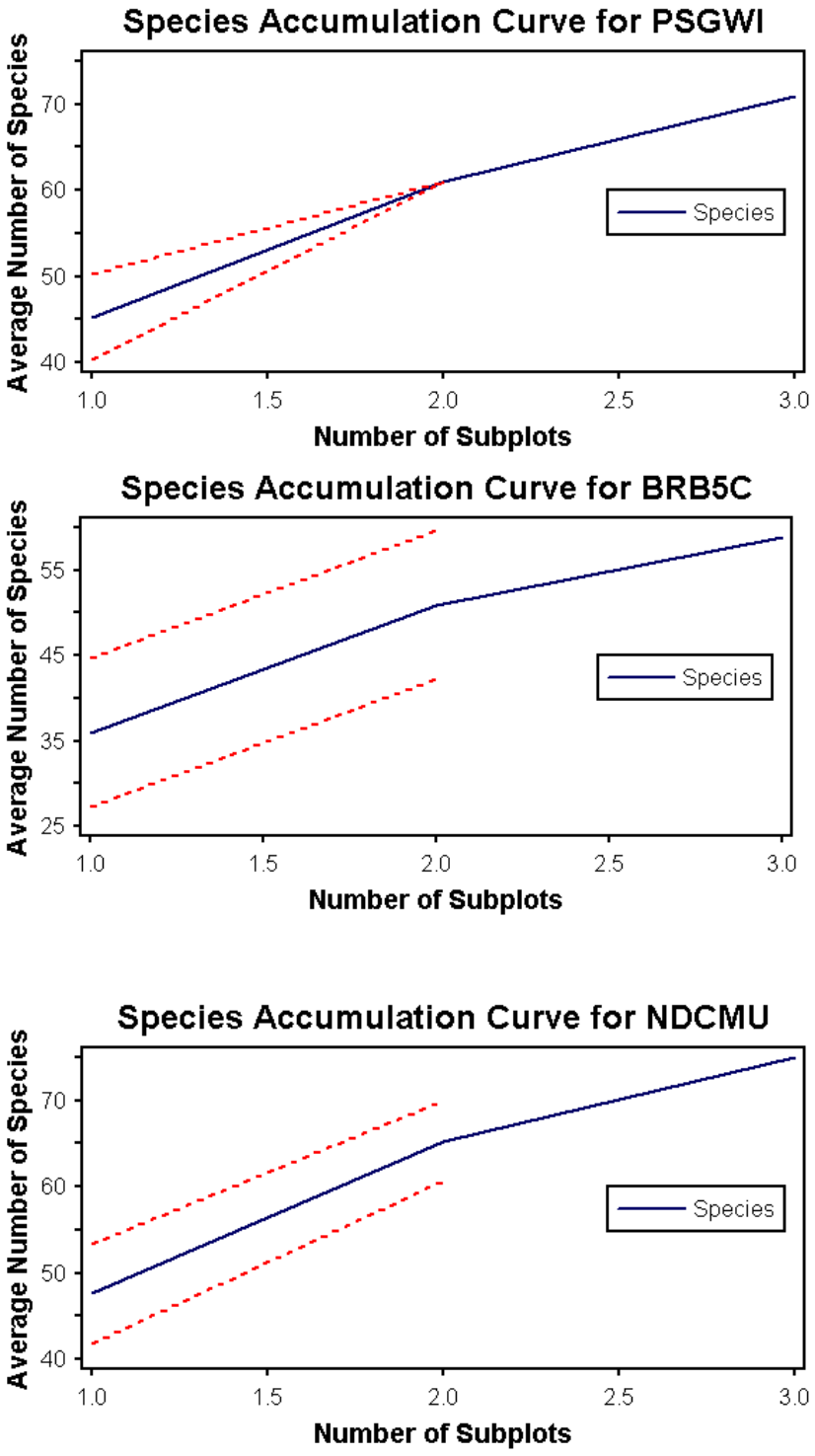


Figure 2.3. Species accumulation curves for the three ponds where replicates were taken. On average 64% of the species found in three samples would be found in the first sample.

Table 2.1. Distance matrix of samples and perceived distance matrix for the Mantel test.

Sorenson Distance Matrix for full data set transposed by ln=1													
	NDC56S1	NDC20S1	NDCMUS3	NDCMUS2	NDCMUS1	BRB4CS1	BRB5CS3	BRB5CS2	BRB5CS1	PSGPTS1	PSGWIS3	PSGWIS2	PSGWIS1
NDC56S1	0	0.378064	0.377443	0.31633	0.339014	0.505673	0.462412	0.519302	0.559611	0.555702	0.629772	0.624491	0.600406
NDC20S1	0.378064	0	0.434729	0.402211	0.485644	0.592808	0.489537	0.567192	0.501072	0.634683	0.648398	0.632358	0.632396
NDCMUS3	0.377443	0.434729	0	0.310297	0.400454	0.450491	0.521803	0.554731	0.586765	0.524822	0.534331	0.577094	0.560286
NDCMUS2	0.31633	0.402211	0.310297	0	0.326772	0.413466	0.415642	0.525162	0.486338	0.550993	0.563429	0.570955	0.567308
NDCMUS1	0.339014	0.485644	0.400454	0.326772	0	0.42285	0.487326	0.495157	0.557727	0.582852	0.601117	0.616314	0.595218
BRB4CS1	0.505673	0.592808	0.450491	0.413466	0.42285	0	0.488218	0.466905	0.514931	0.598703	0.556707	0.558862	0.599516
BRB5CS3	0.462412	0.489537	0.521803	0.415642	0.487326	0.488218	0	0.405671	0.396696	0.625588	0.669693	0.656419	0.651434
BRB5CS2	0.519302	0.567192	0.554731	0.525162	0.495157	0.466905	0.405671	0	0.345067	0.596085	0.699505	0.677115	0.67568
BRB5CS1	0.559611	0.501072	0.586765	0.486338	0.557727	0.514931	0.396696	0.345067	0	0.593488	0.625369	0.609539	0.649673
PSGPTS1	0.555702	0.634683	0.524822	0.550993	0.582852	0.598703	0.625588	0.596085	0.593488	0	0.44739	0.478653	0.489182
PSGWIS3	0.629772	0.648398	0.534331	0.563429	0.601117	0.556707	0.669693	0.699505	0.625369	0.44739	0	0.283388	0.412299
PSGWIS2	0.624491	0.632358	0.577094	0.570955	0.616314	0.558862	0.656419	0.677115	0.609539	0.478653	0.283388	0	0.360687
PSGWIS1	0.600406	0.632396	0.560286	0.567308	0.595218	0.599516	0.651434	0.67568	0.649673	0.489182	0.412299	0.360687	0

Perceived Distance Matrix for relationship between replicates, ponds within complexes and between complexes for use in the Mantel test.													
	NDC56S1	NDC20S1	NDCMUS3	NDCMUS2	NDCMUS1	BRB4CS1	BRB5CS3	BRB5CS2	BRB5CS1	PSGPTS1	PSGWIS3	PSGWIS2	PSGWIS1
NDC56S1	0	0.5	0.5	0.5	0.5	1	1	1	1	1	1	1	1
NDC20S1	0.5	0	0.5	0.5	0.5	1	1	1	1	1	1	1	1
NDCMUS3	0.5	0.5	0	0	0	1	1	1	1	1	1	1	1
NDCMUS2	0.5	0.5	0	0	0	1	1	1	1	1	1	1	1
NDCMUS1	0.5	0.5	0	0	0	1	1	1	1	1	1	1	1
BRB4CS1	1	1	1	1	1	0	0.5	0.5	0.5	1	1	1	1
BRB5CS3	1	1	1	1	1	0.5	0	0	0	1	1	1	1
BRB5CS2	1	1	1	1	1	0.5	0	0	0	1	1	1	1
BRB5CS1	1	1	1	1	1	0.5	0	0	0	1	1	1	1
PSGPTS1	1	1	1	1	1	1	1	1	1	0	0.5	0.5	0.5
PSGWIS3	1	1	1	1	1	1	1	1	1	0.5	0	0	0
PSGWIS2	1	1	1	1	1	1	1	1	1	0.5	0	0	0
PSGWIS1	1	1	1	1	1	1	1	1	1	0.5	0	0	0

Standardized Mantel Statistic of r=.765. The results of the Mantel test rejected the null hypothesis that correlation between the community distance matrix and the hypothesized distance matrix occurred by chance. The MRPP results were also significant with a p-value of .013 and an effect size A of .449. The MRPP test rejected the null hypothesis that the communities of complexes where related by chance.
The 2 dimension solution of the NMS ordination on the entire data set had a low stress value of 8.99 with zero instability. The Monte-Carlo simulation provided a probability that a similar final stress could have been obtained by chance of p=.004. Ordination of the entire ln+1 transformed data set yielded the ordination presented in Figure 2.4. Axes 1 and 2 contain 41% and 48% of the variance, respectively, for a cumulative explained variance of 89%. The axes are relatively orthogonal at 75.6%.

