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CONSUMER-DRIVEN NUTRIENT RECYCLING IN ARCTIC ALASKAN LAKES:
CONTROLS, IMPORTANCE FOR PRIMARY PRODUCTIVITY,
AND INFLUENCE ON NUTRIENT LIMITATION

by

Cody R. Johnson

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Ecology

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2009

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ABSTRACT

Consumer-Driven Nutrient Recycling in Arctic Alaskan Lakes: Controls, Importance for Primary Productivity, and Influence on Nutrient Limitation

by

Cody Ryan Johnson, Doctor of Philosophy

Utah State University, 2009

Major Professor: Chris Luecke
Department: Watershed Sciences

In lakes, fish and zooplankton can be both sources and sinks of nitrogen (N) and phosphorus (P) through the consumption of organic N and P, and subsequent excretion of bioavailable inorganic forms. These source/sink dynamics, known as consumer-driven nutrient recycling (CNR), may, in turn, control the availability of potentially limiting nutrients for algal primary production. In this dissertation I investigate the importance and controls of CNR as a source of inorganic N and P for primary production (Chapter 2). I then examine zooplankton CNR as a mechanism for increasing nutrient mean resident time (MRT) in the mixed layer of lakes (Chapter 3). Finally, I assess whether zooplankton communities dominated by different taxa can affect N versus P deficient conditions for phytoplankton production through differential N and P recycling rates (Chapter 4). Direct excretion of N and P by fish communities was modest in arctic lakes, and accounted for < 4 % of the N and P required for primary production. Recycling of N

and P by zooplankton communities was relatively high, and the fraction of algal N and P demand supplied by zooplankton CNR ranged from 4 – 90% for N and 7 – 107% for P. MRT of ^{15}N , measured in the mixed layer of an arctic lake, was ~16 days, compared to 14 days predicted by a ecosystem model simulation with zooplankton N recycling and 8 days in a model simulation where zooplankton N recycling was absent. The 75% increase in N MRT between model simulations with and without zooplankton recycling suggests that zooplankton N recycling is an important mechanism for retaining N in lake ecosystems. I observed relatively high negative correlations between precipitation and phytoplankton N ($r = -0.33$) and P ($r = -0.30$) deficiencies. I also observed a significant positive correlation ($r = 0.42$, $p = 0.03$) between zooplankton communities with higher copepod biomass, relative to cladoceran biomass, and phytoplankton N-deficient conditions. These results suggest that when precipitation is high N and P deficiency is low in the phytoplankton. When precipitation is low, however, zooplankton communities composed primarily of copepods contribute to N-deficient conditions for phytoplankton production

DEDICATION

I dedicate this work to my father, Richard Johnson, who taught me both the value of pursuing an education and that there is a solution to every problem.

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The help provided by the staff at Toolik Field Station was crucial for conducting my research. Christie Haupert, the Toolik Field Station environmental data manager, collected valuable climate data that I used in my research. She also assisted me with field research, and made sure that key pieces of scientific equipment at Toolik Field Station were always in top working order. Andrew Balsler, Lael Rogan, and Jason Stuckey provided GIS analyses that were incredibly helpful for accomplishing research goals. The science support provided by Scott Houghton, Theresa Trans, Shelby Bakken, and Jorge Noguera made it possible for me to focus on research, while they developed logistic solutions for working at a remote field station.

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CHAPTER 1

BASIS FOR THE QUANTIFICATION OF CONSUMER-DRIVEN NUTRIENT RECYCLING IN ARCTIC LAKES

In arctic lakes biogeochemical processes, such as primary productivity and community respiration, are highly adapted to the extreme environmental fluctuations in temperature and precipitation from the summer to winter months. The arctic region is currently experiencing unprecedented environmental impacts from a changing global climate. One impact of climate change predicted for arctic lake ecosystems is a change in nutrient availability as new precipitation regimes and warmer average air temperatures alter watershed nutrient loading and rates of terrestrial nutrient mineralization and uptake. In order to appreciate the potential changes of biogeochemical processes in arctic lakes, a better understanding of the current sources and sinks of limiting nutrients, as well as drivers of nutrient source/sink dynamics, must be developed. Through my research I addressed the importance of nutrient recycling by fish and zooplankton as a potential source of inorganic nitrogen (N) and phosphorus (P) for phytoplankton production in arctic Alaskan lakes. In addition I investigated whether the presence of a fish community in a lake exerts an important control over nutrient recycling by zooplankton consumer groups. I also examined the importance of nutrient recycling by zooplankton as a mechanism for nutrient retention in the upper mixed layer of lakes. And finally, I examined the relationship between differential recycling of N and P by zooplankton communities and the availability of N and P for phytoplankton production.

Consumers can regulate nutrient availability for primary producers (*e.g.*, Kitchell et al. 1979, Lehman 1980), and in freshwater ecosystems research in this area has focused

primarily on N and P (Vanni 2002). By feeding on lower trophic levels, consumers convert organic N and P into labile inorganic forms. As such consumers can either directly affect N and P availability through nutrient excretion, or indirectly by controlling rates of nutrient excretion from lower trophic levels via predation (Vanni and Layne 1997, Elser and Urabe 1999).

Recycled inorganic nutrients can either be excreted in the same location as the organic nutrients were consumed, or translocated, when organic nutrients are consumed in one location and inorganic nutrients excreted in another (Vanni 2002). The source location of organic nutrients for consumers, and where consumers excreted inorganic forms can have different effects on N and P availability (Vanni et al. 2005). Consumers, such as fish, are capable of moving between habitat boundaries (*e.g.*, benthic and pelagic) (Schindler and Scheuerell 2002), or against directional gradients (*e.g.*, upstream migration) (Schindler et al. 2003). This movement can translocate, or supply 'new' nutrients to a system, and create hotspots of biological activity (McIntyre et al. 2008). However, for this flux of nutrients to be important for the entire system, the mass of translocated N and P must be relatively high compared to other nutrient sources.

In contrast, nutrient recycling in the same location (*e.g.*, the epilimnion of a lake) by consumers may function to impede nutrient loss from a system (Vanni 2002). Or, conversely, the differential conversion of N versus P into new tissue may sequester labile inorganic N and P, and reduce the availability of these nutrients for primary production (Elser et al. 1988). The differential conversion of N and P into new tissue is based on the stoichiometric imbalance between a consumer's demand for N and P for growth, and the

N and P content of its food resources (Sterner et al. 1992). Ecological stoichiometry theory predicts that the variability in the N:P ratio of excreted nutrients can be explained by the difference between a consumers N:P and that of its prey (Sterner and Elser 2002). Thus, a consumer with a relatively high N demand (high N:P) ratio feeding on a relatively low N food resource (low N:P) will superfluously excrete P while retaining N for growth (Elser et al. 2000).

Nutrient recycling by consumer groups has been shown to support a significant fraction of the nutrient demand for primary production across many freshwater ecosystems (Vanni 2002). Controls of consumer nutrient recycling as a nutrient source, and the implications for phytoplankton nutrient availability, however, have rarely been tested at the landscape level for both N and P (Kitchell et al. 1999). My dissertation examines excretion of N and P by consumer groups in several arctic lakes, and seeks to quantify the role consumer nutrient recycling as: 1) a source of bioavailable N and P for phytoplankton production; 2) a driver of differential N versus P water column nutrient deficiency; and 3) a mechanisms for retaining N and P in epilimnetic waters in lake ecosystems. The overall aim of my dissertation research was to quantify the role of nutrient recycling by fish and zooplankton communities as a source of N and P for phytoplankton production. In addition, through my research I attempted to elucidate the importance consumer nutrient recycling at the landscape level, and gain a better understanding of an unexplored nutrient resource in these systems.

My research was conducted in lakes near Toolik Field Station (68°38'N 149°38'W) located in the northern piedmont region of the Brooks mountain range in

arctic Alaska. This research was part of the larger Geomorphic Trophic Hypothesis (GTH) project investigating landscape controls of the trophic structure in these lakes (Hershey et al. 1999). The central tenet of the GTH project is that landscape geomorphology controls the distribution of fish in arctic Alaskan lakes (Hershey et al. 2006), and that fish top-predators control the trophic structure of lake food webs (Hershey et al. 1999). My research expanded on this project by examining how differences in lake trophic structure affect biogeochemical cycling, and ultimately nutrient availability for phytoplankton production.

In my second chapter I quantified the N and P recycling rates by fish and zooplankton consumer groups and determined if these were significant fluxes of nutrients in arctic lake ecosystems. The importance of nutrient recycling by fish and zooplankton in lake nutrient budgets is highly variable between different systems (Vanni 2002), yet this potential nutrient source has not been quantified in arctic Alaskan lakes. Nutrient concentrations in these lakes are characteristically low, and often near the limits of detection (Kling et al. 1992). In addition, phytoplankton production in arctic Alaskan lakes is often nutrient limited by N, P, or co-limited by N and P (Levine and Whalen 2001). Due to the ultra-oligotrophic conditions found in arctic Alaskan lakes, N and P recycling by fish and zooplankton has the potential to be a large, yet unexplored, source of nutrients for phytoplankton production.

In addition, my second chapter examined the potential for fish to control the rates of N and P recycled by zooplankton in my study lakes. Because the presence or absence of a fish community is predictable in lakes near Toolik Field Station (Hershey et al.

2006), understanding how fish control nutrient recycling by lower trophic levels can increase our knowledge of consumer nutrient recycling across the landscape. There are two primary mechanisms by which fish can control nutrient recycling rates by zooplankton (Vanni and Layne 1997). First, fish predation can reduce total zooplankton biomass in a lake, and thus reduce the amount of N and P recycled from this consumer group (Vanni 2002). Second, size selective predation by fish can eliminate larger zooplankton species, and result in zooplankton communities comprised of smaller individuals (Brooks and Dodson 1965). Mass-specific excretion rates are higher for smaller organisms, and a zooplankton community consisting of small bodied species would have higher rates of N and P excretion compared to zooplankton communities with larger individuals (Vanni and Layne 1997). I tested both of these mechanisms in a suite of study lakes near Toolik Field Station.

In my third chapter I used a stable isotope tracer approach to directly measure seston uptake of N excreted by zooplankton. Isotopic tracers have been used in many ecosystem experiments to calculate rates of nutrient transformation (Mulholland et al. 2004), however, to the best of our knowledge a stable ^{15}N isotope tracer has not been used to track the excretion and subsequent uptake of enriched N from zooplankton communities. I conducted a mesocosm experiment using zooplankton from a lake that had been experimentally enriched with ^{15}N during the summers of 2005 – 2008 as part of the Geomorphic Trophic Hypothesis project. In 2007 Lake NE-12 was enriched with 80.9 g of 99% $^{15}\text{NH}_4\text{Cl}$. The $\delta^{15}\text{N}$ of the zooplankton community was subsequently enriched by ~ 175 (‰). I used the zooplankton from Lake NE-12 as the source of the ^{15}N

tracer in a mesocosm field experiment, and measured the ^{15}N enrichment of the seston. From the mesocosm experiment I calculated zooplankton N excretion and seston N uptake rates, and used these rates to model N retention in the upper mixed layer of Lake NE-12 with and without zooplankton excretion. The output from these simulations was compared to the observed ^{15}N decay from the upper mixed layer of Lake NE-12 during the summer of 2007. The observed mean residence time of ^{15}N in the upper mixed layer was ~16 days, compared to 14 day predicted by the model simulation with zooplankton N recycling and 8 days in the model simulation where zooplankton N recycling was absent. The simulation with zooplankton recycling resembled the observed mean residence time of ^{15}N in the NE-12 upper mixed layer during the summer growing season, and suggests that zooplankton N recycling is an important mechanism for retaining N in lake ecosystems.

For my fourth chapter I examined the relationship between zooplankton community composition, specifically the ratio of copepod to cladocerans, and both N and P deficiency in arctic Alaskan lakes. Consumers differentially recycle N and P based on the stoichiometric imbalance between the nutrient composition of food resources and consumer-specific nutrient demands for new tissue (Sterner and Elser 2002). Copepods and cladocerans are two broad taxonomic groups of zooplankton with considerably different demands for N and P, as illustrated by the large differences in the N:P ratio (copepods ≈ 50 , cladocerans ≈ 14) of their tissues (Elser and Urabe 1999). If nutrient recycling by zooplankton is a considerable fraction of the nutrient demand for phytoplankton production then the differential recycling of N and P by distinctive

zooplankton communities may drive lakes toward more N or P limitation of primary production.

The continued observation of N-limitation in freshwater ecosystems is beginning to replace the long-standing paradigm that lakes are P-limited (Lewis Jr. and Wurtsbaugh 2008). Yet clear mechanisms for N-limitation of phytoplankton production are still under investigation. Schindler (1977) proposed that N-fixing organisms would become abundant, and thus alleviate N-limitation, when N concentrations were depleted. Alternatively, Elser et al. (1988) proposed that differential nutrient recycling by consumer groups may selectively sequester N or P, and drive lakes toward more N or P-limitation. Copepods have a relatively high N demand, as indicated by the relatively high tissue N:P ratio. If copepods excrete P at relatively higher rates than N, then lakes with copepod-dominated zooplankton communities would be expected to have higher occurrences of N-limitation for phytoplankton production.

Phytoplankton production in arctic Alaskan lakes is frequently N-limited (Levine and Whalen 2001), yet measured rates of N-fixation are relatively high in these lakes (Gettel 2006). In addition, zooplankton communities in lakes near Toolik Field Station are often typified by high abundances of small copepod species (O'Brien et al. 2004), but the relationship between zooplankton and nutrient limitation of phytoplankton production has not been explored in these lakes.

During the summers of 2007 and 2008 I analyzed N versus P deficiency in a series of lakes using two phytoplankton physiological bioassays, ammonium enhancement response and alkaline phosphatase activity. I then compared the respective

nutrient deficiencies in these lakes to the ratio of copepod:cladoceran biomass in each of these lakes. Both N and P deficiencies showed negative relationships with precipitation, suggesting that when runoff is relatively high phytoplankton nutrient stress can be alleviated. However, there was a positive correlation between the ratio of copepod to cladoceran biomass in a zooplankton community and N-deficiency. This suggests that when copepod biomass is relatively high, P is recycled faster by zooplankton communities while N is sequestered as new tissue, thus contributing to N-deficient conditions for phytoplankton production.

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CHAPTER 2

IMPORTANCE OF NITROGEN AND PHOSPHORUS EXCRETION BY FISH AND ZOOPLANKTON TO PHYTOPLANKTON PRODUCTION IN ARCTIC ALASKAN LAKES¹**Summary**

- 1) Nutrient recycling by freshwater organisms can be a large source of nitrogen and phosphorus required for phytoplankton production. Yet this potential nutrient source has not been quantified in arctic lakes, nor have the controls of nutrient recycling been examined in these systems
- 2) We investigated whether nitrogen and phosphorus excretion by both fish and zooplankton communities was an important inorganic nutrient resource for phytoplankton production in arctic lakes. In addition we tested 3 potential mechanisms of fish control over nutrients recycled by fish and zooplankton consumer groups: 1) direct excretion of nitrogen and phosphorus from fish communities; 2) reduction in zooplankton nitrogen and phosphorus excretion due to lower zooplankton biomass from fish predation; and 3) higher mass-specific nutrient excretion rates from smaller zooplankton resulting from size selective predation by fish.
- 3) Rates of nitrogen and phosphorus excretion by fish communities were small relative to phytoplankton nutrient demand during summer periods. Zooplankton excretion, however, supplied between 4 - 90% and 7 - 107% of the nitrogen and phosphorus required for phytoplankton primary production. Our results indicated that total zooplankton biomass was ~75% lower in lakes with fish. However, rates of

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nitrogen and phosphorus recycling by zooplankton communities in lakes with fish were similar to nutrient recycling rates in lakes where fish were absent.

4) Our research supports compensatory fish controls over nutrient excretion by zooplankton communities. Conversely, we found little evidence that direct excretion of nitrogen and phosphorus by fish was a significant source of nutrient for phytoplankton production.

Introduction

Phytoplankton production in surface waters of arctic lakes are typically nutrient limited by nitrogen (N), phosphorus (P), or co-limited by N and P during the summer growing season (Levine & Whalen, 2001). Bioavailable N and P for phytoplankton production is scarce in these lakes, and concentrations of these nutrients are often at or below detection limits (Kling *et al.*, 1992). Even though nutrient concentrations are very low in arctic Alaskan lakes, there is substantial inter-lake variation in phytoplankton production (Kling *et al.*, 2000). What drives the variability in phytoplankton production, however, has remained unresolved in these lakes. Quantifying different sources of bioavailable N and P in these nutrient-poor environments has the potential to elucidate the controls of lake primary productivity across the arctic landscape.

Currently, our understanding of arctic lake nutrient budgets focuses on the surrounding watershed as an external source of N and P. Nutrients are delivered to lakes during high runoff events such as spring snowmelt (Whalen and Cornwell, 1985), or summer storm events (MacIntyre *et al.*, 2006). However, the extent of winter snow pack and subsequent spring runoff can be inconsistent from year to year, and frequency and

intensity of storm events can have considerable inter and intra-annual variability. During low runoff or low precipitation years, watershed nutrient loading to arctic lakes decreases rapidly as terrestrial plants increase their nutrient uptake (Shaver *et al.*, 1992). As a result, sources of N and P internal to lakes may become increasingly important for phytoplankton production as external sources attenuate during periods of low watershed nutrient loading.

Internal sources include nutrient recycling and remineralization from the sediments. If these processes occur in the upper mixed layer, nutrients are immediately available to phytoplankton. However, if they occur at the sediment-water interface or in the hypolimnion, they will only be available to support growth by phytoplankton if they are mixed vertically into the upper mixed layer (MacIntyre & Melack, 1995).

In lakes, consumer-driven nutrient recycling (CNR) (Elser and Urabe, 1999) is an internal source of N and P, and refers to the consumption of organic nutrients and subsequent excretion of bioavailable inorganic nutrients by higher trophic levels, such as fish and zooplankton (Vanni, 2002). CNR by pelagic fish and zooplankton has the potential to provide nutrients directly to the upper mixed layer. However, whether CNR is an important supplement of N and P for phytoplankton production (Schindler *et al.*, 1993), or comprises only a minor portion of lake nutrient budgets (Sarnelle and Knapp, 2005) is highly variable between systems. The two most common ways of assessing the importance of CNR for phytoplankton production at the lake level are to first quantify the rates of nutrient recycling by consumer groups (*i.e.* fish and zooplankton) and compare these to either rates of nutrient supply from other sources or rates of nutrient uptake

during primary production (Vanni, 2002). Because the common factor in both of these methods is the quantification of consumer recycling rates, understanding the controls of these rates may elucidate the drivers of variability in CNR importance between systems.

The presence of a fish community in a lake may be an important driver of consumer recycling rates, and there are three primary mechanisms by which fish may control CNR (Vanni and Layne, 1997). Figure 2.1 shows a conceptual diagram of these three mechanisms and their potential impacts on the importance of CNR for phytoplankton production. First, N and P excreted directly by fish communities may contribute significantly to phytoplankton nutrient demand (Vanni Layne and Arnott, 1997). If rates of N and P recycling by a fish community are relatively high, then overall lake estimates of CNR will be high as well. Furthermore, CNR by fish may be a source of 'new' nutrients for pelagic primary production if a portion of fish energy resources is derived from benthic or terrestrial habitats (Vander Zanden and Vadeboncoeur, 2002; Mehner *et al.*, 2005).

