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ASSESSING THE BIOLOGICAL CONDITION OF MAINE STREAMS AND RIVERS USING BENTHIC ALGAL COMMUNITIES

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A DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy
(in Ecology and Environmental Science)

The Graduate School

The University of Maine

May, 2010

Advisory Committee:

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ASSESSING THE BIOLOGICAL CONDITION OF MAINE STREAMS AND RIVERS USING BENTHIC ALGAL COMMUNITIES

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Dissertation Advisor: Dr. Cynthia Loftin

An Abstract of the Dissertation Presented
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The purpose of this study was to test and develop algal methods of evaluating the condition of Maine streams and rivers. The primary objective was to develop a statistical model to predict attainment of Maine's aquatic life criteria for water quality classes A, B, and C. I collected 298 samples of algae on rocks from 193 locations across the state. The major pattern in species composition related to conversion of forests to urban, residential, and agricultural land uses. I calculated preferred environmental conditions of 236 algal taxa for 1) concentrations of nitrogen, phosphorus, and dissolved ions in the water, 2) percent of watershed land cover that is not forested, 3) and percent of watershed land cover that is impervious, such as pavement. I then tested and identified algal community metrics that responded to increasing watershed development. Metrics derived from Maine data performed better than metrics developed in other parts of the world. Five biologists with Maine's Department of Environmental Protection (MDEP) grouped samples based on attainment of aquatic life criteria (*i.e.*, A, B, C, and non-attainment) by interpreting algal species abundances and community metrics. I developed a statistical model to replicate biologist assignments, which correctly classified 95% and 91% of samples used to build and test the model. The second objective was to develop models based on algal community composition to estimate concentrations of nitrogen and phosphorus in stream water. A multiple linear regression model and a variation of weighted averaging that weights estimates using localized subsets of data performed the best. The final objective was to use nutrient diffusing substrates to determine if growth of benthic algae in the Sheepscot River was limited by phosphorus or nitrogen. It was co-limited by nitrogen and phosphorus. Although my statistical models have limited transferability to adjacent regions with similar ecological conditions, methods used to build the models have wide transferability. MDEP could use the first model to determine if streams and rivers attain water quality classes A, B, and C. MDEP could then use nutrient inference models and diffusing substrates to better diagnose and manage enrichment of phosphorus and nitrogen.

ACKNOWLEDGMENTS

Funding for this project was from the U.S. Environmental Protection Agency (U.S. EPA), the USGS-Maine Cooperative Fish and Wildlife Research Unit, and the Maine Department of Environmental Protection (MDEP). I would like to thank Jennie Bridge, Al Basile, and Matt Liebman (U.S. EPA), Dr. Cynthia Loftin (USGS), and Dr. David Courtemanch, Norm Marcotte, Tony St. Peter, and Cathy Levesque (MDEP) for helping me to obtain and manage funding for this project.

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I could not have completed this work without the help and support of many of my colleagues at DEP. I would particularly like to thank the other members of the Biological Monitoring Unit, including Leon Tsomides, Jeanne DiFranco, Beth Connors, Susanne Meidel, and Susan Davies. They helped collect, enter, and manage the data, which was an enormous task. They shared their biological expertise and helped me make difficult

decisions. They were also instrumental in developing the model to predict attainment of biological criteria, which was a long and sometime frustrating process. I must thank them for their hard work, dedication, and friendship. I must also thank the Biological Monitoring Unit's seasonal employees, interns, and AmeriCorps volunteers that participated in the project over the years, including Amanda Roy, Emily Cira, Jessica Palmer, Hannah Wilhelm, Rachel Dicker, Heather Hartley, Caitlin Kersten, Sarah Schacter, Nathaniel Meyer, Leanne Kozur, Erin Pekar, John Heist, Allison Moody, Tracy Kreuger, Allison Piper, and Jeremy Deeds. Mike Smith and Christian Halsted were indispensable for helping me with complex GIS projects, for which I am very thankful. Chris Halsted also wrote visual basic script to calculate many of the algal variables. I must also thank the many MDEP staff that volunteered their time to help collect data. More than 50 people from MDEP and other organizations volunteered to do field work over the course of the project, including many regular volunteers. Not only did they help collect data, they also kept the work interesting and exciting and reminded me to appreciate every day in the field. I would like to thank staff from the following organizations for helping conduct the study: Houlton Band of Maliseet Indians, Penobscot Nation, Passamaquoddy Tribe, State of Maine Health and Ecological Testing Laboratory, Manomet Center for Conservation Sciences, Portland Water District, Union River Watershed Association, Downeast Salmon Federation,

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1. INTRODUCTION

1.1 Biological condition of Maine's streams and rivers

In 1986, the Maine State Legislature created the Water Classification Program (Title 38 Maine Revised Statutes Annotated (38 M.R.S.A.) Art. 4-A) to improve the management of the State's waters. Expanding on the language from the Federal Clean Water Act (Public Law 92-500), the Legislature declared that it was the State's objective to restore and maintain the chemical, physical, and biological integrity of the State's waters and to preserve certain "pristine" State waters. The Legislature also recognized that it was unrealistic to assign the same environmental goals to all of the State's fresh surface waters. As a result, the Legislature established four classes for rivers and streams (AA, A, B, and C) with different levels of environmental protection. The WCP defined desired environmental goals by establishing designated uses and established narrative and numeric criteria that must be met to attain the designated uses for each class. The designated use of habitat for fish and other widlife was established for all classes with specific criteria associated with each class (Table 1). This presented a unique approach and a unique opportunity to manage water quality (Courtemanch et al. 1989).

The Maine Department of Environmental Protection (MDEP) is responsible for managing water quality and monitoring streams to determine if they attain the criteria of their respective classes. MDEP uses a set of statistical models to analyze aquatic macroinvertebrate data and predict the likelihood of streams and rivers attaining their aquatic life use criteria (aka, biological criteria) (06-096 Code of Maine Rules Chapter 579, Davies et al. 1993, Davies et al. 1999, Davies and Tsomides 2002). In 2004, MDEP was one of the first states in the country to incorporate its statistical models as numeric biological criteria into state law (USEPA 2002). United States Environmental Protection Agency (USEPA) uses MDEP's and Ohio's water quality programs as models for other states to develop levels of aquatic life designated uses in their water quality standards, termed Tiered Aquatic Life Uses (TALU) (Davies and Jackson 2006).

Table 1.1 Examples of criteria used to assess "habitat for fish and other aquatic life" in rivers and streams established by the Maine Standards for Classification of Fresh Surface Waters (38 M.R.S.A. §465) and excerpt of selected terms from 38 M.R.S.A. §466.

	Dissolved Oxygen Numeric Criteria	**Habitat Narrative Criteria**	**Aquatic Life (Biological) Narrative Criteria**
Class AA	As naturally occurs	Free flowing and natural	Aquatic life . . . shall be as naturally occurs
Class A	7 ppm; 75% saturation	Natural	Aquatic life . . . shall be as naturally occurs
Class B	7 ppm; 75% saturation	Unimpaired	Discharges shall not cause adverse impact to aquatic life in that the receiving waters shall be of sufficient quality to support all aquatic species indigenous to the receiving water without detrimental changes to the resident biological community.
Class C	5 ppm; 60% saturation	Habitat for fish and other aquatic life	Discharges may cause some changes to aquatic life, provided that the receiving waters shall be of sufficient quality to support all species of fish indigenous to the receiving waters and maintain the structure and function of the resident biological community.

Definitions of selected terms from 38 M.R.S.A. §466

1. Aquatic life. "Aquatic life" means any plants or animals which live at least part of their life cycle in fresh water.
2. As naturally occurs. "As naturally occurs" means conditions with essentially the same physical, chemical and biological characteristics as found in situations with similar habitats free of measurable effects of human activity.
3. Community function. "Community function" means mechanisms of uptake, storage and transfer of life-sustaining materials available to a biological community which determines the efficiency of use and the amount of export of the materials from the community.
4. Community structure. "Community structure" means the organization of a biological community based on numbers of individuals within different taxonomic groups and the proportion each taxonomic group represents of the total community.
8. Indigenous. "Indigenous" means supported in a reach of water or known to have been supported according to historical records compiled by State and Federal agencies or published scientific literature.
9. Natural. "Natural" means living in, or as if in, a state of nature not measurably affected by human activity.
10. Resident biological community. "Resident biological community" means aquatic life expected to exist in a habitat which is free from the influence of the discharge of any pollutant. This shall be established by accepted biomonitoring techniques.
11. Unimpaired. "Unimpaired" means without a diminished capacity to support aquatic life.
12. Without detrimental changes in the resident biological community. "Without detrimental changes in the resident biological community" means no significant loss of species or excessive dominance by any species or group of species attributable to human activity.

1.2 Algae of Maine's wadeable streams and rivers

1.2.1 Previous studies

Maine stream algal communities have not been well-studied prior to this project. The U.S. Geological Survey sampled the Little River (Lebanon) and Great Works River (North Berwick) as part of a study of the effect of urbanization on streams in the greater Boston metropolitan area (Coles et al. 2004). Both rivers were selected as least-disturbed sites, and algae were scraped from rocks. Compared to the urban streams, both the Little River and Great Works River had lower taxa richness, Shannon-Wiener diatom diversity, and motile diatom relative abundance. Both rivers had greater Bahl's Pollution Index (Bahls 1993) values suggesting less pollution compared to the urban sites. Interestingly, both sites had much greater relative abundance of *Achnanthidium minutissimum* (Kützing) Czarnecki (>70%) compared to urban sites (<40%). The urban sites in this study were deeper and siltier, which would favor silt-tolerant, motile taxa over *A. minutissimum* and other early successional species that are associated with hard substrates (Peterson and Stevenson 1992). The streams in the Boston study area had more frequent occurrences of salt-intolerant species, such as *Achnanthidium rivulare* Potapova et Ponader, *Eunotia incisa* Smith ex Gregory, and *Tabellaria flocculosa* (Roth) Kützing, compared to Birmingham, Alabama, and Salt Lake City, Utah (Potapova et al. 2005)

Although the Meduxnekeag River in Maine has a history of extensive blooms of attached, filamentous green algae, Fretwell (2006) found nutrient concentrations indicative of oligotrophic conditions (total phosphorus (TP) = 11.8 $\mu g\ L^{-1}$, mean nitrate (NO_3^-) = 0.175 $mg\ L^{-1}$) with nitrogen and phosphorus concentrations increasing upstream to downstream. The absence of "nuisance" algal blooms during the study suggested current watershed management and upgrades to the municipal wastewater treatment facility to control nutrient input into the river.

The Saint John River near Dickey, Maine, is oligotrophic, with low concentrations of TP (median=6 μg L^{-1}), total nitrogen (TN) (median=0.31 mg L^{-1}), and benthic chlorophyll *a* (median=43.7 mg m^{-2}) (Culp et al. 2006). Downstram near Edmunston, New Brunswick, the river is mesotrophic-eutrophic (median TP=74 μg L^{-1}, TN=1.10 mg L^{-1}, and benthic chlorophyll *a* 147 mg m^{-2}) due to eight wastewater treatment plants and two pulp and paper mills. Nutrient enrichment experiments indicated that algal growth was P-limited in the upstream, oligotrophic reaches, and nutrients did not limit algal growth in the eutrophic reaches.

1.2.2 Patterns in algal composition in Maine streams and rivers

From 1999 to 2007, MDEP has collected more than 1,200 algal taxa in 313 rock-scraping samples from 250 locations in Maine. Diatoms (Bacillariophyceae) are the most diverse group in Maine streams, with 800 taxa, followed by 270 green algae (Chlorophyta), 160 blue-green algae or cyanobacteria (Cyanobacteria), 15 euglenoids (Euglenophyceae), 10 yellow-green algae (Xanthophyceae), 6 chrysophytes (Chrysophyceae), 4 red algae (Rhodophyta), 3 dinoflagellates (Dinophyceae), and 1 synurophyte (Synurophyceae) (taxonomy following Guiry and Guiry 2010). More diatoms have been identified, in part, because of the existence of detailed taxonomic keys that distinguish species based on the size, shape, and ornamentation of their silica frustules. Synurophytes have persistent silica scales that assist in their identification, but they are not diverse in Maine streams and rivers. The other groups of algae are collectively called “soft algae”, because they do not have silica scales or frustules, which complicates identification. In addition, taxonomic keys often rely upon reproductive structures that are not always present (*e.g.*, *Oedogonium* spp.), which makes it difficult to identify some soft algae to species-level. Presumably there are more species of soft algae in Maine streams than can be identified using current taxonomic keys.

Most algal taxa are uncommon. Almost a third of the taxa occur in a single sample, almost half of the taxa occur in two or fewer samples, and three quarters of the taxa occur in less than 10 samples (Figure 1.1). Small subsamples and partial diatom valve counts (up to 600) used in this study may underestimate taxa richness compared to earlier algal studies that used larger subsamples and valve counts (*e.g.*, 8,000 valves, Patrick et al. 1954, Patrick 1961). Most of the common diatoms (Figure 1.2) and soft algae (Figure 1.3) have broad ecological tolerances, with the exception of *Tabellaria flocculosa* (diatom) and *Calothrix* spp. (cyanobacteria) which occur mostly in oligotrophic streams. *Achnanthidium minutissimum* is ubiquitous in Maine and occured in all but one sample.

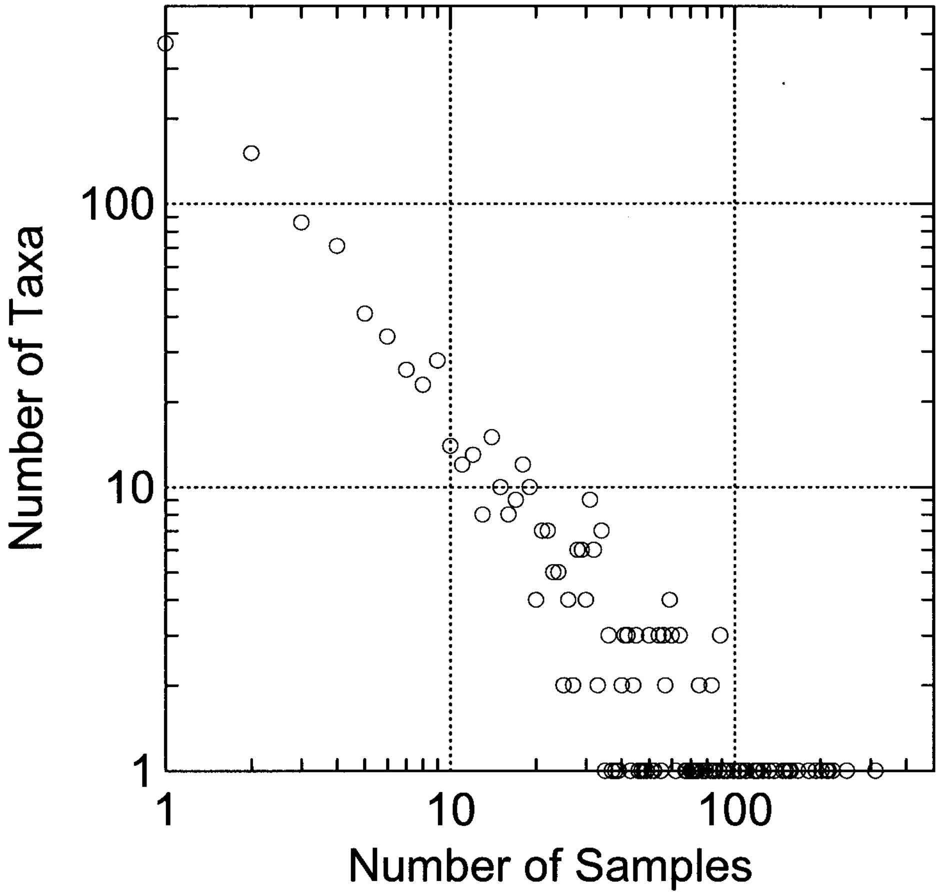


Figure 1.1 Number of taxa occurring at different frequencies in Maine stream and river samples (n=313) collected between 1999-2007.

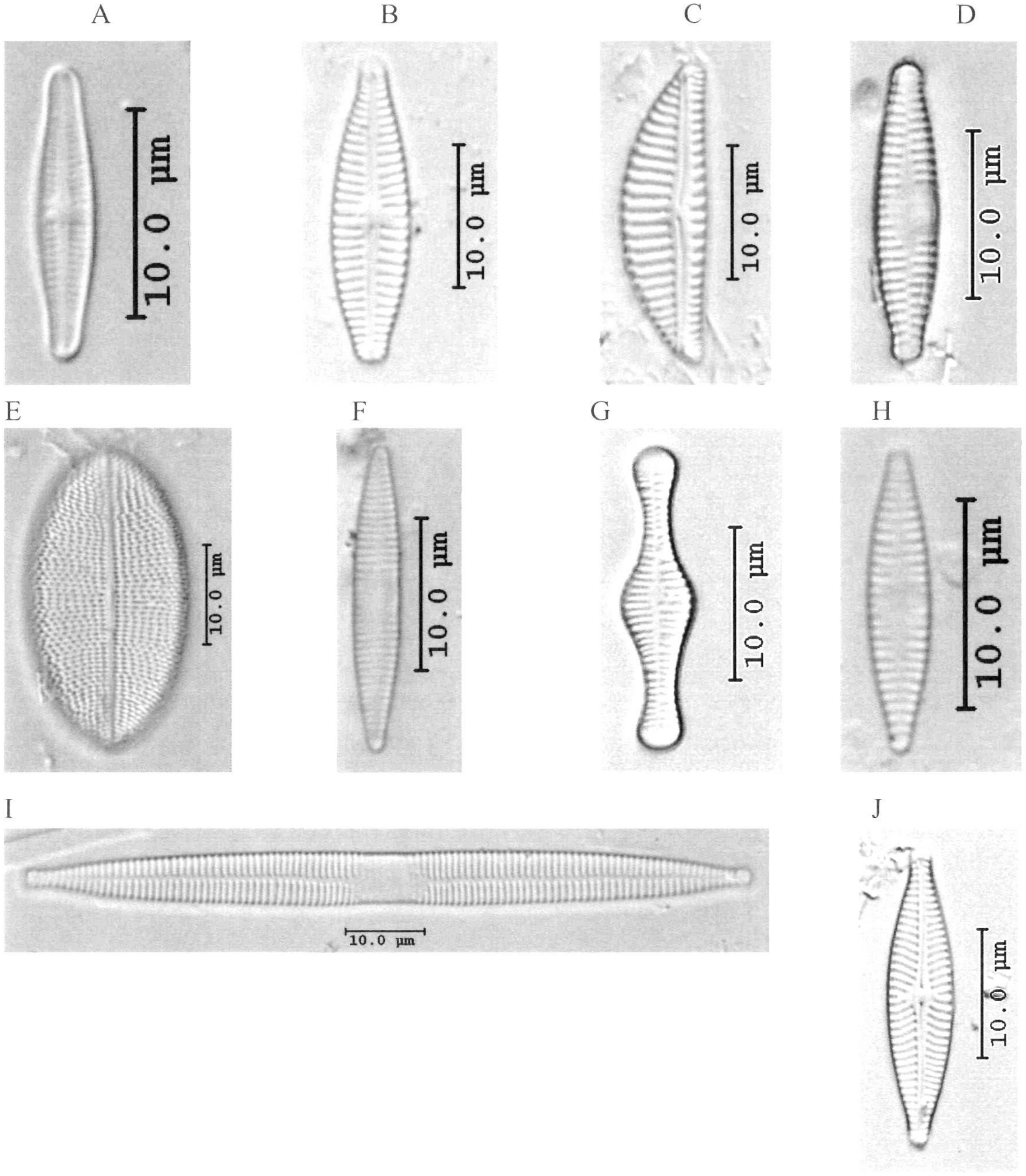


Figure 1.2 Ten most common diatoms collected in epilithic samples (n=313) from Maine streams and rivers. A) *Achnanthidium minutissimum* (Kützing) Czarnecki (n=312), B) *Gomphonema parvulum* (Kützing) Kützing (n=248), C) *Encyonema silesiacum* (Bleisch) Mann (n=221), D) *Fragilaria vaucheriae* (Kützing) Petersen (n=214), E) *Cocconeis placentula var. lineata* (Ehrenberg) Van Heurck (n=213), F) *Synedra rumpens* Kützing (n=210), G) *Tabellaria flocculosa* (Roth) Kützing (n=202), H) *Fragilaria capucina var. gracilis* (Østrup) Hustedt (n=193), I) *Synedra ulna* (Nitzsch) Ehrenberg (n=182), and G) *Navicula cryptocephala* Kützing (n=167).

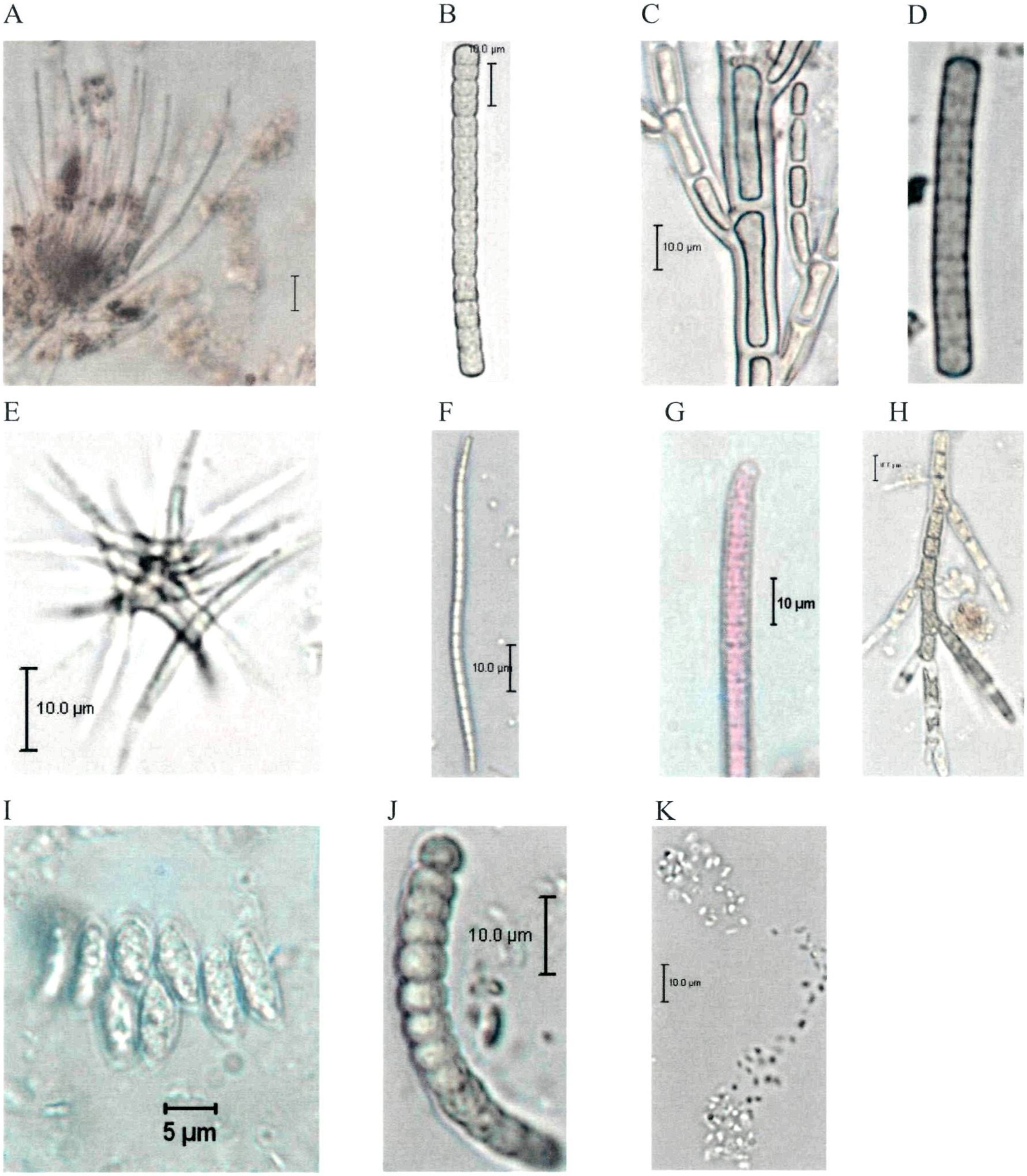


Figure 1.3 Ten most common soft algae collected in epilithic samples (n=313) from Maine streams and rivers. A, B) *Homoeothrix janthina* (Bornet et Flahault) Starmach (cyanobacterium, n=157), C) *Audouinella* spp. (red alga, n=119), D) *Phormidium* spp. (cyanobacterium, n=89), D) *Ankistrodesmus falcatus* (Lundberg) Komárkova-Legnerova (green alga, n=64), E) *Phormidium minnesotense* (Tilden) Drouet (cyanobacterium, n=56), F) *Phormidium formosum* (Bory de Saint-Vincent) Anagnostidis & Komárek (cyanobacterium, n=55), G) *Stigeoclonium* spp. (green alga, n=54), H) *Scenedesmus ecornis* (Ralfs) Chodat (green alga, n=50), I) *Calothrix* spp. (cyanobacterium, n=47), J) *Aphanothece clathrata* West et West (cyanobacterium, n=45).

1.2.3 Algal characteristics

Algal communities reflect combinations of resource availability, substrate type, and disturbance pressure (Biggs 1996). Primary resources for benthic algae include nutrients and light, and primary disturbances include scouring and grazing. Filamentous cyanobacteria (*e.g.*, *Nostoc* spp., *Tolypothrix* spp., *Schizothrix* spp., *Phormidium* spp.) and red algae (*e.g.*, *Audouinella* spp.) tend to respond to low-medium levels of resources and tolerate low levels of disturbance, whereas erect stalked diatoms and filamentous algae (*e.g.*, *Cladophora* spp., *Mougeotia* spp.) respond to abundant resources with low-medium disturbance. Streams with frequent or intense disturbance/grazing often are dominated by low-growing diatoms that tightly adhere to substratum (*e.g.*, *Achnanthidium minutissimum*, *Cocconeis placentula* Ehrenberg) or other taxa capable of rapid colonization/replacement (*e.g.*, *Cymbella* spp., *Fragilaria vaucheriae* (Kützing) Petersen) (Figure 1.4, Steinman 1996). Algal biomass peaks with stable substrate, little shading, abundant nutrients, warm temperature, and infrequent scouring events, where production can greatly exceed consumption by grazing macroinvertebrates. All other factors remaining equal, algal biomass generally is greatest on cobbles, least on fine gravel, and intermediate on boulders, mud, and sand (Cattaneo et al. 1997). Adnate and filamentous algae dominate cobbles and boulders, while cyanobacterial colonies and motile diatoms dominate the sand and mud (Fogg et al. 1973, Cattaneo et al. 1997).

Three long-term temporal patterns in benthic algae biomass accrual occur: (1) constant low biomass, (2) cyclic accrual and sloughing, and (3) seasonal cycles (Biggs 1996). Relatively constant, low algal biomass of disturbance and grazing resistant taxa (*e.g.*, *Achnanthidium*, *Cocconeis*, *Cymbella*, *Synedra*, and basal structures of

Stigeoclonium) can develop in streams with frequent scouring or grazing. Cycles of accrual and sloughing can occur in streams that flood seasonally or frequently and start with rapid colonization of diatoms and eventual encroachment of cyanobacteria and filamentous green algae (Figure 1.5, Peterson 1996). Intense scouring or grazing may remove all or most of an algal mat and restart succession (Figure 1.6, Peterson 1996). Resource availability and magnitude of distrubance may determine how quickly a periphyton mat recovers (Horner and Welch 1981, Horner et al. 1983, Bothwell 1989).

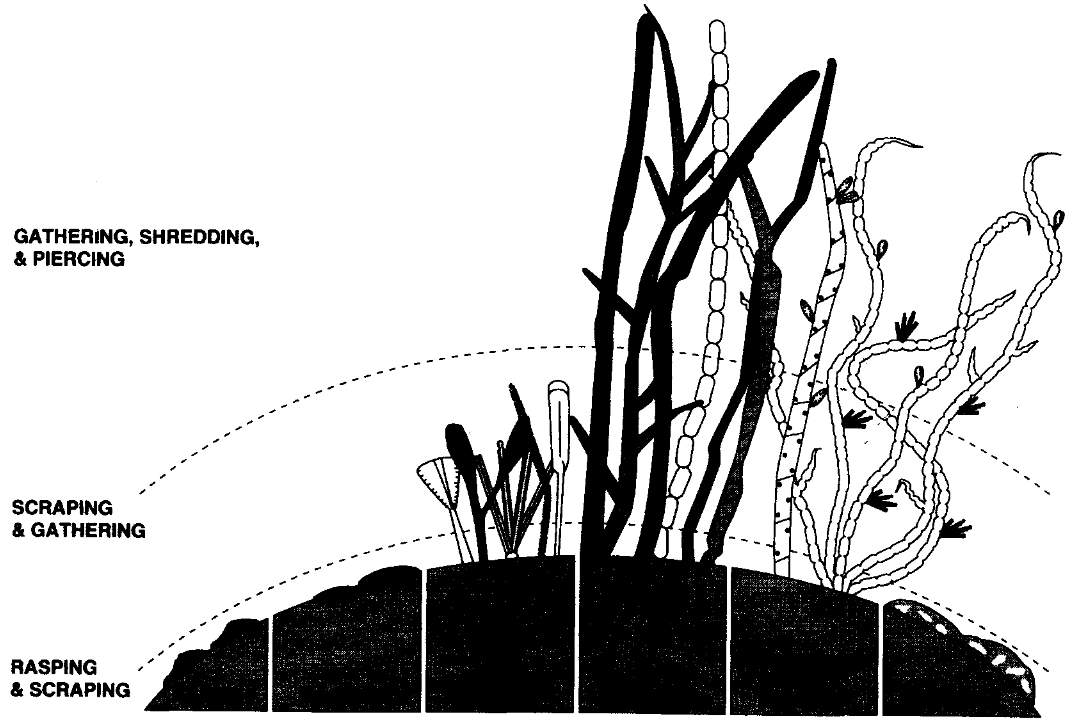


FIGURE 2 Hypothetical schematic representation of key growth forms in benthic algal communities in relation to feeding zones occupied by different types of grazers.

Figure 1.4 Hypothetical schematic representation of key growth forms in benthic algal communities in relation to feeding zones occupied by different types of grazers. (reproduced with permission, Steinman 1996)

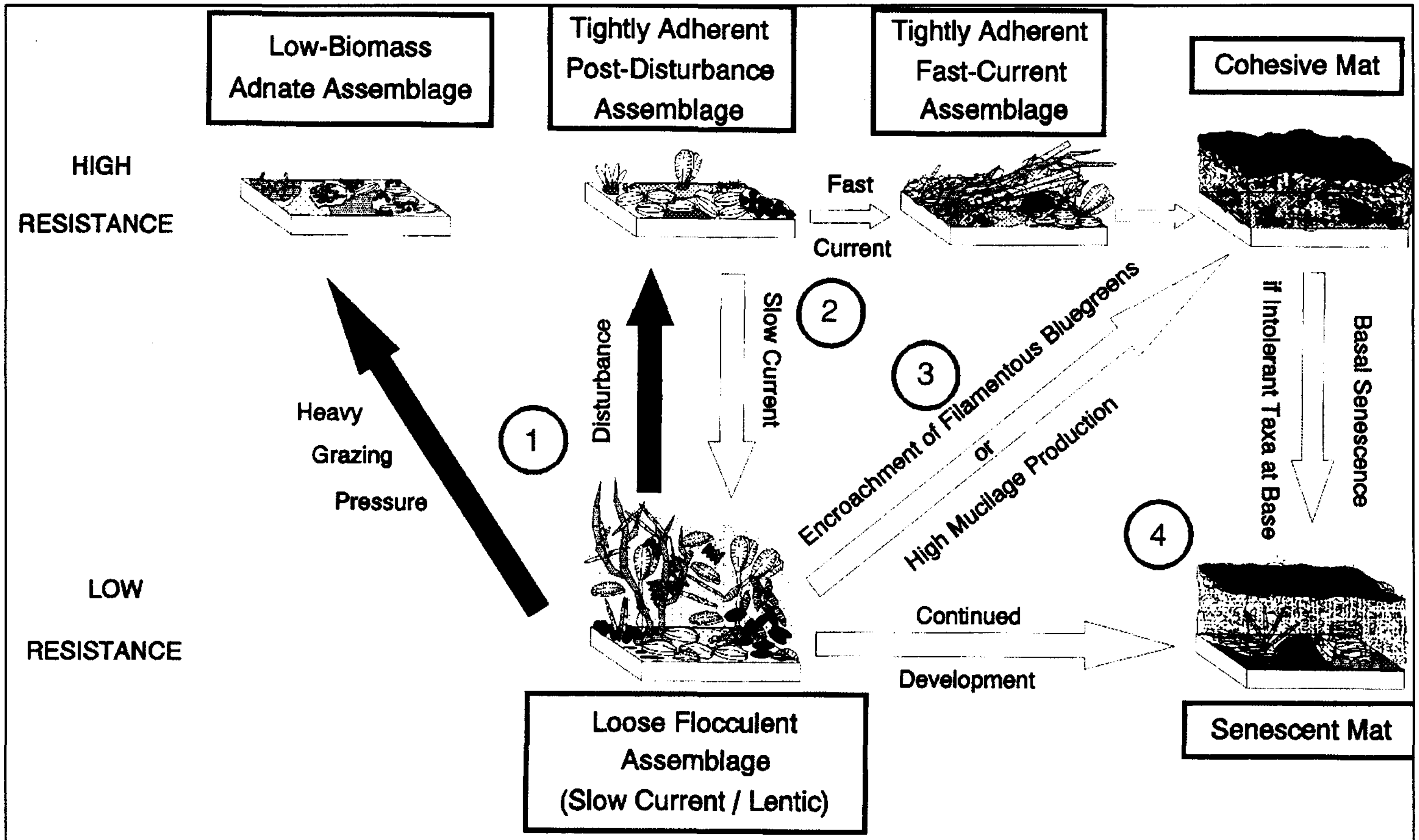


FIGURE 3 Summary of algal community physiognomies that impart high and low resistance to scour disturbance, and the factors that caus transitions between these two states. Numbers demark four transitionary stages referred to in the text.

Figure 1.5 Summary of algal community physiognomies that impart high and low resistance to scour disturbance, and the factors that cause transitions between these two states. Numbers demark four transitionary zones. (reproduced by permission, Peterson 1996)

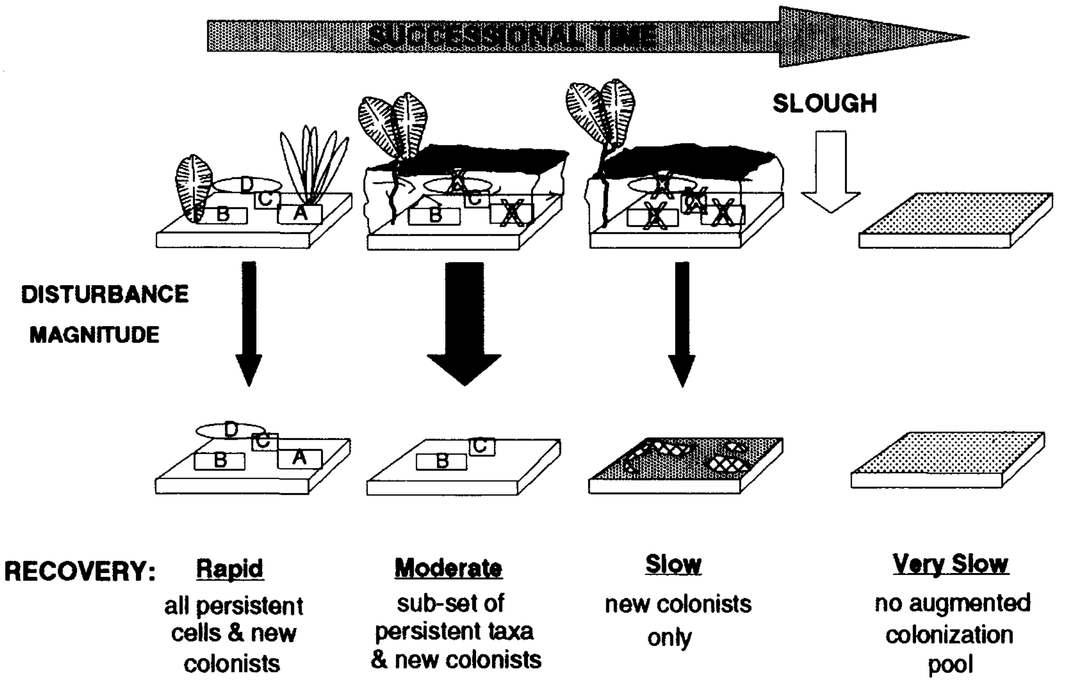


FIGURE 6 Proposed model for successional changes in recovery potential of cells within basal strata of attached algal communities following scour disturbance competent to remove all overlying biomass. The disturbance magnitude required to expose basal cells (indicated by arrow width) varies over successional time because resistance of algal communities to disruption by disturbance changes as with stage of community development (Peterson *et al.,* 1990; Peterson and Stevenson, 1992). Four taxa are depicted in the basal layer (species A–D). An "X" over a species signifies an inability to survive resource limitation at a given stage of development. "Slough" refers to an autogenically induced senescence and detachment of all basal cells.

Figure 1.6 Proposed model for successional changes in recovery potential of cells within basal strata of attached algal communities following scour disturbance competent to remove all overlying biomass. The disturbance magnitude required to expose basal cells (indicated by arrow width) varies over successional time because resistance of algal communities to disruption by disturbance changes as with stage of community development (Peterson et al., 1990; Peterson and Stevenson, 1992). Four taxa are depicted in the basal layer (species A-D). An "X" over a species signifies an inability to survive resource limitation at a given stage of development. "Slough" refers to an autogenically induced senescence and detachment of all basal cells. (reproduced with permission, Peterson 1996)

1.3 Research justification and objectives

1.3.1 Nutrient limitation pilot study

Nutrient enrichment is a threat to the water quality of Maine's streams and rivers (MDEP 2008a) . Historically, it has been assumed that phosphorus was responsible for limiting algal growth in Maine's fresh waters (MDEP 2008b). Recent research indicates that nitrogen can limit algal growth in streams and rivers alone or with phosphorus (Francoeur 2001). Attributing algal growth limitation to N, P, both, or neither would improve stream water quality management. In Chapter 2, I present two methods of determining limiting nutrients: 1) by predicting the limiting nutrient with the ratio of N and P concentrations from water samples (Redfield et al. 1963), and 2) by using nutrient diffusing substrates (NDS, Tank and Dodds 2003, Tank et al. 2006) spiked with N, P, both N and P, and no added nutrients while estimating algal growth with chlorophyll *a* concentrations.

1.3.2 Nutrient inference models for Maine streams and rivers

Managing nutrients and the proliferation of algae in streams and rivers is a problem for water resource managers globally. USEPA has adopted a national strategy to require states to establish nutrient criteria. One approach to this has been the development of models to infer nutrient concentrations in streams and rivers from benthic algae (*e.g.*, Winter and Duthie 2000, Potapova et al. 2004, Ponader et al. 2007, Ponader et al. 2008, Stevenson et al. 2008b) and macroinvertebrates (Smith et al. 2007). In Chapter 3, I develop statistical predictive models to infer TN and TP concentrations of Maine's wadeable streams and rivers based on benthic diatom species composition. The models will be proposed for inclusion in Maine's new nutrient criteria.

1.3.3 Algal metrics for evaluating response of lotic algal communities to watershed disturbance

Anthropogenic disturbances in upstream watersheds alter the species composition of epilithic algal communities of streams and rivers. Algal bioassessments use community metrics to evaluate condition of algal communities and measure departure from regional reference conditions. In Chapter 4, I test novel metrics and metrics used by other algal bioassessments to determine if they distinguish Maine reference sites from non-reference sites and are correlated with the amount of development upstream of sample locations.

1.3.4 Benthic algal model for predicting attainment of biological criteria for Maine streams and rivers

MDEP uses statistical models based on aquatic macroinvertebrates to predict the likelihood of a stream or river attaining Class AA/A, B, or C biological criteria (06-096 Code of Maine Rules Chapter 579). Other state water quality agencies have increased confidence in water quality attainment decisions and improved diagnostic capabilities by evaluating the condition of more than one taxonomic assemblage. In Chapter 5, I develop a model using benthic algae to predict the likelihood that a stream attains legislated biological criteria for the State of Maine. If sufficiently robust in predictive ability, this model will be proposed for inclusion in Chapter 579 as numeric aquatic life criteria.

2. COMPARISON OF METHODS TO DETERMINE LIMITING NUTRIENTS OF THE SHEEPSCOT RIVER, MAINE

2.1 Abstract

Nutrient enrichment is a major threat to water quality of rivers and streams in the United States. The limiting nutrient can be identified with nutrient diffusing substrates (NDS). NDS deployed in the Sheepscot River, Maine, indicated co-limitation by nitrogen (N) and phosphorus (P). Either nutrient added separately caused a 2-fold increase in chlorophyll *a* concentrations. Nitrogen and P added together caused a six-fold increase in chlorophyll *a* concentrations. Chlorophyll *b* concentrations and the relative abundance of green algae (Chlorophyta) also were greatest when N and P were both enriched. The ratio of dissolved inorganic nitrogen (DIN) to soluble reactive phosphorus (SRP) in the water column did not accurately predict the limiting nutrient. Total N: total P correctly predicted co-limitation, however ambient N and P were scarce resulting in less confidence in the predictions. Water quality management activities intended to reduce nutrient loads entering the Sheepscot River and similar oligotrophic rivers should focus on both N and P.

2.2 Introduction

Nutrient enrichment is a major threat to the water quality of streams and rivers in the United States (Carpenter et al. 1998, Smith et al. 1999, Dodds and Biggs 2002, USEPA 2006). Although N and P are essential to aquatic life, excess supply of N and P can cause phytoplankton and periphyton blooms that can harm other aquatic life by competing for habitat, reducing dissolved oxygen, and changing pH (Miltner and Rankin 1998, USEPA 1998, Wang et al. 2007). Benthic algal growth in rivers and streams is influenced by nitrogen (N) and phosphorus (P) concentrations (Patrick 1948, Blum 1956, Welch et al. 1988, Borchardt 1996, Dodds et al. 2002, Stevenson et al. 2006). Until recently phosphorus was assumed the primary nutrient limiting algal growth in fresh waters (Hutchinson 1957, Correll 1999), however, nitrogen limits algal growth either by itself or in combination with phosphorus in some lotic systems (Borchardt 1996, Francouer 2001). Increased knowledge of N or P limitation in lotic systems would improve management of nuisance growth of filamentous algae, discharge licenses issuance, restoration plan development, and best management practices that reduce both point and nonpoint source pollution.

The ratio of total N and total P (TN:TP) has been used to predict the limiting nutrient in streams (Schanz and Juon 1983, Pringle and Bowers 1984, Grimm and Fisher 1986, Hill and Knight 1988, Peterson et al. 1993, Borchardt 1996, Pringle 1997). The TN:TP ratio is based on the molar ratio of carbon, nitrogen, and phosphorus (C:N:P of 106:16:1) (Redfield et al. 1963). If the ratio of nutrients available to algae deviates from the Redfield ratio, one of the nutrients is expected to limit algal growth following the Liebig-Sprengel Law of the Minimum (van der Ploeg et al. 1999). N-limitation is implied when molar N:P ratios are < 10, and P-limitation is implied when ratios are > 16 to 20 (Gregory 1980, Schanz and Juon 1983, Grimm and Fisher 1986, Pringle 1987, Peterson et al. 1993). Nitrogen and P are either co-limiting or not limiting when ratios are between 10-16. Streams with water column TN:TP ratios >15 have greater algal biomass than streams with TN:TP ratios <15 (Dodds et al. 2002). Several studies of nutrient limitation in streams have used the ratio of dissolved inorganic N to soluble reactive P (DIN:SRP) instead of TN:TP (Wold and Hershey 1999, Tank and Dodds 2003).

Nutrient enrichment experiments can confirm nutrient limitation. Nutrients have been added directly to streams, added to artificial streams or flow through systems, and placed into streams within nutrient diffusing substrates (NDS) (see reviews in Borchardt 1996, Francoeur 2001, Tank and Dodds 2003). Early NDS studies used petri dishes, terracotta pots, or terracotta saucers filled with nutrient enriched agar (*e.g.*, Pringle and Bowers 1984, Fairchild et al. 1985, McCormick and Stevenson 1989); however, plastic containers are more commonly used now because terracotta pots can contain minerals that bind P (Brown et al. 2001). In addition, the composition of pots can vary, making it difficult to replicate methods (Pringle and Triska 2006). Plastic containers typically have a perforated lid, and nutrients pass through glass fiber filters (Tank and Dodds 2003), glass fiber frits (Tank et al. 2006), polyester mesh (Biggs et al. 1998, Busse et al. 2006), or thin pieces of wood (Tank and Dodds 2003). The most common NDS experimental design deploys replicate NDSs with one of four treatments: N, P, N and P, plain agar (control, C). Algae colonize the NDS and assimilate the nutrients diffusing from the agar, and the treatment with the greatest chlorophyll *a* concentrations is thought to provide the limiting nutrient or combination of nutrients.

Chlorophyll *b* and *c* are not commonly measured in NDS experiments but also could be evaluated. Chlorophyll *b* is produced by green algae (Chlorophyta), photosynthetic euglenoids (Euglenophyceae), and three cyanobacterial genera (Chlorobacteria) (Graham and Wilcox 2000, Wehr and Sheath 2003, Guiry and Guiry

2010), however euglenoids and especially chlorobacteria are uncommon in Maine streams and rivers (MDEP, unpublished data). Chlorophyll *c* is produced by cryptomonads (Cryptophyta), dinoflagellates (Dinophyceae), haptophytes (Haptophyta), and heterokonts (Heterokontophyta) (Graham and Wilcox 2000, Wehr and Sheath 2003, Guiry and Guiry 2010), but diatoms (Bacillariophyceae) are by far the most diverse and abundant group that produces chlorophyll *c* in Maine streams and rivers (MDEP, unpublished data). Thus, increased chlorophyll *b* or *c* likely signal a response by green algae or diatoms, respectively, which could be confirmed by identifying taxa on the NDS.

This study had three objectives: 1) to predict the limiting nutrient at a site based on the stream water N:P ratio and assess the prediction accuracy with a NDS experiment, 2) to determine the response of diatoms and green algae to nutrient additions indicated by chlorophyll *b* and *c*, and 3) to determine the number of replicates needed to detect a two-fold and four-fold increase in chlorophyll *a* concentrations.

2.3 Methods

2.3.1 Study area

The study site is located on the Sheepscot River, approximately 75 m upstream of the Route 126 bridge in Whitefield, Maine (lat. 452608.08, long. 4896833.22; UTM Zone 19N, NAD 83, meters; Figure 2.1). The 363 km^2 watershed consists of 76% wooded uplands, 9% wetlands, 5% grassland, 4% water, 4% developed, and 2% tilled agriculture (MDEP unpublished data). The Maine Department of Environmental Protection (MDEP) manages the Sheepscot River as a Class AA waterbody; however, the river's benthic macroinvertebrate community does not always attain Class AA/A biological criteria

(Davies et al. 1999). The U.S. Geological Survey (USGS) operates a gauge station (# 01038000) approximately 10 m upstream of the bridge, which records water discharge and temperature.

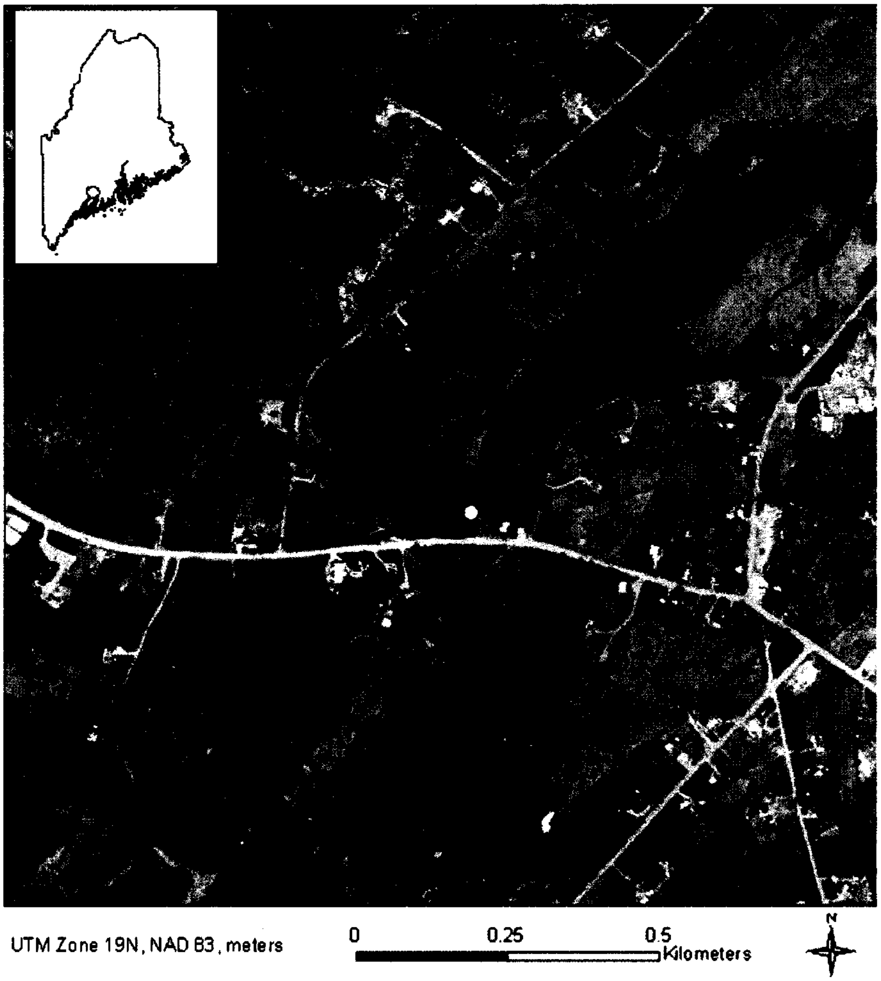


Figure 2.1 Study site location on the Sheepscot River in Whitefield, Maine upstream from the Route 126 bridge. (lat. 452608.08, long. 4896833.22; UTM Zone 19N, NAD 83, meters)

2.3.2 Samples

Algae were collected from natural substrates on July 19, 2006, as part of MDEP's water quality monitoring program. Six transects were established perpendicular to stream flow within the sample reach. Cobbles or small boulders (18) were collected along the near, middle, and far parts of each transect, avoiding eddies, pools, back waters, and areas along the bank where water levels fluctuate. Epilithic algae were removed from a circular area on each rock by a placing a neoprene washer with a 2.54 cm-diameter opening and brushing the area with a stiff-bristled brush. Algae scraped off all rocks were combined into a single sample representing the stream reach and were preserved with 1 mL M^3 preservative per 50 mL of sample (Eaton et al. 2005). A duplicate sample was collected with the same protocols.