The 2 dimension solution of the NMS ordination on the culled data set had a lower stress value of 7.35 with zero instability. The Monte-Carlo simulation provided a probability that a similar final stress could have been obtained by chance of p=.004. Ordination of the culled data set data set yielded the ordination presented in Figure 2.5. Axes 1 and 2 contain 71 and 20% of the variance, respectively, for a cumulative explained variance of 91%. The axes are very orthogonal at 97.6%.

Culling data sets decreases difference scores between sampling units because there are fewer community members that add to the difference scores. The intent of culling species, however, is to reduce the noise by excluding species that do not distinguish one community from another. In this case, the reduction in noise should reduce the variance or breadth of difference scores within our categories of replicates, ponds within complexes, and ponds from different complexes. The box plots in Figure 2.6 provide a graphical comparison of distance score distributions before and after culling for the three categories of replicates, ponds within complexes and ponds between complexes on the culled data set. The box plots for all three categories show smaller

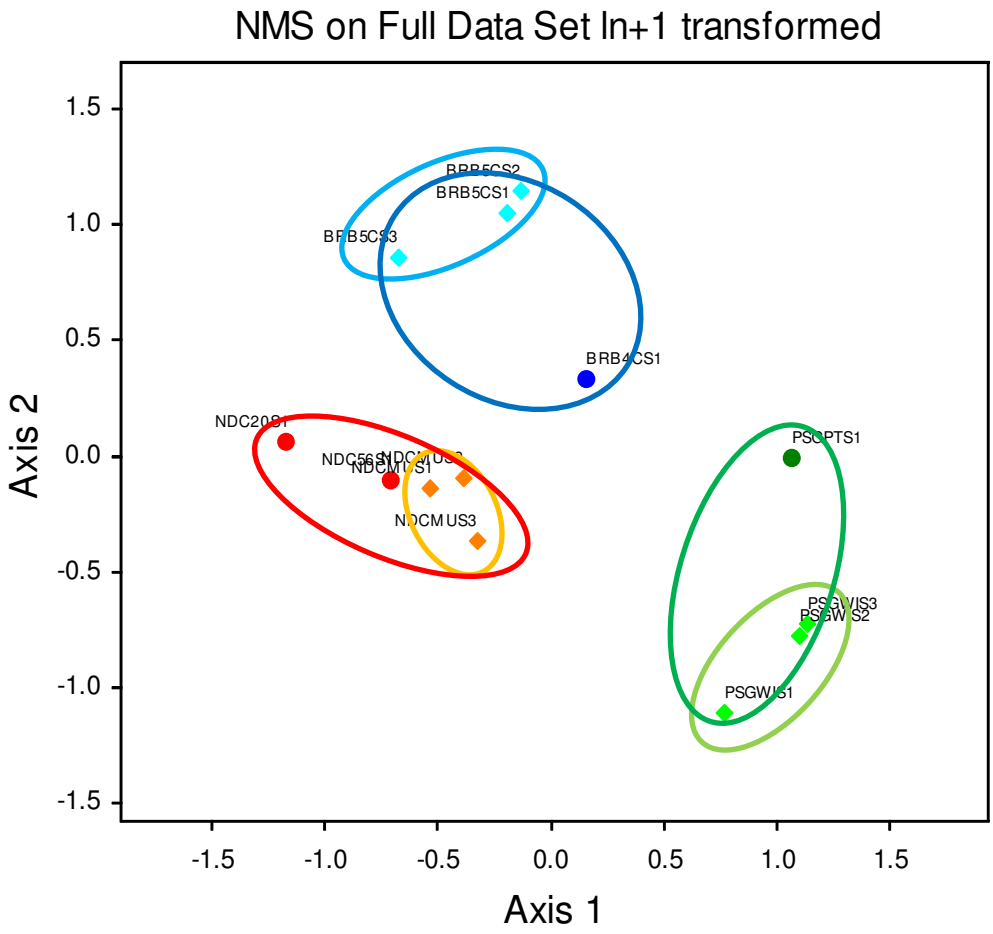


Figure 2.4. Nonmetric Multidimensional Scaling ordination of the ponded wetland diatom assemblages. The full data set was used for this ordination. The diamond shapes in smaller circles are replicates and the larger circles are complexes.

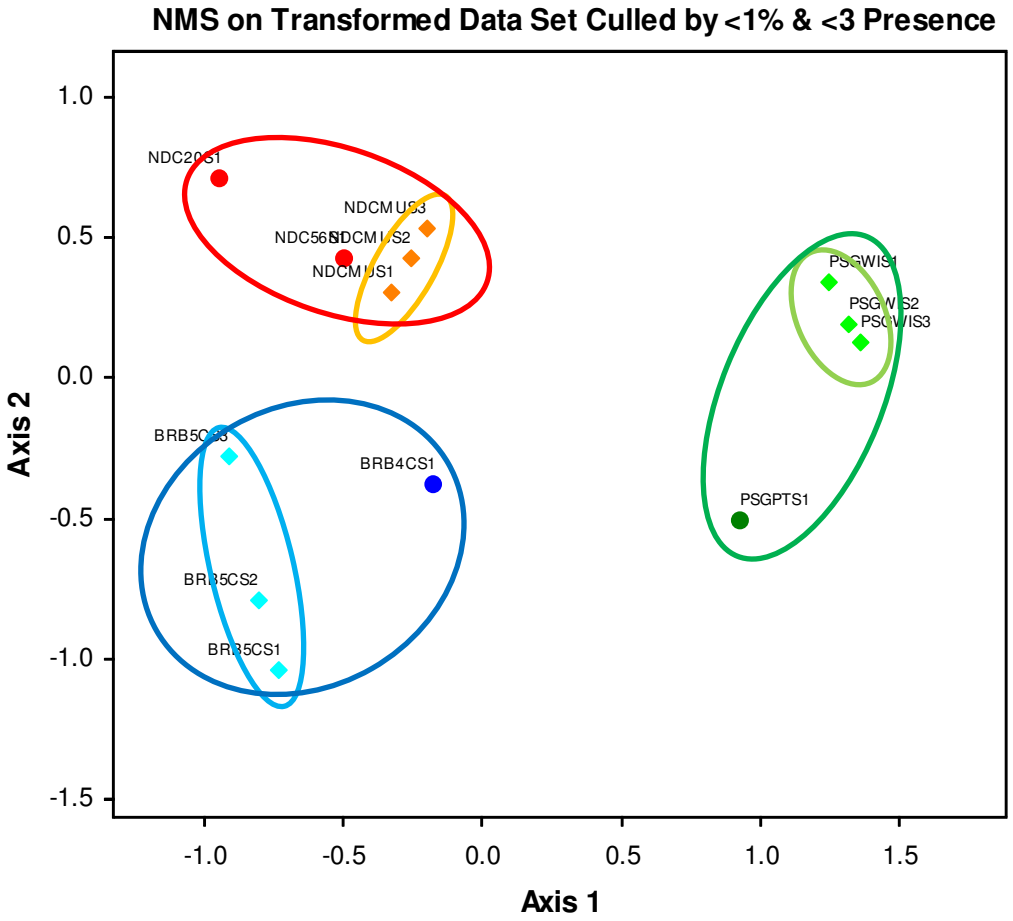
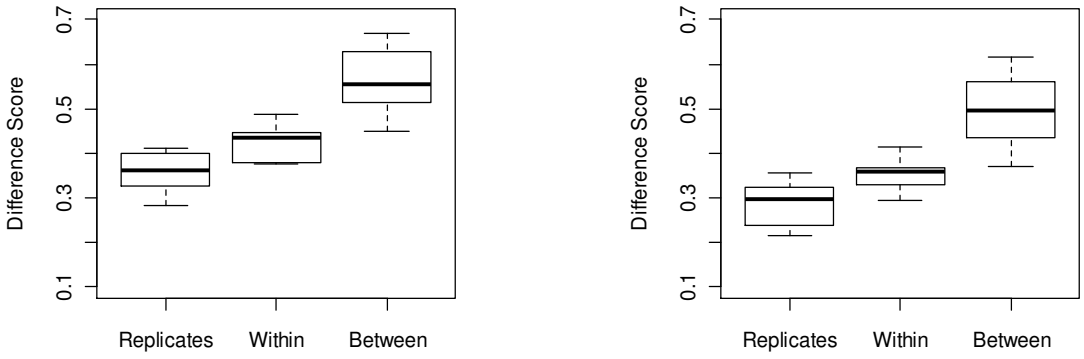


Figure 2.5: Nonmetric Multidimensional Scaling ordination of ponded wetland diatom assemblages after the data set was culled by species that were not represented in 15% of the samples, (i.e. appeared in less than three samples) and species that did not represent more than 1% of the relative abundance in any sample. Diamonds in small circles represent replicates. Complexes are represented by the large circles.



