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ECOLOGICAL STRATEGIES OF BENTHIC DIATOMS FOR NUTRIENT COMPETITION

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ABSTRACT

ECOLOGICAL STRATEGIES OF BENTHIC DIATOMS FOR NUTRIENT COMPETITION

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Diatoms are an important component of stream benthic communities and depend on nutrients and light, which are often limiting resources. The effects of different nutrient concentrations on benthic diatoms were examined using field and laboratory experiments and observations in natural habitats. The field mesocosm experiment was conducted to determine growth rates of benthic diatoms in limiting versus saturated nutrient conditions. In this experiment, pristine stream water was supplied continuously to artificial streams, and nutrients were manipulated to assess effects of nitrogen (N) and phosphorus (P) additions on algae in near-natural stream conditions. Many diatoms were able to grow at very low N and P concentrations. Of the 14 most common species the same species grew fastest in low N and low P conditions. No evidence indicated that there were tradeoffs in species abilities (measured as growth rate) to compete for N and P at low resource levels, or that there were any tradeoffs for highest performance in low and high resource conditions.

Intra- and inter-specific competition of clonal populations of *Achnanthidium minutissimum* and two potential competitors (*Cocconeis placentula* var. *lineata* and *Cymbella cistula*) were examined in laboratory experiments. In circular artificial channels, four treatment combinations of

nutrients and light were created. When light was limiting, nutrient addition did not increase density. Biomass (as total cell surface area) of *A. minutissimum* increased when both nutrients and light were added. Intraspecific competitive effect rather than interspecific competitive effects regulated *A. minutissimum* growth rates. *Cocconeis placentula* var. *lineata* grew very slowly in cultures. *Cymbella cistula* grew well in culture, formed stalks, and overgrew *A. minutissimum*. Facilitation by *C. cistula* stalks, providing substratum for colonization, may have countered interspecific competition on *A. minutissimum.*

Diatom species abundance depends on suitability of ecological conditions and species' ability to disperse. A positive correlation was observed between diatom abundance and distribution of diatom species. *Achnanthidium minutissimum* occurred at all sites and had the highest relative abundance. Relative abundance of the most common species in pristine streams, such as *A. minutissimum*, *Achnanthes deflexa*, *Cymbella affinis*, and *Gomphonema angustatum* decreased with increasing nutrients and chloride concentrations, however they persisted in habitats with high human disturbance. Taxa requiring high nutrients were more diverse than were taxa from pristine streams, but even the high-nutrient taxa with widest distributions occurred at low abundance. Some taxa requiring high nutrients grew faster than taxa from pristine streams when streams were anthropogenically enriched, which caused, partial turnover of the taxa in the natural diatom assemblage that was adapted to low nutrients. Evolutionary strategies of persistence of species adapted to low nutrients were evident in their wide-spread regional distribution.

To my husband

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INTRODUCTION

As suggested by Darwin (1859), complexity of biological systems can be observed in many habitats and conditions. Multispecies benthic algal communities present a great opportunity to understand the role individual species play in structuring complex communities. Benthic algae contribute a large portion of primary production in aquatic systems (Parsson *et al.* 1977), attain high biomass (Moss 1968, Hansson 1992) and are a major food source for grazers (Lamberti *et al.* 1989). Since they are the most abundant and diverse algae in periphyton (Round *et al.* 1990), more research is needed on diatom species and periphyton community natural history. My research contributes to the knowledge of algal diversity and species performance as shaped by their response to different nutrient conditions. This species-specific information is needed since complete, unidirectional species turnover is possible with global nutrient contamination (National Research Council 2000). My goal is to understand if morphological characters and species traits are the result of evolutionary adaptations, ecological strategies or both.

Algae have been used in testing many important ecological theories (Patrick 1967, 1968, Porter 1973, Tilman 1976, 1977, Stevenson 1983, Pringle 1990, McCormick and Stevenson 1991). Lotic algae in particular are very diverse and sensitive to environmental changes (Pan *et al.* 1996). The fundamental questions in algal benthic ecology in streams are similar to fundamental questions in general ecology. What abiotic conditions drive benthic community development and species diversity? Are there species-specific interactions that play a major role in structuring benthic communities? How do some species have great ecological success and dominate a community in one environment, while remain rare in another for a long time? Ultimately, similar to the question posed by Hutchinson (1961): why are there so many taxa in the benthos, when there are only a few limiting resources?

Theoretical background and historic perspective

Environmental heterogeneity leads to the evolution of species ecological strategies that are important for survival under different conditions (Southwood 1977). Evolutionary trade-offs in species' ability to maximize performance under different ecological conditions are a part of those ecological strategies (McCormick 1996). A trade-off according to Tilman (1976, 1977, 1982) is the inverse correlation between species' competitive ability and its capacity to maintain populations under suboptimal conditions (Grime 1979, Keddy 1990). Understanding ecological and evolutionary strategies in diatoms will allow predictions in population dynamics and community development at different environmental scales.

Algae depend on nutrients and light for survival. Nitrogen (N) and phosphorus (P) are important nutrients in development of algal communities (Schindler 1974, Hecky and Kilham 1988, Elser *et al.* 1990) and have unique biochemical roles in cell structure and function (Borchardt 1996). Algae increase in density and abundance with an increase of both nutrients (Stevenson and Glover 1993). At very low concentrations of N and P, non-equilibrium competition results in high species diversity and biomass (Bothwell 1988, 1989, Grover 1991). During periods of intense competition for a limiting resource, tradeoffs in species-specific traits like nutrient uptake ability, reproduction rate, grazer resistance, or dispersal ability are hypothesized because the species that are evolutionarily successful will have allocated most resources and energy to the fitness trait that enables species survival. Species that allocate energy and resources to several traits would, theoretically, be unsuccessful (competitively excluded) because of lack of specialization (Tilman 1976, 1977, Stevenson and Pan 1994). Therefore, competition for resources may restrict a species' allocation of resources to sets of coadapted traits that confer fitness during periods of intense competition for a limiting resource (e.g. nutrient, space, or light).

Lotic algae compete for nutrients and there is compelling evidence that flowing water modifies nutrient delivery, perhaps differentially among algal species (Peterson and Stevenson 1992). Algae in streams have access to more nutrients and have greater growth rates than algae in slow moving water (Stevenson and Peterson 1989). Borchardt (1996) reported that aspects of the benthic growth habitat, especially exposure to greater water motion and the formation of mats have ramifications for nutrient uptake, utilization, recycling and competition that are unique to benthic algae. Therefore, accounting for and controlling these conditions may provide a basis for elucidating the mechanisms and consequences of flowing water to periphytic algal species performance. Stream benthic algae are adapted to a punctuated supply of resources (Biggs 1996).

Algal resource theory and trade-offs (Tilman 1982), have been developed for phytoplankton and have been successful in predicting competitive interactions and successional trajectories among phytoplankton species in lakes (Sommer 1985). Resource trade-offs were also proposed for individual species competition for low nutrients in benthic communities (McCormick 1996) and have been tested with diatom periphyton communities in flasks (Stevenson and Pan 1994), green algae in streams (Borchardt 1996) and among marine benthic species (Sommer 1996). Research with benthic algae demands consideration of factors like attachment, scour resistance, irregular nutrient flow, and intensive grazing. Therefore, in many cases results from phytoplankton and chemostat research cannot be applied directly to benthos. Flow acts as a disturbance agent (Biggs 1995), affects algal colonization, dynamics and immigration patterns (Stevenson and Peterson 1989), and enhances metabolic processes (Stevenson 1984). The ecological and evolutionary role of resource competition in benthic algal assemblages has yet to be critically explored (McCormick 1996).

Diatom communities are dynamic structures, where time is an important component in their development. Patrick (1976) related the succession of benthic diatoms on substrata to Clementsian forest community dynamics in which low prostrate species conditioned substrata and were followed by apically attached and stalked species. On the other hand, Borchardt (1996) suggested that benthic communities were more complex, with exposure to greater water motion and the formation of mats having ramifications for nutrient uptake, utilization, recycling, and competition that were unique to benthic algae. Successional community structure and functions inferred by individual species requirements and tolerance to environmental conditions have not been addressed to date. As in plant communities, changes in the quality and quantity of periphytic algae are known to be influenced by autecological and biological conditions (Pickett *et al.* 1987, Stevenson 1996).

The concept of ecological strategies is based on the assumptions that specific traits confer different survival advantages, traits have a significant energy and resource costs, and these traits require evolutionary choices. Thus, species evolve different sets of traits and become specialized to either live in different habitats or use different energy and material resources within a habitat. Two resource-based strategies for survival are often referred to as r- and K- selection strategies (Biggs *et al.* 1998). With K-selection strategies, species specialize to use resources in very low supply, persist after resources have been exploited, and grow slowly but efficiently in low resource environments. Species could theoretically coexist in low resource environments if they refined traits to use one of several potential resources more effectively in a habitat than another species. With r-selection, species grow rapidly, exploit resources, and produce many offspring when resources are in great supply, and then disperse to new environments where resources are abundant as resource supplies at "home" become limiting. These strategies for benthic algae in streams have been hypothesized during the frequent disturbance and re-colonization stages following rains and floods that scour algae off the stream bottom to restart the colonization process with renewed supplies of nutrient resources (McCormick 1996).

Insight into species preferences and tolerances can be gained with assessment of ecological preferences and distribution of algae over geographic space (Kociolek and Stoermer 2001). Individual traits and ecological strategies determine species regional abundance and distribution (Collins and Glenn 1991). Traditionally, ecological inferences have been constrained to dominant algae in streams (Pan *et al.* 1996), while rare taxa ecology and taxonomy remains unclear. In his classification of species as dominant, subordinate, and transient, Grime suggests that ecosystem functions are determined by the traits of the dominant populations (Grime 1998). The core-satellite hypothesis (Hanski 1982) evaluates rare and dominant taxa simultaneously. It addresses similar ecosystem functions as Grime classification, and relates species abundance to regional distribution. Species are divided into core, intermediate and satellite, where the abundance of core (dominant and common) and satellite (rare and sparse) species relates to the environmental characteristics in each habitat. In both classifications the intermediate (subordinate) species are spatially dynamic (Hanski 1982) and function as biological filters during community assembly (Grime 1998) with a potentially important, but unclear function. If species abundance is related to species growth in specific nutrient conditions, species abundant in low nutrient conditions (reference, native conditions) will be replaced at high nutrient condition (anthropogenically altered).

Experimental work

In nature, environmental variability is composed of numerous physicochemical factors and species interactions (Tilman 1982). Many times partitioning only a few variables that contribute to the community response is inconclusive. Experimental research allows the creation of simpler systems, where different factors can be manipulated and environmental components can be partitioned. Results from experimental studies can provide mechanistic explanation of the events in nature. Experimental studies sometimes are hard to generalize (MacArthur 1972), but the benefit of relating specific environmental conditions to populations and communities is invaluable in understanding natural systems. In periphyton, results from experimental manipulation were comparable to results of periphyton growth in natural streams (Kevern *et al.* 1966). Additionally, experiments allow study of the dynamic properties of communities in a timely manner, i.e. processes that take months and years can be shortened in experimental studies.

In phycological studies, experimental manipulation of phosphorous and nitrogen are often chosen (Fairchild *et al.* 1985, Stevenson 1995) because either one or both nutrients are considered limiting in natural streams (Grimm and Fisher 1986, Borchardt 1996). Moreover, the effect of a given nutrient concentration, as expressed through growth rate and abundance, can be a measure of species performance at those conditions (Stevenson 1995). Artificial streams and substrata are used widely, because they help standardize environmental variables such as light and current, when nutrients are manipulated (Stevenson *et al.* 1991, Tuchman 1996).

Working in a simplified system could be beneficial in unveiling and understanding species interactions in diatoms with controlled variables. Algal biodiversity has been shaped by competition for nutrients, and there is concern that global nutrient contamination and eutrophication will change algal species membership. With a closer look at community growth within a nutrient gradient, at optimal light and temperature and without grazers, one can evaluate the dynamics within a population. Size, growth rates and density-dependence are evolutionary attributes that might give advantage in changing nutrient conditions. Experimental evidence (Austin and Austin 1980) has shown that varying intensities of competition modify the ecological response curves of species in vegetation.

Taxonomic perspective

Taxonomic identification of algae presents opportunities to understand systematic entities like species (Mayr 1942) and to relate them to evolutionary and ecological processes. Correct taxonomic identification provides consistency and transferability of ecological inferences (Kociolek and Stoermer 2001). Species size and distribution limits are important in addressing ecological questions. In order to understand periphyton ecology, diversity attributes, like identity and number of species, growth form, and reproduction, have to be taken into consideration. In streams, rapid growth and dispersal abilities have been reported as advantageous strategies in heterogeneous environments (Patrick 1976).

Morphologically outlining species identity requires careful research on diatom species biology. For example, a very common diatom *Achnanthidium minutissimum* (Kützing) Czarnecki has been reported as widespread and abundant in North America (Patrick and Reimer 1966, Villeneuve and Pienitz 1998, Laing *et al.* 1999). In the General Collection at the Academy of Natural Sciences in Philadelphia, there are records from more than 2500 localities in the United States, where at the time of collection, abundance of *Achnanthes minutissima* Kützing was more than 10%. In the subset of slides that I evaluated, the types of water bodies, different habitats, environmental conditions and times of collection varied (Appendix I), but all slides contained *A. minutissimum*. Similarly, wide distribution in variable environments was reported in a quantitative study from 1109 rivers in the U.S. (U.S. Geological Survey, National Water-Quality Assessment Program NAWQA). *A. minutissimum* was reported from 79% of the 2674 samples counted (Potapova and Charles 2003).

In the literature, *A. minutissimum* is reported as indifferent to nutrients in Europe (van Dam *et al.* 1994). This wide distribution and no indicator value may potentially be due to the fact that this morphological entity potentially contains several different ecodemes able to grow in diverse conditions. Ecophenotypical variation in diatoms has been recognized and reported in seasonal sampling (Kociolek and Stoermer 1988), as well as within genus variation in response to nutrient variability (Geissler 1982). Species-specific phylogenetic variation in response to resource gradients has also been reported (Kilham *et al.* 1996).

Taxonomically, research in diatoms has been difficult because of historical approaches with different objectives. Originally, species were described to fulfill species inventory objectives (Ehrenberg 1843, Hustedt 1967, Patrick and Reimer 1966, 1975) rather than as a part of population studies where ecological optima could be differentiated and linked to morphological entity. Part of the uncertainty comes from the original description. Originally, Kützing (1833, 8:578, pl.16, fig. 54) stated only that *Achnanthes mintutissima* could be four times smaller than *Achnanthes exilis* Kützing. In the line drawing, all Kützing showed were girdle views of curved valves on stalks. Later, Lange-Bertalot (in Krammer and Lange-Bertalot 1991), based on observations of Kützing's type material, added characters to the vague original description such as rounded ends, a straight distal raphe and striae that became finer towards the end. Adding more confusion, Lange-Bertalot stated that the lectotypes he had chosen for *A. minutissima* Kützing and *A. microcephala* Kützing (a completely different diatom until that point) were the same. As a result, morphometric data for routine identification are either not strictly defined (*Achnanthes minutissima* 'Sippen'-complex in Krammer and Lange-Bertalot 1991, size length 5-25 µm usually less than 20, width 2.5 to 4 µm) or well defined, but with wider size ranges (Hustedt 1930, Patrick and Reimer 1966, length 5-40 µm).

Achnanthidium minutissimum (the current name) is an example of the need for interdisciplinary research that combines natural history knowledge, observations and experiments to better elucidate taxonomy. Therefore, diatom taxonomy is evolving and changing rapidly as types and original descriptions are tested with currently existing populations from North America (Morales 2001, 2002, 2003 a, b, Manoylov *et al.* 2003, Potapova and Ponader 2004).

Statement of research goals

The goals of my research were to examine the effect of nutrients on benthic diatoms in controlled experimental settings and in nature. Benthic diatoms were identified to the lowest taxonomic level. Diatom abundances and morphologies were hypothesized to be a result of ecological and evolutionary processes. Species-specific evolutionary adaptations that could help ecological success of one algal species over another were characterized. Those strategies were determined in a flow-through, stream-side, nutrient experiment, tested in the laboratory with a culture-competition study, and confirmed with a study of regional distribution of diatoms in natural streams. In each of the three studies, I concentrated on the species that performed well in the flow-through experiment, which included many algal species from the region.

In my first study (Chapter 1), different species were expected to be best adapted to live in low N and low P concentrations because of specializations for sequestering those nutrients, when N and P are in low supply. I also intended to determine whether trade-off strategies existed among different adaptive traits and whether species traits were complementary in competition for nutrients. The most important variables in the development of the benthic community are still unclear. Finding flowing water with known nutrients, constant light, and no grazers is impossible in nature. I independently studied either N or P gradients, when the other nutrient was at saturated concentration. This approach is a novel one because conditions are usually characterized broadly as oligotrophic, mesotrophic or eutrophic.

Different species were expected to have highest performance (high immigration, growth and reproduction rates, low death rates, and high resistance to sloughing) in low N and low P environments. These species-specific traits were hypothesized to lead to significant differences in community structure in low N environment than in low P environment. Where nutrients in low supply are the prime determinant of algal species selection, interspecific competition is the most significant factor shaping algal communities (Stevenson and Pan 1994). The goals of the first study were to determine whether trade-off strategies existed among different adaptive traits and whether species traits were complementary in competition for nutrients, i.e. to assess growth rates at low N and low P conditions compared to growth rates in low vs. high conditions of the same nutrient.

During the artificial stream experiment, I developed an additional study non-clonal single species populations were compared in different environments and morphological differences were observed and the results have been published (Manoylov and Stevenson 2004). As a result, I decided to study clonal diatom population growth in different environmental conditions and to address biotic interactions. In the second project (Chapter 2), the effects of different nutrient conditions on growth rates of genetically identical populations were studied. Intra- and interspecific competition in four culture combinations under different conditions of variable nutrients and light were assessed. Reduction in competitive interactions was expected with resource availability. For benthic algae, intra- and interspecific competition indices have not been determined in cultures under different nutrient conditions.

Diatom community composition in natural streams with different nutrient conditions were evaluated and related to their abundance and distribution within an ecoregion. In the survey study (Chapter 3), algae growing in pristine streams were compared with algal communities growing where human influence was large. The flow-through, culture and natural streams were limited to the algal species from Kentucky (Mid-Atlantic Eco-region).

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CHAPTER 1

RESOURCE TRADEOFFS AND ADAPTIVE STRATEGIES OF DIATOMS IN DIFFERENT NUTRIENT CONDITIONS

INTRODUCTION

Competition for nutrients is widely considered an important process structuring phytoplankton communities (Tilman 1982, Sommer 1990) and has been generalized to attached communities in the periphyton (McCormick 1996). Even though the factors controlling development of benthic algal communities have been studied for many years (Patrick 1976, Hill and Knight 1988, Blinn 1993, Stevenson 1996, 1997), little experimental work has been conducted to evaluate the role of competition for nutrients in the evolution of benthic diatoms and their growth in streams (Biggs 1996).

Phosphorous and nitrogen are essential nutrients for algal cell growth (Borchardt 1996). Physiologically, algal cells require fixed amounts of nutrients to reproduce (Hecky and Kilham 1988). Resource limitation can constrain algal growth in streams (Grover 1990, 2000, McCormick 1996) and algal competition for nitrogen and phosphorus can increase with algal density on substrata (Stevenson 1990). Concentrations greater than 28 μg P/L (Bothwell 1989) and 55 μg NO_3-N/L (Grimm and Fisher 1986, Stevenson 1995) have been assumed to be saturating concentrations for achieving and maintaining maximum growth. Therefore, in nutrient limited habitats, co-adaptive traits such as species growth rates, nutrient uptake, growth forms, dispersal rates and motility on substrata may be evolutionary important.

Species fitness is conferred in part by "species performance" (*sensu* Pickett *et al.* 1987). Algal performance in low nutrients has been related to different characters, such as size (Round *et al.* 1990), growth forms (Hudon and Legendre 1987, Stevenson *et al.* 1991, Stevenson and Pan 1994), reproduction rate, and immigration rate (Stevenson and Peterson 1991). During periods of intense competition for a limiting resource, tradeoffs are hypothesized because diatom species that allocate energy and resources to several traits would, theoretically, be competitively excluded (Tilman 1982, Grace 1990, Stevenson and Pan 1994, Stevenson 1995). Species growth rates decrease as benthic diatom density increases, which may be caused by competition for nutrients (Stevenson 1983, 1990, Humphrey and Stevenson 1992). Thus, competition for nutrients may be greatest during later stages of benthic community development (Stevenson *et al.* 1991), which coincides with development of morphological adaptations, such as motility and stalk formation.

The concept of ecological strategies is based on the assumptions that specific traits confer different survival advantages, traits have significant energy and resource costs, and these traits require evolutionary choices (Schluter and Ricklefs 1993). Thus, species could evolve different sets of evolutionary traits and specialization. For algae as for other organisms, two resource-based strategies for survival are often identified and referred to as r- and K- selection strategies (MacArthur and Wilson 1967). These strategies have been called: competitive (C-), stress tolerant (S-), and ruderal (R-) strategies by Grime (1979) and for aquatic organisms Categories I, II and III (Reynolds 1984), but regardless of the terminology, those evolutionary strategies are consistent in the prediction of tradeoffs between fast and slow reproduction with high and low nutrient requirements, respectively (Grace 1995). Adaptive strategies have been directly applied to algae (Margalef 1978, Kilham and Kilham 1980, Reynolds 1988, Stevenson *et al.* 1991, Biggs *et al.* 1998). With r-selection, species grow rapidly, exploit resources, produce many offspring, and then disperse to new environments where resources are abundant as resource supplies become limiting at the original habitat (Biggs *et al.* 1998). These r-selection strategies for benthic algae in streams have been hypothesized during frequent disturbance and re-colonization stages (Reynolds 1988, Biggs *et al.* 1998). K-selection strategies allow species to utilize resources in very low supply (Pianka 1970). These species persist after resources have been exploited and grow slowly but efficiently in very low resource environments (McCormick 1989). Species could theoretically coexist in low resource environments if they possess traits to use one of several potential resources more effectively in a habitat than another species (Whittaker 1975, Stevenson and Pan 1994). Experimental confirmation of those relationships with periphyton is needed.

In this study, resource tradeoffs and adaptive strategies of benthic diatoms were addressed by relating diatom species performance in different nutrient conditions. Multispecies competition experiments conducted with diatoms to date (Humphrey and Stevenson 1992, Lamberti and Steinman 1993, McCormick 1996) have not manipulated nutrients along two gradients and removed grazers. In this study, natural stream-like conditions were provided, a diverse species pool was introduced, and nutrient supply was reduced to limiting concentrations of both nitrogen and phosphorus through partial recirculation in stream-side channels with a novel design. The first goal of this experiment was to relate responses of individual diatom populations to nitrogen and phosphorous limitation, while the other nutrient was in saturating supply. I hypothesized that different species would be best adapted to live in low N or low P concentrations because of specializations for sequestering one nutrient or the other, when in low supply (Stevenson and Pan 1994). The second goal of this research was to test for tradeoffs for growth in low and high concentrations of the same nutrient. Maximum growth at very low concentrations theoretically is possible only for the good competitors. Species-specific growth rates were compared in low and high nutrient concentrations and in both low and high density.

MATERIAL AND METHODS

Study area

Artificial streams in the University of Louisville research Stream facility at Bernheim Forest, Kentucky were used for the experiment. Bernheim Forest is a 10,000 acre nature preserve in which the entire watershed of Hart's Run, a pristine third order stream, is located. The relatively small 7.5-km^2 catchment of this stream is 97 % deciduous forested and shaded for most of its length. No fertilizers are used in the open fields (Bernheim Forest Foundation public records, 2002). Hart's Run at base flow has 40 L/s discharge and an average channel width of 6 m. Current velocities are stable, when there are no storms and vary from <5 cm/s in pools to >30cm/s in riffles (Humphrey and Stevenson 1992).

The upper reaches of the stream drain predominantly Devonian oil shale, with the lower reaches cutting into dolomitic limestone. The dominant substrate in Hart's Run is siltstone cobble (Bernheim Forest Foundation public records, 2002). There are no public trails close to the stream, and all access roads are gated.

Experimental design

Manipulations were carried out between May 17 and June 3, 1999 in 36 partially re-circulating streams that consisted of closed 3.5 x 1.5 m loops of 10 cm diameter PVC with the top piece cut lengthwise to allow placement of substrata and algal growth (Fig. 1.1). These loops were filled with stream water from Hart's Run. Six water baths were used to control water temperature. Bottoms of each channel were immersed in water baths. Current was generated in each loop, by forcing air into the bottom of one end of the loop through a hose with a Sweet Water™ model S45 Blower. Air bubbles lifted water and produced current velocities of 25 cm/s. Turbulence was minimized by placing flow stabilizers, made from 15, 1.5 cm diameter plastic tubes, parallel to the flow at the head of each channel. Stream water was continuously exchanged in each bath at a high enough rate to maintain temperatures in the re-circulating channels similar to that of the stream.

Fresh steam water was slowly added to each channel at a rate of 7 ml/s. Water was allowed to overflow through a hole drilled in the ends of each channel. This caused complete turnover of water every 4 hrs. Partial re-circulation allowed microorganisms inhabiting unenriched channels to draw nitrogen and phosphorus below those found in the ambient stream water. This method was shown to be effective by Mulholland *et al.* (1991).

Twenty unglazed ceramic tiles (29.2 cm^2) were placed at the downstream end of each channel where turbulence was minimal. Light levels were reduced by placing two layers of greenhouse shade cloth, supported by 1 cm^2 wire mesh screen over the stream channels. This simulated the light regimes in the nearby streams. The shade allowed 12% of incident photosynthetically active radiation (measured with LiCor™ Model Li 189 light meter) to reach the tiles.

A large species pool of benthic algae from a variety of nutrient conditions were added to each stream on May 17 (one day before the start of nutrient addition). This inoculum was produced by scraping rocks from three streams with different nutrient concentrations: Hart's Run was the low nutrient stream where the experiment was run; Wilson Creek located 5 km north of Hart's Run had moderate concentrations of nitrogen and phosphorus (R. J. Stevenson unpublished data); and the Middle Fork of Beargrass Creek, with an urban watershed had high nutrient concentrations (R. J. Stevenson unpublished data). Scrapings from the three streams were combined in a single container and homogenized before 500 ml aliquots were added to each stream. Algal community in the inoculum was evaluated to ensure large species pool availability.

Stock solutions with different concentrations of $NaNO_3$ and KH_2PO_4 were delivered from 1 L containers to each channel using peristaltic pumps (Manostat™ model STD) set at a drip rate of 0.3 ml/min. Nitrogen and phosphorus were added throughout the experiment at a constant rate that would elevate ambient nutrient concentrations by the following amounts: 0, 16, 64, 256, 512 and 1024 (μg N/L) with 50 μg P/L; and 0, 2, 8, 32, 50 and 128 (μg P/L) with 500 μg N/L prior to uptake within the channels. Three replicate channels were used for each treatment. Twelve nutrient treatments were assigned randomly to the 36 channels. Nutrient addition was started on May 18th, with one day of colonization, because I did not want to affect initial immigration and establishment of a diverse community with nutrient treatments. Benthic algae on tiles in channels were sampled on 6 dates approximately 72 hours apart.

Sample collection

Water samples were collected from the natural stream and from each channel on days 1, 3, 6, 8, 12, and 14 with acid-washed 125 ml polypropylene bottles. Water samples were immediately packed on ice and returned to the lab for analysis. Nitrate + nitrite nitrogen, ammonium nitrogen (NH_4-N), and silicate (Si) concentrations were estimated on a Skalar® auto-analyzer according to standard methods (APHA 1998). Since nitrate + nitrite nitrogen consists mainly of nitrate in this stream (R. Schultz unpublished data), I considered this a measure of nitrate nitrogen (NO_3-N). Soluble reactive phosphorus (SRP) was also determined according to standard methods using a Hitachi® U-200 1 spectrophotometer (APHA 1998). Since nutrient uptake within each channel likely modified treatment conditions, NO_3-N and SRP were measured in the analyses against the targeted valued. After determining that the nutrient manipulation produced the targeted concentrations, those concentrations were used as the independent variable in regression models.

Two tiles (not neighboring and not from the upstream end) were collected from each channel on days 3, 6, 10, 13, and 16 of community development and frozen (APHA 1998). Sampling stopped after day 16 because sloughing was possible. Algae on day one (May 18th) were collected from 6 channels and represented the initial assemblage. Samples were immediately frozen and transported to the laboratory and stored in freezer till processing. One channel in the P gradient of the 8P/500N treatment leaked and lost water so was not used in the analyses. For the experiment, there were 181 samples collected and counted.

At the laboratory, algal material was scraped from the upper surface of the thawed tiles into a beaker with a toothbrush and razor blade. Samples were diluted to known volumes with deionized water, homogenized with a Biospec® model m133\1281-0 biohomogenizer, and then sub-sampled with a mechanical pipette while continuously mixing on a magnetic stir plate (subsamples for chlorophyll *a* and algal enumeration). Chlorophyll *a* was determined spectrophotometerically with Spectronic Genesys®2 spectrophotometer after 24 hours extraction in 90% buffered acetone and correction for phaeophytin (APHA 1998). Subsamples for counts were preserved with M3 for algae counts (APHA 1998). For algal enumeration, semi-permanent syrup mounts were prepared with 0.2 to 2.0 ml sample amount on the cover slip and 4 ml Tuft Mounting syrup (Stevenson 1984).

Initially, a minimum of 300 natural algal units (live unicellular, filamentous and colonial algae) were identified and enumerated to the lowest taxonomic level. For diatoms (*Bacillariophyta*) only frustules with a visible, healthy looking chloroplast, assumed to be physiologically active, were enumerated. Counts were performed at 1000X on a Leica DMLB light microscope. The main diatom floras used for identification were Patrick and Reimer (1966, 1975) and Krammer and Lange-Bertalot (1986, 1988, 1991 a, b). Common taxa in these counts were identified. Then counts were continued until cell densities of the 14 common taxa were estimated precisely (Alverson *et al.* 2003). Common taxa for this experiment were defined as taxa found in all treatments. At least 15 live cells were observed for precise estimate of density. Total community and individual taxa densities were calculated (D, cells cm^{-2}). Log-transformed densities were used to calculate growth rates for the whole algal community and for individual taxa. Densities were log-transformed in order to stabilize the variance for correlation and regression analysis.

Data analysis

The experiment was created to evaluate limiting concentrations of N and P, as well as to evaluate tradeoffs for N and P competitive abilities during successive stages of community development. Tradeoffs for growth at different treatments (nutrient concentrations) were assessed. Treatments usually had 3 statistically independent replicates. Treatment effects (between-subject factor) were tested using repeated measures analyses of variance (RM ANOVA). The univariate approach to RM ANOVA takes into account correlation among sampling dates, which can violate the assumption of independence between samples for regular analysis of variance (ANOVA). In RM ANOVA, there is potential variance violation where a circular variance-covariance matrix might not have the same constant value for the variance of the difference between any two levels of the within-subject factor (see Winer *et al.* 1991). In RM ANOVA, all results were adjusted for circularity with Geisser-Greenhouse epsilon (G-G, von Ende 2001) which is very conservative, measures sphericity and ensures adjustment of the F-statistic degrees of freedom (a circular variance-covariance matrix that is transformed to its normalized orthogonal form is spherical). Time effects on nutrients were evaluated as a within-subject factor in RM ANOVA. Interactions of nutrient and time were reported also. Particular time intervals in which the treatment effects were different were evaluated with polynomial profile analysis (univariate ANOVAs for each contrast: linear, quadratic, etc.). The higher order term was considered first and was dropped from the model if not significant.

Tilman (1982) defines successful competitors as species able to utilize effectively the lowest possible concentration of certain nutrient. Based on this definition, N and P species were defined as the fastest growing in low N and P conditions, respectively.

