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ECOLOGICAL ASSESSMENT OF HUMAN DISTURBANCE TO STREAM ECOSYSTEMS USING ALGAE

presented by

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Ph.D. degree in Zoology/EEBB Program R. Jan Hevens Major Professor's Signature August 4, 2003

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ECOLOGICAL ASSESSMENT OF HUMAN DISTURBANCE TO STREAM ECOSYSTEMS USING ALGAE

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Yi-Kuang Wang

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A DISSERTATION

Submitted to Michigan State University In partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Department of Zoology and the Ecology, Evolutionary Biology, and Behavior Program

ABSTRACT

ECOLOGICAL ASSESSMENT OF HUMAN DISTURBANCE TO STREAM ECOSYSTEMS USING ALGAE

By

Yi-Kuang Wang

Algal attributes responded to land use and environmental variables related to lowlevel human disturbance in streams of glaciated northern Michigan, USA. Diatom species composition and dominant species changed with a human disturbance gradient (HDG), which was defined by covariation among nutrient concentrations and land uses. The percentage of native diatom species was negatively correlated with the HDG. Simpson dominance index and diversity indices were not correlated with the HDG. Average diatom biovolume was positively correlated with the HDG. Relative abundance of species sensitive to trophic status decreased and % trophic tolerant species increased with the HDG, Cl⁻, and conductivity, but did not correlate with nutrient concentrations. Thus changes in diatom assemblages due to nutrient enrichment could be detected, but not related to the narrow range in nutrient concentrations.

Of the three major benthic algal habitats in streams, diatoms on plants were the most sensitive assemblages to the human disturbance. Epiphytes also had the smallest beta diversity and fastest species turnover along an HDG, while epipelon and epilithon had about the same beta diversity and species turnover rate. Epiphytes had the most variance in species composition and species-environment relations explained by CCA. Epiphyton was more sensitive than epilithon and epipelon based on changes in trophic status indicators along the HDG. Species composition of epipelon was distinctly different from the epilithon and epiphyton. Heterogeneity in species composition among habitats did not decrease along the HDG.

A regional pattern in nutrient limitation of periphyton was not observed with experiments in 16 streams despite very low P concentrations. However, species composition did respond to experimental nutrient enrichment. Further examination showed that nutrient enrichments did not change species composition in experiments in the same way that composition varied among streams with different nutrient concentrations. Diatom assemblages from treatment and control clay pots at the same site were more similar than assemblages from the same treatments between sites. Therefore, extrapolation of experimental results from small-scale experiments to large regional scales may be constrained by the local species pools.

A diatom-based Index of Biotic Integrity (IBI) for the Interior Plateau Ecoregion (IPE) of the US was developed using data collected by the Kentucky Division of Water. Diatom attributes at reference and impacted sites were selected for the IBI based on significant differences between site groups, high separation power, and low CV. Seven metrics were selected from a list of 59 attributes to form a diatom IBI, which successfully distinguished reference and impaired sites in both development and test datasets. The IBI scores were significantly related to measures of human disturbance in watersheds. This IBI had more separation power than the other diatom IBIs.

ACKNOWLEDGMENTS

I thank my committee members, Dr. R. Jan Stevenson, Dr. Thomas M. Burton, Dr. Stephen K. Hamilton, and Dr. Bryan C. Pijanowski, for their help on various aspects. Dr. Brian Pijanowski has kindly provided me help on GIS related questions. Drs. Dave Long and Brian Pijanowski have offered assistance on the Grand Traverse Bay Watershed project. I can't complete this dissertation without my major Professor, Dr. R. Jan Stevenson. Jan shares his knowledge, stimulates discussion and thinking, and especially teaches me writing. I have great appreciation to Jan's great support throughout my Ph.D. years.

I like to thank members in my lab, Julie Heinlein, Dr. Vannessa Lougheed, Kalina Manoylova, Chris Parker, Dr. Steve Rier, Scott Rollins, and Lei Zheng, for providing comments on my dissertation. Special thanks to Kalina Manoylova, she taught me algal taxonomy and answered my questions with kids on one hand and a microscope on the other hand.

Lastly, without the support and encouragement of my parents through the long rough years, the completion of this dissertation is totally impossible. I dedicate this dissertation to my parents, Shiu-Yin Chin and Shin-Liang Wang. They have always believed in me and provided warmth to my heart.

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CHAPTER 1 INTRODUCTION

Human activities in watersheds can alter factors directly controlling the structure and function of stream ecosystems (Karr and Dudley 1981, Malmqvist and Rundle 2002). The growing human population and associated water demands accelerate the degradation of stream ecosystems (Baron et al. 2002, Richter et al. 2003). Both urban and agricultural land uses contribute a large amount of nutrients to streams (Paul and Meyer 2001, Smith 2003), subsequently causing algal blooms in rivers, lakes, reservoirs, and even estuaries (Mitsch et al. 2001, Smith 2003). Therefore, an ecologically sound and effective assessment system is needed to evaluate the status and change of stream ecosystems, diagnose stressors, and offer management options to decision makers (Wang and Stevenson 2002). Bioassessment can also provide early warning signs before catastrophic events occur (Cairns and McCormick 1992).

Human induced stresses often change biotic community structure in different ways. Stresses can reverse the autogenic community development (Odum 1985) and induce a series of stress syndromes on ecosystems (Rapport et al. 1985). Decrease in organism size, decrease in species diversity, increase in species dominance, and species prone to diseases are often observed in stressed ecosystems (Odum 1985, Rapport et al. 1985). Stresses also favor r-strategists (Odum 1985), which often are opportunistic species (Rapport et al. 1985, Schindler 1987). Changes in indicator species (tolerant species increase and sensitive species decrease) (Sheehan 1984) and shifts in community structure (Rapport and Whitford 1999, Gunderson 2001) are often observed in stressed ecosystems. From whole lake experiments, Schindler (1987) found that taxonomic

changes are more important than diversity indices, short lifecycle, low dispersal species are most affected, and keystone predators are often lost due to stresses. Loss of native species, invasion of exotic species (Rapport et al. 1985, Rapport and Whitford 1999), and increases in the amplitude of population fluctuations (Rapport 1985) are getting increasing attention in stressed ecosystems.

Biological assessment is actively adopted by agencies around the world for several reasons. It integrates physical, chemical, and biological environments. Organisms incorporate a longer duration of environmental information than a one-time snap-shot water sampling. Biological integrity and biodiversity, important components of bioassessment, are important social values. Bioassessment can detect intermittent sources of pollution and non-point source pollution (Yoder 1995).

Algae have a high intrinsic value in aquatic ecosystems because they are primary sources of energy for grazers (Gullberg et al. 1997, Finlay 2001), play an important role in nutrient cycling (Dodds et al. 2000), and facilitate the growth of microbes (Petit et al. 1999, Romani and Sabater 1999). On the other hand, algal blooms can decrease dissolved oxygen levels, create taste and odor problems, increase difficulty for drinking water processing, produce toxins, deter recreation, and decrease aesthetic values. Therefore, algae have valued ecological attributes from social-economic perspectives and need to be evaluated for bioassessment and management.

From the perspective of water quality assessment, algae have been used extensively to monitor water pollution for over a century due to the following reasons: 1) algae are highly sensitive to pollution (Kolkwitz and Marsson 1908, Sládecková 1962, Patrick 1973, Lowe 1974, Descy 1976); 2) they have rapid reproduction rates and short life

cycles, therefore they respond quickly to perturbation (Stevenson and Lowe 1986); 3) they are easily sampled and quantified (Watanabe et al. 1990); 4) algae are well documented in the taxonomic literature and are relatively easy to identify to species (Stevenson and Pan 1999); 5) a large number of species are sensitive to various environmental factors (Lowe 1974, Round 1981, van Dam et al. 1994); 6) high diversity and species-specific sensitivity to their environments confer diatoms with the power to infer environmental conditions; and 7) sub-cosmopolitan characteristics of algae enable use of indices in many regions (Kelly et al. 1998).

Algal indices can be classified into 4 general types. Two types of indices are based on environmental optima and tolerances of species and help diagnose environmental stressors. The first type uses categorical characterizations of species autecology (often 1-5 or 1-8 ordinal scales) and relative abundance of species to infer the physical and chemical status of ecosystems (e.g. pH, organic enrichment, and nutrients) (Lowe 1974, Watanabe 1990, Prygiel and Coste 1993, van Dam et al. 1994, Kelly and Whitton 1995). The second approach, weighted average models, uses accurate characterization of the optimal environmental conditions of species based on a continuous quantitative environmental scale (ter Braak and van Dam 1989, Cumming and Smol 1993, Pan et al. 1996). The other 2 types of indices provide assessments of biotic integrity, rather than physical and chemical stressors. Diversity indices, the third approach, have been widely used to assess the number of species and evenness of their distributions (Archibald 1972, Patrick 1973, Stevenson 1984). The fourth approach is the similarity in species composition between assessed and designated reference sites and is another measure of biotic integrity (Stevenson and Lowe 1986, Charles 1996, Stevenson and Pan 1999).

Algae indices of biotic integrity (IBIs) are relatively new and only a few examples have been developed (Bahls 1993, Kentucky Division of Water 1993, Hill et al. 2000). The multimetric approach using invertebrates and fish has been used widely for assessing and communicating biotic integrity of streams across the U.S. (Barbour et al. 1996, Barbour et al. 1999). The IBI emphasizes biotic integrity, and uses diversity and similarity indices as well as functional guild metrics and higher taxonomic metrics to form a multimetric scoring index. The IBI scores give a quantitative sense of the ecological health of the biological community and can be statistically effective for evaluating human disturbance (Fore et al. 1994).

Algal biodiversity in streams includes common and unique flora from different habitats. The affinity of algal flora has been documented: for example <u>Achnanthes</u> <u>lanceolata</u>, <u>Achnanthes lauenburgiana</u>, <u>Fragilaria pinnata</u>, and <u>Navicula ignota var</u>. <u>palustris</u> are episammic species (Miller et al. 1987) and species in the genera <u>Asterionella</u>, <u>Melosira</u>, <u>Cyclotella</u>, and <u>Stephanodiscus</u> are planktonic species (Kingston et al. 1983). Epilithon is usually assessed for ecological research and bioassessment. The differences in algae among habitats and applicability of algae in different habitats for bioassessment have seldom been examined.

Though the emphasis has been on community structure in algal bioassessment, some functional assessment methods have been adopted, such as the alkaline phosphatase (Hill et al. 2000), primary production (Niyogi et al. 2002), and nutrient limitation (Wold and Hersey 1999). Nutrient limitation assays are often used to assess whether primary production is limited by P, N, or both (Fairchild et al. 1985, Burton et al. 1991). Nitrogen limitation is common in the Western United States (Grimm and Fisher 1986, Hill and

Knight 1988), while streams are often P limited in Michigan (Pringle and Bowers 1984, Fairchild et al. 1985, Burton et al. 1991). Regional patterns in nutrient limitation may be controlled by the biogeochemistry. Changes in the nutrient limitation pattern may affect biotic integrity. Nutrient limitation assays often use nutrient diffusing substrates (NDS), such as clay pots (Fairchild and Lowe 1985) and filter membranes on test tubes (Wold and Hersey 1999).

Objectives

Many bioassessment studies have focused on highly impacted areas. In chapter 2 to 4, the Grand Traverse Bay Watershed (GTBW), a relatively low impacted area, was studied. The GTBW is approximately 2600 km² and is located in the northwestern part of the lower Michigan peninsula. The Boardman River drains the majority of the GTBW. The Pere Marquette National Forest is located in the upper- and mid-region of the Boardman River watershed and contains a mixed deciduous-coniferous forest. Traverse City, with a summer recreation population of 500,000, is the largest city in the GTBW. Land use/cover in the GTBW is composed of 49% forest, 20% agriculture, 15% herbaceous/shrub/grasslands, 10% water, and 6% urban. Despite a high degree of development in some catchments, land use varies from urbanized and agricultural areas to completely natural areas.

In Chapter 2, a set of hypotheses relating community structure to human disturbance was tested with data from a field survey. The sensitivities of ecological attributes were compared in this low level of human disturbance. Ecological attributes that respond to low human disturbance have the potential to be used in bioassessment and as early warning signs of threats to stream ecosystem health.

In Chapter 3, the similarities among assemblages from multiple habitats were evaluated and characteristic species for each habitat were derived. The sensitivities of diatom assemblages in different habitats were compared with 3 methods. The homogenization of beta diversity among habitats along a disturbance gradient was also evaluated.

Chapter 4 explored the regional nutrient limitation pattern in the GTBW with nutrient diffusing substrates (NDS). To evaluate if short-term small-scale experiments can reproduce community structural responses as observed among assemblages in nutrient enriched streams, similarities of assemblages on clay pots were assessed. The responses of species on nutrient enriched clay pots were compared with their responses to nutrient differences among streams in the region.

In Chapter 5, an index of biotic integrity (IBI) was developed for the Interior Plateau Ecoregion in Kentucky. Attributes tested were selected from those commonly used in bioassessment. The developed IBI was evaluated with land use information, tested with another dataset, and compared to existing IBIs.

CHAPTER 2 ECOLOGICAL RESPONSE OF STREAM ALGAL COMMUNITIES TO HUMAN DISTURBANCE IN THE GRAND TRAVERSE BAY WATERSHEDS

Abstract

Human disturbance in watersheds influences stream ecosystems through alterations of hydrological pathways, water chemistry, habitats, and food sources. A set of hypotheses on ecological responses to human disturbance were tested with algal community data. The following hypotheses were tested: 1) species composition changes along a human disturbance gradient (HDG); 2) dominant species change along the HDG; 3) species diversity decreases along the HDG; 4) the size of organisms decreases along the HDG; 5) native species decrease along the HDG; and 6) indicator species increase along the HDG. Thirty-one sites were sampled in the Grand Traverse Bay Watershed (GTBW) in Fall 2000. The GTBW, located in the Northwestern part of the lower Michigan, is relatively low in development compared to southern lower Michigan. Land use types were correlated with environmental variables. The HDG was formulated as the 1st axis of redundancy analysis and was positively correlated with % urban and % agriculture land uses.

Algal species composition and dominant species changed with the HDG. Diatoms were the dominant algal group. Diatom dominance index and diversity indices were not correlated with the HDG. Average diatom biovolume was positively correlated with the HDG. Relative abundance of trophic sensitive species significantly decreased with the HDG, while % trophic tolerant species significantly increased with the HDG. Both trophic sensitive and tolerant species were significantly correlated with HDG, Cl⁻, and conductivity, but not with nutrient levels. Nutrient levels may be sequestered as resources

by the biota and are often associated with elevated Cl⁻ and conductivity. Hence, diatom species were a better indicator for human disturbance than nutrient concentrations in water samples. The percentages of native species were negatively correlated with the HDG, while % native and trophic sensitive species had a higher correlation with the HDG than % native species. Overall, these algal attributes responded to land use and environmental variables under low-level human disturbance, except for species diversity. Key words: watersheds, human disturbance, species composition change, dominant species, average diatom size, species diversity, indicator species, sensitive species, tolerant species, native species, exotic species.

Introduction

The connections between land use/cover and stream ecosystems have been observed in various watershed experiments (Likens and Bormann 1974, Swift and Swank 1981, Gregory et al. 1991). From field surveys, land use patterns were also strongly correlated with stream ecosystem structure and function (Hynes 1975, Schlosser 1995, Richards et al. 1996, Allen et al. 1997). Human activities in the watersheds can change stream ecosystem structure and function through alteration of nutrients, hydrological regimes, water quality, energy sources, habitat structure, and biotic interactions (Karr 1991).

The relationship between nutrients and algae has been widely studied (Vallentyne 1972, Welch et al. 1988, Chetelat et al. 1999, Biggs 2000). The linkage between watershed disturbance and algae has received less attention until recently. Watershed

disturbance often induces a change in algal species composition (Leland 1995, Leland and Porter 2000, Munn et al. 2002). The responses of algal assemblages to environmental variables are often assessed by ordination and weighted average indicators of environmental conditions. Few algal studies related the effects of human disturbance to valued ecological attributes. A set of hypothesis, referred to as stress syndromes (Rapport et al. 1985) and retrogression characteristics (Odum 1985), have been used to predict the effects of human disturbance on ecosystems (Schindler 1987, Blair 1996, Havens and Carlson 1998). These hypotheses can serve as a unifying ecological framework with which responses of valued attributes of algae can be related to human disturbance.

Six hypotheses or trends of community structural responses to human disturbance were tested in this study. These hypotheses have great literature support and sound ecological bases. They have been observed in different types of ecosystems and under different kinds of stressors and also have both ecological and societal values.

The first hypothesis is that species composition changes along the human disturbance gradient (HDG) (Schindler 1987, Rapport and Whitford 1999, Gunderson 2001). The second hypothesis is that dominant species change along the disturbance gradient (Sheehan 1984). The first hypothesis differs from the second hypothesis by the inclusion of rare species. The basis of these two hypotheses is a combination of environmental requirements of species (niche) (Hutchinson 1957) and differential competitive ability along environmental or resource gradients (Southwood 1977). Changes in species composition along environmental gradients have been observed since Whittaker (1956) and are recognized as a major theme of ecological study (Austin 1985, ter Braak and Prentice 1988).

The third hypothesis is that species diversity decreases with increasing human disturbance, as dominance by one or a few species increases (Odum 1985). Species diversity is a valuable ecological attribute and conservation target (Rozdilsky et al. 2001). Species diversity can have a hump-shaped relationship along the productivity gradient (Mittelbach et al. 2001). Non-resource toxic stressors often have adverse effects on diversity. However, the mix of resources and toxic stressors may have complex effects on diversity. The fourth hypothesis is that organism size decreases with increasing human disturbance gradients (Odum 1985). Human disturbance has been observed to eliminate large species and especially predators (Havens 1994).

Fifth, indicator species have been observed to increase with the increase of relevant environmental gradients (Sheehan 1984). The idea that the presence of certain species can indicate specific environmental conditions has been widely recognized (Cairns 1974). Indicator species can be indicative of the status of other species, key habitats, or the impacts of a stressor (Dale et al. 2000). The environmental preference of species also has its foundation in niche theory (Hutchinson 1957), and has been used in saprobien indicator systems for almost a century and toxicity tests for environmental assessment. Indicator species are often categorized as sensitive or tolerant species to a specific stressor, for example acid tolerant or sensitive species in the plankton (Havens and Carlson 1998).

The sixth hypothesis is that human disturbance can cause loss of native species (Schindler 1987, Rapport et al. 1985, Blair 1996, Rapport and Whitford 1999). Anthropogenically induced changes in environmental conditions can create competitive advantages for non-indigenous species over native species (Byers 2002). Invasive species

have caused serious economic and ecological damage (Leung et al. 2002, Perrings et al. 2002). The relative abundances of native species are often reduced after the invasion of alien species, which is often facilitated by human disturbance. Therefore, invasive species increase their relative abundances along the disturbance gradient.

In this paper, I studied algal community and evaluate the responses of the six ecological attributes listed above to human disturbance. The algal data were collected from a field survey. These attributes have been observed to change under human disturbance, but have seldom been studied together at one time for stream ecosystems, especially in a relatively pristine area such as GTBW.

Materials and Methods

Study Area

Grand Traverse Bay Watershed (GTBW) has an area approximately 2,600 km² and is located in the northwestern part of the lower peninsula of Michigan, US. It has one of the fastest growing populations in the Midwest United States (Grand Traverse County 1996). Traverse City, with a summer recreation population of 500,000, is the largest city in GTBW. Land use/cover in GTBW is composed of 49% forest, 20% agricultural, 15% herbaceous/shrub/grasslands, 10% water, and 6% urban. Despite high development in some catchments, overall the region is mildly developed. The Boardman River drains the majority of GTBW with the Pere Marquette National Forest located in the upper- and mid-region of the Boardman Watershed. Thirty-one sites were selected across a gradient of urban and agriculture land use intensities (Figure 2.1).

Sampling and Sample Processing

Sampling sites were visited between 13 and 20 October 2000, when hydrology is seasonally relatively stable. Biological, physical, and chemical samples were collected. Algae were scraped from 6 rocks with a toothbrush for each site and stored on ice for further lab analysis. The scraped areas on rocks were measured with a ruler. Canopy cover was estimated with a densiometer. Flow regimes were measured with an electronic portable flowmeter (Flo-mate model 2000, Marsh-McBirney Inc.). Conductivity, water temperature, and pH were also measured in the field with a multipurpose probe (Denver Instrument).

