DISSERTATION

ALGAL AND INVERTEBRATE RESPONSES TO ATMOSPHERIC NITROGEN DEPOSITION IN ROCKY MOUNTAIN LAKES

Submitted by

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Graduate Degree Program in Ecology

In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, CO

Fall 2002

COLORADO STATE UNIVERSITY

August 23, 2002

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY BRENDA MORASKA LAFRANCOIS ENTITLED "ALGAL AND INVERTEBRATE RESPONSES TO ATMOSPHERIC NITROGEN DEPOSITION IN ROCKY MOUNTAIN LAKES" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

ALGAL AND INVERTEBRATE RESPONSES TO ATMOSPHERIC NITROGEN DEPOSITION IN ROCKY MOUNTAIN LAKES

In a series of lake surveys and experimental studies we explored how excess nitrogen, alone and in conjunction with phosphorus and/or acid, affects structural and functional aspects of mountain lake ecology in the Colorado Front Range and Wyoming Snowy Range. Our survey of Front Range lakes examined relationships between benthic invertebrate assemblages and environmental characteristics and found that benthic invertebrate composition among lakes was related more to elevation gradients and the presence of fish than to nitrogen or other water chemistry gradients. To understand how increased nitrogen could affect other lake biota (algae, zooplankton) and lakes with low nitrogen, we conducted mesocosm experiments in two Snowy Range lakes. We found that N and N+P additions caused eutrophication responses ranging from increased algal biomass and productivity to marked shifts in phytoplankton composition. Our survey of water chemistry, nutrient ratios and phytoplankton composition in fifteen Snowy Range lakes showed that N regulation of phytoplankton growth and composition may predominate regionally; nitrate and DIN:TP levels were generally low among Snowy Range lakes and phytoplankton composition was tightly linked to N chemistry. Because excess N can lead to both eutrophication and acidification, we conducted mesocosm experiments in a high-nitrate Front Range lake and a low-nitrate Snowy Range lake to

examine responses of lake biota to simultaneous nutrient and acid additions. We found that nutrients and acid altered phytoplankton composition in both lakes, favoring chlorophyte taxa in the low-nitrate lake, and chlorophytes and the dinoflagellate *Gymnodinium* in the high-nitrate lake. Changes in algal biomass were tightly linked to nutrient additions; changes in species composition were related to both nutrients and pH. Overall, zooplankton and benthic algal communities showed inconsistent responses to nutrient or nutrient+acid additions in our mesocosm experiments and may be poorer early indicators of enrichment and acidification than phytoplankton. We conclude that eutrophication effects of N deposition are most likely in low-nitrate lakes such as those of the Snowy Range, and that effects of N and acidification would likely be strong and interactive in lakes of both the Front Range and Snowy Range.

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Fall 2002

ACKNOWLEDGEMENTS

I wish to express my thanks to the individuals, institutions and agencies that made this work possible. I wish to thank my advisors Brett Johnson and Jill Baron for their balanced and boundless supply of enthusiastic support and constructive criticism. I am grateful to Koren Nydick for the ideas and insights she brought to our conversations and for the determination she brought to our fieldwork. Our many interactions as friends and collaborators were instrumental to the development and implementation of this project. I wish to thank Boris Kondratieff for cultivating my interest in taxonomy, and Alan Covich for his helpful suggestions on project methods. The Loch Vale Watershed group provided a forum for sharing research findings and struggles, and often cheerily assisted with our fieldwork. I wish to thank Jorin Botte in particular for his good-natured assistance in both the field and lab. Dan Reuss provided diverse technical help with laboratory procedures and field equipment construction. Sarah Spaulding, Paul Kugrens, Alex Wolfe and Richard Dufford provided generous advice for processing and identifying algal samples. Officials from Rocky Mountain National Park and the U.S. Forest Service, Laramie Ranger District, provided reviews of proposed research projects and granted permission for experimental manipulations. Rocky Mountain National Park also provided logistical support and interpretive information during our experiments in Loch Vale watershed. I am grateful to my parents and sister for their unfailing confidence in my ability to complete this degree and for their encouragement from afar. I wish to thank Marci Koski and others in the Fisheries Ecology Laboratory for being

reliable sources of humor and perspective throughout my graduate studies. Finally, I am indebted to Toben Lafrançois, my husband, packhorse, and zooplankton expert, for his abiding support of all my pursuits and most of all for his contagious joy. Funding for this work was provided by a grant from the U.S. Geological Survey through Dr. Jill Baron at the Natural Resources Ecology Laboratory. A Colorado State University Graduate Fellowship and a Hill Memorial Fellowship from the College of Natural Resources provided additional funding.

PREFACE

PROJECT BACKGROUND

Alteration of the global nitrogen (N) cycle is a significant and well-documented component of global change (Vitousek 1994). Human activities such as fossil fuel combustion, industrial fixation of N for fertilizer and confined livestock operations have led to increased atmospheric N and increased N deposition on both regional and global scales (Vitousek et al. 1997). Because N regulates terrestrial productivity and biomass, excess N deposition can induces fertilization effects in terrestrial ecosystems and alters plant species composition and diversity (Fenn et al. 1998)(Figure P.1). Further, chronic exposure to excess N is hypothesized to cause terrestrial N saturation, a condition characterized by the inability of plants and soils to immobilize further N inputs, a loss of soil base cations and fertility, and the eventual leaching of nitrate into ground and surface water (Aber et al. 1998)(Figure P.1).

High elevation watersheds such as those of the Rocky Mountains may be especially prone to N saturation and leaching, due to their steep slopes, short growing seasons, sparse vegetation and thin soils (Williams et al. 1996). In addition to this predisposition, many such watersheds are also exposed to relatively high levels of atmospheric N deposition; for example, the Colorado Front Range receives 3-5 kg $N \cdot ha^{-1} \cdot year^{-1}$ (Baron et al. 2000), and the Wyoming Snowy Range receives approximately 3 $kg \cdot ha^{-1} \cdot year^{1}$ (Williams et al. 1996), deposition levels comparable to those in parts of the northeastern United States. Further, some Front Range watersheds show characteristic

signs of N saturation, including altered forest foliar and soil chemistry and high surface water nitrate concentrations (Baron et al. 2000).

The effect of terrestrial N saturation on freshwater ecosystems is incompletely understood. Most investigations have focused on the potential for increased nitrate leaching from N-saturated watersheds to cause base cation depletion, loss of lake acid neutralizing capacity (ANC), and acidification (Figure P.1). Such processes may be of particular relevance to mountain waters, which have naturally low ANC. Indeed, the contribution of atmospheric N to both chronic and episodic acidification of freshwater ecosystems is increasingly recognized (Brimblecombe and Stedman 1982, Williams and Tonnessen 2000), and effects of acidification on aquatic processes and biota can be severe (Schindler et al. 1985, Brezonik et al. 1993).

In addition to its acidifying effect, excess nitrate can also contribute to eutrophication (Figure P.1). Despite the prevailing view that freshwaters are P-limited, many oligotrophic lakes appear to be N-limited or co-limited by both N and P (Morris and Lewis 1988, Axler et al. 1994), such that excess N inputs would likely increase algal productivity and biomass and alter species composition. Current indications of N-related effects of eutrophication exist for oligotrophic Lake Tahoe, where atmospheric N deposition is implicated in increased algal biomass, declines in water clarity and shifts in nutrient limitation (Goldman et al. 1993). Additionally, increasing dominance of the diatom taxa *Asterionella formosa* and *Fragilaria crotonensis* in Front Range lakes suggests a shift from oligotrophic to mesotrophic conditions over the past 50 years, concurrent with increases in regional N sources (Wolfe et al. 2001).

Given the likelihood of future increases in N deposition to Rocky Mountain watersheds and the lack of detailed ecological information on how excess N affects regional mountain lakes, it is important to 1) identify aquatic processes, biota, and lake types that are sensitive to N, 2) determine which of these would be most responsive to future N inputs, and 3) explore factors that may interact with lake responses to N. These questions formed the foundation for the collaborative research conducted by Koren Nydick and me from 1998-2002.

We approached the above objectives comprehensively, using research strategies ranging from lake surveys to micro- and meso-scale experiments. We conducted extensive research in two different lake areas (Colorado Front Range and Wyoming Snowy Range), explored differences in N responses among taxonomic groups and habitats, and examined effects of N alone and in conjunction with other potential stressors such as P deposition and acidification. Our dissertations are highly complementary; my work focused on structural aspects such as changes in species composition, community structure and diversity of aquatic biota, and Koren's addressed functional aspects such as changes in productivity, biomass, and nutrient cycling. Our field and analytical efforts are tightly interwoven throughout the chapters of this dissertation, which are summarized briefly below.

OVERVIEW OF RESEARCH FINDINGS

Chapter one of this dissertation explores linkages between water chemistry, physical characteristics, and lake biology in lakes east of the Continental Divide in Rocky Mountain National Park and Indian Peaks Wilderness Area. This study was designed to augment existing water chemistry data from the region, establish a taxonomic baseline

for previously unstudied benthic invertebrate populations, and explore relationships between benthic invertebrate assemblages and environmental characteristics, including lake nitrate concentration. We collected water chemistry and benthic invertebrates from twenty-two lakes and used Canonical Correspondence Analysis (CCA) to relate benthic invertebrates to environmental characteristics. We hypothesized that nitrate concentration was an important factor in determining benthic invertebrate distribution among lakes, but found that benthic invertebrate composition varied most along elevation gradients and according to the presence or absence of fish. Water chemical variables and seasonal variability were of secondary importance to benthic invertebrate presence/absence, and were influential only when relative abundance and density were considered.

We concluded that sampling methodology (semi-quantitative sweep netting, quantitative Hess sampling, or pooled presence/absence analysis) strongly affected our characterization of benthic invertebrate communities. We noted that elevation, fish presence/absence, and seasonal variability should be considered and controlled for in future assessments of N effects on benthic invertebrates. Further, we suggested that more mechanistic investigations into the effects of elevation and fish presence/absence on invertebrate distribution would be instructive. Finally, because relationships of benthic invertebrates to lake nitrate were weak, we concluded that future N-related work in mountain lakes should also examine responses of primary producers, which are more tightly linked to water chemistry.

Chapter two addresses the potential for N to induce eutrophication in mountain lakes, both alone and in conjunction with added P. While experimental N additions did

not cause eutrophication responses in the higher-nitrate lakes of Loch Vale watershed (Nydick et al., submitted 2002), we suspected that responses would differ in low-nitrate lakes. We conducted short-term enclosure experiments in the littoral zones of two low-nitrate Snowy Range lakes, applying pulse treatments of N, P, and N+P. The experiment was repeated twice in the summer ice-free season to address seasonal variability, and special attention was given to algal responses across algal habitat types (phytoplankton, epilithon, and epipelon).

We found that N and N+P additions caused characteristic eutrophication responses in phytoplankton in both early and late-summer experiments, including increased phytoplankton productivity and biomass and shifts in species dominance from chrysophytes toward chlorophytes, cyanophytes and diatoms. Species richness and diversity generally declined with eutrophication. Epilithic algae (simulated on artificial tile substrates) responded less to treatment additions, and only during the late summer experiment. Epipelic (sediment-dwelling) algae dominated algal biomass in the enclosures, but responses to nutrients were inconsistent. Nutrient enrichment did not alter zooplankton biomass, abundance or composition in either experiment. We concluded that increased N deposition can alter multiple components of mountain lake ecology, and that phytoplankton may be the best indicators of future N effects in these lakes.

Chapter three examines the extent of phytoplankton N limitation in lakes of the Snowy Range. While N and N+P additions caused eutrophication effects in previous Snowy Range enclosure experiments (Chapter 2), extrapolation of these results to whole lakes and other lakes in the area required additional study. We collected water chemistry

and phytoplankton once from fifteen Snowy Range lakes soon after ice-out during summer 2001. We used DIN:TP ratios to estimate nutrient limitation among lakes, and redundancy analysis (RDA) to examine the relationship between phytoplankton species composition and water chemistry (particularly nitrogen fractions). Given results from our previous nutrient enrichment experiments, we predicted that most lakes in the area would be N limited or N+P co-limited, and that phytoplankton species composition would vary along nitrogen gradients.

DIN:TP ratios and analysis of phytoplankton species composition across lakes supported our predictions; among fifteen study lakes, all but one were either N or N+P limited. Further, RDA indicated that variation in the concentration of two forms of N (DIN and PN) strongly influenced phytoplankton distribution across the study lakes. As in our Snowy Range mesocosm experiments, high levels of N were associated with greater relative abundance of cyanophytes and chlorophytes, whereas low N levels were associated with chrysophyte taxa. This multi-lake study corroborated our experimental results and provided evidence that phytoplankton N limitation is relatively widespread in Snowy Range Lakes. We conclude that sustained or increased N deposition to the Snowy Range would likely have significant effects on the biomass and composition of lake phytoplankton, particularly if accompanied by increased P deposition.

Chapter four explores how mountain lake biota respond to simultaneous eutrophication and acidification. We used littoral mesocosm enclosures and applied treatments of N, P, N+acid and N+acid+P. We conducted the experiments in two different lakes (a high-nitrate lake in Rocky Mountain National Park and a low-nitrate lake in the Wyoming Snowy Range) to evaluate how lake nutrient status affects

responses to treatment additions. Responses to nutrients (N or P) differed between the two study lakes; N additions to the low-nitrate study lake increased phytoplankton biomass and altered species composition, whereas the high-nitrate lake responded only to P additions. Combined additions of nutrients and acid, however, altered phytoplankton composition in both lakes, favoring chlorophyte taxa in the low-nitrate lake, and chlorophytes and the dinoflagellate *Gymnodinium* in the high-nitrate lake. Thus, changes in algal biomass appeared to relate more to nutrient additions than to acidification, and species composition was affected interactively by both nutrients and pH. We found that phytoplankton and zooplankton responded more consistently to treatment additions than epilithic algae and may be better indicators of future nutrient enrichment and acidification (but see Nydick (2002) for dominance of benthic algae in regulating nutrient retention and alkalinity generation). Finally, since the most dramatic changes occurred following N+acid+P additions in both study lakes, we concluded that greater attention to the effects of multiple interacting stressors on mountain lakes is warranted.

STRUCTURE OF THE DISSERTATION AND ALLOCATION OF EFFORTS

The research described in this dissertation was part of an overall research program designed to integrate aspects of both community and ecosystem ecology. As a result, many findings in the following chapters reflect the combined efforts of both Koren Nydick and me. We worked collaboratively on the design and execution of each study and contributed equally to field work and sample collection (Table P.1). My personal contributions involved assessment of community-level responses to N and other factors (Table P.1). This entailed identifying and enumerating benthic invertebrates, zooplankton, phytoplankton, and benthic algae, as well as calculating community variates

(species richness and diversity) and conducting multivariate statistical analyses. I also participated in the field and lab analysis of water chemistry parameters and algal chlorophyll *a* and photosynthetic rate.

The chapters of this dissertation and that of Nydick (2002) are prepared as a series of scientific journal manuscripts. Due to the broad scope and collaborative nature of this research, they are all coauthored. Division of effort for the work described in chapter two was particularly equal (Table P.1), and order of authorship for the resulting manuscript was determined arbitrarily. This shared chapter was included in both dissertations since its elimination would diminish the continuity of each dissertation as a whole.

The chapters of my dissertation have been submitted to scientific journals or prepared for submission as follows:

Chapter 1: Lafrançois, B. Moraska, D.M. Carlisle, K.R. Nydick, B.M. Johnson, and J.S. Baron. Environmental characteristics and benthic invertebrates in Colorado mountain lakes. Western North American Naturalist, in press.

Chapter 2: Nydick, K.R., B. Moraska Lafrançois, J.S. Baron, and B.M. Johnson. Nitrogen regulation of algal biomass, productivity, and composition in small mountain lakes, Snowy Range, Wyoming, USA. Limnology and Oceanography, in review (Submitted, May 2002).

Chapter 3: Lafrançois, B. Moraska, K.R. Nydick, and B. Caruso. Phytoplankton nitrogen limitation in lakes of the Snowy Range (Wyoming, U.S.A.): a comparative study. Prepared for submission to Arctic, Antarctic and Alpine Research.

Chapter 4: Lafrançois, B. Moraska, K.R. Nydick, B.M. Johnson, and J.S. Baron. Interactive effects of nutrients and pH on plankton and epilithon of two mountain lakes. Prepared for submission to Canadian Journal of Fisheries and Aquatic Science.

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Table P.1. Allocation of efforts for study design and implementation, data processing and analysis, and writing of chapters included in this dissertation. "Community properties" refers to analysis of taxonomic composition, richness, diversity and community structure; "Ecosystem properties" refers to analysis of water chemistry, algal biomass, productivity, and nutrient cycling. "Both" = work conducted by both Koren Nydick and me, "Lafrançois" = work completed entirely by me, "Nydick" = work completed by Koren Nydick.

Aspect of Research	Chapter 1	Chapter 2	Chapter 3	Chapter 4
Study Design	Both	Both	Both	Both
Field Work & Data Collection	Both	Both	Both	Both
Community properties	Lafrançois	Lafrançois	Lafrançois	Lafrançois
Ecosystem properties	--	Nydick	Nydick	Nydick
Data analysis	Lafrançois	Both	Lafrançois	Lafrançois
Writing	Lafrançois	Both	Lafrançois	Lafrançois

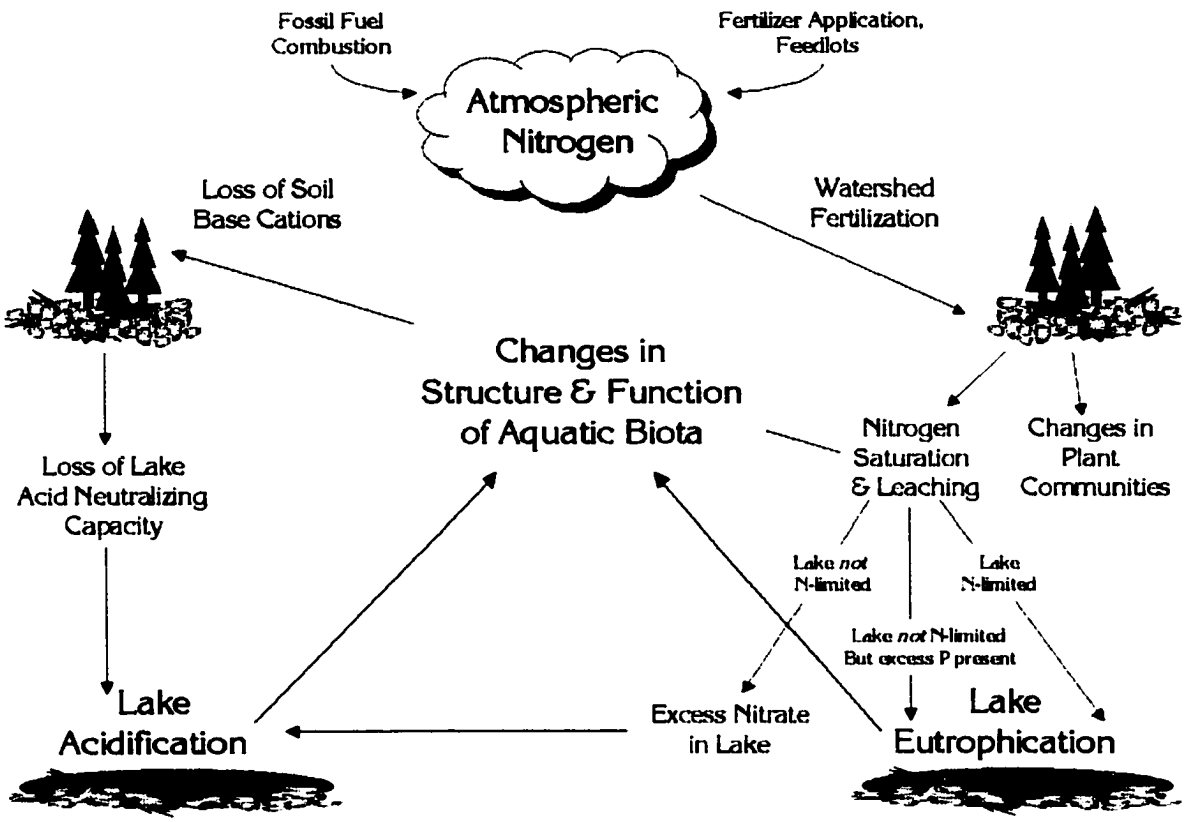

Figure P.1. Pathways of atmospheric N deposition through high elevation watersheds and subsequent effects on mountain lakes. Responses of aquatic biota to N enrichment and acidification were assessed using surveys and experiments in lakes of the Colorado Front Range and Wyoming Snowy Range.

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CHAPTER ONE[1]

Environmental Characteristics and Benthic Invertebrate Assemblages in Colorado Mountain Lakes

[1] This chapter accepted by Western North American Naturalist in 2002 as:

Lafrançois, B. Moraska, D. M. Carlisle, K. R. Nydick, B. M. Johnson and J. S. Baron. Environmental characteristics and benthic invertebrate assemblages in Colorado mountain lakes. Western North American Naturalist, in press.

ABSTRACT

Twenty-two high elevation lakes (>3000m) in Rocky Mountain National Park and Indian Peaks Wilderness Area, Colorado, were surveyed during summer 1998 to explore relationships among benthic invertebrates, water chemistry (particularly nitrate concentrations), and other environmental variables. Water samples were collected from the deepest portion of each lake and analyzed for ions and other water chemistry parameters. Benthic invertebrates were collected from the littoral zone using both a sweep net and Hess sampler. Physical and geographical measurements were derived from maps. Relationships among benthic invertebrate assemblages and environmental variables were examined using Canonical Correspondence Analysis, and the importance of sampling methodology and taxonomic resolution on these relationships was evaluated.

Choice of sampling methodology strongly influenced the outcome of statistical analyses, whereas taxonomic resolution did not. Presence/absence of benthic invertebrate taxa among the study lakes was best explained by elevation and presence of fish. Relative abundance and density of benthic invertebrate taxa were more strongly influenced by sampling date and water chemistry. Nitrate (NO_3^-) concentration, potentially on the rise due to regional nitrogen deposition, was unrelated to benthic invertebrate distribution regardless of sampling method or taxonomic resolution.

INTRODUCTION

The Colorado Front Range is home to a variety of high elevation lakes that invite scientific interest because: 1) they are remote and relatively pristine, making them ideal places for collecting baseline data or establishing long-term monitoring programs, 2) they serve as refugia for taxa adapted to harsh alpine conditions (Grotheer 1995), and 3) they are sensitive to anthropogenic stressors like atmospheric deposition due to low ion concentrations, low acid-neutralizing capacity, sparsely vegetated drainage basins, and rapid spring flushing rates (Eilers et al. 1987, Musselman et al. 1996, Williams et al. 1996, Peterson et al. 1998). Although many studies have examined the relationship between atmospheric deposition (particularly nitrogen deposition) and surface water chemistry in the region (Eilers et al. 1987, Baron et al. 1994, Musselman et al. 1996, Baron and Campbell 1997), few studies have explored the influence of water chemistry and other environmental factors on lake biota, and still fewer have considered benthic invertebrates. Attempts to document the character and distribution of benthic invertebrate populations in high lakes of this region are rare (Bushnell et al. 1987, Stohlgren et al. 1995) or dated (Ward 1904, Shantz 1907, Dodds and Hisaw 1925, Schmitz 1959, Pennak 1966).

The importance of benthic invertebrates to the structure and function of freshwater ecosystems is well established (Klemm et al. 1990, Covich et al. 1999). They are instrumental in processing organic matter and cycling nutrients, and their functional diversity as species and as groups links them tightly to many aspects of aquatic food webs (Merritt et al. 1984). They are long-lived and sedentary relative to most other aquatic life forms, enabling them to integrate variable environmental conditions over time

(Ward and Kondratieff 1992, Cairns and Pratt 1993, Barbour et al. 1999). These relatively low mobilities and long life spans, coupled with taxonomic specificity in terms of habit, habitat preference, and environmental tolerance, makes studies of benthic invertebrates useful for understanding questions of long-term environmental change (Johnson et al. 1993). We chose to examine benthic invertebrates for their diversity, ubiquity, and potential responsiveness to persistent, atmospherically driven changes in water chemistry (Ward and Kondratieff 1992, Hauer and Resh 1996).

Use of benthic invertebrates as biomonitoring tools has gained momentum in recent years, and biomonitoring protocols are under development by various state and federal agencies. Level of taxonomic resolution to be used in monitoring is one important consideration in this process, since sizeable tradeoffs exist between taxonomic resolution and time/effort expenditures. Some studies have indicated that aggregating taxa with similar traits and tolerances may effectively discern certain environmental patterns while limiting sample processing effort (Bowman and Bailey 1997; Clements et al. 2000). However, discerning effects of subtle stressors in heterogeneous environments may require higher levels of resolution. Resolution of the Chironomidae, in particular, may be important to the interpretation of environmental patterns; they are well represented in mountain lakes regionally (Bushnell et al. 1987), and are noted globally for their diversity (Wiederholm 1984, Armitage et al. 1995, Coffman and Ferrington 1996).

Choice of sampling methodology for biomonitoring has implications for the type of habitat accessed, the type of invertebrates collected, and the type of question addressed (Merritt et al. 1996). For example, each sweep net sample covers a fairly large area of

lake substrate and incorporates a high degree of substrate heterogeneity, increasing the likelihood of encountering new taxa. In contrast, each Hess sample covers a much smaller area (perhaps two orders of magnitude less) and is less able to access diverse benthic habitats and taxa. Additionally, mesh size in sweep nets is generally larger than in Hess samplers. While effective at capturing large, motile taxa, sweep nets generally cannot retain the small meiobenthic organisms frequently found in Hess samples. The type of sampling method used may influence not only the type of invertebrates collected and substrates sampled, but also the assessment of their responses to environmental factors/stressors. If the objective is to obtain a representative sample of invertebrates from a series of lakes, sweep net sampling alone may be most efficient (Mackey et al. 1984); however, more quantitative methods may be desirable for other objectives, since sweep net samples yield information only in terms of relative abundance, and Hess samples yield quantitative density data. Detailed inquiries into how sampling methodology affects the outcome of field studies are important to the development of monitoring protocols.

During the summer of 1998 we sampled 22 high elevation (>3000m) lakes in the Colorado Front Range. Our first objective was to conduct a taxonomic inventory of benthic invertebrates across a range of mountain lakes; such information is rare regionally and is necessary for the formation of more detailed research questions. Secondly, we looked for linkages between benthic invertebrate assemblages and various environmental characteristics. Strong effects of elevation on benthic invertebrate distribution were predicted, because earlier work by Pennak (1968) categorized Colorado's mountain lakes according to altitude (alpine, subalpine, or montane) and/or

hydrology (drainage, semi-drainage, or seepage). Strong influence of fish presence/absence was also predicted, because fish are not native to the study lakes and research from other regions suggests that fish introduction has severely impacted native invertebrate assemblages (Bradford et al. 1998, Carlisle and Hawkins 1998, McNaught et al. 1999). Thirdly, relationships between benthic invertebrates and lake nitrate (NO_3^-) concentration were given special attention, because elevated nitrate levels have been implicated in increased diatom biomass and species shifts in some Front Range lakes (Baron et al. 2000, Wolfe et al. 2001), and may translate into altered assemblages and/or densities of organisms at higher trophic levels. Finally, we sought to examine the effect of taxonomic resolution and sampling methodology on the use of benthic invertebrates for biomonitoring studies of mountain lakes.

In this paper we present our findings of: 1) the taxa present in 22 mountain lakes and their relationship to a suite of environmental factors; 2) the relative importance of elevation, fish presence/absence and water chemistry (including nitrate concentration) in shaping benthic invertebrate assemblages; and 3) how taxonomic resolution and sampling methodology affect the use of benthic invertebrates for biomonitoring studies of high elevation lakes.

STUDY AREA

The Colorado Front Range stretches south from the Wyoming state line to the highlands overlooking South Park, Colorado, and is characterized by steep, sparsely vegetated drainage basins with substantial exposed bedrock comprised primarily of granite, gneiss, and schist (Baron and Mast 1992). The study area (Figure 1.1) included lakes in the Big Thompson, St. Vrain, and Boulder Creek watersheds, and was intended

to augment existing water chemistry data from the Rocky Mountain region (Eilers et al. 1987, Musselman et al. 1996, Minear 1998). Lakes included in this survey were predominantly alpine/subalpine drainage lakes, 3000m-3500m in elevation with well-defined inlets and outlets. They ranged from alpine cirque lakes with small rocky watersheds and rocky littoral zones to subalpine drainage or semi-drainage lakes with larger forested watersheds and more organic littoral zones. Fish were present in half the study lakes as a result of previous stocking efforts. Fish species included cutthroat trout (*Oncorhynchus clarki*), rainbow trout (*Oncorhynchus mykiss*) and/or brook trout (*Salvelinus fontinalis*).

METHODS

Water Chemistry and Environmental Variables

Water chemistry samples were collected, and temperature and conductivity were measured once from the deepest portion of each lake between 27 July and 26 September 1998. All water collection bottles were acid-washed and rinsed 5-6 times with deionized water before field use. Water samples were analyzed by the USDA Forest Service Rocky Mountain Station Biogeochemistry Laboratory for major ions, pH, and acid-neutralizing capacity (ANC) following protocols of EPA (1987). Alkalinity, pH, and conductivity were analyzed using the PC-Titrate (COSA) Gran analysis technique. Anions (including NO_3^-) and cations were analyzed using Ion Chromatography (Waters Dionex AS12 A Separator Column for anions, and Waters IC Pak Cation M/D Column for cations).

Several geographical and physical variables were also measured for these analyses. Elevation and lake perimeter measurements (for shoreline development (D_L) calculations, following Wetzel and Likens (1991)) were obtained using USGS

topographical maps. Lake surface area measurements were obtained from Vermillion (2000). Fish presence/absence was determined from communications with local fishery biologists (Bruce Rosenlund, USFWS, pers. comm. and Harry Vermillion, Colorado Division of Wildlife, pers. comm.).

Benthic Invertebrates

Benthic invertebrates from 1-4 (usually two) randomly selected littoral sites away from inlets were sampled once per lake between 27 July and 26 September 1998, using both a D-frame sweep net with 500-μm mesh size and a modified Hess sampler with 150-μm mesh size (Carlisle and Hawkins 1998). Sweep net samples were standardized to 5-minute sampling efforts at each site and generally encompassed a range of substrate types. Depth at sweep net sites varied from 0.5-1 m. Invertebrates were collected in sweep nets, rinsed into Whirl-paks with 70% ethanol, and returned to the lab for identification and enumeration under a dissecting microscope.

Hess samples were collected from each site at a depth of 0.5 m. The sampler was placed 5-10 cm into the lake bottom, and the substrate was agitated and washed into the net by hand. Material on the net was rinsed into Whirl-paks with 70% ethanol. In the lab, Hess samples were separated into >400 μm and 150-400 μm size fractions with a sieve. Individuals from the >400 μm fraction were identified and enumerated under a dissecting microscope without sub-sampling. Material from the 150-400 μm fraction was re-suspended and diluted to 150 ml. Four 5-ml sub-samples were then removed, and individuals were identified and enumerated under a compound microscope at 200x magnification. The >400 μm size fraction was used for abundance and density analysis; the 150-400 μm size fraction was used for presence/absence analysis only.

Specimens of Insecta, Amphipoda, Hirudinea, and Mollusca were identified at least to genus (Pennak 1989, Wiederholm 1989, Ward and Kondratieff 1992, Merritt and Cummins 1996, Wiggins 1997). Copepoda and Cladocera were identified to suborder and family, respectively (Pennak 1989). Taxa of suborder Trombidiformes were all recorded as Hydracarina (water mites) (Pennak 1989). Cnidaria, Nematoda, Nematomorpha, Oligochaeta, Ostracoda, Rotifera, Tardigrada, and Turbellaria were not identified further (Pennak 1989, Thorp and Covich 1991). Relative abundance was estimated for both sweep net and Hess samples. Density (individuals/m^2) was calculated for Hess samples only. Total taxonomic richness was assessed for each study lake and examined for Hess and sweep net samples collectively. Shannon-Wiener taxonomic diversity (Southwood 1978) was also calculated for each study lake, and was examined separately for Hess and sweep net samples.

Statistical Methods

A Pearson product-moment correlation matrix was constructed for environmental variables and community measures using SYSTAT 9.0. P-values were Bonferroni corrected and those ≤ 0.05 were noted. We used Canonical Correspondence Analysis (CCA) to explore environmental gradients and relationships among macroinvertebrates and the environment. Such ordination analyses are useful for finding patterns in community data and revealing which environmental variables are associated with those patterns (ter Braak 1986, Palmer 1993). We used CCA to reveal patterns in taxa distribution, examine relationships between taxa and environmental characteristics, and determine the relative importance of various environmental characteristics. Analyses were performed using CANOCO version 4.0 (ter Braak and Smilaur 1998).

A single environmental data set, consisting of the aforementioned chemical, physical and geographical variables, was used for these analyses (Table 1.1). ANC and conductivity were very highly correlated (r = 0.903) and only ANC was used for the analysis. Ions consistently below detection limit (i.e., phosphate) and ions of unknown ecological importance (i.e., chloride, sodium, potassium) were eliminated from the analysis. Because of variation in scale among measures of environmental variables, all were standardized (mean=0.0, *sd*=1.0) prior to analysis to facilitate comparison.

Six taxa data sets were generated as follows to accomplish our study objectives (Table 1.2). Three sampling methods were analyzed for each of two levels of taxonomic resolution (High = Chironomidae identified to genus, Low = Chironomidae aggregated). Sampling methods included semi-quantitative sweep net sampling, quantitative Hess sampling, and combined sweep net and Hess sampling (qualitative presence/absence).

An initial CCA using the Combined-High data set (both sampling methods, high resolution) was performed to evaluate overarching relationships between invertebrate presence and the various environmental factors using the full extent of our available data. Rare species were downweighted to minimize their influence on the ordination (ter Braak and Smilauer 1998). A forward selection procedure was used in conjunction with 199 permutation tests to select environmental variables for inclusion in the model (ter Braak and Smilauer 1998). This procedure tests the significance of each variable explaining variation in the community composition and functions step-wise, such that the significance of additional information supplied by each variable (given the inclusion of previously selected variables) is tested. Variables with $p>0.1$ were excluded from the model. After selection was completed, an additional Monte Carlo permutation test was

performed to test the significance of the first ordination axis (ter Braak and Smilauer 1998). An ordination plot was generated and included taxa for which >20% of the variation in occurrence was explained by the ordination (ter Braak and Smilauer 1998).

Additional CCA's on each of the other taxa data sets were performed as described above. We compared information gained from these analyses by examining the ranking of important variables, and the total variance in community composition explained by the environmental variables. These comparisons were then used to evaluate how sampling method and taxonomic resolution affected our understanding of the taxa-environment relationship.

