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LINKING GRADIENTS OF PREDATORS AND PRODUCTIVITY TO THE COMPOSITION AND LIMITATION OF BENTHIC ALGAL COMMUNITIES

By

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ABSTRACT

LINKING GRADIENTS OF PREDATORS AND PRODUCTIVITY TO THE COMPOSITION AND LIMITATION OF BENTHIC ALGAL COMMUNITIES

By

Tara Lee Darcy-Hall

Species often exhibit tradeoffs in their abilities to survive in different environments. As a result, tradeoffs among species' traits can lead to shifts in species composition along ecological gradients. Furthermore, compositionally variable communities usually respond differently to changes in the abundance of species within adjacent trophic levels (*i.e.* changes in their food or their predators). In particular, communities along gradients in potential primary productivity can differ in the extent to which they are limited by predators or resources. My work focuses specifically on one tradeoff, between species' abilities to compete for resources and resist predation, and how this tradeoff mediates shifts in benthic algal species composition and consequently alters patterns of nutrient and predator limitation across gradients in productivity.

I investigate the competition-resistance tradeoff within the context of the keystone predation hypothesis (KPH), which predicts that concomitant increases in resources and predators will result in dominance by predator resistant species. Conversely, species that are vulnerable, superior competitors should prevail in environments low in resources and predator biomass. A mathematical model is used to generate predictions of how the keystone predator tradeoff and shifts in species composition might affect patterns of algal nutrient and predator limitation.

A survey of benthic algal communities in 14 southwest Michigan lakes indicated that algal nutrient and predator limitation were similar to predictions of the mathematical model and the KPH. Moreover, several species exhibiting strong correlations with productivity and predator biomass possessed traits consistent with those expected given a tradeoff among competition and resistance.

Similarly, in a large-scale mesocosm experiment focusing on the key role of grazing predators in driving a competition-resistance tradeoff, I demonstrated that ungrazed and grazed benthic algal communities differ substantially in their composition and hence their patterns of nutrient and predator limitation across a productivity gradient. The presence of more predator resistant algal species in grazed communities led to reduced levels of predator limitation and stronger nutrient limitation. These results provided a causative link between the competition-resistance tradeoff inherent in the KPH and patterns of algal limitation.

Additionally, I used series of small and large-scale experiments as well as a unique statistical technique, the fourth-corner analysis, to correlate the key traits of competitive ability and predator resistance to environmental variables, providing a direct test of the predictions of the KPH. The fourth-corner analysis revealed several trait-environment correlations consistent with the KPH; however, results were context dependent and demonstrated that other tradeoffs may also be important.

These results generally attest to the importance of a competition-resistance tradeoff within the benthic algae. More importantly, I have elucidated a link between this tradeoff, benthic algal composition, and patterns of algal nutrient and predator limitation.

Dedicated to my grandparents, William T. Lavey and the late Lorraine D. Lavey

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While social outlets may be limited at KBS, the opportunities for deep and enduring friendships have been anything but scarce. Natalie Dubois, Sarah Grandy, Pamela Geddes, and Kristi Sherfinski are four intelligent, beautiful, and genuine women who have graced me with their friendship. Their companionship has kept a smile on my face and insanity at bay, and their friendship is my most valuable gift from KBS.

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CHAPTER 1

INTRODUCTION

Species exhibit tradeoffs in their ability to use various resources, their ability to allocate biomass to various structures, and/or their ability to compete for resources or colonize new habitats (reviewed in Kneitel and Chase 2004, Grover 1997, Tilman 1988). Moreover, a species' trait selected for in one environment may not prove favorable in a different environment. Thus, species may exhibit tradeoffs between traits that confer success in different environments, and these tradeoffs may drive shifts in species composition along ecological gradients. Here, I focus on a tradeoff among benthic algal species in their ability to procure nutrients and their resistance to predation. A competition-resistance tradeoff has long been recognized as an important phenomenon within ecological communities (Paine 1966, Lubchenco and Gaines 1981, Vance 1978, Holt *et al.* 1994, Grover 1994, Leibold 1996, Chase *et al.* 2000). If prey species (*e.g.* algae) exhibit a competition-resistance tradeoff, then prey community composition might shift to dominance by more predator resistant species as both productivity and predator biomass increase. This potentially critical role of predators in shifting prey community structure has been investigated empirically (*e.g.* Steiner 2001, Leibold 1999) and developed theoretically as the keystone predation hypothesis (KPH; Leibold 1996, Holt *et al.* 1994, Grover 1994).

The keystone predation hypothesis

The keystone predation hypothesis predicts that species compositional shifts across productivity gradients will involve a tradeoff between the ability of species to escape predation and compete for resources. These broadly defined terms actually encompass a number of more specific, physiological or morphological species' traits that qualify as predator avoidance or competitive ability. For example, plants (or algae) can be *resistant* to predators by possessing chemical or structural traits that augment defense. Benthic algal species might be predator resistant by being tightly attached to their substrate, by having a tangled, filamentous morphology that impedes grazing, or by occupying a protected position low in the benthic mat. Algal species can also be predator *tolerant* (*sensu* Chase *et al.* 2000), minimizing the effects of predation by rapid regrowth of affected tissues. Finally, algal species might overcome large predation effects by maintaining a growth rate that is faster than the grazing rate.

Competitive ability in the KPH is defined in the classic sense of resource competition (Tilman 1982); that is, a species is competitive for a particular resource if it can persist on low levels of that resource. A competitive species can be alternatively viewed as one that acquires better access to a key resource (*e.g.* Grime 1979). Both definitions of competition apply within the benthic algae and while both are addressed within the following chapters, I focus primarily on the classic definition, as it is the one used in the keystone predation hypothesis.

The competition-resistance tradeoff implicit in the KPH generates several predictions concerning the nature of species compositional shifts along gradients in productivity and predator biomass. First, the KPH predicts that edible, superior competitors will dominate the prey community at low productivities, while more grazer resistant, inferior competitors will dominate at high productivities. Second, the resultant prey communities across this productivity and predator gradient can differ not only in composition, but also in how they respond to changes within adjacent trophic levels.

It is well established that processes initiated from both the top-down and the bottom-up are critical to community structure and biomass (Power 1992). A strong focus in aquatic communities has been the relative importance of nutrients and herbivores on primary producers within one system. However, surprisingly few studies have explored how the relative effects of top-down (predator limitation) and bottom-up (nutrient limitation) factors shift across communities along a productivity gradient, and no research to date has attempted to attribute a mechanism to such patterns. Osenberg and Mittelbach (1996) hypothesized that patterns of nutrient and predator limitation might relate to herbivore-mediated shifts in prey species composition, thus linking the KPH to broader food web interactions. In my work, I have adopted the definition and metrics of nutrient and predator limitation as defined by Osenberg and Mittelbach (1996). Limitation is measured as the increase in the per capita growth rate of a population upon the removal of a limiting factor (*e.g.* via the addition of nutrients or the removal of predators). The objectives of my dissertation are to test the applicability of the KPH for benthic algal communities, describe natural patterns of nutrient and predator limitation of lake benthic algae, and to experimentally link the herbivore-mediated algal compositional shifts predicted by the KPH to these patterns.

A mathematical model for limitation

I present a mathematical model in Appendix A that outlines the predicted patterns of limitation given the parameters of two competing theories concerning trophic level biomass and composition: the KPH and simple food chain models (Hairston *et al.* 1960, Fretwell 1977, Oksanen *et al.* 1981). Food chain models assume there is one species per trophic level, while the KPH incorporates species heterogeneity within trophic levels.

Below, I refer to these theories as "food chain" and "food web" models, respectively. The mathematics and assumptions resulting in the following predictions are explained in thorough detail in Appendix A.

In food chains, resource (or nutrient) limitation is predicted to substantially decrease along a gradient in nutrient enrichment (Figure 1.1), although this decline is predicted to be slower for less vulnerable prey species (Species 2). Because less vulnerable prey species persist at higher biomass, they deplete resources to a lower level, causing higher resource limitation (Appendix A). Predator limitation of both prey types should increase along a productivity gradient, but again at a slower rate for less vulnerable prey, because mortality due to predation is lower.

In food webs, with compositional shifts to increasingly predator resistant species with enrichment, resource limitation initially decreases, but then remains relatively constant and high across much of the productivity gradient. Resource limitation remains strong for less vulnerable species because these species maintain high biomass and thus deplete their resources more per capita than their more vulnerable counterparts (Appendix A). Therefore, resource limitation in the food web case is expected to be: a) stronger than resource limitation in the food chain case (except at low levels of resource supply), and b) stronger than predator limitation across gradients of enrichment (Figure 1.1). While predator limitation initially increases at low productivities due to the presence of more vulnerable prey species, dominance by increasingly less vulnerable species causes predator limitation to decrease and remain constant (or decrease slowly) and low across a productivity gradient (Figure 1.1). These predictions are examined in

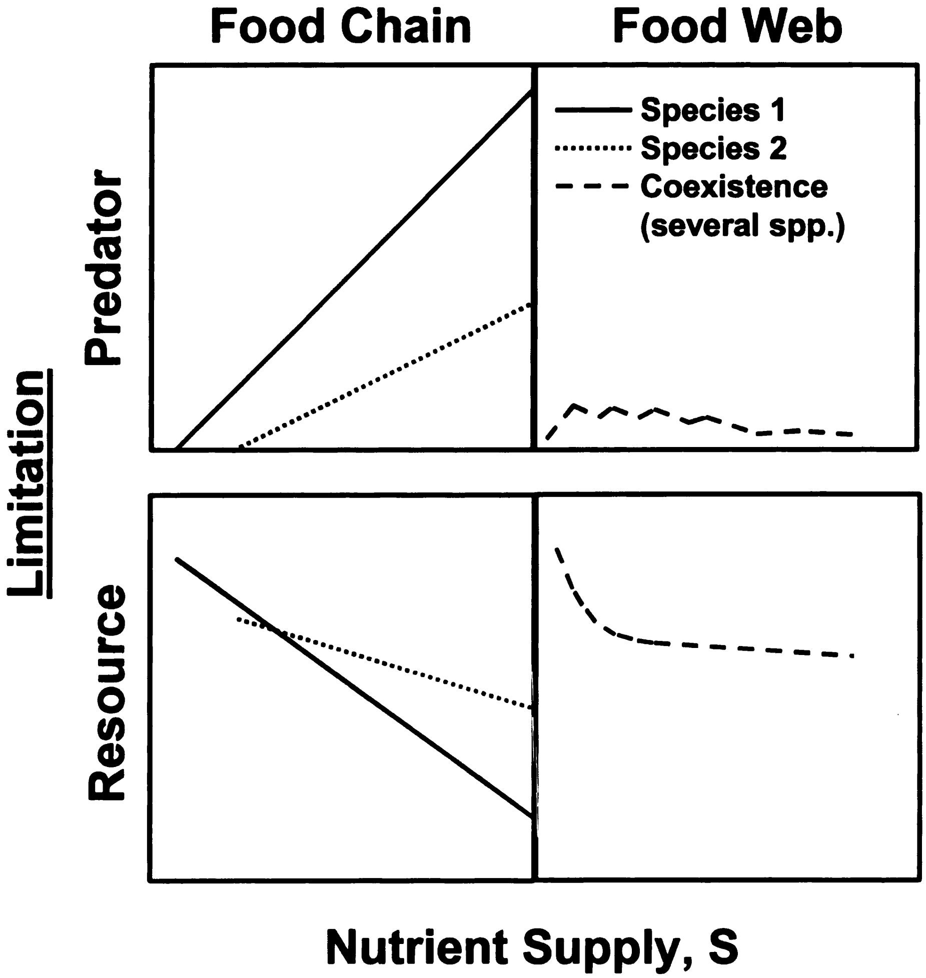

Figure 1.1: Predator and resource limitation over gradients of enrichment in food chains and food webs. Species 1 (solid line) is the more vulnerable, superior resource competitor, while Species 2 (stippled line) is the less vulnerable, inferior resource competitors. The dashed line is a region of coexistence of several species that are increasingly predator resistant with enrichment.

Chapters 2 and 3 using a survey of southwest Michigan lakes and a large-scale mesocosm experiment.

Benthic algae as study organisms

Lake benthic algae are an excellent community for examining predicted changes in species composition and limitation across productivity gradients. Freshwater benthic algae are a diverse community of substrate-bound autotrophs. I have observed over 200 species in local lakes and they are broadly composed of 3 main taxonomic groups: the glass cell-walled diatoms, green algae (chlorophytes), and the prokaryotic blue-green algae (cyanophytes or cyanobacteria). Benthic algae can vastly range in size from small unicellular diatoms (~ 30 μm^3) to relatively large filamentous species (~10,000 μm^3/cell), and they embody a wide range of morphologies. For example, algal species can be unicellular, colonial, gelatinous, mucilaginous, filamentous, or stalked. Furthermore, benthic algae can dominate carbon fixation in many lakes (Wetzel 1996), yet these communities are often ignored when testing current ecological theory (Lowe 1996).

Benthic algal functional form theory (Rosemond 1996, Steinman *et al.* 1992) predicts species compositional shifts that are similar to those predicted by the KPH. For instance, benthic algal species' traits that confer effective nutrient uptake (*e.g.* erect or filamentous forms) render these species vulnerable to herbivores. In general, defended species are inferior competitors, due in part to costs of defense as well as their inherent morphology (*e.g.* adnate). Therefore, there is *a priori* evidence for a competition-resistance tradeoff in the benthic algae, which may drive species compositional shifts and potentially influence species' responses to the removal of limiting factors.

Dissertation synopsis

Chapter 2 of my dissertation describes natural patterns of nutrient and predator limitation of benthic algae across a productivity gradient of 14 lakes in southwest Michigan. In this study, I compare predictions of limitation patterns generated from the KPH and simpler food chain models (*e.g.* Oksanen *et al.* 1981). Because intra-trophic level heterogeneity (*i.e.* the KPH) is a more realistic model assumption, I hypothesized that patterns of benthic algal limitation across lakes would more closely resemble those predicted by keystone predator models. I also surveyed benthic algal species composition across lakes in an effort to relate species compositional shifts to changes in environmental variables and patterns of nutrient and predator limitation. Specifically, I examined whether the KPH predictions of prey species shifts qualitatively matched benthic algal species turnover along a productivity gradient of 13 Michigan lakes.

In Chapter 3, I experimentally link the process of herbivore-mediated species turnover (*i.e.* the KPH) to patterns of benthic algal limitation. In 1000-L, cattle tank mesocosms, I established two sets of algal communities along a productivity gradient: one community that developed in the presence of herbivorous grazers and one that developed in their absence. After several weeks of community establishment, I assayed each community for nutrient and predator limitation and then compared the patterns between historically grazed and ungrazed communities. I hypothesized that algal communities historically exposed to herbivores would exhibit herbivore-mediated shifts in composition, and thus patterns of limitation would be similar to those predicted by the KPH. In contrast, I expected that the composition of historically herbivore-naïve communities would shift solely on the basis of differences in productivity and that predator resistant species would not dominate at higher productivities, generating patterns of limitation resembling predictions from simple food chain models.

In Chapter 4, I evaluate the applicability of the focal KPH tradeoff (*i.e.* competitive ability vs. predator resistance) in benthic algal communities. In a series of small mesocosm experiments, I measured several algal species' traits, including resistance to several herbivores and tolerance to limiting levels of several key resources (*i.e.* nutrients and light). Concurrently, I established algal communities in large mesocosms that varied in their light environments, nutrient concentration, and level of herbivory. Finally, data from these experiments were assembled into matrices (a species x species' trait matrix, an environment x site matrix [abiotic and biotic variables from the large mesocosms], and a species x site matrix [also from the large mesocosms]). These matrices were analyzed using the fourth-corner method, which generates a fourth matrix correlating species' traits to environmental variables (Legendre *et al.* 1997). The KPH predicts that grazer resistance will dominate at high productivities and superior competitive ability will dominate at low productivities. Thus, I hypothesized that the species' traits of resistance to predators would be positively correlated with productivity variables; whereas, traits of superior competitive ability would be negatively correlated with productivity variables.

Findings from my dissertation research demonstrate the importance of a competition-resistance tradeoff within benthic algal communities, not only for altering species composition, but also for affecting patterns of nutrient and predator limitation. Overall, this dissertation research addresses several novel and ecologically relevant questions concerning patterns and mechanisms of algal limitation across environments, while successfully combining rigorous theory, descriptive studies in natural systems, and controlled experiments.

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CHAPTER 2

PATTERNS OF NUTRIENT AND PREDATOR LIMITATION OF BENTHIC ALGAE IN LAKES ALONG A PRODUCTIVITY GRADIENT

INTRODUCTION

After decades of debate, ecologists now concur that bottom-up and top-down forces simultaneously influence trophic level biomass and community structure (Oksanen 1981, McQueen *et al.* 1986, Power 1992, Osenberg and Mittelbach 1996). While many studies have documented the relative effects of these forces within a community or ecosystem (see reviews in Hillebrand 2002, Downing *et al.* 1999, Carpenter and Kitchell 1993, Power 1992), much of this research has focused on the effects of nutrient supply and/or top predators on whole trophic levels within one system, often ignoring heterogeneity within trophic levels and between ecosystems (Power 1992, Hunter and Price 1992). Few studies have attempted to quantify how the relative importance of bottom-up (*i.e.* resource limitation) and top-down (*i.e.* predator limitation) factors changes along environmental gradients (*e.g.* Hillebrand 2001), and none have done so while linking the observed patterns to species heterogeneity (*i.e.* the presence of multiple species) within a focal trophic level.

Simple food chain models (Hairston *et al.* 1960, Fretwell 1977, Oksanen *et al.* 1981) provide a useful benchmark for generating predictions of how resource and predator limitation change across gradients in potential primary productivity. The classic ecosystem exploitation hypothesis (Oksanen *et al.* 1981) posits that top-down and bottom-up limitation of a species or tropho-species will alternate in importance, depending upon the number of trophic levels in a system. For ecosystems with an even number of trophic levels, top-down limitation is predicted to dominate odd-numbered trophic levels (*e.g.* primary producers and secondary consumers), regardless of the overall productivity of the system. Therefore, across systems that vary in productivity, but not in the number of trophic levels, resource (or nutrient) limitation of the primary producer trophic level is expected to decrease in importance as nutrient supply increases, while predator limitation is expected to increase in importance as consumer biomass increases (Darcy-Hall, Chapter 1, Appendix A, Oksanen *et al.* 1981).

Alternative models predict how species heterogeneity will drive patterns of trophic level biomass and community structure across ecosystems that vary in potential primary productivity. For example, Osenberg and Mittelbach (1996) incorporate prey heterogeneity in their predictions of patterns of resource and predator limitation across potential primary productivity gradients. Specifically, they predict that resource limitation should remain strong across systems that vary in productivity, due to a shift in the relative abundances of edible versus inedible prey (Osenberg and Mittelbach 1996). This expectation is in agreement with results of my limitation model, which predicts that resource limitation in a food web context will be strong and positive, but will decrease non-linearly across an enrichment gradient (Darcy-Hall, Chapter 1, Appendix A). Several field studies in aquatic systems have observed a positive correlation between the biomass of adjacent trophic levels (Mills and Schiavone 1982, McNaughton *et al.* 1989, Ginzburg and Akcakaya 1992, Leibold *et al.* 1997, Leibold 1999), indicating that herbivores and primary producers increase concomitantly with productivity. This rise in herbivore biomass can favor dominance by predator resistant forms among the primary producers. These predator resistant forms incur very low levels of predator-induced per-capita mortality rates and can therefore increase in abundance as nutrient levels increase (Osenberg and Mittelbach 1996). Thus, as the nutrient supply of a system increases, one would expect resource limitation to remain important for the producer trophic level and for predator limitation to decrease as more grazer-resistant prey dominate (Darcy-Hall, Chapter 1, Appendix A). However, Osenberg and Mittelbach's (1996) verbal model lacks a mechanism to drive the changes in prey heterogeneity that account for the predicted patterns in resource and predator limitation.

The keystone predation hypothesis (KPH; Holt *et al.* 1994, Grover 1994, Leibold 1996) provides a mechanistic basis for the predictions laid out by Osenberg and Mittelbach (1996). The KPH incorporates a tradeoff among prey species in their competitive ability and resistance to predation and focus on the role of predators in driving turnover among these species (Holt *et al.* 1994, Grover 1994, Leibold 1996). For example, as the biomass of herbivores increases across a nutrient supply gradient, the dominant algal species are expected to shift from edible, superior resource competitors at low productivities to predator resistant, inferior resource competitors at higher productivities. Thus, the KPH tradeoff facilitates predictions of how prey composition should shift across productivity gradients and in turn provides a mechanistic understanding of the consequent changes in resource and predator limitation.

I use the definition and metrics of resource (here, nutrient) limitation and predator limitation as presented by Osenberg and Mittelbach (1996) to examine patterns of benthic algal (*i.e.* periphyton) limitation in 14 southwest Michigan lakes that span a wide gradient in potential primary productivity. Limitation is defined as the extent to which a population's per capita growth rate is reduced by predators or insufficient nutrient supply

(Osenberg and Mittelbach 1996). Limitation can be measured as the increase in the population's per capita growth rate upon the removal of the limiting factor, via the addition of saturating nutrients or the removal of predators. The limitation metric aims to directly measure the change in growth rate due solely to the limiting factor and therefore must be measured in the short-term to avoid confounding the measurement with a numerical (or functional) response of predators or a switch to limitation by another resource (Osenberg and Mittelbach 1996, Downing *et al.* 1999). Downing *et al.* (1999) found that in an experimental setting, limitation was a constant and reliable measure of the change in population per capita growth of marine phytoplankton over a period of 2-7 days, post treatment initiation.

There are several reasons why assemblages of benthic algae are an excellent experimental system with which to address questions of nutrient and predator limitation. First, algal communities have the potential for a rapid response to the removal of limiting factors, since algal division rates are on the order of approximately one generation per day (Downing *et al.*1999). Second, the majority of studies addressing sources of limitation of autotrophs have focused on phytoplankton communities. Finally, it is well established in stream periphyton communities that both resource and predator limitation are important factors affecting community physiognomy (physical structure) and biomass (McCormick and Stevenson 1989, Mulholland *et al.* 1991, Hill *et al.* 1992, Rosemond *et al.* 1993), but these patterns have rarely been investigated in lakes (Lowe 1996).

I compared the relationship between algal nutrient and predator limitation and several productivity variables to assess how natural patterns of algal limitation compare to predictions from classic ecosystem exploitation models and more recent keystone predator models. I hypothesized that patterns of benthic algal limitation across lakes would more closely resemble those predicted by the KPH due to heterogeneity within a trophic level. That is, I expected nutrient limitation of benthic algae to initially decrease at low productivities, but remain strong and positive at higher productivities. Alternatively, I expected predator limitation values to be low and perhaps decline along the productivity gradient of lakes (Darcy-Hall, Chapter 1, Appendix A). I also surveyed benthic algal species composition across lakes in an effort to relate species compositional shifts to changes in environmental variables and patterns of nutrient and predator limitation. Specifically, I examined whether the KPH predictions of prey species shifts qualitatively matched benthic algal species turnover along this productivity gradient of lakes. Finally, I sought to link patterns of algal limitation to changes in algal composition across lakes.

METHODS

Study system - lakes

My primary focus was to investigate patterns of algal limitation across lakes (versus within lakes), therefore, I chose to sample more lakes rather to sample intensively within a few lakes. The 14 study lakes are located within 50 km of the W. K. Kellogg Biological Station (KBS; Hickory Corners, Michigan, USA) and encompass a wide gradient in both total phosphorus (TP: 13.5 – 77.5 μg/L) and total nitrogen (TN: 208.9 - 1869 μg/L), both of which are potentially limiting nutrients for primary productivity in these lakes. I used both of these variables, as well as algal standing crop (*i.e.* chlorophyll *a*), as surrogates for system primary productivity. Total nutrients were sampled in June 2001 in the littoral zone of each lake and measured using spectrophotometry and standard methods (Bachmann and Canfield 1996, Crumpton *et al.* 1992, Prepas and Rigler 1982, APHA 1980). All chlorophyll *a* measurements were conducted using cold ethanol extractions followed by narrow-band fluorometry (*sensu* Welschmeyer 1994). These productivity measures, as well as conductivity, pH, and grazer biomass, were included in ordinations relating environmental variables to benthic algal species composition. All variables except pH and conductivity required log-transformation to meet assumptions of normality. Conductivity and pH measurements for all lakes were obtained from independent data sets (Stephen J. Hamilton, KBS, and Robert Bachmann, University of Iowa, *unpubl. data*), from which 2001 data were included when available, otherwise summer values from other years were used. Prior to the limitation assays, macrograzers were collected from sampling substrates (terracotta clay flowerpots) and preserved in 70% ethanol for later identification and measurement of ash-free dry mass (AFDM). Herbivorous grazers in these lakes are primarily gastropods (*Physa* spp., *Helisoma* sp., *Gyraulus* sp., *Amnicola* sp, and *Bithynia* sp.), which ranged from 1% to 99% of grazer biomass. Other dominant grazers included amphipods (*Hyalla azteca*), mayfly larvae (Family *Caenidae*), caddisfly larvae (order *Trichoptera*), and chironomid midge larvae (family *Chironomidae*). All but one of the study lakes support four trophic levels: benthic primary producers, their invertebrate grazers (insect larvae and gastropods), invertebrate (insect larvae) and vertebrate (fish) predators, and piscivores (one lake, Duck Lake, lacks piscivorous fish).

Limitation assays

Benthic algal communities were established on 10-cm (4-inch) terracotta clay flowerpots in all lakes for 30 days prior to the start of the assays. In each lake, I placed

16 clay pots each in separate 4.7 L(5-quart) plastic buckets which had 3, 15 cm^2-windows cut in them to allow water, algae, and grazers to move through the container. The buckets were attached to floating wooden frames that allowed the clay pots to be submerged in the lake water. Frames were anchored in the littoral zone in a linear array. The clay pots were placed close to the surface in all lakes, thus eliminating differences in light levels across lakes. I monitored algal biomass during a 30-day incubation period to determine when a substantial algal mat had developed for the limitation assays. Each week, one clay pot from each lake was sampled by scraping off of all algae with a toothbrush, razor blade, and deionized water. The resulting algal slurry was homogenized in a blender and then subsampled for chlorophyll *a* analysis.

Identical limitation assays were initiated in all 14 lakes over a 4-day period beginning on July 25, 2001. Due to the distance and travel time between lakes, I was only able to set up these assays in 3-4 lakes per day. Each assay included three, randomly assigned treatments with 4 replicates each: nutrient additions, grazer removals, and controls. In nutrient addition treatments, I added a biologically saturated nutrient solution (NH_4Cl, $NaNO_3$, and KH_2PO_4 in a 16:1 molar N:P ratio) to the interior of the clay pots. The same nutrient solution was added to the clay pots in each lake. The porous clay surface readily diffuses nutrients over time (Darcy-Hall *unpubl. data*, Fairchild *et al.* 1985, Marks and Lowe 1989, Wootton *et al.* 1996). Lake water was added to the interior of control clay pots. Macrograzers (*i.e.* visible to the naked eye) were manually removed from all clay pots and returned to all but the grazer removal treatment. Control treatments mimicked natural conditions in that no nutrients were added and grazers were present. After the treatments had been applied, the clay pots were placed in new plastic buckets with 105-µm mesh covering the windows, with one window removed from all plus-grazer buckets (controls and nutrient additions) to allow grazer migration.

Each limitation assay lasted for 6 days, after which benthic algal communities from each pot were harvested (as described above) and transported on ice back to the laboratory. Any benthic grazers present on the clay pots were collected for future identification and biomass measurements. Algae on clay pots from the same treatment within a lake were combined into one composite sample per treatment; thus, there were three algal slurries (1 per treatment) per lake. In general, composite sampling aims to reduce the effects of spatial variability on composition or biomass among clay pots of the same treatment and also reduces the number of samples that needed to be processed in one day. While this approach did not allow me to measure intra-treatment variability within a lake, my primary question in this study concerned patterns between lakes, therefore I focused sampling intensity across versus within lakes.

Composite algal slurries were subsampled for chlorophyll *a* and community composition. Chlorophyll *a* analyses were conducted on the same day that the algal communities were harvested. The chlorophyll *a* data were used to calculate values of nutrient and predator limitation. Limitation values were calculated using the following equations (Osenberg and Mittelbach 1996, Downing *et al.* 1999):

$$Lim_N = \frac{\ln(NA) - \ln(C)}{t} \tag{1a}$$

$$Lim_P = \frac{\ln(GR) - \ln(C)}{t} \tag{1b}$$

where t is duration of the experiment in days, Lim_N is nutrient limitation and Lim_P is predator limitation, and NA, GR, and C are the chlorophyll *a* measurements of the nutrient addition, grazer removal, and control treatments, respectively. Values of limitation are measures of the change in instantaneous per-capita growth rate of algal communities after the removal of a limiting factor. Positive values of limitation indicate that algal communities cumulatively increased in biomass, while negative values indicate that algal biomass decreased.