Second, fish may lower rates of CNR in a lake by reducing the biomass of lower trophic levels, such as zooplankton, through predation. This mechanism may be 2-fold in that lower zooplankton biomass may result in lower recycling rates of N and P from this consumer group (but see mechanism 3 below), and can also increase phytoplankton biomass, and thus phytoplankton demand for N and P, through cascading trophic interactions (Carpenter and Kitchell, 1988).

Finally, fish may have the reciprocal effect by increasing mass-specific nutrient recycling rates from zooplankton communities through size selective predation (Vanni *et*

al., 1997). In lake pelagic food webs the presence of visually feeding top predators, such as fish, can selectively remove larger zooplankton species, and are often associated with zooplankton communities that are dominated by smaller individuals (Brooks and Dodson, 1965; Carpenter and Kitchell, 1993). Mass-specific rates of nutrient recycling are faster for smaller organisms compared to larger ones based on the allometric relationship between metabolism and body size (Peters, 1983). Therefore, lake zooplankton communities consisting of predominantly smaller organisms may provide a larger internal nutrient source by recycling N and P at faster rates than communities consisting of larger organisms.

The importance of CNR as a nutrient source for phytoplankton production has not been quantified in arctic Alaskan lakes, nor has the hypothesis been tested that fish can control CNR by the manipulation of lower trophic levels. The presence and composition of fish communities can be accurately predicted in these lakes using landscape variables (Hershey *et al.*, 2006), and fish can play an important roll in arctic lake trophic structure (Hershey *et al.*, 1999). Fish feed heavily from the lake benthos (Sierszen McDonald and Jensen, 2003), and can limit the size (Merrick Hershey and McDonald, 1992) and community composition (Goyke and Hershey, 1992) of benthic organisms. However, the impacts of fish predation on pelagic zooplankton communities are less clear (O'Brien *et al.*, 2004, O'Brien Buchanan and Haney, 1979). Therefore our objectives were to: 1) quantify the excretion of N and P from zooplankton and fish communities in arctic Alaskan lakes; 2) determine if the presence of a fish community controlled the community composition and subsequent nutrient excretion by pelagic zooplankton; and

3) calculate the proportion of phytoplankton N and P demand met by zooplankton and fish excretion.

Methods

Site Description

Our research was conducted in lakes near Toolik Field Station (68°38'00"N, 149°36'15"W), site of the Arctic Long Term Ecological Research (LTER) project, in the northern piedmont region of the Brooks Mountain range, arctic Alaska. This area is underlain by continuous permafrost and consists of lakes that are typically shallow (depth between 3 and 20 m) moraine dammed or kettle basins. The lakes near Toolik Field Station are typically dimictic and become thermally stratified during the summer months, between late June and late August, at depths between 4 and 10 m (Arctic LTER database). Shallow lakes (maximum depth < 4 m) may be polymictic, and vertically mix on a diel basis or during storm events (MacIntyre *et al.*, 2006). In addition, lakes in this region are characteristically ultra-oligotrophic with chlorophyll *a* concentrations typically below 5 µg L⁻¹, and concentrations of inorganic N and P near the limits of detection (Arctic LTER database).

Fish species richness is low in these lakes due to geographical barriers to colonization in the south from the Brooks Mountain Range and to the north from the Arctic Ocean (Hershey *et al.*, 1999). Pelagic mesozooplankton species richness is also relatively low, with only 7 common species identified in these lakes (O'Brien *et al.*, 2004). Of these 7 species there are 3 copepod taxa, 2 relatively small species *Cyclops scutifer* and *Diaptomus pribilofensis*, and 1 large invertebrate predator *Heterocope*

septentrionalis (O'Brien *et al.*, 1979). Common cladoceran taxa in lake zooplankton communities consist of 2 relatively large species *Daphnia middendorffiana* and *Holopedium gibberum*, and 2 relatively small species *Daphnia longiremis* and *Bosmina longirostris* (O'Brien *et al.*, 1979).

We selected a series of six lakes that were in close proximity to Toolik Field Station (Figure 2.2). We used these lakes to calculate the contribution of N and P recycled by higher lake trophic levels, and to determine if there were differences in CNR between fish and fishless systems. Three of our study lakes contained resident fish populations (NE-12, GTH 86, Fog-2), and 3 of the lakes were fishless (GTH 114, GTH 99, E-4,) (Table 2.1).

We calculated the contribution of consumer nutrient recycling to arctic lake nutrient budgets by: 1) determining the biomass of the major fish and zooplankton consumer groups in arctic lake food webs; 2) calculating N and P excretion rates from each of these consumer groups; and 3) comparing N and P excreted by consumer groups to phytoplankton nutrient demand.

Fish Biomass

We determined fish biomass in lakes NE-12, GTH 86, and Fog-2 by first estimating fish abundance, using standard mark-recapture techniques with multiple sampling events. We captured fish using a combination of angling, gill nets, and ice fishing. Gill nets were used to capture arctic grayling (*Thymallus arcticus*) and lake trout (*Salvelinus namaycush*), and deployed for a ~0.5 hr intervals to minimize fish mortality. Shoreline angling was used to capture arctic grayling and lake trout during these

intervals. Arctic char (*Salvelinus alpinus*) are difficult to catch using previously described techniques, and we captured fish in Lake Fog-2 by angling under the ice in early spring. Regardless of technique, when fish were captured they were weighed (g), measured (mm), marked using a passive integrated transponder (PIT) tag, and released back into the lake. We used the Schnabel method for calculating fish abundance from multiple mark-recaptures trials within a study period using the following equation:

$$\hat{N} = \frac{\sum_i (C_i M_i)}{\sum_i R_i + 1} \quad \text{Equation 1.}$$

where \hat{N} = the population estimate of fish within a lake, C_i = the number of fish captured during a trial, M_i = the number of marked fish at the time of the trial, and R_i = the number of marked fish captured during the trial (Krebs, 1999). Following our determination of fish abundance, we calculated fish biomass concentration (g m^{-3}) by multiplying the population estimates (\hat{N}) by the average dry weight (g) of each species in each study lake, and dividing by the lake volume (m^3). For our estimate of average individual dry weight we used the mean wet weight of fish captured during our mark-recapture studies in each lake, and assumed that dry mass was 20% of wet mass (Ricker, 1968).

Zooplankton Biomass

We collected zooplankton samples for biomass calculation in early July, following lake stratification when zooplankton biomass and community composition is relatively stable, during the summer of 2008 in both fish and fishless lakes, and all lakes were sampled within a 5-day time period. Zooplankton were collected by taking 3 replicate tows from each lake with an 80- μm mesh plankton net from ~1 m off the

bottom, in the deepest location, to the surface. We immediately preserved zooplankton samples in sugar buffered Lugol's solution and returned them to Toolik Field Station for biomass analysis.

We calculated the zooplankton biomass in each tow by first measuring the length of 10 individuals from each taxon using the ocular micrometer in a dissecting microscope, and then counting the remaining zooplankton in the sample. We converted the length of each measured individual (mm) into mass (g) using length weight regressions for each species (Burkart, 2007). Mean biomass of the 10 measured individuals from each taxa was used as the average individual biomass in each respective population. We then calculated zooplankton biomass concentration (g m^{-3}) for each taxa by multiplying average individual mass by total individuals in the sample and dividing by the tow volume (Wetzel and Likens, 2000).

Fish Excretion Rates

We quantified fish N and P recycling by empirically measuring *in situ* excretion rates of NH_4^+ -N and soluble reactive phosphorus (SRP) for three fish taxa common in arctic Alaskan lakes; arctic grayling, lake trout, and arctic char. Due to logistic constraints, we only measured fish excretion rates for arctic grayling and lake trout in Toolik Lake, and excretion rates for arctic char were only measured in Lake Fog-2. Because we were only able to measure arctic grayling and lake trout excretion rates in Toolik Lake, we needed to make the assumption that measured fish excretion rates were applicable to arctic grayling and lake trout in Lake NE-12. We feel this assumption is valid due to the similarities in epilimnetic water temperatures between these two lakes

(Arctic LTER database), and the consistency in fish diets between arctic Alaskan lakes (Sierszen *et al.*, 2003).

We calculated mass-specific excretion rates from arctic grayling and lake trout in Toolik Lake during the 2005-2007 summers, and arctic char from Lake Fog-2 in 2008. We collected arctic grayling and lake trout using 5 panel experimental gill nets, set for approximately 15-minute intervals, thus minimizing the time fish spent entangled in the net. Arctic char are difficult to catch with gill nets, and we used fyke nets to capture fish in Lake Fog-2. Regardless of capture method, excretion rate measurements were identical for all fish taxa.

Prior to setting our nets we filled 10 L sealable Ziploc® plastic bags with 8 L of surface water that had been strained through 80- μm Nitex mesh to remove meso-zooplankton. Bags were kept in a lightproof cooler to reduce warming and phytoplankton nutrient uptake. When fish were captured they were immediately placed in a pre-filled bag, and incubated in the lake for ~0.5 to 1 hr. Bags were suspended from a surface float, and incubated at ambient epilimnetic water temperatures, usually between 10 and 15 °C.

For all of our nutrient excretion measurements we had both treatment (containing fish) and control (no fish) bags, and an initial (T_0) and final (T_1) sampling event. We took an initial (T_0) nutrient sample from each control bag prior to the incubation, and collected at final (T_1) sample from both treatment and control bags post incubation. We attributed the change between T_1 and T_0 nutrient concentrations in control bags to processes unrelated to fish excretion (*e.g.* photosynthetic uptake or microbial mineralization), and

subtracted the difference from the change in nutrient concentrations of bags containing fish (see nutrient analysis section). In 2006 we measured background NH_4^+ and SRP concentrations by collecting an initial T_0 sample from both treatment and control bags. However, in 2005, 2007, and 2008 we used the T_0 sample collected only from the control bags as a measurement of background NH_4^+ and SRP concentrations, and did not collect T_0 samples from treatment bags.

We collected all T_0 and T_1 nutrient samples in treatment and control bags by filling a 140 cm^3 syringe with water from the respective bag, purging the initial draw, and forcing a second syringe full of water through a 0.45 μm pore size sealed syringe filter into an acid washed high density polyethylene (HDPE) bottle. Bottles were placed in a cooler for less than 3 hours, then returned to Toolik Field Station and frozen until analyzed for nutrient concentrations (see below).

Zooplankton Excretion Rates

We measured zooplankton NH_4^+ and SRP excretion rates experimentally using temperature controlled incubation chambers at Toolik Field Station. As with fish, experimentally measured zooplankton excretion rates collected at one lake were assumed to be valid for other lakes in this study. During the summer of 2007 we measured excretion rates for four meso-zooplankton taxa common to lakes near Toolik Field Station. We used Lake S-11, near Toolik Field Station, as the sampling site because it contains relatively high abundances of the small copepods, *D. pribilofensis* and *C. scutifer*; the large predaceous copepod, *H. septentrionalis*; and the large herbivorous cladoceran, *D. middendorffiana*. We collected zooplankton by taking six replicate tows

from the deepest part of the lake (~10 m) to the surface using a 80- μ m mesh size plankton net. Zooplankton tows were combined in a single 1 L low-density polyethylene (LDPE) bottle and immediately returned to Toolik Field Station.

At Toolik Field Station we separated zooplankton into different treatments by size fractionation. Initially we strained the sample through 500- μ m mesh size Nitex bolting cloth to remove large bodied *D. middendorffiana* and *H. septentrionalis* from small bodied *D. pribilofensis* and *C. scutifer*. *D. middendorffiana* and *H. septentrionalis* were rinsed back into ~1 L of water and divided into two separate treatments using a Folsom plankton splitting wheel. We then visually separated the *D. middendorffiana* individuals from the *H. septentrionalis* treatment and vice versa using a pair of fine tip forceps, thus minimizing the handling of experimental organisms in each treatment. *D. pribilofensis* and *C. scutifer* were too small to separate visually without using a microscope, and handling time to separate these two species would have resulted in high mortality. Therefore we combined these two taxa as one treatment and collected them by straining the remaining sample, post removal of *D. middendorffiana* and *H. septentrionalis*, through 153 μ m mesh size Nitex bolting cloth. Once separated into treatments (hereafter Copepods, Daphnia, and Heterocope), we initially divided each treatment into four replicates using a Folsom plankton splitting wheel. Two replicates from the Heterocope treatment and one repatment were lost during handling, and we had $n = 3, 4,$ and 2 replicates for the Copepod, Cladoceran, and Heterocope treatments respectively. Replicates from each treatment were placed into 250 ml volume LDPE bottles filled with 200 ml of surface water from Toolik Lake that had been previously strained through 80-

μm Nitex mesh. Incubations began < 0.5 hr. following zooplankton collection from the field, and thus zooplankton digestive tracks were representative of natural conditions. We incubated bottles in a temperature controlled facility at Toolik Field Station for ~ 0.5 hr at 15°C for the small copepod and *Daphnia* treatments, however due to logistic constraints the *Heterocope* treatment was incubated at 19°C .

As with fish excretion experiments we had both treatment (containing zooplankton) and control (no zooplankton) bottles and 2 sampling events, and initial T_0 and final T_1 . We took T_0 nutrient samples from control bottles, and assumed they represented background concentrations of NH_4^+ and SRP in treatment as well. We attributed the changes between control bottle T_0 and T_1 concentrations of NH_4^+ and SRP as due to processes unrelated to zooplankton excretion and subtracted the difference from treatment bottles (Equation 2).

Nutrient Analysis

In 2005 and 2006, we analyzed concentrations of NH_4^+ fluorometrically (Holmes *et al.*, 1999) on a Turner Designs 10-AU field fluorometer, and SRP concentrations colorimetrically (Strickland and Parsons, 1972) on a Shimadzu UV-Mini 1240 spectrophotometer at Toolik Field Station. In 2007 and 2008, however, we shipped our frozen nutrient samples to Utah State University, and NH_4^+ and SRP concentrations were analyzed on a Astoria-Pacific 300 series flow injection analyzer following methods outlined in (Eaton *et al.*, 2005).

For all fish and zooplankton nutrient samples, we calculated mass-specific NH_4^+ -N or SRP-P excretion rates using the following equation:

$$E_M = \frac{((C_{treatment} - C_{background}) - \Delta C_{control}) * V_c}{t \times m} \quad \text{Equation 2.}$$

where E_M equals mass-specific excretion rates of either NH_4^+ -N or SRP-P ($\mu\text{g g}^{-1} \text{d}^{-1}$) for each fish or zooplankton treatment, $C_{treatment}$ are the concentrations ($\mu\text{g L}^{-1}$) of NH_4^+ -N or SRP-P in fish or zooplankton treatments at T_1 , $C_{background}$ is the T_0 background concentrations ($\mu\text{g L}^{-1}$) of NH_4^+ -N or SRP-P, $\Delta C_{control}$ is the change in NH_4^+ -N or SRP-P concentrations in control samples without fish or zooplankton, V_c is the volume of water in the incubation container (liters), t is the duration of the incubation (days), and m is the dry mass (g) of either fish or zooplankton in the respective treatment. We calculated mean mass-specific excretion rates for each consumer group using individual fish as replicates (arctic grayling $n = 31$, lake trout $n = 15$, and arctic char $n = 2$), and replicates for zooplankton treatment groups (Copepod $n = 3$, Cladoceran $n = 4$, and Heterocope $n = 2$). Next, we determined lake estimates of fish and zooplankton NH_4^+ -N or SRP-P excretion rates ($\mu\text{g L}^{-1} \text{d}^{-1}$) following the equation:

$$E_L = \frac{E_M \times B}{1000} \quad \text{Equation 3.}$$

where E_L equals lake NH_4^+ -N or SRP-P recycling rates ($\mu\text{g L}^{-1} \text{d}^{-1}$) by either fish or zooplankton communities, E_M is the mean mass-specific excretion rate (Equation 2) for each fish or zooplankton treatment, and B equals the total biomass concentration (g m^{-3}) of fish and/or zooplankton in a respective lake.

Consumer Excretion and Phytoplankton Demand

Because quantifying the rates of nutrient supply by all potential sources to lakes is very difficult, we determined the importance of CNR for phytoplankton production by comparing rates of CNR by fish and zooplankton to rates of N and P uptake during primary productivity (Vanni, 2002). For this method to be valid it must be assumed that all excreted N and P is used for phytoplankton production. We were comfortable using this method because: a) lakes in arctic Alaska are ultra-oligotrophic, and concentrations of inorganic N and P are often at or below detection limits (Kling *et al.*, 1992); and b) lakes in this region are nutrient limited with a high frequency of N and P co-limitation (Levine and Whalen, 2001).

We used lake-specific estimates of NH_4^+ -N or SRP-P excretion rates by fish and zooplankton communities as a measure of consumer nutrient recycling in each of our study lakes. We compared these rates of consumer nutrient recycling to estimates of phytoplankton N and P demand in each lake, to determine the proportion of phytoplankton production that could be supported by consumer nutrient recycling. We used rates of pelagic primary productivity ($\mu\text{gC L}^{-1} \text{d}^{-1}$) that had been previously measured using radioisotope (^{14}C) uptake methods in each of our study lakes as estimates of phytoplankton N and P demand (Evans *unpublished data*, Whalen *et al.*, 2006, Whalen *unpublished data*). All volumetric primary production measurements were sampled at several depths, and incubated for 24 h under natural light and temperature conditions for the sample depth (Wetzel and Likens, 2000). Volumetric production measurements were extrapolated to whole lake carbon uptake by multiplying each point measurement by the

volume of its depth segment and summing across depth segments. Water column carbon uptake was converted to nutrient demand by assuming that phytoplankton mass C:N:P ratios were close to the Redfield ratio by mass (g) of 41:7.2:1. To calculate the percent of phytoplankton nutrient demand potentially supported by CNR in our study lakes, we divided the rates of NH_4^+ -N or SRP-P recycled by fish and/or zooplankton in each lake by the estimates of N and P required for observed rates of phytoplankton production.

Statistical Analyses

We used an analysis of variance (ANOVA) to compare the differences in total zooplankton biomass between lakes with fish and fishless lakes. We also analyzed the difference in the average size of individual zooplankton in both fish and fishless lakes using ANOVA. For this analysis we took the mean length (mm) of individual zooplankton and weighted the mean by the density (individuals L^{-1}) of each taxa in our six study lakes. In comparisons of both total zooplankton biomass and mean length of individuals we used our study lakes as replicates and compared the means from our 3 fish lakes (NE-12, GTH 86, Fog-2) to the means of 3 fishless lakes (E-4, GTH 114, GTH 99) (Table 2.1). We also quantified the zooplankton community composition in each study lake to determine if the presence of a fish community shifted the zooplankton communities to copepod compared to cladoceran dominance. We analyzed zooplankton community composition in each study lake by comparing the percentage of each species biomass from the total zooplankton biomass in each study lake. We graphed the percent of individual taxa present in each study lake and qualitatively determined the differences

between fish and fishless lakes. All statistical analyses were performed using SAS statistical program JMP® 7.0.1.