Growth rates (R) were estimated as daily per capita changes in cell density in each channel and for all time combinations using the following equation:

$R = (\ln D_{(t+1)} - \ln D_{(t)})/(T_{t+1} - T_t)$, where D is algal biomass (total density) and T_t, T_{t+1} are consecutive times. Growth rates ($cells.cell^{-1}.d^{-1}$) were calculated for populations of each common species in each channel for each time period. Growth rates for each period of time were designated as $R_{0\text{-}3}$ for the growth change between day 0 and day 3 and similarly for $R_{3\text{-}6}$, $R_{6\text{-}10}$, $R_{10\text{-}13}$, and $R_{13\text{-}16}$. Densities and growth rates were quite variable among channels and through time; the results are presented without transformation or exclusion of outliers. The question of interest for this part of the study was species population statistics, so the patterns of variability were not considered an obstacle, but were checked for statistical violations.

Maximum growth rate (R_{max}) was estimated using two methods. First, growth rates for each time period were independently related to nitrogen and phosphorus gradients with the Monod (1950) equation: $R=R_{max}*S/(K_s+S)$, where R is growth rate for each period, S is nutrient concentration and the parameters were maximum growth rate (Monod estimate, R_{max} (M)) and the half saturation constant (K_s). The Monod curve was fitted using a nonlinear regression procedure (Wilkinson 1989, in Systat® 10 for Windows® XP). R_{max}(M) and K_s estimates for each taxon were determined. Estimates did not account for density dependence. Estimations of R_{max} and K_s with Monod were not possible after day 10, potentially because the inflow nutrient concentrations and the increase in biomass were not at equilibrium. Second, observed R_{max} at high NP (R_{max} O), was estimated for each treatment as the average growth rate in the two highest nutrient concentrations and could be calculated for all 5 time periods. The results from the estimation of R_{max} (M) and R_{max} (O) were used to assess species competitive abilities.

Growth rate in low nutrient concentrations was calculated 3 ways. K_s from the Monod model was one indicator of species performance in low nutrient conditions, but were too variable. In many cases, statistically stable estimates of K_s and its error variance could not be estimated. Average growth rates in the low N and low P control treatments were also used as estimates of species performance in low nutrients. The third method accounted for expected variation in species abilities to perform in the environments that I created. Species growth rates vary greatly with non-nutrient abiotic factors, so accounting for those effects may provide a better estimate of multiple species performances in one specific habitat. For method 3, average growth rates in low N and low P were divided by the maximum growth rate (R_{max}(O)) for that species in high N and P treatments. Bothwell (1989) used a similar method for normalizing species performances to their optimal performance so that he could compare results of multiple experiments conducted throughout the year, when seasonal variation in performance had to be taken into account. Results of tradeoff analyses were compared using the multiple estimates of performance in low and high nutrient concentrations.

All common taxa were used in the tradeoff analyses. A trade-off relates competitive performance for different traits, because traits require energy and resource allocation. Trade-off hypotheses predict negative correlations between species traits. Therefore, I expected a negative correlation between the growth rates of species in low N and low P conditions if tradeoffs exist for gleaning N and P at low concentrations. A negative correlation between species growth rates in low N and low P conditions would indicate that overall, different species were best at growing in low N and low P conditions. I also expected a negative correlation between species growth rates in low and high N or in low and high P conditions if different species were r- and K-selected (i.e. gleaners and opportunists *sensu* Grover 1990). The questions concerning diatom competitive abilities do require identifying the species, but correlations between species growth, rather than the species themselves imply trade-offs. Correlations were calculated with Spearman rank correlation analyses. All statistical analyses were performed with SYSTAT® 10 (SPSS Inc., Wilkinson 1989). Each growth rate was calculated for the following successional stages: initial colonization ($R_{0\text{-}3}$); accrual phase ($R_{3\text{-}6}$); peak biomass ($R_{6\text{-}10}$) and persistence phases ($R_{10\text{-}13}$ and $R_{13\text{-}16}$). Peak biomass was defined as the maximum areal biomass attained by a periphyton community during development (Bothwell 1989).

RESULTS

Physicochemical characteristics

Water temperatures in the re-circulating channels averaged approximately 20°C which was close to temperatures recorded in Hart's Run. In ambient stream water, NO_3-N ranged from 15 to 38 μg N/L and SRP ranged from 2 to 7 μg P/L. Silicate concentrations were consistently high in Hart's Run ranging from 11 to 12 mg Si/L.

Along the N gradient nitrate concentration in the control treatment was from 0 to 33 μg N/L, SRP was always above 25 μg P/L (Appendix 1.1). Nitrate concentration significantly increased with treatment. Ammonium concentrations ranged from 6 to 98 μg N/L. Total nitrogen varied from 91 to 1113 μg N/L. Soluble reactive phosphorus, after uptake, ranged from 18 to 59 μg P/L. The lowest measure of TP was around 30 μg P/L (TP ranged from 29-168 μg P/L). Silicate was high along the N gradient ranging from 8 to 12 mg Si/L.

Along the P gradient, PO_4-P concentration in the control treatment ranged 2 to 5 μg P/L nitrate was always above 200 μg N/L (Appendix 1.2). Nitrate (NO_3-N) concentration, after uptake, stayed high ranging from 112 to 789 μg N/L. Ammonium concentrations ranged from 7 to 98 μg N/L. Total nitrogen was also high, ranging from 228 to 815 μg N/L. SRP ranged from 2 to 110 μg P/L. TP ranged from 4 to 169 μg P/L. Silicate was also high along the P gradient ranging from 7 to 11 mg Si/L.

Appendices 1.1 and 1.2 present detailed changes in nutrient chemistry in the artificial streams with nutrient treatment and time during the experiment.

Community response in N and P gradient

Total community biomass measured as chlorophyll *a* increased along the two gradients (ANOVA, $p<0.0001$, Fig. 1.2). Chlorophyll *a* increased linearly from 1.0 to 4.9 μg chlorophyll *a* cm^{-2} along the N gradient (Fig. 1.2). Along the P gradient, chlorophyll *a* increased asymptotically from 0.9 to about 4.3 μg chlorophyll *a* cm^{-2} (Fig. 1.2).

Diatoms were the dominant algae in all treatments. Visual differences were observed macroscopically in communities among the nutrient treatments. The algal mat was thinner in the low N compared to thicker golden mat in the low

P treatment. During the later stages of community development, periphyton became a darker color in higher nutrient levels of both N and P gradients.

Cell density of the whole community increased with both nutrients (RM ANOVA, for N and P, $p<0.0001$). Density of green algae (*Chlorophyta*) did not differ significantly with increasing N or P (RM ANOVA, $F_{5,238}=1.328$, $p=0.253$ and $F_{4,164}=1.655$, $p=0.163$ respectively). Bluegreen algae (*Cyanophyta*) did not differ significantly in density with increasing N or P also (RM ANOVA, $F_{5,36}=1.089$, $p=0.366$ and $F_{2,12}=0.054$, $p=0.948$ respectively).

Total diatom density increased with N concentration and time (RM ANOVA, $F_{5,12}=4.11$, $p=0.0209$ and $F_{5,60}=328.58$, $p<0.0001$, respectively, Fig. 1.3). Maximum algal density along the N gradient was 2.1×10^6 ($\pm 0.9 \times 10^6$ SE) cells cm^{-2} and occurred in the 1024N/50P treatment (Fig. 1.3). Adjusted for circularity, the treatment x time interaction was marginally significant (RM ANOVA, $F_{25,60}=2.02$, $p=0.0537$) and the positive effect of nutrients on density seemed to increase during the colonization period (Fig. 1.3). Density increased linearly with N concentration (polynomial profile analyses, $F_{5,12}=6.69$, $p=0.0034$).

Total diatom density increased with P concentration and time (RM ANOVA, $F_{5,11}=5.41$, $p=0.0094$ and $F_{5,55}=317.64$, $p<0.0001$, respectively, Fig. 1.3). Maximum algal density along the P gradient was 1.98×10^6 ($\pm 1.1 \times 10^6$ SE) cells cm^{-2} and occurred in the 500N/50P treatment (Fig. 1.3). The treatment x time interaction after adjustment for circularity was significant (RM ANOVA, $F_{25,55}=2.45$, $p=0.0254$) indicating that diatom density increased faster in high than in low P concentrations. Density increased nonlinearly along the P gradient (polynomial profile analyses: quadratic, $F_{5,11}=7.29$, $p=0.0031$).

Diatom community growth rate (Fig. 1.4 a) increased significantly with increasing N concentration (RM ANOVA, $F_{5,12}=6.56$, $p=0.0037$) and decreased significantly with time (RM ANOVA, $F_{4,48}=24.52$, $p<0.0001$). Growth rates ranged from -0.3 to 0.9 (cells $cell^{-1}day^{-1}$). The treatment x time interaction was not significant (RM ANOVA, $F_{20,48}=0.85$, $p=0.5955$). After day 6, growth rates dropped significantly (polynomial profile analysis quadratic, time $R_{3\text{-}6}$, $F_{5,12}=4.21$, $p=0.0193$) and thereafter did not differ with time (Fig. 1.4 a).

Diatom community growth rate (Fig. 1.4 b) increased significantly with increasing P concentration (RM ANOVA, $F_{5,11}=17.64$, $p<0.0001$) and decreased significantly with time (RM ANOVA, $F_{4,44}=30.90$, $p<0.0001$). Growth rates ranged from -0.4 to above 1 (cells $cell^{-1}$ day^{-1}). The treatment x time interaction was not significant (RM ANOVA, $F_{20,44}=1.96$, $p=0.0649$). Growth rates changed significantly along the P gradient also (polynomial profile analysis quadratic, time $R_{3\text{-}6}$, $F_{5,11}=6.24$, $p=0.0056$, Fig. 1.4 b).

Diatom species responses and evidence for tradeoffs

From 138 taxa observed in the artificial streams, 14 taxa had sufficient densities to allow accurate estimation of growth rates for all days and treatment. Those taxa appeared in all treatments in all channels. Of the 14 common taxa assessed, growth rates of 6 were changed significantly ($p<0.05$) by increasing N concentration (Table 1.1). These taxa were *Achnanthes deflexa, Achnanthidium minutissimum*, *Encyonema minutum, Meridion circulare, Nitzschia linearis*, and

N. palea. Growth rates of 8 of the common diatom taxa changed significantly ($p<0.05$) with increasing P concentration: *Achnanthidium minutissimum*, *Encyonema minutum*, *Meridion circulare*, *Nitzschia acicularis*, *N. dissipata*, *N. linearis*, *N. palea* and *Synedra ulna* (Table 1.2). Growth rates of all taxa, except *Achnanthes deflexa* along the P gradient, decreased significantly with time (Tables 1.1 and 1.2). Along both gradients (treatment x time) interactions were not significant.

Evidence for trade-offs in species ability to compete in low N and low P conditions was not observed (Fig. 1.5 a-e), most correlations were positive rather than the expected negative correlations. During the early stages of colonization $R_{0\text{-}3}$, the same species with highest growth rates in low N also had highest growth rates in low P: *Fragilaria crotonensis*, *F. nanana*, and *F. vaucheriae* (growth rates close to 1, Fig. 1.5 a). Species growth rates in low N and P during initial colonization ($R_{0\text{-}3}$) were positively (Spearman $r = 0.70$, $p=0.01$) correlated. During the accrual phase, there was no significant correlation between species growth in low N or low P ($R_{3\text{-}6}$). Growth rates from day 6 to 9 in low N ranged from 0 to 0.9 (attached taxa like *Achnanthidium minutissimum* and *Achnanthes deflexa*, together with motile *Nitzschia acicularis* and *N. dissipata*) grew at similar high growth (0.9), while growth rates in low P concentrations were less than 0.3 for most diatoms (Fig. 1.5 b). As communities developed toward peak biomass, stalk forming *Cymbella affinis* grew as well as or better than all other taxa in both low N and low P (Spearman $r = 0.55$, $p=0.044$, Fig. 1.5 c). Growth rates after peak biomass phase and during the persistence phase ($R_{10\text{-}13}$, $R_{13\text{-}16}$)

were lower and were similar for all taxa (correlation not significant, Fig. 1.5 d-e). For most periods, small attached achnanthoid taxa, rosette forming *Fragilaria* species, and *Nitzschia acicularis* grew faster than the larger stalk-forming *Cymbella* species.

Evidence for tradeoffs did not increase significantly during community development when density and exploitative depletion of nutrients should increase. The highest positive correlation was observed for the initial colonization period (R_{0-3}), and lowest was usually during later stages of colonization (Fig. 1.6 a). In addition, statistically significant negative correlations were not observed at any day. Correlation among fitness traits remained positive for most of the successional periods rather than dropping to significantly negative close to peak biomass; the same species were able to grow fastest at low N and low P concentration. Similar results were observed with correlations of normalized growth rates (Fig.1.6 b). Diatoms were able to utilize minimum available resources and grow close to their maximum.

Maximum species growth rates R_{max} (M) estimated with the Monod equation decreased with time and estimates did not converge for all taxa after R_{6-9} growth period. Along the N gradient, the model estimated lowest maximum growth rate for *Achnanthes deflexa* (0.339) and highest for *Nitzschia palea* (0.848). Along the P gradient, *A. deflexa* and *Meridion circulare*, had the lowest maximum growth rate (0.302) and *N. palea* had highest growth rate (0.879). The same taxa that had the highest growth rates in low N also had the highest growth rates in low P through peak biomass development (Tables 1.3 and 1.4). The correlation between species maximum growth rates in low and low P with the Monod model was either positive or non-significant (Fig. 1.7).

Observed maximum growth rates R_{max} (O) in the highest nutrient treatments were calculated for all successional phases along each of the 2 gradients (Figs. 1.8 and 1.9). Along the N gradient most taxa growing fast in low N grew fast in high N, but the patterns changed with time. A non-significant negative correlation was observed again only before peak biomass (Fig. 1.8 c). As with the Monod estimation tradeoffs were not indicated by different species growing fastest in low P versus high P conditions during any phase of colonization (Figs. 1.9 b-c). During the persistence phases, the patterns along the N and P gradients were very similar (Figs. 1.8 d-e and 1.9 d-e). Negative correlations, when observed were not significant when the correlations coefficients were plotted for all sampling time periods. More often the observed correlations were positive, so no tradeoffs were observed (Fig 1.10).

DISCUSSION

The experimental conditions created in the re-circulating streams were optimal for testing relationships among species performance traits in different nutrients regimes. Along the two nutrient gradients, diatom communities developed under similar light, temperature and flow conditions. No grazers were observed and no disturbance in the nearby stream was recorded. Nutrients were consistently at the desired concentrations in the water column. The planned low nutrient concentrations were achieved. Study of a benthic community dominated by diatoms created in these experiments allowed estimation of diatom growth parameters in response to known variables. Biomass and densities increased with nutrient addition, saturating around 265 μg N L^{-1} along the N gradient and at 32 μg L^{-1} P along the P gradient. These concentrations are close to saturating levels reported in the periphyton literature (Bothwell 1989, Stevenson 1995). Chlorophyll *a* values increased to similar levels along the two gradients. The negative density effect on growth increased with time and density as seen by others (Stevenson 1983, Bothwell 1989, Stevenson *et al.* 1991).

The first goal of this study was to determine diatom population responses .to increasing N and P. The estimated growth parameters were very similar to values reported in the literature (Sommer 1991, Stevenson 1995). The use of observed maximum growth rates provided estimates of individual taxa performances for all time periods, while maximum growth estimated with the Monod model were possible for three sampling periods only. Species like *Fragilaria vaucheriae*, *F. crotonensis* and *Nitzschia acicularis* together with well established eutrophic species (*N. dissipata* and *N. palea*; Van Dam *et al.* 1994) had the highest observed growth rates. The former three taxa are known as early colonizers and fast immigrators in benthic diatom communities (Hoagland *et al.* 1982, Stevenson 1990, Stevenson and Peterson 1989, 1991), but they do not compete well when biomass increases and nutrient supplies are depleted within periphyton assemblages during late succession (Stevenson and Glover 1993). In the literature, *Fragilaria crotonensis* and the motile species *Nitzschia acicularis* and *N. palea* are reported in nutrient-rich, eutrophic conditions (Kelly and

Whitton 1995, van Dam *et al.* 1994). Contrary to this finding, in another experiment (Stevenson and Pan 1994), *Fragilaria crotonensis* grew faster at any treatment, except in saturated P conditions. In my study, fast growing species like *Fragilaria crotonensis* and *F. vaucheriae* were able to grow at rates higher than any reported for diatoms in both low and high nutrients (Stevenson *et al.* 1991, Stevenson and Pan 1994, Stevenson 1995).

Late succession algae like *Achnanthes deflexa*, *Achnanthidium minutissimum*, *Cymbella affinis*, and *C. cistula* grew faster than other taxa after peak biomass (around day 9), when resources were depleted due to shading and uptake within the mat. Those taxa vertically elevated their position in the benthos by attaching to other diatoms or growing on stalks. In variable nutrient conditions, achnanthoid diatoms have been reported to attach to bare substrate (after disturbance, Biggs *et al.* 1998), to other diatoms (Burkholder 1996), or to macroalgal and plant filaments (Burkholder and Wetzel 1989, Burkholder 1996). Elevating strategies have been reported for stalked diatoms (Stevenson and Peterson 1989, McCormick and Stevenson 1991, Stevenson *et al.* 1991). In this study, population performances within the diatom community were due to species–specific abilities to colonize independent of competitive interactions during the initial stages. With increasing periphyton thickness, density-dependent interactions caused changes in community composition.

Competitive abilities have been hypothesized to be related to size in diatom studies without agreement of which size is evolutionary advantageous (Grover 1989). Small centric diatoms were reported to have higher maximal growth rates than larger elongate species in limiting nutrients (Sommer 1981, 1983). In experiments with phytoplankton, larger diatoms performed as better competitors in competition for phosphorus than smaller cells (Tilman *et al.* 1986), but when small cells were excluded in another experiment, differences in competitive performance were observed for two large diatoms *Synedra* sp. and *Fragilaria crotonensis* (Grover 1988). Growth rates and species performances in this experiment were not a function of size. For example: *Fragilaria vaucheriae* (small), *Nitzschia palea* (medium), and *F. crotonensis* (large) and *N. acicularis* (large) grew fast from day 0 to day 3, while the large *Cymbella affinis* and the small *Achnanthes deflexa* grew slower.

The second objective of this study was to test for tradeoffs in competition for low N and low P, or low and high concentration of the same nutrient. No tradeoffs were observed between species abilities to grow fastest either in low N or low P or grow fastest in low or high concentrations of a nutrient. Theory predicts good competitors will be specialists for gathering one resource or another. In addition, good competitors will have faster growth rates than other taxa at low nutrient concentrations (Tilman 1982). Tradeoffs research is oriented towards finding organisms that are superior performers in sequestering sparse resources and who fare poorly when resources are abundant (Velicer *et al.* 1999). The observed positive relationships between species competitive performances in low versus high concentrations of the same nutrient indicate that same species were able to grow fast at very low concentrations of each nutrient. Regardless of the method of estimation, species tradeoffs for nutrient uptake of limiting resources were not observed, due probably to co-adaptive traits for persistence in the stream periphyton.

Many adaptive strategies may enable coexistence of the great diversity of species and morphological adaptations in stream diatom communities. Diatom community composition may be determined by individual species' ability to immigrate (Stevenson 1983), colonize (Korte and Blinn 1989, McCormick and Stevenson 1991, Tilman and Wedin 1991), utilize unsteady supply of nutrients (Bothwell 1988, Tuchman 1996) and to reproduce rapidly (Sommer 1985), while resisting flow. Changes in species composition of benthic diatom assemblages during community development have been hypothesized to result from succession of r- to K-selected taxa (Biggs *et al.* 1998). However, benthic diatom succession may result from non-interactive processes, such as a shift from fast immigrators to fast reproducers (Patrick 1976; Stevenson and Peterson 1989, 1991; McCormick and Stevenson 1991). Immigration and emigration were not tested in this experiment, but were probably not very important during late stages of succession after settling of the initial inoculum. Fresh stream water was dripped into all channels and re-circulated for few hours and Hart's Run water typically has low density of suspended algae.

Numerous reasons may explain the lack of evidence for competitive tradeoffs in benthic diatoms when compared with results from many findings in phytoplankton ecology, which seem to indicate tradeoffs in nutrient uptake abilities (Tilman 1982, Sommer 1990). In streams, disequilibrium conditions rather than equilibrium conditions (as in lakes) might lead to strong species selection. Competition for nutrients, on an evolutionary time scale, increases the efficiency of population performance by development of co-adaptive traits (Tilman 1982, Stevenson and Pan 1994). Perhaps competition for nutrients was sufficiently great historically and generated characteristics such as size differences, stalks and motility, but disequilibrium conditions have relaxed competitive interactions and constraints on periphyton phenotypes. In streams, periphyton adaptation to simultaneous N and P limitation (and/or any other resource limitation) might functionally constrain the expected nutrient tradeoffs because timing of N and P depletion is the same and rapid. Thus, no evidence was found for tradeoffs because species allocate resources to compete for both N or P simultaneously. Alternatively, good competitors for low N may not be common and therefore may not have occurred in the initial algal inoculum for this experiment if streams in this region are predominantly P-limited. Although empty frustules of *Epithemia* and *Rhopalodia* were found in the initial community, these low-N adapted taxa that can have cyanobacteria endosymbionts (DeYoe *et al.* 1992) were not observed alive in the channels.

Relationships between competition for low nutrients and mechanisms of species coexistence are still not resolved in complex benthic algal communities. Although trade-off responses to limiting factors were not observed, it is evident that diatoms have adopted differential and versatile functional approaches for long-term survival in the complex conditions of the benthos. More research is needed to resolve diatom abilities to grow in the understory and/or overstory of the periphyton mat and the evolutionary adaptations that generated those differences. Within mat changes in nutrients during succession might have been a strong evolutionary force in streams. Few traits that may confer adaptive fitness have been investigated extensively and included in rigorously tested models at a community scale. The value and costs of traits like motility and stalks should be included in research, along with other factors like the genetic regulation of physiological rates through rRNA operons (as found in bacteria by Klappenbach *et al.* 2000).

In conclusion, different nutrient concentrations were added to study effects of nitrogen and phosphorus independently by lowering only one nutrient at a time to a limiting concentration, while controlling for light, temperature and grazers. The rate of biomass production (growth rate) in benthic diatoms depends on the supply of limiting nutrients, but maximum growth rates of common diatom taxa were reached at very low nutrient concentrations. Growth rates decrease greatly during community development and with increasing density on substrata. The same diatom species were able to grow at very low N and low P concentrations and differences in performances in response to different concentrations of the same nutrient were not observed.

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Table 1.1: Summary of repeated measures (ANOVA) results of individual taxa growth rates in N treatment when P saturated at 50 $\mu g\ L^{-1}$. S-nutrient effect, T-time effect, and S x T interaction. *-p value significant at $p<0.05$,**-p value significant at $p<0.001$, ***-p value significant at $p<0.0001$, ns- not significant; all p-values corrected with Greenhouse-Geisser Epsilon for circularity.

effects	S	T	SxT
Taxa	$F_{5,11}$	$F_{4,44}$	$F_{20,44}$
Achnanthes deflexa Reimer	3.15 *	3.51 *	1.23 ns
Achnanthidium minutissimum (Kützing) Czar.	9.25 **	4.21 *	1.86 ns
Cymbella affinis Kützing	0.44 ns	14.29 ***	1.18 ns
Cymbella cistula (Ehrenberg) Kirchner	1.09 ns	11.67 ***	0.72 ns
Encyonema minutum (Hilse) Mann	8.56 *	6.24 *	1.60 ns
Fragilaria crotonensis Kitton	3.03 ns	22.23 ***	1.11 ns
Fragilaria nanana Lange-Bertalot	1.22 ns	20.01 ***	1.64 ns
Fragilaria vaucheriae (Kützing) Petersen	2.38 ns	33.68 ***	0.85 ns
Meridion circulare (Grev.) Ag.	6.02 *	23.70 ***	1.46 ns
Nitzschia acicularis (Kütz.) W. Sm.	1.62 ns	39.36 ***	1.51 ns
Nitzschia dissipata (Kütz.) Grun.	1.02 ns	10.50 **	0.96 ns
Nitzschia linearis (Ag. ex W. Sm.) W. Sm.	3.82 *	16.71 ***	1.01 ns
Nitzschia palea (Kütz.) W. Sm.	5.27 *	33.46 ***	1.24 ns
Synedra ulna (Nitz.) Ehr.	2.79 ns	14.18 ***	0.52 ns

Table 1.2: Summary of repeated measures (ANOVA) results of individual taxa growth rates in P treatment when N saturated at 500 $\mu g\ L^{-1}$. S-nutrient effect, T-time effect and S x T interaction. *-p value significant at $p<0.05$, **-p value significant at $p<0.001$, ***-p value significant at $p<0.0001$, ns – not significant; all p-values corrected with Greenhouse-Geisser Epsilon for circularity.

effects	S	T	SxT
Taxa	$F_{5,11}$	$F_{4,44}$	$F_{20,44}$
Achnanthes deflexa Reimer	2.67 ns	0.84 ns	1.62 ns
Achnanthidium minutissimum (Kützing) Czar.	13.91 **	6.94 *	1.56 ns
Cymbella affinis Kützing	3.12 ns	16.87 ***	1.64 ns
Cymbella cistula (Ehrenberg) Kirchner	2.05 ns	8.45 **	0.76 ns
Encyonema minutum (Hilse) Mann	4.03 *	7.17 *	1.02 ns
Fragilaria crotonensis Kitton	2.41 ns	19.79 ***	1.02 ns
Fragilaria nanana Lange-Bertalot	1.07 ns	19.44 ***	1.00 ns
Fragilaria vaucheriae (Kützing) Petersen	0.96 ns	21.44 ***	0.7 ns
Meridion circulare (Grev.) Ag.	7.05 *	13.28 ***	1.12 ns
Nitzschia acicularis (Kütz.) W. Sm.	4.98 *	26.66 **	0.88 ns
Nitzschia dissipata (Kütz.) Grun.	6.41 *	5.47 *	0.8 ns
Nitzschia linearis (Ag. ex W. Sm.) W. Sm.	8.98 *	10.82 **	1.04 ns
Nitzschia palea (Kütz.) W. Sm.	9.84 ***	23.03 ***	2.06 ns
Synedra ulna (Nitz.) Ehr.	7.4 *	16.00 ***	1.32 ns

		R_{03}			R_{36}			R_{610}		
Common taxa	code	R_{max}	K_s	R^2	R_{max}	Ks	R^2	R_{max}	Ks	R^2
Achnanthes deflexa	AD	0.12	-0.006	ns	0.51	-3.9	ns	0.4	22.4	0.46
Achnanthidium minutissimum	AM	0.5	4.8	0.68	0.28	-9.8	ns	0.34	40.5	0.52
Cymbella affinis	CA	0.53	2.7	0.71	0.53	1.5	0.7	0.02	-61.1	ns
Cymbella cistula	CC	0.7	-0.95	0.79	0.24	1.6	ns	0.13	-41.2	ns
Encyonema minutum	EM	0.65	19.4	0.7	0.26	-7.4	ns	0.2	-39.5	ns
Fragilaria capucina	FC	0.88	3.4	0.81	0.44	0.56	0.5	-0.07	-78	ns
Fragilaria nanana	FN	1	0.3	0.83	0.44	298	ns	0.15	-43.5	0.55
Fragilaria vaucheriae	FV	1.4	0.07	0.84	0.25	-6.1	ns	0.15	-29.6	ns
Meridion circulare	MC	0.78	3.2	0.76	0.3	7.8	0.58	-0.08	-87.2	ns
Nitzschia acicularis	NA	1.2	-0.07	0.79	1.1	3.3	0.66	0.04	-49.2	ns
Nitzschia dissipata	ND	0.61	-1.7	0.65	0.73	4.9	0.53	0.51	5.3	0.59
Nitzschia linearis	NL	0.93	-3.4	0.81	0.31	-2.9	ns	0.54	90.6	0.58
Nitzschia palea	NP	1.3	0.72	0.82	0.83	-0.98	0.67	0.43	82.1	0.5
Synedra ulna	SU	0.87	-1.6	0.78	0.14	0.008	ns	-0.48	3782	ns

Table 1.3: Growth parameters estimated with Monod model along N gradient; ns – not significant, R2 < 0.5

Common taxa	R_{03}			R_{36}			R_{610}		
	R_{max}	K_s	R^2	R_{max}	K_s	R^2	R_{max}	K_s	R^2
Achnanthes deflexa	0.22	na	ns	-0.04	na	ns	0.23	na	ns
Achnanthidium minutissimum	0.29	na	ns	0.95	0.89	0.73	0.29	0.78	0.76
Cymbella affinis	0.6	na	0.64	0.43	na	0.89	-0	na	ns
Cymbella cistula	0.57	na	0.5	2.1	23.3	0.62	-0.01	na	ns
Encyonema minutum	0.57	0.1	0.7	0.24	na	0.69	0.42	1.4	0.6
Fragilaria capucina	0.82	0.03	0.85	0.56	0.56	ns	-0.07	1.24	ns
Fragilaria nanana	0.89	0.02	0.74	0.13	na	0.72	0.09	2.6	ns
Fragilaria vaucheriae	1.3	na	0.76	0.69	1.6	0.78	-0.03	2.6	ns
Meridion circulare	0.65	0.06	0.7	0.29	0.16	ns	na	na	na
Nitzschia acicularis	1	na	0.76	1.3	2.1	0.52	na	na	na
Nitzschia dissipata	0.68	0.57	ns	0.39	na	ns	na	na	na
Nitzschia linearis	0.97	0.17	0.83	0.12	na	ns	na	na	na
Nitzschia palea	1.1	0.52	0.71	0.75	na	0.68	na	na	na
Synedra ulna	0.89	0.4	0.68	0.06	na	ns	na	na	na

Table 1.4: Growth parameters estimated with Monod model along P gradient; na – not applicable; ns – not significant, R2 < 0.5

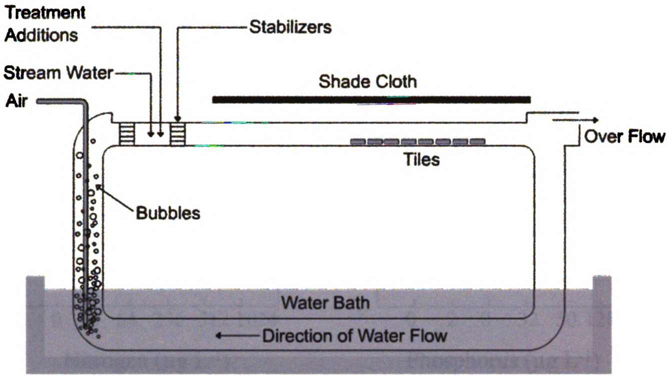

Figure 1.1: Schematic view of each partially re-circulating channel

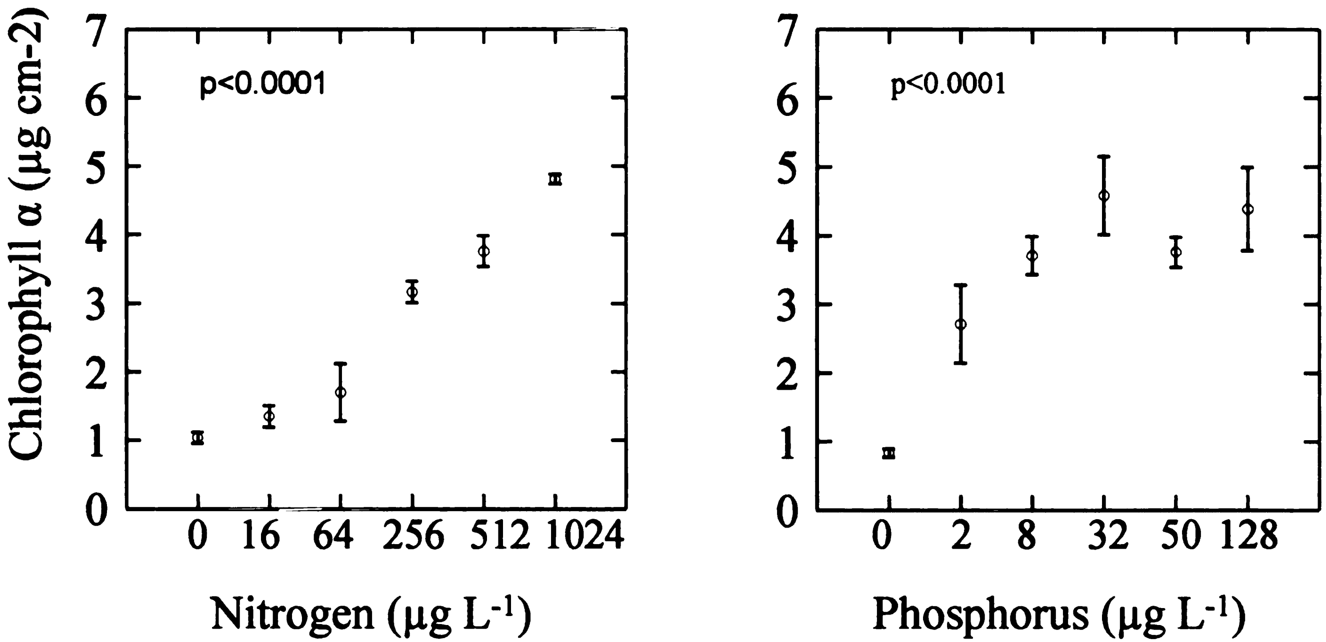

Figure 1.2: Algal biomass (Chlorophyll *a*) change along the a. N gradient; b. P gradient Bars indicate standard error of the treatment means for all times.