Statistical analyses and algal enumeration methods

Average values of nutrient and predator limitation across lakes were calculated for comparison with those observed in other across-system studies (*e.g.* Downing *et al.* 1999, Osenberg and Mittelbach 1996). Bias-corrected 95% confidence intervals around averages of limitation were bootstrapped (*sensu* Dixon 2001) using Matlab v. 5.3 (Mathworks, Inc. 1999). Values of nutrient and predator limitation were regressed against each of the 3 productivity measures (TN, TP, and chlorophyll *a*) as well as a composite variable represented by the first principal components axis (64% explained variance) from a principal components analysis (PCA) on the 3 original productivity variables (PC loadings: TP: 0.79, TN: 0.84, chlorophyll: 0.76). I used the average of chlorophyll *a* on clay pots measured immediately prior to the limitation assays and chlorophyll *a* on control clay pots at the termination of the limitation assays as a productivity measure in statistical analyses (regressions and ordinations). The average of chlorophyll *a* over these two samplings (Appendix B2.1) provides a more accurate representation of algal biomass over the 6-day limitation assay than either sampling alone. The relationships between both sources of limitation and the productivity variables were quantified using simple linear regressions (Systat v.8, SPSS, Inc.), while the means of nutrient and predator limitation were compared with a paired t-test.

Algal communities from control clay pots were used to examine patterns of species compositional change along the gradient of lakes. In one lake, Wintergreen Lake, the control algal composition sample was lost, so algae were enumerated from the grazer removal treatment. Because composition within lakes was more similar than composition between lakes (data not shown), I did not expect the small differences between species relative biovolume in within-lake treatments to change the overall interpretation of results.

Benthic algal species were enumerated and identified using established methods (Lowe and Pan 1996). Semi-permanent wet mounts of a sample were prepared and examined at 1250x magnification on a compound microscope. Three hundred natural units (*e.g.* a cell, filament or colony) were enumerated and identified to the species level (when possible) for each sample. In most cases, a second slide was prepared with acid-cleaned diatoms mounted in Naphrax (Patrick and Reimer 1966) to identify diatoms to the species level. A subsample of 10-15 organisms per species was measured for biovolume calculations, using published geometric forms and volume equations for various algal genera (Hillebrand *et al.* 1999). All algal community composition results presented here are in units of relative biovolume.

Algal species composition was related to lake environmental variables using redundancy analysis (RDA; Canoco v.4). Algal relative biovolume data were arcsine-square root transformed prior to analysis. These data were additionally transformed using a Hellinger distance transformation to avoid violating the strict linearity assumptions of RDA (Legendre and Gallagher 2001).

RDA uses eigenanalysis to partition the variation in algal species composition among several ordination axes (the first 4 in Canoco v.4), which are constrained to represent some combination of the environmental variables. RDA also uses a Monte Carlo permutation method to test whether a significant portion of the variation in algal composition is explained by the ordination axes. Scores of sites (*i.e.* lakes) and species are generated for each ordination axis and these values represent that site or species' correlation with a given axis. Scores from any combination of two axes can be used to graphically represent the results in a biplot. In addition, an RDA generates scores along the ordination axes for each environmental variable and these can be transposed, as vectors, on species' biplots. In a species' biplot, the angles between species' vectors are reflections of the correlations between species (*i.e.* how they often coexist) and the angle between a species' vector and an environmental vector reflects the correlation between that species and a particular environmental variable. However, biplots only show correlations in 2 dimensions (*i.e.* axes), so to better understand the relationship between a species and an environmental variable, I calculated the correlations using the scores from all 4 ordination axes. I use species-environment correlations to explore how algal species composition across lakes relates to predictions of the KPH. Finally, I regress lake site scores against both nutrient and predator limitation to examine how species composition contributed to the observed patterns. Lake site scores are representative of the relative positions of lakes in overall algal species composition and thus provide a univariate measure of species composition.

RESULTS

Limitation vs. lake productivity

There was a generally positive relationship on a log scale between water column TP and average algal biomass accumulation, measured as chlorophyll *a* (Figure 2.1A; r =

0.36, $P > 0.19$). Total phosphorus was also positively correlated with benthic grazer biomass (Figure 2.1B; $r = 0.61$, $P = 0.020$), indicating that the abundances of both primary producers and herbivores were positively correlated with productivity. When a clear statistical outlier (Douglas Lake; studentized residual = -3.7) was removed from the TP-chlorophyll *a* analysis, chlorophyll was more strongly correlated with TP ($r = 0.53$, $P = 0.06$). Further analyses do not include data from Douglas Lake and are restricted to the other 13 lakes.

Values of nutrient and predator limitation as well as environmental variables are listed for each lake in Appendix B2.1. In 13 out of the 14 lakes sampled, nutrient limitation was positive, indicating that algal biomass increased upon the addition of nutrients. The average value (± 95% confidence interval) of nutrient limitation across all lakes was 0.083 ± 0.023/day, which is a value similar to those reported by Downing *et al.* (1999) for marine phytoplankton (average ~ 0.1/day) and by Osenberg and Mittelbach (1996) for freshwater benthic algae (0.098/day). Nutrient limitation did not exhibit a consistent or significant relationship with any of the productivity variables, but, similar to expectations, nutrient limitation did remain strong in several lakes, despite high productivity (Figure 2.2, Table 2.1). In fact, nutrient limitation was significantly stronger than predator limitation (average = –0.019 ± 0.010/day) across nearly all lakes (paired t-test: $t_{12} = 4.38$, $P = 0.001$), which is similar to the pattern observed in a survey of aquatic systems by Osenberg and Mittelbach (1996). Interestingly, average predator limitation was negative (*i.e.* the 95% confidence interval around predator limitation average does not overlap zero; Figure 2.2), indicating that algal biomass decreased after the removal of

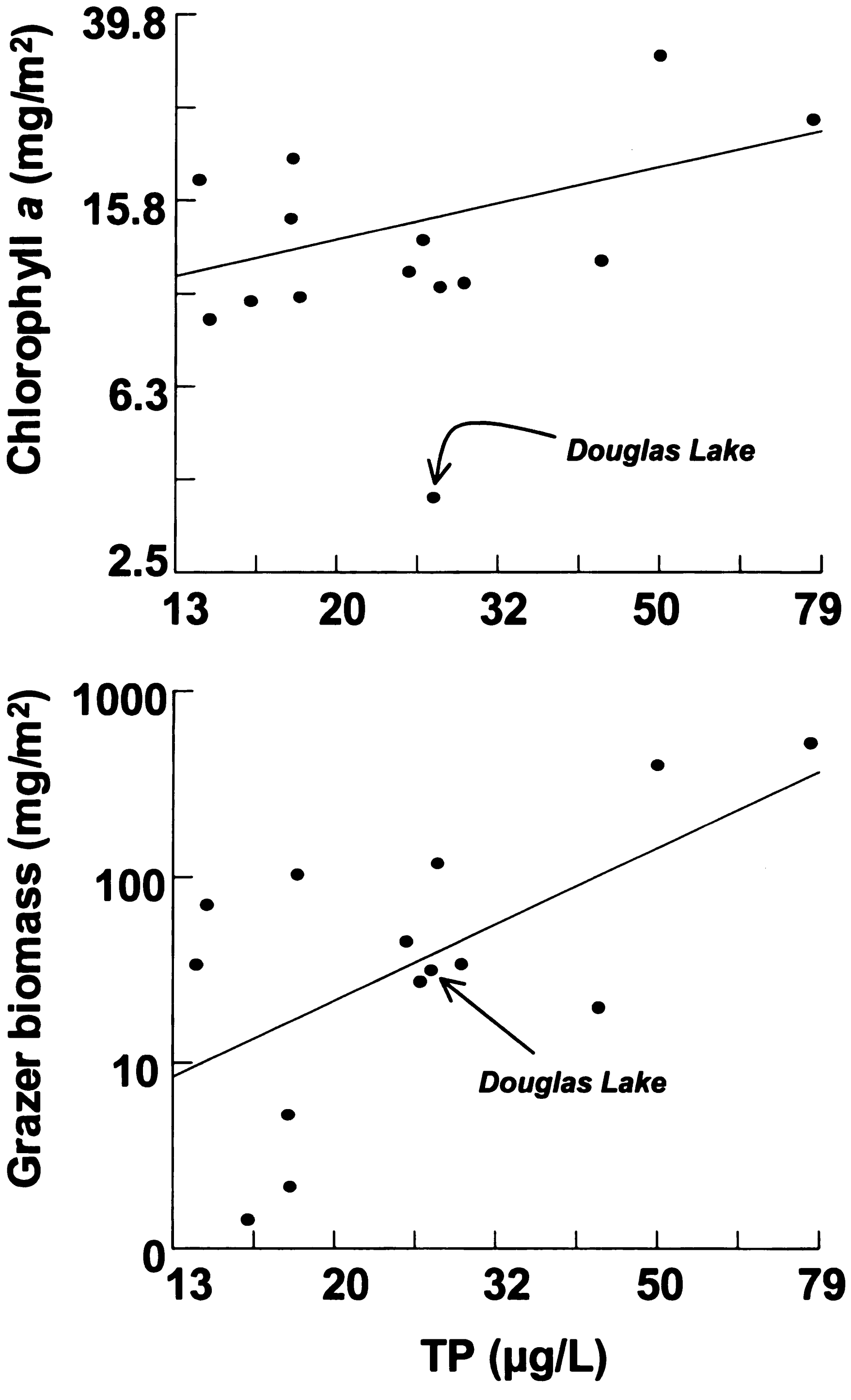


Figure 2.1: Trophic level biomass as a function of primary productivity (total phosphorus – TP) in 14 lakes in southwest Michigan. A) Benthic algal chlorophyll *a* accumulated on control clay flowerpots. B) Benthic grazer biomass (ash-free dry mass). All axes are on log scales, but values are given as non log-transformed values for ease of interpretation. The statistical outlier, Douglas Lake, is labeled. Lines are linear regressions; the regression for (A) does not include Douglas Lake.

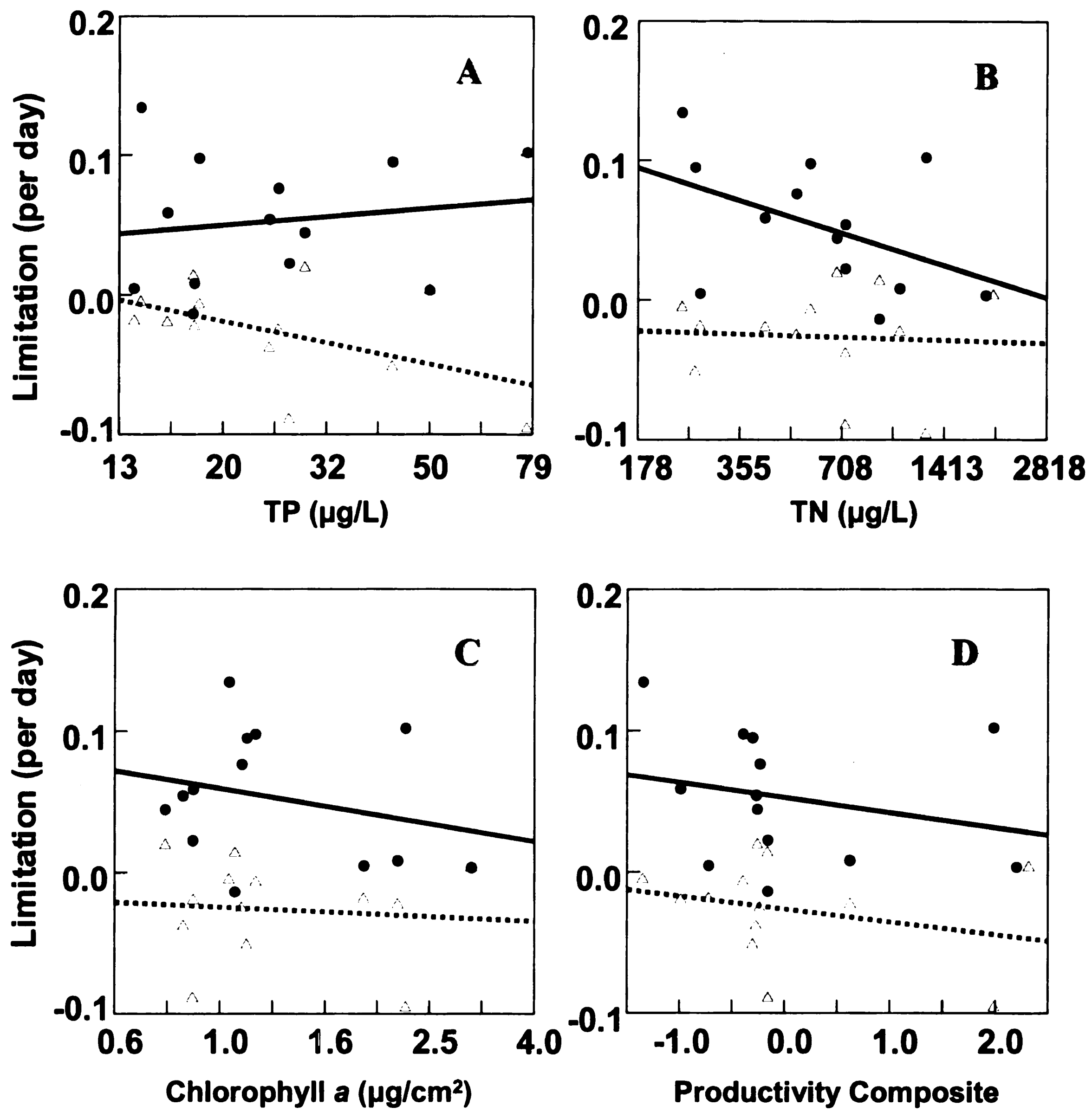


Figure 2.2: Nutrient limitation (closed circles) and predator limitation (open triangles) as functions of 4 measures of lake potential primary productivity. X-axes of A-C are on log scales, but values are given as non log-transformed values for ease of interpretation. Lines are linear regressions of nutrient limitation (solid line) and predator limitation (dashed line). Graphs are limitation versus: A) total phosphorus in the water column, B) total nitrogen in the water column, C) chlorophyll *a* on the clay pots, and D) productivity composite.

Table 2.1: Slopes, variation explained (R^2), and significance of regression statistics for nutrient and predator limitation modeled as a function of 4 explanatory variables: log (total phosphorus) (TP), log (total nitrogen) (TN), log (chlorophyll *a*), and as a composite.

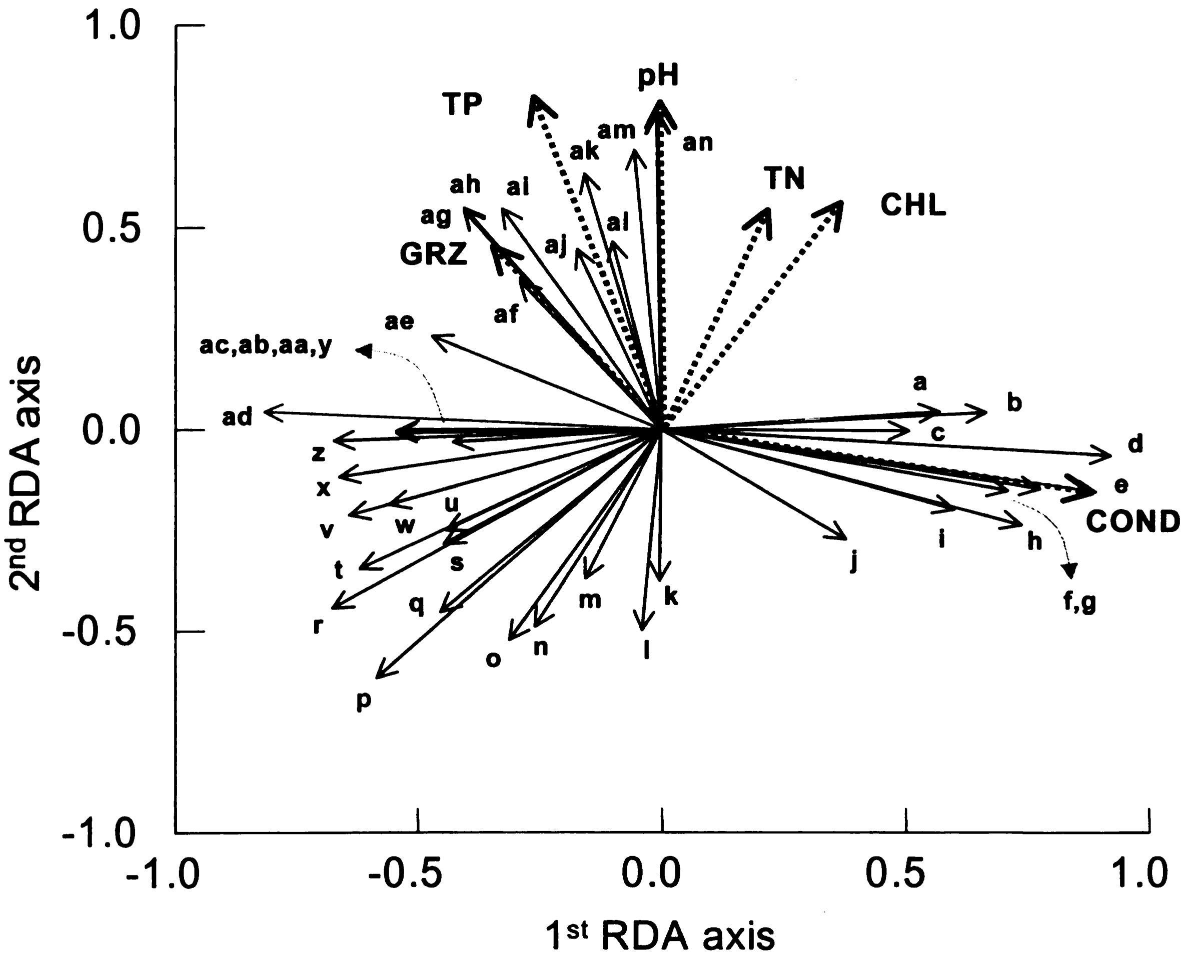
Variable	slope	R^2	F-statistic	P-value
Nutrient Limitation				
log TP	0.03	0.000	0.253	0.625
log TN	-0.078	0.137	2.897	0.117
log chlorophyll *a*	-0.119	0.123	2.687	0.129
composite	-0.019	0.092	2.213	0.165
Predator Limitation				
log TP	-0.076	0.176	3.560	0.086
log TN	-0.007	0.000	0.036	0.854
log chlorophyll *a*	-0.016	0.000	0.071	0.795
composite	-0.012	0.028	1.350	0.270

grazers. Predator limitation did not exhibit significant associations with any of the lake productivity measures, but tended to decline along a gradient in TP (Figure 2.2, Table 2.1). The significance of the regression analyses did not change with the inclusion of the outlier, Douglas Lake.

Species composition and environmental correlations

The first 4 RDA axes explained a significant portion (total = 52.5%) of the variation in algal species composition across lakes (F = 1.998, P = 0.015), accounting for 25%, 11.7%, 9.9%, and 5.9% of the total variation, respectively. The first RDA axis was highly correlated with conductivity (r = 0.86) and thus, algal species primarily sorted across lakes based on levels of conductivity. The second RDA axis correlated positively with the productivity variables (TP: r = 0.81, TN: r = 0.59, and CHL: r = 0.52) as well as with grazer biomass (r = 0.41) and pH (r = 0.74). The third RDA axis correlated negatively with TN (r = -0.55), while the fourth RDA axis correlated positively with CHL (r = 0.67). The RDA biplot (Figure 2.3) uses axis scores from the conductivity and overall productivity-grazer biomass axes (axes 1 and 2, respectively).

Several benthic algal species loaded strongly with the first RDA (conductivity) axis (Figure 2.3). Diatom species exhibited especially strong positive correlations with the conductivity vector. For example, *Achnanthes exilis*, *Denticula keutzingii*, *Encyonopsis microcephala*, *Encyonema* sp., and *Navicula radiosa*, all had correlations over 0.55 with conductivity (see Appendix B2.2 for all species-environment correlations). Alternatively, several chlorophytes demonstrated strong, negative correlations with the conductivity vector. For instance, the filamentous *Stigeoclonium* sp. and the colonial *Ankistrodesmus falcatus*, *Oocystis* spp., *Pediastrum duplex*, and



a) *Navicula radiosa* (D)
b) *Gomphonema clevei* (D)
c) *Cosmarium* sp. B (G)
d) *Encyonema* sp. (D)
e) *Encyonopsis microcephala* (D)
f) *Aphanocapsa* sp. (BG)
g) *Achnanthes exilis* (D)
h) *Leptolyngbya* sp. F (BG)
i) *Denticula keutzingii* (D)
j) *Cymbella cymbiformis* (D)
k) *Mastogloia smithii* (D)
l) *Nitzschia fonticola* (D)
m) *Calothrix* spp. (BG)
n) *Epithemia turgida* (D)
o) *Cylindrospermum stagnale* (BG)
p) *Nostoc* sp. B (BG)
q) *Chroococcus limneticus* (BG)
r) *Aphanothece stagnina* (BG)
s) Medium *Oedogonium* spp. (G)
t) *Rhopalodia gibba* (D)
u) *Amphipleura pellucida* (D)
v) *Nostoc* sp. A (BG)
w) Small *Oedogonium* spp. (G)
x) *Ankistrodesmus falcatus* (G)
y) *Aphanocapsa conferta* (BG)
z) *Oocystis* spp. (G)
aa) *Stigeoclonium* sp. (G)
ab) *Limnothrix redekei* (BG)
ac) *Pediastrum duplex* (G)
ad) *Scenedesmus bijuga* (G)
ae) *Leptolyngbya* sp. A (BG)
af) *Chroococcus minutus* (BG)
ag) Large *Oedogonium* spp. (G)
ah) *Staurastrum* sp. A (G)
ai) *Pediastrum boryanum* (G)
aj) *Scenedesmus quadricauda* (G)
ak) *Fragilaria crotonensis* (D)
al) *Limnothrix lauterbornii* (BG)
am) *Gomphonema truncatum* (D)
an) *Gomphonema parvulum* (D)

Figure 2.3: Biplot of species scores (solid vectors) and environmental scores (dashed vectors) from a redundancy analysis on algal species relative biovolume in 13 lakes. Only species with a score on either axis of over 0.40 are included in the biplot. Species are listed with a taxonomic designation in parentheses: (D) = diatoms, (BG) = blue-green algae (cyanophytes), (G) = green algae (chlorophytes).

Scenedesmus bijuga, all exhibited correlations over –0.60 with the conductivity axis.

A suite of benthic algal species loaded strongly along the productivity/grazer biomass axis (Figure 2.3). The species-environment associations along this axis are particularly important for examining species turnover in a KPH context, so they are presented in more detail. In most cases, the species-productivity (TN, TP, or CHL) correlation and the species-grazer biomass correlation had the same sign and often had approximately the same magnitude (Appendix B2.2). For instance, species that were positively correlated with the productivity variables were also positively correlated with grazer biomass. Figure 2.4 illustrates this trend for the species-TP and species-grazer biomass correlations.

Several interesting compositional patterns emerge when examining the identity of the species showing strong correlations with productivity and grazer biomass (Figure 2.4, Appendix B2.2). First, all of the species strongly negatively correlated with TP and grazer biomass were either small to medium-sized (*i.e.* edible) diatoms or cyanophytes, including several nitrogen-fixers (*Epithemia turgida*, *Calothrix* spp., and *Nostoc* sp. B) (Figure 2.4). Nitrogen-fixers are especially good nutrient competitors, as they are able to persist in low nitrogen conditions (Wehr and Sheath 2003). Second, several chlorophytes, including one grazer resistant filamentous species (*Oedogonium* spp.) were positively correlated with TP and grazer biomass, but none were negatively correlated with these environmental variables. Third, half of the species with strong positive correlations with TP and grazer biomass were plankton species (*Scenedesmus bijuga*, *Fragilaria crotonensis*, *Staurastrum* sp. A, *Scenedesmus quadricauda*, and *Pediastrum boryanum*), indicating that more plankton species tended to settle into the benthic

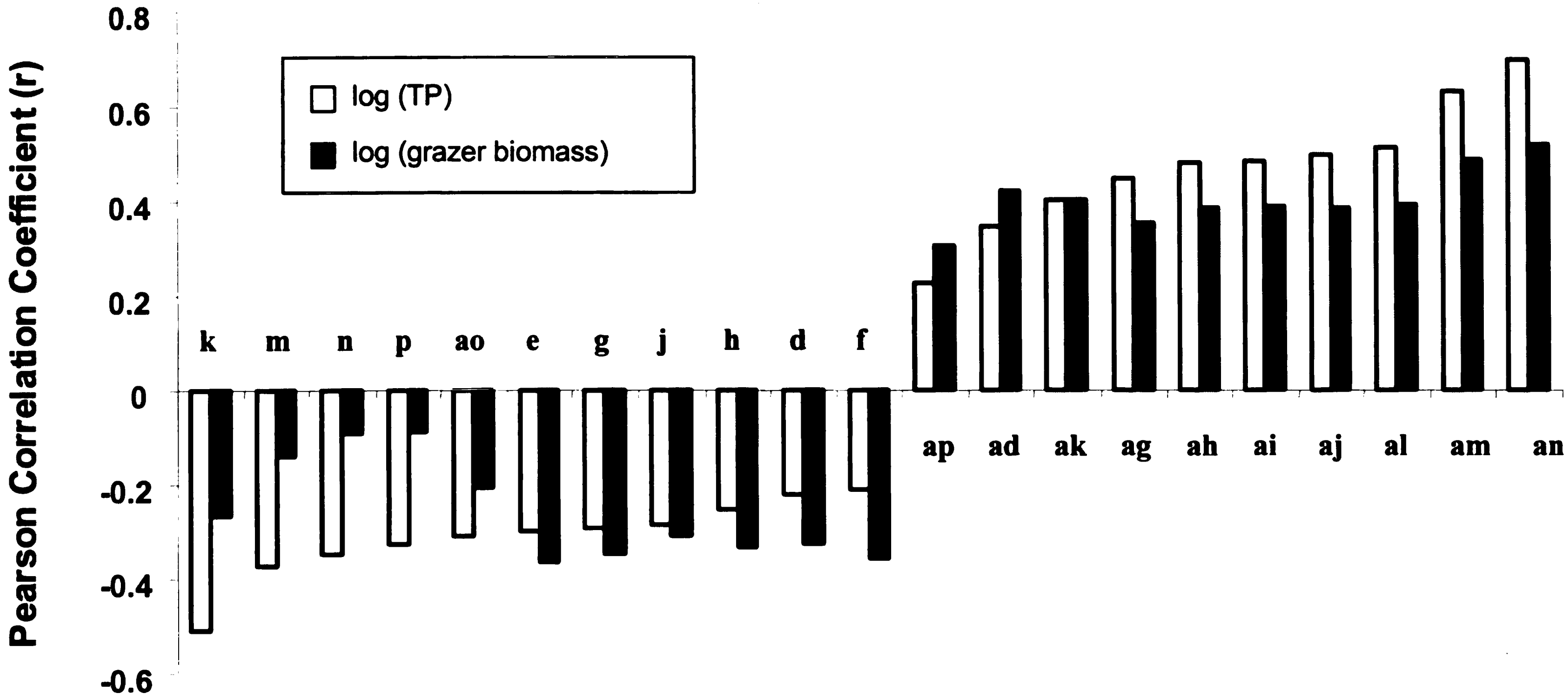


Figure 2.4: Species-environment correlations from RDA for log-transformed total phosphorus (TP; white bars) and log-transformed grazer biomass (black bars). Only those species with correlations over 0.30 are included. Lettering of species is the same as those listed in Figure 2.3, with two additional species: ao) *Anomoneis vitrea* (D) and ap) *Leptolyngbya* sp. B (BG).

correlations with TP and grazer biomass were plankton species (*Scenedesmus bijuga*, *Fragilaria crotonensis*, *Staurastrum* sp. A, *Scenedesmus quadricauda*, and *Pediastrum boryanum*), indicating that more plankton species tended to settle into the benthic community in high productivity lakes. Finally, the species common at high productivity were on average 7 times larger than those at low productivity. In general, larger species are inferior competitors compared to small species (Steinman *et al.* 1992), due to their higher nutrient requirements.

I hypothesized that changes in predator biomass would affect algal species composition, subsequently influencing patterns of limitation across lakes along a productivity gradient. I have shown that grazer biomass and productivity are positively correlated in these lake systems and that algal species turnover occurred along this gradient (Figures 2.1, 2.3, 2.4). In Figure 2.5, I used a univariate measure of species composition (lake site scores from the RDA) to determine if there was any association between species compositional shifts and patterns of nutrient and predator limitation. If a correlation exists, then species composition may be related to limitation. I chose lake site scores from the productivity-grazer biomass RDA axis (axis 2), because this was the primary axis of interest in the context of the KPH. Nutrient limitation was unrelated to lake site scores ($r = 0.03$, $P = 0.93$), whereas predator limitation was significantly negatively correlated to the lake productivity-grazer biomass axis ($r = -0.55$, $P = 0.051$), indicating that algal species composition resulting from gradients in productivity and grazer biomass affected the extent of these communities' responses to the removal of predators.