Full Data Set

Culled Data Set

Figure 2.6. Box plots for difference scores of replicate samples, samples from different ponds within a complex, and samples from ponds in different complexes for the full data set and the culled data set. The figure shows decreased difference scores for all categories and better separation of the categories for the culled dataset.

quartiles for the culled data set and none of the quartiles from the three categories overlap in the culled data set.

Discussion

Weilhoefer and Pan (2006) determined through simulation that species richness of surface sediment diatom samples leveled off at 54 species after five samples were composited from the Rooster Rock wetland in Oregon. Additional species, therefore, would only be found if the project effort was increased by collecting more samples or counting more valves in the sample. The purpose of this study was to determine whether more than one composite sample is necessary to distinguish between diatom communities in similar wetlands.

Biologists have routinely chosen a level of finding 95% of species to adequately characterize the community. Ecologically, 95% was based upon the increased sampling effort required to exceed 95%. The effort required in collecting and analyzing greater than 95% of the species present requires a large sampling effort and depending on the questions being asked, the added information may not be worthy of the effort.

The species accumulation curves for the three ponds that were sampled in replicate provide mathematical evidence that the Weilhoefer and Pan (2006) sampling and analytical methodology does not capture 95% of the species present with one composite sample in these ponded wetlands associated with the Great Salt Lake. The replicates indicate that only about 50% of the jackknife species richness estimates were collected. However, after culling the data set of species that did not occur with a relative abundance of greater than 2%, the triplicate sampling data indicates that tripling the sampling effort, (i.e. three samples are collected and analyzed instead of one), only identifies 2 more species with a relative abundance greater than 2%. The effort and cost of collecting more than one sample to obtain a few more species may not be necessary

because these species add little in the way of relative abundance and therefore little relative information on community composition.

The purpose of this research was to evaluate the ability of the composite sampling protocol to discriminate between diatom community compositions based upon reproducibility and specificity. For this method, reproducibility requires replicate samples to have small community difference scores. Specificity would require that difference scores increase as the difference in environmental gradients increase. This quality is supported by the graphical comparison (Figure 2.6) of difference scores for our three categories. The culled data set provided even greater specificity and separation between the three groups with average difference scores of .29, .35, and .49 for replicates, ponds with a complex, and ponds from different complexes, respectively. Although there is some overlap of scores for each category, the quartiles do not overlap. The ability of the method to measure differences in the environmental gradient with low error is also verified by the Mantel Test. The results indicate the sampling/analysis methodology is capable of separating the communities into the hypothesized relationship.

Error can also occur if the method is incapable of grouping communities with similar environmental attributes since communities living under similar environmental conditions should have similar community composition. Multiresponse Permutation Procedures (MRPP) is an appropriate test for differences among groups and our sampling design consists of samples from groups of ponds within complexes. MRPP tests the null hypothesis that there are no differences between groups and provides an effect size for the grouping variable. The results provided strong support for the effectiveness of the method with a p-value of .013 and an effect size A of .449. Although the p-value is small, small p-values are not necessarily good indicators of separation between communities if the sample size is large. With large sample sizes, p-values of

less than .05 can occur when effects of the grouping may not be ecologically significant. With small sample sizes, however, a large effect size is required to achieve small p-values (McCune and Grace 2002). This data set has a small sample size (n=7) and the MRPP test provides a relatively large effect size of .449. Effect size of <.1 generally indicates a small or no effect of the grouping variable and an effect size of .3 or greater is considered high (McCune and Grace 2002).

The methodology tested herein does demonstrate statistically distinct communities from these ponds but if the relative community differences between ponds are small, the methodology will not provide a significant signal of environmental gradients. A means for assessing the ability of the method to satisfy the requirement of low error and the relative community differences is to graphically analyze the clustering of the ponds in species space. Nonmetric Multidimensional Scaling Ordination of the samples based upon community composition (Figure 2.4) graphically indicates the methods ability to separate ponds between complexes, ponds within complexes and replicates within ponds. Figure 2.4 shows a relatively good separation between complexes and relatively small separation between replicates. Eighty-nine percent of the variance is explained by the two axes indicating a strong relationship between the communities and the ordination. There are, however, large differences between samples within ponds and some replicates are closer to other ponds than they are to their replicates.

As stated earlier, this species data set like most community ecology data sets contains numerous rare species which add stress to the NMS analysis. Although rare species are important when analyzing communities for diversity and richness (Cao, Williams et al. 1998), they add noise to ordination techniques without providing relevant information on environmental gradients (McCune and Grace 2002). Culling rare species

is also common in diatom community analyses because of the large number of rare species in most diatom communities.

To improve the NMS ordination, the data set was culled by eliminating all species that were not present in more than two samples (about 15% of the samples), or did not have a relative abundance of greater than 1% in any sample. Naturally the stress is lower in the culled data set ordination because the ordination contained fewer variables. The ordination is improved and relative separation between the three difference categories is increased with the elimination of rare species. This ordination is also stronger with a low stress of 7.35 and 91% of the variance explained by just two axes that are very orthogonal. None of the complexes overlapped in species space. Replicates appear closer to each other in this ordination while the ponds within complexes are separated by relatively greater distances than the replicates at two of the three complexes. The third replicate sample at NDCMU was 'closer' in community composition to NDC56 than it was to its two replicates even after culling the data set (Figure 2.4 and 2.5). The environmental gradient components for ponds within a complex are the same as between complexes but the distance on those gradients between ponds within complexes are smaller than between complexes. The environmental gradient may not be large between ponds at the Newstate Duck Club and this methodology may not be appropriate to strongly discern between ponds within that complex.

Conclusion

Characterizing biological communities that vary temporally in heterogeneous environments is not a trivial pursuit. Even so, the analysis herein indicates the diatom community may be adequately characterized with this surface sediment composite sampling methodology in waterfowl management areas associated with the Great Salt

Lake. The methodology provides reproducible results with small differences in replicates and strongly differentiates communities in ponded wetlands with different water sources and may differentiate communities on smaller environmental gradients associated with ponds within waterfowl management areas and the same water source. Future studies should incorporate this methodology into an environmental analysis that confirms the power of resolution of the method and relates the communities to environmental gradients.

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CHAPTER 3

CORRELATION OF DIATOM COMMUNITIES AND ENVIRONMENTAL VARIABLES IN PONDED WETLANDS OF THE GREAT SALT LAKE, UTAH, USA

Abstract

This research paper examines the association between environmental variables and diatom assemblages collected by a surface-sediment diatom sampling methodology from ponded wetlands on the eastern edge of the Great Salt Lake, Utah. The sampling methodology provided an assemblage that correlated with biotic and abiotic environmental variables. The Mantel test indicated correlation with water chemistry variables ($r=0.564$, $p=0.028$) after controlling for vegetation variables. Vegetation variables also correlated with the diatom community ($r=0.700$, $p=0.001$) after controlling for chemical variables.

Correlation increases for groups of environmental variables emphasizes the abilities of the Mantel test and BIOENV compared to ordination techniques in ponded wetlands when the factors influencing the community composition are working as a consortium. However, the ordination is still useful because it indicated the diatom community corresponds with our knowledge of shallow lake ecology where biotic and abiotic factors work as a consortium. The sampling methodology could provide wetland managers and environmental regulators with another tool for their environmental

assessment framework that would enhance their ability to deduce the status of a wetland status and provide information that could help promote desired wetland biota.