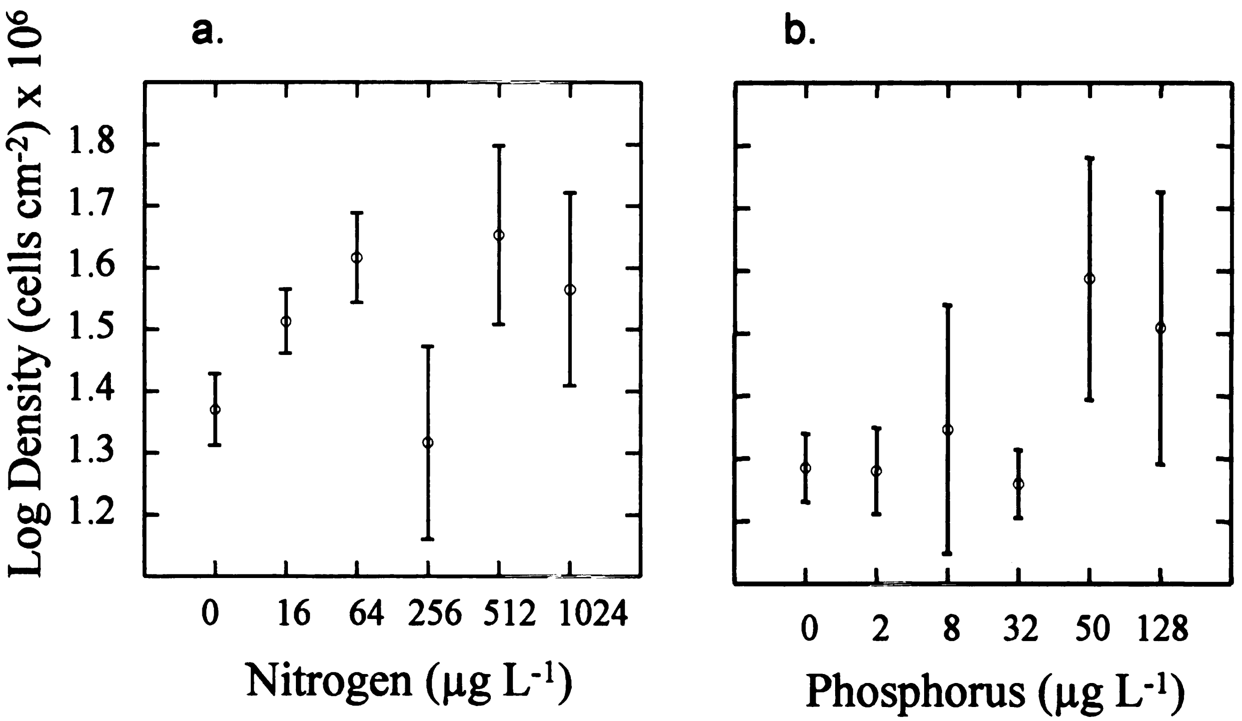

Figure 1.3: Total diatom community density (cells cm2). a. Nitrogen gradient; b. Phosphorus gradient; mean diatom density per treatment; Bars indicate standard error per treatment

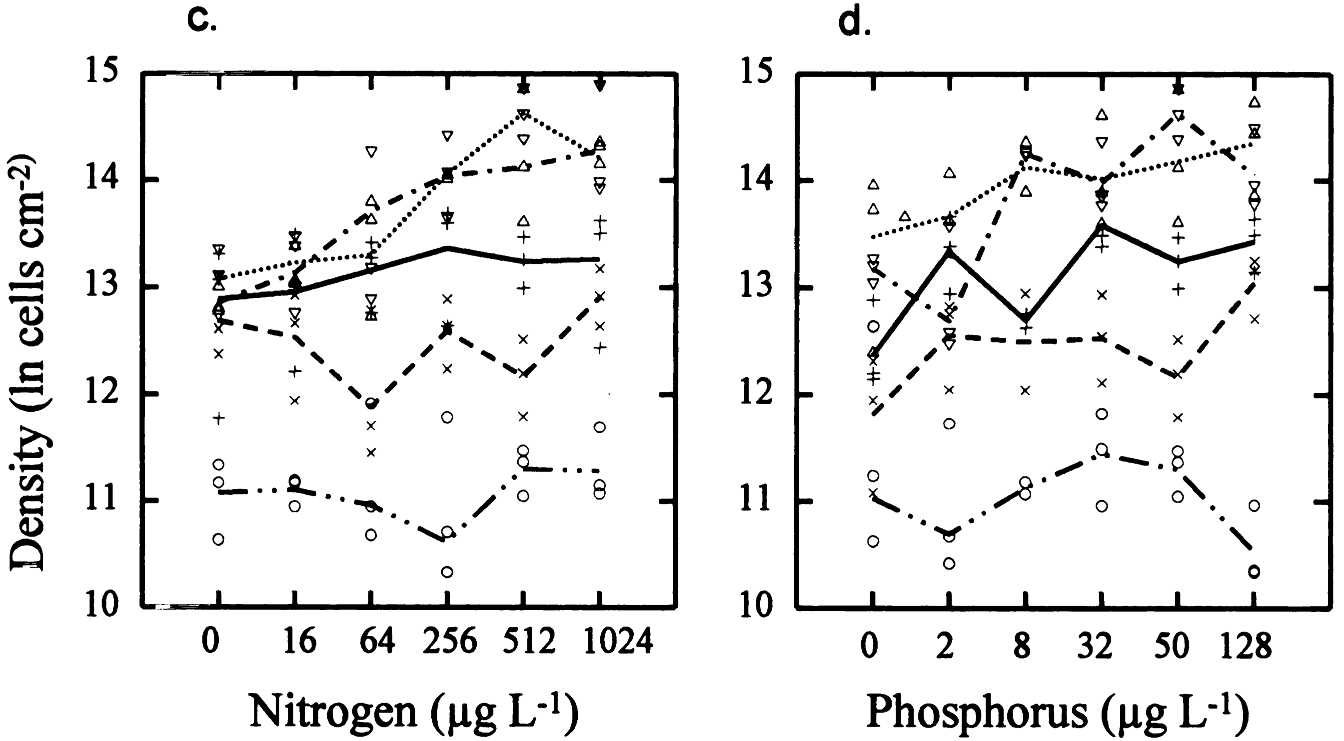

Figure 1.3 (cont'd). Total diatom community density (cells cm2). c. Nitrogen gradient; d. Phosphorus gradient;. c, d. Density with nutrient and time; similar symbols represent replicate days of sampling: D3-circle, D6-x , D10-plus sign, D13-down triangle, D16-up triangle; lines connect the means.

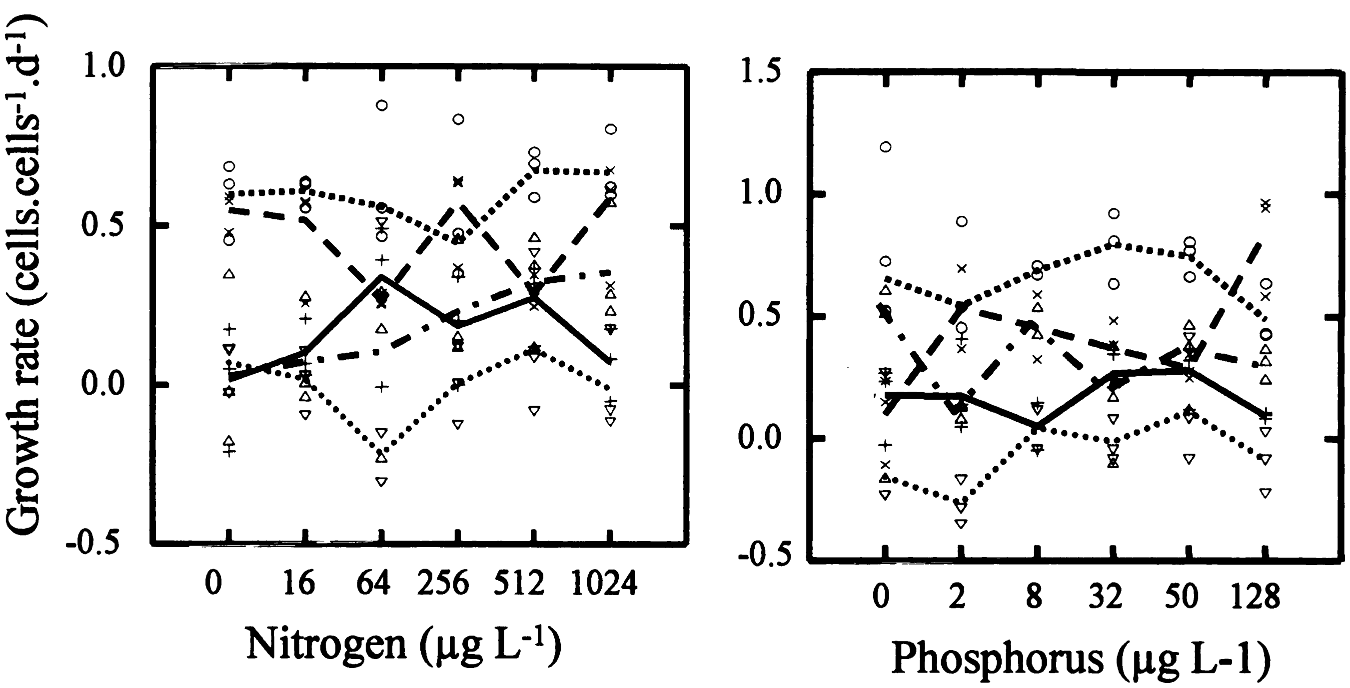

Figure 1.4: Whole community growth rates (cells.cell-1.d-1). a. N gradient, b. P gradient; $R_{0\text{-}3}$-change in growth between day 0 and day 3, small dotted line and circles; $R_{3\text{-}6}$-dashed line and x; $R_{6\text{-}10}$-solid line and plus; $R_{10\text{-}13}$ dash, dot lone and up triangles; $R_{13\text{-}16}$ larger dotted line and down triangles; lines connect the means.

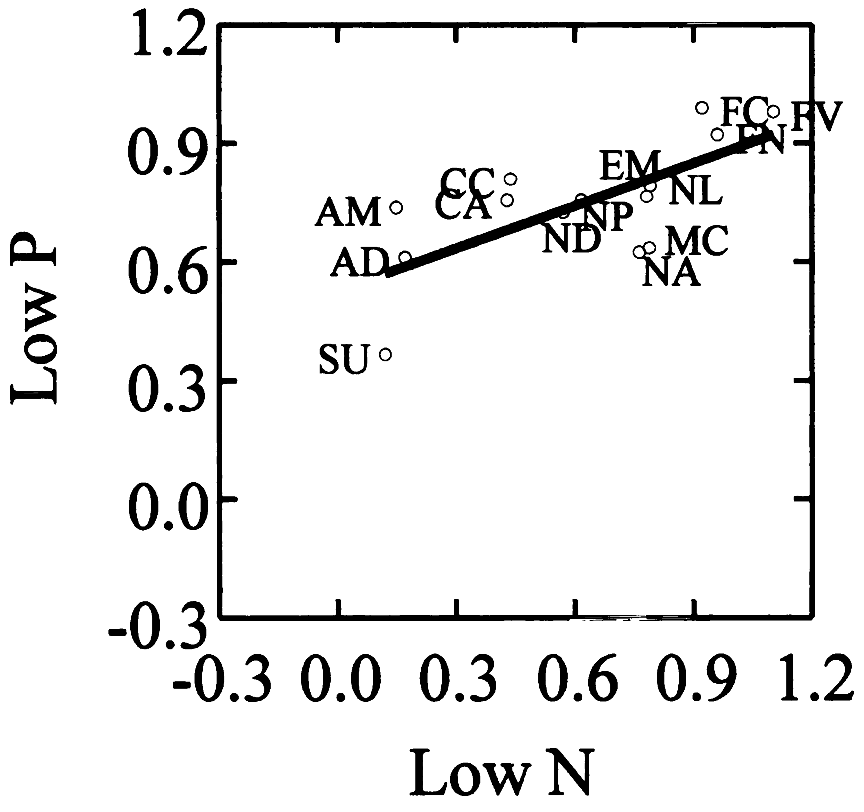

Figure 1.5: Mean growth rates Low N and Low P. a. Time $R_{0\text{-}3}$ r = 0.70 (p=0.01); r - Spearman correlation coefficient; given p values represent significant correlation at 0.05 level (2-tailed), n=14 for all time periods. Taxa abbreviations as in table 1.3.

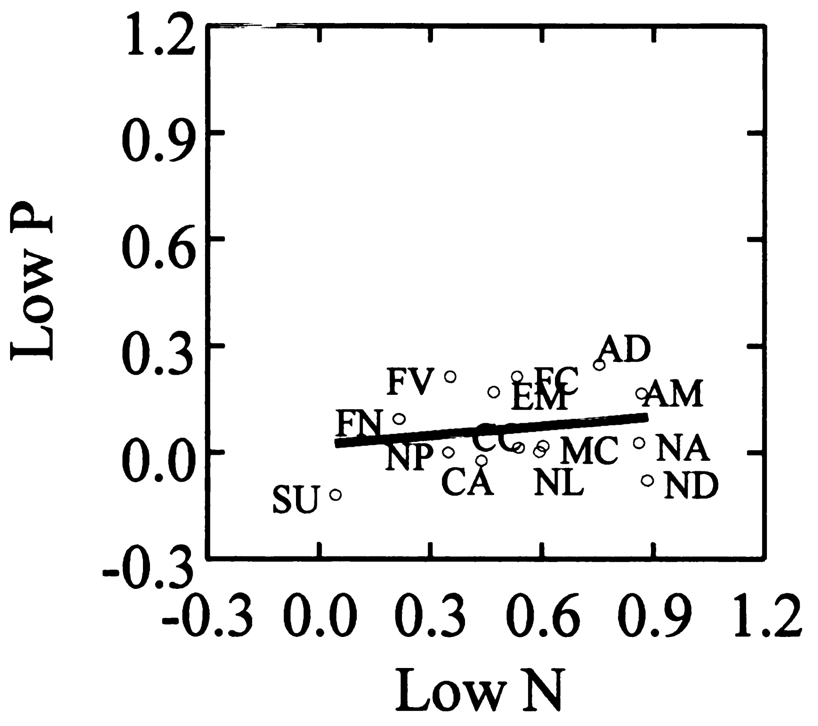

Figure 1.5 (cont'd). Mean growth rates Low N and Low P. b. Time R_{3-6}, r = 0.19 (n.s.); r - Spearman correlation coefficient; given p values represent significant correlation at 0.05 level (2-tailed), n=14 for all time periods. Taxa abbreviations as in table 1.3.

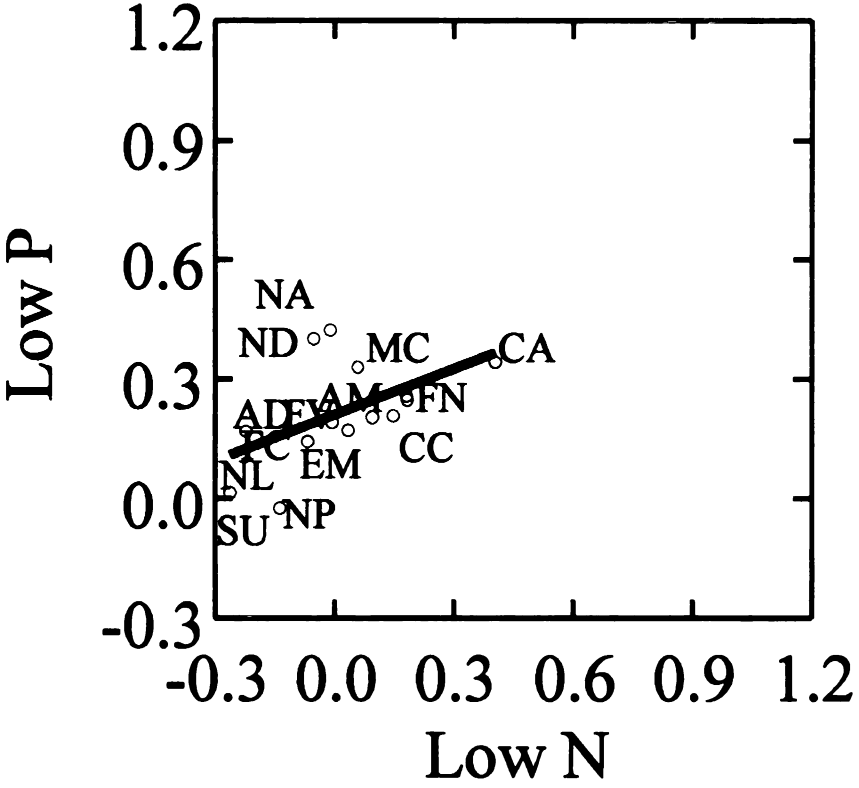

Figure 1.5 (cont'd). Mean growth rates Low N and Low P. c. Time $R_{6\text{-}10}$, r = 0.53 (p=0.044); r - Spearman correlation coefficient; given p values represent significant correlation at 0.05 level (2-tailed), n=14 for all time periods. Taxa abbreviations as in table 1.3.

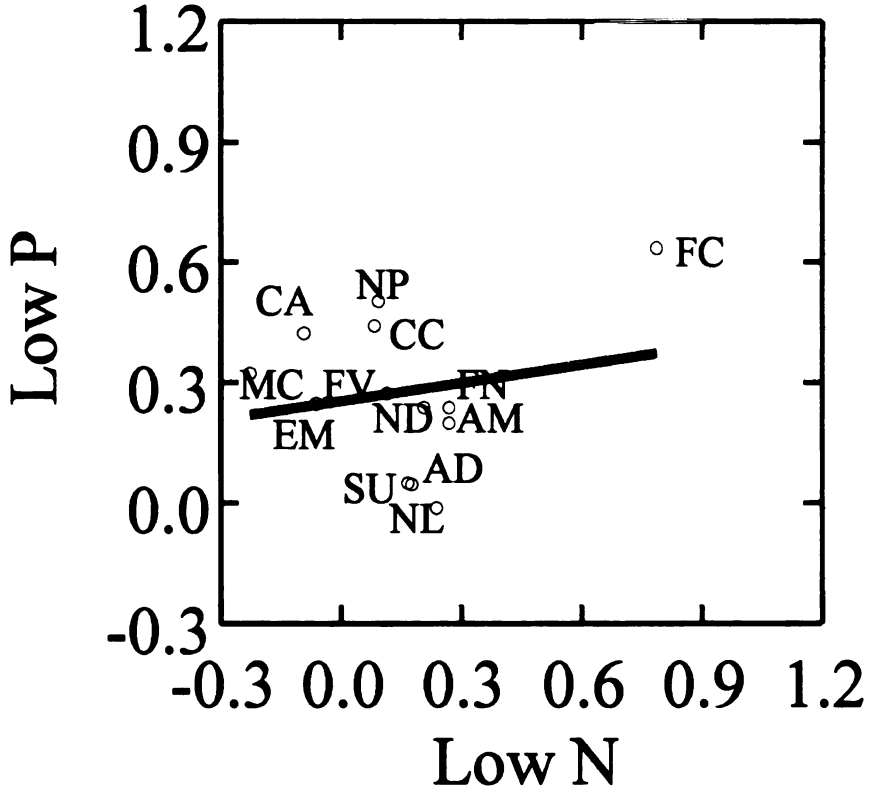

Figure 1.5 (cont'd). Mean growth rates Low N and Low P. d. Time R_{10-13} r = 0.16 (n.s.); r - Spearman correlation coefficient; given p values represent significant correlation at 0.05 level (2-tailed), n=14 for all time periods. Taxa abbreviations as in table 1.3.

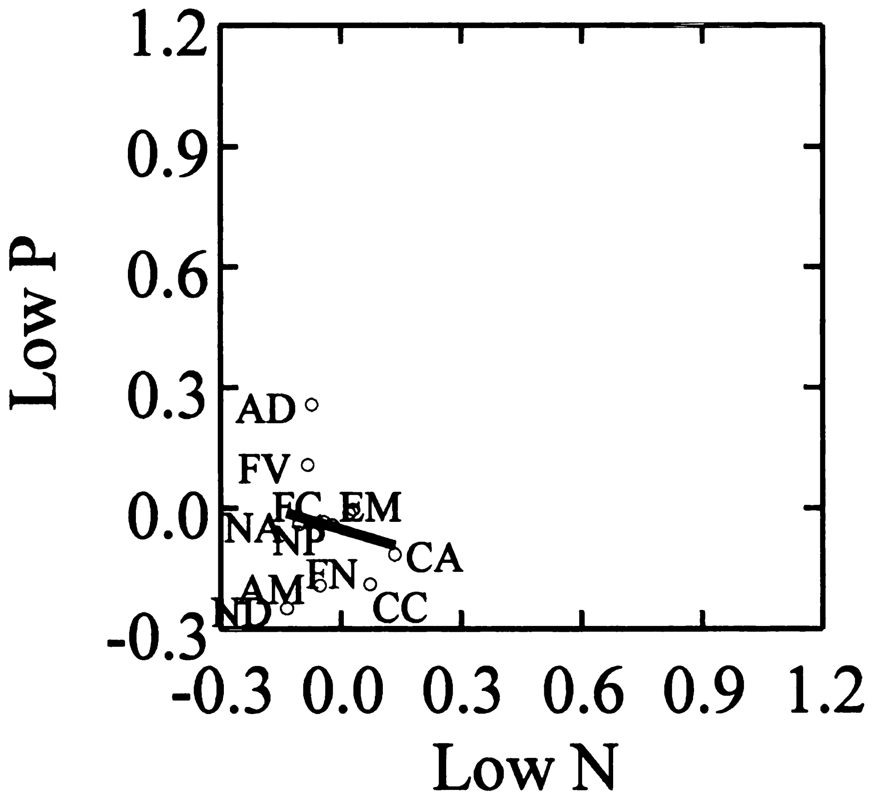

Figure 1.5 (cont'd). Mean growth rates Low N and Low P. e. Time $R_{13\text{-}16}$ r = -0.04 (n.s.); r - Spearman correlation coefficient; given p values represent significant correlation at 0.05 level (2-tailed), n=14 for all time periods. Taxa abbreviations as in table 1.3.

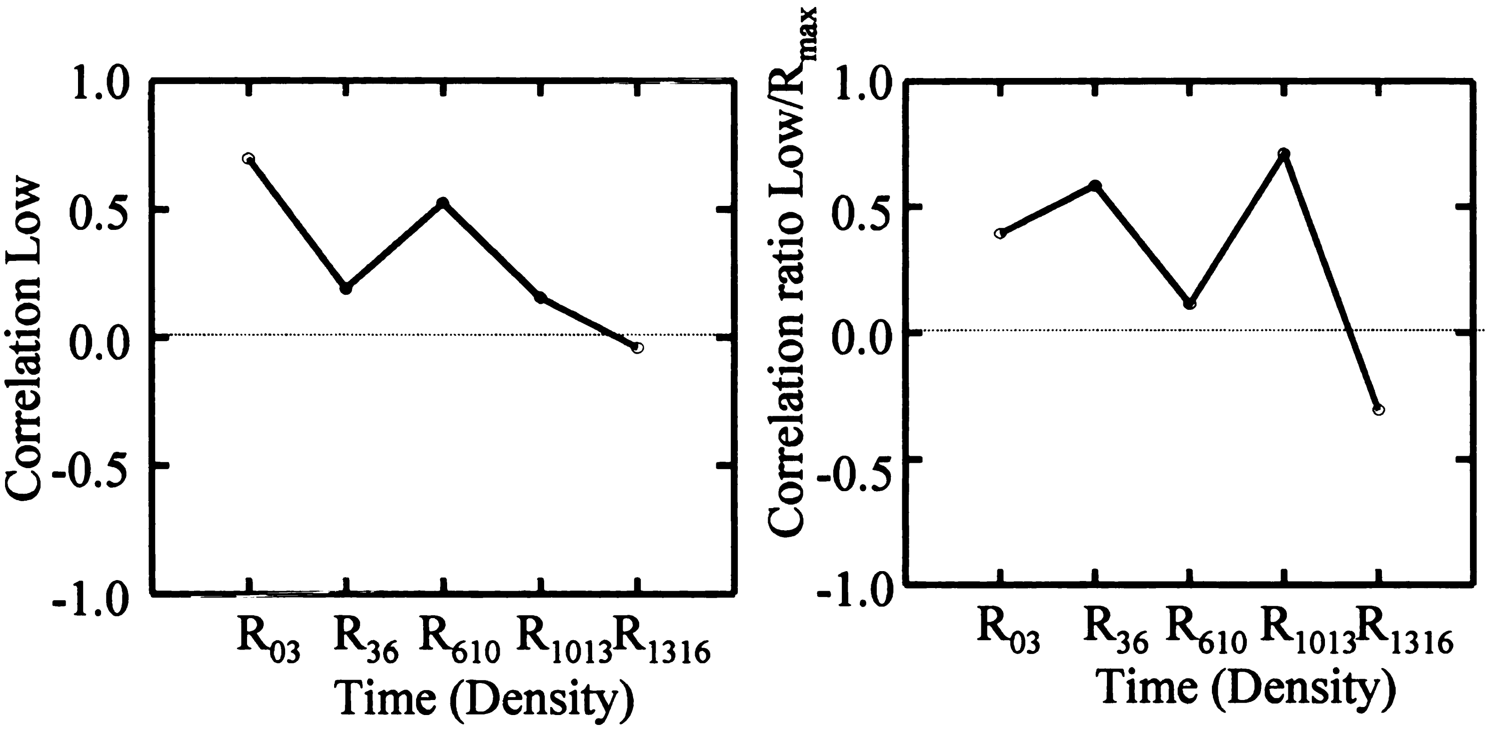

Figure 1.6: Correlation values for performance in limiting nutrient concentration for all 5 sampling periods. a. Low N and Low P. b. Ratios normalized Low N and Low P values with growth at preferable condition. Dotted line separates positive and negative values.

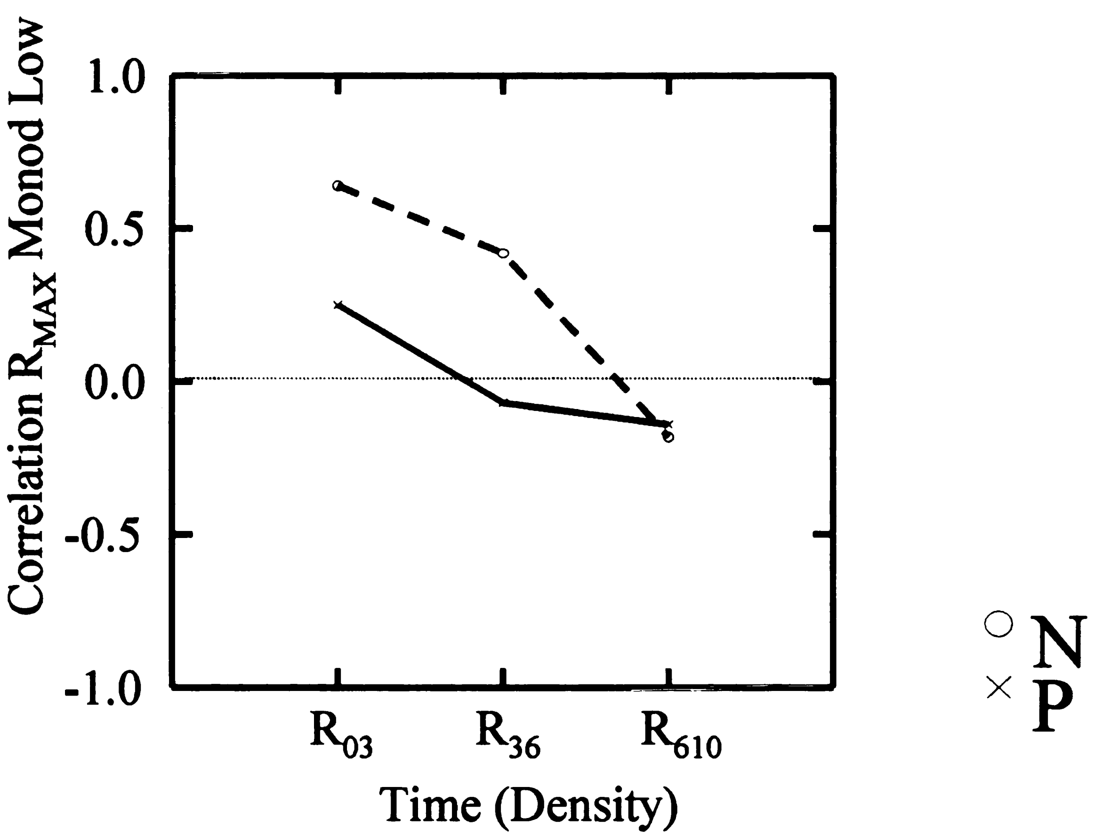

Figure 1.7: Correlation values for maximum growth estimated with the Monod equation in both gradients for all the 3 sampling times prior to peak biomass. Tradeoffs measure for N (o) and P (x). Dotted line separates positive and negative values.