Algal taxa in the two natural substrate samples were identified and enumerated in the form of cell counts and biovolumes following protocols in Charles et al. (2002) by taxonomic specialists in the Patrick Center for Environmental Research of The Academy of Natural Sciences. Soft algae were identified and enumerated, and the numbers of live and dead diatoms were recorded with a Palmer-Maloney counting chamber (Charles et al. 2002). Diatoms were acid cleaned and subsamples of cleaned frustules were permanently mounted with NAPHRAX® or ZYRAX® (The Biology Shop, Hazelbrook, New South Wales, Australia; http://mywebsite.bigpond.com/ thebiologyshop) as mounting medium. Diatoms were identified to the lowest taxonomic level possible, and 600 valves per sample were counted at 1000x magnification with light microscopes with Nomarski Interference Contrast (Charles et al. 2002).

The NDS were 125 mL, cylindrical plastic containers filled with 2% (by weight) agar solution amended with 4 treatments: 0.5 M $NaNO_3$ (N), 0.5 M NaH_2PO_4 (P), both (NP), or neither (control, C). The bottles were filled to overflowing to accommodate shrinkage with cooling. Excess agar was removed, a 2.54 cm diameter Whatman 0.45 μm GF/F glass fiber filter was placed in the center of the agar surface, and the container was topped with caps with a 1.9 cm diameter circle removed from the center similar to Stevenson and Glover (1993). Each replicate (n=12) consisted of one container of each treatment randomly assigned to a position (1, 2, 3, 4) along one long side of a standard clay brick.

The 12 NDS were placed at least 1 m apart in locations with similar water depth and velocity on 2 August 2006 and retrieved on 23 August 2006 after a 3-week incubation period (Tank and Dodds 2003). Water depth and velocity were measured (Global Flow Meter FP101/201) for each NDS replicate on 2, 11, and 23 August. Water samples were collected on 19 and 27 July and 2, 11, 16, and 23 August. Total P (TP), soluble reactive P (SRP), ammonia (NH_3), total Kjeldahl N (TKN), nitrate+nitrite N (NO_3^- and NO_2^-, hereafter referred to as NO_x), total alkalinity (ALK), and dissolved organic carbon (DOC) were analyzed at the State of Maine's Health and Ecological Testing Laboratory. The detection limit was provided as the measurement result when values were less than the detection limit. Dissolved Inorganic Nitorgen (DIN) was estimated by adding NH_3 and NO_x. Total Nitrogen (TN) was estimated by adding TKN and NO_x. Field measurements included water temperature, pH, and specific conductance (Hanna Instruments HI991300) and dissolved oxygen concentration (Hanna Instruments HI9142). Substrate composition was visually estimated as percentage of reach bottom consisting of bedrock, boulder, cobble, gravel, sand, and silt/clay. Bank full width was measured. Canopy cover was estimated with a convex spherical densiometer (Lemmon 1956). Daily water discharge (cfs) and daily mean, maximum, and minimum water temperature (^{o}C) were obtained from the USGS guage station (U. S. Geological Survey 2006).

Ten of the 12 NDS, each consisting of a set of 4 treatments, were successfully recovered on August 23, 2006, but 2 were lost. Nine NDS were used for chlorophyll analysis, and one NDS was used to identify algae on the NDS filters. Filters (n=9) from NDS replicates were collected, frozen, and analyzed for chlorophyll *a*, *b*, and *c* concentrations (μg cm^{-2}) with the trichromatic, spectrophotometric method in Standard Methods (10200 H) (Eaton et al. 2005) at the University of Maine's Sawyer Environmental Research Laboratory, Orono, ME. Macroinvertebrates on the filters and lids were counted and identified in the field. Macroinvertebrates in the scraper and collector-gatherer functional feeding groups (Merritt and Cummins 1996) were combined into a "grazer" group. The filters of one NDS was preserved with M^3, and 4 representative sections of each filter were mounted in a slide-well filled with Karo syrup, which becomes transparent when it hardens (Patrick 1936, Eaton et al. 2005). A different processing method was used for the NDS than the rock samples, because algal cells could not be removed from the glass fiber filters. Algal taxa were identified and enumerated (>600 cells, 400x and 1000x bright light and phase contrast, Nikon Eclipse E200 microscope) from random slide locations (Eaton et al. 2005) with reference to Krammer and Lange-Bertalot (1986, 1988, 1991a, b), Patrick and Reimer (1966, 1975), Prescott (1962), Wehr and Sheath (2003), and Maine's digital image library compiled for MDEP by Michigan State University and the Academy of Natural Sciences (MDEP, unpublished).

2.3.3 Analysis

Water depth and velocities at NDS locations were summarized with descriptive statistics (SYSTAT 11.0, Wilkinson 1990). The Sawyer Lab reported chlorophyll results ($\mu g\ cm^{-2}$) below detection limits and these values were used in calculations rather than arbitrarily using the detection limit or one-half the detection limit in calculations (Helsel and Hirsch 1991). Thus, calculations of means and standard deviations of chlorophyll *a*, *b*, and *c* concentrations (n=9) included some values below detection limits. Chlorophyll values were $\log_{10}$ transformed to approximate a normal distribution and minimize differences in variances. The effects of treatment (C, N, P, NP) and position of treatments on NDS (1, 2, 3, 4) on chlorophyll *a* were determined with a two-factor ANOVA (treatment and position) model (SYSTAT 11.0, Wilkinson 1990). The effects of N and P on chlorophyll *a*, chlorophyll *b*, and the number of grazing macroinvertebrates (grazers) were determined with two factor ANOVA (N and P) models (Dube et al. 1997, Tank and Dodds 2003, Tank et al. 2006). The relationship between chlorophyll *a* and the number of grazers also was evaluated with a scatterplot and Spearman rank correlation (SYSTAT 11.0, Wilkinson 1990). Interpretation of nutrient limitation followed Tank and Dodds (2003) (Table 2.1). A power analysis ($\alpha = 0.05$, power = 0.80) for a two-way completely randomized ANOVA identified the number of replicates needed to detect a two-fold and four-fold increase in chlorophyll *a* concentrations.

Table 2.1 Decision framework for interpreting responses to N and P treatments proposed by Tank and Dodds (2003). A circle represents a significant treatment effect or significant interaction in the two-way ANOVA ($p \leq 0.05$).

Interpretation	N effect	P effect	N x P Interaction
N limited	●		
P limited		●	
N and P colimited			●
N and P colimited	●	●	
N and P colimited	●	●	●
1° N limited, 2° P limited	●		●
1° P limited, 2° N limited		●	●
Not limited by N or P			

2.4 Results

2.4.1 Physical characteristics of study site

Mean water depth and velocity at the NDS locations decreased during the experiment from 45 to 20 cm and 28 to 20 cm sec^{-1} (Table 2.2). Mean daily water temperature collected by the USGS gauge station ranged from 26 to 20 °C and decreased during the study (Figure 2.2). Mean daily discharge decreased from 377 cfs on 8/2/2006 to 67 cfs on 8/19/2006 followed by a small peak at the end of the study period (Figure 2.3). Bank full-width of the study reach was approximately 24 m with a 91% open canopy. The substrate consisted of approximately 60% cobble, 25% boulder, 10% gravel, and 5% sand.

Table 2.2 Average depth and water velocity for NDS replicates (n=10).

	Depth (cm)			Velocity (cm sec^{-1})		
	2-Aug	11-Aug	23-Aug	2-Aug	11-Aug	23-Aug
Minimum	40	23	20	23	16	14
Maximum	50	30	26	33	28	30
Mean	45	25	22	28	22	21
Standard Deviation	3	3	2	4	4	6

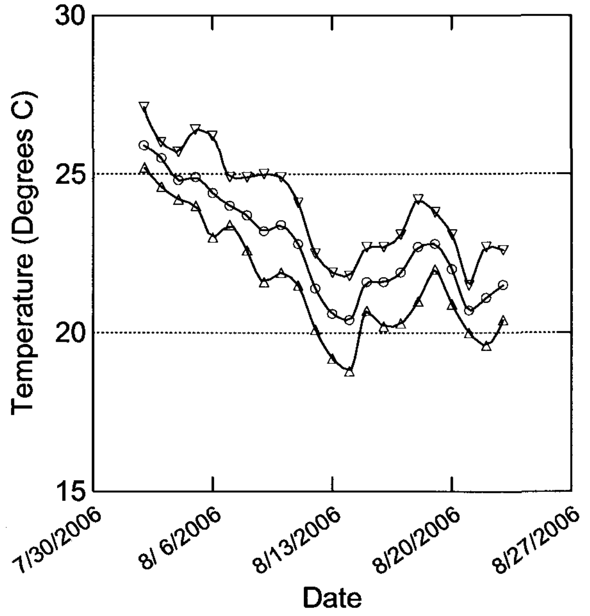


Figure 2.2 Daily mean (O), maximum (▽), and minimum (△) water temperatures recorded at USGS gauging station 01038000 approximately 50 m downstream from NDS from 8/2/2006-8/23/2006.

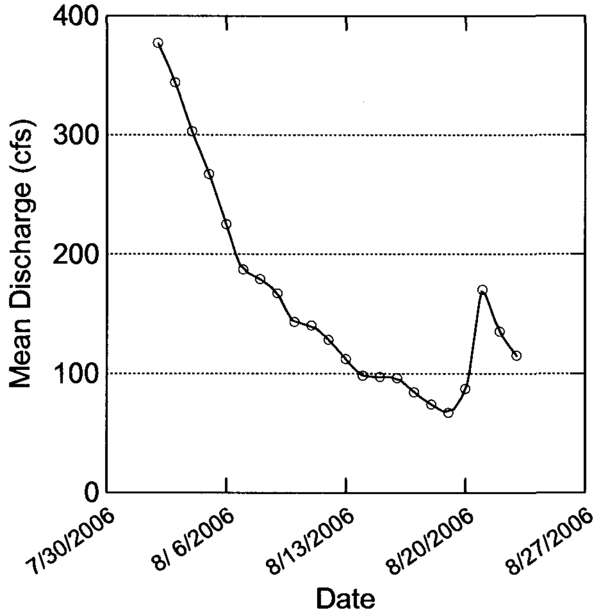


Figure 2.3 Mean daily discharge recorded at USGS gauging station 01038000 approximately 50 m downstream from NDS from 8/2/2006-8/23/2006.

2.4.2 Water chemistry

The NH_3 measurements were consistently at the reporting limit of 10 μg L^{-1} (Table 2.3). TKN ranged from 300 to 500 μg L^{-1} with a mean and standard deviation of 420 and 80 μg L^{-1}. NO_x ranged from 10 to 40 μg L^{-1} with a mean and standard deviation of 23 and 10 μg L^{-1}. Similar to NH_3, SRP was consistently 2 μg L^{-1} and just above the detection limit. TP ranged from 13 to 18 μg L^{-1} with a mean and standard deviation of 15 and 2 μg L^{-1}. The DIN:SRP ratios ranged from 4.5 to 11.3 μg L^{-1} with a mean and standard deviation of 7.6 and 1.7 μg L^{-1}. The TN:TP ratios ranged from 10.4 to 15.0 with a mean and standard deviation of 13.0 and 1.5 μg L^{-1}. ALK ranged from 10-14 mg L^{-1} with a mean and standard deviation of 13 and 2 mg L^{-1}. DOC ranged from 7.1 to 10.0 mg L^{-1} with a mean and standard deviation of 8.1 and 1.1 mg L^{-1}.

Table 2.3 Water chemistry measured before and during the deployment of NDS.

Parameter	N	Reporting Limit	Min	Max	Mean	SD	Method
NH_3 (μg L^{-1})	6	10	10	10	10	0	EPA Method 350.1, Lachat 10-107-06-1-B
TKN (μg L^{-1})	6	100	300	500	420	80	EPA Method 351.2, Lachat 10-107-06-2-E
NOx (μg L^{-1})	6	10	10	40	23	10	EPA Method 353.2, Lachat 10-107-04-1-C
TN (μg L^{-1})	6	--	320	520	440	70	NO_x + TKN
DIN (μg L^{-1})	6	--	20	50	33	8	NO_x + NH_3
SRP (μg L^{-1})	6	1	2	2	2	0	Lachat 10-115-01-1-B
TP (μg L^{-1})	6	1	13	18	15	2	EPA Method 351.2, Lachat 10-107-06-2-E
TN:TP	6	--	10.4	15.0	13.0	1.5	
DIN:SRP	6	--	4.5	11.3	7.6	1.7	
ALK (mg L^{-1})	6	0	10	14	13	2	Standard Method 2320B
DOC (mg L^{-1})	6	1	7.1	10.0	8.1	1.1	EPA Method 505 A

2.4.3 Chlorophyll a, chlorophyll b, and chlorophyll c

Mean chlorophyll *a* concentrations of the NDS ranged from 1.4 μg cm^{-2} (control) to 7.2 μg cm^{-2} (NP treatment) (Figure 2.4). The location of the treatments on NDS (*i.e.*, location of jar on a brick) was not a significant predictor of chlorophyll *a* (Table 2.4a). The N and P treatments and treatment interaction were significant predictors of chlorophyll *a* (Table 2.4b), indicating the stream was co-limited by N and P (Table 2.1). Three replicates are necessary to detect treatment effects, and 4 replicates are necessary to detect interaction effects with a two-fold increase of chlorophyll *a* (power analysis for two-way ANOVA, $power_{treatment}=0.93$, $power_{interaction}=0.91$). The number of necessary replicates was reduced to 2 for both the treatment and interaction effects to detect four-fold increase in chlorophyll *a* (power analysis for two-way ANOVA, $power_{treatment}=0.99$, $power_{interaction}=0.94$).

The NDS filters of the NP group were greener than those of the other groups (Figure 2.5), and chlorophyll *b* concentrations were greatest in the NP treatment (Figure 2.4). Most chlorophyll *b* concentrations were below the detection limit (0.53 μg cm^{-2}), except that all the NP replicates exceeded the detection limit. Results of ANOVA analysis of chlorophyll *b* concentrations may not be reliable because the N, P, and C treatments were all below detection limits, but the N and P treatments and treatment interaction were significant predictors of chlorophyll *b* (Table 2.4c). All chlorophyll *c* values were below the detection limit (1.05 μg cm^{-2}), and no ANOVA analysis was performed (Figure 2.4).

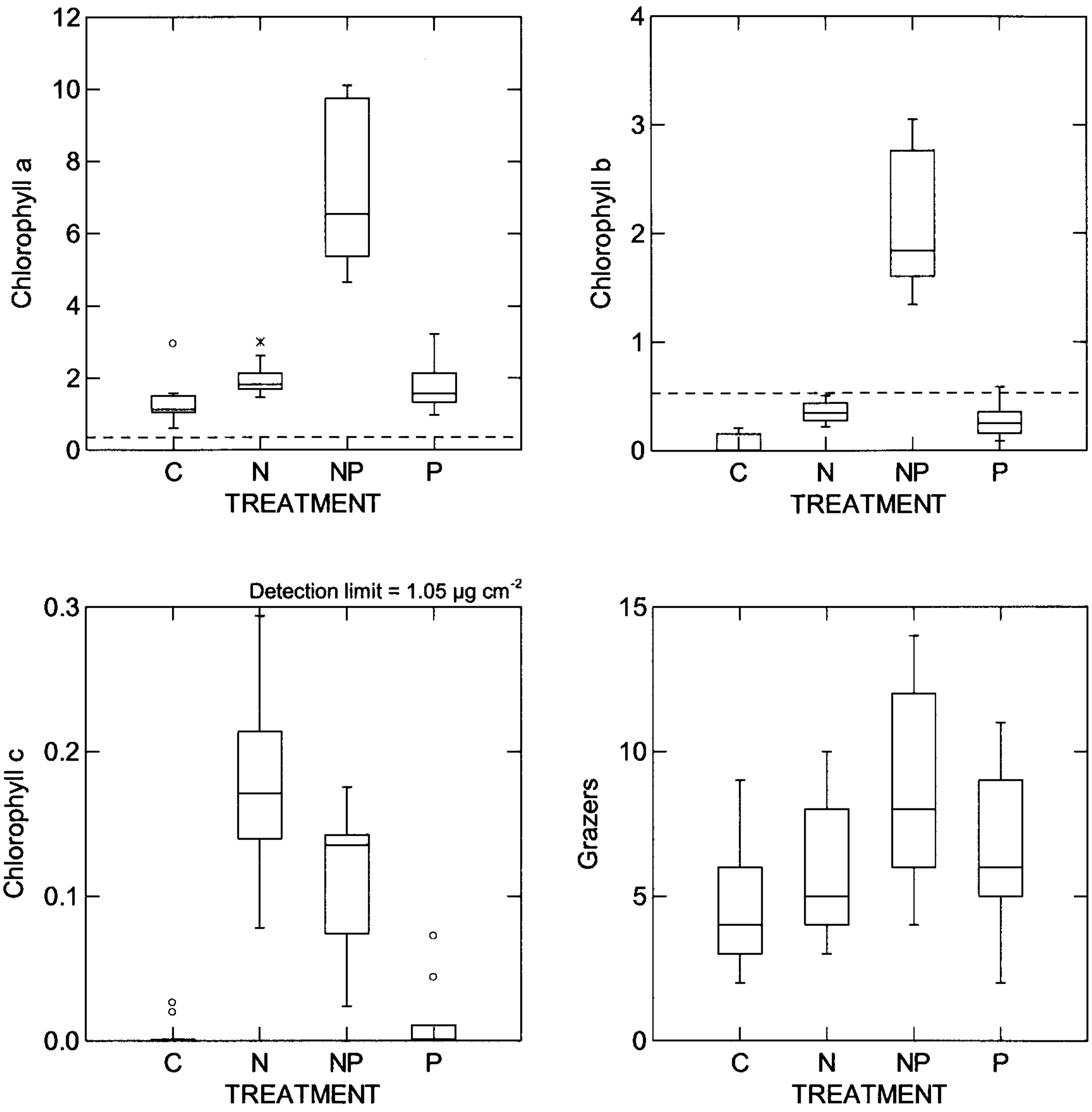


Figure 2.4 Chlorophyll *a*, *b*, and *c* (µg cm^{-2}) and abundance of grazers on the four NDS treatments (n=9) with detection limits displayed as dashed horizontal lines. All chlorophyll c samples were below detection limit.

Table 2.4 ANOVA comparisons of chlorophyll a and chlorophyll b with nutrient treatments, position on nutrient diffusing substrates, and number of grazing macroinvertebrates. ANOVA analyses performed on $\log_{10}$ transformed chlorophyll data.

Source	**Degrees of Freedom**	**F-ratio**	**P-value**
a) Two-way ANOVA of chlorophyll *a* with Treatment (C, N, P, NP) and Position on NDS (1, 2, 3, 4) as predictors (r^2=0.88)			
Treatment	3	61.3	<0.001
Position	3	1.2	0.530
Treatment x Position	9	1.7	0.398
Error	20	1.5	
b) Two-way ANOVA of chlorophyll *a* with N and P as predictors (r^2=0.80)			
N treatment	1	66.8	<0.001
P treatment	1	46.0	<0.001
NxP Interaction	1	18.5	<0.001
Error	32		
c) Two-way ANOVA of chlorophyll *b* with N and P as predictors (r^2=0.74)			
N treatment	1	48.4	<0.001
P treatment	1	37.0	<0.001
NxP Interaction	1	4.5	0.042
Error	32		
d) Two-way ANOVA of the number of grazing macroinvertebrates with N and P as predictors (r^2=0.26)			
N treatment	1	3.8	0.060
P treatment	1	7.1	0.012
NxP Interaction	1	0.3	0.598
Error	32		

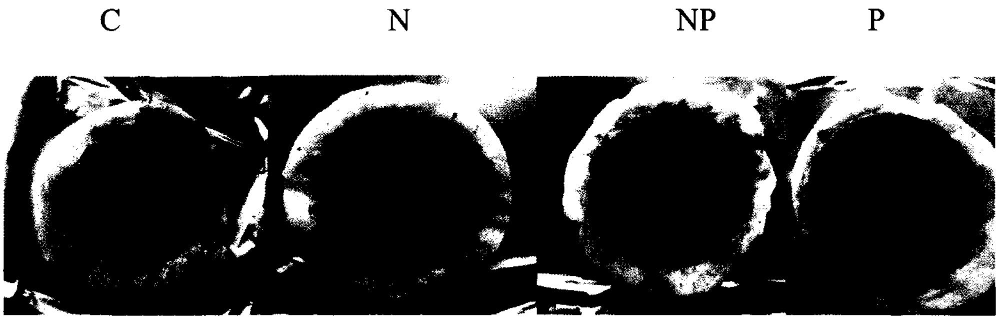


Figure 2.5 Representative filters (2.54 cm diameter) of the four NDS treatments.

2.4.4 Species composition

The duplicate natural substrate samples (NS1 and NS2) had similar taxa richness, and filamentous cyanobacteria were most abundant in both samples (Table 2.5). Taxa richness of NDS filters ranged from 11 (P) to 23 (C). In contrast to the natural substrate samples, cyanobacteria were uncommon, and colonial and unicellular green algae were among the dominant taxa on the NDS filters, especially on the P and NP filters. *Achnanthidium minutissima* and *A. rivulare* were abunant on all the NDS filters except the NP treatment. On the NDS filters, diatoms were the only algae observed that produce chlorophyll *c*, and green algae were the only algae observed that produce chlorophyll *b* (Graham and Wilcox 2000).

2.4.5 Invertebrates on NDS

Most macroinvertebrates on the NDS lids and filters were small, early instar grazers (Table 2.7, Figure 2.4). The mean number of grazers ranged from 4.4 (control) to 8.8 (NP), with *Helicopsyche* sp., early-instar Baetidae, and Hydrobiidae most commonly occurring. The P treatment was a significant predictor of the number of grazers, however the N treatment and interaction were not (Table 2.4c). The number of grazers generally increased with greater chlorophyll *a* (Spearman rank correlation=0.350; Figure 2.6).

Table 2.5 Relative abundance of species observed on filters of the four nutrient treatments (C, N, P, and NP) collected August 23, 2006 compared to 2 natural substrate samples (NS1 and NS2), each consisting of algae scraped from 18 rocks collected July 19, 2006. Taxa richness is included in parenthises. Bold indicates top five relative abundances of each sample.

Taxon Name	**NS 1 (n=55)**	**NS 2 (n=53)**	**C (n=23)**	**N (n=20)**	**P (n=11)**	**NP (n=16)**
Green Algae						
Ankistrodesmus sp.						1.65%
Botryococcus sp.						**4.39%**
Closterium sp.		0.03%	0.31%			
Cosmarium sp.			**11.31%**	0.35%		
Gloeocystis sp.					**10.11%**	**19.34%**
Mougeotia sp.			3.21%			
Sphaerocystis sp.				**7.62%**	**41.17%**	**40.05%**
Undetermined green coccoid 8-10µm (cf. *Chlamydomonas* spp.)						**18.52%**
Cyanobacteria						
Calothrix sp.	1.07%					
Homoeothrix janthina (Bornet et Flahault) Starmach	**10.67%**	**21.86%**		3.01%		**3.02%**
Homoeothrix juliana (Meneghini) Kirchner		**1.43%**				
Homoeothrix varians Geitler	**4.17%**					
Lyngbya sp.						1.51%
Lyngbya martensiana Meneghini	**3.66%**	**4.35%**				
Phormidium autumnale (CA Agardh) Gomont	**67.38%**	**65.33%**				
Phormidium cf. formosum (Bory ex Gomont) Anagnostidis et Komárek	1.22%					
Red Algae						
Audouinella sp.	**6.15%**	0.65%				
Diatoms						
Achnanthes subrostrata var. appalachiana Camburn & Lowe	0.06%					
Achnanthidium deflexum (Rabenhorst) Lange-Bertalot et Ruppel[1]		**1.81%**	1.22%	6.21%		
Achnanthidium minutissimum (Kützing) Czarnecki[1]	0.96%	1.11%	**33.33%**	**35.28%**	**7.84%**	1.51%

Table 2.5 Continued

Taxon Name	**NS 1**	**NS 2**	**C**	**N**	**P**	**NP**
Achnanthidium rivulare Potapova et Ponader[1]	0.75%		**7.65%**	**10.11%**	**22.93%**	
Adlafia suchlandtii (Hustedt) Lange-Bertalot	0.09%	0.08%				
Amphipleura pellucida (Kützing) Kützing	0.02%	0.05%				
Amphora pediculus (Kützing) Grunow	0.01%		1.53%	0.71%	4.56%	3.02%
Aulacoseira ambigua (Grunow) Simonsen	0.03%					0.14%
Brachysira microcephala (Grunow) Compère	0.01%	0.02%		0.89%		
Cocconeis placentula var. lineata (Ehrenberg) Van Heurck	0.20%	0.12%	**8.72%**	2.13%	2.14%	2.20%
Cyclotella meneghiniana Kützing	0.01%		4.89%	2.66%		
Cymbella affinis Kützing	0.01%					
Cymbella cesatii (Rabhenhorst) Grunow ex Schmidt	0.05%					
Cymbella cymbiformis Agardh	0.02%					
Cymbella gracilis (Ehrenberg) Kützing		0.02%	0.31%	0.35%		
Cymbella subturgidula Krammer	0.09%	0.16%				
Cymbella tumida (Brébisson ex Kützing) Van Heurck	0.15%	0.07%				
Diploneis ovalis (Hilse ex Rabenhorst) Cleve		0.01%				
Discostella stelligera (Hustedt) Houk et Klee	0.03%	0.03%				
Encyonema minutum (Hilse) Mann	0.09%	0.14%	**10.40%**	**10.28%**	1.85%	1.65%
Encyonema silesiacum (Bleisch) Mann	0.48%	0.57%	1.53%	4.08%	0.28%	
Eunotia implicata Nörpel, Lange-Bertalot et Alles	0.02%					
Eunotia incisa Smith ex Gregory	0.05%	0.05%	0.46%			
Eunotia pectinalis (Müller) Rabenhorst	0.03%	0.01%				

Table 2.5 Continued

Taxon Name	**NS 1**	**NS 2**	**C**	**N**	**P**	**NP**
Fragilaria capucina Desmazières	0.13%	0.07%	0.92%	3.37%		1.10%
Fragilaria capucina var. gracilis (Østrup) Hustedt	0.38%	0.51%	0.92%	0.89%		
Fragilaria sepes Ehrenberg		0.03%				
Fragilaria vaucheriae (Kützing) Petersen	0.09%	0.19%				
Frustulia crassinervia (Brébisson) Lange-Bertalot et Krammer		0.01%	1.84%			0.27%
Frustulia vulgaris (Thwaites) deToni		0.01%				
Geissleria decussis (Hustedt) Lange-Bertalot et Metzeltin	0.01%					
Gomphonema gracile Ehrenberg emend Van Heurck	0.02%	0.03%				
Gomphonema micropus Kützing		0.03%				
Gomphonema minutum (Agardh) Agardh	0.02%		0.31%	2.13%		
Gomphonema parvulum (Kützing) Kützing	0.33%	0.08%	6.42%	**8.69%**	0.71%	
Gomphonema pumilum (Grunow) Reichardt et Lange-Bertalot	0.09%	0.01%	0.46%			
Gomphonema rhombicum Fricke	0.54%	0.19%				
Gomphonema ventricosum Gregory	0.02%					
Melosira varians Agardh	0.02%					
Meridion circulare var. constrictum (Ralfs) Van Heurck		0.02%				
Navicula antonii Lange-Bertalot	0.14%					
Navicula cryptocephala Kützing		0.01%	0.61%	0.35%		0.14%
Navicula cryptotenella Lange-Bertalot ex Krammer et Lange-Bertalot	0.02%	0.04%	2.14%		**6.70%**	1.51%
Navicula laterostrata Hustedt	0.01%	0.02%				
Navicula mobiliensis Boyer		0.01%				
Navicula radiosafallax Lange-Bertalot			0.92%			

Table 2.5 Continued

Taxon Name	**NS 1**	**NS 2**	**C**	**N**	**P**	**NP**
Navicula recens Lange-Bertalot		0.04%		0.53%		
Navicula rhynchocephala Kützing		0.01%				
Navicula rostellata Kützing	0.01%					
Neidium bisulcatum (Lagerstedt) Cleve	0.01%					
Nitzschia dissipata (Kützing) Grunow	0.01%	0.02%				
Nitzschia frustulum (Kützing) Grunow	0.01%	0.01%			1.71%	
Nupela neglecta Ponader, Lowe et Potapova	0.10%					
Pinnularia gibba Ehrenberg		0.01%				
Pinnularia obscura Krasske		0.03%				
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	0.01%					
Psammothidium chlidanos (Hohn et Hellerman) Lange-Bertalot	0.01%					
Pseudostaurosira trainorii Morales		0.01%				
Reimeria sinuata (Gregory) Kociolek et Stoermer		0.01%				
Rossithidium linearis (Smith) Round et Bukhtiyarova		0.02%				
Sellaphora pupula (Kützing) Meresckowsky		0.01%				
Sellaphora seminulum (Grunow) Mann	0.01%					
Staurosira construens var. venter (Ehrenberg) Hamilton	0.01%	0.02%				
Surirella amphioxys Smith		0.01%				
Synedra acus Kützing		0.37%				
Synedra rumpens Kützing	0.41%					
Synedra ulna (Nitzsch) Ehrenberg	0.04%	0.08%	0.61%	0.35%		
Synedra ulna var. contracta Østrup	0.07%	0.11%				
Tabellaria flocculosa (Roth) Kützing	0.04%	0.03%				

1- Achananthidium species identifications on NDS not certain (*i.e.*, *Achnanthidium cf. deflexum, A. cf. minutissimum*, and *A. cf. rivulare*)

Table 2.6 Mean abundance of grazers on NDS lids or filters. (n=9 per treatment)

Taxon	Group	N treatment	P treatment	NP treatment	C treatment
Rheotanytarsus sp.	Midge	0.78	1.33	0.78	2.00
Limnephilidae*	Caddisfly	0.00	0.11	0.00	0.33
Helicopsyche sp.*	Caddisfly	2.78	3.67	4.67	1.78
Baetidae*	Mayfly	2.33	1.78	3.44	2.11
Ancylidae*	Limpet	0.44	0.44	0.22	0.22
Hydrobiidae*	Snail	0.22	0.44	0.44	0.00
Total		6.56	7.78	9.56	6.44
Grazers		5.78	6.44	8.78	4.44

* - classified as a grazer in this project

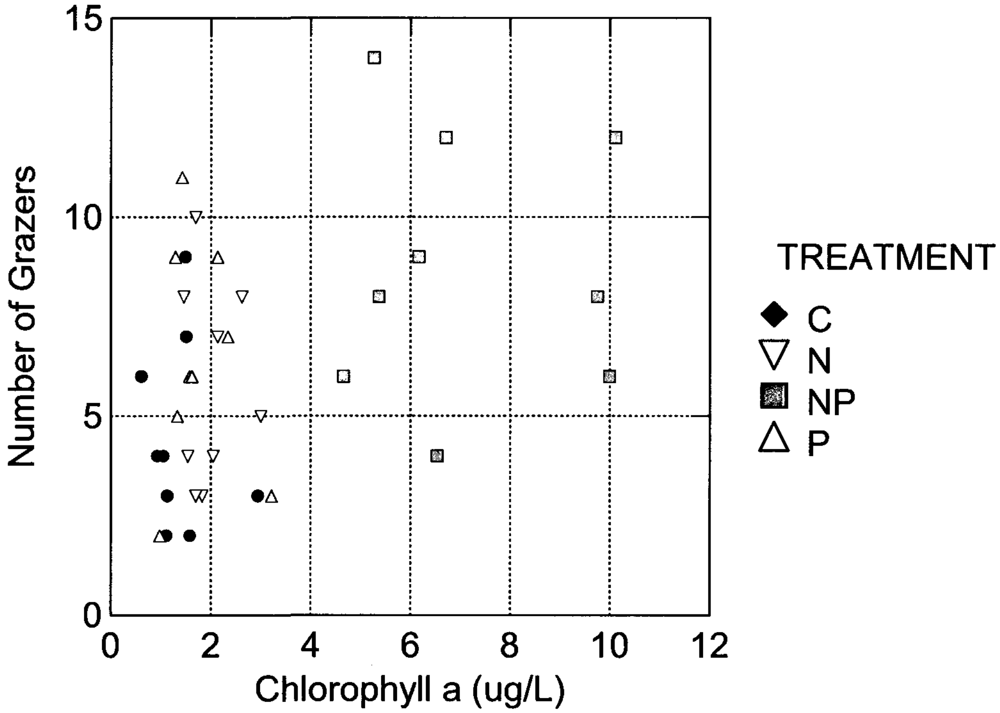


Figure 2.6 Relationship between chlorophyll *a* and the number of "grazer" macroinvertebrates observed on the NDS (n=9) filters and lids of each treatment at the time of collection.

2.5 Discussion

2.5.1 Nutrient ratios

The DIN:SRP ratio consistently was < 10, suggesting that the river was N-limited. In contrast, the TN:TP ratio was consistently 10-16, suggesting that the river was co-limited by N and P, and the NDS experiment concluded that the river was co-limited. The TN:TP ratio conclusion matched the NDS conclusion, but the NDS results probably were attributable to the scarcity of nutrients rather than the nutrient ratio. Nutrient ratios are poor predictors of nutrient limitations when excess nutrients are available (Borchardt 1996, Dodds et al. 2002) or when both N and P are scarce (Tank and Dodds 2003). The Sheepscot River would be categorized as oligotrophic based on Dodds et al. (1998) trophic classification of streams, which defines the oligotrophic-mesotrophic boundary at 700μg L^{-1} TN and 25 μg L^{-1} TP. Concentrations of inorganic nutrients, which are thought to be most readily available to algae (Pringle 1987), were consistently at or slightly greater than reporting limits in this study. Algae colonizing the NDS were opportunistically assimilating any nutrients that were available. SRP, but not DIN, concentrations were below levels shown to saturate algal cell division and biomass in other northern coldwater rivers (Bothwell and Stockner 1980, Bothwell 1985, 1989, Chambers et al. 2000).

Nutrient ratios provide information only about whether a nutrient *might* be limiting (Francouer et al. 1999). Nutrients are only one of many environmental resources or disturbances that control the growth and accumulation of benthic algae (Biggs 1996). Nutrient ratios may be less meaningful if another resource limits algal growth, such as temperature (Cairns 1956, Francoeur et al. 1999) or light availability (Hill and Knight

1988, Winterbourn 1990, Bourassa and Cattaneo 2000, Parkhill and Gulliver 2002). Similarly, nutrient ratios may be poor predictors if disturbances such as scouring (Horner and Welch 1981, Horner et al. 1990) and grazing (McCormick and Stevenson 1989, Power 1990, Rosemond et al. 1993, Hillebrand 2002, Hillebrand et al. 2002) control algal growth and accumulation. Further, nutrient ratios derived from a set of discrete measurements can underestimate availability of nutrients, because nutrient supply is not constant. The discrete set of measurements may fail to capture spikes in nutrient availability after storms, especially for P. Some algae can uptake nutrients when they are available, storing them for later use (Fitzgerald and Nelson 1966). In this study, light was not a limiting resource in this study because there was only 9% canopy cover and the average depth was within the photic zone (Wetzel 1976). Similarly, water temperature in this study was generally favorable to algal diversity and production (DeNicola 1996). Grazing by macroinvertebrates could have reduced algal accumulation on the NDS in this study, but grazer abundance mirrored patterns chlorophyll *a*, and presumably availability in food.

TN:TP ratios may not be appropriate for NDS studies because measurements of TN and TP concentrations include forms of nutrients, such nutrients incorporated in biota or strongly bound to aluminum hydroxides, that are thought to be not readily available to algae during the three-week colonization period (Pringle 1987, Kopatek et al. 2005). TN:TP ratios are more appropriate for studies of periphyton (Dodds 2003), because algae within periphyton mats can produce enzymes to obtain nutrients from sediment, decomposing organic matter, and exudates of organisms (Fitzgerald and Nelson 1966, Tuchman et al. 2006). NDS filters probably do not provide the spatial and temporal complexities of periphyton mats and offer less opportunity to obtain nutrients that are not readily available.

2.5.2 Chlorophyll a, b, and c

Chlorophyll *a* concentrations on the N and P treatments were two times greater and the NP treatments were six times greater than the C treatments. These results clearly indicate that the stream reach was co-limited by N and P during the time period of the study. Chlorophyll *a* often is interpreted as increased algal biomass, but it also can indicate algal cell health and can be affected by light, nutrients, and temperature (Hill et al. 1995, Geider et al. 1998). The concentration of chlorophyll *a* is a common measurement of algal response to nutrient enrichment, but relationships between nutrients and chlorophyll are weaker in streams than in lakes (Dodds et al. 2002). Lake chlorophyll-TP relationships are strong, in part, because the TP includes P contained within the phytoplankton collected in the water sample. Chlorophyll-TP relationships also can be strong in large rivers (Van Nieuwenhuyse and Jones 1996). In contrast, stream algae often occur as periphyton mats that are not collected in water samples and thus do not contribute to stream water chlorophyll measurements. Periphyton mats can assimilate nutrients, reducing nutrients left in the water to low concentrations so that the nutrient enriched condition of the stream water is not apparent (Stevenson et al. 2006). Abundant algal biomass also can occur in nutrient poor conditions if nutrients are retained within the algal mat. Other stream characteristics can confound relationships between water column nutrient concentrations and algal biomass, including: nitrogen fixation by algae (Biggs and Smith 2002), form of nutrients (Chambers *et al.*, 2000; Biggs and Smith, 2002), discharge (Duncan and Blinn 1989), turbidity and shading (Triska et al. 1983, Lowe et al. 1986, Bothwell 1988, Hill and Knight 1988, Duncan and Blinn 1989, Stevenson et al. 1991, Bourassa and Cattaneo 2000, Parkhill and Gulliver 2002), flood disturbance (Humphrey and Stevenson 1992, Lohman et al. 1992, Biggs 2000a, Biggs and Smith 2002), and grazing (McCormick and Stevenson 1989, Steinman et al. 1991, Rosemond et al. 1993, McCormick 1994, Rosemond 1994, Mulholland et al. 1995).

Chlorophyll *b* and *c* concentrations, although not commonly used in NDS studies, can potentially indicate nutrient preferences of different algal groups because not all algal groups produce them. Large chlorophyll *b* concentrations suggest an increase in green algae or euglenoids (Graham and Wilcox 2000). The treatments with the greatest chlorophyll *b* concentrations had the greatest abundance of green algae and no euglenoids, however inferences from my species data are limited because species were identified from only one filter per treatment. Chlorophyll *c* concentrations were below detection limit in our study, but chlorophyll *c* concentrations could be used in a similar way to infer nutrient preferences of diatoms. Future research is needed to better describe patterns in algal community response to different combinations of N and P enrichment.

Glass fiber frits (Tank et al. 2006) and Nitex polyester mesh (Biggs et al. 1998, Biggs and Kilroy 2000) could be used in NDS instead of glass fiber filters because they are more durable and and allow easier removal of algal cells. Representative portions of each NDS could be used for species identification and chlorophyll analysis. Chlorophyll *b* and *c* could be analyzed along with chlorophyll *a* and could be used to identify preferential response of major algal groups. NDS replicates should be anchored in some way to the stream bottom (*e.g.*, Biggs and Kilroy 2000, Tank et al. 2006) to prevent loss of NDS during high flows, however, cross contamination among treatments should be avoided. Grazing pressure could be a confounding factor in NDS experiments and future experiments might include exclosures to prevent grazing (Busse et al. 2006, Ludwig et al. 2008), such as a fine clear, plastic mesh to exclude grazing by macroinvertebrates and minimize shading. Periodic cleaning would be necessary to prevent accumulation of debris. Three to five NDS replicates of each treatment, depending on ambient nutrient concentrations, should be deployed in Maine streams. Fewer replicates would be necessary in oligotrophic streams, where a small increase in available nutrients should elicit a rapid response. An enriched stream may require more replicates to quantify the potentially variable response.

Some caution should be used extrapolating species patterns in NDS experiments to benthic algal communities, because the community composition can be different. In this study, the rock and NDS samples had different community composition, which could be explained in part by differences in processing and identifying samples. The rock samples were processed and identified with more rigorous protocols because of difficulty removing algal cells from the glass fiber filters. It may be possible to use the same methods used for the rock samples for the NDS if nitex mesh or glass frits are used instead of the filters. The algal communities on the NDS also may have fewer taxa and could favor early colonizers (*e.g.*, *Achnanthidium* spp.) because they are at an early successional stage (Peterson 1996). The NDS had fewer filamentous cyanobacteria, stalked diatoms (*e.g.*, *Gomphonema* spp.), and motile diatoms (*e.g.*, *Navicula* and

Nitzschia spp.) than the more mature rock samples. The NDS also could favor initial colonizers because nutrient release from NDS are greatest within the first 48 hours of submersion (Pringle and Bowers 1984, Fairchild et al. 1985, Scrimgeour and Chambers 1997), and release rates decrease log linearly. Luxury uptake of nutrients by early colonizers could provide a competitive advantage over later colonizers (Borchardt 1996). Green algae (*e.g.*, *Sphaerocystis* spp.) could have settled on the nutrient enriched NDS and found a favorable environment with few competitors to grow and reproduce. Finally, natural substrates provide greater surface complexity than NDS, including thicker periphyton mats and presence of aquatic mosses (*e.g.*, *Fontinalis* spp.), which could affect community composition (Pringle 1990). Recognizing differences in species composition, further research is needed to determine if algal communities on NDS have similar response to benthic communities in streams that are being enriched with nutrients.

2.5.3 Management implications

The NDS experiment identified N and P as co-limiting nutrients in the Sheepscot River. MDEP previously managed P inputs to maintain the oligotrophic condition of the Sheepscot River and other Class AA and A rivers and streams. The NDS experiment demonstrated that either N or P alone could increase algal productivity and that simultaneously increasing both could greatly increase algal growth. MDEP can use this information to evaluate and revise management practices to account for the added risk of N enrichment. Further research is needed to evaluate spatial and temporal variability of nutrient limitation in the Sheepscot River, because nutrient limitation of rivers can vary spatially (Chambers et al. 2000) and seasonally (Wold and Hershey 1999).

3. ESTIMATING NUTRIENT CONCENTRATIONS IN MAINE STREAMS BASED ON BENTHIC DIATOM ASSEMBLAGES

3.1 Abstract

This study compares two general approaches for developing inference models to predict total phosphorus (TP) and total nitrogen (TN) concentrations in streams based on benthic algal communities. The first approach is to use Weighted Averaging (WA) transfer functions based on the assumption that species have unimodal response curves to environmental gradients. Several variations of weighted averaging models, including Weighted Averaging – Partial Least Squares (WA-PLS) and Locally-Weighted Weighted Averaging (LWWA) are also considered. The second approach is to use multiple linear regression (MLR) with stepwise selection of diatom species to predict TP concentrations. The WA models consistently had the greatest model bias and worst performance. The MLR models with stepwise selection of species outperformed WA and WA-PLS and had less bias than LWWA. LWWA performed better than WA and WA-PLS. Weighted averaging models may have been hindered because only one third of the species had unimodal response curves for TP or TN. In addition, approximately one third of the algal species were indifferent to TP or TN. However, removing indifferent species from weighted averaging models did not improve model performance. Adding soft algae taxa to weighted averaging models also did not improve model performance. A "slope-snapping" technique was developed to account for model bias associated with weighted averaging models. MLR could be considered as an alternative to weighted averaging

inference models, especially in situations where few taxa have unimodel responses to environmental gradients. LWWA could be a useful alternative to traditional WA and WA-PLS models in other situations.

3.2 Introduction

Managing nutrients and the proliferation of algae in streams and rivers is a problem for water resource managers. The United States Environmental Protection Agency (USEPA) responded to this challenge by adopting a national strategy to establish ecoregional criteria to limit ambient nutrient concentrations (USEPA 1998, 2000). Nutrient concentrations can fluctuate in response to storm events, nonpoint source runoff, stream bank erosion, and point source discharges, but chemical and physical characteristics of streams vary and "cannot be reduced to a simple standard" (Patrick 1949, p. 294). Repeated sampling to account for this variation can be expensive and logistically challenging, especially if locations are spatially dispersed (Cattaneo and Prairie 1995). In addition, nutrients are only one of many environmental factors influencing the accumulation of benthic algae. Resources and disturbances, such as light availability, temperature, grazing, and scouring, can prevent nuisance algal growths (Biggs 1996). Conversely, there can be negative effects from algal growth despite low natural nutrient concentrations. Monitoring programs may underestimate the effects of nutrient enrichment by focusing only on concentrations of nutrients in stream water and missing sporadic pulses of nutrients with scheduled sampling. Some species of algae take up and store nutrients after storm events, and algal mats can remove nutrients from the water column (Stevenson et al. 2006). Relationships between nutrients, algal biomass, and chlorophyll *a* concentrations of benthic algae also vary because of other resources and disturbances (Dodds and Welch 2000, Stevenson et al. 2006).

Benthic diatom assemblages are an alternative to water sampling for monitoring nutrient enrichment in wadeable streams and rivers (or sections thereof) (Winter and Duthie 2000, Potapova and Charles 2007, Porter et al. 2008). Diatom assemblages respond quickly to nutrient enrichment compared to macroinvertebrate and fish assemblages. Diatom indicators are better than single measurements of water chemistry, because they integrate environmental conditions over time (Stevenson 2006) and may be a more cost effective means to infer environmental conditions than multiple measurements of chemical or physical parameters (ter Braak and Barendregt 1986, Philibert et al. 2006). Diatom assemblages integrate nutrient concentrations of the previous 1-5 weeks depending on existing stream trophic state, and one diatom sample can provide the same information as up to 16 TP samples collected over a period of weeks (Lavoie *et al.* 2008).

Several analytical approaches can be used to estimate trophic status or infer nutrient concentrations of rivers and streams based on the composition of algal assemblages. Weighted average indices of categorical values assigned to diatoms based on their trophic preferences have been applied to assess trophic state of rivers (Kelly and Whitton 1995, Kelly 1998a, b, Kelly et al. 2008, Lavoie et al. 2008, Porter et al. 2008). Some algal species traits have been applied nationally or globally (Lowe 1974, van Dam et al. 1994), whereas others are developed for specific regions (Stevenson et al. 2008a). Alternatively, Weighted Averaging (WA) and Weighted Averaging – Partial Least

Squares (WAPLS) inference models have been developed to infer nutrient concentrations of New Jersey high and low gradient streams (Ponader et al. 2007, 2008) northern Piedmont streams (Potapova et al. 2004), the western United States (Stevenson et al. 2008b), and Australia (Philibert et al. 2006). In contrast to indices based on categorical traits, inference models estimate ambient nutrient concentrations with empirically-derived estimates of diatom optima. Inference models based on continuous scales have more predictive capacity than models built on discrete scales (DeNicola et al. 2004). Inference models based on local or regional data sets provide more accurate predictions of stream nutrient condition than those that are created with data from large geographic areas (Pan et al. 1996, Potapova et al. 2004, Charles et al. 2006, Ponader et al. 2008), but are also limited to those geographic areas from which they were developed. Variations of WA and WA-PLS inference models also have been developed for conductivity (Potapova and Charles 2003), pH (Pan et al. 1996), and multiple chemical and land use variables (Stevenson et al. 2008b). Locally-Weighted Weighted Averaging (LWWA) is a recent variation of that uses local means in calculations and performs well with large, heterogeneous data sets (Battarbee et al. 2005, Raunio et al. 2010).

Multiple linear regression (MLR) has been used in several paleoecological inference models of lake diatoms (Charles 1985, Davies and Smol 1985, Flower 1986, Jones et al. 1989) and pollen (Webb and Clark 1977), however, it has not been widely applied to streams. ter Braak and Looman (1986) found that MLR performed better than WA with training data but not with test data. Multiple linear regression is affected by high correlations or multicolinearity among species, however, performance improved with log-transformed diatom abundances (ter Braak and van Dam 1989). In addition, MLR coefficients cannot be interpreted as optima.