RESULTS

Water Chemistry & Environmental Variables

The 22 study lakes varied considerably in physical and chemical characteristics despite their geographical proximity (Table 1.1). Like most Colorado lakes, these were small and high in elevation, ranging from 0.2-16.0 ha in surface area and 3021-3512 m in elevation. Lakes were distributed both above and below treeline. Fish were present in 12 of the 22 study lakes, and were less often present at higher elevations. Water chemistry was variable across the study lakes, although most chemical parameters registered low concentrations, typical of dilute mountain lakes. Phosphate concentrations were below detection in all lakes and pH was generally circumneutral to slightly acidic. Nitrate concentrations were above detection in all but two lakes and surpassed 0.5 mg/L in many lakes.

Significant negative correlations between chemical variables and elevation were common, and positive correlations between ANC, conductivity and most ion

concentrations were noted (Table 1.3). Nitrate was an exception among the measured ions, and was unrelated to either elevation or other chemical parameters. Sampling date, fish presence/absence, and shoreline development (D_L) were not significantly correlated with water chemistry variables or elevation, although fish presence showed weakly positive associations with water chemistry parameters (Table 1.3).

Benthic Invertebrates

A total of 48 invertebrate taxa were identified from all lakes and sampling sites (see Appendix). Five insect orders were represented in the samples, including Coleoptera, Diptera, Ephemeroptera, Hemiptera, and Trichoptera, which were comprised of 4, 17, 2, 2, and 5 genera, respectively. With the exception of dipterans, all insect taxa were collected in sweep net samples only.

The non-insect groups Amphipoda, Hirudinea, and Nematomorpha were also collected only in sweep net samples, and included 2, 1, and 1taxa, respectively. In contrast, taxa of the groups Cladocera, Copepoda, Cnidaria, Rotifera, Tardigrada, and Trombidiformes were collected only in Hess samples. Representatives of Cladocera and Copepoda were comprised of benthic/littoral taxa with representatives of Chydoridae, Harpacticoida, and Cyclopoida (Thorp and Covich 1991); calanoid representatives were likely pelagic incidentals. Sphaeriid molluscs (genus *Sphaerium)*, Nematoda, Oligochaeta, and Ostracoda were found frequently in both sweep net and Hess samples.

Of the 48 taxa found in the study lakes, none was ubiquitous; however, copepods, nematodes, oligochaetes, and ostracods were present in at least 19 of the 22 study lakes. Seven taxa were present in at least half of the lakes; these included Cyclopoida, Harpacticoida, *Procladius* sp., *Sphaerium* sp., Nematoda, Oligochaeta, and Ostracoda.

On average, 15 taxa (+/- 3 *sd*) were collected from each lake, with the maximum number of taxa collected from the Loch (22 taxa), and the minimum number of taxa collected from Blue Lake (9 taxa) (Table 1.4). Taxonomic richness was unrelated to elevation, fish presence/absence, or other environmental variables (Table 1.3). Shannon-Wiener diversity was variable among the lakes, ranging from 0.26-2.06 for Hess and sweep net samples. Hess samples averaged 1.47 (+/- 0.51 *sd*) and sweep net samples averaged 1.25 (+/- 0.49 *sd*) (Table 1.4). Lakes with high sweep net diversity tended to be fishless with higher nitrate concentrations, while lakes with high Hess diversity tended to be at lower elevations below treeline (Table 1.3).

Density estimates from quantitative Hess samples averaged 5,306 individuals/m^2 and varied by an order of magnitude among the lakes, from 1,151 to 15,735 individuals/m^2. Hess densities showed weakly positive correlations with sampling date and lake nitrate concentrations, and appeared higher in lakes with fish (Table 1.3).

Hess sampling and sweep net sampling yielded distinct faunal elements and different numbers of taxa. Roughly half of the total taxa (45%) were collected with the sweep net alone, compared to a quarter of the total taxa (27%) collected with the Hess sampler alone. Another quarter of the specimens (28%) were found in both Hess and sweep net samples. Hess specimens tended to be either early-instar insect taxa or representatives of taxonomically enigmatic groups like nematodes, oligochaetes, tardigrades, and cnidarians. Sweep net specimens, on the other hand, included more mature insect larvae and large, motile taxa like amphipods and adult insects.

Canonical Correspondence Analysis (CCA)

The CCA of the Combined-High data set (our full model including all identified taxa and all sampling methods) explained 51% of the total variation (TVE) in community structure (Table 1.6), and demonstrated that overarching environmental characteristics (i.e., those that explain the most among-lake variation in the taxa data) were elevation and fish presence/absence (Table 1.5). The first ordination axis was correlated with elevation, ANC, and pH, whereas the second ordination axis was chiefly correlated with fish presence/absence (Table 1.6). Taxa found in many lakes (chydorids and nematodes, for example) were situated centrally on the CCA plot, and were not highly correlated with any measured environmental variable (Figure 1.2). Taxa of the insect groups Coleoptera, Ephemeroptera, Hemiptera, and Trichoptera were associated with the absence of fish and somewhat higher elevations. Genera of the Chironomidae (particularly subfamily Chironominae), on the other hand, were associated with fish presence, low elevations, and high shoreline development, ANC, pH and ion concentrations. With respect to invertebrate fauna, the study lakes were widely distributed across the gradient of elevation and chemistry, and reflected a strong fish/no fish dichotomy (Figure 1.2).

The CCA of the Combined-Low data set was similar to results of the Combined-High CCA and explained a similar amount of the total variation in community structure (52%, Table 1.6). Elevation and fish presence/absence together explained over half of the TVE (Table 1.5). The first ordination axis was correlated with elevation, ANC, pH, and calcium, and the second axis was correlated with fish presence/absence (Table 1.6).

CCA's from the sweep net data sets (Sweep-High and Sweep-Low) explained substantially more variation in community structure (61% and 65%, respectively, Table 1.6) and were not influenced strongly by elevation. Instead, sampling date and water chemistry (ANC, pH) explained the majority of benthic invertebrate variation (Table 1.5). Fish presence/absence was a significant factor only in the Sweep-High analysis, in which chironomids were identified to genus. Ordination axes were, however, correlated with elevation and fish presence/absence as well as sampling date and ANC (Table 1.6).

CCA's of Hess sample data sets (Hess-High and Hess-Low) explained slightly less variation in community structure (47% and 50%, respectively, Table 1.6) and were influenced strongly by elevation and sampling date, which together accounted for most of the TVE (Table 1.5). Ordination axes were correlated with sampling date and water chemistry, as well as elevation and to a lesser extent fish presence/absence (Table 1.6).

TVE values, eigenvalues, and significant environmental variables varied greatly among the sampling methodologies, but not between the two levels of taxonomic resolution. Thus, collection method, but not taxonomic resolution, substantially influenced our assessment of dominant environmental gradients and taxa-environment relationships. Highest TVE values were derived from the sweep net data sets; lowest TVE values were derived from Hess data sets. Elevation, fish presence/absence, and sampling date were the most frequently significant environmental variables from all CCA's; however, water chemistry (particularly ANC) explained substantial variation in sweep net CCA's. Nitrate did not explain significant variation in invertebrate assemblages in any of the CCA's.

many chironomid taxa (Bushnell et al. 1987). All of these factors contribute either directly or indirectly to benthic invertebrate distribution.

Fish are highly selective predators of invertebrates, shaping assemblages in both pelagic (Carpenter and Kitchell 1993, Parker et al. 2001) and benthic zones (Power 1990). Further, effects of fish may extend beyond their direct effects on structure (taxonomic composition) to affect ecosystem function (altered grazing, turnover of primary producers, and nutrient cycling). Fish predation is commonly implicated in the structuring of invertebrate assemblages in mountain lakes (Bradford et al. 1998, Carlisle and Hawkins 1998, McNaught et al. 1999). Direct mechanisms for the influence of fish on taxonomic composition relate to predation as well as size and habits of the invertebrate prey. For example, the relatively small size of chironomids, coupled with their propensity for burrowing and tube building, makes them inconspicuous and may limit fish predation. Studies of the Sierra Nevada and the Uinta mountains showed that smaller-bodied invertebrates like chironomids were abundant where fish were present, whereas larger, more conspicuous invertebrates like hemipterans, trichopterans and ephemeropterans were consistently found in fishless lakes (Bradford et al. 1998, Carlisle and Hawkins 1998). We found that as a group chironomids were associated with fish (Figure 1.2), suggesting better ability to persist in the presence of fish predators. In marked contrast, their non-chironomid insect counterparts were strongly associated with the absence of fish (Figure 1.2).

Nitrate did not significantly affect benthic invertebrate community structure regardless of the data set used in the analysis. This result provides little support for our initial hypothesis that increased algal biomass and altered algal composition in high

nitrate lakes (Baron et al. 2000, Wolfe et al. 2001) would result in altered benthic invertebrate communities. Low nitrate lakes were poorly represented in our study (only Finch Lake and Pear Lake had nitrate levels below detection limit) resulting in a nitrate gradient skewed toward high nitrate lakes and limiting our ability to infer taxa-nitrate relationships from CCA. However, the positive association between density of Hess-sampled organisms and nitrate concentrations found in our study is corroborated by experimental work in arctic and temperate lakes (Hershey et al. 1992, Blumenshine et al. 1997), and may indicate potential for density or biomass measurements in future examinations of N-impacts on mountain lake biota.

Chironomids are frequently noted for their richness and functional diversity (Armitage et al. 1995, Coffman and Ferrington 1996), and in our study were the most taxa-rich group of invertebrates evaluated, exceeding Trichoptera (the next richest group) by 13 taxa. Chironomid genera comprised over a third of the total taxa collected and averaged 25% of the total abundance per lake, reflecting the group's richness globally and its numerical importance locally (Bushnell et al. 1987). In spite of all this, increasing resolution of Chironomids to genus did not enhance our ability to assess dominant environmental gradients and taxa-environment relationships. The fact that 15 of the 18 chironomid taxa collected belonged to either subfamily Orthocladiinae (widespread collector-gatherers and tube-builders in oligotrophic lakes) or subfamily Chironominae (widespread collector-gatherers and burrowers in lake littloral zones) may imply that the chironomid genera encountered in our survey were largely high-elevation generalists with environmental tolerances beyond the ranges found in our study lakes. Additionally, half the chironomid taxa were found in both sweep net and Hess samples, despite differences

in the nature of the sampling devices. This result indicates a lack of specificity in habit and habitat among chironomids in these lakes, and may help explain why increasing taxonomic resolution did not alter the outcome of our analyses.

Choice of sampling methodology places a variety of well-established constraints on the type of substrate sampled, the type of organisms collected, and the type of question addressed. Less clear in the current literature is the manner in which those constraints affect the outcome of a given field study. In our case, comparison of CCA results among data sets from several sampling methods (sweep net vs. Hess sampler vs. both combined) showed that differences in sampling methodology resulted in marked differences in the characterization of invertebrate communities.

We attribute these differences to the nature of the measurement provided by each method. Sweep net sampling measured relative abundance and Hess sampling measured density, whereas their combination allowed only presence/absence assessment. Presence/absence of invertebrate taxa was influenced by persistent environmental factors like elevation and fish presence, whereas relative abundance and density showed a strong effect of seasonal variability. Mountain lake environments are sharply seasonal, marked by long winters under thick ice cover, strong snowmelt hydrology, and brief ice-free seasons (Baron 1992). Mountain lake fauna thus must accomplish important parts of their life histories during the narrow window of opportunity afforded by the milder summer months. Seasonal variation in water chemistry, temperature, and food availability likely resulted in the seasonal changes in invertebrate density and relative abundance exhibited in our data, but did not affect invertebrate presence/absence.

Factors controlling presence/absence of invertebrate taxa are apparently different from those that control abundance and density.

CONCLUSIONS

Several recommendations for future research and management can be generated from this study. First, it is clear that choice of sampling method affected our characterization of benthic invertebrate communities. The types of invertebrates collected, the total amount of variance explained by CCA model, and the dominant environmental gradients all varied substantially by sampling method. Thus, careful consideration of objectives, target taxa, and environmental variability is essential to selecting appropriate sampling methods for biomonitoring. In our study, the highest amount of explained variation resulted from sweep net analyses without high chironomid resolution. By this criterion, sampling by sweep net and lumping the Chironomidae was most effective at discerning important environmental gradients and taxa-environment relationships, and was the least labor-intensive. While more repeatable and quantitative than the sweep net samples, Hess samples were considerably more expensive in terms of field and lab efforts, captured fewer taxa, and captured taxa that were more difficult to identify and less informative with respect to measured environmental variables. Wwe conclude that sweep net samples will yield more information per effort spent (Resh and Jackson 1993) in cases where 1) field and lab methods limit number of Hess samples per lake or degree of taxonomic resolution (thus compromising the Hess advantage of quantifiability); or 2) the main study objective is to obtain a representative sample of invertebrates from a series of lakes.

Secondly, the usefulness of detailed information on chironomids for describing gradients of organic and inorganic pollutants is well-established (Wiederholm 1984); however, in our study, level of resolution of the Chironomidae had little effect on our assessment of taxa-environment relationships. It appears that for general evaluations of mountain lake environmental gradients (where the range in most environmental factors is relatively narrow), it suffices to lump Chironomidae into one group or identify them to subfamily or tribe. Nonetheless, specific inquiries about taxa-environment relationships derived from this initial characterization would doubtless benefit from identification of chironomids to genus or species.

Thirdly, results of our CCA analyses underscore the importance of seasonal variability in environmental assessments using benthic invertebrates. While presence/absence of taxa was not affected by sampling date, density and abundance invariably were. Future mountain lake research addressing questions of density and/or relative abundance must consider seasonal variation regardless of what sampling method is employed or what portion of the invertebrate assemblage is under investigation. Controlling for such variation by confining studies to a particular portion of the ice-free season, repeating studies over the course of the ice-free season, or including sampling date as a covariable may expedite our understanding of other factors influencing invertebrate distribution and density.

Finally, several specific questions relating to two dominant environmental gradients (elevation and fish) may be generated from this work. For example, what factors constrain the distribution of invertebrate taxa at higher vs. lower elevations, and what are the implications of those distribution patterns for ecological functions like

organic matter processing and nutrient cycling? Further, given the strength of the elevation gradient in our study and the likely scenario of anthropogenic climate change in mountainous regions (Houghton et al. 2001), we might also ask what influence a change in treeline elevation would have on littoral substrate quality, light conditions, water chemistry, and ultimately benthic invertebrate distribution. With respect to the effects of introduced fish on lake biota, it is clear from ours and other mountain lake studies that fish presence significantly influences the character of benthic invertebrate assemblages, and that future research into other anthropogenic impacts (such as nitrogen deposition) must be placed against this important backdrop. Examining what factors make fish presence so influential for mountain lake invertebrates (i.e., size or group-specific predation, alteration of water chemistry and nutrient cycling) and what aspects of invertebrate assemblages are influenced most (i.e., taxa richness, density, composition, life history) would provide important information for the management/restoration of Colorado mountain lakes.

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Table 1.1. Sampling date, location, presence (P) or absence (A) of fish, morphometric and chemical characteristics for lakes (n=22) in Rocky Mountain National Park and Indian PeaksWilderness Area, Colorado, sampled for benthic invertebrates and water chemistry during summer 1998. D_L = Shoreline Development Index (a measure of the relative extensiveness of the lake littoral zone), ANC = acid neutralizing capacity, COND = conductivity, sd= standard deviation. Variables included in environmental data set used for all Canonical Correspondence Analyses denoted with (*).

Lake	Lake #	Latitude (dms)	Longitude (dms)	*Sampling Date	*Elevation (m)	*Lake Area (ha)	* D_L	*Fish (P/A)	*pH
Blue	1	400520	1053711	8/9/98	3450	10.4	1.10	A	6.29
Bluebird	2	401129	1053917	8/16/98	3346	8.9	1.30	A	6.39
Coney	3	400701	1053639	8/7/98	3231	3.6	1.01	P	6.73
Embryo	4	401714	1053945	9/26/98	3139	0.2	1.00	A	6.50
Falcon	5	401342	1053927	8/11/98	3371	3.1	1.00	A	6.23
Finch	6	401059	1053534	8/20/98	3021	3	1.01	P	6.77
Glass	7	401653	1053955	9/25/98	3298	1.9	1.09	P	6.40
Isabelle	8	400408	1053706	8/28/98	3313	12.1	1.52	P	6.40
Lion 1	9	401355	1053818	8/12/98	3373	2.3	1.16	A	6.60
Lion 2	10	401416	1053829	8/12/98	3475	1.7	1.00	A	6.58
Loch	11	401732	1053925	9/25/98	3109	5.9	1.25	P	6.30
Long	12	400421	1053530	8/28/98	3207	16	1.52	P	6.64
Mitchell	13	400516	1053547	8/9/98	3267	5.6	1.24	P	6.42
Ouzel	14	401157	1053755	8/17/98	3051	2.6	1.03	P	6.74
Pear	15	401034	1053733	8/29/98	3225	6.7	1.11	A	6.69
Pipit	16	401133	1054007	8/26/98	3479	5.2	1.02	A	6.53
Rainbow 1	17	400044	1053506	8/4/98	3085	1.2	1.00	A	6.70
Rainbow 2	18	400044	1053506	8/4/98	3103	1.2	1.00	P	6.66
Sky Pond	19	401640	1054007	9/25/98	3325	4.5	1.16	P	6.41
Snowbank	20	401425	1053840	8/12/98	3512	3	1.40	A	6.59
Thunder	21	401320	1053849	8/13/98	3225	6.7	1.08	P	6.57
Upper Coney	22	400628	1053730	8/6/98	3335	6.5	1.01	P	6.60
mean					3270	5.1	1.14		6.53
minimum					3021	0.2	1.00		6.23
maximum					3512	16.0	1.52		6.77
sd					144	3.9	0.17		0.16

Table 1.1., continued.

Lake #	*ANC (μeq/L)	COND (μS/cm^2)	*Nitrate (mg/L)	Phosphate (mg/L)	*Sulfate (mg/L)	Chloride (mg/L)	*Calcium (mg/L)	Magnesium (mg/L)	Sodium (mg/L)	Potassium (mg/L)
1	24.70	6.80	0.52	0.00	1.01	0.05	0.88	0.08	0.25	0.06
2	46.40	8.90	0.58	0.00	0.62	0.10	1.01	0.09	0.35	0.09
3	117.70	20.20	0.74	0.00	3.38	0.12	3.34	0.28	0.49	0.15
4	74.80	16.20	0.33	0.00	2.85	0.27	1.87	0.33	0.91	0.17
5	38.10	7.10	0.40	0.00	1.33	0.05	1.09	0.16	0.41	0.07
6	177.20	19.00	0.00	0.00	0.96	0.04	1.71	0.49	2.82	0.09
7	34.50	10.20	1.24	0.00	1.53	0.08	1.21	0.18	0.43	0.11
8	46.70	11.20	0.36	0.00	2.41	0.05	1.52	0.12	0.31	0.10
9	58.40	10.50	0.55	0.00	1.23	0.07	1.14	0.30	0.62	0.08
10	44.90	8.30	0.54	0.00	0.91	0.06	0.90	0.18	0.49	0.07
11	48.50	11.10	0.99	0.00	1.58	0.14	1.33	0.21	0.54	0.17
12	87.40	14.00	0.12	0.00	2.21	0.06	1.82	0.19	0.48	0.14
13	68.10	13.50	0.42	0.00	2.19	0.07	1.98	0.19	0.50	0.10
14	90.90	13.10	0.56	0.00	1.49	0.08	1.76	0.28	0.71	0.11
15	86.20	11.20	0.00	0.00	0.95	0.08	1.51	0.23	0.46	0.10
16	36.30	6.70	0.45	0.00	0.67	0.13	0.76	0.10	0.37	0.11
17	146.70	20.70	0.37	0.00	2.90	0.07	3.21	0.31	0.80	0.26
18	134.70	21.10	0.56	0.00	3.24	0.12	3.34	0.31	0.79	0.32
19	37.40	10.00	1.07	0.00	1.29	0.08	1.02	0.14	0.38	0.12
20	46.80	8.90	0.59	0.00	0.97	0.07	1.04	0.19	0.53	0.08
21	61.70	9.30	0.52	0.00	0.76	0.04	1.14	0.16	0.49	0.06
22	72.70	13.20	0.57	0.00	2.06	0.17	2.20	0.12	0.39	0.15
mean	71.85	12.33	0.52	0.00	1.66	0.09	1.63	0.21	0.61	0.12
minimum	24.70	6.70	0.00	0.00	0.62	0.04	0.76	0.08	0.25	0.06
maximum	177.20	21.10	1.24	0.00	3.38	0.27	3.34	0.49	2.82	0.32
sd	40.39	4.52	0.30	0.00	0.86	0.05	0.78	0.10	0.52	0.06

Table 1.2. Denotations for taxa data sets used in Canonical Correspondence Analysis (CCA). Three combinations of sampling method were combined with two levels of taxonomic resolution to generate six taxa data sets. Effects of sampling methodology and Chironomid resolution on our analysis of dominant environmental gradients and taxa-environment relationships were evaluated by comparing CCA results from these data sets.

	Resolution	
Sampling Method	High (Chironomidae identified)	Low (Chironomidae lumped)
Sweep net (semi-quantitative)	Sweep-High	Sweep-Low
Hess (quantitative)	Hess-High	Hess-Low
Sweep net + Hess (qualitative)	Combined-High	Combined-Low

Table 1.3. Pearson product-moment correlation coefficients for environmental variables and community measures from lakes (n=22) in Rocky Mountain National Park and Indian Peaks Wilderness Area, Colorado, sampled for benthic invertebrates and water chemistry during summer 1998. Bonferroni-corrected p-values < 0.05 are denoted (*). D_L = Shoreline Development Index (a measure of the relative extensiveness of the lake littoral zone), ANC = acid neutralizing capacity, COND = Conductivity, Sweep H' =Shannon-Wiener diversity from sweep net samples, Hess H' = Shannon-Wiener diversity from Hess samples.

	Date	Elev	Area	DL	Fish	pH	ANC	COND	Nitrate	Sulfate
Sampling Date	1.000									
Elevation	-0.152	1.000								
Lake Area	-0.002	0.171	1.000							
DL	0.169	0.196	0.712*	1.000						
Fish	0.175	-0.489	0.198	0.157	1.000					
pH	-0.351	-0.425	-0.237	-0.243	0.139	1.000				
ANC	-0.312	-0.755*	-0.262	-0.300	0.267	0.761	1.000			
COND	-0.168	-0.756*	-0.288	-0.241	0.371	0.625	0.903*	1.000		
Nitrate	0.393	0.194	-0.252	-0.056	0.277	-0.415	-0.455	-0.216	1.000	
Sulfate	-0.065	-0.470	-0.127	-0.058	0.344	0.235	0.482	0.784*	0.006	1.000
Chloride	0.394	-0.186	-0.281	-0.258	-0.042	0.007	0.019	0.263	0.145	0.408
Calcium	-0.328	-0.603	-0.227	-0.251	0.341	0.533	0.765*	0.922*	-0.124	0.876*
Magnesium	-0.029	-0.714*	-0.527	-0.344	0.131	0.637	0.832*	0.747*	-0.338	0.336
Sodium	-0.020	-0.559	-0.308	-0.262	0.171	0.465	0.727*	0.518	-0.408	-0.004
Potassium	-0.017	-0.551	-0.275	-0.212	0.208	0.300	0.558	0.749*	0.064	0.752*
Taxa Richness	0.299	-0.249	-0.031	0.397	-0.052	0.113	0.023	0.046	0.000	0.012
Sweep H'	-0.181	0.148	0.040	-0.090	-0.507	0.138	-0.029	-0.149	-0.314	-0.126
Hess H'	0.264	-0.433	-0.139	0.248	0.215	0.372	0.288	0.372	0.012	0.248
Hess Density	0.512	-0.159	0.196	0.224	0.363	-0.261	-0.215	-0.033	0.422	0.156

Table 1.3., continued.

	Chloride	Calcium	Magnesium	Sodium	Potassium	Taxa Richness	Sweep H'	Hess H'	Hess Density
Sampling Date									
Elevation									
Lake Area									
DL									
Fish									
pH									
ANC									
COND									
Nitrate									
Sulfate									
Chloride	1.000								
Calcium	0.256	1.000							
Magnesium	0.117	0.501	1.000						
Sodium	-0.051	0.199	0.829*	1.000					
Potassium	0.427	0.800*	0.368	0.091	1.000				
Taxa Richness	0.082	-0.038	0.218	-0.011	0.093	1.000			
Sweep H'	-0.035	-0.069	-0.131	-0.154	0.004	0.017	1.000		
Hess H'	0.013	0.208	0.434	0.220	0.114	0.584	-0.249	1.000	
Hess Density	0.028	-0.058	-0.095	-0.179	-0.025	0.293	0.040	0.272	1.000

Table 1.4. Community measures for lakes (n=22) in Rocky Mountain National Park and Indian Peaks Wilderness Area, Colorado, sampled for benthic invertebrates and water chemistry during summer 1998. Taxa richness represents all individuals captured in Sweep net and Hess samples combined. H' = Shannon-Wiener diversity, calculated separately for Sweep net and Hess samples.

Lake	Taxa Richness	H' Sweep	H' Hess
Blue	9	1.55	0.80
Bluebird	15	1.41	1.25
Coney	13	0.76	1.60
Embryo	17	1.39	2.06
Falcon	13	1.83	0.26
Finch	13	0.83	1.62
Glass	15	1.24	1.73
Isabelle	13	0.80	1.72
Lion 1	18	0.91	1.46
Lion 2	15	1.00	1.97
Loch	22	0.33	1.71
Long	20	1.84	1.77
Mitchell	17	0.64	1.76
Ouzel	17	1.99	1.94
Pear	16	1.51	1.54
Pipit	14	1.91	0.41
Rainbow 1	18	1.97	1.98
Rainbow 2	12	0.95	1.15
Sky	13	0.91	1.45
Snowbank	19	1.64	1.79
Thunder	15	0.77	1.68
UpperConey	11	1.29	0.67
mean	15	1.25	1.47
minimum	9	0.33	0.26
maximum	22	1.99	2.06
sd	3	0.49	0.51

Table 1.5. Results of forward selection of environmental variables in six CCA models used to examine taxa-environment relationships among 22 lakes in Rocky Mountain National Park and Indian Peaks Wilderness Area, Colorado. Only those variables with $p<0.1$ are included. % variation = the percentage of the total variation in species data explainable by each variable given the variables already in the model; ANC = acid neutralizing capacity.

Model	Environmental variable	F	p	% variation
Combined-High	elevation	1.779	0.010	16
	fish	1.395	0.060	12
Combined-Low	elevation	2.312	0.005	20
Sweep-High	sampling date	2.232	0.005	17
	ANC	2.373	0.015	16
	fish	1.661	0.055	11
Sweep-Low	sampling date	2.935	0.005	20
	ANC	3.085	0.015	18
	calcium	1.958	0.035	11
	pH	1.477	0.095	8
Hess-High	sampling date	1.952	0.045	19
	elevation	1.629	0.065	15
Hess-Low	sampling date	2.203	0.005	20
	elevation	1.726	0.030	15

Table 1.6. Environmental variables (Var.) and their weighted correlations (Corr.) with each species axis in six canonical correspondence analyses performed on environmental and taxa data sets from a survey of 22 lakes in Rocky Mountain National Park and Indian Peaks Wilderness Area during summer 1998. TVE (%) indicates the percent of the total variation in community structure that is "explained" by all environmental variables; ANC = acid neutralizing capacity.

	Eigenvalues		Axis I		Axis II		
Data set	axis I	axis II	Var.	Corr.	Var.	Corr.	TVE (%)
Combined-High	0.225	0.148	Elevation	0.655	Fish	0.531	51
			ANC	-0.526	No Fish	-0.532	
			pH	-0.450			
			Date	-0.404			
Combined-Low	0.185	0.108	Elevation	-0.755	Fish	0.575	52
			ANC	0.664	No Fish	-0.575	
			pH	0.518			
			Calcium	0.424			
Sweep-High	0.704	0.669	Elevation	0.662	ANC	0.580	61
			Date	-0.609			
			Fish	-0.583			
			No Fish	0.583			
Sweep-Low	0.666	0.658	ANC	0.675	Date	-0.674	65
					Fish	-0.532	
					No Fish	0.532	
					Elevation	0.562	
Hess-High	0.348	0.325	Elevation	0.496	ANC	0.589	47
			Fish	-0.467	Date	-0.578	
			No Fish	0.467	pH	0.548	
					Calcium	0.477	
Hess-Low	0.269	0.237	Date	-0.771	Elevation	0.554	50
			ANC	0.530			
			Calcium	0.526			
			pH	0.500			

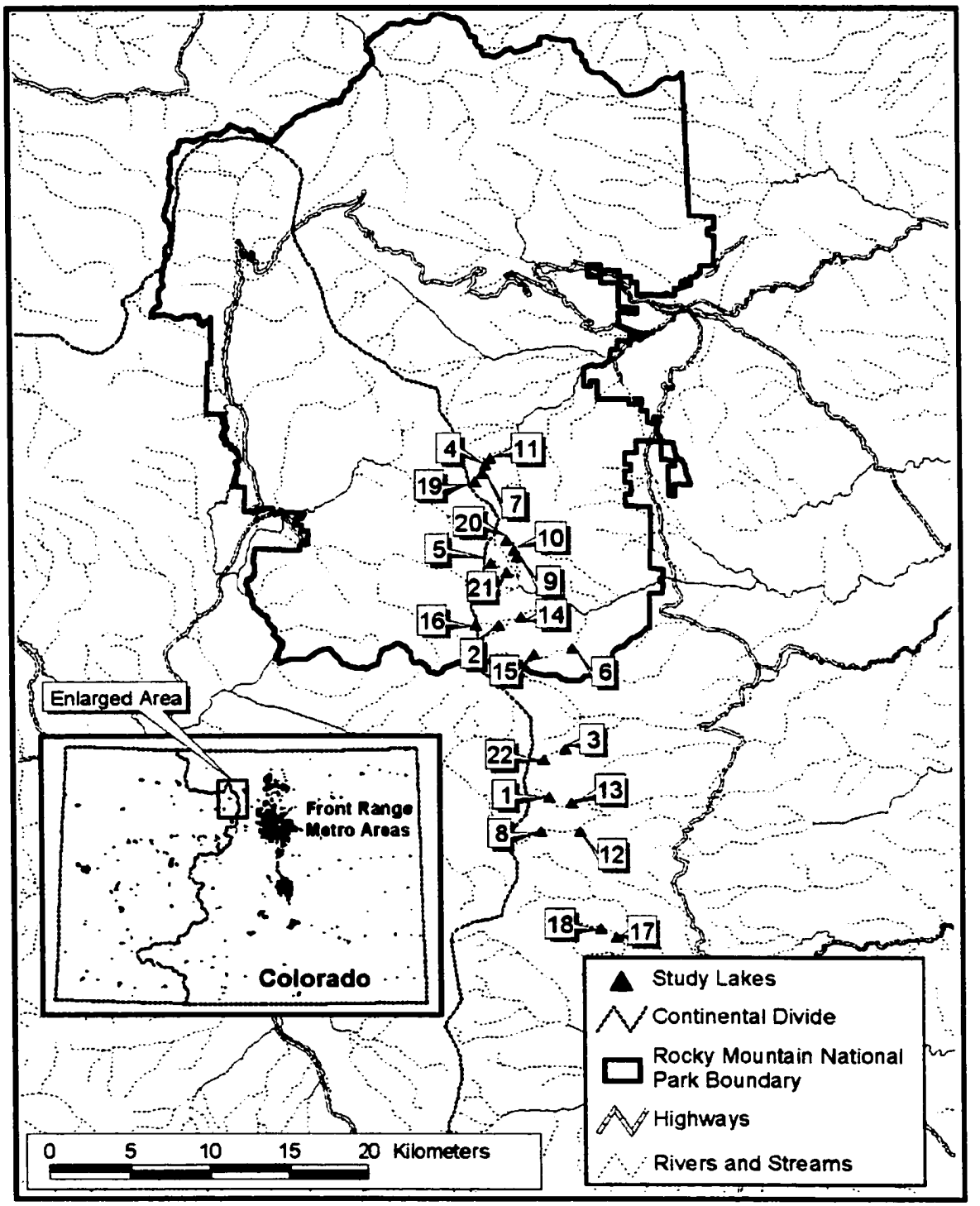

Figure 1.1. Map of study area detailing Rocky Mountain National Park and Indian Peaks Wilderness Area, Colorado, and study lake locations where water chemistry and benthic invertebrate samples were collected during summer 1998.

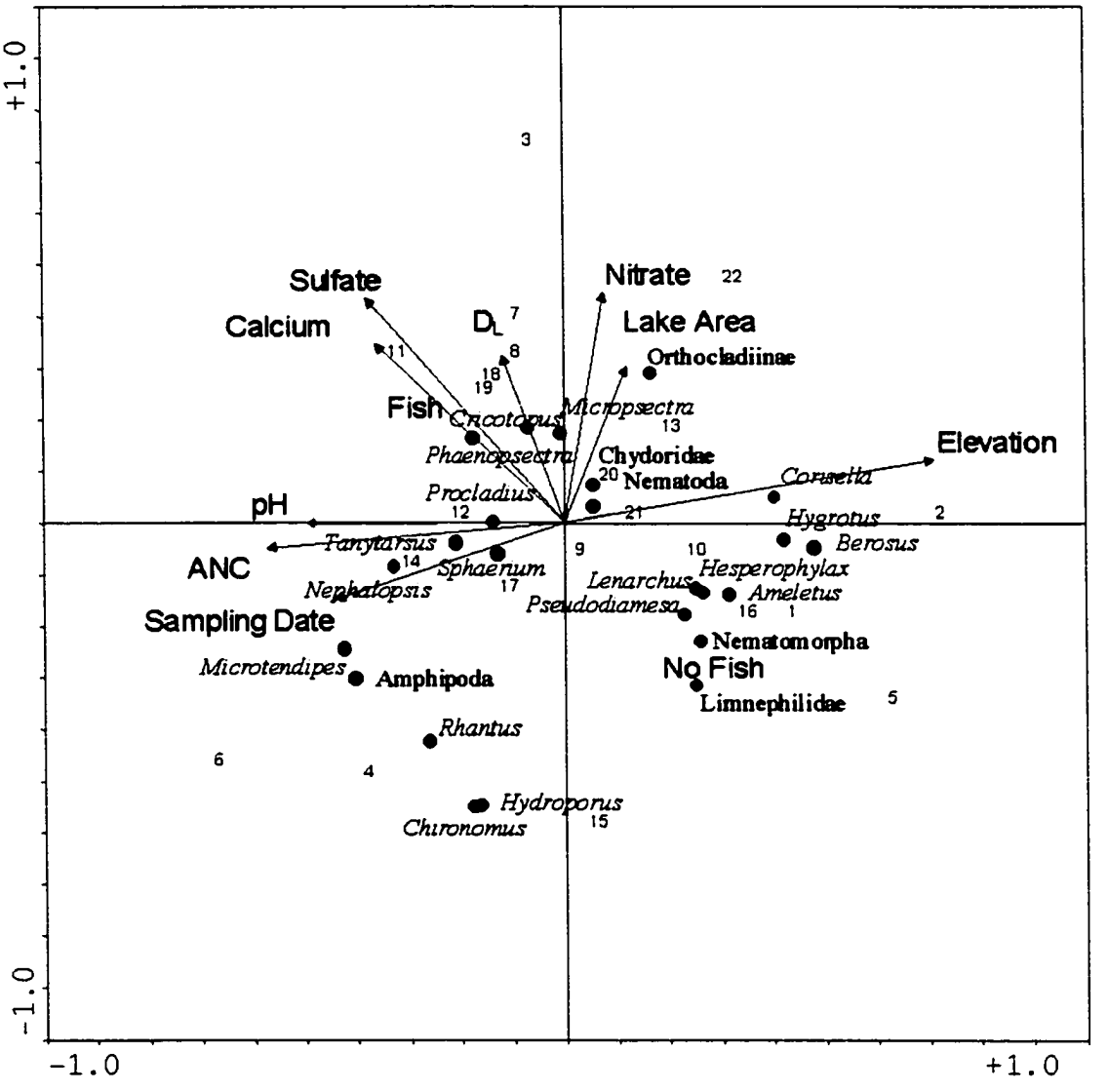

Figure 1.2. Association of benthic invertebrate taxa, study lakes, and environmental variables based on canonical correspondence analysis (CCA) for the Combined-High data set only. Data were generated from a survey of 22 lakes in Rocky Mountain National Park and Indian Peaks Wilderness Area, Colorado, during summer 1998. D_L = Shoreline Development Index, a measure of littoral zone development, ANC = acid neutralizing capacity. Study lakes are denoted by numbers; corresponding names are found in Table 1.1.