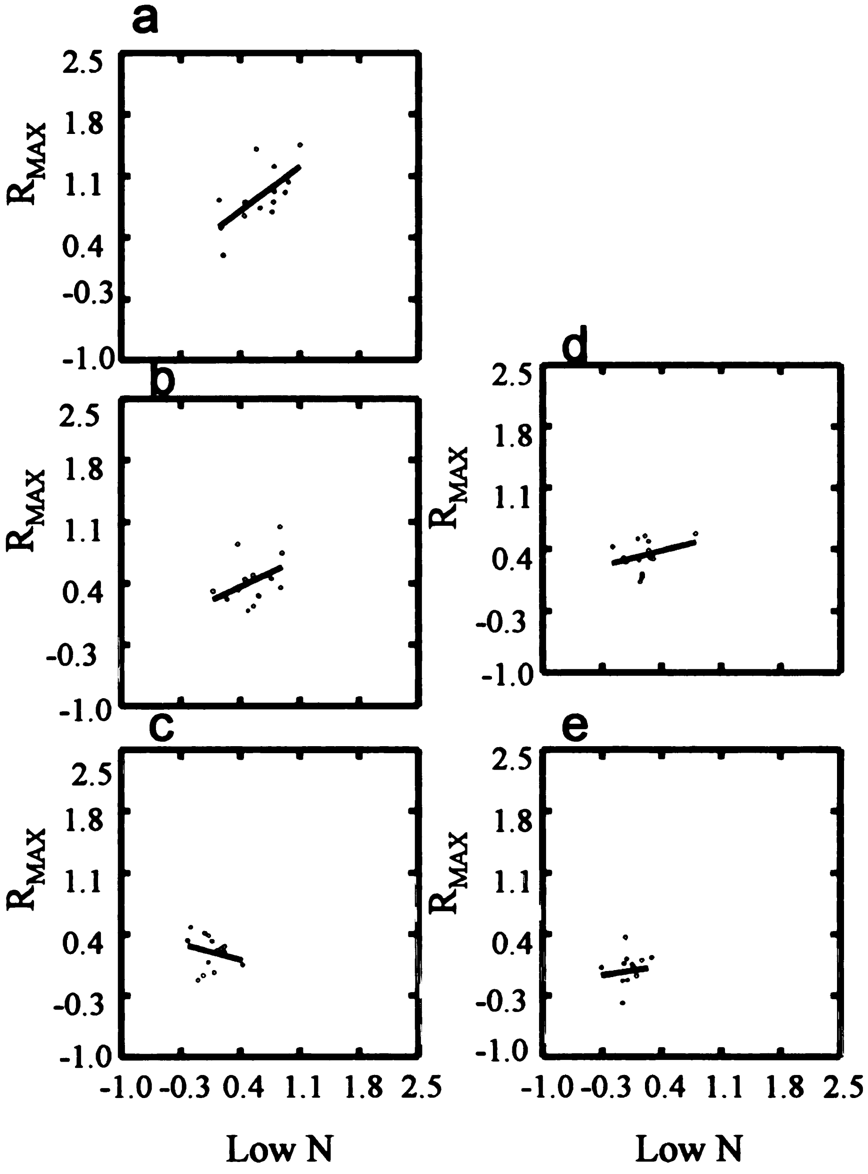

Figure 1.8: Relationships between maximum observed growth rates in high N and in low N conditions. a. Time $R_{0\text{-}3}$, r = 0.66 (p=0.022); b. Time $R_{3\text{-}6}$, r = 0.40 (n.s); c. Time $R_{6\text{-}10}$, r = -0.22 (n.s.); d. Time $R_{10\text{-}13}$ r = 0.32 (n.s.); e. Time $R_{13\text{-}16}$ r = 0.21 (n.s.); r - Spearman correlation coefficient; given p values represent significant correlation at 0.05 level (2-tailed), n=14 for all time periods

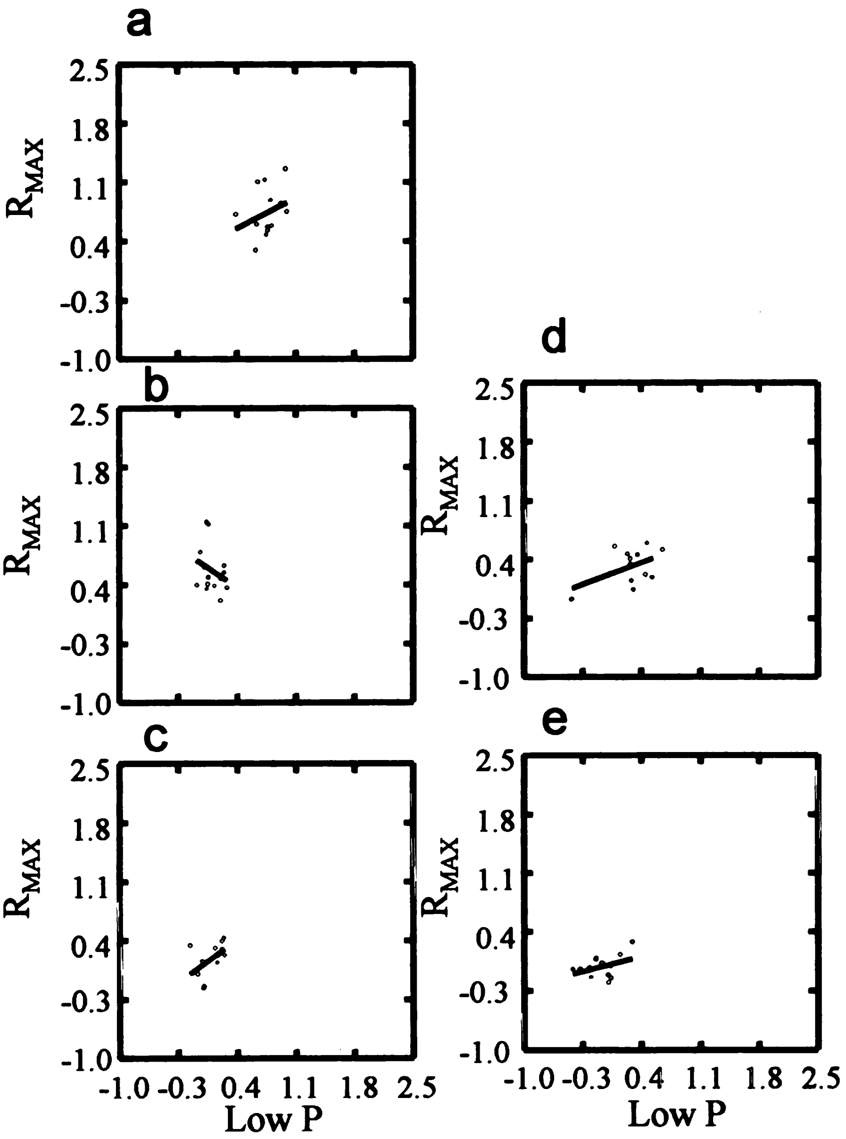

Figure 1.9: Relationships between maximum observed growth rates in high P and in low P conditions. a. Time R_{0-3}, r = 0.29 (n.s.); b. Time R_{3-6}, r = - 0.28 (n.s.); c. Time R_{6-10}, r=0.52 (p=0.044). d. Time $R_{10-1}3$ r = 0.50 (p=0.05); e. Time R_{13-16} r = 0.36 (n.s.); r - Spearman correlation coefficient; given p values represent significant correlation at 0.05 level (2-tailed), n=14 for all time periods

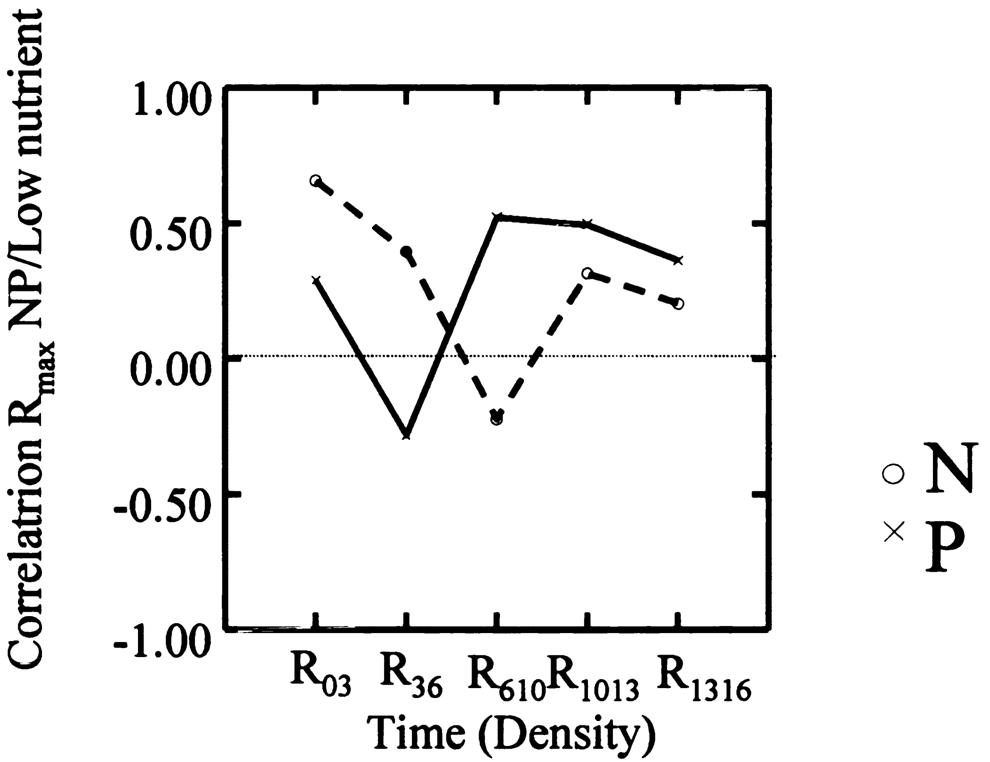

Figure 1.10: Correlation values for maximum observed growth along the two gradients for all the 5 sampling times with increase in density. Tradeoffs measure for N (o) and P (x). Dotted line separates positive and negative values.

CHAPTER II

INTRA- AND INTERSPECIFIC COMPETITION FOR NUTRIENTS AND LIGHT IN DIATOM CULTURES

INTRODUCTION

Few studies on species interactions within periphyton mats have been reported in the literature (Patrick 1968, Hoagland *et al.* 1982, Stevenson *et al.* 1991, Stevenson 1995) and there is a need for experimental validation of negative (competitive) or possible positive interactions between algal species (McCormick and Stevenson 1991). In diatoms, as in plant communities, patterns of abundance could be shaped by competitive interactions. Interspecific competition for nutrients was reported in the literature as a major determinant of algal community structure (Kilham 1971). Theory predicts that under stable conditions (no stress or disturbance) species with similar requirements can coexist when intraspecific competition is stronger than interspecific competition (MacArthur and Levins 1967, Tilman and Pacala 1993). How this applies to benthic diatoms is still unclear (McCormick 1996).

Benthic diatoms attach to the substrates available in streams and limitation or abundance of shared resources like nutrients and light affect their growth. There is a large amount of literature on the constraints on algal growth in streams caused by limiting nutrients (Bothwell 1985, Grimm and Fisher 1986, Pringle 1987), light (Triska *et al.* 1983, Steinman and McIntire 1987, Hill 1996) or both variables (Hill and Knight 1988, Hill and Harvey 1990, Rosemond 1993, 1994). Most studies show diatoms as the dominant algal group in streams, therefore concentrating on diatom populations can lead to important insights to periphyton dynamics in naturally complex systems. Nutrient concentrations had different effects on algal growth in shaded (< 40 µmol m^{-1} s^{-1}, Fisher and Likens 1973, Hill and Knight 1988, Hill *et al.* 1995) and unshaded streams (full sun light around 100 µmol m^{-1} s^{-1}, Bothwell 1985, Grimm and Fisher 1986).

Light requirements by individual benthic species are rarely studied (Hill 1996). Light attenuation by water, inorganic particles (Kuhl and Jorgensen 1994) and sediment can leave little light available for the periphytic communities (5-6 µmol m^{-2} s^{-1}, Richardson *et al.* 1983). On a broad community scale, the outcome of diatom population interactions for limiting nutrients measured as growth in different light regimes is unknown. In natural communities, nonmotile adnate algae (e.g., *Achnanthidium, Cocconeis* and other monoraphid genera) are hypothesized to be at a disadvantage when an overstory develops vertically (Hoagland *et al.* 1982, Steinmen 1996). Species from these genera are expected to decrease in relative abundance (Hill 1996), while biraphid diatoms (e.g., *Cymbella* and *Nitzschia*) are expected to increase in abundance. Biraphid diatoms can regulate their position along a light gradient through phototaxis (either gliding or stalk formation, Faure-Fremiet 1951, Hoagland *et al.* 1982). Light effects have been related to algal size (Laws 1975), where in low light conditions, larger diatom cells grew faster than small cells due to lower respiration loss per unit biomass (but see Michelson *et al.* 1979). Simultaneous nutrient and light dependence has also been reported (Hannson 1992).

Clonal diatom populations have been used to define phytoplankton growth patterns in controlled environments (Harrison *et al.* 1976, Tilman 1977, Grover 1988, 1989, 1990). Diatom experimental work has been performed in chemostats, where limitation to nutrients lead to competitive exclusion (Taylor and Williams 1975) or coexistence in planktonic diatoms (Epply *et al.* 1971). Diatom growth rate and competition for nutrients have been addressed with water column algae in chemostats (Michelson *et al.* 1979, Tilman 1982), with benthic algae in culture chambers (Stevenson 1995), and with marine benthic algae in nature (Sommer 1996). Genetically identical populations of periphyton diatoms in controlled environments are excellent systems to address similar growth constraints in benthic diatoms (Mann 1999). In natural and experimental studies with multispecies membership, morphologically identical cryptic species might obscure ecological differentiation among species. Manipulating nutrients and light in a controlled environment allows isolation and determination of biomass responses of diatom populations to common environmental variables (Manoylov and Stevenson 2004).

When resources are limited, populations interact in ways leading to decline in their performance measured as growth, reproduction, or survival (Begon *et al.* 1996). The complexity of species competitive ability (ability to reduce resources for others and tolerate reduction of resources by others) has been related to evolutionary theory of coexistence when resources are limited (Aarssen 1983). The ability of a diatom to reduce the performance (growth, reproduction or survival) of other diatoms is termed competitive effect (following Goldberg and Fleetwood 1987, Goldberg 1996). Competitive effect examines the role different neighbor species have on the same target species. Competitive effect can be assessed with estimation of competitive indices (reviewed by Weigelt and Jolliffe 2003). Competitive indices incorporate the effect of neighbor's growth on a target species (Goldberg and Barton 1992). The ability of a diatom species to grow well with competition from other diatoms is termed competitive response (i.e. response of different target species to the same neighbor). Competitive response requires several target species to grow in mono and mixed cultures. Competitive effect and competitive response can be unique or complementary proxies in assessing species-specific competitive ability (Goldberg and Werner 1983, Goldberg 1996).

Intra- and interspecific competition can be studied in many ways (Goldberg and Barton 1992). In plant competition, several studies compared species performance in low and high densities of the same species ('community density series' Goldberg *et al.* 1995). Alternatively, species performance in monocultures has been compared with species performance in mixtures with other species (Campbell and Grime 1992, Goldberg and Fleetwood 1987). In this experiment, I used a combination of the two models: first, a target species was grown at low and high initial density in limiting or luxurious resource (nutrient and light) levels; second, the same target species was grown with another diatom species at varying resource levels.

This study addressed performance of clonal populations of *Achnanthidium minutissimum* (Kützing) Czarnecki in different densities and environmental conditions and with other diatom species. The first objective of this study was to assess growth rate of *A. minutissimum* in four environmental conditions created with low and high levels of nutrients and light. Higher growth rates were expected when nutrients and light were high. Strong intraspecific competition was expected at limiting levels of nutrients and light, and those negative interactions should be reduced with higher resource availability (Tilman 1982). The second objective of the study was to assess the competitive effect of contraspecific diatom on *A. minutissimum*. Interspecific competition was examined with another adnate diatom, *Cocconeis placentula* var. *lineata* Krasske and with a larger, stalk-forming diatom, *Cymbella cistula* (Ehrenberg) Kirchner. These species are abundant and widely distributed in North America (Patrick and Reimer 1966) and occur together in natural assemblages (personal observations).

MATERIAL AND METHODS

Diatom cultures were purchased from Loras College Freshwater Diatom Culture Collection (maintained and operated by Dr. D. Czarnecki). *Achnanthidium minutissimum* (strain identification LI468), *Cocconeis placentula* var. *lineata* (023), and *Cymbella cistula* (LI550) were used in the experiment. Taxa in the mixed treatments were selected because of their morphological and growth traits. *Achnanthidium minutissimum* and *Cocconeis placentula* var. *lineata* were assumed to have similar modes of attachment to the substratum (adnate), but to differ in shape and size. The smaller, *A. minutissimum*, is lanceolate and the larger, *Cocconeis placentula* var. *lineata*, is oval. The second mixture of *A. minutissimum* and *Cymbella cistula* was chosen because those two species have different modes of attachment, shape and size. *Cymbella cistula* grows on stalks. It is wedge shaped and larger.

Experimental design

The experiment was conducted May 16 to June 6, 2004. The cultures were grown in modified Bold's medium (Sigma-Aldrich Scientific, Inc) for two weeks prior to inoculation of the experimental channels. The medium was diluted 10X and 28.4 mg/L sodium silicate ($Na_2SiO_3.9H_2O$) was added. The experiment was conducted in a controlled environmental chamber (Percival Scientific, Inc. Model I-36LL). Temperature was kept at 18°C, 14 h light to 10 h dark cycle, and humidity was 90 ± 5%. Full light in the chamber was 96-100 μmol m^{-2} s^{-1}. Shaded (low light) treatments were created with the use of 0.9 neutral density filter (LEE Filters, Burbank CA, item # 211), which reduced the full light to 10%. A remote alarm system signaled any deviation from the expected conditions.

Custom circular channels (Plas Labs, Inc., Lansing, MI) were constructed with 49 cylinders (diameter 10 cm, height 6 cm) of clear plastic (3 mm thick), glued onto a 70 x 70 cm clear plastic (7 mm thick) platform (Figure 2.1 a, b). Within each cylinder, a smaller cylinder 4 cm in diameter was centered and glued to create a 3 cm wide circular channel between the cylinders. Six unglazed tiles (2 x 2 cm) were scrubbed, washed with distilled water, and arranged flat in the channels (Figure 2.1 c). Each circular channel was a closed system. A clear plastic lid (3 mm thick) covered the whole structure to slow evaporation. The platform was placed on a rotary shaker (45 rpm, EL600 Heavy-Duty Orbital-Reciprocal Shaker, Eberbach, Inc.) to move water in the channels. The current velocity in the channels was 5 cm s^{-1}. The shaker and the platform were positioned within the incubator. The peripheral 24 channels on the artificial structure (Figure 2.1) were selected for the shading. Within each of the 24 shaded or full light channels, nutrient treatments and species combinations were randomly assigned in triplicate. Forty-eight of the available 49 channels were used.

Bold's algal medium (Bolds, Sigma-Aldrich Scientific) was modified by adding silica (as sodium silicate) and introducing nitrogen (N, as cobalt nitrate and sodium nitrate) and phosphorus (P, as potassium phosphate monobasic and potassium phosphate dibasic) in different concentrations (Table 2.1) to create two nutrient treatments: control (C) and high nutrient (NP). In the C treatment, nutrient concentrations were roughly equivalent to those considered to be growth-limiting for periphytic diatoms (Bothwell 1989), 25 µg N/L and 3 µg P/L. 250 µg N/L and 35 µg P/L were used in the NP treatment, which was designed to satisfy nutrient demand in the cultures. Before each use, the culture medium was filter-sterilized using 0.45 µm filters (APHA 1998). Each circular channel was filled with 80 ml modified medium, which was 2 cm deep over the tiles. Twenty ml of fresh medium was added every other day to replenish nutrients depleted by algal uptake.

Four species combinations were used (Table 2.2): 1. a monoculture of *Achnanthidium minutissimum* (A); 2. monoculture with a double initial density of *A. minutissimum* (AA); 3. mixture of *A. minutissimum* and *Cocconeis placentula* var. *lineata* (AC); and 4. mixture of *A. minutissimum* and *Cymbella cistula*

(ACM). In the later two cases, species were inoculated in approximately equal biosurface (density x surface area) at the beginning of the experiment.

To monitor nutrient concentrations, water samples were taken before periphyton sampling every 3 days from randomly selected channels (2 from shaded and 2 from full light, n = 4). NO_3-N and PO_4-P concentrations were measured by automated cadmium reduction and automated ascorbic methods, respectively (Scalar, Inc.). Periphyton was sampled by collecting 1 tile at random from the same position in each channel every 3 days for 6 consecutive sampling dates. After sampling, the diatoms were preserved with M3 (APHA 1998). The removed tile was replaced with a clean tile to preserve flow conditions. Diatoms were scraped from the tiles with razor blades and toothbrushes. Cell densities were enumerated and measured using a Palmer-Maloney counting chamber at 400X magnification (Leica DMLB microscope). This method allowed detection of the cytoplasm in the cells and good resolution of cell wall structure of the 3 diatom species. At least 300 cells with an intact chloroplast were identified and enumerated in each channel for each sampling date. Standard diatom morphological measurements were taken from individuals in each treatment. For all cultures the following measurements were performed: cell apical length, transapical width, frustule depth in the middle and for the purposes of taxonomic evaluation striae in 10 µm (mainly in *Cymbella cistula*). At least 15 specimens were measured at random in each channel.

For all inoculum and sampled populations, cell volumes (V, μm^3) and cell surface area (S, μm^2) in each treatment were calculated using formulae from

Hillebrand *et al.* (1999). Volume and surface area for *A. minutissimum* and *Cocconeis placentula* var. *lineata* were calculated with the following formulae: $V=(\pi/4)*a*b*c$ and $S=(\pi/4)*(a*b+[a+b]*c)$, where a is the apical length, b is transapical width and c is the depth of the mantle. The volume of *Cymbella cistula* was calculated using the formula: $V=4/6*\pi*b^2*a*(\beta/360)$ and cell surface area was calculated using $S= ((\pi*b*a)/2)+c*((b+((a/2)^2/((sqrt\ (a/2)^2)-b^2))*\sin^{-1((2*sqrt(a/2)-2)-b2)/a)})$, where a is the apical length, b is transapical width, c is the pervalvar axis on dorsal side and β is the angle between the two transapical sides, calculated as $\sin \beta/2=c/(2*b)$. Cell surface area calculation and use in biosurface calculation (total cell surface area, mm^2) was necessary to offset the potential overestimation of the larger *C. cistula* and underestimation of the smaller *A. minutissimum* that could result from using biovolume.

Absolute cell densities (D, cells mm^{-2}) were calculated for each treatment. Total biosurface (B) was calculated as the average cell surface area x cell density in each treatment. Growth rates were calculated based on species density for each treatment. Growth rates (R) were estimated as daily per capita changes in cell density (in each channel): $R = (\ln D_{(t+1)} - \ln D_{(t)})/3$, where $D_{(t)}$ and $D_{(t+1)}$ are total densities on consecutive times and 3 was the uniform number of days between sampling days. Growth rates (ln cells $cell^{-1}$ d^{-1}) were calculated for each species in each channel, for each time period. Growth rates for each period were designated as R_{xy} for the growth change between community development day x and day y.

Treatment effects on growth rates were tested using repeated measures analysis of variance (RM ANOVA) in each culture combination. The univariate approach to RM ANOVA takes into account temporal autocorrelation within each channel, which can violate the assumption of independence among samples. All results were adjusted for circularity with Geisser-Greenhouse epsilon (G-G, von Ende 2001) which is the most conservative correction of degrees of freedom for independence among samples within a channel. If the treatment effect was significant, time effects were evaluated with polynomial, profile analyses in RM ANOVA, in which differences are constructed from adjacent levels of the time factor.

I assessed growth of clonal *A. minutissimum* in limiting concentrations of nutrient and very low light. Addition of nutrients in low light was expected to increase growth rate and competition for light between taxa in this treatment, where ability to produce stalks by *C. cistula* should be advantageous. Nutrient enrichment in low light was expected to similarly stimulate *A. minutissimum* and *Cocconeis placentula* var. *lineata*. With the addition of light in low nutrients, *A. minutissimum* was expected to increase growth rate, as a good competitor for limiting nutrients (reported as indifferent in Van Dam, 1994), while growth rate of *Cocconeis placentula* var. *lineata* should decline (reported as eutraphentic, Butcher 1947, Van Dam 1994). Throughout the time periods of community development I hypothesized that addition of both nutrients and light would alleviate all competitive interactions.

Competitive effect

A regression based method for measuring the competitive effect of conspecific and/or contraspecific individuals on a 'target' species was developed by Goldberg and Werner (1983). In my modified model, the slope of a regression line (relating growth rate as a function of biosurface) is a measure of the performance of the target species as a function of the biosurface of all neighboring individuals. Competitive effects were estimated with competitive indices assessing the proportional competitive effects of neighbors (other diatoms either conspecific or contraspecific) on the growth rate of the target species, *A. minutissimum* (GR_A). The following model was used:

$$GR_A = C + (\alpha_A * B_A) + (\alpha_{CC} * B_{CC}) + (\alpha_{CM} * B_{CM}),$$

Here, C is an estimate of maximum growth rate and B_A, B_{CC}, B_{CM} are biosurface of *Achnanthidium minutissimum*, *Cocconeis placentula* var. *lineata* and *Cymbella cistula*, respectively. The following were estimates of competitive indices: α_A is a measure of the competitive performance of *A. minutissimum* in each culture combination; α_{CC} performance of C. *placentula* var. *lineata* in the AC culture combination; and α_{CM} performance of *Cymbella cistula* in the ACM culture combination. In the mixed cultures, together with conspecific influence, performance of *A. minutissimum* proportionally measures the interspecific influence of the other taxa. If α_A, α_{CC} and α_{CM} are equal to 0, there was no competition. In each species combination, intra- and interspecific competition respectively was low when slopes were close to 0 and not significantly different from each other (slope comparison, Sokal and Rohlf 1995). Any $\alpha > 0$ indicated facilitation and $\alpha < 0$ indicated competition. All coefficients have variance associated with them, which allowed comparison of the competitive response of the target species in each culture combination. α estimates were graphically presented for high biosurface gradient only in each treatment, where stronger competition was expected. All statistical analyses were performed with SYSTAT® 10 (Wilkinson 1989).

RESULTS

Nutrient concentrations in the control channels were low, 19-25 µg NO_3-N/L and 1.5-3 µg PO_4-P/L. Adding enriched medium to the high nutrient treatments resulted in an eight to ten-fold increase in N and P concentrations (Fig. 2.2).

Observations of cells on tiles showed cells attached to the tile surface and covered it completely by the end of the experiment. *Achnanthidium minutissimum* did not grow in a uniform pattern in any treatment, it was observed in single and multiple cells, within and around mucilage globules. Those globular structures protruded above the tile surface, remained adhered to the tiles and kept growing in size. The protruded in the water column flocks of cells, attached at one end to the tile, potentially were a source for immigrating cells. Judging from the healthy appearance of the chloroplasts of the cells in all those structures, growth was equally successful in the different growth structures. This diversity in extracellular mucilage production was not observed for *Cocconeis placentula* var. *lineata*. Cells remained attached close to the surface without elevating over the tiles and without visible aggregation of cells. Healthy looking single cells of *Cocconeis placentula* var. *lineata* were observed within the mucilage globules created by *A. minutissimum*. Cells of *Cymbella cistula* initially remained close to the substrate, but with increasing time and density, short and long stalks formed and elevated the cells above and around the mucilage aggregates of *A. minutissimum*.

Total density increased for all species combinations with nutrients addition ($p<0.0001$, Fig. 2.3). The increase in density was greatest at the early time periods when density was low. Total density increased to 8000-9000 cells^{-1} mm^{-1} after day 9 of community development. Total density was consistently higher in the shaded treatment (Fig. 2.3). Total biosurface of *Achnanthidium minutissimum* did not differ significantly with addition of nutrients in low light, decreased with higher light availability in low nutrient treatments and increased with nutrient addition in high light treatments (Fig. 2.4).

Growth rates of *A. minutissimum* decreased with density and time in almost all treatments. Growth rate of *A. minutissimum* varied significantly with treatment in most culture combinations (Table 2.3, Figs 2.5, 2.6). There were no significant treatment x time effects for any culture combination (Table 2.3). Polynomial analysis of the time period that contributed to the significant treatment or time effect revealed that growth rate from day 6 to 9 contributed (cubic trend significant $p=0.0175$ ANOVA, Fig. 2.5) to the significance when all treatments and consecutive times were analyzed together.

Nutrient or light addition did not have a significant effect on *Achnanthidium* growth rate when *A. minutissimum* grew alone or with *Cocconeis placentula* var. *lineata* (Fig. 2.5). During the initial growth period (Fig. 2.5 open bars) monocultures of *A. minutissimum* grew at the same rate regardless of the initial density (Fig. 2.5). Growth rates in monocultures were always higher than growth rate of *A. minutissimum* in mixture with another diatom. Addition of nutrients significantly increased *A. minutissimum* growth at higher density only when *A. minutissimum* was mixed with *Cymbella cistula* and in low light (Fig. 2.5 dark bars). Growth rate at higher density was independent of nutrient addition with excess light (Fig. 2.5).

Most regression coefficients (competition coefficients), whether measured using the whole biomass gradient or just high biomass conditions were negative when they were significantly different from 0 (Table 2.4, Fig. 2.6). Within each culture combination, the steepest slopes were observed in the low light-low nutrient treatment (Table 2.4). Because of the similarities in the direction of the regression coefficient estimates along the whole biomass gradient and at high biomass, competition coefficients were studied only in high biomass conditions. With addition of nutrients and light, biosurface tripled (compare range in biosurface values in Figs.2.6 a. with 2.6 d.).

Low nutrients and light had strong effects on intraspecific competition in three of the culture combinations (Fig. 2.7), with coefficients of -2.4, -0.95, and -0.59 in cases: A, AC and ACM respectively. When nutrients were abundant, competitive interactions in populations of clonal *A. minutissimum* were not detected, regardless of the neighbor identity (Fig. 2.7).

Cocconeis placentula var. *lineata* grew slower than *A. minutissimum* when nutrients were limiting, (Fig. 2.8 a and Table 2.4), the strongest competitive index

(-2.14) was estimated in low nutrient and high light conditions. No interspecific competitive interactions between *A. minutissimum* and *Cymbella cistula* were observed in comparison with the very little evidence observed with *Cocconeis placentula* var. *lineata*. Most slope estimates were not different from 0 and positive (Fig. 2.8 b and Table 2.4).

DISCUSSION

Intraspecific competitive effects in all culture combinations regulated growth of *Achnanthidium minutissimum*. Growth rate of *Achnanthidium minutissimum* decreased with increasing biosurface of algae in all treatments. Initial growth rate of *Achnanthidium minutissimum* were reduced by presence on interspecific competitors, reduction of growth rate was independent of treatment Intraspecific competition was strongest when nutrients and light were low and *A. minutissimum* was in monocultures. Intraspecific competition was relieved with addition of nutrients or both nutrients and light. Light availability relieved constraint on *Cocconeis placentula* var. *lineata* growth and enabled strong interspecific competitive effects on *A. minutissimum*. Addition of nutrients significantly increased *A. minutissimum* growth at higher density only when *A. minutissimum* was mixed with *Cymbella cistula*. The observed potential facilitation of the presence of *C. cistula* on the growth of the target species in low resources suggests potential coexistence in nature. The observed cell densities were comparable to other studies where competitive interactions were observed in nature (Butcher 1947, Rosemond 1993).

When initial densities of *Achnanthidium minutissimum* were higher intraspecific competition at low resources was not as strong as observed in treatments with lower initial density. Growth rates of *A. minutissimum* in monocultures were always higher than its growth rate in mixture with another diatom. These results need further research with the same diatom and different diatoms in monocultures with varied densities. For example, chemical signals in the denser initial monoculture might have influenced diatom growth.

Achnanthidium minutissimum has been reported from many ecologically diverse environments (Potapova and Charles 2003). In *in situ* nutrient enrichment experiments, Cholnoky (1968) and Kawecka (1981) reported high growth rates for *A. minutissimum* in both low and high nutrient conditions. Consistently, this taxon has been reported as indifferent to nutrient concentrations (Van Dam *et al.* 1994). Clones from the same species have shown great affinity for nutrients, depending on the natural conditions. Diatoms potentially adapted to growth in low nutrients were able to grow at very low nutrient concentrations (Chapter 1 of this dissertation, Carpenter and Guillard 1971). In this study, using genetically identical populations, I characterized response of one phenotype that might differ from the response of other populations of the same species isolated from different natural habitats. However, many of the above observations for *A. minutissimum* indicate it is a good competitor for nutrients when they are in low supply.

Species-specific diatom responses to light has not been studied in relationship to competition, but in freshwater benthic systems total community response to light has been reported (DeNicola *et al.* 1992, Hill 1996, Wellnitz and

Rinne 1999). One possible mechanism explaining the observed higher biomass in shaded streams in this study compared to full light is that the species I manipulated may have low photosynthetic optima. Shade adaptation increases carbon uptake at low light, and it is common response of phytoplankton (Richardson *et al.* 1983). This has been reported in single culture studies (Dubinsky *et al.* 1986, Falkowski and LaRoche 1991) and multispecies communities (Hill and Boston 1991, Hill *et al.* 1995). At high light, photoinhibition might have been possible among 'shade-tolerant' diatom species (Hill *et al.* 1995) manipulated in this experiment.