The first objective of this study is to compare inferences of nutrient concentrations in Maine's wadeable streams with MLR models and WA, WA-PLS, and LWWA inference models. The second objective is to evaluate performance of inference models with the addition of some green algae (Chlorophyta), red algae (Rhodophyta), yellow-green algae (Xanthophyceae), and cyanobacteria (Cyanophyta) (Guiry and Guiry 2010). The third objective is to test several methods to remove inference model bias. Persistent model bias (*i.e.*, overestimating high values and underestimating low values) can be problematic, especially when managing high quality waters with low nutrient concentrations.

3.3 Methods

3.3.1 Study Sites

Samples (n=298) were collected from 193 locations distributed across Maine spanning Level-3 ecoregions in the Acadian Plains and Hills (n=136), Northeastern Highlands (n=35), and Northeastern Coastal Zone (n=22) (Figure 3.1, Omernik 1987, Griffith et al. 2009). The Northeastern Highlands is mountainous, primarily acidic soils and forested. The Acadian Plains and Hills is a mixture of rolling hills and glacial deposits. Some areas within the coastal zone are flat and dominated by glacial or marine deposits. Most of the Acadian Plains and Hills ecoregion is forested, but much of the state's population is located within several urban areas in the southern and central portion of this ecoregion. Tilled agriculture, pasture, and blueberry barrens are present where

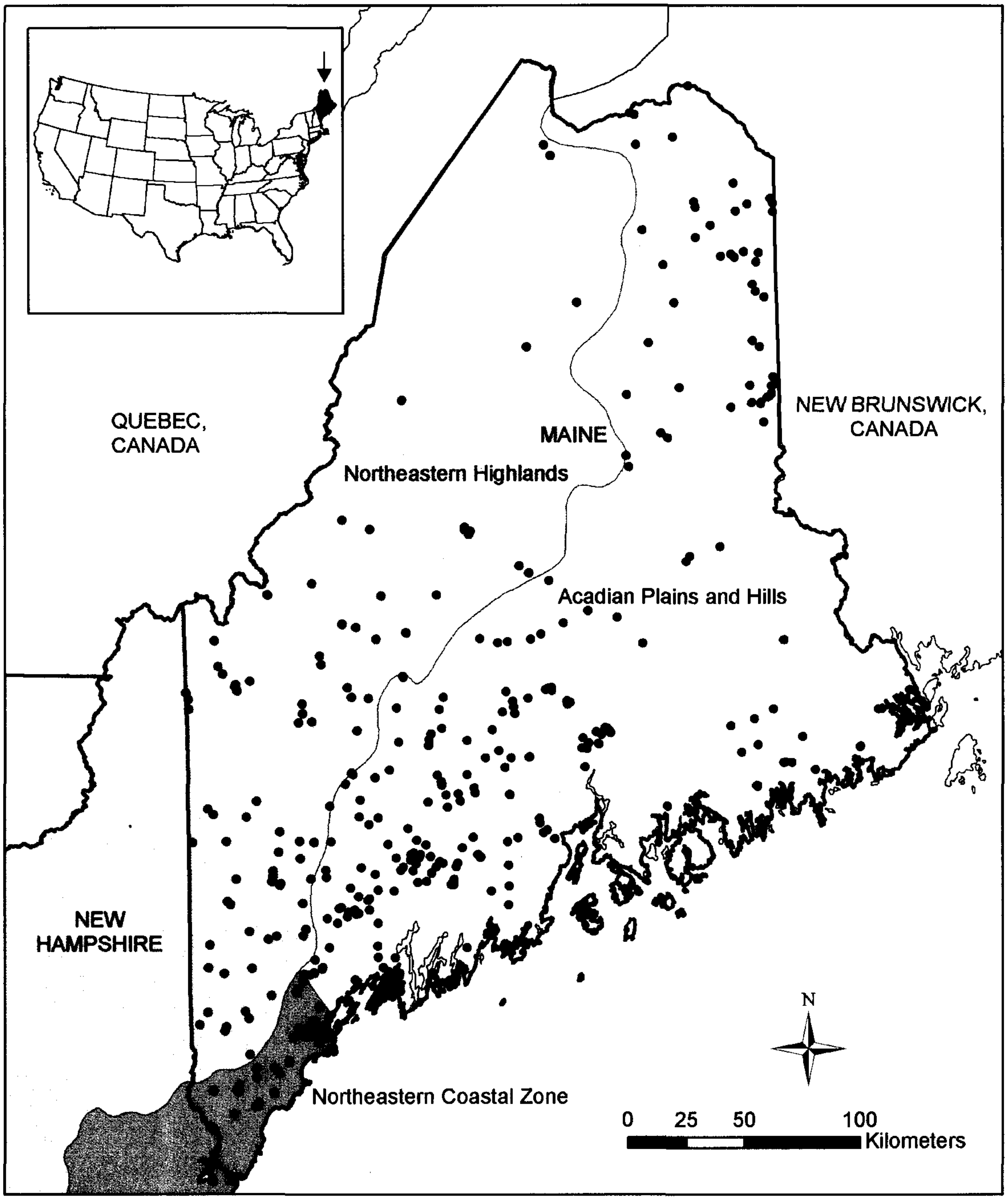


Figure 3.1 Sample locations and ecoregions (Omernik 1987, Griffith et al. 2009).

conditions are suitable. Soils in some portions of the Acadian Plains and Hills are calcium-rich and there is some intensive agriculture, however, most of the ecoregion is forested. The Northeastern Coastal Zone has sandier soils and a mosaic of forest, urban, rural, and residential areas. The Northeastern Coastal Zone has the state's largest city and is the most developed ecoregion.

I selected study sites to represent a range of natural conditions and also a range or watershed disturbance. Drainage areas of sample locations ranged from 0.17 to 3,660 km^2. Minimally disturbed sites represented reference conditions (Stoddard et al. 2006), with (1) >95% of upstream watershed consisting of forest or wetland, (2) no upstream dams, and (3) no point source discharges such as wastewater treatment plants or fish hatcheries. Logging occurs in the watersheds of some minimally disturbed sample locations, and many stream channels were altered by historic log drives. Acid rain and atmospheric deposition of chemicals, such as mercury, occur across the state. Other sample locations occur in watersheds with intermediate to severe disturbance due to increasing urbanization, agriculture, or point source discharges of pollutants.

3.3.2 Field and laboratory procedures

Algal samples were collected from riffles or runs in late June or July 1999-2006. Six transects were established within a stream reach and perpendicular to stream flow. Cobbles or small boulders (n=18) were collected along the near, middle, and far parts of stream reaches, avoiding eddies, pools, back waters, and areas along the bank where benthos could be exposed by fluctuating water levels. Epilithic algae were removed with a wire brush from a circular area on each rock by placing a neoprene washer with a 2.54-cm diameter opening and brushing the area with a stiff-bristled brush. Algae scraped off all rocks were combined into a single sample representing the stream reach. Samples were preserved with 1 mL M^3 per 50 mL of sample (Eaton et al. 2005). Diatoms and soft algae were identified and enumerated by taxonomic specialists (Michigan State University and The Patrick Center for Environmental Research of The Academy of Natural Sciences) following methods described by Charles et al. (2002). Algal counts were expressed as cell densities (cells/cm^2 substrate) and cell biovolumes (mm^3 cells/cm^2 substrate). Water samples and measurements of study site characteristics were collected concurrently with algal samples (Table 3.1). Watersheds upstream of sample locations were digitized from elevation contours from USGS 1:24,000 scale quadrangles (ESRI® ArcMap™ 9.2, Redlands, CA). Percent of land area within watersheds consisting of impervious surfaces (MeGIS 2004a) and forest, wetland, tilled agriculture (MeGIS 2004b) were calculated (Table 3.1). Percent developed watershed was estimated by subtracting percent forest and percent wetland from 1.

Table 3.1 Environmental parameters collected at 190 independent samples during 1999-2006 in Maine.

Parameter	**Code**	**Min**	**Max**	**Mean**	**1st quart.**	**Median**	**3rd quart.**	**Method**	**Detection Limit**
Watershed Area (km^2)	AREA	0.7	3,660	217	18	82	211	calculations in ArcMap	--
% Forest and Wetland	FORWET	6	100	80	74	88	96	calculations in ArcMap	
% Wetland	WET	0	45	7	3	6	10	calculations in ArcMap	--
% Developed	DEV	0	94	20	4	12	26	calculations in ArcMap (1 - FORWET)	--
% Impervious	IMP	0	46	5	0	2	4	calculations in ArcMap	--
% Tilled Agriculture	AG	0	71	6	0	2	5	calculations in ArcMap	--
UTM longitude (m)	UTM_X	338019	650703	470486	396507	486240	558029	Garmin Etrex, UTM Zone 19N, NAD 83	--
UTM latitude (m)	UTM_Y	4790631	5244806	4973692	4904439	4962147	5022636	Garmin Etrex, UTM Zone 19N, NAD 83	--
Elevation (m)	ELEV	3	713	134	32	79	134	Digitized 1:24,000 elevation contours	--
% Canopy Cover	COVER	0	66	22	9	19	33	spherical densiometer	--
Width (m)	WIDTH	0	282	16	3	9	20	representative bank width	--
Depth (cm)	DEPTH	1	140	32	18	29	40	average depth from 5 locations where rocks were collected in reach	--
Velocity (cm/sec)	VEL	0	120	36	20	35	61	Global Water, Inc. FP 101, depth integrated average of 5 locations in reach	--

Table 3.1 Continued

Parameter	**Code**	**Min**	**Max**	**Mean**	**1st quart.**	**Median**	**3rd quart.**	**Method**	**Detection Limit**
% Fines	FINES	0	98	32	18	5	10	Visual estimate of percent sand, silt, and clay in reach	--
Dissolved Oxygen (mg/l)	DO	3.9	14.1	8.8	8.1	8.8	9.4	Hanna Instruments® HI9142	1
Temperature (°C)	TEMP	9.8	27.8	21.1	18.8	21.5	23.9	Hanna Instruments® HI991300	0
pH	pH	5.9	9.0	7.1	6.8	7.1	7.5	Hanna Instruments® HI991300	0.1
Specific Conductance (μS/cm)	SPC	7	2,930	164	29	59	164	Hanna Instruments® HI991300	0
Alkalinity as $CaCO_3$ (mg/L)	ALK	2	206	37	8	16	45	Standard Method 2320B	0
Dissolved organic carbon (mg/L)	DOC	1.4	20.0	6.5	4.6	6.2	8.2	EPA Method 505 A	1
Total P (μg/L)	TP	3	870	28	11	18	30	EPA Method 351.2, Lachat 10-107-06-2-E	1
Soluble reactive P (μg/L)	SRP	1	130	6	1	2	5	Lachat 10-115-01-1-B	1
Total N (mg/L)	TN	0.13	3.99	0.56	0.31	0.45	0.72	Nitrate + Nitrite + TKN	0.11
Nitrate + Nitrite (mg/L)	NOx	0.01	1.6	0.16	0.02	0.06	0.22	EPA Method 353.2, Lachat 10-107-04-1-C	0.01
Total Kjeldahl N (mg/L)	TKN	0.1	3.7	0.4	0.3	0.4	0.5	EPA Method 351.2, Lachat 10-107-06-2-E	0.1

3.3.3 Analytical methods

Species and Environmental Patterns. Nutrient data were divided into a training set (123 independent samples) and a validation set (75 samples; 42 independent samples and 33 samples from training set sites from different sample years). Major patterns in environmental variables were correlated with the patterns in species composition with nonmetric multidimensional scaling (NMS) (PC-ORD v. 5, McCune and Mefford 1999). NMS is an unconstrained ordination technique that is particularly useful for ecological data with a large number of taxa and many zero counts (McCune and Grace 2002). A subset of 81 samples with data for all environmental variables (Table 3.1) was included in the NMS analysis. Algal species occurring in <7 training set samples were excluded to improve ordination performance (McCune and Grace 2002), resulting in 182 species. The influence of numerically abundant, ubiquitous taxa such as *Achnanthidium minutissimum* (Kützing) Czarnecki and *Gomphonema parvulum* (Kützing) Kützing was reduced by transforming all taxa abundances to square roots of percent abundances. The "slow and thorough" method (McCune and Grace 2002) was used with Sorenson distance to recommend the number of axes that should be used in the final solution by cycling through 500 Monte Carlo permutations comparing results of 1-6 axes. After selecting the number of axes in the final solution, NMS performed 250 runs with real data and 250 runs with randomized data to avoid local minima. Performance of the final solution was expressed as the final stress and instability. The secondary matrix included environmental variables (Table 3.1) displayed on NMS graphs as vectors, with line length representing axis correlation strength. The resulting NMS graphs were rotated to the most correlated environmental variable to improve interpretation of results.

Species Responses to TP and TN. General linear models were estimated to describe response of species' square root percent abundances to $\log_{10}$ transformed TP or TN. General linear models were fit (Canoco 4.55, CanoDraw 4.14) with the Poisson distribution and stepwise addition of linear and quadratic terms to the null model (ter Braak and Smilauer 2002). Akaike's Information Criteria was used to select the best fitting model from three options: quadratic (second-order), linear (first-order), no response (flat line) (Hastie and Tibshirani 1990). Significant quadratic models imply symmetrical parobolic or Gaussian response curves, and significant linear models imply monotonically increasing or decreasing responses curves (Potapova et al. 2004). General linear models can describe non-linear relationships despite adding variables in linear combinations. Linear (first-order) general linear model curves appear to look like exponential curves when plotted because of log-link functions (Potapova et al. 2004).

Weighted averaging optima for 211 diatom species were calculated with 163 training and independent validation samples (C2 version 1.5, Juggins 2007). TN and TP were $\log_{10}+1$ transformed, and taxa relative abundances were square-root transformed to reduce the influence of *Achnanthidium minutissimum* and other ubiquitous, abundant species (Dieffenbacher-Krall et al. 2007). The optima were back-transformed and were used in conjunction with the general linear model curves to describe species' responses to nutrient enrichment. A Pearson's correlation was performed on the species' TN and TP optima. Taxa optima were compared to optima of epilithic diatoms in the eastern North America (Winter and Duthie 2000, Potapova et al. 2004, Ponader et al. 2007) and to nutrient indicator species for Glaciated North region of the United States (Potapova and Charles 2007).

Inference Models. Weighted averaging (WA), Weighted Averaging – Partial Least Squares (WA-PLS), and Locally-Weighted Weighted Averaging (LWWA) transfer functions were developed for TN and TP with the training data (n=123) and tested with the validation data (n=75) (C2 version 1.5, Juggins 2007). TN and TP were $\log_{10}+1$ transformed, and taxa percent abundances were square-root transformed to reduce the influence of *Achnanthidium minutissimum* (Kützing) Czarnecki and other ubiquitous, abundant species (Dieffenbacher-Krall et al. 2007). Taxa present in < 7 training samples were excluded from analysis resulting in 209 species. WA sample estimates were adjusted with inverse deshrinking, because preliminary analysis showed that classical deshrinking and WA with tolerance downweighting did not significantly improve model performance. WA-PLS models based on more than one component were used only if the added component significantly improved root mean squared error of prediction (RMSEP) as shown by a randomization t-test and a P-value < 0.10 (Juggins 2007). The LWWA model used classical deshrinking. Models were cross-validated with 1,000 bootstrap permutations (Ponader et al. 2007).

Multiple linear regressions (MLR) with the same diatom data were performed (SYSTAT v. 11.0, Wilkinson 1990) as an alternative to the WA transfer functions. Species were added in forward stepwise manner with a probability-to-enter value of 0.075. Unlike past MLR inference models that combined species into guilds with similar species traits (Charles 1985, Davies and Smol 1985, Flower 1986, Jones et al. 1989), I kept species separate, because preliminary attempts using species guilds did not work well (not shown). Stepwise selection was used instead of Akaike's Information Criteria because preliminary tests using Akaike's Information Criteria failed to select a subset of variables, probably because of the large number of zero counts in the data set. Outliers were examined and a sample with large studentized residuals was removed. Species with final P-values > 0.10 or correlated ($r>0.50$) with another species were identified, and the less-frequent species was removed. The final model performed 1,000 bootstrap permutations of r^2 and RMSE (boot, R v. 2.6.2, R Development Core Team 2008). The r^2 and the adjusted r^2 values were reported for the MLR model. A simple linear regression with TP or TN as the independent variable and the model estimates as the dependent variable was performed on each model to estimate the slope (β_1) and the intercept (β_0) of the relationship between observed and estimated values.

The following criteria were used to identify the best performing model for each variable: 1) the largest coefficient of determination (r^2), 2) the smallest root mean square error (RMSE), 3) the largest r^2 based on cross-validation (*i.e.*, r^2_{boot}), 4) the smallest root mean square error of prediction (RMSEP) based on cross-validation, 5) the smallest average bias (bias_{avg}) and maximum bias (bias_{max}), and 6) the largest slope of a linear regression (β_1) (Birks 2003, Ponader et al. 2007). Performance with the validation set was assessed with r^2, RMSE, β_1 and β_0 estimated by linear regression of observed and expected values of environmental variables.

Three methods were used to improve performance and remove bias of WA, WA-PLS, and LWWA inference models. The first approach was to exclude diatoms that showed no response to an environmental variable or had inverse quadratic response curves. The second approach was to recalculate inference models after adding soft algal taxa (n=43) and recalculating square-root percent abundances. I developed the third approach, “slope snapping,” to remove model bias by rotating the distribution of estimated values until the slope equals 1.

Slope snapping is based on the simple equation for a line (Equation 1) and uses the slope (β_1) and y-intercept (β_0) of a linear regression of estimated (Y) and observed (X) values to rotate the distribution of estimated values at the pivot point (A) until $\beta_1=1$ (Figure 3.2). The pivot point is at the point where both the observed value (X) and the estimated value (Y) equal A, or more simply where the regression line intersects the line where $\beta_1=1$. Equation 3.2 includes the formulas for the pivot point (A) and line segments B, C, and D in Figure 3.2. Slope snapping adjusts an estimated value (Y) by adding an amount (D), which is determined by the slope of the line (β_1) and the distance from the pivot point (B) (Equation 3.3). Values of estimates close to the pivot point change only a little, whereas, estimates furthest from the pivot point change the most. The equation works equally well for positive and negative bias. The values of individual estimates do not change with respect to other estimates, and sample residuals remain unchanged. Estimated values of samples not in the training set are adjusted with Equation 3.4.

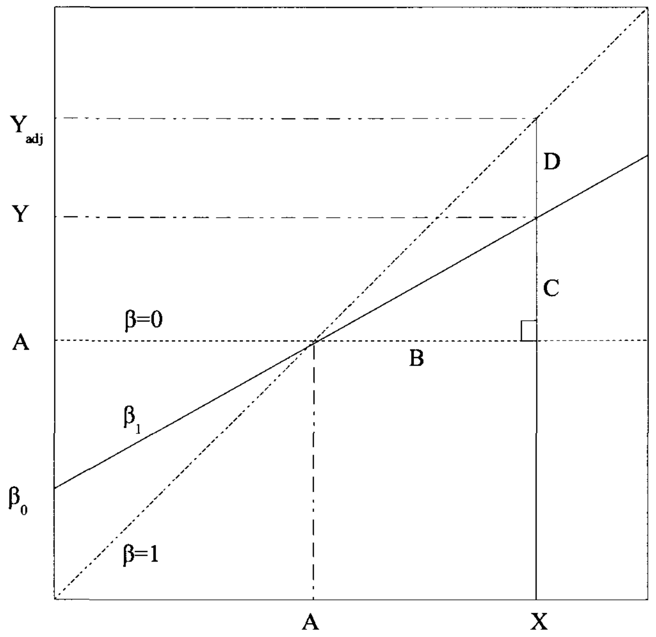


Figure 3.2 Geometry used to derive slope snapping equation.

Equation 3.1 Equation of a line as shown in Figure 3.2.

$Y= \beta_1 X+\beta_0$ where X is the observed value, Y is the estimated value, β_1 is the slope, and β_0 is the intercept.

Equation 3.2 Equations of pivot point (A) and line segments B, C, and D in Figure 3.2.

$$A=\beta_0 (1-\beta_1)^{-1}$$

$$B = X-A = X-\beta_0(1 - \beta_1)^{-1}$$

$$C = \beta_1 B$$

$$D= B-C = B-\beta_1 B = B(1-\beta_1) = (X-\beta_0(1-\beta_1)^{-1})(1-\beta_1) = X(1-\beta_1)-\beta_0$$

Equation 3.3 The slope snapping equation for adjusting the estimated value (Y) of samples in the training set as shown in Figure 3.2. Y_{adj} is the adjusted estimate.

$$Y_{adj} = Y+D = Y+X(1-\beta_1)-\beta_0$$

Equation 3.4 Equation for adjusting the observed values for samples not in the training set.

$$X=(Y- \beta_0)\, \beta_1^{-1}$$

β_1 and β_0 are the foundation of the slope snapping equations; slope snapping should not be used if β_1 and β_0 are unstable. The 95% confidence intervals of β_1 and β_0 were estimated by calculating the 97.5th and 2.5th percentiles of 2,500 bootstrap permutations (boot, R v. 2.6.2, R Development Core Team 2008). The steepest line (97.5th percentile of β_1, 2.5th percentile of β_0) and the shallowest line (2.5th percentile of β_1, 97.5th percentile of β_0) were applied to Equation 3.3, and the results were used to graphically display potential variability of the slope snapping. Finally, a likelihood ratio test was performed (lmtest, R v. 2.6.2, R Development Core Team 2008) to determine if the rotated model was significantly different from the original model.

3.4 Results

3.4.1 Diatom assemblage patterns

NMS analysis resulted in a 3-dimensional solution ($P<0.001$) with final instability of <0.0001 and final stress of 16.6, within the range 10-20 considered to be suitable for ecological data (McCune and Grace 2002) Axis 1, 2, and 3 explained 23%, 17%, and 37% of the variation in species composition and cumulatively explained 77% of variation (Figure 3.3). NMS does not order the axes by amount of variation explained. Axis 3 was correlated with following watershed land cover percentages: % forest and wetlands, % developed, % impervious sufaces, and % grassland (Figure 3.3). Axis 3 was also correlated to the following chemistry parameters: total nitrogen, total phosphorus, nitrate + nitrite, soluble reactive phosphorus, specific conductance, and alkalinity. Axis 2 was not strongly correlated with environmental variables but was correlated with *Achnanthidium minutissimum* (r=-0.74), *A. deflexum* (Rabenhorst) Lange-Bertalot et Ruppel (r=0.65), *Cymbella tumida* (Brébisson ex Kützing) Van Heurck (r=0.53), and

Nitzschia frustulum (Kützing) Grunow (r=0.57). Axis 1 represented a north-south gradient of sites, with those in the north being influenced primarily by tilled agriculture and sites in the south being influenced primarily by urban development. Aggregation of samples by ecoregion also reflected this pattern in land use. Most of the samples from the Northeastern Coastal Zone were on the disturbed end of Axis 3 and none were categorized as minimally disturbed. In contrast, most of the samples in the sparsely populated Northeastern Highlands were aggregated on the least disturbed end of Axis 3. Ecoregion patterns reflected unbalanced spatial distribution of development and agricultural land uses. Several environmental variables were not correlated with a major pattern in species composition, including longitude, watershed area, channel width, average water depth, water velocity, percent canopy cover, percent fine substrate, pH, TKN, percent tilled agriculture in watershed, and percent wetlands in watershed.

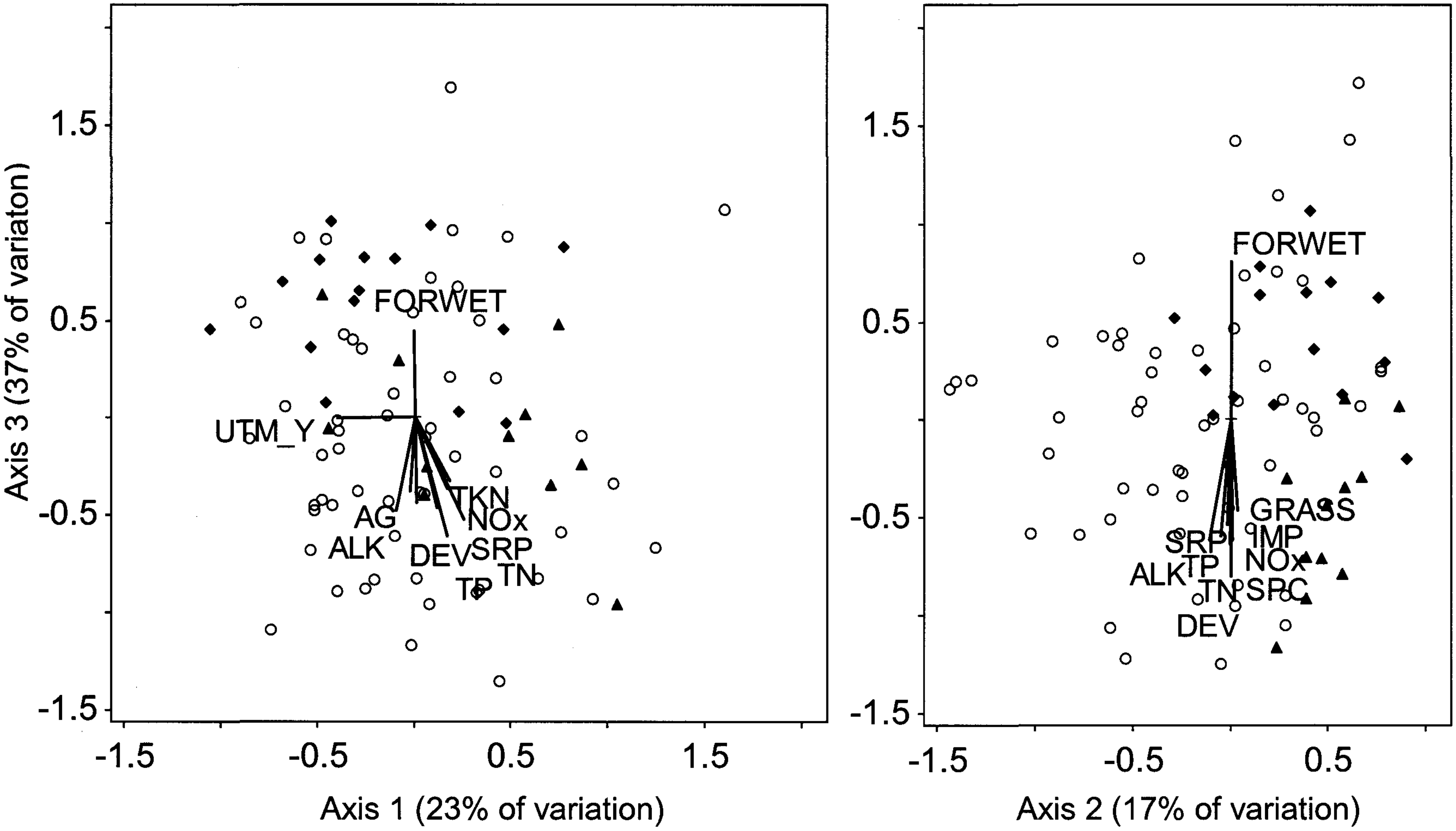


Figure 3.3 Nonmetric multidimensional scaling (NMS) biplots of species composition with correlated environmental variables shown as vectors. Labels described in Table 3.1. Samples are grouped by ecoregion (Omernik 1987, Griffith et al. 2009).

○ = Acadian Plains and Hills, ▲ = Northeastern Coastal Zone, ◆ = Northeastern Highlands

3.4.2 Diatom response curves, optima, and nutrient indicators

General linear models were selected with Akaike's Information Criteria to describe algal species response curves of five types: quadratic, inverse quadratic, linear decreasing, linear increasing, and indifferent with no relationship (*i.e.*, a flat line). *Gomphonema kobayasii* Kociolek et Kingston represents a species with a quadratic response to TP (Figure 3.4). *Rossithidium linearis* (Smith) Round et Bukhtiyarova was indifferent to TP, *Cymbella gracilis* had a decreasing relationship, *Navicula minima* (NAminima) had an increasing relationship, and *Brachysira microcephala*, *Achnanthidium deflexum*, and *Rhoicosphenia abbreviata* had quadratic curves with different TP optima (Figure 3.5). Overall, 70 species had quadratic models, 4 species had an inverse quadratic model, 27 species had linear decreasing models, 54 species had linear increasing models, had 58 species were indifferent and had no relationship with TP (Table A.1). For TN, 70 algal species were best described by quadratic models, 5 species by inverse quadratic models, 20 species by linear decreasing models, 52 species by linear increasing models, and 66 species were indifferent (Table A.1).

Weighted averaging optima for TP ranged from 7 μg L^{-1} (*Eunotia muscicola var. tridentula* Nörpel et Lange-Bertalot*)* to 46 μg L^{-1} (*Navicula viridulacalcis* (Hustedt) Lange-Bertalot*)*. The 25th, 50th, and 75th percentiles were 16, 23, and 29 μg L^{-1} respectively. The most common taxa with TP optima <16 μg L^{-1} were *Tabellaria flocculosa*, *Brachysira microcephala, Cymbella gracilis, Encyonopsis microcephala* (Grunow) Krammer, *Fragilaria sepes* Ehrenberg, *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst, *Gomphonema acuminatum* Ehrenberg, and *G. gracile* Ehrenberg emend Van Heurck. The most common diatoms with TP optima >29 μg L^{-1} were *Rhoicosphenia* *abbreviata*, *Navicula trivialis* Lange-Bertalot, *Planothidiumrostratum* (Østrup) Lange-Bertalot, *Nitzschia inconspicua* Grunow, and *N. fonticola* Grunow. The five most common taxa, as measured by Hill's N2, were *Achnanthidium minutissimum, Gomphonema parvulum, Fragilaria vaucheriae* (Kützing) Petersen, *Encyonema silesiacum* (Bleisch) Mann, and *Synedra rumpens* Kützing, and their TP optima ranged from 17 to 21 μg L^{-1}.

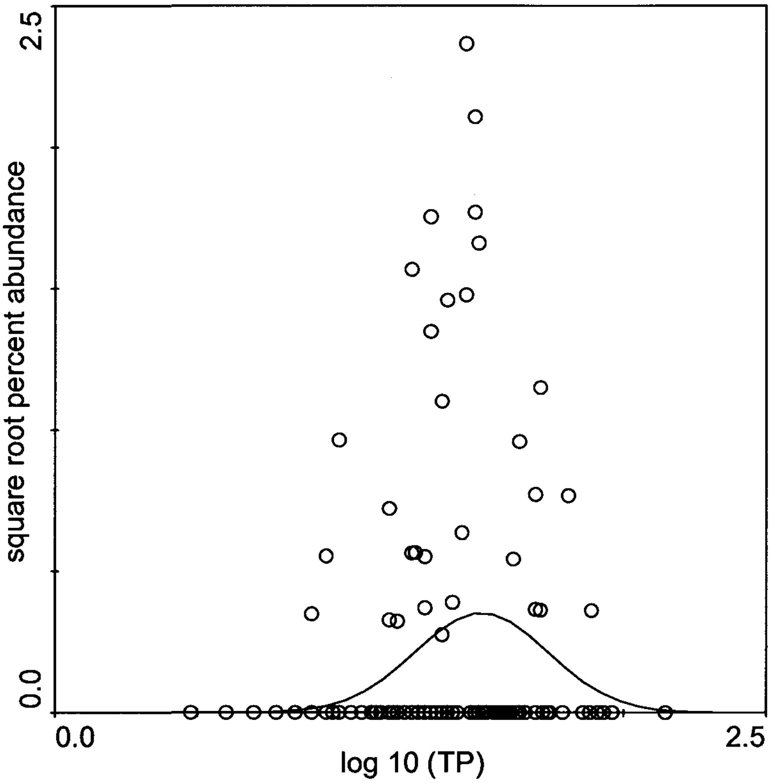


Figure 3.4 General linear model response curve for *Gomphonema kobayasii* Kociolek et Kingston showing values of training samples (n=123).

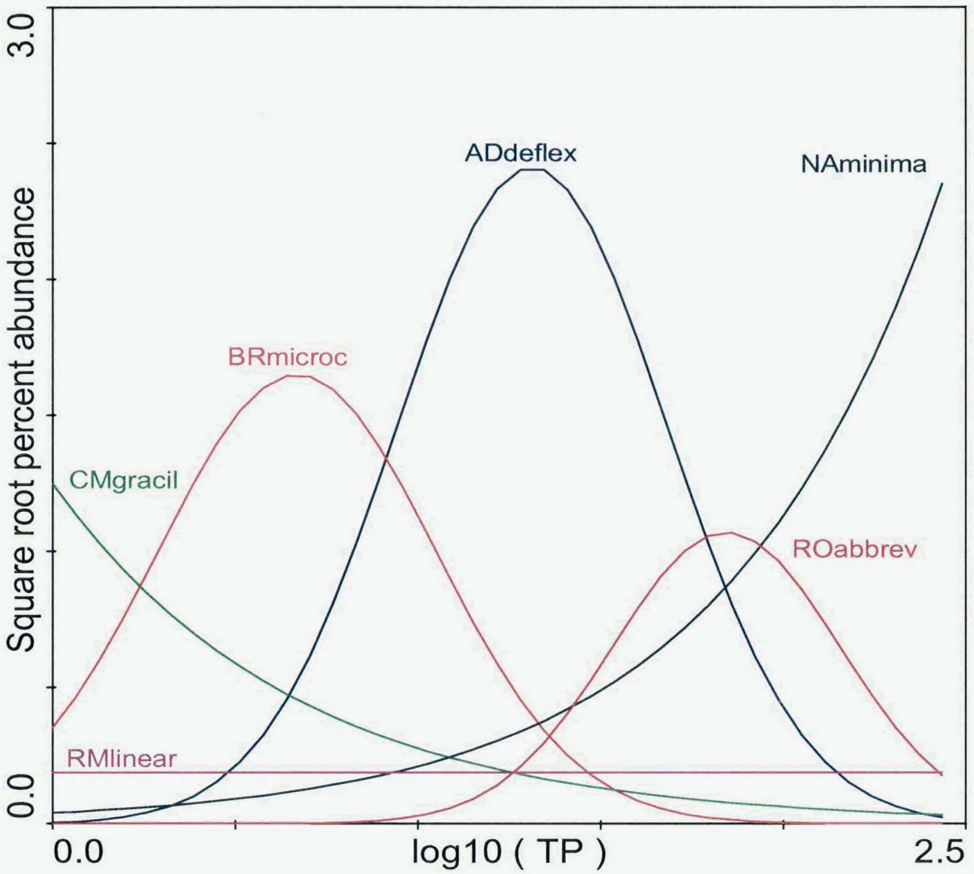


Figure 3.5 Examples of different general linear model response curves for diatom square root percent abundance in relation to increasing total phosphorus (TP). Rossithidium linearis (RMlinear) was indifferent to TP. Cymbella gracilis (CMgracil) had a linear decreasing response. Navicula minima (NAminima) had a linear increasing response. Brachysira microcephala (BRmicroc), Achnanthidium deflexum (ADdeflex), and Rhoicosphenia abbreviata (ROabbrev) had quadratic responses with different optima.

The TN optima ranged from 235 μg L^{-1} *(Eunotia muscicola var. tridentula)* to 995 μg L^{-1} (*Surirella brebissonii* Krammer et Lange-Bertalot*)*. The 25th, 50th, and 75th percentiles were 419, 548, and 652 μg L^{-1} respectively. The most common species with TN optima <419 μg L^{-1} were *Eunotia pectinalis* (Müller) Rabenhorst*, Brachysira brebissonii* Ross*, B. microcephala, Gomphonema acuminatum*, and *Tabellaria flocculosa*. The most common species with TN optima >652 μg L^{-1} were *Planothidium* *lanceolatum* (Brébisson ex Kützing) Lange-Bertalot, *P. lanceolatum var. omissum* (Reimer) Andresen, Stoermer et Kreis, *Navicula tripunctata* (Müller) Bory, *Nitzschia tubicola* Grunow ex Cleve et Grunow, and *Navicula lanceolata* (Agardh) Ehrenberg. The optima of the five most common taxa were ranged from 437 to 508 μg L^{-1}.

TN optima were correlated with TP optima (r=0.84), however, the relationship was heteroskedastic toward greater optima (Figure 3.6). The following group of taxa diverged from the general trend by having proportionally greater TP optima compared to other taxa with similar TN optima (Figure 3.6): *Craticula molestiformis* (Hustedt) Lange-Bertalot (CRmolest), *Gomphonema subclavatum* (Grunow) Grunow (GOsubcla), *Navicula antonii* Lange-Bertalot (NAantoni), *N. trivialis* (NAtrivia), *N. viridulacalcis* (Hustedt) Lange-Bertalot (NAvirlin), *Nitzschia acicularis* (Kützing) Smith (NIacicul), *Planothidium rostratum* (Østrup) Lange-Bertalot (PLrostra), and *Staurosira construens* Ehrenberg (STconstr). In contrast, the following taxa had proportionally smaller TP optima compared to other taxa with similar TN optima (Figure 3.6): *Achnanthidium kranzii* (Lange-Bertalot) Round et Bukhtiyarova (ADkranz), *Diadesmis perpusilla* (Grunow) Mann (DSperpus), *Diploneis oblongella* (Näegeli ex Kützing) Ross (DPoblong), *Encyonema reichardtii* (Krammer) Mann (ENreicha), *Navicula laterostrata* Hustedt (NAlatero), *N. reichardtiana* Lange-Bertalot (NAreicha), *N. tripunctata* (NAtripun), and *Planothidium lanceolatum* (PLlanceo).

Many optima in this study (*e.g.*, TP 7-46 μg L^{-1}, TN 235-995 μg L^{-1}) were less than corresponding optima from Southern Ontario (Winter and Duthie 2000), Northern Piedmont ecoregion consisting of parts of Pennsylvania, Maryland, Delaware, and New Jersey (Potapova et al. 2004), New Jersey (Ponader et al. 2007), and the Mid-Atlantic

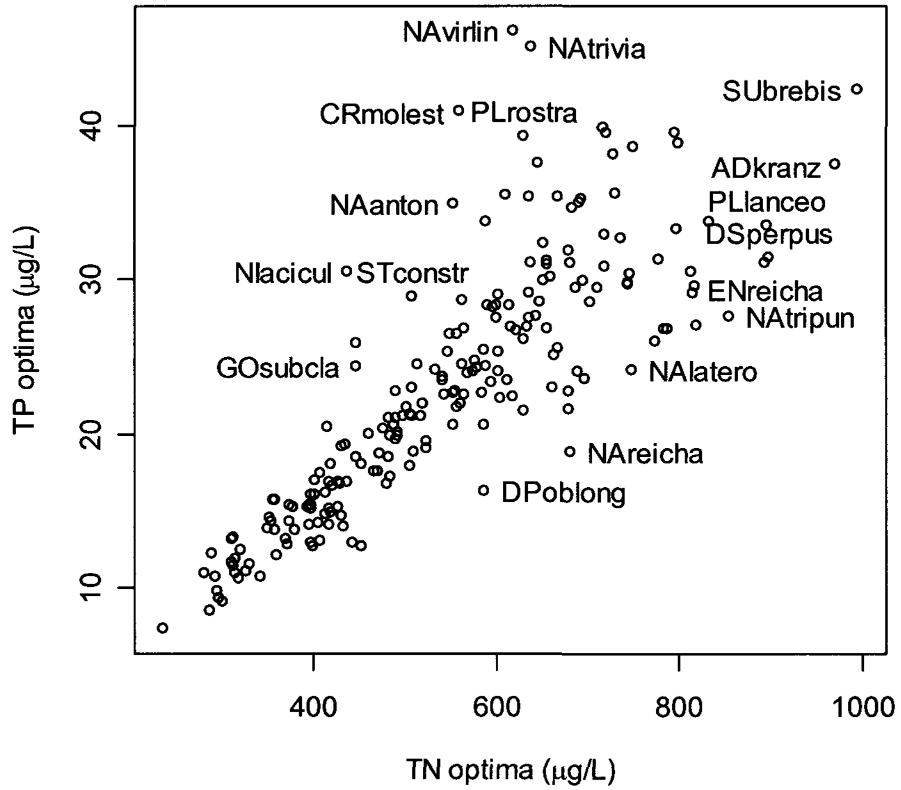


Figure 3.6 Relationship between species (n=211) TN optima and TP optima. ADkranz=Achnanthidium kranzii, CRmolest=Craticula molestiformis, DPoblong=Diploneis oblongella,GOsubcla=Gomphonema subclavatum, DSperpus=Diadesmis perpusilla, ENreicha=Encyonema reichardtii, NAantoni=Navicula antonii, NAlatero=Navicula laterostrata, NAreicha=N. reichardtiana, NAtripun=N. tripunctata, NAtrivia=N. trivialis, NAvirlin=N. viridulacalcis, NIacicul= Nitzschia acicularis, PLlanceo=Planothidium lanceolatum, PLrostra=Planothidium rostratum, STcontr=Staurosira construens, and SUbrebis= Surirella brebissonii.

Highlands region of Pennsylvania, Maryland, Virginia, and West Virginia (Pan et al. 1996). *Fragilaria vaucheriae*, for example, occurred in 124 Maine samples and had TP and TN optima of 20 and 484 µg L^{-1}. In contrast, *F. vaucheriae* occurred in 17 New Jersey samples with TP and TN optima of 76 and 1,704 µg L^{-1} (Ponader et al. 2007), and 31 Northern Piedmont samples with TP and TN optima of 66 and 1,297 µg L^{-1} (Potapova et al. 2004). Confidence intervals for most optima in this study, however, overlapped confidence intervals of optima from the other studies. Other species with smaller optima in Maine included *Achnanthidium exiguum* (Grunow) Czarnecki, *Cocconeis placentula var. euglypta* (Ehrenberg) Grunow, *Cyclotella meneghiniana* Kützing, *Frustulia vulgaris* (Thwaites) deToni, *Gomphonema gracile, G. kobayasii, Navicula minima, N. rostellata* Kützing, *Nitzschia amphibia* Grunow, *N. capitellata* Hustedt, *N. inconspicua*, and *Rhoicosphenia abbreviata*. Many species with the largest TP and TN optima in New Jersey, Northern Piedmont, and Mid-Atlantic Highlands were uncommon or absent in Maine. Similarly, many of the species with the least optima in Maine were uncommon or absent in other studies.

Several common Maine taxa were identified as indicators of low TP concentrations in the Glaciated North region (Potapova and Charles 2007). A group of the species identified as low TP indicator species of the Glaciated North had TP optima <20 µg L^{-1} in Maine, including *Achnanthidium minutissimum, Brachysira brebissonii, B. microcephala, Cymbella affinis* Kützing, *C. delicatula* Kützing, *Eunotia exigua, E. implicata* Nörpel, Lange-Bertalot et Alles, *E. incisa* Smith ex Gregory, *E. pectinalis, Fragilaria capucina var. gracilis* (Østrup) Hustedt, *Gomphonema acuminatum, G. apuncto* Wallace, *Navicula notha* Wallace, and *Tabellaria flocculosa*. Another group of species identified as low TP indicators had TP optima ranging from 20 to 25 µg L^{-1} in Maine, including *Achnanthidium deflexum, A. rivulare, Encyonema minutum, Frustulia amphipleuroides* (Grunow) Cleve-Euler, *Gomphonema olivaceoides var. hutchinsoniana* Patrick, *Stauroforma exiguiformis* (Lange-Bertalot) Flower, Jones et Round, and *Reimeria sinuata* (Gregory) Kociolek et Stoermer. Overall, the Maine TP optima correspond well with the regional indicators of low TP, however, some of the indicator taxa are not common in Maine, and there are many species in Maine with low TP values that are not identified as indicators for the Glaciated North.

The Maine TP optima did not correspond as well with the list of species identified as high TP indicator species for the Glaciated North (Potapova and Charles 2007). Several species had Maine optima <29 μg L^{-1}, the 75th percentile. *Navicula reichardtiana* and *Navicula tripunctata,* for example, had unimodal responses with TP optima of 19 and 28 μg L^{-1}, respectively. *Gomphonema angustatum* (Kützing) Rabenhorst did not respond to TP in Maine, with an optimum of 25 μg L^{-1}. *Nitzschia perminuta* (Grunow) Peragallo had an increasing response to TP in Maine with an optimum of 27 μg L^{-1}. Several indicator species for the Glaciated North had TP optima above the 75th percentile in Maine. *Cyclotella meneghiniana, Mayamaea agrestis* (Hustedt) Lange-Bertalot, and *Navicula gregaria* Donkin for example, all had unimodal responses to TP in Maine with a optimum of 30 μg L^{-1}. *Luticola mutica* (Kützing) Mann and *Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski had increasing response curves with optima of 31 and 35 μg L^{-1} respectively. Many of the taxa in the upper quartile of Maine's TP optima were not identified as high TP indicator species in the Glaciated North.

Most of the soft algae taxa had small to medium optima compared to the range observed for the diatoms. The TP optima of the 43 soft algal taxa ranged from 9 μg L^{-1} for *Bulbochaete* spp. to 37 μg L^{-1} for *Microspora* spp. (Table A.2). Thirteen taxa had optima less than the 25th percentile of the diatom optima (15 μg L^{-1}), and only 5 taxa had optima greater than the mean of the diatom TP optima (23 μg L^{-1}). The TN optima ranged from 267 μg L^{-1} for *Zygnema* spp. to 835 μg L^{-1} for *Pseudanabaena* spp. (Table A.2). Thirteen taxa had optima less than 25th percentile of the diatom optima (419 μg L^{-1}), and only 4 taxa had optima greater than the 75th percentile of the diatom optima (650 μg L^{-1}).

3.4.3 Inference models

The best performing models for phosphorus were multiple linear regression (MLR), Locally-Weighted Weighted Averaging (LWWA), and LWWA adjusted with slope-snapping ($LWWA_{adj}$) with $r^2 \geq 0.88$ and RMSE$\leq$0.110 (Table 3.2 and Figure 3.7). The worst performing models for phosphorus were Weighted Averaging (WA) (r^2=0.64, RMSE=0.221) and Weighted Averaging – Partial Least Squares (WA-PLS) using the second component (P=0.067) (r^2=0.80, RMSE=0.134). The best performing models for nitrogen were LWWA, $LWWA_{adj}$, and MLR with $r^2 \geq 0.88$ and RMSE$\leq$0.075 (Table 3.2 and Figure 3.7). The worst performing models for nitrogen was the WA model (r^2=0.65, RMSE=0.149). The WA-PLS (2nd component) model did not perform significantly better than the WA model. The MLR model performed slightly better than the other models for both TP and TN training data (Table 3.2). The LWWA models had smaller RMSE than the MLR models when applied to the validation data, but had more bias indicated by the β_1 estimates (Table 3.3). Final MLR models for TN and TP were based on 42 and 31 species, respectively (Table 3.4).

Table 3.2 Performance of best TP and TN inference models with training data.

Model	r^2	RMSE	r^2_{boot}	RMSEP	β_1	β_0
TP						
Locally-Weighted Weighted Averaging	0.88	0.110	0.61	0.201	0.920	0.147
Locally-Weighted Weighted Averaging adjusted with slope snapping	0.91	0.110	0.61	0.201	1.000	~0.000
Multiple Linear Regression	0.93 (0.90^a)	0.097	0.94	0.083	0.927	0.092
TN						
Locally-Weighted Weighted Averaging	0.88	0.090	0.60	0.169	0.897	0.305
Locally-Weighted Weighted Averaging adjusted with slope snapping	0.91	0.090	0.61	0.201	1.00	~0.000
Multiple Linear Regression	0.94 (0.91^a)	0.075	0.97	0.056	0.942	0.157

a - adjusted r^2

Table 3.3 Performance of best TP and TN inference models with validation set.