Results

Consumer Biomass and Community Composition

The fish abundance estimates were higher in lakes NE-12 and Fog-2 compared to GTH 86 (Figure 2.3a). However, total fish biomass concentrations (g m^{-3}) were similar, and had overlapping 95% confidence intervals, in all three of our study lakes with fish (Figure 2.3b). There were trends toward lower zooplankton biomass and lower individual zooplankton lengths in lakes with fish (Figure 2.4). However with only three replicate lakes in each category we had low statistical power, and these trends were not statistically significant ($p > 0.05$ *d.f.* 5) between lakes with and without fish for both analyses of total zooplankton biomass and mean length of zooplankton. Total zooplankton biomass was lower in all three lakes with fish communities compared to those without (Table 2.2). The average zooplankton biomass was 0.13 g m^{-3} in lakes without fish compared to only 0.04 g m^{-3} in lakes where fish were present, an almost 4-fold difference between fish and fishless lakes (Figure 2.4), though high variance made this result non-significant. In general zooplankton communities were composed of smaller individuals in lakes with fish, and the mean length of individuals, weighted by density, was 0.8 mm in fish lakes compared to 1.2 mm in lakes without fish (Figure 2.4).

The presence of a fish community also had an effect on zooplankton community composition. In general, lakes with fish had higher abundances of the two small bodied copepod species *C. scutifer* and *D. pribilofensis*, while lakes without fish had higher

abundances of the larger cladoceran taxa *D. middendorffiana* and *H. gibberum* (Figure 2.5). Fish did not completely eliminate larger zooplankton taxa, however, and we found relatively high abundances of the typically larger species *D. middendorffiana*, *H. gibberum*, and *H. septentrionalis* in two of our lakes with fish, Fog-2 and GTH 86 (Figure 2.5). In addition, *D. pribilofensis* had relatively high abundances in two fishless lakes GTH 114 and E-4 (Figure 2.5).

Consumer Excretion Rates

Rates of fish and zooplankton NH_4^+ -N or SRP-P excretion showed a negative relationship with organism mass (Figure 2.6) consistent with allometric growth and metabolism. Major zooplankton taxa demonstrated higher mass-specific excretion rates for both N and P compared to fish (Figure 2.6). Additionally, smaller copepods had higher mass-specific excretion rates compared to larger *Daphnia* (Figure 2.6). The somewhat higher excretion rates of N and P measured for *Heterocope* might be the result of higher incubation temperature for the *Heterocope* treatment.

Consumer Excretion and Phytoplankton Uptake

Fish excretion of N and P was low and contributed minimally to phytoplankton nutrient demand in our study lakes. Fish supplied between <1% and ~3% of both N and P demand in our 3 study lakes with fish populations (Table 2.2). In contrast, zooplankton excretion of N and P was greater than fish excretion by over an order of magnitude in the six study lakes (Figure 2.7). Nutrient excretion by zooplankton showed the potential to be a large source of N and P for phytoplankton production, but also showed higher

variability between lakes (Figure 2.7). Among lakes with fish populations, zooplankton nutrient recycling in lake NE-12 and Fog-2 could supply ~90% and ~36% of N demand, as well as ~107% and 50% of P demand for phytoplankton production, respectively, but accounted for only ~7% and ~10% of phytoplankton N and P demand in lake GTH 86 (Table 2.2). In the fishless lakes, E-4 zooplankton excretion was a smaller source of N and P compared to phytoplankton demand, supporting ~4% and 7% of N and P demand respectively. However, zooplankton N and P excretion was higher in two other fishless lakes, GTH 114 and GTH 99, and had the potential to supply ~17% and ~31% of phytoplankton N demand and ~26% and ~70% of P demand in these lakes (Table 2.2).

Discussion

We tested 3 potential mechanisms by which fish communities could control CNR as an internal source of nitrogen and phosphorus for phytoplankton production in arctic lakes. We found the first mechanism, direct excretion of nitrogen and phosphorus by fish communities, to be an insignificant source of nutrients for primary production. Even though the biomass of fish and the biomass of zooplankton were comparable in study lakes with fish populations (Table 2.2), excretion rates were over an order of magnitude lower for fish than for zooplankton in these lakes (Figure 2.7).

Our research supported both the second and third mechanisms (Figure 2.1), and showed that fish communities have large, but opposite, effects on CNR by: a) lowering CNR from zooplankton communities through a reduction in total zooplankton biomass; and b) increasing CNR from zooplankton communities through higher mass-specific excretion rates. Although differences in zooplankton biomass were not statistically

significant in our assessment of 6 lakes, the 4-fold difference in biomass we report is similar to statistically significant differences found in surveys of zooplankton biomass from larger numbers of lakes (O'Brien *et al.*, 2004). The lower crustacean zooplankton biomass sampled in lakes with fish, coupled with the higher mass-specific excretion rates measured in our experiments resulted in our conclusion that rates of CRN were similar for both fish and fishless lakes (Figure 2.7).

The large bodied cladoceran *D. middendorffiana* comprised >90% of the total zooplankton biomass in the fishless lake GTH 99 (Figure 2.5), and as such mass-specific excretion from the zooplankton community in GTH 99 would have been the lowest (Figure 2.6). However, total zooplankton biomass in GTH 99 was ~2x greater than the lake with the next highest total zooplankton biomass (Lake E-4), and between ~6 and 12x greater than any of the lakes with fish (Table 2.2). As a result, total nitrogen and phosphorus excretion rates were the highest in Lake GTH 99 (Figure 2.7).

The importance of CNR showed high variability between our study lakes, and this variability was primarily due to differences in rates of phytoplankton primary productivity (Table 2.2). It is possible that these differences arise, in part, because we did not measure phytoplankton nutrient demand coincident with our estimates of zooplankton biomass and community composition. Inter-lake and temporal variability of primary production in arctic Alaskan lakes requires finer resolution to precisely quantify the importance of consumer nutrient recycling with these lakes. The variability we observed in our measurements of phytoplankton primary production was likely the result of bioavailable N and P supplied by other sources.

Two of our study lakes, GTH 86 and E-4, had relatively high phytoplankton production compared to CNR (Table 2.2), and it is likely that these lakes receive relatively large nutrient inputs in addition to CNR. Lake GTH 86 has a relatively large watershed area compared to the surface area of the lake (Table 2.1), and also has 2 continuously running inlet streams, one draining a lake higher in the watershed. We did not measure watershed nutrient loading in our study lakes. It is likely, however, that GTH 86 receives significant nutrient inputs from the two inlet streams, which may stimulate the relatively high phytoplankton productivity observed in this lake. Lake E-4 is relatively shallow it is likely polymictic, thus upwelling of nutrients from the sediment water interface into surface waters could account for the relatively high rates of primary production in this lake. GTH 99 is also a relatively shallow lake, but has a surface area that is ~6x smaller than E-4 (Table 2.1) and is sheltered by the topography of the surrounding landscape. As such the potential for wind to mix GTH 99 completely is much less than for E-4.

We showed that CNR by zooplankton communities may be a substantial internal nutrient source in lakes both with and without fish, and potentially supplies up to 90% of N and 107% of P required for sustaining phytoplankton production through the summer growing season in arctic lakes (Figure 2.7). Vanni (2002) summarized nutrient excretion experiments for fish and zooplankton in several lake systems. Zooplankton nutrient excretion rates varied from ~1.5 to ~25 ($\text{mg N m}^{-2} \text{d}^{-1}$) and 0.07 to ~5 ($\text{mg P m}^{-2} \text{d}^{-1}$), supporting between 0.5 to 160% for N and 1 to 58% for P of phytoplankton nutrient demands (Vanni 2002). Our measurements of zooplankton nutrient excretion and

percentage of phytoplankton nutrient demand supported by zooplankton excretion were towards the high end of the wide range reported in Vanni (2002). It should be noted, however, that our study did not include nutrient recycling by microzooplankton or protozoa, and may be an underestimate of the importance of CNR in these lakes. In addition we found as much variability in the percentage of phytoplankton demand supported by zooplankton CNR in our six study lakes, located in the same geographic region, as Vanni (2002) reported between lakes located across vast geographic distances.

Nutrient budgets for arctic lakes have focused primarily on external sources of N and P from the surrounding watershed, and CNR has not previously been quantified in these lakes. Our research suggests that CNR can be an important driver of phytoplankton productivity during the summer, and that nutrient recycling within the water column needs to be considered in whole lake nutrient budgets. By investigating lakes both with and without fish we were able to compare 3 separate mechanisms for fish control of CNR in our study lakes. We found strong support for 2 of these mechanisms, with reciprocal effects on the importance of CNR. Our study showed that the increase in mass-specific excretion rate from zooplankton communities with smaller individuals (mechanism 3) offsets the decrease of nutrient excretion rates from lower total zooplankton biomass in lakes with fish (mechanisms 2) (Figure 2.1). Because these 2 mechanisms have a compensatory relationship our research showed the highest rates of zooplankton CNR from a lake where total zooplankton biomass was one of the lowest observed (Table 2.2).

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Table 2.1. Study lake depth, surface area, volume, watershed area, watershed to lake surface area (W:L) ratio, and fish community

Lake	Maximum Depth (m)	Surface Area (m²)	Volume (m³)	Watershed Area (m²)	W:L	Fish Community
Fog-2	20	56,500	469,000	460,000	8.1	arctic char
NE-12	17	74,500	566,000	1,250,000	16.8	arctic grayling, lake trout
GTH 86	10	34,000	119,500	1,400,000	41.2	arctic grayling
GTH 114	6	39,500	87,000	592,000	6.8	none
GTH 99	4	7,000	14,500	130,000	18.6	none
E-4	4	39,500	82,500	461,000	5.6	none

Table 2.2. Consumer biomass, primary productivity, phytoplankton nutrient demand, consumer nutrient excretion rates, and percent of nutrient demand supplied by each consumer group for our 6 study lakes. * from Evans *unpublished data*, † from Whalen *unpublished data*, ‡ from Whalen et al. 2006.

Lake	Consumer	Biomass (g m ⁻³)	Primary Productivity (µgC L ⁻¹ d ⁻¹)	Phytoplankton Nutrient Demand (µg L ⁻¹ d ⁻¹)		Consumer Nutrient Excretion (µg L ⁻¹ d ⁻¹)		% of Nutrient Demand	
				Nitrogen	Phosphorus	Nitrogen	Phosphorus	Nitrogen	Phosphorus
NE-12	Fish	0.038	8.79 [‡]	1.55	0.21	0.052	0.006	3.3	2.6
	Zooplankton	0.043						90.7	107.4
GTH 86	Fish	0.025	137.07 [‡]	24.09	3.34	0.044	0.005	<1	<1
	Zooplankton	0.038						6.8	9.7
Fog-2	Fish	0.019	7.07 [*]	1.24	0.17	0.014	0.002	1.1	1.2
	Zooplankton	0.022						35.7	49.5
GTH 114	Fish	----	52.80 [†]	9.28	1.29	----	----	----	----
	Zooplankton	0.050						17.3	26.5
E-4	Fish	----	264.24 [‡]	46.44	6.44	----	----	----	----
	Zooplankton	0.129						3.9	6.8
GTH 99	Fish	----	45.60 [‡]	8.01	1.11	----	----	----	----
	Zooplankton	0.239						2.52	0.77

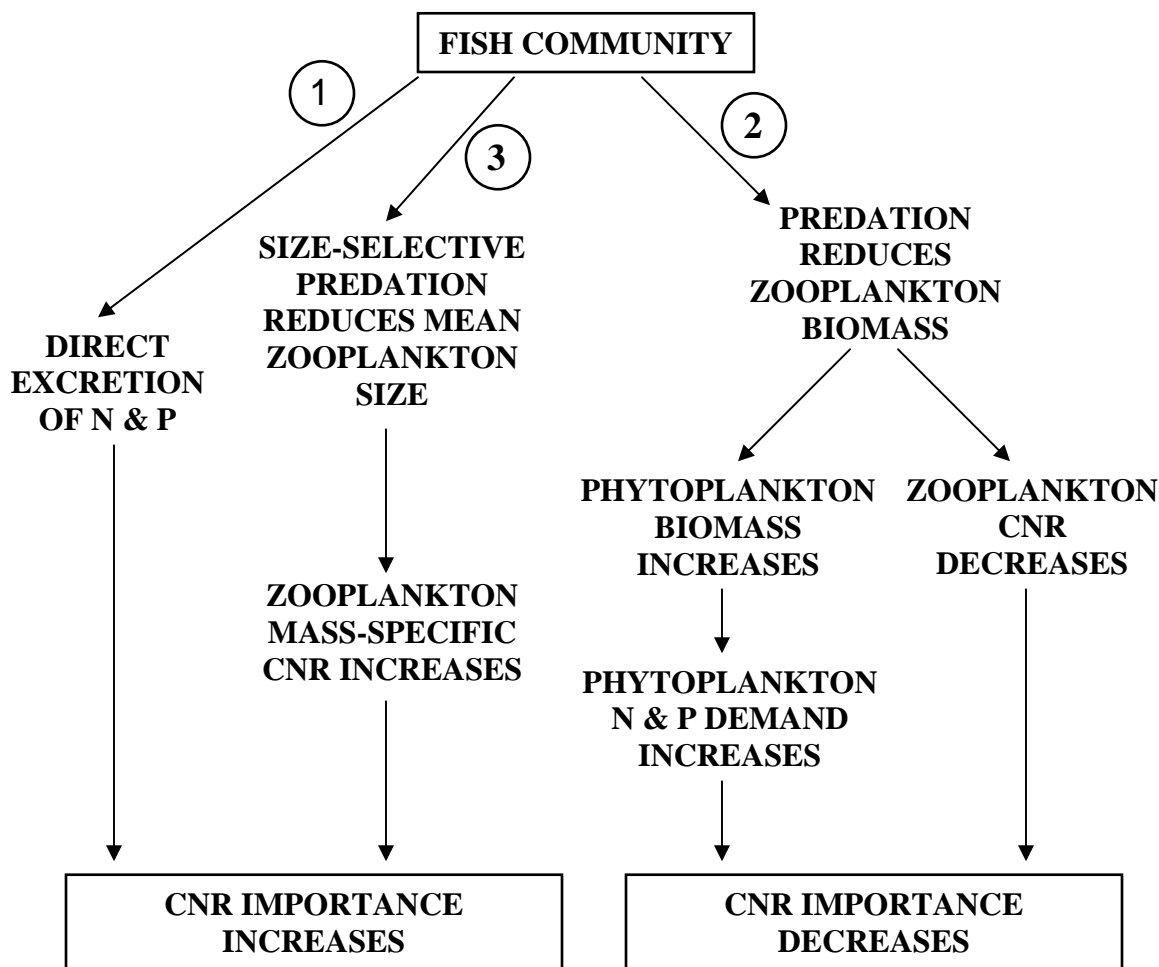


Figure 2.1. Conceptual diagram of potential fish controls of consumer-driven nutrient recycling in lakes. Numbers 1, 2 and 3 correspond with the order mechanisms were presented. Importance of CNR is derived by comparing rates of nutrient excretion to rates of nutrient uptake during primary production. Diagram was modified from Figure 1 in (Vanni and Layne, 1997).

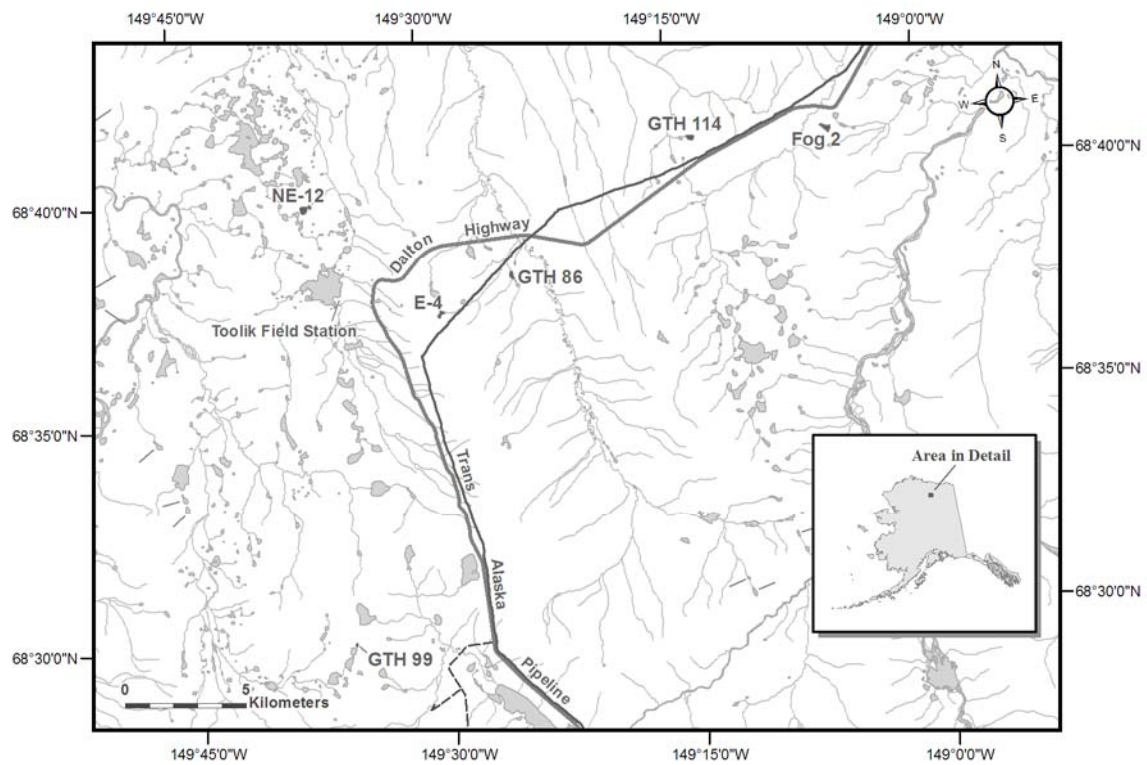


Figure 2.2. Research area and location of study lakes in relation to Toolik Field Station, arctic Alaska.

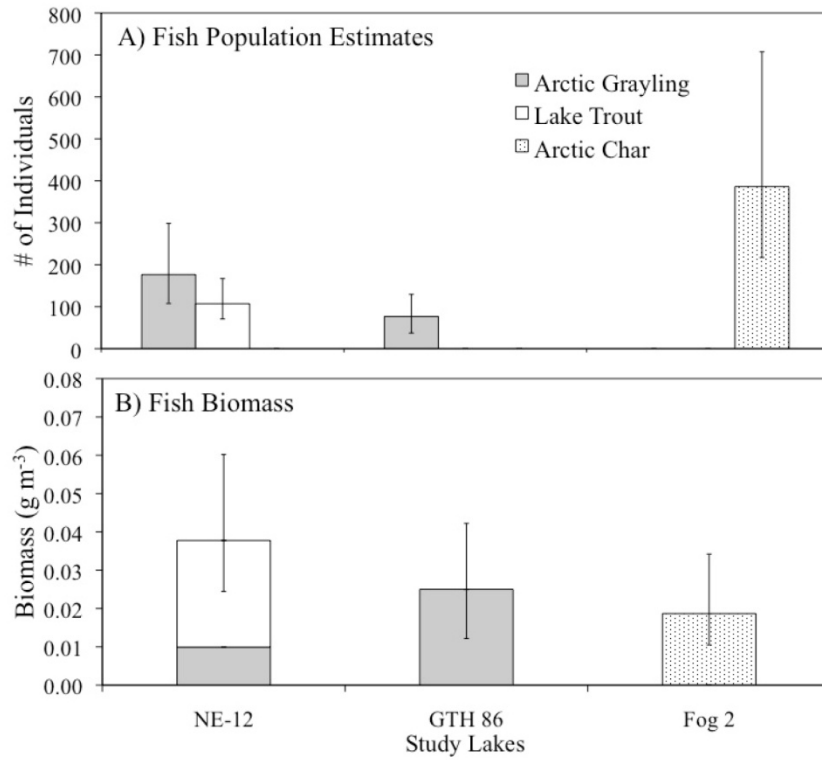


Figure 2.3. Estimates of fish abundance (a) and total fish biomass (b) in 3 study lakes containing fish populations. Error bars are 95% confidence intervals.