Algal samples were subsampled in the lab for chlorophyll analysis and algae enumeration. Diatoms were digested with the boiling nitric acid method and catalyzed with potassium dichromate. Soft algae were preserved with M3 solution (APHA 1998).

Diatoms were identified based on Krammer and Lang-Bertalot (1986, 1988, 1991a, 1991b); Patrick and Reimer (1966, 1975) were used as supplements. Prescott (1951) was used as the primary reference for soft algae identification; Taft and Taft (1990) was used as a supplement. Both diatom and soft algae were identified to the lowest level possible with a Leica microscope under 1000X and 400X magnification respectively. Biovolumes of soft algae were measured from samples. The biovolumes of common diatom species were calculated from size measurements based on designated shapes (Charles et al. 2002), while the biovolumes of rare species came from past lab data or average sizes from taxonomic references.

Water chemistry analysis was done with a Technicon Auto-analyzer III following standard methods (APHA 1998). NH₃-N was analyzed with an automated phenate



Figure 2.1. The map of sampling sites in the Grand Traverse Bay Watersheds and their locations in Michigan.

method, NO₃-N with an automated Cd-reduction method, and total N (TN) was digested with persulfate before using the nitrate method. Soluble reactive phosphorus (SRP) was measured with the automated ascorbic acid method, while total P (TP) was measured using the SRP method after persulfate digestion and the sample was not filtered. Cl⁻ was measured by the automated ferricyanide method, while SiO₂-Si was measured by an automated method for molybdate-reactive silica. Chlorophyll a (Chl a) was extracted by dissolving the membrane filter in 90% aqueous acetone and measured following standard methods (APHA 1998) with a spectrophotometer (Spectro Instrument). Chl a concentration was calculated as mg/cm^2 .

Land use/cover information was obtained from the 1980 Michigan Resource Information System (MIRIS) with Anderson land use/land cover level 1 classification. A watershed was delineated as the sourceshed above the sampling point in the catchment. Digital elevation modeling with ArcInfo software was used for watershed delineation.

A major environmental gradient (MEG) was obtained from redundancy analysis (RDA) of environmental variables with land use as constraining variables to maintain the independence of the MEG from biological data. RDA is derived from multiple linear regression, followed by principal component analysis (Legendre and Legendre 1998). The MEG represented the major human disturbance gradient (HDG) in RDA and was used for future statistical analysis in this paper.

Data Analysis

Shifts in species composition along the HDG were evaluated by similarity to reference sites. The top 25% of low-developed sites along the HDG were selected as reference sites. The HDG was formulated from land use and environmental variables, and

no species data were involved to avoid circularity. The average Bray-Curtis similarity with reference sites was correlated with the HDG to test for significant relationship.

The change of dominant species along the HDG was evaluated with the top 2 species from each site. The correlation between relative abundances of top 2 species and the HDG was evaluated with Pearson correlation analysis. Dominance pattern was also evaluated by the correlation between Simpson's dominance index (Simpson 1949). The correlations between the HDG and relative biovolumes of major algal groups, such as % filamentous green and % blue green algae, were also evaluated with Pearson correlation analysis.

The relationships between organism size and species diversity measures with the HDG were assessed by Pearson correlation analysis. Diatom species diversity was separated into species richness and evenness measures. Species richness was defined as the average number of species per 100 diatom count, to offset the phenomenon of increasing number of species with increasing diatom counted. Evenness was calculated using Pielou's evenness index (Pielou 1975).

Sensitive species were defined as species that decreased in relative abundances with increasing disturbance; on the contrary, tolerant species increased in their abundances with increasing disturbance. Trophic sensitive and tolerant species were taken from 3 compiled lists: Bahls (1993), van Dam et al. (1994), and MAIA trophic index (Stevenson's unpublished US EPA Mid-Atlantic region diatom trophic index). Species with score 3 in Bahls's list, 1 in van Dam's trophic and saprobity lists, and 1 and 2 (< 10 μ g/L TP) in Stevenson's list were selected as trophic sensitive species. Species with a score of 1 on Bahls's list, 5 and 6 in van Dam's trophic list (eutrophic species), 4

and 5 in van Dam's saprobity list (poly-saprobous species), and 6, 7, and 8 (> 25 μ g/L TP) in Stevenson's list were selected as trophic tolerant species. Species listed in both sensitive and tolerant lists were thrown out. The tendencies of relative abundance of trophic sensitive and tolerant species with the HDG were evaluated with Pearson correlation analysis. Nitrogen, pH, and salinity indicator species were also tested and were derived from van Dam's N, pH, and salinity indices respectively. Species in the lower 2 categories in these van Dam's indices were selected as sensitive species, while those in the top 2 categories were considered as tolerant species. Percent nitrogen, pH, and salinity sensitive and tolerant species and % motile diatom genera (Wang and Stevenson unpublished manuscript) were evaluated along the HDG with Pearson correlation analysis.

Native species are those that live at a site prior to human disturbance. Nonindigenous species are invaders from outside the region. Sites in top 25th percentile of the first RDA site scores were selected as reference sites. Species at reference sites were designated as native species. Species on both native and sensitive species lists were defined as sensitive and native species. Percent native species at reference sites were evaluated with native species in other reference sites, and the same procedure was used for % sensitive and native species. Relative abundance of non-indigenous species was calculated as 1 minus the relative abundance of native species. The relationships between relative abundances of native species, non-indigenous species, and sensitive and native species with the HDG were evaluated with Pearson correlation analysis.

Results

Physical and chemical characteristics were quite variable among sites (Table 2.1). Nonetheless, these variables were generally indicative of pristine to moderately impacted sites. The watershed is largely dominated by forested land (49%) with lesser amounts of agriculture (22%) and urban (5%) development. Water temperature ranged from 7.9 to 16.1 °C, while pH levels were mildly basic. NO₃-N ranged from non-detectable to 2.01 mg/L, TN ranged from 0.13 to 2.7 mg/L with 0.65 mg/L average, which indicated mild enrichment in some streams. TP and SRP levels were relatively low (< 5 μ g/L). Cl⁻, an indicator of human presence in watersheds, ranged from 0.5 to 21.4 mg/L with a mean 7.9 mg/L. Conductivity ranged from 253 to 605 with a mean of 407 μ S/cm. Stream order spanned from 1st to 4th order.

Urban land use was significantly correlated with conductivity and Cl⁻ (Table 2.2). Agricultural land use was positively correlated with TP, conductivity, water temperature, and Cl⁻, and negatively correlated with % forest, stream order and pH. Wetland cover in the watershed was positively correlated with SRP and NH₃-N. Forest cover was positively correlated with stream order and pH, but negatively correlated with % agriculture, % urban, conductivity, TP, TN, Cl⁻, and water temperature.

The first 2 RDA axes explained 54.6 and 0.2% of environmental variable variance respectively, so only the first RDA axis was used as the HDG (Table 2.3). The 1st RDA axis was positively correlated with % agriculture, % urban, TP, Si, Cl⁻, and conductivity, and negatively correlated with % forest and pH. The HDG served as a watershed disturbance gradient from high forest cover to relatively high development and nutrient

	Mean	SD	Minimum	Maximum
% Urban	6.97	9.95	0	43
% Agriculture	22.19	19.97	0	67
% Forest	49.00	26.78	9	93
% Wetland	4.26	5.75	0	27
Order	1.81	0.91	1	4
Canopy	38.08	28.62	0.5	95.5
Water Temp. (°C)	10.5	2.0	7.9	16.1
Conductivity (µS/cm)	407.06	96.17	253	605
рН	8.15	0.13	7.8	8.4
NO ₃ (mg/L)	0.45	0.43	N.D.	2.01
NH₄-H (mg/L)	0.01	0.02	N.D.	0.11
Total N (mg/L)	0.65	0.56	0.13	2.70
Soluble Reactive P (µg/L)	1.53	0.67	0.1	3.3
Total P (µg/L)	1.75	1.04	0.1	4.7
Si (mg SiO ₂ /L)	8.89	2.12	3.2	15.7
Cl (mg/L)	7.87	5.43	0.5	21.4

Table 2.1. Mean, SD, and range of land use and environmental variables. N.D. is the short for non-detectable.

% UrbanConductivityCl% Urban0.36*0.35*Total PConductivity% Agriculture0.62***0.56***	5*								
% Urban 0.36* 0.35* Total P Conductivity % Agriculture 0.62*** 0.56***	5*	Si							
Total PConductivity% Agriculture0.62***0.56***		0.33							
% Agriculture 0.62*** 0.56***	ctivity Strea	am Order	Hd	Vater Temp	ū	Total N			
)- ***).50**	-0.48**	0.44*	0.42*	0.33			
SRP NH3	13	Si/P	Water Temp						
% Wetlands 0.59*** 0.50**	- **(0.42*	0.32						
% Agriculture Conductivity	ctivity T	otal P	CI	Order	% Urban	Water Temp	Ηd	Total N	Si
% Forest -0.79*** -0.73***	()- ***{	.62***	-0.52**	0.49**	-0.43*	-0.42*	0.41*	-0.39*	-0.34

Table 2.2. The correlations between land use types and environmental variables. All selected correlations have a p < 0.10. r is the Pearson correlation coefficient. All correlations shown have p < 0.10. * indicates p < 0.05, ** for p < 0.01, *** for p < 0.001.

	Axis 1	All 4 axes			
Cumulative percentage variance					
of species data	54.6	54.8			
of species-environment relation	99.6	100			
F	28.86	7.28			
p	0.001	0.001			
Correlation with land use					
% Urban	0.48*				
% Agriculture	0.48*				
% Forest	-0.75*				
% Wetlands	0.07				
Correlation with environmental variables					
NO ₃ (mg/L)	0.34				
NH ₄ -H (mg/L)	0.13				
Total N (mg/L)	0.30				
Soluble reactive P (μ g/L)	0.12				
Total P (µg/L)	0.54*				
Si (mg SiO ₂ /L)	0.43*				
Cl (mg/L)	0.52*				
Water Temp. (°C)	0.35				
Conductivity (μ S/cm)	0.77*				
рН	-0.37*				
Canopy	-0.29				

Table 2.3. RDA of environmental variables with land use and the correlation between RDA axes and relevant variables. * indicates statistical significance, p < 0.05.

concentrations. The rest of the RDA axes explained a small fraction of variance in environmental variables.

Diatom species composition, measured as % similarity to reference condition, changed along the HDG (r = -0.48, p = 0.007) (Table 2.4), and was also negatively correlated with % urban, conductivity, SRP, and Si. Diatoms were the dominant algal group and had a mean relative biovolume of 94% (SD 8%) of cells in counts. Centric diatoms were a relatively small proportion with a mean relative biovolume of 1%. No major algal taxonomic group was significantly correlated with the HDG. Of diatom species, <u>Achnanthidium minutissimum</u> Kützing, <u>Achnanthidium biasolettiana</u>, and <u>Amphora pediculus</u> (Kützing) Grunow were the 3 most common species based on relative abundance, while <u>Synedra ulna</u> (Nitzsch) Ehrenberg, <u>Navicula tripunctata</u> (Müller) Bory, and <u>Achnanthidium minutissimum</u> were the 3 most dominant in relative biovolume.

Only 2 of 21 dominant species were significantly correlated with the HDG (Figure 2.2). Percentages of <u>Cymbella delicatula</u> and <u>Gomphonema pumilum</u> were positively correlated with the HDG (r = 0.95, p = 0.003; r = 0.65, p = 0.049). The Simpson dominance index, ranging from 0.05 to 0.70, was not correlated with the HDG. Though diatom species richness, evenness, and Shannon diversity were not significantly correlated with the HDG, they were positively correlated with pH and negatively with Si concentrations.

Average diatom biovolume ranged from 61.8 to 620.2 μ m³ and was positively correlated with the HDG (r = 0.39, p = 0.04) (Table 2.4). The highest average biovolume occurred at one site due to a large proportion of <u>Navicula lanceolata</u> (Agardh) Ehrenberg,

a relatively large diatom. <u>Achnanthidium minutissimum</u>, a very small and abundant diatom, may have constrained the average biovolume at several sites.

The relative abundance of trophic sensitive species, ranging from 0.09 to 0.97, significantly decreased with the HDG (r = -0.51, p = 0.005), was positively correlated with % wetlands, but was negatively correlated with % agriculture, Cl⁻, and conductivity (Table 2.4). The relative abundance of trophic tolerant species, ranging from 0.09 to 0.84, significantly increased with the HDG (Pearson correlation, r = 0.48, p = 0.009). Both trophic sensitive and tolerant species were significantly correlated with Cl⁻ and conductivity, but not with nutrients. Percent motile diatom genera was significantly correlated with the HDG (r = 0.43, p = 0.02) and conductivity, but negatively correlated with % forest. While % high N species was not significantly correlated with the HDG, % low N species was significantly correlated with the HDG (r = -0.45, p = 0.01). Low N species positively correlated with % wetlands and negatively correlated with conductivity. Percent high salinity species was correlated with the HDG (r = 0.44, p = 0.01), while % low salinity species was not correlated with the HDG. Percent high salinity species was positively correlated with % urban, Cl⁻, and conductivity; on the contrary, low salinity species was negatively correlated with those. None of pH indicators (% high pH, % low pH species, and % acidophilic genera) was significantly correlated with the HDG.

Percent native species, ranging from 62 to 100, was negatively correlated with the HDG (r = -0.46, p = 0.01), while % non-native species was positively correlated with the HDG (r = 0.46, p = 0.01). Percent native species at all sites had a mean of 94% and a SD of 9%. After restricted to trophic sensitive species, % trophic sensitive and native species, ranging from 62 to 1, had a highly significant correlation with the HDG (r = -0.55, p =

0.002). Percent native and sensitive species wwere positively correlated with % forest and negatively with % agriculture, conductivity and Cl⁻.

Discussion

Results of this study demonstrated that algal assemblages responded to human disturbance in watersheds, even with the low levels of disturbance in the Grand Traverse Bay Watershed. All tested community retrogressive characteristics and stress syndromes responded to the low gradient of human disturbance except species diversity measures. These attributes have been widely used for ecological studies and assessments, and have seldom been tested together for stream ecosystems. Current bioassessment tools often use these characteristics without addressing their ecological bases. Putting bioassessment into an ecological framework can strengthen its foundation, increase transferability among types of ecosystems, and facilitate acceptance and communication among biologists. All hypotheses were tested with field survey data, which is often the scale of study where ecological patterns are observed (MacArthur 1957, Fischer 1960, Brown 1999) and generalizations about nature can be made (Naeem 2001). The results of lab bioassays and microcosm studies are difficult to extrapolate to nature (Cairns and McCormick 1992). Ecosystem experiments often are not replicated, which makes generalization difficult (Hurlbert 1984, Carpenter et al. 1998). However, the experimental approach can provide causal relationships to support survey findings. This study provides the first intensive survey of algae in low nutrient streams in this region.
Table 2.4. The correlations between diversity measures, evenness, dominance, average
size, native species, indicator guilds with $RDA 1^{st}$ axis scores, land use and
environmental variables. All correlations shown have p < 0.10. * indicates p <0.05, ** for
p < 0.01, *** for p < 0.001.

Change of species-0.48**0.42composition-0.22-0.42Species richness-0.22Evenness0.06Evenness0.05Annon Diversity-0.13Dominance-0.13Average diatom biovolume0.39*% Trophic sensitive species-0.51**% Native species-0.51**% Native species-0.55**% Native species-0.55**% Native species-0.55**	0.42*		% Wetlands	Order	Canopy	Conductivity
Species richness-0.22Evenness0.06Evenness0.06Shannon Diversity-0.13Dominance-0.02Average diatom biovolume0.39*Of Trophic sensitive species-0.37*% Trophic sensitive species-0.37*% Native species-0.46*% Native species-0.55**% Native species-0.55**		-0.37*	-0.33	0.32	0.50**	-0.44*
Evenness0.06Shannon Diversity-0.13Dominance-0.02Average diatom biovolume0.39*Average diatom biovolume0.39*Chlorophyll a0.39*% Trophic sensitive species-0.37*% Native species0.48**% Native species-0.55**% Native species0.55**				0.41*	0.37*	-0.31
Shannon Diversity-0.13Dominance-0.02Average diatom biovolume0.39*Average diatom biovolume0.39*Chlorophyll a0.39*% Trophic sensitive species-0.37*% Native species0.46*% Native species-0.46*% Native species-0.35**% Native species0.55**						
Dominance-0.02Average diatom biovolume0.39*Chlorophyll a0.39*% Trophic sensitive species-0.31**% Trophic tolerant species0.48**% Native species0.46*% Native species-0.35**% Native species0.35**						
Average diatom biovolume0.39*Chlorophyll a0.39*Chlorophyll a0.39*% Trophic sensitive species-0.51**% Trophic tolerant species0.48**% Native species-0.46*% Native species-0.35**% Native species0.35**						
Chlorophyll a0.39*-0.3% Trophic sensitive species-0.51**-0.37*0.38% Trophic tolerant species0.48**-0.46*0.46*% Native species-0.46*-0.39*0.45% Native species-0.55**-0.39*0.45						0.35
% Trophic sensitive species-0.51**-0.37*0.38% Trophic tolerant species0.48**-0.46*% Native species-0.46*-0.39*0.42% Native species-0.55**-0.39*0.42	-0.32	0.50**			-0.31	0.41*
% Trophic tolerant species 0.48** % Native species -0.46* % Native species -0.55** -0.39* % sensitive species -0.55** -0.45	* 0.38*		0.31	0.39**		-0.56**
% Native species -0.46* % Native and trophic -0.55** -0.39* 0.42 sensitive species						0.35*
% Native and trophic -0.55** -0.39* 0.43 sensitive species						
	* 0.43*			0.37*		-0.58**
% Non-native species 0.46*						
% Motile diatom 0.43* -0.4	-0.41*			-0.31		0.61***
% Low N species -0.45* -0.3			0.48**	0.31		-0.46**
% High salinity species 0.44*		0.46**		0.31	-0.42*	0.38*

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Table 2.4

	μd	NO3-N	NH4-N	Total N	SRP	Total P	Si	C
Change of species composition					-0.38*		-0.38*	-0.32
Species richness	0.56**						-0.49**	
Evenness	0.56**						-0.37*	
Shannon Diversity	0.60***						-0.45*	
Dominance	-0.57**						0.36*	
Average diatom biovolume								0.38*
Chlorophyll a			0.61***					0.37*
% Trophic sensitive species	0.54**							-0.39**
% Trophic tolerant species								0.43*
% Native species								-0.31
% Native and trophic sensitive species	0.50**							-0.44*
% Non-native species								0.31
% Motile diatom								
% Low N species	0.32	-0.31						
% High salinity species								0.49**



Figure 2.2. The relationships between dominant species and the HDG. A. Cymbella delicatula, r = 0.95, p = 0.003; B. Gomphonema pumilum, r = 0.65, p = 0.049.

In GTBW, human disturbance was a driver of species composition change. The change of community structure or species composition along environmental gradients has become one of the most studied relationships in ecology (Whittaker 1975, Austin 1985, ter Braak and Prentice 1988). Changes in community composition along environmental gradients have been observed widely in various ecosystems, such as coral reefs (Ostrander et al. 2002), coastal plants (La Peyre et al. 2001), fish assemblages (Rodriguez and Lewis 1997), oak savannas (Leach and Givnish 1999), and arthropod communities (Progar and Schowalter 2002). It has also been observed in stream ecosystems (Leland and Porter 2000, Griffith et al. 2001, Munn et al. 2002).

Comparison of species composition at reference and test sites was used to determine community composition change along the HDG. The pollutants from agriculture and urban land use often include both resources and physiological stressors (Cooper 1993, Paul and Meyer 2000). Therefore, multivariate statistics (ordination) were used to characterize the HDG and to evaluate the species composition changes along multivariate environmental gradients. The assessment of the overall change in species composition relative to reference condition is advantageous not only in indicating the degree of changes at community and ecosystem levels, but also can be easily conveyed to the general public if quantified. Biological attributes described with complex multivariate ordination are difficult for the public to understand.