Model	r^2	RMSE	β_1	β_0
TP				
Locally-Weighted Weighted Averaging	0.47	0.192	0.601	0.506
Locally-Weighted Weighted Averaging adjusted with slope snapping	0.47	0.201	0.653	0.390
Multiple Linear Regression	0.50	0.225	0.768	0.304
TN				
Locally-Weighted Weighted Averaging	0.34	0.167	0.589	1.098
Locally-Weighted Weighted Averaging adjusted with slope snapping	0.34	0.183	0.648	0.910
Multiple Linear Regression	0.31	0.242	0.810	0.487

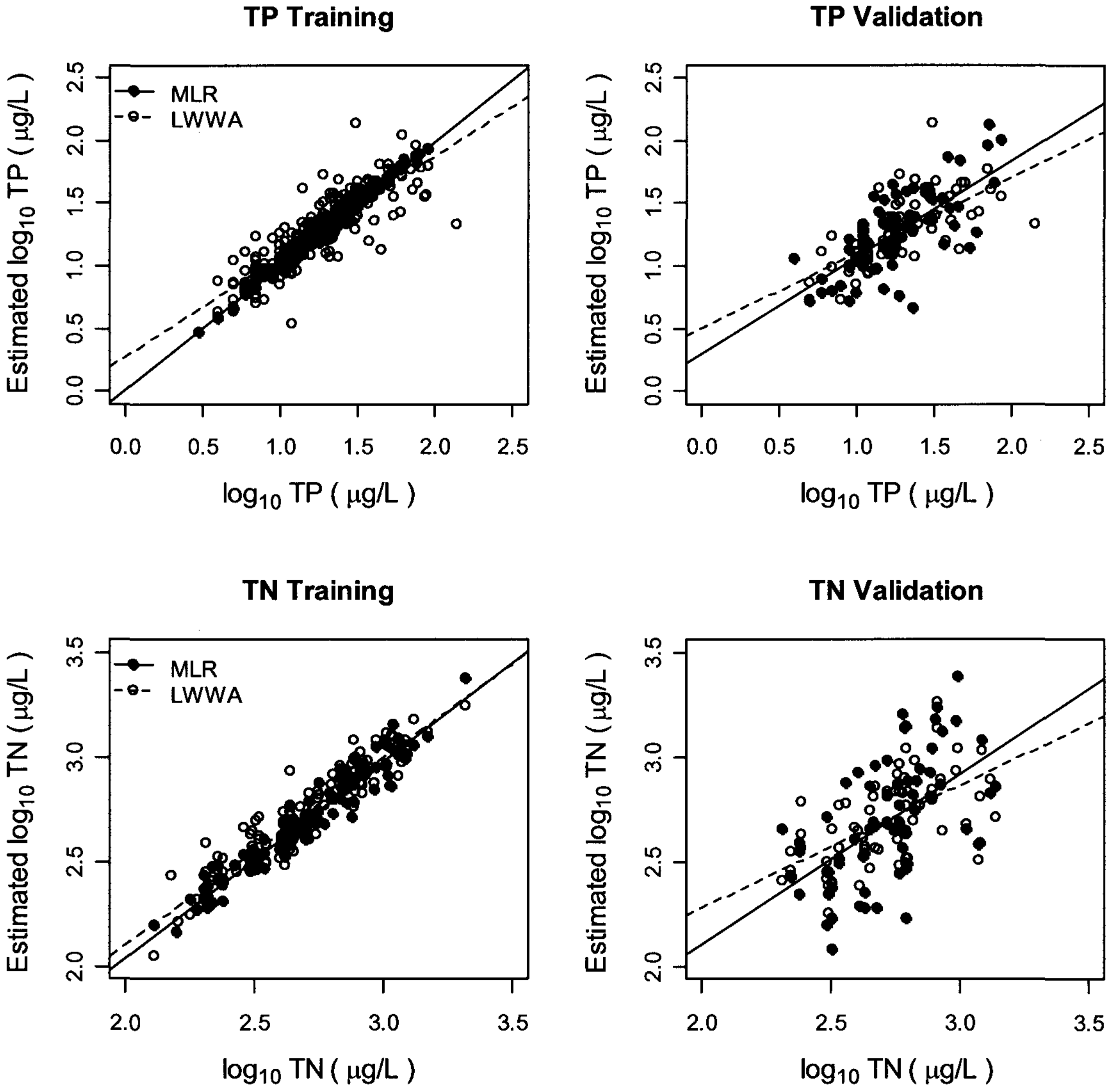


Figure 3.7 Plots of estimated versus observed $\log_{10}$ TP (µg L^{-1}) and $\log_{10}$ TN (µg L^{-1}) with the training and validation sets. MLR=multiple linear regression method (●), LWWA=locally-weighted weighted averaging method (○).

Table 3.4 Coefficients of diatom species used in stepwise linear regression models to infer nutrient concentrations in Maine streams. Significant coefficients are indicated † (P<0.10), * (P<0.05), ** (P<0.01), and *** (P<0.001).

Full name	**TN**	**TP**
Achnanthes oblongella Østrup	0.173293 ***	0.142281 ***
Achnanthidium deflexum (Rabenhorst) Lange-Bertalot et Ruppel	0.040581 ***	
Achnanthidium minutissimum (Kützing) Czarnecki	0.018376 ***	-0.028851 ***
Achnanthidium rivulare Potapova et Ponader	0.050075 ***	
Adlafia bryophila (Petersen) Lange-Bertalot		-0.07318 **
Amphipleura pellucida (Kützing) Kützing		-0.167188 ***
Amphora copulata (Kützing) Schoeman et Archibald	-0.297057 ***	-0.20777 *
Amphora pediculus (Kützing) Grunow	0.110615 ***	
Aulacoseira ambigua (Grunow) Simonsen		0.14403 **
Aulacoseira subarctica (Müller) Haworth		-0.309249 ***
Caloneis bacillum (Grunow) Cleve	0.275167 ***	
Cocconeis pediculus Ehrenberg		0.097392 ***
Cocconeis placentula var. euglypta (Ehrenberg) Grunow	-0.043675 **	
Craticula submolesta (Hustedt) Lange-Bertalot	-0.063395 †	
Cymbella affinis Kützing	-0.122131 **	
Cymbella delicatula Kützing		-0.076764 **
Cymbella gracilis (Ehrenberg) Kützing	0.082538 ***	
Cymbella naviculiformis Auerswald ex Héribaud		0.200185 **
Diploneis oblongella (Näegeli ex Kützing) Ross	0.516098 ***	
Discostella pseudostelligera (Hustedt) Houk et Klee	-0.24269 ***	
Encyonopsis microcephala (Grunow) Krammer		-0.155503 ***
Eucocconeis laevis (Østrup) Lange-Bertalot	-0.083052 **	
Eunotia exigua (Brébisson ex Kützing) Rabenhorst	0.066465 **	
Eunotia muscicola var. tridentula Nörpel et Lange-Bertalot	-0.509509 ***	-0.415916 ***
Eunotia naegelii Migula	0.120606.	
Eunotia paludosa Grunow	-0.133429 **	-0.327318 ***
Eunotia pectinalis var. undulata (Ralfs) Rabenhorst		-0.053001 **
Fragilaria capucina var. gracilis (Østrup) Hustedt		0.02382 *
Fragilaria sepes Ehrenberg	0.046787 *	
Fragilaria vaucheriae var. capitellata (Grunow) Ross	0.091822 **	
Frustulia krammeri Lange-Bertalot et Metzeltin	0.079925 **	

Table 3.4 Continued

Full name	**TN**	**TP**
Geissleria decussis (Hustedt) Lange-Bertalot et Metzeltin	-0.092402 **	
Gomphonema angustatum (Kützing) Rabenhorst	0.08028 **	
Gomphonema drutelingense Reichardt		-0.112772 *
Gomphonema kobayasii Kociolek et Kingston	0.107094 ***	
Gomphonema minutum (Agardh) Agardh	0.057565 ***	
Gomphonema olivaceoides var. hutchinsoniana Patrick		0.086564 **
Gomphonema parvulum (Kützing) Kützing		0.035445 **
Gomphonema truncatum Ehrenberg		0.073149 **
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski		0.12347 **
Karayevia suchlandtii (Hustedt) Bukhtiyarova		0.268543 ***
Navicula capitatoradiata Germain	0.07829 **	
Navicula cryptocephala Kützing	0.107229 ***	0.176553 ***
Navicula cryptotenella Lange-Bertalot ex Krammer et Lange-Bertalot	-0.047946 ***	
Navicula cryptotenelloides Lange-Bertalot		0.092992 ***
Navicula hintzii Lange-Bertalot	-0.280382 ***	
Navicula notha Wallace	-0.070592 ***	
Navicula schmassmanni Hustedt		-0.24594 ***
Navicula tenelloides Hustedt	0.122624 †	
Navicula trivialis Lange-Bertalot		0.170725 ***
Nitzschia acidoclinata Lange-Bertalot	0.120971 ***	
Nitzschia amphibia Grunow	-0.088765 **	
Nitzschia inconspicua Grunow		0.144145 ***
Nitzschia lacuum Lange-Bertalot	0.161536 **	
Nitzschia palea (Kützing) Smith		0.055 **
Nitzschia paleacea Grunow ex Van Heurck	0.164201 **	
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	0.115348 ***	
Planothidium stewartii (Patrick) Lange-Bertalot		0.572415 ***
Psammothidium bioretii (Germain) Bukhtiyarova et Round		-0.123658 ***
Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round	0.07577 ***	
Pseudostaurosira brevistriata (Grunow) Williams et Round Round	-0.131968 ***	

Table 3.4 Continued

Full name	**TN**	**TP**
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	0.027763 *	
Staurosira elliptica (Schumann) Williams et Round		-0.194351 ***
Staurosirella pinnata (Ehrenberg) Williams et Round	0.099565 ***	
Surirella amphioxys Smith	0.095076 **	
Synedra rumpens Kützing	0.019816 *	
Tabellaria flocculosa (Roth) Kützing		-0.039732 ***

The three approaches (*i.e.*, excluding indifferent diatoms, including soft algae, slope-snapping) for removing bias differed in performance. Excluding indifferent diatoms and including soft algae taxa did not improve model performance. In contrast, slope-snapping removed bias (slopes=1.00), increased r^2 values, and retained original standard errors. For example, slope snapping increased the TN WA model r^2 (0.61 to 0.81) and β_1 (0.605 to 1.00), while RMSE (0.149) did not change (Figure 3.8). The β_1 and β_0 for the TN WA model were close to the mean of the bootstrap estimates. The range of potential error associated with the slope snapping technique, for this example, was small compared to the original model bias (Figure 3.8).

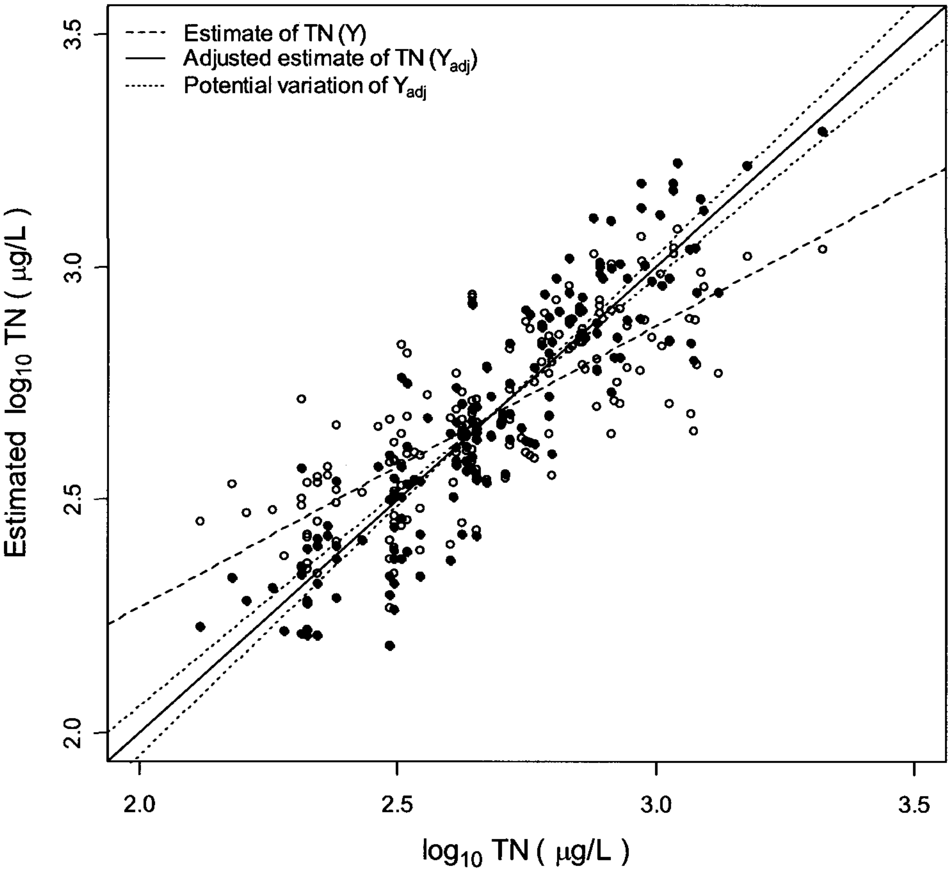


Figure 3.8 Estimates of TN (Y) based on WA model (open circles) compared to adjusted estimates of TN (Y_{adj}) from slope snapping method (solid circles). Variation in Y_{adj} was estimated by replacing β1 with the 5th and 95th percentiles of bootstrapped β1.

3.5 Discussion

3.5.1 Species responses to increasing TP concentrations

Diatom species exhibited a variety of responses to N and P gradients. Similar to observations in the Northern Piedmont ecoregion, approximately one third of the species had unimodal response curves for TN and TP (Potapova et al. 2004). Approximately one third of the species were indifferent to TN or TP. Similar to the Northern Piedmont, indifferent species had optima close to the average of all species (Potapova et al. 2004).

The remaining species had linear decreasing or increasing responses. Species with linear decreasing response curves are most abundant in oligotrophic streams, and their optima accurately represent their nutrient preferences. Species with linear increasing response curves may have truncated unimodal response curves with optima beyond the range observed in Maine. As a result, the Maine optima likely underestimate preferred nutrient conditions of some species with linear increasing response curves. For example, *Nitzschia inconspicua* has one of Maine's largest TP optima (39 $\mu g\ L^{-1}$), which is less than the optima observed in the Northern Piedmont (68 $\mu g\ L^{-1}$, Ponader et al. 2007), New Jersey (76 $\mu g\ L^{-1}$, Potapova et al. 2004), and Southern Ontario (115 $\mu g\ L^{-1}$, Winter and Duthie 2000). Similarly, *Navicula minima* had an increasing response to TN and an optimum of 643 $\mu g\ L^{-1}$ compared to a New Jersey optimum of 1,627 $\mu g\ L^{-1}$.

Regional differences in geology, climate, vegetation, and land use influence water chemistry and likely cause most of the discrepancies in a species optima across geographic regions (Poff 1997). Lower TP optima of diatom taxa in Maine than in other parts of the Eastern North America (Pan et al. 1996, Winter and Duthie 2000, Potapova et al. 2004, Ponader et al. 2007) reflect the abundance of oligotrophic and scarcity of eutrophic streams. Some common eutraphentic diatoms of other regions of North America were absent or not common in Maine, such as *Craticula accomoda* (Hustedt) Mann, *Craticula cuspidata* (Kutzing) Mann, *Diadesmis confervacea* Kützing, *Gyrosigma scalproides* (Rabenhorst) Cleve, *Stephanodiscus hantzschii* Grunow, *Tryblionella apiculata* Gregory, *Tryblionella calida* (Grunow) Mann, and *Tryblionella hungarica* (Grunow) Mann (Pan et al. 1996, Potapova and Charles 2007, Porter 2008).

Regional differences in other environmental variables, such as alkalinity, pH, specific conductance, and % fines could contribute to discrepancies in optima or absence of some eutraphentic taxa in Maine. Nutrient optima treat each environmental variable independently, however, many of these environmental conditions are highly correlated with nutrients in Maine and other regions of North America (Winter and Duthie 2000, Ponader et al. 2007, Stevenson et al. 2008b). Potapova et al. (2007) found that diatom indicator status of oligotrophic and eutrophic conditions varied in different regions of the country. Some diatoms may respond to a variable that is correlated with nutrient concentrations, such as alkalinity or specific conductance, more than to the nutrients themselves. The % fines can vary regionally, and large % fines could favor motile taxa such as *Navicula* sp., *Nitzschia* sp., and *Surirella* sp. Further, regional patterns in nutrient limitation could enhance the effect of one nutrient over others on diatom communities (Francouer 2001, Tank and Dodds 2003).

3.5.2 Inference models

Weighted Averaging (WA), Weighted Averaging – Partial Least Squares (WA-PLS), and Locally-Weighted Weighted Averaging (LWWA) assume that species' response curves are unimodal with respect to environmental gradients and include all species in calculations. The large proportion of species without unimodal response curves could affect weighted average inference model performance. Indifferent species had optima near the middle of the gradient in our study and in the Northern Piedmont despite occurring across the full gradient (Potapova et al. 2004). Excluding indifferent species did not improve model performance in this study.

The Multiple Linear Regression (MLR) models with stepwise selection of species outperformed WA and WA-PLS and had less bias than LWWA and LWWA adjusted with slope snapping ($LWWA_{Adj}$). In contrast to weighted average inference models, MLR assumes linear responses and includes only a subset of statistically selected algal species. I used a stepwise process to select the best combination of species to estimate nutrient concentrations based on their combined cooccurrence. The stepwise selection process did not consider the shape of response curves, and the MLR models included a few indifferent species and several species with significant unimodal response curves, which also had significant linear response curves. Inclusion of indifferent species presumably improved predictive power due to co-occurrence with other species.

Relationships between environmental variables could confound inference models for individual environmental variables. Similar to other studies, I found that the major pattern in diatom community composition was related to a gradient of watershed disturbance representing nutrients and a variety of other environmental variables (Winter and Duthie 2000, Ponader et al. 2007, Stevenson et al. 2008b). Covariation of nutrients with other indicators of watershed disturbance, such as specific conductance, pH, and % fines, can hinder development of nutrient inference models (Potapova et al. 2004, Stevenson et al. 2008b), especially when one of the other indicators influences diatom community composition more than nutrients, such as pH in some regions (Pan et al. 1996). It is possible that an inference model for one variable could actually be an inference model for another variable in disguise. The availability of non-nutrient resources (*e.g.*, light, temperature) and the amounts of disturbances (*e.g.*, scouring, substrate stability, grazing) also influence benthic algal communities (Biggs 1996) and could confound nutrient inference models. Increased nutrients can alter macroinvertebrate communities and increase grazing pressure, which in turn can change diatom community structure (McCormick and Stevenson 1991, Bourassa and Cattaneo 1998).

The performance of my inference models may have been hindered by several factors. First, most of my samples were represented by single nutrient measurements rather than the mean of several measurements. Single measurements may not accurately represent the dynamic variability in nutrient concentrations over time (Cattaneo and Prairie 1995). Taking the mean of multiple nutrient samples would improve inference model accuracy, because diatoms in a sample more closely represent the availability of nutrients in the previous weeks than the concentration of nutrients found at the time the samples are collected (Philibert et al. 2006, Lavoie et al. 2008). Second, the data set included more oligotrophic streams than eutrophic streams, which could cause poor predictive capability for nutrient enriched streams (Philibert et al. 2006). Winter and Duthie (2000) had the opposite problem of not enough oligotrophic sites. Adding streams to balance the distribution across the nutrient condition gradient could improve estimates of species optima, decrease bias in estimated nutrient concentrations, and improve overall performance of models. Third, my data set was diverse and had many infrequent species, resulting in a large percentage of zero counts that can decrease performance of some statistical methods (McCune and Grace 2002). There was great variation in community structure and many samples in the validation set introduced new combinations of species and relative abundances not similar to samples in training set, which could explain the large drop in r^2 values when models were applied to the validation set. Finally, stream nutrient inference models may simply be less predictive than inference models for non-resource variables, such as pH because of the temporal and spatial variability of supply and form of nutrients (*i.e.*, organic, inorganic, particulate, dissolved) (Pan et al. 1996).

3.5.3 Methods of improving performance and removing bias

Models that overestimate low nutrient values are problematic to water quality programs attempting to protect waterbodies with low nutrient concentrations. The WA models consistently had the greatest model bias with β_1 (the slope of the linear regression of estimated and observed values) <0.65, whereas LWWA and MLR had the least bias with β_1 >0.90. Model bias could be caused by an unequal distribution of sites across an environmental gradient (Don Charles, personal communication.). For example, our data set included fewer eutrophic sites than oligotrophic sites.

Excluding indifferent taxa from WA, WA-PLS, and LWWA calculations did not improve model performance. The large diversity in species and many zero counts could have reduced model performance. Some of the indifferent taxa were among the most common species, and removing them could have exacerbated the effect of numerous zero counts. The five most abundant species often exceeded 80% of the diatom valves, and some samples contained a single species representing more than 80% of the valves. Indifferent species were sometimes among the dominant species and removing them could have increased the influence of remaining species.

Adding soft algae to the inference models also did not improve model predictive power. Diatom inference models may have poor predictive power for eutrophic sites, because the models do not capture potential shifts in community structure from diatoms to green or bluegreen algae (Pan et al. 1996, Winter and Duthie 2000). In our study, most of the soft algae had small to intermediate tolerance values and would not improve prediction of eutrophic sites. *Cladophora glomerata* occurred in several enriched, urban streams but was not common enough for inclusion in the models. I also did not observe predictable increases in biovolume or percent biovolume of green algae or cyanobacteria across the full disturbance gradient. Some minimally disturbed streams had large relative biovolumes of sensitive, soft algae and some urban streams were dominated by diatoms, presumably because of frequent scouring events. Filamentous algae in enriched streams can provide habitat for epiphytic diatoms, such as *Cocconeis* spp., thereby increasing diatom counts.

The slope snapping technique of removing model bias could be useful to water resource agencies when no alternatives are available. The TN WA model, for example, improved after the application of slope snapping. The distribution of sample estimates relative to each other and the SE_{reg} remained the same, however, the bias was removed, and the r^2 increased from 0.60 to 0.81, a 33% improvement. One advantage of the slope snapping technique is that it does not require subjective decisions about how much to rotate the distribution of sample estimates. The slope snapping equation automatically rotates the distribution of samples until the slope reaches 1.00. This method should only be used if the estimates of β_1 and β_0 are shown to be stable through bootstrap permutations or an alternative resampling technique. The steepest and flattest lines of the distribution of sample estimates from the bootstrap estimates could be used to graphically display the range of potential variation caused by the slope snapping (*e.g.*, Figure 3.8). Further, application of the method could be restricted to situations when the 95%

confidence interval of the linear regression of the original model falls outside of the range of potential variation caused by the slope snapping. Applying slope snapping to new data in model development could be similar to a second deshrinking step. Further research is needed to test the method, especially when applying models to new data.

3.5.4 Selecting the best model

Ultimately, the MLR were selected because of they had less bias, were more parsimonious, and were easier to calculate and explain than the LWWA models. The MLR models performed slightly better with the training data than the LWWA models because the LWWA models were more biased. Ultimately, selection of the MLR model reflects how the models will be used; there is great value to regulatory agencies, such as MDEP, for using models that are parsimonious, simple to calculate, and easy to explain to legislators, the public, and the regulated community.

Diatom community composition in Maine is strongly influenced by a generalized disturbance gradient that encompasses nutrients, specific conductance, and watershed development. Covariation among environmental variables may hinder development of nutrient inference models. Most diatoms sampled in this study did not respond unimodally to TN and TP gradients, invalidating a primary assumption of WA inference models. Attempts to improve model performance and reduce model bias by adding soft algae or by excluding indifferent diatoms were not successful. Further research is needed to determine if slope snapping is a viable alternative for reducing model bias.

4. EFFICACY OF STREAM ALGAL METRICS FOR DETECTING IMPAIRMENT OF WADEABLE STREAMS AND RIVERS

4.1 Abstract

The relationship of metrics based on benthic algal communities was evaluated with a general land use disturbance gradient affecting wadeable streams and rivers in Maine, USA. Epilithic algal samples (n=298) were collected from 193 sample locations across the State. Patterns in species composition were computed with Non-metric Multidimensional Scaling and displayed with correlated environmental variables. The major pattern in species composition was related to land use development, nutrient enrichment, and increased specific conductance. Maine Stream Tolerance values were computed with Principal Components Analysis for common algal taxa by determining major patterns in species weighted average optima for total phosphorus, total nitrogen, specific conductance, percent developed watershed, and percent impervious surfaces. Taxa were assigned to tolerance categories of sensitive, intermediate, and tolerant based on their Maine tolerance values and response to disturbance as shown by general linear models. Algal community attributes used in other multimetric indexes, and novel attributes based on Maine data, were assessed by 1) plotting attribute response to percent developed watershed in scatterplots with LOWESS regression lines, 2) determining strength of Spearman rank correlations with percent developed watershed, and 3) testing ability to distinguish reference from non-reference sites with Mann-Whitney U tests. Few community structure attributes (*e.g.*, total species richness) were effective metrics in Maine. Most metrics with the strongest relationship with percent developed watershed were based on Maine data, such as the relative richness of species characterized as being

sensitive in Maine. Bioassessment programs could benefit from testing attributes before including them in multimetric indices (*e.g.*, Index of Biotic Integrity) or multivariate statistical models. This process to test attributes and identify metrics could be replicated in other regions.

4.2 Introduction

Human population growth and concurrent changes in land use, pollution, water supply, and fluvial geomorphology threaten water quality of streams and rivers. These changes increasingly require that water quality monitoring programs apply rigorous biological assessments to evaluate the condition of aquatic resources, determine attainment of water quality standards, and inform management decisions that maintain and restore water quality (Karr 1991, Courtemanch 1995, Yoder and Rankin 1998). Biological assessment methods for lotic fish and macroinvertebrate communities are commonly used by state water quality agencies (USEPA 2002), however, few monitoring programs have identified algal metrics for flowing waters and integrated them into multimetric indices of biotic integrity (Bahls 1993, Fore and Grafe 2002, KDEP 2002, Fore 2003, Passy and Bode 2004, Wang et al. 2005). Algal bioassessments are a good alternative or supplement for monitoring and assessing the condition of rivers and streams (Patrick 1949, McCormick and Cairns 1994, Stevenson and Bahls 1999).

The Biological Condition Gradient (BCG) is a mechanism to "(1) assess aquatic resources more uniformly and directly and (2) communicate more clearly to the public the current status of aquatic resources and their potential for restoration" (Davies and Jackson 2006: 1251). The BCG was originally developed for fish and benthic macroinvertebrate communities of permanent, hard-bottomed streams exposed to increasing temperature, nutrients, and fine sediments attributable to watershed development (Davies and Jackson 2006). Applications of the BCG framework to other ecosystems and biological assemblages are incomplete, yet the BCG framework has great potential for application to a broad range of ecosystems and taxonomic groups. The BCG uses six tiers of biological condition, developed from fish and macroinvertebrate community responses, to indicate changes in 10 ecological attributes along an environmental stress gradient, ranging from natural condition (Tier 1) to severe alteration of structure and function (Tier 6). BCG Attributes II-V evaluate the representation of taxa that are highly sensitive, sensitive, intermediate, and tolerant to disturbance. Water quality professionals must identify taxa that are sensitive to disturbance (Attributes II and III), are of intermediate tolerance (Attribute IV), or are tolerant to disturbance (Attribute V) when incorporating the BCG into the development of a new bioassessment method (Davies and Jackson 2006). Computed variables based on the tolerance groupings can then measure departure from regional reference conditions (Stoddard et al. 2006).

This study evaluated the effectiveness of benthic algal community attributes for detecting a general disturbance gradient in Maine's rivers and streams. First, I determined if algal species composition reflected a general disturbance gradient. Second, I applied Maine stream and river algal data to metrics from existing algal IBIs to determine their applicability to Maine. Third, I computed Maine Stream Tolerance (MST) values for algae, assigned algal taxa to BCG attributes II-V, and generated metrics based on these attributes. Finally, I evaluated algal community attributes to identify metrics that respond to environmental stress and detect deviation from Maine reference conditions.

4.3 Methods

The sampling area, study sites, and definition of reference sites are described in Section 3.3.1. Field and laboratory procedures are described in Section 3.3.2. Major patterns in species composition were identified and correlated with environmental variables as described in Section 3.3.3.

4.3.1 Maine Stream Tolerance values

Maine stream tolerance values were calculated for common taxa to represent an overall tolerance to watershed disturbance (Table A.3). First, weighted average optima were calculated (C2 v. 1.5.0, Juggins 2007) for 195 diatoms and 41 soft algal taxa for total phosphorus (n=167), total nitrogen (n=166), specific conductance (n=166), percent developed watershed (*i.e.*, no longer forest and wetland) (n=186), and percent impervious surfaces in watershed (n=186). These five environmental variables were selected because they were strongly correlated with the primary NMS axis (Section 3.4.1 and Figure 3.3). Diatom optima were calculated with square roots of percent abundances to downweight the influence of ubiquitous, dominant taxa and taxa occurring in <7 samples were excluded from analysis. The optima for soft algal taxa were calculated separately from diatoms and were based individually on $\log_{10}$ densities, because large densities of some cyanobacteria would confound relative abundances. Major patterns of the taxa optima were identified with Principle Components Analysis (PCA) based on taxa optima of the five environmental variables (PC-ORD v. 5.0). Taxa scores on the primary PCA axis were rescaled to 1-100, with 1 representing the most sensitive taxon and 100 representing the most tolerant taxon.

Algal taxa were categorized as sensitive, intermediate, or tolerant based on their Maine tolerance values and general linear models (Canoco 4.55 and CanoDraw 4.14, ter Braak and Smilauer 2002) of taxa square root percent abundances to increasing percent developed watersehd. Akaike's Information Criteria was used to select the best fitting model from three options: quadratic (second-order), linear (first-order), and null model (flat line) (Hastie and Tibshirani 1990). Sensitive taxa were identified as having small tolerance values and linearly declining response curves. Intermediate taxa had intermediate tolerance values and either quadratic or flat response curves. Tolerant taxa had large tolerance values and either quadratic or linearly increasing response curves. I grouped BCG Attributes II (sensitive rare taxa) and III (sensitive ubiquitous taxa), because some of the most sensitive taxa were not rare. Intermediate and tolerant taxa correspond with BCG Attributes IV and V, respectively. *Cladophora spp.* and *Cladophora glomerata* (Linnaeus) Kützing were not sufficiently common to compute tolerance values, however, they were dominant in several urban streams with high conductivity and were assigned an tolerance value of 80.

4.3.2 Metric calculation and selection

I tested a combination of algal community attributes from multimetric indexes developed elsewhere and others based on Maine data to determine if a predictable and empirical relationship with percent developed watershed existed (Table 4.1). Attributes were examined for their suitability as "metrics" (Karr and Chu 1999) by 1) plotting attribute response to DEV (LOWESS regression lines, R v. 2.6.2, R Development Core Team 2008), 2) determining strength of relationships with percent developed watershed (Spearman rank correlations, R v. 2.6.2), and 3) determining the difference between median values of reference (n=42) and non-reference (n=148) sites (Mann-Whitney U test, SYSTAT 11.0, Wilkinson 1990). Scatterplots displaying attribute response are valuable, because although rank correlations alleviate problems with non-normal data, they do not describe the shape of a relationship (Van Sickle 2003). Triplots also were used to display sensitive, intermediate, and tolerant attributes and deviation from reference conditions. Variables were grouped into the categories of community composition, tolerance/intolerance, nutrient and organic enrichment, and individual condition (Karr and Chu 1999). Predicted responses of metrics used in previous studies were summarized.

Diatom autecology was based on van Dam et al. (1994) and included species indicator values for pH, salinity, nitrogen uptake metabolism, dissolved oxygen requirements, saprobity, trophic state, and moisture. Attributes based on autecological guilds were expressed in terms of 1) richness, 2) relative richness, or 3) relative abundance. Diatoms without autecological values were excluded from both the numerator and denominator of computations. For this study, alkaphilic diatoms were defined as having pH indicator values of ≥4. Acidophilic diatoms had pH indicator values of 1. Diatoms that require high oxygen concentrations had indicator values of 1 and diatoms that tolerate low oxygen concentrations had indicator values of ≥3. Salt-tolerant diatoms had salinity values ≥3. Diatoms tolerant of dry conditions had moisture values of ≥4. Nitrogen autotrophic and nitrogen heterotrophic diatoms had nitrogen uptake values of of 1 and ≥3, respectively. Oligosaprobic and polysaprobic were defined as having saprobity values of 1 and ≥4, respectively. Oligotraphentic and eutraphentic diatoms were defined as having trophic indicator values of 1 and ≥5, respectively. Diaom with trophic indicator values of 7 were excluded from trophic computations.

Attributes based on sensitive taxa, intermediate taxa, and tolerant taxa included all diatom and non-diatom taxa with Maine tolerance values. Taxa without Maine tolerance values were excluded from both the numerator and denominator of computations. The Pollution Tolerance Index was based on pollution tolerance values assigned to diatoms by Bahls (1973). The relative richness and relative abundance of erect, prostrate, stalked, and unattached diatom growth forms also were tested (Fore 2003, Wang et al. 2005). Two novel metrics were the relative richness and relative abundance of the families Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS), which are associated with streams in disturbed watersheds in Maine. Relative richness and relative abundance were computed for the sum of *Brachysira*, *Eunotia*, *Tabellaria*, and *Anomoeoneis* (BETA), which are genera associated with minimally disturbed sites in Maine. The relative biovolume of sensitive soft algae included *Batrachosperma* (Rhodophyta), *Mougeotia* (Chlorophyta), *Ulothrix* (Chlorophyta), *Zygnema* (Chlorophyta), *Tolypothrix* (Cyanophyta), and Rivulaceae (Cyanophyta). Several attributes from other studies were not tested in Maine because of a lack of data, such as phosphotase production and percent deformed diatom valves. Several attributes based on similarity of taxa composition of reference sites, such as the Diatom Similarity Index, were not tested in Maine because of great variation in taxa composition of reference sites.

Table 4.1 Algal attributes tested to determine response to increasing percent developed watershed using Maine data. Attributes expressed as richness, such as diatom richness, are shown in the *Richness* column. Attributes expressed as relative richness, such as relative richness of erect diatoms, are shown in the *Relative Richness* column. Attributes expressed as relative abundance, such as relative abundance of erect diatoms, are shown in the *Relative Abundance* column. Other metrics are self explanatory and are included in the *As Described* column. Algal attribute footnotes indicate the attribute source. Cell contents include 1) predicted response in other algal bioassessment papers (↑increase, ↓ decrease, footnotes indicate relevant citations, † indicates studies that define sensitive and tolerant taxa differently from this study), 2) Spearman rank correlation of algal attribute with DEV (only significant correlations ($P<0.05$) are shown, bold = $\rho > 0.50$, -- = not significant, * = $P<0.001$), and significance of Mann-Whitney U test algal attributes grouped by reference (n=42) and non-reference (n=148) sites (+ = $P<0.05$, -- = not significant).

Algal Attribute	CODE	As Described	Richness (R)	Relative Richness (RR)	Relative Abundance (RA)
COMMUNITY STRUCTURE					
Total Species Richness	TOT		--,--		
Diatom Species Richness	DIA		↓[4,6],0.14,--		↓[5], --,--
Diatom Genera Richness	DIAG		↓[6],--, --		
Generic Richness / Expected Richness		↓[5]			
Diatom Divisions	DIAD		↓[6],--,--		
% Green Algae	GRN		---,--		--,--
% Red Algae	RED		--,--		--,--
% Cyanobacteria	CYA		--,--		↑[5],--,--
Shannon Diversity Index	SHAN	↓[1,6],0.17,--			
% Dominant Diatom	PDOM				↑[4,5],--,--
% *Achnanthidium minutissimum*	PACH				↑[1],--,--
Diatom Similarity Index[1] & Average Similarity to Reference[7]	SIM	↓[1,7]			
Erect Diatoms[3,7]	EREC		-0.42*,--	**-0.57***,+	**-0.51***,--
Prostrate Diatoms[3,7]	PROS		0.44*,+	**0.59***,+	0.39*,+

Table 4.1 Continued

Stalked Diatoms^{3,7}	STAL		--,--	--	--,+
Unattached Diatoms^{3,7}	UNA		--,--	-0.23*,+	-0.30*,+
TOLERANCE/INTOLERANCE					
Pollution Tolerance Index^{9}	PTI	↓^{1,6,7},-0.25*,+			
Fragilaria Group	FRAG		↓^{6},-0.19,--		
Cymbella Group	CYMB		↓^{6},--,--	↓^{7},0.32*,+	
Navicula species	NAV			↑^{7},**0.57***,+	
% *Achnanthes* / (*Achnanthes* + *Navicula*)	ACHNAV				↓^{1,7},**-0.51***,+
Alkaphilic or Acidobiontic Diatoms^{8}	ALK		↓^{2}↑^{3},--,--		↓^{4},--,--
Acidophilic Diatoms^{8}	ACID		-0.35*,+	-0.39*,+	↑^{5},-0.37*,+
High Oxygen Diatoms^{8}	HIGH		-0.28*, --	**-0.67***,+	-0.45*,+
Low Oxygen Diatoms^{8}	LOW		**0.57***,+	**0.54***,+	0.33*,+
Salt Tolerant Diatoms^{8}	SALT		**0.66***,+	**0.66***,+	**0.64***,+
Dry Tolerant Diatoms^{8}	DRY		--,--	-0.27*,--	--,--
Motile Diatoms^{2,7}	MOT		**0.50***,+	**0.63***,+	↑^{1,2,3,4,5},**0.53***,+
Relative Biovolume Sensitive Soft Algae (Maine)	SNSFT_RB	-0.39*,+			
Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae	BCRS		**0.63***,+	**0.69***,+	**0.70***,+
Brachysira, *Eunotia*, *Tabellaria*, and *Anomoneis*	BETA		**-0.63***,+	**-0.74***,+	**-0.70***,+
Sensitive Taxa†	SEN		↓^{7},**-0.69***,+	↓^{7},**-0.81***,+	↓^{2,3},**-0.62***,+
Intermediate Tolerance Taxa	INT		0.16,--	--,--	--,--
Tolerant Taxa	TOL		**0.77***,+	**0.80***,+	↑^{2†},**0.76***,+
Relative Biovolume Sensitive Taxa	SEN_RB	**-0.64***,+			
Relative Biovolume Intermediate Taxa	INT_RB	--,+			

Table 4.1 Continued

NUTRIENT AND ORGANIC ENRICHMENT					
Chlorophyll *a*	CHL	↑[5,6]			
Ash Free Dry Mass	AFDM	↑[4,5,6],--,--			
Total Biovolume	TOT_BV	--,--			
Diatom Biovolume	DIA_BV	--,--			
Soft Algae Biovolume	SFT_BV	--,--			
Total Density	TOT_DEN	--,--			
Diatom Density	DIA_DEN	0.20,--			
Soft Algae Density	SFT_DEN	--,--			
N-Autotrophic Diatoms[8]	NAUT		-0.43*,+	**-0.72***,+	↓[2,3],**-0.53***,+
N-Heterotrophic Diatoms[8]	NHET		**0.60***,+	**0.58***,+	0.36*,+
Oligosaprobic Diatoms[8]	OSAP		-0.43*,+	**-0.65***,+	,+
Polysaprobic Diatoms[8]	PSAP		**0.50***,+	0.39*,+	0.19,+
Oligotraphentic Diatoms[8]	OTRO		-0.44*,--	**-0.58***,+	-0.42*,+
Eutraphentic Diatoms[8]	EUTR		**0.71**,+	**0.74***,+	↑[3,4,5],**0.63***,+
INDIVIDUAL CONDITION					
% Deformed Diatom Valves	DEAD	↑[2]			
OTHER ECOLOGICAL ATTRIBUTES					
Phosphotase Production	PHOS	↑[4,5]			

Sources: [1] Bahls (1993), [2] Fore and Grafe (2002), [3] Fore (2003), [4] Hill et al. (2003), [5] Hill et al. (2002), [6] KDEP (2002), [7] Wang et al. (2005), [8] van Dam et al. (1994), [9] Bahls (1973)

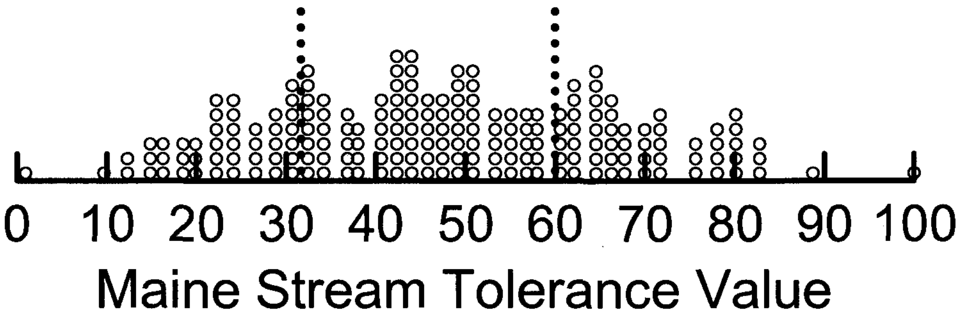
4.4 Results

4.4.1 Maine stream tolerance values

Weighted average optima for total phosphorus, total nitrogen, specific conductance, percent developed watershed, and percent impervious surfaces for individual taxa were correlated ($r>0.70$, $P<0.001$). The major pattern in taxa optima (PCA 1-axis solution, eigenvalue=4.28, P=0.001, 80% of variation) was correlated with optima for total phosphorus, total nitrogen, specific conductance, percent developed watershed, and percent impervious surfaces ($r>0.90$, $P<0.001$). Approximately 25% of taxa, including 11 soft algal taxa, were classified as sensitive because they had Maine tolerance values <32.2 and most had decreasing responses (quadratic or linear, $P<0.05$) to percent developed watershed as shown by general linear models (Figure 4.1). For example, *Tabellaria flocculosa* (Roth) Kützing (TAfloccu) had a tolerance value of 18.7, a decreasing response, and was classified as sensitive (Figure 4.2). Approximately 25% of taxa, including only 2 soft algal taxa, were classified as tolerant because they had Maine tolerance values >60.0 and most had increasing responses (quadratic or linear, $P<0.05$) to percent developed watershed (Figure 4.1). *Gomphonema kobayasii* Kociolek et Kingston (GOkobaya), for example, had a tolerance value of 71.2, an increasing response ($P<0.05$) to percent developed watershed, and was classified as tolerant (Figure 4.2). The tolerance values of the remaining taxa ranged from 32.2 to 60.0 and were classified as intermediate (Figure 4.1). Intermediate taxa exhibited a variety of response curves. For example, *Reimeria sinuata* (Gregory) Kociolek et Stoermer (REsinuat) had a unimodal response (Figure 4.2). *Fragilaria vaucheriae* (Kützing) Petersen (FRvauche) did not have a significant quadratic or linear response resulting in the default null model (*i.e.*, flat line) (Figure 4.2).

Most soft algae taxa were sensitive or intermediate. Sensitive taxa included the green algae *Bulbochaete, Mougeotia, Tetraedron minimum* (Braun) Hansgirg, *Ulothrix,* and *Zygnema*, the red algae *Audouinella* (including unidentified chantransia stage Floridophyceae) and *Batrachospermum*, and the cyanobacteria *Calothrix* and *Chroococcus minor* (Kützing) Nägeli. The only tolerant soft taxa were *Cladophora spp.* and *Cladophora glomerata*.

a) Diatoms - Sensitive Intermediate Tolerant



b) Soft Algae - Sensitive Intermediate Tolerant

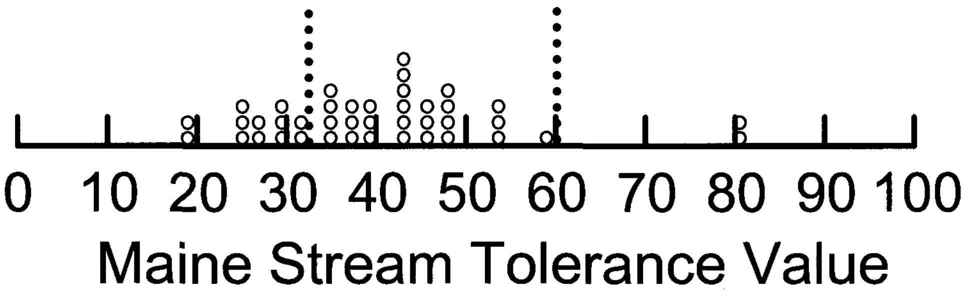


Figure 4.1 Distribution of Maine Stream Tolerance (MST) values for a) diatoms and b) soft algae grouped by tolerance. Senstive taxa had MST<32.2 and either decreasing response to a gradient of watershed development or unimodal response with low optima. The MST of intermediate taxa ranged from 32.2 to 60.0. Intermediate taxa had unimodal or flat responses to watershed development. Tolerant taxa had MST >60.0 and increasing response to watershed development.

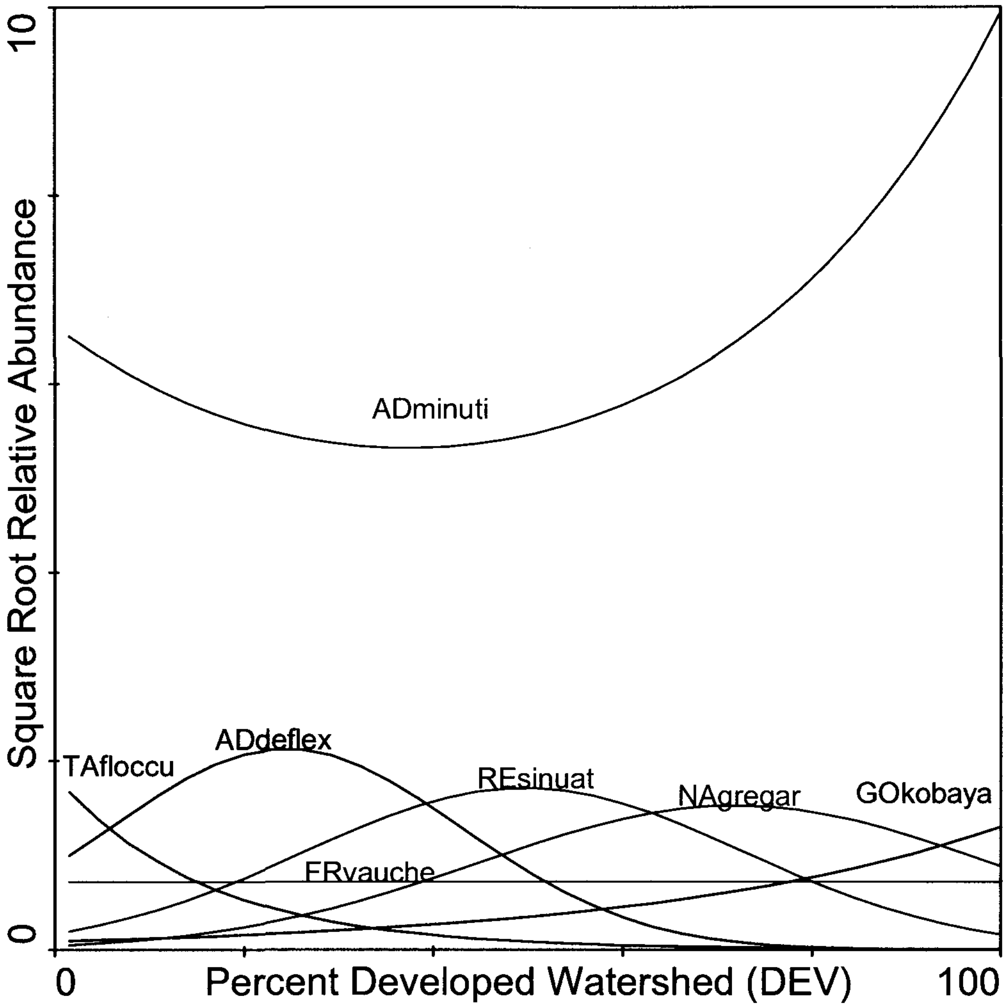


Figure 4.2 Examples of diatom species with differently shaped general linear model curves in response to increasing watershed development. *Tabellaria floculossa* (TAfloccu) had a decreasing response and *Gomphonema kobayasii* (GO kobaya) had an increasing response to percent developed watershed (DEV) (*i.e.*, no longer forest and wetland). *Fragilaria vaucheriae* (FRvauche) was indifferent and *Achnanthidium minutissimum* (ADminuti) had an inverse quadratic response to DEV. *A. deflexum* (ADdeflex), *Reimeria sinuata* (REsinuat), and *Navicula gregaria* (NAgregar) had unimodal responses with different optima.

4.4.2 Algal community metrics

Most attributes that qualified as metrics [*i.e.*, moderately correlated (r>0.50) with percent developed watershed and discriminating reference and non-reference sites] were in the Tolerance/Intolerance or Nutrient and Organic Enrichment categories (Table 4.1). The relative richness of erect diatoms, relative abundance of erect diatoms, and relative richness of prostrate diatoms were the only metrics from the Community Structure category. No attributes related to the richness, relative richness, diversity, density, or biovolume of the entire algal community or major groups (*e.g.*, diatoms, green algae) were metrics in Maine. Many metrics were correlated (Table 4.2). Some metrics and attributes had non-linear responses to DEV. For example, the relative abundance of sensitive (SEN_RA), intermediate (INT_RA), and tolerant (TOL_RA) algae had non-linear decreasing, unimodal, and increasing response curves, respectively (Figure 4.3). Triplots were used to display proportions relative richness, relative abundance, and relative biovolume of sensitive, intermediate, and tolerant taxa. (*e.g.*, Figure 4.4).