Appendix. Taxa sampled during a 1998 survey of lakes in Rocky Mountain National Park and Indian Peaks Wilderness Area, Colorado. Presence of a taxon or group is noted by "S ", if found among study lakes in sweep net samples only, "H" if found in Hess samples only, and "B" if found using both methods. Study lakes are denoted by numbers; corresponding names are listed in Table 1.1.

	Lake Number																					
Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
AMPHIPODA						S											S					
Hyalella azteca						S																
Gammarus lacustris																	S					
COLEOPTERA		S		S	S	S			S	S					S	S						S
Hygrotus sp.					S											S						S
Rhantus sp.				S		S			S						S							
Hydroporus sp.				S											S							
Berosus sp.		S								S												
CLADOCERA (Chydoridae)		H	H		H																	
COPEPODA	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Calanoida				H						H		H								H		
Cyclopoida		H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H
Harpacticoida	H	H	H	H	H		H	H		H	H	H	H	H	H	H	H	H	H	H	H	
CNIDARIA						H																H
DIPTERA	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
Chironomus sp.				S											S							
Corynoneura sp.			S							S		S					S				S	S
Cricotopus sp.			H	H	H						H	H						H	H	H		
Diplocladius sp.												B	B									
Heterotrissocladius sp.		B			B		B	B			B	B	B						B	B	B	
Hydrobaenus sp.							B	B		B	B	B	B	B	B		B		B			
Micropsectra sp.		B	B				B	B			B		B		B		B	B	B			
Microtendipes sp.				H		H	H															
Orthocladiinae		S	S										S						S			

Appendix., continued

Taxa	Lake Number																					
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Pagastia sp.												S		S		S						
Parakiefferiella sp.											H			H						H		
Paratanytarsus sp.				B							B	B	B									
Phaenopsectra sp.			B				B	B	B	B		B					B		B	B		
Polypedilum sp.											H						H					
Procladius sp.			B	B		B	B	B	B	B	B	B		B	B		B	B		B		
Psectrocladius sp.							S				S			S								
Pseudodiamesa sp.	B	B							B			B			B	B						
Tanytarsus sp.				B		B	B		B		B	B		B					B	B	B	
EPHEMEROPTERA	S	S	S		S			S	S	S				S	S	S		S		S	S	
Ameletus sp.	S				S											S				S	S	
Siphlonurus occidentalis		S	S					S	S	S				S	S			S		S		
HEMIPTERA		S				S				S						S						S
Corisella sp.		S								S						S						S
Notonecta sp.						S														S		S
HIRUDINEA (*Nephalopsis obscura*)						S					S	S		S			S					
MOLLUSCA (*Sphaerium* sp).				B		B	B		B		B	B	B	B	B		B	B	B	B	B	B
NEMATODA	B	B	B	B	B		B	B	B	B	B	B	B	B	B	B	B	B	B	B	B	B
NEMATOMORPHA		S		S	S																S	
OLIGOCHAETA		B	B	B	B	B	B		B	B	B	B	B	B	B	B	B	B	B	B	B	B
OSTRACODA	B	B	B	B		B	B	B	B	B	B	B	B	B	B		B	B	B	B	B	
ROTIFERA									H				H									
TARDIGRADA								H					H			H						

Appendix., continued

	Lake Number																				
Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
TRICHOPTERA	S			S	S				S		S	S		S	S	S				S	S	S
Grammotaulius lorettae									S		S											
Hesperophylax sp.	S				S				S							S				S	S	
Lenarchus sp.	S				S				S											S	S	
Onocosmoecus unicolor				S	S							S		S	S							S
Psychoglypha subborealis												S		S	S							S
TROMBIDIFORMES (Hydracarina)				H		H	H			H	H		H	H		H	H			H		
TURBELLARIA	B															B		B				

CHAPTER TWO[2]

Nitrogen Regulation of Algal Biomass, Productivity and Composition in Small Mountain Lakes of the Wyoming Snowy Range

[2] This chapter submitted to Limnology and Oceanography as:

Nydick, K. R., B. Moraska Lafrançois, J. S. Baron and B. M. Johnson. Nitrogen regulation of algal biomass, productivity and composition in small mountain lakes, Snowy Range, Wyoming, USA. Submitted, Limnology and Oceanography, May 2002.

ABSTRACT

Nitrogen may be an important regulator of mountain lake ecology, especially where ambient nitrate levels are low and future increases in atmospheric N deposition are likely. We investigated the effects of increased N inputs, alone and in combination with phosphorus, using enclosure experiments in small mountain lakes of the Snowy Range, Wyoming. Each set of experiments lasted two weeks in the early and late summer, during which we measured a suite of ecosystem and community variables with special attention to algal responses across habitat types (phytoplankton, epilithon, and epipelon). Phytoplankton responded most strongly to N and N + P enrichment, showing increased photosynthetic rate, chlorophyll *a*, particulate carbon (PC), and cell density. N and N+P enrichment also led to more alkaline and light-limited environments, and phytoplankton composition shifted markedly from chrysophytes to cyanophytes, chlorophytes, and diatoms. Epilithon responded to a lesser degree and only in the late summer experiment. Epipelon dominated chl *a* stock, but replicates were highly variable and responses inconsistent. Nutrient enrichment did not alter zooplankton biomass, abundance or composition. The relative increases in photosynthetic rate, chl *a*, and PC suggest that a large degree of photosynthetic carbon gain was lost via respiration or excretion in the dark, and that a portion of phytoplankton chl *a* gain and probably all of the epilithic chl *a* gain was physiological and not a result of increased algal biomass. These results suggest that increased N deposition could substantially alter multiple components of mountain lake ecology.

INTRODUCTION

Alpine watersheds are characterized by steep slopes, sparse vegetation, shallow soils and short growing seasons that limit their capacity to immobilize nutrients and heighten their sensitivity to atmospheric pollutants (Musselman et al. 1996; Williams et al. 1996). This sensitivity has important implications for mountain lakes, many of which lie close to urban and agricultural sources of nitrogen (N) emissions in the western United States. For example, high-elevation sites on the eastern flank of the Colorado Front Range, closer to the Denver-Fort Collins urban corridor, receive mean annual wet deposition of about 3.5 kg inorganic N ha^{-1} y^{-1}, compared with less than half that in more remote locations in Colorado (NADP 1999). Furthermore, N deposition has been increasing at several high-elevation sites in the Colorado Front Range since monitoring began in the early to mid-1980's (Baron et al. 2000).

Even slight increases in N deposition are associated with measurable changes in mountain lake community and ecosystem properties (Jassby et al. 1994; Interlandi and Kilham 1999; Baron et al. 2000). In the Colorado Front Range east of the Continental Divide, forests show signs of N saturation, many lakes have elevated nitrate (NO_3) concentrations, and episodic acidification is reported to occur in some headwaters (Williams et al. 1996; Baron et al. 2000; Williams and Tonnessen 2000). Analysis of sediment cores from two alpine lakes in this region shows that changes in algal community composition occurred by about 1950-1970, coincident with intensified population growth, fertilizer usage, and cattle production in the Colorado Front Range (Wolfe et al. 2001). Diatoms shifted from a typical oligotrophic assemblage to dominance by disturbance species *Asterionella formosa* and *Fragilaria crotonensis*.

Diatom valve concentrations and biovolumes also increased dramatically, suggesting increased productivity.

Despite historical biological responses, however, current high NO_3 conditions likely inhibit further fertilization effects due to N alone. For example, nutrient enrichment experiments in Rocky Mountain National Park (RMNP) lakes with up to 1 mg l^{-1} ambient NO_3 suggest that additional N inputs will alter phytoplankton community composition, but will not increase phytoplankton biomass without commensurate phosphorus (P) inputs (Nydick et al. submitted 2002). Similarly, nutrient ratios of dissolved inorganic N to total P (DIN:TP) in RMNP lakes indicate that limitation by N alone is rare regionally (Sickman 2001).

How continued increases in N inputs will affect present-day mountain lakes depends on a variety of additional factors, including abundance of grazers, lake hydrology, and interactions between benthic and pelagic responses. Trophic structure, particularly the presence/absence of large zooplankton and benthic grazers, may influence the fate of excess N by limiting algal standing stock and altering species composition (Carpenter et al. 1987; Vanni 1987; Cottingham and Schindler 2000) or regulating nutrient supply via recycling (Sterner 1986; Elser et al. 1987). Hydrological differences among lakes also have implications for nutrient cycling. For example, denitrification rates are generally higher in shallow lakes with short hydraulic residence times (Kelly at al. 1987). Additionally, morphometric differences among mountain lakes result in variation in the relative importance of benthic versus pelagic habitat. Differences in species' nutrient requirements and life histories between these habitats (Vinebrooke and Leavitt 1998), as well as competition for nutrients between habitats

(Blumenshine et al. 1997; Hansson 1990), may affect the fate of excess N and its impact on lake ecology.

We used littoral mesocosm enclosures to examine the effects of nutrient additions to shallow mountain lakes with low NO_3 concentrations in order to: 1) explore potential effects of N deposition on low NO_3 lakes, 2) determine the relative impacts of N and P, alone and in combination, on low NO_3 lakes, and 3) investigate competition for nutrients between the benthos and water column. We hypothesized that addition of NO_3 would increase algal biomass and productivity and alter community composition in a manner similar to changes documented in the fossil record since the 1950's (Wolfe et al. 2001), and that N+P additions would amplify those patterns. We measured both phytoplankton and benthic responses to investigate possible shifts in habitat dominance, and predicted that nutrient additions would favor epilithic algae growing on hard substrates (Axler and Reuter 1996; Blumenshine et al. 1997; Vinebrooke and Leavitt 1998).

MATERIALS AND METHODS

Study area

We conducted enclosure (mesocosm) experiments in Shelf Lakes 4 and 5, located adjacent to one another at 3,313 m in the Snowy Range, Medicine Bow National Forest, Wyoming, about 55 km west of Laramie. We selected these lakes because they had very low NO_3 concentrations (unpublished data) and were relatively accessible via hiking trails. The lakes share essentially the same watershed, which drains the NW slope of a quartzite ridge composed chiefly of talus and exposed bedrock, as well as some meadow vegetation and krummholz (Engleman spruce and subalpine fir). Spring snowmelt dominates lake hydrology, with surface water inflow/outflow diminishing throughout the

summer. The ice-free season lasts from early July to mid-October, and cool temperatures, high winds and summer frosts are typical during these months (Musselman 1992). Hikers and anglers rarely visit either lake. No record of fish stocking exists for either lake, but brook trout (*Salvelinus fontinalis*) are present and naturally reproducing in both lakes. Surface areas are 1.2 and 0.4 ha and maximum depths are 3.4 and 1.8 m, for Shelf Lakes 4 and 5, respectively. Littoral zones are extensive and are characterized by large rocks and substantial fine sediment. A fisheries survey documented summer alkalinities at or less than 100 $\mu eq\ l^{-1}$, circumneutral pH's, and Secchi depths reaching the lake bottoms (Snigg 1989).

Experimental Design

We conducted two mesocosm experiments during the summer of 2000. The early summer experiment in Shelf Lake 4 lasted four weeks (11 July – 8 Aug). Because NO_3 and phosphate (PO_4) concentrations fell to near-ambient levels in enriched mesocosms after two weeks during this experiment, we conducted the late summer experiment in Shelf Lake 5 for two weeks only (22 Aug – 5 Sep). We performed the experiments in 500-l cylindrical enclosures, approximately 1.5 m deep. Frames were constructed with vertical PVC tubing and horizontal plastic hula-hoops. PVC was attached on the bottom to a semi-rigid plastic cylinder that extended at least 10 cm into the sediments. Clear, cylindrical polyethylene plastic was fitted tightly around the cylindrical frame and attached to the top hula-hoop. The enclosure extended about 10-20 cm above water level. Plastic tarps formed a skirt around the bottom of the enclosure, and rocks were piled on the tarps to secure the enclosures in place. We documented enclosure effects by

including a "Lake" (La) treatment, consisting of the frame only (i.e. no polyethylene plastic).

Mesocosms were installed in three blocks of five, and treatments were randomly assigned within each block. Care was taken to minimize sediment disturbance during installation and enclosures were allowed to sit for one day (early experiment) and three days (late experiment) prior to taking initial measurements to permit settling of fine sediments. Nitrogen (N) and phosphorus (P) enclosures received a single pulse of approximately 1 mg l^{-1} NO_3-N and 0.1 mg l^{-1} PO_4-P, respectively, at the beginning of the experiment. Combined N and P (NP) mesocosms received both additions in a N:P molar ratio of about 22. Treatments were added to the water surface and mixed carefully with a clean boat paddle. Specific conductance was measured concurrently and mixing was continued until conductivity at 1 m depth reached a steady value.

Sample collection, processing, and analysis

Field measurements, incubations, and sampling were conducted just prior to adding treatments and at regular intervals thereafter (every two weeks and every week for the early and late experiments, respectively). Sampling included epilithic algae, which were colonized on unglazed ceramic tiles (2" x 2") glued to trays. The tile trays had been placed in the lakes one month prior to each experiment. Tile trays were randomly assigned to mesocosms and initial samples were collected prior to lowering trays to the bottom of each enclosure with ropes. Each sampling event lasted two days; pelagic samples (water and plankton) were taken on the first day, followed by benthic samples on the second. Nitrate, PO_4, ammonium (NH_4), and conductivity were also measured immediately after adding treatments. Zooplankton were sampled at the beginning and

end of each experiment. Samples of epipelic algae and epi-plastic algae (growing on mesocosm walls) were collected at the end of each experiment.

Samples were collected and processed using standard Loch Vale Watershed project methods (Allstott et al. 1999). Nitrate, PO_4, and NH_4 were determined colorimetrically with a Perstorp Analytical Alpkem Spectrophotometer (Model 3590) using the cadmium reduction, salicylate, and ascorbic acid methods, respectively. Silica was analyzed colorimetrically with the molybdate method modified by Hach, Inc. DOC was analyzed on an Oceanographics International Model 700 Carbon Analyzer (U.S. Geological Survey, Boulder, CO). Alkalinity was measured by titration (APHA 1995). Temperature, specific conductance, dissolved oxygen (DO) and pH were measured with meters (Orion Model 128, Hach Sension6, VWR Model 8000).

Depth-integrated water samples for phytoplankton analyses were collected by hand-pump. Epilithic algae was scraped from tiles with a razor blade and rinsed with deionized water. One tile was collected per response variable on each sampling date. Samples for both epilithic and phytoplankton chlorophyll *a* analyses were immediately filtered through Whatman GF/C filters. Filters were frozen in dark containers and chl *a* was extracted with methanol (Riemann 1980). Chl *a*, corrected for phaeophytin, was determined with a Sequoia-Turner Model 450 Digital Fluorometer. Particulate C on filters was oxidized and measured with a LI-COR Model LI-6252 CO_2 Analyzer (Lampman et al. 2001). Photosynthetic rate was measured using the ^{14}C light/dark technique (Pregnall 1991). Clear borosilicate bottles suspended at 1m depths were used for phytoplankton incubations. Clear Whirlpaks™ containing lake water and an algae tile were employed to measure epilithic photosynthesis. Incubations lasted four to six hours

after which containers were kept cold and dark in coolers while they were transferred to the lab.

Samples for both phytoplankton and epilithic taxonomic analyses were preserved with Lugol's Iodine in a 1:100 ratio. For phytoplankton taxonomic analyses, samples were allowed to settle in sedimentation chambers (Utermöhl 1958). Several transects were counted under low magnification (150x or 480x) and high magnification (1500x) using a Leitz Diavert inverted microscope with phase contrast and oil immersion. A minimum of 400 cells was enumerated per sample and identified to genus and species where possible. Species richness and diversity (Simpson's *D*, Washington 1984) were calculated for all samples.

For epilithic taxonomic analyses, samples scraped from tiles were sonicated for 1-2 minutes to homogenize contents. Three replicate aliquots were removed from each sample, diluted as necessary and settled in Utermöhl chambers. They were then enumerated as described above for phytoplankton, and identified to Division.

Zooplankton were collected twice from each mesocosm (initial and final sampling dates) by pumping mesocosm water with a bilge pump into a four liter container. Contents of the container were filtered onto 80 μm mesh, rinsed into Whirlpaks™ and preserved in 4% sugared buffered formalin. In the lab, samples were poured through a 40 μm sieve, rinsed into 50 ml plastic centrifuge tubes, diluted to 15 ml with deionized water and shaken to suspend. From this suspended sample, five 1-ml aliquots were placed in Sedgewick-Rafter cells for identification and enumeration. Zooplankton biomass was estimated using length-weight regression (Copepoda and Cladocera) or biovolume

equations (Rotifera), following Bottrell et al. (1976), Dumont et al. (1975), and McCauley (1984).

At the end of each experiment we collected epipelic and epi-plastic algal samples. We sampled epipelic algae from the sediment of each mesocosm by cutting a hole in the plastic enclosure and slowly embedding a plastic petri dish into the sediment. A thin plastic plate was slid under the petri dish. The sample was retrieved and sealed in the petri dish (Vinebrooke and Leavitt 1998). The sample included the top 1.0 cm of epipelon. In the lab, a sub-sample was thoroughly mixed with 100 ml deionized water and 10 ml of this homogeneous mixture was filtered onto a Whatman GF/C filter. Epi-plastic algae growing on the enclosure walls were also sampled. Both filters and plastic strips were analyzed for chl *a* as described above for epilithic algae and phytoplankton.

Statistical Analyses

To facilitate comparisons between the two experiments of different durations, we analyzed results from only the first two weeks of the early experiment. Each experiment was analyzed separately because they occurred in different lakes and at different times. Repeated-measures analyses of variance (RM-ANOVA) were used to test the effects of treatments (excluding "Lake" mesocosms) on response variables that were measured at regular time intervals. If the treatment main effect or the treatment*week interaction was significant at the $\alpha = 0.1$ level, we used a priori comparisons of each treatment with the control (ls means) and set the significance level at $\alpha = 0.05$. If more than one treatment was different than the control, we compared these treatments to each other to determine if a certain treatment had a significantly larger response than the other treatment. An analysis of variance (ANOVA) was used to test for treatment effects on final epipelic and

epi-plastic chl *a*. A Tukey correction for multiple comparisons was used to compare individual treatments. We also used RM-ANOVA's and ANOVA's to identify enclosure effects by comparing response variables between control and "Lake" mesocosms. Data were transformed when necessary to meet the assumptions of normality and homogeneity of variance. Analyses were performed with SAS Version 8e (SAS Institute, 1999-2001).

Statistical analyses of phytoplankton community data were performed using CANOCO version 4 (Ter Braak and Smilauer 1998). Principal response curves (PRC) were used to examine algal community response to experimental manipulations over time (Van Den Brink and Ter Braak 1998, 1999). PRC is a derivation of partial redundancy analysis (RDA) designed to overcome visual limitations of RDA and display major treatment effects on species composition over time. The resulting diagram plots time (week of experiment) on the horizontal axis and treatment effect (expressed as deviation from control) on the vertical axis. Controls (in this case, "Lake" mesocosms) were set to zero and used as the baseline in order to simultaneously compare treatment response curves and examine potential container effects. Individual species scores identify which taxa were most involved in the observed community response patterns. Species abundance data were log-transformed prior to analysis. Significance of the first axis of the PRC diagram was tested by performing 199 Monte Carlo permutations of the mesocosms using an *F*-type, eigenvalue-based test statistic (Van Den Brink and Ter Braak 1999). The null hypothesis predicts no deviation of treatment communities relative to lake controls. Diagrams of second and higher order axes were also constructed and tested to examine residual variance (Van Den Brink and Ter Braak 1999).

RESULTS

Ambient Lake Conditions

Ambient lake conditions were similar for the early experiment in Shelf Lake 4 and the late experiment in Shelf Lake 5 (Table 2.1). Temperature averaged 15.7 °C in the early experiment and 11.4 °C in the late experiment. Both lakes experienced low ambient ion concentrations, and low conductivity and alkalinity. Both had NO_3 concentrations below detection limit and circumneutral pH. Phytoplankton chl *a* concentrations were characteristic of oligotrophic lakes, while TP levels were indicative of slightly mesotrophic systems. Phytoplankton photosynthetic rate during the late experiment was nearly double that of the early experiment, but epilithic photosynthetic rates were comparable.

Enclosure Effects

Conditions in the control mesocosms varied slightly from the "Lake" environments, although differences were orders of magnitude less than treatment responses. For the early summer experiment in Shelf Lake 4, NH_4, PO_4, conductivity, DOC, alkalinity, pelagic PC, and phytoplankton chl *a* and photosynthetic rate were all slightly, yet significantly, greater in the controls than in the "Lake" for at least one of the two sampling dates (Table 2.2). Silica concentration was slightly, yet significantly, lower after two weeks in the control. Temperature was marginally cooler (about 0.1-0.3 °C) in the control during initial sampling. Nitrate, pH, DO, and epilithic tile chl *a* and photosynthetic rate did not differ between controls and the "Lake". Significantly more chlorophytes and fewer cryptophytes were present in control mesocosms after two weeks, but no significant enclosure effects were noted for zooplankton abundance or biomass.

For the late experiment in Shelf Lake 5, temperature was slightly cooler in the controls for all three sampling dates (Table 2.2). DO was significantly greater in the controls for initial conditions only. Control phytoplankton photosynthetic rate and pelagic PC were greater, but the difference with the "Lake" diminished over the course of the experiment. Conductivity was greater in the controls after both weeks one and two. Significantly higher densities of planktonic diatoms and benthic cyanophytes were found in control mesocosms; no enclosure effects were detected for other response variables including zooplankton. Differences were attributed to release of nutrients due to sediment disturbance during installation, lack of mixing, and slight shading by the mesocosm plastic.

Treatments

Initial mean NO_3-N and PO_4-P concentrations were < 5 and 8 $\mu g\ l^{-1}$ respectively, prior to adding treatments. Mean NO_3-N concentrations after adding treatments were 1,041 and 1,090 $\mu g\ l^{-1}$ for N and NP treatments in the early experiment and 999 and 927 $\mu g\ l^{-1}$ for the late experiment. Nitrate-N levels were < 5 $\mu g\ l^{-1}$ after two weeks for the NP treatments, but were 17 and 125 $\mu g\ l^{-1}$ in the N treatments for the early and late experiments, respectively (Table 2.3). Phosphate-P concentrations after adding treatments were 127 and 123 $\mu g\ l^{-1}$ for the P and NP treatments in the early experiments and 96 and 115 $\mu g\ l^{-1}$ in the late experiment. Phosphate was reduced to 17 and 12 $\mu g\ l^{-1}$ P for the NP treatments and 38 and 13 $\mu g\ l^{-1}$ P for the P treatments after two weeks for the early and late experiments, respectively.

Water Quality Responses

Prior to adding nutrient amendments, water quality conditions among the enclosures (excluding "Lake" mesocosms, but see *Enclosure Effects* above) did not differ significantly among treatments for either experiment with the exception of alkalinity at the onset of the late summer experiment. Temperature measurements did not differ significantly among the enclosures during either experiment. Temperature across treatments increased during the early summer experiment, however, and decreased during the late summer experiment (Tables 2.4 and 2.5). Ammonium, silica, and DOC concentrations did not differ among treatments for either experiment. Ammonium increased over time during the early summer experiment, but decreased during the late summer experiment. Silica decreased over time in both experiments while DOC increased in just the early experiment.

Alkalinity and pH in the N and NP treatments were greater than control and P treatments at the end of both experiments, reaching concentrations of about 7 and 9 mg l^{-1} $CaCO_3$ (i.e. pH's above 9; Figure 2.1). As mentioned above, initial alkalinity concentrations differed among treatments for the late experiment, but by the end of the late experiment, the N and NP treatments clearly had gained more alkalinity than both the control and P treatments. DO (percent saturation) did not vary among treatments nor over time during the early experiment. Levels remained at about 90% saturation. DO saturation increased dramatically from about 100 to 115% for the N and NP treatments during the first week of the late experiment. By the end of two weeks, DO saturation had further increased to over 140% in response to the NP amendment, but remained at week one levels in the N mesocosms.

Phytoplankton

Phytoplankton did not differ among enclosed mesocosm treatments prior to either experiment, with the exception of fewer phytoplankton cells in P treatments at the start of the early experiment and larger chl *a*:C ratios in the N and NP treatments prior to the late experiment. N and NP amendments increased total phytoplankton abundance, chl *a*, photosynthetic rate, and PC over controls (Tables 2.4 and 2.5, Figure 2.2) by the end of both experiments. The patterns of change in chl *a*, photosynthetic rate, and pelagic C were very similar, although photosynthetic rate increased more than chl *a*, yielding higher photosynthetic efficiency (C uptake per unit chl *a)* by the end of both experiments. Similarly, chl *a* increased more than pelagic PC, so that chl *a*: C ratios increased (far beyond the differences present at the start of the late experiment). Addition of PO_4 alone did not cause any significant responses.

In the early summer experiment, the N and NP treatments showed similar results, although N responses were greater for some variables. Responses were also similar between N and NP treatments for the first week of the late summer experiment, but chl *a*, photosynthetic rate, and PC continued to increase in the NP treatment and were significantly different than the N treatment after two weeks (similar to the DO response during this experiment). Means, after two weeks during early summer, reached about 3.2 x 10^5 and 1.6 x 10^5 cells ml^{-1} (respectively for N and NP), 11 $\mu g\ l^{-1}$ chl *a*, 400 mg C m^{-3} h^{-1}, and 0.55 and 0.96 mg C l^{-1} (respectively for N and NP). Responses to N and NP treatments reached approximately 2.7 and 3.9 x 10^5 cells ml^{-1}, 7 and 17 $\mu g\ l^{-1}$ chl *a*, 230 and 760 mg C m^{-3} h^{-1}, and 0.73 and 1.06 mg C l^{-1}, respectively, after two weeks during late summer.

Dramatic changes in phytoplankton composition also accompanied N and N+P additions. Phytoplankton in the "Lake", control and P enclosures were relatively sparse and dominated by small chrysophytes throughout both experiments. N and NP treatments, on the other hand, caused declines in chrysophyte abundance and significant increases in chlorophytes, cyanophytes, diatoms, and total cell abundance in both experiments (ls means, $\alpha = 0.05$). Species shifts in N and NP treatments were accompanied by significant declines in species richness in the early experiment and declines in both richness and diversity in the late experiment (ls means, $\alpha = 0.05$).

PRC analysis of the early experiment explained significant variation in phytoplankton composition ($F=6.596$, $p=0.005$) and displayed the strong divergence of phytoplankton in N and NP enclosures from phytoplankton in "Lake", control, and P enclosures (Figure 2.3a). Time (differences between weeks) explained 25% of the total variance in phytoplankton composition (Table 2.6). Treatment regime (time*treatment interaction) explained another 40% of the variance (Table 2.6). Species with high positive weights showed a strong affinity for the patterns displayed in the PRC diagram, indicating that they increased in abundance in N and NP treatments relative to the "Lake". Conversely, negative weights denoted species that decreased in relative abundance in N and NP treatments. Only taxa with weights greater than $|1|$ were included in diagram. Taxa that increased with N and NP enrichment tended to be small colonial Chroococcales (Cyanophyta) and chlorophytes, whereas taxa that diminished in relative importance were mainly chrysophytes and two species of *Monoraphidium* (Figure 2.3a). The second PRC explained a significant amount of the residual variance ($p=0.005$), but explained less of the treatment variance than the first axis (Table 2.6).

PRC analysis for the late experiment explained significant variation in phytoplankton composition ($F=7.900$, $p=0.005$) and, as in the early experiment, displayed a strong divergence of N and NP treatments from "Lake", control and P mesocosms (Figure 2.3b). Time (differences between weeks) explained 21% of the total variance in phytoplankton composition (Table 2.6). Treatment regime (the time*treatment interaction) explained another 36% of the variance (Table 2.6). Taxa negatively associated with the PRC diagram (i.e., those that decreased in abundance in N and NP treatments) were again chrysophytes, notably the initially dominant *Ochromonas* sp. With the exception of *Nitzschia acicularis* and a colonial *Cryptomonas* sp., all taxa that increased with N and NP enrichment were chlorophytes, whereas taxa that diminished most in relative importance were chrysophytes (Figure 2.3b). The second PRC for the late experiment explained a significant amount of the residual variance ($p=0.01$), but explained less of the treatment variance than the first axis (Table 2.6).

Zooplankton

Zooplankton abundance and biomass did not differ among mesocosms at the start of either experiment (Tables 2.4 and 2.5). Additionally, no significant differences in abundance, biomass, richness, or diversity were detected across treatments at the end of either experiment. Instead, time accounted for the majority of changes in zooplankton variables, particularly biomass (Tables 2.4 and 2.5).

Pre-treatment zooplankton assemblages for the early experiment were dominated by copepods, mainly nauplii and *Paracyclops* sp. By week four, zooplankton assemblages in all mesocosms were dominated by *Keratella* sp. Abundance and biomass (particularly of rotifers) increased across all treatments from beginning to end (Table

2.4), and diversity, but not richness, increased ($p < 0.0001$ for all treatments). In the late experiment, pre-treatment zooplankton consisted of a mixed assemblage of copepod nauplii and rotifers (*Polyarthra* sp. and *Keratella* sp.). By week two, overall zooplankton abundance and biomass had decreased across all treatments (Table 2.5), and diversity had increased slightly over pre-treatment samples.

Epilithic Algae

In the early summer experiment, epilithic chl *a* did not vary among treatments nor over time (Table 2.4, Figure 2.4). A slight, yet significant, decrease in photosynthetic rate occurred in the P treatment. This caused a decrease in photosynthetic efficiency for the P amendments. This ratio increased in the N and NP treatments, however. The chl *a*:pheophytin ratio was similar among treatments although it increased significantly over time. PC and community composition were not measured in the early experiment.

After two weeks in the late summer experiment, both N and NP treatments had significantly greater epilithic chl *a* and total cell abundance than the control (Table 2.5, Figure 2.4). Magnitude of the mean NP response was twice as large as the N response, but variability among replicate mesocosms precluded detection of significant difference between N and NP treatments. Photosynthetic rate increased for the NP treatment only during the first week and was significantly different from the control, but this effect was temporary. The chl *a*: phaeophytin ratio was significantly greater in the NP treatment, suggesting more vigorous growth or less senescence. Epilithic PC did not respond to nutrient enrichments, however, yielding greater chl *a*:C ratios in both the N and NP treatments after two weeks.

Epilithic algal taxonomic responses were comparable to phytoplankton at the Division level. Significant increases in cyanophyte and chlorophyte abundance occurred in N and NP treatments (ls means, α=0.05), contributing to the increase in overall epilithic algal abundance in N and NP treatments by week two.

Epipelic Algae

Mean epipelic chl *a* concentrations in the top 1.0 cm of sediment were 150 ± 7.6 and 156 ± 9.1 mg m^{-2} across all mesocosms at the end of the early summer (four weeks) and late summer (two weeks) experiments, respectively (mean ± 1 SE). Variability was substantial, even among replicates, and individual concentrations ranged from 16 to 478 mg m^{-2}. At the end of the early experiment, means were not significantly different among treatments (p=0.7726). At the end of the late experiment, chl *a* was marginally different among treatments (p=0.0569). Means for the N, NP, and "Lake" treatments (246, 213, and 189 mg m^{-2}) were much larger than the control and P treatments (86 and 48 mg m^{-2}), but variability among replicates prevented detection of significant differences among individual treatments.

Epi-plastic Algae

Mean epi-plastic chl *a* concentrations across all mesocosms were 0.58 ± 0.03 and 0.26 ± 0.01 mg m^{-2} at the end of the early and late experiments, respectively (mean ± 1 SE). Individual concentrations ranged from 0.128 to 1.207 mg m^{-2}. We detected significant treatment differences for epi-plastic chl *a* at the end of both the early and late summer experiments (p=0.0026 and 0.0126, respectively). The direction of these differences was opposite, however. In the early trial, both the N and NP treatments were significantly lower than the control (p=0.0101 and 0.0427, respectively), probably

because the plastic strips were sampled at four weeks, after excess nutrients had been consumed and attached algae could have sloughed off. In the late experiment, NP was significantly greater than the control ($p=0.0162$). The N treatment was intermediate; N was not significantly different than the control, but also only marginally different from NP ($p=0.0691$).

Algal Responses Across Habitats

When scaled to mesocosm size (assuming half of the benthos is epipelon and half is epilithon), epipelic chl *a* clearly dominated over phytoplankton, epitlithic algae, and epi-plastic algae in all treatments for both experiments (Figure 2.5a). When we consider algal responses as percent of control at the end of the experiments, however, epipelic algae did not experience much *relative* chl *a* gain from N or N+P enrichment compared to other algal types. After two weeks in the early summer experiment, phytoplankton chl *a* was about 4.5 times greater than the control in both the N and NP treatments, but only a maximum of about two times greater than the control for epilithon, epipelon (after four weeks), and "epi-plaston" (after four weeks; $p=0.0147$; Figure 2.5a). In the late summer experiment, there were negligible differences in relative chl *a* gain among algal types for the N treatment, but both phytoplankton and epilithic algae gained significantly more relative chl *a* than epipelic or epi-plastic algae in the NP treatment ($p=0.0031$; Figure 2.5a). If we consider the photosynthetic rate of treatments compared to control, however, phytoplankton clearly dominated relative gain in C uptake compared to epilithic algae for both experiments ($p=0.0161$ and $p<0.0001$ for early and late experiments, respectively; Figure 2.5b). Furthermore, phytoplankton particulate C at the end of the late summer experiment was two-thirds and two times greater than the control in the N and NP

treatments, respectively, while epilithic PC was similar among all treatments (Figure 2.5c).