Community composition changes can also be broken down into the responses of characteristic species or indicator taxonomic groups for statistical tests. Both sensitive and tolerant indicator species were responsive to human disturbance in this study. Species often have preferences for different levels of environmental conditions, such as oxygen,

salinity, pH, and temperature. Sensitivity and tolerance are species traits with roots in basic biology and ecology, such as shade tolerant species in forests (Canham 1988, Porter 1999), pH sensitive and tolerant zooplankton species in lakes (Brezonik et al. 1993, Locke and Sprules 1993, Fischer et al. 2001) and salinity and anoxia tolerant species in salt marshes (Bertness and Ellison 1987). Noss (1990) and Kremen (1994) have proposed using indicator species to measure environmental changes or impacts for conservation programs. A well-developed database of species autecology may provide valuable information for ecological and environmental research.

Trophic sensitive, trophic tolerant species, and low N species were significantly correlated with the HDG and conductivity, but not with any specific nutrient measured. Streams in the study region are groundwater fed and relatively stable hydrologically (Boutt et al. 2001). Consequently, mild addition of nutrients in watersheds may not show up in stream water because of hyporheic microbial activities, riparian vegetation uptake, and especially rapid uptake by instream biota. Hence, algal trophic indicators indicated increased nutrients, even though correlations with nutrients were not observed. Non-resource pollutants associated with nutrient sources, such as conductivity, pH, and Cl⁻, were also significantly correlated with nutrient indicator species. The change in relative abundances of species indicative of nutrients enabled the diagnosis that nutrients were important factors affecting diatom assemblages in this region.

Percent native and % invasive species were significantly responsive to the HDG. When % native species was constrained with sensitive species, it was more sensitive to the HDG than % native species alone. Anthropogenic changes in environmental conditions can increase invasion success of non-indigenous species by creating new

habitats for colonization, introducing propagules, decreasing populations of native species, and shifting the competitive advantage to non-indigenous over native species (Byers 2002). Native species respond to stressors in watersheds and are considered a valuable ecological attribute for conservation and assessment purposes (Zampella and Bunnell 1998, Diamond and Serveiss 2001). However, caution should be exercised to recognize that not all native species are sensitive and some species may tolerate the human disturbance. Tolerant native species may positively respond to the HDG and mask the important loss of sensitive native species.

Diatom species richness and diversity measures were not significantly correlated with the HDG. Algal species richness can be negatively correlated with SRP and soluble inorganic N, especially in the lower range of nutrients (Biggs and Smith 2002). On the contrary, Dodson et al. (2000) found a unimodal response of phytoplankton richness to pelagic productivity. van Dam (1982), however, found that algal species diversity decreased with nutrients. The fixed count (300 algal colonies or 600 diatom valves) of algal species does not fully assess algal species diversity (Stevenson and Lowe 1986). Types of pollutants (Patrick 1973) and levels of pollutants may also control the change in species richness, evenness, and dominance.

The average diatom size was positively correlated with the HDG. Contrary to the hypothesis of Odum (1985), Peters (1983) stated that increased resources may lead to individuals in communities with larger sizes. The difference in responses of organism size to human disturbance may come from the different effects between resources and toxic stressors. Pollutants from urban and agriculture land use often include nutrients. Contrary to toxic stressors such as heavy metals and herbicides, nutrients are resources to

algae and stimulate algal growth. Hence, cell size may increase instead of decrease due to enrichment. Nutrient enrichment can even ease the effect of a toxic stressor (Lozano and Pratt 1994). Average diatom size was also observed to increase along a phosphorus gradient in streams and lake littoral zones (Cattaneo 1987, Cattaneo et al. 1995). Nutrient enrichment can shift community composition from diatom dominated to big-cell filamentous green algae (Dodds and Gudder 1992). However, nutrients may have opposite effects on different taxonomic groups. Excessive cover of algae usually reduces O₂ levels, so sensitive and large aquatic insects and fish may respond differently than algae.

Valued ecological attributes are desired assessment endpoints and should be applicable to set ecological protection and resource conservation criteria (Campbell 2001). Stream water quality protection depends on the designated use, as different streams have different designated usages and are subject to different standards. Different community attributes respond to environmental gradients with different sensitivities. Hence, different valued ecological attributes have the potential to be used as the criteria for different designated uses. For example, % trophic sensitive and native species and % native species had different sensitivities along the HDG. For comparison, values of these attributes were plotted against the HDG with a standardized range from 0 to 1 (Figure 2.3). If a 50% decrease in the attribute value is the criterion, % native species had a higher corresponding level on the environmental gradient than % trophic sensitive and native species species. Percent native and trophic sensitive species decreased faster along the HDG and was more sensitive to the HDG than % native species. In other words, % trophic sensitive and native species can be used to set a higher standard than % native

species. This is useful to set different criteria to protect valued ecological attributes when there are different desired protection levels.

In summary, algal assemblages responded to the HDG according to patterns proposed by Odum (1985) and others in this low impacted area, except for diversity measures. Average organism size responded positively to the HDG. Species composition change as well as native species and non-indigenous species were good indicators of human disturbance. Indicator species, widely used by ecological studies and environmental assessment, have also been supported as sensitive to human disturbance in this study. Comparison of species autecology across regions may reveal some interesting ecological and taxonomical properties and may result in indicator species lists that are applicable to wide regions. Despite our structural focus, we encourage the test of responses of functional attributes to human disturbance as proposed by Odum (1985) and the study of linkages among structural and functional attributes and ecosystem services that are important for protecting stream ecosystems.



Figure 2.3. Differential responses of % trophic sensitive and native species and % native species to the human disturbance gradient. The lower line is % trophic sensitive and native species, % native species is the top line. Dashed lines represent the corresponding environmental gradient values when attribute values are at 50%.

CHAPTER 3 RESPONSES AND SIMILARITIES OF DIATOM ASSEMBLAGES FROM MULTIPLE HABITATS ALONG ENVIRONMENTAL GRADIENTS FROM MICHIGAN STREAMS

Abstract

Biodiversity in stream ecosystems may be supported by habitat diversity. Epilithon is the most studied algal assemblage for ecological and bioassessment research in streams. In this study, diatoms were sampled from multiple habitats, including plants, rocks, and sediments, in streams through the Grand Traverse Bay Watershed during October 2000. The similarities among the 3 assemblages were evaluated with detrended correspondence analysis (DCA), while the responses of assemblages in multiple habitats to an environmental gradient were evaluated with canonical correspondence analysis (CCA), beta diversity, and the MAIA trophic index. The reduction of beta diversity along the disturbance gradient was also evaluated. The major disturbance gradient (MEG) was defined as the site scores on the 1st axis of a principal component analysis, which was positively correlated with total N, total P, Si, Cl⁻, and conductivity. Epipelon was distinctly different from the epilithon and epiphyton cluster in a DCA plot. Assemblages in each habitat had characteristic species, while epipelon had the most unique species. Epiphytes had the most % species variance explained and % species-environment relation variance explained from CCA, while epipelon was ranked 2nd among the 3 assemblages. Epiphytes also had the smallest beta diversity and fastest species turnover along the MEG, while epipelon and epilithon had about the same beta diversity and species turnover rate. Further tests showed that epiphyton was more sensitive than epilithon and epipelon when the MAIA trophic index was applied to 3 assemblages. Similarities among habitats were

not increased along the MEG. Natural habitats, besides rock, deserve more study for biodiversity and bioassessment.

Introduction

Streambeds are composed of many types of habitats for the colonization and growth of algae, such as fine organic matter, sand, rock, and plant. Each of these habitats supports the diversity of algae that contribute to the overall function of stream ecosystems. Rocks in riffles are the most studied habitat for algae in streams (Round 1991). In many streams, however, other substrata are dominant, and riffles often are rare. The study of algal communities on substrata other than rock has been limited (Miller et al. 1987, Stevenson and Hashim 1989). Different habitats often have unique algal flora (Douglas 1958, Kingston et al. 1983, Stevenson and Hashim 1989, Jüttner et al. 1996, Lim et al. 2001). However, some studies have shown no consistent distinct assemblages among different habitats (Sullivan 1982, Winter and Duthie 2000).

Eutrophication has the potential to reduce the heterogeneity of assemblages among multiple habitats and homogenize inter-habitat diversity (*sensu* McKinney and Lockwood 1999, Rahel 2002). Differences in assemblages among multiple habitats come from the differences in the nutrient environment (release of nutrients) and stability of substrata. Elevated nutrients in stream water may reduce the difference in nutrients among habitats. Therefore, eutrophication may increase the similarity among assemblages from different habitats in each site. The total diversity in a site or region is composed of the average species diversity in habitats and the change of species composition among habitats (interhabitat diversity) (Loreau 2000). The total diversity may suffer from the reduction of inter-habitat diversity, while the local diversity in each habitat is intact (Loreau 2000, Rahel 2002).

From a bioassessment perspective, sampling the habitat where algae are most sensitive to environmental gradients is preferable for the effectiveness of the monitoring program. The most sampled habitat for algal bioassessment is also the rocks. The sensitivities of algal flora to stressors by human disturbance are largely unknown in habitats other than rocks. Only a few studies compared the sensitivities of algal assemblages in different habitats (e.g. Winter and Duthie 2000). Even when rocks are present, epilithon may not be the most sensitive flora. Winter and Duthie (2000) found that epiphyton was more sensitive than epilithon. Beta diversity, a component of the regional diversity, is the variation in species composition among localities or the extent of species replacement or biotic change along environmental gradients (Whittaker 1972). It is a measurement of species turnover along environmental gradients, hence it can detect the sensitivities of assemblages to the disturbance gradient.

In this study, algae were sampled in sediment, plant, and rock habitats. I studied the distinctness of algal assemblages in 3 habitats and analyzed the characteristic diatom assemblage from each habitat. The sensitivity of response to human disturbance among 3 habitats was compared with 3 methods: CCA, beta diversity, and MAIA trophic index. I also tested the hypothesis that the similarity of diatom assemblages among 3 habitats increased along a human disturbance gradient.

Materials and Methods

Sampling Area

The Grand Traverse Bay Watershed (GTBW) with an area of approximately 2,600 km² is located in the northwestern part of the lower Michigan peninsula and has one of the fastest growing populations in the Midwestern region of the U.S. (Grand Traverse County 1996). Traverse City, with a summer recreation population of 500,000, is the largest city in GTBW. Land use/cover in GTBW is composed of 49% forest, 20% agricultural, 15% herbaceous/shrub/grasslands, 10% water, and 6% urban. Despite high development in some catchments, a wide range of development exists throughout GTBW. The Boardman River drains the majority of GTBW, while the Pere Marquette National Forest is located in the upper- and mid-region of the Boardman River watershed.

Sampling and Sample Processing

Algae were sampled from rock, aquatic plant, and sediment (fine organic matter) from stream margins, which often do not occur together at the same site. Sediments were sampled at 37 sites, rocks at 35 sites, and plants at 15 sites during early October 2000 (Figure 3.1). Twelve sites had all 3 habitats sampled. Algae were collected from 6 rocks in each site and were scraped with a toothbrush into a white pan. Aquatic plants were cut off and stored in whirl-pac plastic bags for epiphyte sampling, and included a wide variety of large plants, such as *Myriophyllum* spp., *Potamogeton* spp., and overhanging grasses that dipped into the stream. Epipelon was sampled with a petri dish inverted to the sediments, then sealed by a spatula, and stored in whirl-pac plastic bags. Diatoms were sampled by swirling algae off sands in a container



Figure 3.1. Locations of the sampling sites in Michigan.

then decanting suspended algae into a beaker 10 times. Samples were stored on ice until further analysis in the lab.

Samples were subsampled in the lab for diatom enumeration. Diatoms were digested with boiling nitric acid method and catalyzed with potassium dichromate. Sampled plants were also digested with the same method to get digested epiphytic diatoms. Identification of diatoms was based on Krammer and Lange-Bertalot (1986, 1988, 1991a, 1991b); Patrick and Reimer (1966, 1975) were used as supplements. Diatoms were identified to the lowest level possible and usually to species level.

Canopy cover in the streams was estimated with a densiometer. Flow regimes were measured with an electronic portable flowmeter (Flo-mate model 2000, Marsh-McBirney Inc.). Conductivity, water temperature, and pH were also measured in the field with a multipurpose probe (Denver Instrument).

Water chemistry analysis was done in the lab with a Technicon Auto-analyzer III following standard methods (APHA 1998). NH₄⁺-N was analyzed with an automated phenate method, NO₃⁻-N with an automated Cd-Reduction method, and total N (TN) was digested with persulfate before using the nitrate method. Soluble reactive phosphorus (SRP) was measured with the automated ascorbic acid method, while total P (TP) was measured using the SRP method after persulfate digestion and the sample was not filtered. Cl⁻ was measured by the automated ferricyanide method, while SiO₂-Si was measured by an automated method for molybdate-reactive Silica.

Data Analysis

The distinctness of diatom assemblages in each habitat was analyzed with detrended correspondence analysis (DCA). The site scores on the 1st DCA axis were plotted against

those on the 2nd DCA axes with different symbols for each habitat. Similarity of assemblages from different habitats can be evaluated from the DCA biplot.

Species unique to each habitat were analyzed by Indicator Species Analysis (ISA) (Dufrene and Legendre 1997). The indicator value (IV) is calculated by dividing the mean relative abundance of a species in one habitat by its average in all habitats, then multiplied by the proportional frequency of the species in that habitat (Dufrene and Legendre 1997). The highest IV among habitats (IVmax) represents the indicator value from the associated indicator group for that species. For example, if a species has a significant IV max for the epiphyte group, then it is an indicator species of epiphyte. Statistical significance of the indicator value was estimated by a Monte Carlo method. It is done by randomly reassigning sites to groups 1,000 times, then calculating IV, and selecting IVmax each time. The null hypothesis is that the simulated IVmax is no larger than would be expected by chance. The probability of type I error is based on the proportion of occurrences that the IVmax from the randomized data set equals or exceeds the IV max from the actual data set. The significance level was set at 0.05. In this study, indicator species were further restricted with the following procedures because the proportional frequency was not compared among groups. If the habitat in which a species had its highest relative abundance was different from the habitat with the highest proportional frequency of observation, then the species was eliminated from the indicator species list. A second indicator value was calculated as the relative abundance in the maximum group multiplied by the difference between the maximum proportion and average proportion divided by two-thirds of the maximum proportion. If the 2nd indicator value was < 23.5, which was the average of the 1st indicator values at p = 0.05 level, the species was discarded.

Environmental variables from all sampling sites were reduced to a composite environmental variable by principal component analysis (PCA). The 1st axis of PCA usually explains the most variation in environmental data, hence it was used as the major environmental gradient (MEG) among sampling sites. Site scores along PCA axis 1 were used to characterize environmental conditions at each site.

The precision of diatom assemblage response from each habitat to the MEG was compared with % variance explained by the first and all 4 axes from canonical correspondence analysis (CCA). NO₃⁻ and Cl⁻ were excluded from analysis due to a variation inflation factor > 20 (ter Braak and Verdonschot 1995). Each habitat had a different sample size; while epiphytes had the smallest sample size of 15. For a fair comparison, 15 sites were randomly selected for each run from epilithic and epipelic samples separately for CCA to calculate % variance explained until the means of % variance explained stabilized. Sample selection for runs was performed with 2 random functions to emulate the real random process. A stabilized mean was defined as when the means of % variance explained changed by < 5 % for 10 consecutive runs after at least 50 runs.

Beta diversity was calculated to compare the sensitivities of different assemblages to the MEG. For each habitat assemblage, pair-wise comparison was calculated with Bray-Curtis similarity and the difference of scores on the MEG (Pielou 1975). Bray-Curtis dissimilarity was converted to similarity values by subtracting dissimilarity from 1.0. The rate of change in assemblage similarity along the MEG distance was estimated with

linear regression. Beta diversity was calculated as the change of distance on the MEG that corresponded to a 50% change in community similarity. In a second approach, community similarity was calculated as the mean Bray-Curtis similarity with reference sites. Reference sites were those in the lowest one-third on the MEG for each habitat. Regression analysis was also applied to estimate the rate of change in assemblage similarity to the reference condition.

Due to unequal sample size among habitats, random sampling was used to compare beta diversities from different substrata. Fifteen sites were randomly selected from sites with epilithon and epipelon 1,000 times to calculate beta diversity. The probability of eipelon or epilithon with beta diversity smaller than epiphyton's beta was calculated as the proportion of the number of times that those habitats had beta < epiphyton's beta out of 1,000 runs.

Sensitivity of habitats was further evaluated by the slopes of regression analysis between MAIA trophic index (Stevenson's unpublished US EPA Mid-Atlantic region diatom trophic index) and site scores on the MEG. The slope of the regression model was used as the indicator of sensitivity. For a fair comparison with epiphyton, 15 samples from epipelon and epilithon were randomly selected for 1000 runs. The 1000 slopes generated for epilithon and epipelon were compared with the slope of epiphyton.

To evaluate the reduction of inter-habitat diversity along the MEG, similarity of species composition among the 3 habitats within each of 12 sites with all 3 habitats sampled was calculated with Bray-Curtis and Jaccard indices. Bray-Curtis dissimilarity was converted to similarity values by subtracting dissimilarity from 1.0. Changes in inter-habitat similarity were examined along the MEG with Pearson correlation analysis. A

second inter-habitat diversity, Whittaker's β diversity, was calculated as total species diversity divided by the mean species diversity among habitats in each site (Whittaker 1960). The relationship between Whittaker's β diversity and the MEG was examined with Pearson correlation analysis.

Results

Generally, studied watersheds had low levels of human land use except for several sites having either moderate agricultural activity or urban development (Table 3.1). Nutrient levels were relatively low, especially phosphate. The median TP concentration was 1.4 μ g/L, while median SRP was 1.3 μ g/L. TN concentration spanned from 0.10 to 2.70 mg/L, NO₃⁻-N ranged from non-detectable to 2.01 mg/L, and NH₄⁺-N was generally low with a maximum level of 0.11 mg/L. Most pH levels were above 8. Most sites had conductivity above 300 μ s/cm. Si had a range from 3.2 to 15.7 mg/L; while Cl⁻ was between 0.5 and 21.5 mg/L.

Species compositions of samples from 3 habitats were grouped by DCA into two clear clusters (Figure 3.2). Epiphytic and epilithic samples were inter-dispersed among each other, however epipelic samples aggregated into a distinct cluster.

Indicator species analysis showed that each habitat had its own characteristic species (Table 3.2). For example, epiphytes were characterized by <u>Gomphonema pseudotenellum</u> L-B., <u>Cocconeis placentula</u> var. <u>lineata</u> (Ehr.) V. H. Sm, <u>Eunotia arcus Ehr.</u>, and <u>Gomphonema affine Kütz</u>, while epilithon was represented by <u>Fragilaria elliptica</u>

Variables	Minimum	Maximum		Percentiles	
			25th	50th	75 th
% Agriculture	0	67	9	18	40
% Forest	9	93	21	46	69
% Wetlands	0	27	1	2	5
% Urban	0	43	1	3	8
NO3 ⁻ -N (mg/L)	0.000	2.010	0.155	0.280	0.740
NH_4^+-N (mg/L)	0.000	0.110	0.002	0.004	0.008
TN (mg/L)	0.100	2.700	0.300	0.420	0.930
SRP (µg/L)	0.7	3.3	0.9	1.3	1.8
TP (μg/L)	0.0	4.7	1.0	1.4	2.0
SiO ₂ -Si (mg/L)	3.20	15.70	7.75	9.10	9.95
Cl (mg/L)	0.50	21.50	4.85	6.00	9.15
Water Temp °C	7.9	16.1	9.1	10.5	11.3
Conductivity (μ S/cm)	253	605	313	373	476
pH	7.8	8.4	8.1	8.2	8.2

Table 3.1. The minimum, maximum, 25th, median, and 75th percentile of environmental variables. N = 41.



DCA axis 1

Figure 3.2. A DCA biplot for all sites from 3 habitats. ▲ represents epipelic samples; ■ for epilithic samples; * for epiphyte samples.