Table 4.2 Spearman rank correlation (ρ) matrix of attributes that were identified as potential metrics. Shaded cells ar

Richness of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_R), Relative Abundance of
of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_RR), Richness of *Brachysira*, *Eunoti*
and *Anomoneis* (BETA_RA), Relative Richness of of *Brachysira, Eunotia*, *Tabellaria,* and *Anomoneis* (BETA_RR
Wetland Disturbance Index (DWDI), Relative Abundance of Erect Diatoms (EREC_RA), Relative Richness of Erect
of Eutraphentic Diatoms (EUTR_RR), Relative Richness of High Oxygen Diatoms (HIGH_RR), Relative Biovolume
Abundance of Motile Diatoms (MOT_RA), Relative Richness of Motile Diatoms (MOT_RR), Relative Abundance o
Relative Abundance of N-Heterotrophic Diatoms (NHET_RA), Relative Richness of Oligosaprobic Diatoms (OSAP_
Relative Richness of Prostrate Diatoms (PROS_RR), Richness of Polysaprobic Diatoms (PSAP_R), Relative Richnes
Relative Richness of Salt-Tolerant Diatoms (SALT_RR), Relative Biovolume of Sensitive Algae (SEN_RB), Relativ
Relative Biovolume of Sensitive Soft Algae (SNSFT_RB), Relative Biovolume of Tolerant Algae (TOL_RB), Relati

	BCRS_R	BCRS_RA	BCRS_RR	BETA_R	BETA_RA	BETA_RR	DSCI	DTPI	DWDI	EREC_RA	EREC_RR	EUTR_RA	EUTR_RR	HIGH_RR	INT_RB	LOW_RR	MOT_RA	MOT_RR
BCRS_R	1																	
BCRS_RA	**0.88**	1																
BCRS_RR	**0.94**	**0.85**	1															
BETA_R	-0.37	-0.44	-0.55	1														
BETA_RA	-0.48	-0.52	-0.60	**0.89**	1													
BETA_RR	-0.59	-0.63	-0.68	**0.93**	**0.89**	1												
DSCI	0.62	0.64	**0.71**	**-0.75**	**-0.78**	**-0.81**	1											
DTPI	**0.73**	**0.78**	0.69	-0.40	-0.57	-0.57	0.58	1										
DWDI	0.69	**0.72**	**0.72**	-0.58	-0.67	**-0.70**	**0.85**	**0.74**	1									
EREC_RA	-0.26	-0.32	-0.38	**0.71**	0.69	0.67	-0.64	-0.28	-0.52	1								
EREC_RR	-0.47	-0.51	-0.58	0.84	**0.74**	**0.86**	**-0.70**	-0.38	-0.53	**0.77**	1							
EUTR_RA	0.51	0.60	0.51	-0.40	-0.52	-0.49	0.53	0.69	0.67	-0.32	-0.36	1						
EUTR_RR	0.59	0.62	0.69	**-0.73**	**-0.74**	**-0.77**	**0.77**	0.61	0.76	-0.52	-0.65	0.63	1					
HIGH_RR	-0.59	-0.62	-0.66	0.64	**0.70**	0.68	-0.68	-0.68	**-0.70**	0.42	0.55	-0.64	**-0.89**	1				
INT_RB	0.12	0.10	0.11	-0.16	-0.28	-0.18	0.12	0.28	0.08	-0.13	-0.12	0.25	0.17	-0.21	1			
LOW_RR	0.49	0.55	0.58	-0.55	-0.55	-0.58	0.52	0.52	0.55	-0.43	-0.54	0.48	**0.72**	-0.66	0.15	1		
MOT_RA	0.64	**0.77**	0.56	-0.24	-0.27	-0.43	0.46	0.63	0.56	-0.19	-0.36	0.44	0.42	-0.44	-0.06	0.32	1	
MOT_RR	**0.84**	**0.83**	**0.83**	-0.39	-0.45	-0.57	0.62	**0.71**	0.69	-0.30	-0.49	0.53	0.56	-0.55	0.05	0.51	0.72	
NAUT_RA	-0.29	-0.32	-0.40	0.59	0.69	0.54	-0.55	-0.47	-0.56	0.44	0.42	-0.60	-0.62	0.60	-0.27	-0.47	-0.12	-0.3
NAUT_RR	-0.58	-0.63	-0.65	0.67	**0.75**	**0.73**	**-0.70**	**-0.73**	**-0.76**	0.47	0.56	-0.63	**-0.87**	**0.90**	-0.25	-0.66	-0.44	-0.5
NHET_RR	0.57	0.60	0.68	-0.62	-0.62	-0.65	0.59	0.52	0.56	-0.47	-0.59	0.44	**0.76**	-0.68	0.11	**0.85**	0.38	0.5
OSAP_RR	-0.52	-0.55	-0.61	0.69	**0.72**	**0.72**	-0.69	-0.62	-0.69	0.45	0.59	-0.57	**-0.89**	**0.90**	-0.19	-0.63	-0.39	-0.4
OTRO_RR	-0.39	-0.41	-0.50	**0.70**	0.68	0.67	-0.68	-0.38	-0.55	0.52	0.63	-0.47	**-0.76**	**0.73**	-0.19	-0.51	-0.26	-0.3
PROS_RR	**0.74**	**0.78**	**0.74**	-0.51	-0.57	-0.65	0.57	**0.74**	0.64	-0.43	-0.60	0.56	0.56	-0.57	0.19	0.55	0.62	**0.8**
PSAP_R	0.67	0.69	0.58	-0.33	-0.39	-0.52	0.46	0.58	0.50	-0.24	-0.41	0.51	0.60	-0.60	0.13	0.65	0.56	0.5
PSAP_RR	0.29	0.37	0.38	-0.53	-0.50	-0.49	0.42	0.34	0.36	-0.37	-0.44	0.42	0.65	-0.63	0.14	**0.78**	0.21	0.2
SALT_RA	0.68	0.65	0.69	-0.47	-0.50	-0.60	0.67	0.64	0.66	-0.32	-0.51	0.53	0.68	-0.62	0.12	0.53	0.53	0.6
SALT_RR	0.67	0.61	**0.72**	-0.59	-0.58	-0.68	**0.72**	0.60	**0.70**	-0.40	-0.56	0.49	**0.79**	-0.68	0.11	0.59	0.45	0.6
SEN_RB	-0.48	-0.56	-0.53	0.59	0.69	0.64	-0.65	-0.63	-0.64	0.48	0.51	-0.62	-0.64	0.63	-0.61	-0.48	-0.40	-0.5
SEN_RA	-0.42	-0.47	-0.49	0.60	**0.71**	0.63	-0.64	-0.56	-0.68	0.48	0.47	-0.56	-0.67	0.65	-0.38	-0.46	-0.27	-0.4
SEN_RR	-0.66	**-0.72**	**-0.74**	**0.78**	**0.84**	**0.86**	**-0.84**	**-0.76**	**-0.80**	0.59	**0.70**	-0.64	**-0.82**	**0.78**	-0.29	-0.62	-0.51	-0.6
SNSFT_RB	-0.29	-0.36	-0.34	0.42	0.45	0.44	-0.41	-0.35	-0.38	0.31	0.37	-0.36	-0.44	0.41	-0.36	-0.39	-0.18	-0.3
TOL_RB	**0.73**	**0.80**	**0.76**	-0.64	**-0.73**	**-0.78**	**0.79**	**0.72**	**0.80**	-0.53	-0.66	0.57	**0.73**	-0.69	0.07	0.55	0.61	**0.7**
TOL_RA	**0.76**	**0.84**	**0.78**	-0.63	-0.69	**-0.78**	**0.77**	**0.75**	**0.79**	-0.51	-0.65	0.59	**0.73**	-0.67	0.10	0.57	0.68	**0.7**
TOL_RR	**0.78**	**0.82**	**0.82**	-0.67	**-0.76**	**-0.81**	**0.81**	**0.79**	**0.84**	-0.51	-0.64	0.65	**0.84**	**-0.81**	0.17	0.65	0.59	**0.7**

trics. Shaded cells are ρ≥0.70.

lative Abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_RA), Relative Richness
f *Brachysira* , *Eunotia* , *Tabellaria* , and *Anomoneis* (BETA_R), Relative Abundance of *Brachysira* , *Eunotia, Tabellaria,*
nomoneis (BETA_RR), Diatom Specific Conductance Index (DSCI), Diatom Total Phosphorus Index (DTPI), Diatom
ive Richness of Erect Diatoms (EREC_RR), Relative Abundance of Eutraphentic Diatoms (EUTR_RA), Relative Richness
), Relative Biovolume of Intermediate Algae (INT_RB), Relative Richness of Low Oxygen Diatoms (LOW_RR), Relative
elative Abundance of N-Autotrophic Diatoms (NAUT_RA), Relative Richness of N-Autotrophic Diatoms (NAUT_RR),
obic Diatoms (OSAP_RR), Relative Richness of Oligotraphentic Diatoms (OTRO_RR),
_R), Relative Richness of Polysaprobic Diatoms (PSAP_RR), Relative Abundance of Salt-Tolerant Diatoms (SALT_RA),
e (SEN_RB), Relative Abundance of Sensitive Algae (SEN_RA), Relative Richness of Sensitive Algae (SEN_RR),
ae (TOL_RB), Relative Abundance of Tolerant Algae (TOL_RA), and Relative Richness of Tolerant Algae (TOL_RR).

INT_RB	LOW_RR	MOT_RA	MOT_RR	NAUT_RA	NAUT_RR	NHET_RR	OSAP_RR	OTRO_RR	PROS_RR	PSAP_R	PSAP_RR	SALT_RA	SALT_RR	SEN_RB	SEN_RA	SEN_RR	SNSFT_RB	TOL_RB	TOL_RA	TOL_RR
1																				
0.15	1																			
-0.06	0.32	1																		
0.05	0.51	0.72	1																	
-0.27	-0.47	-0.12	-0.33	1																
-0.25	-0.66	-0.44	-0.57	0.68	1															
0.11	**0.85**	0.38	0.53	-0.45	-0.67	1														
-0.19	-0.63	-0.39	-0.48	0.66	**0.92**	-0.66	1													
-0.19	-0.51	-0.26	-0.35	0.60	**0.70**	-0.58	**0.80**	1												
0.19	0.55	0.62	**0.88**	-0.39	-0.60	0.56	-0.46	-0.33	1											
0.13	0.65	0.56	0.58	-0.26	-0.58	0.62	-0.55	-0.43	0.54	1										
0.14	**0.78**	0.21	0.28	-0.44	-0.59	**0.73**	-0.62	-0.56	0.33	**0.73**	1									
0.12	0.53	0.53	0.65	-0.34	-0.60	0.51	-0.57	-0.50	0.58	0.56	0.33	1								
0.11	0.59	0.45	0.66	-0.47	-0.68	0.58	-0.65	-0.53	0.58	0.51	0.37	**0.88**	1							
-0.61	-0.48	-0.40	-0.51	0.54	0.67	-0.47	0.60	0.52	-0.59	-0.41	-0.38	-0.54	-0.55	1						
-0.38	-0.46	-0.27	-0.43	0.64	0.69	-0.45	0.65	0.56	-0.49	-0.32	-0.37	-0.52	-0.56	**0.79**	1					
-0.29	-0.62	-0.51	-0.67	0.61	**0.82**	-0.68	**0.77**	0.66	**-0.72**	-0.53	-0.46	-0.69	**-0.73**	**0.79**	**0.75**	1				
-0.36	-0.39	-0.18	-0.34	0.36	0.46	-0.34	0.38	0.32	-0.41	-0.26	-0.31	-0.33	-0.35	0.61	0.63	0.49	1			
0.07	0.55	0.61	**0.76**	-0.45	**-0.73**	0.59	-0.65	-0.53	**0.75**	0.55	0.36	0.69	**0.71**	-0.65	-0.65	**-0.83**	-0.51	1		
0.10	0.57	0.68	**0.77**	-0.43	**-0.72**	0.64	-0.66	-0.52	**0.77**	0.62	0.42	0.67	**0.70**	-0.66	-0.56	**-0.83**	-0.41	**0.90**	1	
0.17	0.65	0.59	**0.79**	-0.53	**-0.83**	0.69	**-0.76**	-0.60	**0.77**	0.66	0.50	**0.75**	**0.79**	**-0.71**	-0.69	**-0.89**	-0.47	**0.91**	**0.90**	1

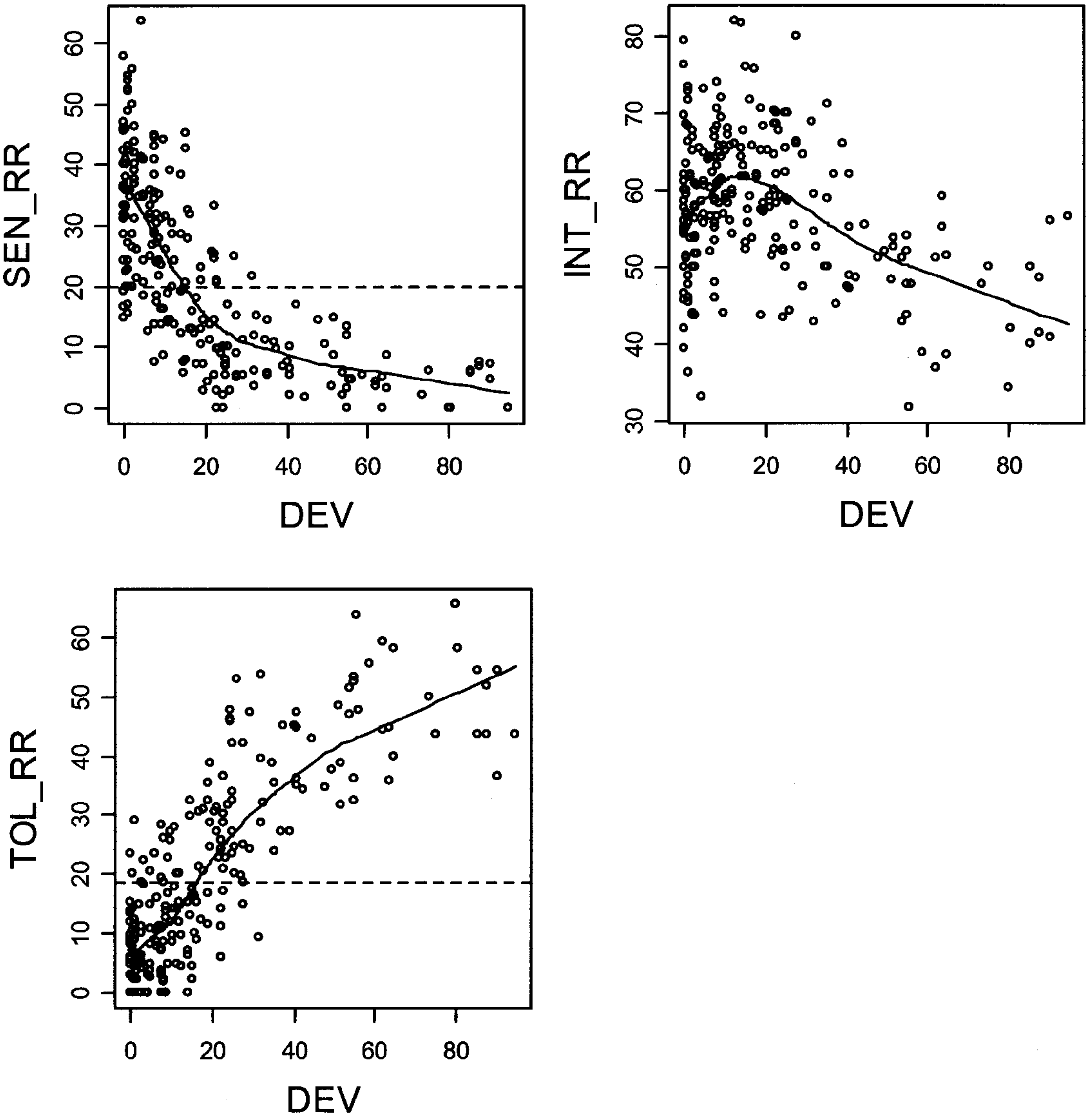


Figure 4.3 Examples of attributes with decreasing (relative richness of sensitive taxa, SEN_RR), unimodal (relative richness of intermediate taxa, INT_RR), and increasing (relative richness of tolerant taxa, TOL_RR) responses to percent of watershed land cover that is developed (DEV) and not forest and wetland. The trend lines are locally-weighted regression (LOWESS) lines.

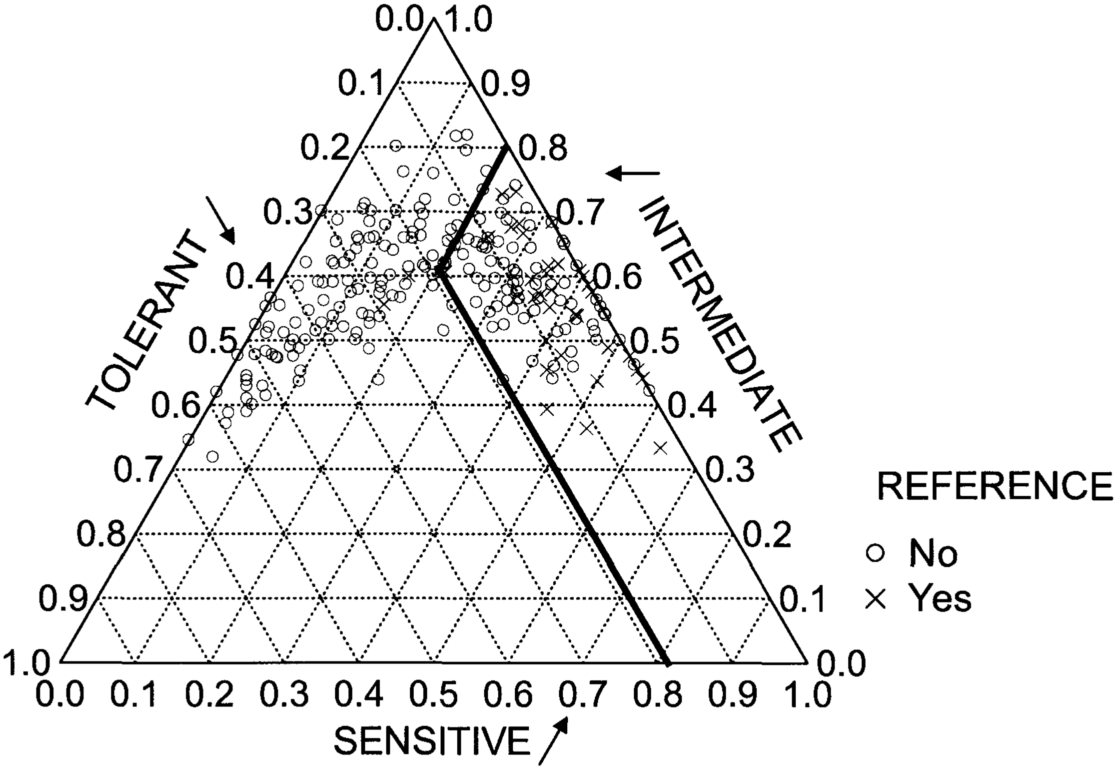


Figure 4.4 Relative richness of sensitive, intermediate, and tolerant taxa collected from reference (n=42) and non-reference (n=148) sites. Bold lines indicate the 90th percentiles of reference sites and define the range of "natural condition". Arrows indicate direction of grid lines for each axis.

4.5 Discussion

4.5.1 Algal taxa tolerance values and responses to an environmental disturbance gradient

The major pattern in species composition reflected a gradient of human disturbance (Section 3.4.1). The major pattern of species composition was related to a general disturbance gradient associated with urbanization and increases in nutrients, specific conductance, and impervious surfaces (Figure 3.3). Width, depth, and canopy were not correlated with the major pattern in species composition, presumably because of the standardized site selection and sampling of wadeable segments with partly open to open canopies. Water velocity, pH, and percent fines also were not correlated with the major pattern in species composition, which was somewhat surprising. I expected sites with fast moving water to favor adnate, non-motile diatoms and early colonizing species and slow moving water to favor later successional mats consisting of erect, stalked, and motile taxa (McCormick 1996). Velocity, however, is highly variable; velocity measurements were not always collected at base flows; and impervious surfaces can alter flow regimes. I expected percent fines in a stream reach to favor motile species (Bahls 1993, Kutka and Richards 1996), but standardized sampling methods of collecting samples from rocks may have reduced the importance of this variable. Quantifying the sediment adhering to the rocks may reveal a relationship between % fines and abundance of motile species. Algal communities are shaped by pH (Hustedt 1939, Nygaard 1956, Patrick and Reimer 1966, Planas 1996), however, most streams in Maine are somewhat acidic to circumneutral. Although pH problems can alter algal communities in Maine, they apparently are not common enough to shape statewide patterns in species composition.

Synthesizing information from both Maine tolerance values and general linear model response curves provides an empirical method for identifying sensitive, intermediate, and tolerant algal taxa and assigning taxa to Biological Condition Gradient (BCG) Attributes II-V. Species classified as Attribute II (sensitive-rare) or Attribute III (sensitive-ubiquitous) were pooled into a sensitive group, because taxa with the lowest MST values were not always rare in terms of abundance and number of samples. Specimen identifications were limited to 600 diatom valves extracted from subsamples, which likely underrepresented sensitive-rare taxa. Historic practices of identifying several thousand valves (*e.g.*, 8,000) may increase detections of rare Attribute II taxa (Patrick et al. 1954, Patrick 1961). An alternative approach is to base Attributes II and III on sensitivity and not on rarity, which will reduce a potential influence of sample size on categorization of the stream condition as well as reduce effort required to discriminate sites at the less-disturbed end of the BCG. Attribute II and III taxa could be distinguished based on occurrence restricted to less disturbed sites versus occurrence across the full disturbance gradient. For example, *Brachysira brebissonii* has a Maine tolerance value of 16.29 and is restricted in occurence to watersheds with little watershed disturbance (Figure 4.5) and could be categorized as an Attribute II taxon. Although *Brachysira microcephala* has a Maine tolerance value of 18.00, it occurs in streams with a broad range of watershed disturbance (Figure 4.5) and could be categorized as an Attribute III taxon. Assignments could be reevaluated periodically with additions of new samples to confirm patterns of occurence.

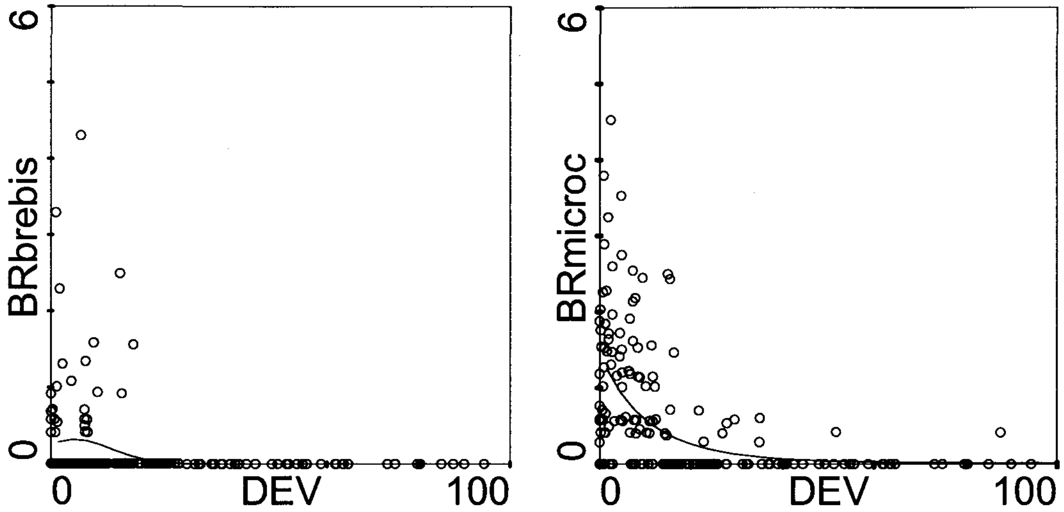


Figure 4.5 The square-root percent abundance of *Brachysira brebissonii* (BRbrebis) and *B. microcephala* (BRmicroc) in relation to percent developed watershed (DEV). Response curves are based on best performing general linear models.

The intermediate tolerance category combined two ecologically different groups. All intermediate taxa had intermediate Maine tolerance values, but for different reasons. Taxa in the first group were truly of intermediate tolerance because they had unimodal responses to watershed development, nutrient enrichment, and specific conductance. Taxa in the second group were seemingly indifferent to the watershed development and the range of water quality observed in this study, as shown by flat response curves. One option would have been to exclude these taxa from assignment of tolerance values and categories. The indifferent taxa were retained in this study because they were common, often abundant, and thought to be important components of the algal communities.

Further research is needed to determine if excluding indifferent taxa would improve the response of community attributes based on relative richness, relative abundance, or relative biovolume of sensitive, intermediate, and tolerant taxa.

Several patterns of tolerance appeared with multi-species diatom genera. Most *Brachysira, Cymbella, Eunotia*, *Fragilaria*, *Frustulia*, *Psammothidium*, *Synedra* species had MST values <50 and included many sensitive species. Many species in these genera are associated with oligotrophic to mesotrophic, oligosaprobic, and well oxygenated streams that range from acidic to circumneutral (*i.e.*, Maine reference sites). In addition, most species in these genera are not motile and susceptible to sedimentation observed in degraded streams in urban and agricultural settings. Many species in the genera *Achnanthidium*, *Gomphonema*, *Karayevia*, *Meridion*, and *Staurosira* were of intermediate tolerance with fewer sensitive and tolerant species, which is reflected by a broader range in tolerances in the literature (van Dam et al. 1994, Porter 2008). Most species in the genera *Amphora, Cocconeis, Encyonema, Mayamaea, Navicula, Nitzschia, Planothidium,* and *Surirella* were generally tolerant or intermediate. Many species in these genera tolerate increased nutrient enrichment, chloride concentrations, and specific conductance (van Dam et al. 1994, Potapova and Charles 2003, Porter 2008). These species' traits presumably provide greater resistance to stressors associated with the dominant disturbance gradient in Maine, such as nutrient enrichment and increased specific conductance. Maine streams with greatest specific conductance are in urban settings with greatest application of road salts, which could favor species tolerant of chloride (Rott et al. 1998, Leland and Porter 2000, Fore 2003). In addition, species of *Amphora*, *Navicula*, *Nitzschia*, and *Surirella* are motile and are thought to be tolerant of sedimentation, which has been observed in degraded streams in urban and agricultural settings (Bahls 1993, Kutka and Richards 1996). Species in the genera *Achnanthes, Diatoma*, and *Pinnularia* were distributed equally among sensitive, intermediate, and tolerant categories and, therefore as a group, are not reliable indicators of stream condition.

4.5.2 Evaluation of metric validity and limitations

Metric assessment. I evaluated attributes and identified as metrics those with increasing or decreasing response curves (*e.g.*, Figure 4.3), significant correlation with DEV, and that distinguish reference and non-reference sites (Table 4.1). Karr and Chu (1999, page 47) distinguish attributes that are "any measurable part or process of a biological system" from metrics that are attributes "empirically shown to change in value along a gradient of human influence". Attribute response to a disturbance gradient should be evaluated before applying the attribute as a metric, however, many studies that use algal multimetric indices do not indicate if the metrics have been evaluated prior to their use (Bahls 1993, Hill et al. 2000, KDEP 2002, Hill et al. 2003). Although there are examples of algal multimetric indexes that did confirm metric response to disturbance (e.g,, Fore and Grafe 2002, Fore 2003, Wang et al. 2005), there is regional variation in metric response. The Shannon-Weiner Diversity Index was expected to decrease with disturbance in Kentucky (KDEP 2002), however, the metric increased when evaluated empirically in Kentucky (Wang et al. 2005) and had no response in Maine. Similarly, diatom species richness was expected to decrease with human disturbance in the Mid-Atlantic (Hill et al. 2000, Hill et al. 2003) but increased when empirically evaluated (Fore 2003). Formal tests of attributes also can compare performance of variations in the way that attributes are expressed, such as richness, relative richness, and relative abundance. Although relative richness metrics are uncommonly used, most attributes based on relative richness and disturbance performed as well or better than corresponding attributes based on richness (Table 4.1). A formal process of evaluating metrics is necessary to confirm that attributes actually respond as theorized.

Geographic limitations of metrics. Monitoring programs can improve algal bioassessments by using local data to develop novel metrics and test metrics developed in other regions of the world. Empirically testing and developing metrics with local data is important, because regional variation in climate, geology, topography, and vegetation influence the algal community composition of minimally disturbed streams, resulting in different expectations of regional reference conditions in dissimilar ecoregions (Grenier et al. 2006). Most metrics that were strongly correlated with Maine's disturbance gradient ($r \geq 0.70$) were developed with Maine data, such as the Maine tolerance values. In addition, regional variation in land use and human activities can impose different stressors that cause regional variation in metric response (Griffith et al. 2002, Wang et al. 2005). For example, relative abundance of acidophilic diatoms increased and relative abundance of acidobiontic diatoms decreased in the Mid-Atlantic where mining activities caused acidification (Hill et al. 2000, Hill et al. 2003). In contrast, Maine reference sites often were more acidic because of geology or association with wetlands compared to some impaired streams in urban and agricultural settings with greater geologic and anthropogenic alkalinity. Applying metrics to a new region with different geology, climate, etc. without verifying response could confound interpretation of resource condition. For example, the relative abundance of the dominant diatoms showed no response to disturbance in Maine, however, an increase with disturbance was expected in the Mid-Atlantic region (Hill et al. 2000, Fore 2003, Hill et al. 2003) and a decrease with disturbance in Kentucky (Wang et al. 2005). Monitoring programs can avoid erroneous interpretation of resource condition by using local data to develop novel metrics and to test metric response.

Limitations of community attributes as metrics. Most of the community structure attributes based on richness, diversity, and relative abundances of algal groups were not metrics in Maine because of variability of species response to stressors within genera or groups (Table 4.1). Most community richness attributes (*e.g.*, total richness, diatom richness, *Cymbella* richness) were not correlated with percent developed watershed because of great variability and replacement of species by more tolerant species. Diatom taxa richness response to disturbance varies greatly across studies and actually increased in response to disturbance in regions where reference sites were naturally oligotrophic (Fore 2003). The relative abundance of diatoms was expected to decrease and the relative abundance of cyanobacteria was expected to increase in Mid-Atlantic streams in response to disturbance (Hill et al. 2000, Hill et al. 2003), however, both attributes showed no response to disturbance in Maine. Many reference streams in Maine contained large relative abundance and relative biovolume of sensitive and intermediate cyanobacteria, such as *Calothrix* and *Oscillatoria*. Impaired streams had similar relative abundance of cyanobacteria, but no sensitive species. Diversity and richness metrics may work in other regions, however, they should be tested to confirm predicted response.

Several attributes were not metrics in Maine because of inconsistent relationships between land use, availability of resources (*e.g.*, nutrient, light), and magnitude and frequency of disturbances (*e.g.*, scouring, grazing) (Biggs and Hickey 1994, Biggs 1996). Variability in resource supply and disturbances caused great variation in biomass attributes (*e.g.*, AFDM, total biovolume) and several other attributes. For example, the relative abundance of *Achnanthidium minutissimum* was expected to increase in Montana (Bahls 1993, Peterson and Tuchman 1994), but it was not a metric in this study or in Idaho (Fore and Grafe 2002). Some Maine reference streams were oligotrophic, high gradient streams with periodic scouring events enhanced by gradient. In contrast, some urban streams were heavily grazed by macroinvertebrates or periodically scoured by surges of water off of impervious surfaces. Grazing and scouring could explain the inverse quadratic response of *A. minutissimum* to percent developed watershed in addition to competition from other *Achnanthidium* species of intermediate tolerance (*e.g.*, *A. deflexum*) (Figure 4.2).

Combining multiple metrics for assessment. Attributes with unimodal responses can be valuable for interpreting the BCG when combined with other metrics. The relative richness of intermediates (INT_RR) had a unimodal, subsidy-stress response to disturbance in Maine with moderate values for Tiers 1 and 2, higher values for Tiers 3 and 4, and lower values at Tiers 5 and 6 (Odum et al. 1979) (Figure 4.3). Most multimetric indexes would not include INT_RR as a metric, because it has a small correlation with percent developed watershed, and it would be complicated to score (*e.g.*, 1, 3, 5) and integrate into an additive index. For example, a small INT_RR value could be associated with a reference site or an impaired site but would likely receive the same metric score (*e.g.*, 1). However, biologists can use judgment and non-additive multivariate models to interpret INT_RR in context with other metrics. Plotting the relative richness of sensitive, intermediate, and tolerant taxa together (Figure 4.4) more clearly defines reference conditions and illustrates departure from reference conditions than creating three separate graphs (*e.g.*, Figure 4.3). The interaction of the three variables provides a clearer signal of community condition than any of the metrics alone. Simultaneously evaluating multiple metrics can improve assessments of resource condition (Gerritsen 1995, Karr and Chu 1999).

Bioassessment programs often use land use indicators as primary disturbance gradients, but could benefit from examining attribute response to other disturbance gradients, especially when point source discharges and isolated sources of pollution confound metric response to land use disturbance. For example, localized inputs of nutrients and organic material from poorly managed wastewater discharges, agriculture, or other sources can damage streams in mostly forested watersheds (Sosiak 2002). The relative abundance of polysaprobic diatoms was not a metric when tested against percent developed watershed because organic pollution did not always accompany land use disturbance. The relative abundance of polysaprobic diatoms was quite large, however, at some sites with mostly forested watersheds because of isolated sources of organic pollution. Similarly, very large relative abundance of acidophilic diatoms in combination with low diatom richness and no non-diatom taxa were observed at a site damaged by historic iron mining. Although the relative abundances of polysaprobic and acidophilic diatoms were not identified as metrics, atypically large values could indicate damaged conditions. Similar patterns may exist in other heavily forested regions with isolated sources of pollution (Griffith et al. 2002). Bioassessment programs could consider adding these types of metrics as 1) part of a multimetric index or model, 2) as diagnostic metrics to help identify sources of impairment, or 3) as a component of best professional judgment and evaluation of attainment of water quality standards.

4.5.3 Management applications

The metrics identified in this study will provide the foundation of a multivariate model that will predict attainment of Maine's water quality standards. Maine has four classes of streams and rivers in its water quality standards. Classes AA and A have similar biological expectations and are equivalent to BCG Tiers 1 and 2. Classes B and C approximate BCG Tiers 3 and 4, respectively. BCG Tiers 5 and 6 would not attain minimum state water quality standards. The BCG framework describes the transformation of stream fish and macroinvertebrate communities in response to disturbance from BCG Tier 1 to 6 with the loss of sensitive taxa (BCG Attributes II and III), subsidy-stress response of taxa of intermediate tolerance (BCG Attribute IV), and increase in tolerant taxa (BCG Attribute V) (Davies and Jackson 2006). The methods of developing Maine tolerance values for individual taxa and evaluating species response curves introduced in this paper provide an empirical process for assigning algal taxa to BCG Attributes. Locally derived tolerance values and metrics provide more accurate assessments than application of tolerance values and metrics developed in other parts of the world (*e.g.*, van Dam et al. 1994, Potapova et al. 2005, Newall et al. 2006). Biologists can use metrics based on local tolerance values, such as the relative richness of sensitive, intermediate, and tolerant taxa in combination with other metrics to interpret the transition of stream algal communities from Tiers 1 to 6 and characterize the condition of individual samples. Although the Maine tolerance values and metrics may cautiously be applied to adjacent regions with similar ecoregions, the *process* of developing Maine tolerance values and testing metrics is more widely transferable and could be replicated in regions with different reference expectations or dominant disturbances.

MDEP biologists could use metrics to interpret narrative aquatic life criteria (Table 1.1). The range of metric values associated with reference sites could be used to determine if algae are "as naturally occurs" for Class AA and Class A. A variety of metrics based on the relative richness and relative abundance of algal taxa that require cold, clean water could be used to interpret the Class B requirement of supporting "all aquatic species indigenous species . . . without detrimental changes to the resident biological community", such as sensitive taxa, *Brachysira, Eunotia, Tabellaria, and Anomoneis* (BETA), nitrogen autotrophic diatoms, high oxygen diatoms, and oligotraphentic diatoms. Class C waterbodies must "maintain the structure and function of the resident biological community". Structure could be evaluated with the proportions of sensitive, intermediate, and tolerant taxa. Class C waters are characterized by a substatial decrease in sensitive taxa relative to Class A waters, but some sensitive taxa are still present. In addition, intermediate taxa make up a greater proportion of richness and abundance than tolerant taxa. Function could be evaluated with the relative richness and relative abundance of motile, erect, prostrate, nitrogen autotrophic, nitrogen heterotrophic, oligosaprobic, and polysaprobic diatoms.

Final selection of metrics can be assisted with scatterplots with LOWESS regression trend lines (*e.g.*, Figure 4.3), metric correlations with percent developed watershed (Table 4.1), correlations with other metrics (Table 4.2), and identification of metrics with great overlap in species included in metric computations. Conceptually redundant metrics (*e.g.*, relative richness and relative abundance of sensitive taxa) can be prioritized based on strength of response to disturbance illustrated with scatterplots and correlations with percent developed watershed or ability to distinguish reference streams from modified streams (e.g, Fore 2003, Wang et al. 2005). Multimetric indices or multivariate statistical models with metrics representing multiple categories (*e.g.*, community composition, tolerance/intolerance, nutrient and organic enrichment, individual condition, and other ecological attributes) provide a more comprehensive indication of stream condition (Karr and Chu 1999, Fore and Grafe 2002). Most of the metrics in this study were in the categories tolerance/intolerance and nutrient and organic enrichment, therefore, it would be beneficial to select metrics that represent multiple types of stressors (Wang et al. 2005). Many of the highly correlated metrics were probably responding to the same dominant pattern of land use disturbance and concomitant increase in specific conductance, nutrient supply, sedimentation, and habitat disturbance (Wang et al. 2005). Therefore, it could be beneficial to evaluate attributes specific to certain stressors and include them in assessments of condition and attainment of water quality standards.

Algal metrics also provide information about the type of stressor affecting a waterbody in addition to being used in overall assessments of resource condition. Algal metrics and inference models have been used to diagnose effects of sedimentation (Bahls 1993, Kutka and Richards 1996, Cuffney et al. 1997, Detenbeck et al. 2000, Fore and Grafe 2002, Fore 2003), nutrient enrichment (Cuffney et al. 1997, Leland and Porter 2000, Fore 2003, Wang et al. 2005, Ponader et al. 2007, Ponader et al. 2008, Porter et al. 2008, Stevenson et al. 2008a), increased salinity or specific conductance (Fore 2003, Potapova and Charles 2003, Stevenson et al. 2008b), organic enrichment (Fore and Grafe 2002, Fore 2003, Kelly et al. 2008), and acidification (Hill et al. 2000, Hill et al. 2003, Stevenson et al. 2008b). Combinations of diagnostic metrics have been used to distinguish the effects of agricultural land use from urban land use (Fore 2003) and mining (Pan et al. 1996). Combinations of diagnostic metrics can also distinguish the effects of organic and inorganic effluents on diatom communities (Kelly 1998a, b, Rott et al. 1998, Leland and Porter 2000). MDEP could benefit from supplementing the model that predicts attainment of water quality standards (*i.e.*, Classes AA, A, B, and C) with diagnostic metrics and simultaneous evaluation of multiple metrics to help determine the cause(s) of impairment. Completion of an algal bioassessment method will improve the management of water quality of Maine's streams and rivers.

5. A BENTHIC ALGAL COMMUNITY MODEL FOR PREDICTING THE ATTAINMENT OF BIOLOGICAL CRITERIA FOR MAINE STREAMS AND RIVERS

5.1 Abstract

A statistical model was developed with benthic algal data to predict attainment of Maine's biological criteria of Classes AA/A, B, and C. The algal bioassessment is based on interpretation of algal community data with Maine's narrative biological criteria and the U.S. Environmental Protection Agency's Biological Condition Gradient (BCG). The BCG framework, originally applied to fish and macroinvertebrates, was modified to make it applicable to Maine stream algae. A novel method was developed to empirically derive tolerance values and assign algal taxa to sensitive, intermediate, and tolerant categories. Novel metrics and metrics used by other algal bioassessments were tested to determine if they could distinguish Maine reference sites from non-reference sites and were correlated with percent developed watershed. Locally-derived metrics were more effective than metrics developed in other regions. Finally, a Discriminant Analysis model was developed to replicate *a priori* class (*i.e.*, AA/A, B, C) attainment decisions made by a panel of Maine biologists. The model correctly predicted 95% of classes for training samples (n=150) and 91% of classes for validation samples (n=80).

5.2 Introduction

The objective of the United States Federal Water Pollution Control Act or "Clean Water Act" is to "restore and maintain the chemical, physical and biological integrity of the Nation's waters" (Public Law 92-500, Section 101). In response to this goal, state water quality programs increasingly emphasize fish, macroinvertebrate, and algal communities as water quality indicators (USEPA 2002). The Biological Condition Gradient (BCG) is a mechanism to "(1) assess aquatic resources more uniformly and directly and (2) communicate more clearly to the public the current status of aquatic resources and their potential for restoration" (Davies and Jackson 2006, p. 1251). The BCG describes changes in 10 ecological attributes along an environmental stress gradient with 6 tiers of biological condition of fish and macroinvertebrate communities, ranging from natural condition (Tier 1) to severe alteration of structure and function (Tier 6). It was originally developed for permanent, hard-bottomed streams exposed to increasing temperature, nutrients, and fine sediments. Applications of the BCG framework to other ecosystems and biological assemblages are incomplete, yet the BCG framework has great potential for application to a broad range of ecosystems and taxonomic groups.

A natural extension of the BCG is to integrate tiers of biological condition into water quality standards in the form of Tiered Aquatic Life Uses (Courtemanch 1995, Yoder and Rankin 1998, Davies and Jackson 2006). Maine's water quality standards recognize four classes of streams and rivers with narrative biological criteria describing the aquatic life goals of each class (Courtemanch et al. 1989, Courtemanch 1995). Class AA and A waters have the same biological expectations, which may be represented as BCG Tiers 1 and 2, Class B corresponds to BCG Tier 3, and Class C corresponds with BCG Tier 4. Streams that do not attain Class C are called non-attainment (NA) and correspond to BCG Tiers 5 and 6. The Maine Department of Environmental Protection (MDEP) predicts class attainment with a discriminant analysis model of benthic macroinvertebrate data (Davies et al. 1993, Davies and Tsomides 2002). The tiered classes provide flexibility to manage streams and rivers at multiple levels of risk and condition.

Although most states use fish or macroinvertebrates to assess the biological condition of rivers and streams, algae also are good indicators of water quality (Stevenson and Bahls 1999). Algal indices have been developed for nutrient and organic pollution (Kolkwitz and Marsson 1908, Pantle and Buck 1955, Watanabe 1962, Palmer 1969, Descy 1979, Lange-Bertalot 1979, Kelly et al. 1995, Kelly 1998a, b, Potapova and Charles 2007), and inference models have been developed to estimate levels of nutrients and other water quality parameters (Pan et al. 1996, Winter and Duthie 2000, Potapova et al. 2004, Ponader et al. 2007, Ponader et al. 2008, Stevenson et al. 2008b). These assessments often lack a direct link to reference conditions and emphasize single water chemistry or enrichment gradients. Several states and regions of the United States have developed algal multimetric indices of biotic integrity to quantify the condition of algal communities with respect to reference conditions (Bahls 1973, Fore and Grafe 2002, KDEP 2002, Fore 2003, Passy and Bode 2004, Wang et al. 2005). Algal indices also have been developed in Australia (Chessman et al. 1999, Chessman et al. 2007), Canada (Belore et al. 2002, Lavoie et al. 2006), and Europe (Kelly et al. 2009).

The purpose of this study was to develop a model with benthic algal community data to predict the probability of a stream attaining biological criteria of its assigned Maine water classification (*i.e.*, AA/A, B, C). Monitoring multiple taxonomic assemblages improves the ability to detect environmental degradation and diagnose stressors (Patrick 1949, Yoder and DeShon 2003, Hering et al. 2006). MDEP biologists assigned Maine classes to samples by interpreting algal community data and variables with Maine's narrative aquatic life criteria and the BCG framework. Interpretation was based on a combination of novel variables and variables from the literature that were empirically shown to respond to environmental disturbance. I developed a statistical model to replicate assignments made by the biologists. I assessed model performance with an independent data set and interpretation by independent, professional biologists. Lastly, I compared model performance to the existing macroinvertebrate model and examined patterns in model agreement.

5.3 Methods

The sampling area, study sites, and definition of reference conditions are described in Section 3.3.1. Field and laboratory procedures are described in Section 3.3.2.

5.3.1 Algal community patterns

Major patterns in environmental variables were correlated with patterns in species composition with nonmetric multidimensional scaling (NMS, Kruskal 1964, Mather 1976) using Sorenson distance measure, random starting configurations, 250 runs with real data, and 250 runs with randomized data (PC-ORD v. 5.0, McCune and Mefford 1999). NMS is an unconstrained ordination technique that is particularly useful for ecological data with a large number of taxa and many zero counts (McCune and Grace 2002). The first of two NMS ordinations used 42 samples from minimally disturbed streams to determine if natural physical factors, such as watershed size or stream width, were correlated with major patterns in species composition. NMS plots were examined to determine if samples formed clusters based on ecoregion. The second NMS ordination used 81 samples with values for all environmental variables (Table 3.1) to identify environmental variables most correlated with major patterns in species composition. Species occurring in < 7 training set samples were excluded from both ordinations. The influence of dominant, ubiquitous taxa, such as *Achnanthidium minutissimum* (Kützing) Czarnecki and *Gomphonema parvulum* (Kützing) Kützing, was reduced in both NMS ordinations with square roots of percent abundances for all taxa.

Multi-response permutation procedures (MRPP, Mielke Jr. and Berry 2001) were used with Sørenson (Bray–Curtis) distance measure to determine if species composition varied by ecoregion (PC-ORD v. 5.0, McCune and Mefford 1999). MRPP is a non-parametric procedure that tests if groups of samples have different species composition and is similar in concept to Analysis of Similarity (ANOSIM, Clarke 1993), but uses differences in actual distances instead of ranks. MRPP computes 1) a *T*-statistic that describes separation between groups, 2) a P-value for evaluating the likelihood of an observed difference being due to chance and 3) a chance-corrected within-group agreement (*A*) for evaluating the ecological importance of the difference. The more difference there is between groups, the more negative *T* gets (McCune and Grace 2002). The statistic *A* equals 1 when samples within groups are identical, 0 when heterogeneity within groups equals expectation by chance, and <0 when heterogeneity within groups is more than expected by chance. *A* values commonly range from 0.1 to 0 for community data (McCune and Grace 2002).

5.3.2 Metric calculation and selection

The set of metrics (Table 5.1) used to assign Maine water quality classes and BCG tiers combined species traits obtained from literature sources and metrics empirically derived using Maine data (Chapter 4). Literature metrics were based on diatom motility ratings (Fore and Grafe 2002, Wang et al. 2005), diatom growth forms (Fore 2003, Wang et al. 2005), and diatom preferences for organic enrichment, nitrogen uptake, eutrophication, salinity, and oxygen requirements (van Dam et al. 1994). Maine metrics were based on tolerance to a general disturbance gradient (Chapter 4). Metrics were screened to ensure that they had good correlation with the percent of watershed land cover that was not forest or wetland and they could distinguish reference sites from non-reference sites. Some correlated variables were retained to allow biologists flexibility to determine which were most important for assigning BCG tiers.

The Diatom Total Phosphorus Index (DTPI), Diatom Specific Conductance Index (DSCI), and Diatom Watershed Development Index (DWDI) were generated (C2 v. 1.5.0, Juggins 2007) with weighted average – partial least squares (2nd components) and were added to the set of variables used by biologists to assign BCG tiers and Maine Classes. The DTPI, DSCI, and DWDI were built with square root of percent abundances of 209 diatom species that occurred in 7 or more samples and with 167, 166, and 186 samples respectively.

A report describing the metrics was prepared and given to MDEP biologists involved in the study. The metrics were grouped into the categories of Community Structure, Tolerance/Intolerance, Nutrient and Organic Enrichment, Specific Conductance, and Watershed Disturbance (Table 5.1). The report included the formulas used to compute metrics, metric codes used in graphs, the expected range of metric values for minimally disturbed reference sites, the overall range of metric values observed in all study sites, and the predicted response to watershed development (Table 5.1). Quantile plots were produced for each metric with samples grouped as reference and non-reference to display ranges of values for the two groups of samples (SYSTAT 11.0, Wilkinson 1990). Plots of metrics and the percent of upstream watershed land cover that is developed (*i.e.*, no longer forest and wetland) were produced with locally weighted regression (LOWESS) lines (SYSTAT 11.0, Wilkinson 1990) (Figure 5.1). The upper limit of "natural" conditions was defined by selecting the 90th-95th percentile of reference samples for variables that increased with watershed disturbance (Table 5.1, Figure 5.1). The lower limit of "natural" conditions was defined by selecting the 5th-10th percentile of reference samples for variables expected to decrease with watershed disturbance (Table 5.1, Figure 5.1). This approach was not appropriate for several variables with widely ranging values for reference sites. Although the percentile approach could not distinguish samples collected at reference sites from samples collected at non-reference sites, most of the samples with large values had little watershed disturbance. These metrics were retained as indicators of low watershed disturbance (LWD) and greater metric values indicated good conditions. Lesser values of LWD metrics, however, were not reliable indicators of poor conditions.

Table 5.1 Metrics supplied to MDEP biologists for assigning Maine classes and BCG tiers via expert judgement. Included in the table are 1) formulas for computing metrics, 2) metric codes used in subsequent graphs and tables, 3) range of values observed in reference sites, 4) overall range of values observed in all study sites, and 5) predicted response to watershed development (↓ = decrease, ↑ increase). MST = Maine stream tolerance values. LWD indicates metrics of low watershed disturbance as described in Section 5.3.3.2.