Introduction

Ponded wetlands associated with the Great Salt Lake, Utah, are critical habitat to migrating waterfowl and important centers of biodiversity (Aldrich and Paul 2002). Functional assessment using biological criteria of these Utah wetlands and wetlands nationally has recently been the focus of state and national agencies responsible for implementing the clean water act (USEPA 1998; Utah DWQ 2009). Characterizing biological assemblages in wetlands is challenging because of their internal spatial heterogeneity and the large variation in wetland types. Confounding the complexity of heterogeneous habitat, biological communities of wetlands experience diurnal fluctuations and in temperate latitudes also experience seasonal heterogeneity. Weilhoefer and Pan (2006) determined that surface-sediment diatom richness in samples collected at the Rooster Rock wetland in Oregon leveled off with a composite of five surface-sediment cores. Through mathematical simulation techniques with 29 samples that contained a total of 159 species from the Rooster Rock wetland, the methodology estimated that 54 species (about 1/3) would be identified in a composite of five core samples.

The performance of the Weilhoefer and Pan (2006) methodology was evaluated with individual and replicate samples from ponded wetlands in three different wetland waterfowl management areas associated with the Great Salt Lake, Utah (Chapter 2). The methodology resulted in the identification of about 50% of estimated species richness in these ponded wetlands. However, the rare species added little to the total relative abundance and the method provided sufficient abundance information to unambiguously characterize the different diatom community assemblages. The

research in this chapter evaluates whether samples collected by the Weilhoefer and Pan (2006) methodology can be related to environmental gradients in ponded wetlands associated with the Great Salt Lake, Utah.

Diatoms have been used in freshwater research to assess environmental conditions in aquatic systems for over a century (Kolkwitz and Marssln 1908). Historically the focus has been to use benthic algae in riverine systems for assessing environmental conditions associated with point source pollution (Hansmann and Phinney 1973; Lowe and McCullough 1974; Cooper and Wilhm 1975; Morgan 1987; Biggs 1989; Stevenson, Bothwell et al. 1996). Numerous autecological tables exist for lake and riverine systems and in general, the ecological preferences of many diatoms are available (Van Dam, Mertens et al. 1994; Kelly and Whitton 1995). These indices, however, are generally not correlated to specific abiotic variables. They are more frequently correlated with specific abiotic states, also known as trophic states that classify aquatic ecosystems by nutrient and productivity rankings. It would be of great value to wetland scientists and managers to correlate diatom guilds with actual concentration gradients of abiotic characteristics such as individual nutrients, organic carbon, depth, or water retention time.

Although essential work has been done on biological assessment procedures in rivers and streams that respond to environmental gradients (USEPA 2002), wetlands are a substantially different environment. Wetlands are generally considered harsh environments and the fauna and flora associated with them is tolerant of harsh wetland environments that can exhibit wide abiotic fluctuations (Mitsch and Gosselink 2000). Therefore, the response of such fauna and flora communities to environmental gradients may not be particularly variable as the community is composed largely of tolerant species. If diatom communities in these wetlands respond significantly to environmental

gradients wetlands, they could help quantify wetland sensitivity to anthropogenic influences.

Shallow lake ecology consists of complicated biotic and abiotic interactions that can result in alternative steady states (Scheffer 1998). The alternative states are, 1) a clear water state dominated by submergent aquatic vegetation (SAV) or, 2) a turbid state dominated by total suspended solids and chlorophyll a. Shallow lakes can exhibit hysteric behavior between these two states and the ponded wetlands in this study exhibit alternate states even though they are extensively managed for a clear water state (Hoven and Miller 2009). They are managed for the clear water state since SAV provides an essential food source for a variety of water fowl (Aldrich and Paul 2002).

According to Gibson (2000), metrics used to determine trophic status, i.e. environmental trophic indicators, should fulfill the following five requirements: a) be related to biological integrity, b) respond monotonically to environmental stresses, c) be measurable with a low error, d) be cost effective, and e) be environmentally benign to measure. The sampling protocol outlined by Weilhoefer and Pan (2006) accomplishes the last two requirements. The protocol has also been previously shown to fulfill the third requirement: measurable with low error (Chapter 2). The purpose of this chapter is to evaluate the first and second requirements, the ability of the composite sampling protocol to collect a diatom assemblage that responds to changes in environmental variables.

Materials and Methods

Study site

Sediment samples were collected in the fall of 2008 at three ponded wetland complexes on the eastern shores of the Great Salt Lake (Figure 3.1). All three complexes contain several connected ponded wetlands and are managed to enhance

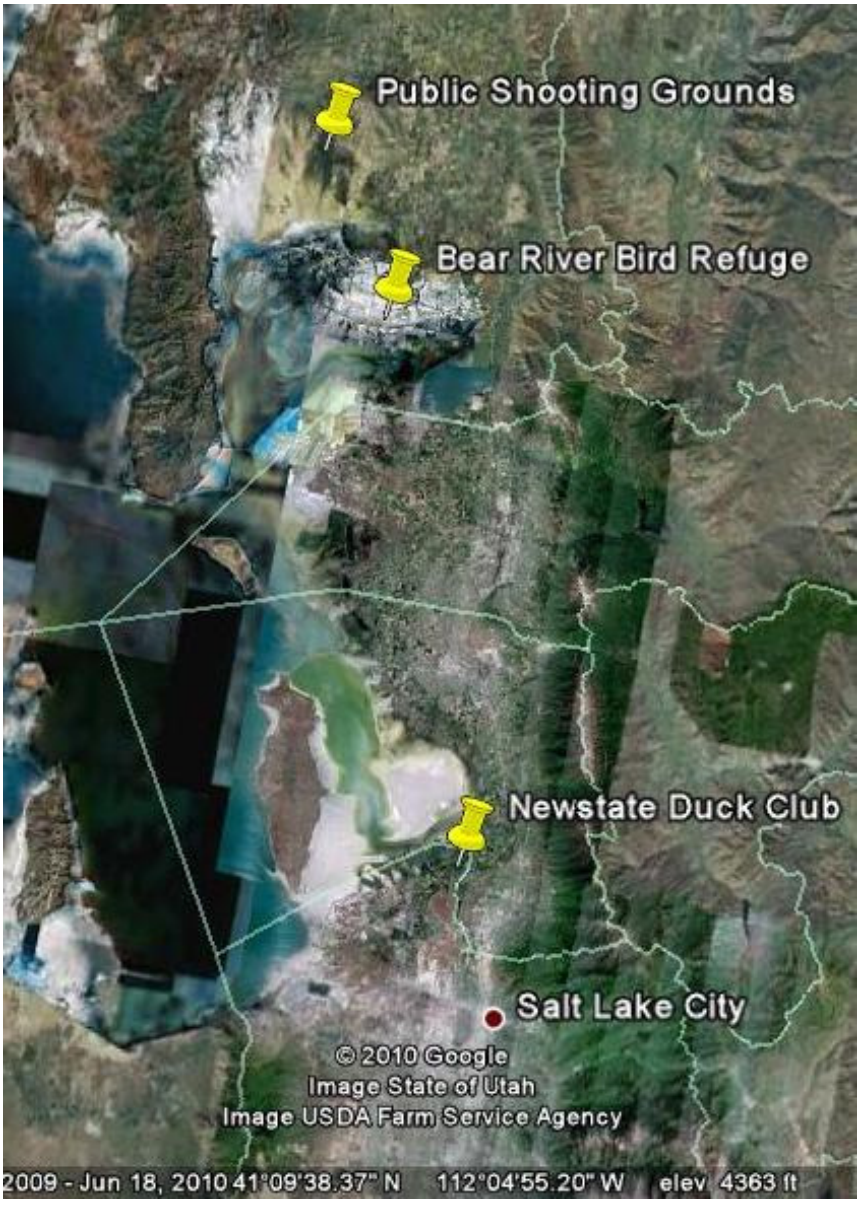

Figure 3.1.Location of ponded complexes along the eastern shore of the Great Salt Lake studied in this research**.**

waterfowl habitat. A total of seven ponds were sampled, three at the private Newstate Duck Club, two at the federal Bear River Migratory Bird Refuge, and two at the State of Utah Public Shooting Grounds (Figure 3.1). Although the three complexes are managed similarly, they have different source water with differing water quality and therefore should have differing diatom community assemblages.