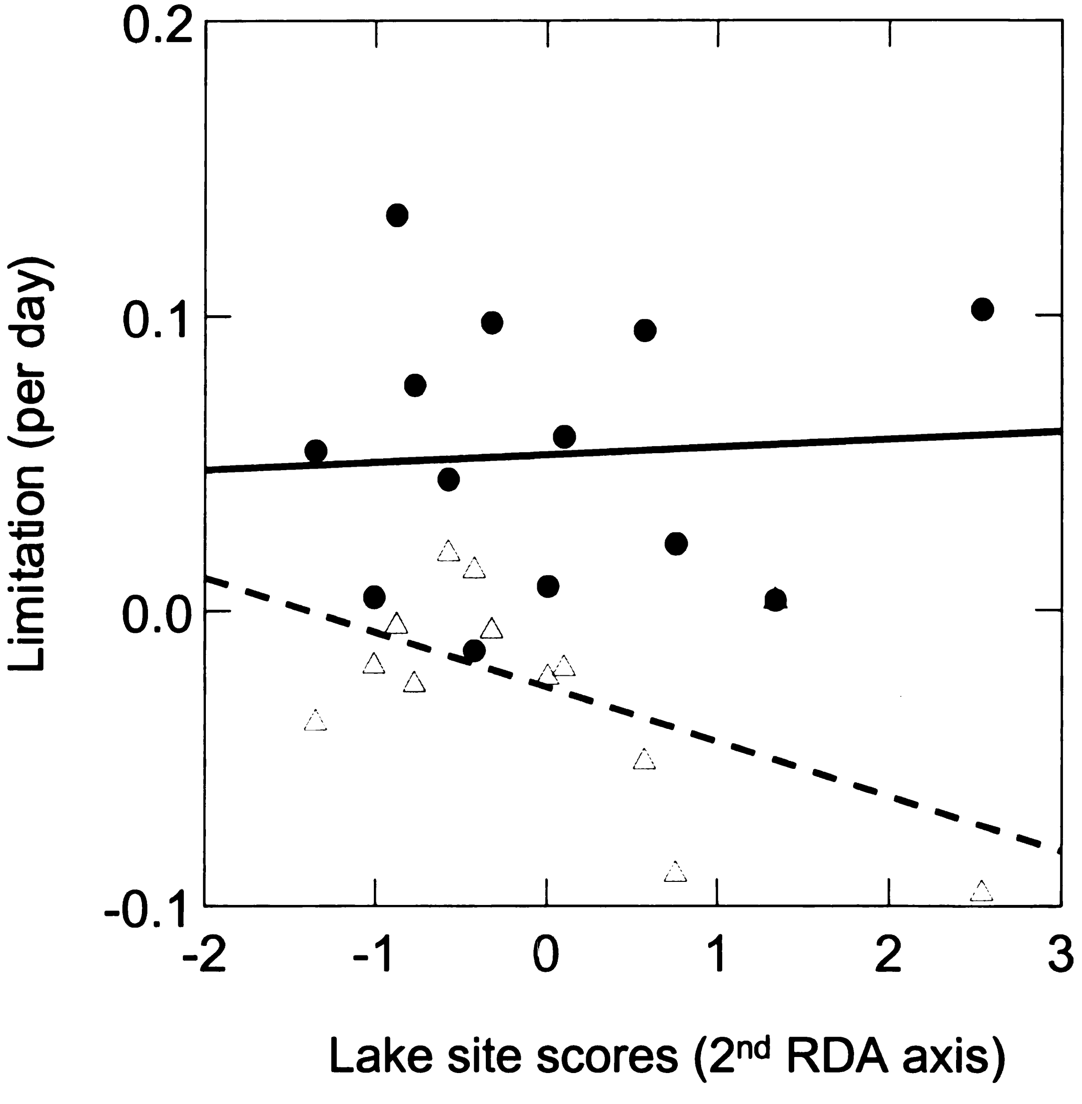


Figure 2.5: Nutrient limitation (closed circles) and predator limitation (open triangles) versus the lake site scores from the 2nd RDA axis (productivity-grazer axis). Lines are linear regressions for nutrient limitation (solid line) and predator limitation (dashed line).

DISCUSSION

Patterns of nutrient and predator limitation

The limitation model predicted that in food chains, nutrient and predator limitation should strongly decrease and increase, respectively, as potential primary productivity increases (Darcy-Hall, Chapter 1, Appendix A). Alternatively, the limitation model predicted that for food webs (*i.e.* KPH context), nutrient limitation should decline non-linearly, remaining strong at high productivities, while predator limitation should be low but also a declining function of productivity (Darcy-Hall, Chapter 1, Appendix A). The results of the benthic algal limitation assays conducted across these lakes more closely matched predictions from the KPH than those of simple food chain models. First, nutrient limitation remained stronger than predator limitation across all lakes. Second, large values of nutrient limitation were observed along the entire gradient in TP and chlorophyll. Lastly, while neither nutrient nor predator limitation varied significantly with productivity, predator limitation tended to decrease across a gradient in TP, contrary to what would be expected from the ecosystem exploitation hypothesis (Oksanen *et al.* 1981).

While nutrient limitation did not significantly decline with any of the productivity variables, one could argue that I lacked power to detect a significant decline of nutrient limitation along a gradient of TN (Figure 2.2). Algal communities in especially low TN lakes (*e.g.* Shaw and Bassett) responded more to the addition of nutrients (Whitford Lake is an exception to this). Hillebrand and Kahlert (2001) similarly noted that lake periphyton responded less to the addition of nutrients in higher TN systems; however, they were unable to detect any significant relationships between abiotic variables and the strength of top-down or bottom-up effects in their 3 lake systems. Similarly, in a meta-analysis of 85 experiments manipulating nutrient enrichment and herbivore presence in the benthic algae, Hillebrand (2002) found no significant relationship between productivity and top-down or bottom-up effect size. A potential decline in nutrient limitation with TN enrichment is not necessarily supportive of simple food chain model predictions. In fact, a decline in nutrient limitation with any productivity variable cannot be attributed to either food chain models or the KPH in the present study, because it is impossible to determine where these particular lakes fall along the hypothetical nutrient supply gradient in the limitation model (Darcy-Hall, Chapter 1, Appendix A). However, as mentioned above, other evidence points to the KPH (or food web) expectations as being more realistic. For example, one might expect from simple food chain models that the importance of predator limitation would surpass that of nutrient limitation in more productive systems (Darcy-Hall, Chapter 1, Appendix A, Figure A1.2). Regardless of the productivity measure used, nutrient limitation was always as strong as or stronger than predator limitation and predator limitation did not increase across lakes.

I had hypothesized that predator limitation would decline with grazer biomass and productivity, because of the presence of more predator resistant species at higher productivities. The strongly negative predator limitation values in high productivity lakes indicated that algal community biomass decreased greatly upon the removal of grazers. A decrease in algal biomass following the removal of grazers was unexpected, but not unprecedented, as both theory (de Mazencourt *et al.* 1998) and short- (Sterner 1986, Leibold *unpubl. data*) and long-term experiments (McCormick and Stevenson 1989, McCormick and Stevenson 1991) have observed the stimulation of algal growth by grazers via nutrient regeneration or removal of competitors. Both the KPH (Holt *et al.* 1994, Grover 1994, Leibold 1996) and models of defense theory (de Mazancourt and Loreau 2000, de Mazancourt *et al.* 2001), assume that better defended species tradeoff predator resistance with the ability to acquire nutrients. Thus, grazer resistant species may often be nutrient limited. In the context of a benthic algal mat, grazer resistant, nutrient-limited species can benefit from herbivory, either by utilizing a limiting nutrient excreted by herbivores as they consume more vulnerable species (de Mazancourt *et al.* 1998, de Mazancourt and Loreau 2000) or by gaining closer access to water column nutrients and light via the removal of edible overstory species (McCormick and Stevenson 1991, McCormick 1994, Steinman 1996). Given that I expected and observed more grazer resistant species in lakes with higher grazer biomass (see below), it is not surprising that it was within these lakes that predator limitation was often strong and negative. Similarly, in a Canadian lake, Graham and Vinebrooke (1998) observed a shift from a net negative to a net positive effect of grazers on benthic algal biomass, as algal composition shifted to more grazer resistant (*i.e.* prostrate, firmly attached species) forms throughout the summer.

Benthic algal compositional shifts

The 13 lakes in this study varied widely in conductivity and pH (Appendix B2.1), which appeared to be important abiotic variables affecting the distribution of algal species amongst lakes. This is not surprising, as many algal species, especially diatoms, are sensitive to levels of conductivity or alkalinity (Lowe 1996, Patrick and Reimer 1966). The strong relationship between conductivity and species composition was likely driven by several lakes in this survey, particularly Lawrence, Tamarack, and Three Lakes

2, which are high conductivity, marl lakes with very similar algal compositions (data not shown). As I had no *a priori* interest in exploring the relationship between conductivity or pH and algal composition and as neither variable is important within a KPH context, I have focused my discussion of algal compositional changes to those related to gradients in productivity and grazer biomass.

The KPH predicts that in high productivity, high grazer biomass lakes, grazer resistant forms of benthic algae should dominate; whereas, at low productivities, grazer vulnerable, superior nutrient competitors should prevail. The patterns of benthic algal composition across my study lakes lend some support to these hypotheses. Low productivity lakes were dominated by predator-vulnerable, small to medium-sized diatoms and cyanophytes. Several of the species at low productivity were undoubtedly superior nutrient competitors (*e.g.* the nitrogen fixers), and the generally small size of these low productivity species may imply stronger competitive abilities when compared to the larger species observed in high productivity lakes (Steinman *et al.* 1992).

A variety of algal species, over half of which were planktonic, exhibited strong positive correlations with TP and grazer biomass. Only one of the benthic species, the tightly attached, filamentous chlorophyte, *Oedogonium* spp., displays a grazer resistant morphology (Steinman *et al.* 1992, Graham and Vinebrooke 1998). The two other benthic species, *Gomphonema parvulum* and *G. truncatum*, are edible, stalked diatoms that are likely epiphytic on *Oedogonium*. The presence of edible species at high productivities is not unexpected; in fact, edible species must be present in order to support high populations of benthic grazers. The remaining species associated with high productivity lakes are planktonic (or unknown in the case of the cyanophytes), several of which (*e.g. Fragilaria crotonensis*, *Pediastrum boryanum*) are commonly observed in eutrophic systems (Wehr and Sheath 2003). In addition, most of these plankton species are likely to be resistant to zooplankton grazing: *F. crotonensis* and *P. boryanum* on the basis of size and *Staurastrum* sp. A and *Scenedesmus quadricauda* due to the presence of arms or spines. The persistence of these plankton species in the benthic mat indicates that they are somehow avoiding benthic predation. I propose two possible mechanisms of predator avoidance. First, traits conferring grazer resistance in the plankton may also apply within the benthos. Alternatively, nutrient-rich lakes may produce a constant re-supply of phytoplankton to the benthos, resulting in phytoplankton immigration rates that surpass the rate of benthic grazing. Given the descriptive nature of the present study, it is not possible to distinguish between these two alternative explanations. Regardless of the mechanism, these phytoplankton species ultimately escaped benthic predation and should be considered grazer resistant.

Tradeoffs in competitive ability and herbivore resistance have been documented before in the periphyton (Graham and Vinebrooke 1998, Rosemond *et al.* 1993, Steinman *et al.* 1992). However, these researchers define competitiveness as better access to water-column nutrients and not as the ability to persist when nutrients are scarce (*sensu* Tilman 1982), the definition adopted by the KPH. The former view of competition may be more appropriate for vertically structured benthic communities. Despite the semantic differences in defining competition, the KPH continues to be an appropriate context for exploring benthic algal ecology. The fundamental tenet of the KPH is that predators mediate shifts in prey composition, due to a tradeoff among prey species in their ability to resist predation and compete for resources. If the tradeoff exists, regardless of how the key species' traits of competition and resistance are defined, the KPH remains applicable.

The overall pattern of species composition across the lake productivity gradient provides support for the predictions of the KPH. There were more predator-vulnerable, superior competitors strongly associated with low productivity lakes, while larger, grazer resistant species dominated in high productivity systems. However, this study is correlative in nature and thus I cannot conclusively state that the patterns in species composition were caused by changes in herbivore biomass. I address this question experimentally in Chapter 3. In addition, there is not enough known about individual species' traits to interpret these patterns as a broad demonstration of the KPH. I provide a more thorough treatment of algal species' traits in the context of the KPH in Chapter 4.

Linking species turnover to patterns of limitation

Different algal communities vary in the identity and the traits of the dominant species. Variation in dominant algal species and species' traits across communities should lead to differences in the community-wide response to the removal of limiting factors (*i.e.* limitation). As I have described above, species composition varied greatly across the study lakes, and many species demonstrated strong associations with certain environmental factors (*e.g.* TP or grazer biomass), indicating that species' traits might also vary across lakes. Given this variation in species' traits, the composition of an algal community in a particular lake should affect how that community responds to the addition of nutrients or the removal of grazers. Among these lakes, algal species composition along the productivity-grazer biomass gradient was clearly related to predator limitation, but not to nutrient limitation.

One possible scenario to explain the predator limitation-species composition correlation is that the presence of more grazer resistant species in lakes with high grazer biomass translated into a less predator-limited community that in turn responded negatively to the removal of grazers. This counterintuitive response was perhaps due to the tendency of grazer resistant species to be nutrient limited, because of their size, position in the algal mat, or the costs associated with defenses such as mucilaginous exteriors (de Mazancourt and Loreau 2000). Nutrient limitation, on the other hand, did not appear to be related to species composition along the productivity-grazer biomass axis. This is not surprising as nutrient limitation was not significantly related to any of the productivity variables, and nutrient limitation did not significantly correlate with lake site scores from any of the other three axes (data not shown). It is possible that nutrient limitation may indeed be related to species composition, but along some unmeasured gradient. Alternatively, the strength of nutrient limitation could be more affected by abiotic variables and less by species composition and individual species' traits. Finally, it is also possible that with only 13 lakes, I lacked the power necessary to observe a relationship between nutrient limitation and species composition.

While the analysis of benthic algal composition provided support for predictions of the KPH, it is obvious that it cannot fully account for the variability in algal species composition across these lakes. The fundamental keystone predator model parameters, herbivore biomass and potential primary productivity (and pH) accounted for only 27.9% of the variation in algal species composition. Conductivity alone accounted for nearly as much (25%). The low variation explained by the variables of interest is hardly surprising, given that the survey of algae is conducted in natural systems, which are inevitably highly variable in all components of the ecosystem. Therefore, it is crucial to recognize that while the KPH tradeoff may be an important influence affecting limitation patterns and species composition, it is only one of several simultaneously occurring mechanisms. In Chapter 3, I use a more controlled, experimental approach to examine the linkage between herbivore biomass, productivity, and patterns of algal limitation.

Conclusions

Inter-ecosystem patterns of nutrient and predator limitation of benthic algae in this study mirrored predictions of both verbal (Osenberg and Mittelbach 1996) and mechanistic keystone predator models (Holt *et al.* 1994, Grover 1994, Leibold 1996). Nutrient limitation was stronger than predator limitation and remained so across a lake productivity gradient. In these lakes, predator limitation did not increase across the productivity and grazer biomass gradient, contradicting predictions of simple food chain models (Darcy-Hall, Chapter 1, Appendix A, Oksanen *et al.* 1981). Additionally, changes in benthic algal species composition across lakes and the traits of common species qualitatively complied with the keystone predation hypothesis. This research illustrates the importance of heterogeneity both within and among systems to the elucidation of interactions between a community of organisms and the surrounding abiotic and biotic environments.

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CHAPTER 3

LINKING HERBIVORE-MEDIATED SPECIES TURNOVER TO PATTERNS OF BENTHIC ALGAL NUTRIENT AND PREDATOR LIMITATION

INTRODUCTION

Predators can play an important role in structuring communities by directly affecting the species composition of prey communities. When predation pressure is strong, we expect prey communities to be composed of more predator resistant, well-defended species. In aquatic systems, there are numerous examples of herbivorous predators causing shifts to more predator resistant algal prey communities. For instance, herbivore-mediated effects have been observed in freshwater periphyton (or benthic algae) (*e.g.* Rosemond *et al.* 1993, Lamberti *et al.* 1995), phytoplankton (*e.g.* Leibold 1999, Steiner 2001) and marine macroalgae (*e.g.* Lubchenco and Cubit 1980). However, there are costs to defense, and it has been hypothesized that organisms, particularly primary producers, will exhibit tradeoffs in their ability to compete for resources and resistance to predation (Paine 1966, Lubchenco and Gaines 1981, Vance 1978, Holt *et al.* 1994, Grover 1994, Leibold 1996, Chase *et al.* 2000). For instance, benthic algal species with traits that confer superior competitive abilities (*e.g.* small size or erect forms) are vulnerable to herbivores. Alternatively, defended species tend to be inferior competitors, due in part to costs of defense as well as their inherent morphology (*e.g.* large or adnate) (Steinman *et al.* 1992, Rosemond 1996). Despite an abundance of anecdotal observations, direct evidence for this tradeoff is limited.

If prey species exhibit a tradeoff in their ability to acquire resources and their resistance to predation, then theory predicts that prey community composition should shift toward dominance by predator resistant species with changes in both predator density and resource supply (Leibold 1996, Holt *et al.* 1994, Grover 1994). The first prediction is straightforward: higher intensities of predation favor more predator resistant prey species. The second prediction involves an upward trophic cascade: higher ecosystem resources result in higher abundances of herbivores (Leibold 1999, Ginzburg and Akcakaya 1992, McNaughton *et al.* 1989, Mills and Schiavone 1982), that in turn exert more selection for resistance in their primary producer prey. This phenomenon has been coined the keystone predation hypothesis (KPH; Leibold 1996, Holt *et al.* 1994, Grover 1994) and it focuses on the role of predators in shifting prey community structure, contributing to the positive correlations between the biomass of adjacent trophic levels.

The KPH predicts that vulnerable, superior competitors will dominate at low productivities, while more predator resistant, less resource efficient taxa will dominate at high productivities (Leibold 1996, Holt *et al.* 1994, Grover 1994). As a result, resource limitation (*i.e.* the extent to which prey increase upon augmentation of resources [*sensu* Osenberg and Mittelbach 1996]) of prey is expected to remain strong across a gradient in resource supply (Darcy-Hall, Chapter 1, Appendix A). For instance, competitively inferior but defended species will be less limited by predators, yet still limited by nutrients. Therefore, resource limitation of prey will remain strong along a productivity gradient: at low productivities, resources are scarce but taxa are good resource competitors, while at high productivities, resources are plentiful, but dominant taxa are less efficient at consuming those resources. Alternatively, predator limitation (the extent to which producer biomass increases after the removal of predators) is expected to be low and potentially decrease across a productivity gradient, as more predator resistant species dominant at higher productivities (Darcy-Hall, Chapter 1, Appendix A). These general predictions of the KPH are in sharp contrast with predictions from simple (*i.e.* one species/trophic level) food chain models (Hairston *et al.* 1960, Oksanen *et al.* 1981). For instance, these models posit that in a 2-trophic level system, herbivores will increase with productivity, but primary producers will not. Consequently, the producer species is expected to become less nutrient limited but more predator limited as potential primary productivity and the biomass of herbivores increases (Darcy-Hall, Chapter 1, Appendix A). Field studies of primary producers have observed consistent and strong nutrient limitation across a productivity gradient of aquatic systems (Darcy-Hall, Chapter 1, Downing *et al.* 1999, Osenberg and Mittelbach 1996). However, there has been no direct effort to link herbivore-mediated shifts in prey composition to patterns of resource and predator limitation.

One approach to linking herbivore-mediated species turnover to patterns of limitation is to compare such patterns in communities that have developed with and without herbivore pressure. I hypothesize that benthic algal communities historically exposed to herbivores ("grazed" communities) will exhibit herbivore-mediated shifts in composition, and thus patterns of limitation will be similar to those predicted by the KPH. In contrast, I expect that the composition of historically herbivore-naïve ("ungrazed") algal communities will shift solely on the basis of differences in nutrient supply rate, and due to the absence of any grazing pressure, predator resistant species will not dominate at higher productivities. Thus, I predict that at high productivities, ungrazed communities will exhibit stronger predator limitation and less nutrient limitation than historically grazed communities, a pattern resembling predictions from simple food chains. Patterns at low productivity are less predictable, as the limitation model indicates that limitation for both food chains and food webs may be similar at low enrichment (*i.e.* high resource limitation and low predator limitation) (Darcy-Hall, Chapter 1, Appendix A). I do not expect limitation in ungrazed communities to perfectly mimic the expectations of simple food chain models, as these communities will actually be food webs and will likely exhibit more complex patterns than those predicted for food chains in the limitation model (Darcy-Hall, Chapter 1, Appendix A). Despite this, I expect that limitation in ungrazed communities will be measurably different than in grazed communities and in the directions predicted. Hence, this experiment aims to directly examine the herbivore-mediated changes in algal composition and how these shifts might drive patterns of algal limitation.

METHODS

Limitation metric

Limitation is defined as the extent to which a population's per capita growth rate is reduced by predators or insufficient nutrient supply and is measured as the increase in the population's per capita growth rate upon the removal of a limiting factor (*e.g.* via the addition of saturating nutrients or the removal of predators) (Osenberg and Mittelbach 1996). Limitation is measured in the short-term to avoid confounding its direct effects with the indirect effects caused by a numerical (or functional) response of predators or a switch to limitation by another resource (Osenberg and Mittelbach 1996, Downing *et al.* 1999). Downing *et al.* (1999) found that in an experimental setting, limitation was a constant and reliable measure of the change in algal population per capita growth over a period of 2-7 days, post treatment initiation.

Study system

The experiment was conducted in 1000-L cattle tank mesocosms during the summer of 2003 at Michigan State University's W. K. Kellogg Biological Station (KBS) experimental pond lab facility (Hickory Corners, MI, USA). Cattle tanks are known to effectively represent pond environments (Leibold *et al.* 1997) and previous experiments have demonstrated that a large subset of lake benthic algae persist in the mesocosm environment (Darcy-Hall, Chapter 4). For example, a 2001 lake survey in southwest Michigan, I identified 214 benthic algal species, 120 of which were observed in a cattle tank experiment the following year (Darcy-Hall, Chapters 2, 4). The mesocosm experiment was conducted in two phases: an initial, 3-month community establishment period (Phase 1) followed by 1-week limitation assays (Phase 2).

Phase 1: Community establishment

Initial grazed and ungrazed algal communities were established in early May 2003 within a simple experimental context. Four levels of nutrient supply were crossed with the presence or absence of a benthic grazer community, yielding 8 treatments that were replicated 4 times. Cattle tank mesocosms were acid-washed, filled with well water, and covered with a 1-mm mesh lid, composed of fiberglass window screening. Fourteen 3" (7.6 cm) terracotta clay flowerpots in each mesocosm served as sampling and experimental substrates for benthic algal communities. Nitrogen (as ammonium nitrate) and phosphorus (as potassium phosphate) were added to the water column to establish four levels of productivity. Target nutrient supply concentrations were 10 (LOW), 30 (MED), 75 (HIGH), and 200 (XHIGH) μg/L total phosphorus (TP), with nitrogen added in a 50:1 molar ratio. These productivity levels encompassed natural variation in TP and

TN of both lakes and ponds local to KBS (Hall *et al. in press*, Darcy-Hall, *unpubl. data*). Nutrients were added every 5 days throughout the experiment to maintain the approximate target nutrient concentrations. Benthic grazers included three species of gastropods (*Physa* sp., *Helisoma* sp., and *Gyraulus* sp.) as well as amphipods (*Hyallela azteca* and *Gammarus* sp.). In nature, consumer densities increase concomitantly with increasing producer density (reviewed Leibold *et al.* 1997), so I initially established an increasing density of grazers among the four levels of nutrient supply. Grazer density data from lake surveys (Darcy-Hall, Chapter 2) were used to determine the densities at which to add grazers to mesocosms. Grazers were added at 0.09, 0.24, 0.63, 1.64 g dry mass/m^2, densities approximate to those expected at increasing levels of nutrient supply.

Arrays of clay pots were established in the littoral zone of seven lakes to grow communities of algae for seeding the mesocosm experiment. The lakes were chosen to span a wide gradient of TP (13.5 – 77.5 μg/L) and TN (208.9 - 1869 μg/L). To inoculate mesocosms, benthic algae were scraped from several clay pots in all lakes and mixed with deionized water. Resulting algal slurries were transported to the lab, sieved through a 125 μm sieve to remove micro- and macrograzers, combined and mixed in a carboy, and then distributed in 100 mL aliquots to each cattle tank. Algal species composition among lakes was expected to vary through time, so this inoculation process was repeated once every 2 weeks for the duration of Phase 1 to allow continued colonization opportunities for a diverse algal assemblage.

Benthic algal species were allowed to establish in these experimental environments for 15 weeks (approximately 50 algal generations) prior to the initiation of Phase 2. One week prior to the limitation assays, the algal communities from two clay pots per cattle tank were combined and subsampled to measure pre-Phase 2 chlorophyll *a*, grazer densities, and algal species composition. Algae were removed from clay pots as described above. Grazers were preserved in 70% ethanol and algae for species identification were preserved in 10% formalin. All chlorophyll *a* measurements were conducted on the same day as sampling, using cold ethanol extractions followed by narrow-band fluorometry (*sensu* Welshmeyer 1994). In addition, water from the mesocosms was sampled to measure TP and TN content. Total nutrients were measured using spectrophotometry and standard methods (Bachmann and Canfield 1996, Crumpton *et al.* 1992, Prepas and Rigler 1982, APHA 1980)

After sampling, the remaining 12 clay pots from each ungrazed cattle tank were shifted into a grazed cattle tank (Figure 3.1) within the same nutrient supply treatment. For example, clay pots from a no-grazer, LOW mesocosm were shifted into a randomly chosen plus-grazer, LOW mescosm. Ultimately, 16 mesocosms containing both grazed and ungrazed sets of algal communities were used in the limitation assays. Resident clay pots in the grazed mesocosms were also lifted out of the water and moved to a new location within the same tank. Predator limitation by definition measures the effect of the *removal* of grazers on a communities' per capita growth rate. Therefore, the ungrazed algal communities required a period of grazing prior to the onset of the limitation assays. From previous experiments, one week was determined to be long enough for grazers to have an effect on biomass but not on community composition (Darcy-Hall, *unpubl. data*).

Phase 2: Limitation assays

Seven-day limitation assays were initiated on August 25, 2003. Due to the scale of these experiments, I was only able to set up the assays in 8 cattle tanks per day;

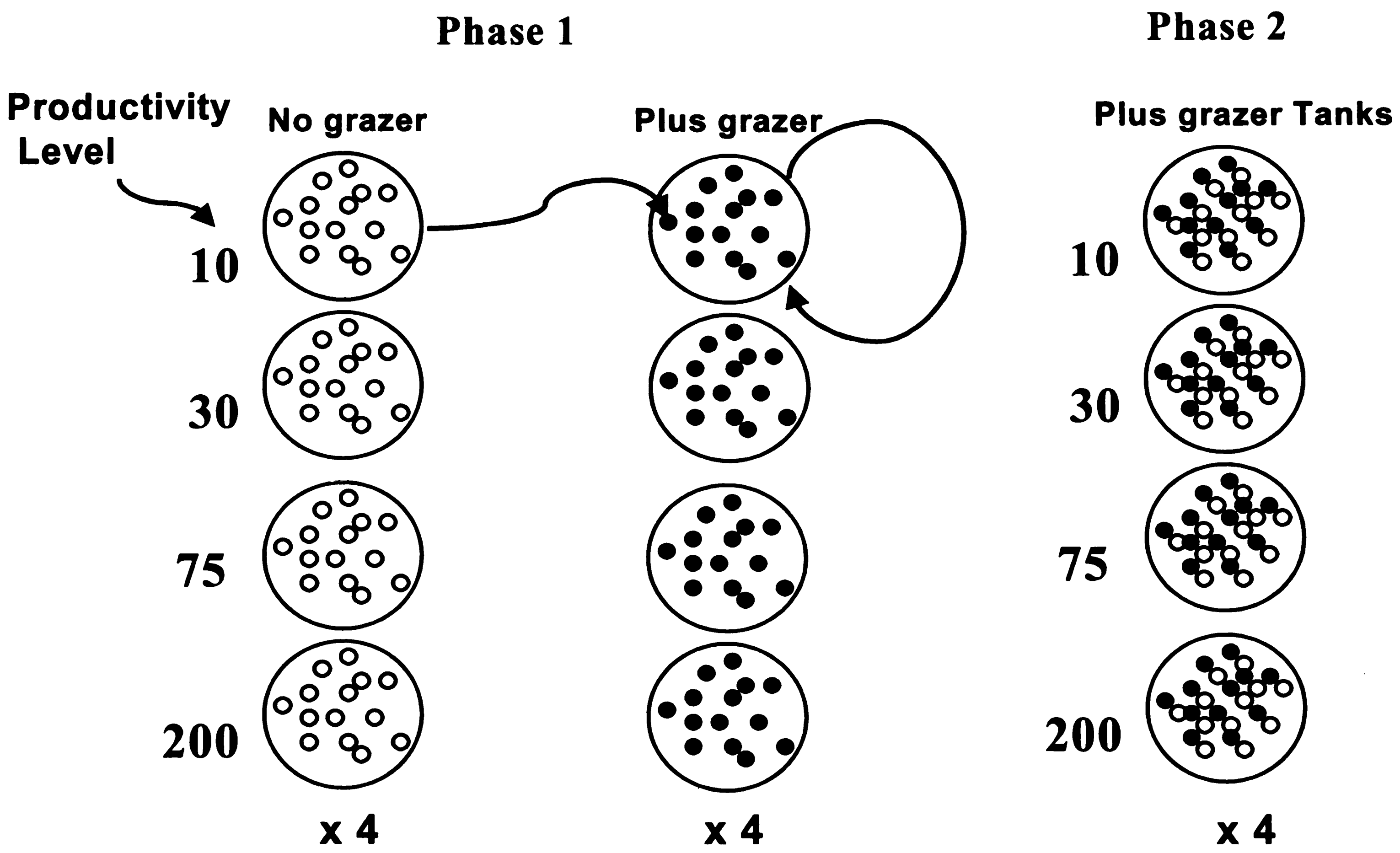


Figure 3.1: Experimental design for Phase 1 (community establishment) and Phase 2 (limitation assays). Open and closed circles represent terracota clay flowerpot sampling substrates. Arrows represent the movement of clay pots at the start of Phase 2.

therefore, half of the assays began on August 26 while the remaining half began on August 28. Within each cattle tank, 2 limitation assays took place: one within the ungrazed set of clay pots and the other within the grazed set of clay pots. Each assay included four, randomly assigned treatments, with 3 replicates each: nutrient additions alone, grazer removals alone, nutrient additions and grazer removals, and controls. Control treatments were those that resembled natural conditions, that is, no nutrients added and grazers present. The nutrient addition treatment involved removing 25 ml of water from the interior of the clay pot and either returning it (to controls and grazer removal treatments) or replacing it with 25 mL of a saturated nutrient solution (NH_4NO_3, and KH_2PO_4 in a 2:1 molar N:P ratio). The nutrient solution was approximately 1500 times more concentrated at a molar level than the highest nutrient supply rate in the experiment (200 μg/L TP). Nitrogen was added at such a low N:P ratio to avoid adding toxic levels of nitrogen to the algal and grazer communities. The porous clay surface of the flowerpots readily diffuses nutrients over time (Darcy-Hall *unpubl. data*, Fairchild *et al.* 1985, Marks and Lowe 1989, Wootton *et al.* 1996). Grazers were manually removed from all clay pots and returned to all but the grazer removal treatment. Care was taken to evenly redistribute the grazers among the nutrient additions and control treatments. Once treatments had been applied, all clay pots were placed within an overturned 2-pint (~ 1-L) clear, plastic deli container with 2, 105-μm mesh windows which retained herbivores but allowed water movement.