Metric (Formula)	Code	Range of Values for Reference Sites	Overall Range of Values	Predicted Response to Watershed Development
Community Structure				
Relative Richness of Erect Diatoms[1] *(number of diatom species with erect growth form / total number of diatom species)*	EREC_RR	>12%^{LWD}	0-42%	↓
Relative Abundance of Erect Diatoms[1] *(number of diatom species with erect growth form / total number of diatom species)*	EREC_RA	>9%^{LWD}	0-88%	↓
Tolerance / Intolerance				
Relative Richness of Sensitive Taxa* *(number of taxa with MST values < 32.2 / total number of taxa with MST values)*	SEN_RR	>20%	0-63%	↓
Relative Richness of Tolerant Taxa *(number of taxa with MST values > 60 / total number of taxa with MST values)*	TOL_RR	<18.5%	0-64%	↑
Relative Abundance of Sensitive Taxa *(density of taxa with MST values < 32.2 / total density of taxa with MST values)*	SEN_RA	>5%^{LWD}	0-92%	↓
Relative Abundance of Tolerant Taxa* *(density of taxa with MST values > 60 / total density of taxa with MST values)*	TOL_RA	<2%	0-62%	↑
Relative Biovolume of Sensitive Taxa *(biovolume of taxa with MST values <32.2 / total biovolume of taxa with MST values)*	SEN_RB	>10%	0-98%	↓
Relative Biovolume of Tolerant Taxa *(biovolume of taxa with MST values > 60 / total biovolume of taxa with MST values)*	TOL_RB	<4%	0-97%	↑
Relative Richness of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae *(number of diatom species in these 4 families / total number of diatom species)*	BCRS_RR	<10%	0-41%	↑
Relative Abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae *(density of diatom species in these 4 families / total diatom density)*	BCRS_RA	<4%	0-42%	↑
Relative Biovolume of Sensitive Soft Algae *(biovolume of taxa in Batrachosperma, Mougeotia, Rivulaceae, Tolypothrix, Ulothrix, and Zygnema / total algal biovolume)*	SNSFT_RR	>3%^{LWD}	0–94%	↓
Relative Richness of *Brachysira, Eunotia, Tabellaria,* and *Anomeoneis* *(number of diatom species in these 4 genera / total number of diatom species)*	BETA_RR	>7%	0-45%	↓

Table 5.1 Continued

Metric (Formula)	Code	Range of Values for Reference Sites	Overall Range of Values	Predicted Response to Watershed Development
Relative Abundance of *Brachysira, Eunotia, Tabellaria,* and *Anomeoneis* *(density of diatom species in these 4 genera / total number of diatom species)*	BETA_RA	>2%	0-96%	↓
Nutrient and Organic Enrichment				
Relative Richness of Diatoms that Require High Dissolved Oxygen Concentrations[2] *(# of diatom species with oxygen values of 1 / # of all diatom species with oxygen values)*	HIGH_RR	>42%	6-72%	↓
Relative Richness of Polysaprobic Diatoms[2]* *(# of diatom species with saprobic values of 4 or 5 / # of all diatom species with saprobic values)*	PSAP_RR	<18%	0-44%	↑
Relative Abundance of Polysaprobic Diatoms[2]* *(density of diatom species with saprobic values of 4 or 5 / density of all diatom species with saprobic values)*	PSAP_RA	<17%	0-49%	↑
Relative Richness of Nitrogen Autotrophic Diatoms[2] *(# of diatom species with organic-N uptake values of 1 / # of diatom species with organic-N uptake values)*	NAUT_RR	>37%	0-75%	↓
Relative Richness of Eutraphentic Diatoms[2]* *(# of diatom species with trophic values of 5 or 6 / # of diatom species with trophic values)*	EUTR_RR	<40%	0-92%	↑
Relative Abundance of Eutraphentic Diatoms[2]* *(# of diatom species with trophic values of 5 or 6 / # of diatom species with trophic values)*	EUTR_RA	<33%	0-97%	↑
Diatom TP Index	DTPI	<18	4-73	↑
Specific Conductance				
Relative Richness of Salt-Tolerant Diatoms[2]* *(# of diatom species with salinity values of 3 or 4 / # of diatom species with salinity values)*	SALT_RR	<10%	0-26%	↑
Diatom Specific Conductance Index	DSCI	<100	4-1,772	↑
Watershed Disturbance				
Diatom Watershed Disturbance Index	DWDI	<20	0-81	↑
Relative Richness of Motile Diatoms[3]* *(# of motile or highly motile diatom species / total # of diatom species)*	MOT_RR	<38%	4-70%	↑
Relative Abundance of Motile Diatoms[3]* *(density of motile or highly motile diatom species / total density of diatom species)*	MOT_RA	<18%	0-69%	↑

Sources: 1 - (Fore 2003, Wang et al. 2005). 2 - (van Dam et al. 1994). 3 - (Fore and Grafe 2002, Wang et al. 2005)

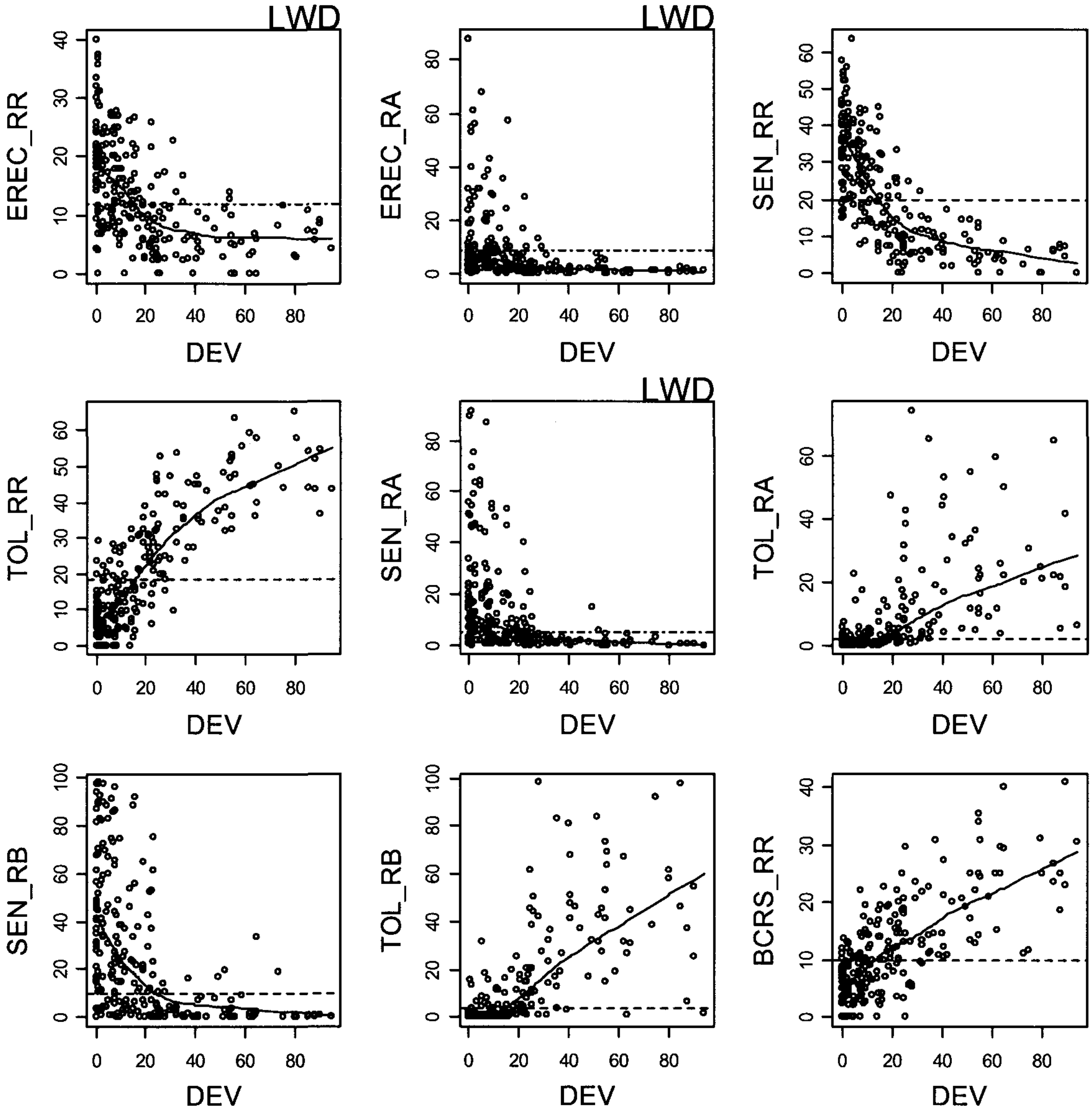


Figure 5.1 Plots of metrics supplied to MDEP biologists for assigning Maine classes and BCG tiers with land cover that is developed (DEV) (*i.e.*, no longer forest and wetland). Dashed horizontal lines indicate limit of expected reference conditions. LWD indicates metrics that are indicators of low watershed disturbance as described in Section 5.3.3.2. The metrics include relative richness of erect diatoms (EREC_RR), relative abundance of erect diatoms (EREC_RA), relative richness of sensitive taxa (SEN_RR), relative richness of tolerant taxa (TOL_RR), relative abundance of sensitive taxa (SEN_RA), relative abundance of tolerant diatoms (TOL_RA), relative biovolume of sensitive taxa (SEN_RB), relative biovolume of tolerant taxa (TOL_RB), and relative richness of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_RR).

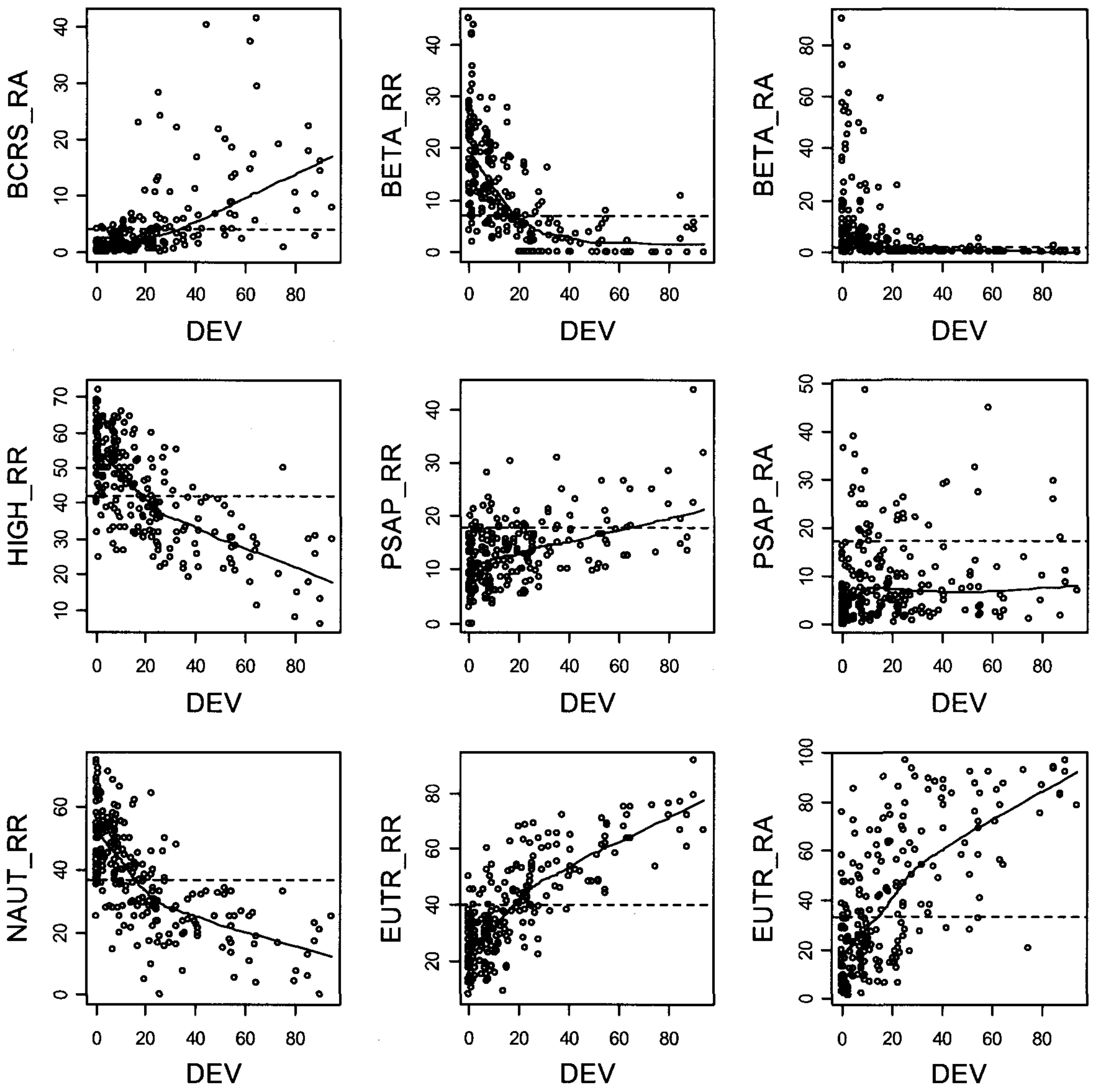


Figure 5.1 Continued

The metrics include of relative abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_RA), relative richness of Brachysira, Eunotia, Tabellaria, and Anomeoneis (BETA_RR), relative abundance of Brachysira, Eunotia, Tabellaria, and Anomeoneis (BETA_RA), relative richness of diatoms that require high dissolved oxygen concentrations (HIGH_RR), relative richness of polysaprobic diatoms (PSAP_RR), relative abundance of polysaprobic diatoms (PSAP_RA), relative richness of nitrogen autotrophic diatoms (NAUT_RR), relative richness of eutraphentic diatoms (EUTR_RR), and relative abundance of eutraphentic diatoms (EUTR_RA).

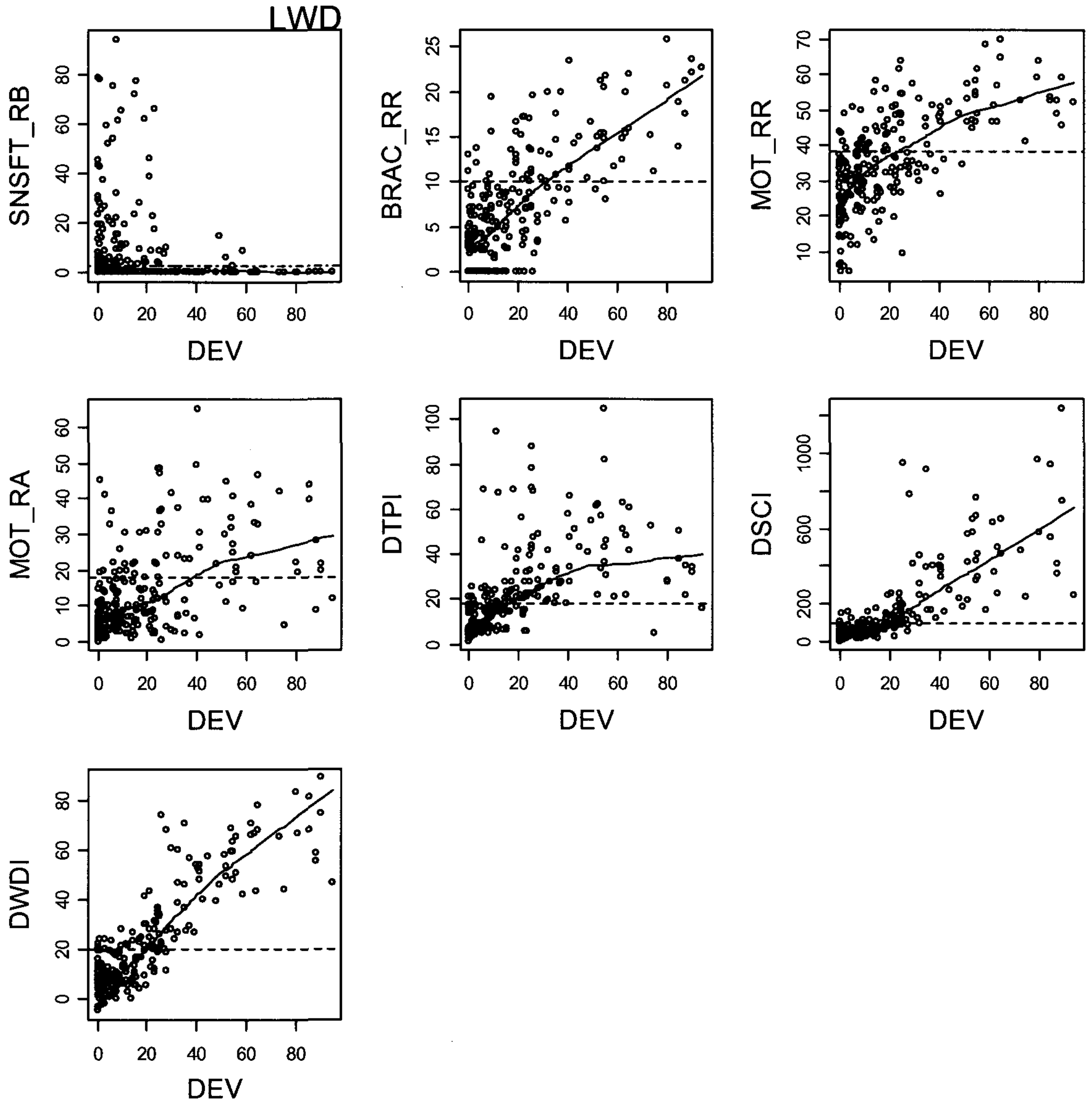


Figure 5.1 Continued
The metrics include relative biovolume of sensitive soft algae (SNSFT_RB), relative richness of salt-tolerant diatoms (SALT_RR), relative richness of motile diatoms (MOT_RR), relative abundance of motile diatoms (MOT_RA), Diatom Total Phosphorus Index (DTPI), Diatom Specific Conductance Index (DSCI), and Diatom Watershed Development Index (DWDI).

5.3.3 Assignment of Maine water quality classes and BCG tiers

Five MDEP biologists (Beth Connors, Tom Danielson, Jeanne DiFranco, Caitlin Kersten, and Leon Tsomides) independently assigned Maine water quality classes and BCG tiers to 230 samples. Samples were identified with random numbers and no study site information was provided to facilitate "blind" assignments based only on biological information. Biologists referred to the stream algal BCG framework (Table 5.2; adapted from Davies and Jackson 2006), narrative biological criteria, sample taxa lists, computed metrics for each sample, and a report summarizing each metric's response to DEV. Taxa lists for each sample included taxa desnsities, relative abundances, biovolumes, relative biovolumes, Maine tolerance values, diatom motility ratings (Fore and Grafe 2002, Wang et al. 2005), diatom growth forms (Fore 2003, Wang et al. 2005), and diatom preferences for organic enrichment, nitrogen uptake, eutrophication, salinity, and oxygen requirements (van Dam et al. 1994). Biologists compared independent assignments, agreed on consensus assignments (Biologist Classifications), and calculated average BCG tiers for each sample. The panel of biologists was asked to recommend additional variables to be included in the linear discriminant model (Table 5.3). Representatives from The Academy of Natural Sciences (Don Charles and Marina Potapova) replicated the process and assigned Maine classes and BCG tiers to a subset of 40 samples selected in a stratified random design to include the range of BCG tiers.

Table 5.2 BCG framework for Maine stream algae.

	BCG Tier					
	Tier 1 Natural Condition	**Tier 2** Minimal changes in community structure	**Tier 3** Evident changes in community structure and minimal changes in ecosystem function	**Tier 4** Moderate changes in community structure and minimal changes in ecosystem function	**Tier 5** Major changes in community structure and moderate changes in ecosystem function	**Tier 6** Severe changes in community structure and major changes in ecosystem function
	Corresponding Maine Water Class					
BCG Attributes	**AA/A**	**AA/A**	**B**	**C**	**NA**	**NA**
II Highly Sensitive Taxa & III Sensitive Taxa	Sensitive taxa usually represent >20% of species and >10% of biovolume. Most metrics are within the range of "natural condition".	Sensitive taxa usually represent >20% of species and >10% of biovolume. Most metrics are within the range of "natural condition".	Sensitive taxa usually represent >15% of species and >5% of biovolume. Most metrics are within or near the range of "natural condition".	Richness and biovolume of sensitive taxa usually well below the range of natural conditions. Several sensitive taxa metrics are low.	Absent or richness and biovolume very low. Occasionally can have a sensitive filamentous alga with high biovolume. Most or all sensitive taxa metrics are low.	Absent
IV Opportunistic Taxa of Intermediate Tolerance	Intermediate and indifferent taxa usually represent between 30-70% of richness and 0-90% of biovolume.	Intermediate and indifferent taxa usually represent between 30-70% of richness and 0-90% of biovolume.	Intermediate and indifferent taxa usually represent between 50-85% of richness and 25-99% of biovolume	Intermediate and indifferent taxa usually represent between 50-80% of richness and 40-99% of biovolume	Intermediate and indifferent taxa usually represent between 30-60% of richness and 0-70% of biovolume	Richness <30%
V Tolerant Taxa	Tolerant taxa usually represent less than 18.5% of taxa richness, 2% of cell density, and 4% of biovolume. Most tolerant metrics are within range of natural condition.	Tolerant taxa usually represent less than 18.5% of taxa richness, 2% of cell density, and 4% of biovolume. Most tolerant metrics are within range of natural condition.	Tolerant taxa usually represent less than 25% of taxa richness and 10% of biovolume. Some tolerant taxa metrics may be elevated.	Tolerant taxa usually represent less than 40% of taxa richness and 50% of biovolume. Several tolerant taxa metrics are elevated.	Usually more than 40% of taxa richness and 50% of biovolume. Ratio of the number of tolerant taxa to sensitive taxa is high. Most tolerant taxa metrics are elevated.	Usually comprise the majority of the assemblage; often at either very low or very high densities. Most tolerant taxa metrics are highly elevated.

Table 5.2 Continued

BCG Attributes	**BCG Tier**					
	Tier 1 Natural Condition	**Tier 2** Minimal changes in community structure	**Tier 3** Evident changes in community structure and minimal changes in ecosystem function	**Tier 4** Moderate changes in community structure and minimal changes in ecosystem function	**Tier 5** Major changes in community structure and moderate changes in ecosystem function	**Tier 6** Severe changes in community structure and major changes in ecosystem function
	Corresponding Maine Water Class					
	AA/A	**AA/A**	**B**	**C**	**NA**	**NA**
VI Non-native taxa	Very little is known about which species of algae are native or non-native to Maine. The greatest known risk is from the diatom *Didymosphenia geminata,* which has been observed in Vermont and New Hampshire. It can form extensive mats that smother the stream bottom. The greatest risk is to high quality streams and rivers because it has the potential to colonize many oligotrophic-mesotrophic streams and rivers and can be transported between sites by fishermen.					
Diatom Community Inference Models	Predict very low TP, specific conductance, and % developed watershed	Inferred TP, specific conductance, and % developed watershed are usually less than 18, 100, and 20. One may be elevated.	One or more of the inference models is elevated above the range of natural conditions.	Variable. Inference models may be somewhat elevated. One or more may be much higher than expected.	Predict high TP, SPC, and % developed watershed	Predict high TP, SPC, and % developed watershed
VIII Ecosystem Function	Typically well oxygenated because of cold water, riffles, and/or low to moderate algal growth.	Typically well oxygenated because of cold water, riffles, and/or low to moderate algal growth.	Increased algal growth may increase food supply for algivores and begin to alter habitat for benthic organisms.	Excessive algal growth may cause alter habitat and/or cause aesthetic problems. Oxygen concentrations may greatly increase during the day and decrease at night. Abundant cyanobacteria may raise concern of cyanotoxins.	Excessive algal growth may cause alter habitat and/or cause aesthetic problems. Oxygen concentrations may greatly increase during the day and decrease at night. Abundant cyanobacteria may raise concern of cyanotoxins.	Algal community may no longer be dominant and may be replaced by "sewage fungus".

Table 5.3 Additional variables added for potential inclusion in the algal discriminant analysis model at the recommendation of MDEP biologists after making a priori Maine Class (*i.e.*, A, B, C, NA) and Biological Condition Gradient tier (*e.g.*, 1-6) assignments. (↑=increase, ↓=decrease, ∩=unimodal).

Metric (Formula)	Code	Predicted Response to DEV
Relative Richness of Diatoms that Tolerate Low Dissolved Oxygen *(number of diatom species with oxygen value of # / number of diatom species with oxygen values)*	LOW_RR	↑
Sensitive – Tolerant Richness Ratio *(Number of taxa with MTS values <32.2 / 1 + Number of taxa with MTS value > 60)*	SENTOL	↓
Intermediate – Tolerant Richness Ratio *(Number of taxa with MTS values between 32.2 and 60 / 1 + Number of taxa with MTS values > 60)*	INTTOL	↓
Relative Richness of Intermediate Taxa *(Number of taxa with MTS values between 32.2 and 60 / number of taxa with MTS values)*	INT_RR	∩
Relative Abundance of Intermediate Taxa *(Density of taxa with MTS values between 32.2 and 60 / density of taxa with MTS values)*	INT_RA	∩
Relative Biovolume of Intermediate Taxa Biovolume of taxa with *MTS values between 32.2 and 60 / density of taxa with MTS values)*	INT_RB	∩
Richness of Erect Diatoms *(number of diatom species with erect growth form)*	EREC_R	↓
Richness of Sensitive Taxa *(number of taxa with MST values < 32.2)*	SEN_R	↓
Richness of Intermediate Diatoms *(number of diatom species with MST values between 32.2 and 60)*	INTD_R	∩
Richness of Tolerant Diatoms *(number of diatom species with MST values > 60)*	TOLD_R	↑
Richness of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae *(number of diatom species in these 4 families)*	BCRS_R	↑
Richness of Polysaprobic Diatoms *(number of diatom species with saprobic values of 4 or 5)*	PSAP_R	↑
Richness of Nitrogen Autotrophic Diatoms* *(number of diatom species with organic-N uptake values of 1)*	NAUT_R	↓
Richness of Eutraphentic Diatoms* *(number of diatom species with trophic values of 5 or 6)*	EUTR_R	↑
Richness of Salt-tolerant Diatoms *(number of diatom species with salinity values of 3 or 4)*	BRAC_R	↑
Richness of Motile Diatoms *(number of motile or highly motile diatom species)*	MOT_R	↑

5.3.4 Algal discriminant analysis model

Variables in Tables 5.2 and 5.4 were transformed using several methods (*e.g.*, square root, 4th root, arcsine, arcsine square root, log) to normalize variance or improve homoscedacity. As a potential alternative to an algal discriminant analysis model, samples in the training set (n=150) were objectively assigned to 4 groups based on the algal metrics in Tables 5.1 and 5.3. Resulting cluster assignments were compared to *a priori* Biologist Classifications (K-Means Clustering, Euclidean distance, SYSTAT 11.0, Wilkinson 1990). A preliminary discriminant analysis model was created with samples in the training set (n=150) using automatic backward stepwise selection of variables with a probability of 0.05 and tolerance of 0.001 (DISCRIM, SYSTAT 11.0, Wilkinson 1990). The stepwise selection process excludes some variables that individually may predict groups better than than some variables included in the model, but are redundant with one or more variables already in the model. Within pool correlations of model variables were screened to identify correlated metrics. The final set of variables was selected by removing variables with large within pool correlations ($|r|>0.70$) and iteratively adding and dropping additional variables. Canonical factor scores of samples in the training set were plotted with ellipses centered on group means with a standard deviation of 0.6827. The ability of final metrics to distinguish *a priori* Biologist Classifications was tested using Tukey's post hoc pairwise comparisons between groups (ANOVA, SYSTAT 11.0, Wilkinson 1990). Samples in the validation set were not used to build the model, however, the model predicted their group membership. Model predictions of the training and validation samples were compared to *a priori* Biologist Classifications to calculate the percent of correct predictions. One sample in the validation set was excluded from calculations because of atypically small taxa richness (n=11). Tables of agreement between the algal LDM and *a priori* Biologist Classifications were produced for the training and validation data sets. Strength of agreement agreement between the model and Biologist Classifications was measured with Cohen's κ (Cohen 1960) and Kendall's τ_B (Kendall 1938, Kruskal 1958) (SYSTAT 11.0, Wilkinson 1990). Cohen's κ values >0.75 indicate strong agreement, and Kendall's τ_B is a measure of association of two ranked, ordinal variables similar to a correlation. Symmetry of disagreements above and below the diagonal line of agreement was tested using McNemar's χ^2 test of symmetry (SYSTAT 11.0, McNemar 1947, Wilkinson 1990) . Finally, algal model predictions were compared to paired macroinvertebrate model predictions (n=147) (Davies and Tsomides 2002, Davies and Jackson 2006).

5.4 Results

5.4.1 Patterns in algal communities

Major patterns in species composition of minimally disturbed reference sites were not strongly related to ecoregions. The NMS 3-axis solution described 83% of variation (final stress=14.9, instability<0.00001, P<0.001) and samples from the Northeastern Highlands and Acadian Plains and Hills ecoregions broadly overlapped. No reference sites were samples in the Northeastern Coastal Zone. The species compositon of reference sites in the Northeastern Highlands was statistically different than the species composition of reference sites in the Acadian Plains and Hills (MRPP, T=-2.55, P=0.017, A=0.013), but the effect size (A) was small and of questionable ecological importance.

Therefore I developed a single, statewide bioassessment model with data compiled across the ecoregions. The major pattern in species composition of all sites reflected a gradient of human disturbance (Section 3.3.3.1).

5.4.2 a priori Biologist Classifications

The *a priori* Biologist Classifications were 105 Class A, 46 Class B, 46 Class C, and 33 non-attainment. Of the 230 samples in the training and test sets, 53% of samples had unanimous class assignments, 42% differed by one class, and there was disagreement by more than one class for 12 samples. Many of the samples without unanimous agreement had assignments that differed by only one class, such as four biologists assigning one class and the fifth biologist assigning the next lower class. Assignments by the national experts differed from each other as well as with the Biologist Classifications. Assignments by one expert agreed with MDEP biologists for 35 of 40 (87.5%) samples. The five sample assignments that did not match the MDEP biologists' evaluation were samples for which the MDEP biologists reached concensus assignment with difficulty. Assignments by the other expert agreed with MDEP biologists for 15 of 45 (37.5%) samples and consistently were greater water quality classes (*i.e.*, less impacted) than MDEP biologists.

5.4.3 Algal Discriminant Analysis model

The samples in the training set were objectively split into four groups (K-Means Clustering, Pearson Chi-square=163.825, df=9, P<0.001), but the groups did not distinguish *a priori* Biologist Classifications effectively (Figure 5.2). Therefore, MDEP decided to use a discriminant analysis model. The algal Discriminant Analysis model based on automatic backward selection process resulted in 11 variables: Diatom Specific

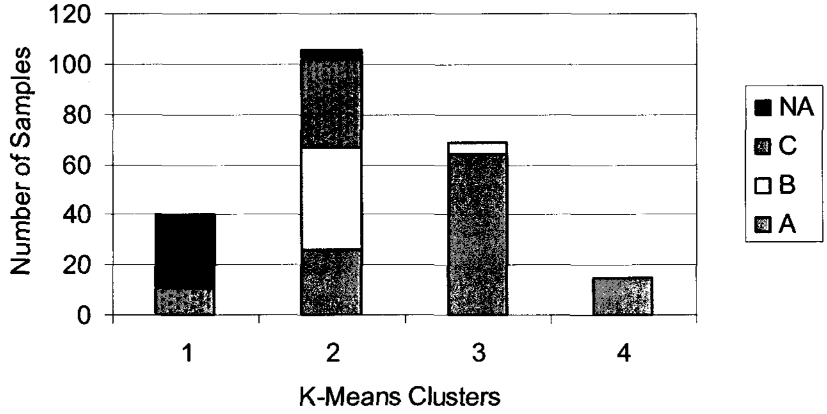


Figure 5.2 Number of samples in four K-Means cluster analysis groups showing sample membership in *a priori* Biologist Classifications.

Conductance Index (DSCI), relative abundance of erect diatoms (EREC_RA), richness of diatoms that require high oxygen concentrations (HIGH_R), relative richness of intermediate taxa (INT_RR), relative biovolume of intermediate taxa (INT_RB), relative richness of sensitive taxa (SEN_RR), relative biovolume of sensitive taxa (SEN_RB), intermediate-tolerant richness ratio (INTTOL), sensitive-tolerant richness ratio (SENTOL), relative richness of tolerant taxa (TOL_RR), and relative biovolume of tolerant taxa (TOL_RB). Metric calculations are described in Tables 5.1 and 5.3. The relative richness and relative biovolume of tolerant taxa were removed because of large within pool correlations ($|r|>0.80$) with other metrics. The Diatom Specific Conductance Index also was removed and replaced with the relative abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_RA) to avoid including an inference model in the model (Tables 5.4 and 5.5). Each of the 9 variables in the final algal model effectively distinguished one or more *a priori* Biologist Classes (one-way ANOVA, F-ratio>18.494, df=3, 226, $P<0.001$ and at least one Tukey pairwise comparison $P<0.05$) (Table 5.6, Figure 5.3). All Tukey pairwise comparisons of

BCRS_RA, INTOL, SEN_RB, SEN_RR, and SENTOL were significant (P<0.001). HIGH_R distinguished NA from A, B, and C (P<0.001). INT_RB distinguished A and NA from B and C (P<0.001). All pairwise comparisons of INT_RR were significant (P<0.01) except for the A and C groups.

Table 5.4 Classification functions of nine variables in the final algal discriminant analysis model using samples in training set (n=150). The nine variables are relative abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_RA), relative abundance of erect diatoms (EREC_RA), richness of diatoms that require high oxygen concentrations (HIGH_R), relative richness of intermediate taxa (INT_RR), relative biovolume of intermediate taxa (INT_RB), intermediate-tolerant richness ratio (INTTOL), relative biovolume of sensitive taxa (SEN_RB), relative richness of sensitive taxa (SEN_RR), and sensitive-tolerant richness ratio (SENTOL).

Variable	**Transformation**	**A**	**B**	**C**	**NA**
Constant		-402.743	-345.655	-271.173	-212.396
BCRS_RA	4th root	103.154	101.749	99.952	112.145
EREC_RA	4th root	-22.778	-20.192	-21.129	-14.504
HIGH_R	square root	-0.355	0.008	0.269	-2.056
INT_RB	arcsine	64.054	63.318	53.664	30.441
INT_RR	arcsine	52.328	73.567	47.320	25.235
INTTOL	4th root	540.168	488.664	444.500	408.181
SEN_RB	4th root	87.324	86.118	74.211	45.088
SEN_RR	arcsine square root	1749.161	1580.800	1394.386	1244.261
SENTOL	4th root	-631.965	-576.899	-519.906	-468.459

Table 5.5 Discriminant analysis canonical discriminant functions of nine variables in algal model using samples in training set (n=150). The nine variables are relative abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_RA), relative abundance of erect diatoms (EREC_RA), richness of diatoms that require high oxygen concentrations (HIGH_R), relative richness of intermediate taxa (INT_RR), relative biovolume of intermediate taxa (INT_RB), intermediate-tolerant richness ratio (INTTOL), relative biovolume of sensitive taxa (SEN_RB), relative richness of sensitive taxa (SEN_RR), and sensitive-tolerant richness ratio (SENTOL).

Variable	Factor 1 eigenvalue=13.046 conical correlation=0.964 dispersion propor.=0.920	Factor 2 eigenvalue=1.043 conical correlation=0.715 dispersion propor.=0.074	Factor 3 eigenvalue=0.088 conical correlation=0.284 dispersion propor.=0.006
Constant	21.377	1.056	-6.288
BCRS_RA	0.522	2.769	5.301
EREC_RA	0.672	0.608	3.944
HIGH_R	-0.099	-0.553	-0.926
INT_RB	-2.966	-4.396	-2.410
INT_RR	-2.371	-10.096	16.331
INTTOL	-13.922	6.515	-0.610
SEN_RB	-3.728	-5.437	-3.248
SEN_RR	-52.912	15.007	14.576
SENTOL	16.998	-4.458	-1.217

Table 5.6 ANOVA analysis of nine variables in algal discriminant analysis model using *a priori* defined water quality groups as classification variable and samples in the training and validation sets (n=230). The nine variables are relative abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_RA), relative abundance of erect diatoms (EREC_RA), richness of diatoms that require high oxygen concentrations (HIGH_R), relative richness of intermediate taxa (INT_RR), relative biovolume of intermediate taxa (INT_RB), intermediate-tolerant richness ratio (INTTOL), relative biovolume of sensitive taxa (SEN_RB), relative richness of sensitive taxa (SEN_RR), and sensitive-tolerant richness ratio (SENTOL).

Variable	**r^2**	**degrees of freedom**	**F-ratio**	**P-value**	**Tukey pairwise comparison groups (P<0.05)**
BCRS_RA	0.485	3, 226	71.082	<0.001	A, B, C, NA
EREC_RA	0.286	3, 226	30.128	<0.001	A, B, C-NA
HIGH_R	0.212	3, 226	20.283	<0.001	A-B, B-C, NA
INT_RB	0.197	3, 226	18.494	<0.001	A-NA, B-C
INT_RR	0.234	3, 226	22.984	<0.001	A-C, B, NA
INTTOL	0.687	3, 226	165.041	<0.001	A, B, C, NA
SEN_RB	0.556	3, 226	94.390	<0.001	A, B, C, NA
SEN_RR	0.800	3, 226	301.548	<0.001	A, B, C, NA
SENTOL	0.773	3, 226	256.351	<0.001	A, B, C, NA

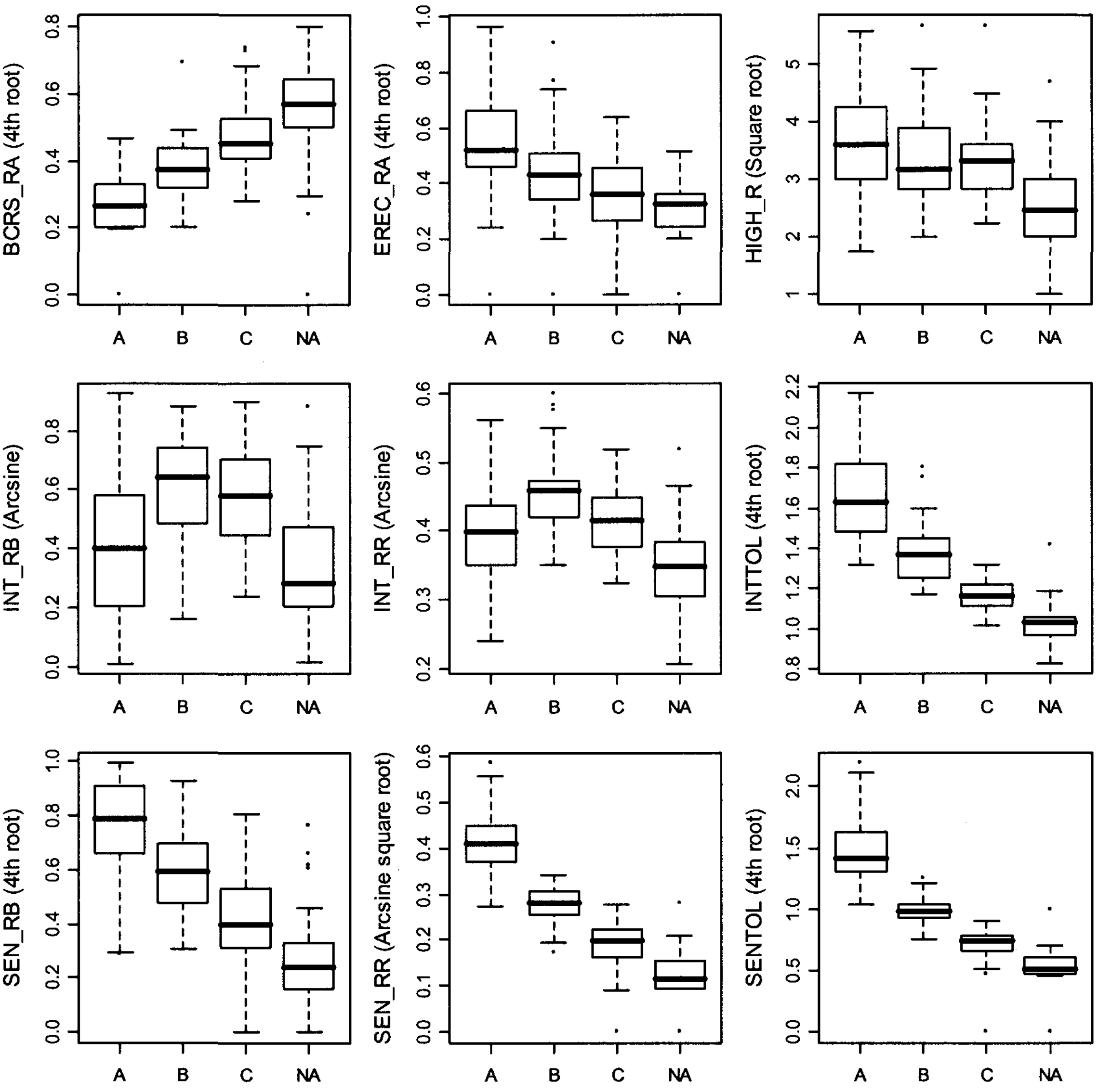


Figure 5.3 The distribution of nine variables within and between *a priori* defined water quality groups (*i.e.*, A, B, C, NA) in the four-way algal linear discriminant model. The nine variables are relative abundance of Bacillariaceae, Catenulaceae, Rhoicospheniaceae, and Surirellaceae (BCRS_RA), relative abundance of erect diatoms (EREC_RA), richness of diatoms that require high oxygen concentrations (HIGH_R), relative richness of intermediate taxa (INT_RR), relative biovolume of intermediate taxa (INT_RB), intermediate-tolerant richness ratio (INTTOL), relative biovolume of sensitive taxa (SEN_RB), relative richness of sensitive taxa (SEN_RR), and sensitive-tolerant richness ratio (SENTOL).

The algal model correctly classified 95% of training samples (Discriminant Analysis, Wilk's λ=0.032, approximate F=33.6, df=27, 403, P<0.0001) (Table 5.7a). The best quality (Class A) samples were correctly classified 97% of the time, and 100% of the worst quality (NA) samples were correctly classified (Table 5.7a). The algal model correctly classified 90% of Class B samples and 93% of Class C samples. Agreements between the model and Biologist Classifications were strong (Cohen's κ=0.932 with SE=0.025 and Kendall's τ_B=0.968 with SE=0.012). Disagreements between the model and Biologist Classifications were symmetrical (McNemar's χ^2=4.33, df=6, P=0.632). Canonical factor scores of training samples formed distinct clusters with little overlap (Figure 5.4). The jackknife analysis of training data yielded correct classification of 93% of samples (Table 5.7b). Classes assigned by the algal model are listed in Table A.4.

The classification of validation samples yielded correct classification of 91% of samples (Table 5.7c). Agreements between the model and Biologist Classifications were strong (Cohen's κ=0.855 with SE=0.048 and Kendall's τ_B=0.919 with SE=0.029). Disagreements between the model and Biologist Classifications were symmetrical (McNemar's χ^2=2.667, df=6 , P=0.849). The *a priori* class sample that was excluded because of atypically small taxa richness (n=11) was NA but the model predicted that it was Class B.

The *a priori* Biologist Classifications and macroinvertebrate model results were in agreement for 59% of the 147 paired algal and macroinvertebrate samples (Table 5.8). The algal and macroinvertebrate models had moderate agreement (Cohen's κ=0.441 with SE=0.055 and Kendall's τ_B=0.569 with SE=0.058). Disagreements between the models assignments were not strongly asymmetrical (McNemar's χ^2=7.444, df=6, P=0.282). The

Table 5.7 Performance of algal liner discriminant model compared to consensus class assignments of biologists (*a priori*) with a) training data set (n=150), b) jackknife analysis of training data set, and c) validation data set (n=80). Row percents with numbers of samples in parentheses.

a) Performance of algal model with training data (95% correct)				
	algal model Predicted Class			
	A	B	C	NA
a priori Class A	97% (67)	3% (2)	--	--
a priori Class B	3% (1)	90% (27)	7% (2)	--
a priori Class C	--	--	93% (28)	7% (2)
a priori NA	--	--	--	100% (21)
b) Performance of algal model with jackknife analysis of training data (93% correct)				
	algal model Predicted Class			
	A	B	C	NA
a priori Class A	96% (66)	4% (3)	--	--
a priori Class B	3% (1)	90% (27)	7% (2)	--
a priori Class C	--	--	93% (28)	7% (2)
a priori NA	--	--	10% (2)	90% (19)
c) Performance of algal model with validation data (91% correct)				
	algal model Predicted Class			
	A	B	C	NA
a priori Class A	97% (35)	3% (1)	--	--
a priori Class B	13% (2)	81% (13)	6% (1)	--
a priori Class C	--	13% (2)	88% (14)	--
a priori NA	--	-- (*1**)	9% (1)	91% (10)

* This sample was excluded from calculations because of atypical total richness (n=11).

Table 5.8 Comparison of algal model predicted classes and macroinvertebrate model predicted classes (n=147). Row percents with numbers of samples in parentheses.

	Macroinvertebrate model determination			
	Class A	Class B	Class C	NA
algal model Class A	82% (51)	10% (6)	6% (4)	2% (1)
algal model Class B	29% (8)	54% (15)	14% (4)	4% (1)
algal model Class C	28% (9)	28% (9)	28% (9)	16% (5)
algal model NA	12% (3)	16% (4)	24% (6)	48% (12)

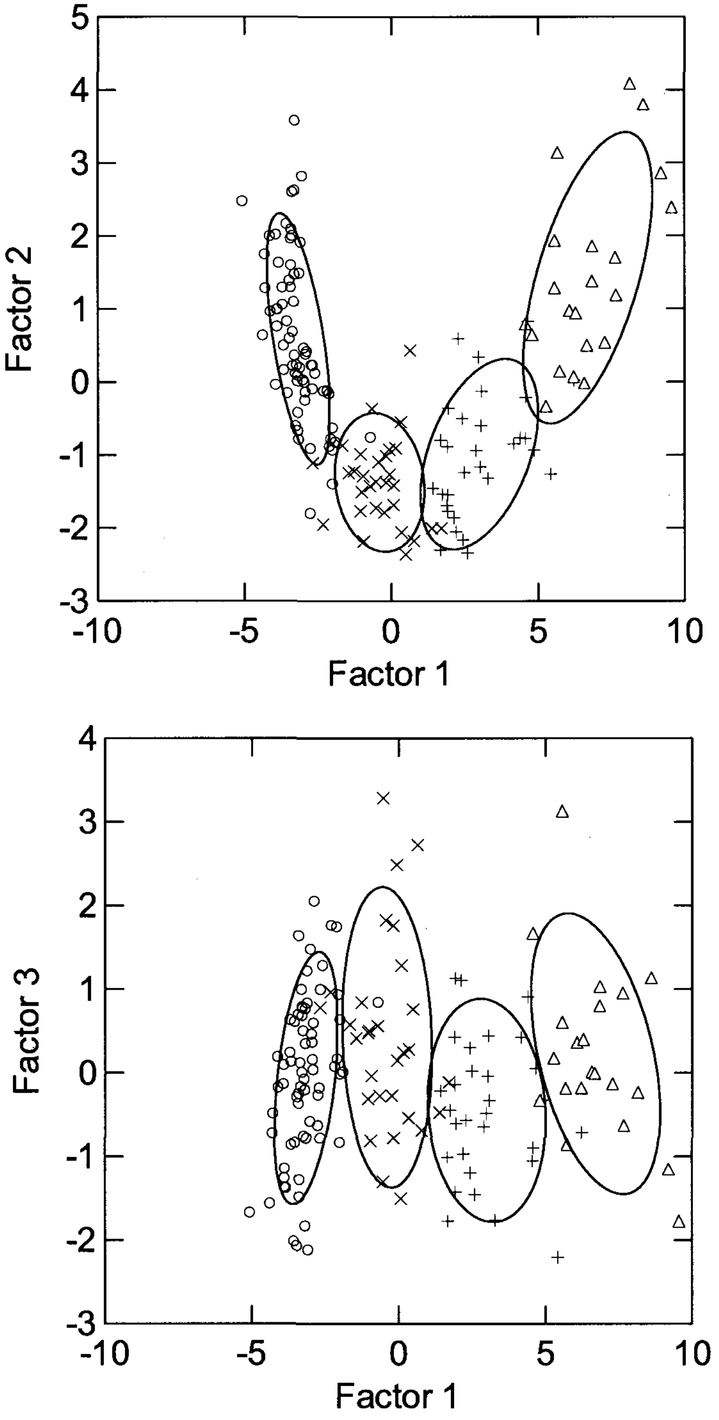


Figure 5.4 Canonical scores for training set samples (n=150) included in the algal discriminant analysis model (○ = Class A, × = Class B, + = Class C, △= NA). Ellipses are centered on group means with a standard deviation of 0.6827. The proportion of dispersion explained by factors 1, 2, and 3 are 0.920, 0.074, and 0.006 respectively.

macroinvertebrate model predicted a better quality class than the algal class for 27% of the samples. These streams tended to be in rural areas with nutrient enrichment from point sources such as treatment plants or non-point sources such as agriculture (MDEP unpublished data). The macroinvertebrates predicted poorer water quality classes than algal classes for 14% of the samples. These samples were collected from streams in urban areas or in systems with an abundance of particulate organic matter that increased abundance of collector-filterer caddisflies, such as *Hydropsyche* spp. and *Cheumatopsyche* spp. (MDEP unpublished data).

5.5 Discussion

5.5.1 Patterns in algal communities

Stream biological communities are influenced by abiotic and biotic factors occurring across multiple spatial and temporal scales (Poff 1997). Hierarchical landscape features at the region, watershed, reach, and microhabitat scales have landscape attributes (*e.g.*, urbanization, water chemistry) that "filter" or constrain species in stream algal communities (Table 5.9, adapted from Poff 1997). Spatial and temporal patterns in the types, frequency, duration, magnitude, and co-occurrence of these filters determine the algal species present in a stream. Effects of certain filters or combinations of filters may act as a limiting resource, disturbance, or constraint (Taylor 1934, van der Ploeg et al. 1999). For example, a heavily shaded stream may have little algal growth despite having abundant nutrients (Borchardt 1996).