DISCUSSION

N limitation of freshwater productivity is now considered more common than previously thought (Elser et al. 1990; Guildford and Hecky 2000). In our study of shallow mountain lakes, we found N to be the nutrient most limiting to algal biomass and productivity in both the early and late summer. P was secondarily limiting during late summer as ambient productivity increased and inflow diminished. Additionally, a survey of 15 lakes in the Snowy Range found that N regulation was common regionally (Lafrançois et al., submitted 2002). N limitation has also been documented by experimental enrichments in lakes in Yellowstone National Park, Wyoming (Interlandi and Kilham 1999), the Sawtooth Mountains, Idaho (Wurtsbaugh et al. 1997), the Sierra Nevada Mountains, California (Axler et al. 1981; Goldman et al.1993), and in montane lakes on the western slope of the Front Range, Colorado (Morris and Lewis 1988). Mountain lakes present harsh environments that may constrain the growth of N-fixing cyanobacteria and increase the occurrence of N limitation (Elser et al. 1990; Reuter and Axler 1992).

We found that although epipelon dominated algal biomass in our mesocosms, phytoplankton responded most consistently to N enrichment. Several other studies have also found that the linkage between water column nutrients and algal biomass was stronger for phytoplankton rather than periphyton (Cattaneo 1987, Hansson 1992, O'Brien et al. 1992). Light limitation, resulting from pronounced increases in phytoplankton biomass, may restrict periphyton response to nutrients (Hansson 1992;

O'Brien et al. 1992). This scenario may have occurred in our mesocosms. N and NP mesocosms were noticeably more turbid than the "Lake", control, and P mesocosms. Tiles on the N and NP substrates were not visible, and the Secchi disk depth would have been less than 1.5 m. Greatly increased productivity also led to very high pH levels and, therefore, low CO_2 concentrations. The dramatic decline in epilithic productivity in the NP treatment after the second week in our late summer experiment could be explained by shading and dissolved inorganic carbon (DIC) limitation (Turner et al. 1994), since phytoplankton biomass reached its peak at this time.

Other studies contrast with our findings and document increased periphytic biomass with nutrient enrichment. Specifically, N + P amendments fertilized epilithon and, to a lesser extent, epipelon, rather than phytoplankton in shallow, alpine Pipit Lake (Vinebrooke and Leavitt 1998). Similarly, accumulation of chl *a* in epi-plastic algae greatly exceeded that of phytoplankton and epipelon for shallow mesocosms in an oligo-mesotrophic lake (Blumenshine et al. 1997). There are several factors that may contribute to contrasting results among studies. First, adsorptive properties of the sediment may differ among lakes, altering transport of nutrients to the benthos. The form of N amendment may also affect its affinity toward the benthos since NH_4, but not NO_3, binds to clay sediments (Horne and Goldman 1994). Our N amendments were as a nitrate salt (KNO_3), so as to mimic N deposition transformed by the terrestrial environment, while NH_4NO_3 or NH_4Cl have typically been used in other studies. Differences in grazer density and composition might also influence pelagic versus benthic responses to nutrient enrichment. Enclosures in our study lakes had low populations of planktonic copepods and rotifers, which are inefficient phytoplankton

grazers compared to *Daphnia*. Copepods and rotifers also predominated in Pipit Lake, however, where phytoplankton biomass did not respond to nutrients (Vinebrooke and Leavitt 1998). Benthic grazers, not sampled in our study, may similarly regulate the magnitude of benthic algal response to nutrient enrichment, but Blumenshine et al. (1997) found that epi-plastic chl *a* increased following nutrient enrichment despite increases in epi-plastic invertebrate density.

Although the factors determining pelagic versus benthic responses to nutrient enrichment are not fully understood, our results as well as others (Blumenshine et al. 1997; Vinebrooke and Leavitt 1998) indicate differences within benthic algal types. Epilithic/epi-plastic algae responded more frequently or to a greater degree than epipelic algae. Epipelon benefit from access to nutrients diffusing upward from the sediment, while algae attached to inert surfaces such as rocks, tiles, and plastic rely on nutrients from the water column or from regeneration within the mat itself (Burkholder 1996; Wetzel 1996). Thus, nutrient availability most likely rarely limits epipelon growth.

Most other mesocosm nutrient experiments did not include multiple indices of algal biomass/productivity. Chl *a* is most often used as an index for algal biomass, but it is well known that factors such as light and physiological cell condition can alter chl *a* concentrations without impacting biomass (Laws and Bannister 1980, Chapra 1997). Particulate C is not a perfect indicator of algal biomass either because it can include non-algal C, although we suspect that in our experiments increased PC following nutrient enrichment was due to accumulation of algal C. By combining indices, our measurements of chl *a*, PC, and photosynthetic rate highlight certain factors not often considered. First, increased chl *a*:C ratios suggest that a portion of the phytoplankton chl

a gain was due to a physiological response to increased nutrients followed by reduced light availability. Epilithic PC did not increase, and it is likely that all of the epilithic chl *a* gain was physiological, and that nutrient enrichment did not cause any actual biomass gain. The increase in epilithic cell density likely reflects the proliferation of small cyanophytes. Second, photosynthetic efficiency (i.e. C uptake per unit chl *a*) was higher for phytoplankton than epilithon. Means ± 1 SE across all mesocosms and all sampling dates were 18.9 ± 0.2 and 11.0 ± 0.2 mg C mg Chl a^{-1} h^{-1}, respectively for phytoplankton and epilithon. Differences in photosynthetic efficiency between benthic and pelagic habitats may reflect gradients within the epilithic mat that reduce DIC, nutrient, and light availability at depth (Lowe 1996). We expect that algal cells lower in the mat would, therefore, be less photosynthetically efficient despite containing living chl a. Thus, chl *a* measurements might be misleading in terms of both biomass gain and ecological function. Thirdly, gains in photosynthetic rate greatly exceeded gains in PC, suggesting that much of the additional C uptake was lost to dark respiration, excretion or grazing. Because zooplankton biomass did not increase, we suspect that, at least for phytoplankton, most of this loss occurred via dark respiration or excretion. Since DOC levels were not altered, it is likely that the microbial component, not measured in this study, was greatly augmented by nutrient enrichment.

Changes in phytoplankton community composition and diversity with nutrient enrichment have been described from nutrient gradient studies across many lakes (Ilmavirta 1990; Watson et al. 1997; Reynolds 1998) as well as experimental nutrient additions. We found that addition of N and N+P to mesocosm enclosures caused a shift from chrysophytes toward assemblages dominated by cyanophytes and/or chlorophytes

and diatoms. These shifts were accompanied by declines in species richness and diversity. Similar responses have followed experimental additions of N and P to whole oligotrophic lakes (Schindler 1975; Findlay and Kasian 1987; Cottingham et al. 1998b) and to mesocosm enclosures in other regions (Paul et al. 1995; Vanni 1987). Increases in chlorophytes and small Chroococcales (Cyanophyta) also followed experimental additions of N to water from several Michigan lakes (Hough and Thompson 1996). In the absence of a strong grazing effect, these division-level shifts may be explained largely by competitive differences among algal divisions. Chrysophytes, the dominant pre-treatment phytoplankton in the above studies and ours, are good competitors in low nutrient, low pH conditions due to their small size and large surface area:volume ratios. Increased N and N+P availability following treatment addition led to replacement of chrysophyte taxa by larger cyanophyte and chlorophyte taxa with generally higher N optima (Reynolds 1984). Increased NO_3 uptake resulted in higher pH levels and, consequently, lower availability of free CO_2. These conditions favored cyanophytes and chlorophytes, which unlike chrysophytes can utilize bicarbonate in photosynthesis (Shapiro 1973, 1990) and thrive in high pH environments (Moss 1973; Levine and Schindler 1999). Increased abundance of diatoms following N enrichment in our experiments has been documented in the fossil record of other mountain lakes (Wolfe et al. 2001) and may relate to the high competitive ability of diatoms at elevated N:P ratios (Tilman et al. 1986). Similarly, paleolimnological studies in various Connecticut lakes showed that *Nitzschia acicularis*, which responded positively to N and N+P in our experiments, was most often found lakes with high total N content (Siver 1999). Overall, phytoplankton community responses to N and N+P in our experiments closely matched

eutrophication responses observed at different spatial and temporal scales and in lakes of different geographic regions. Individual species responses, while somewhat similar in our two experiments, appear to be lake- and region-specific, and remain difficult to predict (Cottingham et al. 1998).

Previous studies have also noted changes in benthic algal composition in response to nutrient enrichment, although these have generally been slight (Peterson et al. 1993; Vinebrooke and Leavitt 1998). Unlike other investigations, which have demonstrated increased density of benthic diatoms (Vinebrooke and Leavitt 1998; Waters et al. unpublished manuscript) with enrichment, we found no evidence of increased diatom density among our treatments. Instead, benthic chlorophytes and cyanophytes responded most to N and NP additions. Although shading by phytoplankton likely limited epilithic biomass in N and NP treatments, it may have also protected chlorophytes and cyanophytes from UV light damage and accounted for their relative increase in those treatments (Vinebrooke and Leavitt 1999).

Zooplankton and phytoplankton biomass are often closely coupled (McCauley and Kalff 1981), and bottom-up effects on zooplankton biomass and density following algal nutrient enrichment have been reported (Vanni 1987; Paul et al. 1995; Cottingham et al. 1997). Nonetheless, zooplankton have also been shown to *not* respond significantly to nutrient enrichment (Brett and Goldman 1997; Vinebrooke and Leavitt 1998), to respond unevenly among taxa (Hansson and Carpenter 1993) or even to respond negatively (Malley et al. 1988), indicating some weakness in the phytoplankton-zooplankton relationship. Factors limiting the ability of zooplankton to respond to increased nutrients and algal biomass include composition and size structure of the

zooplankton community (Bergquist et al. 1985; Cottingham and Schindler 2000; Carpenter et al. 2001), shifts toward poorer quality food items such as cyanophytes and chlorophytes (Brett et al. 2000), chemical stress related to greatly elevated primary productivity (Malley et al. 1988), and phytoplankton responses that are too rapid or large for zooplankton populations to effectively graze (Scheffer et al. 1993). Additionally, slow growing copepods may not have responded due to the limited duration of the experiments. A combination of the above factors likely accounted for the insignificant effect of nutrient enrichment on zooplankton biomass and composition in our lake enclosures. Sparse populations of copepods and rotifers dominated enclosures in both study lakes, rather than Cladocera, whose parthenogenetic reproduction and large body size enable them to respond efficiently to increased algal biomass (Elser and Goldman 1991; Cottingham and Schindler 2000). Likewise, shifts in phytoplankton composition from highly edible chrysophytes toward cyanophytes and chlorophytes, accompanied by pH levels exceeding nine in fertilized mesocosms, may have dampened effects of enrichment on zooplankton biomass. Further, the magnitude of the phytoplankton response (which involved four to eleven-fold increases in algal chl *a*) may have simply exceeded the grazing capacity of sparse zooplankton assemblages in our enclosures.

Enclosure experiments occupy small spatial and temporal scales that complicate generalization to whole lake ecosystems (Carpenter 1996). Our experiments took place in only two lakes and lasted only two weeks, during the relatively warm and calm summer months. If N supply were instead to increase during periods of rapid hydrologic flushing, effects on algal biomass and productivity would likely be reduced due to loss of phytoplankton via lake outlets (Keefer and Pennak 1977; Reynolds 1984), and sloughing

of benthic algal mats (Peterson 1996; Vinebrooke and Leavitt 1996). Further, if nutrient increases were sustained over longer time periods, changes in slower-growing benthic algal mats and responses of invertebrate grazers may become more apparent (Fairchild and Sherman 1992; Hershey 1992).

Similarly, applicability of our results to other mountain lakes depends on inter-lake differences in chemistry, morphometry, and food web structure. Many mountain lakes already have high NO_3 levels due to atmospheric inputs (Baron et al. 2000), and NO_3 addition to these lakes would not be expected to alter algal biomass or composition as it did in our enclosures (Nydick et al., submitted 2002). Further, our study indicates that even primarily N limited lakes may quickly become P limited with continued N additions. Differences in lake morphometry affect the magnitude of benthic versus pelagic responses, and nutrient losses to the hypolimnion of stratified lakes may alter apparent enrichment effects (Proulx et al. 1996). Finally, the presence of fish in our study lakes minimized zooplankton grazing pressure. Other mountain lakes are fishless and dominated by large daphnid grazers, which could place greater constraints on algal nutrient responses.

Despite the limitations of our mesocosm approach, several factors suggest that other low-NO_3 mountain lakes are likely to respond in a manner similar to our enclosures and that the nature of the response may be generally predictable. First, enclosure effects were minimal compared to nutrient enrichment responses. Secondly, N limitation has been documented in other western mountain lakes and is quite common among Snowy Range lakes (Lafancois et al., submitted 2002). Additionally, we found very similar responses to N addition in two mountain lakes and during two different time periods,

demonstrating that strong effects of N addition may override lake-to-lake differences and seasonal variation in water chemistry and community composition. Furthermore, changes in algal biomass and composition following N and N+P additions in our experiments were consistent with P or N+P enrichment studies from geographically diverse regions and whole-lake experiments. We conclude that increased N influx will alter algal productivity and composition in many N limited lakes of the mountain West, and may trigger eutrophication responses both alone and in conjunction with additional P.

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Table 2.1. Ambient lake conditions during the two experiments. Variables are reported as mean ± 1 SE for all measurements taken in "Lake" mesocosms. $n = 9$ for most variables (3 replicates x 3 sampling dates). Epipelic chl *a* is the mean of 3 replicates at the end of each experiment.

VARIABLE	UNIT	EARLY EXPERIMENT SHELF LAKE 4	LATE EXPERIMENT SHELF LAKE 5
Temperature	°C	15.7 ± 0.2	11.4 ± 0.5
Conductivity	$\mu S\ cm^{-1}$	12.5 ± 0.3	14.3 ± 0.6
pH		7.1 ± 0.1	7.1 ± 0.1
Alkalinity	mg l^{-1} as $CaCO_3$	4.35 ± 0.09	5.45 ± 0.16
Dissolved Oxygen	mg l^{-1}	6.88 ± 0.32	8.64 ± 0.09
NO_3-N	$\mu g\ l^{-1}$	≤ 5	≤ 5
NH_4-N	$\mu g\ l^{-1}$	43 ± 21	18 ± 8
PO_4-P	$\mu g\ l^{-1}$	4 ± 1	5 ± 1
TP	$\mu g\ l^{-1}$	20 ± 0.5	26 ± 0.9
Silica	mg l^{-1}	2.85 ± 0.23	2.56 ± 0.15
DOC	mg l^{-1}	2.18 ± 0.09	2.74 ± 0.20
Pelagic Particulate C	mg l^{-1}	0.44 ± 0.03	0.38 ± 0.03
Epilithic (tile) Particulate C	mg m^{-2}	- -	254 ± 39
Phytoplankton Chl a	$\mu g\ l^{-1}$	1.41 ± 0.12	1.87 ± 0.15
Epilithic (tile) Chl a	mg m^{-2}	0.84 ± 0.33	1.32 ± 0.29
Epipelic Chl a	mg m^{-2}	125 ± 76	189 ± 98
Phytoplankton Photosynthetic Rate	mg C $m^{-3}\ h^{-1}$	16.7 ± 2.7	35.6 ± 3.8
Epilithic (tile) Photosynthetic Rate	mg C $m^{-2}\ h^{-1}$	10.2 ± 1.6	9.03 ± 1.98

Table 2.2. Enclosure, time, and enclosure*time effects for chemical and biological variables during early and late summer mesocosm experiments. Differences between controls and un-enclosed "Lake" mesocosms are reported as p-values; values ≤ 0.05 are in bold.

	EARLY EXPERIMENT			LATE EXPERIMENT		
VARIABLE	ENCL	TIME	ENCL* TIME	ENCL	TIME	ENCL* TIME
NH_4-N	0.0884	**0.0055**	0.0884	0.8996	**0.0149**	0.9625
NO_3-N	All below 5 μg l^{-1}			All below 5 μg l^{-1}		
PO_4-P	**0.0200**	0.2787	0.6200	0.3574	0.2063	0.4985
DOC	0.0680	**0.0046**	0.1562	0.4948	0.8872	0.7779
Silica	0.0611	0.0549	0.1309	0.4381	**<0.0001**	0.6375
Alkalinity	0.1078	**0.0106**	**0.0295**	0.7564	**0.0103**	0.1297
pH	0.9509	0.0594	0.9074	0.1431	0.2946	0.1600
DO	0.3532	0.0910	0.8598	0.0734	**<0.0001**	**0.0038**
Temperature	0.0810	**0.0395**	0.6030	**0.0176**	**<0.0001**	0.1626
Conductivity	**0.0292**	**0.0061**	**0.0381**	**0.0301**	**0.0029**	0.0752
Pelagic Particulate C	**<0.0001**	**0.0040**	0.0769	0.2586	**0.0003**	**0.0032**
Phytoplankton Chl *a*	**0.0155**	0.6230	0.1978	0.2285	**0.0125**	0.5220
Phytoplankton Photosynthesis	**0.0063**	**<0.0001**	**0.0179**	**0.0311**	**<0.0001**	0.5561
Pelagic Chl *a*:C	0.5936	0.0587	0.2344	0.1299	0.0908	0.1538
Phytoplankton Photosynthetic Efficiency	0.7177	**0.0009**	**0.0432**	0.1004	0.2399	0.6487
Phytoplankton Cell Abundance	0.5013	**0.0050**	0.5843	0.2088	0.1597	0.1421
Epilithic Particulate C	--	--	--	0.9919	**0.0119**	0.0571
Epilithic (tile) Chl *a*	0.7280	0.5057	0.3376	0.4583	**<0.0001**	0.1121
Epilithic (tile) Photosynthetic Rate	0.4926	**0.0439**	0.1307	0.1600	**<0.0001**	0.9739
Epilithic Chl *a* : C	--	--	--	0.5611	0.0862	0.1105
Epilithic Photosynthetic Efficiency	0.5934	0.3047	0.1045	0.9275	0.1912	0.0829
Epiiithic Cell Abundance	--	--	--	**0.0087**	**0.0348**	0.1868
Zooplankton Abundance	0.1219	0.2381	0.2381	0.6433	0.1868	0.8683
Zooplankton Biomass	0.0969	**<0.0001**	0.0958	0.9202	0.2730	**0.0170**

Table 2.3. Nitrate, ammonium and phosphate concentrations at the start and end of the 14-d period for A) the early summer experiment in Shelf Lake 4, and B) the late summer experiment in Shelf Lake 5. Units are μg l^{-1}. Numbers in brackets show SE (n = 3).

		START of EXPERIMENT		END of EXPERIMENT	
	Treatment	NO_3-N	PO_4-P	NO_3-N	PO_4-P
A)	Lake	5 (0)	5 (0)	5 (0)	2 (0)
	Control	5 (0)	10 (2)	5 (0)	8 (3)
	N	1,041 (71)	11 (3)	17 (9)	8 (3)
	P	5 (0)	127 (2)	5 (0)	38 (10)
	NP	1,090 (17)	123 (3)	5 (0)	17 (7)
B)	Lake	5 (0)	5 (2)	5 (0)	5 (0)
	Control	5 (0)	9 (3)	5 (0)	5 (0)
	N	999 (23)	11 (2)	125 (171)	7 (2)
	P	93 (88)	96 (21)	5 (0)	13 (7)
	NP	927 (145)	115 (7)	5 (0)	12 (2)

Table 2.4. Treatment effects for the early summer experiment in Shelf Lake 4.

RESPONSE VARIABLE	WEEK 0 (lsmeans)	WEEK 2 (lsmeans)	TIME MAIN EFFECT
Temperature	Ø	Ø	↑
NH_4	Ø	Ø	
Silica	Ø	Ø	↓
DOC	Ø	Ø	↑
pH	Ø	N↑ NP↑	↑
Alkalinity	Ø	N↑ NP↑	↑
Dissolved Oxygen (% Saturation)	Ø	Ø	
Phytolankton Cell Abundance	P↓	N↑ > NP↑	↑
Pelagic Particulate C	Ø	N↑ > NP↑	
Phytoplankton Chl *a*	Ø	N↑ NP↑ P↓	↑
Phytoplankton Photosynthesis	Ø	N↑ NP↑	↑
Pelagic Chl *a* : C	Ø	N↑	↑
Phytolankton Photosynthetic Efficiency	Ø	N↑ NP↑	↑
Epilithic Chl *a*	Ø	Ø	
Epilithic Photosynthesis	Ø	P↓	↑
Epilithic Photosynthetic Efficiency	Ø	N↑ NP↑ P↓	
Epilithic Chl *a* : Pheophytin ratio	Ø	Ø	↑
Zooplankton Abundance	Ø	Ø	
Zooplankton Biomass	Ø	Ø	↑
Epipelic Chl *a* (after four weeks)	--	Ø	--
Epi-plastic Chl *a* (after four weeks)	--	N↓ NP↓	--

Ø = no significant differences among treatments (see methods – statistical analyses). For individual week responses, letters indicate which treatment means were different than control (N=nitrate, NP = nitrate + phosphate, P= phosphate). Arrow indicates direction of difference (↑ = greater than control, ↓ = less than control). N↑ > NP↑ indicates that N response was greater than NP response, although both were significantly greater than the control. For time main effect, arrow indicates direction of change (↑ = means increase over time).

Table 2.5. Treatment effects for the late summer experiment in Shelf Lake 5.

RESPONSE VARIABLE	WEEK 0 (lsmeans)	WEEK 1 (lsmeans)	WEEK 2 (lsmeans)	TIME MAIN EFFECT
Temperature	Ø	Ø	Ø	↓
NH_4	Ø	Ø	Ø	↓
Silica	Ø	Ø	Ø	↓
DOC	Ø	Ø	Ø	
pH	Ø	N↑ NP↑	N↑ < NP↑	↑
Alkalinity	N↑ NP↑ P↑	N↑ NP↑	N↑ NP↑	↑
Dissolved Oxygen (% Saturation)	Ø	N↑ NP↑	N↑ < NP↑	↑
Phytoplankton Cell Abundance	Ø	Ø	N↑ < NP↑	↑
Pelagic Particulate C	Ø	N↑ NP↑ P↑	N↑ < NP↑	↑
Phytoplankton Chl *a*	Ø	N↑ NP↑	N↑ < NP↑	↑
Phytoplankton Photosynthesis	Ø	N↑ NP↑	N↑ < NP↑	↑
Pelagic Chl *a* : C	N↑ NP↑	N↑ NP↑	N↑ < NP↑	↑
Phytolankton Photosynthetic Efficiency	Ø	Ø	N↑ NP↑	↑
Epilithic Cell Abundance	Ø	P↓	N↑ < NP↑	↑
Epilithic Particulate C	Ø	Ø	Ø	↓
Epilithic Chl *a*	Ø	NP↑	N↑ NP↑	↑
Epilithic Photosynthesis	Ø	NP↑	Ø	↓
Epilithic Chl *a* : C	Ø	Ø	N↑ < NP↑	
Epilithic Photosynthetic Efficiency	Ø	Ø	Ø	↓
Epilithic Chl *a* : Pheophytin ratio	Ø	NP↑	NP↑	↑
Zooplankton Abundance	Ø	Ø	Ø	
Zooplankton Biomass	Ø	Ø	Ø	↓
Epipelic Chl *a*	--	--	Ø	
Epi-plastic Chl *a*	--	--	NP↑	

Ø = no significant differences among treatments (see methods – statistical analyses). For individual week responses, letters indicate which treatment means were different than control (N=nitrate, NP = nitrate + phosphate, P= phosphate). Arrow indicates direction of difference (↑ = greater than control, ↓ = less than control). N↑ < NP↑ indicates that N response was less than NP response, although both were significantly greater than the control. For time main effect, arrow indicates direction of change (↑ = means increase over time).

Table 2.6. Percentages of the total variance attributed to time and treatment regime in Principal Response Curves (PRC) for phytoplankton assemblages during the early and late experiments. The treatment component includes time x treatment interactions; variance remaining after time and treatment effects are summed is residual. The fraction of treatment variance captured by the first and second PRC's is also noted and tested, with respective p-values in parentheses.

	% Variance accounted for by:		% Variance explained by treatment regime in PRC:	
Experiment	Time	Treatment	First PRC	Second PRC
Early	25	40	47 (p=0.005)	20 (p=0.005)
Late	21	36	46 (p=0.005)	13 (p=0.01)

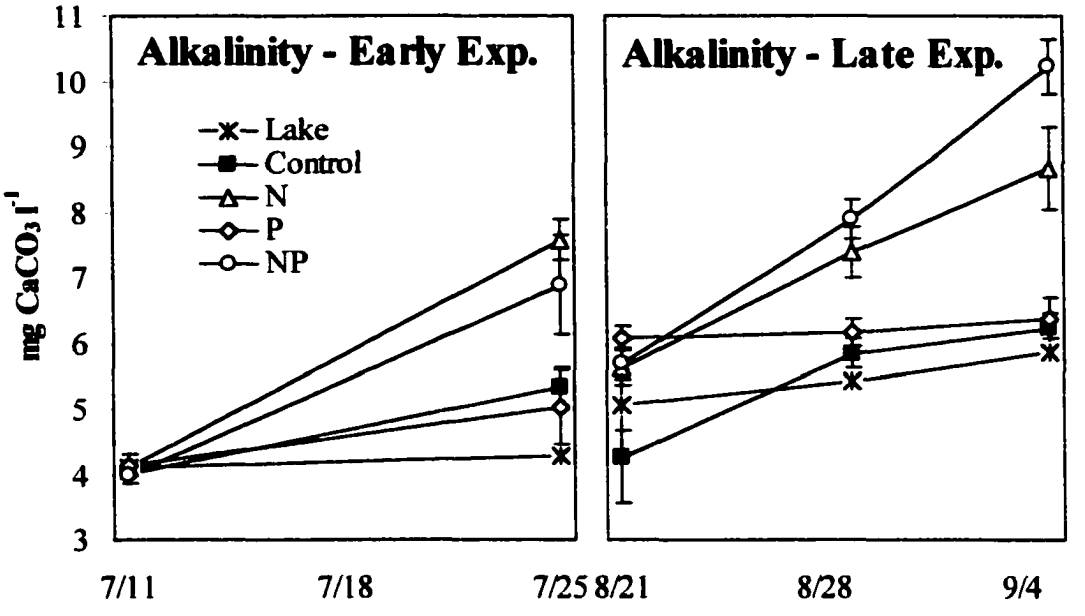


Figure 2.1. Effects of nitrate (N), phosphate (P), and combined nitrate + phosphate (NP) fertilization on alkalinity. Bars indicate ±1 SE (n = 3).

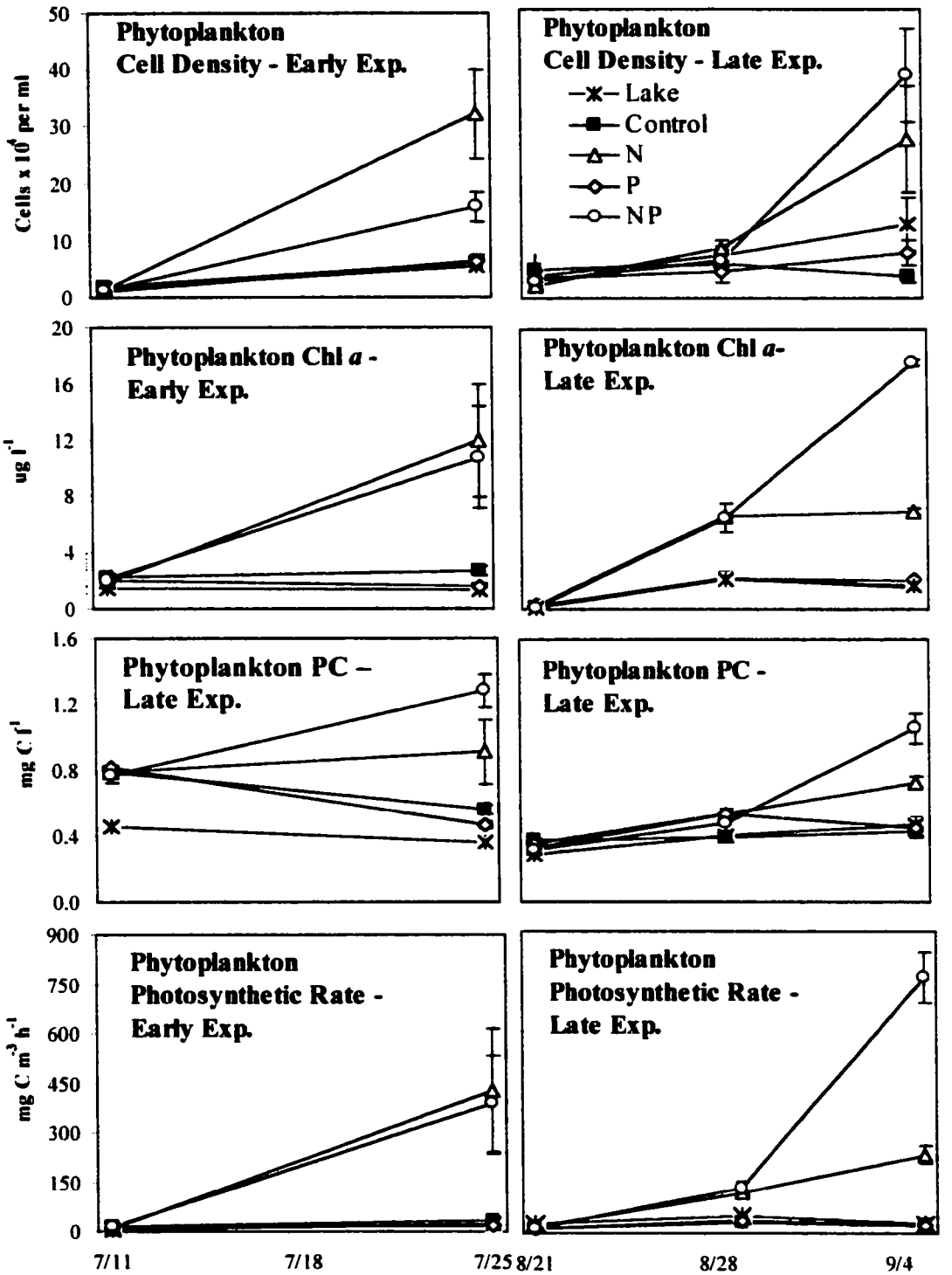


Figure 2.2. Effects of nitrate (N), phosphate (P), and combined nitrate + phosphate (NP) fertilization on phytoplankton cell density, chl *a*, pelagic particulate C, and photosynthetic rate. Bars indicate ±1 SE ($n = 3$).

2.3 (a)

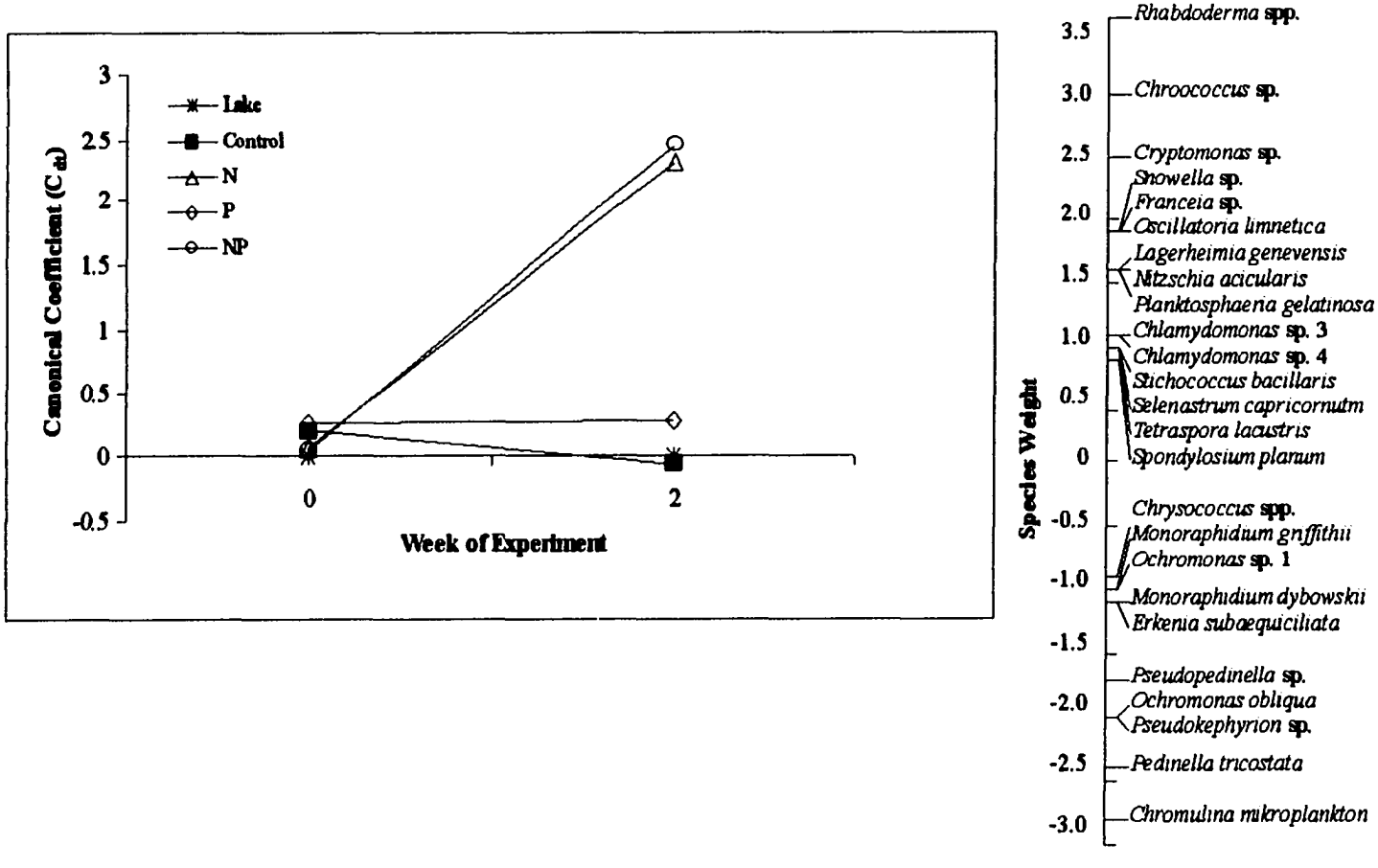
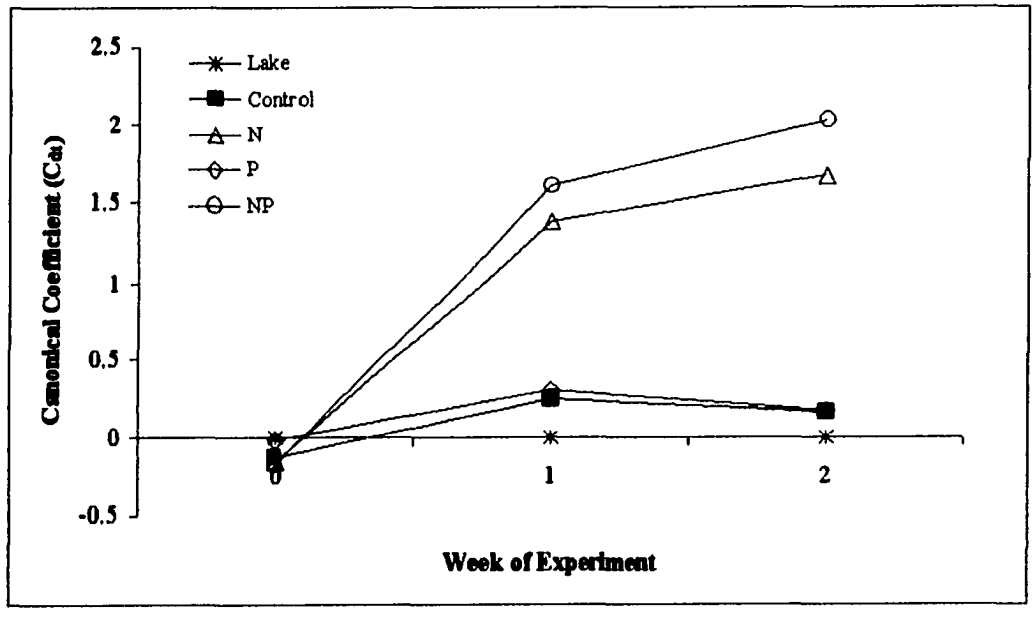
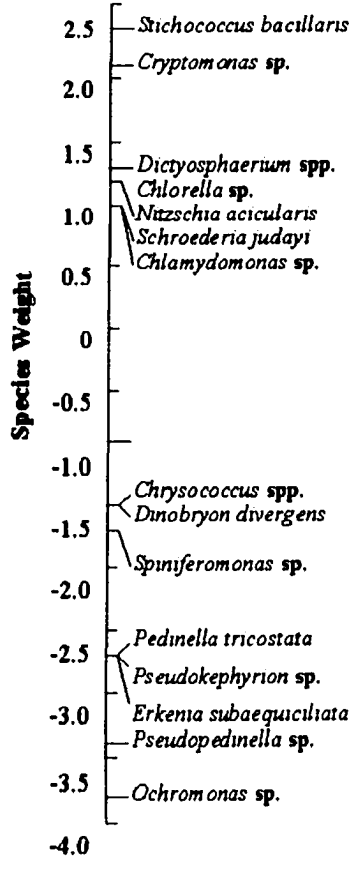


Figure 2.3. First Principal Response Curves (PRC) with species weights for the early experiment (a) and late experiment (b) phytoplankton, showing strong effects of N and N+P additions on phytoplankton composition. Percentages of variance accounted for and significance levels are found in Table 2.6. See text for discussion of species weights.