Species	Habitat	IV	р	2nd IV
Gomphonema pseudotenellum L-B.	Epiphytes	35.3	0.001	86.6
Cocconeis placentula var. lineata (Ehr.) V. H. Sm	Epiphytes	80.8	0.001	27.9
Eunotia arcus Ehr.	Epiphytes	13.7	0.012	82.3
Gomphonema affine Kutz.	Epiphytes	18.6	0.013	62.1
Cymbella caespitosa (Kutz.) Brun	Epiphytes	23.9	0.014	60.8
Fragilaria elliptica Schumann	Epilithon	36.4	0.001	100.0
Nitzschia dissipata (Kutz.) Grun.	Epilithon	60.6	0.002	50.9
Navicula stroemii Hust.	Epilithon	28.7	0.004	78.2
Fragilaria capucina var. gracilis (Oestr.) Hust.	Epilithon	40.9	0.009	47.9
Opephora olsenii Mull.	Epilithon	24.5	0.011	80.0
Achnanthes linearis (W. Sm.) Grun.	Epilithon	20.2	0.013	88.2
Fragilaria leptostauron (Ehr.) Hust.	Epilithon	31	0.015	45.3
Nitzschia dissipata var. media (Hantz.) Grun.	Epilithon	31.3	0.047	29.1
Cymbella prostrata (Berk.) Cl.	Epilithon	21.2	0.051	65.3
Navicula costulata Grun.	Epipelon	61.7	0.001	79.1
Stauroneis smithii Kram.	Epipelon	66.4	0.001	74.8
Navicula menisculus Schum.	Epipelon	65.8	0.001	59.2
Fragilaria construens var. venter (Ehr). Grun.	Epipelon	60.9	0.001	42.0
Nitzschia recta Hantz. ex Rabh.	Epipelon	54.9	0.001	30.1
Fragilaria brevistriata Grun.	Epipelon	56.4	0.001	29.5
Navicula reinhardtii (Grun.) Grun.	Epipelon	33.3	0.002	100.0
Navicula viridula (Kutz.) Ehr.	Epipelon	34.7	0.002	88.0
Cocconeis neothumensis Kram.	Epipelon	59.9	0.002	59.8
Amphora libyca Ehr.	Epipelon	42.4	0.002	52.9
Cocconeis disculus (Schum.) Cl.	Epipelon	42.4	0.003	57.0
Achnanthes conspicua A. Mayer	Epipelon	54.5	0.003	26.7
Navicula libonensis Schoeman	Epipelon	28.1	0.004	80.1

Table 3.2. Indicator species, indicative habitats, and their indicator values (IV). p (probability) was for the first IV.

Table 3.2. Continued.

Species	Habitat	IV	p *	2nd IV
Fragilaria construens (Ehr.) Grun.	Epipelon	48.4	0.004	39.6
Achnanthes laevis Schimanski	Epipelon	32.4	0.005	47.3
Sellaphora pupula (Kutz.) Meresckowsky	Epipelon	34.4	0.006	53.6
Navicula capitata Ehr.	Epipelon	41.9	0.006	52.6
Navicula decussis Oestr.	Epipelon	28.2	0.007	73.4
Amphora thumensis (A. Mayer) Krieger	Epipelon	22.2	0.009	102.3
Navicula trivialis Lange-Bert.	Epipelon	30.4	0.009	60.0
Navicula subrotundata Hust.	Epipelon	31.3	0.01	46.3
Navicula veneta Kutz.	Epipelon	35.1	0.01	43.0
Diploneis oblongella (Naeg. ex Kutz.) Ross	Epipelon	37.7	0.01	41.9
Navicula cincta (Ehr.) Ralfs	Epipelon	22.2	0.013	102.3
Gomphonema innocens	Epipelon	22.5	0.014	75.6
Planothidium peragalli Brun & Herib.	Epipelon	34.2	0.014	43.2
Navicula gregaria Donk.	Epipelon	41	0.014	26.9
<i>Nitzschia linearis</i> var. <i>subtilis</i> (Grun.) Hust.	Epipelon	20.9	0.015	89.7
Fragilaria leptostauron var. dubia (Grun.) Hust.	Epipelon	46.5	0.015	23.7
Cymbella sp. K	Epipelon	35.4	0.016	65.5
Navicula pseudoscutiformis Hust.	Epipelon	23	0.02	66.7
Achnanthes exigua Grun.	Epipelon	38.1	0.021	25.2
Navicula tenelloides Hust.	Epipelon	19.3	0.024	83.0
Nitzschia heufleriana Grun.	Epipelon	20.2	0.026	54.8
Navicula reichardtiana Grun.	Epipelon	39.7	0.026	24.3
Meridion circulare (Grev.) Ag.	Epipelon	27.1	0.027	37.5
Planothidium lanceolata var. dubia (Grun.) L-B.	Epipelon	34.1	0.029	34.7
Navicula cari Ehr.	Epipelon	27.7	0.033	35.8
Surirella angusta W. Smith	Epipelon	16.7	0.034	67.9
Planothidium joursacense Heri.	Epipelon	19.8	0.042	57.1
Amphora montana Kras.	Epipelon	13.2	0.05	81.4

Schumann, <u>Nitzschia dissipata</u> (Kütz.) Grun., <u>Navicula stroemii</u> Hust., <u>Fragilaria</u> <u>capucina</u> var. <u>gracilis</u> (Oestr.) Hust., and <u>Achnanthes linearis</u> (W. Sm.) Grun. Epipelon had a long list of characteristic species and featured <u>Navicula costulata</u> Grun., <u>Stauroneis</u> <u>smithii</u> Kram., <u>Navicula menisculus</u> Schumnann, <u>Fragilaria construens</u> var. <u>venter</u> (Ehr). Grun., <u>Nitzschia recta</u> Hantz. ex Rabh.

Other than <u>Achnanthidium minutissimum</u> and <u>Fragilaria pinnata</u>, no overlap occurred in the 5 most common taxa in each habitat. The top 5 dominant species from epiphyton were <u>Achnanthidium minutissimum</u>, <u>Cocconeis placentula</u> var. <u>lineata</u>, <u>Cocconeis placentula</u> var. <u>euglypta</u>, <u>Gomphonema pumilum</u>, and <u>Navicula lanceolata</u>. The top 5 epilithon species were <u>Achnanthidium minutissimum</u>, <u>Achnanthidium</u> <u>biasolettiana</u>, <u>Amphora pediculus</u>, <u>Navicula cryptotenella</u>, and <u>Fragilaria pinnata</u>. <u>Fragilaria pinnata</u>, <u>Fragilaria construens</u>, <u>Achnanthidium minutissimum</u>, <u>Melosira varians</u>, and <u>Fragilaria construens</u> f. <u>venter</u> were the dominant epipelon species.

More variation in species composition of epiphyton can be explained by environmental variables in CCA than in the cases of epipelic and epilithic assemblages. From 110 CCA runs of simulation, no overlap was present between epiphyton and the box plots of epiplithon and epipelon in % species variance and % species-environment variance explained from 4 axes (Figure 3.3). Values of % variance explained for epiphytes, 52.2% species variance explained and 66.8% species-environment variance explained, were greater than the highest values of the other 2 habitats for all 4 axes. The mean % species variance and % species-environment variance explained by all 4 axes stabilized and had values of 39.8 and 58.6% respectively for epilithon (Table 3.3). The



Figure 3.3. Box plots for comparing CCA results from 3 habitats. Both epilithon and epipelon had 110 CCA runs, while epiphyton had 1 run. A. % species variance explained; B. % species-environment variance explained. \bigcirc represents outliers.

Α.

В.

	Axis 1	4 Axes total
Epiphyte (n = 15)		
% Species variance		
	19.2	52.2
% Species-environment	nt variance	
	24.6	66.8
Epipelon $(n = 15)$		
% Mean species variat	nce	
	17.0	45.8
% Mean species-envir	onment var	iance
	22.6	61.0
Epilithon $(n = 15)$		
% Mean species varia	nce	
	14.1	39.8
% Mean species-envir	onment var	iance
	20.8	58.6

Table 3.3. Variances explained by CCA axes in different habitats. Fifteen sites were randomly selected from epipelon and epilithon 110 times for CCA runs. All variances are cumulative.

means were stabilized after 110 runs for epipelon and yielded means of 45.8 and 61.0 % for species variance and % species-environment variance explained for all 4 axes.

The 1st PCA axis explained 28.7% of the variance in environmental variables, which was about twice the variance explained by the 2nd axis (Table 3.4). The 1st principal component, as the major environmental gradient (MEG), was positively correlated with conductivity, TP, TN, Cl⁻, Si, and NO₃, and negatively correlated with pH, stream order, and canopy. Sites with epiphytic samples spanned 80 % of the 1st PCA axis (Figure 3.4), while sites with epipelon or epilithon sampled were found along the whole MEG.

From beta diversity along the MEG distance, epiphytes had the highest absolute slope values and smallest beta diversity, which were -0.132 and 1.87 respectively (Table 3.5). Epiphytic assemblages had faster species turnover along the environmental gradient than the other 2 habitat assemblages. Epilithon had about the same slope value and beta diversity as epipelon. After 1,000 runs of simulation on 15 randomly selected sets of samples, the chance that epilithon's beta diversity < epiphyton's beta diversity was 0.001, and the probability that epipelon's beta diversity < epiphyte's beta diversity was 0.001 (Figure 3.5A). The frequency distribution of epilithon's beta diversity was about the same as the distribution of epipelon's beta diversity.

When similarity was calculated as average similarity with reference sites, the absolute values of slopes increased (Table 3.5). The results were consistent with pairwise beta diversity outcomes. Epiphytes had the highest slope -0.13 and smallest beta diversity 1.96 among 3 assemblages. Epilithon's beta diversity was close to epipelon's beta diversity. After 1,000 runs of 15 randomly selected samples, the chance that

	Axis 1	Axis 2
% Variance explained	28.69	14.93
NO ₃	0.58*	0.75*
NH ₃	-0.01	0.05
Total N	0.63*	0.72*
Soluble Reactive P	0.11	-0.13
Total P	0.60*	0.16
Si	0.52*	-0.22
Cl	0.54*	-0.36*
Temperature	0.33	-0.50*
Conductivity	0.80*	-0.37*
рН	-0.59*	0.13
Canopy	-0.53*	0.27
Order	-0.63*	-0.03

Table 3.4. Correlations of PCA axes with environmental variables and % variance explained for PCA axes. * indicates statistical significance.



Figure 3.4. A plot of PCA axis 1 vs. axis 2. \bullet represents for epiphytic sites, \bigcirc is for non-epiphytic sites.

	n	β	Intercept	Slope	SE	r ²	р
Pair-wise sim	ilarity						
Epiphyton	105	1.87	0.493	-0.132	0.024	0.215	0.0001
Epilithon	595	6.46	0.31	-0.024	0.007	0.018	0.001
Epipelon	666	6.33	0.481	-0.038	0.005	0.072	0.0001
Similarity with	th referen	ce sites					
Epiphyton	15	1.96	0.342	-0.13	0.039	0.431	0.005
Epilithon	35	4.37	0.291	-0.04	0.014	0.166	0.009
Epipelon	37	4.57	0.46	-0.06	0.013	0.352	0.0001

Table 3.5. Regression statistics for similarity changes along the MEG with pair-wise comparison and similarity with reference sites approaches. Sample sizes (n) were the numbers of possible pair-wise comparison.



Β.



Figure 3.5. The comparison of epiphyton's beta diversity and 1,000 re-sampled beta diversity of epipelon and epilithon with box plots. A. pair-wise beta diversity; B. beta diversity calculated from average similarity with reference approach. \bigcirc represents extreme values, and \bigcirc is for outliers.

epilithon's beta diversity < epiphyton's beta diversity was 0.001, and the probability that epipelon's beta diversity < epiphyte's beta diversity was 0.001 (Figure 3.5B). The frequency distribution of epilithon's beta diversity was about the same as the distribution of epipelon's beta diversity.

The rate of change in MAIA trophic index with the change in the MEG was greater for epiphyton (0.685) than epilithon (0.131) and epipelon (0.130). From 1,000 simulations of epilithon's and epipelon's slopes, no case of epilithon and 3 cases of epipelon had slopes greater than the slope of epiphyton (Figure 3.6). The median slopes of epipelon and epilithon were about the same.

No evidence indicated that nutrients or human disturbance homogenized assemblages from multiple habitats in this study. Similarity in species composition among habitats within each site (inter-habitat diversity) was not significantly correlated with the MEG for both Jaccard and Bray-Curtis indices. Whittaker's β in each site was also not significantly correlated with the MEG. The lowest Whittaker's β was 1.6 and the average among sites was 1.8.

Epipelon and epilithon had about the same number of species recorded from 600 valve counts from 12 sites where all habitats were sampled, 229 and 221 respectively. Epiphyton had the lowest number of species recorded, 138. On average, epipelon had 58.2 species recorded for each site, 42 for epilithon, and the 36.9 for epiphyton.

Discussion



Figure 3.6. A comparison of the slopes of 3 habitat assemblages with box plots. The slopes were derived from the regression between MAIA trophic index and the MEG. Epilithon and epipelon were randomly sampled for 15 sites 1,000 times. \bigcirc represents for outliers.

Streams, just like terrestrial ecosystems, consist of a mosaic of different habitats. Habitats sampled in this study had unique algal assemblages. Epipelon was especially different from epiphyton and epilithon. The unique algal assemblages from different habitats contributed to the overall diversity of stream ecosystems. Diatom assemblages in all 3 habitats were capable of indicating the changes in environmental gradients. However, the beta diversity was not reduced along the MEG.

Epipelon had a quite distinct set of species, while epiphyton and epilithon had more similar species composition. However, Winter and Duthie (2000) showed that epilithon was most distinct from epipelon and epiphyton in a study from southern Ontario. From another study in the Maple River in Michigan, Stevenson and Hashim (1989) found that epipelon deviated from the episammon, epilithon, epiphyton, and plankton aggregate. These different results may reflect stream- and region-specific factors affecting assemblages.

Species have varying degrees of affinity to habitats (Lim et al. 2001), so the affinity species to habitats may not be clear-cut. The significance test of indicator species analysis provides a basis for assessment of habitat affinity. The species in the dominant habitat may affect the abundance of species in other habitats. In this study, the dominance of sand substrata in sampled streams may contribute to the dominance of sand-adapted species in epipelon and epilithon. Cosmopolitan species were also found dominating in all habitats, such as <u>Achnanthidium minutissimum</u>.

Epiphyton had the smallest beta diversity among 3 habitats, which indicated a fast species turnover along the MEG. Epipelon and epilithon had similar species turnover rates in both analyses. The within-site Whittaker's β had a range from 1.62 to 1.95, which

indicated that sampling a single habitat would miss at least 62 % of the total number of species in a site. Therefore, sampling multiple habitats is required to reveal the overall biodiversity in streams. The pattern of beta diversity has seldom been studied for algae in aquatic ecosystems.

The inter-habitat diversity was not reduced along the MEG. The low nutrient levels in sampled streams may not have a significant effect on beta diversity. The ecological pattern of reduction in inter-habitat diversity of algae in streams has not been studied, so there is no basis for comparison.

From both beta diversity calculations, assemblages in all habitats strongly responded to human disturbance as indicated by the MEG, thus all have the potential to indicate water quality. Epiphytic assemblages had the strongest response to the MEG in both CCA variance explained and beta diversity. Epipelon were the 2nd most responsive to the MEG in CCA approach. Winter and Duthie (2000) also found that epipelon and epiphyton were slightly more responsive to the environmental gradient than epilithon in southern Ontario. From littoral habitats of the St. Lawrence River, Reavie and Smol (1997) showed that the inference models from diatom assemblages on <u>Cladophora</u> and macrophytes out-performed the model of epilithon. Rott et al. (1998) indicated that both epilithon and epiphyton responded to water quality, however no comparison was made.

In this study, various aquatic plants and over-hanging grass were collected to sample epiphyton. Despite the effects of different aquatic plants on diatom species composition and dominance (Blindow 1987, Shamsudin and Sleigh 1995), epiphytes responded strongly to the environmental gradient, which was also reported by Winter and Duthie (2000). The small species number in epiphyton did not reduce its sensitivity to the

environmental gradient, and may be controlled by the constant movement in the flow, less refugia on the plant surface, and possible toxicants released by plants (Blindow 1987).

Epipelon grows on deposition zones of rich organic materials and nutrients. The instability of fine substrata and the different chemical environment deter the use of epipelon for biomonitoring. However, epipelon was used to assess water quality change where organic sediments were the dominant substrata in an Argentina study (Gomez 1998). Winter and Duthie (2000) reported that epipelon was more sensitive than epilithon, which was true in our CCA results.

Rocks and other hard surfaces are commonly recommended for diatom sampling to monitor water quality (e.g. Kelly et al. 1998). However, epilithon was not the most sensitive assemblage in this study. Many potential factors may constrain the response of epilithon to environmental variables. Crevices on the rock surface provide a good shelter for live diatoms where the chemical property of the substrata may dominate (Hiebert and Bennett 1992). Rocks, relatively stable substrata, often support dense algal mats, while the chemical environment inside thick algal mats may be different from outside the mats (Stevenson and Glover 1993). In hydrologically stable streams, grazers are capable of clearing overstory algae from the rock surface, and thereby constrain algal species composition to grazer resistant taxa (Jacoby 1987, Steinman 1992). In such a situation, epiphyton or epipelon would deserve more consideration for biomonitoring.

Algae on substrata other than rocks were more sensitive to the environmental change in this study. For many streams in our study region, hard surfaces are not naturally available. Under this situation, Kelly et al. (1998) recommended using introduced
(artificial) substrata for biomonitoring. Natural assemblages, such as epiphyton and epipelon, are sensitive, and require no colonization period, and deserve consideration. When assemblages from multiple habitats were combined for analysis, the sensitivity increased over any one habitat alone (Winter and Duthie 2000). A similar result was also found in a study of algae in Maine wetlands (Wang and Stevenson unpublished results). For assessment purposes, the sensitivities of epiphyton and epipelon need to be established with more studies. The autecology of species in different habitats need to be compared, thus habitat effects can be understood and transfer functions may be developed. Other natural substrata, such as sand, dominant in certain streams, and wood, a stable dominant substratum in certain streams, are worthy of study for bioassessment.

CHAPTER 4 EFFECTS OF SMALL-SCALE NUTRIENT ENRICHMENT ON ALGAL COMMUNITY COMPOSITION IN THE GRAND TRAVERSE BAY WATERSHED

Abstract

The goal of ecological experiments is to understand how natural ecosystems work. Due to problems associated with experiments, such as lack of natural complexity, small spatial-temporal scales, and insufficient experimental duration, extrapolation is required for predicting events at the large scale. To study regional patterns in nutrient limitation, clay pots with nutrient enriched agar were placed in 16 streams for 2 weeks in the Grand Traverse Bay Watershed. Five treatments (N, P, Si, N+P, N+P+Si) and one control were applied. No consistent regional pattern in nutrient limitation was observed for periphyton biomass. However, species composition responded to nutrient enrichment as shown by the significant differences in detrended correspondence analysis (DCA) scores between treatments and controls. The plot of the first 2 axes of DCA also revealed that nutrient enrichments did not change species composition on clay pots similar to those assemblages in high nutrient streams. Community assemblages from treatment clay pots in the same site were more similar than assemblages on clay pots from the same treatments across sites. Comparison between the rankings of species response to nutrient enrichments and rankings of species environmental optima indicated no consistent correlation pattern. Also no correlation was found between rankings of species response to nutrient enrichments and rankings of species environmental optima from another regional survey or species indicator values in van Dam's trophic index. Therefore,

extrapolation of experimental results from small-scale experiments to large regional scales is probably constrained by the local species pools in streams.

Introduction

The ultimate goal of ecological experiments is to understand how the natural world works. Experiments can examine the processes and mechanisms important to the research topic and infer causal relationships. However, the results of experiments may differ when the physical size of experiments changes (Petersen et al. 1999). Field experiments or model ecosystem studies suffer from the problems of scaling-up and artifacts of experimental systems (Petersen et al. 1999, Wiens 2001). Experimental systems often have problems such as no replication for whole ecosystem experiments, lack of natural complexity (Sarnelle 1997), insufficient experiment duration (England and Cooper 2003), and wall effects (Bloesch et al. 1988, Lyche et al. 1996). Hence, the validity of extrapolation of their results to large scales is often in doubt (Carpenter 1996, Schindler 1998).

Maintaining the regional patterns of nutrient limitation may protect ecological integrity and biodiversity. Within a region, the understanding of nutrient limitation may help control nuisance algal blooms (Conley 1999). Nutrient-diffusing substrate (NDS) experiments often are short-term and smaller than 1 m² in spatial scale, and have been widely applied to evaluate the limiting factor for algal growth (Fairchild et al. 1985, Carrick et al. 1988, Wold and Hershey 1999, Scrimgeour and Chambers 2000). A recent review of NDS experiments showed that both N and P were of the same importance in

limiting algal growth in streams (Francoeur 2001). The pattern of regional nutrient limitation is often controlled by biogeochemistry. Nitrogen limitation is common in the Western United States (Grimm and Fisher 1986, Hill and Knight 1988). In Michigan, streams were found P limited (Pringle and Bowers 1984, Fairchild et al. 1985, Burton et al. 1991). In contrast, two regional studies found that there was no uniform regional pattern in nutrient limitation (Wold and Hershey 1999, Snyder et al. 2002).