Each limitation assay lasted for one week, after which benthic algal communities from each pot were harvested as described above and subsampled for chlorophyll *a* analysis and algal species identification. Any grazers present on clay pots were also harvested and preserved in 70% ethanol for biomass measurements. Chlorophyll *a* analyses were conducted on the same day that the algal communities were harvested. The chlorophyll *a* data were used to calculate values of nutrient and predator limitation. Limitation values were calculated using the following equations:

$$Lim_N = \frac{\ln(NA) - \ln(C)}{t} \tag{1a}$$

$$Lim_P = \frac{\ln(GR) - \ln(C)}{t} \tag{1b}$$

where t is duration of the experiment in days, Lim_N is nutrient limitation and Lim_P is predator limitation, and NA, GR, and C are the chlorophyll *a* measurements of the nutrient addition, grazer removal, and control treatments, respectively. Values of limitation are measures of the change in instantaneous per-capita growth rate of algal communities after the removal of a limiting factor. In general positive values of limitation are expected, as algal communities should increase in biomass with the removal of the limiting factor.

At the start of the limitation assays, diel dissolved oxygen patterns (a surrogate for ecosystem productivity) were measured in the mesocosms to obtain a direct measure of overall community metabolism. Dissolved oxygen (DO) measurements were measured with a YSI® oxygen probe and were taken at dusk, dawn, and dusk, during a 24-hour period at the start of the limitation assays. Net ecosystem productivity was calculated as the difference in DO between dawn and dusk (in units of mg/L/hour), and it was these values that were used as a system productivity measure in regressions of limitation.

Algal species composition at the end of the limitation assays was also assessed in communities in the LOW and XHIGH productivity mesocosms. Composition was enumerated for the control, nutrient addition, and predator removal limitation assay treatments, but only data for the control treatment are presented.

Algal enumeration methods

Benthic algal communities were enumerated and identified using established methods (Lowe and Pan 1996). Semi-permanent wet mounts of a sample were prepared and examined at 1250x magnification on a compound microscope. Three hundred natural units (*e.g.* a cell, filament or colony) were enumerated and identified to the species level (when possible) for each sample. In most cases, a second slide was prepared with acid-cleaned diatoms mounted in Naphrax (Patrick and Reimer 1966) to identify diatoms to the species level. A subsample of 50 organisms per species was measured for biovolume calculations, using published geometric forms and volume equations for various algal genera (Hillebrand *et al.* 1999). All algal community composition results presented here are in units of relative biovolume, which were arc-sine square-root transformed prior to analyses.

Statistical analyses

The relationship between nutrient and predator limitation of grazed and ungrazed algal communities and net primary productivity (DO) were explored qualitatively with LOWESS regression (Trexler and Travis 1993; Systat, v.8, SPSS Inc.1998). In addition, limitation values in historically grazed versus ungrazed communities were compared quantitatively using a 2-way ANOVA, treating nutrient supply and grazing history (ungrazed or grazed) as factors. Each type of limitation was first tested together in a MANOVA, and then separately as the global hypothesis of no difference was rejected in the MANOVA. Slices of significant interaction terms were conducted in SAS v.8 (SAS Institute).

Shifts in algal community composition due to the Phase 1 treatments were analyzed using several methods. First, I ran a permutational MANOVA (perMANOVA; McCardle and Anderson 2001, Anderson 2001, Anderson 2004) to test whether the nutrient supply and grazer treatments significantly affected algal community composition. The perMANOVA uses permutation tests on a distance matrix of raw data to provide F-values and test statistics for each treatment factor, their interaction, uncorrected post-hoc treatment comparisons, and slices of interaction terms (Anderson 2001). It relaxes the assumption of multivariate normality, which is usually an unrealistic assumption with community data, due to the presence of many zeroes in the data set. The perMANOVA also allows the user to choose a distance measure other than the Euclidean distance, which is an assumption of traditional MANOVA. Here, I use the Bray-Curtis distance matrix, as it is known to perform especially well with species abundance data (Anderson and Willis 2003, McCune and Grace 2002, Legendre and Legendre 1998).

In addition, I used a canonical analysis of principal coordinates (CAP) analysis (Anderson and Robinson 2003, Anderson and Willis 2003, Anderson 2002) to provide a visual ordination of the community composition data and to explore which individual species were most affected by the treatments. Unlike many other ordination methods, the CAP analysis takes into account the correlation structure between variables (*i.e.* species) (Anderson and Willis 2003). The CAP analysis also deals well with situations where the number of species is greater than the number of samples or experimental units, which is the case here (Anderson and Willis 2003). The CAP analysis first performs an unconstrained principal coordinates analysis (PCoA) on a Bray-Curtis distance matrix of the transformed algal biovolume data and then uses the resulting principal coordinates (PCO axes) in a constrained ordination. The type of constrained ordination performed depends upon the hypothesis of interest. Here, a 2-way crossed design provided the structure for the hypotheses concerning algal shifts in response to experimental treatments. Therefore, a matrix of dummy variables coding for the experimental ANOVA design constrained the ordination of algal data in a canonical correlation analysis (CCorA). The CAP analysis produces canonical axes that are representative of the ANOVA treatments, the strength of which is measured as a squared canonical correlation coefficient (δ^2) that is comparable to an R^2 value. It also generates canonical scores of each mesocosm for use in a biplot of the relative locations of each mesocosm's algal community along the ordination axes as well as individual species' correlations with the canonical axes.

RESULTS

Phase 1 – Algal community composition

The applied nutrient supply treatments created a gradient in overall productivity (Figure 3.2), with higher nutrient supply resulting in increased TP (Figure 3.2a) and TN (Figure 3.2b) in the water column, higher chlorophyll *a* on clay pots (Figure 3.2c) and higher net ecosystem productivity measured by DO (Figure 3.2d). Differences in TP and chlorophyll across nutrient supply treatments depended upon the presence or absence of grazers (interaction terms: $F_{TP:3,24}$=6.7, P=0.002; $F_{chl:3,24}$=3.3, P=0.038), while differences in TN and DO were affected only by nutrient supply (nutrient supply: $F_{TN:3,24}$=92.7, P<0.0001, $F_{DO:3,24}$=44.48, P<0.0001).

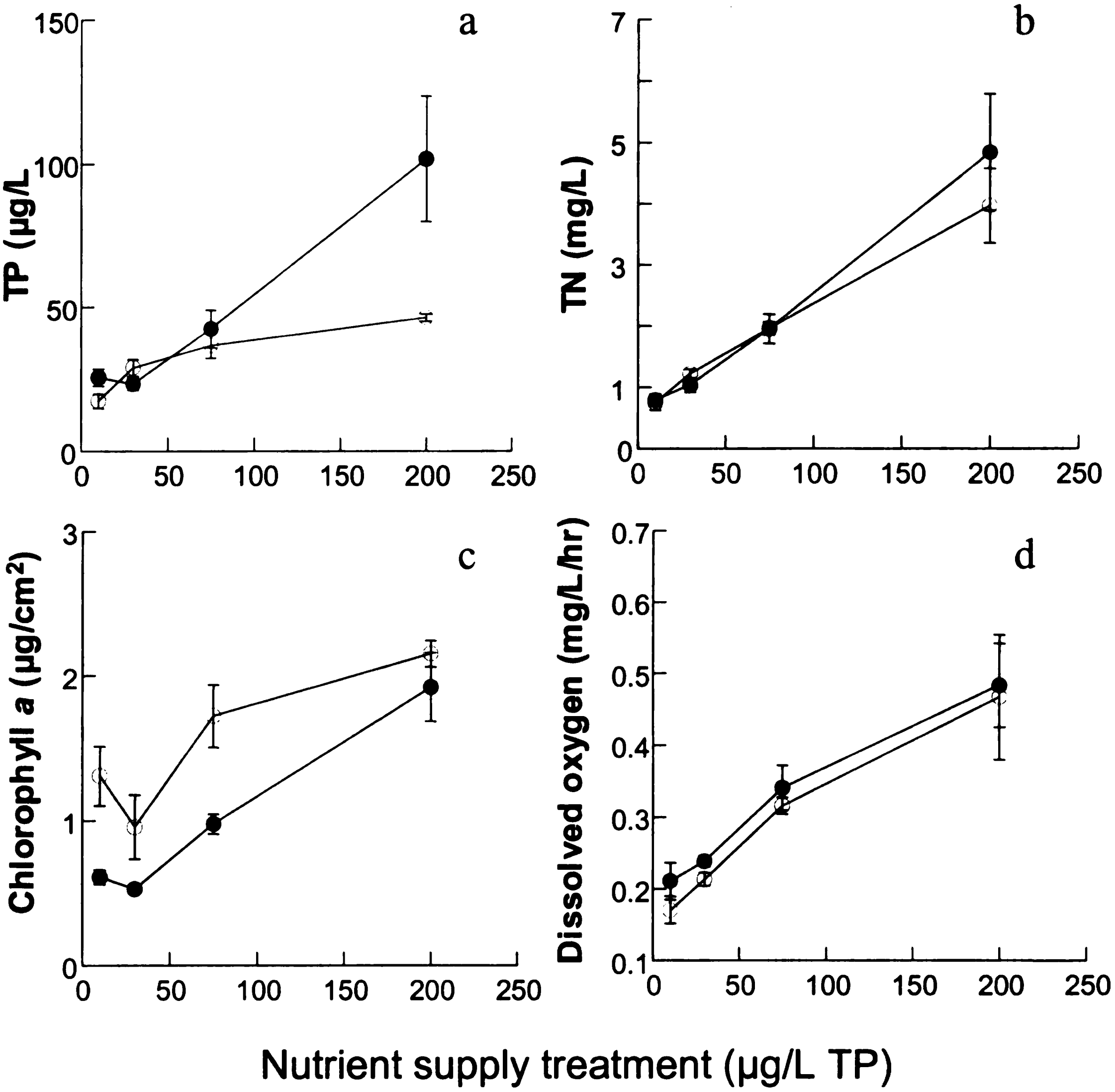


Figure 3.2: Means ± 1 S.E. of a) Total phosphorus, b) total nitrogen, c) chlorophyll *a* of periphyton on terracota clay flowerpots and d) dissolved oxygen in cattle tank mesocosms after 3 months of applied nutrient supply treatments. Closed circles are systems containing herbivores and open circles are systems lacking herbivores.

Benthic algal species composition clearly differed across the productivity and grazer treatments. A biplot of the CAP analysis' canonical scores (Figure 3.3) shows clear separation between both nutrient supply and grazer treatments. The first and second CAP axes represent variation due to the nutrient supply treatment ($\delta^2_1 = 0.82$) and the grazer treatment ($\delta^2_2 = 0.46$), respectively. Thus, lower nutrient supply mesocosms plot negatively along the 1st CAP axis, while mesocosms from higher nutrient supply plot positively (Figure 3.3). In addition, mesocosms containing herbivores tended to be negative along the 2nd CAP axis, while ungrazed communities are positive (Figure 3.3).

A perMANOVA of algal species composition revealed a significant interaction between the nutrient supply and grazer presence/absence treatments ($F_{3,24} = 1.76$, $P = 0.027$). Slices of this interaction showed a significant difference between grazed and ungrazed communities in the LOW and XHIGH treatments (Appendix C3.1). In addition, among the ungrazed communities, composition in the XHIGH treatment was significantly different from all other nutrient supply treatments (Appendix C3.1). Among the grazed communities, composition in the XHIGH treatments differed significantly from only the LOW and MED treatment while the HIGH treatment also differed from the LOW treatment (Appendix C3.1).

The algal composition of major taxonomic groups also exhibited dramatic changes in relative biovolume across treatments (Figure 3.4a). A correlation biplot of benthic algal species (Figure 3.4b) provided species-specific correlations with nutrients (1st CAP axis) and herbivore presence/absence (2nd CAP axis). Many of the species present at the ends of the productivity gradient were strongly correlated with the nutrient supply and grazer CAP axes (Figure 3.4b). In general, filamentous chlorophytes

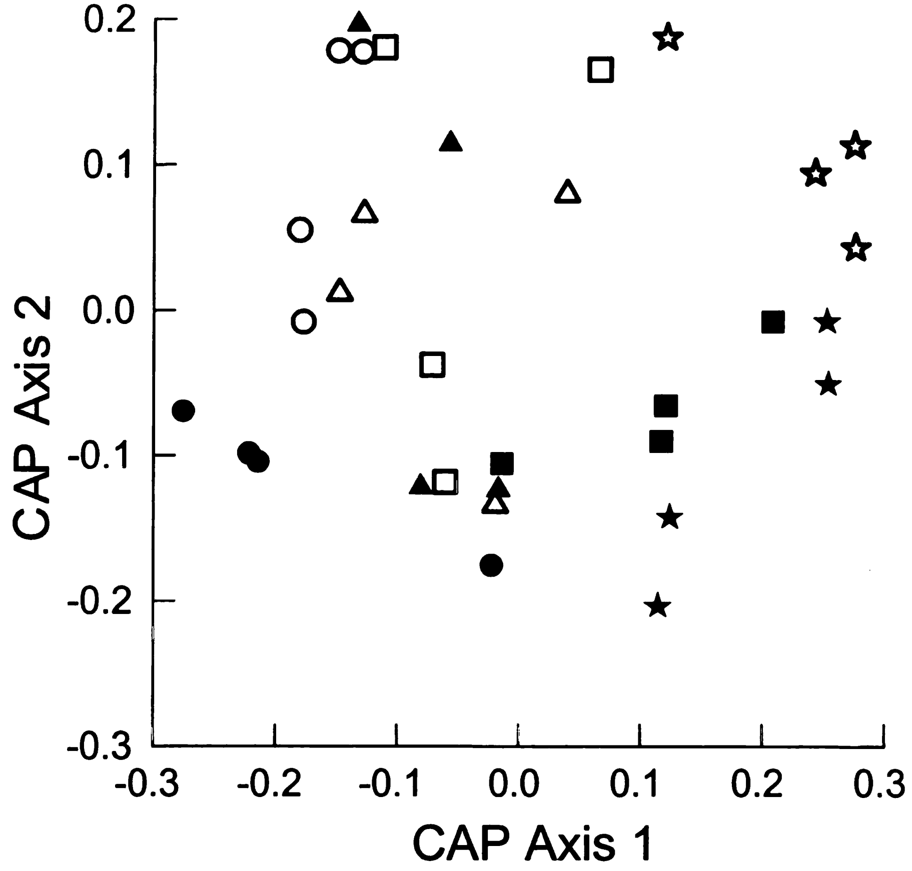


Figure 3.3: Response of experimental benthic algal communities to various nutrient supply concentrations (CAP Axis 1) and the presence/absence of herbivores (CAP Axis 2). Ordination of site scores from CAP analysis. Open symbols are ungrazed mesocosms and closed symbols are grazed mesocosms. Nutrient supply treatments are represented by different shapes: LOW (circles), MED (triangles), HIGH (squares), and XHIGH (stars).

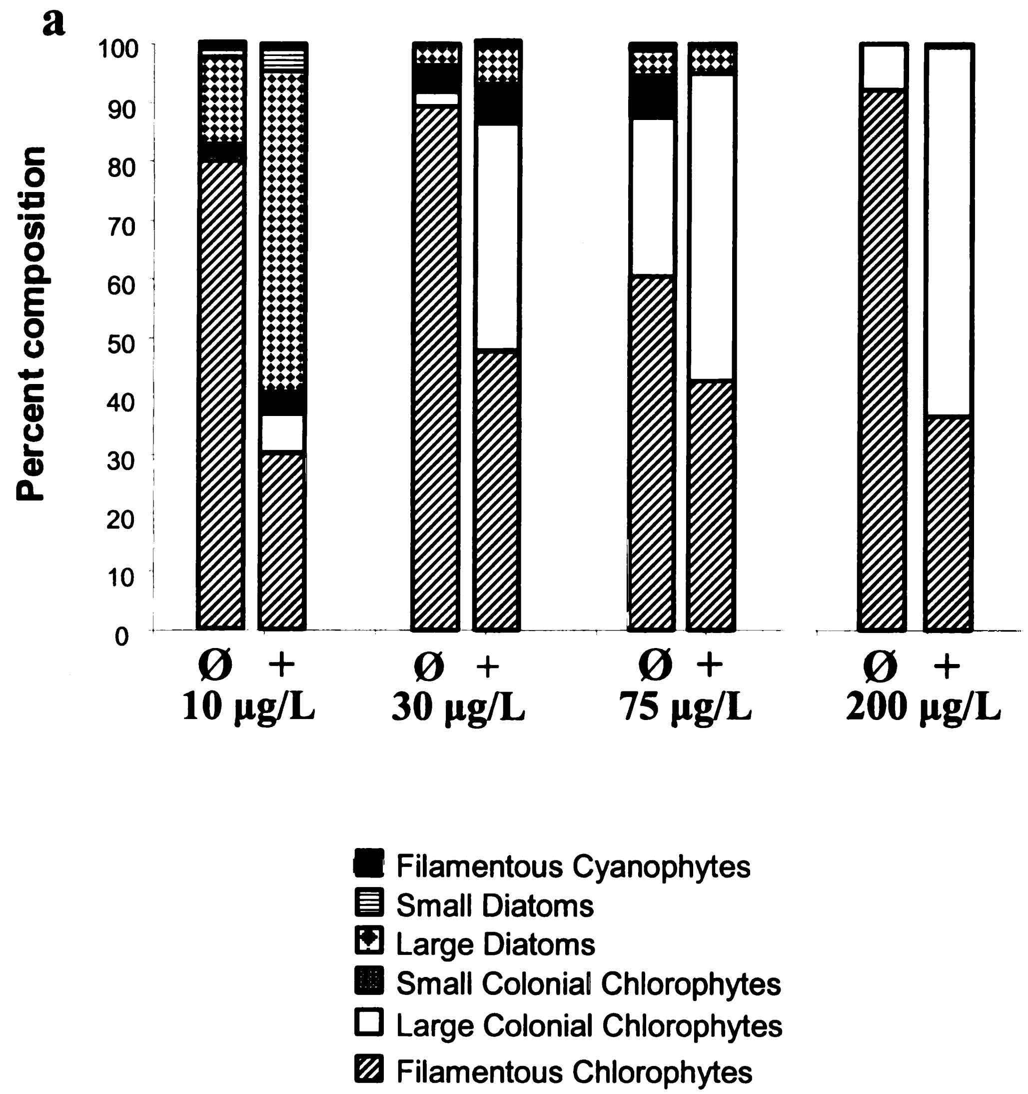
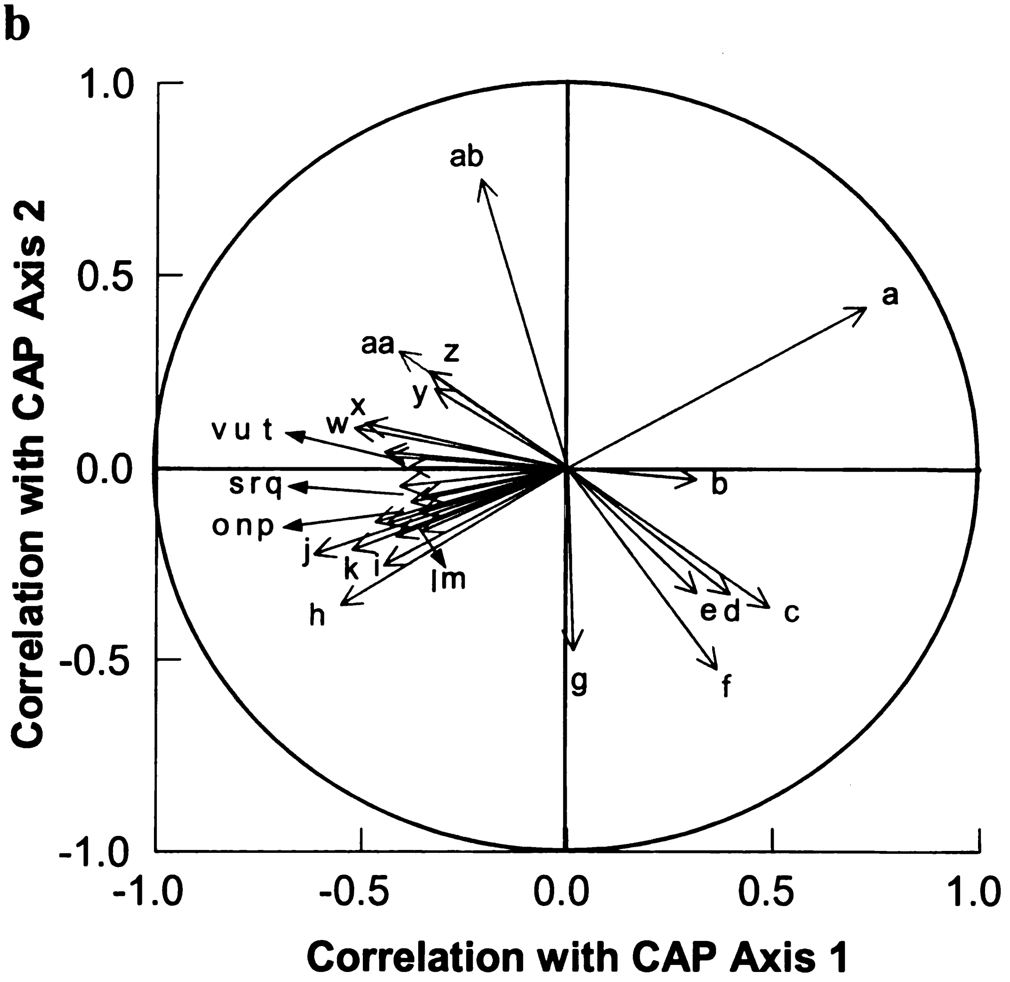


Figure 3.4: a) Benthic algal composition divided into broad morphological and taxonomic groups for ungrazed (Ø) and grazed (+) communities at each nutrient supply level. b) Correlations of algal species with each CAP axis. Only species with correlations of over 0.35 are included. Species are listed with a taxonomic designation in parentheses: (D) = diatoms, (BG) = blue-green algae (cyanophytes), (G) = green algae (chlorophytes).

Figure 3.4 (cont'd).



a) Large *Oedogonium* spp. (G)
b) *Scenedesmus brasiliensis* (G)
c) *Cosmarium* sp. B (G)
d) *Heteroleibleinia* sp. (BG)
e) *Pediastrum boryanum* (G)
f) *Stigeoclonium* sp. (basal cells) (G)
g) *Rhizoclonium* sp. (G)
h) *Epithemia adnata* (D)
i) *Encyonopsis microcephala* (D)
j) *Achnanthidium minutissimum* (D)
k) *Epithemia turgida* (D)
l) *Leptolyngbya* sp. F (BG)
m) *Tetraedron minimum* (G)
n) *Cosmarium* sp. A (G)
o) *Leptolyngbya* sp. D (BG)
p) *Achnanthes exilis* (D)
q) *Chroococcus minor* (BG)
r) *Oocystis parva* (G)
s) *Geminella* sp. (G)
t) *Pseudanabeana* sp. D (BG)
u) *Calothrix* spp. (BG)
v) *Pseudanabaena* sp. E (BG)
w) *Scenedesmus bijuga* (G)
x) *Leptolyngbya* sp. G (BG)
y) *Mougeotia* spp. (G)
z) *Rhopalodia gibba* (D)
aa) *Bulbochaete* spp. (G)
ab) *Cladophora* sp. (G)

dominated the biovolume of ungrazed algal communities. The identity of these filamentous chlorophytes shifted from species such as *Mougeotia* spp., *Cladophora* sp., and *Rhizoclonium* sp. at low and intermediate productivities (*i.e.* these species showed strong negative correlations with the 1st CAP axis; Figure 3.4b) to primarily large *Oedogonium* species at high productivities (*i.e.* this species had a strong positive correlation with the 1st CAP axis; Figure 3.4b).

The most striking impact of grazers was the drastic reduction in the relative biovolume of filamentous chlorophytes, regardless of system nutrient supply (Figure 3.4a). The understory species that benefited from the removal of the filamentous overstory differed along the productivity gradient. At low productivity, the relative biovolume of diatoms (small-celled *Achnanthes exilis*, *Achnanthidium minutissimum*, and *Encyonopsis microcephala* and large-celled *Epithemia adnata*, *E. turgida*, and *Fragilaria crotonensis*) increased in the presence of grazers (Figure 3.4). Alternatively, at very high productivity, the biovolume of the large, planktonic chlorophytes *Cosmarium* sp. and *Pediastrum boryanum*, and the predator tolerant basal cells of *Stigeoclonium* sp. increased with herbivory (Figure 3.4). Thus, at low productivity, herbivory favored an understory of generally edible diatom species, although at least one predator tolerant species was present in low numbers (*Stigeoclonium* sp. – the large colonial chlorophyte in Figure 3.4a). In contrast, in high productivity systems, herbivory generally favored more predator resistant (or tolerant) species (mainly *Stigeoclonium* sp.).

Overall, herbivores tended to shift algal composition from a community dominated by a vulnerable, filamentous overstory to one primarily composed of less vulnerable understory species. The herbivore treatment clearly resulted in drastically different algal communities that were then introduced into a common environment and tested for differences in the degrees of nutrient and predator limitation. I expected that nutrient limitation of ungrazed algal communities would decrease across the productivity gradient while predator limitation would increase, adherent with predictions from food chain models. In contrast, I expected that grazed algal communities would exhibit strong, but declining nutrient limitation and declines in predator limitation across the productivity gradient, due to an herbivore-mediated shift toward more predator resistant, less resource efficient taxa at high productivities.

Phase 2 – Limitation assays

As predicted, the algal communities that developed under different herbivore and nutrient environments exhibited different degrees of nutrient and predator limitation (Figure 3.5); however, the extent of these differences varied with overall system productivity (DO). An initial MANOVA (using the Phase 1 experimental design) on nutrient and predator limitation demonstrated a significant global hypothesis test (Pillai's trace = 0.915, $F_{2,23}$=124.2, P<0.0001). A separate ANOVA on nutrient limitation revealed a significant interaction ($F_{3,24}$ = 3.49, P=0.031) between nutrient supply level and grazing history (*i.e.* ungrazed communities versus grazed communities). Slices of the interaction show that nutrient limitation of grazed and ungrazed communities differed significantly at LOW nutrient supply ($F_{1,24}$ = 6.86, P = 0.015) and marginally at XHIGH ($F_{1,24}$ = 3.23, P = 0.085), the same two treatments with the largest differences in composition (Figure 3.3, Appendix C3.1). Although the patterns of limitation were complex, in general the ungrazed algal communities at high productivities showed a stronger decline in nutrient limitation than the grazed communities (Figure 3.5a).