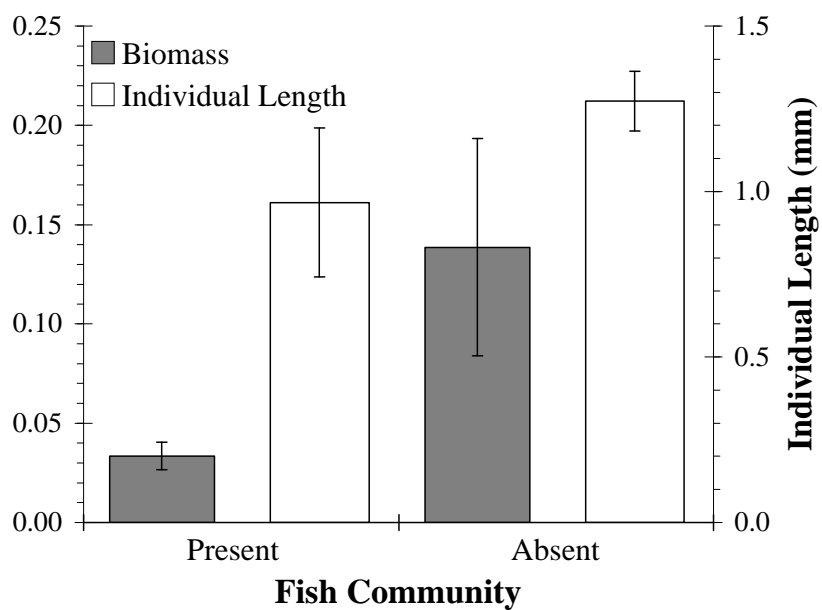


Figure 2.4. Difference in total zooplankton biomass (shaded bars) and mean zooplankton length, weighted by zooplankton density, (open bars) between lakes where fish are present and absent. Differences were not statistically significant ($p > 0.05$ *d.f.* 5), but show trends toward lower values in lakes where fish were present. Error bars are ± 1 standard error.

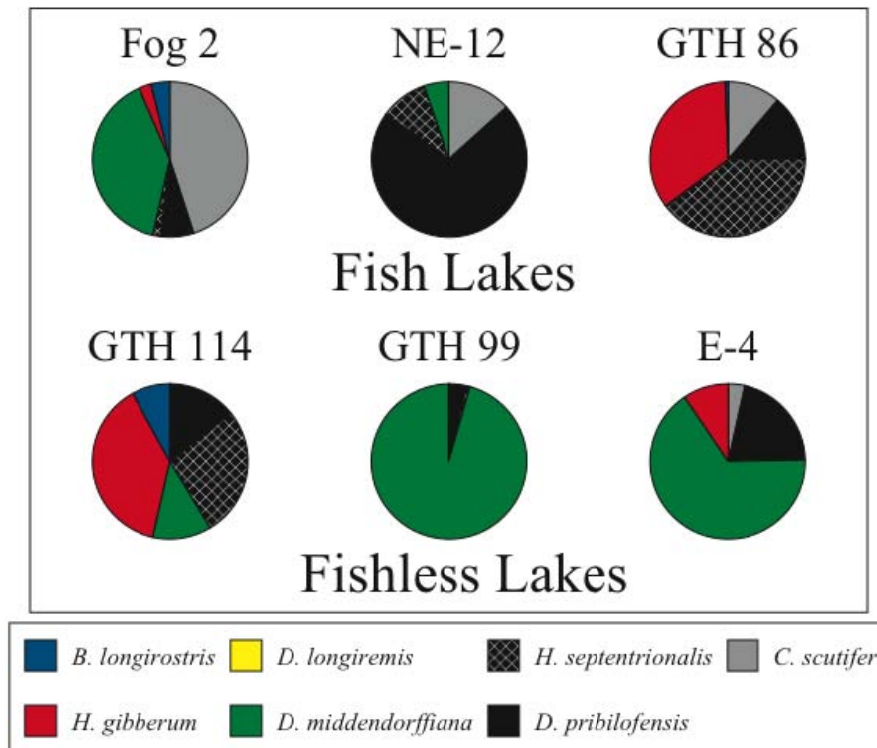


Figure 2.5. Zooplankton community composition in fish and fishless lakes. Wedges are percent biomass of individual taxa. Black and gray wedges are copepod taxa and colored wedges are cladoceran taxa.

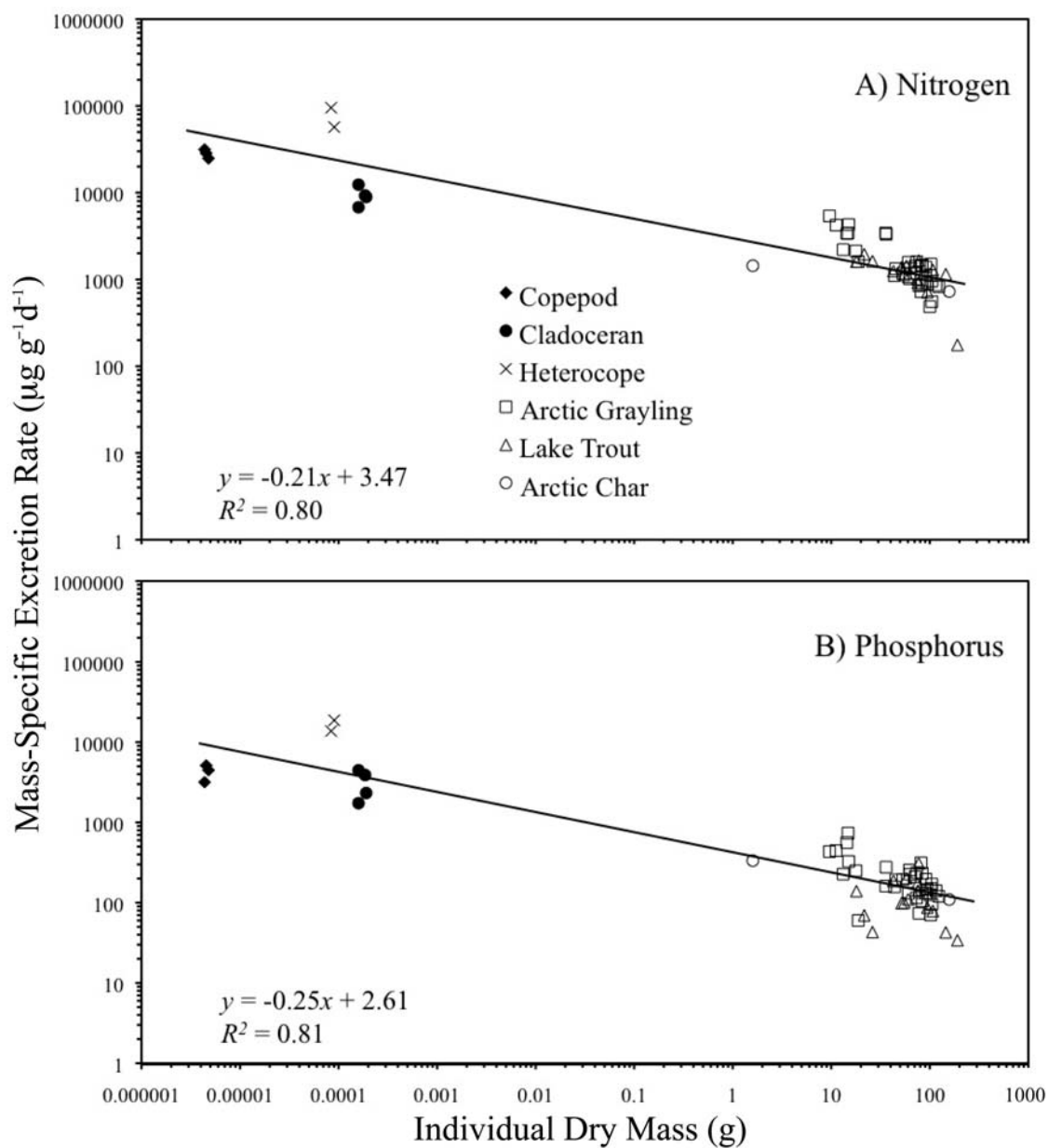


Figure 2.6. Log x log plot of mass-specific excretion rates ($\mu\text{g g}^{-1}\text{d}^{-1}$) of (A) $\text{NH}_4^+\text{-N}$ and (B) SRP-P by individual dry mass for fish and zooplankton consumer groups.

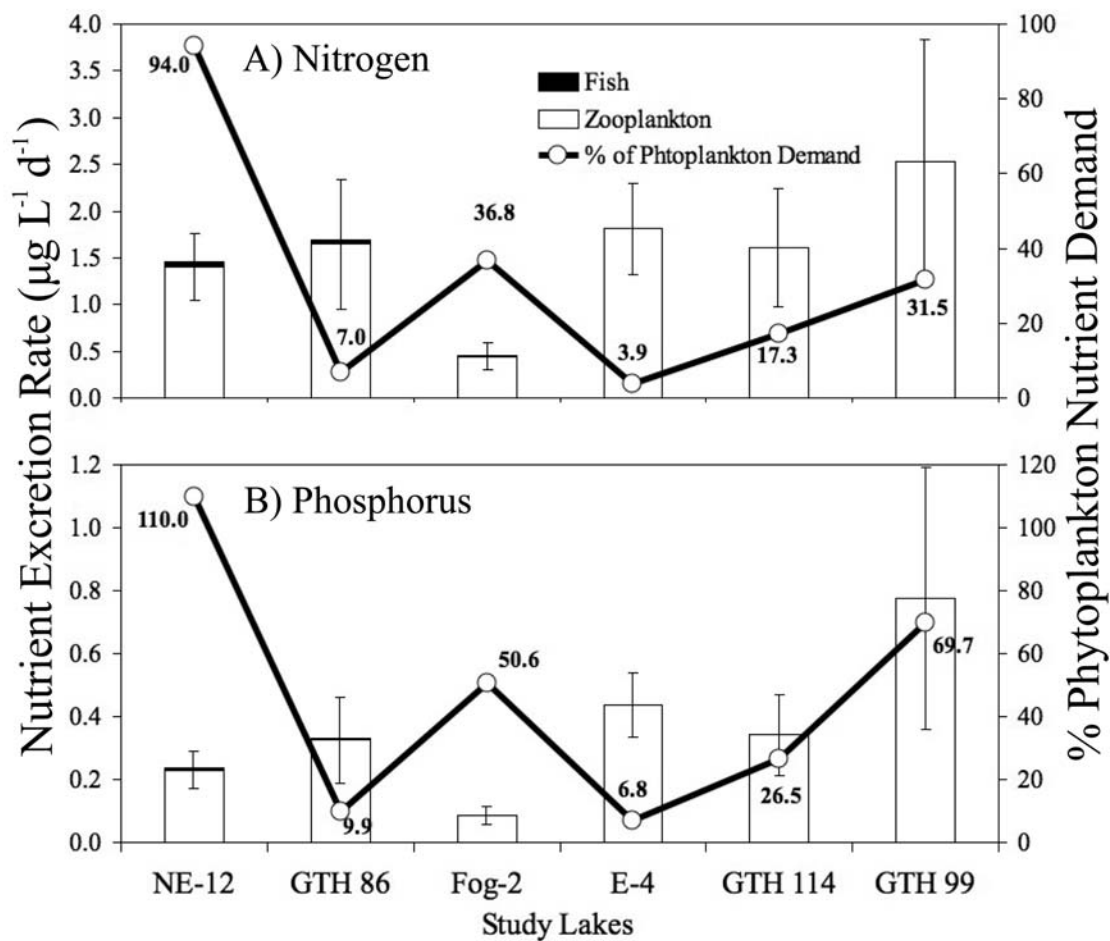


Figure 2.7. (A) NH₄⁺-N and (B) SRP-P excretion rates (μg g⁻¹ d⁻¹) for fish (black bars) and zooplankton (white bars) consumer groups compared to percent of phytoplankton nutrient demand (open circles) supplied by total consumer-driven nutrient recycling (fish + zooplankton).

CHAPTER 3

ZOOPLANKTON NITROGEN RECYCLING IMPEDES THE LOSS OF
EPIIMNETIC NITROGEN AS SHOWN USING A ^{15}N TRACER¹**Abstract**

We used an isotopic tracer, set up in a mesocosm field experiment, to calculate the mass flux of nitrogen excreted from zooplankton into seston biomass. Zooplankton for this experiment were taken from a lake that had been experimentally enriched with ^{15}N , and used as the source of our isotopic tracer. By measuring the enrichment of the seston pool, and assuming first order kinetics, we were able to calculate the loss rate of ^{15}N from the zooplankton pool and the rate of ^{15}N assimilation into seston biomass. Nitrogen loss rate from the zooplankton pool was $0.25 \text{ (d}^{-1}\text{)}$, and seston became isotopically enriched in ^{15}N showing a mean uptake rate of $0.81 \text{ (d}^{-1}\text{)}$. We used the rates of zooplankton nitrogen excretion and seston assimilation to build a model of nitrogen retention in the mixed layer of a small arctic lake. From the model we ran two simulations, with and without zooplankton excretion, and compared the mean residence time of mixed layer nitrogen in both simulations to the observed ^{15}N retention in the lake following the addition of $\sim 80 \text{ g}$ of 99% $^{15}\text{NH}_4\text{CL}$. The mean residence time of nitrogen in the model simulation with zooplankton nitrogen excretion was 14 days, and closely resembled the observed nitrogen mean residence time of 16 days in the mixed layer of the arctic lake during the summer of 2007. The mean residence time of nitrogen from our model simulation without zooplankton excretion was approximately 8 days, a 50% decrease compared to the simulation run with zooplankton excretion. This output from

¹ Coauthored by Cody R. Johnson, Chris Luecke, Anne E. Hershey, and Lindsey Pollard.

our model simulations provides evidence that zooplankton nitrogen recycling is an important mechanism for nitrogen retention in lakes.

Introduction

Higher trophic levels in aquatic systems recycle inorganic nutrients as products of metabolic waste that then become available for uptake by primary producers (Kitchell et al. 1979). However, whether or not these nutrients are a significant supplement for primary production (Brabrand et al. 1990; Sarnelle and Knapp 2005) or comprise only a small portion of nutrient budgets (Nakashima and Leggett 1980; Wurtsbaugh 2007) is still largely unresolved. The different conclusions from nutrient recycling studies conducted to date are often due to the variability in productivity rates and consumer communities between the systems under investigation, but a considerable driver of these discrepancies may be due to the methods by which ‘importance’ is determined.

Vanni (2002) summarized the 3 primary methods by which the importance of nutrient excreted by consumer groups to phytoplankton production has been assessed. Two of these methods use empirically measured nutrient excretion rates from consumer groups and compare them to either 1) nutrients supplied by other sources or 2) the nutrient demand by phytoplankton for primary production (Vanni 2002). A third approach is to experimentally isolate the effects of either fish or zooplankton consumer groups and measure changes in phytoplankton abundance, phytoplankton community composition, and/or concentrations of dissolved nutrients as primary response variables (Vanni and Layne 1997; Vanni et al. 1997). These 3 approaches have proved to be useful tools in understanding food web effects on phytoplankton production. An additional

function of nutrient recycling by consumers, not reviewed in Vanni (2002), may be to retain nutrients within a system, and thus be important to phytoplankton production by increasing the mean residence time of bioavailable nutrients.

Nutrients may be permanently lost from the upper mixed layer of lakes when sedimenting organic matter falls below a thermocline or is exported into outlet streams (Rydin et al. 2008). However, if organic matter is consumed and remineralized by zooplankton, the loss of bioavailable nutrients from lake surface waters may be impeded. Increasing the residence time of nutrients in surface waters allows for greater nutrient transformations, and can sustain lake primary production even when watershed nutrient inputs are minimal. Yet we know of no study that specifically considers zooplankton nutrient recycling as a mechanism for lake nutrient retention.

Isotopic tracers have been implemented in many ecological studies to give high-resolution measurements of nutrient transformation and uptake rates (Cole et al. 2006; Mulholland et al. 2004; Stark 2000). Isotopic tracers methods are advantageous because they directly measure the gross transfer rates of elements between various pools within an ecosystem. By experimentally enriching a pool with stable or radioactive isotopes the fate of products derived from that pool can be unambiguously quantified. This could provide additional insights into the importance of consumer-supplied nutrients by directly tracking nutrients excreted from consumer into phytoplankton pools.

In food web studies isotopic tracers have been used to quantify food resources for higher trophic levels (Vander Zanden and Vadeboncoeur 2002; Pace et al. 2004; Carpenter et al. 2005). Phytoplankton production rates have also been reliably

calculated by quantifying the fixation of radioactively labeled inorganic ^{14}C -carbon (Wetzel and Likens 2000). In addition, isotopic tracers have been used in metabolic studies to quantify the loss, or decay rate ($-k$), of nutrients from consumers giving direct measurements of nutrient excretion and tissue turnover rates (Bosley et al. 2002; He and Wang 2007) However, to the best of our knowledge isotopic tracers have not been used in aquatic ecosystems to simultaneously quantify nutrient excretion from consumer pools and subsequent phytoplankton uptake of excreted nutrients.

We used an isotopic tracer approach to assess the importance of N recycling by zooplankton as a mechanism for N retention in the upper mixed layer of lake ecosystems. To this end, our objectives were to: 1) quantify the rates of N loss from a zooplankton pool via excretion and N uptake by phytoplankton; and 2) Using the rates of N loss and uptake thus calculated, build an ecosystem model for the upper mixed layer of a lake to determine the importance of N recycling by a zooplankton community as a mechanism for retaining nutrients.

To accomplish our first objective, we designed a mesocosm experiment using zooplankton that were isotopically enriched ($\delta \sim 170 \text{‰}$) in ^{15}N as the source pool for a isotopic tracer. We then measured the subsequent enrichment of $\delta^{15}\text{N}$ in our mesocosm seston pool, and used a feed forward model (Chapra 1997) fit to our data to calculate zooplankton excretion and seston assimilation rates of ^{15}N . We then used the calculated zooplankton excretion and seston assimilation rates from our mesocosm experiment to build an ecosystem model of the upper mixed layer in Lake NE-12, a lake in arctic Alaska that was part of a whole-lake ^{15}N enrichment experiment. We parameterized our

model with data from NE-12, and: 1) tested our model output to see if it resembled the observed enrichment, and subsequent decay, of ^{15}N in the upper mixed layer of NE-12; and 2) ran a model simulation predicting the enrichment and decay of ^{15}N in the upper mixed layer of NE-12 when zooplankton N recycling was absent. The comparison of two model simulations (with and without zooplankton N recycling), along with the observed data in NE-12, allowed us to determine the impact of zooplankton nutrient recycling on the retention of N in the upper mixed layer of NE-12.