2.3 (b)





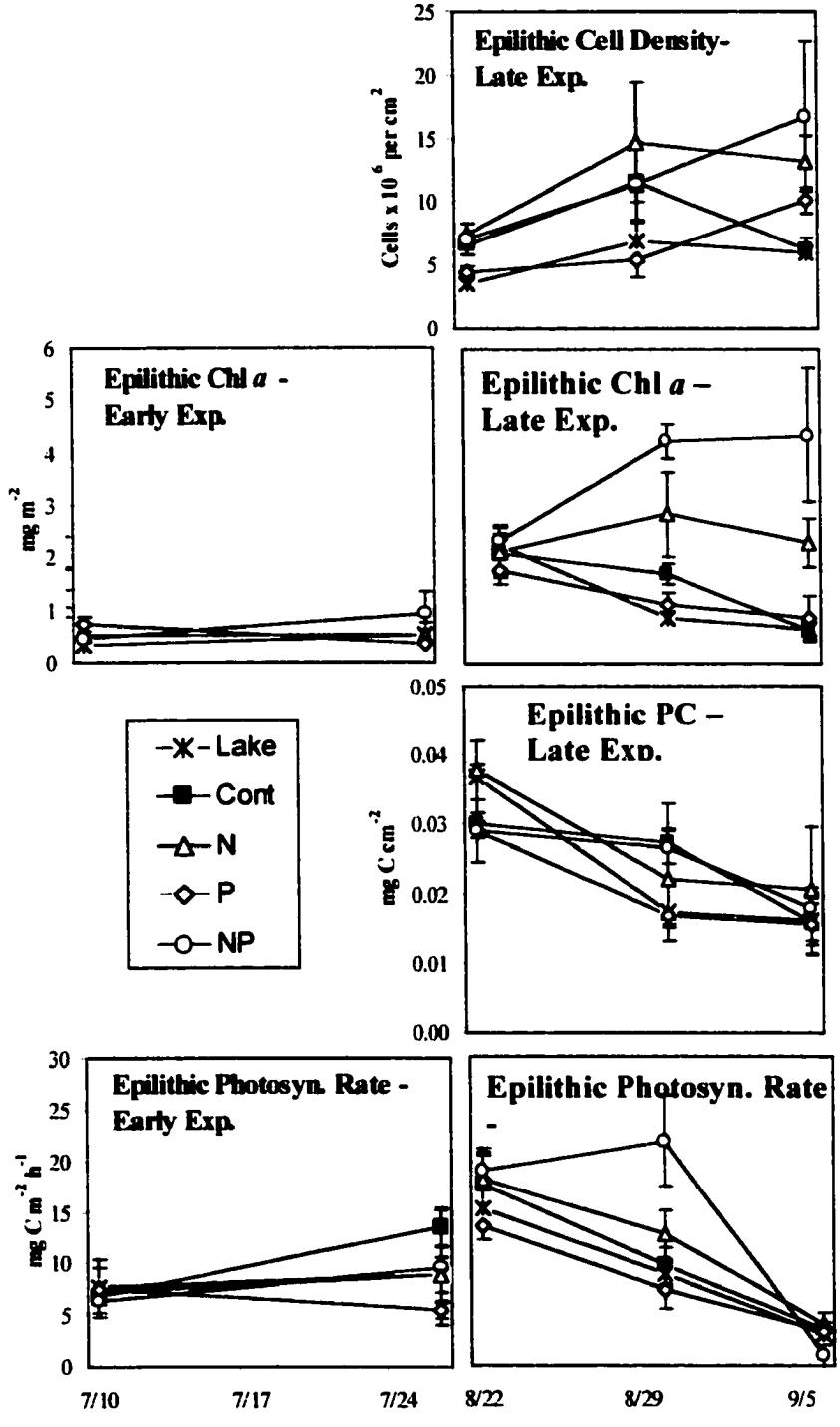


Figure 2.4. Effects of nitrate (N), phosphate (P), and combined nitrate + phosphate (NP) fertilization on epilithic algal cell density, chl *a*, particulate C, and photosynthetic rate. Bars indicate ±1 SE ($n = 3$).

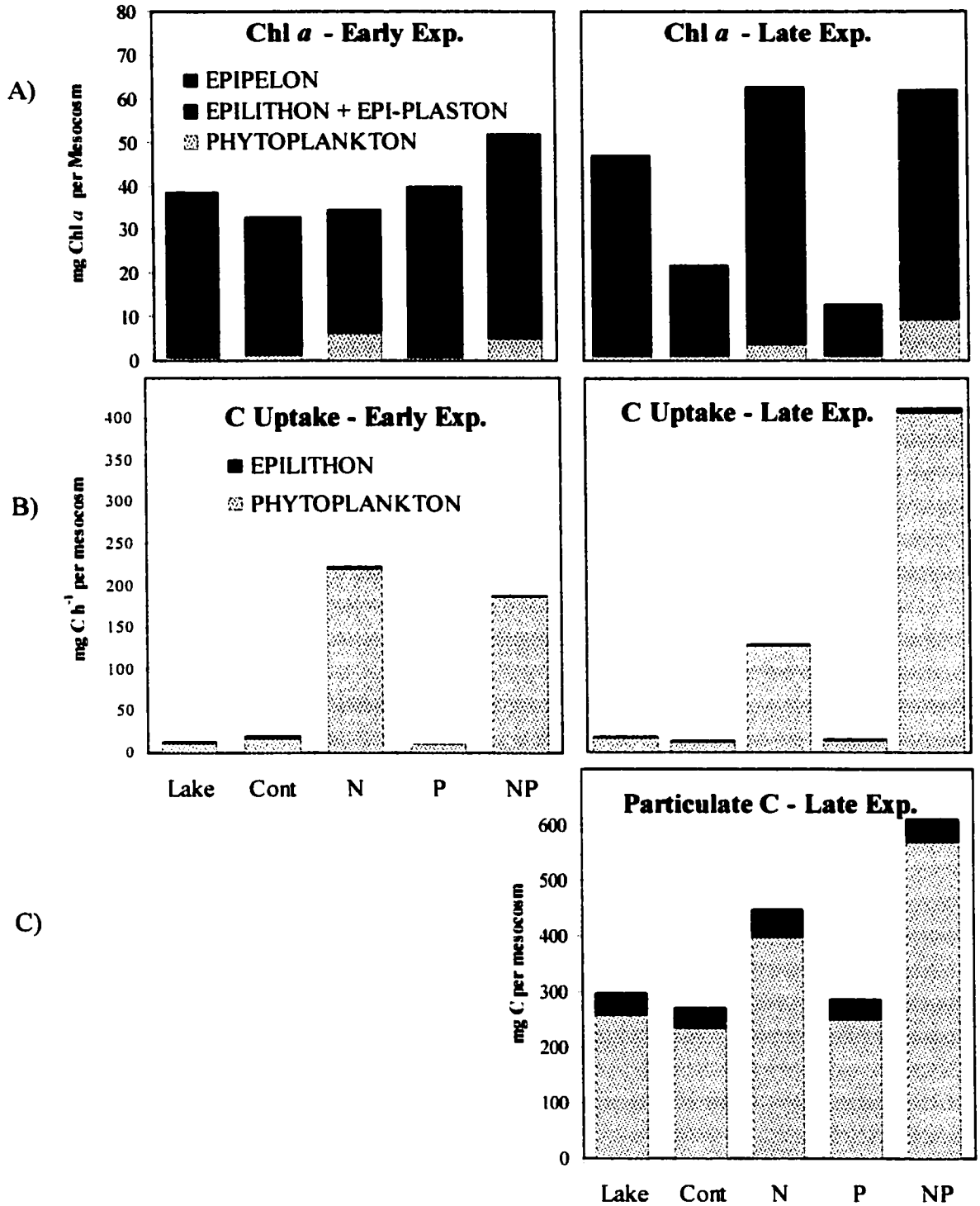


Figure 2.5. Mesocosm-scale budgets of different algal types at the end of the experiments for A) chl *a*, B) photosynthetic rate, and C) particulate carbon.

CHAPTER THREE[3]

Phytoplankton Nitrogen Limitation in Lakes of the Snowy Range (Wyoming,USA): A Comparative Study

[3]This chapter prepared for Journal of Arctic, Antarctic, and Alpine Research:

Lafrançois, B. Moraska, K. R. Nydick, and B. Caruso. Phytoplankton nitrogen limitation in lakes of the Snowy range (Wyoming, USA): a comparative study. Submitted, Journal of Arctic, Antarctic and Alpine Research, September 2002.

ABSTRACT

Nitrogen (N) deposition has been implicated in changes in surface water chemistry and algal composition in several dilute mountain lakes of the western United States. Lakes of the Snowy Range (Medicine Bow National Forest, Wyoming) appear to have low nitrate concentrations currently, and have shown strong eutrophication responses to N or N+phosphorus (P) additions in previous mesocosm experiments. In this study, we explored the regional extent of phytoplankton N limitation by examining nutrient ratios (dissolved inorganic nitrogen: total phosphorus) and phytoplankton species-environment relationships across fifteen Snowy Range lakes. As expected, we found that phytoplankton of Snowy Range lakes were largely N limited or N+P co-limited. Additionally, redundancy analysis (RDA) demonstrated strong relationships between phytoplankton species composition and N gradients, with chrysophyte taxa favored in low-N lakes and cyanophytes and chlorophytes favored in higher-N lakes. These survey results corroborated earlier mesocosm experiments and indicated that future increases in N loading to Snowy Range lakes would likely cause increased phytoplankton biomass and significant alterations in phytoplankton community structure.

INTRODUCTION

Mountain ecosystems are thought to be especially sensitive to atmospheric nitrogen (N) deposition due to steep, sparsely vegetated watersheds, thin soils, short growing seasons, and dilute waters with low acid neutralizing capacity (Eilers et al. 1988, Marchetto et al. 1995, Baron et al. 2000). Nitrogen deposition is currently implicated in high surface water nitrate levels and altered diatom composition in Colorado Front Range lakes (Baron et al. 2000, Wolfe et al. 2001), and in shifts in water clarity and nutrient status of oligotrophic Lake Tahoe (Goldman et al. 1993). The Wyoming Snowy Range receives levels of N deposition similar to the Colorado Front Range (Williams et al. 1996) but lakes in the area generally have low nitrate concentrations (Musselman 1994), suggesting that the capacity for watershed and in-lake N uptake in the Snowy Range has not been exceeded (Aber et al. 1989).

Nutrient addition experiments in two Snowy Range lakes documented significant increases in phytoplankton biomass and productivity as well as marked changes in species composition in response to N or N+phosphorus (P) (Nydick et al., submitted 2002a). Such striking responses indicate that N regulates phytoplankton growth and composition in Snowy Range lakes. N limitation of freshwater ecosystems has received increasing attention in recent years (Elser et al. 1990) and has been noted in other mountain lakes (Axler and Reuter 1996, Interlandi et al. 1999); however, the extent of phytoplankton N limitation among lakes of the Snowy Range is unknown. Additionally, examples of phytoplankton N limitation have rarely been integrated with community ecology, such that relationships between N and phytoplankton composition across lakes are unclear.

We examined nutrient limitation patterns and phytoplankton species-environment relationships in Snowy Range lakes by conducting a lake survey soon after spring ice-out in 2001. Previous sampling efforts in Snowy Range lakes have focused on basic limnological parameters (Secchi depth, water temperature, dissolved oxygen, pH, alkalinity, turbidity, hardness) and physical habitat characteristics for fisheries management (Snigg 1989). Our survey results supplement this data set by incorporating several chemical and biological characteristics that have not been measured previously (including silica concentrations, chemical fractions of N, P and carbon, as well as phytoplankton chlorophyll-*a*, richness, diversity and species composition). Based on results from previous mesocosm experiments (Nydick et al., submitted 2002a, Lafrançois et al., submitted 2002), we predicted that phytoplankton of Snowy Range lakes would be N limited or N+P co-limited. Further, we expected that phytoplankton species composition across lakes would be more strongly related to N than to other chemical characteristics, with chrysophyte taxa favored in low-N lakes and chlorophytes and cyanobacteria more common in higher-N lakes.

METHODS

Study Site

The Snowy Range is a broad quartzite massif situated east of Laramie, Wyoming, in the Medicine Bow National Forest. Catchments are comprised of rocks, krummholz conifers, tundra and wet meadow vegetation (Musselman 1994). Ice cover typically persists from late October through mid-June; annual hydrology is driven by spring snowmelt. We selected study lakes from a set of 99 lakes sampled during a previous Snowy Range fisheries survey (Snigg 1989). In order to focus on high elevation lakes

with low productivity, we eliminated from the selection pool all lakes <3100 m in elevation, and lakes with very high alkalinity (>300 $\mu eq \cdot l^{-1}$) and/or rooted macrophytes. From the remaining pool of 75 lakes, 14 were randomly selected for our survey. Shelf Lake 4, the site of previous nutrient enrichment experiments (Nydick et al., submitted 2002a), was also included in the survey for comparative purposes.

Field Methods and Laboratory Analysis

Chemistry and phytoplankton were sampled once from each lake from July 1 through July 6 in 2001. Measurements of lake physical characteristics (elevation, surface area, mean depth, maximum depth and volume) were derived from Snigg (1989). Epilimnetic water chemistry samples were collected from a depth of 1 m over the deepest part of each lake and processed using standard Loch Vale Watershed project methods (Allstott et al. 1999). Nitrate (NO_3-N), phosphate (PO_4-P) and ammonium (NH_4-N) were determined colorimetrically using a Perstorp Analytical Alpkem Spectrophotometer (Model 3590) using the cadmium reduction, salicylate, and ascorbic acid methods, respectively. Dissolved organic P (DOP) was oxidized to orthophosphate with a potassium persulfate digest and analyzed as described above for PO_4-P. Dissolved organic carbon (DOC) was analyzed on an Oceanographics International Model 700 Carbon Analyzer (U.S. Geological Survey, Boulder, CO). Silica was analyzed colorimetrically with the molybdate method modified by Hach, Inc. Alkalinity was measured by titration (APHA (American Public Health Association) 1995). Temperature, specific conductance and pH were measured with meters (Orion Model 128, VWR Model 8000). Particulate N (PN), particulate P (PP), and particulate C (PC) were collected on pre-combusted filters and oxidized with a potassium persulfate digest

(Lampman et al. 2001). NO_3-N and PO_4-P in the digest were determined colorimetrically as described for water samples, and particulate carbon was measured with a LI-COR Model LI-6252 CO_2 Analyzer.

Phytoplankton nutrient limitation was assessed using the ratio of DIN:TP in ambient water, which estimates N and P available to phytoplankton and has been shown to reliably predict the effects of nutrient enrichment (Morris and Lewis 1988, Axler et al. 1994). DIN:TP ratios ≤ 0.5 indicated N limitation, DIN:TP ratios $\geq$ 4.0 indicated P limitation, and intermediate DIN:TP ratios indicated co-limitation by both N and P (Sickman 2001). The timing of the survey was chosen conservatively; since mountain lake nitrate concentrations generally reach their annual peak during snowmelt (Baron 1992, Lafrançois and Nydick, unpublished data), N limitation is least likely at this time and estimates of N limitation are expected to underestimate the actual occurrence of N limitation in Snowy Range lakes.

Phytoplankton samples were collected once from each lake for analysis of chlorophyll *a* and species composition. Samples for chlorophyll *a* were immediately filtered through Whatman GF/C filters. Filters were frozen in dark containers and chlorophyll was extracted with methanol (Reimann 1980). Chlorophyll *a* corrected for phaeophytin was determined with a Sequoia-Turner Model 450 Digital Fluorometer. Samples for phytoplankton taxonomic analyses were preserved with Lugol's Iodine in a 1:100 ratio and settled in Utermöhl chambers for identification (Utermöhl 1958). Several transects were counted under low magnification (150x or 480x) and high magnification (1500x) using a Leitz Diavert inverted microscope. A minimum of 400 cells were enumerated per sample and identified to genus and species where possible. Species

richness and diversity (Simpson's *D*, (Washington 1984)) were calculated for each sample.

Statistical Analysis

Pearson correlations were calculated for environmental variables using SYSTAT version 9 (SPSS 1999). The resulting matrix was used to identify patterns in the environmental data and evaluate which environmental characteristics were most strongly correlated with DIN:TP ratios (i.e., nutrient limitation). Bonferroni-corrected *p*-values were used to identify statistically significant correlations between environmental variables; correlations $\geq |0.5|$ were used to explore relationships with potential biological significance.

Relationships between phytoplankton composition and environmental variables were assessed using redundancy analysis (RDA, CANOCO version 4), a linear ordination method (ter Braak and Smilauer 1998). The taxonomic data set consisted of phytoplankton cell densities from each lake, identified to species when possible. Phytoplankton <3 μm in greatest linear dimension were eliminated from the analysis due to taxonomic ambiguity. Total densities for each algal Division (Chlorophyta, Cyanophyta, Chrysophyta, Dinophyta, Bacillariophyta, and Cryptophyta) were included as supplementary taxonomic variables (i.e., no influence on the ordination plot), and all taxonomic data were log-transformed to stabilize variance and reduce the influence of dominant taxa on the ordination (ter Braak 1995).

The full environmental data set included physical and water chemistry variables listed in Table 3.1. Species richness, diversity, and chlorophyll *a* were excluded from the environmental data set. Additionally, particulate carbon (PC) and silica were unavailable

for one study lake and could not be used in the RDA. The remaining variables were standardized (mean=0, standard deviation=1) and subjected to a data screening procedure in order to reduce the number of environmental variables and minimize redundancy within the environmental data set (Hall and Smol 1992). This screening procedure identified groups of correlated variables, tested which variable(s) from each group explained significant variation in species composition, and generated a modified environmental data set (see Table 3.1). An RDA using the modified environmental data set was performed using forward selection to identify the variables that best explained phytoplankton species composition. Variables with p-values > 0.1 were excluded from the model. A final RDA was conducted using environmental variables selected during forward selection, and the significance of the first ordination axis was determined using Monte Carlo permutation testing (199 permutations). An ordination triplot was generated, and included taxa for which at least 25% of the variation was explained by the ordination.

RESULTS

Environmental Variables

The study lakes showed considerable variation in physical characteristics but tended to be small and shallow, averaging 6.40±5.16 ha in surface area and 3.7±2.9 m in mean depth (Table 3.1). The lakes were all situated above 3100 m above sea level, and spanned an elevation gradient of <200 m. Elevation was not significantly correlated with any lake physical characteristics (Table 3.2).

Typical of mountain lakes, the survey lakes were cold and relatively dilute. Epilimnetic water temperature averaged 13.8±3.6 °C, and conductivity and alkalinity

ratios ≥ 4.0) (Figure 3.1). Nine lakes were co-limited by both N+P (i.e., had intermediate DIN:TP ratios). DIN:TP was not significantly correlated with other environmental variables (Table 3.2). In general, however, DIN:TP was positively related to both forms of inorganic nitrogen (NH_4-N and NO_3-N), and negatively correlated with temperature and DOC.

Phytoplankton Composition

A total of 96 phytoplankton taxa were encountered in the study lakes (Appendix B). The majority of phytoplankton taxa belonged to the divisions Chlorophyta and Chrysophyta; fewer representatives were identified from Bacillariophyta and Cyanophyta, and very few Cryptophytes or Dinoflagellates were encountered. Species richness and Simpson's *D* averaged 27±8 and 0.75±0.15, respectively, across the study lakes (Table 3.1). Neither measure was significantly correlated with any environmental variable; however, both showed weakly negative relationships with NO_3-N, DIN, and DIN:TP (Table 3.2).

The RDA using forward selection and the screened environmental data yielded two significant environmental variables, PN and NO_3-N (Table 3.4). Together, these two variables accounted for approximately 32% of the variation in the phytoplankton species data (Table 3.4, Figure 3.2). The first RDA axis (λ_1=0.148) was significant (p=0.005), and described a gradient of N concentration (both DIN and PN). Taxa associated with high concentrations of DIN and PN were primarily cyanophytes and chlorophytes, whereas taxa associated with low N concentrations were almost exclusively chrysophytes (Figure 3.2, Table 3.5). The second RDA axis (λ_2=0.108) accounted for slightly less of the variation in species data, and contrasted taxa in high PN lakes with taxa in high DIN

lakes. Taxa associated with high DIN but relatively lower PN included the snow alga *Chlamydomonas nivalis* Wille, the cryptophyte *Chroomonas* sp. Ehrenberg, and a small chrysophyte flagellate. Conversely, taxa associated with low DIN but higher PN included several cryptophytes, a centric diatom and the cyanophyte *Anabaena* sp. Bory (Figure 3.2). N-limited lakes tended to be chrysophyte-cryptophyte dominated (Figure 3.2); P limited and N+P co-limited lakes had less distinct assemblage types.

DISCUSSION

Our analysis of nutrient ratios (DIN:TP) supported experimental findings that phytoplankton biomass is regulated by N or N+P in Snowy Range lakes (Nydick et al., submitted 2002a). We found that one third of the surveyed lakes were primarily N-limited, one lake was primarily P-limited, and the majority of lakes were co-limited by N+P. Other cases of N limitation in western mountain lakes have been reported from Yellowstone National Park, Wyoming (Interlandi and Kilham 1998), the Sawtooth Mountains, Idaho (Wurtsbaugh et al. 1997), the Sierra Nevada Mountains, California (Axler et al. 1981, Goldman et al. 1993), and the western slope of the Colorado Front Range (Morris and Lewis 1988). Subalpine lakes on the eastern slope of the Colorado Front Range, however, do not appear to be N limited (Nydick et al., submitted 2002b). Further, Snowy Range lakes showed a gradient of nutrient limitation from highly N limited to N+P or slightly P limited in our early summer survey.

Variation in nutrient limitation among mountain lakes is commonly linked to regional atmospheric deposition as well as localized watershed characteristics. Deposition of atmospheric N, for example, is implicated in a gradual shift from N limitation toward P limitation in Lake Tahoe (Goldman et al. 1993), and a similar process

may be responsible for the P and N+P limitation found in lakes on the eastern slope of the Colorado Front Range (Nydick et al., submitted 2002b). Variation in N deposition among Snowy Range lakes, however, has never been addressed and the role of N deposition in determining nutrient limitation of these waters is unclear.

Watershed characteristics, particularly slope and vegetation cover, have also been effectively invoked to explain nutrient limitation in mountain lakes. Lakes with steep, rocky watersheds tend to have higher N concentrations and higher proportions of nitrate-N than lakes with gently sloping, vegetated watersheds (Marchetto et al. 1995, Kamenik et al. 2001); such lakes are unlikely to be N limited (Kopáček et al. 2000). Conversely, lakes with vegetated catchments tend to have higher organic carbon and TP concentrations, and are more likely to be N limited (Kopáček et al. 2000). In our study, higher dissolved organic carbon and dissolved P concentrations, typical of lakes with vegetated watersheds, corresponded with low DIN:TP ratios and N limitation, offering indirect evidence that watershed characteristics affected nutrient limitation in our study.

Consistent with our initial hypotheses, RDA analysis indicated that phytoplankton composition was closely associated with N availability across Snowy Range lakes. PN and DIN accounted for more of the variation in species composition than any other environmental characteristic. Further, phytoplankton taxa were distributed predictably across N gradients, with higher relative abundance of chrysophytes occurring in low N conditions and higher relative abundance of chlorophytes and cyanophytes at higher N levels. Such taxa distributions are similar to composition changes noted in N-addition mesocosm experiments in two Snowy Range lakes (Nydick et al., submitted 2002a) and whole-lake fertilization experiments in other areas (Schindler 1975, Findlay and Kasian

1987, Cottingham et al. 1998). These patterns are generally attributed to differences in nutrient physiology; chrysophytes commonly dominate in nutrient-poor conditions due to their small size and large surface area:volume ratios (Sandgren 1988), whereas cyanophytes and chlorophytes have higher N optima and thrive in higher nutrient conditions (Reynolds 1984).

Our analysis also demonstrated a difference in how phytoplankton composition was related to two forms of N (DIN versus PN) across lakes. In general, high DIN was associated with isolated chrysophyte and cryptophyte taxa and the snow alga *Chlamydomonas nivalis* Wille. DIN likely affected phytoplankton composition directly, via N uptake and increased abundance of taxa with high N requirements as described above. Alternatively, high DIN was also associated with low water temperatures and characteristically cold-water algal taxa among our study lakes. The close association between DIN and the snow alga *Chlamydomonas nivalis* Wille, in particular, points toward recent hydrologic inputs from nearby snow and ice fields and suggests that the strong relationship of DIN to phytoplankton composition may be partially attributable to corresponding low water temperatures and early snowmelt stage.

High PN, on the other hand, was associated with a suite of taxa overwhelmingly similar to those that responded positively to nitrate enrichment in previous mesocosm experiments (Nydick et al., submitted 2002a). Since algae are unable to utilize PN as a source of N (Morris and Lewis 1988), the link between phytoplankton composition and PN was most likely indirect. Positive correlations between PN, PP and algal biomass (as chlorophyll *a*) suggest that high availability of DIN prior to our survey increased the accumulation of phytoplankton biomass and shifted in phytoplankton composition toward

chlorophytes and cyanophytes. Similarly, nutrient enrichment/acidification experiments in Snowy Range lakes showed greater algal biomass accumulation, higher PN, and more pronounced species shifts in N-treated mesocosms versus control and P-treated enclosures (Nydick 2002).

In summary, this survey indicated that N and N+P limitation are prevalent among Snowy Range lakes and revealed close relationships between N chemistry and phytoplankton species composition. These findings corroborate results from previous nutrient addition experiments in the Snowy Range and suggest that increases in N deposition would have significant effects on phytoplankton biomass and community structure in many Snowy Range lakes. Further, our experimental and survey work in the Snowy Range has spanned time periods ranging from early to late summer, indicating that phytoplankton N limitation and strong species-N relationships may prevail throughout the ice-free season. Given the predominance of N limitation, the relatively high ambient levels of available P and the potential for strong N+P interactions, future trends in N deposition and watershed N retention in the Snowy Range should be closely monitored.

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Table 3.1. Mean (± 1 standard deviation), minimum and maximum values for environmental variables measured for lakes of the Wyoming Snowy Range (n=15). Variables selected in redundancy analysis (RDA) data screening procedure are denoted (*). Min. = minimum; Max. = maximum.

Variable	Abbreviation	Unit	Mean	Min.	Max.
Elevation *	ELEV	m	328±47	3159	3345
Surface Area *	SA	ha	6.40±5.16	1.15	17.94
Maximum Depth	Dmax	m	9.5±7.8	0.9	25.0
Mean Depth	Dmean	m	3.7±2.9	0.4	9.0
Volume	VOL	$m^3 x10^5$	2.96±4.09	0.11	12.10
Surface Area:Volume	SA:VOL		0.84±1.08	0.11	4.13
Conductivity *	COND	$\mu S \cdot cm^{-1}$	11.33±6.16	6.10	28.50
Alkalinity *	ALK	$\mu eq \cdot l^{-1}$	94.18±59.82	28.25	257.74
pH *	pH		7.40±0.46	6.78	8.35
Temperature *	TEMP	°C	13.8±3.6	5.5	20.4
Dissolved Organic Carbon *	DOC	$mg \cdot l^{-1}$	1.86±1.20	0.50	4.66
Particulate Carbon	PC	$\mu g \cdot l^{-1}$	627±293	189	1212
Silica	Si	$mg \cdot l^{-1}$	1.30±0.85	0.06	2.78
Ammonium*	NH_4-N	$\mu g \cdot l^{-1}$	18±12	3	47
Nitrate-N	NO_3-N	$\mu g \cdot l^{-1}$	11±22	0	84
Dissolved inorganic nitrogen *	DIN	$\mu g \cdot l^{-1}$	29±26	0.003	101
Particulate nitrogen *	PN	$\mu g \cdot l^{-1}$	108±62	36	262
Phosphate-P	PO_4-P	$\mu g \cdot l^{-1}$	4±8	0	28
Dissolved organic phosphorus	DOP	$\mu g \cdot l^{-1}$	8±11	0	45
Total dissolved phosphorus	TDP	$\mu g \cdot l^{-1}$	12±19	1	73
Particulate phosphorus	PP	$\mu g \cdot l^{-1}$	18±10	7	40
Total phosphorus	TP	$\mu g \cdot l^{-1}$	30±20	8	86
DIN:TP *	DIN:TP		1.19±1.11	0.14	4.39
Chlorophyll *a*	CHL-a	$\mu g \cdot l^{-1}$	6.24±5.25	0.89	16.57
Species Richness			27±8	15	41
Simpson's *D*			0.75±0.15	0.37	0.90

Table 3.2. Pearson correlation matrix for all environmental variables measured in lakes (n=15) of the Wyoming Snowy Range during early summer 2001. Bonferroni-corrected p-values < 0.05 are noted (*). See Table 3.1 for variable abbreviations. Rich = Species richness; D = Simpson's D diversity.

	ELEV	SA	Dmax	Dmean	VOL	SAVOL	COND	ALK	pH	TEMP	DOC	PC	Si
ELEV	1.000												
SA	0.257	1.000											
Dmax	0.166	0.683	1.000										
Dmean	-0.108	0.522	0.939	1.000									
VOL	0.242	*0.843	*0.920	0.794	1.000								
SAVOL	-0.058	-0.024	-0.516	-0.535	-0.464	1.000							
COND	-0.765	-0.309	-0.069	0.193	-0.139	-0.235	1.000						
ALK	-0.672	-0.377	-0.074	0.185	-0.185	-0.199	*0.961	1.000					
pH	-0.464	-0.378	0.004	0.223	-0.214	-0.410	0.559	0.519	1.000				
TEMP	-0.430	-0.475	-0.538	-0.374	-0.556	0.096	0.363	0.336	0.243	1.000			
DOC	-0.127	-0.294	-0.563	-0.494	-0.457	0.161	0.065	0.000	-0.013	0.807	1.000		
PC	0.341	0.176	0.323	0.295	0.284	-0.453	-0.055	-0.072	0.073	-0.450	-0.245	1.000	
Si	-0.714	-0.321	-0.330	-0.101	-0.343	0.288	0.425	0.404	0.266	0.380	0.285	-0.603	1.000
NH_4-N	0.374	0.018	-0.255	-0.349	-0.264	0.769	-0.472	-0.336	-0.584	-0.163	0.078	-0.088	-0.041
NO_3-N	0.289	0.414	0.245	0.107	0.440	-0.067	-0.273	-0.311	-0.420	-0.741	-0.444	0.407	-0.136
DIN	0.419	0.365	0.097	-0.065	0.261	0.289	-0.449	-0.421	-0.627	-0.714	-0.348	0.312	-0.136
PN	0.769	0.007	0.055	-0.092	0.015	-0.215	-0.551	-0.468	-0.107	-0.143	0.186	0.574	-0.625
PO_4-P	-0.067	-0.117	-0.524	-0.528	-0.325	0.379	-0.049	-0.064	-0.225	0.530	0.712	-0.546	0.530
DOP	0.005	-0.133	-0.554	-0.579	-0.359	0.348	-0.093	-0.107	-0.216	0.552	0.745	-0.449	0.410
TDP	-0.025	-0.127	-0.544	-0.560	-0.346	0.362	-0.075	-0.090	-0.221	0.546	0.735	-0.491	0.461
PP	0.688	0.394	0.288	0.071	0.402	-0.281	-0.523	-0.528	-0.414	-0.542	-0.163	0.752	-0.707
TP	0.303	0.066	-0.383	-0.502	-0.140	0.213	-0.320	-0.337	-0.408	0.264	0.625	-0.112	0.104
DINTP	0.075	0.257	0.144	0.092	0.091	0.591	-0.292	-0.232	-0.412	-0.577	-0.532	0.005	-0.008
CHL-a	0.618	0.160	0.261	0.126	0.181	-0.281	-0.606	-0.592	0.089	-0.415	-0.083	0.608	-0.495
Rich	0.041	-0.235	-0.321	-0.321	-0.347	-0.019	0.292	0.321	0.419	0.361	0.147	-0.043	-0.266
D	-0.450	-0.400	-0.449	-0.330	-0.564	0.294	0.511	0.522	0.425	0.584	0.299	-0.321	0.157

Table 3.2, continued.