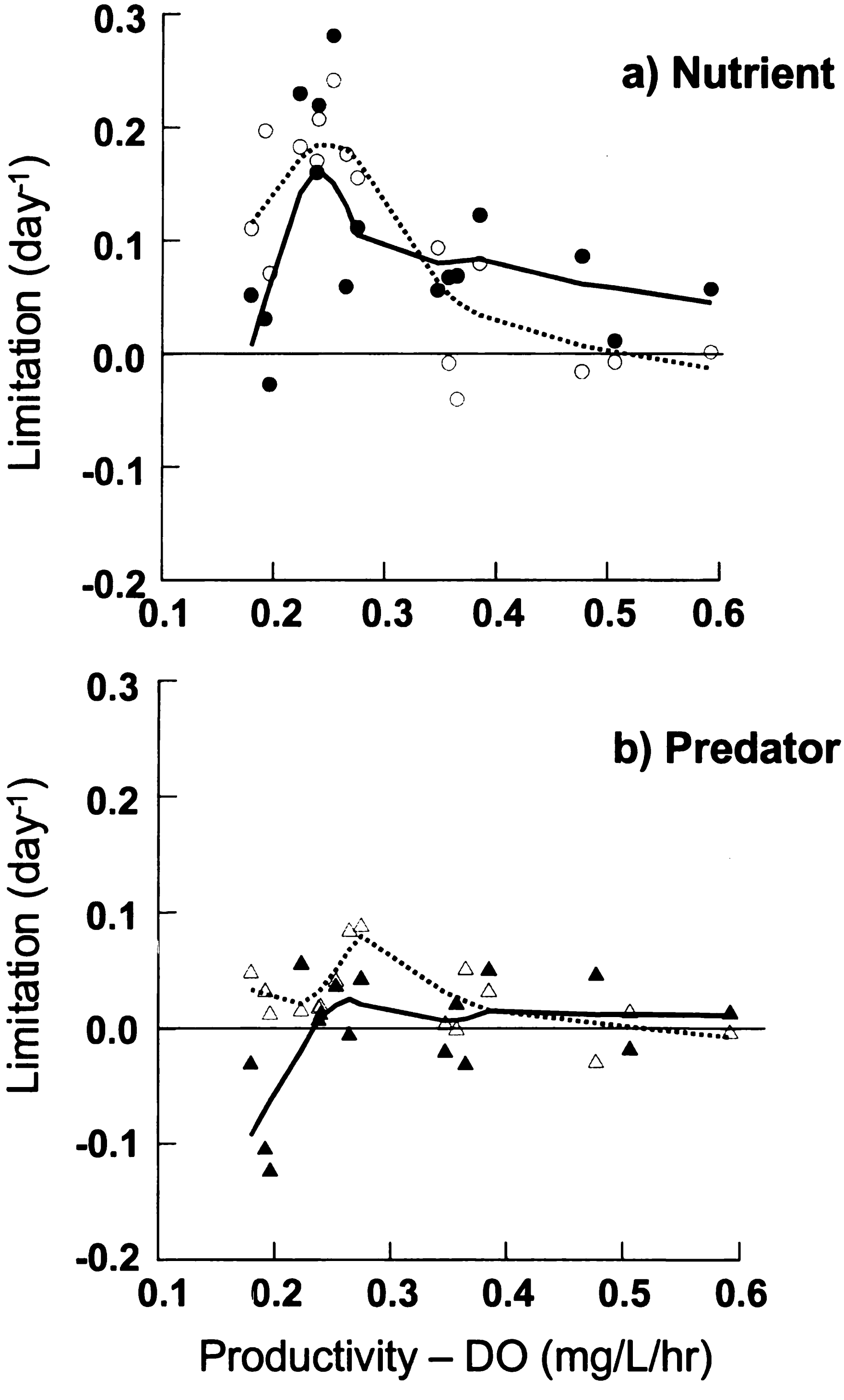


Figure 3.5: a) Nutrient and b) predator limitation of historically grazed (closed symbols) and ungrazed (open symbols) benthic algal communities. Regression lines are LOWESS fits (tolerance = 0.6) for grazed (solid lines) and ungrazed (dashed lines) relationships with net primary productivity (dissolved oxygen – DO).

In the nutrient addition treatments of the limitation assays, I observed 25-50% grazer mortality that may have confounded the nutrient limitation results. To assess the effect of grazer mortality, I used an alternative method of calculating nutrient limitation in the absence of grazers (*i.e.* by subtracting chlorophyll values in predator removal treatments from chlorophyll in treatments where nutrients were added *and* predators removed) and compared the results to those using the original method (see *Methods*). The only change resulting from the alternative method (data not shown) was that nutrient limitation no longer differed between LOW grazed and ungrazed communities. Because this difference may be confounded by grazer mortality, I do not discuss this result further.

On average, predator limitation in ungrazed algal communities was higher than in grazed communities, especially at low productivities (Figure 3.5b). An ANOVA on grazed and ungrazed predator limitation demonstrated a significant interaction between nutrient supply rate and grazing history ($F_{3,24} = 4.28$, $P = 0.015$). Slices of this interaction indicated that predator limitation differed significantly between LOW ungrazed and grazed communities ($F_{1,24} = 14.45$, $P = 0.0009$).

To measure predator limitation in ungrazed algal communities, these algae had to be exposed to grazers one week prior to the initiation of the one-week limitation assays. One concern might be that this period of grazing rendered the ungrazed and grazed communities so similar that no differences in limitation would be apparent. To address this issue, I compared algal species composition at the end of Phase 1 to composition two weeks later, in control communities at the end of the limitation assays. I tested the effects of time and treatment (4 levels: LOW and XHIGH, grazed and ungrazed) on algal composition in a two-way perMANOVA. The perMANOVA revealed a significant time by treatment interaction ($F_{3,24} = 2.25$, $P = 0.01$), slices of which indicated that only the XHIGH ungrazed communities exhibited a significant effect of time ($t_1 = 2.80$, $P = 0.03$). Thus, by the end of the limitation assay, the species composition of ungrazed XHIGH communities was significantly different than when grazers were first introduced.

DISCUSSION

The presence of herbivores clearly altered benthic algal community composition and in turn affected how the community responded to the removal of limiting factors. However, differences in the limitation of ungrazed and grazed communities depended upon the underlying productivity of the ecosystem. At intermediate productivities, grazed and ungrazed algal communities did not differ in the extent of nutrient or predator limitation. In contrast, these factors differed most in the endpoint communities; that is, in the communities where nutrient supply rate differed by more than an order of magnitude (10 and 200 μg/L). Thus, I will focus on the algal composition and limitation differences in these communities.

Algal composition and the keystone predation hypothesis

The algal compositional shifts observed along productivity and herbivore gradients were consistent with the expectations of the keystone predation hypothesis. Herbivore-free communities were dominated by vulnerable, filamentous chlorophytes, and more predator resistant species benefited from herbivory in high productivity systems. In most cases, filamentous chlorophytes are considered predator resistant, but they can be vulnerable if herbivory occurs before filaments outgrow the edible, early stages of development (Dudley and D'Antonio 1991, DeNicola *et al.* 1990). Because grazers were present in the mesocosms before substantial benthic algal mats had developed, it is highly probable that herbivory prevented significant filamentous chlorophyte establishment.

At low productivity, herbivore grazing of the filamentous overstory benefited the understory community of diatoms. In this experiment, the indirect, positive effects of grazing (*i.e.* the removal of the overstory) on diatoms likely outweighed the direct, negative effects on these morphologically edible species. In contrast, at higher productivities, herbivore consumption of the filamentous chlorophytes resulted in the dominance of a predator tolerant species (*sensu* Chase *et al.* 2000), the basal cells of *Stigeoclonium*, and potentially predator resistant planktonic chlorophytes, *Cosmarium* sp. and *P. boryanum* (see Darcy-Hall, Chapter 2).

While the distribution of predator resistant algal species along the productivity gradient fits the predictions of the KPH, it is not clear whether the same is true of competitive (*sensu* Tilman) algal species. In general, filamentous chlorophyte species have higher nutrient requirements than most species, (due to their large size; Steinman *et al.* 1992), so it may appear that grazing fell more heavily on the inferior nutrient competitor (contrary to expectations). However, in vertically structured benthic algal communities, superior competitors may be more appropriately viewed as those species that more effectively acquire nutrients. Because the overstory of filamentous chlorophytes would have direct contact with water-column nutrients, they were, in this case, the competitively superior species (Rosemond 1996, Steinman *et al.* 1992). Therefore, herbivory fell on the competitive dominant, which is generally consistent with the predictions of the KPH. Others have observed a similar tradeoff between access to nutrients and predator resistance among benthic algal species (*e.g.* Graham and

Vinebrooke 1998, Rosemond *et al.* 1993, Steinman *et al.* 1992). For example, Graham and Vinebrooke (1998) found that competitive growth forms of periphyton (*i.e.* tall, loosely attached, overstory species) were most abundant when grazers were absent, but that species composition shifted to firmly attached, short and tall growth forms when herbivores were present.

Patterns of limitation of grazed and ungrazed communities

Nutrient and predator limitation differed in ungrazed and grazed communities, likely due to grazer-mediated compositional differences. These compositional differences between grazed and ungrazed communities were most dramatic at low and very high productivities (see Figure 2.3), resulting in concomitantly larger differences in the extent of nutrient and predator limitation. In addition, the direction of these differences in nutrient and predator limitation supported the predictions of the KPH.

Nutrient Limitation – Nutrient limitation in both grazed and ungrazed communities declined with enrichment, and similar to the predictions of the limitation model (Darcy-Hall, Chapter 1, Appendix A), ungrazed nutrient limitation declined more strongly than grazed nutrient limitation. As described above, algal communities coexisting with herbivores were comprised of more predator tolerant, competitively inferior algal species that were likely nutrient limited. For example, dominant *Stigeoclonium* basal cells increased in the nutrient addition treatments of the limitation assays (data not shown), potentially contributing to higher values of nutrient limitation in grazed communities.

The shape of the relationship between nutrient limitation and productivity was surprisingly similar in ungrazed and grazed treatments. Interestingly, nutrient limitation exhibited a non-linear response to variation in net primary productivity, peaking at intermediate productivity and declining thereafter. While the non-linearity was unexpected in ungrazed communities, the response is consistent with expectations for grazed communities. For instance, the predicted relationship between resource limitation and productivity in food webs (see Figure 1.1, Darcy-Hall, Chapter 1) corresponds to a portion of the relationship between nutrient limitation and productivity in Figure 3.2 (the segment of the graph after ~0.23 mg/L/hr DO). It is possible that the range of mesocosm productivity in this experiment includes lower levels than those used for the limitation model parameters.

The peak in nutrient limitation at intermediate productivity may be due to the low algal biomass of communities at corresponding levels of productivity in this study. Hillebrand (2002) found that the effect of nutrient additions decreased with initial algal biomass. Similarly, nutrient limitation (the result of a nutrient addition) was particularly high when pre-limitation assay chlorophyll *a* was low (compare Figure 3.2c and Figure 3.5a). Thus, initial algal biomass clearly affected the extent to which the community responded to the addition of nutrients. However, initial algal biomass did not explain the entire response, because nutrient limitation of grazed and ungrazed communities differed at high productivities, but initial chlorophyll *a* values did not.

Predator Limitation – Predator limitation in communities with and without herbivores differed strongly at low productivity, yet converged at higher productivities. Similar to predictions of the limitation model, predator limitation in low productivity, grazed communities was lower than in ungrazed communities, likely due to the presence of more grazer resistant species. Indeed, there were higher percentages of *Stigeoclonium* sp. basal cells, which on average, responded negatively to the removal of herbivores (data not shown). However, there were more predator resistant species in grazed communities along the entire productivity gradient, thus one might expect predator limitation in all grazed mesocosms to surpass that of ungrazed communities.

The absence of a difference between predator limitation in grazed and ungrazed communities at higher productivities may be potentially explained in two ways. First, the large difference in predator limitation at low productivity may involve a positive effect of nutrient recycling by herbivores. This hypothesis is supported by the fact that predator limitation in grazed communities is negative at low productivity. Yet at higher productivities, the net effect of predators in the historically grazed communities declined and predator limitation was generally positive and low. Second, contrary to my predictions for simple food chain models, predator limitation in historically ungrazed communities did not increase across the productivity gradient. In fact, predator limitation in ungrazed communities at intermediate to high productivities did not differ from that of grazed communities. This may be due in part to the timing of herbivory and the composition of these XHIGH communities. Grazed XHIGH communities were primarily composed of the predator tolerant basal cells of *Stigeoclonium* sp.; thus, it was not surprising that predator limitation values were low. Ungrazed XHIGH communities were dominated by filamentous chlorophytes, which were vulnerable to herbivory in grazed communities, because grazing occurred at early stages of the filaments' development. However, when the ungrazed clay pots, with their fully developed filamentous communities, were exposed to herbivores prior to the limitation assays, these filaments of *Oedogonium* spp. were almost certainly resistant (Darcy-Hall, Chapter 2, Steinman *et al.*

1992). Therefore, at the time of the limitation assays, both ungrazed and grazed algal communities were effectively predator resistant, perhaps causing predator limitation values to be low in both treatments.

Comparisons to other studies - This study illustrates that herbivores can significantly alter the species composition of benthic algal communities, but that the magnitude of this effect varied with overall system productivity. The variation in the size of the herbivore effect was nonlinear since the largest effects were observed at the lowest and highest nutrient supply rates, but not at intermediate levels. Very few studies have examined the relationship between productivity and herbivore effects on composition, although Chase *et al.* (2000) found decreasing effects of large herbivores on grassland plant species composition as the productivity (*i.e.* precipitation) of grasslands increased.

Only a handful of studies have experimentally examined the relative effects of resources and predators in habitats that vary in productivity (*e.g.* Hillebrand and Kahlert 2001), and only one (Darcy-Hall, Chapter 2) examines patterns of short-term limitation. Hillebrand and Kahlert (2001) did not find a conclusive pattern of nutrient versus grazer effects across a productivity gradient of lakes; however, their gradient included only 3 systems.

In some respects the pattern of limitation of grazed communities in this study and the one observed in 13 southwest Michigan lakes (Darcy-Hall, Chapter 2) were similar. First, in both cases nutrient limitation was stronger than predator limitation across the entire productivity gradient studied. Additionally, negative values of predator limitation were observed in both cases; however, the most negative values of predator limitation in lakes were in high productivity systems, while the opposite was true in the mesocosm experiment. It is no surprise though that strong relationships between limitation and productivity were easier to detect in a simpler, experimental context than in the complex and highly variable environment of lake ecosystems.

Conclusions

This research demonstrates a clear effect of herbivores in shifting benthic algal species composition along gradients in system nutrient supply. Furthermore, these herbivore-mediated shifts in composition lead to differences in the extent of algal nutrient and predator limitation in historically grazed versus ungrazed communities. In general, nutrient limitation in grazed algal communities was stronger than in ungrazed algal communities, especially in systems with high nutrient supply. In low nutrient supply systems, predator limitation in historically ungrazed algal communities appeared to be stronger than in grazed communities. These differences in nutrient and predator limitation were consistent with predictions of the limitation model and the keystone predator hypothesis. Thus, my results provide evidence for a link between keystone predator-mediated compositional shifts and patterns of benthic algal nutrient and predator limitation in habitats along a productivity gradient.

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CHAPTER 4

EVIDENCE FOR THE KEYSTONE PREDATION HYPOTHESIS IN THE BENTHIC ALGAE: LINKING SPECIES' TRAITS TO ENVIRONMENTAL VARIABLES IN A FOURTH-CORNER ANALYSIS

INTRODUCTION

Species are adapted to particular environments and as a result, traits that confer high fitness in one environment may not necessarily prove favorable in a different environment. In many cases, traits exhibit functional tradeoffs, preventing species from being well adapted to multiple environments. When this occurs, species composition is predicted to change along ecological gradients. For example, Gleeson and Tilman (1990) demonstrated a tradeoff among plant species in their relative allocation of biomass to above and below-ground structures along a successional gradient, and Tilman (1988) also argues for a general tradeoff between allocation to roots and shoots along gradients in soil fertility. Species may also exhibit tradeoffs in their ability to compete for resources and their resistance to predation along gradients in potential primary productivity (Paine 1966, Lubchenco 1978, Vance 1978, Holt *et al.* 1994, Grover 1994, Leibold 1996, Chase *et al.* 2000). Increases in ecosystem resource supply (*i.e.* potential primary productivity) often lead to concomitant increases in both primary producer and consumer biomasses (Leibold 1999, Ginzburg and Akcakaya 1992, McNaughton *et al.* 1989, Mills and Schiavone 1982). Thus, if primary producer-prey species exhibit a competition-resistance tradeoff, then prey community composition might shift to dominance by more predator-resistant prey as both productivity and predator biomass increase. This phenomenon has been termed the keystone predation hypothesis (KPH; Leibold 1996, Holt *et al.* 1994, Grover 1994), which focuses on the role of predators in shifting prey community structure.

The keystone predation hypothesis (Leibold 1996, Holt *et al.* 1994, Grover 1994) predicts how species' traits should vary across systems of different resource supply. At low productivities, when predator levels are also low, vulnerable prey species with superior resource efficiency are expected to dominate. Alternatively, as resources and predators increase, prey communities will shift in dominance to more predator invulnerable species. By the nature of the implicit tradeoff, these resistant species should be relatively poor resource competitors.

A number of studies have documented the predicted predator-mediated shifts in prey species composition along productivity gradients (Darcy-Hall, Chapters 2, 3, Steiner 2003, Chase *et al.* 2000, Bohannan and Lenski 1999, 2000); however, only one of these studies (Steiner 2003) has directly examined the traits of the resultant prey communities. This is surprising, as a key component to testing the KPH model is that the prey species favored under specific conditions (*e.g.* high productivity-high predator biomass) exhibit the hypothesized traits (*e.g.* predator resistance). Instead, most studies provide anecdotal evidence that species' traits match those predicted by the competition-resistance tradeoff (*e.g.* Darcy-Hall Chapter 2, Hillebrand 2000, Rosemond *et al.* 1993). We still lack direct evidence for strong associations between the traits of competitive ability and resistance and the expected environmental contexts.

Here, I examine this question within a predator-prey system composed of benthic algal prey and benthic grazer predators. A competition-resistance tradeoff has been suggested in the periphyton (*e.g.* Hillebrand 2000, Graham and Vinebrooke 1998, Rosemond *et al.* 1993), but has never been directly addressed. To investigate benthic algal trait-environment correlations, I combine short-term assays measuring algal traits of competitive ability and grazing resistance with a long-term, outdoor mesocosm study of algal species composition across an array of environmental conditions. The key algal traits of competitive ability and resistance are measured as relative growth rates under various conditions of resource limitation or grazing regime, respectively. Data from these experiments are analyzed using the fourth-corner analysis, a statistical technique that correlates species' traits to environmental variables (Legendre *et al.* 1997). This technique has been used successfully by other researchers examining trait-environment correlations (*e.g.* Hooper *et al. in press*, Legendre *et al.* 1997). I hypothesize that benthic algal species' traits of superior resource competitive ability will be most common in low nutrient supply environments and will thus be negatively correlated with higher productivity environments. In contrast, I expect algal grazing resistance traits to be more common at high nutrient supply (and thus high grazer environments) and to therefore exhibit positive correlations with high productivity.

Empirical and theoretical studies of the KPH have generally focused on shifts in prey composition along gradients in nutrient supply. However, primary producers may also compete for light, yet I know of no studies examining how primary producers (*i.e.* algae) might tradeoff in their ability to compete for light and to resist predators. Most studies that have examined species' abilities to compete for light have focused on tradeoffs between light and nutrients (*e.g.* Huisman *et al.* 1999, Grover 1997). As part of this experiment, I also included treatments measuring algal light competition to gain a preliminary understanding of how light availability may function within a keystone-predator context. This effort will motivate future research concerning the nature of benthic algal tradeoffs involving light, nutrients, and resistance to predators.

METHODS

Overview of the experimental approach

The fourth-corner analysis uses three matrices to generate a species' traits by environment correlation matrix (the "D-matrix"): a species x site matrix (the "A-matrix"), a species by species' trait matrix (the "B-matrix"), and a site x environment matrix (the "C-matrix"). In this study, the A- and C-matrices were generated from a field experiment using cattle tank mesocosms (*i.e.* the sites) in which I created a wide-range of environmental conditions (based on variation in nutrient supply, herbivore biomass, and light levels) across which a regional species pool of benthic algae were allowed to sort. At the conclusion of the mesocosm experiment, algal species composition data and various abiotic and biotic environmental variables from each mesocosm were compiled to create the A- and C-matrices. Algal traits for the B-matrix were measured in two shorter-term bioassays, which measured changes in algal species' composition in several resource-limiting or grazing environments. The change in each species' absolute biovolume (also referred to as a species' relative growth rate) in each treatment of the two bioassays was used to represent these species' traits for the B-matrix. Below, I describe each of these experiments and bioassays in detail as well as the specifics of the fourth-corner analysis.

Outdoor mesocosm experiment

The experiment used to develop the A- and C-matrices was conducted in 1000-L cattle tank mesocosms during the summer of 2002 at the W. K. Kellogg Biological

Station's experimental pond lab facility (Hickory Corners, MI, USA). Cattle tank mesocosms were acid-washed and filled with well water prior to the initiation of the experimental design. All cattle tanks were affixed with a 1-mm mesh lid, composed of fiberglass window screening. The main treatments were four nutrient supply concentrations [LOW1 (13:1 N:P ratio), LOW2 (50:1 N:P ratio), MED, and HIGH (both 50:1 N:P ratio)] and 2 light treatments (shaded/unshaded, at LOW2 and HIGH nutrient concentrations). Each of these six treatments was crossed with the presence or absence of a grazer community, yielding 12 treatments that were replicated 4 times. Six 4-inch (~10-cm) terracotta clay flowerpots served as sampling substrates for the benthic algal communities in each mesocosm. Nitrogen (as ammonium nitrate) and phosphorus (as potassium phosphate) were added to the water column to establish the four levels of productivity. Target nutrient supply concentrations were 10 (LOW1 and LOW2), 30 (MED), and 75 (HIGH) µg/L total phosphorus (TP), with nitrogen added in a 50:1 molar ratio (or 13:1 in the LOW1 treatment). The design included a low N:P ratio treatment to provide species with an opportunity to invade a low nitrogen environment. These productivity levels encompassed natural variation in TP and total nitrogen (TN) of local lakes and ponds near KBS (Hall *et al. in press*, Darcy-Hall, *unpubl. data*). Nutrients were added every 5 days throughout the experiment to maintain the approximate target nutrient concentrations. Low light treatments were achieved by adding shadecloth to the lids of the low light mesocosms. Shadecloth blocked approximately 95% of ambient light entering the cattle tank. Benthic grazers were added at approximately 0.5 g dry mass/m^2 and included 3 species of gastropods (*Physa* sp., *Helisoma* sp., and *Gyraulus* sp.) as well as amphipods (*Hyallela azteca*). Zooplankton (*Dahpnia* spp.) were also added to each cattle tank to control populations of phytoplankton that could potentially shade periphyton communities.

Arrays of clay pots were established in the littoral zone of six lakes for accumulation of algal inoculum for the mesocosm experiment. The lakes were chosen to span a wide gradient of TP (13.5 – 77.5 μg/L) and TN (208.9 - 1869 μg/L). To seed the mesocosms, benthic algae were scraped from several clay pots from each lake and mixed with deionized water. Resulting algal slurries were sieved through a 125-μm sieve to remove micro- and macrograzers, combined and mixed in a carboy, and then distributed in 100-mL aliquots to each cattle tank. The inoculation process was repeated once every 3 weeks for the duration of the experiment to incorporate natural temporal variation in algal species composition.

Algal species were allowed to sort among these experimental environments for 16 weeks, after which endpoint communities were sampled twice: on 3-4 September, 2002, and 17-18 September, 2002. Only data from the first sampling period are presented here. During each sampling, the algal and grazer communities from three clay pots per cattle tank were combined and subsampled to measure chlorophyll *a*, algal species composition, and grazer densities. Algae were removed from clay pots as described above, and samples for species identification were preserved in 10% formalin. Algal enumeration was conducted as in previous work (Darcy-Hall, Chapters 2, 3), and all analyses of species compositional shifts were conducted using arc-sine, square-root transformed species relative biovolume data. Chlorophyll *a* measurements were taken on the same day as sampling, using cold ethanol extractions followed by narrow-band fluorometry (*sensu* Welshmeyer 1994). Grazers were preserved in 70% ethanol and biomass was calculated using length-mass regressions (C. W. Osenberg, *unpubl. data*). In addition, several abiotic variables were measured during the first sampling period. Total (TP and TN) and dissolved nutrients (ammonia, nitrate, soluble reactive phosphorus, and silica) were measured using spectrophotometry and standard methods (Bachmann and Canfield 1996, Crumpton *et al.* 1992, Prepas and Rigler 1982, APHA 1980). Temperature, conductivity, and pH were measured using a Hariba® multi-sensor probe. Dissolved oxygen (DO) measurements were measured with a YSI® oxygen probe and were taken at dusk, dawn, and dusk, during a 24-hour period. Net ecosystem productivity was calculated as the difference in DO between dawn and dusk (in units of mg/L/hour). Additionally, light measurements (μmol/m^2/s) at the bottom of each cattle tank were sampled with a LI-COR® light meter (LI-COR Biosciences, Lincoln, NE, USA).

Resource limitation bioassay

Resource limitation bioassays were conducted to measure the response of algal species to limiting levels of several key resources. Algae were exposed to four limiting resource environments (P, N, silica, and light) and a control with all resources at saturating levels. Each experimental treatment included the target limiting resource at very low levels, with the other resources maintained at saturating levels. The bioassay was conducted in 19-L plastic buckets, and target limiting nutrient values were ascertained from the literature (Borchardt 1996): 3 μg/L P, 100 μg/L N, and 100 μg/L Si. Because the nutrient content of the laboratory well water often exceeded these values, buckets were filled with 1-L well water and 8-L deionized water, and nutrients were added back at saturating levels where necessary. Saturating levels of key nutrients were:

50 μg/L P, 1350 μg/L N (50:1 N:P ratio), and 5000 μg/L Si. The light-limiting environment was created using 95% shadecloth. Each of the five treatments was replicated 4 times, for a total of 20 experimental units. Experimental arrays were arranged on two tables in the pond lab in a randomized, complete block design, with each table as a block. All buckets were placed under full-spectrum fluorescent lights and exposed to a 16-hour light, 8-hour dark cycle.

A hyperdiverse slurry of algae collected from local lakes and one clay pot-substrate were added to each bucket. Algae quickly consumed the added nutrients (Darcy-Hall, *unpubl. data*); so to maintain the treatment conditions, nutrients were added to the buckets every 2 days to maintain saturating levels where necessary (*i.e.* there were no additions of target limiting nutrients). Loading rates were determined from pilot experiments that examined how quickly levels of soluble reactive phosphorus (SRP), ammonia (NH_4^+), nitrate (NO_3^-) and silica (Si) decreased (Darcy-Hall, *unpubl. data*). Algae from clay pots were sampled after two weeks (as described above) for chlorophyll *a* and species composition, and the water column of each bucket was sampled for dissolved nutrient analyses.

Grazer resistance bioassay

Algal communities that had established on clay pots in two treatments of the cattle tank experiment were brought into the laboratory for the grazer resistance assay. The two source mesocosm treatments were the grazed, 10 μg/L nutrient supply (LOW +) and the grazed, 75 μg/L nutrient supply (HIGH +) treatments. One clay pot from three replicates of each source treatment was exposed to three grazing treatments (two grazer species and a no-grazer control) in the laboratory. The two herbivores were species of gastropod snails (*Physa* sp. and *Helisoma* sp.). Thus, the experimental design crossed two source environments with three grazer treatments, each replicated three times for a total of 18 experimental units (19-L plastic buckets). All grazers were added at 0.1 μg dry mass/m^2, a value based on natural densities in a high-grazer biomass lake (Darcy-Hall, Chapter 2). Clay pots and grazers were placed in buckets filled with well water and N and P were added to each bucket to achieve a medium level of productivity (~ 30 μg/L TP). Clay pots were exposed to a 16-hour light, 8-hour dark cycle via full spectrum fluorescent lights in the laboratory. After 10 days, clay pots were sampled (as above) for chlorophyll *a*, algal community composition, and grazer density.

Algal traits were calculated the same way in both bioassays. The absolute biovolumes of each species in the control and experimental treatments (*e.g.* Low P or *Helisoma*) were entered into the equation for limitation (see Darcy-Hall, Chapter 2 & 3). The resultant value is identical to a relative growth rate (Harper 1977) for each species in each limiting condition. Below, I will refer to these traits as, for example, "phosphorus competition" or "resistance to *Helisoma* in HIGH+ environments".

Statistical analyses

My main goal was to test for correlations between nutrient competition traits and productivity variables, as predicted by the KPH. Therefore, I did not include light treatments in initial analyses of species compositional shifts and the fourth-corner analysis. Instead, I focused on nutrient and herbivore effects on species composition in all experiments. In subsequent analyses, I examined species trait correlations associated with all three treatments (nutrient supply, herbivore presence/absence, and light), and I present an abbreviated analysis of these results. A more detailed examination of the effects of light on algal species composition will be treated elsewhere.

Tests of treatment establishment – Treatment effects of log (x+1)-transformed productivity variables and grazer biomass in all experiments were tested initially by MANOVA (when necessary; SAS, v.8, SAS Institute) on several variables of interest, followed by univariate ANOVA's (Systat, v.8, SPSS, Inc.) of each variable when appropriate. The long-term mesocosm experiment was not set-up in a fully crossed design, thus two subsets of data, each containing a fully crossed design, were tested separately. The first subset was a 2-way analysis testing the effects of nutrient supply (low N:P ratio and 10, 30, 75 μg/L TP) and herbivore presence/absence. The second subset was a 3-way analysis testing the significance of nutrient supply (10 μg/L and 75 μg/L TP), herbivore presence/absence, and light (shaded/unshaded) treatments.

Analyses of species composition – My general approach to examining the responses of species to experimental treatments was first to test for significant treatment effects with permutational MANOVA (perMANOVA; see Darcy-Hall Chapter 3, McCardle and Anderson 2001, Anderson 2001, Anderson 2004, Jones 2002, for details) and then conduct an ordination of species composition to visualize treatment separation and/or individual species' responses. The cattle tank mesocosm data set was divided into two subsets, as described above. Each perMANOVA used 10,000 permutations to test for significant effects and *a-posteriori* pairwise comparisons were tested when appropriate.