Methods

Study site

Lake NE-12 (68° 39.73' N., 149° 37.21' W.) is a relatively large (surface area = 74,500 m²), deep (maximum depth = 17 m, mean depth = 7.6 m), kettle basin lake, located ~2.5 km north of Toolik Lake in the northern piedmont region of the Brooks Mountain range, arctic Alaska. NE-12 is dimictic, and is typically thermally stratified, at a depth of ~ 4 m, from mid June, shortly after ice off, to early September. NE-12 is in a relatively sheltered basin, with an inlet and outlet streams on the eastern and western shores, respectively. Both the inlet and outlet can run continuously from the start of spring runoff through the summer growing season, but may dry up completely in July and August during dry years.

The upper mixed layer of Lake NE-12 has been experimentally enriched with a pulse of 99% $^{15}\text{NH}_4\text{Cl}$ during the early part of the summer growing season from the year 2005 to 2008. In 2007, 80.9 g of 99% $^{15}\text{N-NH}_4\text{Cl}$ was added to the upper mixed layer of Lake NE-12 on 26-Jun-2007. Following the enrichment, the biomass (g) and $\delta^{15}\text{N}$ (‰)

of zooplankton, seston, and organic material sedimenting out of the upper mixed layer was sampled approximately every seven days until the end of July.

Field sampling

We collected all zooplankton samples from Lake NE-12 by straining the water column from a depth of 15 m to the surface with a 30-cm diameter, 80- μ m mesh Wisconsin plankton net (WILDCO[®] Wildlife Supply Company). On each sampling day, following enrichment, three replicate zooplankton tows were collected for the calculation of zooplankton biomass. Zooplankton tows collected for biomass were rinsed into separate 250 ml low-density polyethylene (LDPE) bottles, and immediately preserved with sugar buffered Lugol's solution. We also collected samples for the analysis of zooplankton $\delta^{15}\text{N}$ by taking six zooplankton tows, and combining all of the tows into a single 1 L LDPE bottle. All zooplankton samples were returned to Toolik Field Station within 3 hrs of collection.

We calculated the zooplankton biomass in each tow by first measuring the length of 10 individuals from each taxon using the ocular micrometer in a dissecting microscope, and then counting the remaining zooplankton in the sample. We converted the length of each measured individual (mm) into mass (g) using species-specific length weight regressions (Burkart 2007). Mean biomass of the 10 measured individuals from each species was used as the average individual biomass in each respective population. We then calculated total zooplankton biomass (g) for each sample by multiplying average species-specific individual mass by total number of individuals from each species present in the sample, and summed the biomass of each species.

Zooplankton collected for analysis of $\delta^{15}\text{N}$ were separated from lake water, and placed in deionized water for ~ 1 hr. to allow the contents of their guts to be evacuated. Afterward, we separated zooplankton from the water by straining through 80- μm mesh bolting cloth. We separated zooplankton by species under a dissecting microscope, and encapsulated individuals of the same species in Costech 4 x 6 mm tin capsules. Trays of encapsulated zooplankton were sent to the UC Davis Stable Isotope Facility (<http://stableisotopefacility.ucdavis.edu>), where they were analyzed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ (‰), carbon (C) and N mass (μg), and C:N ratio on a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope mass spectrometer.

We measured the mass of N and the $\delta^{15}\text{N}$ in the seston from the upper mixed layer by collecting 4 L of water, from a depth of 1 m, in Lake NE-12. Water samples were kept in lightproof containers, and returned to Toolik Field Station within 3 hrs of collection. We filtered upper mixed layer water through 47 mm, pre-combusted GFF filters, recorded the volume filtered, then dried the filters at 60 °C for ~ 48 hrs. We sent all of our seston filters to the Colorado Plateau Stable Isotope Laboratory (CPSIL) located at Northern Arizona University (<http://www.mpcer.nau.edu/isotopelab>), where they were analyzed for $\delta^{15}\text{N}$ (‰) and total N mass (μg) on a Thermo-Finnigan Delta^{plus} Advantage mass spectrometer coupled with a Costech Analytical ECS4010 elemental analyzer.

We measured the net sedimentation rate (g d^{-1}) of ^{15}N from the upper mixed layer of NE-12 using a cluster of four sediment traps deployed 4 m below the surface of the water, at approximately the bottom of the mixed layer. Water from all four sediment

traps was collected ~ weekly, following the enrichment of Lake NE-12, coincident with the sampling of seston ^{15}N . Water from each sediment trap was forced through a 47 mm, pre-combusted, GFF filter, and dried at 60 °C for ~ 48 hrs. Filters were analyzed for $\delta^{15}\text{N}$ (‰) and mass N (μg) at CPSIL as described above.

Mesocosm field experiment

We conducted a mesocosm field experiment to calculate the rate constants for zooplankton excretion and seston assimilation of N. The mesocosm experiment was carried out from 13 to 24 July 2007 in a small pond (~ 1.5 m deep and ~ 900 m² surface area) located ~ 20 m from the southern shore Toolik Lake (68° 38' N, 149° 38' W). We used six 113 L opaque plastic trashcans as our mesocosms, and filled each mesocosm with 100 L of water from the pond. The water in each mesocosm was strained through 80- μm mesh bolting cloth to remove meso-zooplankton.

We used Lake NE-12 as the source of ^{15}N enriched zooplankton for the mesocosm field experiment. On 13-Jul-2007 we collected 3 sets of zooplankton samples from Lake NE-12 (described above). Zooplankton were collected: 1) as a source of ^{15}N for our mesocosm experiment, by taking 3 replicate vertical tows, and rinsing each tow into a separate 250 ml LDPE bottle; 2) to estimate the biomass of zooplankton added to each mesocosm, by taking three replicate vertical tows, rinsing each tow into a separate 250 ml LDPE bottle, then adding sugar buffered Lugol's solution to each bottle as a preservative; and 3) to determine the initial $\delta^{15}\text{N}$ of the zooplankton used in the mesocosm experiment, by taking six replicate vertical tows, and rinsing each vertical tow into a single 1 L LDPE bottle.

At Toolik Field Station we prepared the zooplankton treatments for use in the mesocosm experiment by first straining them from the lake water through 80- μm mesh bolting cloth. Zooplankton were immediately placed in clean bottles filled with deionized water for ~ 1 hr to allow them to evacuate the contents of their guts. We then exchanged the water by again straining out zooplankton using 80- μm mesh bolting cloth, and replaced them in deionized water in a clean bottle. After preparing the zooplankton treatments they were kept refrigerated for < 0.5 hr until added to the mesocosm experiment.

We used the mean zooplankton biomass from three replicate tows, preserved in sugar buffered Lugol's solution, as an estimate of the initial zooplankton biomass added to each mesocosm. Zooplankton collected for initial $\delta^{15}\text{N}$ analysis were prepared and sent to the UC Davis Stable Isotope Facility, following the procedure described above.

The experimental design of the mesocosm experiment consisted of two treatments, 'zooplankton' and 'no zooplankton', with three replicate mesocosms randomly assigned to each treatment. In all zooplankton treatment mesocosms we added zooplankton from Lake NE-12 that had been enriched with ^{15}N , while nothing was added to no zooplankton treatment mesocosms.

The six mesocosms were mounted in two rows of three to a floating frame that was anchored in the middle of the pond. The frame kept the top of the mesocosms above the surface of the pond, but suspended them at the level of the water in each container. Suspending the mesocosms in a small pond kept the water in each of our mesocosms at

the ambient temperature of the pond, and pond water temperature was measured intermittently throughout the duration of the experiment.

We collected initial (T_0) and final (T_1) 8 L water samples from all six mesocosms by filling two 4 L containers, by hand, from just below the surface of the water. The T_0 water samples were collected prior to adding the zooplankton treatments, and 8 L of pond water, strained through 80- μm mesh bolting cloth to remove meso-zooplankton, was added back to each mesocosm following the sample collection. After we collected the T_0 water sample, a bottle containing a ^{15}N -enriched zooplankton from Lake NE-12 was added to each zooplankton treatment mesocosm. We estimated from zooplankton tows taken in Lake NE-12 that each bottled contained ~ 17 mg of meso-zooplankton, for an estimated initial zooplankton biomass of 0.17 g m^{-3} in each mesocosm. This estimate was approximately 4 x higher than the observed ambient zooplankton biomass in Lake NE-12 on the same day zooplankton were collected for the mesocosm experiment. The volume of deionized water added with the zooplankton treatment was considered to be negligible compared to the volume of the mesocosm as a whole.

We allowed the experiment to run for 11 days untouched, and then collected the final T_1 samples from all mesocosms. Quantitative samples for final zooplankton biomass were collected from each zooplankton treatment mesocosm prior to taking the T_1 water samples. Zooplankton were collected by lowering a small 80- μm mesh, 20-cm diameter, plankton net to the bottom of each container, measuring the depth the net was lowered below the surface of the water, and vertically raising the net to the surface through the center of the mesocosm. We collected a single zooplankton tow from each

zooplankton treatment mesocosm, and each sample was immediately preserved in sugar buffered Lugol's solution until analyzed for zooplankton biomass (as described above).

We analyzed both T_0 and T_1 water samples in each mesocosm for NH_4^+ and chlorophyll *a* concentrations ($\mu\text{g L}^{-1}$), as well as the $\delta^{15}\text{N}$ (‰), mass of N (g), and C:N ratio of the seston. From each water sample we forced ~ 60 ml of water for NH_4^+ analysis through 0.45- μm pore size, sterilized, mixed cellulose ester filters into acid washed LDPE bottles. The water samples were immediately frozen and shipped to the Utah State University Aquatic Biogeochemistry Laboratory <http://www.biology.usu.edu/labsites/bakerlab/abl.html> where they were analyzed for NH_4^+ concentration on a Astoria-Pacific 300 series flow injection analyzer following methods outlined in (Eaton et al. 2005).

We filtered 300 ml aliquots from each T_0 and T_1 water sample for chlorophyll *a* analysis through GFF filters, and extracted chlorophyll *a* from the filters in 95% acetone for 18 hrs. Following the extraction, chlorophyll *a* concentrations were analyzed fluorometrically, with correction for phaeopigments, following the methods in Wetzel and Likens (2000) using a Turner Designs 10-AU field fluorometer. We also filtered ~ 1.5 L and ~ 3.5 L of water from T_0 and T_1 water samples, respectively, for seston $\delta^{15}\text{N}$, mass of N, and C:N ratio. Seston samples were prepared and sent to C.P.S.I.L., as described above.

Rate constant calculations

We used the ^{15}N enrichment of the seston in our 'zooplankton' treatment mesocosms to determine the flux of $^{15}\text{N-NH}_4^+$ from zooplankton into seston, and

calculate rate constants for zooplankton excretion and seston assimilation of N. First, we calculated the total N ($^{15}\text{N} + ^{14}\text{N}$) in zooplankton, seston, and dissolved NH_4^+ pools in our mesocosms. The zooplankton total N pool was calculated using the following the equation:

$$N_Z = B_{\text{ZOOPLANKTON}} \times F_N \quad 1$$

where N_Z is the total N mass (μg) in the zooplankton pool, $B_{\text{ZOOPLANKTON}}$ = total zooplankton biomass (μg), and F_N is the fraction of N in zooplankton dry mass. The fraction of N in zooplankton dry mass was calculated by dividing the mass of N in zooplankton isotope samples by the total sample mass. Zooplankton dry mass was $\sim 9\%$ N.

We calculated the mass of N in our seston pool from the samples we collected for seston isotope analysis using the following equations:

$$N_{\text{sample}} = \frac{N_{\text{filter}}}{V_{\text{filter}}} \quad 2$$

$$N_P = N_{\text{sample}} \times V_{\text{mesocosm}} \quad 3$$

where N_{sample} is the concentration of total N in the sample ($\mu\text{g L}^{-1}$), N_{filter} is the mass of total N on the filters analyzed at CPSIL, V_{filter} is the volume of water forced through the filter, N_S is the total mass (μg) of N in the seston pool, and V_{mesocosm} is the volume of the mesocosm (L).

We calculated the mass of the dissolved NH_4^+ -N pool using the following the equation:

$$N_A = (N)\text{NH}_4^+ \times V_{\text{mesocosm}} \quad 4$$

where N_A = the total mass (μg) of N in the dissolved NH_4^+ pool, $(\text{N})\text{NH}_4^+$ is the concentration of N as NH_4^+ ($\mu\text{g L}^{-1}$), and V_{mesocosm} is the volume of the mesocosm (L).

Next, we converted the $\delta^{15}\text{N}$ values from zooplankton and T_0 and T_1 seston pools into the mole fraction, or molar proportion, of ^{15}N from total N ($^{15}\text{N}/(^{15}\text{N}+^{14}\text{N})$) in each sample using the following equation (Mulholland et al. 2004):

$$\frac{^{15}\text{N}}{^{15}\text{N}+^{14}\text{N}} = \frac{\left(\frac{\delta^{15}\text{N}}{1,000} + 1\right) \times 0.0036765}{1 + \left[\left(\frac{\delta^{15}\text{N}}{1,000} + 1\right) \times 0.0036765\right]} \quad 5$$

Hereafter the ^{15}N mole fraction for zooplankton T_0 and seston T_0 , and T_1 pools will be referred to as MF_Z , MF_{S0} , and MF_{S1} , respectively. We then calculated the mass of ^{15}N in each of these respective pools using the equation:

$$^{15}\text{N}_i = \text{MF}_i \times N_i \quad 6$$

where $^{15}\text{N}_i$ = the mass of ^{15}N in the respective pool (μg), MF_i is the mole fraction for the pool, and N_i = the total mass of N in the pool. Because we assumed there was negligible fractionation of ^{15}N during seston uptake we calculated the mass of the $^{15}\text{N}\text{-NH}_4^+$ pools at T_0 and T_1 using equation 6 and substituting MF_{S0} and MF_{S1} for the ^{15}N mole fraction of NH_4^+ at each respective time point.

Figure 3.1 shows a box and arrow diagram of our mesocosm field experiment. Our calculations required that we make several key assumptions: 1) flux of N between zooplankton, NH_4^+ , and seston pools followed first order kinetics; 2) there was no isotopic fractionation of ^{15}N during either zooplankton excretion or seston assimilation of N; 3) all excreted ^{15}N from zooplankton was $^{15}\text{N}\text{-NH}_4^+$; and 4) the volume of water in the

mesocosms did not change over the duration of the experiment. The validity of these assumptions is discussed below. We calculated the flux of ^{15}N in each of our zooplankton treatment mesocosms, and used the mean from our no zooplankton mesocosms as the background ^{15}N flux. We then used the mean excretion (k_e) and uptake (k_u) rates from our 3 treatment mesocosms, and calculated the error around the two rate constants.

We used first order kinetics to model the uptake of N from zooplankton excretion using the change in ^{15}N in the seston pool (Figure 3.1). The flux of tracer ^{15}N from zooplankton into seston is expressed in the following equations:

$$\partial^{15}N_Z/\partial t = -k_e^{15}N_Z + k_g^{15}N_S \quad 7$$

$$\partial^{15}N_D/\partial t = k_e^{15}N_Z - k_u^{15}N_D \quad 8$$

$$\partial^{15}N_S/\partial t = k_u^{15}N_D - k_g^{15}N_S \quad 9$$

where $^{15}N_Z$, $^{15}N_D$, and $^{15}N_S$ are the respective masses (μg) of ^{15}N in the zooplankton, dissolved NH_4^+ , and seston pools, k_e is the first order rate constant for zooplankton excretion, and k_u is the first order rate constant for seston uptake (assimilation), and k_g is the first order rate constant for zooplankton grazing. We held the zooplankton grazing rate at 0.11 d^{-1} (Bowie et al. 1985), and used the ‘solver’ optimization tool in a Microsoft Excel spreadsheet model and solved equations 7, 8, and 9 for k_e and k_u values that would match the observed enrichment of the seston and NH_4^+ pools at T_1 .

Modeling nitrogen mean residence time

We used the first order rate constants calculated from the mesocosm field experiment to build two models of N retention in the upper mixed layer of Lake NE-12. The first model (Figure 3.2A) calculated N retention in the upper mixed layer with

zooplankton N recycling. In the second model simulation (Figure 3.2B), however, we removed zooplankton N recycling.

The upper mixed layer of Lake NE-12 is not a closed system, and we included losses of ^{15}N from the upper mixed layer to sedimentation of organic matter, and outflow stream discharge in both of our models. We calculated the first order rate constant for sedimentation (k_s) from measured sedimentation rates in Lake NE-12 following the equation:

$$k_s = \frac{\partial^{15}\text{N}_{se\ dim\ ent} / \partial t}{^{15}\text{N}_{seston}} \quad 10$$

where k_s is the first order rate constant (d^{-1}) for sedimentation of organic ^{15}N ,

$\partial^{15}\text{N}_{se\ dim\ ent} / \partial t$ is the net ^{15}N sedimentation rate (g d^{-1}) measured from sediment traps in Lake NE-12 (described above), and $^{15}\text{N}_{seston}$ is the mass of seston ^{15}N (g) measured in the upper mixed layer of NE-12 (described above).

Outflow stream discharge was only measured once during the summer of 2007, on July 18th. Therefore, we made the assumption that discharge rate did not change over the course of the model simulations. We calculated the first order rate constant for outflow stream discharge following the equation:

$$k_o = \frac{\partial^{15}\text{N}_{outflow} / \partial t}{(^{15}\text{N}_{ammonium} + ^{15}\text{N}_{seston})} \quad 11$$

where k_o is the rate constant (d^{-1}) for ^{15}N loss to stream outflow, $\partial^{15}\text{N}_{outflow} / \partial t$ is the rate that ^{15}N is lost (g d^{-1}) from the upper mixed layer to stream outflow (discharge x upper

mixed layer ^{15}N concentration), and $(^{15}\text{N}_{\text{ammonium}} + ^{15}\text{N}_{\text{seston}})$ is the mass (g) of ^{15}N in the NE-12 upper mixed layer dissolved NH_4^+ and seston pools.

We parameterized our models with the mass of ^{15}N in the zooplankton, NH_4^+ , and seston pools in the upper mixed layer of NE-12, measured on 26-June-2007, when the lake was enriched. We used 26-June as the start of our model simulations (day 0), and ran the model for 32 days. Pools, fluxes, and equations for both models are shown in Table 3.1. We measured the mass of ^{15}N in the upper mixed layer of NE-12 (zooplankton + seston + NH_4^+) on 26-June, 3-July, 9-July, 16-July, 23-July, and 28-July, corresponding with days 0, 7, 13, 20, 27, and 32 of our model simulations.

Results

Mesocosm field experiment

The zooplankton communities in our mesocosms were composed entirely of two small copepod species, the calanoid *Diaptomus pribilofensis* and the cyclopoid *Cyclops scutifer*. This combination was consistent with the zooplankton community typically found in Lake NE-12 (Chapter 2). Total zooplankton biomass, in each respective zooplankton mesocosm, was 0.19, 0.39, and 0.13 g m^{-3} at the conclusion of the field experiment, which was ~ 3 - 9 fold greater than the ambient zooplankton biomass in Lake NE-12 on 26-June-2007, the day the zooplankton were collected. Mean zooplankton biomass from the three zooplankton mesocosms at the conclusion of the field experiment, however, was not significantly different from the estimate of zooplankton biomass added to each mesocosm at the beginning of the experiment (Figure 3.3).