Most studies reported only responses of algal chlorophyll to nutrient enrichment (Lohman et al. 1991, Chessman et al. 1992, Scrimgeour and Chambers 2000, Mosisch et al. 2001, Dodds et al. 2002). Algal species have different and sensitive responses to different nutrients (Fairchild et al. 1985, Carrick et al. 1988, Keithan et al. 1988, Marks and Lowe 1993). The increase in total biomass due to a specific nutrient does not indicate the responses for all algal species. Only few studies described responses of algal species composition to nutrient amendments (Pringle and Bowers 1984, Fairchild et al. 1985, Carrick et al. 1988, Marks and Lowe 1993). Shifts in species composition are probably more sensitive to nutrient enrichment than changes in biomass, especially when biomass may be regulated by grazers or physical disturbances.

The responses of the algal community to NDS experiments have not been compared to algal assemblages in nutrient enriched streams at the regional scale. If small-scale nutrient enrichment experiments can change community structure on clay pots similar to those in high nutrient streams, no extrapolation is needed for predicting large-scale events from small-scale experiments. Alternatively, if the algal assemblages on nutrient enriched clay pots are more similar to assemblages on control clay pots than those in high nutrient streams, the validity of small-scale experiments for inferring nutrient effects on

community structure is in question and the design of experiments may need to be modified. Species responding to nutrient enrichments at the experimental scale may not respond to nutrient enrichments at the regional scale because of constraints on immigration of species adapted to exploit high levels of nutrients. The comparison of species responses from experimental and regional scales may illuminate linkages between community structure and function across scales.

This study evaluates the pattern of regional nutrient limitation for the Grand Traverse Bay Watershed (GTBW) where sandy substrata, high grazing pressure, and low nutrient levels make this evaluation challenging. NDS were used to determine whether N, P, or Si limits algal biomass accrual and regulates species composition. I also evaluated whether nutrient enrichment can change diatom assemblages similar to those in high nutrient streams. Responses to nutrient enrichment by individual species were assessed, and then the responses of species to small-scale experimental nutrient enrichment were compared to their responses to nutrient availability at the regional scale.

Materials and Methods

Study Area

GTBW, with an area of approximately 2,600 km², is located in the northwestern part of the lower Michigan peninsula (Figure 4.1). It has one of the fastest growing populations in the Midwest United States. Traverse City, with a 500,000 summer recreation population, is the largest city in the watershed. Land use in GTBW is composed of 49% forest, 20% agricultural, 15% herbaceous/shrub/grasslands, 10% water, and 6% urban. The Boardman River drains the majority of the land of GTBW.

Sampling and Processing

Sixteen sites were selected for nutrient enrichment experiments with nutrientdiffusing clay pots, which had a sampling area of 75.6 cm² and an internal volume of 100 ml. Clay pots were soaked in deionized water for at least 24 hours to wash off potential toxic contaminants. A plastic petri dish was stuck to the mouth of each pot with silicon glue. Five types of enriched agar were made: N (NaNO₃, 1 mol/L), P (K₂HPO₄, 1mol/L), Si (SiO₂, 1 mol/L), N + P (NaNO₃, 1 mol/L; K₂HPO₄, 1mol/L), and N + P + Si (NaNO₃, 1 mol/L; K₂HPO₄, 1mol/L; SiO₂, 1 mol/L). Chemicals were dissolved in 2 % hot agar, then poured into clay pots. Each clay pot was sealed with a rubber stopper (size 000). Control pots were filled with pure agar. For each stream, 2 clay pots were made for each treatment and control. Four randomly selected clay pots were glued to a tile to anchor the pots into streams. Clay pots were kept moist before placement in streams.

Clay pots were placed in streams on 28 and 29 September 2000 and collected 14 days later. The material on clay pots from the 2 pots for each treatment was scraped off with a toothbrush, stored in a whirl-pac plastic bag, and kept on ice. Canopy cover above clay pots was estimated with a densiometer. Current velocities were measured with an electronic portable flowmeter (Flo-mate model 2000, Marsh-McBirney Inc.). Conductivity, water temperature, and pH were also measured in the field (AP50, Denver Instrument).

Water chemistry was analyzed with a Technicon Auto-analyzer III by following standard methods (APHA 1998). NH₃-N was analyzed with an automated phenate method, NO₃-N with an automated Cd-Reduction method, and total N (TN) was digested with persulfate before using the nitrate method. Soluble reactive phosphorus (SRP) was



Figure 4.1. Site locations in the Grand Traverse Bay Watersheds, Michigan.

measured with the automated ascorbic acid method, while total P (TP) was measured using the SRP method after persulfate digestion and the sample was not filtered. Cl⁻ was measured with the automated ascorbic acid method, while total P (TP) was measured using the SRP method after persulfate digestion and the sample was not filtered. Cl⁻ was measured by the automated ferricyanide method, while SiO₂-Si was measured by an automated method for molybdate-reactive Silica.

Algal samples were subsampled in the lab for chlorophyll analysis, diatom enumeration, and soft algae enumeration. Chlorophyll *a* (Chl *a*) was extracted by dissolving the filter membrane and algal pigments in 90 % aqueous acetone and measured following standard methods (APHA 1998) with a spectrophotometer (Spectro Instrument). Chl *a* concentration was calculated as mg/cm². Diatoms were digested with the boiling nitric acid method, and catalyzed with potassium dichromate. Diatoms were identified primarily based on Krammer and Lang-Bertalot (1986, 1988, 1991a, 1991b), and Patrick and Reimer (1966, 1967) were used as supplements. Diatoms were identified to the lowest level possible, often species.

The regional nutrient limitation pattern was evaluated for algal biomass, measured as Chl *a*, by the relation between a nutrient limitation index and stream nutrient concentrations, nutrient ratios, surrounding land uses, current velocity, and canopy cover from 16 sites. The nutrient limitation index was calculated as the ratio of the difference between the Chl *a* level of a treatment and the Chl *a* of the control divided by the Chl *a* of the control. To test the hypothesis that streams in the GTBW were generally limited by one nutrient or another, the mean value of the nutrient limitation index for each treatment was compared to 0 with a one-sample t test. To test the hypothesis that nutrient limitation

was related to nutrients and other environmental factors, the relationships among nutrient limitation indices for 5 treatments and nutrients, nutrient ratios, flow, and canopy were evaluated by Pearson correlation analysis. Si/DIN (NO₃ +NH₃) and TN/TP were calculated as molar ratios. I assumed that N:P > 23 indicates P limitation, while both Si:P < 16 and Si:N < 1 indicate Si limitation (Redfield 1958).

Detrended correspondence analysis (DCA) was used to determine whether nutrient enrichment with NDS can change diatom species composition in a direction similar to assemblages in high nutrient streams. Relative abundances of species were used for analysis after log-transformation. The correlations between the first 2 DCA axes and environmental variables were analyzed with the Pearson correlation analysis. To evaluate the relationship between changes in species composition and treatment effects, the mean difference of the first 2 DCA axes scores between a treatment and the control was compared with zero by a 2-tailed one-sample t test.

To evaluate the response of species at the experimental scale, the species response index was calculated by dividing the difference in species relative abundance between a treatment and the control with relative abundance in the control. The absolute values of this indicator were log-transformed before statistical analysis to meet the assumption of parametric statistics and control for potential overestimation of common species responses. The relative abundance of species not found in 600 valve-counts were substituted by 1/1,200, assuming that species were in low abundance and only 1valve could be found in 1,200 valve-count. In the one-sample t test, the mean response of a species from 16 clay pots was compared to zero. The mean value of species response index was used to rank the strength of species response.

To assess the responses of species at regional scale, species appearing in > 3 sites on control clay pots were chosen for weighted average calculation. TN, TP, Si, and Cl concentrations were selected for analysis. Cl level has been used as an indicator for human disturbance (Herlihy et al. 1998) and was used as a surrogate for trophic state in this study. Species optima for each specific nutrient were then ranked among species.

The patterns of species response at experimental and regional scales were compared. The ranks of species responses at both scales were evaluated with the Pearson correlation analysis to see whether species with strong responses at the experimental scale also had high ranks at the regional scale. The ranks of species responses to N, P, and Si treatments were compared to the ranks of species TN, TP, and Si optima respectively. The ranks of species responses to N+P and N+P+Si treatments were compared to the ranks of species Cl optima. The top 10 ranked species from each treatment were examined for differential responses between experimental and regional scales. Species responses at the experimental scale were also compared with the ranks of species Cl optima from another regional survey, in which epilithon was sampled from 31 sites in GTBW. The correlation between species ranks in N+P and N+P+Si treatments at experimental scale and species indicator categories in van Dam's trophic index (van Dam et al. 1994) was evaluated. van Dam's indices for taxa were developed at a global geographical scale and therefore represent a categorization of species at a broad scale.

Results

Generally, sampling sites had very low phosphorus and higher nitrogen

concentrations (Table 4.1). The maximum TN was 1.46 mg/L, whereas the minimum TN was 0.08 mg/L. Ammonium levels were relatively low compared to nitrate levels. NO₃-N ranged from 0.01 to 1.18 mg/L, while NH₃-N varied from non-detectable to 0.03 mg/L. The maximum TP was very low (2.4 μ g/L), while the minimum was close 0 μ g/L. pH ranged from 7.7 to 8.3, while conductivity spanned from 253 through 587 μ s/cm. Stream order spanned from 1 to 4. Only 3 sites had canopy cover < 50%. Four sites had about 50% agricultural land in the watershed, while urban development in watersheds was below 15%.

The NDS failed to show evidence for nutrient limitation of algal biomass accrual. The Chl a on control clay pots had a range of 0 to 524.4 μ g/cm². The means of nutrient limitation indices from all 5 treatments were not significantly different from 0 (Figure 4.2). No regional pattern in nutrient limitation was observed based on algal biomass. However, based on water chemistry, all sites should be P limited because all DIN/SRP ratios were > 30 and TN/TP ratios > 138. The nutrient limitation indices were not correlated with any nutrient, land use types, canopy, flow, or other environmental variables.

A plot of the first 2 axes of DCA showed that changes in species composition of low nutrient streams caused by NDS nutrient enrichment did not resemble assemblages in high nutrient streams (Figure 4.3). Species composition on all clay pots from the same site was more similar to each other than to assemblages on clay pots from the same treatments across sites. The 1st DCA axis was significantly correlated with NO₃, NH₃,

Variables	Minimum Maximum				
			25th	50th	75th
% Urban	0	15	0	2	8
% Agriculture	0	64	3	16	45
% Forest	15	93	25	53	79
% Wetlands	0	27	1	2	5
Conductivity (μ S/cm)	253.0	587.0	308.3	345.5	467.3
рН	7.7	8.3	8.1	8.2	8.2
Water Temp (°C)	7.9	13.0	9.2	10.5	11.2
Canopy	0.5	73.0	7.1	18.0	48.7
Stream Order	1	4	1	1.5	2
$NO_3^{-}N$ (mg/L)	0.01	1.18	0.06	0.14	0.51
NH_4^+ -N (mg/L)	N.D.	0.03	0.00	0.01	0.01
TN (mg/L)	0.08	1.46	0.19	0.27	0.91
SRP (ug/L)	0.8	1.7	0.8	1.0	1.2
TP (ug/L)	0.3	2.4	0.9	1.2	1.5
Si (mg/L)	7.7	17.2	8.3	9.0	9.6
Cl (mg/L)	1.2	21.4	3.2	5.1	10.1
TN/TP	138	3592	311	674	2305
DIN/SRP	32	3103	100	330	1147
Si/DIN	4	344	10	32	62

Table 4.1. The summary statistics for land use, environmental variables, and nutrient ratios. N.D. is short for non-detectable.



Figure 4.2. Box plots of nutrient limitation indices. \bullet represents outliers, while \circ represents extreme values.

and TN (Table 4.2). The 2nd axis was positively correlated with SRP, but negatively correlated with % forest. Therefore, nutrients affected species composition more on a regional scale among streams than among experimentally nutrient enriched treatments within each stream.

Species composition of diatom assemblages did, however, respond consistently to experimental nutrient enrichment even though they did not correspond well to regional relationships between biomass, species composition, and nutrient enrichments. Comparison between species composition in treatments and controls on the 1st DCA axis showed that the differences between N, P, NP, and NPSi versus controls had means significantly > 0 (Table 4.3). On the 2nd DCA axis, the difference between Si, P, NP, and NPSi versus controls had means significantly > 0.

One-sample t tests showed that the relative abundances of 13 species increased compared to controls in N treatments (Table 4.4). However, <u>Nav. veneta</u> decreased in N treatments. Eighteen species increased their relative abundances compared to controls in P treatments, while 9 species increased their relative abundance compared to controls in Si treatments. Six species decreased from control clay pots in Si treatments. In NP treatments, 20 species had increases in relative abundances, while <u>Cyc. distinguenda</u>, <u>Fra. delicatissima</u>, and <u>Fra. nanana</u> decreased in their relative abundances. In NPSi treatments, 13 species increased their relative abundance except <u>Amp. libyca</u>, which decreased its relative abundance compared to controls.

Species responding to nutrient enrichment experiments did not respond to nutrient enrichment at the regional scale. No correlation was found between ranks of species



Figure 4.3. A plot of the first 2 DCA axes scores of all clay pots. Symbols represent different sites. The first DCA axis was positively correlated with NO_3^- and TN, and negatively correlated with NH₃. The second DCA axis was positively correlated with SRP and negatively with % forest.

Correlated environmental variables					
DCA axis 1	NO ₃	NH ₃	TN		
г	0.71**	-0.70**	0.54*		
DCA axis 2	% Forest	SRP			
r	-0.51*	0.50*			

Table 4.2. Correlation between DCA axes of clay pot diatom species and environmental variables. r is Pearson correlation coefficient. DCA1 is the short for DCA 1st axis; DCA2 for DCA 2nd axis. * indicates 0.01 ; ** indicates <math>0.001 .

	t	Mean Difference	p (1-tailed)
DCA axis 1			
N – C	1.98	0.06	0.04 *
NP – C	2.74	0.14	0.007 *
NPS – C	1.85	0.10	0.03 *
P – C	2.27	0.09	0.01 *
S – C	0.99	0.05	0.17
DCA axis 2			
N – C	1.47	0.04	0.07
NP – C	2.47	0.10	0.01 *
NPS – C	2.82	0.19	0.008 *
P – C	1.79	0.07	0.03 *
S – C	4.03	0.17	0.0001 *

Table 4.3. The comparison of the difference between a treatment and the control to 0 on 2 DCA axes with t test.

Species	N	Р	Si	NP	NPSi
Achnanthes conspicua A. Mayer	*		*	-	
Ach. exigua Grun.		*	*		
Ach. laevis Schimanski				*	
Ach. lauenburgiana Hust.		*			*
Ach. nollii Bock					*
Amphora libyca Ehr.					_*
Amp. pediculus (Kutz.) Grun.			*		
Cocconeis neothumensis Kram. Coc. placentula var. euglypta (Ehr.) Cl.		*	*		
Coc. placentula var. lineata (Ehr.) V. H.	**	*	*		
Cyclotella distinguenda Hust.				_*	
Cyc. meneghiniana Kutz.	*	*		*	
Diploneis oblongella (Naeg. ex Kutz.) Ross	*				*
Eunotia bilunaris (Ehr.) Mills	*			*	
Fragilaria capucina var. rumpens		*		*	
(Kutz.) Lange-Bert. Fra. capucina var. vaucheriae (Kutz.) Lange-Bert.	*				
Fra. construens f. venter (Ehr).			*		
Grun. Fra. delicatissima (W. Sm.) Lange- Bert				_*	
Fra. leptostauron var. dubia (Grun.) Hust.			_*		
Fra. nanana Lange-Bert.	*			_*	
Gomphonema acuminatum Ehr.				*	*
Gom. angustatum (Kutz.) Rabh.				*	
Gom. clavatum Ehr.		*	*		
Gom. micropus Kutzing		*		**	*
Gom. parvulum Kutz.	*	*			*
Navicula capitatoradiata Germain		*			
Nav. costulata Grun.		*			**
Nav. cryptocephala Kutz.	*			**	

Table 4.4. The responses of species to nutrient enrichment treatments compared to control clay pot. T-tests were performed on the relative abundance difference between treatments and the control. * indicates p < 0.05 and ** represents p < 0.01.

Table 4.4. Continued.

Species	N	Р	Si	NP	NPSi
Nav. gregaria Donk.			_*		
Nav. menisculus var. grunowii				*	*
Lange-Bert.					
Nav. radiosa Kutz.		*			
Nav. reichardtiana Grun.			_*	*	
Nav. seminulum Grun.		*		*	*
Nav. subrotundata Hust.	*				
Nav. tripunctata (O. F. Mull.) Bory		*			*
Nav. veneta Kutz.	_**				
Nitzschia dissipata (Kutz.) Grun.		*		*	
Nit. gracilis Hantz. ex Rabh.		*			*
Nit. incognita Krasske	*			*	
Nit. linearis (Ag. ex W. Sm.) W.			_**		
Sm.	44			-1-	.1.
Nit. palea (Kutz.) W. Sm.	*			*	*
Nit. palea var. debilis (Kutz.) Grun.		*		**	
Nit. pura Hust.	*				
Nit. recta Hantz. ex Rabh.		*		*	
Planothidium lanceolata var. dubia				*	*
(Grun.) Lange-Bert.					
Pla. peragalli Brun et Herib.			*		
Reimeria sinuata (Greg.) Kocio. &			*	*	
Stoer.					
Stauroneis smithii Kram.				*	
Surirella angusta W. Smith				*	
Synedra ulna W. Sm.			_*		

response from treatments and ranks of regional species environmental optima (Figure 4.4). No correlation was observed between ranks of species response from treatments and the ranks of species Cl optima from another regional survey (Figure 4.5) and van Dam's species classification (Figure 4.6).

From those species ranked in the top 10 from each treatment, 3 species ranked about the same at both experimental and regional scales: <u>Nav. radiosa</u>, <u>Nav. costulata</u>, and <u>Nit.</u> <u>sociabilis</u>. Eight species had ranks at the experimental scale close to ranks at the regional scale (within 5 ranking difference): <u>Ach. conspicua</u>, <u>Ach. lauenburgiana</u>, <u>Ach. exigua</u>, <u>Cym. microcephala</u>, <u>Nav. cryptocephala</u>, <u>Nit. recta</u>, <u>Nit. dissipata</u>, and <u>Pla. lanceolata</u> var. <u>dubia</u>. All other species ranked much lower at the regional scale than at the experimental scale.

Discussion

The N:P ratios and low P concentrations in stream water showed a regional pattern of P limitation in the GTBW, but this was not confirmed with NDS experiments. Our results showed no correlation between nutrient limitation indices and canopy cover or flow. Short experimental periods, low colonization rates, and high grazing may have limited algal accumulation and manifestation of nutrient limitation with NDS. Spatial heterogeneity of nutrient limitation has been observed in Minnesota streams (Wold and Hershey 1999), Alberta streams (Scrimgeour and Kendall 2002), and Idaho rivers (Snyder et al. 2002). Spatial heterogeneity of nutrient limitation may come from heterogeneity of biogeochemistry, point sources, and non-point sources. Anthropogenic



Ranks of species response from N treatments



Ranks of species response to P treatments



Ranks of species response to Si treatments



Ranks of species response in NP treatments



Ranks of species response to NPSi treatment

Figure 4.4. Relationship between species environmental optima and species responses to treatments. The lines indicate 1 to 1 ratio.



Ranks of species response in NP treatments

Β.



Ranks of species response in NPSi treatments

Figure 4.5. The relationship between ranks of species Cl optima from a regional survey with A. ranks of species response to the NP treatment, B. ranks of species response to the NPSi treatment. The lines indicate 1 to 1 ratio.



Ranks of species response in NP treatments



Α.



Ranks of species response in NPSi treatments

Figure 4.6. The relationship between van Dam's category and A. ranks of species in NP treatments, and B. ranks of species in NPSi treatments.

inputs of nutrients can change nutrient ratios in streams (Vitousek et al. 1997). Arbuckle and Downing (2001) found that pasture land led to a low N:P ratio and row-crop agriculture contributed to a high N:P ratio. Scrimgeour and Chambers (2000) also showed that point-source inputs could change nutrient limitation patterns. Other factors, such as light (Hill and Knight 1988, Steinman et al. 1989) and grazers (Rosemond et al. 1993), can regulate algal growth rates.