	NH_4-N	NO_3-N	DIN	PN	PO_4-P	DOP	TDP	PP	TP	DINTP	CHL-a	Rich	*D*
ELEV													
SA													
Dmax													
Dmean													
VOL													
SAVOL													
COND													
ALK													
pH													
TEMP													
DOC													
PC													
Si													
NH_4-N	1.000												
NO_3-N	0.065	1.000											
DIN	0.507	0.893	1.000										
PN	0.304	0.027	0.160	1.000									
PO_4-P	0.210	-0.096	0.012	-0.097	1.000								
DOP	0.212	-0.110	0.001	0.026	0.982	1.000							
TDP	0.212	-0.105	0.005	-0.024	0.994	0.997	1.000						
PP	0.141	0.538	0.529	0.672	-0.210	-0.112	-0.153	1.000					
TP	0.270	0.156	0.256	0.296	*0.850	*0.899	*0.883	0.329	1.000				
DINTP	0.633	0.418	0.647	-0.147	-0.316	-0.366	-0.347	-0.008	-0.335	1.000			
CHL-a	0.133	0.213	0.244	0.840	-0.267	-0.171	-0.211	0.666	0.115	0.000	1.000		
Rich	-0.182	-0.528	-0.538	0.106	0.039	0.127	0.091	-0.145	0.018	-0.458	-0.103	1.000	
D	0.020	-0.683	-0.580	-0.201	0.128	0.180	0.160	-0.546	-0.107	-0.166	-0.363	0.720	1.000

Table 3.3. Names and abbreviations for lakes of the Wyoming Snowy Range (n=15) sampled once for chemistry and phytoplankton during early summer 2001.

Lake Abbreviation	Lake Name
CLA	Class
HRT	Heart
LIB	Libby
LTL	Little Telephone
MSFC	Middle South Fork Rock Creek
MUT	Mutt
NGAP	North Gap
RES	Reservoir
SGAP	South Gap
SH1	Shelf 1
SH2	Shelf 2
SH4	Shelf 4
SHP	Sheep
TLN	Twin Lake North
WKL	West Klondike

Table 3.4. Results of forward selection of modified environmental data in a redundancy analysis (RDA) model used to examine taxa-environment relationships among lakes (n=15) in the Wyoming Snowy Range. Only those variables with $p < 0.1$ are included. % variation explained = percentage of the total variation in species data explainable by each variable given the variables already in the model; DIN=dissolved inorganic nitrogen, PN=particulate nitrogen.

Variable Name	F	p	% variation explained
DIN	1.974	0.010	16.4
PN	2.010	0.005	14.9

Table 3.5. Abbreviations and full taxonomic names for phytoplankton taxa and groups included in the redundancy analysis triplot (Figure 3.2) generated from a survey of Wyoming Snowy Range lakes (n=15).

Taxa Abbreviation	Taxa
Anabaena	*Anabaena* sp.
Centric 1	*Indeterminate Centrales 1*
Chlam 5	*Chlamydomonas* sp. 5
Chlam niv	*Chlamydomonas nivalis*
Chromul	*Chromulina* sp.
Chroo min	*Chroococcus minimus*
Chroomon	*Chroomonas* sp.
Chrys par	*Chrysochromulina parva*
Chrys pun	*Chrysococcus punctiformis*
Chryso 1	*Indeterminate chrysophyte 1*
Cryptom	*Cryptomonas* sp.
Erken sub	*Erkenia subaequiciliata*
Komma	*Komma* sp.
Mallamon	*Mallamonas* sp. 3
Mono cont	*Monoraphidium contortum*
Mono grif	*Monoraphidium griffithii*
Ochro 1	*Ochromonas* sp. 1
Oscillat	*Oscillatoria* sp.
Pedinella	*Pedinella* spp.
Pennate	*Indeterminate Pennales 1*
Plank gel	*Planktosphaeria gelatinosa*
Pseudana	*Pseudanabaena* sp.
Schro jud	*Schroederia judayi*
Spinifero	*Spiniferomonas* sp.
Stich bac	*Stichococcus bacillaris*
CHRYSO	Chrysophytes (total)
CYANO	Cyanophytes (total)
CHLORO	Chlorophytes (total)

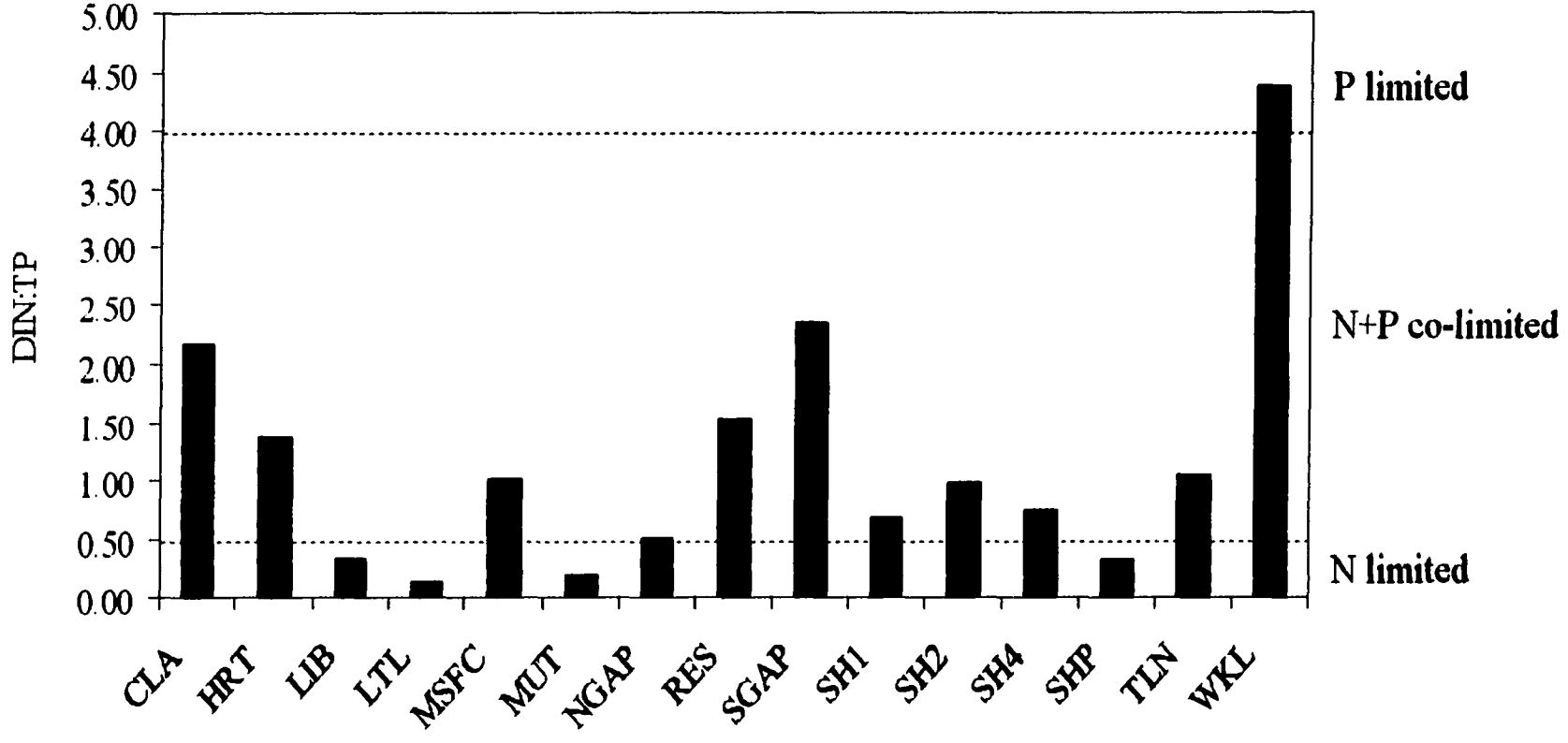

Figure 3.1. DIN:TP ratios measured from the water column of fifteen Wyoming Snowy Range lakes soon after ice-out in spring 2001. Dotted lines delineate nutrient limitation cut-offs (Sickman 2001). Lakes with DIN:TP ratios ≤ 0.5 were primarily N limited, whereas lakes with DIN:TP ratios ≥ 4.0 were primarily P-limited. Lakes with intermediate DIN:TP ratios were considered co-limited by both N and P. Lake abbreviations are found in Table 3.3.

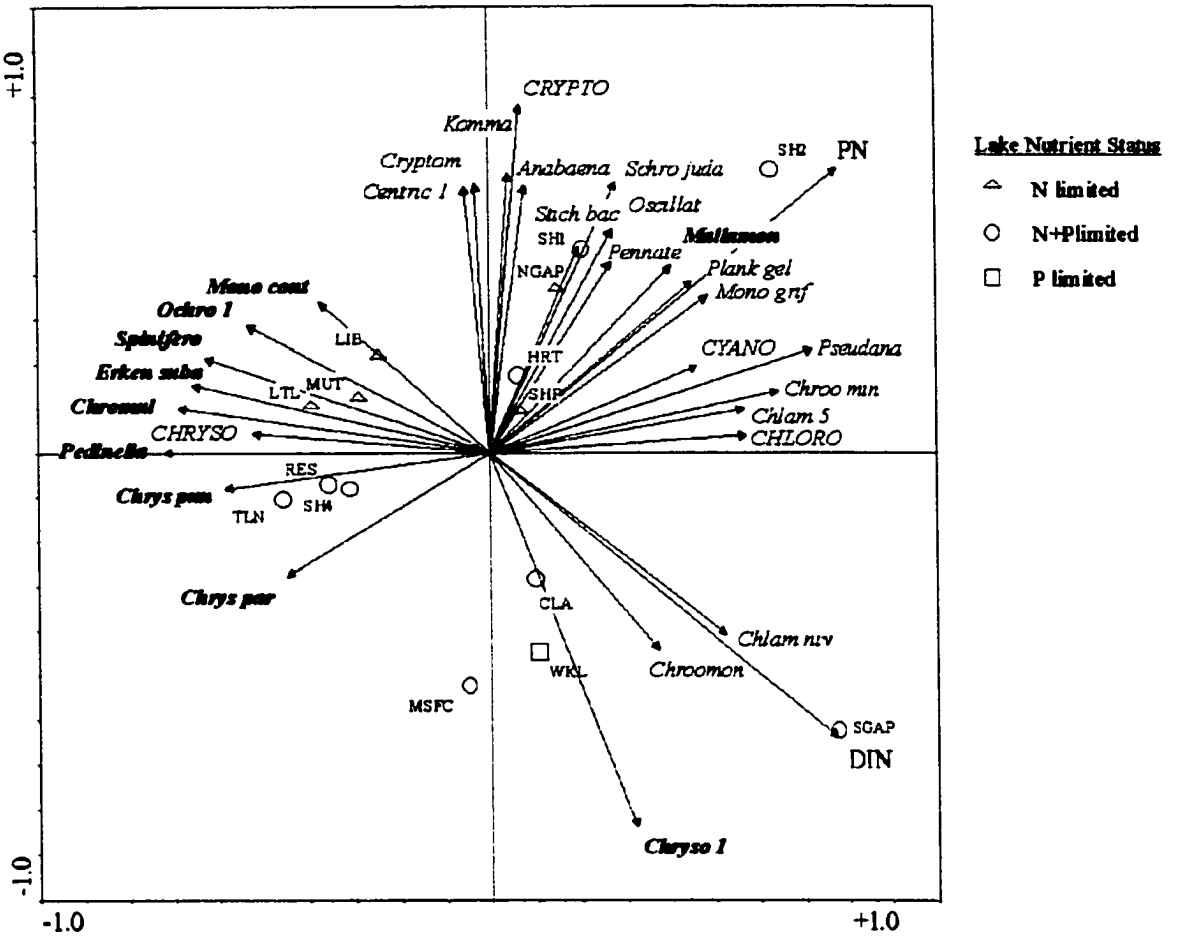

Figure 3.2. Triplot of species, lakes and environmental variables derived from redundancy analysis (RDA) of phytoplankton species data from lakes (n=15) in the Wyoming Snowy Range. Lake and taxa abbreviations are found in Tables 3.3 and 3.5, respectively. Chrysophyte taxa are noted in bold print; algal Divisions are plotted as supplementary variables. Nutrient limitation status of each lake (based on DIN:TP ratios; see Figure 3.1) is denoted symbolically.

Appendix A. Characteristics of Wyoming Snowy Range lakes (n=15) surveyed for water chemistry and phytoplankton in early July 2001. Abbreviations for environmental variables and lakes are found in Tables 3.1 and 3.3, respectively.

Variable	ELEV	SA	Dmax	Dmean	VOL	SA:VOL	COND	ALK	pH	TEMP	DOC	PC	Silica
Units	m	ha	m	m	m3		$\mu S \cdot cm^{-1}$	$\mu eq \cdot l^{-1}$		°C	$mg \cdot l^{-1}$	$\mu g \cdot l^{-1}$	$mg \cdot l^{-1}$
CLA	3273	1.42	4.57	1.02	14358	0.99	12.90	125.12	7.15	12.50	0.70	543	0.89
HRT	3274	4.90	13.72	5.50	269476	0.18	6.70	39.21	8.35	11.00	1.00	644	1.88
LIB	3277	10.32	12.20	4.85	500665	0.21	17.40	168.08	8.02	13.90	1.16	581	1.08
LTL	3262	3.40	3.66	1.65	58837	0.58	18.70	129.62	7.85	15.70	2.30	682	0.78
MSFC	3270	1.57	0.91	0.70	10990	1.43	12.10	112.66	7.18	12.20	1.81	265	2.78
MUT	3241	6.52	2.13	1.14	74440	0.88	7.60	35.41	7.21	19.40	3.57	474	1.56
NGAP	3317	13.04	20.43	6.32	823058	0.16	6.90	52.77	7.21	11.10	1.32	842	0.06
RES	3284	11.70	25.00	9.03	1054666	0.11	10.60	89.16	7.14	13.70	1.20	189	1.42
SGAP	3338	17.94	21.34	6.75	1210281	0.15	6.10	28.25	6.78	5.50	0.50	1212	0.41
SH1	3345	1.15	8.84	3.66	42172	0.27	7.60	88.74	7.46	15.00	1.38	880	0.44
SH2	3345	2.35	5.49	1.90	44676	0.53	7.10	65.68	7.32	14.30	3.53	940	0.54
SH4	3313	1.17	3.35	1.49	17417	0.67	11.00	80.55	7.04	14.10	1.70	--	--
SHP	3284	7.49	1.22	0.45	33526	2.23	10.70	86.44	7.20	20.40	4.66	253	2.20
TLN	3159	3.28	13.41	8.08	264863	0.12	28.50	257.74	8.11	16.80	2.10	817	2.65
WKL	3277	9.72	6.10	2.41	23547	4.13	6.10	53.20	6.96	11.10	0.92	450	1.45

Appendix A., continued.

	NH4-N $\mu g \cdot l^{-1}$	NO3-N $\mu g \cdot l^{-1}$	DIN $\mu g \cdot l^{-1}$	PN $\mu g \cdot l^{-1}$	PO4-P $\mu g \cdot l^{-1}$	DOP $\mu g \cdot l^{-1}$	TDP $\mu g \cdot l^{-1}$	PP $\mu g \cdot l^{-1}$	TP $\mu g \cdot l^{-1}$	DIN:TP	CHL-a $\mu g \cdot l^{-1}$	Richness	Simpson's *D*
CLA	21	27	48	90	0	5	5	17	22	2.16	3.26	28	0.88
HRT	9	16	25	136	0	3	3	15	18	1.38	16.57	23	0.69
LIB	6	0	6	99	1	4	5	12	17	0.35	4.61	41	0.88
LTL	3	0	3	67	1	5	6	15	21	0.14	2.36	38	0.90
MSFC	22	29	51	44	15	20	35	15	50	1.01	1.39	21	0.58
MUT	7	1	8	83	8	15	23	17	40	0.20	4.66	22	0.68
NGAP	20	1	21	168	0	5	5	36	41	0.52	12.19	30	0.74
RES	12	1	13	53	1	0	1	7	8	1.54	1.34	18	0.61
SGAP	17	84	101	143	0	3	3	40	43	2.34	11.44	15	0.37
SH1	20	0	20	186	0	5	5	24	29	0.68	9.15	31	0.66
SH2	32	3	35	262	2	7	9	27	36	0.97	15.08	25	0.71
SH4	15	1	16	58	2	8	10	11	21	0.75	2.06	40	0.89
SHP	29	0	29	127	28	45	73	13	86	0.34	4.28	31	0.90
TLN	9	0	9	36	0	1	1	8	9	1.05	0.89	22	0.86
WKL	47	9	56	73	0	1	1	12	13	4.39	4.34	24	0.83

Appendix B. Taxa list, categorized according to algal Division, generated from a survey of Wyoming Snowy Range lakes (n=15) conducted in early July 2001.

Chlorophytes

Ankistrodesmus falcatus (Corda) Ralfs
Carteria sp. Diesing
Chlamydomonas nivalis Wille
Chlamydomonas sp.1 Ehrenberg
Chlamydomonas sp.2 Ehrenberg
Chlamydomonas sp.3 Ehrenberg
Chlorella sp. Beijerinck
Chlorella vulgaris Beijerinck
Coelastrum astroideum de Notaris
Cosmarium bioculatum Brébisson
Cosmarium spp. Corda
Dictyosphaerium spp. Nägeli
Dictyosphaerium subsolitarium Van Goor
Elakotothrix genevensis(Reverdin) Hindák
Eudorina elegans Ehrenberg
Franceia droescheri (Lemmermann) G. M. Smith
Gonium sociale(Dujardin) Warming
Kirchneriella sp. Schmidle
Lagerheimia genevensis Chodat
Micractinium pusillum Fresenius
Monoraphidium contortum (Thuret) Komárková-Legenerová
Monoraphidium griffithii (Berkeley) Komárková-Legenerová
Oocystis sp. Nägeli
Planktosphaeria gelatinosa G. M. Smith
Quadrigula sp. Printz
Scenedesmus bicellularis Chodat
Scenedesmus spp. Meyen
Schroederia sp. Lemmermann
Selenastrum capricornutum Printz
Sphaerocystis schroeteri Chodat
Spondylosium planum (Wolle) W. & G. S. West
Staurastrum sp. Meyen
Stichococcus bacillaris Nägeli
Tetraspora lemmermannii Fott
Westella sp. de Wildeman

Appendix B., continued.

Chrysophytes

Chromulina mikroplankton (Pascher) Pascher
Chrysamoeba sp. Klebs
Chrysochromulina parva Lackey
Chrysococcus punctiformis Pascher
Chrysococcus spp. Klebs
Chrysolykos sp. Mack
Diacronema spp. Prauser
Diceras sp. Reverdin
Dinobryon cylindricum Imhof
Dinobryon divergens Imhof
Epipyxis sp. Ehrenberg
Erkenia subaequiciliata Skuja
Kephyrion boreale Skuja
Mallamonas akrokomos Ruttner
Mallamonas sp. 1 Perty
Mallamonas sp. 2 Perty
Mallamonas sp. 3 Perty
Ochromonas miniscula Conrad
Ochromonas sp. 1 Wyssotzki
Ochromonas sp. 2 Wyssotzki
Pedinella tricostata Rouchijainen
Pseudokephyrion ellipsoideum (Pascher) Schmid
Pseudopedinella sp. Carter
Spiniferomonas spp. Takahashi
Synura sp. Ehrenberg
Indeterminate Chrysophyte 1
Indeterminate Chrysophyte 2

Cryptophytes

Chroomonas sp. Hansgirg
Cryptomonas sp. Ehrenberg
Komma sp.

Cyanophytes

Anabaena spp. Bory

Appendix B., continued.

Aphanothece sp. Nägeli
Aphanocapsa sp. Nägeli
Chroococcus distans (G. M. Smith) Komárková-Legenerová
Chroococcus minimus (Kiessler) Lemmermann
Microcystis sp. Kützing
Nostoc sp. Vaucher
Oscillatoria spp. Vaucher
Pseudanabaena spp. Lauterborn
Rhabdoderma spp. Schmidle et Lauterborn
Spirulina spp. Turpin
Synechococcus sp. Nägeli

Diatoms (Bacillariophytes)

Asterionella formosa Hassall
*Cocconeis*sp. Ehrenberg
Cymbella sp. Agardh
Diatoma sp. Bory de St. Vincent
Fragilaria spp. Lyngbye
Gomphonema sp. Ehrenberg
Meridion sp. Agardh
Nitzschia sp. Hassall
Rhizosolenia sp. Ehrenberg
Surirella sp. Turpin
Synedra sp. Ehrenberg
Tabellaria sp. Ehrenberg ex Kützing
Indeterminate Centric Diatom 1
Indeterminate Centric Diatom 2
Indeterminate Pennate Diatom 1
Indeterminate Pennate Diatom 2

Dinoflagellates (Dinophytes)

Gymnodinium sp. 1 Stein
Gymnodinium sp. 2 Stein

CHAPTER FOUR[4]

Interactive Effects of Nutrients and pH on the Plankton and Epilithon of Two Mountain Lakes

[4]This chapter prepared for Canadian Journal of Fisheries and Aquatic Sciences:

Lafrançois, B. Moraska, K. R. Nydick, B. M. Johnson and J. S. Baron. Interactive effects of nutrients and pH on the plankton and epilithon of two mountain lakes. Submitted, Canadian Journal of Fisheries and Aquatic Sciences, September 2002.

ABSTRACT

We conducted enclosure experiments to examine effects of nutrients, alone and in conjunction with acidification, on plankton and epilithon in two mountain lakes of differing nutrient status. We found that effects of nutrient additions (N or P) differed between the study lakes; N additions to the low-nitrate lake increased phytoplankton biomass (as chlorophyll *a*) and altered species composition, whereas neither N nor P additions caused significant changes in the high-nitrate lake. Combined additions of nutrients and acid altered phytoplankton composition in both lakes, favoring chlorophyte taxa in the low-nitrate lake, and chlorophytes and the dinoflagellate *Gymnodinium* in the high-nitrate lake. Algal biomass appeared tightly linked to nutrient additions, whereas species composition was affected interactively by both nutrients and pH. Phytoplankton and zooplankton were most responsive to treatments; epilithic algal responses were highly variable. The most dramatic changes in species composition and biomass occurred following combined additions of N, P and acid in both study lakes, indicating the potential for strong interactions among multiple anthropogenic stressors in mountain lakes.

INTRODUCTION

Many mountain lakes of the western United States are sensitive to atmospheric pollutants due to their steep, exposed watersheds and dilute chemistry (Marchetto et al. 1995, Kamenik et al. 2001). Atmospheric nitrogen (N) deposition, in particular, has been implicated in elevated nitrate levels (Baron et al. 2000), altered nutrient ratios (Jassby et al. 1994) and changes in algal species composition (Interlandi and Kilham 1998, Wolfe et al. 2001). Atmospheric phosphorus (P) deposition is often poorly measured, but relatively high levels have been detected periodically in the Colorado Front Range (Lewis et al. 1985), and may be responsible for increased occurrence of N limitation in lakes of the Sierra Nevada of California (Sickman 2001). Further, atmospheric P is known to contribute significantly to primary production in some remote oligotrophic lakes (Cole et al. 1990, Jassby et al. 1994). Atmospheric N compounds contribute to acid deposition and watershed acidification (Brimblecombe and Stedman 1982, Grennfelt and Hultberg 1986), and are likely involved in the episodic acidification of surface waters of the Front Range (Williams and Tonnessen 2000) and the Sierra Nevada (Williams and Melack 1991).

Effects of nutrient (N and P) enrichment on lake biology are well established, and generally include increased algal biomass and productivity and shifts in plankton species composition (Schindler 1975, Smith et al. 1999). The ability of nitrogen alone to affect such changes is often overlooked; however, co-limitation by N and P has been documented in many mountain lakes (Morris and Lewis 1988, Elser et al. 1990), and experimental and anthropogenic additions of N alone have generated marked algal responses in several low-nitrate mountain lakes (Goldman 1988, Axler and Reuter 1996,

Interlandi and Kilham 1998)(Nydick et al. submitted 2002a). High-nitrate mountain lakes, on the other hand, appear less likely to respond to further increases in nitrogen inputs without commensurate phosphorus inputs (Nydick et al., submitted 2002b), so the effects of further nutrient deposition on lakes of differing nutrient status merits further investigation.

Extensive lake acidification literature has linked pH declines with decreases in species diversity and dramatic changes in aquatic species composition across taxonomic groups (Stokes 1986, Turner et al. 1991, Brezonik et al. 1993, Locke et al. 1994, Engle and Melack 1995, Findlay 1999). The combined effects of nutrients and acidity, however, have seldom been addressed and are likely interactive. In one sense, interactions of nutrient and acid additions may be predictable based on well-established responses to either nutrients or acid alone. Species richness and diversity, for example, tend to decline following either eutrophication or acidification (Schindler 1990), as does chrysophyte abundance (Schindler et al. 1985a, Stokes 1986). Effects on other parameters may be less predictable because their responses to nutrients and acidity differ. Phytoplankton productivity and biomass, for instance, commonly increase during eutrophication (Smith et al. 1999) but may decrease or remain unchanged during acidification (Shearer and DeBruyn 1986, Stokes 1986). Moreover, the response of algae to nutrient enrichment may itself modify pH via biological alkalinity production and mediate the effects of acidification (Schindler et al. 1985b).

We conducted mesocosm experiments to examine the individual and interactive effects of nutrients and pH on plankton and epilithon in mountain lakes of the Colorado Front Range and Wyoming Snowy Range. Our objectives were to 1) assess the effects of

several plausible chemical disturbances on lake biota; 2) examine how low-nitrate vs. high-nitrate lakes differ in their response to these treatments; and 3) identify taxa and habitats most sensitive to nutrients and acidity. We predicted that effects of treatment additions would differ between low-nitrate and high-nitrate study lakes. We expected the study lakes to respond to N or P additions in a manner consistent with eutrophication literature and their nutrient status; accordingly, the low-nitrate lake was expected to respond strongly to N additions while the high-nitrate lake was expected to respond more to P additions. Additionally, we predicted declines in species diversity and shifts in species composition following combined additions of nutrients and acid to both lakes. We expected acid-related changes to be more pronounced in the high-nitrate lake, where biological nitrate uptake and pursuant alkalinity production were less likely.

METHODS

Study Sites

Two mountain lakes were selected for mesocosm experiments (Table 4.1). Both are located above 3,000 m and are accessible only by foot trail. Both have relatively short ice-free seasons, snowmelt-dominated hydrology, and dilute oligotrophic waters that differ chiefly in nitrate concentration. Shelf Lake 4 is small low-nitrate lake located at the base of a large quartzite ridge in the Medicine Bow National Forest, Snowy Range, Wyoming. Summer nitrate (NO_3-N) concentrations and alkalinity average ≤ 5 $\mu g \cdot L^{-1}$ and 87 $\mu eq \cdot L^{-1}$ as HCO_3^-, respectively (Nydick et al., submitted 2002a). Its watershed is small and composed of talus and exposed bedrock, with some meadow and krummholz vegetation (Engelmann spruce and subalpine fir). Surface area is 1.2 ha and mean depth

is 1.5 m. No record of fish stocking exists for the lake, but brook trout (*Salvelinus fontinalis*) are present and naturally reproducing (Snigg 1989).

The Loch is a shallow high-nitrate drainage lake located in Rocky Mountain National Park, Colorado. Nitrate concentrations average 229 $\mu g \cdot L^{-1}$ NO_3-N, and acid neutralizing capacity averages only 55 $\mu eq \cdot L^{-1}$ (Baron 1992). Watershed geology is predominantly Precambrian granite, schist, and gneiss. Subalpine vegetation is comprised of Engelmann spruce and subalpine fir; tundra and permanent snowfields occur at higher elevations in the watershed. Surface area is 5.0 ha and mean depth is 1.5 m. Although originally fishless, The Loch was stocked with greenback cutthroat trout (*Oncorhychus clarki stomias*), Colorado River cutthroat (*Oncorhynchus clarki pleuriticus*), and rainbow trout (*Oncorhynchus mykiss*) in the early to mid 1900's (Rosenlund and Stevens 1990) and maintains a population of rainbow x cutthroat trout hybrids.

Experimental Design

Mesocosm enclosures were cylindrical structures 0.7 m in diameter and 1 m deep (~500 L). Polyethylene sheeting was fixed around a frame made from PVC, plastic hoops and a rigid plastic base. Mesocosms were inserted approximately 10 cm into the sediment in the littoral zone of each lake, and secured to the lake bottom with a fitted tarp and rocks. In each lake, mesocosms were installed in two blocks of five and treatments were randomly assigned within each block. Care was taken to minimize sediment disturbance during installation, and enclosures were allowed to sit for several days prior to initial sampling to permit settling of fine sediments. One mesocosm frame without plastic was also placed in each lake so that ambient lake conditions could be monitored

throughout the experiments; this enclosure is referred to hereafter as the "lake" enclosure. Treatments included a control, to which no nutrients or acid were added, N (ambient NO_3-N plus 1 $mg \cdot L^{-1}$ NO_3-N as KNO_3), P (ambient PO_4-P plus 0.01 $mg \cdot L^{-1}$ PO_4-P as KH_2PO_4), NA (N treatment plus HCl to pH 4.5-5.0), and NAP (N treatment plus P treatment plus HCl to pH 4.5-5.0). Treatments were added weekly and were intended to characterize future lake conditions, should deposition of nitrogen and acid continue to rise and phosphorus deposition occur concurrently. Following treatment addition, sampling took place weekly for four weeks in both lakes, although a large storm event flooded The Loch enclosures after the second week. Weekly sampling included water chemistry, phytoplankton, zooplankton, and epilithic algae from unglazed ceramic tiles pre-colonized in each lake for four weeks prior to the start of the experiments. Epilithic tiles on trays were suspended 5-10 cm above the sediment surface in each enclosure to minimize grazing by benthic invertebrates.

Water Chemistry

Water samples were collected from the "lake" and mesocosms with a depth-integrating PVC tube sampler that was acid-washed and rinsed three times with sample water prior to collection. Water chemistry samples were processed using standard Loch Vale Watershed project methods (Allstott et al. 1999). Nitrate, phosphate, and ammonium were determined colorimetrically with a Perstorp Analytical Alpkem Spectrophotometer (Model 3590) using the cadmium reduction, salicylate, and ascorbic acid methods, respectively. Silica was analyzed colorimetrically with the molybdate method modified by Hach, Inc. Dissolved organic carbon (DOC) was analyzed on an Oceanographics International Model 700 Carbon Analyzer (U.S. Geological Survey,

Boulder, CO). Temperature, specific conductance, dissolved oxygen (DO) and pH were measured with meters (Orion Model 128, Hach Sension6, VWR Model 8000).

Phytoplankton

Phytoplankton samples were collected with the depth-integrating tube sampler and analyzed for photosynthetic rate, chlorophyll *a* (referred to hereafter as phytoplankton biomass), and species composition. Samples for chlorophyll *a* analyses were immediately filtered through Whatman GF/C filters. Filters were frozen in dark containers and chlorophyll was extracted with methanol (Reimann 1980). Chlorophyll *a* corrected for phaeophytin was determined with a Sequoia-Turner Model 450 Digital Fluorometer. Photosynthetic rate was measured weekly in one replicate of each treatment, using the ^{14}C light/dark technique (Pregnall 1991). Clear borosilicate bottles suspended at 1m depth and incubated for four to six hours, after which containers were kept cold and dark in coolers while they were transported to the lab. Samples for phytoplankton taxonomic analyses were preserved with Lugol's Iodine in a 1:100 ratio, and settled in Utermöhl chambers (Utermöhl 1958). Several transects were counted under low magnification (150 or 480x) and high magnification (1500x) using a Leitz Diavert inverted microscope with phase contrast. A minimum of 400 cells were enumerated per sample and identified to genus and species where possible, following Smith (1950), Prescott (1962), Starmach (1985), Tikkanen (1986), Cox (1996), Dillard (1999), and Komárek and Anagnostidis (1999). Species richness and diversity (Simpson's *D*, (Washington 1984)) were calculated for all samples.

Epilithic Algae

One epilithic tile was collected from the suspended trays weekly for epilithic chlorophyll *a* (referred to hereafter as epilithic biomass) and every second week for taxonomy and measurement of photosynthetic rate. For chlorophyll *a* analysis, algae was scraped from tiles with a razor blade, rinsed with deionized water and filtered. Clear Whirlpaks™ containing lake water and an algae tile were used to measure epilithic photosynthesis in one enclosure of each treatment, using the ^{14}C light/dark technique as described above. Epilithic algal samples for taxonomic analysis were scraped from tiles and preserved in Lugol's Iodine. In the lab, epilithic samples were sonicated briefly to homogenize contents. An aliquot was removed from the suspended sample, diluted as necessary, and settled in an Utermöhl chamber. Samples were enumerated and identified as described above for phytoplankton, and species richness and diversity were calculated.

Zooplankton

Zooplankton were collected from ambient lake water on each sampling date to examine temporal changes throughout the experiment, and from each mesocosm on the last sampling date to assess differences among treated mesocosms. Loch samples from the final sampling date were damaged during transport from field to lab and are not presented here. Zooplankton were captured using a plexiglass tube sampler approximately 3.5 L in volume. Sample water was poured through a filtering apparatus with 80-μm mesh; 3-5 replicate tube samples were filtered from each mesocosm. Samples were rinsed into Whirl Paks and preserved in 4% sugared buffered formalin. In the lab, samples were poured into a 40-μm sieve, rinsed into 50 mL plastic centrifuge tubes, diluted and shaken to suspend. From this suspended sample, 1-mL aliquots were

removed and placed in Sedgewick-Rafter cells for identification and enumeration using a Zeiss compound microscope. Identification followed Pennak (1989) and Thorp and Covich (1991). Zooplankton biomass was estimated using length-weight regression (Copepoda and Cladocera) and biovolume equations (Rotifera) (Dumont et al. 1975, Bottrell et al. 1976, McCauley 1984). Zooplankton species richness and diversity (Washington 1984) were also calculated for each sample.