The changes in concentrations of NH_4^+ and chlorophyll *a*, well as $\delta^{15}\text{N}$ and C:N ratio of seston between T_1 and T_0 in both zooplankton and no zooplankton treatments are given in Table 3.1. Of the four parameters measured in each mesocosm on T_1 and T_0 , significant differences were only observed in $\delta^{15}\text{N}$ ($p = 0.0007$ *d.f.* = 5) and the concentration of NH_4^+ ($p = 0.0009$ *d.f.* = 5) in the zooplankton mesocosms, while no significant differences were found in the no zooplankton treatment. The average $\delta^{15}\text{N}$ enrichment of seston in the zooplankton treatment mesocosms was ~41 ‰, but the other variables measured during the experiment showed either no or modest change between T_1 and T_0 (Figure 3.3). NH_4^+ showed a significant increase between T_1 and T_0 , but was only a small change of $2.3 \mu\text{g L}^{-1}$ in the actual concentration of NH_4^+ . Average C:N ratios were relatively high in zooplankton and no zooplankton treatments on both T_0 and T_1 (Table 3.2). Assuming that C:N > 5.69 (redfield ratio 41:7.2:1 by mass) is indicative of N limitation in the phytoplankton, we had N limiting conditions through the extent of the experiment.

From the observed ^{15}N enrichment of the seston in our mesocosm field experiment, we calculated mean rate constants (d^{-1}) for zooplankton excretion (k_e) of 0.25 and seston assimilation (k_u) of 0.81, with 0.10 and 0.09 95% confidence intervals around the respective means (Figure 3.4).

Modeling nitrogen mean residence time

We used the rate constants for zooplankton excretion and seston assimilation calculated in our mesocosm field experiment in our models of N retention in the Lake NE-12 upper mixed layer. In addition, we used a zooplankton grazing rate constant of

0.11, taken from the literature (Bowie et al. 1985), and calculated sedimentation and stream outflow rate constants of 0.09 and 0.01, respectively. We parameterized our models with zooplankton and seston ^{15}N pools (g), 2.9 and 5.0 respectively, measured on 26-June-2009 in Lake NE-12, just prior enriching the upper mixed layer with 80.9 g of 99% $^{15}\text{NH}_4\text{Cl}$. Because natural abundance of ^{15}N in the upper mixed layer NH_4^+ pool was very small, we used 81 g as our initial $^{15}\text{NH}_4^+$ pool size.

Model simulations, with and without zooplankton recycling of N, both showed an exponential decay of N from the upper mixed layer of Lake NE-12 (Figure 3.5). The model simulation without zooplankton N recycling, however, showed a steeper slope and approached the x-axis asymptote faster than the model simulation where zooplankton recycling of N is present (Figure 3.5). Decay rates for ^{15}N in the upper mixed layer were -0.07 and -0.12 (d^{-1}) for model simulations with and without zooplankton recycling, respectively. The mean residence time ($\text{MRT} = 1/|k|$) of ^{15}N in the model simulation with zooplankton N recycling was 14 days, compared to a MRT of 8 days in the model simulation without zooplankton N recycling.

Discussion

Due to relatively short growing seasons, high winter snowfall, and low summer precipitation, the nutrient budgets in many high latitude and high elevation lakes are dominated by an early season pulse of nutrients from snowmelt runoff (Whalen and Cornwell 1985; Brown et al. 2008). Mechanisms of nutrient retention in lakes receiving early season nutrient pulses can be important for driving primary production when summer nutrient loading to lakes is relatively low. We developed a model to determine if

N recycling by zooplankton in an arctic lake was an important factor for nutrient retention.

Using an isotopic tracer approach we were able to directly measure seston assimilation of N that was derived from zooplankton excretion. An isotopic approach also allowed us to calculate the first order rate constants for zooplankton N loss through excretion and assimilation of N into seston biomass. The rate constant for zooplankton excretion of N calculated from our mesocosm field experiment ($0.25 \text{ (d}^{-1}\text{)}$) was similar to other reported rates (Bowie et al. 1985; He and Wang 2007). The mesocosm field experiment also showed that excreted N was assimilated rapidly ($\sim 80\%/day$) by seston, which would be expected in waters where primary production is limited by N availability.

From the rate constants calculated in the mesocosm field experiments we constructed two models of N retention, with and without zooplankton N recycling, in the upper mixed layer of Lake NE-12. Both models were relatively simple interpretations of the nutrient transformations occurring in the upper mixed layer of the actual lake. However, the mean residence time of N in the upper mixed layer predicted by our model with zooplankton N recycling (14 days) was close to the observed N mean residence (16 days) calculated using a whole lake ^{15}N addition. Without zooplankton N recycling our model predicted that the mean residence time of N in the upper mixed layer was 8 days, almost a week shorter than the mean residence times observed in the NE-12 upper mixed layer and predicted from the model with zooplankton N recycling.

Sedimentation of organic N was the largest loss of mixed layer ^{15}N in Lake NE-12. Also, because sediment traps were sampled \sim weekly, some portion of the organic

^{15}N in the traps would have been remineralized between trap sampling, and thus our measurements of sedimentation are likely an underestimate. In marine systems, fast sinking zooplankton fecal pellets have been considered a major flux of nitrogen to the deep ocean (Knauer et al. 1979), and would expedite the loss of mixed layer N. Recent research, however, has shown that zooplankton corprophagy can retain the majority of fecal pellets in mixed layer waters, and reduce the loss of N through fecal sedimentation (Gonzalez 1994; Elser et al. 1995; Iversen and Poulsen 2007). From our model, zooplankton impede sedimentation of particulate matter, increasing N mean residence time, via grazing on seston. In our relatively simple model zooplankton fecal pellets would be sampled in the mixed layer seston pool, and given the similarity of our model output and observed values, we feel our model included zooplankton corprophagy reasonably well.

Diel vertical migration of zooplankton from epilimnetic to hypolimnetic waters has also been shown to contribute to the removal of nutrients from the mixed layer (Hannides et al. 2009). Buchanan and Haney (1980), however, showed that arctic zooplankton do not vertically migrate during summer periods (between late May and late July) of 24 hr sunlight. Therefore physical transport of nutrients out of mixed layer waters by zooplankton is unlikely.

From our model it is apparent that recycling of N by the zooplankton community increased the mean residence time of N in the upper mixed layer of Lake NE-12. Even though the pattern of ^{15}N decay from the model output was similar to the observed values of ^{15}N in the upper mixed layer. Several mechanisms could account for these differences.

The assimilation of N from sources other than the ^{15}N added to the UML of NE-12 would dilute the mass of ^{15}N in the phytoplankton pool. Our model also did not account for the potential of phytoplankton to fix atmospheric N_2 into new biomass, or the potential fractionation of added $^{15}\text{NH}_4\text{Cl}$ during nitrification. In addition, our single measurement of discharge likely did not accurately represent the loss of ^{15}N to the stream outflow. More precise measurements of N loss and transformation would help reduce the error associated with our model and observed data.

Our model used several key assumptions to solve for k_e : 1) total zooplankton biomass did not change over the duration of the experiment; 2) there was no isotopic fractionation of ^{15}N during either zooplankton excretion or phytoplankton assimilation; 3) all excreted ^{15}N from zooplankton was $^{15}\text{N-NH}_4^+$; and 4) the volume of water in the mesocosms did not change over the duration of the experiment. Because analysis of zooplankton biomass necessitates the sacrifice of the organisms we were not able to directly measure the biomass of zooplankton added to each mesocosm prior to the experiment. Therefore we collected analogous samples for initial zooplankton biomass estimates coincident with the collection of zooplankton for our experiment. We then compared the initial estimates with the mean zooplankton biomass from our mesocosms following completion of the experiment. Estimates of mean zooplankton biomass were higher following the completion of our experiment, but this difference was not statistically significant using a paired t-test ($P = 0.47$ *d.f.* = 5). Following this evidence we concluded that our assumption of no change in zooplankton biomass during our experiment was valid.

Addressing our second assumption, fractionation of ^{15}N would not occur during photosynthesis if N is limiting (Fry 2006), and C:N ratios in our experiment indicated that N was likely limiting or co-limiting. During excretion ^{15}N has a isotopic fractionation of $\sim 9\text{‰}$ (Fry 2006). However, when pools have been experimentally enriched relatively small isotope fractionation can be considered insignificant by comparison when calculating isotopic flux (Fry 2006). Our zooplankton pool had an enrichment of $\sim \delta 175\text{‰}$, and an isotopic fractionation of 9‰ is $\sim 5\%$ of the total enrichment. We believe that this fractionation compared to our enrichment is small enough to be an insignificant source of error when calculating our flux, and was thus disregarded.

For our third assumption we assumed that all excreted ^{15}N was in the form of NH_4^+ . NH_4^+ is the dominant form of nitrogen released during excretion (Miller and Roman 2008), and is the most bioavailable form of N recycled by the consumers.

Finally, we assumed that the volume of water did not change through the duration of the experiment. While this is most certainly not true, and water volume would have been lost to evaporation, we felt that the experiment was conducted over a short enough time period to make this loss insignificant.

Summer phytoplankton production in arctic Alaskan lakes has a high frequency of N limitation (Levine and Whalen 2001). Rates of primary production, however, have considerable variation in lakes across the landscape (Whalen et al. 2006). The main drivers of this variability are still unknown, yet it is clear that zooplankton have the potential to account for a substantial portion of phytoplankton nutrient demand in some

lakes (Johnson et al. *submitted*). From our experiment it is clear that zooplankton nutrient turnover rates are quite high, as are subsequent nutrient recycling rates. However, how important zooplankton recycled nutrient are to lake primary productivity will ultimately be a function of what controls the mass of nutrients recycled (*e.g.* zooplankton biomass) compared to other drivers of lake productivity (*e.g.* external nutrient loading, light attenuation, temperature, lake mixing, etc.).

Pulses of inorganic N and P are delivered to lakes during snowmelt runoff (Whalen and Cornwell 1985) and summer storm events (MacIntyre et al. 2006). In contrast, during relatively calm periods, terrestrial vegetation can retain close to 100% of mineralized N (Shaver et al. 1992), and watershed N loading to lakes can be quite low. By increasing the mean residence time of N in the UML of lakes, zooplankton N recycling can be essential for retaining N required for pelagic primary production in arctic lakes.

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Table 3.1. Pools, rate constants and equations for ecosystem models of mixed layer nitrogen mean residence with zooplankton nitrogen recycling and without zooplankton nitrogen recycling.

Simulation	Pools (g)	Rate Constants (d ⁻¹)	Model Equations
With Recycling	NH ₄ ⁺ (¹⁵ N _{ammonium})	Uptake (k _u)	$\frac{\partial^{15}N_{ammonium}}{\partial t} = k_e^{15}N_{zooplankton} - k_u^{15}N_{ammonium} - k_o^{15}N_{ammonium}$
	Seston (¹⁵ N _{seston})	Grazing (k _g)	$\frac{\partial^{15}N_{seston}}{\partial t} = k_u^{15}N_{ammonium} - k_g^{15}N_{seston} - k_s^{15}N_{seston} - k_o^{15}N_{seston}$
	Zooplankton (¹⁵ N _{zooplankton})	Excretion (k _e)	$\frac{\partial^{15}N_{zooplankton}}{\partial t} = k_g^{15}N_{seston} - k_e^{15}N_{zooplankton}$
	Sedimentation (¹⁵ N _{sediment})	Sedimentation (k _s)	$\frac{\partial^{15}N_{sediment}}{\partial t} = k_s^{15}N_{seston}$
	Outflow (¹⁵ N _{outflow})	Outflow (k _o)	$\frac{\partial^{15}N_{outflow}}{\partial t} = k_o^{15}N_{seston} + k_o^{15}N_{ammonium}$
Without Recycling	NH ₄ ⁺ (¹⁵ N _{ammonium})	Uptake (k _u)	$\frac{\partial^{15}N_{ammonium}}{\partial t} = -k_u^{15}N_{ammonium} - k_o^{15}N_{ammonium}$
	Seston (¹⁵ N _{seston})		$\frac{\partial^{15}N_{seston}}{\partial t} = k_u^{15}N_{ammonium} - k_s^{15}N_{seston} - k_o^{15}N_{seston}$
	Sedimentation (¹⁵ N _{sediment})	Sedimentation (k _s)	$\frac{\partial^{15}N_{sediment}}{\partial t} = k_s^{15}N_{seston}$
	Outflow (¹⁵ N _{outflow})	Outflow (k _o)	$\frac{\partial^{15}N_{outflow}}{\partial t} = k_o^{15}N_{seston} + k_o^{15}N_{ammonium}$

Table 3.2. Initial, final, and change in variables measured in both control and zooplankton treatment mesocosms over the duration of the experiment. Mean change in variables between T_0 and T_1 were compared using a paired t-test, NS = not significant, $d.f. = 5$ in all comparisons.

Experimental Group	Initial T_0 (S.E.)				Final T_1 (S.E.)				Δ (P – value)			
	NH_4^+ ($\mu\text{g L}^{-1}$)	Chlorophyll <i>a</i> ($\mu\text{g L}^{-1}$)	$\delta^{15}\text{N}$ Phytoplankton (‰)	C:N (mass)	NH_4^+ ($\mu\text{g L}^{-1}$)	Chlorophyll <i>a</i> ($\mu\text{g L}^{-1}$)	$\delta^{15}\text{N}$ Phytoplankton (‰)	C:N (mass)	NH_4^+ ($\mu\text{g L}^{-1}$)	Chlorophyll <i>a</i> ($\mu\text{g L}^{-1}$)	$\delta^{15}\text{N}$ Phytoplankton (‰)	C:N (mass)
Zooplankton	4.63 (0.22)	1.49 (0.27)	5.7 (2.1)	7.07 (0.33)	6.93 (0.15)	1.19 (0.15)	46.5 (4.2)	6.10 (0.26)	2.30 (0.0009)	-0.30 (NS)	40.8 (0.0007)	-0.97 (NS)
No Zooplankton	5.13 (1.25)	1.18 (0.04)	4.0 (0.5)	7.27 (0.11)	5.97 (0.68)	0.80 (0.02)	2.9 (0.3)	6.14 (0.15)	0.84 (NS)	-0.38 (NS)	-1.1 (NS)	-1.13 (NS)

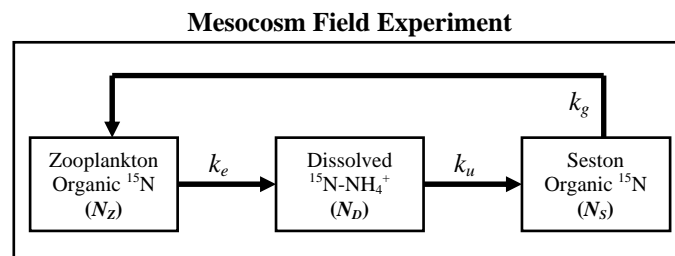


Figure 3.1. Box and arrow diagram of the ^{15}N flux between zooplankton, NH_4^+ , and seston pools in the mesocosm field experiment

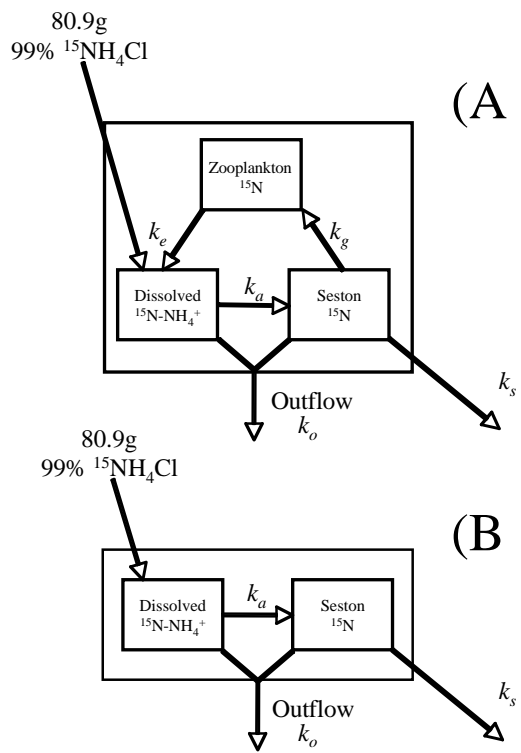


Figure 3.2. Box and arrow diagram of the pools and fluxes in an ecosystem model of mixed layer nitrogen mean residence time with A) zooplankton nitrogen recycling and B) without zooplankton nitrogen recycling.

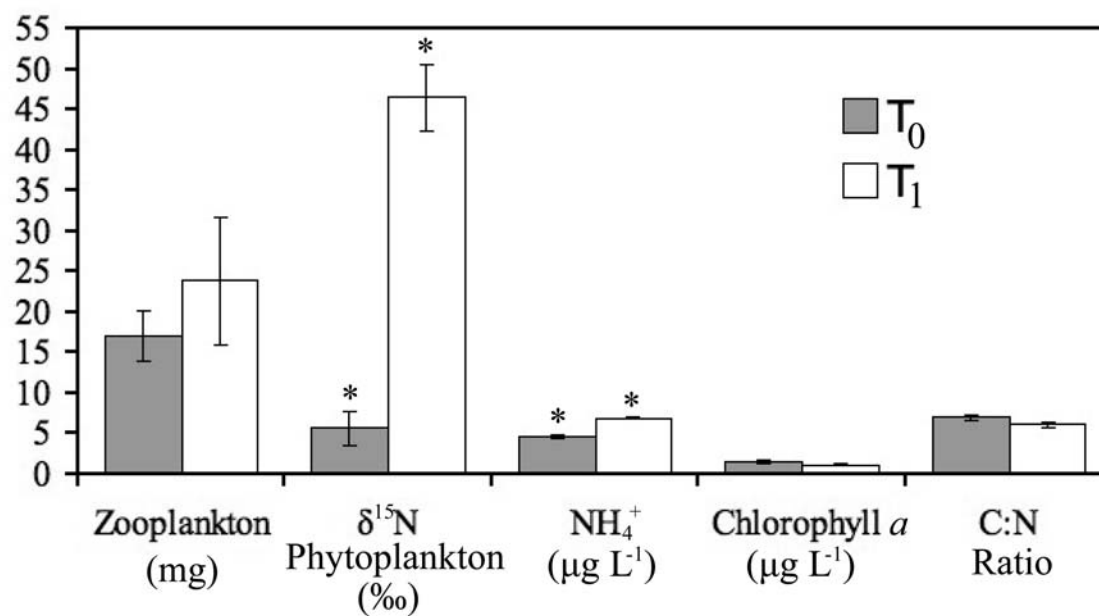


Figure 3.3. Change between initial (T₀) and final (T₁) variables in the zooplankton treatment mesocosms sampled in the experiment. Error bars are ± 1 standard error, * = statistically significant difference ($P < 0.05$ *d.f.* = 5) between T₀ and T₁.