Relationships between algal species composition and environments within the mesocosm experiment were analyzed with a redundancy analysis ordination (Canoco v.4;

see Legendre and Legendre 1998, ter Braak and Šmilauer 1998, Darcy-Hall, Chapter 2, for details). Algal compositional data were Hellinger-distance transformed (see Darcy-Hall Chapter 2 and Legendre and Gallagher 2001) and were constrained using five environmental variables from the cattle tank experiment (DO, TP, TN, N:P ratios, and grazer biomass), all of which were also included in the C-matrix of the fourth-corner analysis. I used the same variables in both analyses to facilitate comparisons between fourth-corner results and individual species' responses in the cattle tanks.

Species compositional changes in the resource competition assay were analyzed with a 1-way perMANOVA (4 levels), followed by post-hoc treatment comparisons (Jones 2002). Individual species' responses and separation of treatments were analyzed using a canonical analysis of principal coordinates (CAP; see Anderson and Robinson 2003, Anderson and Willis 2003, Anderson 2002, Darcy-Hall Chapter 3, for details). Composition data were analyzed to maximize separation between the four treatments (*i.e.* a discriminant analysis was run on principal coordinates) and 10,000 permutations were used to test for the significance of the first canonical axis.

I used a 2-way perMANOVA on algal relative biovolume data in the grazer resistance bioassay to test for effects of grazer type (3 levels) and source of algal community (2 levels). The only significant factor affecting algal composition was the community's source mesocosm, thus I did not perform an ordination of species' data.

The fourth-corner analysis – To avoid an undefined solution to the matrix equation, only those species that occurred in all treatments of both the grazer and resource bioassays were included in the fourth-corner analysis. The data pertaining to this suite of species were assembled into the A- (species x site) and B- (species x species' trait) matrices. The A-matrix was composed of 1's and 0's only, representing either presence or absence, respectively, of each species in each cattle tank. The B-matrix contained the relative growth rates of each species under each treatment of the two bioassays. My main hypothesis concerned trait correlations with environmental variables that might encompass system primary productivity, so the C-matrix (environmental variables x site) was composed of 5 abiotic and biotic productivity variables (DO, TP, TN, N:P, and light levels [in a second analysis only]), and grazer biomass for each of the 32 experimental units of the mesocosm experiment. These 3 matrices were entered into a FORTRAN program (Legendre 2002), which performed the matrix algebra necessary to produce the D-matrix. In addition, 10,000 permutations of columns of the A-matrix were performed (Model 2; Legendre *et al.* 1997) to determine the statistical significance of the D-matrix correlations between algal traits and environmental variables. Holm's procedure (Rice 1989) was employed to assess the table-wide significance ($\alpha = 0.05$) of the D-matrix.

RESULTS

Cattle-tank mesocosm experiment (the A- and C-matrices)

The nutrient supply treatments created a gradient in overall productivity, with higher nutrient supply resulting in increased TP, TN, and N:P ratios in the water column, higher chlorophyll *a* (chl) on clay pots, and higher net ecosystem productivity measured by DO (see Appendix D4.1 for MANOVA and ANOVA results). Grazer biomass did not significantly differ along the established productivity gradient ($F_{2,17} = 1.011$, $P = 0.385$). Light levels at the bottom of shaded mesocosms (average = 49.6 μmol/m^2/sec ± 0.49

S.E.) were reduced by 92% compared to light reaching the bottom of unshaded cattle tanks (average = 650.0 $\mu mol/m^2/sec$ ± 12.7 S.E.).

Benthic algal community composition differed due to variation in nutrient supply, herbivore biomass, and (marginally) their interaction (perMANOVA results, Table 4.1A) The first 4 axes of a redundancy analysis constraining variation of benthic algal relative biovolume to the environmental variables found in the mesocosms (Figure 4.1) explained a significant amount of variation (21.9% total) in species composition ($F = 1.516$, $P = 0.034$). The first RDA axis represents 10.6% of the variation in species composition and correlates strongly with general productivity ($r_{DO} = 0.62$, $r_{TN} = 0.60$, $r_{N:P} = 0.42$, $r_{TP} = 0.40$). The 2nd RDA axis represents 5.7% of the variation in species composition and correlates strongly with grazer biomass ($r_{GRZ} = 0.54$). Species scoring negatively along the 1st RDA axis dominated in low productivity environments, while species scoring positively along this axis dominated in high light environments. A suite of species, composed primarily of diatoms and cyanobacteria, dominated low productivity environments, while one species (large species of the filamentous chlorophyte *Oedogonium*) correlated strongly with high DO (Figure 4.1). A complete list of species-environment correlations is in Appendix D4.2. Two species (basal cells of *Stigeoclonium* and *Scenedesmus acutiformis*) exhibited grazer resistance and correlated strongly with high herbivore biomass, while several species (*e.g.* the filamentous chlorophytes *Bulbochaete* spp. and *Geminella* spp.) prospered in environments with low grazer biomass (Figure 4.1).

A perMANOVA of the subset of mesocosm data including the light treatment indicated that light explained a significant portion of variation in species composition and

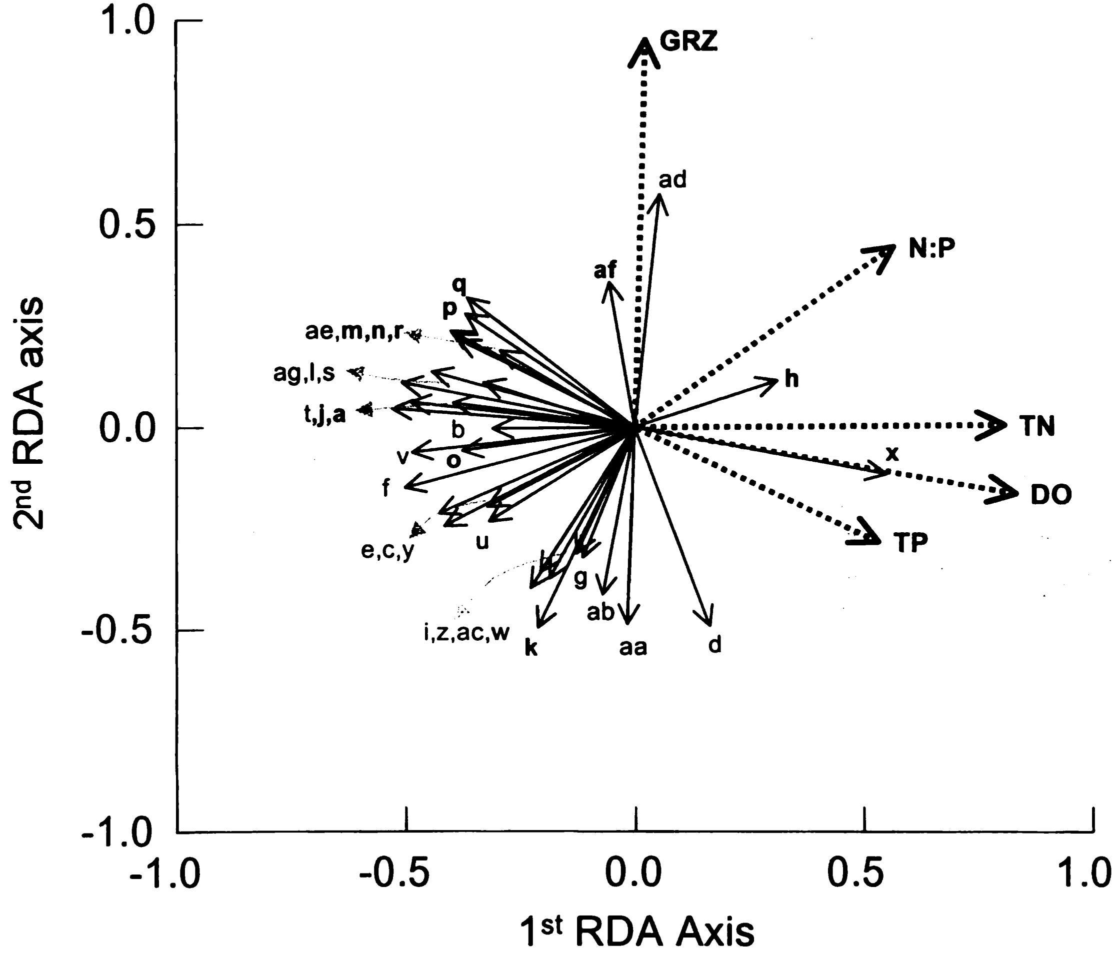
Table 4.1: Results of permutational MANOVAs on the relative biovolume of benthic algal species composition for two subsets of the mesocosm experiment: A) the nutrient supply-light subset and B) the nutrient supply-N:P ratio.

A

Factor	df	F-statistic	P-value
Nutrient supply	3	1.685	0.050
Grazer	1	2.540	0.022
Nutrient supply * grazer	3	1.611	0.062
Error	24		
Significant post-hoc comparisons			
Nutrient supply levels 1 and 4	1	1.765	0.001

B

Factor	df	F-statistic	P-value
Nutrient supply	1	1.130	0.314
Light	1	7.200	0.001
Grazer	1	0.995	0.379
Nutrient supply * light	1	0.621	0.616
Nutrient supply * grazer	1	1.059	0.345
Light * grazer	1	3.634	0.021
Nutrient supply * light * grazer	1	1.205	0.280
Error	24		



a	***Achnanthidium minutissimum*** (D)
b	*Aphanocapsa hyalina* (BG)
c	*Aphanocapsa sp.* (BG)
d	*Bulbochaete* spp. (G)
e	*Calothrix* spp. (BG)
f	*Chroococcus minutus* (BG)
g	*Cladophora* sp. (G)
h	***Cosmarium* sp. B (G)**
i	*Encyonopsis microcephala* (D)
j	***Epithemia adnata*** (D)
k	***E. turgida***
l	*Geminella* sp. (G)
m	***Gomphonema gracile*** (D)
n	***G. angustatum***
o	***G. pseudotenellum***
p	***G. clevei***
q	***G. subtile***
r	***Leptolyngbya* sp.** E (BG)
s	***Leptolyngbya* sp. A**
t	*Leptolyngbya* sp. B
u	*Leptolyngbya* sp. C
v	*Limnothrix* sp. A (BG)
w	*Limnothrix* sp. B (BG)
x	**Large *Oedogonium* spp.** (G)
y	*Oocystis* sp. (G)
z	*Pseudanabaena* sp. A (BG)
aa	*Pseudanabaena* sp. D
ab	*Pseudanabaena* sp. E
ac	*Rhopalodia gibba* (D)
ad	*Scenedesmus acutiformis* (G)
ae	*Scenedesmus obliquus* (G)
af	***Stigeoclonium* sp. basal cells** (G)
ag	*Tetraedron minimum* (G)

Figure 4.1: Biplot of species scores (solid vectors) and environmental scores (dashed vectors) from a redundancy analysis on algal species relative biovolume in the cattle tank mesocosm experiment. Species are listed with a taxonomic designation in parentheses: (D) = diatoms, (BG) = blue-green algae (cyanophytes), (G) = green algae (chlorophytes). Only species with a correlation over 0.30 with either axis is included in the biplot. Species in bold are those included in the fourth-corner analysis.

there was a significant light by grazer interaction, but no significant main effects of nutrient supply and grazers (Table 4.1B). When light was included in the RDA, an additional 6.6% of variation in algal species composition was explained and light correlated strongly (r = -0.71) with the 1st RDA axis (data not shown). Appendix D4.2 includes species-light correlations from this RDA.

The overall goal of the mesocosm experiment was to provide a diversity of environments across which a heterogeneous species pool of algae could sort. The presence or absence of these species in the particular environments (the A-matrix) provided the foundation for linking algal traits to environmental variables.

Resource competition and grazer resistance trait assays (the B-matrix)

Resource competition assay – Two experimental units were lost from the resource competition assay due to a mix-up in experimental application. Thus, the low P and low light treatments were only replicated 3 times. The low nutrient treatments of the competition assay resulted in reduced levels of the key, limiting nutrient (compared to controls), but not always to target limiting levels. Mean levels (± 1 S. E.) of NH_4^+ and NO_3^- in low nitrogen treatments were 180 µg/L ± 6.4 and 75.7 µg/L ± 0.33, respectively, compared to 1146 µg/L NH_4^+ ± 512 and 87.0 µg/L NO_3^- ± 0.67 in controls (an 84% reduction). Dissolved silica in low silica treatments averaged 2.3 mg/L ± 0.20, compared to 4.6 mg/L ± 1.7 in controls (a 50% reduction). Soluble reactive phosphorus levels in low phosphorus treatments averaged 8.4 µg/L ± 0.79, compared to 10.1 µg/L ± 1.8 in controls (a 17% reduction). Control experimental units received nutrient additions every 2 days to maintain saturating levels, but these nutrients were often quickly consumed, contributing to the apparent similarity between nutrient levels (particularly SRP) in control and experimental treatments. Even though the low nutrient treatments never received additional inputs of the target nutrient throughout the assay, levels did not always decline as expected. The P and Si treatments in particular might not have reached a truly limiting condition, and this corresponded to there being no observed difference in chlorophyll *a* on clay pots in P or Si treatments relative to controls (Appendix D4.3). Chlorophyll *a* on clay pots differed significantly among treatments ($F_{3,11} = 50.39$, $P<0.0001$), but this was primarily due to strong declines in the low nitrogen treatment (Appendix D4.3).

Algal species composition did not differ significantly among the nutrient limitation treatments (perMANOVA results: $F_{3,14} = 1.22$, $P = 0.247$) and a CAP analysis of algal species relative biovolume did not show significant separation among the 4 treatments (Figure 4.2a, canonical test statistic of 1st CAP axis, $\delta_1^2=0.64$, $P = 0.104$), corroborating the perMANOVA results. A correlation biplot of algal species (Figure 4.2b) indicates that many algal species thrived under the saturating conditions in the controls, but only a few (*e.g.* large species of *Oedogonium* in low N treatments) benefited from limiting nutrient conditions (Figure 4.2b).

If the low light treatment is included in the analysis, the 1st CAP axis explains a significant portion of the variation between groups ($\delta_1^2 = 0.84$, $P = 0.004$), and a perMANOVA also exhibits significant differences among treatments ($F_{3,14} = 1.569$, $P = 0.051$). Post-hoc comparisons revealed that these differences were mainly due to differences between communities at low light and the other experimental treatments and controls (Appendix D4.3). Several species (primarily diatoms) dominated in low light conditions in both the resource competition assay and in the mesocosm experiment;

A

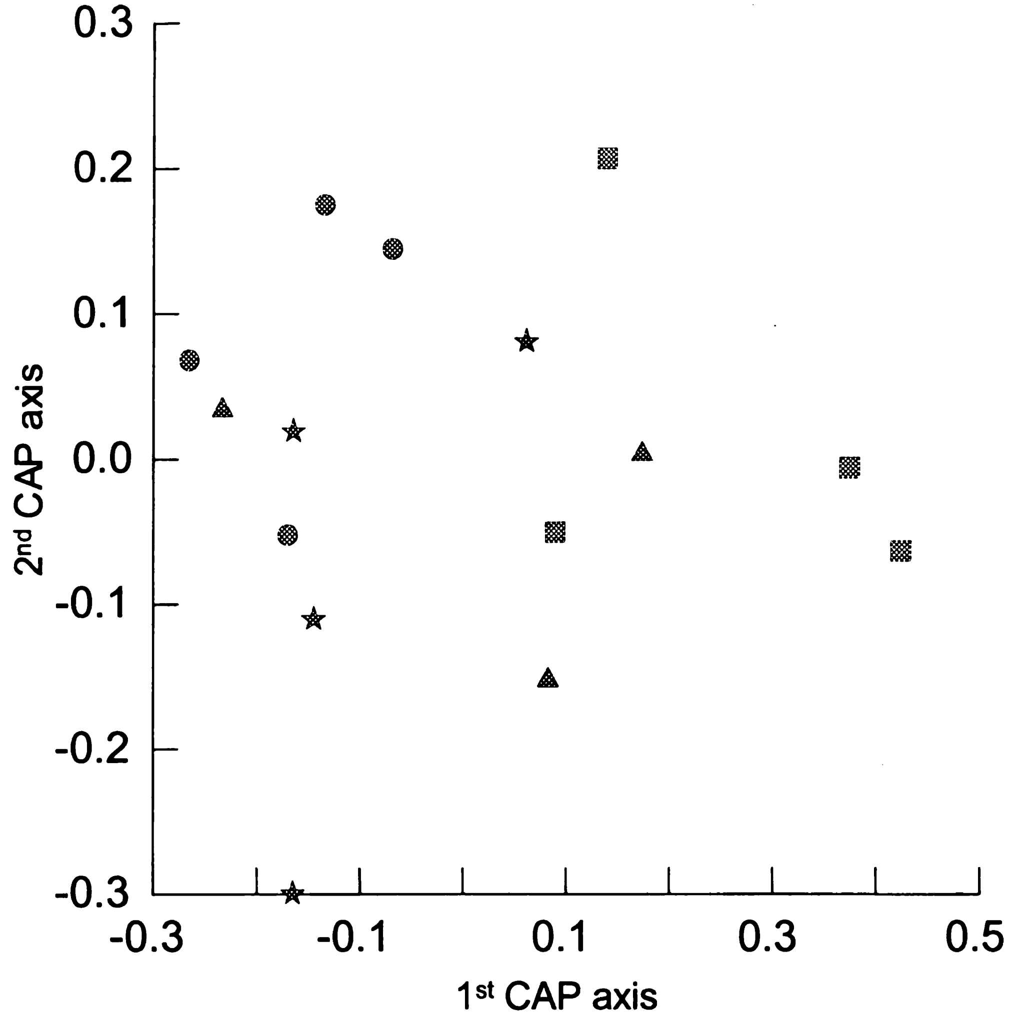
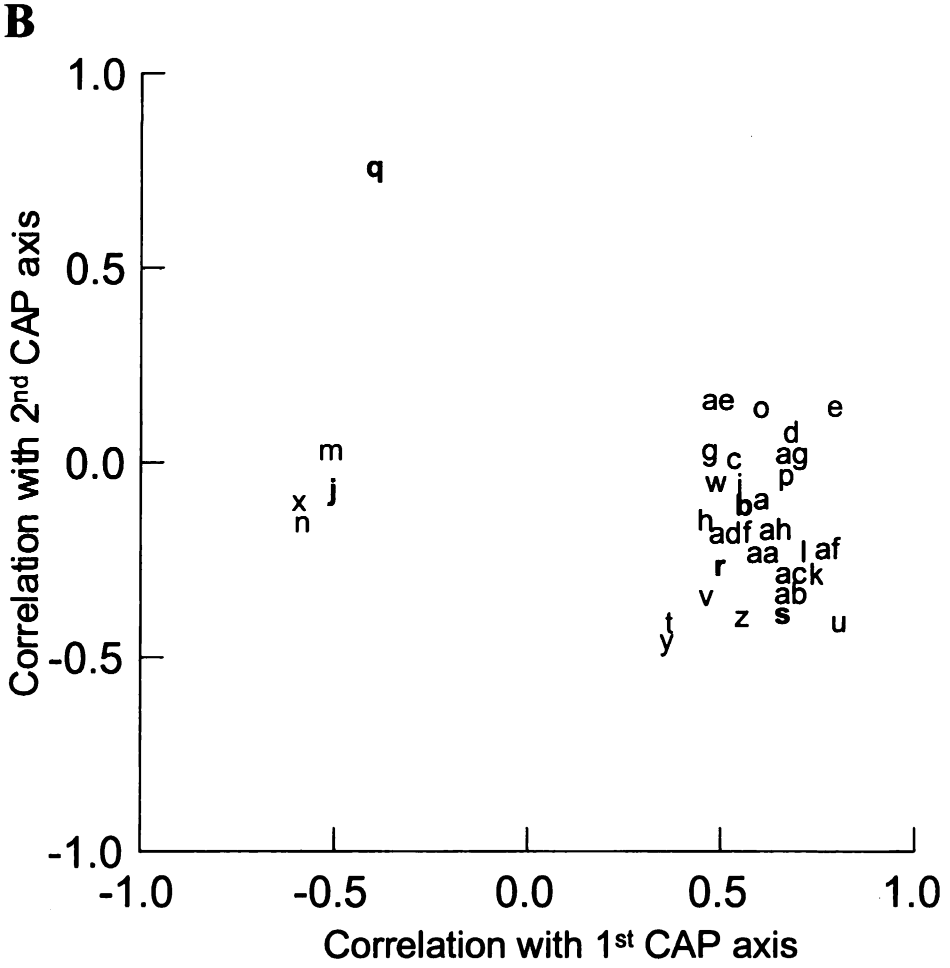


Figure 4.2: Results of CAP analysis on algal species relative biovolume from resource competition bioassay: A) Biplot of experimental treatments: low P (triangles), low Si (stars), low nitrogen (circles), and controls (squares). B) Biplot of species correlations with the CAP axes. Species are listed with a taxonomic designation in parentheses: (D) = diatoms, (BG) = blue-green algae (cyanophytes), (G) = green algae (chlorophytes). Species in bold are those included in the fourth-corner analysis.

Figure 4.2 (cont'd).



a	*Ankistrodesmus falcatus* (G)	r	**Medium *Oedogonium* spp.**
b	***Chroococcus* sp.** (BG)	s	**Small *Oedogonium* spp.**
c	Small *Cosmarium* spp. (G)	t	*Pediastrum boryanum* (G)
d	*Fragilaria brevistriata* (D)	u	*Pseudanabaena catentata* (BG)
e	*F. capucina*	v	*Pseudanabaena* sp. D
f	*F. construens*	w	*Pseudanabaena* sp. E
g	*Geitlerinema tenue* (BG)	X	*Rhopalodia gibba* (D)
h	*Gloeocystis ampla* (G)	y	*Scenedesmus abundans* (G)
i	*Gloeocystis vesiculosa* (G)	z	*S. bijuga*
j	***Gomphonema angustatum*** (D)	aa	*S. incrassatulus*
k	*Leptolyngbya* sp. C (BG)	ab	*S. obliquus*
l	*Limnothrix* sp. B (BG)	ac	*S. serratus*
m	*Limnothrix lauterbornii*	ad	*Staurosira construens* (D)
n	*Mougeotia* spp. (G)	ae	*Staurosira construens* var. *venter*
o	*Navicula cryptotenellum* (D)	af	*Staurastrum* spp. (G)
P	*Nitzschia palea* (D)	ag	*Tetraedron* spp. (G)
q	**Large *Oedogonium* spp.** (G)	ah	*Tychonema* sp. (BG)

however, the identities of the species were not necessarily the same in both experiments. While *Achnanthidium minutissimum* exhibited similar responses in both experiments *Achnanthes exilis* and *Encyonopsis microcephala* did not. These results highlight the context dependency of individual species' responses.

Grazer resistance assay – Chlorophyll *a* levels was significantly higher on clay pots from HIGH+ mesocosms (source: $F_{1,12} = 7.96$, $P = 0.015$), but was not significantly affected by herbivory (grazer type: $F_{2,12} = 0.429$, $P = 0.661$; Figure 4.3). A perMANOVA on algal species relative biovolume indicated that only the identity of the source mesocosm community (and not grazer type) significantly affected algal composition ($F_{1,212} = 4.647$, $P = 0.003$). Despite this, the effect of *Helisoma* and *Physa* snails on certain algal groups depended on the source mesocosm community (Figure 4.3). For instance, diatom relative biomass increased with *Helisoma* and *Physa* predation in algal communities from LOW+ cattle tanks, but declined due to predation in communities from HIGH+ cattle tanks (Figure 4.3). For this reason, I calculated separate values for the B-matrix traits from each of the source communities in the grazer resistance bioassay. More specifically, for each of the species included in the B-matrix, I calculated 4 average trait values, one from each of the source communities (LOW+ and HIGH+) for both *Helisoma* and *Physa* grazing.

The fourth corner analysis

A total of 14 species were present in all treatments of the trait assays and thus data for these species were assembled into matrices for the fourth-corner analysis. The fourth-corner analysis produced a matrix of correlations between the algal species' traits (resource competitive ability/resistance to predation) and environmental conditions

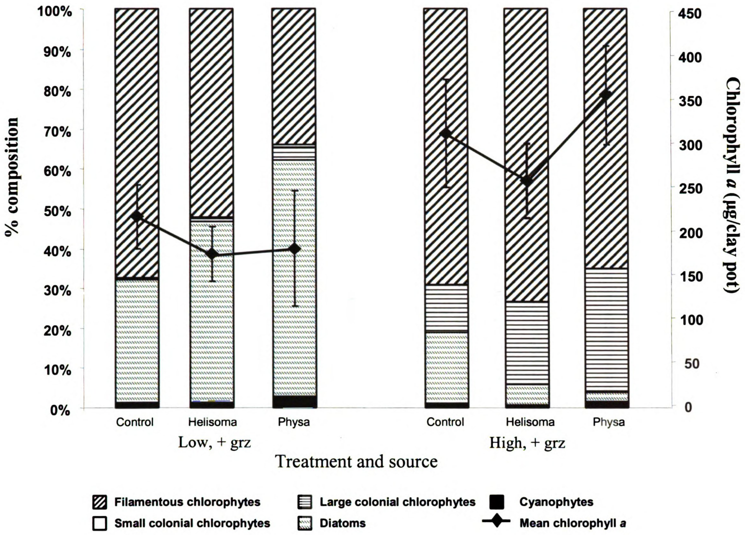


Figure 4.3: Algal community composition and mean chlorophyll *a* (± 1 S.E.) in the grazer resistance bioassay.

within the cattle tank mesocosms. The KPH predicts that algal traits of superior competitive ability should be associated with low productivity environments while resistance to grazing should be associated with high productivity environments. Therefore, I expected negative correlations between competition traits and productivity and positive correlations between resistance traits and productivity. In general, when traits differ in the sign of the correlation for the same environmental variable, this may indicate a tradeoff between those traits.

The D-matrix from the fourth corner analysis indicated that several correlations between algal traits and environmental variables matched these *a priori* expectations (Table 4.2A). For instance, several measures of system productivity (DO, TN, N:P ratio) in the mesocosms were significantly negatively correlated with phosphorus competition, but significantly positively correlated with resistance to *Helisoma* grazing (although this was dependant on the source of the algal community). In addition, grazer biomass was significantly positively correlated with both *Helisoma* and *Physa* grazing (in HIGH+ treatments), indicating that the ability to resist grazing by these two snail species became a more common trait with high grazer biomass. However, several environment-trait correlations did not support the predictions of the KPH. For example, nitrogen competition was positively correlated with DO, and resistance to *Helisoma* and *Physa* by algal species in LOW+ source mesocosms was negatively correlated with several productivity variables (*e.g.* DO and TN). It is not surprising that LOW+ algal communities did not adhere to the competition-resistance tradeoff because these algal communities likely experienced less grazing pressure in mesocosms and as a result were composed of less grazer resistant species (*e.g.* large colonial chlorophytes [basal cells of

Table 4.2: D-matrix from a fourth-corner analysis, associating algal species' traits from trait bioassays to the environmental variables in the cattle tank mesocosm experiment. All values are correlation statistics r(i,j) and probabilities (P) tested using 10,000 permutations. Values in bold are significant at the $P<0.05$ level. None of the correlations were statistically significant at the table-wide level. A) Fourth-corner analysis without light treatments. B) Fourth-corner analysis including light treatments.

A

Environmental variable		**Trait**						
		Low P	Low N	Low Si	Hel, low +	Hel, high +	Phy, low +	Phy, high +
DO (mg/L/hr)								
	r(i,j)	**-0.059**	**0.052**	0.016	**-0.114**	**0.112**	**-0.132**	0.028
	P	0.021	0.037	0.255	0.016	0.020	0.007	0.281
TP (μg/L)								
	r(i,j)	-0.041	0.000	0.015	-0.067	0.055	-0.077	0.005
	P	0.086	0.492	0.276	0.104	0.161	0.087	0.466
TN (μg/L)								
	r(i,j)	**-0.072**	-0.003	0.003	**-0.109**	**0.108**	**-0.127**	0.044
	P	0.005	0.456	0.460	0.018	0.024	0.010	0.167
molar N:P ratios								
	r(i,j)	**-0.057**	-0.006	-0.018	-0.078	**0.093**	**-0.093**	0.065
	P	0.024	0.418	0.231	0.070	0.042	0.046	0.072
Grazer biomass (mg/clay pot)								
	r(i,j)	-0.007	-0.039	-0.002	-0.065	**0.104**	-0.078	**0.120**
	P	0.414	0.102	0.472	0.111	0.029	0.083	0.003

Table 4.2 (cont'd).