The three complexes have similar elevations and are proximate enough to have similar climate. The three complexes have similar hydrology and are managed for water fowl. Managers of each facility control pond depth to cultivate *Stuckenia* and *Ruppia*. Ponds are drained in late January to control nuisance fish species (common carp) and filled during spring runoff in late February or early March. The rest of the year pond depths are generally maintained around 16 inches (40.6 cm).

Field Sampling

Diatom samples were collected by wading transects parallel to impoundment dikes approximately 50 meters from the pond's controlling culvert. Each sample from a pond was a composite of five core grab samples. Five random numbers between 1 and 100 were generated for sampling along 100 meter transects. Core samples were collected by pushing a 2" ID clear plastic tube into the sediment approximately 30 cm. The top 1 cm of the core was placed in an appropriately labeled polyethylene container. The process was repeated at the next four random points along the transect and the composite of five surface sediment samples was then blended with a putty knife, capped and frozen until analysis. To obtain three samples (replicates) at one pond, 15 cores were collected along one transect by generating three random sets of five numbers and the three composite samples of five cores were collected during one pass along the transect to avoid collecting at a disturbed location. A total of 13 samples each containing 5 cores was collected.

Water quality samples and field measurements were also collected at each controlling culvert near the transect prior to surface-sediment sampling. These ponds have also been historically sampled for water quality as part of a long-term monitoring project performed by the State of Utah Division of Water Quality. The ponds were sampled three times during the productive season and once in the late fall with the surface-sediment sampling. Water samples were collected and preserved according to EPA methods.

Wetland characteristic variables for vegetation and physical attributes, the ponds structural components, were also collected three times during the 2008 productive season by Heidi Hoven of The Institute for Watershed Sciences. Sampling locations along transects were selected similarly to the surface sediment sampling. Five random numbers between 0 and 100 were generated for each pond and each sampling date. Physical data, observations and samples were collected at each of the five points on the transect with the exception of light extinction which was collected at three of the five sites.

The wetland water chemistry and structural variables collected are presented in Table 3.1. The percent surface mat and SAV cover were estimated by the sampler for every measurement. The categorical measurement for epiphyte density was estimated by the same sampler at all sites as rare, rare-common, common, common-abundant, or abundant and coded 1 through 5, respectively. Light extinction coefficients (K_d) were determined by measuring the light intensity at the surface, top of the SAV canopy, and at the bottom. Light attenuation is logarithmically distributed based upon depth and The Lambert Beer K_d was found by rearranging the equation $I_z = I_0 e^{-Kd\,z}$ where z is the depth, I_0 = light intensity at surface and I_z = is the light intensity at depth z (Lind 1985). The above- and below-ground biomass samples were collected with a corer. The above-ground vegetation and below-ground tubers were separated from sediment and other

Table 3.1. The annual average water chemistry and wetland structural characteristics used in this study of the seven ponded wetlands.

Wetland Water Chemistry Characteristics								
	Chlorophyll a	Nitrite + Nitrate as N	Ammonia as N	Dissolved Organic Nitrogent	Total Phosphorus as P	Dissolved Total Phosphorus as P	Salinty	Total Suspended Solids
NST56	43.73	1.77	0.32	2.71	0.66	0.56	0.93	75.38
NST20	6.50	1.63	0.39	2.65	0.70	0.65	0.82	34.63
NSTMU	19.05	0.12	0.09	1.22	0.14	0.09	0.86	32.75
BRB4C	15.28	0.05	0.20	1.44	0.08	0.04	2.77	43.68
BRB5C	16.50	0.05	0.24	0.67	0.14	0.05	1.23	62.83
PSGPT	3.72	0.05	0.07	0.97	0.02	0.01	2.10	17.48
PSGWI	3.36	0.05	0.18	2.29	0.29	0.17	4.62	12.70

Wetland Environmental Structural Characteristics								
	% Surface mat cover	EpiphyteDensity on SAV	% ground cover SAV	Surface to Canopy of SAV light attenuation as Kd	Surface to Sub-canopy light attenuation as Kd	Above Ground Biomass g/m2	Below ground biomass g/m2	Pond Depth cm
NST56	12.73	2.27	74.07	0.031	0.477	286.11	6.57	39.70
NST20	11.22	2.46	93.20	0.031	0.118	392.59	15.06	36.80
NSTMU	16.07	2.32	75.75	0.059	0.180	206.21	7.81	35.90
BRB4C	0.07	2.13	94.47	0.045	0.238	260.24	31.79	45.70
BRB5C	0.00	1.90	98.20	0.142	0.334	264.88	23.40	29.00
PSGPT	0.20	1.80	81.67	0.084	0.246	652.60	5.65	28.15
PSGWI	21.53	2.33	94.13	0.042	0.124	595.52	2.09	25.60

materials before storing them in a plastic bag. Depths where measured with a calibrated PVC pipe.

Laboratory Analysis

Strewn mounts were prepared by following the methods of Sgro and Johansen (1995). Microscopy was performed using an Olympus BX51 research microscope equipped with differential interference phase contrast optics. Permanent strewn mounts were examined along linear, horizontal transects on the slide. Each species with at least 50% of a single valve present was counted until a minimum total of 600 valves were enumerated. Identifications were performed using standard taxonomic works and personal reference slide collections.

The 13 samples contained a total of 130 species. Forty-one species appeared in only one sample, and each sample contained a large number of species with only one individual identified. These singletons provide a large number of the total species present in the samples, but account for a small proportion of the total relative abundance.

A Hydrolab® insitu-mulitiprobe was used in the field to determine pH, temperature, specific conductance, salinity, dissolved oxygen and percent dissolved oxygen saturation. Salinity is the only field parameter used in this analysis because the other field parameters fluctuate diurnally and instantaneous measurements are not representative of a pond's water chemistry.

Water samples for laboratory analysis were collected and analyzed by the following methods: nitrate+nitrite, USEPA 353.2; ammonia,USEPA 350.1; total and dissolved total phosphorus, USEPA 365.1; Chlorophyll-a, Standard Methods 10200H;and total suspended solids, USEPA 160.2. Samples for dissolved total phosphorus were filtered in the field prior to preservation with a 45 µm Millipore® type HA

filter. Chlorophyll samples were also collected in the field on a Millipore® glass fiber prefiltration filter.

Above- and below-ground biomass were determined as dry-weight by a gravimetric method.

Data Analysis

Data on species counts were converted to relative abundance (RA) by dividing individual species counts by total number counted in the samples. As with most diatom communities, these samples contained a large number of individuals from a few abundant species and very few individuals from a large number of rare species. Rare species that did not account for greater than 1 percent relative abundance in any sample and species that did not occur in more than two samples were culled from the data set to reduce their influence and improve the analyses. Relative abundance data were transformed to decrease the distributional skew by taking the natural logarithm of the relative abundance plus 1 (ln (RA+1)). The wetland characteristic vegetation percent data for submergent aquatic vegetation (SAV) and surface mat cover were square root arcsine transformed and multiplied by $2/\pi$ to decrease the distributional skew and obtain distributions that ranged from 0 to 1. Above-ground and below-ground biomass and chemical data were transformed by taking their natural logarithm. The categorical variable for epiphyte density on SAV was converted to a numerical variable by converting rare through abundant scores to the values 1 through 5, respectively. All water chemistry and structural data for the year were averaged for each variable since water chemistry and structural components vary seasonally. The diatom sampling methodology was designed to account for seasonal heterogeneity by collecting a composite of the community that had settled to the bottom during the previous year.

Multiresponse Permutation Procedures (MRPP) were performed to show the relative and significant differences of complexes based upon the diatom community, water chemistry variables and structural characteristics. MRPP is a nonparametric test and does not require assumptions of normality and equal variance that are often absent in community data sets. The software for the analysis was PC-ORD 6.0 which uses methods described in Mielke and Berry (2001). Bray-Curtis difference scores were calculated with PC-ORD 6.0 on the diatom community to compare dissimilarity. The Bray-Curtis difference calculation was used throughout this research for the diatom community difference score matrix because it does not overemphasize large differences in community data sets that are characterized by a few abundant species and numerous rare species. MRPP tests the null hypothesis that there is no difference between grouped communities. Groups were defined by the complexes, Public Shooting Grounds, Bear River Bird Refuge and Newstate Duck Club.