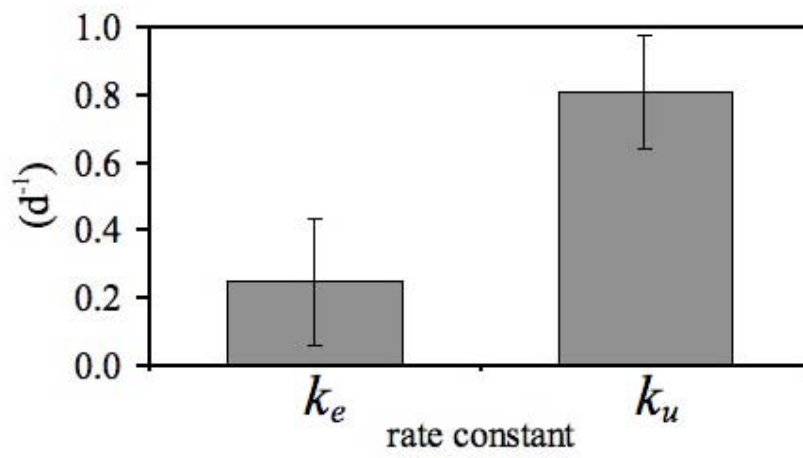


Figure 3.4. Mean excretion (k_e) and uptake (k_u) rate constants in the zooplankton treatment mesocosms calculating using the model. Error bars are ± 1 standard error.

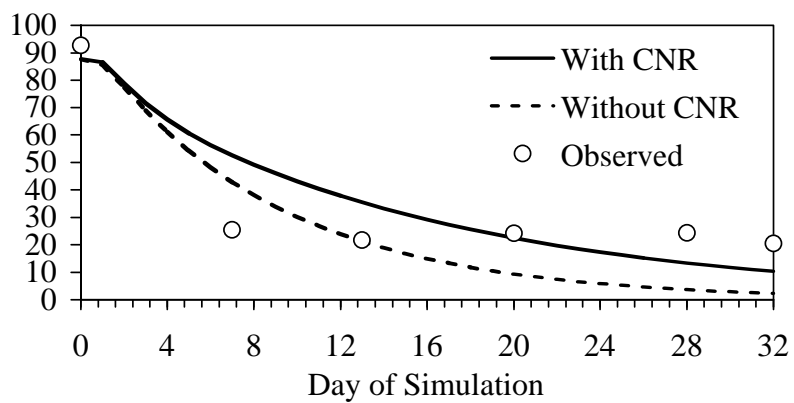


Figure 3.5. Model simulations, with and without zooplankton nitrogen recycling, and observed values of the change in upper mixed layer ^{15}N (g) in lake NE-12 following the addition of $^{15}\text{N-NH}_4\text{Cl}$.

CHAPTER 4

COPEPOD DOMINANCE CONTRIBUTES TO NITROGEN DEFICIENT
CONDITIONS FOR PHYTOPLANKTON PRODUCTION IN
LAKES DURING PERIODS OF LOW PRECIPITATION**Abstract**

We investigated whether consumer-driven nutrient recycling (CNR) by zooplankton was a mechanism for nitrogen limitation of primary production in arctic Alaskan lakes. Copepods and cladocerans, two major zooplankton taxonomic groups, differentially recycle inorganic nutrients based on their own nutrient demands for new tissue. Thus we hypothesized that zooplankton communities dominated by copepods, would sequester bioavailable nitrogen as new tissue, and lead to nitrogen, relative to phosphorus, deficient conditions for primary production of phytoplankton. Conversely, we hypothesized that zooplankton communities dominated by cladocerans would result in phosphorus limitation of phytoplankton growth. We observed a significant positive relationship ($p = 0.03$) between zooplankton communities with higher copepod relative to cladoceran biomass and levels of water column nitrogen deficiency. We also observed a relatively strong negative relationship between total precipitation over the 10 days prior to our sampling and metrics of both nitrogen and phosphorus deficiency. This supported our hypothesis that periods of relatively high watershed nutrient loading can overwhelm internal nutrient source sink dynamics. The relationship between copepod biomass and nitrogen deficiency provides a potential mechanism for the high frequency of nitrogen limitation in arctic Alaskan lakes.

Introduction

Following the publication of Schindler (1977), phosphorus (P) was considered to be the primary limiting macronutrient in most lake ecosystems. Schindler (1977) proposed that when lake nutrient ratios favored nitrogen (N) limitation, phytoplankton communities would become dominated by N-fixing cyanobacteria and convert atmospheric N₂ into bioavailable forms. More recent research has shown the importance of both N and P as limiting nutrients for primary production in freshwaters (Francoeur 2001). The environmental inhibition of N-fixing organisms is often considered the mechanism for N limitation in lakes (Gettel et al. 2007, Marcarelli and Wurtsbaugh 2007). In contrast, the stoichiometric relationship of N and P between primary producers and nutrients recycled by higher trophic levels, or consumer-driven nutrient recycling (CNR), has been proposed as an alternative mechanism driving N-limitation in lakes (Elser et al. 1988, Sterner and Elser 2002).

The stoichiometric theory of CNR states that consumers will differentially recycle nutrients (typically N and P) based on their demands for somatic growth (Sterner 1990). Consumer demand for N and P is thought to be relatively homeostatic and illustrated by the relatively constant N:P ratios in tissue of groups such as zooplankton, insects, and fish (Sterner and Elser 2002). Therefore if two consumer groups with different tissue N:P ratios (one high and one low) are feeding on the same food resource, the group with a high N:P ratio will sequester N as new tissue and excrete dissolved nutrients with a relatively low N:P ratio. The opposite holds true for a consumer group with a relatively high P demand (low N:P ratio) for growth. In the second case the low N:P consumer

group will selectively retain P while excreting dissolved nutrients with a high N:P ratio (Sterner and Elser 2002). In lakes, nutrient recycling by crustacean zooplankton can be an important resource for primary production (Vanni 2002), and differential recycling of N and P by consumer groups can effect the bioavailability of these potentially limiting nutrients for algal production (Elser et al. 1988).

Lake meso-zooplankton communities are typically dominated by copepods and cladocerans, two major taxonomic groups that have considerably different physiological demands for N and P (Elser and Urabe 1999). Copepods characteristically have a relatively high N and low P demand for development (N:P ~30-50), while continuously reproducing cladocerans have a relatively high P demand (N:P ~14) driven by high rates of nucleic acid production (Sterner and Elser 2002). Based on the stoichiometric theory of CNR, a zooplankton community dominated by copepods should sequester N as tissue and excrete dissolved inorganic nutrients with a relatively low N:P ratio, while the converse would be predicted in lakes dominated by cladoceran zooplankton. Consequently, lakes with predominantly copepods should have a higher frequency of N-limitation, while cladoceran dominated communities should lead to more P-limitation (Elser et al. 1988).

Lakes near the Toolik Field Station in Arctic Alaska are an ideal setting to test the hypothesis that CNR is a mechanism for the prevalence of N-limitation of primary productivity at the landscape level. The area surrounding Toolik Field Station is characterized by a large number of relatively pristine lakes that exhibit both N and P limitation, as well as N and P co-limitation of phytoplankton primary production (Levine

and Whalen 2001). In addition, Toolik Field Station is the site of the Arctic Long Term Ecological Research (LTER) project, and lake zooplankton communities in this area are well characterized (O'Brien et al. 2004).

We tested the hypothesis that zooplankton community composition was related to differences in N and P nutrient deficiency for phytoplankton production by conducting physiological nutrient deficiency experiments on the phytoplankton communities in a suite of lakes during the summers of 2007 and 2008. Large differences in precipitation in 2007 and 2008 also allowed us to assess the relative importance of watershed nutrient loading compared to CNR. Watershed loading can overwhelm the importance of internal sources and sinks of inorganic nutrients when runoff is high, such as during snowmelt or rain events (Whalen and Cornwell 1985, MacIntyre et al. 2006). Conversely, when runoff is low, arctic soils and vegetation can retain close to 100% of mineralized inorganic nutrients (Shaver et al. 1992), and watershed loading of nutrients to lakes is negligible. Therefore, we hypothesized that when precipitation was high, watershed nutrient loading would alleviate N and P stress in the phytoplankton communities. However, when precipitation was low internal nutrient recycling would be the primary driver of nutrient availability for phytoplankton production.

Methods

Site Description

Our research was based out of Toolik Field Station site of the Arctic LTER (68⁰ 38' N, 149⁰ 38' W), in the foothills of the Brooks Mountain range in northern Alaska (Figure 4.1). This area is underlain by continuous permafrost and consists of lakes that

are typically shallow (depth between 3 and 20 m) moraine dammed or kettle basins. The lakes near Toolik Field Station are typically dimictic and become thermally stratified during the summer months, between late June and late August, at depths < 4 m (Arctic LTER database <http://ecosystems.mbl.edu/ARC/>). Shallow lakes (maximum depth < 4 m) may be polymictic, and vertically mix on a diel basis or during storm events (MacIntyre et al. 2006). In addition, lakes in this region are characteristically ultra-oligotrophic with chlorophyll *a* concentrations typically below 5 $\mu\text{g L}^{-1}$, concentrations of dissolved nutrients are frequently below analytical detection limits, and so are not helpful in inferring patterns of nutrient limitation (Arctic LTER database <http://ecosystems.mbl.edu/ARC/>).

Loading from the surrounding watershed is the largest source of inorganic nutrients to lakes in this region, and occurs in pulses during snowmelt runoff (Whalen and Cornwell 1985) or precipitation events (MacIntyre et. al 2006). The timing and intensity of these pulse events, however, is highly variable between years for snowmelt, and both between years and within a given growing season for precipitation (Arctic LTER database <http://ecosystem.mbl.edu/ARC/>).

We performed experiments during the summer growing seasons in 2007 and 2008. In 2007 we selected a total of six study lakes (GTH 86, E-1, S-11, E-4, GTH 114, and NE-9B), and all six lakes were sampled on three separate intervals (late-June, mid-July, and late-July) (Table 4.1). In 2008 we repeated the study on the same six lakes, but study lakes were sampled only once during mid-July (Table 4.1). During the three sampling intervals in 2007, and the single sampling interval in 2008, we sampled all

study lakes within six days of each other. Lakes were chosen based on their proximity to Toolik Field Station and a sufficient maximum depth for thermal stratification during the summer (Figure 4.1).

Precipitation

We did not directly measure watershed nutrient loading during our study, thus we used the total precipitation (mm) from the previous 10 days prior to lake sampling as an index of watershed runoff. High runoff events deliver a pulse of nutrients to these arctic lake ecosystems (MacIntyre et al. 2006), and we made the assumption that nutrient loading to our study lakes during the summer growing season would be directly related to the amount of rain. Precipitation data was obtained from the Toolik Field Station Environmental Data Center (http://www.uaf.edu/toolik/env_data/index_env_data.html).

Zooplankton biomass

We sampled pelagic zooplankton for calculation of biomass concentration by straining the water column using a 30 cm diameter, 80 μ m mesh Wisconsin zooplankton net (Wildco®). We took three replicate tows from the deepest portion of the lake to the surface in 2007 and five replicate tows in 2008. We preserved all zooplankton samples in sugar buffered Lugol's solution until samples were analyzed.

Biomass concentrations of major zooplankton taxa were calculated by measuring the length of 10 indiscriminately selected individuals from each species in each tow using an optical micrometer on a dissecting microscope. We converted the length of each individual (mm) into biomass (μ g) using species-specific length weight regressions

(Burkart 2007), and calculated the mean individual biomass for each species. Following the calculation of average biomass we counted the remaining individuals of each species in each tow. We then multiplied the number of individuals by the average biomass for each species then divided by the tow volume to give us biomass concentration ($\mu\text{g L}^{-1}$) of each species in each lake.

Nutrient deficiency analysis

We used the physiological bioassays, ammonium enhancement response (AER) and alkaline phosphatase activity (APA) to assess the respective N and P deficiencies in each of our study lakes. We collected epilimnetic water for physiological bioassays from all study lakes, coincident with zooplankton sampling, from a depth of 1 m. Water was kept in 4 L, lightproof containers, for < 1 hr before returning to Toolik Field Station to run the analyses.

When phytoplankton communities experience N-deficient conditions, they produce exoenzymes that allow for uptake of NH_4^+ in the dark accompanied by carbon fixation that can be measured using H^{14}CO_3 uptake (Amand et al. 1998). For each study lake we filled seven glass 300 ml bottles with epilimnetic water. Three bottles were supplemented with $3.5 \mu\text{M NH}_4^+$ while four bottles were left at ambient NH_4^+ concentrations, and all bottles received $3 \mu\text{Ci H}^{14}\text{CO}_3$. Carbon fixation was immediately measured in one ambient NH_4^+ bottle for an initial (T_0) value. The Following a 5 hr incubation in the dark, carbon fixation was measured in both supplemented and ambient bottles, subtracting off T_0 values from each bottle, and a paired t -test used to determine if carbon fixation was statistically different ($p < 0.05$) between the two treatments. If

results were statistically significant, AER was calculated by dividing the carbon fixation in supplemented bottles by fixation in ambient bottles. If the difference between supplemented and ambient treatments were not statistically significant we assigned the AER calculation a value of 1. Values significantly > 1 showed N deficiency in the phytoplankton community (Yentsch et al. 1977).

Phytoplankton communities in P-deficient conditions excrete the exoenzyme alkaline phosphatase that allows for the cleaving of phosphate groups from larger organic phosphorus molecules (Pettersson 1980). Using the APA technique, we measured the production of alkaline phosphatase by phytoplankton communities that were supplemented with a solution of 165 μM 4-methylumbelliferyl-phosphate (MUP). The MUP solution is a saturating concentration of a phosphate-containing organic complex, where the phosphate group is cleaved by the alkaline phosphatase enzyme produced in the lake water (Amand et al. 1998). The APA measurements were normalized to the concentration of chlorophyll *a* present in the sample of lake water, and high production of alkaline phosphatase by phytoplankton communities was indicative of P-deficient conditions.

Using enzyme analyses allows for finer resolution of phytoplankton nutrient status. Typical nutrient enrichment bioassays that are run for periods of days or weeks may not accurately depict nutrient limitation in systems where ambient N and P concentrations are very low (Elser et al. 1988). In such systems phytoplankton may switch between N and P limitation, as well as N and P co-limitation, relatively rapidly. Temporal resolution of traditional bioassays is too coarse to capture these changes, but

phytoplankton enzyme activity analyses offer an instantaneous indication of phytoplankton nutrient stress. However, it should be noted that even though metrics of enzyme activity are precise indicators of nutrient stress, these analyses indicate nutrient deficiency and not traditional nutrient limitation (Elser et al. 1988).

Statistical analyses

We analyzed the relationship between zooplankton community composition as an internal driver of N versus P deficiency, and watershed nutrient loading as an external driver of lake nutrient deficiency. The relationships between internal and external drivers of nutrient deficiency are complex, and may be highly variable within a lake throughout the season, as well as between lakes across a landscape. In order to quantify these complex relationships, we used a path analysis to 1) consider complicated relationships between the independent variables, and 2) quantify the relationship of these variables on multiple dependent variables (Mitchell 2001). Path analysis combines multiple regression and multivariate statistical techniques to clarify complicated causal schemes between multiple dependent and independent variables (Mitchell 2001). The relationships between these variables are first organized in a diagram based on *a priori* hypotheses (Figure 4.5A). A path analysis can then be used to determine if the relationships (positive or negative) match those hypothesized, as well as to quantify the strength of the correlations between these relationships. Path analysis also provides an estimate of unexplained error associated with the dependent variables, designated as E in the path diagrams (Figure 4.5). Relatively large unexplained error terms can be

indicative of potentially statistically significant relationships not included in the analysis (Mitchell 2001).

Our *a priori* hypotheses were; 1) during periods of high precipitation watershed nutrient loading would dominate lake nutrient availability, and subsequent internal biotic drivers would be insignificant, and 2) when precipitation was low, watershed nutrient loading to lakes would be minimal, and internal drivers of nutrient availability would dominate (Figure 4.5A). Thus we predicted that precipitation would have a negative relationship with both AER and APA, and show a negative relationship with copepod:cladoceran ratio (Figure 4.5A). We also predicted that a higher proportion of copepods relative to cladocerans would be positively correlated with N-deficiency (*e.g.*, as copepod:cladoceran ratio increases, AER increases), and that a relatively higher proportion of cladocerans would be positively correlated with P-deficiency (*e.g.*, as copepod:cladoceran ratio decreases, APA increases) (Figure 4.5A). For all statistical analyses we used the program JMP® 7.0.1 (SAS Institute Inc.). Because of the small number of lakes used in these analyses, we choose an *a priori* alpha level of 0.10 for statistical significance for all tests reported.

Results

The mean of total precipitation at Toolik Field Station during the months of June and July from 1988 – 2005 is 135 mm, but only 91 mm of precipitation was recorded at Toolik Field Station for the months of June and July in 2007. In contrast, higher than average precipitation fell during the summer of 2008, and total June and July precipitation at Toolik Field Station measured 194 mm

(<http://ecosystems.mbl.edu/arc/weather/tl/index.shtml>). There was an ~30 fold difference in the total precipitation that fell 10 days prior to the sampling events in 2007 and 2008 (Figure 4.2). In 2007 precipitation ranged from ~2 mm in mid-July 2007 to ~20 mm in late-June and late-July 2007. Precipitation in mid-July 2008 was ~60 mm, however, and was considerably higher than all sampling events in 2007 (Figure 4.2).

The proportion of copepod versus cladoceran biomass was variable in our study lakes, and showed high seasonal and annual variation within a given lake (Figure 4.3). GTH 86 had consistently high copepod biomass during all sampling 2007 and 2008 sampling events, while other lakes in our study shifted between copepod and cladoceran dominance depending on the sampling date. In 2007, the zooplankton community in lake NE-9B was comprised almost entirely of cladocerans, but was dominated by copepods during July 2008 (Figure 4.3).

Phytoplankton N and P deficiencies, as indicated by AER and APA analyses, were variable between lakes during all sampling events in 2007 (Figure 4.4). N deficiency, however, was generally lower and showed less variability between lakes during early-June, compared to the mid-July and late-July 2007 (Figure 4.4A). Of the six lakes sampled in 2007, only three (GTH 86, GTH 114, and NE-9B) showed a response to AER in late-June, and the response was relatively low ($AER \approx 1.2$) in these three lakes. In contrast, N deficiency was highest during mid-July, and all lakes, except for NE-9B, showed a response to AER ranging from ~1.2 to ~2.2 (Figure 4.4A).

APA analysis showed that P deficiency generally increased through the summer growing season in all of the lakes sampled in 2007, but showed a high degree of

variability between study lakes during all sampling events (Figure 4.4B). Lake E-4 showed the highest response to APA, ranging from ~20 to ~85 nmol [$\mu\text{g chlorophyll } a$] $^{-1}$ min $^{-1}$ over the course of the 2007 summer, yet showed little or no response to AER analysis. This indicates a high degree of P deficiency, with little or no N deficiency, in lake E-4 during 2007. By comparison, GTH 86 showed very little response to APA analysis, but had relatively high responses to AER during all 2007 sampling events (Figure 4.4). Therefore, it is likely that phytoplankton production in GTH 86 was N-limited. Other lakes in our study (*e.g.* S-11) showed relatively high responses to both AER and APA analyses during the summer of 2007, suggesting N and P co-limitation of phytoplankton production (Figure 4.4).