B

Environmental variable	**Trait** Low P	Low N	Low Si	Low light	Hel, low +	Hel, high +	Phy, low +	Phy, high +
DO (mg/L/hr)								
r(i,j)	**-0.049**	0.043	0.008	-0.022	**-0.093**	0.054	**-0.095**	-0.006
P	0.045	0.081	0.247	0.389	0.014	0.131	0.022	0.446
TP (μg/L)								
r(i,j)	-0.017	-0.026	-0.005	-0.029	-0.026	0.028	-0.051	0.014
P	0.281	0.206	0.185	0.432	0.281	0.289	0.147	0.372
TN (μg/L)								
r(i,j)	-0.021	-0.020	0.000	-0.033	-0.044	0.050	-0.074	0.026
P	0.235	0.261	0.151	0.495	0.159	0.156	0.062	0.281
Light at mesocosm bottom (μmol/m^2/sec)								
r(i,j)	0.039	**0.060**	**0.073**	**0.055**	0.026	-0.066	**0.102**	-0.036
P	0.086	0.026	0.042	0.004	0.280	0.092	0.015	0.210
molar N:P ratios								
r(i,j)	-0.016	0.005	0.008	-0.020	-0.050	0.061	-0.070	0.032
P	0.291	0.434	0.273	0.387	0.130	0.104	0.075	0.240
Grazer biomass (mg/clay pot)								
r(i,j)	0.012	-0.028	0.019	-0.001	-0.039	0.068	-0.032	**0.086**
P	0.334	0.178	0.482	0.261	0.185	0.078	0.263	0.027

Stigeoclonium] in Figure 4.3). Although there was evidence for a competition-resistance tradeoff in the D-matrix, this tradeoff did not operate in all contexts or in isolation of other potential tradeoffs.

Most of the D-matrix environment-trait correlations were driven by several abundant species that exhibited strong, corresponding responses in both the trait experiments and the cattle tank mesocosms. For example, the positive correlation between productivity (DO and TN) and resistance to *Helisoma* (source HIGH+) was largely driven by species that responded positively to *Helisoma* grazing in the grazer resistance assay *and* were found in high productivity environments in the mesocosms (*e.g.* large *Oedogonium* species and *Cosmarium* sp. B). Other species, which exhibited similar, corresponding responses in the trait assays and the mesocosm experiment included *Cosmarium* sp. A, *Leptolyngbya* sp. A, *Rhopalodia gibba*, and the basal cells of *Stigeoclonium* sp. Table 4.3 lists the traits and environmental correlations of these key species. Several of these key species had competition and resistance traits that matched the predictions of the keystone predation tradeoff, but only in some environments. For example, *Cosmarium* sp. B was a superior nutrient competitor and vulnerable to predation, but only in treatments from LOW+ source mesocosms (Table 4.3). Similarly, *Leptolyngbya* sp. A was an inferior nutrient competitor and grazer resistant, but only not in all source environments (Table 4.3).

If the trait of light competition is included in the fourth-corner analysis, less trait-environment correlations are significant (Table 4.2B), but those that are (*e.g.* trait-DO correlations), exhibit the same approximate magnitudes and signs as the analysis without light. Light reaching the bottom of the mesocosms was significantly positively correlated

Table 4.3: Trait values (relative growth rates – RGR) and environmental correlations (from the RDA) for algal species whose responses in the trait bioassays and the outdoor mesocosm experiment drive the significant correlations between algal traits and environmental variables in the fourth-corner analysis.

Species	RGR Low P	RGR Low N	RGR Low Si	RGR Low light	RGR Hel, LOW+	RGR Hel, HIGH+	RGR Phy, LOW+	RGR Phy, HIGH+
Cosmarium sp. A	0.130	0.119	0.188	0.012	-1.241	0.572	0.358	0.700
Cosmarium sp. B	0.067	0.121	0.235	-0.012	-1.256	0.659	-1.256	0.700
Leptolyngbya sp. A	-0.057	-0.207	-0.029	-0.353	1.054	-0.999	1.066	0.100
Large *Oedogonium* spp.	-0.476	0.012	-0.099	-0.508	-1.547	0.496	-1.547	-0.102
Rhopalodia gibba	0.013	0.134	0.090	-0.029	1.278	-1.079	1.148	-1.079
Stigeoclonium basal cells	0.242	0.003	0.315	-0.118	0.066	0.024	-0.001	-0.010

	DO correlation	TP correlation	TN correlation	N:P correlation	Grazer correlation	Light correlation
Cosmarium sp. A	-0.052	0.151	0.058	-0.134	0.111	0.488
Cosmarium sp. B	0.230	0.004	0.192	0.325	0.102	0.303
Leptolyngbya sp. A	-0.573	-0.244	-0.330	-0.193	0.089	0.446
Large *Oedogonium* spp.	0.46	0.24	0.40	0.323	-0.11	0.32
Rhopalodia gibba	-0.106	-0.200	-0.268	-0.149	-0.391	-0.489
Stigeoclonium basal cells	-0.26	-0.02	0.08	0.160	0.34	0.214

with the traits of light, N, and Si-competition, as well as the ability to resist *Physa* in LOW+ treatments (Table 4.2B). Both *Cosmarium* species and *Stigeoclonium* basal cells and *Leptolyngbya* sp. A affected these correlations (Table 4.3).

DISCUSSION

Linking species' traits to environmental contexts is key to elucidating the mechanisms and generality of ecological tradeoffs. Through the design and implementation of independent experiments as well as the use of a novel statistical approach, I was able to directly correlate species' traits and environmental variables. Results of the fourth-corner analysis provided some evidence in support of the keystone predation tradeoff in the benthic algae. Significant negative correlations between competition traits and mesocosm productivity and concomitant positive correlations between resistance traits and productivity indicate that several algal species tradeoff in their ability to procure resources and their resistance to predation; thus contributing to compositional shifts along gradients in resource supply and herbivore presence.

Earlier studies of species compositional shifts along productivity (and often predator) gradients in a variety of habitats have revealed evidence for the keystone predation tradeoff (Darcy-Hall, Chapter 3, Steiner 2003, Bohannan and Lenski 2000, Chase *et al.* 2000, Hillebrand 2000, Leibold 1999, Leibold *et al.* 1997). Most of these studies either directly or indirectly documented predator-mediated prey shifts from presumably vulnerable to less vulnerable species as system primary productivity increases. However, only Steiner (2003) and the present study go the step further and attempt to independently demonstrate that resultant prey communities exhibit the expected traits. Steiner (2003) demonstrated that smaller (*i.e.* edible) phytoplankton size fractions exhibited higher growth rates than larger (*i.e.* inedible) size fractions and that the larger fractions were more resistant to zooplanktivorous predators. Despite his coarse examination of algal traits, Steiner's (2003) results support those of the present study's individual species-based test of keystone predator tradeoffs.

While several of the significant correlations in the D-matrix supported the predictions of the KPH, it is clear that algal traits (as defined here) were context dependent. In many instances, the same species exhibited differential responses to herbivory in LOW+ and HIGH+ communities. For example, *Cosmarium* sp. B and *Rhopalodia gibba* had opposite responses to herbivory in LOW+ versus HIGH+ communities (Table 4.3). Differential species' responses in LOW+ and HIGH+ communities were potentially the result of the large differences in community composition in the two nutrient environments (Figure 4.3). The response to herbivory by a focal species would undoubtedly be affected by the identity of and responses to herbivory of neighboring algal species, which are different in each environment. In addition, several species, such as *Cosmarium* sp. A and *Stigeoclonium* responded positively to one species of grazer, but negatively to the other species (Table 4.3). These results highlight that the trait of grazer resistance is context dependent, and that different species will benefit from herbivory, depending on the species of grazing predator and the composition and abundance of their competitors.

The context dependence of species' responses to herbivory has precedence in both plant and algal communities. For example, in a meta-analysis of plant species and their traits' responses to herbivory along a productivity gradient, Pakeman (2004) concluded that only about 40% of species' and species' traits responses were consistent. The other

60% of species and trait responses were either context dependent, complex, or random (Pakeman 2004). Similarly, Darcy-Hall (Chapter 3), Steiner (2003), and Hillebrand (2000) found that the algal response to herbivory depended upon the productivity of the habitat, which is consistent with the present results. Differential responses to herbivory appear to be common in benthic algae. In a literature review, Steinman (1996) concluded that factors such as grazer type, timing of the grazer-algal interaction, and habitat can affect not only algal compositional responses but also biomass and diversity responses. Similarly, I found clear evidence that various grazer types can have opposing effects on algal composition. These examples of the context dependency of algal responses to herbivory can be at least partially explained by the effects of different grazer species as well as by shifts in relative species composition in habitats of different productivity.

There was also evidence of other tradeoffs in the D-matrix. For instance, P-competition correlated negatively with productivity (DO), but N-competition correlated positively with DO. These opposing relationships were driven primarily by large species of *Oedogonium*, which appeared to be poor competitors for phosphorus, but good competitors for nitrogen. The mechanism of this response is unclear, but as has been well documented in the phytoplankton literature, tradeoffs are common within and among species in their ability to efficiently use various resources (reviewed in Grover 1997).

The inclusion of light treatments in both the mesocosm experiment and the resource competition assay highlighted the importance of light in shifting algal species composition. In both experiments, the light treatment was particularly strong, resulting in over 90% light attenuation in low light treatments. The large difference between high and low light treatments likely contributed to the drastic compositional effects, relative to nutrient supply and herbivore treatments. Therefore, it is evident that subsequent models of competition-resistance tradeoffs should incorporate a more thorough treatment of light competition.

Future work concerning algal traits in the context of a competition-resistance tradeoff may also benefit from a broader definition of competition than the one used here. The resource competition assays were designed to measure competition for a limiting nutrient, hence I only measured classic, Tilman-type competition for resources. As evidenced by previous results (Darcy-Hall, Chapters 2, 3), competition in benthic algal communities can also be viewed as access to nutrients rather than survival in a limiting condition. While I found evidence for a competition-resistance tradeoff among the benthic algae using the classic measure of resource competition, it is possible that the incorporation of a broader view of competition would strengthen the results.

In summary, the fourth-corner analysis provided support for the trait-environment associations predicted by the KPH. The algal trait of P-competition correlated negatively with several productivity variables, while the trait of resistance to *Helisoma* and *Physa* grazing (in HIGH+ source communities) correlated positively with productivity. Therefore, the competition-resistance tradeoff inherent to keystone predator models was at least one of several potential mechanisms influencing benthic algal species composition and coexistence. In addition, comparisons both within and among these experiments highlight the context dependency of species' responses – a phenomenon that deserves more direct examination and review.

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CHAPTER 5

SUMMARY

The research reported here provides a thorough investigation into the nature and mechanisms driving patterns of benthic algal composition and limitation along gradients in potential primary productivity. The results combined a mathematical model, data from a field survey of natural lakes, controlled mesocosm experiments and rigorous multivariate analyses to provide convincing evidence for the importance of a competition-predation resistance tradeoff among benthic algae. From a conceptual standpoint, I was able to theoretically and empirically link questions from two important ecological areas, one pertaining to keystone predator models of competition-resistance tradeoffs and another concerning the relative effects of top-down and bottom-up factors on trophic level biomass and community structure. In general, the benthic algae are an under-studied group in lentic systems, despite their significant contribution to primary production. This fact alone provides an important justification to the present research.

Generally, my results demonstrate that the competition-resistance tradeoff implicit in keystone predator models can impact compositional shifts of benthic algae and hence patterns of nutrient (bottom-up) and predator (top-down) limitation of algal growth across habitats (*i.e.* lakes and mesocosms in southwest Michigan) that vary in potential primary productivity. More specifically, my results provide evidence for several key predictions of the keystone predator model. First, I demonstrated that both natural and experimental patterns of nutrient and predator limitation of benthic algae are consistent with expectations of the keystone predation hypothesis (KPH). Second, I show that predators mediate benthic algal compositional shifts in the manner predicted by the KPH.

Finally, I provide support for the expected species' trait-environment associations under the KPH.

In Chapter 2, I investigated how benthic algal composition and limitation varied across lakes along a wide productivity gradient. I found that inter-ecosystem patterns of nutrient and predator limitation of benthic algae were similar to predictions of the "food web" case (*i.e.* KPH) in the limitation model, which incorporates species heterogeneity within trophic levels. Nutrient limitation remained strong across a productivity gradient of lakes, while predator limitation remained low and declined across the productivity and grazer biomass gradient. These results contradicted predictions of classic, trophically homogeneous food chain models. Additionally, benthic algal species composition across lakes and traits of key species qualitatively complied with expectations of the KPH. This research illustrated the importance of heterogeneity both within and among systems to interactions between a community of organisms and the surrounding abiotic and biotic environment.

In Chapter 3, I showed that herbivore grazing favored algal communities dominated by grazer resistant species. Similar to predictions from the KPH, systems without herbivores were dominated by vulnerable, superior competitors, while systems with herbivores were dominated by less vulnerable, inferior nutrient competitors. In addition, these herbivore-mediated shifts in algal composition led to differences in the extent of nutrient and predator limitation in historically grazed versus ungrazed communities. Nutrient limitation of historically ungrazed communities was lower than that of historically grazed communities, but primarily in high productivity mesocosms. Predator limitation, on the other hand, was higher in ungrazed communities but only in low productivity mesocosms. These results qualitatively matched predictions from the KPH. Thus, this experiment provided evidence for a link between herbivore-mediated compositional shifts and patterns of benthic algal nutrient and predator limitation in habitats along a productivity gradient.

In my final chapter, I used a recently developed statistical technique (the fourth-corner analysis) to test whether the algal traits of resource competitive ability and predation resistance were correlated with the environments as predicted by the keystone predation hypothesis. Keystone predator models predict that algal competitive ability for resources should be associated with low productivity environments while resistance to grazing should be associated with high productivity environments. Therefore, I expected negative correlations between competition traits and productivity and positive correlations between resistance traits and productivity. Indeed, algal phosphorus and light-competition correlated negatively with several measures of productivity, while resistance to *Helisoma* and *Physa* grazing correlated positively with several productivity variables (although this effect depended on the source of algal community exposed to grazers). However, several species' trait-environment correlations did not match the predictions of the KPH, indicating that other trade-offs may be important within benthic algal communities and deserve further attention. In general, the competition-resistance tradeoff inherent to keystone predator models was at least one of several potential mechanisms influencing benthic algal species composition and coexistence.

Future research concerning tradeoffs within benthic algal communities should focus in several key areas. First, the context dependency of algal species' responses to grazing should be investigated more specifically and more thoroughly. Research should focus on how and why individual species' responses vary with productivity of the habitat, herbivore type, identities of dominant, coexisting species, and interactions between all of these factors. One potential analytical method to address such questions might involve partial multivariate analyses. Analyses such as partial redundancy analysis or partial canonical correspondence analysis partition out variation due to certain variables while controlling for other variables with known effect.

Second, the idea of competition within benthic algal communities should be more rigorously investigated. Ideally, experiments involving individual or small groups of species would measure relative competitive abilities in low and high nutrient environments to assess both R* competition and nutrient acquisition competition. These experiments could be paired with predator resistance assays to explore the competition-resistance tradeoff in a population versus a community context.

Third, the role of light in affecting algal species composition remains understudied, especially in the context of ecological tradeoffs such as the KPH. Significant priorities should involve studies focusing on the relative importance of light, nutrients, and herbivores on species compositional shifts in natural and experimental settings.

Finally, I would like to pursue questions relating to the relative edibility of algal communities resulting from various levels and/or types of herbivory. It remains to be demonstrated that presumably grazer resistant algal communities are poor sources of food for herbivorous grazers. I would like to investigate the growth rates of various grazer types on algal communities from sources varying in productivity and/or the presence or absence of herbivores, perhaps from both natural and experimental systems.

APPENDICES

APPENDIX A

THE LIMITATION MODEL

THE LIMITATION MODEL

with Spencer Hall

Model structure and description

Consider the familiar diamond food web model. Two plants compete for a limiting resource and can potentially coexist with a generalist herbivore (Holt *et al.* 1994, Leibold 1996, Grover 1997). This model mixes competition for a limiting resource, *R*, among the two plants, N_j (Tilman 1982) with apparent competition among plants which must withstand herbivory from a grazer, *P* (Holt 1977). This model can be described with a simple system of equations representing the growth rates of the herbivore, plants, and freely available resource as the net result of trophic fluxes and nutrient recycling (Grover 1997):

$$\frac{dP}{dt} = P\left(\sum_j e_j f_j N_j - d\right) \tag{1.a}$$

$$\frac{dN_j}{dt} = N_j\left(u_j(R) - m_j - f_j P\right) \tag{1.b}$$

$$\frac{dR}{dt} = a(S - R) - Q_j N_j \sum_j \left(u_j(R) - m_j\right) + P\left(\sum_j \left(Q_j - e_j q\right) f_j N_j + dq\right) \tag{1.c}$$

In this model, growth rate of the herbivore (equ. 1.a) is the balance between gains from feeding on prey item *Nj* (following a linear functional response at grazing rate f_j and conversion efficiency e_j), and losses at rate *d*. Because the herbivore's functional responses for both prey items are linear, the prey items are linearly substitutable (Tilman 1982), and the model produces stable dynamics at equilibrium (Grover 1997). The growth rate of plant *j* is the balance of gains from resource acquisition, at rate $u_j(R)$, and losses from predatory (f_jP) and non-predator (m_j) sources. Finally, the change in free nutrient concentration is the balance between gains and losses from dilution (at rate a), where S is the total concentration of the incoming nutrient resource, the net effect of nutrient uptake and instantaneous recycling of dead plants (where Q_j is the nutrient content of the producer per unit carbon), recycling of producers eaten but not converted into new grazer tissue (where q is the nutrient content of the grazer per unit carbon), and recycling of dead producers. Although this free nutrient pool is open to gains and losses from the outside environment, one can use the mass balance constraint (MBC) technique to simplify the analysis. For this model, the MBC becomes:

$$R = S - \sum_j Q_j N_j - qP. \tag{2}$$

Now free resource dynamics follow an algebraic representation as the difference between S, which becomes the total concentration of nutrients in the system, and those nutrients locked up in plant ($\sum_j Q_j N_j$) and grazer (qP) tissues (Grover 1997).

Initial progress is best made by making some preliminary assumptions about the plants and grazer. First, we consider the case of linear uptake of nutrients by the plant, such that $u_j(R) = u_j R$. This keeps the system simpler algebraically (and will be relaxed elsewhere). Next, we assume that plant N_1 is a superior nutrient competitor in the sense that it can persist at the lowest concentration of freely available nutrients in the absence of grazing (*i.e.*, it has the lowest R_j^* requirement, or $R_1^* < R_2^*$, following Tilman 1982), where:

$$R_j^* = \frac{m_j}{u_j}. \tag{3}$$

(Thus, $m_2 u_1 > m_1 u_2$). Yet, the herbivore prefers N_1 to N_2 as it has a higher feeding rate on the former (*i.e.*, $f_1 > f_2$). As long as defense against herbivory is costly, we can then assume that plant N_1 trades off ability to compete for nutrients (low R_j^*) with its ability to resist predation (high f_j). In mathematical terms, this tradeoff ensures that the nullclines (with slope u_j / f_j) of the plant can cross in *R*-*P* space. The cross eventually occurs because the nullcline for N_2 in *R*-*P* space is steeper than that for N_1 (*i.e.*, $u_1/f_1 < u_2/f_2$, or $u_1 f_2 < u_2 f_1$) while the *R*-axis intercept (R_j^*) is lower for N_1 than N_2 (Grover 1997). This cross of nullclines is critical for species turnover and potential coexistence along enrichment gradients.

In this well-known case, the herbivore can mediate shifts in plant composition along gradients of resource supply, *S*, and facilitate coexistence of the plants at intermediate *S*. Assuming sufficient resource supply to support the herbivore, at low and high nutrient supply, the food chain comprised of the herbivore, plant, and resource comes to an equilibrium:

$$N_{j,P}^* = \frac{d}{e_j f_j} \tag{4.a}$$

$$P^* = \frac{u_j\left(S - Q_j N_j^*\right) - m_j}{f_j + q u_j} \tag{4.b}$$

$$R_{j,P}^* = \frac{f_j\left(S - Q_j N_j^*\right) + m_j q}{f_j + q u_j} \tag{4.c}$$

This equilibrium requires that nutrient supply exceeds a minimum level, $S > Q_j N_{j,P}^* + R_j^*$. If the herbivore is adaptive, it prefers to consume the prey for which it is the best competitor (lowest $N_{j,P}^*$). Thus, the herbivore can drive biomass of its preferred prey (N_1) to lower levels than its other prey (N_2), *i.e.*, since $N_1^* < N_2^*$, $e_1 f_1 > e_2 f_2$. It follows then, that unless there are large differences in plant stoichiometry (Q_j) among species, this minimal nutrient level required for invasion of the grazer is lower for the superior nutrient competitor N_1 than for the predation-resistant species N_2 (since $R_1^* < R_2^*$ and $N_{1,P}^* < N_{2,P}^*$). Once this nutrient level is surpassed, plant biomass ($N_{j,P}^*$) does not change with further enrichment but is instead regulated by the grazer's traits. However, the herbivore (P^*) and resource ($R_{j,P}^*$) both increase with nutrient supply (Figure A1.1). One can readily show that both P^* and $R_{j,P}^*$ increase in a food chain with the more vulnerable species (N_1) than with the less vulnerable species (N_2), as the slopes of the relationships between P^* and S and between $R_{j,P}^*$ and S are positive functions of f_j (Figure A1.1).

At intermediate nutrient supply in the food web, the two plants can potentially coexist with the predator at an additional equilibrium:

$$R^{**} = \frac{m_2 f_1 - m_1 f_2}{u_2 f_1 - u_1 f_2} \tag{5.a}$$

$$P^{**} = \frac{m_2 u_1 - m_1 u_2}{u_2 f_1 - u_1 f_2} \tag{5.b}$$

$$N_1^{**} = \frac{dQ_2 - e_2 f_2\left(S - R^{**} - qP^{**}\right)}{e_1 f_1 Q_2 - e_2 f_2 Q_1} \tag{5.c}$$

$$N_2^{**} = \frac{dQ_1 + e_1 f_1\left(S - R^{**} - qP^{**}\right)}{e_1 f_1 Q_2 - e_2 f_2 Q_1} \tag{5.d}$$

where now both free nutrient (R^{**}) and predator biomass (P^{**}) do not change with increasing nutrient supply. Instead, increasing nutrient becomes reallocated among the plants as N_1 decreases and N_2 increases (Figure A1.1). Coexistence requires that nutrient supply falls within the key range:

$$qP^{**} + Q_2N_2^{*} + R^{**} < S < qP^{**} + Q_1N_1^{*} + R^{**} \tag{6}$$

(thresholds *e* and *f* of Figure A1.1, respectively) but also that the plants individually impact the factor that most limits their own growth. In this model, the plant impacts its predator at rate e_jf_j and its resource at ratio Q_j. Therefore, coexistence requires that $e_1f_1/e_2f_2 > Q_1/Q_2$. Once these various requirements are met (intermediate resource supply, tradeoff in competitive ability versus grazing resistance, and each species has a greater relative impact on the factor most limiting its growth), coexistence is possible (Leibold 1996, Grover 1997).

Resource and predator limitation in food chains and food webs

Whether the plant coexists with the predator alone or with its competitor and predator, it is simultaneously limited by both resources and predation. Following Osenberg and Mittelbach (1996), we define limitation as the effect on per capita growth rate of the plant (or plant assemblage) as one of the limiting factors is removed. Mathematically, this means that predator limitation (Lim_P) and resource limitation (Lim_R) can be written generally as:

$$Lim_P = \left.\frac{dN_j}{N_jdt}\right|_{N = N_j^{*}, R = R^{*}, P = 0} \quad \text{and} \tag{7a}$$

$$Lim_R = \left.\frac{dN_j}{N_jdt}\right|_{N = N_j^{*}, R = \hat{R}, P = P^{*}} . \tag{7b}$$

Figure A1.1: Biomass and nutrient partitioning along nutrient supply gradients in food chains and food webs with plants and a grazer at equilibrium. In the food chain example, two examples are shown: species 1 is a superior nutrient competitor (*i.e.*, lower R_j^*) but is also more vulnerable to predation. As nutrient supply exceeds the plant's R_j^* (level *a* for species 1 and level *b* for species 2), biomass of the plant increases with enrichment until the predator can invade (at level c for more vulnerable species, and level d for the less vulnerable species). Once these thresholds are reached, plant biomass remains constant (at the predators N_j^*) with enrichment as predator biomass (P^*) and free nutrient levels (R^*) increase. The rate at which P^* and R^* increase with enrichment is higher for species 1 (more vulnerable) than species 2 (less vulnerable). In the food web, coexistence is possible at intermediate resource supply (between thresholds *e* and *f*). In this region, P^* and R^* remain constant with enrichment, as increased nutrient supply is reallocated among species 1 (declining) and species 2 (increasing).

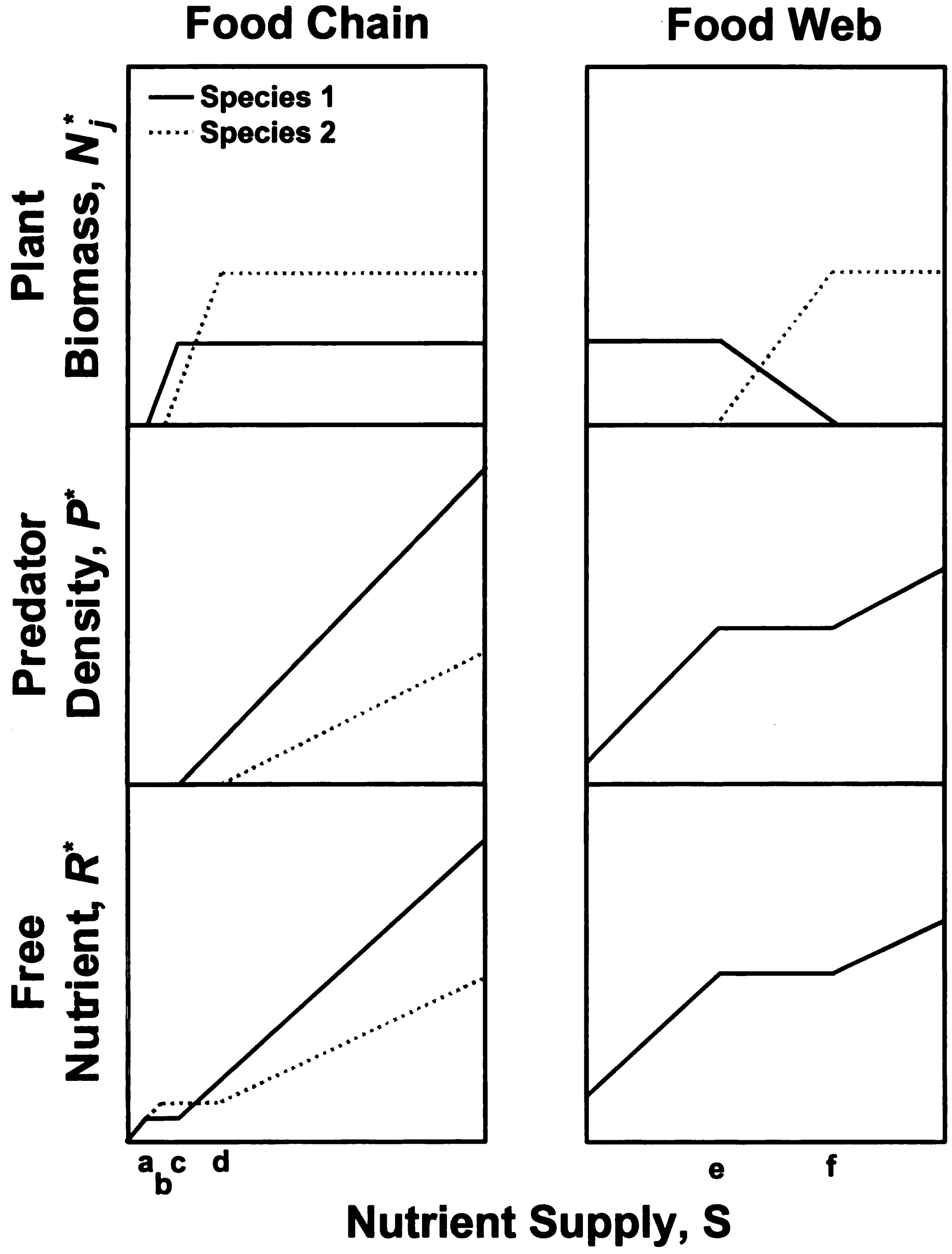
Food Chain
Food Web
Species 1
Species 2
Plant Biomass, N_j^*
Predator Density, P^*
Free Nutrient, R^*
a
b
c
d
e
f
Nutrient Supply, S

Figure A1.1

Predator limitation is evaluated as predators are instantly removed from the plant-herbivore system at equilibrium (*i.e.*, predators are reduced to P=0). Meanwhile, resource limitation is measured with instant elevation of free nutrient levels to $\hat{R}$ which greatly exceeds equilibrial free nutrient concentration, $R_{j,P}^*$.

In the food chain case, predator limitation becomes:

$$Lim_P = u_j R_{j,P}^* - m_j \tag{8}$$

which is a positive function of nutrient supply (S) since:

$$\frac{\partial(Lim_P)}{\partial S} = \frac{f_j u_j}{f_j + qu_j}. \tag{9}$$

This response of the predator limitation index to nutrient enrichment is identical to the slope at which predator biomass increases with enrichment (following equation (4.b)). Additionally, this slope is a positive function of feeding rate f_j. Thus, at a given nutrient concentration, predator limitation is larger and increases more quickly for the more vulnerable prey (N_1) than for the less vulnerable prey (Figure A1.2). This result makes biological sense since the more vulnerable prey is depleted to a lower level, supports a higher predator population, and enjoys higher available nutrient concentrations than the more resistant prey (Figure A1.1). Therefore, removing predators removes a large source of mortality for the more vulnerable species but a smaller source of mortality for the more resistant species. Furthermore, this predator limitation index's S-axis intercept is equal to the minimal S required to support the food chain (*i.e.*, $Q_j N_{j,P}^* + R_j^*$). Since this minimal S is higher for the more resistant species, predator limitation is always higher for the more vulnerable species along gradients of nutrient supply.