Species composition in short-term nutrient enrichments did not resemble communities in nutrient-enriched streams. Diatom assemblages from treatment clay pots tended to cluster more by site than by treatment. Synder et al. (2002) found similar results and showed that assemblages in treatments were closer together within the same watersheds than within the same treatments. Hence, they suggested that large-scale factors are more important in determining the potential diatom assemblage than the small-scale, proximate nutrient enrichment provided by NDS. Kohler and Wiley (1997) found that the changes of community composition in microcosm experiments, where the caddisfly <u>Glossosoma</u> was manually removed, were different from the changes in community structure when <u>Glossosoma</u> was naturally wiped out by parasites. Brown et al. (2001) also found that short-term observation or experiments did not detect complex dynamics of rodent populations found by long-term study. Therefore, the results of smallscale short-term experiments may not be extrapolated to natural ecosystems.

Most species that responded to nutrient enrichments at the experimental scale did not respond to nutrient enrichment at the regional scale. However, diatom species did respond consistently across the region to nutrient enrichments with consistent changes in DCA scores when comparing treatment to control NDS assemblages. Such subtle

responses may only have validity at the experimental scale, and not at the regional scale. This scale effect is apparent in the DCA plot where diatom assemblages from treatment clay pots tended to cluster by site than by treatment.

I also found little similarity between species responses on NDS and species responses to nutrients reported in the literature. Autecological profiles provide responses of species at the regional scale. Therefore, indices, such as the trophic state index, may serve as references for predicting ecological changes imposed by human disturbance or climate change. However, disagreement among indices in different literature sources impeded their applicability to my results. Among those species ranked closely at both scales, all had disagreement in their trophic-state associations in the literature. Ach. exigua and Nav. cryptocephala were ranked as widely tolerant by van Dam, eutrophic by Lange-Bertalot, but sensitive by Bahls. <u>Nav. cryptocephala</u> was also ranked as eutrophic by Lowe (1974). Ach. conspicua and Nit. recta were categorized as widely tolerant in both van Dam's and Lange-Bertalot's trophic indices, but were classified as sensitive by Bahls. Ach. lauenburgiana was classified as oligotrophic by van Dam and Bahls, but eutrophic by Lange-Bertalot. Cym. microcephala was categorized as mesotrophic (less tolerant) by van Dam and Bahls, but as wide tolerant by Lange-Bertalot; while Nit. dissipata was classified as eutrophic by both Lowe and Lange-Bertalot, mesotrophic by van Dam, and sensitive by Bahls. Among 3 species ranked about the same at both scales, disagreement on their autecology persisted in the literature. Nit. sociabilis was classified as a eutrophic species by van Dam et al. (1994), widely tolerant by Lange-Bertalot (1995), and less tolerant by Bahls (1993). While Nay, radiosa was classified as mesotrophic by

van Dam and eutrophic by Lange-Bertalot, it was categorized as oligotrophic (sensitive) by Bahls. <u>Nav. costulata</u> was classified as less tolerant by Bahls.

Though Bahls' diatom tolerance index was developed in the U.S. and has an extensive list of species found in the U.S., it had little agreement with species autecology found in this study. Despite accepted applicability of van Dam's indices, they have been criticized for not checking the validity of indices when applied in different regions. Differences in species autecology among regions may arise due to biogeographic variability among populations. Therefore, species autecology profiles and indices developed from a region may be required to appropriately compare species responses to environmental change in experiments and across regions.

The design of experiments and extrapolation of experimental results may need to consider factors associated with the species pools involved and regional heterogeneity (Englund and Cooper 2003). The lack of resemblance in species composition at experimental and regional scales is probably because the local species pool controls the community structure of short-term small-scale experiments. In microcosm or clay pot experiments, colonized species are from the local species or supplied species pool. Without enough time and the release of competition for species colonization from other species pools, the end community structure is decided by the local species pool or supplied species pool.

Community structure changes when disturbance occurs. The local community structure is controlled by species pools from both local and regional scales. The regional species pool changes the community structure when a press perturbation occurs over a large spatial-temporal extent. In a stream, the local community is colonized by the local

species pool drifting from upstream. Species from a local species pool are adapted to the local in-stream environment and can out-compete species from the regional species pool that are colonizing through aerial dispersal or introduction by animals. When a large area is affected by a press perturbation, invasive species from the regional species pool may out-compete the local species, change the community structure, and become established in the local species pool (Byers 2002). For example, phosphorus enrichment due to the change of land use shifted the Florida Everglades from a sawgrass dominated ecosystem to a cattail dominated marsh (Newman et al. 1998). Predator addition enabled invasive zooplankton species to establish in experimental ponds (Shurin 2001).

Only with the knowledge of the species pool involved can we predict the expected change of the impacted ecosystems (*sensu* Kolar and Lodge 2002). Through understanding the autecology of species involved, we may more precisely predict the magnitude and outcome of impact on ecosystem structure and function from a specific event. Uncertainty is much higher when inferring the structural and functional changes of ecosystems without knowing species involved. Therefore, small-scale experiments need to include the regional species pool, instead of only the local species pool, to extrapolate to real ecosystems and over large spatial and temporal scales.

CHAPTER 5 DEVELOPMENT AND EVALUATION OF A DIATOM-BASED INDEX OF BIOTIC INTEGRITY FOR THE INTERIOR PLATEAU ECOREGION

Abstract

Environmental assessments should measure valued ecological attributes, stressors that likely affect valued ecological attributes, and human activities that produce stressors. Many modern diatom indicators used in bioassessments infer abiotic conditions (such as nutrient concentration, pH, or salinity), which are usually defined as stressors. In this research I developed a diatom-based Index of Biotic Integrity (IBI) for the Interior Plateau Ecoregion (IPE) of the US that more closely assesses effects of human disturbance on the biotic condition of periphyton, a valued ecological attribute. Benthic diatoms were collected and characterized by the Kentucky Division of Water. Diatom attributes at reference and impacted sites were selected for the IBI based on significant differences between site groups using the Mann Whitney-U test, high separation power, and low CV. The 7 metrics selected from a list of 59 attributes tested were the Kentucky (KY) Diatom Tolerance Index, % species characterized as sensitive in KY, number of distinct reference species, average similarity of species composition with reference sites, % Achnanthes / (% Achnanthes + % Navicula), % Cymbella species of all species, and % Navicula species of all species. IBIs were calculated by summing metrics for a site after transforming metrics to a discrete 1, 3, 5 scale and a continuous 0-10 scale. Both scaling systems successfully separated reference and impaired sites and IBI scores were significantly related to measures of human disturbance in watersheds. The diatom IBI was tested using a second dataset collected from IPE streams and processed using slightly different methods and taxonomy. Sites were again classified as reference or impaired

sites based on the same criteria for the development dataset. Diatom IBI scores differed significantly between reference and impaired streams in the second dataset and they classified 80 % of these sites correctly. Compared to other diatom IBIs, this IBI had more separation power than the others. Thus diatom indicators of ecological condition can provide valuable characterizations of biotic condition and human disturbance in stream ecosystems.

Introduction

According to a report by the United States Environmental Protection Agency (USEPA 1998), 40% of streams and rivers surveyed by states in 1996 were threatened or polluted. Agriculture, modification of flow regimes, urban runoff, and storm sewers were identified as primary sources of pollution (USEPA 1998), and all of these are known to have significant effects on stream ecosystem structure and function (Karr and Chu 1999, Carpenter et al. 1998, Mac et al. 1998). Pressure on aquatic resources through water use and habitat alteration is expected to increase due to increasing population (Mac et al. 1998). To maintain sustainable ecosystems, effective methods of assessing the health of our stream ecosystems are urgently needed.

Indices of biotic integrity (IBI) have been developed to detect structural and functional differences in valued ecological attributes between a test community and the reference condition (*sensu* Cairns 1977, Karr and Dudley 1981, Barbour et al. 1999, Stevenson and Hauer 2002). These valued ecological attributes are important for establishing criteria to protect and restore ecosystems. IBIs usually are composed of structural attributes that respond to human disturbance (i.e., metrics), such as diversity indices, relative abundance of species, and species in taxonomic or functional groups that are sensitive to pollution (Barbour et al. 1999). This multimetric approach is statistically effective for evaluating human disturbance (Fore et al. 1994) and has been used widely for assessing and communicating biotic integrity of streams across the US (Barbour et al. 1996, Barbour et al. 1999).

Diatoms have a long history of use for environmental monitoring in Europe, North America, and to some extent worldwide (Stevenson and Smol 2002), but development and use of diatom-based IBIs are relatively new and restricted to the US (Bahls 1993, KDOW 1993, Hill et al. 2000). Bahls (1993) used the sum or minimum of 4 metrics to evaluate sites. The Kentucky Division of Water used 5 metrics to assess biotic integrity (KDOW 1993). Hill et al. (2000) selected 10 periphyton metrics and demonstrated their relationship to environmental variables.

Identifying and solving environmental problems requires assessing valued ecological attributes such as biotic condition as III as the stressors and human activities causing stressors. Most diatom indicators, used both individually and in an IBI, have been developed to indicate stressors in aquatic ecosystems, such as trophic status (Pan et al. 1996), salinity (Blinn 1993), pH (Dixit et al. 1999), and climate change (Pienitz and Vincent 2000). Bioindicators of stressor conditions are valuable complements to measuring abiotic stressors (Stevenson and Smol 2002), however emphasis should also be placed on developing diatom indicators of biotic condition to help assess, protect, and restore valued ecological attributes.

The objectives of this study were to develop and test diatom indicators of biotic condition and a multimetric diatom IBI. I tested the hypothesis that diatom attributes reflecting elements of biotic condition were capable of separating streams with and without extensive human activity in watersheds using a dataset from the Interior Plateau Ecoregion (IPE) collected by the Kentucky Division of Water (KDOW). I selected a set of diatom metrics for a multimetric IBI and tested the capability of the IBI to detect effects of human disturbance with the KDOW dataset, as is usually done in IBI assessments. To test the diatom IBI more rigorously, I then compared diatom IBI values from reference and disturbed streams with a different dataset from the same ecoregion, but collected and analyzed for another project using slightly different methods. Finally, I compared the performance of existing periphyton IBIs with the test dataset.

Materials and Methods

IBI development dataset

The dataset used for developing the diatom-based IBI was generated by the biological sampling program of the KDOW. Data from 19 impaired and 7 reference sites were selected from the IPE (Figure 5.1). Land use in the IPE was dominated by cropland and pasture with some woodland and forest (USEPA 1997). The geology of the IPE is mostly composed of limestone with some sandstone and shale distributed in the south and West part of the region (McDoIII 1986). Most soils in the region have formed from limestones (Bailey and Winsor 1964). Mean annual rainfall ranges from 127 cm in the



Figure 5.1. Study area and locations of sampling sites in Kentucky (USA) and the Interior Plateau Ecoregion. \bullet indicates sites sampled in development dataset, while \circ represents sites sampled in test dataset.

south to 102 cm in the north; whereas mean annual average temperature is 14 °C (USDA 1938).

KDOW data collected between 1992 and 1995 and between 20 April and 15 June were chosen for analysis because of data availability and because spring is one of the most biologically diverse seasons in Kentucky streams. Diatoms were collected qualitatively by scraping algae off rocks with a toothbrush. The diatoms were acidcleaned, identified based mainly on Patrick and Reimer (1966, 1975), and 500 valves were counted for each sample. Since multiple samples were collected from the same sites during this period, one sample from each site was selected randomly for the dataset used to develop the IBI.

Streams were designated as reference and impacted sites based on criteria in Mills et al. (1997). Reference sites were initially selected based upon the past records of an undisturbed riparian zone, absence of towns or communities along the stream bank, absence of resource extraction activities in the watershed, absence of hydrologic modification in the watershed, and absence of major sewage treatment dischargers. The second phase of site selection was based on cross-reference with past state biological and chemical data. The reference sites were confirmed by field reconnaissance by KDOW staff. Additional criteria based on land use were added: > 50 % forest in the watershed and within 100-meter buffer, and < 20 % of agriculture, urban and suburban combined in the watershed. All other sites were classified as impacted sites.

Attributes Tested

The development of the diatom IBI was based on the recommended methods in USEPA rapid bioassessment protocols (Barbour et al. 1999) with minor modifications.

First, a large pool of diatom attributes (potential metrics) was compiled because relatively few attributes have been evaluated in literature. Attributes were classified into 7 categories of indicators (Table 5.1): biotic indices, diversity indices, growth form, sensitive species, tolerant species, similarity, and community structure. As described in detail later, potential metrics were selected from a pool of biotic community attributes when they were related to human disturbance (Karr and Chu 1999), as indicated by Mann-Whitney U tests (Mann and Whitney 1947). Final metrics were selected for high separation power and low coefficient of variation (CV).

The first class of attributes evaluated, biotic indices, was calculated based on the relative abundances and autecological values of species. Species autecological characteristics were determined by review of literature documenting habitat characteristics in which taxa were most abundant (LoI 1974, KDOW 1993, Bahls 1993). The Kentucky Diatom Pollution Tolerance Index (KYDPTI) (KDOW 1993) and the Montana Diatom Pollution Index (MTDPI) (Bahls 1993), 2 indices commonly used in the US (Stevenson and Pan 1999), are expected to decrease with impairment. They are the sum of products of species pollution tolerance values (PTVs) (θ_i) and their proportional relative abundance (p_i) for all species ($\sum p_i \theta_i$). These values are available from the authors upon request.

In the diversity index category, I tested the Shannon diversity index (Shannon and Weaver 1949), the evenness index (Pielou 1966), species richness, and the number of diatom genera. Species diversity and evenness was hypothesized to decrease with human disturbance (Sheehan 1984, Odum 1985).

Metric	Taxonomic level	Description	R
Biotic Index			
Kentucky Diatom Pollution	Species	Weighted average of average abundance	
Tolerance Index (KYDPTI)	-	and tolerance value	
Montana Diatom Pollution	Species	Weighted average of relative abundance	_
Index (MTDPI)	-	and tolerance value	
Diversity Index			
Shannon Diversity Index	Species	A measure of evenness and richness	
Evenness Index	Species	A measure of species composition evenness	_
Species Richness	Species	Number of species in the count	—
No. of Genera	Genus	Number of Genera	
Growth Form			
% Prostrate individuals	Genus	Relative abundance of prostrate genera	+
% Erect individuals	Genus	Relative abundance of erect genera	_
% Stalked individuals	Genus	Relative abundance of stalked genera	V
% Unattached individuals	Genus	Relative abundance of unattached genera	+
% Motile individuals	Genus	Relative abundance of motile genera	+
Sensitive Species			
KY % sensitive species	Species	Relative abundance of KYDPTI sensitive species	-
KY no. of sensitive species	Species	Number of KYDPTI sensitive species	_
KY % no. of sensitive species	Species	Percent of number of sensitive species to	_
		the total number of species in the count	
MO % sensitive individuals	Species	Relative abundance of MODPI sensitive species	
MO no. of sensitive species	Species	Number of MODPI sensitive species	
MO % no. of sensitive species	Species	Percent of number of sensitive species to	_
		the total number of species in the count	
No. of distinct reference species	Species	No. of species found primarily in reference sites	—
Tolerant Species			
KY % tolerant individuals	Species	Relative abundance of KYDPTI tolerant species	+
KY no. of tolerant species	Species	Number of KYDPTI tolerant species	+
KY % no. of tolerant species	Species	Percent of number of tolerant species to	+
•	•	the total number of species in the count	
MO % tolerant individuals	Species	Relative abundance of MODPI tolerant	+
	-	species	
MO no. of tolerant species	Species	Number of MODPI tolerant species	+
MO % no. of tolerant species	Species	Percent of number of tolerant species to	+
-	-	the total number of species in the count	

Table 5.1. Algal population and community attributes, their descriptions, and their expected response to impairment. + represents attribute increasing with impairment, - represents attribute decreasing with impairment, and V represents variable.

Table J.T. Communucu

Metric	Taxonomic level	Description	R
Similarity			
Average similarity with	Species	Average of Jaccard similarity to all	_
reference sites		reference sites	
% reference species	Species	Percent of species found in reference sites	_
No. of distinct reference species	Species	No. of species found primarily in reference sites, not in impaired sites	_
Community Composition		, ,	
% Achnanthes minutissima	Species	Relative abundance of Achnanthes	_
individuals	-	minutissima	
% Achnanthes/	Genus	The ratio of Achnantes to Achnanthes and	
(Achnanthes+Navicula)		Navicula combined	
% Cymbellal	Genus	The ratio of Cymbella to Navicula	—
(Cymbella+Navicula)		and Cymbella combined	
% Dominant species	Species	Relative abundance of dominant species	+
% Achnanthes individuals	Genus	Relative abundance of Achnanthes	—
% Amphora individuals	Genus	Relative abundance of Amphora	+
% Cocconeis individuals	Genus	Relative abundance of Cocconeis	+
% Cyclotella individuals	Genus	Relative abundance of Cyclotella	+
% Cymbella individuals	Genus	Relative abundance of Cymbella	
% Fragilaria individuals	Genus	Relative abundance of Fragilaria	—
% Frustulia individuals	Genus	Relative abundance of Frustulia	—
% Gomphonema individuals	Genus	Relative abundance of Gomphonema	
% Navicula individuals	Genus	Relative abundance of Navicula	+
% Rhoicosphenia individuals	Genus	Relative abundance of Rhoicosphenia	_
% Surirella individuals	Genus	Relative abundance of Surirella	+
% Synedra individuals	Genus	Relative abundance of Synedra	_
% No. of Achnanthes species	Genus	Percent of No. of Achnanthes species	_
% No. of Amphora species	Genus	Percent of No. of Amphora species	+
% No. of Cocconeis species	Genus	Percent of No. of Cocconeis species	_
% No. of Cyclotella species	Genus	Percent of No. of Cyclotella species	—
% No. of Cymbella species	Genus	Percent of No. of Cymbella species	—
% No. of Fragilaria species	Genus	Percent of No. of Fragilaria species	_
% No. of Frustulia species	Genus	Percent of No. of Frustulia species	-
% No. of Gomphonema species	Genus	Percent of No. of Gomphonema species	
% No. of Navicula species	Genus	Percent of No. of Navicula species	+
% No. of Rhoicosphenia species	Genus	Percent of No. of Rhoicosphenia species	-
% No. of Surirella species	Genus	Percent of No. of Surirella species	+
% No. of Synedra species	Genus	Percent of No. of Synedra species	
Diatom growth forms represent morphological adaptations to environmental conditions and community successional stages (Peterson and Stevenson 1992, Peterson 1996) (Table 5.2). Metrics in this category were all based on genus-level identification because species in a genus usually have the same morphological adaptations. Motile diatoms have a raphe, can move around in silt particles, and may show increased abundances in streams with high siltation. Erect and stalked diatoms are susceptible to severe hydraulic disturbance. Conversely, prostrate diatoms grow flat within the boundary layer and may indicate heavy grazing, hydraulic disturbance, or early developmental status of a diatom community (Stevenson 1996). Stalked individuals are believed to be adapted to overgrow and outcompete prostrate diatoms for nutrients and light (Hudon and Legendre 1987). Finally, high abundance of unattached growth forms can indicate stagnant water.

Sensitive species occur more frequently in reference than polluted streams and may represent native species. Pollution tolerant taxa occur in relatively low numbers in reference streams and thrive in polluted streams. I hypothesized that sensitive species would decrease and tolerant species would increase with human disturbance (sensu Odum 1985, Rapport and Whitford 1999). Species sensitivies and tolerances to pollution were characterized based on assigned PTVs in species tables of the KYDPTI and MTDPI. Species with the highest PTVs were assigned as sensitive species. Similarly, tolerant species were those with the loIst PTVs. Percent relative abundance of individuals, number of species, and % of species with specific environmental preferences are 3 ways to calculate metrics relating species composition of assemblages

Genera	Growth form	Genera	Growth form
Achnanthes	Р	Gyrosigma	P, M
Amphipleura	Р	Hannaea	Ε
Amphora	Р	Hantzschia	P, M
Anomoeoneis	Р	Melosira	U
Asterionella	U	Meridion	Ε
Biddulphia	Е	Navicula	Р
Caloneis	Р	Neidium	Р
Cocconeis	Р	Nitzschia	P, M
Cyclostephanos	U	Opephora	Ε
Cyclotella	U	Pinnularia	Р
Cymatopleura	P, M	Rhoicosphenia	S
Cymbella	S	Rhopalodia	Р
Denticula	Р	Simonsenia	Р
Diatoma	U	Stauroneis	Р
Diploneis	Р	Stenopterobia	P, M
Epithemia	Р	Stephanodiscus	U
Eunotia	Ε	Surirella	P, M
Fragilaria	V	Synedra	Ε
Frustulia	Р	Tabellaria	U
Gomphoneis	S	Thalassiosira	U
Gomphonema	S		

Table 5.2. Diatom genera growth form and motility. P = prostrate, E = erect, S = stalked, U = unattached, V = variable, M = highly motile diatoms.

and species autecologies (Barbour et al. 1999). All 3 were calculated for both sensitive and tolerant species indices.