In the experiment, *Achnanthidium minutissimum* in limited light treatments produced mucilage layers, with very healthy growth within them. Taxa from *Achnanthidium* and *Cocconeis* usually attach close to the substrate and do not produce long chains, but the ability to produce mucilage might have been advantageous for *A. minutissimum* in the low light and low nutrient environment. *C. placentula* var. *lineata* grew slower than *A. minutissimum* in this experiment potentially due to inability to elevate itself when it was light and nutrient limited. In the other interspecific competition treatment, *Cymbella cistula* had the ability to extend well above the surface in the shaded treatments (DC and DNP), potentially reducing shading effects and increasing surface area for attachment. However, because *A. minutissimum* was able to attach to the stalks of *C. cistula* or aggregate in mucilage globules, *A. minutissimum* was elevated over the substrate also. These results suggest a potential for *A. minutissimum* to competitively exclude *C. placentula* var. *lineata*, but coexistence with *C. cistula* was possible.

Intra- and interspecific response to competition was relieved when nutrients and light were available. It had been hypothesized that microalgae would not be successful competitors at both low and high resource concentrations, but instead they would pursue one strategy or the other (McCormick 1996). This experimental research presented a few successful ecological strategies of one common diatom in different taxa combinations and nutrient concentrations. This experiment is the first reported in the literature where competitive effects of other diatoms was evaluated on clonal populations of *Achnanthidium minutissimum*. Evolutionary traits like small size might be more important in interspecific competition than nutrient uptake efficiency. Competitive abilities are restricted by the morphological differences of the competing species (e.g. size and bio-surface).

In conclusion, this was the first attempt to characterize the competitive ability of *Achnanthidium minutissimum* in a controlled setting. I was able to create periphyton communities in culture conditions and to evaluate the ecological effects of other diatoms on clonal populations of *A. minutissimum*. Light had a strong negative effect on *A. minutissimum* growth when nutrients were limiting and had a positive effect when nutrients were available. The prediction of competitive effects being strongest at limiting resources was confirmed in this experiment. The mechanisms to compete for low nutrients and low light of these morphologically diverse diatoms may be different. *Achnanthidium minutissimum* and *Cocconeis placentula* var. *lineata* are monoraphid diatoms, but the former had potentially lower nutrient requirements than the latter. The morphologically different pair: *A. minutissimum* and *Cymbella cistula* were both good competitors for limiting resources. The stalks produced by *C. cistula* provided substratum for *A. minutissimum* colonization.

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Component	formula	in medium C (mg/L)	element	in C µg/L	in medium NP (mg/L)	element	in NP µg/L
Boric acid	H_3BO_3	0.0007613			0.0007613		
Calcium chloride dihydrate	$CaCl_2.2H_2O$	0.0016667			0.0016667		
Cobalt nitrate • $6H_2O$	$Co(NO_3)_2 \bullet 6H_2O$	3.267E-05			0.0001304		
			N	0.00314		N	0.0125
Cupric sulfate • $5H_2O$	$CuSO_4 \bullet 5H_2O$	0.0001047			0.0001047		
EDTA (free acid)	$C_{10}H_{16}O_8N$	0.0033333			0.0033333		
Ferrous sulfate • $7H_2O$	$FeSO_4 \bullet 7H_2O$	0.000332			0.000332		
Magnesium sulfate • $7H_2O$	$MgSO_4 \bullet 7H_2O$	0.005			0.005		
Manganese chloride • $4H_2O$	$MnCl_2 \bullet 4H_2O$	0.000096			0.000096		
Molybdenum trioxide	MoO_3	4.733E-05			4.733E-05		
Nickel chloride • $6H_2O$	$NiCl_2 \bullet 6H_2O$	0.0000002			0.0000002		
Potassium hydroxide	KOH	0.0020667			0.0020667		

Table 2.1: Medium used for culture growth

Component	formula	in medium C (mg/L)	element	in C µg/L	in medium NP (mg/L)	element	in NP µg/L
Potassium iodide	KI	0.0000002			0.0000002		
Potassium phosphate monobasic	KH_2PO_4	0.0116667			0.0925		
			P	2.6		P	21.1
Potassium phosphate dibasic	K_2HPO_4	0.005					
			P	0.889		P	10.9
Sodium chloride	NaCl	0.002			0.002		
Sodium nitrate	$NaNO_3$	0.148			1.51		
			N	24.5		N	250
Sodium selenite	Na_2SeO_3	6.667E-07			6.667E-07		
Stannic chloride	$SnCl_4$	3.333E-07			3.333E-07		
Vanadium sulfate • $3H_2O$	VS• $3H_2O$	7.333E-07			7.333E-07		
Zinc sulfate • $7H_2O$	$ZnSO_4$• $7H_2O$	0.00294			0.00294		
Sodium silicate• 9H2O	Na_2SiO_3•$9H_2O$	0.0051			0.0051		

Table 2.1: Cont'd

Table 2.2: Culture combination of the initial communities (n=15). Species cell biovolume (μm^3) (mean ± SE) and cell biosurface mm^2 (mean ± SE).

Case	Taxon	Density $cells^{-1} ml^{-1}$	Biovolume (μm^3)	Biosurface (μm^2)
A	*Ac. minutissimum*			
	mean	1002 ± 3	68.7 ± 5	111 ± 6
AA	*Ac. minutissimum*			
	mean	2000 ± 5	68.7 ± 5	111 ± 6
AC	*Ac. minutissimum*			
	mean	1002 ± 3	68.7 ± 5	111 ± 6
	C. placentula var. *lineata*			
	mean	200 ± 1	408 ± 66	466 ± 52
ACM	*Ac. minutissimum*			
	mean	1002 ± 3	68.7 ± 5	111 ± 6
	Cymbella cistula			
	mean	180 ± 5	505 ± 45	1459 ± 149

Table 2.3: Repeated measures ANOVA for differences in growth rate of *Achnanthidium minutissimum* when grown in different case combinations: case A - *A. minutissimum* only; AA- *A. minutissimum* in double initial density; with *Cocconeis placentula* var. *lineata* (AC); and in combination with *C. cistula* (ACM). Between treatment effect – treatment combination and time and treatment interaction tested as within channel effect. For each effect: F statistics, df-degrees of freedom, and p- value adjusted for circularity.

Case	Taxon	Source of variation	df	Mean Square	F ratio	$P_{F\,adj}$
A	*Ac. minutissimum*	Treatment	3	0.029	4.86	0.0328
		Error	8	0.006		
		Time	5	1.328	47.91	<0.0001
		Treatment*Time	15	0.02	1.00	ns
		Error	40	0.03		
AA	*Ac. minutissimum*	Treatment	3	0.029	10.06	0.0043
		Error	8	0.003		
		Time	5	1.19	35.35	<0.0001
		Treatment*Time	15	0.03	0.75	ns
		Error	40	0.034		
AC	*Ac. minutissimum*	Treatment	3	0.007	1.21	ns
		Error	8	0.006		
		Time	5	0.95	43	<0.0001
		Treatment*Time	15	0.019	0.87	ns
		Error	40	0.022		
	C. placentula var. *lineata*	Treatment	3	0.003	0.75	ns
		Error	8	0.004		
		Time	5	2.13	1.58	ns
		Treatment*Time	15	0.05	2.3	ns
		Error	40	0.025		
ACM	*Ac. minutissimum*	Treatment	3	0.033	21.2	0.0004
		Error	8	0.001		
		Time	5	1.01	49.06	<0.0001
		Treatment*Time	15	0.046	2.23	ns
		Error	40	0.02		
	Cymbella cistula	Treatment	3	0.004	1.09	ns
		Error	8	0.003		
		Time	5	0.065	4.16	0.0273
		Treatment*Time	15	0.029	1.92	ns
		Error	40	0.015		

P_F – probability that F ratio would be equal to or greater than what is due to chance alone.

Table 2.4: Competitive effects of diatoms on *Achnanthidium minutissimum* (target). Intraspecific competition (column α_A). Intraspecific competition input from the neighbors are given under other (competitive indices estimations of the neighbor species in mixed cultures, α_{CC} α_{CM}). Values are the intercept (maximum growth rate, GR_{max}) and regression coefficient 'α's from the linear regression analysis, Growth rate as a function of B- total neighbor biosurface. Growth rate of *Achnanthidium minutissimum* when grown in different case combinations: case A - *A. minutissimum* only, without competition; AA - *A. minutissimum* in double initial density; AC -with *Cocconeis placentula* var. *lineata*); and ACM with *Cymbella cistula*. Indices estimates given with standard error given (SE), slopes different from 0 are given in bold. Estimates of intercept and slope compared (P_F – probability that F ratio would be equal to or greater than what is due to chance alone); ns- non significantly different, different at $p<0.05$). Designated same letter indicated non significant means.

Table 2.4:

	Whole range biomass			High biomass		
	GR_{max} (SE)	α_A (SE)		GR_{max} (SE)	αA (SE)	
Case/Treatment						
A		target	other		target	other
DC	0.48 (±0.35)	**-1.95 (±0.70)**^{a}		0.47 (±0.06)^{a}	**-2.40 (±0.51)**^{a}	
DNP	0.39 (±0.17)	-0.32 (±0.18)^{b}		0.17 (±0.03)^{b}	-0.06 (±0.06)^{b}	
LC	0.20 (±0.13)	0.00 (±0.13)^{b}		0.13 (±0.05)^{b}	-0.01 (±0.09)^{b}	
LNP	0.31 (±0.08)	**-0.14 (±0.06)**^{b}		0.18 (±0.05)^{b}	-0.07 (±0.05)^{b}	
	ns	$p<0.001$		$p<0.001$	$p<0.001$	
AA						
DC	0.30 (±0.09)	-0.36 (±0.59)		0.13 (±0.05)	-0.07 (±0.30)	
DNP	0.29 (±0.08)	-0.16 (±0.27)		0.17 (±0.06)	-0.08 (±0.18)	
LC	0.19 (±0.08)	-0.03 (±0.14)		0.23 (±0.06)	**-0.25 (±0.12)**	
LNP	0.33 (±0.08)	**-0.24 (±0.09)**		0.11 (±0.05)	-0.10 (±0.05)	
	ns	ns		ns	ns	
AC			α_{CC} (SE)			α_{CC} (SE)
DC	0.39 (±0.08)	**-1.20 (±0.45)**	0.1 (±0.07)	0.27 (±0.05)^{a}	**-0.95 (±0.27)**^{a}	-0.20 (±0.85)
DNP	0.48 (±0.16)	-0.91 (±0.56)	-0.20 (±0.10)	0.12 (±0.10)^{ab}	-0.05 (±0.33)^{b}	1.55 (±0.88)
LC	0.41 (±0.12)	-0.57 (±0.31)	0.06 (±0.07)	0.36 (±0.10)^{ac}	**-0.51 (±0.25)**^{a}	**-2.14 (±0.76)**
LNP	0.42 (±0.09)	**-0.36 (±0.13)**	0.00 (±0.06)	0.32 (±0.08)^{a}	**-0.25 (±0.11)**^{b}	-0.12 (±0.64)
	ns	ns	ns	$p=0.04$	$p=0.01$	ns
ACM			α_{CM} (SE)			α_{CM} (SE)
DC	0.31 (±0.12)	-0.27 (±0.44)	**0.24 (±0.08)**	0.25 (±0.04)	**-0.59 (±0.16)**^{a}	0.20 (±0.18)
DNP	0.28 (± 0.12)	-0.07 (±0.29)	-0.18 (±0.09)	0.17 (±0.11)	0.11 (±0.25)^{b}	0.05 (±0.32)
LC	0.39 (±0.09)	**-0.26 (±0.10)**	0.13 (±0.07)	0.28 (±0.08)	-0.17 (±0.09)^{b}	0.48 (±0.39)
LNP	0.44 (± 0.08)	**-0.23 (±0.06)**	0.13 (±0.09)	0.32 (±0.07)	**-0.16 (±0.05)**^{b}	0.05 (±0.45)
	ns	ns	ns	ns	$p<0.001$	ns

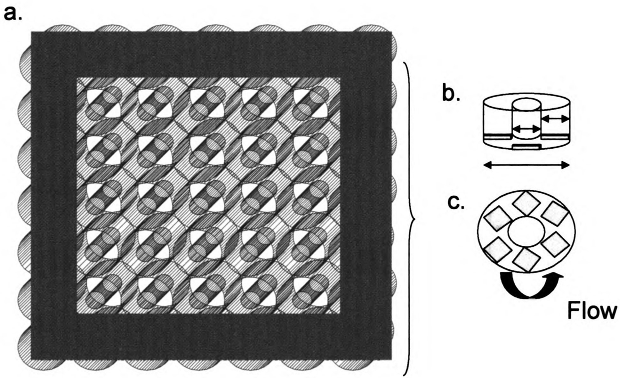

Figure 2.1: Schematic representation of the closed experimental circular channels; a. whole view; b. side view, circular channel diameter = 3 cm; c. view from top, where tile arrangement can be seen. Arrow indicates flow direction (flow = 5 cm.s-1)

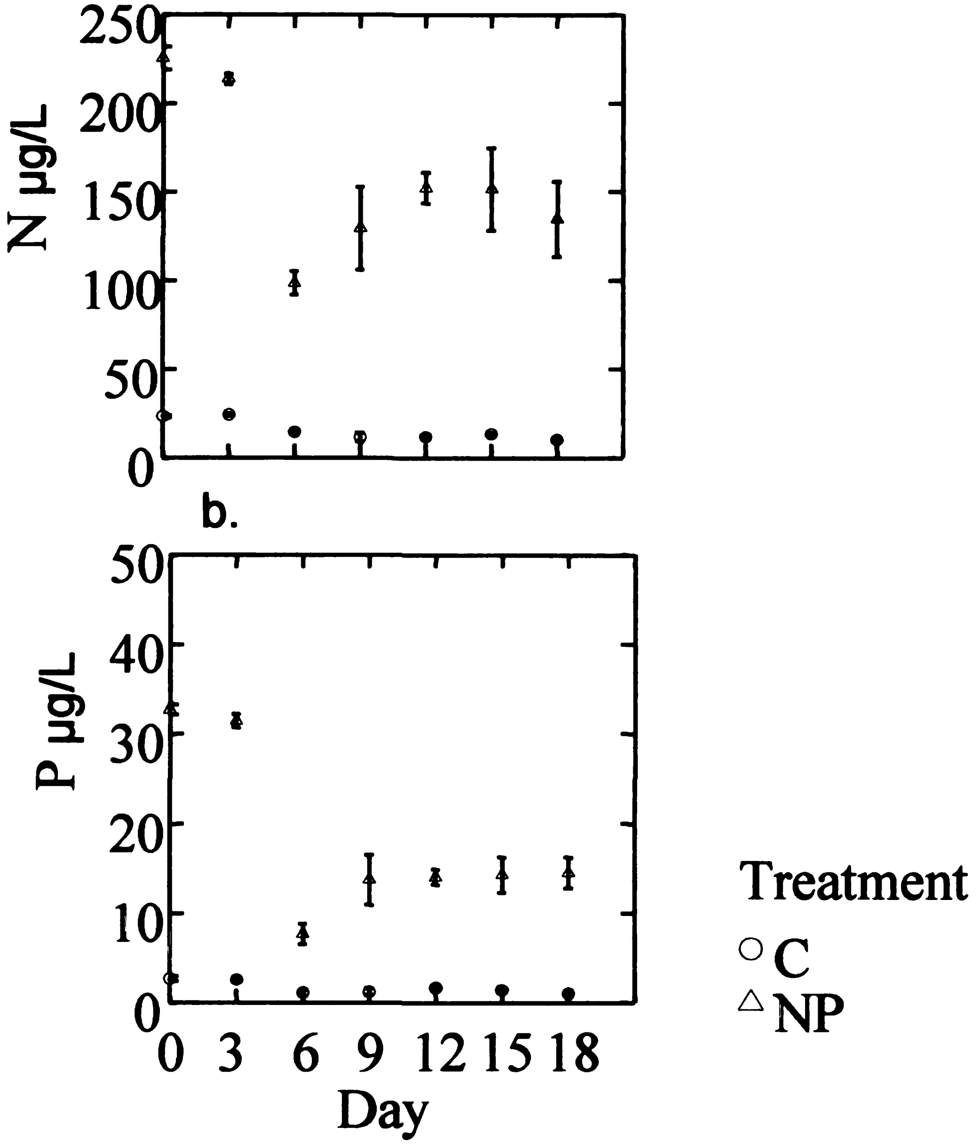

Figure 2.2: Nutrient concentrations measured (µg/L); (a.) N and (b.) P values in the control and NP treatments (n=4). Error bars around the means represent standard error

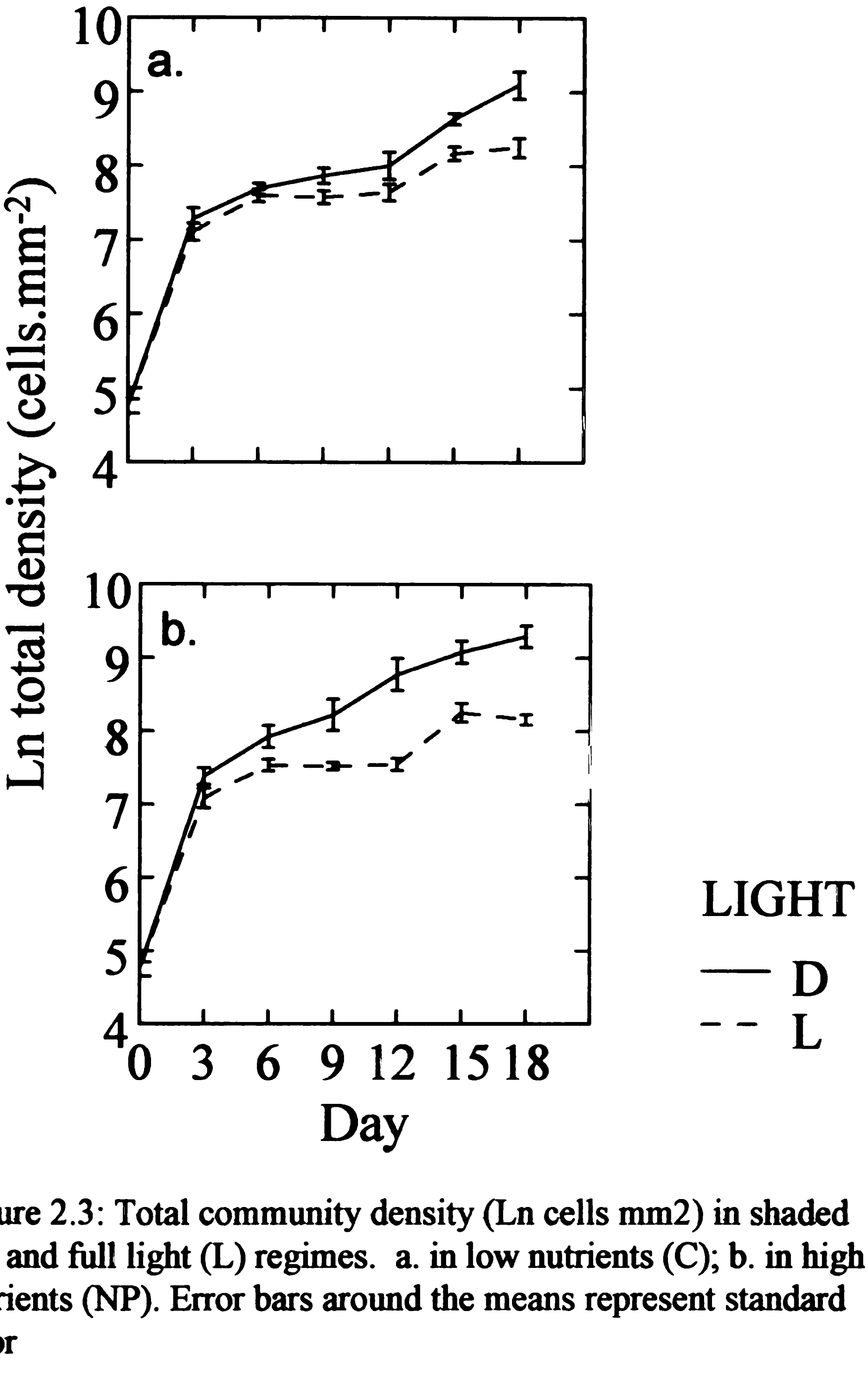

Figure 2.3: Total community density (Ln cells mm2) in shaded (D) and full light (L) regimes. a. in low nutrients (C); b. in high nutrients (NP). Error bars around the means represent standard error

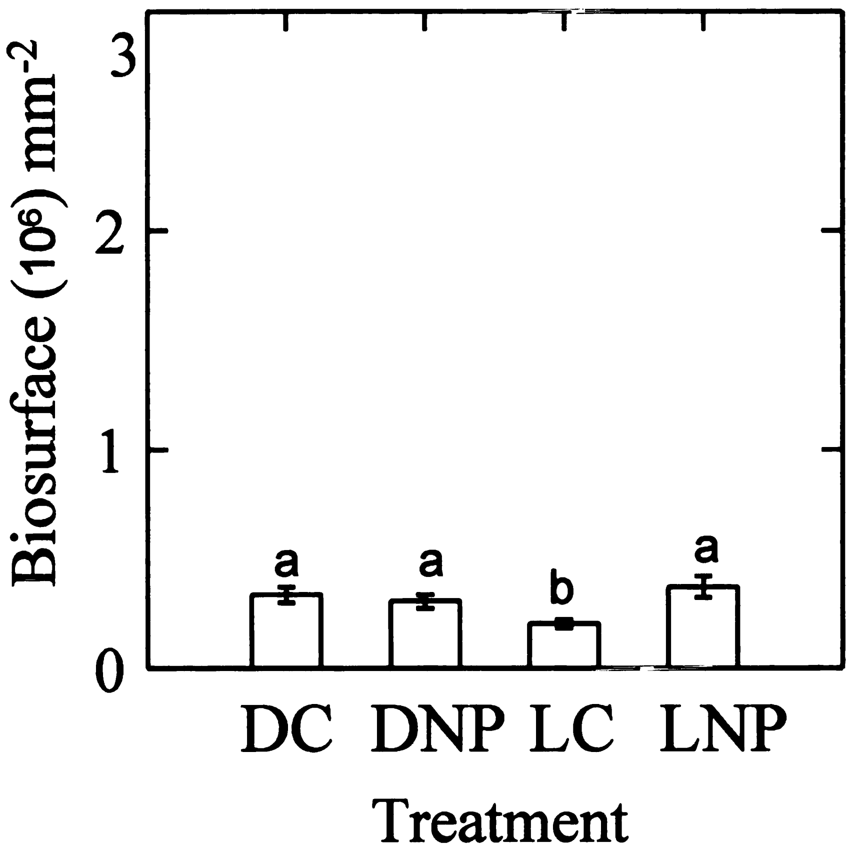

Figure 2.4: Total community biosurface (106 total cell surface area, mm-2) in each treatment combination. Bars (means ± standard error) with the same letters are not significantly different

Figure 2.5: Growth rate of ***Achnanthidium minutissimum*** in culture combination. a. DC; b. DNP; c. LC; d. LNP; open bars- initial growth (difference in days of community development 0 to 3); shaded bars high biomass growth (difference in days of community development 6 to 9; RM ANOVA polynomial test result, significant time period change); Bars (means ± standard error) with the same * (for the initial growth) or same letter (for the high biomass growth) not significantly different

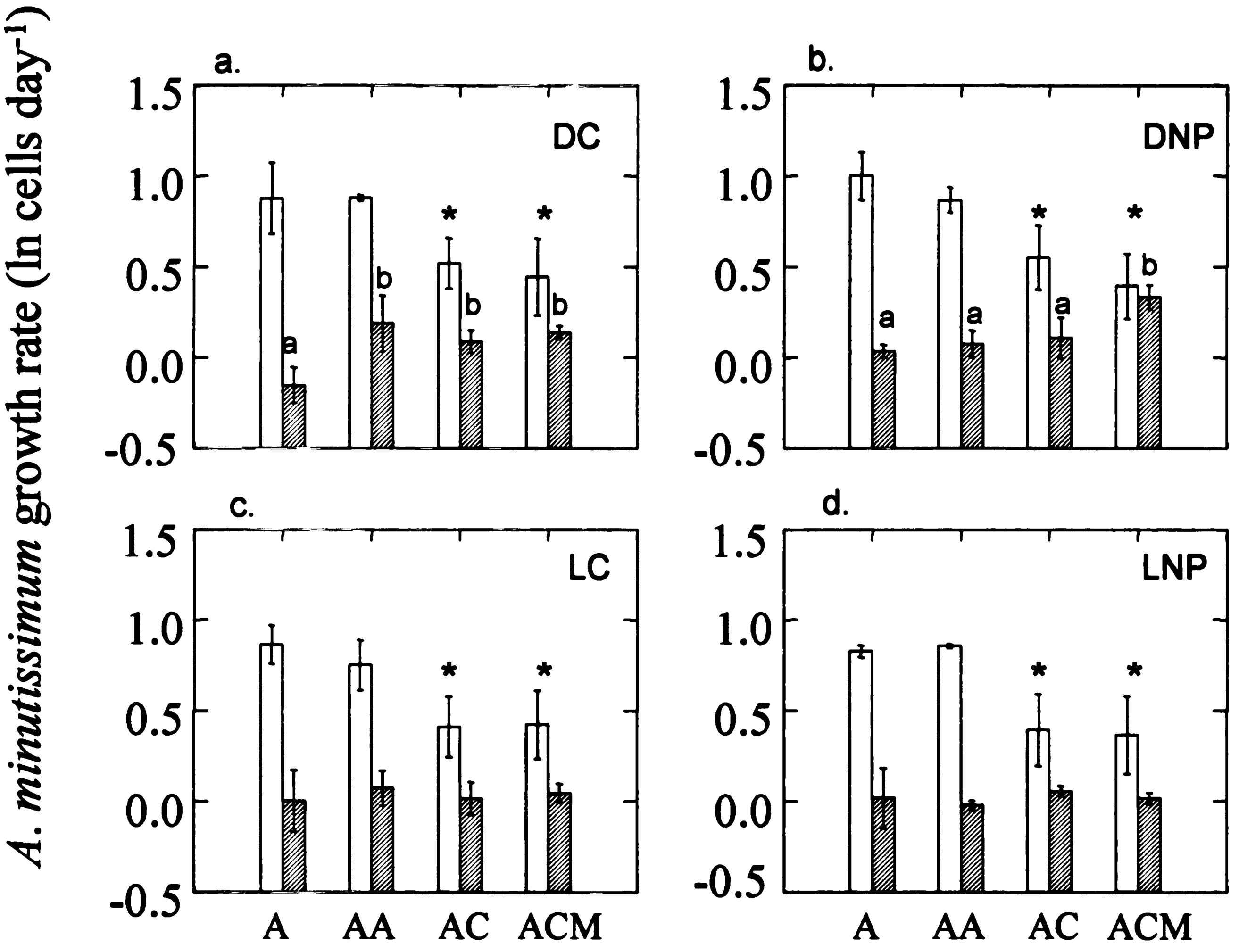
A. minutissimum growth rate (ln cells day-1)
a.
DC
b.
DNP
c.
LC
d.
LNP
1.5
1.0
0.5
0.0
-0.5
A
AA
AC
ACM
a
b
*

Figure 2.5

Figure 2.6: Growth rate relationships of *Achnanthidium minutissimum* per biomass estimate in culture combination (Case); A- dashed line, AA-dashed & dotted line, AC - dotted line, and ACM-solid line in each treatment combination; a. DC; b. DNP; c. LC; d. LNP

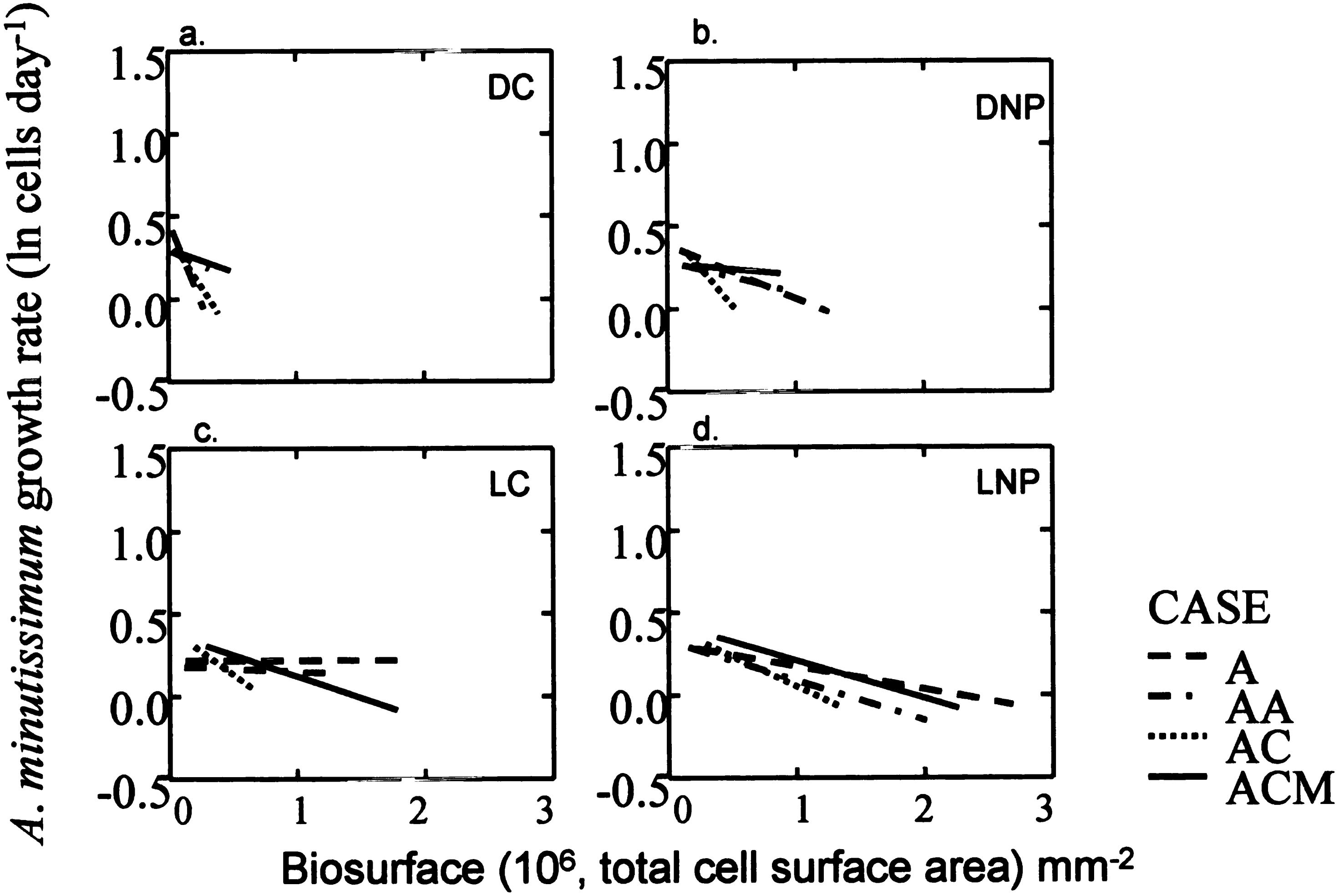
A. minutissimum growth rate (ln cells day-1)
a.
DC
b.
DNP
c.
LC
d.
LNP
1.5
1.0
0.5
0.0
-0.5
0
1
2
3
Biosurface (10^6, total cell surface area) mm^-2
CASE
A
AA
AC
ACM

Figure 2.6

Figure 2.7: Intraspecific competition indices (α) estimates in high biomass of the target species *Achnanthidium minutissimum* in each culture and treatment combination. Bars (model slope estimates, means ± standard error)