Similarly, nutrient limitation of the food chain becomes:

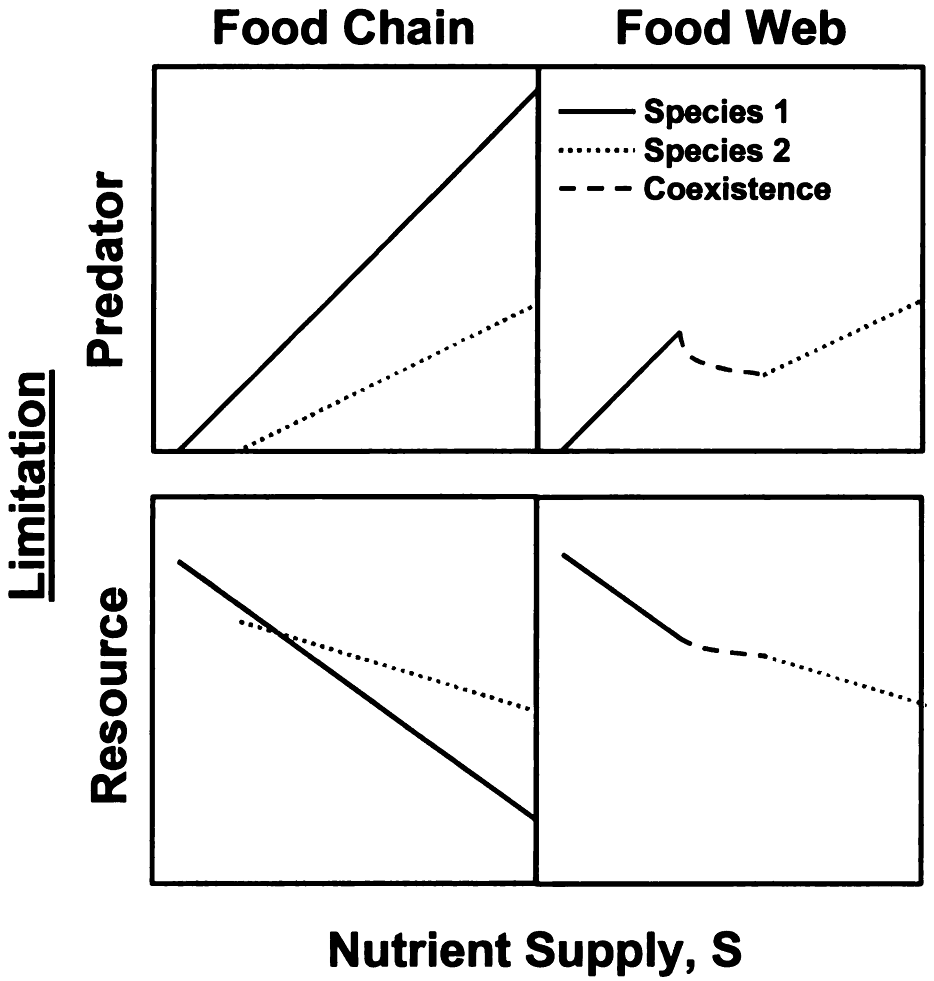

Figure A1.2: Predator and resource limitation over gradients of enrichment in food chains and food webs. In food chains, predator limitation is always higher for more vulnerable species than less vulnerable species. Yet, resource limitation declines more rapidly over the nutrient supply gradient for the more vulnerable species. At intermediate resource supply, coexistence of the plants separates a low nutrient regime, dominated by the superior nutrient competitor, to one dominated by the more resistant plant. In the region of coexistence, predator limitation typically would decrease with enrichment while resource limitation always does.

$$Lim_R = u_j\hat{R} - m_j - f_jP^* \tag{10}$$

where, again, $\hat{R}$ is the level to which nutrients are increased to relieve nutrient limitation ($\hat{R} >> R_{j,P}^*$). Resource limitation is a negative function of S, since:

$$\frac{\partial(Lim_R)}{\partial S} = -\frac{f_j u_j}{f_j + qu_j} \tag{11}$$

Thus, resource limitation decreases more quickly for the more vulnerable species (N_1) than for the less vulnerable species (N_2). Mathematically, this result arises because the slope of the relationship between Lim_R and S is a negative function of increasing f_j (Figure A1.2). Biologically, this result stems from the fact that the less vulnerable species persists at higher biomass, supports few predators, but depletes available resources more than the more vulnerable species. Thus, the less vulnerable species is simultaneously more resource limited and less predator limited than its competitor (Figure A1.2).

In the food web region of resource supply in which both producers coexist, one must examine limitation of the entire trophic level, N_{tot}. If $N_{tot} = N_1 + N_2$, then the aggregate plant trophic level increases with nutrient supply because:

$$\frac{\partial N_{tot}^*}{\partial S} = \frac{e_1 f_1 - e_2 f_2}{e_1 f_1 Q_1 - e_2 f_2 Q_2} > 0 \tag{12}$$

which is true given the assumptions that species have higher impacts on the factor most limiting them (ensuring a positive denominator) and that the predator is adaptive (ensuring a positive numerator). The growth rate of the plant assemblage, then, is:

$$\frac{dN_{tot}}{dt} = \frac{dN_1}{dt} + \frac{dN_2}{dt} \tag{13}$$

following the chain rule from calculus. A bit of analysis reveals that predator limitation of the plant assemblage can increase or decrease with enrichment. First, per capita response of the growth rate of N_{tot} to the removal of the predator is positive, since each individual plant population's growth rate increases with predator removal. Change in this response to increases in S, following the chain rule, is:

$$\frac{\partial f/\partial S}{N_{tot}} - \frac{f(\partial N_{tot}/\partial S)}{N_{tot}^{\;2}} \tag{14}$$

where $f \equiv dN_{tot}/dt$. The right-hand term is positive, but the left-hand term can be positive or negative since:

$$\frac{\partial f}{\partial S} = \frac{f_1 f_2 (e_1 - e_2)(u_1 m_2 - u_2 m_1)}{(e_1 f_1 Q_2 - e_2 f_2 Q_1)(u_2 f_1 - u_1 f_2)} \tag{15}$$

which is positive if $e_1 > e_2$ and negative otherwise (given the stipulations already placed on the other terms in parentheses of equation (15) above). Thus, when the conversion efficiency of the less preferred prey (e_2) is less than the conversion efficiency of the more preferred prey (e_1), predator limitation of the plant trophic level always decreases with enrichment. This case will likely occur when the predator is adaptive (since $e_1 f_1 > e_2 f_2$). When $e_2 > e_1$, predator limitation can remain as a negative function of plant biomass if the right-hand portion of equation (14) exceeds the left-hand portion. This result makes sense biologically because total plant biomass increases while predator biomass remains constant in the coexistence region (Figure A1.2). Thus, the plant trophic level experiences less predation per capita as S increases. Interestingly, nutrient limitation of the plant assemblage is always a negative function of nutrient enrichment. Following similar logic as above (equation (14)), it is straightforward to show that resource limitation of the plant assemblage, while positive, is a non-linear, decreasing function of enrichment as long as $\hat{R} > R^{**}$ (which ensures that both left and right hand terms of equation (14) become negative). This result echoes the change in species composition of the plant assemblage. As the superior nutrient competitor decreases with increasing nutrient supply, it becomes less nutrient-limited. Meanwhile, the increasing population of inferior nutrient competitor along the enrichment gradient becomes more nutrient-limited. Yet, apparently, the net balance of these factors ensures that nutrient limitation decreases with enrichment in the coexistence zone.

Over a resource supply gradient, then, changes in species composition yield higher resource limitation and lower predator limitation than expected from a simple food chain. This fact becomes apparent after comparing a food chain with a superior competitor-highly vulnerable species (Figure A1.2, "Food Chain" case) and a food web with many species displaying the keystone-predation tradeoff between competitive ability and predation resistance ("Food Web" case of Figure A1.2; Figure A1.3). In the food chain, predator limitation would steadily increase while resource limitation decreased along an enrichment gradient (Figure A1.2). Species turnover, however, greatly attenuates this increase in predator limitation. Along a gradient of enrichment, species turnover switches plant composition from a highly vulnerable species alone to two species coexisting to a less vulnerable species alone to another species pair coexisting, and so forth (Leibold 1996). As composition of the plant assemblage changes, the slope of predation limitation in each single-species section becomes increasingly shallow (since more resistant species eventually dominate). Meanwhile, predator limitation declines in regions of plant coexistence (Figures A1.2, A1.3). Therefore, over a broad gradient of enrichment, a zigzag pattern emerges (Figure A1.3). Furthermore, nutrient limitation of

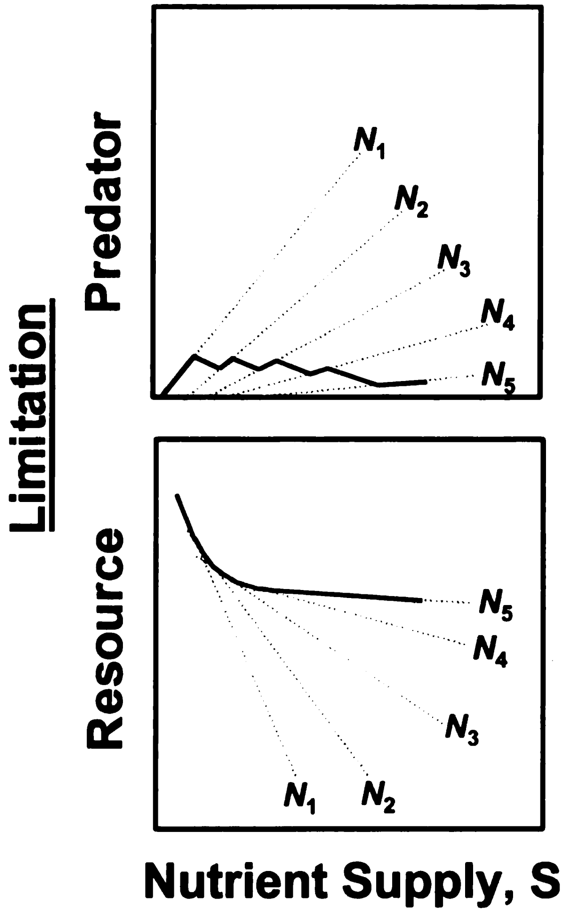

Figure A1.3: Predator and resource limitation in food webs with many plants displaying the keystone-predation tradeoff of competitive ability versus predation resistance. Over an enrichment gradient, plant assemblages become increasingly dominated by resistant species. As a result, predation limitation remains low over a broad gradient of nutrient supply (as compared to the food chain case). These increasingly resistant species maintain a high level of resource limitation of the plant assemblage over this same enrichment gradient.

plant assemblages remains much higher with species turnover than expected from the simple food chain. Here, species of decreasing vulnerability support less predator biomass per capita but also deplete their resources more per capita than the highly vulnerable species (Figures A1.2, A1.3). Resource limitation always remains high for a plant assemblage displaying the keystone-predation tradeoff.

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APPENDIX B

CHAPTER 2 APPENDICES

Appendix B2.1: Abiotic and biotic variables measured in the 14 study lakes. Grazer biomass is measured as ash-free dry mass (AFDM).

Lake	TP (µg/L)	TN (µg/L)	pH	Conductivity (µS/cm)	Chlorophyll *a* (mg/m^2)	Grazer AFDM (mg/m^2)	Nutrient Limitation (per day)	Predator Limitation (per day)
Whitford (Wtf)	13.5	271.6	7.8	343	17.5	33.7	0.005	-0.019
Shaw	13.9	239.8	8.3	370	8.8	70.4	0.134	-0.005
Wamer (Wnr)	15.6	421.0	8.5	217	9.6	1.4	0.059	-0.020
Three Lakes 2 (3L2)	17.5	912.5	8.3	403	14.5	5.2	-0.014	0.014
Lawrence (Lwn)	17.6	1047.7	8.0	413	19.5	2.1	0.008	-0.023
Tamarack (Tam)	18.0	572.3	7.7	480	9.8	102.5	0.098	-0.007
Middle Crooked (MC)	24.5	725.0	6.8	261	11.1	44.8	0.054	-0.038
Baker (Bak)	25.5	521.0	8.2	263	13.0	27.3	0.076	-0.025
Douglas (Dgl)	26.3	208.9	7.9	386	3.6	31.4	0.320	0.005
Lower Crooked (LC)	26.8	725.0	9.0	175	10.3	117.5	0.022	-0.090
Duck	28.7	684.7	7.6	188	10.5	33.9	0.044	0.019
Bassett (Bas)	42.5	262.0	8.5	320	11.8	19.8	0.095	-0.051
Lagoon (Lag)	50.2	1869.8	8.3	362	32.5	394.6	0.003	0.003
Wintergreen (Wgn)	77.5	1249.7	9.2	254	23.7	519.2	0.102	-0.096

Appendix B2.2: Correlations of the algal species in Figures 2.3 and 2.4 with the six environmental variables included in the RDA. Correlations were calculated using the species and environmental scores from the first 4 ordination axes.

Species	**TP** (µg/L)	**TN** (µg/L)	**chlorophyll *a*** (mg/m^2)	**grazer biomass** (mg/m^2)	**conductivity** (µS/cm)	**pH**
Achnanthes exilis	-0.292	-0.102	-0.053	-0.346	0.608	-0.019
Amphipleura pellucida	-0.043	-0.044	-0.422	0.000	-0.485	-0.213
Ankistrodesmus falcatus	0.132	0.066	-0.357	0.162	-0.706	-0.122
Anomoneis vitrea	-0.308	-0.317	-0.257	-0.209	0.077	-0.219
Aphanocapsa sp.	-0.209	0.178	0.027	-0.358	0.696	-0.117
Aphanocapsa conferta	0.053	-0.267	-0.091	0.173	-0.394	0.168
Aphanothece stagnina	-0.065	-0.137	-0.220	0.102	-0.552	-0.505
Calothrix spp.	-0.374	-0.701	-0.273	-0.142	-0.028	-0.014
Chroococcus limneticus	-0.082	0.147	-0.237	-0.024	-0.371	-0.720
Chroococcus minutus	0.129	-0.111	-0.332	0.087	-0.373	0.549
Cosmarium sp. B	-0.059	0.109	0.289	-0.113	0.500	-0.067
Cylindrospermum stagnale	-0.192	-0.098	-0.038	-0.041	-0.136	-0.691
Cymbella cymbiformis	-0.284	-0.148	-0.266	-0.310	0.311	-0.054
Denticula keutzingii	-0.232	-0.053	0.132	-0.248	0.573	-0.155
Encyonopsis microcephala	-0.298	-0.019	0.051	-0.366	0.724	-0.138
Encyonema sp.	-0.220	0.095	0.240	-0.326	0.855	-0.086
Epithemia turgida	-0.348	-0.607	-0.146	-0.092	-0.055	-0.260
Fragilaria crotonensis	0.405	0.046	0.527	0.404	-0.090	0.616
Gomphonema clevei	0.047	0.371	0.232	-0.126	0.490	-0.008
Gomphonema parvulum	0.698	0.686	0.828	0.521	-0.036	0.427
Gomphonema truncatum	0.630	0.603	0.754	0.487	-0.066	0.357
Leptolyngbya sp. A	0.201	-0.089	0.214	0.293	-0.308	0.172
Leptolyngbya sp. F	-0.252	0.033	0.002	-0.332	0.609	-0.145
Leptolyngbya sp. B	0.228	-0.045	0.449	0.305	-0.078	0.224
Limnothrix lauterbornii	0.514	0.480	0.466	0.393	-0.193	0.308
Limnothrix redekei	0.084	-0.189	-0.352	0.147	-0.591	0.230
Mastogloia smithii	-0.509	-0.823	-0.287	-0.268	0.202	0.002
Navicula radiosa	-0.133	-0.130	0.233	-0.144	0.551	0.160
Nitzschia fonticola	-0.292	-0.163	-0.711	-0.293	-0.217	-0.216
Nostoc sp. A	-0.087	-0.411	-0.333	0.097	-0.547	-0.027
Nostoc sp. B	-0.327	-0.444	-0.427	-0.089	-0.414	-0.554
Large *Oedogonium* spp.	0.449	0.333	0.234	0.356	-0.346	0.379
Medium *Oedogonium* spp.	-0.096	-0.205	-0.233	0.027	-0.353	-0.229
Small *Oedogonium* spp.	0.046	0.063	-0.185	0.105	-0.475	-0.338
Oocystis spp.	0.238	0.255	-0.207	0.229	-0.694	-0.191
Pediastrum boryanum	0.485	0.233	0.043	0.389	-0.543	0.631
Pediastrum duplex	0.160	0.075	-0.308	0.162	-0.617	0.026
Rhopalodia gibba	-0.216	-0.509	-0.425	-0.005	-0.472	-0.135
Scenedesmus bijuga	0.349	0.150	0.054	0.419	-0.768	-0.064
Scenedesmus quadricauda	0.497	0.417	0.346	0.385	-0.289	0.361
Staurastrum sp. A	0.481	0.229	0.037	0.386	-0.544	0.628
Stigeoclonium sp.	0.165	0.074	-0.309	0.165	-0.621	0.038

APPENDIX C

CHAPTER 3 APPENDIX

Appendix C3.1: Slices of a significant nutrient supply by grazer history interaction term in the permutational MANOVA on the relative biovolume of benthic algal species composition in Phase 1 (community establishment) mesocosms.

Factor	df	t-statistic	P-value
Slices of nutrient supply (ungrazed)			
LOW vs. MED	1	1.288	0.202
LOW vs. HIGH	1	1.306	0.111
LOW vs. XHIGH	1	2.296	0.026
MED vs. HIGH	1	1.139	0.286
MED vs. XHIGH	1	1.840	0.030
HIGH vs. XHIGH	1	1.959	0.029
Slices of nutrient supply (grazed)			
LOW vs. MED	1	1.332	0.144
LOW vs. HIGH	1	1.682	0.057
LOW vs. XHIGH	1	2.139	0.024
MED vs. HIGH	1	1.109	0.200
MED vs. XHIGH	1	1.628	0.030
HIGH vs. XHIGH	1	0.901	0.513
Slices of grazer histroy at:			
LOW	1	1.551	0.058
MED	1	1.186	0.260
HIGH	1	1.406	0.108
XHIGH	1	2.364	0.031

APPENDIX D

CHAPTER 4 APPENDICES

Appendix D4.1: Two-way (A) and three-way (B) MANOVA's and ANOVA's from the cattle tank mesocosm experiments. MANOVA F-statistics and P-values are Pillai's Trace.

	MANOVA				Univariate ANOVA											
					chlorophyll *a*			DO		TP		TN		N:P		
Factor	num df	denom df	F-statistic	P-value	df	F-statistic	P-value	F-statistic	P-value	F-statistic	P-value	F-statistic	P-value	F-statistic	P-value
A) Nutrient supply x grazer															
Nutrient supply	15	66	3.66	0.0001	3	18.5	0.0002	20.64	<0.0001	7.53	0.001	27.37	<0.0001	3.13	0.044
Grazer	5	20	7.13	0.0006	1	0.57	0.639	17.06	0.0004	2.39	0.135	0.01	0.932	5.16	0.032
Nutrient supply * grazer	15	66	1.08	0.391	3	1.43	0.259	1.05	0.388	0.61	0.612	2.2	0.115	1.33	0.288
Error					24										
B) Nutrient supply x light x grazer subset															
Nutrient supply	4	21	37.67	<0.0001	1	0.330	0.571	17.28	0.0004	82.02	<0.0001	138.55	<0.0001		
Light	4	21	6.54	0.001	1	9.68	0.005	9.52	0.0051	11.05	0.003	14.31	0.0009		
Grazer	4	21	4.65	0.008	1	9.96	0.004	3.57	0.071	0.22	0.644	1.56	0.223	N/A	
Nutrient supply * light	4	21	5.94	0.002	1	0.01	0.930	3.23	0.085	21.72	<0.0001	22.88	<0.0001		
Nutrient supply * grazer	4	21	2.10	0.117	1	5.76	0.025	2.88	0.102	0	0.957	0.55	0.465		
Light * grazer	4	21	2.45	0.078	1	1.94	0.177	2.38	0.136	5.12	0.033	3.00	0.096		
Nutrient supply * light * grazer	4	21	3.48	0.025	1	0.66	0.426	0.23	0.637	0.10	0.751	7.63	0.011		
Error					24										

Appendix D4.2: Species-environment correlations from RDA of cattle tank mesocosm experiment. Species in bold are those included in the fourth-corner analysis. Species with * were only present in the RDA including light treatments. Species are listed with a taxonomic designation in parentheses: (D) = diatoms, (BG) = blue-green algae (cyanophytes), (G) = green algae (chlorophytes).

Species	**DO** (mg/L/hr)	**TP** (µg/L)	**TN** (µg/L)
*Achnanthidium exigua** (D)	-0.469	0.235	0.297
Achnanthes exilis (D)	-0.168	-0.028	-0.158
*Achnanthidium lanceolata** (D)	-0.187	0.303	0.363
Achnanthidium minutissimum (D)	-0.485	-0.316	-0.431
*Amphora pediculus** (D)	-0.170	0.230	0.176
Aphanocapsa sp. (BG)	-0.201	-0.343	-0.486
Aphanocapsa hyalina (BG)	-0.254	-0.326	-0.341
Bulbochaete spp. (G)	0.224	0.337	0.183
Calothrix spp. (BG)	-0.415	-0.358	-0.477
Chroococcus aphanocapsoides (BG)	-0.071	-0.027	0.123
Chroococcus minutus (BG)	-0.334	-0.198	-0.364
Chroococcus prescotti (BG)	-0.425	-0.479	-0.343
***Chroococcus* sp.** (BG)	-0.204	-0.291	-0.112
Cladophora sp. (G)	-0.028	0.122	-0.052
Closterium sp.* (G)	-0.337	0.118	0.171
***Cosmarium* sp. A (G)**	-0.052	0.151	0.058
***Cosmarium* sp. B (G)**	0.230	0.004	0.192
Encyonopsis microcephala (D)	-0.309	-0.169	-0.325
***Epithemia* spp.** (D)	-0.040	-0.083	-0.066
Geminella spp. (G)	0.028	0.005	-0.211
***Gomphonema* spp.** (D)	-0.023	-0.061	-0.102
***Leptolyngbya* sp. A** (BG)	-0.573	-0.244	-0.330
Leptolyngbya sp. B (BG)	-0.430	-0.389	-0.381
***Leptolyngbya* sp. D** (BG)	-0.097	-0.043	-0.208
Leptolyngbya sp. E (BG)	0.054	0.078	0.029
Leptolyngbya sp. F (BG)	-0.415	-0.280	-0.317
Limnothrix lauterbornii (BG)	-0.059	0.049	-0.125
Limnothrix sp. A (BG)	-0.287	-0.164	-0.386
Limnothrix sp. B (BG)	-0.213	-0.288	-0.232
Microcystis sp.* (BG)	0.007	-0.154	-0.142
Mougeotia spp. (G)	-0.142	-0.133	-0.078
Nostoc sp. (BG)	-0.176	-0.168	-0.200
Large *Oedogonium* spp. (G)	0.456	0.240	0.402
Medium *Oedogonium* spp. (G)	0.104	0.290	0.341
Small *Oedogonium* spp. (G)	0.121	-0.059	-0.075
Oocystis spp. (G)	-0.163	-0.212	-0.350
Pseudanabaena sp. A (BG)	-0.003	0.006	-0.222
Pseudanabaena sp. C (BG)	0.041	0.014	-0.047
Pseudanabaena sp. D (BG)	0.035	0.036	-0.062
Pseudanabaena sp. E (BG)	0.018	0.098	-0.057
Rhizoclonium sp.* (G)	-0.027	-0.265	-0.277
***Rhopalodia gibba* (D)**	-0.106	-0.200	-0.268
Scenedesmus acutiformis (G)	-0.136	-0.149	0.079
Scenedesmus obliquus (G)	-0.289	-0.202	-0.231
***Stigeoclonium* sp. basal cells** (G)	-0.256	-0.021	0.075
Tetraedron minimum (G)	-0.309	-0.173	-0.252

Appendix D4.2 (cont'd).

Species	**molar N:P**	**grazer biomass** (μg/pot)	**light** (μmol/m^2/s)
*Achnanthidium exigua** (D)	0.261	-0.001	-0.548
Achnanthes exilis (D)	-0.229	0.059	0.404
*Achnanthidium lanceolata** (D)	0.286	-0.127	-0.582
Achnanthidium minutissimum (D)	-0.254	0.017	-0.291
*Amphora pediculus** (D)	-0.022	-0.171	-0.760
Aphanocapsa sp. (BG)	-0.305	-0.270	0.250
Aphanocapsa hyalina (BG)	-0.083	-0.029	-0.021
Bulbochaete spp. (G)	-0.204	-0.453	0.311
Calothrix spp. (BG)	-0.267	-0.179	0.483
Chroococcus aphanocapsoides (BG)	0.254	-0.102	0.487
Chroococcus minutus (BG)	-0.320	-0.227	0.281
Chroococcus prescotti (BG)	0.150	-0.082	0.359
***Chroococcus* sp.** (BG)	0.257	0.076	-0.086
Cladophora sp. (G)	-0.276	-0.294	0.359
Closterium sp.* (G)	0.186	-0.108	-0.318
***Cosmarium* sp. A (G)**	-0.134	0.111	0.488
***Cosmarium* sp. B (G)**	0.325	0.102	0.303
Encyonopsis microcephala (D)	-0.298	0.140	0.308
***Epithemia* spp.** (D)	0.014	0.121	0.419
Geminella spp. (G)	-0.369	-0.328	0.194
***Gomphonema* spp.** (D)	-0.082	0.009	0.112
***Leptolyngbya* sp. A** (BG)	-0.193	0.089	0.446
Leptolyngbya sp. B (BG)	-0.056	0.010	0.412
***Leptolyngbya* sp. D** (BG)	-0.291	-0.225	0.241
Leptolyngbya sp. E (BG)	-0.071	0.014	0.558
Leptolyngbya sp. F (BG)	-0.114	-0.085	0.102
Limnothrix lauterbornii (BG)	-0.289	-0.099	0.319
Limnothrix sp. A (BG)	-0.411	-0.049	0.287
Limnothrix sp. B (BG)	0.046	-0.368	0.385
Microcystis sp.* (BG)	-0.041	-0.010	0.331
Mougeotia spp. (G)	0.072	-0.007	-0.526
Nostoc sp. (BG)	-0.086	-0.077	0.320
Large *Oedogonium* spp. (G)	0.323	-0.109	0.408
Medium *Oedogonium* spp. (G)	0.137	0.031	0.139
Small *Oedogonium* spp. (G)	-0.037	0.157	0.250
Oocystis spp. (G)	-0.274	-0.200	0.147
Pseudanabaena sp. A (BG)	-0.388	-0.369	-0.005
Pseudanabaena sp. C (BG)	-0.102	-0.054	-0.337
Pseudanabaena sp. D (BG)	-0.160	-0.482	-0.207
Pseudanabaena sp. E (BG)	-0.248	-0.397	-0.644
Rhizoclonium sp.* (G)	-0.148	-0.265	0.353
***Rhopalodia gibba* (D)**	-0.149	-0.391	-0.489
Scenedesmus acutiformis (G)	0.364	0.545	0.300
Scenedesmus obliquus (G)	-0.087	0.185	0.058
***Stigeoclonium* sp. basal cells** (G)	0.160	0.343	0.214
Tetraedron minimum (G)	-0.168	0.103	0.341

Appendix D4.3: Post-hoc pairwise comparisons in the resource competition bioassay for A) chlorophyll *a* (Tukey's HSD), B) chlorophyll *a* (Tukey's HSD) with low light treatment, C) species composition (perMANOVA pairwise comparisons) with low light treatment

A

Factor	df	Mean difference	P-value
Chlorophyll *a* Pairwise comparisons			
Control vs. Low Si	1	0.121	0.219
Control vs. Low N	1	0.659	<0.0001
Control vs. Low P	1	0.134	0.202
Low P vs. Low Si	1	0.013	0.997
Low P vs. Low N	1	0.525	<0.0001
Low N vs. Low Si	1	0.538	<0.0001

B

Factor	df	Mean difference	P-value
Chlorophyll *a* Pairwise comparisons			
Control vs. Low Si	1	0.121	0.801
Control vs. Low light	1	0.956	<0.0001
Control vs. Low N	1	0.659	<0.0001
Control vs. Low P	1	0.134	0.787
Low P vs. Low Si	1	0.013	1.000
Low P vs. Low light	1	0.822	<0.0001
Low P vs. Low N	1	0.525	0.005
Low N vs. Low Si	1	0.538	0.002
Low N vs. Low light	1	0.297	0.147
Low light vs. Lo Si	1	0.835	<0.0001

C

Factor	df	t-statistic	P-value
Species composition pairwise comparisons			
Control vs. Low Si	1	1.096	0.227
Control vs. Low light	1	1.578	0.029
Control vs. Low N	1	1.430	0.173
Control vs. Low P	1	0.792	0.692
Low P vs. Low Si	1	0.863	0.758
Low P vs. Low light	1	1.359	0.038
Low P vs. Low N	1	1.186	0.244
Low N vs. Low Si	1	1.104	0.245
Low N vs. Low light	1	1.637	0.094
Low light vs. Lo Si	1	1.422	0.018