Correlations of diatom abundance with vegetation and water chemistry variables were determined with the BIO-ENV method using R 2.12.1 (R Development Core Team, 2010) with the vegan package (Oksanen, Blanchet et al. 2011). The method's long title "*Best Subset of Environmental Variables with the Maximum (Rank) Correlation with Community Dissimilarities*" is a generic description of the function originally developed by Clarke and Ainsworth (1993) that determines the maximal correlation of the biotic difference matrix with difference matrices from subsets of the abiotic variables.

The Mantel test with asymptotic approximation was used to determine if diatom communities in these ponds were related to environmental variables (Urban, Goslee et al. 2002). The Mantel procedure tests the null hypothesis that two distance matrices of equal dimension are not related. In this context, it is used to test the diatom community distance matrix against distance matrices for environmental variables. The first matrix was the Bray-Curtis difference scores and the second matrix was a Euclidian difference

matrix of a suite of environmental variables. Euclidean difference scores were used to calculate the difference matrices for chemical and structural variables. Euclidean difference scores are appropriate and necessary for environmental variables whose values were transformed to achieve normality and contain negative values. A partial Mantel test was also used in this analysis to determine correlation of just the chemical or structural variables while controlling for the structural or chemical variables, respectively.

Ordination of the entire data set was performed with Nonmetric Multidimensional Scaling (NMDS) to graphically display and determine the significance of the individual environmental variables on the ordination of sites in species space. NMDS does not assume normality or linear relationships among variables (McCune and Grace 2002). The ordination was performed with the "metaMDS" function from the Vegan Package (Oksanen, Blanchet et al. 2011) in R.12.1 (R Development Core Team, 2010).

Correlation between environmental factors and assemblage composition was assessed by fitting environmental vectors onto the NMDS ordination with the "envfit" function in the R vegan package (Oksanen, Blanchet et al. 2011). After calculating a "goodness of fit statistic" (squared correlation coefficient r^2), the function uses a permutation procedure (1000 permutations) to define the significance of each environmental factor on all axes conjointly.

Results

One hundred and thirty-one taxa were identified in the 13 composite samples. The species richness of the samples was large in all samples from all complexes with a median of 46 and a range of 29 to 52. The samples had an average Shannon-Wiener diversity index of 2.99 and ranged from 2.09 to 3.34. Sample evenness averaged 0.79 and ranged from 0.60 to 0.86. The relative abundance in the samples has the characteristic distribution typically found in ecological community surveys. A few species

in each sample represent the majority of the relative abundance and a large number of species are represented by few individuals.

The difference score matrices for the diatom community, water chemistry, and structural characteristics are shown in Table 3.2. The MRPP test rejected the null hypothesis that the diatom communities in complexes were related by chance ($p=0.014$), with an effect size A of 0.469. The structural characteristics were also significant ($p=0.009$), with an effect size of 0.469. The water chemistry results were marginally significant ($p=0.084$), with an effect size of 0.244.

The Mantel test rejected the null model of no relationship between the community difference matrix and the difference matrix for all 16 environmental variables ($p<0.05$) with a Mantel correlation coefficient (r) of 0.47. Further understanding was acquired with the Mantel test by separating the chemical and vegetation environmental variables. The difference matrix for the eight structural variables was highly correlated with the community difference matrix ($r=0.590$, $p<0.015$), whereas the correlation between the chemical variables showed a nonsignificant trend ($r=0.370$, $p=0.120$).

The partial Mantel test controls for one set of environmental variables while comparing the correlation between a different set of variables and the community difference matrix. The partial mantel test indicated that the differences in the diatom community are strongly correlated with differences in water chemistry variables when variation in structural characteristics are controlled for ($r=0.564$, $p=0.028$). The partial Mantel test indicated that the difference in the diatom community was also very strongly correlated with structural characteristics when variation in water chemistry is controlled for ($r=0.700$, $p=0.001$). The Mantel test for correlation of chemical and vegetation

Table 3.2. Matrices of difference scores for the diatom community, water chemistry, and structural characteristic variables. The Bray-Curtis difference calculation was used for the diatom community matrix and the Euclidean difference calculation was used for the water chemistry and structural variable matrices.

Diatom Community Difference Score Matrix							
	NDC56S1	NDC20S1	NDCMUSu	BRB4CS1	BRB5CSu	PSGPTS1	PSGWISu
NDC56S1	0	0.3281	0.2078	0.4379	0.421	0.5029	0.5539
NDC20S1	0.3281	0	0.352	0.5287	0.4428	0.5703	0.5826
NDCMUSu	0.2078	0.352	0	0.3239	0.3851	0.4698	0.4561
BRB4CS1	0.4379	0.5287	0.3239	0	0.3895	0.5539	0.5016
BRB5CSu	0.421	0.4428	0.3851	0.3895	0	0.5083	0.5615
PSGPTS1	0.5029	0.5703	0.4698	0.5539	0.5083	0	0.3451
PSGWISu	0.5539	0.5826	0.4561	0.5016	0.5615	0.3451	0

Water Chemistry Difference Score Matrix							
	NDC56S1	NDC20S1	NDCMUSu	BRB4CS1	BRB5CSu	PSGPTS1	PSGWISu
NDC56S1	0	2.079673	4.084963	5.298956	4.893152	7.082819	5.236201
NDC20S1	2.079673	0	4.159233	5.337121	4.971859	6.673319	4.439187
NDCMUSu	4.084963	4.159233	0	1.997091	1.702926	3.374033	3.07152
BRB4CS1	5.298956	5.337121	1.997091	0	1.385349	2.594898	2.930873
BRB5CSu	4.893152	4.971859	1.702926	1.385349	0	3.32735	3.238749
PSGPTS1	7.082819	6.673319	3.374033	2.594898	3.32735	0	3.928904
PSGWISu	5.236201	4.439187	3.07152	2.930873	3.238749	3.928904	0

Structural Characteristic Difference Score Matrix							
	NDC56S1	NDC20S1	NDCMUSu	BRB4CS1	BRB5CSu	PSGPTS1	PSGWISu
NDC56S1	0	0.99485	0.48968	1.633471	1.413284	1.067314	1.486233
NDC20S1	0.99485	0	0.948082	0.967511	0.911233	1.339807	2.055518
NDCMUSu	0.48968	0.948082	0	1.487737	1.282294	1.350407	1.734149
BRB4CS1	1.633471	0.967511	1.487737	0	0.615164	2.04734	2.925522
BRB5CSu	1.413284	0.911233	1.282294	0.615164	0	1.700471	2.614527
PSGPTS1	1.067314	1.339807	1.350407	2.04734	1.700471	0	1.182679
PSGWISu	1.486233	2.055518	1.734149	2.925522	2.614527	1.182679	0

variables was not statistically significant (p=0.57), and gave a negative Mantel correlation coefficient of -0.14.

The BIO-ENV function was used to determine the magnitude of the correlation between environmental variables and the community assemblage. The BIO-ENV function does not provide probability statistics. Chemical and structural variables were separated because they correlated better with the diatom community when they were analyzed separately. The results for the vegetation variables determined that four variables were responsible for the greatest correlation with the community assemblage. The four variables, in order from greatest to least contribution to the correlation, were: above-ground biomass, below-ground biomass, water depth and density of epiphytes on SAV. The results for the chemical variables also determined four variables were responsible for the greatest correlation with the community assemblage. In the order from greatest to least contribution to the correlation, they were: salinity, total suspended solids (TSS), dissolved-total phosphorus and chlorophyll a.

The partial Mantel test statistic of correlation for the best four chemical variables with the community assemblage when correlation with the effect of the best four vegetation variables was removed was 0.569 (p=0.010), which is an improvement from the analysis of all chemical variables. However, the Mantel test statistic for correlation was not improved for the best four vegetation variables while controlling for the best four chemical variables (r=0.657, p=0.002. The Mantel statistic was r= 0.657 with a p value of .002.