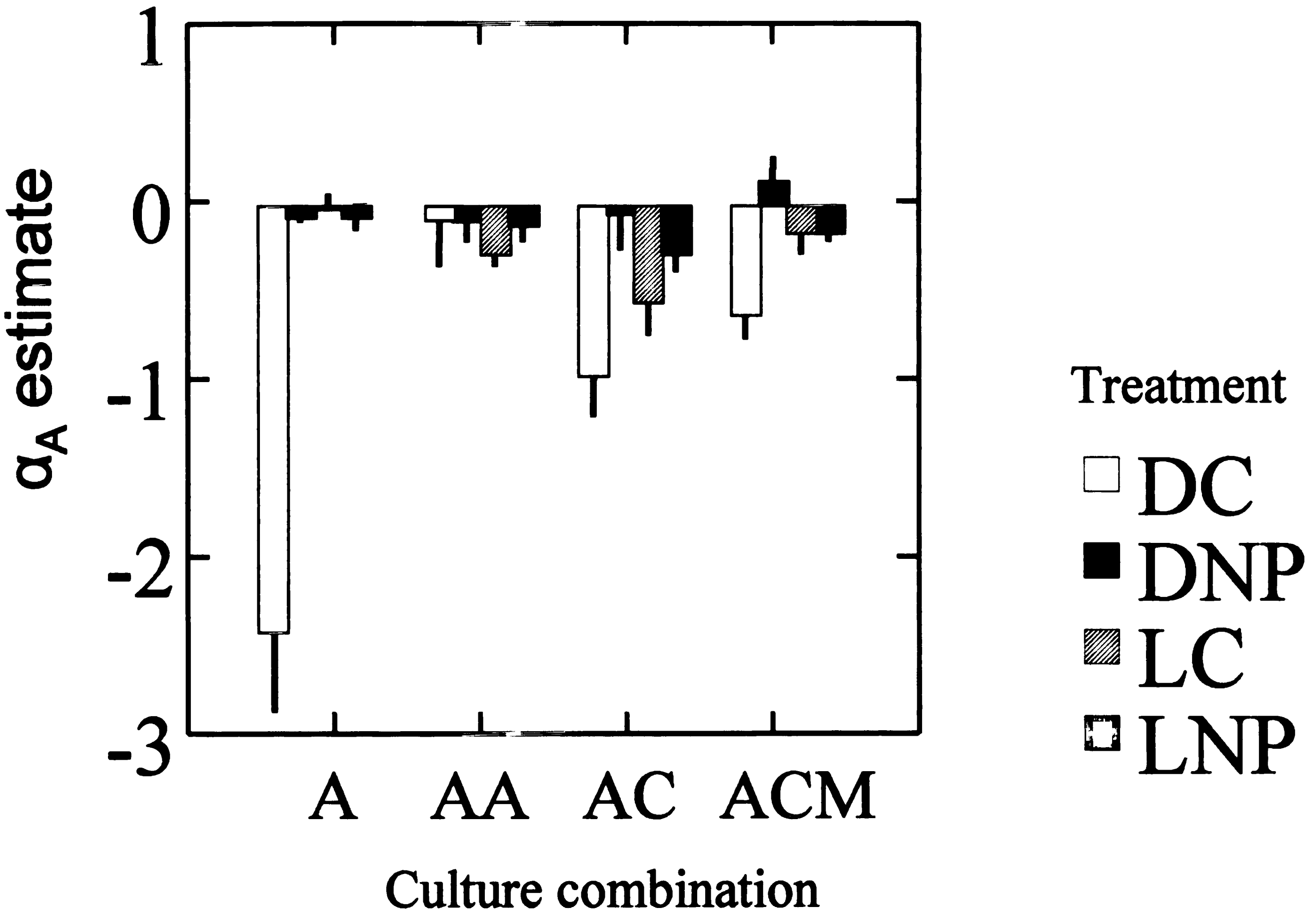
1
0
-1
-2
-3
α_A estimate
A
AA
AC
ACM
Culture combination
Treatment
□ DC
■ DNP
▨ LC
▣ LNP

Figure 2.7

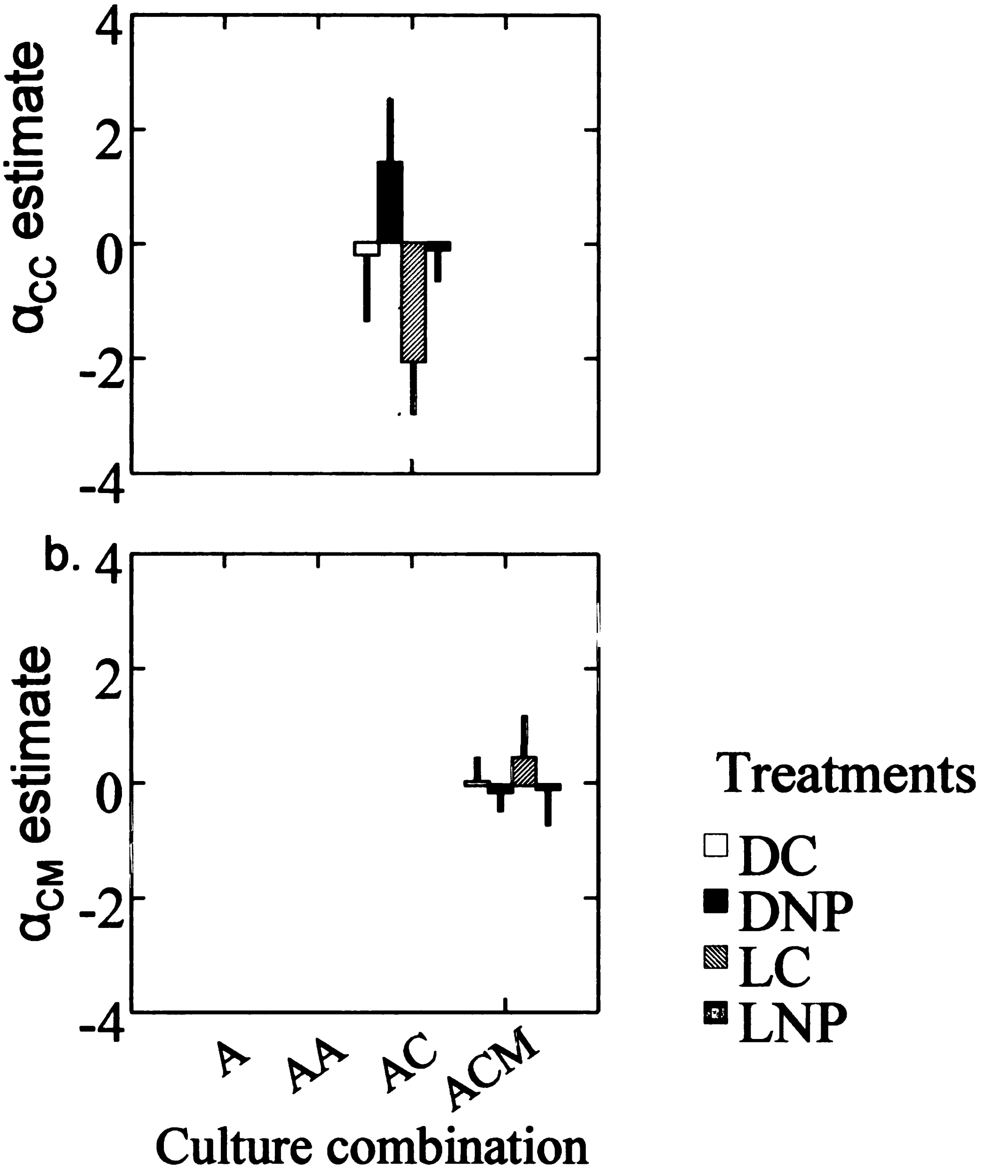

Figure 2.8: Interspecific competition indices (α) estimates in high biomass of neighbor species a. *Cocconeis placentula* var. *lineata*; b. *Cymbella cistula* in each culture and treatment combination. Bars (model slope estimates, means ± standard error)

CHAPTER 3

COMMUNITY ORGANIZATION AND DISTRIBUTIONAL PATTERNS OF BENTHIC DIATOMS FROM STREAMS IN KENTUCKY

INTRODUCTION

The relationship between species abundance (relative, absolute or percent cover) and distribution has been studied for many years (Adrewartha and Birch 1954, Brown 1984, Krebs 1978, Hengeveld and Haeck 1982, Gotelli and Simberloff 1987, Gaston and Blackburn 2000) on the premise that dynamics of resource exploitation by species in local communities are connected with their regional distribution. Almost without exception (Hanski *et al.* 1993), species with wide distribution, i.e. present at a large proportion of sites in a defined region, tend to be more abundant than species with restricted distribution (McNaughton and Wolf 1970, Hanski 1982a). In this context, abundance is the average abundance at sites, where the species population is observed. Either positive (Hanski 1982a) or no (Levins 1969) correlation between abundance and distribution of species has been observed in many groups of organisms (Hanski *et al.* 1993). A positive correlation between species abundance and distribution contradicts the presumed relation expected if ecological specialization was important. Specialization is the ability of a species to exploit and maintain populations in unique environmental conditions (McNaughton and Wolf 1970). Specialists will have higher mean abundance in unique sites, which will result in no or negative correlations between species abundance and distribution in a region.

Study of the abundance-distribution relationship in periphyton may be valuable because species abundance changes greatly along environmental gradients and relative abundance is often reported in large-scale surveys (Pan *et al.* 1996). Most survey studies report algal species (distributional) patterns, but distribution here refers to correspondence between abundance and environmental characteristics. In those studies, usually dominant species are weighted more than species with lower abundance in an ecoregion (Patrick 1961, Lewis 1977, Peterson *et al.* 1993). Algal community structure is a result of complex interactions with the habitat condition (Stevenson 1997) and addressing relationships between species abundance and distribution (as frequency of occurrence) could be valuable. Periphyton abundance and distribution are influenced by disturbance (Peterson *et al.* 1990, Humphrey and Stevenson 1992), hydrology (Biggs and Stokseth 1996), substrate conditions (Peterson and Stevenson 1989), grazing (Hill and Knight 1988, Steinman 1996), and nutrient concentrations and ratios (Peterson and Grimm 1992).

Diatoms are an excellent group for testing the abundance-distribution relationships, because they are mostly microscopic, widely distributed and every species has the potential of establishing populations under a given set of environmental conditions. Diatom species composition is sensitive to environmental changes (van Dam 1982, Winter and Duthie 2000) and diatom community attributes, like species abundance, are used in ecological assessment (Pan *et al.* 1996, Stevenson and Smol 2003). Consideration of both ecological preferences and historical occurrences could provide the accurate understanding of diatom abundance and distribution (Stoermer and Julius 2003). The abundance and distribution relationship in periphyton diatoms within an ecoregion have not been addressed.

The shape of abundance-distribution relationship for diatoms as other organisms might be regulated by one of the following paradigms of community organization: fundamental niche differentiation (FND, Whittaker 1975), shifting competitive hierarchy (Keddy 1989), or balanced continuum (Austin 1985). The FND predicts niche differentiation along a gradient according to limits in species physiological optima (for temperature, pH, alkalinity, and conductivity for example). In the shifting competitive hierarchy (SCH), all taxa have physiological optima at resource availability, but different competitive abilities at resource limitation. Tradeoffs are expected, and poor competitors are displaced to undesirable habitats. The continuum concept (CC) incorporates both theories and predicts gradual changes in variables and gradual changes in communities depending on the nature of the gradients.

According to the FND hypothesis, diatoms that are most abundant at limiting nutrient concentrations will not perform at relatively high rates when nutrients are abundant. Distinguishing between species performance as a limitation due to poor physiological functioning at a given resource level or performance limitation due to resource depletion by neighboring populations in the communities is needed in SCH and CC (Bigelow and Canham 2002). It is unclear which one or combination of the three paradigms is most applicable to diatoms. If the gradient in question provides the full spectrum from limiting conditions to saturation, different species are expected to perform best at the different concentrations (Tilman 1982). Abundance-distribution patterns along those gradients will be easy to distinguish. Species separation according to nutrient preference in streams is expected (Biggs 1996, Stevenson 1995).

The above mentioned patterns and paradigms provide hypotheses that can be tested with several models. The abundance-distribution patterns have been addressed with static niche-based models (Brown 1984, Collins and Glenn 1991, Hanski and Gyllenberg 1993, Sheiner and Rey-Benayas 1997), with mechanistic models (Collins and Glenn 1997) and with metapopulation models (Levins 1969, Hanski 1982a, Gotelli 1992, Tokeshi 1992, van Rensburg *et al.* 2000). The static niche-based model tests whether the positive correlation between abundance and distribution is a sampling artifact. It is assumed that rare species are more difficult to detect than abundant species (McArdle 1990). The abundance-distribution patterns have been addressed with mechanistic models like the model for ecological specialization (Brown 1984). This model is based on the testable hypothesis that ecological generalists are both widely distributed and locally abundant, while specialized species are restricted in distribution and with low or high average abundance. The metapopulation model relates dispersal abilities of species, for a given average abundance, to distribution. For the given average abundance, it could be tested whether species with high ability to disperse have wider distribution (Hanski *et al.* 1993).

Species in streams with minimal human impact can be defined as reference species (Stevenson *et al.* in press). Diatoms from those streams are likely the reference diatoms of the area or region. Those diatoms presumably existed in streams prior to anthropogenic alterations. Reference species are expected to have wide distribution under reference conditions, while specialists (potentially introduced to a region) will have narrow distribution, according to the FND paradigm. In recent years, reference diatom communities have been recognized as important in regional river and stream assessment (the European Union Document 2000). Diatoms are a very diverse group of algae with estimated species of tens to hundreds of thousands (Gordon and Drum 1994, Mann 1999). It is unrealistic to expect a few diatom species to be declared 'most valuable' for protection, rather in current bioassessment, the desire to protect and restore reference communities, regardless of their diversity, is considered an important ecological goal (Karr and Dudley 1981, Stevenson, personal communication).

The goals of this study were to describe and understand the relationship between relative abundance and distribution of diatom species in streams. Diatoms with high abundance were expected to have a narrow distribution at a small number of sites due to specialization. As nutrient loading, conductivity and chloride increase with human disturbance, a loss of reference taxa was expected, according to the niche differentiation hypothesis. Sampling error, specialization and dispersal ability were tested as the possible explanation of the abundance-distribution pattern in diatoms. Diatom communities in reference streams were predicted to be similar, while diatom communities between reference and non-reference streams were expected to differ. Ultimately, I hypothesized that relative abundance and distribution differences could be explained by responses of species-specific growth rate to nutrient enrichment. Therefore, results from the survey, were compared with experimental growth rate results in the same ecoregion.

MATERIAL AND METHODS

Samples from 69 samples from the Knobs ecoregion (northwestern Kentucky and southeastern Indiana) were collected during the April – June periods in 1996 and 1997 (Appendix 3.1). Streams of this region have rapid runoff due to steep watershed topography, which is related to the underlying limestone bedrock formation (McGrain 1983). Streams sampled ranged from 1st to 4th order (Strahler 1957) with most streams being 2nd and 3rd order (Stevenson *et al.* in press). Streams in this region have minimal invertebrate grazing compared to other regions (Riseng *et al.* 2004). Four streams were designated as reference sites and were sampled during both years. Those sites were completely enclosed in nature preserves or human disturbance in the watersheds was low.

Each stream was visited weekly for 8 weeks. At each visit, pH and conductivity (CON) were measured with a YSI meter (YSI Incorporated, Yellow Springs, Ohio, USA); water temperature was determined with a thermometer; and canopy cover (CAN) was assessed with a spherical canopy densiometer. Samples for chloride and nutrients were collected in 2 125-mL acid-rinsed polyethylene bottles. Water in one sample was filtered in the field through 0.45 μm pore-size filters to measure dissolved nutrients. Nutrient samples were stored on ice until returning to the lab where they were frozen until analysis.

Benthic algae were sampled during 1 of the 8 weeks of sampling, which was timed so that algae had greater than 7 days to recover after the last storm event. Benthic algae were sampled from 15 rocks in riffles (3 rocks at 5 random locations) with a spoon and toothbrush into a container. The composite sample was split for different analysis. In this project, subsamples for chlorophyll *a* and enumeration were analysed. Estimates of rock surface areas from which benthic algae were scraped were made in the field by measuring the upper surface of rocks (an exact outline of the cleared from algae surface was estimated with a ruler in the field). Subsamples for chlorophyll *a* were stored on ice until returning to the lab, where they were frozen until processing. Algal enumeration samples were preserved with M3 in the field (APHA 1998).

Laboratory assays

Water samples were analyzed for chloride, nitrate (NO_3-N), and ammonium (NH_4-N) using a Skalar® auto-analyzer. Soluble reactive phosphorus (SRP) was measured using a Hitachi® U-2001 spectrophotometer. Alkalinity (ALK) was assessed according to standard methods (APHA 1998). Silica was determined with ascorbic acid method on a Skalar® auto-analyzer (APHA 1998). To determine total phosphorus (TP) and total nitrogen (TN) concentrations, particulate matters in water samples were oxidized with persulfate and analyzed for SRP and NO_3-N. Chlorophyll *a* was measured spectrophotometrically after extraction from the periphyton samples with 90 % buffered acetone (APHA 1998).

Algal subsamples were analyzed for diatom species composition. Permanent diatom slides were prepared by acid-cleaning to increase the clarity of observing diatoms (Patrick and Reimer 1966) and mounting in Naphrax resin (RI 1.74, Northern Biological Supplies L., Ipswich, UK). A minimum of 600 diatom valves were identified and counted on each slide. The main diatom floras used for identification were Patrick and Reimer (1966, 1975), Krammer and Lange-Bertalot (1986, 1988, 1991 a, b). Additional taxonomic sources like Hustedt (1930 a, b, 1959 – 1966), Lange-Bertalot and Metzeltin (1996) were available at the R. J. Stevenson algal laboratory library (at Michigan State University) and were consulted when needed. Correct taxonomic identification in the dataset was ensured following a quality assurance/quality control (QAQC) protocol on the original counts. A second analyst independently counted samples from the reference streams and four random streams in each year. Relative abundances of diatom taxa in each sample were determined and mean relative abundance for the sites a species occurred was calculated.

The following community characteristics were calculated for each sample: Richness (total number of taxa observed in a site); Shannon-Weaver diversity index $H= - \sum_{(i-s)} p_i \log(p_i)$, where p_i is the proportion of the total count arising from the *i*th species (Shannon and Weaver 1949); evenness (equitability)=H'/ln(richness) (Pielou 1969) and Simpson's dominance index (Simpson 1949), which measures the likelihood of two randomly chosen individuals in a sample to be the same species. If Simpson's dominance index is 0, there is complete evenness and low dominance in a site. For simplicity, diatom species that appeared in more than 10 % of the samples were defined as common. In this study, diatom species that occurred in less than 7 samples were defined as rare. Taxa that appeared as auxospores or a single broken valve without central area were not included in the analyses.

In the reference sites, human disturbance is considered negligible (Stevenson *et al.* in press), so diatoms from those sites were defined as reference taxa. Those species are the species that occur naturally in Kentucky (or not introduced) and in many water bodies, fossil records and habitats. Extensive counts were performed to account for as many rare taxa as possible in the four reference streams. For all reference sites and 8 random non-reference sites, after the original 600 count and the QAQC count, a third count continued until at least five taxa were enumerated with 100 valves (which in diverse sites reached a count of >2500 valves). This protocol ensured accounting for all dominant taxa (more than 5 % relative abundance in each count), allowed observation of more rare taxa in the count and was independent of a predetermined number count.

Experiment

Species-specific growth rates were estimated in different nutrient conditions with an experiment in artificial streams and then compared to survey results. I tested the hypothesis that reference species were growing at higher rates in low nutrient conditions than in high nutrient sites.

Artificial streams at the University of Louisville Stream Facility in Bernheim Forest, Kentucky, were used to manipulate nutrient concentrations (Manoylov, Chapter 1, Figure 1.1). Those streams were built alongside a pristine

3rd order stream, Hart's Run, which was completely enclosed within Bernheim Nature Preserve. Three artificial channels had ambient N and P from Hart's Run (had 17.5-38 µg NO_3-N/L, 6.8-51 µg NH_4-N/L, 1.5-4.4 µg SRP-P/L, and 10.5-11.9 mg SiO_2-Si/L). Three channels had 1024 µg N/L with 50 µg P/L and three channels had 500 µg N/L with 128 µg P/L. Stock solutions with different concentrations of $NaNO_3$ and KH_2PO_4 were delivered from 1 L containers to each channel using peristaltic pumps (Manostat™ model STD). The pumps were set at a different drip rate to achieve the desired nutrient concentration. Nutrient supply was kept at a constant rate throughout the experiment. Light in the experiment was reduced by covering the channels with greenhouse shade cloth that lowered the full sunlight to 10 % incident light, which was comparable to light levels in many habitats of Hart's Run. The experiment was carried out in May 1999, with stream water temperature around 20°C.

Periphyton communities were created by algal colonization of 29.2 cm^{-2} unglazed ceramic tiles, which provided uniform habitat surface simulating rocks in natural streams. Tiles were placed at the lower end of each channel where water flow was most stable. To ensure algal diversity, the initial algal inoculum was gathered from three streams in the Mid-Atlantic Ecoregion. The streams were collected in the survey and had different trophic status: urban Beargrass Creek (with nuisance growths of *Cladophora*), agriculturally impacted Wilson Creek, and the pristine Hart's Run (reference site). Equal amounts of algal suspension from the three streams were mixed, and 250 ml aliquots were placed in each re-circulating experimental stream channel.

A sample of two random tiles (non-adjacent and not from the upstream end, where disturbance from flow was possible) were collected for 16 days of community development in the controlled conditions. The sample was immediately frozen after collection. At the laboratory, algae were scraped from the tiles with a scalpel and toothbrushes. A 10-ml subsample was preserved with M3 (APHA 1998) for algae counts. Semi-permanent syrup mounts were prepared with Tafts Mounting Medium (Stevenson 1984), which allows good resolution of cell wall structure of diatoms without cleaning. Total algal community counts were performed. At least 300 live algal cells (natural units as colonies, filaments etc. in soft algae and diatom frustules) were identified to the lowest taxonomic level and counted. Diatoms dominated the community. Only frustules with a visible, healthy-looking chloroplast were counted. Counts were performed at 1000X on a LEICA DMLB light microscope. Species relative abundances were averaged per treatment.

Careful monitoring and consecutive sampling every 3 days allowed a good estimate of peak biomass. Absolute cell densities (D, cells mm^2) were estimated. Growth rates were calculated based on species density in response to each nutrient treatment. Growth rates (R) were estimated as daily per capita changes in cell density in each circular channel using the following equation: $R = (\ln D_{(10)} - \ln D_{(3)})/(T_{10} - T_3)$, where D is total density, T_3 and T_{10} are days of community development prior to peak biomass, this corresponded to the time of sampling in the survey. Growth rates ($\ln$ cells $cell^{-1}$ d^{-1}) were calculated for each species in control and the NP channels.

Data analysis

The relationship between species abundance and distribution was tested with regression analysis. Following the terminology of the core-satellite hypothesis (Hanski 1982a), a core species was a common diatom that appeared in more than 90 % of the sites, while a satellite species appeared in less than 10 % of the sites (rare). Species in the middle (found in more than 10 % and less than 90 % of the sites) were called intermediate. Based on the number of species in each category, different distribution patterns are possible (skewed to the left or right, unimodal, or bimodal). All categories can vary in abundance.

Several analyses: sampling error, ecological specialization and dispersal abilities were performed to explain the observed patterns of abundance and distribution. In sampling microorganisms, there is a need to test for possible aggregation of species at a subset of sampling sites. The role of sampling error due to diatom aggregation was tested with regression analyses. Parameters of the sampling model were expected to differ from zero (Hanski *et al.* 1993). The probability (p_o) for a taxon to occupy all available sites (69 sites) was calculated with the following equation:

$$\mathrm{Ln}(-\ln(p_o)) \approx \ln(\ln x_{all})-2 * \ln(CV),$$

where x_{all} is the mean species relative abundance for all available sites; CV is species specific coefficient of variation (CV), when measuring mean species relative abundance for all available sites with the observed mean relative abundance (i.e. mean relative abundance for the actual sites observed). With the decrease of a species average abundance (x_{all}) for all available sites and increase of spatial variation (CV for all taxa), the fraction of empty sites in a region will increase (hence the regional distribution (p) will decrease).

For a given abundance, more dispersive species should have wider distribution than less dispersive species (Hanski *et al.* 1993). Phylogenetic relationships between diatom species were not considered in this study. Taxonomic identification of diatoms is usually based on morphological characters (size, shape, attachment etc.) and smaller size has been assigned to good dispersal abilities (Biggs *et al.* 1998). A metapopulation model was tested with multiple regression analysis with two dependent variables, average abundance (for the sites the taxon was found, $\log_e$-transformed) and size, which I assumed was related to dispersal ability. Size was assigned to each diatom species in the following rank order from highest dispersal ability: small length (5-15 μm), medium (15-40 μm) and large (>40 μm, for some *Nitzschia* and *Synedra* species even >100 μm). The independent variable was number of sites (arcsin-sqrt-transformed, method follows Hanski *et al.* 1993). Using ANOVA, the dispersal effect tests whether residuals from the abundance-distribution relationship can be explained with dispersal ability (size).

The physicochemical differences between reference and non-reference sites were tested with a Mann-Whitney nonparametric test. Spearman rank correlations were used to identify relationships between physico-chemical variables in reference and non-reference sites. Only significant relations ($p<0.05$) were reported.

Average species similarity (aveS) within reference and non-reference sites or dissimilarity (aveδ) between the two groups of sites was evaluated with SIMPER procedure (Primer-E Ltd., Clarke and Gorley 2001). This allowed examining the contribution of each diatom species to either similarity within or dissimilarity between reference and non-reference sites. Standard deviation of the means were estimated for S and δ, respectively SD(S) and SD(δ). For both measures, how consistently a diatom species contributes to either aveS or aveδ was measured with the ratios aveS/SD(S) and aveδ/SD(δ) respectively. When SD(S) and SD(δ) were small, the ratios were large and the respective diatom species not only contributed much for the similarity or dissimilarity, but was also consistent in doing so. Diatom species with high discriminating ratio aveδ/SD(δ) were called discriminating species (Clarke and Warwick 2001) and are reported.

The effect of ecological specialization (for all taxa observed) in the abundance-distribution relationship was tested with ANOVA, where ecological specialization was defined in three categories. All common diatoms were included in the analyses. Common reference taxa were hypothesized to be generalists for the region. If there were no human alterations of the habitat, the reference taxa are expected to be widely distributed and with high mean relative abundance. Taxa from non-reference sites (where human influence is expected) are expected to be more specialized. Being interested in the identity of taxa occurring in reference sites not by chance, I took the following approach: if a taxon was found in more than 3 reference sites and some non-reference sites, regardless of its abundance, it was not included in the specialists' number from non-reference sites. Non-reference taxa were expected to have a narrower distribution with high abundance at a few sites. The third category was termed random taxa (appeared in less than 0.5 % of the sites, either reference or non-reference). Those taxa were considered in the sites by chance and no eco-specialization was assigned. Species distribution (proportion of sites in which a species occurred) was arcsin-sqrt-transformed and mean abundance was $\log_e(x + 1)$-transformed.

Presence-absence of all taxa observed in the experiment was compared with the survey. If a diatom species was present in a reference site, but not necessarily in a non-reference site, growth rate was evaluated in the experiment. In the experiment, growth rates were assessed in control (ambient low nutrients) and enriched channels. The potential of higher growth rates of taxa with increase in nitrogen and phosphorus concentration was tested in the experiment. The Spearman correlation coefficient for the relationship between species growth rates in low and high nutrients was determined. If reference taxa grew best at low nutrient concentrations, growth rates of reference taxa were expected to decrease in high nutrients. Statistical analyses were performed with SYSTAT® version 10 (Wilkinson 1990) unless stated otherwise.

RESULTS

Species abundance-distribution relationship, sampling error, aggregation, and dispersal

A positive relationship was observed between diatom species abundance and distribution in the survey, i.e. more abundant species occupied a larger percent of the sites than less abundant species (regression analysis: $r^2 = 0.68$, $p<0.01$; Figure 3.1 a). A total of 184 diatom species were recorded. Three taxa were observed in more than 90 % of the sites sampled. *Achnanthidium minutissimum* was observed at all sites (Table 3.1). This diatom had the highest mean relative abundance for the survey (20.8 %). The other two core species in this study, *Nitzschia dissipata* and *Gomphonema angustatum*, had 6.5 and 15.5 % mean relative abundances, respectively. Sixty-nine percent of the observed taxa were defined as rare because they appeared in less than 10 % of all sites. The highest observed mean relative abundance for a rare taxon was 4.2 % (Figure 3.1 b, Table 3.1).

The observed positive relationship between abundance and distribution was not due to sampling error or aggregation. The coefficient of variation was negatively related to species distributions (Table 3.2). Diatoms with high abundance were found in more sites and had smaller coefficient of variation. The analysis showed that the parameters differed from zero.

In this study, diatom dispersal abilities were not dependent on diatom size ($p=0.75$). High, medium and low dispersal abilities (based on species size) were evenly distributed between sites. Abundance was size dependent ($p<0.0001$, Table 3.3), the most abundant diatom was small, but small diatoms were observed in all categories.

Relation of core-satellite species to ecological conditions

Temperature, canopy cover (measure of light) and pH were not different between the reference and non-reference streams. Silica was higher in the reference streams. All other physico-chemical variables in the reference sites were significantly lower compared to sites with human influence (Table 3.4). Conductivity and chloride were 1.5 times higher in the non-reference sites compared to the reference sites. Nutrients were several orders of magnitude higher also.

In reference sites, TN was positively correlated with N:P ratio and chloride, while TP was negatively correlated with silica and conductivity. N:P ratios were positively related to chloride concentration. Silica was positively correlated to alkalinity and conductivity (Table 3.5). Different correlations between the measured physico-chemical variables were observed in the non-reference streams. TN was positively correlated with TP and chloride. Alkalinity and conductivity increased in the same direction also (Table 3.5). The lower nutrient availability in the reference sites corresponded to lower chlorophyll *a* compared to the non-reference streams (Table 3.6). With the increase in nutrients, chlorophyll *a* increased 3-fold in non-reference sites.

Shannon diversity (Table 3.6) and Simpson's dominance index were lower in the reference sites also (mean 1.6 and mean 0.67 respectively) compared to non-reference sites, where the mean value of Shannon diversity was 2.2 and the mean value of Simpson's index was 0.81. In reference streams 15 to 26 taxa were observed (evenness 0.41 to 0.66), while in sites with human influence, 14 to 45 diatom taxa were observed (evenness 0.36 to 0.82). For the 8 available reference sites, diatom species richness was less than at all sites (Table 3.1).

Average similarity among reference streams was 48 %. Four taxa contributed to 90 % of the similarity, with over 60 % contributed by *A. minutissimum* alone. *Achnanthidium minutissimum* and *Gomphonema angustatum* were the two discriminating species in reference sites, because of their high similarity and low standard deviation measures (discriminating ratios 16.44 and 8.38 respectively, Table 3.7).

Within non-reference streams, average similarity among sites was lower, 34 % (Table 3.8). Thirteen taxa accounted for 90 % of the accounted similarity. *Achnanthidium minutissimum* accounted for only 30.6 % of similarity among the non-reference sites. Three discriminating species in non-reference sites had high similarities and low standard deviations: *A. minutissimum*, *G. angustatum* and *Cymbella affinis* (discriminating ratios 9.15, 6.75 and 5.60 respectively, Table 3.8).

The observed differences in species richness between reference and non-reference sites contributed to the high 68 % dissimilarity between the two groups. Twenty-seven taxa (15 % of all identified) contributed for 90 % of the dissimilarity, with 50 % of it contributed by four taxa (Table 3.9). The highest discriminating species in the between sites dissimilarity was *Cymbella affinis* (ratio 8.67). Additional dissimilarity was contributed by *Gomphonema olivaceum*, *Nitzschia inconspicua*, *Rhoicosphenia abbreviata* and *Navicula gregaria* (Table 3.9). All four discriminating taxa appeared in both reference and non-reference taxa.