Table 5.9 Generalized relationships among hierarchical landscape features, system attributes, landscape filters, and associated algal traits (adapted from Poff 1997).

Spatial Scale	**Landscape Features**	**System Attributes**	**Constraint / Filter**	**Algal Traits**
Region	History	Species evolution and dispersal	Biogeography	Taxonomic distribution
	Climate	Hydroclimatology	Seasonality of flow	Life history
	Climate and Geology	Hydrologic Regime	Flow variability	Attachment strategies
			Drought frequency	Drought tolerance and adaptations
		Thermal regime	Temperature extremes	Thermal tolerance
		Water chemistry	pH, alkalinity, nutrients	pH, alkalinity, & nutrient preferences & sensitivity
Watershed	Confinement/ slope	Flood power	Flood intensity	Resistance to sloughing, fast reproduction
	Lithology	Sediment size range	Litho-habitat	Substrate preference
	Land use	Riparian condition	Organic inputs	Trophic group
		Agriculture	Enrichment	Nutrient requirements and tolerance
			Siltation	Silt tolerance
		Urbanization	NPS pollution	Pollution tolerance
			Pavement	Thermal tolerance
			"Flashy" flows	Flood and drought tolerance
		Mining	Acid mine drainage	pH tolerance
			Metal toxicity	Metal tolerance
	Channel morphology	Bankfull geometry	Flood intensity	Disturbance tolerance
		Thermal regime	Temperature extremes	Thermal tolerance
	Wetlands	Dissolved organic carbon (tannins)	Light penetration	Light requirements
Reach	Morphometry	Channel hydraulics	Water velocity	Current preference
		Bank stability	Sedimentation	Sedimentation tolerance
	Riparian zone	Trees	Shading	Light requirements
	Substrate size distribution	Substrate requirements	Colonization potential	Substrate preference, motility and attachment strategies
		Turbidity	Light penetration	Light requirements
Microhabitat	Water depth, velocity	Nearbed hydraulics	Hydraulic stress	Flow exposure group
	Particle size	Substrate type	Substrate requirement	Substrate preference
	Mosses, plants, macroalgae	Substrate type	Substrate requirement	Substrate preference
		Nutrient sources	Nutrient forms	Nutrient uptake mechanisms
	Periphyton mat	Successional stage	Shading	Light requirements and motility
	Organic matter		Carbon source	Heterotrophy
	Upstream inputs	Nutrient inputs	Grazers	Resistance to grazing
			Amount of nutrients	Nutrient requirements

Reference conditions for benthic algal communities are expected to be different in ecoregions with dissimilar hierarchical filters, such as different alkalinity caused by regional variation in geology (Grenier et al. 2006). Ecoregional characteristics tend to exert the greatest influence on algal communities when differences between ecoregions are great (*e.g.*, large change in topography or geology), and their influence may be very subtle when landscape attributes in the ecoregions impose similar filters on algal communities (Whittier et al. 1988, Pan et al. 1999, Pan et al. 2000). In Maine, reference sites from the Acadian Plains and Hills and Northeastern Highland ecoregions had similar biological communities because the ecoregions had similar hierarchical filters. Many of the taxa that were common in Maine reference streams also were common in low-alkalinity reference streams in Quebec, Canada (Grenier et al. 2006), such as *Achnanthidium minutissimum*, *Tabellaria flocculosa* (Roth) Kützing, *Brachysira microcephala* (Grunow) Compère, *Encyonopsis microcephala* (Grunow) Krammer, *Diatoma moniliformis* Kützing, *Navicula notha* Wallace, *Staurosira construens var. venter* (Ehrenberg) Hamilton, *Eunotia pectinalis* (Müller) Rabenhorst, several forms of *Fragilaria capucina* Desmazières. Disturbed conditions also may not be regionally consistent. Differences in the type, magnitude, and spatial distribution of anthropogenic stressors can result in dissimilar degraded algal assemblages. For example, algal communities from streams damaged by acid mine drainage are different from those from streams damaged by agricultural activities (Pan et al. 2000); however they could be equally damaged in terms of departure from their regional reference conditions.

Anthropogenic stressors can impose filters that overwhelm the influence of natural filters on algal communities (Leland and Porter 2000, Pan et al. 2000, Fore 2003).

The major patterns of diatom community composition of Maine streams were most influenced by nutrient enrichment, increased specific conductance, and sedimentation caused by human activities at the regional and watershed scales. In Maine, the Northeastern Highland ecoregion had few degraded algal communities and the Northeast Coastal Zone ecoregion had few healthy algal communities reflecting the unequal distribution of development and agricultural activities. Similarly, in New Zealand and the Mid-Atlantic region of the United States, human activities at the watershed scale (*e.g.*, urbanization) and reach scale (*e.g.*, riparian alteration) influenced algae more than regional conditions such as climate, geology, soil, and vegetation (Pan et al. 1999, Biggs 2000b, Pan et al. 2000). Species composition of streams with primarily forested watersheds in the Washington Yakima River basin varied with basin geology, but the species composition of agricultural streams was shaped primarily by anthropogenic stressors affecting local conditions, such as enrichment, turbidity, and embeddedness (Leland and Porter 2000).

Streams and rivers may not uniformly provide reference conditions for every taxonomic assemblage, because the same set of landscape features and system attributes could impose different filters on different taxonomic groups. The watershed of the Bull Branch Sunday River in Newry, Maine for example, is entirely forested. The river is oligotrophic, has good water quality, and was included as a reference site for this project, however its channel morphology is still recovering from historic log drives. Brook trout (*Salvelinus fontinalis*) are more sensitive to alteration of channel morphology because they require a variety of substrates, habitats, and hydrogeomorphic conditions at different stages of their lives for spawning, resting, feeding, and overwintering (Smith 1985, Morinville and Rasmussen 2006, Johnson 2008). Although the river provides reference conditions for algae, it might not provide reference conditions for brook trout and other fish.

5.5.2 Algal bioassessment model

I developed an algal-based predictive bioassessment model that integrates professional judgement based on interpretation of the BCG, narrative biocriteria, and departure of algal metrics from regional reference conditions. The model correctly classified 95% of samples in the training data, 91% of the validation data set samples, and had minimal disagreement with class assignments by MDEP biologists. The BCG framework from Davies and Jackson (2006), originally developed for stream macroinvertebrate communities, was adjusted to be applicable to Maine stream algal communities (Table 5.2). The adjustment reflects that BCG Tier 1 and 2 samples typically have a large relative abundance of sensitive macroinvertebrates, however, the same locations often do not have an abundance of sensitive algae. Minimally disturbed sites in Maine typically have a large richness of sensitive algae, but not necessarily a large relative density. The relative abundance of ubiquitous, eurytopic taxa, such as *Achnanthidium minutissimum*, can exceed 80% in some Maine reference sites. Therefore, the algal BCG places greater emphasis on the relative richness of sensitive algae, which was strongly correlated with percent developed watershed (Chapter 4).

Locally derived metrics and indices better measure the deviation of algal communities from local reference conditions than attributes that are developed in other parts of the country or world (Kelly et al. 1998, Pipp 2002, Rott et al. 2003, DeNicola et al. 2004, Potapova et al. 2005, Newall et al. 2006). Most of the algal community attributes that were shown to respond predictably to a disturbance gradient in Maine, and thus served as metrics (Karr and Chu 1999), were locally derived (Chapter 4). Further, the algal bioassessment model consisted of mostly local metrics. Potapova et al. (2005) concluded that bioassessments must be regionally specific because the variety of anthropogenic disturbances in different regions result in a variety of algal community responses. Region-specific metrics also more accurately measured the effects of nutrient enrichment than metrics developed for other geographic areas (Potapova and Charles 2007). Differences in class assignments by the national experts and MDEP biologists reflect contrasting reliance on local metrics to inform the assignments: both used local metrics to inform their evaluations, but the national expert with less agreement with MDEP biologists' placed greater emphasis on literature-based metrics and expectations of other regions of the country. It is also possible that other regions of the country have more degraded streams. Differences in class assignments may increase in regions with great diversity of anthropogenic stressors and algal community responses. Knowledge of local algal communities and availability of locally derived metrics can improve the development and performance of algal bioassessment models.

Maine's algal bioassessment model differs from multimetric indices mainly in how it incorporates professional judgment, combines metrics, and relates to water quality standards. Multimetric indices incorporate professional judgment in selecting metrics, assigning metric scores (*e.g.*, 1, 3, 5), computing indices, defining tiers of condition (*e.g.*, good, fair, poor), and interpreting how to relate the index to the BCG or water quality standards (Karr 1981, Barbour et al. 1995, Gerritsen 1995). Multimetric indices have been criticized for assuming linear metric responses, arbitrarily setting scoring thresholds, combining metrics that can change at different rates, and adding metrics to create a single index score (Suter 1993, Norris 1995). Norris (1995) asserted that the shortcomings of multimetric indices can be overcome by using the metrics in a multivariate statistical analysis. In contrast to multimetric indices, Maine's approach relied on biologists with knowledge of regional algal communities to interpret the non-linear responses and interrelationships of metrics, assign Maine classes and BCG tiers, and construct a statistical model to replicate professional judgment. A key advantage is that the output of Maine's bioassessment model seamlessly integrates with Maine's tiered aquatic life uses, which are used to manage water quality (Courtemanch et al. 1989, Courtemanch 1995, Barbour et al. 2000, Davies and Jackson 2006). Although, multivariate analysis has been criticized because of perceived complexities in calculations (Gerritsen 1995, Karr and Chu 2000), Maine's bioassessment model uses a linear combination of metrics, similar to a multimetric index. The key difference is that rather than adding metric scores (*e.g.*, 1, 3, and 5), Maine's model includes coefficients that are statistically derived to weight metrics in a way that best replicates the judgment of the biologists. Multivariate analysis also has been criticized because of perceived difficulties in explaining the results to the public (Gerritsen 1995, Karr and Chu 2000); however explanation of the probability of a stream attaining its designated class is relatively simple, and Maine's model provides transparency in interpretation of stream condition that is absent in multimetric index scoring.

5.5.3 Comparison of algal model with macroinvertebrate model

Disagreements between classifications based on algae and macroinvertebrates reflect different sensitivities to environmental degradation: algae are influenced more by water quality, nutrient enrichment, and land uses that alter water quality, whereas, macroinvertebrates are more sensitive to oxygen depletion, changes to hydrology and habitat, and some toxic substances (Passy et al. 2004, Hering et al. 2006, Johnson et al. 2006). Sites found to be in higher class condition by the macroinvertebrate model (*i.e.*, better water quality) than the algal model had moderate nutrient enrichment that caused a functional replacement of sensitive algae adapted to low nutrient concentrations by intermediate algae. Nutrients increased stream productivity at those sites, but abundant dissolved oxygen may have prevented negative effects to macroinvertebrate communities (Odum et al. 1979). Many sites that supported better quality algal communities than macroinvertebrate communities were in urban watersheds or located downstream of lake outlets or fish hatcheries (MDEP unpublished data). Some of the urban streams have altered hydrogeomorphology that could affect sensitive macroinvertebrates more than algae. Algae also might recolonize more quickly after disturbances than many sensitive and intermediate macroinvertebrates because of rapid reproduction and recolonization (Peterson 1996). Some sites downstream of lake outlets or fish hatcheries had hyperdominance by caddisflies filter feeding on plankton, zooplankton, and particulate organic matter, which are sources of nutrients not readily available to benthic algae (MDEP unpublished data). Additional research is needed to compare responses of algae and macroinvertebrates to environmental degradation.

5.5.4 Management applications

Biological monitoring data can be the foundation of water quality management if assessment tools are coordinated with water quality standards and criteria (Karr 1991, Courtemanch 1995, Yoder and Rankin 1998, Barbour et al. 2000). MDEP uses bioassessment results to identify impaired waterbodies in need of restoration, target high quality waters for conservation, and improve management of dams, point source discharges, stormwater, and non-point source pollution. Biological assessments also can improve total maximum daily load decision making (Karr and Yoder 2004). MDEP has used attainment of aquatic life criteria as the endpoint of concern in several urban stream TMDLs (Meidel and MDEP 2003a, b, Meidel and Evers 2007). All of these management activities will be improved with the addition of the algal bioassessment model. Evaluations based on both algae and macroinvertebrates can more comprehensively assess waterbody condition and detect effects of a broader range of stressors than evaluations based on only one assemblage (Barbour et al. 1999). In addition, consistent algal model and macroinvertebrate model predictions of class attainment could provide greater confidence in management decision, and differing predictions in class assignment could help diagnose stressors (Yoder and DeShon 2003).

Algal bioassessment programs could benefit from adding best professional judgment review of several key attributes that are not represented in a multimetric index or model to the process of reviewing bioassessment results and determining if streams attain biological criteria. Multimetric indices and models that emphasize metric response to a generalized disturbance gradient can overlook effects of stressors that are not correlated with the general disturbance gradient (Wang et al. 2005). Most metrics included in the algal model are greatly correlated with the general land use disturbance gradient represented by percent developed watershed (Chapter 4). Although large relative abundances of polysaprobic or acidophilic diatoms could indicate damaged algal communities, neither attribute was correlated with percent developed watershed because land use development was not always accompanied by organic pollution or acidification, and infrequent problems with these stressors usually occurred in mostly forested watersheds. As a result, the algal model might not detect impairment caused by the effets of localized sources of acidification (*e.g.*, mines) or organic pollution (*e.g.*, poorly managed agriculture, hatcheries, and wastewater discharges). For example, the model predicted that Blood Brook in Katahdin Ironwork Township, attained Class A because of a predominance of taxa sensitive to increased development, nutrient enrichment, and specific conductance. Although Blood Brook is oligotrophic and its watershed is almost entirely forested, it is damaged by acidification from historic mining activities resulting in atypically low diatom richness and large relative abundance of acidophilic diatoms. MDEP and other bioassessment programs could add best professional judgment review of several diagnostic attributes, such as the relative abundance of polysaprobic diatoms, to the process of reviewing algal bioassessment results and determining if streams attain biological criteria. MDEP could also include the presence of invasive species in the process of reviewing the algal model results because it is included in the original BCG framework but not well-represented in the algal model. Although not observed in Maine, the diatom *Didymosphenia geminata* (Lyngbye) M. Schmidt occurs in nearby states (Vermont and New Hampshire) and Canadian provinces (Quebec and New Brunswick) and could colonize many Maine oligotrophic and mesotrophic streams and rivers (MDEP

2010, VTDEC 2010). It is beneficial to recognize that no model is perfect and to account for model weaknesses when making final decisions about attainment of water quality standards.

The addition of the algal model and specific diagnostic metrics will improve MDEP's ability to identify stressors and manage water quality. Including multiple biological assemblages in a biological monitoring program can improve diagnostic capabilities (Patrick 1949, Paavola et al. 2003, Yoder and DeShon 2003, Passy et al. 2004), especially when incorporated into a formal process such as the U.S. EPA's Stressor Identification and Evaluation process (Cormier et al. 2003). Individual algal metrics and inference models, that might not be included in overall assessments of resource condition, can help diagnose effects of sedimentation (Bahls 1993, Kutka and Richards 1996, Cuffney et al. 1997, Detenbeck et al. 2000, Fore and Grafe 2002, Fore 2003), nutrient enrichment (Cuffney et al. 1997, Leland and Porter 2000, Fore 2003, Wang et al. 2005, Ponader et al. 2007, Ponader et al. 2008, Porter et al. 2008, Stevenson et al. 2008a), increased salinity or specific conductance (Fore 2003, Potapova and Charles 2003, Stevenson et al. 2008b), organic enrichment (Fore and Grafe 2002, Fore 2003, Kelly et al. 2008), and acidification (Hill et al. 2000, Hill et al. 2003, Stevenson et al. 2008b). Combinations of diagnostic metrics have been used to distinguish the effects agricultural land use from urban land use (Fore 2003) and mining (Pan et al. 1996) as well as distinguishing the effects of organic and inorganic effluents on diatom communities (Kelly 1998a, b, Rott et al. 1998, Leland and Porter 2000). MDEP can improve diagnosis of stressors damaging a stream or river by simultaneously evaluating algal and macroinvertebrate diagnostic metrics and indices. The addition of algal model, inference models (*e.g.*, Diatom Total Phosphorus Index), and diagnostic metrics (*e.g.*, relative richness of motile diatoms) will improve the management of water quality in Maine's streams and rivers.

6. NOVEL CONTRIBUTIONS AND MANAGEMENT CONSIDERATIONS

6.1 Introduction

The goal of this research was to provide the Maine Department of Environmental Protection (MDEP) with new tools to improve management of Maine's streams and rivers. MDEP manages water quality and monitors streams and rivers to determine attainment of water quality standards. Maine has four Classes of streams and rivers (AA, A, B, and C) with different environmental expectations and conditions of aquatic life communities (Courtemanch et al. 1989, Courtemanch 1995, Davies and Jackson 2006). MDEP currently collects benthic macroinvertebrate samples and uses the data in a linear discriminant model to predict the likelihood of a sample attaining Class AA/A, B, and C biological criteria. If a stream does not attain its class, then MDEP must develop a plan to restore its water quality.

The objectives of this study were to:

1) conduct a pilot study to determine if algal growth in the Sheepscot River was limited by nitrogen (N) or phosphorus (P) (Chapter 2),
2) develop models with benthic algal data to infer nutrient concentrations of stream water (Chapter 3),
3) identify algal community metrics that distinguish reference sites from disturbed sites and that predictably respond to watershed disturbance (Chapter 4), and
4) develop a statistical model that uses species composition of benthic algae to predict attainment of biological criteria (*i.e.*, AA/A, B, C) (Chapter 5).

6.2 Key Findings

6.2.1 Nutrient limitation

MDEP previously assumed that P was often the limiting nutrient in fresh waters and N was often the limiting nutrient in marine waters. I tested two methods to determine if algal growth in the Sheepscot River was limited by N or P. The first method used ratios of N and P concentrations in stream water to infer nutrient limitation. The ratio of total N to total P (TN:TP) in stream water implied that the Sheepscot River was co-limited, and the ratio of dissolved inorganic N and soluble reactive P (DIN:SRP) implied that algal growth in the Sheepscot River was strongly limited by N during the study period. Overall, the nutrient ratios were of limited use, because nutrients in stream water were scarce during the study. The second method was to conduct an experiment with four different nutrient diffusing substrate (NDS) treatments: enriched with N, enriched with P, enriched with both, enriched with neither. The NDS experiment indicated co-limitation by N and P in the Sheepscot River. Adding either nutrient alone doubled chlorophyll *a,* while adding both N and P, resulted in a six-fold increase in chlorophyll *a* concentrations.

6.2.2 Nutrient inference models

I applied several approaches to model development for inferring nutrient concentrations of streams and rivers based on the species composition of benthic algal samples. I developed models to infer TP and TN concentrations with weighted averaging (WA), weighted averaging – partial least squares (WA-PLS) (*e.g.*, Potapova et al. 2004, Ponader et al. 2007, Ponader et al. 2008, Stevenson et al. 2008b), locally-weighted weighted averaging (LWWA), and multiple linear regressions (MLR). I also evaluated three methods of reducing model bias of weighted averaging models, including 1) excluding indifferent taxa, 2) including soft algae in addition to diatoms, and 3) applying a novel slope-snapping technique. The best performing models were MLR and LWWA. Excluding indifferent taxa and including soft algae in inference models did not improve performance or reduce bias of WA, WA-PLS, and LWWA models. In contrast, slope-snapping successfully removed bias in the training set and reduced bias when applied to validation data. MLR, LWWA, and LWWA with slope-snapping were improvements over traditional weighted average inference models. MLR ultimately was selected because it had less bias than LWWA when applied to validation data and was more transferrable (*i.e.*, easier to calculate and explain to the public).

6.2.3 Algal community metrics for Maine's streams and rivers

I evaluated relationships of benthic algal metrics with a general land use disturbance gradient affecting wadeable streams and rivers in Maine, USA. Epilithic algal samples (n=298) were collected from 193 sample locations across the state. Patterns in species composition were computed with non-metric multidimensional scaling (NMS) and displayed with correlated environmental variables. The major pattern in species composition was related to land use development, nutrient enrichment, and increased specific conductance. Maine Stream Tolerance (MST) values were computed with Principal Components Analysis (PCA) for common algal taxa by determining major patterns in species weighted average optima for total phosphorus, total nitrogen, specific conductance, percent developed watershed, and percent impervious surfaces. Taxa were assigned to tolerance categories of sensitive, intermediate, and tolerant based on their MST values and response to disturbance as shown by general linear models. Algal community attributes used in other multimetric indexes and novel attributes based on Maine data were assessed by 1) plotting attribute response to DEV in scatterplots with LOWESS regression lines, 2) determining strength of Spearman rank correlations with percent developed watershed, and 3) testing ability to distinguish reference from non-reference sites with Mann-Whitney U tests. Few community structure attributes (*e.g.*, total species richness) were effective metrics in Maine. Most metrics with the strongest relationship with percent developed watershed were based on Maine-specific data, such as the relative richness of species characterized as being sensitive in Maine.

6.2.4 Algal bioassessment model for Maine streams and rivers

I built a model to predict attainment of Maine's biological criteria (*i.e.*, AA/A, B, C) based on the community composition of benthic algal samples. This linear discriminant analysis model uses a subset of statistically selected algal community metrics and represents a first application of the Biological Condition Gradient (BCG, Davies and Jackson 2006) to stream algae. It also is the first stream algal model to explicitly tie the evaluation of biological condition to Tiered Aquatic Life Uses in water quality standards. The model performed very well, correctly classifying 95% of samples in the training set and 91% of samples in the validation set.

6.3 Management Recommendations

6.3.1 Nutrient limitation

Algal growth was co-limited by N and P in the Sheepscot River, suggesting that MDEP's assumption that P is the primary limiting nutrient in Maine streams and rivers is not uniformly accurate. Caution is warranted when temporally or spatially extrapolating nutrient limitation assessments, because the heterogeneous distributions of natural and anthropogenic sources of nutrients may influence nutrient limitation. In addition, other resources (*e.g.*, light, temperature) and disturbances (*e.g.*, substrate stability, grazing, scouring) can limit algal growth even with sufficient nutrients.

DIN:SRP or TN:TP ratios alone are probably not reliable indicators of nutrient limitation in Maine's streams and rivers, especially for oligotrophic waterbodies and when nutrient concentrations are very small or very large. Further research is needed to determine if the nutrient ratios are reliable in mesotrophic streams and rivers because nutrient supply, storage, transport, and transformation can be complex and confound inferences made by nutrient ratios.

6.3.2 Nutrient inference models

Multiple linear regression inference models to infer TP and TN concentrations in Maine streams and rivers outperformed the traditional weighted averaging approaches and had less model bias. Inference models of the algal community represent a time-integrated estimate of stream nutrients and may be more reliable than a series of water samples. The models could be improved with model recalibration with more eutrophic samples in the training data. Model results should not be extrapolated beyond the range of data used to build the models. The inference models could be used to identify streams and rivers with nutrient enrichment problems and evaluate the success and incremental improvements of management activities designed to reduce nutrient inputs.

6.3.3 Algal community metrics for Maine streams and rivers

Algal community attributes confirmed as metrics in Maine formed the foundation of the algal bioassessment model. Samples were collected primarily from stream and river reaches with rocky substrate and open canopy. As such, the metrics might not be transferable to reaches that are shaded, sandy, or mucky. Paired samples collected from adjacent sunny and shady reaches and paired rock scrapings, surface sediment cores, or artificial substrates will provide a comparative data set to determine if the metrics are transferable to shady conditions and alternative substrates. Validation of the metrics will provide an indication of the robustness of the sample techniques in a variety of stream conditions.

MDEP could use a similar process of computing Maine tolerance values and testing attribute performance when developing biological assessment methods for other waterbody types, such as wetlands. The process of computing Maine tolerance values could be particularly valuable for wetlands, because little is known about the ecological tolerances of wetland algae and macroinvertebrates in New England. A comprehensive approach would evaluate novel attributes and attributes used in other wetland bioassessments to determine those that best distinguish reference sites from non-reference sites and those that respond to anthropogenic disturbances to the wetlands and their watersheds.

6.3.4 Algal bioassessment model for Maine streams and rivers

Implementing the algal bioassessment model in conjunction with the existing macroinvertebrate model could provide a more comprehensive assessment of stream attainment class (*i.e.*, AA/A, B, C). The algal and macroinvertebrate models are independent indicators, however, so that impaired waterbodies are identified if either model indicates non-attainment of the assigned class. When both models indicate attainment or nonattainment, there is increased confidence in determinations of class attainment. Model results and individual metrics also may provide diagnostic information of causes of impairment when invertebrate-based and algal-based predictions of attainment class do not agree. The algal model likely will identify more streams impaired by nutrient enrichment than the macroinvertebrate model.

MDEP should consider incorporating the new algal model into the existing bioassessment framework to maintain consistency. MDEP could benefit from maintaining consistency between the algal model and macroinvertebrate model by making it easier to write computer code, structure reports, integrate the algal model into numeric biological criteria, and communicate with the public. In addition, the existing bioassessment framework (06-096 Code of Maine Rules Chapter 579, Davies et al. 1993, Davies et al. 1999, Davies and Tsomides 2002) provides several provisions that could improve the reliability of the algal model. For example, MDEP requires taxa richness>15 and total abundance>50 for running macroinvertebrate samples through the macroinvertebrate model. A similar provision for taxa richness could be used for algal data. MDEP also uses professional judgment to review indeterminate predictions (*i.e.*, probability of attaining a class between 0.40 and 0.60) for the macroinvertebrate model. For example, qualified staff review indeterminate macroinvertebrate model predictions to either raise the finding, lower the finding, or determine that a site needs to be resampled (Davies and Tsomides 2002). MDEP could apply the same protocols when interpreting algal model probabilities of class attainment between 0.40 and 0.60, although the algal model was decisive and resulted in only 13 of 298 (4%) samples with probabilities within that range. MDEP could consider officially incorporating the algal model into state rule (06-096 Code of Maine Rules Chapter 579) if it is determined to be sufficiently robust. Finally, MDEP could consider recalibrating the model with a larger data set in the future.

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APPENDIX

Table A.1 Diatom taxa frequency (n), maximum square root percent abundance (Max), Hill's N2 measure of diversity (Hill 1973), weighted average optima (opt), weighted average tolerance (tol), and shape of generalized linear model (GLM) curves fitted to the response of taxa square root percent abundance to total phosphorus (TP) and total nitrogen (TN). Optima and tolerances are log10 µg/L. Response curve shapes are as follows: Q = quadratic, QI = inverse quadratic, D = decreasing, I = increasing, and N = flat line.

Taxon Name	Code	N	Max	Hill's N2	TN opt	TN tol	TN GLM	TP opt	TP tol	TP GLM
Achnanthes nollii Bock	ACnollii	10	1.07	8.6	2.61	0.07	Q	1.15	0.10	Q
Achnanthes oblongella Østrup	ACoblong	7	3.06	3.6	2.83	0.29	I	1.37	0.33	N
Achnanthes subhudsonis var. kraeuselii (Cholnoky) Cholnoky	ACsubkra	17	2.31	14.6	2.84	0.20	Q	1.56	0.26	I
Achnanthidium affine (Grunow) Czarnecki	ADaffine	8	1.24	7.1	2.84	0.11	N	1.40	0.16	Q
Achnanthidium deflexum (Rabenhorst) Lange-Bertalot et Ruppel	ADdeflex	95	7.92	57.9	2.69	0.19	Q	1.32	0.24	Q
Achnanthidium exiguum (Grunow) Czarnecki	ADexigua	17	0.80	16.1	2.78	0.20	N	1.40	0.24	Q
Achnanthidium kranzii (Lange-Bertalot) Round et Bukhtiyarova	ADkranz	6	3.26	3.1	2.99	0.12	Q	1.58	0.20	Q
Achnanthidium minutissimum (Kützing) Czarnecki	ADminuti	162	9.31	147.2	2.67	0.24	D	1.27	0.29	D
Achnanthidium rivulare Potapova et Ponader	ADrivula	49	8.21	28.0	2.80	0.20	Q	1.35	0.22	Q
Adlafia bryophila (Petersen) Lange-Bertalot	ALbryphl	17	2.01	13.3	2.68	0.29	N	1.25	0.27	N
Adlafia suchlandtii (Hustedt) Lange-Bertalot	ALsuchla	7	1.87	6.0	2.57	0.17	Q	1.18	0.24	N
Amphipleura pellucida (Kützing) Kützing	APpelluc	26	1.24	22.1	2.69	0.18	N	1.31	0.27	N

Table A.1 Continued

Taxon Name	Code	N	Max	Hill's N2	TN opt	TN tol	TN GLM	TP opt	TP tol	TP GLM
Amphora copulata (Kützing) Schoeman et Archibald	AMcopula	9	1.09	7.5	2.70	0.23	N	1.36	0.17	Q
Amphora pediculus (Kützing) Grunow	AMpedcls	44	4.09	26.9	2.89	0.23	I	1.51	0.25	Q
Aulacoseira alpigena (Grunow) Krammer	AUalpige	12	0.95	10.9	2.74	0.28	N	1.33	0.24	N
Aulacoseira ambigua (Grunow) Simonsen	AUambigu	14	1.79	11.3	2.63	0.16	Q	1.25	0.19	Q
Aulacoseira subarctica (Müller) Haworth	AUsubart	6	1.65	4.7	2.46	0.11	Q	0.98	0.18	Q
Brachysira brebissonii Ross	BRbrebis	22	3.29	14.5	2.49	0.13	Q	1.10	0.19	Q
Brachysira microcephala (Grunow) Compère	BRmicroc	72	4.53	48.8	2.50	0.16	Q	1.06	0.19	Q
Brachysira vitrea (Grunow) Ross	BRvitrea	9	3.77	5.5	2.58	0.26	N	1.21	0.18	Q
Caloneis bacillum (Grunow) Cleve	CAbacill	20	1.18	17.7	2.86	0.23	I	1.53	0.25	I
Cavinula cocconeiformis (Gregory ex Greville) Mann et Stickle	CJcoccon	14	1.03	12.7	2.70	0.21	N	1.35	0.25	N
Chamaepinnularia bremensis (Hustedt) Lange-Bertalot	CKbremen	8	2.31	5.5	2.86	0.17	I	1.56	0.20	I
Chamaepinnularia mediocris (Krasske) Lange-Bertalot	CKmedioc	9	1.85	6.6	2.83	0.28	I	1.35	0.26	N
Cocconeis pediculus Ehrenberg	CCpedcls	15	3.76	11.3	2.91	0.19	I	1.48	0.25	Q
Cocconeis placentula var. euglypta (Ehrenberg) Grunow	CCplaeug	25	3.17	17.1	2.78	0.20	I	1.42	0.24	Q
Cocconeis placentula var. lineata (Ehrenberg) Van Heurck	CCplalin	113	5.26	70.2	2.76	0.22	I	1.40	0.26	Q
Craticula molestiformis (Hustedt) Lange-Bertalot	CRmolest	7	1.01	6.3	2.75	0.22	Q	1.62	0.28	I

Table A.1 Continued

Taxon Name	Code	N	Max	Hill's N2	TN opt	TN tol	TN GLM	TP opt	TP tol	TP GLM
Craticula submolesta (Hustedt) Lange-Bertalot	CRsubmol	10	2.16	7.3	2.58	0.17	N	1.17	0.22	D
Ctenophora pulchella (Ralfs ex Kützing) Williams et Round	CTpulche	10	6.52	2.7	2.80	0.13	Q	1.56	0.20	Q
Cyclotella bodanica var. lemanica Müller	CYbodlem	8	1.20	6.6	2.48	0.17	Q	1.00	0.18	Q
Cyclotella meneghiniana Kützing	CYmenegh	52	4.71	28.3	2.84	0.20	N	1.49	0.24	Q
Cyclotella michiganiana Skvortzow	CYmichig	8	0.84	7.3	2.60	0.19	N	1.21	0.25	N
Cymbella affinis Kützing	CMaffins	19	1.83	15.1	2.62	0.21	N	1.18	0.24	D
Cymbella cistula (Ehrenberg) Kirchner	CMcistul	12	1.49	9.5	2.54	0.17	Q	1.07	0.21	D
Cymbella delicatula Kützing	CMdelcat	31	5.03	17.4	2.60	0.19	Q	1.14	0.25	D
Cymbella gracilis (Ehrenberg) Kützing	CMgracil	53	2.29	41.2	2.57	0.20	Q	1.15	0.22	D
Cymbella naviculiformis Auerswald ex Héribaud	CMnavicu	11	0.83	10.2	2.74	0.15	Q	1.42	0.26	N
Cymbella subturgidula Krammer	CMsubtur	7	2.20	5.8	2.50	0.16	Q	1.15	0.10	Q
Cymbella tumida (Brébisson ex Kützing) Van Heurck	CMtumida	45	2.24	33.4	2.75	0.16	Q	1.47	0.29	I
Diatoma mesodon (Ehrenberg) Kützing	DAmesodo	11	1.53	9.1	2.47	0.23	D	1.07	0.31	D
Diatoma moniliformis Kützing	DAmonili	34	7.02	14.7	2.68	0.23	N	1.33	0.32	N
Diatoma tenuis Agardh	DAtenuis	19	1.89	15.4	2.69	0.27	N	1.33	0.31	N
Diatoma vulgaris Bory	DAvulgar	7	1.42	5.5	2.86	0.08	Q	1.61	0.21	Q
Diploneis oblongella (Näegeli ex Kützing) Ross	DPoblong	6	1.35	4.5	2.77	0.17	N	1.24	0.22	N
Discostella pseudostelligera (Hustedt) Houk et Klee	DOpseudo	14	1.29	12.3	2.60	0.19	Q	1.23	0.34	N
Encyonema minutum (Hilse) Mann	ENminutu	72	4.08	44.5	2.75	0.21	I	1.38	0.26	I

Table A.1 Continued

Taxon Name	Code	N	Max	Hill's N2	TN opt	TN tol	TN GLM	TP opt	TP tol	TP GLM
Encyonema prostratum (Berkeley) Kützing	ENprostr	9	1.13	8.0	2.89	0.17	I	1.43	0.24	I
Encyonema reichardtii (Krammer) Mann	ENreicha	7	2.95	5.3	2.95	0.20	I	1.51	0.20	Q
Encyonema silesiacum (Bleisch) Mann	ENsilesi	121	5.92	83.0	2.71	0.21	Q	1.35	0.25	Q
Encyonopsis cesatii (Rabenhorst) Krammer	EScesati	15	2.87	9.8	2.66	0.24	N	1.13	0.25	D
Encyonopsis falaisensis (Grunow) Krammer	ESfalais	6	4.18	4.0	2.56	0.23	N	1.12	0.30	D
Encyonopsis microcephala (Grunow) Krammer	ESmicroc	52	1.96	41.1	2.60	0.21	D	1.14	0.22	Q
Eucocconeis laevis (Østrup) Lange-Bertalot	EClaevis	39	1.29	34.9	2.62	0.21	D	1.20	0.27	D
Eunotia arcus var. bidens Grunow	EUarcbid	9	1.74	6.9	2.47	0.15	Q	1.01	0.18	Q
Eunotia bilunaris (Ehrenberg) Mills	EUbiluna	48	1.90	37.3	2.66	0.20	Q	1.32	0.27	N
Eunotia exigua (Brébisson ex Kützing) Rabenhorst	EUexigua	43	4.27	24.5	2.56	0.28	D	1.17	0.35	QI
Eunotia flexuosa Brébisson ex Kützing	EUflexuo	8	1.58	6.5	2.49	0.11	Q	1.09	0.18	Q
Eunotia implicata Nörpel, Lange-Bertalot et Alles	EUimplic	48	3.35	35.0	2.60	0.19	Q	1.23	0.22	Q
Eunotia incisa Smith ex Gregory	EUincisa	62	4.80	37.0	2.57	0.22	D	1.21	0.27	N
Eunotia minor (Kützing) Grunow	EUminor	42	2.10	33.2	2.74	0.26	N	1.37	0.31	I
Eunotia muscicola var. tridentula Nörpel et Lange-Bertalot	EUmustri	8	1.01	7.3	2.37	0.29	D	0.92	0.33	D
Eunotia naegelii Migula	EUnaegel	10	0.81	9.4	2.51	0.26	N	1.13	0.25	N
Eunotia paludosa Grunow	EUpaludo	12	1.33	10.3	2.57	0.33	D	1.14	0.37	D

Table A.1 Continued

Taxon Name	Code	N	Max	Hill's N2	TN opt	TN tol	TN GLM	TP opt	TP tol	TP GLM
Eunotia pectinalis (Müller) Rabenhorst	EUpectin	31	6.72	15.5	2.45	0.15	D	1.08	0.15	D
Eunotia pectinalis var. undulata (Ralfs) Rabenhorst	EUpecund	14	4.95	7.6	2.55	0.20	D	1.19	0.19	N
Eunotia rhomboidea Hustedt	EUrhombo	14	3.46	8.7	2.49	0.26	D	1.15	0.31	D
Eunotia subarcuatoides Alles, Nörpel et Lange-Bertalot	EUsubarc	13	1.97	9.3	2.64	0.38	QI	1.17	0.34	N
Fragilaria capucina Desmazières	FRcapuci	52	4.53	30.2	2.60	0.23	D	1.25	0.21	Q
Fragilaria capucina var. gracilis (Østrup) Hustedt	FRcapgra	108	5.88	79.4	2.60	0.21	Q	1.21	0.27	D
Fragilaria sepes Ehrenberg	FRsepes	38	1.63	32.5	2.61	0.20	D	1.18	0.26	D
Fragilaria tenera (Smith) Lange-Bertalot	FRtenera	26	2.22	20.4	2.64	0.20	N	1.19	0.25	D
Fragilaria vaucheriae (Kützing) Petersen	FRvauche	124	2.65	98.1	2.69	0.23	N	1.32	0.27	N
Fragilaria vaucheriae var. capitellata (Grunow) Ross	FRvaucap	15	2.91	9.9	2.75	0.28	N	1.36	0.37	N
Fragilariforma virescens (Ralfs) Williams et Round	FAviresc	19	3.68	11.1	2.60	0.24	N	1.21	0.26	N
Frustulia amphipleuroides (Grunow) Cleve-Euler	FSamphip	11	0.85	10.2	2.75	0.21	N	1.40	0.30	N
Frustulia crassinervia (Brébisson) Lange-Bertalot et Krammer	FScrassi	35	3.79	19.8	2.60	0.19	Q	1.21	0.24	Q
Frustulia krammeri Lange-Bertalot et Metzeltin	FSkramme	15	3.07	7.6	2.60	0.16	N	1.18	0.22	N
Frustulia vulgaris (Thwaites) deToni	FSvulgar	18	1.03	16.0	2.82	0.21	I	1.42	0.22	I
Geissleria acceptata (Hustedt) Lange-Bertalot et Metzeltin	GAignacc	7	1.05	6.0	2.76	0.29	N	1.40	0.42	N

Table A.1 Continued

Taxon Name	Code	N	Max	Hill's N2	TN opt	TN tol	TN GLM	TP opt	TP tol	TP GLM
Geissleria decussis (Hustedt) Lange-Bertalot et Metzeltin	GAdecuss	26	1.82	20.6	2.69	0.24	N	1.38	0.28	N
Gomphonema acuminatum Ehrenberg	GOacumin	27	1.69	22.5	2.52	0.20	Q	1.08	0.24	D
Gomphonema affine Kützing	GOaffine	19	1.17	17.1	2.63	0.21	N	1.21	0.29	N
Gomphonema angustatum (Kützing) Rabenhorst	GOangust	28	2.37	21.3	2.75	0.23	I	1.41	0.30	I
Gomphonema angustum Agardh	GOangstm	20	2.58	15.4	2.70	0.23	N	1.27	0.32	N
Gomphonema clavatum Ehrenberg	GOclavat	16	2.07	12.2	2.71	0.17	Q	1.30	0.25	N
Gomphonema clevei Fricke	GOclevei	15	3.72	11.2	2.55	0.24	D	1.18	0.24	D
Gomphonema drutelingense Reichardt	GOdrutel	8	1.05	7.6	2.71	0.15	Q	1.41	0.27	N
Gomphonema gracile Ehrenberg emend Van Heurck	GOgracil	30	4.25	18.0	2.54	0.14	Q	1.17	0.23	D
Gomphonema kobayasii Kociolek et Kingston	GOkobaya	31	2.40	22.7	2.80	0.18	Q	1.43	0.20	Q
Gomphonema micropus Kützing	GOmicrop	35	3.11	24.3	2.68	0.20	N	1.29	0.24	N
Gomphonema minutum (Agardh) Agardh	GOminutu	40	3.32	27.6	2.77	0.25	I	1.39	0.29	I
Gomphonema olivaceoides Hustedt	GOolivco	19	2.30	14.8	2.90	0.19	I	1.53	0.26	I
Gomphonema olivaceoides var. hutchinsoniana Patrick	GOolihut	7	3.17	5.5	2.77	0.24	N	1.42	0.19	Q
Gomphonema parvulum (Kützing) Kützing	GOparvul	145	4.44	109.2	2.69	0.22	N	1.32	0.28	I
Gomphonema pseudotenellum Lange-Bertalot	GOpseten	8	2.59	5.3	2.61	0.33	N	1.26	0.40	N
Gomphonema pumilum (Grunow) Reichardt et Lange-Bertalot	GOpumilu	51	3.65	36.8	2.67	0.25	N	1.27	0.25	N

Table A.1 Continued

Taxon Name	**Code**	**N**	**Max**	**Hill's N2**	**TN opt**	**TN tol**	**TN GLM**	**TP opt**	**TP tol**	**TP GLM**
Gomphonema pumilum var. rigidum Reichardt et Lange-Bertalot	GOpumilu	51	3.65	36.8	2.67	0.25	N	1.27	0.25	N
Gomphonema rhombicum Fricke	GOrhombi	33	4.13	23.4	2.62	0.16	Q	1.20	0.19	Q
Gomphonema subclavatum (Grunow) Grunow	GOsubcla	7	1.55	5.2	2.65	0.23	N	1.40	0.40	N
Gomphonema truncatum Ehrenberg	GOtrunca	40	2.74	30.8	2.62	0.23	N	1.25	0.32	N
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski	HPcapita	25	1.27	21.6	2.83	0.15	Q	1.56	0.28	I
Karayevia clevei (Grunow) Bukhtiyarova	KAclevei	17	0.96	15.0	2.75	0.16	N	1.44	0.25	I
Karayevia laterostrata (Hantzsch) Bukhtiyarova	KAlatero	20	1.47	16.1	2.80	0.20	I	1.51	0.32	I
Karayevia suchlandtii (Hustedt) Bukhtiyarova	KAsuchla	18	2.69	11.8	2.81	0.26	I	1.52	0.33	QI
Luticola mutica (Kützing) Mann	LUmutica	13	0.60	12.4	2.82	0.19	I	1.51	0.29	I
Mayamaea agrestis (Hustedt) Lange-Bertalot	MYagrest	7	1.16	5.6	2.91	0.22	I	1.48	0.07	Q
Mayamaea atomus var. permitis (Hustedt) Lange-Bertalot	MYatoper	18	1.73	14.3	2.87	0.21	I	1.50	0.26	I
Melosira varians Agardh	MEvarian	40	3.32	28.8	2.87	0.23	I	1.49	0.19	Q
Meridion circulare (Greville) Agardh	MDcircul	42	2.42	29.7	2.72	0.27	QI	1.36	0.34	QI
Meridion circulare var. constrictum (Ralfs) Van Heurck	MDcircon	26	1.44	22.3	2.75	0.24	I	1.37	0.29	N
Navicula angusta Grunow	NAangust	7	1.05	6.2	2.50	0.19	D	1.08	0.21	D
Navicula antonii Lange-Bertalot	NAanton	11	2.29	7.7	2.74	0.21	N	1.56	0.27	I
Navicula capitatoradiata Germain	NAcaprad	27	2.22	20.9	2.85	0.21	I	1.47	0.24	I
Navicula cryptocephala Kützing	NAcrypto	91	2.89	71.0	2.78	0.20	I	1.45	0.27	Q

Table A.1 Continued

Taxon Name	Code	N	Max	Hill's N2	TN opt	TN tol	TN GLM	TP opt	TP tol	TP GLM
Navicula cryptotenella Lange-Bertalot ex Krammer et Lange-Bertalot	NAcryten	100	3.45	74.5	2.69	0.23	N	1.34	0.28	I
Navicula cryptotenelloides Lange-Bertalot	NAcrytot	13	3.75	8.3	2.83	0.24	I	1.51	0.23	Q
Navicula erifuga Lange-Bertalot	NAerifug	10	1.78	7.4	2.77	0.24	N	1.54	0.30	I
Navicula gregaria Donkin	NAgregar	52	3.79	35.2	2.87	0.21	I	1.49	0.24	Q
Navicula hintzii Lange-Bertalot	NAhintzi	11	1.06	10.3	2.62	0.19	D	1.33	0.28	N
Navicula lanceolata (Agardh) Ehrenberg	NAlanceo	32	2.85	21.8	2.91	0.16	Q	1.50	0.18	Q
Navicula laterostrata Hustedt	NAlatero	6	1.25	5.2	2.87	0.27	I	1.40	0.26	N
Navicula leptostriata Jörgansen	NAleptos	11	0.83	10.3	2.71	0.27	N	1.34	0.24	N
Navicula menisculus Schumann	NAmenscl	18	3.04	12.4	2.82	0.25	I	1.42	0.19	Q
Navicula minima Grunow	NAminima	63	5.08	38.6	2.81	0.24	I	1.46	0.32	I
Navicula notha Wallace	NAnotha	45	4.73	27.8	2.63	0.23	QI	1.25	0.26	N
Navicula radiosa Kützing	NAradios	20	0.91	18.0	2.72	0.19	N	1.31	0.27	N
Navicula radiosafallax Lange-Bertalot	NAradiof	13	1.44	10.7	2.66	0.21	N	1.28	0.22	N
Navicula reichardtiana Lange-Bertalot	NAreicha	13	1.35	11.6	2.83	0.18	Q	1.30	0.18	Q
Navicula rhynchocephala Kützing	NArhynch	49	1.49	40.8	2.82	0.21	Q	1.49	0.24	Q
Navicula rostellata Kützing	NArostel	26	1.55	20.9	2.77	0.19	Q	1.47	0.26	I
Navicula schmassmanni Hustedt	NAschmas	11	2.59	7.1	2.78	0.21	N	1.47	0.26	I
Navicula schroeteri var. escambia Patrick	NAschroe	13	1.17	11.5	2.83	0.12	Q	1.55	0.14	Q
Navicula stroemii Hustedt	NAstroem	7	0.78	6.6	2.65	0.19	N	1.14	0.12	Q
Navicula tenelloides Hustedt	NAtendes	8	2.79	4.3	2.82	0.15	Q	1.38	0.20	D
Navicula tripunctata (Müller) Bory	NAtripun	14	3.77	10.6	2.93	0.21	I	1.46	0.24	Q
Navicula trivialis Lange-Bertalot	NAtrivia	19	2.42	12.0	2.81	0.12	Q	1.66	0.28	I

Table A.1 Continued

Taxon Name	Code	N	Max	Hill's N2	TN opt	TN tol	TN GLM	TP opt	TP tol	TP GLM
Navicula veneta Kützing	NAveneta	19	1.60	16.4	2.84	0.15	I	1.39	0.19	Q
Navicula viridulacalcis (Hustedt) Lange-Bertalot	NAvirlin	8	2.79	4.9	2.79	0.14	Q	1.67	0.23	Q
Nitzschia acicularis (Kützing) Smith	NIacicul	7	1.50	5.3	2.64	0.17	N	1.50	0.38	I
Nitzschia acidoclinata Lange-Bertalot	NIacidoc	29	1.53	24.7	2.80	0.23	I	1.45	0.26	I
Nitzschia amphibia Grunow	NIamphib	21	2.28	15.8	2.85	0.20	I	1.48	0.27	I
Nitzschia archibaldii Lange-Bertalot	NIarchi	7	0.91	6.3	2.78	0.12	Q	1.48	0.13	Q
Nitzschia capitellata Hustedt	NIcapite	18	1.20	16.4	2.82	0.16	Q	1.51	0.25	Q
Nitzschia dissipata (Kützing) Grunow	NIdissip	58	2.73	43.8	2.79	0.19	Q	1.47	0.25	Q
Nitzschia dissipata var. media (Hantzsch) Grunow	NIdismed	23	1.31	20.4	2.84	0.18	I	1.48	0.16	Q
Nitzschia fonticola Grunow	NIfontic	15	4.57	7.2	2.86	0.20	I	1.59	0.28	I
Nitzschia frustulum (Kützing) Grunow	NIfrustu	42	1.85	35.8	2.73	0.18	N	1.40	0.24	Q
Nitzschia gracilis Hantzsch ex Rabenhorst	NIgracil	16	0.86	15.2	2.75	0.25	QI	1.36	0.34	QI
Nitzschia inconspicua Grunow	NIincons	21	2.65	14.8	2.88	0.20	I	1.60	0.28	I
Nitzschia lacuum Lange-Bertalot	NIlacuum	8	1.37	6.8	2.90	0.16	Q	1.44	0.10	Q
Nitzschia liebethruthii Rabenhorst	NIliebrt	9	1.98	6.1	2.89	0.24	I	1.44	0.21	Q
Nitzschia linearis (Agardh ex Wm. Smith) Wm. Smith	NIlinear	13	1.22	11.3	2.77	0.19	Q	1.37	0.30	N
Nitzschia palea (Kützing) Smith	NIpalea	87	2.69	67.4	2.80	0.21	I	1.45	0.26	Q
Nitzschia palea var. debilis (Kützing) Grunow	NIpaldeb	21	3.48	14.7	2.74	0.15	N	1.44	0.31	I
Nitzschia paleacea Grunow ex Van Heurck	NIpaleac	11	0.93	10.2	2.78	0.26	N	1.47	0.35	I
Nitzschia perminuta (Grunow) Peragallo	NIpermin	19	1.25	16.4	2.82	0.20	N	1.44	0.31	I
Nitzschia pumila Hustedt	NIpumila	10	1.04	9.1	2.79	0.21	I	1.45	0.25	I