The NMDS ordination placed sites from within the same complex proximal to each other and it appears that the complexes do not overlap in species space. The ordination had a final stress of 5.1 and a linear fit r^2 of 0.981. The ordination shown in Figure 3.2 includes the correlation vectors for the chemical characteristics salinity, TSS,

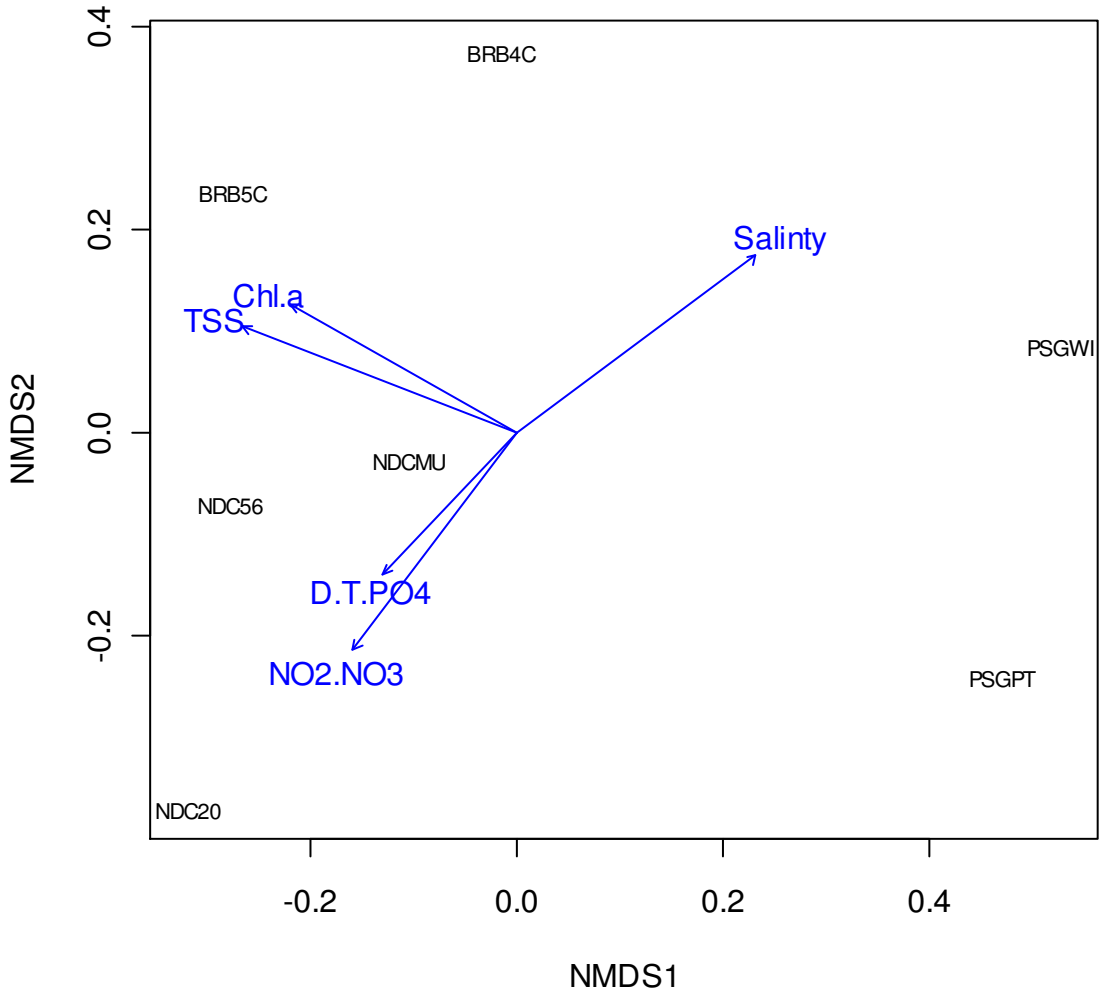

Figure 3.2. NMDS ordination of ponded wetland sites in diatom species assemblage space overlaid with vectors of NO2+NO3 and "best 4" chemical variables

chlorophyll a, dissolved-total phosphorus, and Nitrate +Nitrite. The ordination shown in Figure 3.3 includes correlation vectors for the above- and below-ground biomass, water depth, and epiphyte density on SAV. The correlation and significance of all 16 variables are listed in Table 3.3.

Discussion

The intent of this research was to determine if the sampling methodology used in this study collected a diatom community that was sensitive to environmental gradients. The diatom community in these ponded wetlands appears to be related to biotic factors of wetland vegetation and the abiotic factors associated with water chemistry. This conclusion is drawn since the results of BIO-ENV and the Mantel tests indicate a high correlation of the diatom community and some environmental variables. The high correlation between environmental variables and the diatom community ordination in species space provides further support for this conclusion. Furthermore, the results of the tests on the diatom community assemblage indicate the community components correlate with our current understanding of ponded wetland ecology.

According to Scheffer (1998), the underlying ecology of shallow lakes is governed by complicated dynamics between biotic and abiotic interactions which tend to reinforce one of two steady states. One state is comprised of clear water and the flora of the pond is dominated by submergent aquatic vegetation (SAV). The other state is comprised of turbid water and the flora is dominated by phytoplankton. Figure 3.4 shows the correlation of the elements associated with turbidity, TSS and Chlorophyll a, and the element associated with clear water, above-ground biomass (SAV) imposed on the ordination of diatom communities in species space. The vectors are pointed in opposite directions indicating the relationship between the two steady states may also be exhibited in the diatom community.

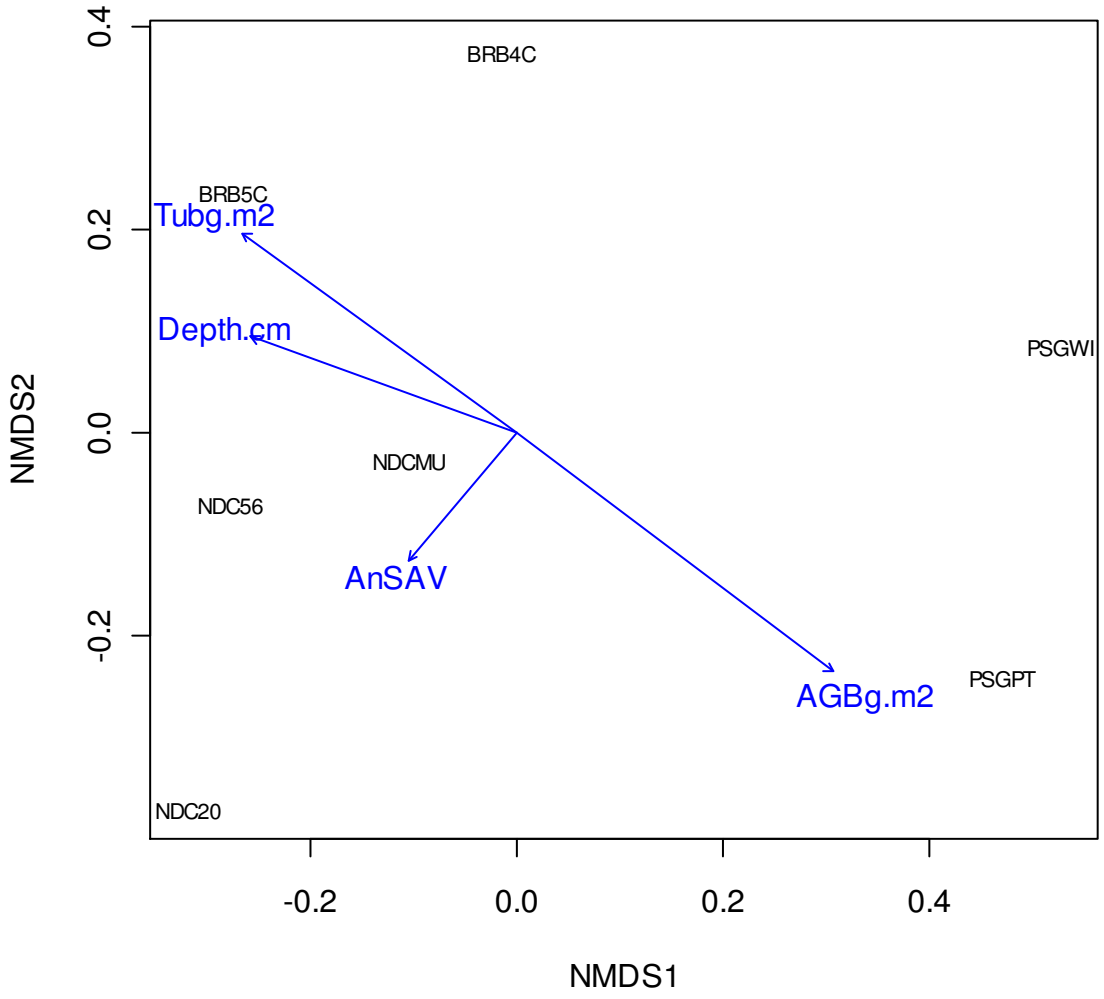

Figure 3.3. NMDS ordination of ponded wetland sites in diatom species assemblage space overlaid with vectors of “best 4” structural variables