Cymbella affinis occurred in 80 % of the samples, but had mean relative abundance of 14.9 % and 8.8 % in reference and non-reference sites, respectively (Tables 3.1 and 3.9). *Achnanthes deflexa* and *Achnanthidium minutissimum* were the most abundant taxa in reference streams. *Achnanthes deflexa* appeared in 62 % of non-reference and 88 % of reference sites (mean relative abundance 5.9 % and 14.9 % respectively). Only *A. minutissimum* and *Cymbella* sp. *K* appeared in all reference and non-reference samples, but differed in mean relative abundance (in reference sites 38 % to 2.8 % and in non-reference sites 18.6 % and 1.8 % respectively for the 2 species).

For the whole dataset, reference taxa had greater average abundance and wider distribution than non-reference taxa (Table 3.10). Non-reference taxa had low average abundance regardless of the distribution pattern. For example: *Rhoicosphenia abbreviata* had 2.8 % mean relative abundance and was found in 54 % of the non-reference sites; *Amphora pediculus* was distributed in 78 % of the sites and also had 2.8 % mean relative abundance, *Navicula lanceolata*, *N. subminuscula*, and *Sellaphora seminulum* appeared in about 17 % of the sites with similar mean relative abundance (2.1-3.2 % mean relative abundance). The abundance of *Achnanthes deflexa* and *Achnanthidium minutissimum* was reduced in half in non-reference sites (Tables 3.1 and 3.9).

In the experiment, growth rates were calculated for 11 taxa. In the survey, those taxa were mostly reference taxa, only *Nitzschia acicularis* and *Fragilaria nanana* were found in the non-reference sites only. The hypothesis of reference taxa growing faster at low nutrients compared to high nutrient conditions was rejected. The potential of higher growth rates of non-reference taxa with increasing nitrogen and phosphorus concentration was confirmed in the experiment, where growth rates of the reference taxa decreased in general or did not change with enrichment (taxa like: *Achnanthidium minutissimum*, *Achnanthes deflexa*, *Encyonema minutum*, *Fragilaria nanana*, *F. vaucheriae*, and *Cymbella cistula*). In low nutrient concentrations, representatives of the genus *Nitzschia* (*N. dissipata*, *N. palea*, and *N. acicularis*) had lower growth rates than the other taxa from reference sites in the survey, but their growth rate increased more than reference taxa with increasing nutrients (Figure 3.2).

DISCUSSION

In this study, a positive abundance-distribution relationship was observed for benthic diatoms. *Achnanthidium minutissimum* had highest mean relative abundance in the survey and was found at all sites. Core diatom species comprised only 2 % of the diatom community, while the majority of diatoms observed were satellite species. The large number of rare taxa observed did not change the positive correlation between abundance and distribution. Diatom communities from reference streams had lower diversity and higher similarity than non-reference stream communities. Reference and non-reference diatom communities were highly dissimilar. A relatively small number of diatom taxa contributed to the observed dissimilarity. Diatoms observed in the reference sites were observed in many non-reference sites, but in significantly lower abundance. All non-reference diatoms were in lower abundance regardless of the number of sites in which they appeared.

With an increase in nutrients, eutrophic species grow faster when compared with diatom species from the reference community. The robustness of the pattern was confirmed with the experimental results. In the experiment, conductivity, temperature and chloride were constant; only nutrient concentrations were manipulated. Reference species like *Achnanthidium minutissimum* and *Achnanthes deflexa* had relatively high growth rates in low nutrients and continued to grow with increasing nutrients, but growth of non-reference taxa, like representatives of *Nitzschia*, were stimulated more than the achnanthoid diatoms in higher nutrients. Thus, reference taxa could occur in non-reference conditions, but non-reference taxa were not likely to occur in reference conditions because they could not grow well in low nutrient conditions. This finding implies nestedness of the diatom taxa from reference streams within communities in non-reference streams, as patterns of nested subsets of species can be related in this study to nested physiological tolerances and colonization ability, but not to motility as found in other organisms (Greve *et al.* 2005).

In the abundant literature addressing the abundance-distribution relationships, only one relates to algae. Lewis (1977) found an asymmetric relationship for phytoplankton, which was skewed to the right with most taxa in a few sites. That distribution was independent of mean abundance and resource supply. The relationship was specific for green algae, bluegreen algae, and diatoms and varied with grazer conditions. In the present study, I found a strong positive relationship between abundance and distribution, common taxa were most abundant and had wider distribution.

Biologically, the appearance of *Achnanthidium minutissimum* at all sites and with high mean relative abundance can be explained with better colonization, the relatively fast growth of this diatom and high tolerance for low nutrient concentrations. Tradeoff theory suggests that there should be optimal conditions, so if *A. minutissimum* was at optimal growth in the reference sites, different taxa should have high abundance at the non-reference sites. The mean abundance of *A. minutissimum* in the reference sites was significantly higher compared to all sites. In the literature, *Achnanthidium minutissimum* was found in 79 % of samples throughout the United States, which include great variability in environmental conditions (Potapova and Charles 2003). In areas with minimum human disturbance like Alaska, *A. minutissimum* was the most abundant species in a four year study and community composition did not change significantly with nutrient increase (Peterson *et al.* 1993).

The significant decrease in relative abundance of reference taxa with increasing human influence was due to increases in abundance of non-reference species like, *Nitzschia dissipata* and *N. inconspicua*. None of the observed diatoms had high abundance and low distribution. Several species were observed only in non-reference sites: *Amphora pediculus*, *Diatoma vulgaris*, *Naviucla cryptotenella*, and *Rhoicosphenia abbreviata*. Those species were distributed in up to 79 % of the sites, but their mean abundance was never above 7 % for any site. None of the rare diatoms had more than 5 % mean abundance for any site observed.

Niche differentiation was potentially influenced by nutrients, but was not influenced by light, temperature, and pH, because those were the same in the reference and non-reference sites. Effects of grazing on taxa abundance and distribution were assumed to be low for all natural streams in Kentucky (Riseng *et al.* 2004) and were not directly tested in this survey. *Achnanthidium minutissimum* and *Achnanthes deflexa* are understory species, resistant to grazing (Steinman 1996). If grazing was important, differences in abundance of overstory taxa like *Gomphonema angustatum*, *G. olivaceum*, *Cymbella affinis* and other stalked diatoms would be expected. Those taxa were abundant in the reference and non-reference sites in similar pattern with the experimental study where grazers were excluded. The same relationship of high abundance of the grazer-resistant *Achnanthidium minutissimum* and grazer-sensitive *C. affinis* in all experimental channels was observed.

The observed pattern did not follow completely the core-satellite hypothesis (Hanski 1982a), which predicts a positive abundance-distribution relationship and a bimodal pattern in species numbers in the core and satellite categories. This pattern has been observed in different organisms and along multiple gradients (Gibson *et al.* 1999, McGeoch and Gaston 2002), but it was not evident for benthic diatoms. Diatoms with high abundance were found in more sites and had a smaller coefficient of variation. Thus, the prediction of ecological specialization was not supported.

Sample collection, sampling area, and the number of samples were appropriate to study the abundance-distribution relationship (McGeogh and

Gaston 2002). However, the degree of environmental heterogeneity was minimized by sampling only riffles, rocks, and streams within an ecoregion. Environmental heterogeneity among sampled habitats may not have been sufficient to observe ecological specialization of diatom taxa. Different species composition would be expected if, for example, depositional sampled from streams and wetlands were sampled at different temporal and spatial scales. Diatom specialization has been observed in wetlands, springs and streams with metal or acid contamination (Stevenson *et al.* 1996). In diatom studies, specialization has been based on single species (Raschke 1993), on groups of indicator species (Schoeman 1976) or on the whole community (Pan *et al.* 1996). Depending on the scale of the ecological assessment, diatoms respond rapidly to environmental changes and provide useful assessment of the biotic integrity of streams.

Distribution based on dispersal ability was not different for the different diatom size groups. Diatom species differ in size (from 2 µm to 2 mm in some marine forms, Round *et al.* 1990) and all have potentially exceptional powers of dispersal (Round 1981). Air, insects and birds are considered effective dispersal agents. Direct research on diatom dispersal is old and was not directed to streams (Schlichting 1960, 1964, 1969). Circumstantial evidence of the limitless dispersal abilities of diatoms can be obtained from the literature (Behre and Schwabe 1970 in Mann 1999). Diverse freshwater diatom communities were observed on an island only 5 years after volcanic eruption in the ocean. Diatom dispersal is poorly understood, but in this research, dispersal was not related to size. In this study, size was related to mean abundance, as the most abundant and distributed diatom was small, but several small diatoms were found in the rare category. Smaller diatoms are known to have faster immigration (r-selection), but *A. minutissimum* (a small diatom) was a K-selection species because of the persistence pattern observed in this study.

This research did confirm the prediction of the fundamental niche differentiation hypothesis, that diatom that were most abundant in limiting nutrient concentrations would not perform as well with increasing nutrients. Reference species persisted in sites with human influence, but were less abundant. Changes in species membership followed the shifting competitive hierarchy hypothesis: non-reference species grew faster, compared to reference taxa with increasing nutrient supplies. The relationship predicted by the continuum concept with gradual changes in variables and gradual changes in communities would also explain the observed relationships. The direction of negative change (predicted by the continuum concept hypothesis) as mean relative abundance of reference taxa gets lower with increase of human influence, does not relate to distribution yet. Brown (1984) introduced the ecological specialization hypothesis, where one group of species utilizes more abundant and widespread resources than a second group of species, suggesting low specialization for group one. For the non-reference taxa, lower observed abundance might still warrant future dominance in a site, because the transition period between competitive exclusion of high nutrient species in diatoms is unknown.

In conclusion, diatom species with high mean relative abundance had the widest distribution. Species from non-reference sites had narrower distribution and lower abundance. Mean relative abundance of reference species decreased with increasing nutrients and chloride. Non-reference sites had higher diversity, lower evenness and lower richness than reference sites. Growth rates of non-reference taxa increased faster than reference taxa with increasing nutrient enrichment related to anthropogenic influence. Complete taxa turnover and competitive exclusion or physical loss of the reference taxa was not observed, however partial turnover in community composition was observed. These patterns were due to the species-specific abundance and distribution relationships of a relatively small number of diatom species.

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Table 3.1: Taxa names and abbreviation for diatoms found in the survey of all 69 sites and 8 reference sites; N-ascending number of sites; mean RA-mean relative abundance.

		All sites		Reference	
Taxon	ABBR	N	mean RA	N	mean RA
Achnanthidium minutissimum (Kütz.) Carn.	ACM	69	20.8	8	38.0
Nitzschia dissipata (Kütz.) Grun.	NID	64	6.5	5	1.5
Gomphonema angustatum (Kütz.) Rabh.	GOA	62	15.5	7	19.0
Navicula minima Grun.	NAM	59	2.6	7	0.4
Gomphonema olivaceum (Lyngb.) Kütz.		58	7.6	5	3.2
Cymbella affinis Kütz.	CMA	57	10.5	6	11.2
Navicula veneta Kütz.		56	4.0	5	1.8
Surirella minuta Bréb.	SUM	52	2.2	2	1.5
Achnanthes deflexa Reimer	ACD	50	9.3	7	14.9
Cymbella sp.K	CMK	50	2.7	8	2.8
Amphora pediculus (Kützing) Grun.	AMP	48	2.8		
Planothidium lanceolatum (Bréb. ex Kütz.) L.-B.		47	0.9	2	0.1
Diatoma vulgaris Bory		45	1.8		
Navicula cryptotenella Lange-Bert.	NAC	43	3.5		
Reimeria sinuata (Greg.) Kocio. & Stoer.		43	1.6	2	0.5
Navicula gregaria Donk.		39	3.0	2	0.2
Nitzschia sociabilis Hust.		39	1.2		
Caloneis bacillum (Grun.) Cl.		38	1.0	2	0.6
Encyonema silesiacum (Bleisch) Mann		37	1.5	7	2.6
Gomphonema minutum (Ag.) Ag.		36	1.8	4	1.6
Meridion circulare (Grev.) Ag.		35	1.0	6	1.7
Synedra ulna (Nitz.) Ehr.	SYU	35	1.2	3	1.2
Rhoicosphenia abbreviata (Kütz.) Mann	ROC	33	6.5		
Nitzschia inconspicua Grun.	NII	32	8.5	2	0.5
Navicula tripunctata (O. F. Müll.) Bory		31	0.8		
Nitzschia palea (Kütz.) W. Sm.	NIP	31	1.3	2	0.7
Cocconeis pediculus Ehr.		24	1.9	2	0.2
Nitzschia frustulum (Kütz.) Grun.		23	2.6	2	0.2
Navicula arvensis Hust.		22	0.4	3	0.5
Navicula meniscus Schumann		22	1.0		
Nitzschia perminuta (Grun.) Peragallo		22	0.5		
Gomphonema parvulum (Kütz.) Kütz.		21	7.7	2	0.7
Nitzschia palea var. tenuirostris Grun.		21	0.9		
Nitzschia palea var. debilis (Kütz.) Grun.		20	0.9	1	0.2
Nitzschia amphibia Grun.		18	0.7		
Fragilaria vaucheriae (Kützing) Petersen	FRV	17	1.3	1	6.0
Cymbella delicatula Kütz.		15	1.7	5	2.9
Navicula capitatoradiata Germain		15	0.6		
Nitzschia linearis (Ag. ex W. Sm.) W. Sm.		14	0.5		
Achnanthes conspicua Mayer		12	1.0		
Encyonopsis microcephala (Grunow) Krammer		12	1.3	2	2.4
Navicula cryptocephala Kütz.		12	0.3	1	0.3

Table 3.1: cont'd

		All sites		Reference	
Taxon	ABBR	N	mean RA	N	mean RA
Navicula lanceolata (Ag.) Ehr.	NAL	12	3.2		
Navicula subminuscula Mang.	NASM	12	2.1		
Nitzschia solita Hust.		12	0.6		
Cocconeis placentula var. lineata (Ehr.) V. H.		11	0.8		
Sellaphora seminulum (Grun.) Mann	NASL	11	3.1		
Nitzschia acicularis (Kütz.) W. Sm.	NIA	11	1.2		
Achnanthes ventralis (Krasske) Lange-Bert.		9	2.5	2	6.6
Eucocconeis laevis (Oestrup) Lange-Bertalot		9	0.7	4	1.0
Craticula molestiformis (Hustedt) Lange-Bert.		9	0.4		
Nitzschia sinuata var. tabellaria (Grun.) G.inV.H.		9	0.2		
Surirella angusta Kütz.		9	0.3		
Mayamaea atomus (Kützing) Lange-Bertalot		8	0.9		
Cocconeis placentula Ehr.		7	0.2		
Cyclotella meneghiniana Kütz.		7	0.2		
Fragilaria capucina var. gracilis (Østr.) Hust.		7	0.7		
Navicula exilis Kütz.		7	0.8	2	0.2
Aulacoseira granulata (Ehr.) Simonsen		6	0.3	1	0.1
Cocconeis placentula var. euglypta (Ehr.) Cl.		6	0.3		
Encyonema minutum (Hilse) Mann	ENM	6	1.4	1	5.3
Fragilaria capucina var. rumpens (Kütz.) L.-B.	FRR	6	4.2	3	7.4
Nitzschia heufleriana Grunow		6	0.3		
Cymbella cistula (Ehr.) Kirchn.	CMC	5	0.9	3	1.0
Fragilaria capucina Desm.		5	0.2	2	0.2
Synedra delicatissima W. Sm.		5	0.2		
Navicula cincta (Ehr.) Ralfs		5	0.4		
Navicula recens Lange-Bert.		5	0.2		
Cyclostephanos invisitatos (H.& H.)Th.,Sto.& H.		4	0.2		
Melosira varians Ag.		4	0.3		
Mayamaea atomus var. permitis (Hustedt) L.-B.		4	0.7		
Fistulifera pelliculosa (Brébisson ex Kütz.) L.-B.		4	0.2		
Nitzschia pusilla Grun.		4	0.3		
Synedra acus Kütz.		4	1.2		
Amphora montana Krasske		3	0.1		
Encyonopsis falaisensis (Grunow) Krammer		3	0.5		
Cymbella hebridica Grunow ex Cleve		3	0.1		
Fragilaria nanana Lange-Bert.	FRN	3	1.1		
Psammodictyon constrictum (Gregory) Mann		3	0.2		
Psammothidium chlidanos (H. et Heller.) L.-B.		2	1.2		
Achnanthidium exilis (Kützing) Round et Bukh.		2	1.6		
Achnanthidium affine (Grunow) Czarnecki		2	1.0		
Achnanthes gracillima Hustedt		2	0.3	2	0.3
Cocconeis placentula var. pseudolineata Geitl.		2	0.2		
Cyclotella stelligera (Cl. & Grun.) V. H.		2	0.1		

Table 3.1: cont'd

		All sites		Reference	
Taxon	ABBR	N	mean RA	N	mean RA
Cymbella mesiana Choln.		2	0.2		
Denticula kuetzingii Grun.		2	0.4		
Eunotia bilunaris var. mucophila L.-B. & Nör.		2	0.3		
Fragilaria bidens Heiberg		2	0.5		
Fragilaria capucina var. mesolepta Rabh.		2	0.6		
Tabularia fasciculata (Ag.) Williams and Round		2	1.1		
Gomphonema affine Kütz.		2	1.1		
Gomphonema truncatum Ehr.		2	0.2		
Navicula harderii Hust.		2	0.3		
Fallacia monoculata (Hustedt) Mann		2	0.2		
Navicula subrotundata Hust.		2	0.2		
Nitzschia capitellata Hust.		2	0.3		
Nitzschia fonticola Grun.		2	0.2		
Nitzschia perspicua Choln.		2	0.5		
Fragilaria tenera (Smith) Lange-Bertalot		2	0.3		
Karayevia clevei (Grunow) Kingston		1	0.2		
Eucocconeis flexella (Kützing) Cleve		1	0.3		
Planothidium hauckianum (Grunow) R.et Bukht.		1	1.5		
Psammothidium lauenburgianum (Hust.) R.et Bukht.		1	0.2		
Psammothidium marginulatum (Grun) R.et Bukht.		1	0.2		
Achnanthes minutissima var. jackii (Rab.) L.-B.		1	1.0		
Achnanthidium saprophila (Kob.et May.)R.et Bukht.		1	0.3		
Rossithidium pusillum (Grunow) R.et Bukht.		1	0.5		
Achnanthidium kranzii (L.-B.) R.et Bukht.		1	1.8		
Amphora ovalis (Kütz.) Kütz.		1	0.2		
Asterionella formosa Hassal		1	0.2		
Aulacoseira alpigena (Grun.) Krammer		1	0.2		
Caloneis tenuis (Greg.) Kram.		1	0.2		
Cymbella amphioxys (Kütz.) Cl.		1	0.8		
Cymbella obscura Krasske		1	0.3		
Encyonema prostratum (Berkeley) Kützing		1	0.3		
Cymbella simonsenii Krammer		1	0.1	1	0.1
Denticula tenuis Kütz.		1	0.2		
Diploneis ovalis (Hilse ex Rabh.) Cl.		1	0.1		
Epithemia adnata (Kütz.) Bréb.		1	0.2		
Eunotia bilunaris (Ehr.) Mills		1	0.1		
Eunotia exigua (Bréb. ex Kütz.) Rabh.		1	0.2		
Eunotia subarcuatoides Alles et al.		1	0.1		
Fragilaria capucina var. distans (Grun.) Lange-Bert.		1	0.2		
Fragilaria parasitica var. subconstricta Grun.		1	0.2		
Fragilaria capucina var. radians (Kütz.) Lange-Bert.		1	0.5		
Fragilaria crotonensis Kitton		1	0.7		

Table 3.1: cont'd

		All sites		Reference	
Taxon	ABBR	N	mean RA	N	mean RA
Gomphonema sarcophagus Greg.		1	1.0		
Gomphonema augur var. sphaerophorum (Ehr.) L.-B.		1	0.1		
Gomphonema gracile Ehr. emend. V. H.		1	0.3		
Gomphonema olivaceoides Hustedt		1	0.9		
Gyrosigma eximium (Thwaites) Boyer		1	0.2		
Melosira undulata (Ehr.) Kütz.		1	0.3		
Craticula accomoda (Hustedt) Mann		1	0.2		
Diadesmis confervacea Kützing		1	0.2		
Navicula ingenua Hust.		1	1.0		
Navicula lenzii Hust.		1	0.3		
Navicula germainii Wallace		1	0.3		
Navicula salinarum Grun.		1	0.2		
Geissleria schoenfeldii (Hustedt) L.-B. et Metzeltin		1	0.3		
Navicula stroemii Hust.		1	0.3		
Navicula tantula Hust.		1	0.3		
Navicula tenelloides Hust.		1	0.3		
Navicula trivialis Lange-Bert.		1	0.3		
Nitzschia amphibioides Hust.		1	0.1		
Nitzschia bacillum Hust.		1	0.3		
Nitzschia clausii Hantz.		1	0.2		
Tryblionella hungarica (Grun.) Mann		1	0.3		
Nitzschia recta Hantz. ex Rabh.		1	0.3		
Nitzschia subconstricta Grunow		1	0.3		
Nitzschia tropica Hust.		1	0.2		
Pinnularia gibba var. mesogongyla (Ehr.) Hust.		1	0.2		
Stauroneis thermicola (Peters.) Lund		1	0.2		
Stephanodiscus minutus H. L. Sm.		1	1.1		
Stephanodiscus tenuis Hust.		1	0.5		
Surirella brebissonii var. kuetzingii Kr. & L.-Bert.		1	0.3		
Thalassiosira pseudonana Hasle & Heimdal		1	0.2		

Table 3.2: Test of the influence of sampling on the abundance-distribution relationship for Kentucky stream diatoms. Species-specific coefficient of variation (CV) and average abundance (x_{all}) were calculated across all sites. SE- standard error, *p* – significance level.

Variable	Estimate	SE	*p*
Intercept	0.04	0.09	0.66
$\ln(\ln(x_{all}))$	0.99	0.03	<0.0001
$-2*\ln(CV)$	1.99	0.03	<0.0001

Regression analysis: Model: R^2=0.98, $F_{2,188}$=5095.22, p<0.0001

Table 3.3: Effect of species dispersal abilities on abundance and distribution relationship for Kentucky stream diatoms.

Variable	Estimate	SE	*p*
Intercept	-4.62	2.04	0.02
Abundance	0.14	0.01	<0.0001
Dispersal ability	0.28	0.87	0.75

Multiple regression analysis: Model $R^2=0.43$, $F_{2,188}=71.93$, $p<0.0001$

	Reference sites				Non-reference sites				
Variables	mean	±SE	min	max	mean	±SE	min	max	*p*
air temperature °C	15.9	2.1	6	32	15.8	0.7	3	36	n.s
water temperature °C	13.6	1	6.8	19.7	14.3	0.5	5	25.9	n.s
canopy cover (%)	24.36	3.69	0	87	29.02	1.3	0	88	n.s.
conductivity (µS cm-1)	220	17.9	107	322	345	16	105.2	804	<0.001
alkalinity (mg L-1)	75.3	7.6	51	120	113.5	6.3	12	208	0.02
pH	8	0	8	8	8.1	0	7	9	n.s
Chloride (meq L-1)	2.5	1	0.8	7.1	7.3	0.7	1.6	21.3	0.01
NO_3-N (µg N/L)	204.7	67.9	27.8	789.6	957.0	123.9	19.2	6645.2	0.01
NH_4-N (µg NH_4/L)	12.7	2.4	2.1	32.6	23.9	3.5	3.8	263.0	<0.001
TN (µg N/L)	308.1	75.5	2.6	876.0	1131.8	131.0	38.0	7358.0	0.04
SRP (µg P/L)	6.6	1.4	1.0	20.6	31.6	11.7	0.0	940.9	<0.001
TP (µg P/L)	9.3	1.9	2.6	27.1	51.1	16.7	2.3	1379.9	0.01
SI (mg L-1)	8.9	0.8	5.0	12.0	6.5	0.3	1.0	17.0	0.01
NP (molar ratio)	86.3	24.5	0.5	298.0	107.0	18.2	4.6	688.0	n.s

Table 3.4: Physicochemical measurements in reference and non-reference sites. *p*= significance level

Table 3.5: Correlations between physicochemical variables in reference and non-reference streams; (*) significance level $p<0.05$.

	Reference							
	TN	TP	NP	Si	Chloride	ALC	CON	CAN
TN	1							
TP	0.19	1						
NP	0.91*	-0.17	1					
Si	-0.31	-0.97*	0.02	1				
Chloride	0.96*	0.06	0.90*	-0.19	1			
ALC	-0.58	-0.62	-0.33	0.75*	-0.55	1		
CON	-0.41	-0.88*	-0.16	0.90*	-0.24	0.53	1	
CAN	-0.05	0.06	0.07	-0.07	-0.27	0.22	-0.35	1
	Nonreference							
TN	1							
TP	0.65*	1						
NP	0.13	-0.3	1					
Si	-0.22	-0.17	-0.05	1				
Chloride	0.73*	0.55	-0.05	-0.22	1			
ALC	0.27	0.2	-0.05	-0.55	0.29	1		
CON	0.49	0.5	-0.12	-0.49	0.55	0.83*	1	
CAN	0.18	0.07	-0.2	0.17	0.1	0.21	0.03	1

	Reference sites				Non-reference sites				
Attributes	mean	±SE	min	max	mean	±SE	min	max	*p*
Chlorophyll *a* (µg cm^{-2})	5.04	4.7	0	33.2	15.8	1.72	0.16	257.9	0.03
Richness	20	2.38	12	26	28.3	0.88	14	45	<0.001
Evenness	0.55	0.03	0.41	0.66	0.67	0.01	0.36	0.82	<0.001
Diversity	1.6	0.13	1.3	2.2	2.2	0.05	1.1	2.9	<0.001
Simpson's dominance	0.67	0.03	0.58	0.78	0.81	0.01	0.48	0.92	<0.001

Table 3.6: Community attributes in reference and human disturbance sites. *p*= significance level

Taxa	aveS	SD(S)	aveS/SD(S)	?(aveS)%	Cum%
Achnanthidium minutissimum (Kützing) Czar.	29.26	1.78	**16.44**	61.5	61.5
Achnanthes deflexa Reimer	6.86	0.9	7.62	14.4	75.9
Gomphonema angustatum (Kütz.) Rabh.	6.03	0.72	**8.38**	12.7	88.6
Cymbella sp. K	0.82	0.48	1.71	1.7	90.3

Table 3.7: Diatom species average similarity (aveS) in reference sites, contribution from each species; species are ordered in decreasing contribution with 90 % cumulative contribution (Cum %) as a cut-off percentage; (SD)S – standard deviation of the S (*i*th species) values; aveS/SD(S) - discriminating ratio, highest in bold; ∑(aveS) % - average percent contribution to similarity by the (*i*th species).

Table 3.8: Diatom species average similarity (aveS) in non-reference sites, contribution from each species; species are ordered in decreasing contribution with 90 cumulative contribution (Cum %) as a cut-off percentage; (SD)S – standard deviation of the S (*i*th species) values; aveS/SD(S) - discriminating ratio, highest in bold; ∑(aveS) % - average percent contribution to similarity by the (*i*th species).

Taxon	aveS	SD(S)	aveS/SD(S)	? aveS%	Cum%
Achnanthidium minutissimum (Kützing) Cza	10.43	1.14	**9.15**	30.62	30.6
Gomphonema angustatum (Kütz.) Rabh.	4.86	0.72	**6.75**	14.28	44.9
Nitzschia dissipata (Kütz.) Grun.	3.21	0.93	3.45	9.44	54.3
Cymbella affinis Kützing	2.91	0.52	**5.60**	8.55	62.9
Gomphonema olivaceum (Lyngb.) Kütz.	2.57	0.68	3.78	7.53	70.4
Achnanthes deflexa Reimer	1.79	0.49	3.65	5.26	75.7
Navicula veneta Kütz.	1.14	0.6	1.90	3.36	79.1
Navicula cryptotenella Lange-Bert.	0.71	0.52	1.37	2.09	81.1
Navicula minima Grun.	0.66	0.49	1.35	1.92	83.1
Cymbella sp. K	0.62	0.54	1.15	1.81	84.9
Surirella minuta Bréb.	0.59	0.62	0.95	1.74	86.6
Nitzschia inconspicua Grun.	0.59	0.22	2.68	1.73	88.3
Amphora perpusilla (Grun.) Grun.	0.59	0.46	1.28	1.73	90.1

Table 3.9: Diatom species average dissimilarity (ave δ) between reference and non-reference sites, contribution from each species; species are ordered in decreasing contribution with 90 % cumulative contribution (Cum %) as a cut-off percentage; meanRAnr- mean relative abundance in non-reference sites, compare with mean RA in reference sites Table 3.4; (SD)δ – standard deviation of the δ (*i*th species) values; aveδ/SD(δ) - discriminating ratio, highest in bold; ∑(aveδ) % - average percent contribution to similarity by the (*i*th species).

Taxon	meanRAnr	ave δ	ave δ (SD)	δ/δSD	∑ave δ%	Cum%
Achnanthidium minutissimum	18.6	12.67	1.79	**7.08**	18.7	18.7
Gomphonema angustatum	11.6	8.42	1.08	**7.80**	12.5	31.2
Cymbella affinis	8.8	6.85	0.79	**8.67**	10.1	41.3
Achnanthes deflexa	5.9	5.97	1.36	**4.39**	8.8	50.1
Gomphonema olivaceum	7	3.27	0.75	**4.36**	4.8	54.9
Nitzschia dissipata	6.6	3.04	0.93	3.27	4.5	59.5
Nitzschia inconspicua	4.5	2.25	0.41	**5.49**	3.3	62.8
Rhoicosphenia abbreviata	6.5	1.75	0.47	3.72	2.6	65.4
Navicula veneta	3.5	1.75	0.67	2.61	2.6	68
Cymbella sp. K	1.8	1.57	1	1.57	2.3	70.3
Fragilaria capucina var. rumpens	0.1	1.41	0.59	2.39	2.1	72.4
Navicula cryptotenella	3.5	1.22	0.6	2.03	1.8	74.2
Navicula minima	2.5	1.17	0.52	2.25	1.7	75.9
Amphora pediculus	2.8	1.13	0.53	2.13	1.7	77.6
Navicula gregaria	1.9	0.95	0.25	**3.80**	1.4	79
Encyonema silesiacum	0.6	0.94	1	0.94	1.4	80.4
Surirella minuta Bréb.	1.8	0.92	0.66	1.39	1.4	81.8
Achnanthes ventralis	0.2	0.86	0.56	1.54	1.3	83
Cymbella delicatula	0.2	0.77	0.92	0.84	1.1	84.2
Gomphonema minutum	1	0.69	0.48	1.44	1	85.2
Meridion circulare	0.4	0.68	0.84	0.81	1	86.2
Diatoma vulgaris	1.8	0.64	0.45	1.42	1	87.1
Reimeria sinuata	1	0.52	0.73	0.71	0.8	87.9
Nitzschia frustulum	1	0.49	0.31	1.58	0.7	88.6
Fragilaria vaucheriae	0.3	0.48	0.47	1.02	0.7	89.3
Synedra ulna	0.7	0.45	0.51	0.88	0.7	89.9
Nitzschia sociabilis	1.2	0.37	0.64	0.58	0.6	90.6

Table 3.10: Effect of ecological specialization on the average abundance and distribution of Kentucky stream diatoms. The table gives means ± standard error.