Table A.1 Continued

Taxon Name	Code	N	Max	Hill's N2	TN opt	TN tol	TN GLM	TP opt	TP tol	TP GLM
Nitzschia recta Hantzsch ex Rabenhorst	NIrecta	30	1.56	25.3	2.71	0.20	Q	1.38	0.30	I
Nitzschia subacicularis Hustedt	NIsubaci	7	1.24	5.8	2.79	0.09	Q	1.37	0.21	N
Nitzschia supralitorea Lange-Bertalot	NIsupral	34	3.52	21.9	2.81	0.22	QI	1.47	0.24	Q
Nitzschia tubicola Grunow ex Cleve et Grunow	NItubico	10	1.32	8.7	2.92	0.22	I	1.54	0.25	I
Pinnularia obscura Krasske	PIobscur	9	1.04	8.3	2.73	0.15	Q	1.39	0.23	N
Pinnularia subcapitata Gregory	PIsubcap	9	1.04	8.0	2.55	0.17	Q	1.22	0.25	N
Pinnularia viridis (Nitzsch) Ehrenberg	PIviridi	9	0.91	8.3	2.79	0.28	N	1.56	0.35	I
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	PLfreque	58	4.58	36.9	2.87	0.19	Q	1.53	0.26	Q
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	PLlanceo	18	1.49	14.4	2.95	0.20	I	1.54	0.27	I
Planothidium lanceolatum var. omissum (Reimer) Andresen, Stoermer et Kreis	PLlanceo	18	1.49	14.4	2.95	0.20	I	1.54	0.27	I
Planothidium rostratum (Østrup) Lange-Bertalot	PLrostra	18	4.04	10.8	2.80	0.17	Q	1.61	0.25	I
Planothidium stewartii (Patrick) Lange-Bertalot	PLstewar	9	1.44	7.1	2.75	0.25	N	1.44	0.28	I
Platessa conspicua (Mayer) Lange-Bertalot	PVconspi	7	1.01	6.2	2.65	0.17	N	1.29	0.12	N
Psammothidium bioretii (Germain) Bukhtiyarova et Round	PSbioret	24	1.91	18.9	2.62	0.20	N	1.28	0.32	N
Psammothidium chlidanos (Hohn et Hellerman) Lange-Bertalot	PSchlida	30	1.47	25.3	2.67	0.27	N	1.29	0.29	N

Table A.1 Continued

Taxon Name	Code	N	Max	Hill's N2	TN opt	TN tol	TN GLM	TP opt	TP tol	TP GLM
Psammothidium grischunum fo. daonensis (Lange-Bertalot ex Lange-Bertalot et Krammer) Bukhtiyarova et Round	PSgridao	15	1.10	13.3	2.69	0.24	N	1.26	0.20	Q
Psammothidium grischunum fo. daonensis (Lange-Bertalot ex Lange-Bertalot et Krammer) Bukhtiyarova et Round	PSgridao	15	1.10	13.3	2.69	0.24	N	1.26	0.20	Q
Psammothidium marginulatum (Grunow) Bukhtiyarova et Round	PSmargin	16	1.09	14.4	2.57	0.27	D	1.18	0.30	D
Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round	PSsubato	50	3.45	30.4	2.76	0.23	I	1.41	0.29	I
Psammothidium ventralis (Krasske) Bukhtiyarova et Round	PSventra	10	1.84	7.2	2.78	0.26	I	1.37	0.26	N
Pseudostaurosira brevistriata (Grunow) Williams et Round Round	PTbrevis	28	1.87	22.2	2.62	0.23	N	1.21	0.28	D
Pseudostaurosira parasitica (Smith) Morales	PTparasi	7	0.72	6.7	2.77	0.29	N	1.33	0.18	Q
Reimeria sinuata (Gregory) Kociolek et Stoermer	REsinuat	83	5.56	50.5	2.79	0.20	Q	1.44	0.27	Q
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	ROabbrev	30	5.52	15.9	2.90	0.15	Q	1.60	0.20	Q
Rossithidium linearis (Smith) Round et Bukhtiyarova	RMlinear	36	4.21	19.5	2.63	0.20	N	1.30	0.25	N
Sellaphora pupula (Kützing) Meresckowsky	SEpupula	32	1.55	27.6	2.74	0.24	I	1.37	0.31	I
Sellaphora seminulum (Grunow) Mann	SEseminu	38	1.99	29.4	2.79	0.27	I	1.39	0.28	I

Table A.1 Continued

Taxon Name	Code	N	Max	Hill's N2	TN opt	TN tol	TN GLM	TP opt	TP tol	TP GLM
Stauroforma exiguiformis (Lange-Bertalot) Flower, Jones et Round	SQexigui	12	1.99	8.9	2.77	0.25	N	1.40	0.21	Q
Stauroneis anceps Ehrenberg	SSanceps	10	0.72	9.6	2.73	0.23	N	1.39	0.26	N
Stauroneis kriegeri Patrick	SSkriege	19	2.79	12.7	2.80	0.22	I	1.48	0.23	I
Staurosira construens Ehrenberg	STconstr	15	4.12	7.6	2.71	0.14	Q	1.48	0.40	I
Staurosira construens var. venter (Ehrenberg) Hamilton	STconsve	73	2.75	57.7	2.68	0.23	N	1.34	0.30	I
Staurosira elliptica (Schumann) Williams et Round	STellipt	19	1.77	16.6	2.72	0.23	N	1.30	0.29	N
Staurosirella pinnata (Ehrenberg) Williams et Round	SLpinnat	56	1.80	46.4	2.70	0.22	N	1.35	0.30	N
Surirella amphioxys Smith	SUamphio	18	1.53	15.3	2.83	0.15	Q	1.52	0.23	Q
Surirella angusta Kützing	SUangust	12	0.85	11.2	2.86	0.18	I	1.50	0.19	I
Surirella brebissonii Krammer et Lange-Bertalot	SUbrebis	8	3.55	4.0	3.00	0.20	I	1.64	0.18	I
Surirella minuta Brébisson	SUminuta	9	0.72	8.5	2.91	0.16	I	1.45	0.15	I
Synedra acus Kützing	SYacus	31	3.54	19.5	2.62	0.16	Q	1.23	0.23	N
Synedra delicatissima Smith	SYdelica	7	0.83	6.6	2.56	0.23	D	1.22	0.37	N
Synedra rumpens Kützing	SYrumpen	107	3.42	82.4	2.64	0.23	D	1.25	0.27	D
Synedra ulna (Nitzsch) Ehrenberg	SYulna	119	5.37	70.5	2.62	0.22	Q	1.25	0.28	D
Synedra ulna var. oxyrhynchus (Kützing) Van Heurck	SYulnoxy	9	2.02	6.9	2.50	0.19	D	1.11	0.17	Q
Tabellaria flocculosa (Roth) Kützing	TAfloccu	104	7.32	51.9	2.52	0.19	Q	1.10	0.25	D

Table A.2 Soft algal taxa North American Diatom Ecological Database (NADED) identification numbers (Porter 2008), frequency (N), Hill's N2 measure of diversity (Hill 1973), weighted average optima (opt), and weighted average tolerance (tol) for total phosphorus (TP) and total nitrogen (TN).

Taxon Name	**NADED**	**N**	**N2**	**TP opt**	**TP tol**	**TN opt**	**TN tol**
Cyanobacteria							
Anabaena spp.	803001	13	12.41	1.45	0.26	2.75	0.19
Aphanocapsa elachista West et West	807003	8	7.97	1.17	0.20	2.55	0.27
Aphanothece clathrata West et West		33	31.87	1.29	0.29	2.70	0.25
Calothrix spp.	814002	29	27.53	1.24	0.29	2.59	0.21
Chroococcus minor (Kützing) Nägeli	820014	8	7.91	1.21	0.36	2.64	0.19
Geitlerbactron spp.		23	22.36	1.28	0.27	2.71	0.24
Homoeothrix janthina (Bornet et Flahault) Starmach	852004	99	96.66	1.31	0.27	2.69	0.24
Leptolyngbya spp.	863016	14	13.69	1.26	0.32	2.67	0.21
Lyngbya	860008	23	22.65	1.37	0.20	2.79	0.22
Merismopedia tenuissima Lemmermann	875000	13	12.78	1.28	0.31	2.76	0.21
Phormidium spp.	890025	119	114.68	1.31	0.28	2.69	0.24
Planktothrix prolifica (Gomont) Anagnostidis et Komárek	891003	7	6.68	1.19	0.31	2.73	0.20
Pseudanabaena spp.	897003	5	4.93	1.42	0.17	2.95	0.26
Xenococcus spp.		9	8.96	1.36	0.33	2.86	0.30
Red Algae (Rhodophyta)							
Audouinella spp.	1502001	68	66.19	1.36	0.21	2.74	0.21
Unknown Rhodophyte Florideophycidae (chantransia)	1599006	68	66.19	1.36	0.21	2.74	0.21
Batrachospermum spp.	1505001	26	25.34	1.18	0.27	2.59	0.24
Green Algae (Chlorophyta)							
Ankistrodesmus	261004	13	12.89	1.32	0.35	2.67	0.23
Ankistrodesmus falcatus (Corda) Ralfs	261000	40	38.83	1.30	0.28	2.68	0.24

Table A.2 Continued

Taxon Name	NADED	N	N2	TP opt	TP tol	TN opt	TN tol
Bulbochaete spp.	282000	18	17.50	1.10	0.28	2.54	0.26
Chlamydomonas spp.	298000	9	8.82	1.28	0.24	2.74	0.29
Closterium ehrenbergii Meneghini	316018	7	6.77	1.55	0.16	2.87	0.14
Closterium moniliferum Ehrenberg	316000	11	10.49	1.21	0.22	2.61	0.22
Closterium parvulum Nägeli	316026	11	10.83	1.20	0.19	2.65	0.17
Cosmarium angulosum Brébisson	327022	19	18.44	1.16	0.18	2.58	0.16
Gloeocystis spp.	389004	9	8.91	1.29	0.19	2.66	0.20
Gongrosira spp.	401000	18	17.76	1.32	0.22	2.63	0.18
Microspora spp.	441003	11	10.77	1.54	0.21	2.90	0.23
Mougeotia spp.	444000	31	30.11	1.21	0.29	2.54	0.20
Oedogonium spp.	455000	51	48.93	1.27	0.27	2.66	0.22
Pediastrum tetras (Ehrenberg) Ralfs	467002	13	12.73	1.31	0.39	2.59	0.26
Scenedesmus acuminatus (Lagerheim) Chodat	510006	14	13.74	1.34	0.33	2.74	0.31
Scenedesmus bijuga (Turpin) Lagerheim	510013	9	8.78	1.28	0.26	2.72	0.18
Scenedesmus dimorphus	510000	8	7.87	1.38	0.40	2.56	0.14
Scenedesmus ecornis (Ralfs) Chodat	510002	35	33.78	1.34	0.35	2.67	0.24
Scenedesmus quadricauda (Turpin) Brébisson	510001	13	12.72	1.28	0.33	2.68	0.27
Scenedesmus spinosus Chodat	510005	15	14.57	1.25	0.28	2.70	0.21
Spirogyra spp.	533000	10	9.62	1.24	0.31	2.65	0.22
Staurastrum alternans (Brébisson) Ralfs	538009	7	6.96	1.23	0.25	2.58	0.20
Staurastrum orbiculare (Ehrenberg) Ralfs	538004	6	5.95	1.30	0.34	2.70	0.23
Stigeoclonium spp.	543002	52	50.59	1.28	0.30	2.67	0.25
Tetraedron minimum (Braun) Hansgirg	553002	15	14.70	1.10	0.14	2.57	0.15
Ulothrix spp.	569000	15	14.60	1.18	0.28	2.62	0.22
Zygnema spp.	582000	7	6.86	1.05	0.17	2.43	0.15

Table A.3 Algal taxa with their Maine Stream Tolerance (MST) values and North American Diatom Ecological Database (NADED) identification numbers (Porter 2008).

Taxon Name	**NADED**	**MST**
Cyanobacteria		
Anabaena spp.	803001	53.11
Aphanocapsa elachista West et West	807003	34.38
Aphanothece clathrata West et West		43.10
Calothrix spp.	814002	29.11
Chroococcus minor (Kützing) Nägeli	820014	30.09
Geitlerbactron spp.		39.35
Homoeothrix janthina (Bornet et Flahault) Starmach	852004	43.04
Leptolyngbya spp.	863016	38.27
Lyngbya spp.	860008	48.50
Merismopedia tenuissima Lemmermann	875000	47.59
Phormidium spp.	890025	42.62
Planktothrix prolifica (Gomont) Anagnostidis et Komárek	891003	34.79
Xenococcus spp.		53.40
Diatoms (Bacillariophyceae)		
Achnanthes nollii Bock	2153	26.93
Achnanthes oblongella Østrup	2105	62.17
Achnanthes subhudsonis var. kraeuselii (Cholnoky) Cholnoky	2132	57.01
Achnanthidium affine (Grunow) Czarnecki	1011	64.71
Achnanthidium deflexum (Rabenhorst) Lange-Bertalot et Ruppel	2176	43.50
Achnanthidium exiguum (Grunow) Czarnecki	1024	51.12
Achnanthidium minutissimum (Kützing) Czarnecki	1010	41.16
Achnanthidium rivulare Potapova et Ponader	1036	49.90
Adlafia bryophila (Petersen) Lange-Bertalot	204001	38.22
Adlafia suchlandtii (Hustedt) Lange-Bertalot	204004	29.09
Amphipleura pellucida (Kützing) Kützing	6001	43.83
Amphora copulata (Kützing) Schoeman et Archibald	7075	53.47
Amphora pediculus (Kützing) Grunow	7043	76.18
Aulacoseira alpigena (Grunow) Krammer	10028	43.36
Aulacoseira ambigua (Grunow) Simonsen	10008	34.52
Brachysira brebissonii Ross	18005	16.29
Brachysira microcephala (Grunow) Compère	18013	18.00
Brachysira vitrea (Grunow) Ross	18006	32.00
Caloneis bacillum (Grunow) Cleve	12001	64.95
Cavinula cocconeiformis (Gregory ex Greville) Mann et Stickle	195001	37.50
Chamaepinnularia bremensis (Hustedt) Lange-Bertalot	212002	64.44
Chamaepinnularia mediocris (Krasske) Lange-Bertalot	212005	41.87
Cocconeis pediculus Ehrenberg	16011	72.28
Cocconeis placentula var. euglypta (Ehrenberg) Grunow	16005	58.16
Cocconeis placentula var. lineata (Ehrenberg) Van Heurck	16003	51.52
Craticula molestiformis (Hustedt) Lange-Bertalot	21015	67.22
Craticula submolesta (Hustedt) Lange-Bertalot	21007	26.63
Ctenophora pulchella (Ralfs ex Kützing) Williams et Round	201001	49.74

Table A.3 Continued

Taxon Name	**NADED**	**MST**
Cyclotella bodanica var. lemanica Müller	20034	14.31
Cyclotella meneghiniana Kützing	20007	72.58
Cyclotella michiganiana Skvortzow	20008	27.50
Cymbella affinis Kützing	23073	36.44
Cymbella cistula (Ehrenberg) Kirchner	23005	23.65
Cymbella delicatula Kützing	23072	31.33
Cymbella gracilis (Ehrenberg) Kützing	23117	24.01
Cymbella naviculiformis Auerswald ex Héribaud	23016	51.58
Cymbella subturgidula Krammer	23814	23.61
Cymbella tumida (Brébisson ex Kützing) Van Heurck	23068	57.83
Diatoma mesodon (Ehrenberg) Kützing	27002	9.68
Diatoma moniliformis Kützing	27008	52.82
Diatoma tenuis Agardh	27012	46.79
Diatoma vulgaris Bory	27013	88.74
Discostella pseudostelligera (Hustedt) Houk et Klee	2506002	28.59
Encyonema minutum (Hilse) Mann	110004	50.19
Encyonema prostratum (Berkeley) Kützing	110013	80.90
Encyonema reichardtii (Krammer) Mann	110014	76.13
Encyonema silesiacum (Bleisch) Mann	110005	46.95
Encyonopsis cesatii (Rabenhorst) Krammer	203001	38.01
Encyonopsis microcephala (Grunow) Krammer	203002	34.81
Eucocconeis laevis (Østrup) Lange-Bertalot	187002	29.52
Eunotia arcus var. bidens Grunow	33002	18.93
Eunotia bilunaris (Ehrenberg) Mills	33185	37.57
Eunotia exigua (Brébisson ex Kützing) Rabenhorst	33015	21.71
Eunotia flexuosa Brébisson ex Kützing	33019	16.65
Eunotia implicata Nörpel, Lange-Bertalot et Alles	33168	28.33
Eunotia incisa Smith ex Gregory	33026	24.80
Eunotia minor (Kützing) Grunow	33183	42.43
Eunotia muscicola var. tridentula Nörpel et Lange-Bertalot	33169	1.00
Eunotia naegelii Migula	33036	22.28
Eunotia paludosa Grunow	33083	19.92
Eunotia pectinalis (Müller) Rabenhorst	33039	12.94
Eunotia pectinalis var. undulata (Ralfs) Rabenhorst	33041	24.34
Eunotia rhomboidea Hustedt	33051	15.75
Eunotia subarcuatoides Alles, Nörpel et Lange-Bertalot	33245	28.62
Fragilaria capucina Desmazières	34006	32.90
Fragilaria capucina var. gracilis (Østrup) Hustedt	34098	30.58
Fragilaria sepes Ehrenberg	34212	32.08
Fragilaria tenera (Smith) Lange-Bertalot	34105	31.94
Fragilaria vaucheriae (Kützing) Petersen	34030	44.20
Fragilaria vaucheriae var. capitellata (Grunow) Ross	34082	50.33
Fragilariforma virescens (Ralfs) Williams et Round	192008	23.01
Frustulia amphipleuroides (Grunow) Cleve-Euler	35036	44.46

Table A.3 Continued

Taxon Name	**NADED**	**MST**
Frustulia crassinervia (Brébisson) Lange-Bertalot et Krammer	35024	24.75
Frustulia krammeri Lange-Bertalot et Metzeltin	35039	20.30
Frustulia vulgaris (Thwaites) deToni	35011	51.66
Geissleria acceptata (Hustedt) Lange-Bertalot et Metzeltin	210001	50.83
Geissleria decussis (Hustedt) Lange-Bertalot et Metzeltin	210003	44.52
Gomphonema acuminatum Ehrenberg	37001	26.66
Gomphonema affine Kützing	37002	34.28
Gomphonema angustatum (Kützing) Rabenhorst	37003	57.99
Gomphonema angustum Agardh	37125	37.10
Gomphonema clavatum Ehrenberg	37126	43.55
Gomphonema clevei Fricke	37060	31.28
Gomphonema drutelingense Reichardt	37302	53.99
Gomphonema gracile Ehrenberg emend Van Heurck	37007	21.86
Gomphonema kobayasii Kociolek et Kingston	37197	71.24
Gomphonema micropus Kützing	37168	38.99
Gomphonema minutum (Agardh) Agardh	37178	54.56
Gomphonema olivaceoides Hustedt	37062	70.80
Gomphonema olivaceoides var. hutchinsoniana Patrick	37075	51.56
Gomphonema parvulum (Kützing) Kützing	37010	42.17
Gomphonema pseudotenellum Lange-Bertalot	37172	40.80
Gomphonema pumilum (Grunow) Reichardt et Lange-Bertalot	37096	43.57
Gomphonema pumilum var. rigidum Reichardt et Lange-Bertalot	37194	43.57
Gomphonema rhombicum Fricke	37080	32.06
Gomphonema spp.	37889	15.05
Gomphonema subclavatum (Grunow) Grunow	37029	44.70
Gomphonema truncatum Ehrenberg	37022	36.49
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski	213001	66.82
Karayevia clevei (Grunow) Bukhtiyarova	125001	54.31
Karayevia laterostrata (Hantzsch) Bukhtiyarova	125002	58.98
Karayevia suchlandtii (Hustedt) Bukhtiyarova	125009	53.53
Luticola mutica (Kützing) Mann	130002	62.17
Mayamaea agrestis (Hustedt) Lange-Bertalot	211001	67.77
Mayamaea atomus var. permitis (Hustedt) Lange-Bertalot	211004	65.27
Melosira varians Agardh	44073	66.95
Meridion circulare (Greville) Agardh	45001	46.45
Meridion circulare var. constrictum (Ralfs) Van Heurck	45002	43.73
Navicula angusta Grunow	46002	11.77
Navicula antonii Lange-Bertalot	46893	65.42
Navicula capitatoradiata Germain	46661	68.50
Navicula cryptocephala Kützing	46014	56.30
Navicula cryptotenella Lange-Bertalot ex Krammer et Lange-Bertalot	46527	44.59
Navicula cryptotenelloides Lange-Bertalot	46749	66.01

Table A.3 Continued

Taxon Name	**NADED**	**MST**
Navicula erifuga Lange-Bertalot	46648	66.46
Navicula gregaria Donkin	46023	79.60
Navicula hintzii Lange-Bertalot	93187	42.08
Navicula lanceolata (Agardh) Ehrenberg	46859	77.53
Navicula leptostriata Jörgansen	46095	45.28
Navicula menisculus Schumann	46373	64.26
Navicula minima Grunow	46039	56.98
Navicula notha Wallace	46044	37.39
Navicula radiosa Kützing	46056	41.83
Navicula radiosafallax Lange-Bertalot	93172	36.33
Navicula reichardtiana Lange-Bertalot	46666	55.66
Navicula rhynchocephala Kützing	46154	62.11
Navicula rostellata Kützing	46896	66.11
Navicula schmassmanni Hustedt	46066	45.36
Navicula schroeteri var. escambia Patrick	46394	67.29
Navicula stroemii Hustedt	46812	48.65
Navicula tenelloides Hustedt	46401	75.83
Navicula tripunctata (Müller) Bory	46104	69.44
Navicula trivialis Lange-Bertalot	46774	78.55
Navicula veneta Kützing	46504	79.74
Navicula viridula (Kützing) Kützing emend. Van Heurck	46408	70.67
Navicula viridulacalcis (Hustedt) Lange-Bertalot	46895	58.90
Nitzschia acidoclinata Lange-Bertalot	48347	51.64
Nitzschia amphibia Grunow	48004	79.84
Nitzschia archibaldii Lange-Bertalot	48417	60.05
Nitzschia capitellata Hustedt	48006	75.66
Nitzschia dissipata (Kützing) Grunow	48008	60.87
Nitzschia dissipata var. media (Hantzsch) Grunow	48099	64.73
Nitzschia fonticola Grunow	48011	82.24
Nitzschia frustulum (Kützing) Grunow	48013	50.40
Nitzschia gracilis Hantzsch ex Rabenhorst	48015	46.90
Nitzschia inconspicua Grunow	48122	83.70
Nitzschia lacuum Lange-Bertalot	48377	64.03
Nitzschia liebethruthii Rabenhorst	48156	65.76
Nitzschia linearis (Agardh ex Wm. Smith) Wm. Smith	48023	54.17
Nitzschia palea (Kützing) Smith	48025	60.34
Nitzschia palea var. debilis (Kützing) Grunow	48228	53.37
Nitzschia paleacea Grunow ex Van Heurck	48165	61.33
Nitzschia perminuta (Grunow) Peragallo	48126	56.67
Nitzschia pumila Hustedt	48027	71.18
Nitzschia recta Hantzsch ex Rabenhorst	48029	46.63
Nitzschia supralitorea Lange-Bertalot	48312	65.38
Nitzschia tubicola Grunow ex Cleve et Grunow	48349	81.23
Pinnularia obscura Krasske	52049	47.05

Table A.3 Continued

Taxon Name	**NADED**	**MST**
Pinnularia subcapitata Gregory	52059	19.46
Pinnularia viridis (Nitzsch) Ehrenberg	52071	66.84
Planothidium frequentissimum (Lange-Bertalot) Lange-Bertalot	155017	65.05
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	155003	72.50
Planothidium lanceolatum var. omissum (Reimer) Andresen, Stoermer et Kreis	155024	72.50
Planothidium rostratum (Østrup) Lange-Bertalot	155018	56.59
Planothidium stewartii (Patrick) Lange-Bertalot	155035	40.61
Platessa conspicua (Mayer) Lange-Bertalot	2508001	49.76
Psammothidium bioretii (Germain) Bukhtiyarova et Round	186001	31.23
Psammothidium chlidanos (Hohn et Hellerman) Lange-Bertalot	186011	32.48
Psammothidium grischunum fo. daonensis (Lange-Bertalot ex Lange-Bertalot et Krammer) Bukhtiyarova et Round	186002	30.16
Psammothidium marginulatu (Grunow) Bukhtiyarova et Round	186005	24.64
Psammothidium subatomoides (Hustedt) Bukhtiyarova et Round	186008	42.42
Psammothidium ventralis (Krasske) Bukhtiyarova et Round	186009	53.34
Pseudostaurosira brevistriata (Grunow) Williams et Round Round	73001	32.24
Pseudostaurosira parasitica (Smith) Morales	73010	49.60
Reimeria sinuata (Gregory) Kociolek et Stoermer	55002	60.92
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	57002	83.40
Rossithidium linearis (Smith) Round et Bukhtiyarova	189002	32.00
Sellaphora pupula (Kützing) Meresckowsky	170006	46.84
Sellaphora seminulum (Grunow) Mann	170014	51.46
Stauroforma exiguiformis (Lange-Bertalot) Flower, Jones et Round	193001	48.04
Stauroneis anceps Ehrenberg	62002	40.11
Stauroneis kriegeri Patrick	62008	48.35
Staurosira construens Ehrenberg	172001	46.82
Staurosira construens var. venter (Ehrenberg) Hamilton	172006	41.30
Staurosira elliptica (Schumann) Williams et Round	172007	44.94
Staurosirella pinnata (Ehrenberg) Williams et Round	175005	44.18
Surirella amphioxys Smith	65069	61.47
Surirella angusta Kützing	65002	65.40
Surirella brebissonii Krammer et Lange-Bertalot	65068	100.0
Surirella minuta Brébisson	65048	79.72
Synedra acus Kützing	66042	31.66
Synedra delicatissima Smith	66046	24.65
Synedra rumpens Kützing	66016	34.30
Synedra ulna (Nitzsch) Ehrenberg	66024	34.21
Synedra ulna var. oxyrhynchus (Kützing) Van Heurck	66059	18.33
Tabellaria flocculosa (Roth) Kützing	67004	18.76
Green Algae (Chlorophyta)		
Ankistrodesmus falcatus (Corda) Ralfs	261000	45.73
Ankistrodesmus spp.	261004	47.47
Bulbochaete spp.	282000	25.09

Table A.3 Continued

Taxon Name	**NADED**	**MST**
Chlamydomonas spp.	298000	54.46
Cladophora glomerata (Linnaeus) Kützing	309000	80.00
Cladophora spp.	309001	80.00
Closterium moniliferum Ehrenberg	316000	35.86
Closterium parvulum Nägeli	316026	31.66
Cosmarium angulosum Brébisson	327022	25.97
Gloeocystis spp.	389004	39.59
Gongrosira spp.	401000	37.29
Microspora spp.	441003	59.00
Mougeotia spp.	444000	26.91
Oedogonium spp.	455000	37.60
Pediastrum tetras (Ehrenberg) Ralfs	467002	35.16
Scenedesmus acuminatus (Lagerheim) Chodat	510006	44.01
Scenedesmus bijuga (Turpin) Lagerheim	510013	32.32
Scenedesmus ecornis (Ralfs) Chodat	510002	39.88
Scenedesmus quadricauda (Turpin) Brébisson	510001	46.40
Scenedesmus spinosus Chodat	510005	45.72
Spirogyra spp.	533000	42.56
Staurastrum alternans (Brébisson) Ralfs	538009	25.66
Stigeoclonium spp.	543002	42.63
Tetraedron minimum (Braun) Hansgirg	553002	19.54
Ulothrix spp.	569000	28.94
Zygnema spp.	582000	18.98
Red Algae (Rhodophyta)		
Audouinella spp. / Unknown Rhodophyte Florideophycidae (chantransia)	1502001	47.47
Batrachospermum spp.	1505001	27.56

Table A.4 Sites (n=243) sampled from 1999-2006 with statutory classes and algal discriminant analysis model class predictions. Statutory classes are the goals that were assigned by the Maine State Legislature through a public process. Classes AA and A have the same biological criteria and attain water quality goals if the algal model predicts Class A. Duplicate samples in parentheses. Coordinates are based on the Universal Transverse Mercator (UTM) Zone 19N, North American Datum of 1983, and meters.

Site Name	**Town**	**UTM X**	**UTM Y**	**Statutory Class**	**Class Predicted by Algal Model**							
					1999	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Allagash River - Station 750	Allagash	495511	5214406	A						A (A)		
Aroostook River - Station 118	Masardis	548863	5150222	AA						A		
B Stream - Station 464	Houlton	589029	5109084	B				B				
Barberry Creek - Station 672	South Portland	396507	4830770	C					C	C		
Birch Stream - Station 691	Bangor	515141	4963351	B			NA		NA (NA)			C
Blood Brook - Station 666	Katahdin Iron Works Township	486240	5032372	A					A			
Bond Brook - Station 597	Augusta	437660	4908484	B				C				
Bond Brook - Unnamed Tributary - Station 618	Augusta	435179	4907472	B				NA				
Boyden Stream - Station 822	Perry	650704	4981650	B								A
Bull Branch Sunday River - Station 659	Riley Township	347777	4929568	A					A	A	A	A

Table A.4 Continued

Site Name	Town	UTM X	UTM Y	Statut-ory Class	Class Predicted by Algal Model							
					1999	2000	2001	2002	2003	2004	2005	2006
Capisic Brook - Station 257	Portland	394517	4836092	C					NA	NA		
Card Brook - Station 815	Ellsworth	546217	4931028	B								B
Caribou Stream - Station 935	Caribou	575350	5189996	B						C		
Carlisle Brook - Station 792	Lyman	368772	4813076	B							B	
Carrabassett River - Station 606	Kingfield	407767	4982551	AA				A (A)				
Chandler River - Station 503	Jonesboro	609878	4946870	A						B		
Chase Mills Stream - Station 113	East Machias	629142	4956959	B			A					A
Chase Mills Stream - Station 114	East Machias	629482	4956969	B			A					A
China Lake Outlet Stream - Station 604	Winslow	451171	4930501	B				C				
Collyer Brook - Station 228	Gray	397381	4860664	B							NA	
Coloney Brook - Station 733	Fort Fairfield	591476	5189841	B						NA		
Concord Gully - Station 498	Freeport	411224	4855364	B			NA					
Cove Brook - Station 681	Winterport	510173	4948031	AA					C			

Table A.4 Continued

Site Name	Town	UTM X	UTM Y	Statutory Class	Class Predicted by Algal Model							
					1999	2000	2001	2002	2003	2004	2005	2006
Crooked Brook - Station 510	Corinth	495871	4982477	B			C (C)					C
Crooked River - Station 673	Waterford	359943	4899004	AA					A			
Crooked River - Station 800	Naples	373946	4872825	AA							A	
Cupsuptic River - Station 677	Lower Cupsuptic Township	353880	4988315	AA					A			
Dennys River - Station 740	Dennysville	637126	4973264	AA						A	A	
Dennys River - Station 741	Dennysville	638785	4973372	AA						A	A	
Dickey Brook - Station 688	T17 R05 WELS	548307	5222431	B					C			
East Branch Penobscot River - Station 823	T03 R07 WELS	529231	5078610	AA								A
East Branch Wesserunsett Stream - Station 486	Athens	449186	4978347	B			A			A (A)		
East Branch Wesserunsett Stream - Station 487	Cornville	447369	4973068	B			A					
Fifteenmile Stream - Station 602	Benton	462709	4938939	B				C				

Table A.4 Continued

Site Name	Town	UTM X	UTM Y	Statut-ory Class	Class Predicted by Algal Model							
					1999	2000	2001	2002	2003	2004	2005	2006
Fish Brook - Station 422	Fairfield	448308	4941585	B			NA					
Fish Brook - Station 423	Fairfield	448644	4940219	B			NA					
Fish River - Station 373	Wallagrass	532379	5219142	B						A		
Fish Stream - Station 738	Patten	543234	5093360	B						A		
French Stream - Station 310	Exeter	485589	4977861	B			B					
French Stream - Station 505	Exeter	493300	4981669	B			C					C
Gardner Brook - Station 689	Wade	558078	5178621	B					B	B		
Grand Lake Stream - Station 492	Grand Lake Stream Plantation	596212	5003436	A						A (A)		
Grand Lake Stream - Station 493	Grand Lake Stream Plantation	596456	5003297	A						B		
Great Works River - Station 439	North Berwick	358915	4797479	B					A		A (A)	
Greeley Pond Brook - Station 614	Chelsea	444193	4901656	B				C				
Halfmoon Stream - Station 603	Thorndike	477869	4935934	B				C				

Table A.4 Continued

Site Name	Town	UTM X	UTM Y	Statut-ory Class	Class Predicted by Algal Model							
					1999	2000	2001	2002	2003	2004	2005	2006
Hart Brook - Station 663	Lewiston	403798	4880102	B					NA	NA		
Henderson Brook - Station 557	Littleton	591650	5117808	B	NA							
Henderson Brook - Station 558	Littleton	591971	5117605	B	C							
Hill Brook - Station 555	Houlton	590791	5113978	B	NA							
Hill Brook - Station 556	Houlton	591204	5113925	B	NA							
Jamie's Stream - Station 790	Hallowell	432024	4904439	B							B	
Kenduskeag Stream - Station 145	Kenduskeag	503775	4976074	B								C
Kenduskeag Stream - Station 508	Corinth	494286	4982246	B			C					
Kenduskeag Stream - Station 563	Corinth	495897	4981221	B			B					
Kenduskeag Stream - Station 568	Corinth	502572	4976687	B			B					
Kenduskeag Stream - Station 828	Bangor	517303	4962147	C								C
Kennebago River - Station 685	Rangeley	359134	4982799	AA					A (A)			
Kennebunk River - Station 270	Kennebunk	376360	4806756	B							C	

Table A.4 Continued

Site Name	Town	UTM X	UTM Y	Statutory Class	Class Predicted by Algal Model							
					1999	2000	2001	2002	2003	2004	2005	2006
Kennedy Brook - Station 613	Augusta	437605	4905831	B				NA				
Kennedy Brook - Station 646	Presque Isle	574989	5169818	B						NA		
Limestone Stream - Station 47	Limestone	591520	5193494	C						NA (NA)		
Limestone Stream - Station 732	Limestone	590353	5195508	C						C		
Little Androscoggin River - Station 43	Paris	378864	4896535	C					A (A)			
Little Androscoggin River - Station 660	Paris	379236	4897074	A					A			
Little Androscoggin River - Station 79	Paris	378731	4896440	C					B			
Little Black River - Station 725	Allagash	492629	5219041	A						A		
Little Heald Brook - Station 574	Caratunk	433242	5006267	A			A	A	A			
Little Heald Brook - Station 575	Caratunk	433027	5006451	A			A	A	A			
Little Machias River - Station 735	Ashland	544230	5166642	A						A		
Little Madawaska River - Station 230	Caribou	580376	5193180	B						B		
Little Madawaska River - Station 730	Connor Township	574561	5202221	A						A		

Table A.4 Continued

Site Name	Town	UTM X	UTM Y	Statutory Class	Class Predicted by Algal Model							
					1999	2000	2001	2002	2003	2004	2005	2006
Little Ossippee River - Station 446	Limington	365477	4838662	B							A	
Little Ossippee River - Station 447	Limerick	355608	4834989	B							A (A)	
Little Ossippee River - Station 671	Limerick	354988	4833247	B					A (A)			
Little River - Station 440	Lebanon	350149	4807542	B					A			
Little River - Station 821	Columbia Falls	599653	4949907	A								A
Long Creek - Station 580	South Portland	392849	4832314	C							C	
Long Creek - Station 793	South Portland	392783	4832910	C							C	
Machias River - Station 495	Northfield	604427	4961342	AA								A
Machias River - Station 499	T31 MD BPP	591703	4973262	AA					A (A)			A
Martin Stream - Station 609	Fairfield	446478	4944364	B				B				
Martin Stream - Station 679	Dixmont	486308	4952486	A								B
Martin Stream - Station 755	Dixmont	486208	4952256	A								B
Martin Stream - Station 756	Dixmont	486196	4952282	A								B

Table A.4 Continued

Site Name	**Town**	**UTM X**	**UTM Y**	**Statut-ory Class**	**Class Predicted by Algal Model**							
					1999	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Mattakeunk Stream - Station 661	Winn	554195	5037428	A					A			
Mattawamkeag River - Station 662	Mattawam-keag	555734	5039683	AA					A (A)			
Meduxnekeag River - Station 365	Houlton	590287	5109591	B						B		
Meduxnekeag River - Station 367	Houlton	586370	5106160	B				B		B		
Meduxnekeag River - Station 647	Houlton	590932	5110501	B				B[1]				
Meduxnekeag River - Station 649	Houlton	592276	5114850	B				NA		C		
Merriland River - Station 670	Wells	369655	4802012	A					A		A	
Merrill Brook - Station 350	Newry	350143	4927174	A					A	A (A)	B	A
Merritt Brook - Station 742	Presque Isle	578971	5172286	B						NA		
Middle Branch Pleasant River - Station 667	T05 R09 NWP	494754	5028958	A					A			A
Mile Brook - Station 448	Casco	377493	4874350	B					B			
Moose Brook - Station 466	Houlton	586515	5106799	B	B							

1 – This sample is indeterminate because of taxa richness<15.

Table A.4 Continued

Site Name	**Town**	**UTM X**	**UTM Y**	**Statut-ory Class**	**Class Predicted by Algal Model**							
					1999	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Moose Brook - Station 467	Ludlow	581556	5114196	B	B					B		
Mopang Stream - Station 501	T30 MD BPP	583650	4969101	AA					A			
Mousam River - Station 391	Sanford	361040	4807362	C						B		
Mousam River - Station 739	Sanford	360086	4807919	C						A		
Mt Hope Brook - Station 615	Bangor	521309	4962977	B				C				
Narraguagus River - Station 111	Deblois	577987	4954460	AA								A
Narraguagus River - Station 112	Beddington	573506	4966020	AA					A			A
Narraguagus River - Station 564	Cherryfield	584619	4940098	B			A					
Narraguagus River - Station 565	Cherryfield	585118	4939905	B			A					
Narraguagus River - Station 566	Cherryfield	585050	4939902	B			A					
Narraguagus River - Station 567	Cherryfield	585244	4939501	B			A					A
Nason's Brook - Station 638	Portland	393649	4836146	C					NA	NA		
Nonesuch River - Station 788	Gorham	384804	4830701	B							B	

Table A.4 Continued

Site Name	**Town**	**UTM X**	**UTM Y**	**Statut-ory Class**	**Class Predicted by Algal Model**							
					1999	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
North Branch Meduxnekeag River - Station 780	TC R02 WELS	582757	5133617	A						A		
North Branch Presque Isle Stream - Station 687	Mapleton	569078	5170286	A					C	C		
Passadumkeag River - Station 668	Passadum - keag	535373	5001812	AA					A			
Passagassawakeag River - Station 430	Belfast	492576	4919517	B		B						
Passagassawakeag River - Station 431	Belfast	494027	4920510	B		B						
Penjajawoc Stream - Station 314	Bangor	520389	4964051	B			NA	NA				C
Penjajawoc Stream - Station 315	Bangor	521249	4962887	B				C				NA
Penjajawoc Stream - Station 511	Bangor	519890	4964873	B			NA					C
Penjajawoc Stream - Station 513	Bangor	520604	4963665	B			C					
Perley Brook - Station 727	Fort Kent	531808	5232026	B						C		
Piper Brook - Station 507	Kenduskeag	502662	4975157	B			C					
Piscataqua River - Station 787	Falmouth	395803	4843375	B							NA	

Table A.4 Continued

Site Name	Town	UTM X	UTM Y	Statutory Class	Class Predicted by Algal Model							
					1999	2000	2001	2002	2003	2004	2005	2006
Piscataquis River - Station 135	Sangerville	472665	5002114	C								B
Piscataquis River - Station 152	Dover-Foxcroft	486886	5003638	B			B			C		B
Piscataquis River - Station 559	Guilford	476760	5002485	B			A					
Piscataquis River - Station 561	Sebec	491256	5005891	B			B					
Piscataquis River - Station 83	Abbot	464831	5003901	A			A			A (A)		A
Pleasant River - Station 293	T18 MD BPP	585218	4957797	AA								A
Pleasant River - Station 825	Columbia	596124	4949960	AA								A
Pleasant River - Station 394	Gray	391964	4857661	B	B	B					B	
Pleasant River - Station 544	Windham	385871	4850113	B	C						B	
Pleasant River - Station 548	Windham	386490	4851241	B		B						
Pleasant River - Station 549	Windham	388703	4854829	B	C	C						
Prestile Stream - Station 690	Westfield	582772	5158164	A					NA	NA		
Prestile Stream - Station 734	Easton	584285	5167974	A						NA		

Table A.4 Continued

Site Name	Town	UTM X	UTM Y	Statut-ory Class	Class Predicted by Algal Model							
					1999	2000	2001	2002	2003	2004	2005	2006
Pretty Brook - Station 458	Mars Hill	584121	5155058	B						C		
Rangeley River - Station 136	Rangeley	360031	4980945	B					A			
Rangeley River - Station 678	Rangeley	359947	4981198	A					A			
Riggs Brook - Station 599	Augusta	439465	4909797	B				C				
Rocky Brook - Station 375	Mars Hill	587819	5152613	B						NA		
Royal River - Station 226	Gray	397209	4860014	B							C	
Sabattus River - Station 629	Sabattus	411365	4885014	C					C			
Sabattus River - Station 630	Sabattus	411720	4884076	C					C	C		
Salmon Brook - Station 376	Perham	558029	5191927	B	A							
Salmon Brook - Station 377	Washburn	564613	5183891	B	C							
Salmon Falls River - Station 276	Berwick	348832	4791883	B							A	
Salmon Falls River - Station 52	Berwick	350478	4790631	B							A	
Sanderson Brook - Station 576	Lincoln Plantation	338296	4979990	A			A	A	B			

Table A.4 Continued

Site Name	**Town**	**UTM X**	**UTM Y**	**Statut-ory Class**	**Class Predicted by Algal Model**							
					1999	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
Sanderson Brook - Station 577	Lincoln Plantation	338020	4980039	A			A	A	A			
Sandy River - Station 17	Phillips	392382	4967777	A				A				
Sandy River - Station 572	Farmington	410253	4944564	B				B				
Sandy River - Station 616	Farmington	409425	4945412	B				A				
Sandy River - Station 617	New Sharon	419604	4943336	B				B (B)				
Schoodic Brook - Station 766	Medford	511631	5016082	A								A (A)
Sebec River - Station 827	Milo	501051	5010588	B								A
Seboeis River - Station 737	T06 R07 WELS	528277	5110132	AA						A		
Seboeis Stream - Station 665	Howland	524316	5013088	A					A			A
Shaw Brook - Station 480	Hermon	511927	4958478	B			C					C
Sheepscot River - Station 393	Palermo	464311	4909902	B					A			A
Sheepscot River - Station 74	Whitefield	452608	4896833	AA				A	B	B	B	B (A)
Sheepscot River - Station 826	Whitefield	453359	4897664	AA								A

Table A.4 Continued

Site Name	Town	UTM X	UTM Y	Statut-ory Class	Class Predicted by Algal Model							
					1999	2000	2001	2002	2003	2004	2005	2006
Souadabscook Stream - Station 290	Hampden	509040	4956194	A								B
Souadabscook Stream - Station 291	Hampden	511257	4956013	A								B
South Branch Sandy River - Station 600	Phillips	386757	4967352	A				A				
St John River - Station 368	Madawaska	554960	5244806	C						A		
St John River - Station 8	Fort Kent	529867	5232772	A						A		
Stetson Brook - Station 356	Lewiston	405408	4886796	B						B		
Stroudwater River - Station 789	Gorham	387019	4834539	B							C	
Sucker Brook - Station 657	Hampden	515950	4958097	B					NA			
Sucker Brook - Station 658	Hampden	516200	4957892	B					NA			
Swan Pond Brook Tributary - Station 786	Biddeford	376317	4817360	B							NA	
Ten Mile River - Station 669	Porter	347959	4860416	A					A			
Togus Stream - Station 610	Chelsea	444341	4901824	B				B				

Table A.4 Continued

Site Name	Town	UTM X	UTM Y	Statut-ory Class	Class Predicted by Algal Model							
					1999	2000	2001	2002	2003	2004	2005	2006
Togus Stream - Station 611	Chelsea	444321	4901666	B				A				
Togus Stream - Station 612	Pittston	442130	4898121	B				C				
Trout Brook - Station 302	South Portland	399180	4831930	C					NA	NA		
Unnamed Stream - Station 743	Presque Isle	573406	5171252	B						C		
Unnamed Brook- Station 543	Kibby Township	373453	5022636	A			A	A	A			
Unnamed Brook - Station 551	Kibby Township	373228	5022804	A			A	A	A			
Unnamed Brook - Station 552	Upper Cupsuptic Township	350302	5002677	AA			A	A	A			
Unnamed Brook - Station 554	Upper Cupsuptic Township	350373	5003014	AA			B	A	A			
Wassataquoik Stream - Station 812	T03 R07 WELS	528126	5083427	AA								A
Wesserunsett Stream - Station 488	Cornville	448413	4964893	B			A					
West Branch Mattawamkeag River - Station 736	Moro Plantation	550935	5113299	A						A		

Table A.4 Continued

Site Name	**Town**	**UTM X**	**UTM Y**	**Statut-ory Class**	**Class Predicted by Algal Model**							
					1999	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**
West Branch Nezinscot River - Station 664	Sumner	387488	4908168	A					A			
West Branch Pleasant River - Station 686	Katahdin Iron Works Township	481931	5035402	AA					A			B
West Branch Sheepscot River - Station 268	China	457668	4912874	AA				A	B	B	B	A
West Branch Sheepscot River - Station 550	Whitefield	454067	4899074	AA		B		B	B	A	B	B
West Branch Sheepscot River - Station 553	Windsor	454805	4904601	AA		B						
West Branch Sheepscot River - Station 585	China	462164	4917335	AA		B						
Western Little River - Station 820	Columbia	597082	4950300	AA								A
Whitney Brook - Station 601	Augusta	438936	4908532	B				NA				
Wild River - Station 674	Batchelders Grant TWP	341189	4914967	AA					A	A	A	A
Wilson Stream - Station 34	Wilton	407103	4940492	C							B	

BIOGRAPHY OF THE AUTHOR

Tom Danielson was born in Northampton, Massachusetts and graduated from Amherst Regional High School in Amherst, Massachusetts. He pursued his undergraduate studies at the University of Massachusetts, Amherst where he graduated *magna cum laude* in 1993 with a Bachelor of Science in Wildlife Biology and a Bachelor of Business Administration in Finance. He graduated from Duke University in 1996 with a Master of Environmental Management in Water Resources and a Master of Public Policy. During the summer of 1995, he received a National Network for Environmental Management Studies fellowship and worked with the U.S. Environmental Protection Agency, Wetlands Division in Washington, D.C.

After graduating in 1996, he received a Presidential Management Fellowship and worked as a Biologist and later an Ecologist with the U.S. Environmental Protection Agency, Wetlands Division in Washington, D.C. until 2000. He helped states develop water quality standards for wetlands and coordinated the Biological Assessment of Wetlands Workgroup (BAWWG), which was dedicated to developing tools to evaluate the biological condition of wetlands. In June 2000, he was hired as a Biologist for the Maine Department of Environmental Protection, Biological Monitoring Program where he assesses the biological condition of Maine's streams, rivers, and wetlands. He is responsible for developing new tools to improve the evaluation and management of water quality. He is also responsible for coordinating the Department's development of new aquatic life criteria and nutrient criteria for fresh surface waters.

Tom began his dissertation research in 2003, advised by Dr. Cythia Loftin, while working at the Maine Department of Environmental Protection. His dissertation research focused on developing methods to better evaluate the condition and improve the management of Maine's streams and rivers. The University of Maine's Wildlife Ecology Scholarship Committee awarded Tom the 2010 Horace Bond Award for outstanding graduate research in fisheries or aquatic science. The North American Benthological Society awarded Tom a 2010 General Endowment Award to support his research. Tom is a candidate for the Doctor of Philosophy degree in Ecology and Environmental Science from The University of Maine in May, 2010.