I hypothesized that similarity in species composition between reference and impaired sites would decrease with increasing human impairment (Sheehan 1984, Odum 1985, Rapport and Whitford 1999). Similarity with reference sites was calculated using the average of Bray-Curtis dissimilarity index (Bray and Curtis 1957) between a test site and all reference sites. The % reference species index was the relative abundance of all species observed in all reference sites. In contrast, the number of distinct reference species was the number of species that appeared primarily in reference sites and not in impaired sites as identified by indicator species analysis (Dufrene and Legendre 1997, McCune and Grace 2002). In indicator species analysis, indicator values (IV) were calculated for each species based on the exclusiveness of species occurrences in a group (the relative abundance of species in that group versus all sites) and the probability that the species would be observed at sites in that group. The maximum IV (IVmax) was then determined for each species to assign them to a group. A Monte Carlo method was used to determine the statistical significance of IVmax and designation of a species as an indicator for that specific group.

Species and genus-level community structure were hypothesized to change with human disturbance (Sheehan 1984, Rapport and Whitford 1999). Environmental responses of taxonomic groups may be regulated by genetic and phenotypic similarities among species within genera, families, and higher-level taxa (e. g. % Ephemeroptera, Plecoptera, Trichoptera). <u>Achnanthes minutissima</u> (Kützing) is a very common species in samples and is expected to decrease with human disturbance (Stevenson and Bahls 1999).

Genus-based attributes summarize the average response of con-generic species to human disturbance. The proportion of all individuals that are in one genus can be used as a genus-level attribute of biotic condition. For genera with many species in a sample, the percentage of species in a sample from that one genus can also be used as an attribute. Achnanthes (Kützing), Cocconeis (Ehrenberg), Cymbella (Agardh), Fragilaria (Lyngbye), Frustulia (Rabenhorst), Gomphonema (Agardh), Rhoicosphenia (Grunow), and Synedra (Ehrenberg) were considered to be clean water genera (Bahls 1993). <u>Amphora</u> (Ehrenberg), Navicula (Bory), Nitzchia (Hassall), and Surirella (Turpin) were thought to be neutral genera (Bahls 1993). <u>Achnanthes</u> and Navicula classification was based on Achnanthes and Navicula sensu lato (i.e., as defined before recent separation of some species into other genera (Round 1990)). Percent <u>Achnanthes</u> was divided by the sum of percentage of <u>Achnanthes</u> and <u>Navicula</u> in the denominator to reduce the variance of the attribute. Percent <u>Cymbella</u> was similarly standardized by the sum of percent <u>Cymbella</u> and <u>Navicula</u>.

Metric selection

Attributes were selected and excluded based on the following sequence of analyses. Attributes with medians of 0 for both reference and impaired sites were eliminated first because low values would prevent the identification of differences. The ability of remaining attributes to separate reference and impaired sites was evaluated with the Mann-Whitney U test (Mann and Whitney 1947). When tests showed statistically significant differences ($\alpha < 0.05$) between site groups, potential metrics were identified. Separation power of these potential metrics was evaluated with box plots, where separation power was defined by the overlap of box plots for reference and impaired sites

(Barbour et al. 1996, Barbour et al. 1999). The box marks the range between the 25th and the 75th quartiles of an attribute. The highest power of 3 was assigned when there was no overlap between boxes of reference and impaired sites. A separation power of 2, 1, and 0 were assigned respectively when interquartile ranges overlapped but did not reach the medians, when only one median was within the interquartile range of the other box, and when both medians were within the range of the other boxes.

The final metrics were selected from the set of potential metrics based on separation power and CV (Table 5.3). Metrics with separation power < 3 and CV > 1 were excluded. Similar metrics, such as % sensitive species and % number of sensitive species, were evaluated to select one based on highest separation power and loIst CV.

Scaling systems and IBI calculation

Two scaling systems were used to normalize the range of metrics. The 1, 3, 5 scaling system has been commonly used with fish and macroinvertebrate IBIs (Karr 1981, Keran and Karr 1994, Barbour et al. 1999). Metrics decreasing with impairment were scored 5 if values exceeded the 25th percentile of reference sites, 3 if values were between the 25th percentile of reference and the 50th percentile of impaired sites, and 1 if the metric value was less than the 50th percentile of impaired sites. Metrics increasing with impairment were assigned 5 if the metric value was < 75th percentile of reference sites, 3 if the value was between the 75th percentile of reference and the 50th percentile of reference sites, 3 if the value was between the 75th percentile of reference and the 50th percentile of reference sites, 3 if the value was between the 75th percentile of reference and the 50th percentile of impaired sites.

A 0-10 system (Hill et al. 2000) was included to compare with the 1, 3, 5 system. For metrics decreasing with impairment, the score was calculated by dividing the metric value for each site by the 90th percentile from reference sites, then multiplied by 10. The

90th percentile was used to avoid the distortion of scores by the possible extreme maximum value. For metrics increasing with impairment, the metric value was divided by the 90th percentile of impaired sites, subtracted from 1, and then multiplied by 10. IBI values were the sum of metric scores.

The relationship between the IBI and land use in watersheds was examined by linear regression. Land use coverage was obtained from the US Geological Survey with a resolution of 1:250,000. The coverage was classified according to Level II categories of the land use and land cover classification system (Anderson et al. 1976). The watershed was defined as the hydrologically contributing land surface upstream of the sampling site. Watershed boundaries were derived from 30 by 30 meter digital elevation grids through digital hydrological modeling with Arc/Info software (Version 7; ESRI, Inc., Redlands, CA). Percent land use was square-root transformed to normalize the data.

Independent testing of metrics and IBI

The IBI was tested with a dataset from a project designed to evaluate relations among stream algae, invertebrates, and nutrients (SAIN Project). Thirty SAIN sites from the IPE were sampled during spring 1996. Diatoms were cleaned with nitric acid. Six hundred valves were counted and identified following the taxonomy of Kramer and Lange-Bertalot (1986, 1988, 1991a, 1991b). SAIN reference sites were defined using the same criteria as used for KDOW reference sites.

Tests were conducted to determine if the developed IBI could distinguish reference and impaired sites in the SAIN dataset. From the development dataset, both the 25th percentile of IBI scores at reference site and the average of IBI scores for the 75th percentile of the impaired sites and the 25th percentile of reference site were subjectively set as the minimum values for reference sites.

I also tested diatom indices of Hill et al. (2000), KDOW (Kentucky Division of Water 1993), and Bahls (1993) with the SAIN test dataset to determine the relative performance of the IBI developed in this study. Tests were performed using box separation ratios, which were the distances between boxes divided by the medians of IBI scores from reference sites. Five diatom metrics used by Hill at al. were included in our metric selection process (diatom taxa richness, % dominant taxa, % acidophilic genera, % motile genera, and % eutrophentic genera). All 5 metrics of KDOW (diatom taxa richness, Shannon diversity, KYDPTI, percent similarity with reference sites, and % sensitive species) and all of Bahl's metrics (Shannon diversity index, diatom pollution index, siltation index, and similarity index) were included in our tests.

Results

After initial evaluation, 29 of 55 attributes were classified as potential metrics (Table 5.3). Percent <u>Cyclotella</u> and % <u>Frustulia</u> attributes were eliminated because both had medians of 0 for reference and impaired sites. Twenty-four metrics were rejected due to non-significant differences between reference and impaired sites based on Mann-Whitney U tests. For example, species richness and number of diatom genera were unable to separate reference from impaired sites. Additionally, Shannon diversity and evenness indices were eliminated because both increased with impairment, which contradicted the commonly predicted responses.

Table 5.3. Selected algal metrics and their separation power, coefficient of variation, response to impairment, and reasons for exclusion. + represents metric increasing with impairment caused by human disturbance, - represents metric decreasing with impairment.

Metrics	Separation Power	CV	Response to impairment	Reasons for exclusion
KYDPTI	3	0.15	-	
MTDPI	3	0.16	—	High correlation
KY % sensitive species	2	1.32		Low power
KY % tolerant species	2	0.9	+	Low power
MT % tolerant species	2	1.08	+	High CV
KY no. sensitive species	3	0.87		High correlation
KY % no. sensitive species	3	0.81	—	
MT no. tolerant species	2	0.43	+	Low power
MT % no. tolerant species	1	0.41	+	Low power
No. distinct reference species	3	0.6	—	
Shannon Diversity index	3	0.15	+	Opposite response
Evenness index	3	0.1	+	Opposite response
% Reference species	2	0.1	_	Low power
Average similarity to reference	3	0.26	—	
% Achnanthes minutissima	3	1.32	—	High CV
% Achnanthes /(Achnanthes+Navicula)	3	0.9	-	
% Cymbella /(Cymbella+Navicula)	3	1.4		High CV
% Achnanthes	2	1.0	_	Low power
% Amphora	2	1.0	+	Low power
% Cocconeis	3	1.56	+	High CV
% Cymbella	3	1.64	—	High CV
% Gomphonema	2	1.03	+	Low power
% Navicula	3	0.79	+	High correlation
% Synedra	3	1.29	-	High CV
% No. Cocconeis species	3	0.63	+	Few species
% No. Cymbella species	3	0.81	-	
% No. Navicula species	3	0.79	+	
% No. Synedra species	3	1.06		High CV
% Erect individuals	3	1.29	_	High CV

Potential metrics were screened based on separation power, CV, and redundancy. Kentucky (KY) % sensitive species, KY % tolerant species, Montana (MT) number of tolerant species, MT % number of tolerant species, % reference species, % <u>Achnanthes</u>, % <u>Amphora</u>, and % <u>Gomphonema</u> were removed because of low separation power. MT % tolerant species, % <u>Achnanthes minutissima</u>, % <u>Cymbella</u> / (% <u>Cymbella</u> + % <u>Navicula</u>), % <u>Cocconeis</u>, % <u>Cymbella</u>, % <u>Synedra</u>, % number <u>Synedra</u> species and % erect individuals were eliminated due to CV > 1. Both MTDPI and KYDPTI performed equally III, but MTDPI was removed because of its redundancy with KYDPI and because the KYDPI was based on the accumulated knowledge of regional flora. KY % number of sensitive species was selected over KY number of sensitive species because of its loIr CV and redundancy. Percent number of <u>Cocconeis</u> species was excluded because only a few species were present in the area. Percent number of <u>Navicula</u> species was selected over % <u>Navicula</u> because I expected that % number of <u>Navicula</u> is more transferable among regions.

Seven metrics were selected for the IBI. The KYDPTI, KY % number of sensitive species, number of distinct reference species, average similarity with reference sites, % <u>Achnanthes / (Achnanthes + Navicula)</u>, and % number of <u>Cymbella</u> species decreased with impairment. Percent number of <u>Navicula</u> species increased with impairment (Table 5.4). Average similarity with reference sites had the smallest distribution range, while % <u>Achnanthes / (% Achnanthes + % Navicula</u>) had the widest range. Box plots for each metric showed good separation between reference and impaired sites (Figure 5.2).

IBI, based on the seven metrics with both the 1, 3, 5 and 0-10 scaling systems, clearly separated quartiles of reference sites from quartiles of impaired sites (Figure 5.3). The

		Score		Stat	istics
Metrics	5	3	1	min	90th
KYDPTI	>2.8	2.8-2.3	<2.3	2.1	3.5
KY % No. sensitive species	>16.2	16.2-5.7	<5.7	0	21.2
No. distinct reference species	>5	5-3	<3	0	8
Average similarity with reference sites	>0.46	0.46-0.35	<0.35	0.24	0.54
% Achnanthes I(Achnanthes+Navicula)	>27.9	27.9-11.7	<11.7	0.4	85
% No. Cymbella species	>15.0	15.0-3.0	<3.0	0	18.2
% No. Navicula species	<25.0	25.0-32.0	>32.0	9.1	38.5

Table 5.4. Scoring range and 90th percentile and minimum values for 10 selected metrics of IBI.



Figure 5.2. Box plots of diatom metrics for reference (Ref) and impaired (Imp) sites of development dataset. Boxes are interquartile ranges (between 25th percentile and 75th percentile); middle lines are medians; and whiskers are 1.5 interquartile ranges beyond the boxes.



Figure 5.3. Box plots of total IBI scores of reference sites versus impaired sites from development dataset. A. 1-3-5 scaling system. B. 0-10 scaling system.

averages of the 75th percentile of the impaired sites and the 25th percentile of reference site were 39.5 and 25.0 for the 0-10 system and 1,3,5 system, respectively. The 25th percentiles of reference sites were 47.5 and 29 for the 0-10 system and 1,3,5 system, respectively. The IBI increased significantly with % forest cover in watersheds in the development data set (linear regression, $r^2 = 0.67$ and p < 0.001) (Figure 5.4). The correlation between IBI and % forest cover was higher than all component metrics except % number of <u>Cymbella</u> species (Table 5.5). KY % number of sensitive taxa was the second highest metric correlated with % forest cover and % number of <u>Navicula</u> species was the third (Table 5.5).

After application of the selected IBI to the SAIN dataset, IBIs using both scaling systems were significantly different between reference and impaired sites with a high separation power of 3 (Figures 5.5, 5.6). The average of the IBI scores for the 75th percentile of the impaired sites and the 25th percentile of reference site was used as a criterion to distinguish reference and impaired sites because it correctly classified more sites as reference and impaired than a criterion based on the 25th percentile of IBI scores at reference sites. Many reference sites were misclassified as impaired when the 25th percentile of the reference condition was used as a criterion. Only 18 of 30 sites were correctly classified under the 1, 3, 5 scoring system. With the 0-10 system, 20 of 30 sites were and the 25th percentile of reference sites are correctly categorized. With the average of the 75th percentile of the impaired sites and the 25th percentile of reference site as the criterion, the 1, 3, 5 system correctly classified 25 of 30 sites. Three reference sites were classified as impaired sites. The 0-10 system correctly classified 24 of 30 sites with 4 impaired sites classified as reference sites.



Figure 5.4. Relation between index values of biotic integrity from the KDOW data set and % forest cover in watersheds (adjusted $r^2 = 0.616$, p < 0.001). Percent forest was arcsine squared-root transformed.

Metrics	Pearson r	р
IBI total score	0.82	0.000*
KYDPTI	0.69	0.000*
KY % No. sensitive species	0.8	0.000*
No. distinct reference species	0.64	0.000*
Average similarity with reference sites	0.52	0.006*
Ach. / (Ach. + Nav.)	0.55	0.004*
% No. of Cymbella species	0.84	0.000*
% No. of Navicula species	0.69	0.000*

Table 5.5. Pearson correlation coefficients between IBI metrics and % forest cover in watersheds. * denotes statistical significance, $\alpha < 0.05$. Ach. is short for Achnanthes; Nav. for Navicula.



Figure 5.5. Box plots of diatom metrics for reference (Ref) and impaired (Imp) sites of test dataset. Boxes are interquartile ranges (between 25th percentile and 75th percentile); middle lines are medians. \bullet = outliers.



Β.



Figure 5.6. Box plots of total IBI scores of reference sites versus impaired sites from test dataset. A. 1-3-5 scaling system. B. 0-10 scaling system.

All 3 tested IBIs from the literature were significantly different between reference and impaired sites, however, box separation ratios of these 3 IBIs were lower than the IBI developed in this project (Table 5.6). The Shannon diversity index and species richness, which were included in KDOW's (1993) IBI, gave the opposite of expected responses to human disturbance and showed higher values for impaired sites. The diatom pollution tolerance index and similarity with reference sites both had separation power of 3. Except for the Shannon diversity index, the metrics in Bahls's (1993) IBI (similarity to reference sites, siltation index, and MTDTI) were able to separate reference from impaired sites. Two metrics from the 5 Hill's IBI metrics (Hill et al. 2000) (taxa richness and acidophilic genera) had separation power of 1. Percent dominant taxa had higher values for reference sites than for impaired sites. Eutraphentic genera and motile genera had high separation power (3).

Discussion

The diatom IBI developed for the IPE of Kentucky was able to separate reference from impaired sites in both the dataset in which it was developed and in a test dataset generated with slightly different sampling and analytical methods. IBI scores were a good indicator of human disturbance regime in watersheds as measured by % forest cover. The diatom IBI developed for the IPE was also more powerful than 3 pre-existing indices when applied to streams in the IPE.

Several misclassified cases during tests of our IBI with a new dataset were underappraisals of biotic integrity. The metric for number of distinct reference species failed to

IBI	Box separation ratio
0-10 system	0.14
1, 3, 5 system	0.15
Balhs IBI	0.03
KY diatom IBI	0
Hill's IBI	0

Table 5.6. Box separation ratios of developed IBIs, Bahls IBI, KY diatom IBI, and Hill's IBI on test dataset.

separate reference and impaired sites in the test dataset using both 1, 3, 5 and 0-10 systems. Samples in the KYDOW and SAIN projects were analyzed by different technicians, who followed different taxonomic references. Rapid changes in nomenclature present a challenge to ecologists (Stoermer 2001, Kociolek and Stoermer 2001). Taxonomic consistency should improve metrics that use a few species that are sensitive to different identification. Cross-referenced taxonomic databases with names, pictures, and literature citations can improve taxonomic consistency and can be established and regularly updated through venues like the taxonomy workshops sponsored by USGS National Water Quality Monitoring Program. Despite potential taxonomic discrepancies between projects, our IBI separated impaired from reference sites with higher statistical significance and consistency than other algal IBIs.

Our misclassified cases included several impaired sites classified as reference sites, which reflects a type II statistical error. Type II errors with multimetric IBIs can be checked by 2 methods. First, they can be checked if a certain metric has a value below a critical value or percentile. For example, with the 1, 3, 5 system I could correct 2 misclassified sites if I checked for KYDPTI values below 3. Second, I could tally the number of selected metrics below certain values or percentiles for each site. For example, I could correct classification of 2 sites if I checked for sites with 3 or more metrics with low scores (e.g., score 1 in 1, 3, 5 system).

Another type of error was characterizing unimpaired sites as impaired. Some highly correlated metrics for the test dataset may be the cause of this failure. This type of error is difficult to check, but may be discovered with diagnostic metrics if no specific stressors

exceed criteria. Multiple lines of evidence, such as fish and invertebrate data, could also help provide correct classification.

I included both species and genus-based attributes in our metrics. The advantage of genus-based metrics is their ease of use because of easier identification than species. The limitation of genus-based metrics is the masking of individual species responses. Congeneric species may have different environmental optima, sensitivity, and tolerance levels. Hill et al. (2001) found no significant loss of power for several genus-based metrics of environmental conditions when compared to species-based metrics, but found fewer environmental conditions can be inferred by genera than by species. Genus-based metrics such as % number of Cymbella species were good indicators of biotic condition in the IPE. Genus-based metrics may be most suitable for specific types of aquatic habitats and small regions, rather than for national or international use because the number of species per genus is likely positively related to the size and habitat diversity of the region. The likelihood of all taxa in a genus responding similarly to human disturbance should be inversely related to the sizes of regions studied. Thus, genus-based metrics may not be as transferable among regions as species-based metrics. For example, % Achnanthes decreased with impairment in our study; holver, Kelly (1998) classified Achnanthes as a median nutrient genus. Genus based metrics do have potential for application, particularly for some stressors and within regions where sets of species within genera respond similarly to human disturbance (Hill et al. 2001).