Statistical Methods

Experiments from Shelf Lake 4 and The Loch were analyzed separately. Five sampling dates (weeks 0 through 4) were analyzed for Shelf Lake 4; the large storm event and flooding precluded analysis beyond week two for The Loch. Repeated-measures analysis of variance (RM-ANOVA) was used to test the effects of treatments (excluding ambient lake mesocosms) on chemical response variables, phytoplankton and epilithic algal biomass, cell density of phytoplankton and epilithic algae and abundance of major algal groups, abundance and biomass of zooplankton and of major zooplankton groups, and richness and diversity for algae and zooplankton. Photosynthetic rate measurements were conducted for only one replicate of each treatment and were not analyzed statistically. If the treatment main effect or the treatment*week interaction was significant at the $\alpha = 0.1$ level, we used F-test protected least squared means to compare responses among treatments separately for each sampling date. We used *a priori* comparisons of each treatment with the control and set significance level at $\alpha = 0.05$. If more than one treatment was different than the control, we compared these treatments to each other to determine if a certain treatment had a significantly larger response than the other treatment. An analysis of variance (ANOVA) was used to test for treatment effects

on zooplankton collected on the final sampling date. A Tukey correction for multiple comparisons was used to compare individual treatments. Data were transformed when necessary to meet the assumptions of normality and homogeneity of variance. Analyses were performed with SAS Version 8e (SAS Institute, 1999-2001). Phytoplankton and epilithic algal photosynthetic rate were not analyzed statistically since only one replicate from each treatment was measured.

Analysis of algal community data was performed using CANOCO version 4 (ter Braak and Smilauer 1998). We used principal response curves (PRC), a derivation of partial redundancy analysis (RDA), to display major treatment effects on species composition over time (Van den Brink and ter Braak 1998, 1999). Species abundance data were log-transformed prior to analysis to stabilize variance (Jongman et al. 1995). Controls (in this case ambient "lake" control) were set to zero and used as the baseline for comparing treatment response curves. Individual species weights demonstrated which taxa are most involved in the observed community response patterns; only taxa with species weights $> |1|$ were included in the PRC diagram. Taxa with high scores exhibited treatment response patterns similar to those displayed in the PRC diagram. Significance of the first axis of the PRC diagram was tested by performing 199 Monte Carlo permutations of the mesocosms using an *F*-type, eigenvalue-based test statistic (Van den Brink and ter Braak 1999); the null hypothesis predicted no deviation of treatment community composition relative to the lake and controls. Diagrams of second and higher order axes were also constructed and tested to examine residual variance (Van den Brink and ter Braak 1999). Since equal replication of treatments is not required for PRC, one nitrogen treatment mesocosm in Shelf Lake 4 and one control mesocosm in

The Loch were eliminated from the analysis due to evidence of leakage (Nydick et al., submitted 2002c).

RESULTS

Ambient Lake Conditions

Shelf Lake 4 and The Loch showed substantial similarities in overall water chemistry conditions, but differed in biological characteristics during our experiment. Alkalinities were low in both lakes, 90.77 $\mu eq \cdot L^{-1}$ in Shelf Lake 4 and 37.91 $\mu eq \cdot L^{-1}$ in The Loch (Table 4.1). pH ranged from 6.96 to 7.11 in Shelf Lake 4, and from 6.68 to 6.81 in the Loch, averaging 7.04 and 6.74, respectively (Table 4.1). Conductivity and DOC concentrations were low for both lakes, as were chlorophyll *a* and photosynthetic rates. Shelf Lake 4 was the warmer of the two lakes, averaging 14.7 °C during the experiment compared with 11.5 °C for The Loch. Ion concentrations differed mainly with respect to ambient NO_3-N, which averaged two orders of magnitude less in Shelf Lake 4 (≤ 3 $\mu g \cdot L^{-1}$) than in The Loch (215 $\mu g \cdot L^{-1}$). Ambient PO_4-P, on the other hand, was greater in Shelf Lake 4 than in The Loch (Table 4.1).

Algal composition differed between lakes throughout the experiment. Phytoplankton assemblages in Shelf Lake 4 were composed of diverse chrysophyte taxa. Phytoplankton assemblages in The Loch, on the other hand, were dominated by the diatoms *Asterionella formosa* and *Fragilaria crotonensis*, with small chrysophytes and chlorophytes also common. Shelf Lake 4 epilithon composition consisted primarily of *Anabaena* sp., chroococcoid cyanobacteria, and diatoms, whereas The Loch epilithon was dominated by chlorophytes and diatoms.

Zooplankton abundance, biomass and composition differed between lakes. In Shelf Lake 4, zooplankton abundance was approximately eight times higher than in The Loch, and differences in biomass were even more pronounced (Table 4.1). Zooplankton assemblages in Shelf Lake 4 were comprised of cladocerans (*Daphnia* spp.) and copepods (*Diaptomus shoshone* and small cyclopoid taxa) with low densities of rotifers throughout the experiment. Zooplankton assemblages in The Loch, in contrast, were comprised mainly of rotifers (*Keratella* sp. and *Trichotria* sp.), with occasional cyclopoid copepods and nauplii also present.

Treatments

Mean NO_3-N concentrations for all post-treatment samples in Shelf Lake 4 approximated the intended 1,000 $\mu g \cdot L^{-1}$ above ambient NO_3-N concentration for N, NA and NAP treatments, and ranged from 1,000-1,400 $\mu g \cdot L^{-1}$ (Table 4.2). Mean PO_4-P concentrations from post-treatment samples were 36 and 14 $\mu g \cdot L^{-1}$ in P and NAP treatments, respectively. Post-treatment pH averaged 5.7 and 5.9 for NA and NAP treatments, respectively.

As in Shelf Lake 4, Loch NO_3-N concentrations for all post-treatment samples averaged 1,000 $\mu g \cdot L^{-1}$ greater than ambient N, ranging from 1,000-1,200 $\mu g \cdot L^{-1}$ (Table 4.2). Mean PO_4-P concentrations from post-treatment samples were 16 and 11 $\mu g \cdot L^{-1}$ for P and NAP treatments, respectively. Post-treatment pH averaged 5.5 and 5.7 for NA and NAP treatments, respectively.

Water Quality Responses to Treatments

Temperature did not differ among the treatments for either lake, but decreased significantly throughout the experiment in Shelf Lake 4 and increased significantly in

The Loch (Table 4.3). DOC concentrations did not differ among treatments for either lake but increased throughout the experiment in both lakes. Ammonium concentration and dissolved oxygen (DO) percent saturation increased significantly in N, NA and NAP treatments in Shelf Lake 4. In The Loch, ammonium increased mainly in the N treatments, and DO percent saturation increased in N, NA and NAP treatments after two weeks. In Shelf Lake 4, alkalinity increased steadily (though not significantly) to >200 $\mu eq \cdot L^{-1}$ HCO_3^- in N treatments (Figure 4.1a). In The Loch, alkalinity increased slightly in control, N and P enclosures, reaching 70-80 $\mu eq \cdot L^{-1}$ HCO_3^- by the end of the experiment (Figure 4.1b). Alkalinity was significantly lower in NA and NAP treatments than controls for all post-treatment sampling dates, declining to approximately 0 $\mu eq \cdot L^{-1}$ HCO_3^- in both lakes (Table 4.3, Figure 4.1). Details of water chemistry responses, including alkalinity generation and N fate, are reported elsewhere (Nydick et al., submitted 2002c).

Biological Responses to Nutrients

Phytoplankton

Phytoplankton biomass and productivity showed positive responses to N but little response to P in Shelf Lake 4. In N treatments phytoplankton biomass and photosynthetic rate increased 2 to 5-fold, but phytoplankton cell density was not affected (Table 4.4a, Figure 4.2a). P treatments, on the other hand, did not increase phytoplankton biomass or photosynthetic rate, and significantly *lower* phytoplankton cell densities were noted in P treatments on two sampling dates (Table 4.4a, Figure 4.2a).

Similarly, changes in Shelf Lake 4 phytoplankton taxonomic composition occurred in N but not P treatments. PRC analysis of phytoplankton species data revealed

that roughly half (53%) of the total variance in phytoplankton composition was explained by the overall treatment regime (i.e., time x treatment interaction); of this, the first axis explained a significant portion and described treatment response patterns (Table 4.5). The second axis explained a significant portion of the residual variance. The PRC plot for Shelf Lake 4 indicated that N treatments consistently had different phytoplankton composition than the lake, P treatments or controls (Figure 4.3a). In contrast, phytoplankton composition in P treatments deviated only slightly from the lake, and was very similar to control enclosures for all sampling dates. Taxa with high species weights were predominantly cyanophytes and chrysophytes, indicating that taxa of these groups decreased in relative abundance in N treatments (Figure 4.3a). Conversely, taxa with negative species weights were almost invariably chlorophytes (Figure 4.3a), which increased in relative abundance with N additions. As a group, chlorophyte abundance was marginally higher in N treatments than controls for all post-treatment sampling dates, and significantly higher than controls for the first week of the experiment (Table 4.4a). Dinoflagellate abundance, on the other hand, was lower in N treatments than controls for the first two weeks of the experiment (Table 4.4a).

Phytoplankton species richness and diversity (Simpson's *D*) showed variable responses to treatment additions in Shelf Lake 4. Species richness was significantly lower in N treatments on two sampling dates and in week-two P treatments (Table 4.6a). Diversity, on the other hand, was lower in P treatments for the final two weeks of the experiment but was not altered by N treatments.

Unlike Shelf Lake 4, Loch phytoplankton were largely unaffected by additions of N alone and instead showed weakly positive responses to P additions. Phytoplankton

photosynthetic rate was >3 times higher in P treatments than in controls for the first week of the experiment, and cell density was approximately two times higher in P treatments than in controls by the end of the experiment (Table 4.4b, Figure 4.2b).

Although PRC analysis demonstrated significant overall treatment effects on Loch phytoplankton composition (Table 4.5), effects of N or P on composition were small. Overall, 55% of the variation in phytoplankton composition was explained by the treatment regime (i.e., the time x treatment interaction). Of this, 24% could be accounted for by the first axis, which described variation due to experimental treatments (mainly variation NA and NAP treatments); the second PRC axis described a significant amount of residual variance (Table 4.5). The PRC plot showed that phytoplankton composition in P enclosures deviated slightly from the lake at week one, coincident with slight increases in photosynthetic rate; composition in control and N enclosures, on the other hand, was very similar to the lake (Figure 4.3b). Taxa with positive species weights were a taxonomically diverse group that exhibited treatment response patterns similar to those shown in the PRC plot and thus did not respond markedly to either N or P additions (Figure 4.3b).

As in Shelf Lake 4, species richness and diversity responded inconsistently to treatments in The Loch. Species richness was initially higher in P treatments than in controls, but by the end of the experiment was significantly lower than controls for both N and P treatments (Table 4.6b). Species diversity, on the other hand, was significantly lower than controls for N treatments only (Table 4.6b).

significantly to either N or P additions (Table 4.4b). Likewise, neither richness nor diversity of Loch epilithic algae was significantly affected (Table 4.6b).

Zooplankton

On the final sampling date, zooplankton collected from Shelf Lake 4 mesocosms showed significant differences in biomass and composition across treatments (Table 4.4a, Figure 4.5). Total zooplankton biomass was significantly lower in N treatments than controls, and significantly higher in P treatments (Table 4.4a). Cladoceran biomass, in particular, increased in P treatments (mainly *Daphnia pulex* and *D. schoedleri)* and decreased in N treatments (Table 4.4a). Copepod biomass (mainly *Diaptomus shoshone* and *Diacyclops* sp.) was significantly greater in P treatments than in N treatments, but did not differ significantly from controls. Rotifer biomass was significantly higher in N treatments than controls (ls means, α=0.05). Neither richness nor diversity differed significantly from controls for N or P treatments (Table 4.6a).

Biological Responses to Nutrients Combined with Acid

Phytoplankton

In Shelf Lake 4, phytoplankton biomass, productivity and cell density all showed strong responses to NA and NAP treatments after two weeks (Table 4.4a). In fact, by the end of the experiment biomass in NA and NAP treatments greatly exceeded that of controls (Figure 4.2a). There were no discernable biomass differences between N, NA and NAP treatments for any sampling date (ls means, α=0.05). Trends in photosynthetic rate paralleled those of phytoplankton biomass, with higher productivity occurring in NA and NAP enclosures than in controls for post-treatment samples (Figure 4.2a). Similarly,

phytoplankton cell density in NA and NAP treatments increased significantly over controls (Table 4.4a, Figure 4.2a).

PRC analysis of Shelf Lake 4 phytoplankton data revealed significant changes in composition in NA and NAP treatments (Table 4.5). While some effect of N alone was apparent, response patterns for NA and NAP treatments clearly dominated the PRC plot throughout the experiment (Figure 4.3a). Response curves were nearly identical for NA and NAP treatments, indicating that similar species changes occurred whether or not P was included in N + acid treatments. Taxa with high species weights included mainly cyanophytes and chrysophytes; these groups decreased in relative abundance in NA and NAP treatments (Figure 4.3a). Conversely, taxa with negative species weights were almost invariably chlorophytes, notably *Micractinium pusillum, Gonium sociale,* and *Tetraspora lemmermannii* (Figure 4.3a). As a group, chlorophytes increased significantly in NA and NAP treatments (Table 4.4a). Cyanophytes, cryptophytes and diatoms, on the other hand, tended to decrease in NA and NAP treatments (Table 4.4a).

Phytoplankton species richness and diversity (Simpson's *D*) showed variable responses to nutrient and acid additions in Shelf Lake 4. Species richness was initially greater in NA and NAP treatments than controls, but was significantly lower in NAP treatments than controls after one week (Table 4.6a). Species diversity, on the other hand, was significantly different *higher* in NAP treatments than controls on one sampling date (Table 4.6a).

While Loch phytoplankton was unaffected by additions of N or P alone, it did respond to the combined NAP treatment. Biomass, photosynthetic rate and cell density were all significantly higher in NAP treatments on the final sampling date (Table 4.4b,

Figure 4.2b), exceeding controls by approximately seven times. Loch NA treatments did not cause comparable increases in phytoplankton biomass or photosynthetic rate.

PRC analysis described significant treatment-related changes in Loch phytoplankton species composition (Table 4.5), and these patterns were derived mainly from NA and NAP treatment responses. Phytoplankton composition in these two treatments differed substantially from the lake and all other treatments, particularly at week one (Figure 4.3b). As in Shelf Lake 4, NA and NAP treatments resulted in similar species composition. Taxa with highly positive species scores exhibited treatment response patterns similar to those shown in the PRC plot and decreased in relative abundance in NA and NAP treatments. These included *Asterionella formosa* and *Fragilaria crotonensis*, two dominant diatom taxa in The Loch (Figure 4.3b). Taxa with strongly negative species weights increased in relative abundance in NA and NAP treatments. These included several chlorophytes (including the small, unicellular *Choricystis* sp. and the desmid *Closterium* sp.), chrysophytes, and the dinoflagellate *Gymnodinium* sp. (Figure 4.3b). As taxonomic groups, algae that generally increased in abundance in NA and NAP treatments included chlorophytes, chrysophytes, and dinoflagellates (Table 4.4b), whereas groups that responded negatively to NA and NAP treatments included diatoms and cryptophytes (Table 4.4b).

Both species richness and diversity were significantly lower in Loch NAP treatments than controls at the end of the experiment (Table 4.6b). In contrast, NA treatments did not affect richness significantly, and affected diversity inconsistently (Table 4.6b).

Epilithic Algae

NA and NAP treatments did not significantly affect epilithic algal biomass, photosynthetic rate, cell density or composition in Shelf Lake 4 (Table 4.4a, Figure 4.4a). PRC analysis indicated that the treatment regime explained some of the variance in epilithon composition, but the first PRC axis explained little of this and was not significant (Table 4.7). Both the nitrogen-fixing *Anabaena* sp. and epilithic diatoms as a group showed significantly lower abundance in NA and NAP treatments for post-treatment sampling dates, as they did for N treatments (α=0.05 and Table 4.4a). Neither NA nor NAP treatments altered species richness or diversity, although both measures decreased significantly over time (Table 4.6a).

Unlike Loch phytoplankton, neither cell density nor biomass of Loch epilithon showed a significant effect of NA and NAP treatments, although epilithic algal biomass in NAP treatments exceeded that of controls by approximately seven times after two weeks (Table 4.4b, Figure 4.4b). Algal photosynthetic rate showed no clear treatment trends but was lowest in the NAP enclosures after two weeks (Figure 4.4b). PRC analysis indicated that the treatment regime explained 42% of the variance in epilithic algal composition; Monte Carlo permutation tests found neither axis to be significant, indicating that the majority of variability in Loch epilithon was unrelated to the treatment regime (Table 4.7). Nevertheless, the PRC plot of the first axis showed a slight deviation of epilithon composition in NA and NAP treatments from that of the lake. Neither NA nor NAP caused significant changes in abundance of any major algal group (Table 4.4b); species richness and diversity were likewise unaffected (Table 4.6b).

Zooplankton

Shelf Lake 4 zooplankton showed significant differences in biomass (but not abundance) and composition in NA and NAP treatments versus controls on the final sampling date (Table 4.4a, Figure 4.5). Total zooplankton biomass was significantly lower in NA and NAP treatments than in controls (ls means, α=0.05), as was cladoceran biomass (mainly *Daphnia pulex* and *D. schoedleri)* (Table 4.4a). Copepod biomass (mainly *Diaptomus shoshone* and *Diacyclops* sp.) was significantly lower in NA and NAP treatents than in P treatments, but did not differ significantly from controls. Relative rotifer biomass was significantly higher than control in NA and NAP treatments (ls means, α=0.05). Neither richness nor diversity differed significantly from controls for either treatment (Table 4.6a).

DISCUSSION

Effects of nutrients alone

Addition of limiting nutrient(s) to oligotrophic waters is known to cause increased algal biomass and productivity, as well as declines in species richness and diversity and shifts toward taxa common to nutrient enriched waters (Smith et al. 1999). Nutrient enrichment may also have both direct (Paul et al. 1995) and indirect (Malley et al. 1988) effects on the structure and biomass of zooplankton communities. In this study, we predicted that effects of nutrient additions would differ according to the nutrient status of the study lakes, such that low-nitrate Shelf Lake 4 would respond to N alone and the high-nitrate Loch would respond to P alone.

As expected, we documented clear phytoplankton responses to N treatments in Shelf Lake 4, including increased biomass and photosynthetic rate, and a shift from

chrysophyte to chlorophyte dominance. This shift toward larger, more chlorophyll-rich taxa may account for the difference in chlorophyll *a* versus cell density responses to N. Though less dramatic, these N responses were consistent with those documented in previous nutrient addition experiments in Shelf Lakes 4 and 5 (Nydick et. al, submitted 2002a) and with lake eutrophication literature in general (Schindler 1975). Additions of P, on the other hand, did not increase phytoplankton biomass and photosynthetic rate or alter species composition. In fact, P treatments had *lower* phytoplankton cell densities than controls on two sampling dates, likely due to the higher biomass of cladoceran zooplankton in P treatments (Elser and Goldman 1991).

Our prediction that The Loch phytoplankton would respond to additions of P alone was weakly supported by this experiment, despite substantial evidence of P-limitation from previous nutrient bioassays (Nydick et al., submitted 2002b). P treatments caused only slight increases in phytoplankton photosynthetic rate and cell density, and effects on phytoplankton composition were small. A simultaneous ^{15}N tracer study indicated that a considerable fraction of the nitrate in Loch enclosures was immobilized in the benthos and thus rendered unavailable to phytoplankton (Nydick et al., submitted 2002c). This process, coupled with isolation of mesocosm water from lake and watershed sources of new nitrate, may have caused phytoplankton to shift toward limitation by both N and P. Stronger phytoplankton responses to P alone may be expected if P were to increase on a whole-lake scale in the presence of readily replenished nitrate from lake inlets.

In contrast to phytoplankton, neither biomass nor photosynthetic rate of epilithic algae responded to additions of N or P in either lake, and effects on epilithic algal

composition were weak. This is in partial agreement with previous enrichment experiments in the Snowy Range, which indicated no response of epilithic biomass to P alone, and variable responses to N alone (Nydick et. al, submitted 2002a). While we found no dramatic shifts in overall epilithic algal composition for either lake, the decline of the N-fixing *Anabaena* sp. in Shelf Lake 4 N treatments was likely due to the reduced competitive ability of cyanophytes when N:P ratios are high (Smith 1983). Weak and/or species-specific responses of periphyton to nutrients have been noted in other studies (Fairchild et al. 1985) and may indicate that periphyton biomass is collectively limited by both N and P, or that most periphyton taxa are simply less sensitive to water column nutrient concentrations than phytoplankton (Cattaneo 1987, Fairchild and Sherman 1993). Factors limiting sensitivity of periphyton to nutrient additions generally include light limitation (resulting from increased phytoplankton biomass, as in Shelf Lake 4 N treatments)(Hansson 1992), inorganic carbon limitation (Turner et al. 1994), and benthic grazing (Stein 1996), not measured in this study.

While phytoplankton biomass is often positively correlated with zooplankton biomass across nutrient gradients (McCauley and Kalff 1981), increased phytoplankton biomass corresponded with *lower* zooplankton biomass in Shelf Lake 4 N treatments. This negative relationship likely resulted from shifts in phytoplankton species composition (from highly edible chrysophytes toward less palatable chlorophytes and cyanophytes) in N treatments. Conversely, higher zooplankton biomass in Shelf Lake 4 P treatments showed that while P failed to cause dramatic changes in algal species composition, it may have enhanced algal food quality and augmented growth of Cladocera (Brett et al. 2000). It is evident from ambient Loch samples that zooplankton

assemblages were sparse and dominated by small rotifer taxa; consequently, it is unlikely that zooplankton grazing or nutrient recycling significantly affected the response of Loch algae to N or P.

Interactive effects of nutrients and acid

Lake acidification is known to cause relatively predictable shifts in the species composition of phytoplankton, periphyton, and zooplankton, decreased species richness and diversity across taxonomic groups, and unpredictable changes in algal biomass and productivity (Stokes 1986, Brett 1989, Schindler 1990, Turner et al. 1991). Based on established response patterns for acidification and nutrient enrichment in isolation, we predicted that additions of acid and nutrients *together* would cause marked shifts in species composition and declines in species richness and diversity across all taxonomic groups. We expected that acid-related changes would be less pronounced in Shelf Lake 4, where biological nitrate uptake was more likely to generate alkalinity and diminish the effects of acid additions.

As predicted, combined acid and nutrient treatments had clear effects on phytoplankton species composition but variable effects on phytoplankton biomass. In Shelf Lake 4, NA and NAP treatments increased phytoplankton biomass and productivity *and* caused shifts in composition from a diverse chrysophyte assemblage toward dominance by chlorophytes. In The Loch, biomass and productivity of Loch phytoplankton increased in NAP treatments only, but both NA and NAP treatments caused phytoplankton composition to shift from a distinct diatom-chrysophyte assemblage toward a new assemblage of chrysophytes, chlorophytes (particularly the unicellular chlorophyte *Choricystis* sp.) and the dinoflagellate *Gymnodinium* sp. Thus,

effects of nutrients and acid on phytoplankton biomass differed between lakes, and appeared to be linked to nutrient availability rather than to pH. In contrast, effects of nutrient and acid on species composition were similar between lakes and appeared to relate interactively to both acid and nutrients. Similarly, Havens and Carlson (1998) noted that while plankton taxonomic composition differed markedly along a gradient of lake pH, biomass varied according to lake nutrient levels.

The shift toward chlorophytes in both lakes following nitrate and acid additions was unsurprising; chlorophytes have high N requirements and are good competitors at high N levels (Reynolds 1984). Additionally, enrichment experiments in acidified waters have demonstrated that chlorophyte abundance (particularly *Chlorella* sp. and *Choricystis* sp.) increased following nutrient additions (Wilcox and DeCosta 1982, Findlay et al. 1999). Shifts toward dinoflagellate dominance are commonly reported in lake acidification literature; however, we found increased dinoflagellate abundance only in The Loch acid treatments. Findlay et al. (1999) proposed that while high dissolved inorganic carbon (DIC) and high nitrate conditions favor chlorophytes, dinoflagellates are favored when DIC is lower. If, as they suggest, atmospheric CO_2 invasion (and thus DIC) decreases with pH, a dinoflagellate response would indeed have been most likely in The Loch, which was slightly more acidic than Shelf Lake 4 following treatment additions.

Several notable phytoplankton taxa decreased in relative abundance in NA and NAP treatments in both lakes. In Shelf Lake 4, cyanophyte and diatom species that had responded positively to N or N+P in previous Shelf Lake experiments (Nydick et al., submitted 2002a) showed a negative response to combined nutrient and acid treatments.

This discrepancy indicates that effects of acidification were in opposition to those of nutrient enrichment for these taxa, and is consistent with their physiological preference for less acidic conditions (Shapiro 1973, Dixit et al. 1988, Findlay and Shearer 1992). Similarly, in The Loch, the dominant diatoms *Asterionella formosa* and *Fragilaria crotonensis* responded negatively to NA and NAP treatments, which corresponds well with their alkaliphilic distributions in other regions (Charles 1985, Findlay and Shearer 1992).

We found little evidence that combined additions of nutrients and acid altered epilithic algal biomass or community composition in either study lake. Further, the shift toward filamentous chlorophytes, characteristic of acidic lakes (Stokes 1986), did not occur during our short-term experiments. Abundance of *Anabaena* sp. and epilithic diatoms in Shelf Lake 4 NA and NAP treatments were lower than in controls, but did not differ significantly from N treatments, indicating that changes in these species likely related more to N enrichment than to pH. Similarly, in The Loch we found marginally increased epilithic algal biomass in NAP but not NA treatments, suggesting that biomass responded more to cumulative nutrient effects of N and P than to pH. Effects of simultaneous nutrient addition and acidification on periphyton have rarely been addressed in other studies; however, pH gradients do appear to influence periphyton responses to nutrient addition across lakes (Fairchild and Sherman 1992, 1993), such that nutrient enrichment seldom enhances periphyton growth or photosynthetic rate in lakes with alkalinity <100 $\mu eq \cdot L^{-1}$ (Fairchild and Sherman 1992, Turner et al. 1994). Further, carbon limitation of epilithic algae increased during a lake acidification experiment (Turner et al. 1987), and additions of DIC stimulated epilithic growth in a low alkalinity

lake (Turner et al. 1991). Accordingly, DIC limitation resulting from naturally low-alkalinity conditions (and artificially low-alkalinity conditions induced by NA and NAP treatments) may account for the small response of epilithic algae in our experiments.

Zooplankton assemblages indicated a strong response to nutrient and acid additions in Shelf Lake 4. We found that the initially mixed assemblage of cladocerans, copepods and rotifers shifted toward rotifer dominance in acid treatments. Similar patterns were noted in the Sierra Nevada, California (Barmuta et al. 1990, Bradford et al. 1998), and are common themes in lake acidification literature (Brett 1989). Nonetheless, no major zooplankton group responded substantially more to NA or NAP treatments than to N-alone in our experiments, which suggests that zooplankton responses may have had more to do with N-related changes in phytoplankton composition than with pH declines.

As predicted, N additions did increase biological alkalinity production and mediate water chemistry responses to NA and NAP treatments in Shelf Lake 4 (Nydick et al., submitted 2002c); however, N enrichment did not effectively moderate the effects of acidification on biological processes and composition. In fact, phytoplankton biomass and productivity responses to NA treatments were generally *more* dramatic in Shelf Lake 4 than in The Loch, and phytoplankton composition changes were more severe and longer-lived. Nonetheless, the nature of the phytoplankton composition changes in Shelf Lake 4 (shifting toward chlorophytes, which are generally more alkaliphilic than the chrysophytes they replaced) versus The Loch (shifting toward chrysophytes, dinoflagellates, and acidophilic chlorophytes/desmids) offered evidence that phytoplankton in Shelf Lake 4 acid treatments were generally less acid-stressed than those of the Loch.

CONCLUSION

Our experiments demonstrated that effects of nutrient additions differed according to lake nutrient status, with mild eutrophication and plankton species shifts following N additions to the low-nitrate study lake and P additions to the high-nitrate study lake. Effects of nutrients and acid on phytoplankton biomass differed between lakes, and appeared to be linked more tightly to nutrient availability than to pH. In contrast, effects of nutrient and acid on species composition were similar between lakes, and were evidently linked interactively to both nutrients and pH.

In both lakes, combined nutrient and acid treatments favored shifts toward phytoplankton taxa that were larger and of poorer food quality than the previously dominant chrysophytes and diatoms. The simultaneous shift toward smaller, less efficient zooplankton grazers suggests that nutrients and acidity may increase phytoplankton biomass synergistically. Such changes in species composition and trophic relations may have significant and potentially long-term implications for mountain lake food webs.

We noted variability in the responses of different taxonomic groups and variables to our treatment additions. Phytoplankton and zooplankton were more sensitive to treatments than epilithon, and may thus serve as better indicators of future nutrient enrichment and acidification. Responses of biomass and composition were frequently similar in character, although phytoplankton species shifts occurred in The Loch without significant changes in biomass. Thus, while biomass and composition generally provided similar insights, neither was a perfect substitute for the other. Species richness and diversity did not respond consistently to experimental treatments, nor did they always

reflect major changes in species composition. These community-level measures appeared to be unreliable indicators of short-term chemical stress despite their ability to reflect long-term declines following enrichment and acidification in other regions.

Finally, human population growth and increased use of fossil fuels and fertilizers across western North America contribute increasingly to atmospheric N and P deposition and to the gradual acidification of sensitive high elevation watersheds. Our experiments provided evidence that nutrients, alone and in conjunction with acidification, can significantly alter various aspects of mountain lake ecology. We found that in both study lakes the combined N, acid and P treatment triggered the most striking changes in biomass and community composition. Given these strong experimental responses and the potential for nutrient enrichment and acidification to co-occur in many mountain lakes, we suggest that greater attention to the interactions of multiple anthropogenic stressors is warranted.

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Table 4.1. Comparison of physical and chemical characteristics for Shelf Lake 4 and The Loch. Chemical parameters are means (± 1 standard error) from "lake" mesocosms for the weeks of the experiment (n=8 for Shelf Lake 4, n=5 for the Loch).

	Units	Shelf Lake 4	The Loch
Elevation	m	3313	3050
Mean depth	m	1.49	1.50
Surface area	ha	1.2	5.0
pH		7.04 ± 0.02	6.74 ± 0.02
Alkalinity	$\mu eq \cdot L^{-1}$ as HCO_3^-	90.77±2.15	37.91 ± 1.15
Temperature	° C	14.7 ± 0.3	11.5 ± 0.8
Conductivity	$\mu S\ cm^{-1}$	12.64 ± 0.44	10.48 ± 0.07
NO_3-N	$\mu g \cdot L^{-1}$	≤ 3	215 ± 8
PO_4-P	$\mu g \cdot L^{-1}$	4 ±1	1 ± 0
NH_4-N	$\mu g \cdot L^{-1}$	15 ± 6	10 ± 3
DOP	$\mu g \cdot L^{-1}$	7 ± 1	3 ± 2
TDP	$\mu g \cdot L^{-1}$	12 ± 1	5 ± 2
DOC	$mg \cdot L^{-1}$	1.99 ± 0.05	0.82 ± 0.08
Phytoplankton Chlorophyll *a*	$\mu g \cdot L^{-1}$	1.47 ± 0.34	1.74 ± 0.43
Phytoplankton Photosynthetic Rate	$mg\ C \cdot L^{-1} \cdot hour^{-1}$	0.01 ± 0.00	0.01 ± 0.00
Epilithic Chlorophyll *a*	$\mu g \cdot cm^{-2}$	0.32 ± 0.10	0.57 ± 0.16
Epilithic Photosynthetic Rate	$mg\ C \cdot cm^{-2} \cdot hour^{-1}$	28.48 ± 8.42	10.84 ± 3.40
Zooplankton Abundance	$Number \cdot L^{-1}$	24 ± 8	3 ± 1
Zooplankton Biomass	$\mu g \cdot L^{-1}$	109 ± 31	0.44 ± 0.10

Table 4.2. Treatment means (± 1 standard error) for pH, NO_3-N and PO_4-P, derived from all post-treatment samples for Shelf Lake 4 and the Loch. Units for NO_3-N and PO_4-P are given in $\mu g \cdot L^{-1}$.

	Shelf Lake 4			The Loch		
Treatment	pH	NO_3-N	PO_4-P	pH	NO_3-N	PO_4-P
Lake	7.05	2	5	6.75	210	2
Control	7.24 ± 0.08	1 ± 1	4 ± 1	6.89 ± 0.06	48 ± 9	3 ± 0
N	8.72 ± 0.21	1060 ± 294	2 ± 1	6.94 ± 0.04	1208 ± 28	2 ± 0
P	7.31 ± 0.02	0 ± 0	36 ± 9	7.00 ± 0.09	40 ± 4	16 ± 2
NA	5.73 ± 0.13	1433 ± 113	2 ± 0	5.50 ± 0.05	1103 ± 73	2 ± 0
NAP	5.94 ± 0.26	1396 ± 1	14 ± 0	5.74 ± 0.07	1070 ± 100	11 ± 2

Table 4.3: Responses of chemical variables for Shelf Lake 4 (a), and the Loch (b). Ø = No significant differences among treatments (see methods). For individual week responses, letters indicate which treatment means were different than control (N=nitrate, P= phosphate, NA=nitrate+acid, NAP=nitrate+acid+phosphate). Arrow indicates direction of difference (↑ = greater than control, ↓ = less than control). Main time effects are given as *p*-values; arrow indicates direction of change (↑ = means increase over time).

4.3 (a)

	Week 0	Week 1	Week 2	Week 3	Week 4	Main Time Effect
Temperature	Ø	Ø	Ø	Ø	Ø	<0.0001↓
Conductivity	Ø	N, NA, NAP ↑	N, NA, NAP ↑	N, NA, NAP ↑	N, NA, NAP ↑	<0.0001↑
Alkalinity	Ø	NA, NAP ↓	NA, NAP ↓	NA, NAP ↓	NA, NAP ↓	<0.0001↓
Dissolved Oxygen	Ø	N ↑	N, NA, NAP ↑	Ø	N, NAP ↑	<0.0001↑
pH	Ø	NAP ↓	N ↑	N ↑	N ↑	0.0001 ↑
NO_3-N	Ø	NA, NAP ↑	NA, NAP ↑	N, NA, NAP ↑	N, NA, NAP ↑	<0.0001↑
PO_4-P	Ø	P ↑	P ↑	P ↑	P ↑	0.0011↑
NH4-N	Ø	Ø	NAP ↑	N, NA, NAP ↑	N, NA, NAP ↑	<0.0001↑
DOP	Ø	N ↓	Ø	Ø	Ø	<0.0001↓
TDP	Ø	P ↑	P ↑	P ↑	P ↑	0.0003↑
DOC	Ø	Ø	Ø	Ø	Ø	0.0003 ↑

4.3 (b)

	Week 0	Week 1	Week 2	Main Time Effect
Temperature	Ø	Ø	Ø	<0.0001↑
Conductivity	Ø	N, NA, NAP ↑	N, NA, NAP ↑	<0.0001↑
Alkalinity	Ø	NA, NAP ↓	NA↓	0.0003 ↓
Dissolved Oxygen	Ø	NAP ↑	N, NA, NAP ↑	<0.0001↑
pH	Ø	NA, NAP ↓	N, NA, NAP ↓	0.0422 ↓
NO_3-N	Ø	N, NA, NAP ↑	N, NA, NAP ↑	<0.0001↑
PO_4-P	Ø	Ø	Ø	0.8512
NH4-N	N, P ↑	N ↑	N ↑	0.0005 ↓
DOP	Ø	Ø	Ø	0.0271↓
TDP	Ø	Ø	Ø	0.7428
DOC	Ø	Ø	Ø	<0.0001↑

Table 4.4. Responses of biological variables to treatment additions in Shelf Lake 4 (a) and the Loch (b). Ø = No significant differences among treatments (see methods). For individual week responses, letters indicate which treatment means were different than control (N=nitrate, P= phosphate, NA=nitrate+acid, NAP=nitrate+acid+phosphate). Arrow indicates direction of difference (↑ = greater than control, ↓ = less than control). Main time effects are given as *p*-values; arrow indicates direction of change (↑ = means increase over time). "--" indicates weeks for which data were not collected or are unavailable due to sample damage.