Category	Average $\log_e$-abundance	Distribution	n
reference (gen)	1.18 ± 0.12	0.74 ± 0.06	37
random	0.33 ± 0.02	0.14 ± 0.00	113
nonreference (sp)	0.61 ± 0.07	0.45 ± 0.03	39
ANOVA:			
R^2	0.38	0.65	
F	57.66	175.26	
p	<0.001	<0.001	
df_{model}/df_{total}	2/188	2/188	

Figure 3.1: Relationship between diatom species abundance and distribution (as percent of sites occupied) in the survey for all sites (n=69); a. A scatter plot of mean relative abundance for the sites occupied; b. A histogram number of species and percent sites occupied (second y axes represents the proportion of taxa in the bars) against their distribution

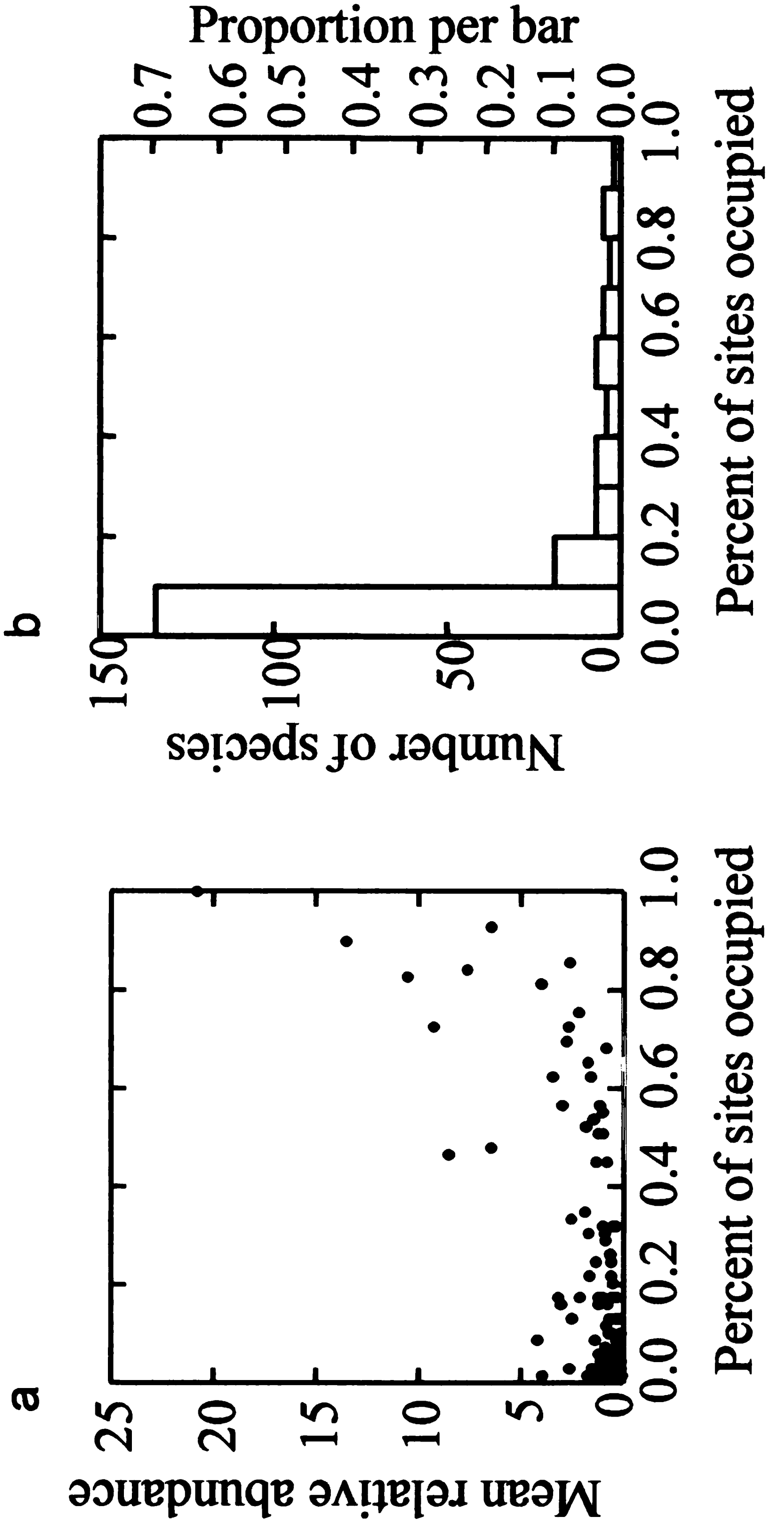
a
Mean relative abundance
25
20
15
10
5
0
0.0
0.2
0.4
0.6
0.8
1.0
Percent of sites occupied
b
Number of species
150
100
50
0
Proportion per bar
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.2
0.4
0.6
0.8
1.0
Percent of sites occupied

Figure 3.1.

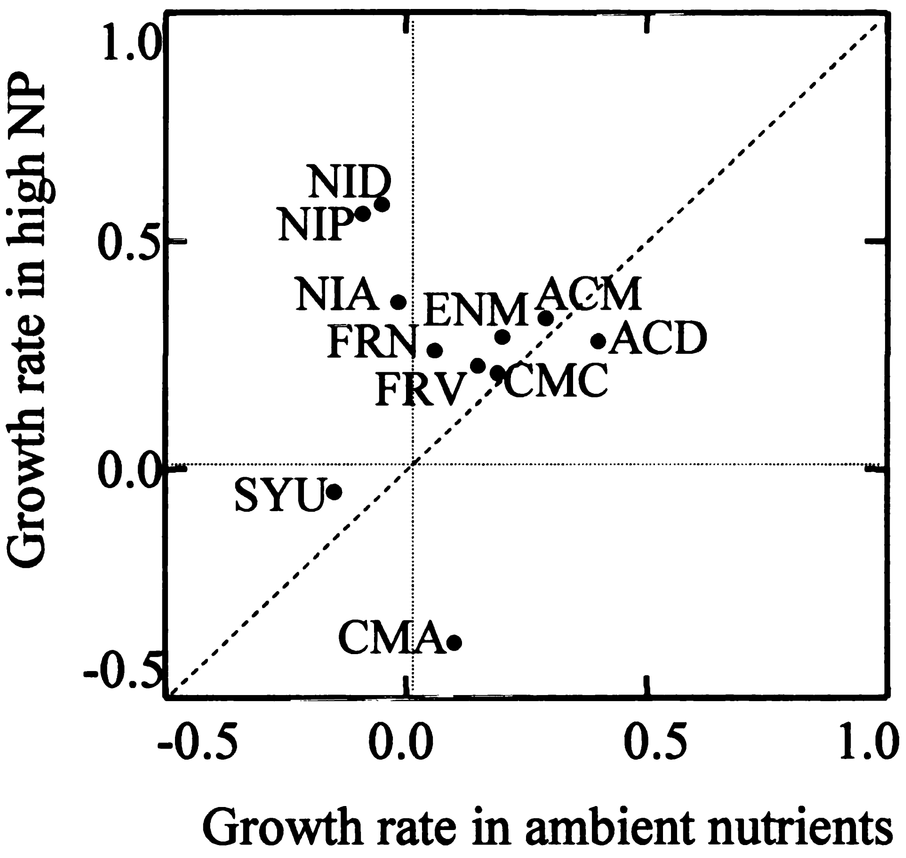

Figure 3.2: Species growth rates in ambient and high NP experimental channels. Abbreviations as in Table 3.1

GENERAL CONCLUSIONS

The goals of my research were to evaluate ecological and evolutionary strategies of benthic diatoms and relate them to nutrient conditions. Diatom abundances and morphologies observed in nature are hypothesized to be a result of ecological constraints and evolutionary processes. Many ecological factors affect benthic algae, and competition for nutrients is likely one of the most important. Species-specific evolutionary strategies involving physiological and morphological traits develop to ensure advantages of individual algal species at different environmental conditions. I tested the likelihood that sets of co-adaptive traits do exist and that they improve performance of taxa with a combination of experiments and surveys in the laboratory and in natural stream settings. The results of these studies broaden our understanding of how periphyton communities are structured.

In a near-natural setting (Chapter 1), stream water from a pristine watershed was supplied to experimental streams in which nutrients were manipulated. Recirculating experimental streams were used in a novel approach when controlling for effects of nitrogen and phosphorus, providing constant stream conditions, broad species pool, and nutrient supply of nitrogen and phosphorus at limiting concentrations. Different species were expected to grow best at low N and low P or at low and high concentrations of one nutrient. A broad species pool was inoculated and a gradient of resource conditions among channels was established in which only one nutrient was limiting at a time.

Responses of diatom species to each nutrient condition were measured as differences in growth rates and density.

Diatoms were able to grow at very low P and N concentrations. The same species were adapted to growing fastest in low N and low P conditions, so no evidence was observed for tradeoffs. Positive rather than negative correlations were observed among species growth rates in low and high P concentrations, or low and high N concentrations. Thus, the same species grew fastest in low and high concentrations. The same diatom species may be co-adapted to live in both low N and low P concentrations due to common co-occurrence of low concentrations of both nutrients in streams.

Even though species growth rates were positively correlated in low and high nutrient conditions (for both nutrients) the magnitude of those relations decreased with increasing algal density on substrata. Some evidence for density-dependent growth in competitive hierarchies was evident. Differences in N and P utilization and storage during colonization of substrata may explain the different strategies for N and P competition. Maximum growth rate of most taxa was significantly influenced by the growth of other taxa, especially initial colonizers like *Acnanthes deflexa*. Functional response of diatoms was species specific and time dependent. Abilities to utilize low nutrient concentrations and grow rapidly seemed to be co-adaptive traits for benthic diatoms in streams. This may be due to the likelihood that both low N and P supply constrain benthic diatom growth in many habitats.

The rate of biomass production (growth rate) in benthic diatoms does depend on the supply of limiting nutrients, but maximum growth rates of common diatom taxa were reached at very low nutrient concentrations. Little evidence for tradeoffs in resource specialization was observed. These results are inconsistent with the resource competition theory (Tilman 1982) in which a species able to grow at low concentration of one nutrient was not a good competitors at low concentrations of another nutrient. This work demonstrates that in stream benthic communities when diatoms are the dominant algal group, some diatom species are adapted to grow rapidly and to utilize very low resources.

Intra- and interspecific competition of clonal populations of *Achnanthidium minutissimum* were examined in low and high levels of nutrients and light (Chapter 2). Growth rate of *Achnanthidium minutissimum* was compared at low and high densities and when in competition with another monoraphid diatom (*Cocconeis placentula var. lineata*) or with a large stalk-forming *Cymbella cistula* species. Circular artificial channels were designed as a novel experimental apparatus for use within an incubator. In this study, I was able to create periphyton communities of genetically identical populations to evaluate the ecological effects of other diatoms on clonal populations of *A. minutissimum*. Nutrients limited growth of *A. minutissimum*. *Achnanthidium minutissimum* growth decreased with increase of diatom density and that decrease was dependent on the culture combinations. Light had a strong negative effect when nutrients were limiting but had a positive effect when nutrients were available. The mechanisms to compete for low nutrients and low light of those morphologically diverse diatoms may be different. *A. minutissimum* and *C. placentula* var. *lineata* are both monoraphid diatoms, but the latter had very slow growth rates in cultures. Although *C. cistula* grew well in culture, formed stalks, and overgrew *A. minutissimum*, the competitive effect on *A. minutissimum* may have been reduced by stalks providing substratum for it's colonization. The prediction of strong intraspecific competition in limiting resources was confirmed in this experiment. The importance of intraspecific competition regulating *A. minutissimum*, the most common diatom in North American streams, is an important contribution for the knowledge about competition regulating composition of stream periphyton. In addition, this study presents evidence of possible evolutionary adaptation to low light by common diatoms in natural streams.

Evolutionary strategies of several diatom species were evident in the large scale pattern observed in nature (Chapter 3). Species would be expected to be most abundant in relatively few habitats in a region if species specialized with optimal performance in specific environmental conditions. Contrary to this prediction, a positive correlation was observed between diatom abundance and breadth of their distribution in the Knobs ecoregion of Kentucky and southern Indiana. A positive relationship between relative abundance and breadth of species distribution was observed. Few taxa were abundant and they had broad distributions, the majority (79%) of the observed diatom species had low abundance and appeared in few sites (less that 10% of sites). Findings on relative abundance changes with nutrients in experimental work were successfully linked with the patterns from the survey. Mean relative abundance of species from pristine streams decreased with increasing nutrient and chloride concentrations, but distribution remained wide. These results do warrant concern in regards to conservation of the native low nutrient, low diversity sites in the Knobs ecoregion.

To evaluate ecological constraints on abundant and rare taxa, species abundance and frequency of occurrence were related to variation in ecological factors, which was dominated by variability in nutrients and chloride associated with an human disturbance gradient. Species that were most common and most abundant were species that occurred in streams in low disturbance watersheds. Relative abundance of these species decreased with increasing nutrient and chloride concentrations, however they persisted in habitats with high human disturbance. Taxa requiring high nutrients were more diverse than taxa from pristine streams, but even those high-nutrient taxa with widest distributions had low abundance. Some taxa requiring high nutrients grew faster than taxa from pristine streams when streams were anthropogenically enriched, which caused, partial turnover of taxa in the natural diatom assemblage. Evolutionary strategies of several diatom species were evident in the large scale pattern observed in nature. Integration of results provided a more certain description of the ecological strategies and adaptations employed by diatoms when multiple research approaches were used rather than any one approach alone.

The observed patterns were due to the species-specific abundance and distribution relationship of relatively small number of diatom species. Increase in nutrients cannot have a direct negative effect on diatoms, because algae depend on nutrients. One of the important findings in my work was that higher nutrients have species specific effects. Diatom species that were able to grow at very low nutrient concentrations grew equally well when nutrients were not limiting, but the high nutrient species grew faster with nutrient increases and were limited from low nutrient habitats. Correspondence between observed natural patterns on regional scales and experimental results in my dissertation indicate that diatoms have adapted to nutrient-related environmental conditions. On a larger scale in streams, potential tradeoffs for slow and fast growth in different environmental conditions have enabled specialization of taxa.

In the three studies, one diatom *Achnanthidium minutissimum* was an important species in the periphyton communities. My experimental study confirmed the ability of this diatom to grow at very low nutrients and low light, both common conditions in natural steams. *Achnanthidium minutissimum* employed different strategies in different conditions. This diatom grew fast at low nutrient concentrations. It was strongly regulated by intraspecific competition but not interspecific competition. *Achnanthidium minutissimum* was able to attach to other diatoms, and to produce mucilaginous support structures under some conditions. The observed results suggest that diatoms have great abilities to adapt to environmental conditions and that in the process they adopt evolutionary strategies, like changes in growth rate, changes in growth form or both, that can explain community and regional patterns commonly observed in nature.

LITERATURE CITED

Tilman, D. 1982. *Resource competition and community structure*. Princeton University Press, Princeton, 296 pp.

APPENDICES

APPENDIX I : *Achnanthidium minutissimum* distribution. Habitat and locality from selected records (more than 10% reported abundance) in USA. Source: General Collection (GC) at the Academy of Natural Sciences, Philadelphia, PA.

Habitat type	Collection locality	State	Collected by	Year	GC number
Clear mountain stream	Cave Creek, Coronalo National Forest	Arizona	C. Reimer	1944	6736a
Stagnant pool	near Beaver Dam	Arizona		1934	6728a
Clear mountain stream	White River	Arkansas			61616a
Plankton	San Joaquin River	California	R.R. Grant Jr	1962	62012a
Waterfall		California	R. Patrick	1952	6778
Plankton	Sacremento River	California		1956	8013
Plankton	Lake Tahoe	California			62220a
Warm sluggish stream	South Boulder Creek	California			8848b
Periphyton	North fork of Colorado River	Colorado			8974a
Epiphytes on grass & weeds	Roudre River	Colorado			8986b
Stagnant pool	Thompson River	Colorado			8843b
Cold muddy stream bed	Tonahutu creek	Colorado			9001b
Rock scrub	East River	Colorado			62521
hot spring	above Copper lake	Colorado	J. Lennox	1967	62584
Surface scum	Coal Creek	Colorado			62570
Periphyton	Red Clay Creek	Delaware	M.H. Hohn	1956	44090
periphyton	Delaware River	Delaware	M.H. Hohn	1957	44226b
epiphytes	Silver springs	Florida			44479
Plankton	Lake Okechobee	Florida			55160a
Periphyton	Escambia River	Florida			43850
Periphyton	Savannah River	Georgia			43929
Periphyton	Loon Creek	Idaho			64306a
Plankton	Lake Michigan	Illinois			46919
Raised bog	Cabin Creek	Indiana			45669
Plankton	Kansas River	Kansas			8052
Periphyton	Green River	Kentucky			55859a
Plankton	Lake Maurepas	Lousiana			48526a
Periphyton	Kennebec River	Maine			56723a
Periphyton	Potomak River	Maryland			45426a
Periphyton	Center creek	Missuri			45147a
Plankton	Flathhead Lake	Montana			62224
Periphyton	Assunpink River	New Jersey			44888
Periphyton	Auglaize River	Ohio			45184a
Periphyton	Burpee Station	Oregon	J. L. Parker	1969	62744
Periphyton	Slippery Rock Creek	Pennsylvania	R.R. Grant Jr	1967	50884
Periphyton	Brandywine Creek	Pennsylvania	C. Reimer	1964	48716
Periphyton	Susquehanna River	Pennsylvania	R.R. Grant Jr	1970	52169
Diatoms on mud flat	Savannah River	South Carolina	C.Reimer	1965	48869a-b
Mat, scraped from stone	Savannah River	South Carolina	Joy Morrill	1972	52882a
Periphyton	Holston River	Tennessee	R. R. Grant J	1965	48925a
On rock near shore	Cumberland River	Tennessee	C. Reimer	1973	53661a
On cypress log	Sabine River	Texas	R. R. Grant J	1962	62036a
Periphyton - composit	Guadalupe River	Texas	R.R. Grant Jr	1962	62193a
Periphyton	Providence Creek	Utah	G. Piranian	1936	6775a
Floating in stream from hot	Lincoln Beach	Utah	Edna Snow	1930	60428a
Diatom tuft in riffle	Holston River	Virginia	R. R. Grant J	1974	56409a
Periphyton - composit	Potomac River	Virginia	C.Reimer	1973	53365a
Periphyton	Quinault River	Washington DC	C.D. McIntin	1967	60990

APPENDIX 1.1: Nutrient concentrations μg L^{-1} along N gradient (mean ± SD, n=3).

day	N conc	NO_3(±SD)	NH_4(±SD)	TN (±SD)	SRP (±SD)	TP (±SD)	Si (±SD)
1	0	33 (±1)	8 (±3)	137 (±3)	25 (±2)	28 (±3)	11 (±1)
1	16	38 (±2)	9 (±3)	122 (±5)	26 (±1)	30 (±2)	11 (±1)
1	64	67 (±3)	16 (±10)	148 (±5)	23 (±2)	27 (±3)	11 (±1)
1	256	167 (±5)	16 (±12)	233 (±7)	25 (±1)	30 (±2)	11 (±1)
1	512	219 (±4)	8 (±1)	340 (±3)	23 (±1)	31 (±0.5)	11 (±0)
1	1024	500 (±33)	15 (±14)	578 (±38)	25 (±3)	29 (±3)	11 (±1)
3	0	12 (±2)	33 (±2)	139 (±3)	29 (±6)	33 (±8)	11 (±0)
3	16	22 (±3)	20 (±4)	143 (±3)	24 (±3)	31 (±3)	11 (±0)
3	64	74 (±26)	29 (±2)	184 (±18)	59 (±35)	64 (±37)	11 (±0)
3	256	188 (±24)	24 (±6)	273 (±24)	32 (±12)	35 (±12)	11 (±0)
3	512	242 (±28)	34 (±8)	332 (±29)	26 (±2)	33 (±2)	11 (±0)
3	1024	503 (±71)	34 (±5)	636 (±56)	22 (±2)	27 (±2)	11 (±0)
6	0	0 (±0)	9 (±1)	126 (±5)	25 (±1)	49 (±1)	12 (±0)
6	16	2 (±1)	9 (±1)	126 (±12)	26 (±2)	46 (±2)	12 (±0)
6	64	30 (±16)	10 (±1)	152 (±9)	26 (±10)	46 (±12)	12 (±0)
6	256	289 (±130)	14 (±3)	423 (±174)	49 (±29)	68 (±33)	10 (±1)
6	512	401 (±36)	11 (±1)	552 (±53)	24 (±5)	43 (±5)	10 (±0)
6	1024	898 (±153)	13 (±1)	1045 (±121)	27 (±7)	41 (±10)	10 (±0)
8	0	5 (±0)	13 (±2)	91 (±21)	26 (±7)	50 (±3)	10 (±0)
8	16	9 (±1)	12 (±1)	106 (±36)	21 (±4)	46 (±1)	10 (±1)
8	64	21 (±3)	16 (±5)	121 (±20)	20 (±63)	44 (±2)	9 (±0)
8	256	185 (±129)	16 (±3)	312 (±131)	26 (±11)	56 (±32)	9 (±0)
8	512	280 (±122)	17 (±1)	437 (±93)	18 (±6)	40 (±9)	9 (±0)
8	1024	668 (±504)	18 (±1)	1113 (±336)	27 (±8)	46 (±15)	9 (±0)
10	0	4 (±1)	6 (±0)	106 (±3)	47 (±24)	55 (±25)	11 (±0)
10	16	6 (±2)	8 (±5)	107 (±7)	32 (±8)	39 (±8)	10 (±0)
10	64	11 (±8)	9 (±2)	128 (±16)	36 (±4)	39 (±8)	9 (±2)
10	256	85 (±3)	58 (±10)	254 (±46)	37 (±113)	45 (±13)	10 (±0)
10	512	229 (±34)	62 (±8)	473 (±76)	41 (±10)	52 (±14)	10 (±0)
10	1024	802 (±331)	49 (±15)	997 (±397)	30 (±5)	50 (±9)	9 (±1)
12	0	3 (±1)	14 (±2)	162 (±5)	40 (±2)	52 (±5)	10 (±0)
12	16	6 (±2)	20 (±8)	142 (±12)	37 (±3)	50 (±1)	10 (±0)
12	64	16 (±2)	31 (±3)	189 (±30)	42 (±3)	60 (±7)	9 (±2)
12	256	87 (±23)	42 (±13)	273 (±21)	44 (±6)	54 (±8)	9 (±0)
12	512	220 (±37)	98 (±10)	502 (±75)	46 (±12)	54 (±13)	8 (±0)
12	1024	760 (±55)	51 (±17)	931 (±64)	36 (±3)	44 (±4)	8 (±0)
14	0	3 (±1)	12 (±1)	129 (±7)	58 (±60)	88 (±66)	10 (±1)
14	16	9 (±2)	12 (±1)	99 (±2)	21 (±7)	42 (±4)	10 (±0)
14	64	15 (±4)	12 (±1)	152 (±49)	50 (±46)	51 (±5)	9 (±1)
14	256	244 (±255)	15 (±2)	442 (±311)	41 (±35)	79 (±49)	9 (±1)
14	512	283 (±56)	14 (±2)	477 (±79)	14 (±6)	41 (±9)	9 (±0)
14	1024	723 (±19)	14 (±1)	928 (±53)	20 (±6)	168 (±227)	9 (±0)

APPENDIX 1.2: Nutrient concentrations μg L^{-1} along P gradient (mean ± SD, n=3).

day	P conc	NO_3	NH_4	TN	SRP	TP	Si
1	0	234 (±0)	7 (±0)	254 (±4)	5 (±1)	8 (±0)	11 (±0)
1	2	245 (±39)	11 (±7)	321 (±13)	8 (±1)	10 (±1)	11 (±0)
1	8	227 (±8)	19 (±18)	352 (±39)	9 (±0)	12 (±1)	11 (±1)
1	32	219 (±5)	12 (±7)	323 (±22)	17 (±3)	20 (±3)	11 (±1)
1	50	214 (±4)	8 (±1)	340 (±3)	23 (±1)	31 (±1)	11 (±0)
1	128	125 (±103)	8 (±2)	228 (±94)	30 (±30)	35 (±31)	11 (±0)
3	0	306 (±69)	27 (±4)	410 (±93)	5 (±1)	8 (±3)	11 (±0)
3	2	159 (±116)	21 (±2)	263 (±100)	5 (±2)	7 (±2)	11 (±0)
3	8	442 (±369)	32 (±7)	610 (±338)	8 (±4)	11 (±3)	11 (±0)
3	32	328 (±197)	21 (±1)	436 (±222)	21 (±9)	26 (±11)	11 (±0)
3	50	242 (±28)	34 (±8)	332 (±29)	26 (±2)	33 (±2)	11 (±0)
3	128	205 (±21)	30 (±6)	321 (±35)	52 (±1)	59 (±3)	11 (±0)
6	0	423 (±79)	11 (±1)	815 (±404)	4 (±0)	5 (±1)	11 (±0)
6	2	451 (±78)	11 (±1)	579 (±100)	5 (±1)	10 (±0)	10 (±0)
6	8	426 (±39)	13 (±4)	483 (±103)	10 (±7)	20 (±8)	9 (±3)
6	32	381 (±64)	13 (±1)	493 (±48)	17 (±4)	24 (±3)	10 (±0)
6	50	401 (±36)	11 (±1)	551(±53)	24 (±5)	43 (±5)	10 (±0)
6	128	347 (±11)	12 (±1)	456 (±19)	87 (±12)	118 (±18)	10 (±0)
8	0	493 (±47)	19 (±2)	620 (±43)	3 (±2)	4 (±1)	10 (±0)
8	2	406 (±8)	12 (±1)	549 (±25)	3 (±1)	4 (±1)	9 (±0)
8	8	221 (±139)	12 (±0)	314 (±142)	5 (±0)	7 (±0)	9 (±0)
8	32	300 (±70)	14 (±0)	471 (±59)	12 (±4)	26 (±4)	9 (±0)
8	50	280 (±122)	17 (±1)	437 (±93)	18 (±6)	40 (±9)	9 (±0)
8	128	203 (±83)	16 (±1)	398 (±86)	62 (±43)	90 (±41)	9 (±0)
10	0	497 (±64)	16 (±9)	580 (±51)	3 (±2)	7 (±2)	10 (±0)
10	2	301 (±105)	15 (±3)	447 (±74)	2 (±1)	6 (±1)	9 (±0)
10	8	560 (±401)	22 (±2)	674 (±394)	4 (±0)	9 (±0)	8 (±1)
10	32	529 (±562)	23 (±4)	724 (±640)	21 (±14)	32 (±21)	9 (±1)
10	50	229 (±34)	62 (±8)	473 (±76)	41 (±10)	52 (±14)	10 (±0)
10	128	112 (±93)	39 (±10)	303 (±164)	51 (±43)	65 (±51)	9 (±1)
12	0	459 (±31)	18 (±6)	613 (±23)	3 (±1)	4 (±1)	9 (±0)
12	2	496 (±158)	40 (±15)	680 (±199)	2 (±1)	5 (±2)	9 (±0)
12	8	370 (±65)	40 (±7)	563 (±93)	4 (±0)	7 (±1)	8 (±1)
12	32	267 (±46)	38 (±19)	457 (±23)	21 (±4)	28 (±3)	9 (±1)
12	50	220 (±37)	98 (±10)	502 (±75)	46 (±12)	54 (±13)	8 (±0)
12	128	120 (±102)	40 (±31)	298 (±158)	68 (±64)	78 (±72)	9 (±2)
14	0	514 (±104)	15 (±4)	591 (±101)	2 (±1)	4 (±1)	10 (±0)
14	2	518 (±168)	12 (±0)	632 (±160)	4 (±1)	6 (±1)	8 (±3)
14	8	323 (±28)	11 (±0)	468 (±6)	5 (±0)	8 (±0)	9 (±0)
14	32	789 (±720)	14 (±2)	801 (±881)	10 (±4)	44 (±16)	6 (±3)
14	50	283 (±56)	14 (±2)	477 (±79)	14 (±6)	41 (±9)	9 (±0)
14	128	478 (±401)	16 (±2)	660 (±437)	110 (±72)	169 (±79)	7 (±3)

APPENDIX 3.1: Site locality and identification sampled for 1996 and 1997, sites in bold are considered reference.

ID	SITE NAME	site	WATERSHED	COUNTY	LAT	LONG	1996	1997
1	Barebone Creek	BBCR	Ohio River	Trimble	38.59	85.4		•
2	Bird Hollow	BIHO	Little Blue River	Crawford	38.36	86.77	•	•
3	Bluelick Creek	BLCR	Ohio River	Bullitt	38.03	85.69	•	
4	Bogard Creek	BOCR	Little Blue River	Crawford	38.29	86.43	•	•
5	**Brushy Creek**	**BRCR**	**Little Blue River**	**Crawford**	**38.31**	**86.44**	•	•
6	Brooks Run	BRRU	Ohio River	Bullitt	38.05	85.69	•	
7	Brownstown Creek	BTCR	Little Blue River	Crawford	38.35	86.48	•	•
8	Buck Creek	BUCR	Buck Creek	Harrison	38.14	86.04	•	•
9	Camp Fork Creek	CAFO	Little Blue River	Crawford	38.34	86.41	•	•
10	Cain Run	CARU	Ohio River	Jefferson	38.07	85.87	•	
11	Cedar Creek #1	CEC1	Salt River	Bullitt	38.06	85.65	•	
12	Cedar Creek #2	CEC2	Salt River	Bullitt	38.42	85.45	•	•
13	Corn Creek	COCR	Indian River	Harrison	38.33	86.04		•
14	Crandall Branch	CRBR	Indian River	Harrison	38.29	86.07	•	•
15	Crooked Creek	CRCR	Salt River	Bullitt	37.89	85.72	•	•
16	Dog Creek	DOCR	Little Blue River	Crawford	38.36	86.45	•	•
17	Goose Creek	GOCR	Ohio River	Jefferson	38.3	85.62	•	•
18	Hardy Creek	HRCR	Ohio River	Trimble	38.61	85.27		•
19	Harrison Fork	HAFO	Ohio River	Nelson	37.87	85.6		•
20	**Harts Run**	**HARU**	**Salt River**	**Bullitt**	**37.86**	**85.61**	•	•
21	Hite Creek	HTCR	Ohio River					•
22	Jersey Park Creek	JPCR	Indian River	Floyd	38.37	85.96	•	•
23	**Knob Creek**	**KNCR**	**Ohio River**	**Bullitt**	**38.04**	**85.79**	•	•
24	Little Indian Creek	LIIN	Indian River	Floyd	38.31	85.91	•	
25	Long Lick Creek	LLCR	Salt River	Bullitt	37.93	85.71	•	•
26	Lower Wilson Creek	WICR	Salt River	Nelson	37.87	85.61	•	•
27	Lickskillet Creek	LSCR	Salt River	Bullitt	37.93	85.65	•	•
28	MB Harrods Creek	HACR	Ohio River	Oldham	38.45	85.42		•
29	MF Beargrass Creek	MFBG	Ohio River	Jefferson	38.24	85.7	•	•
30	MF Blue River	MFBL	Blue River	Washington	38.59	85.98		•
31	MF Buck Creek	MFBU	Buck Creek	Harrison	38.14	86.04	•	•
32	MF Indian Creek	MFIN	Indian River	Floyd	38.36	85.96		•
33	NF Harrods Creek	NFHA	Ohio River	Oldham	38.42	85.45		•
34	Otter Creek	OTCR	Little Blue River	Crawford	38.3	86.53	•	•
35	**Overalls Creek**	**OVCR**	**Salt River**	**Bullitt**	**37.87**	**85.6**	•	•
36	Pryors Fork	PRFO	Ohio River	Trimble	38.06	85.41		•
37	Racoon Branch	RABR	Indian River	Harrison	38.28	86.1	•	•
38	Richland Creek	RICR	Indian River	Floyd	38.32	86.01	•	•
39	SF Blue River	SFBR	Blue River	Washington	38.53	85.92		•
40	Stinking Fork	STFO	Little Blue River	Crawford	38.23	85.53	•	•
41	Turkey Fork	TUFO	Little Blue River	Crawford	38.2	86.4	•	•
42	Upper Wilson Creek	UWIL	Salt River	Nelson	37.87	85.6		•
43	Whiskey Run	WHRU	Ohio River	Washington	38.52	85.92		•
44	Yellow Fork	YEFO	Indian River	Floyd	38.35	85.91		•