Species diversity is commonly used as a metric in diatom assessments, even though it is poorly correlated to environmental change. Archibald (1972) and van Dam (1982) found that Shannon diversity index increased with impairment as in our study. Conversely, Stevenson (1984) found diversity indices changed unpredictably with increasing pollution. However, diversity indices are still used in several IBIs (Bahl 1993, KDOW 1993, Hill et al. 2001). Response of diversity indices to stressors depends on the type and severity of pollution and may be too unpredictable for general assessment. Although high diversity is commonly considered a valued ecological attribute, the use of diversity in algal bioassessment is cautioned. Species numbers in a 600-valve count poorly reflect the number of taxa in a habitat (Stevenson and LoI 1986). In addition, high numbers of species may not be characteristic of natural conditions, such as those in low nutrient habitats (McCormick and O'Dell 1996).

I recommend using a 0-10 scaling system as proposed by Hill et al. (2000). No distinct difference was observed in correct assessment of streams for the 1, 3, 5 and 0-10 scaling systems, however the 0-10 system was analytically simpler and easier to communicate with public. The choice of categorical scaling systems (1, 2, 3, and 2, 4, 6) is often considered arbitrary.

The advantage of a multimetric system over a univariate assessment is the assumed greater certainty of detecting impairment and transferability among habitats in a region and potentially between regions. The multimetric diatom IBI had a higher separation power and lower CV in our test dataset than all metrics except the KYDPTI, which had similar power and CV with the IBI. Multimetric systems can provide more thorough integration of the overall system condition (Gerritsen 1995) because no one metric can respond to all types of human impact on ecological systems (Karr 1999). They can account for erroneous responses of a few metrics in assessment projects and may include

metrics related to multiple ecological attributes valued by both decision makers and stakeholders.

In the future, I recommend developing an approach in which greater weight is given to one or more metrics, in addition to the IBI scores. Examining the response of IBI to human disturbance can mask the response of individual metrics because of the averaging feature of a multimetric system (Suter 1993). Fewer streams would have been misclassified if I had used more than the multimetric IBI in the assessment. Individual criteria could be set based on a metric indicating a valued ecological attribute that has sufficient public or ecological concern that it merits specific management attention. For example, the minimum scores of 3 or the 75th percentile for metrics highly correlated with % forest cover, such as KYDPTI and % number of sensitive taxa, can be used as criteria for protection. The number of metrics not meeting pre-defined benchmarks could also be used as another criterion. Different management actions could be triggered by different kinds of violations, ranging from more thorough study to implementing restoration or protection plans. Scores of multimetric indices should be used primarily to summarize the overall ecological conditions and communicate results.

In conclusion, the diatom-based IBI for the IPE derived from a development dataset classified reference and impaired sites in an independent dataset with higher accuracy than other multimetric indices. The IBI values highly reflected the degree of human disturbance in watersheds. Ecologically significant metrics should be examined individually and should have minimum acceptable criterion. More extensive study of periphyton assemblages with all algae and algal functional groups could provide indices of periphyton function. I need to more directly address the valued ecological structures

and functions of assemblages and develop means of assessing those attributes. Distinguishing between metrics of valued ecological attributes and metrics diagnosing stressors provides management with specific information, targeting reasons, and strategies for action.

CHAPTER 6 CONCLUSION

Various ecological attributes respond to pollutants imposed by human activities as summarized by Sheehan (1984), Odum (1985), Rapport et al. (1985), Schindler (1987), and Rapport and Whitford (1999). These attributes have wide literature support and provide biological and ecological basis for bioassessment. Therefore, they should be used to develop bioassessment tools with different kinds of organisms and ecosystems.

In chapter 2, 6 hypotheses of community structural response to stress were tested. These 6 attributes are valued ecological attributes. From correlation analyses, land use types had significant relationships with environmental variables. Overall, these algal attributes responded to land use and environmental variables under low-level human disturbance except for species diversity. Algal species composition and dominant species changed with the human disturbance gradient (HDG). Average diatom biovolume was positively correlated with the HDG. Relative abundance of trophic sensitive species significantly decreased with the HDG, while % trophic tolerant species significantly increased with the HDG. Percent native species was negatively correlated with the HDG, while % trophic sensitive and native species had a higher correlation with the HDG than % native species. Both trophic sensitive and tolerant species were significantly correlated with HDG, Cl⁻, and conductivity, but not with nutrient levels. Nutrient inputs, which are often correlated with elevated Cl⁻ and conductivity, were reduced by biotic uptake. Hence, diatom species were a better indicator for human disturbance than nutrient levels in streams.

cha env dif sp lis Ej vv s s Rock is often the main sampling substratum for algal study and bioassessment. In chapter 3, the sensitivities of epilithic, epiphytic, and epipelic diatom assemblages to an environmental gradient were compared. Species composition of epipelon was most different from epilithon and epiphyton. Each assemblage has its own characteristic species list as explored by indicator species analysis. Epiphyton had the shortest species list from counts, while epilithon and epipelon had about 220 species in the counts. Epiphytes had the most % species variance explained and % species-environment relation variance explained from CCA, while epipelon was ranked 2nd. Epiphytes also had the smallest beta diversity and fastest species turnover along the MEG, while epipelon and epilithon had about the same beta diversity and species turnover rate. Further tests showed that epiphyton was more sensitive than epilithon and epipelon when MAIA trophic index applied to the 3 assemblages. Inter-habitat diversity was not homogenized along the MEG. Natural habitats, besides rock, deserve more study for biodiversity and bioassessment research.

Understanding regional nutrient limitation is another kind of assessment that can contribute to stream management. Small-scale nutrient enrichment experiments conducted in 16 streams showed no consistent regional pattern in nutrient limitation. However, species responded to nutrient enrichment as shown by the significant differences between treatments and controls on their detrended correspondence analysis (DCA) scores. The plot of first 2 axes of DCA also revealed that nutrient enrichments did not change species composition on clay pots similar to those assemblages in high nutrient streams. Community assemblages from treatment clay pots in the same site were more similar than assemblages on clay pots from the same treatments across sites. Comparison

between the rankings of species response to nutrient enrichments and rankings of species environmental optima indicated no consistent correlation pattern. Also no correlation was found between rankings of species response to nutrient enrichments and rankings of species environmental optima from another regional survey and between rankings of species response to nutrient enrichments and species indicator values in van Dam's trophic index. Therefore, extrapolation of experimental results from small-scale experiments to large scales needs to consider regional species pools and regional species autecology.

In chapter 5, a diatom index of biotic integrity (IBI) was developed for the Interior Plateau Ecoregion in Kentucky. Based on IBI development protocols (US EPA 1999) and ecological attributes tested, 7 metrics were selected: the Kentucky (KY) Diatom Tolerance Index, % species characterized as sensitive in KY, number of distinct reference species, average similarity of species composition with reference sites, % Achnanthes / (% Achnanthes + % Navicula), % number of Cymbella species, and % number of Navicula species. IBIs were calculated by summing metrics for a site after transforming metrics to a discrete 1, 3, 5 scale and continuous 0-10 scale. Both scaling systems successfully separated reference and impaired sites and IBI scores were significantly related to measures of human disturbance in watersheds. The diatom IBI was tested using a second dataset collected from IPE streams and processed using slightly different methods and taxonomy. Sites were again classified as reference or impaired sites based on the same criteria for the development dataset. Diatom IBI scores differed significantly between reference and impaired streams in the second dataset and they classified 80 % of these sites correctly. Compared to other diatom IBIs, this IBI had more separation power

than the others. Thus diatom indicators of ecological condition can provide valuable characterizations of biotic condition and human disturbance in stream ecosystems.

Future directions

Community structural attributes have been supported as sensitive in this study. However, the temporal variability and uncertainty of these attributes to human disturbance have not been explored. The understanding of uncertainty of these attributes can provide a foundation for risk analysis in decision-making processes. Hence, the risks associated with different levels of biocriteria can be communicated to managers, stakeholders, and the public.

Despite the sensitivity of community structure to human disturbance, ecosystem functions are also valued ecological attributes and related to ecosystem services directly. Structural and functional ecological attributes represent different ecological and societal values and can be used as different endpoints for assessment and management. Therefore, both need to be studied together to have direct comparison for future research.

Changes of community structure can be the product of the interactions between local and regional species pools. Experiments for evaluating community structural changes need to consider the impacts of species from the regional species pool. The knowledge of species pool involved and species autecology can also facilitate the realism of ecological forecasting and prediction. Understanding of roles of species pool may provide linkages to current knowledge in ecology.

APPENDICES

1X

Species	TN opt	TN rank	TP opt	TP rank	Si opt	Si rank	Cl opt	Cl rank
ACbiasol	0.51	13	1.02	13	2.94	11	2.48	38
ACclevei	0.65	38	1.07	22	2.99	23	2.27	23
ACconspi	0.73	50	1.19	58	3.21	59	2.70	52
ACexigua	0.58	26	1.22	64	3.30	63	2.32	25
AClauenb	0.75	55	1.20	61	2.92	6	2.00	8
ACminuti	0.69	45	1.09	30	3.08	45	2.47	34
ALpelluc	0.68	43	1.19	57	3.41	65	2.81	53
AMlibyca	0.70	47	1.17	54	3.19	56	2.36	28
AMpedcls	0.68	44	1.10	31	3.04	36	2.65	48
CCneothu	0.61	32	1.07	24	3.02	31	2.48	37
CCplaeug	0.61	30	1.08	27	3.04	37	2.48	36
CCplalin	0.65	37	1.17	52	3.12	47	2.39	31
CCpsethu	0.55	23	1.06	20	2.99	21	2.17	17
CMaffins	0.46	3	0.94	3	2.95	12	2.31	24
CMcesati	0.49	6	1.00	11	3.05	40	2.50	39
CMcistul	0.44	2	0.94	4	2.97	19	2.01	9
CMmicroc	0.52	16	1.13	39	3.18	55	2.47	35
CMminuta	0.51	14	1.08	26	2.97	18	2.33	26
Cmsilesi	0.65	39	1.25	65	3.62	68	2.70	51
Cydistin	0.49	10	1.04	16	3.05	39	2.81	54
Datenue	0.41	1	1.06	18	2.83	1	1.83	3
Frbrevis	0.55	24	1.07	23	2.94	10	2.10	14
FRcapgra	0.59	28	1.04	15	2.90	4	1.96	7
FRcaprum	0.50	12	1.15	45	2.92	9	2.08	11
FRcapvau	0.53	19	1.00	12	2.89	3	2.02	10
FRconstr	0.52	15	1.10	32	2.92	8	1.92	4
FRconven	0.64	35	1.07	25	2.99	24	2.17	19
FRdelcat	0.52	17	0.97	9	2.92	5	1.81	1
FRlepdub	0.55	25	1.04	17	2.96	16	2.17	18
FRnanana	0.53	20	1.22	63	3.29	62	2.15	16
FRparasi	0.96	66	1.18	56	3.00	26	2.54	44
FRpinnat	0.77	56	1.12	37	2.96	17	2.19	20
FRtenera	0.53	18	0.95	6	3.07	43	2.26	22

Appendix A. Species optima and ranks for TN (mg/L), TP (μ g/L), Si (mg/L), and Cl (mg/L). Species optima were calculated from species on control clay pots and appeared in > 3 sites. opt is the short for optima.

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Species	TN opt	TN rank	TP opt	TP rank	Si opt	Si rank	Cl opt	Cl rank
GOgracil	0.64	34	1.27	67	3.60	67	2.52	40
GOkobaya	0.73	51	1.21	62	3.04	35	3.24	62
GOmicrop	0.48	5	0.86	1	3.01	30	2.54	45
GOolivcm	0.61	31	1.13	41	2.98	20	2.62	47
GOparvul	0.53	22	0.97	10	3.08	44	2.53	43
GOpumilu	0.46	4	0.96	7	2.96	14	2.13	15
GOtrunca	0.49	9	0.95	5	2.92	7	1.96	5
MDcircul	0.95	65	0.94	2	3.16	51	2.92	58
NAcapita	0.78	59	1.17	55	3.16	53	3.41	64
NAcaprad	0.74	53	1.16	49	3.02	32	2.68	50
NAcostul	0.74	52	1.19	60	3.01	29	2.52	41
NAcrypto	0.91	64	1.16	48	3.12	46	3.16	61
NAcryten	0.64	36	1.17	53	3.23	61	2.60	46
NAgregar	0.67	41	1.10	35	3.07	41	3.27	63
NAignacc	0.65	40	1.13	38	2.99	22	2.23	21
NAlanceo	0.69	46	1.08	29	3.20	58	3.66	67
NAmengru	0.99	67	1.19	59	3.07	42	2.84	55
NAradios	0.59	27	1.26	66	3.40	64	2.37	29
NAreicha	0.75	54	1.15	46	3.00	25	2.53	42
NAtripun	0.63	33	1.08	28	2.95	13	2.33	27
NAveneta	0.68	42	1.16	50	3.20	57	3.79	68
NIamphib	0.70	48	1.36	68	3.44	66	2.38	30
NIdissip	0.77	58	1.14	42	3.04	34	3.04	59
NIlinear	0.88	63	1.02	14	3.18	54	3.41	65
NIpaldeb	0.79	60	1.14	44	3.05	38	2.85	56
NIpalea	0.61	29	1.10	33	3.01	28	2.43	32
NIrecta	0.71	49	1.06	19	3.14	49	3.07	60
NIsociab	0.81	61	1.13	40	3.16	52	3.44	66
NIsupral	0.49	7	1.12	36	3.01	27	2.09	12
PTlandub	1.00	68	1.14	43	3.14	48	2.90	57
PTlanfre	0.82	62	1.15	47	3.15	50	2.46	33
PTlanros	0.77	57	1.17	51	3.22	60	2.68	49
PTperaga	0.50	11	1.10	34	3.02	33	2.10	13
REsinuat	0.53	21	1.06	21	2.88	2	1.82	2
SYulna	0.49	8	0.97	8	2.96	15	1.96	6

Appendix A. Continued.

Species code	Cl Opt	Cl rank	VD trophic
ACbiasol	5.75	76	3
ACclevei	5.97	70	4
ACconspi	7.11	43	7
ACexigua	5.56	82	7
AClauenb	9.36	22	2
ACminuti	7.23	42	7
ALpelluc	8.14	31	2
AMlibyca	6.65	57	
Ampedcls	7.32	39	5
CCplaeug	8.15	30	5
CCplalin	5.26	89	5
CCpsethu	5.36	87	
CMaffins	5.60	80	5
CMcistul	5.48	84	5
CMmicroc	6.58	60	4
CMminuta	6.78	53	
CMsilesi	4.53	94	7
DAtenue	5.21	90	5
FRbrevis	3.75	96	7
FRcapgra	5.57	81	2
FRcaprum	7.09	44	2
FRcapvau	5.39	86	5
FRconstr	5.93	72	4
FRconven	5.76	75	4
FRlepdub	6.81	51	
FRparasi	6.47	63	4
FRpinnat	7.43	37	7
GOgracil	7.32	40	3
GOkobaya	12.84	8	
GOolivcm	14.65	3	5
GOparvul	9.77	18	5
GOpumilu	8.72	27	7

Appendix B. Species Cl optima, Cl ranks, and indicator categories from van Dam's trophic index. Cl optima were from an epilithon survey in the GTBW. Species listed appeared in > 3 sites in both control clay pots and the epilithon survey.

Species code	Cl Opt	Cl rank	VD trophic
MDcircul	8.88	25	7
NAcapita	12.84	7	4
NAcaprad	5.94	71	5
NAcrypto	6.14	67	7
NAcryten	10.18	15	7
NAgregar	12.68	10	5
NAignacc	6.74	54	
NAlanceo	19.22	1	5
NAmengru	6.92	48	
NAradios	6.80	52	4
NAreicha	8.50	28	
NAtripun	9.65	20	5
NAveneta	6.26	65	5
NIdissip	8.85	26	4
NIlinear	7.08	45	4
NIpalea	11.24	14	6
NIrecta	5.80	73	7
NIsociab	11.66	12	5
PTlandub	7.60	35	
PTlanfre	6.56	61	7
PTlanros	6.86	50	5
PTperaga	7.66	34	1
REsinuat	7.39	38	3
SYulna	6.59	59	7

Appendix B. Continued.

Species code	Species name
ACbiasol	Achnanthes biasolettiana (Kutz.) Grun.
ACclevei	Achnanthes clevei Grun.
ACconspi	Achnanthes conspicua A. Mayer
ACexigua	Achnanthes exigua Grun.
AClauenb	Achnanthes lauenburgiana Hust.
ACminuti	Achnanthes minutissimum Kutz.
ALpelluc	Amphipleura pellucida Kutz.
AMlibyca	Amphora libyca Ehr.
Ampedcls	Amphora pediculus (Kutz.) Grunow
CCneothu	Cocconeis neothumensis Kram.
CCplaeug	Cocconeis placentula var. euglypta (Ehr.) Cl.
CCplalin	Cocconeis placentula var. lineata (Ehr.) V. H.
CCpsethu	Cocconeis pseudothumensis Reich.
CMaffins	Cymbella affinis Kutz.
CMcesati	Cymbella cesatii (Rabh.) Grun. ex A. S.
CMcistul	Cymbella cistula (Ehr.) Kirchn.
CMmicroc	Cymbella microcephala Grun.
CMminuta	Cymbella minuta Hilse ex Rabh.
CMsilesi	Cymbella silesiaca Bleisch
CYdistin	Cyclotella distinguenda Hust.
DAtenue	Diatoma tenue Ag.
FRbrevis	Fragilaria brevistriata Grun.
FRcapgra	Fragilaria capucina var. gracilis Hust.
FRcaprum	Fragilaria capucina var. rumpens (Kutz.) Lange-Bert.
FRcapvau	Fragilaria capucina var. vaucheriae (Kutz.) Lange-Bert.
FRconstr	Fragilaria construens (Ehr.) Grun.
FRconven	Fragilaria construens f. venter (Ehr). Grun.
FRdelcat	Fragilaria delicatissima
FRlepdub	Fragilaria leptostauron var. dubia (Grun.) Hust.
FRnanana	Fragilaria nanana Lange-Bert.
FRparasi	Fragilaria parasitica (W. Sm.) Grun.
FRpinnat	Fragilaria pinnata Ehr.
FRtenera	Fragilaria tenera (W. Sm.) Lange-Bert.
GOgracil	Gomphonema gracile Ehr. emend. V. H.

Appendix C. The correspondence between species codes and species names.

Appendix C. Continued.

Species code	Species name
GOkobaya	Gomphonema kobayasii Kocio. & Kings.
GOmicrop	Gomphonema micropus Kutzing
GOolivcm	Gomphonema olivaceum (Lyngb.) Kutz.
GOparvul	Gomphonema parvulum Kutz.
GOpumilu	Gomphonema pumilum (Grun.) Reich. & Lange-Bert.
GOtrunca	Gomphonema truncatum Ehr.
MDcircul	Meridion circulare (Grev.) Ag.
NAcapita	Navicula capitata Ehr.
NAcaprad	Navicula capitatoradiata Germain
NAcostul	Navicula costulata Grun.
NAcrypto	Navicula cryptocephala Kutz.
NAcryten	Navicula cryptotenella Lange-Bert.
NAgregar	Navicula gregaria Donk.
NAignacc	Navicula ignota var. acceptata (Hust.) Lange-Bert.
NAlanceo	Navicula lanceolata (Ag.) Kutz.
NAmengru	Navicula menisculus var. grunowii Lange-Bert.
NAradios	Navicula radiosa Kutz.
NAreicha	Navicula reichardtiana Grunow
NAtripun	Navicula tripunctata (O. F. Mull.) Bory
NAveneta	Navicula veneta Kutz.
NIamphib	Nitzschia amphibia Grun.
NIdissip	Nitzschia dissipata (Kutz.) Grun.
NIlinear	Nitzschia linearis (Ag. ex W. Sm.) W. Sm.
NIpaldeb	Nitzschia palea var. debilis (Kutz.) Grun.
NIpalea	Nitzschia palea (Kutz.) W. Sm.
NIrecta	Nitzschia recta Hantz. ex Rabh.
NIsociab	Nitzschia sociabilis Hust.
NIsupral	Nitzschia supralitorea Lange-Bert.
PTlandub	Planothidium lanceolata var. dubia (Grun.) Lange-Bert.
PTlanfre	Planothidium lanceolata subsp. frequentissima Lange-Bert.
PTlanros	Planothidium lanceolata subsp. rostrata (Oestrup) Lange-Bert.
PTperaga	Planothidium peragalli Brun & Herib.
REsinuat	Reimeria sinuata (Greg.) Kocio. & Stoer.
SYulna	Synedra ulna W. Sm.
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