4.4 (a)

	Week 0	Week 1	Week 2	Week 3	Week 4	Main Time Effect
Phytoplankton Chlorophyll *a*	--	Ø	NA, NAP ↑	N, NA, NAP ↑	NA, NAP ↑	<0.0001 ↑
Phytoplankton Cell Density	Ø	Ø	P ↓, NA ↑	P ↓, NA ↑	NA, NAP ↑	0.0002 ↑
Phytoplankton Chlorophytes	Ø	N, NA, NAP ↑	P ↓, NA, NAP ↑	NA, NAP ↑	NA, NAP ↑	<0.0001 ↑
Phytoplankton Desmids	Ø	Ø	Ø	Ø	Ø	0.0002 ↑
Phytoplankton Cyanophytes	Ø	Ø	Ø	NA, NAP ↓	NAP ↓	<0.0001↑
Phytoplankton Chrysophytes	Ø	Ø	Ø	Ø	Ø	0.0018 ↓
Phytoplankton Cryptophytes	Ø	NAP ↓	NAP ↓	NAP ↓	NA, NAP ↓	0.0584
Phytoplankton Diatoms	Ø	Ø	Ø	NA, NAP ↓	NAP ↓	0.9220
Phytoplankton Dinoflagellates	Ø	N ↓	N ↓	Ø	Ø	0.0002 ↓
Epilithic Chlorophyll *a*	--	Ø	Ø	Ø	Ø	0.0047 ↓
Epilithic Cell Density	Ø	--	Ø	--	Ø	<0.0001 ↓
Epilithic Chlorophytes	Ø	--	Ø	--	Ø	<0.0001 ↓
Epilithic Cyanophytes	Ø	--	Ø	--	Ø	<0.0001 ↓
Epilithic Diatoms	Ø	--	Ø	--	N, NA, NAP ↓	<0.0001 ↓
Epilithic Desmids	Ø	--	Ø	--	Ø	0.0036 ↓
Zooplankton Abundance	--	--	--	--	Ø	--
Zooplankton Biomass	--	--	--	--	P ↑, N, NA, NAP ↓	--
Cladoceran Biomass	--	--	--	--	P ↑, N, NA, NAP ↓	--
Copepod Biomass	--	--	--	--	P> N, NA, NAP	--
Rotifer Biomass	--	--	--	--	Ø	--

4.4 (b)

	Week 0	Week 1	Week 2	Main Time Effect
Phytoplankton Chlorophyll *a*	Ø	Ø	NAP ↑	<0.0001↑
Phytoplankton Cell Density	Ø	NAP ↑	P, NAP ↑	<0.0001↑
Phytoplankton Chlorophytes	Ø	N ↓, NA, NAP ↑	NA, NAP ↑	<0.0001↑
Phytoplankton Desmids	Ø	N, NAP ↑	N, NAP ↑	0.3361
Phytoplankton Cyanophytes	Ø	Ø	Ø	0.0006 ↑
Phytoplankton Chrysophytes	Ø	NA, NAP ↑	NA ↑	<0.0001↑
Phytoplankton Cryptophytes	Ø	NA, NAP ↓	Ø	0.0454
Phytoplankton Diatoms	Ø	NA, NAP ↓	NAP ↓	<0.0001↓
Phytoplankton Dinoflagellates	Ø	NA, NAP ↑	NA ↑	0.0045 ↑
Epilithic Chlorophyll *a*	Ø	Ø	Ø	<0.0001↑
Epilithic Cell Density	Ø	--	N ↑	0.0207 ↑
Epilithic Chlorophytes	Ø	--	Ø	0.1259
Epilithic Cyanophytes	Ø	--	Ø	0.0088 ↑
Epilithic Diatoms	Ø	--	Ø	0.0966
Epilithic Desmids	Ø	--	Ø	0.7536
Zooplankton Abundance	--	--	--	--
Zooplankton Biomass	--	--	--	--
Cladoceran Biomass	--	--	--	--
Copepod Biomass	--	--	--	--
Rotifer Biomass	--	--	--	--

Table 4.5. Percentages of the total variance attributed to time and treatment regime in Principal Response Curves (PRC) for phytoplankton assemblages of Shelf Lake 4 and the Loch. The treatment component includes time x treatment interactions; variance remaining after time and treatment effects are summed is residual. The fraction of treatment variance captured by the first and second PRC's is also noted and tested, with respective p-values in parentheses.

	% variance accounted for by:		% variance explained by treatment regime in PRC:	
Lake	Time	Treatment	First PRC	Second PRC
Shelf Lake 4	25	53	20 (p=0.005)	12 (p=0.045)
The Loch	24	55	24 (p=0.005)	13 (p=0.005)

Table 4.6. Species richness ("richness") and diversity (Simpson's *D*) for phytoplankton, epilithon and zooplankton in (a) Shelf Lake 4 and (b) The Loch. Ø = No significant differences among treatments (see methods). For individual week responses, letters indicate which treatment means were different than control (N=nitrate, P= phosphate, NA=nitrate+acid, NAP=nitrate+acid+phosphate). Arrow indicates direction of difference (↑ = greater than control, ↓ = less than control). For time main effect, arrow indicates direction of change (↑ = means increase over time). "--" indicates weeks for which data were not collected or are unavailable due to sample damage.

4.6 (a)

	Week 0	Week 1	Week 2	Week 3	Week 4	Main Time Effect
Phytoplankton richness	NA, NAP ↑	NAP ↓	N, P, NAP ↓	N, NAP ↓	Ø	<0.0001 ↓
Phytoplankton diversity	Ø	Ø	Ø	P ↓, NAP ↑	P ↓	<0.0001 ↓
Epilithic richness	Ø	--	Ø	--	Ø	0.0330 ↓
Epilithic diversity	Ø	--	Ø	--	Ø	0.0478 ↓
Zooplankton richness	--	--	--	--	P>NA	--
Zooplankton diversity	--	--	--	--	Ø	--

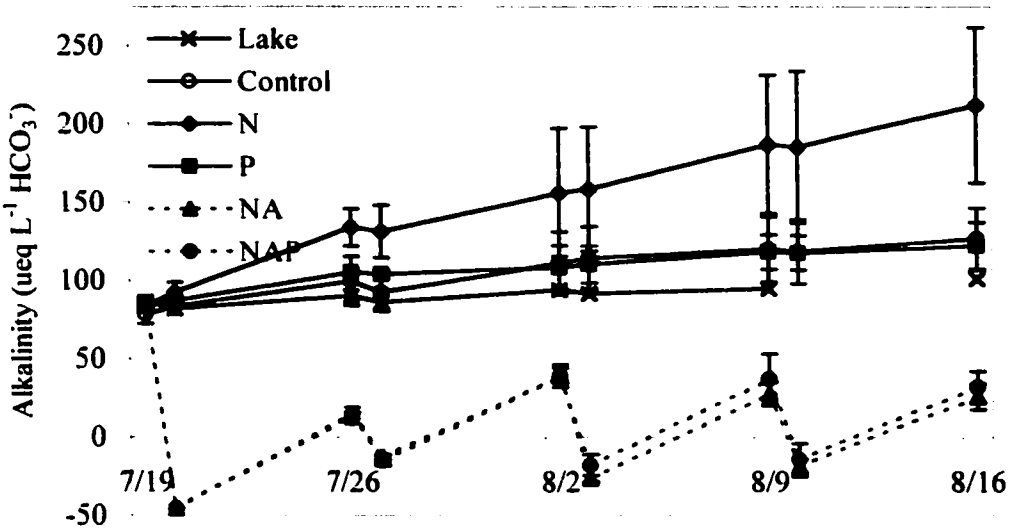
4.6 (b)

	Week 0	Week 1	Week 2	Main Time Effect
Phytoplankton richness	P ↑	Ø	N, P, NAP ↓	<0.0001 ↓
Phytoplankton diversity	Ø	NA ↓	N, NA ↑, NAP ↓	0.0087 ↓
Epilithic richness	Ø	--	Ø	0.1060
Epilithic diversity	Ø	--	Ø	0.1816
Zooplankton richness	--	--	--	--
Zooplankton diversity	--	--	--	--

Table 4.7. Percentages of the total variance attributed to time and treatment regime in Principal Response Curves (PRC) for epilithon assemblages of Shelf Lake 4 and the Loch. The treatment component includes time x treatment interactions; variance remaining after time and treatment effects are summed is residual. The fraction of treatment variance captured by the first and second PRC's is also noted and tested, with respective p-values in parentheses.

	% variance accounted for by:		% variance explained by treatment regime in PRC:	
Lake	Time	Treatment	First PRC	Second PRC
Shelf Lake 4	24	47	15 (p=0.290)	13 (p=0.035)
The Loch	19	42	19 (p=0.860)	17 (p=0.955)

4.1 (a)



4.1 (b)

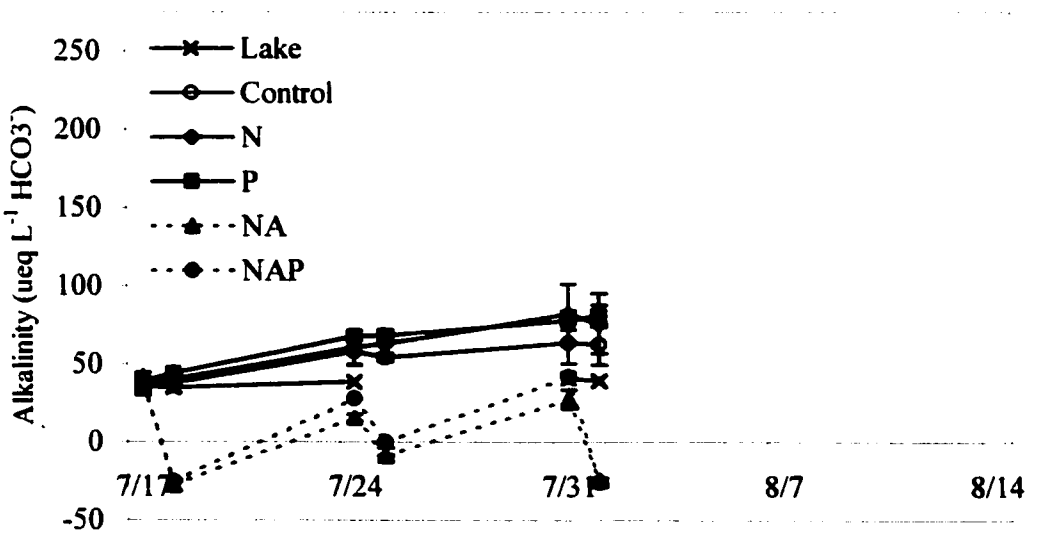


Figure 4.1. Effects of treatment additions on alkalinity (given in $\mu eq \cdot L^{-1}$ $HCO3^-$) in Shelf Lake 4 (a), and The Loch (b). Bars represent ±1 standard error ($n = 2$).

4.2 (a) 4.2 (b)

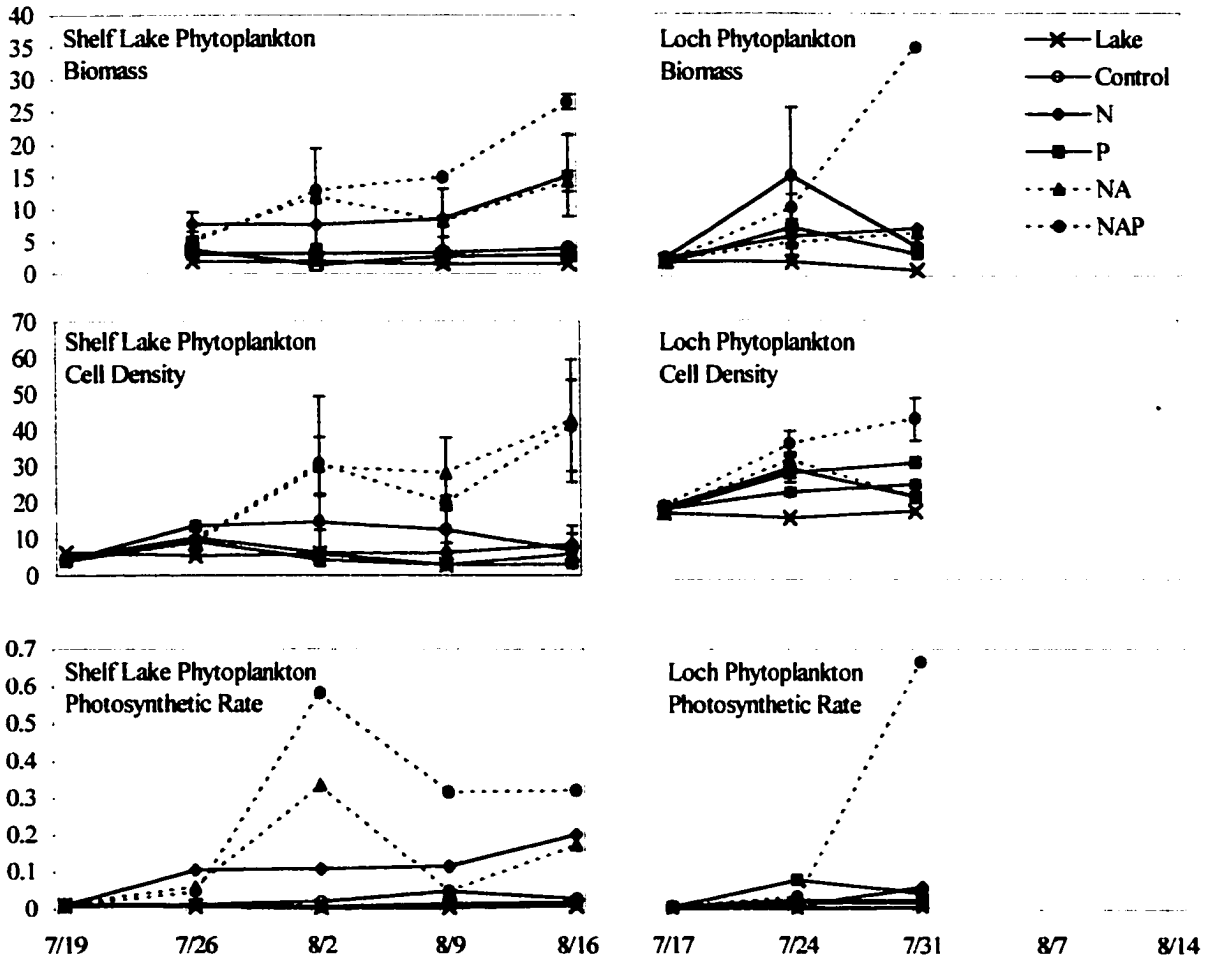


Figure 4.2. Phytoplankton responses to treatment additions for Shelf Lake 4 (a) and The Loch (b). Phytoplankton biomass is given as $\mu g \cdot L^{-1}$ chlorophyll *a*; cell densities are given in number of cells x $10^4 \cdot mL^{-1}$; photosynthetic rates are given in mg $C \cdot L^{-1} \cdot hour^{-1}$. Bars represent ± 1 standard error ($n = 2$).

4.3 (a)

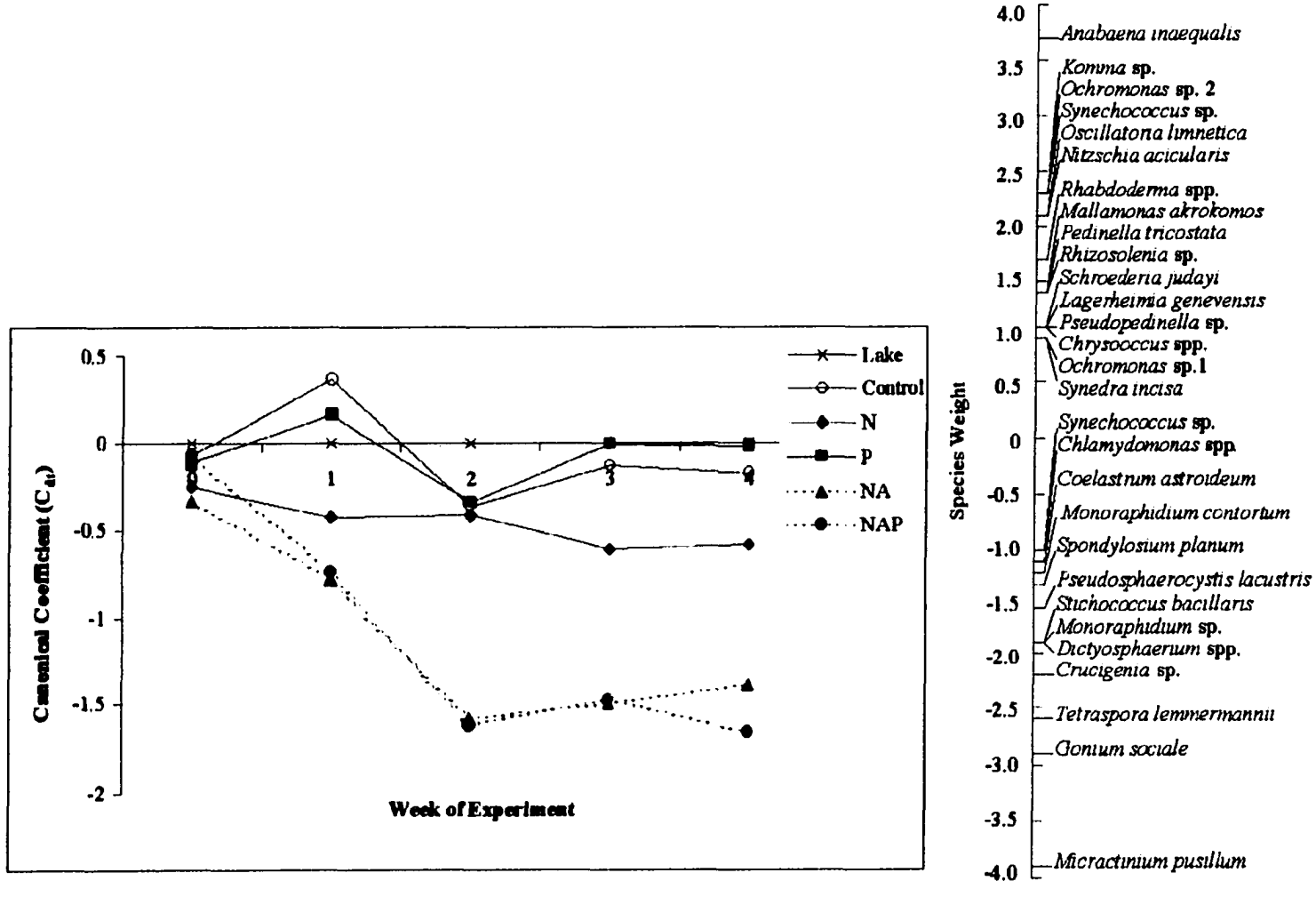
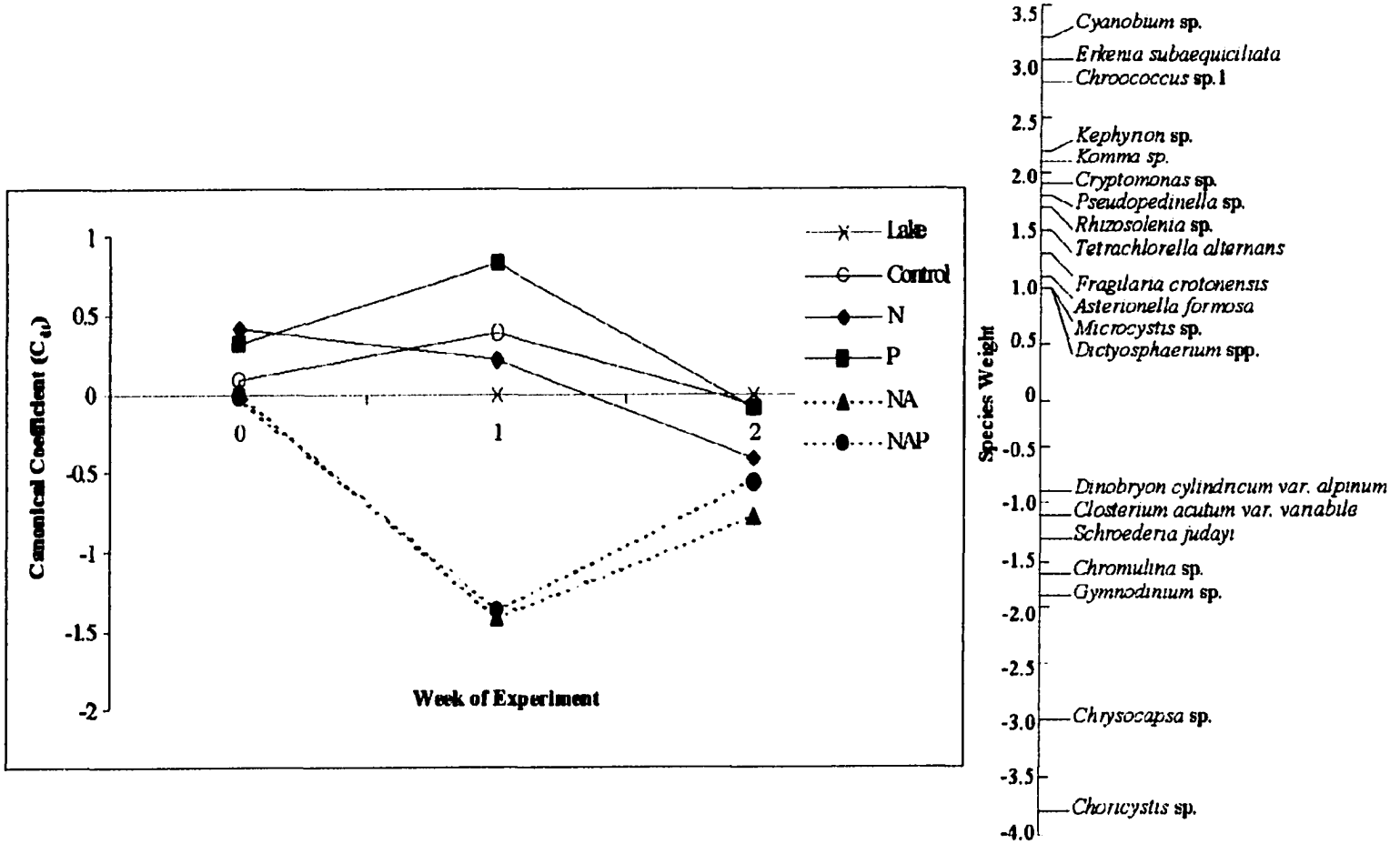


Figure 4.3. Principal Response Curves (PRC) with species weights for Shelf Lake 4 phytoplankton (a) and The Loch phytoplankton (b). Percentages of variance accounted for and significance levels are found in Table 4.5. See text for discussion of species weights.

4.3 (b)



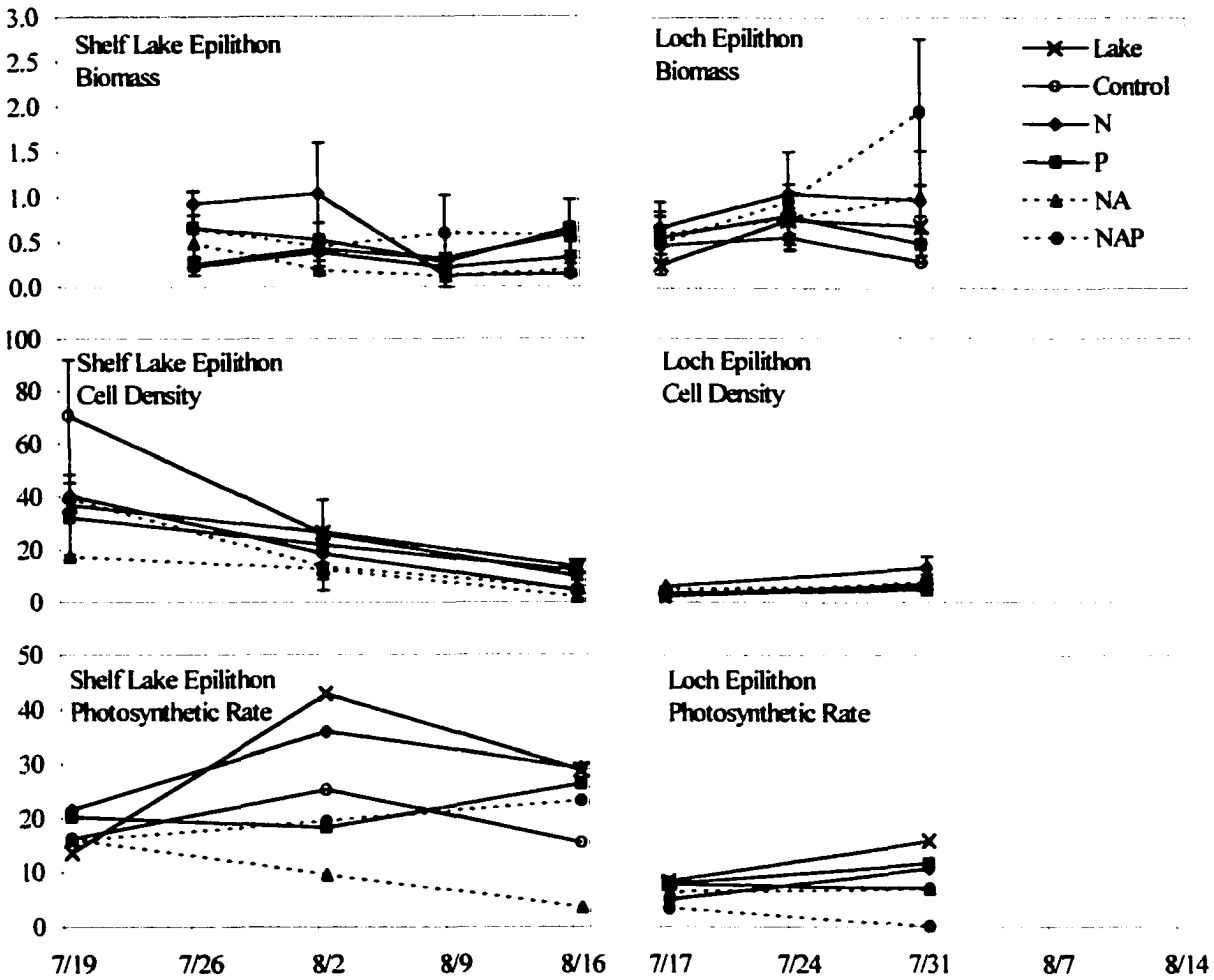


Figure 4.4. Responses of epilithon to experimental treatments in Shelf Lake 4 (a) and the Loch (b). Epilithic biomass is given in $\mu g \cdot (cm^2)^{-1}$ chlorophyll *a*; cell densities are given in number of cells x $10^5 \cdot (cm^2)^{-1}$; photosynthetic rates are given in mg $C \cdot (cm^2)^{-1} \cdot hour^{-1}$. Bars represent ± 1 standard error ($n = 2$).

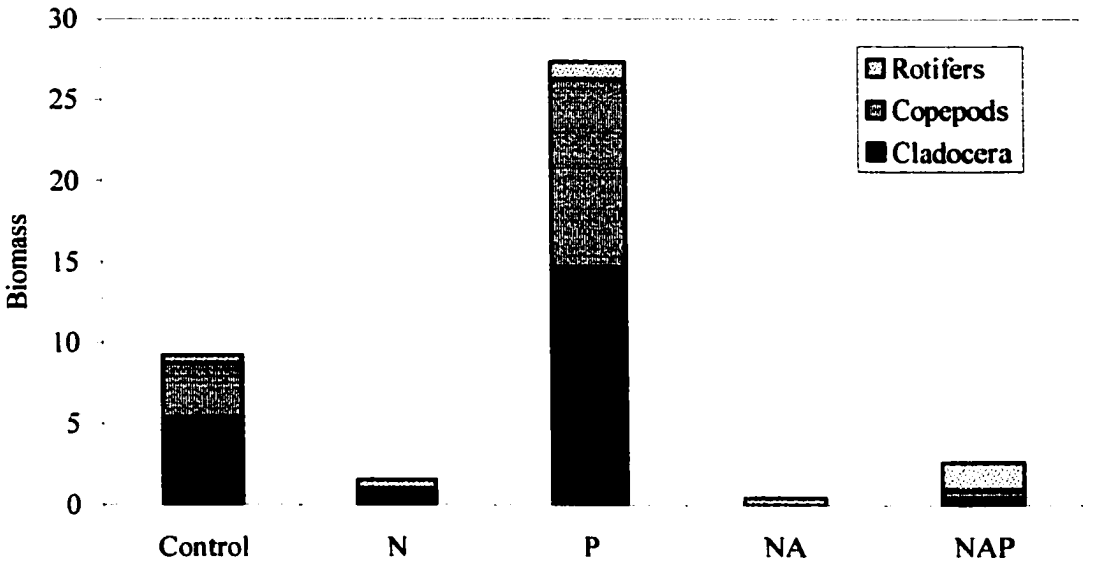


Figure 4.5. Biomass of zooplankton ($\mu g \cdot L^{-1}$) and major zooplankton groups for Shelf Lake 4 on the final sampling date.

CONCLUSIONS

The preceding chapters detail our efforts to understand how nitrogen (N) deposition affects ecological structure and function in Rocky Mountain lakes. We used lake surveys to explore relationships of benthic invertebrates and phytoplankton to N and regional environmental gradients, and a series of experiments to assess the effects of N alone and in conjunction with other stressors on several taxonomic groups, lakes and aquatic habitats. While findings of each phase of our dissertation work are summarized elsewhere (see Chapter 1), some conclusions may be drawn from the project as a whole.

First, we found that the potential for N deposition to cause eutrophication is likely limited to lakes that currently have nitrate-N concentrations at or near detection limits (i.e., ≤ 0.005 mg·l^{-1}). Small-scale N enrichment bioassays in Loch Vale watershed (Colorado Front Range), where ambient surface water nitrate-N may reach 0.2-0.3 mg·l^{-1}, caused neither increases in phytoplankton biomass nor shifts in species composition unless P was also added (see Chapter 1, Nydick 2002). In contrast, additions of 1 mg l^{-1} nitrate-N to mesocosms in two low-nitrate Wyoming Snowy Range lakes altered phytoplankton species composition and caused marked increases in algal biomass and productivity without additional P.

Two lake surveys indicated that these experimental patterns were regionally applicable. Lakes surveyed on the east side of the Colorado Front Range had relatively high nitrate concentrations and, like those of Loch Vale watershed, would be unlikely to show a eutrophication response to future inputs of N (Chapter 2). Lakes surveyed in the Wyoming Snowy Range, on the other hand, tended to have lower nitrate concentrations and low DIN:TP ratios indicative of N limitation or N+P co-limitation. These lakes

likely *would* respond to increased N deposition and undergo eutrophication effects similar to those noted in our mesocosm N-addition experiments.

While the effects of additions of N alone varied seasonally and among lakes, our work showed that additions of N and P *together* consistently induced eutrophication responses despite differences in experiment duration, scale or lake type. We found that cases of phytoplankton limitation by P alone were rare and that cases of N limitation tended to be short-lived and followed by N+P co-limitation at longer time scales. Additionally, we documented a high proportion of N+P co-limited lakes in our spring survey of Snowy Range lakes. Effects of N and P on algal growth and composition thus appear to be highly interactive in mountain lakes, and efforts to monitor future atmospheric deposition of both N *and* P are critical.

Effects of nutrients and acid appear to be equally interactive, as indicated by our mesocosm enrichment/acidification experiments in the Colorado Front Range and Wyoming Snowy Range (Chapter 4, Lafrançois 2002 and Chapter 3, Nydick 2002). We found that addition of limiting nutrients (N or N+P in the Snowy Range lake and N+P in the Front Range lake) caused increases in phytoplankton productivity and biomass and altered species composition as described above. Addition of limiting nutrients plus acid, however, caused similar changes in productivity and biomass but altered phytoplankton species composition in a manner different from that of nutrients alone. We concluded that productivity and biomass relate directly to the availability of limiting nutrients, whereas species composition is interactively affected by both nutrients and pH.

Furthermore, we noted that the most dramatic changes in all measured parameters occurred following additions of nutrients *and* acid in both study lakes; greater attention to multiple interacting stressors in mountain lakes is thus warranted.

Throughout our work we assessed effects of N and other stressors on benthic versus pelagic habitats, community versus ecosystem properties, and different types of biota. In general, we found benthic habitats to be more heterogeneous and less responsive to nutrients or acidification than pelagic habitats. There was no discernable relationship between benthic invertebrate composition and lake nitrate concentration among lakes surveyed in eastern Rocky Mountain National Park, and responses of benthic algae to nutrients and acidification in short-term mesocosm experiments were negligible compared with those of phytoplankton. Secondly, we found that coherence between community and ecosystem responses was common but not universal in our studies. In general, biomass or productivity changes were accompanied by changes in species diversity or community structure; however, changes in phytoplankton biomass and composition were not tightly linked in our seasonal bioassay experiments in Rocky Mountain National Park (Chapter 1, Nydick 2002), and changes in diversity were not always consistent with changes in biomass or productivity in our nutrient/acidification experiments. Finally, we noted differences in the relative sensitivity of phytoplankton, benthic algae and zooplankton to our experimental manipulations. In general phytoplankton responded more strongly and consistently to nutrients and nutrients+acid than benthic algae or zooplankton, and appear to be superior indicators of short-term chemical stress in mountain lakes. Nonetheless, the importance of benthic habitats as

structural and functional responses to N and other stressors, and learn from one another's area of expertise. Without question, the net result of our joint labors is a more complete and insightful understanding of the effects of N deposition on mountain lakes than either of us could have accomplished individually. Given the complex nature of ecological questions, it may be in the best interest of both science and society to foster more such collaborations among graduate students.

REFERENCES

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