Table 3.3. Regression coefficients and probabilities for the 16 environmental variables overlaid on NMDS ordination of site diatom species assemblages. * indicates a significance of p<.005 and . indicates a significance of p<0.10

Variable	r2	Pr(>r)
Chl.a	0.6694	0.10789
NO2.NO3	0.7352	0.07892 .
Amm	0.4255	0.28971
OrgN	0.1435	0.70829
T.PO4	0.3359	0.42058
D.T.PO4	0.378	0.36264
Salinty	0.879	0.01199 *
TSS	0.8573	0.03497 *
a.TMat	0.135	0.6993
AnSAV	0.1406	0.72128
aT..SAV	0.2039	0.63337
Kd_S_C	0.0998	0.84016
Kd_S_SC	0.1915	0.66933
AGBg.m2	0.7811	0.07193 .
Tubg.m2	0.5755	0.17183
Depth.cm	0.3986	0.4036

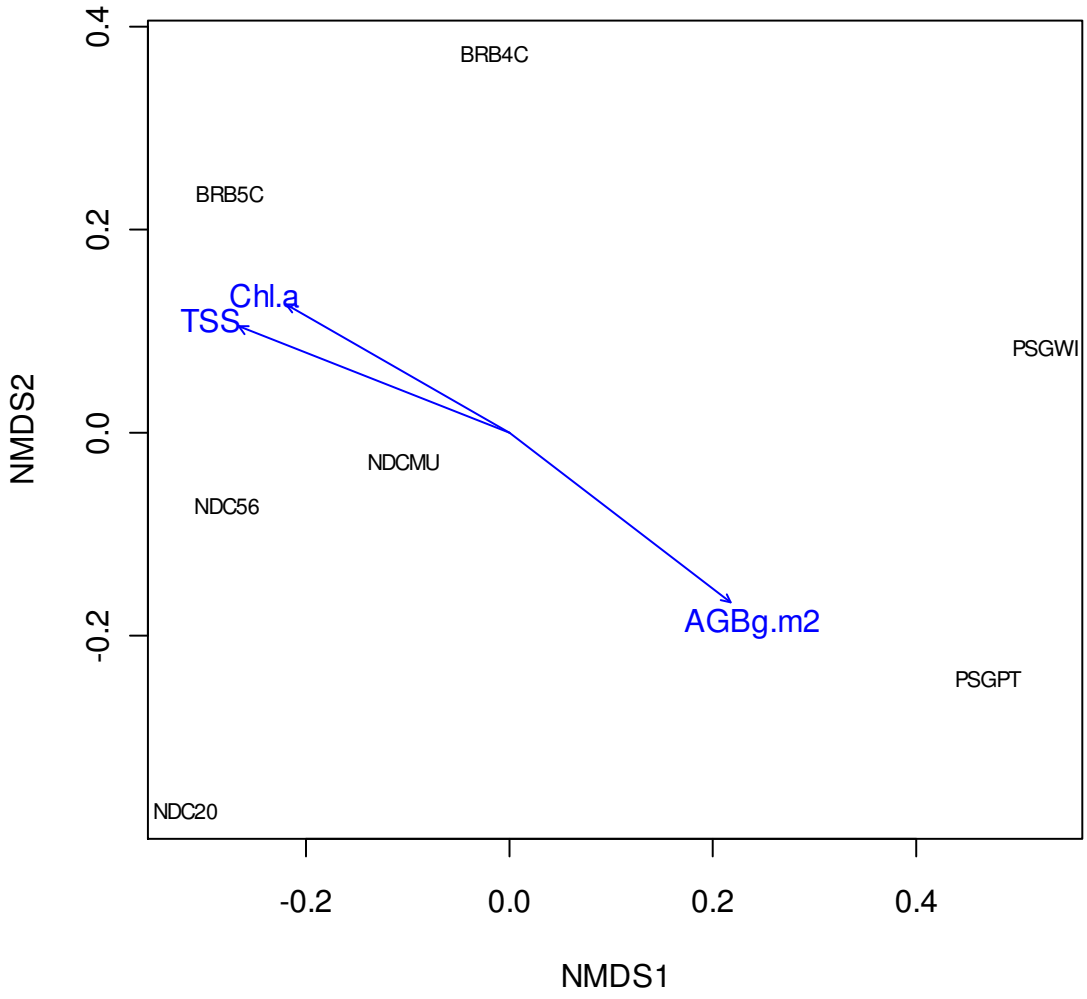

Figure 3.4: NMDS ordination of ponded wetland sites in diatom species assemblage space overlaid with vectors of Chlorophyll a, TSS, and Above-Ground Biomass. The opposite directions of above-ground biomass to chlorophyll a and total suspended solids correlation with species space corresponds to the alternate steady state ecology of shallow lakes.

Water chemistry in these wetlands is subject to diurnal variations which makes the determination of absolute concentrations difficult to assess. Furthermore, the biogeochemistry of nutrients, especially phosphorus, in wetlands is complicated and variable (Reddy and DeLaune 2008). Even with this perplexity, however, the correlation of water chemistry variables with the diatom community was significant after the correlation with vegetation was removed. The ordination results indicated statistically significant correlation of salinity and TSS, and marginally significant correlation of nitrate+nitrite. Although it is interesting that several chemical constituents appear to be correlated with the structure of the diatom community, the cumulative correlation as reflected in the Mantel test statistic was larger for a difference matrix that contained all chemical variables and the larger Mantel correlation statistic suggests these chemicals are operating in concert with each other relative to the diatom community. This corresponds with our knowledge of shallow lake ecology where abiotic and biotic factors are interacting to determine the community components. Results for vegetation were similar according to the Mantel test with a few components having significant correlation but the significance and correlation of the combined vegetation variables was much greater.

Scheffer's (1998) compilation of ecological dynamics of shallow lakes provides background for understanding the abiotic and biotic interactions on the shallow lake ecosystems. Shallow lakes like other wetlands are both allogenic and autogenic, succession is driven by both abiotic components and by biological components (Mitsch and Gosselink 2000). Wetlands exhibit self-design where biotic factors reinforce the current ecological conditions and stabilize the community during abiotic perturbations (Jeppesen, Søndergaard et al. 1998; Mitsch and Gosselink 2000). The larger Mantel coefficient for vegetation variables compared to water chemistry variables could indicate

that in the current state of these ponds, the vegetation is a greater determinant of the structure of the diatom community assemblage than are the chemical variables.

NMDS and the partial Mantel test provide slightly different results. The differences between them are probably due to the fact that NMDS treats each environmental variable independently, and we know this is not true. NMDS can determine the correlation of individual environmental variables with a set of sites organized in species space; however, it cannot determine the correlation of a group of factors that correlate with each other and are working in concert on the community assemblage. The Mantel test improved the correlation of the suite of chemical variables after removing correlation from the structural variables.

Conclusion

Diatoms have been useful environmental indicators for a variety of ecological applications (Stoermer and Smol 1999; Stevenson and Smol 2003) and our results indicate they also respond to environmental gradients in these ponded wetlands. Showing a statistically significant relationship of salinity and TSS to an ordination of diatom communities from several ponded complexes is not surprising. However, what is startling is that it was done with such a small sample size (n=7). This research was not an attempt to unambiguously determine the major environmental factors driving the diatom community composition in these ponded wetlands. That certainly would take larger a sample size. This research was proposed to determine if this sampling methodology could be used to collect a community assemblage that responded to environmental gradients.

The analysis discussed above has shown that complicated relationships between environmental factors and the diatom communities in these ponded wetlands can be resolved by this simple and cost effective sampling methodology. Furthermore, even

with correlating variables the relative strength of environmental variables as forcing factors can be assessed. Wetland managers and environmental regulators could incorporate this tool into an environmental assessment framework that would enhance their ability to deduce the wetland's status and manage to promote desired wetland biota.

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