Both N and P deficiencies were lower in all of our study lakes in mid-July 2008 compared to 2007 (Figure 4.4). Of the six lakes we sampled in 2008, only two (GTH 86 and GTH 114) showed a response to AER, indicating N deficiency in the phytoplankton communities (Figure 4.4A). P deficiency in 2008 showed less variability between study lakes, and was lower in all lakes compared to the same lakes sampled in 2007 (Figure 4.4B).

The observed positive and negative relationships between precipitation, zooplankton community composition, N deficiency and P deficiency in our path analysis were consistent with our *a priori* hypotheses (Figure 4.5). However, only three of the relationships demonstrated relatively high correlations between variables (Figure 4.5B). We found negative correlations between precipitation and both AER, ($r = -0.33$, $p = 0.09$), and APA ($r = -0.30$, $p = 0.16$), indicating that as precipitation increased both N and

P deficiency decreased in our study lakes (Figure 4.5B). In addition, we found a positive correlation ($r = 0.42$, $p = 0.03$) between the ratio of copepod:cladoceran biomass in lake zooplankton communities and AER (Figure 4.5B). This shows that when copepod relative to cladoceran biomass was high in our lakes, phytoplankton communities experienced N-deficient conditions. For both AER and APA, residual unknown error was the largest correlation, indicating considerable unknown variability within our data (Figure 4.5B).

Discussion

The prevalence of N limitation of phytoplankton production is challenging the long-standing paradigm that P limits primary production in freshwater ecosystems (Francoeur 2001, Levine and Whalen 2001, Lewis Jr. and Wurtsbaugh 2008). The mechanisms driving N limitation in many of these systems, however, are still unknown. Our research supports the hypothesis that zooplankton community composition can contribute to the deficiency of N for phytoplankton production through differential CNR, but only when environmental conditions favor internal controls of lake nutrient dynamics.

During periods of high runoff, watershed nutrient loading is the dominant driver of freshwater N and P availability (Whalen and Cornwell 1985, MacIntyre et al. 2006, McNamara et al. 2008), and it is unlikely that zooplankton CNR would impact nutrient limitation during these events. This was evident during the summer of 2008, when precipitation during the 10 days prior to our lake sampling was ~30 times higher than the precipitation recorded on similar dates in 2007. The high precipitation in 2008 coincided with our lowest measurements of both N and P deficiency of the phytoplankton

communities in our study lakes. In contrast, 2007 was an exceptionally dry year, and total precipitation 10 days prior to our mid-July sampling was only ~2 mm. The relatively dry period in 2007 coincided with the highest measurements of N deficiency in our study lakes.

When watershed nutrient loading is minimal, internal drivers of nutrient limitation may become more important. We observed a significant positive relationship between higher copepod biomass in lake zooplankton communities and N-deficient conditions for phytoplankton production, supporting our hypothesis that differential CNR by copepods may sequester bioavailable N relative to P (Figure 4.5B). No relationship, however, was observed between P-deficiency and zooplankton community composition. The bioavailability of P in lake ecosystems can be complex, and include composition of parent material, chemical adsorption and desorption, and biological cycling (Wetzel 2001). Soils and sediments in lakes near Toolik Field Station are characterized by high iron concentrations that can sorb to P and sequester it in lake sediments (Kling et al. 1992). Therefore bioavailability of P for primary production may depend largely on chemical rather than biological processes.

Conclusion

Arctic lakes are characteristically ultra-oligotrophic (Kling et al. 1992), and phytoplankton production is frequently N-limited (Levine and Whalen 2001). We demonstrated that zooplankton communities dominated by copepods can be an effective sink of available N, relative to P, during periods of low runoff, and can push lakes to

more N-deficient conditions. Thus, our research provides support for CNR as a mechanism for N-limitation in lakes across this landscape.

We observed both intra and inter-annual variation in copepod versus cladoceran dominance in our study lakes, yet the drivers of zooplankton community composition in arctic lakes are still unclear (O'Brien et al. 2004). While the controls of zooplankton community composition were beyond the scope of this research, future research focusing on these controls may further elucidate the drivers of lake nutrient availability.

Finally, nutrient availability for primary production at the landscape level is dynamic, and a function of physical, chemical, and biological processes, none of which are mutually exclusive. Our research showed that nutrient deficiency decreased across lakes during a summer with higher than average precipitation. Annual precipitation is predicted to increase in the Arctic, however this increase expected to occur mainly in the autumn and winter (Arctic Climate Impact Assessment 2004). Watershed nutrient loading to lakes will likely increase in these 'shoulder' seasons as well, but remain relatively lower during the peak of the summer growing season. Understanding the changing controls nutrient availability over the course of the short arctic summers will allow us to better grasp the currently observed and predicted changes in ecosystem function as a response to climate changes across this region.

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Table 4.1. Date sampled, maximum depth, surface area, volume, watershed area. Watershed area not available for lake S-11

Lake	Date Sampled (dd-mmm)				Maximum Depth (m)	Surface Area (m ²)	Volume (m ³)	Watershed Area (m ²)
	Late-June 2007	Mid-July 2007	Late-July 2007	Mid-July 2008				
GTH 86	23-Jun	07-Jul	25-Jul	14-Jul	10	34,000	120,000	1,400,000
E-1	27-Jun	10-Jul	26-Jul	18-Jul	12	33,000	327,000	918,000
S-11	28-Jun	10-Jul	26-Jul	15-Jul	10	4,000	28,000	NA
GTH 114	27-Jun	12-Jul	27-Jul	14-Jul	6	39,500	87,000	592,000
E-4	25-Jun	07-Jul	25-Jul	14-Jul	4	40,000	82,500	461,000
NE-9B	29-Jun	12-Jul	21-Jul	15-Jul	9	3,500	14,000	138,000

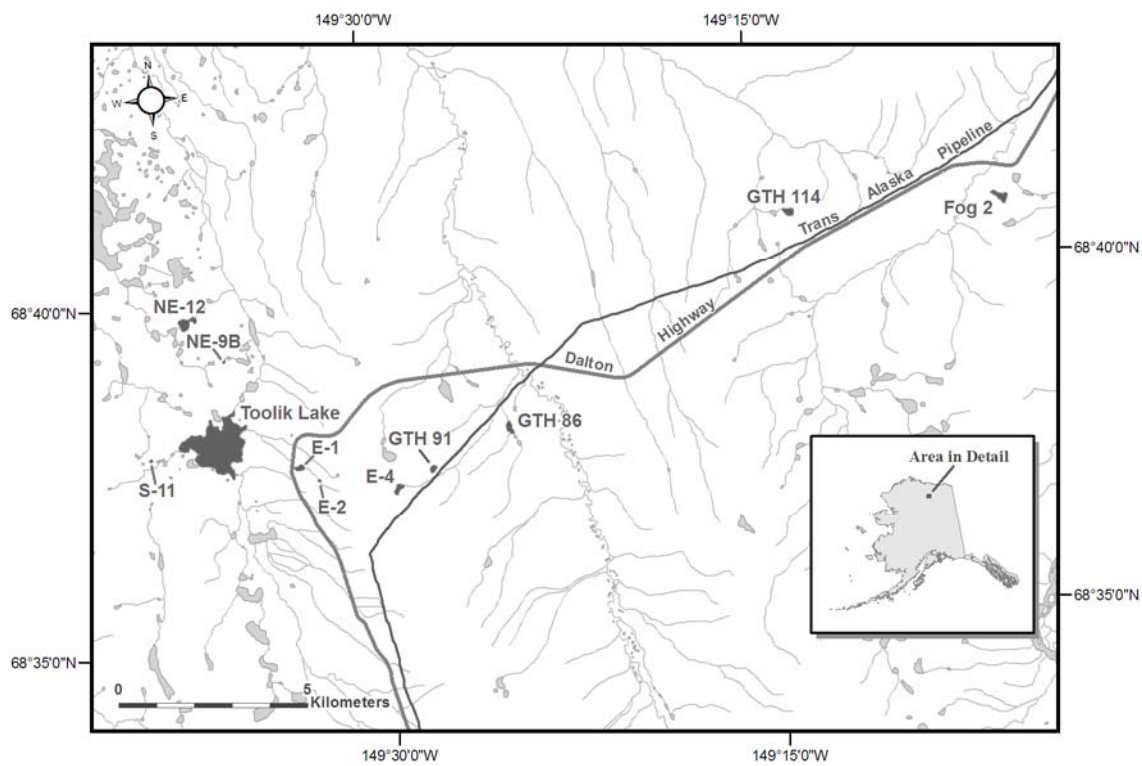


Figure 4.1. Research area and location of study lakes in relation to Toolik Lake, arctic Alaska.

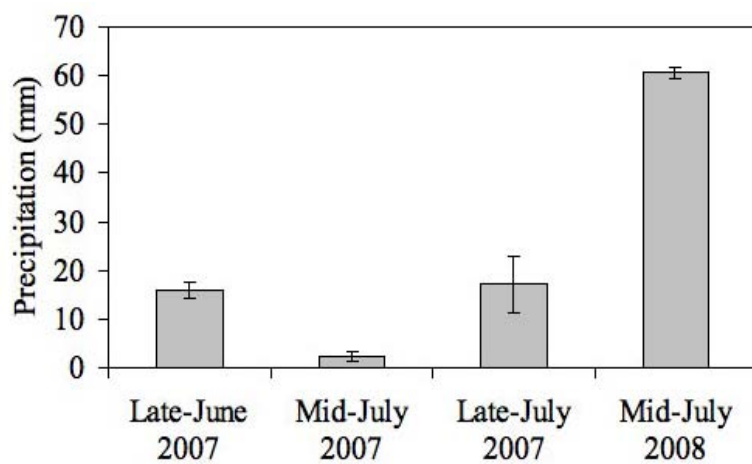


Figure 4.2. Mean of the total precipitation that fell during the previous 10 days prior to our sampling of each lake during each sampling event. Error bars are ± 1 standard error.

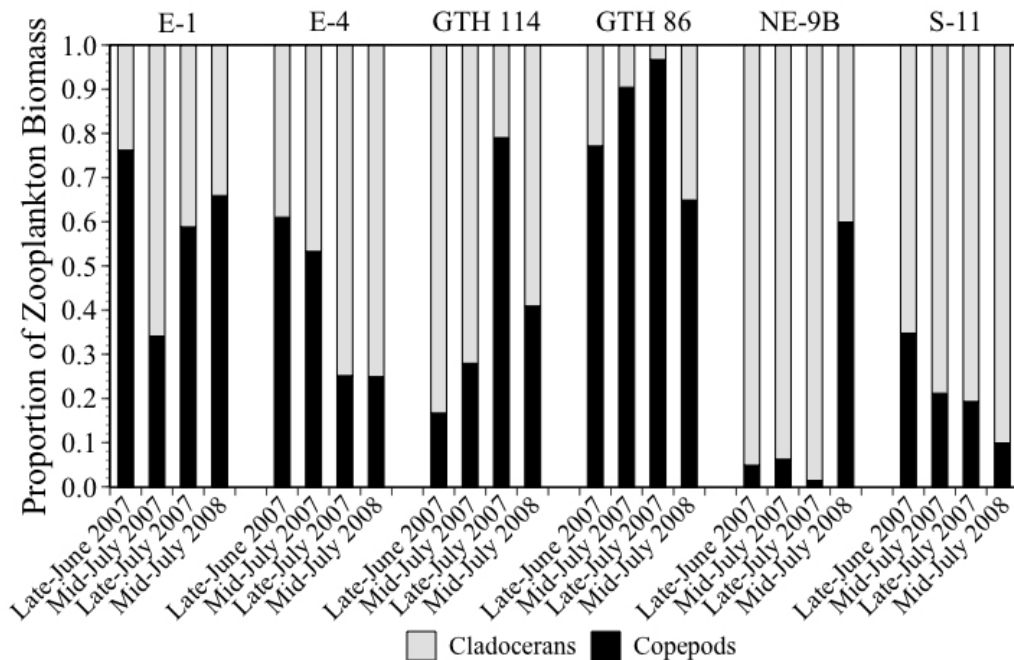


Figure 4.3. The proportion of total zooplankton biomass comprised of copepods (black bars) and cladocerans (gray bars) in our study lakes (upper x-axis) during each sampling event (lower x-axis).

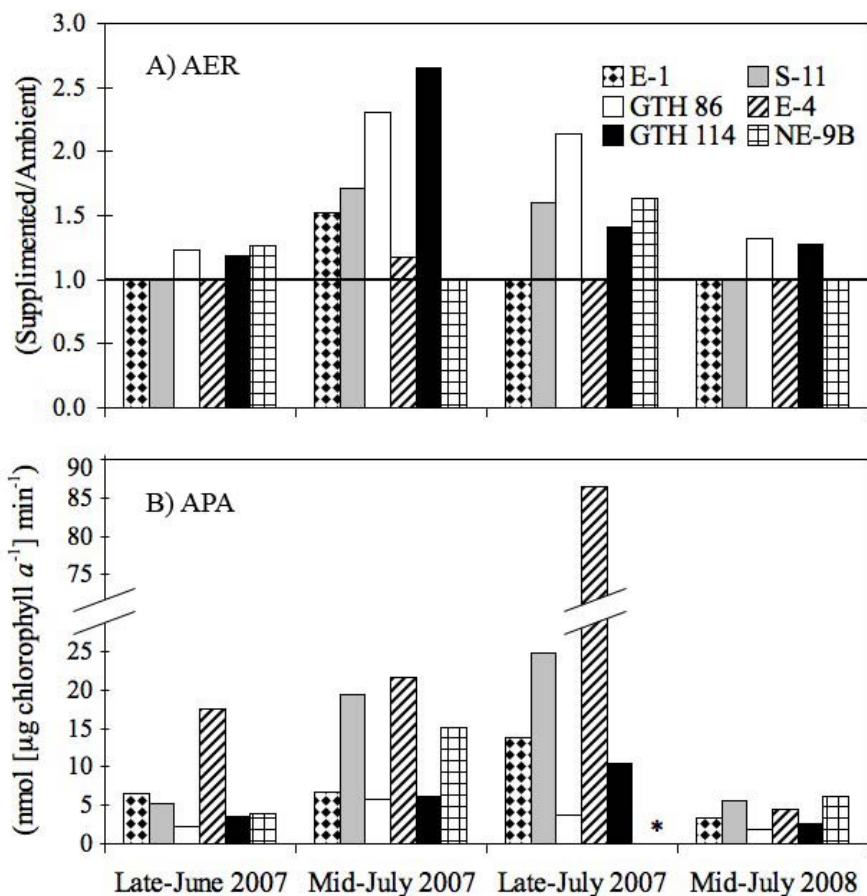


Figure 4.4. Comparison of A) AER and B) APA nutrient deficiency assays in study lakes during 2007 late-June, mid-July, and late-July and 2008 mid-July sampling events. Values below the horizontal line in panel (A) indicate no response to AER. * late-July 2007 sample for NE-9B was lost.

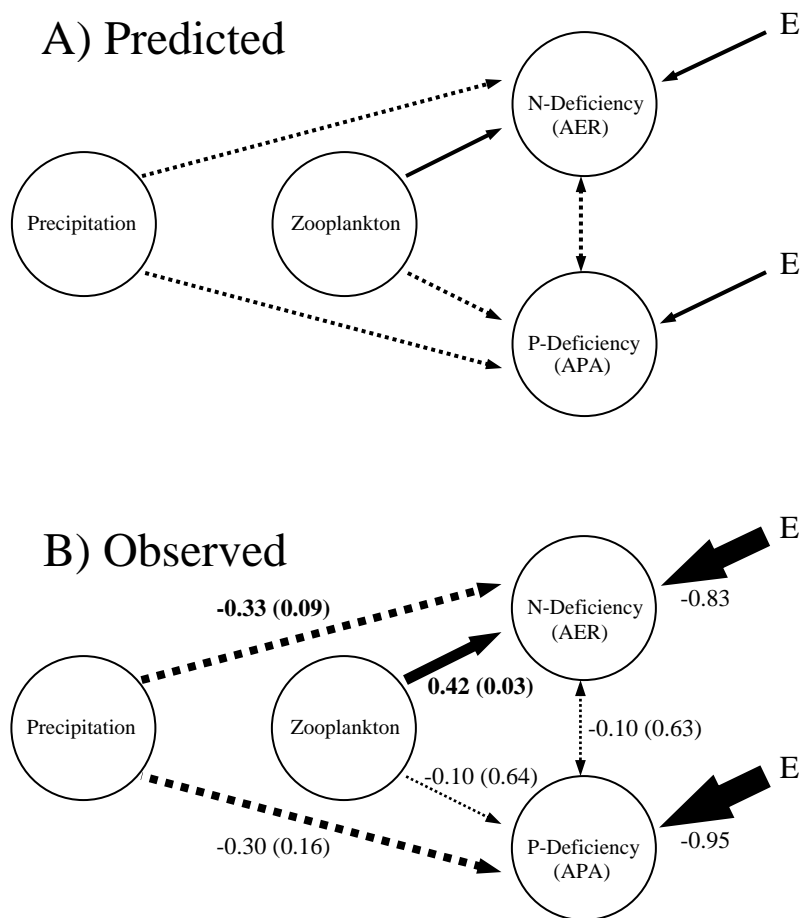


Figure 4.5. The A) predicted and B) observed relationships between independent variables precipitation and copepod:cladoceran ratio (zooplankton), and dependent variables of phytoplankton N-deficiency (AER), and P-deficiency (APA) using a path analysis. Solid and dashed lines indicate positive and negative relationships, respectively, between variables. The values in our observed data are the correlations (r) and (p -values) between variables in our model. Bold values are statistically significant at the $p < 0.10$ level, and arrow width shows the strength of the correlation

CHAPTER 5

SUMMARY, CONCLUSIONS, AND FUTURE RESEARCH.

Top-down versus bottom-up regulation of primary production has received considerable attention in limnological research (McQueen et al. 1989; Gliwicz 2002;). Bottom-up nutrient bioavailability, often nitrogen (N) and phosphorus (P), has been shown to limit rates of primary production and algal standing stocks in many lake systems (Schindler 1977; Levine and Whalen 2001; Lewis and Wurtsbaugh 2008;). In contrast, top-down controls of producer biomass (*sensu* trophic cascade) has been a well documented regulator of production at the base of the food web (Carpenter and Kitchell 1988; Vanni and Findlay 1990). For many years top-down versus bottom-up regulators were viewed as competing mechanisms for the control of lake productivity. Relatively recently, however, consumer-driven nutrient recycling (CNR) has been investigated as a synthesis of these two hypotheses (Lehman 1980; Vanni 2002; Hargrave 2006). When consumers feed on lower trophic levels, they not only exert top-down pressure on prey biomass and community composition, but also excrete nutrients in labile inorganic forms as byproducts of metabolic waste (Elser and Urabe 1999). Nutrient excretion by consumers can supply potentially limiting N and P, and can therefore affect bottom-up resource availability for lake primary productivity.

Although CNR theory provides an elegant coupling between traditional top-down/bottom-up mechanisms of lake productivity (Vanni 2002), research investigating the importance of CNR as a resource for phytoplankton production, and the controls over CNR has been highly variable between different lake ecosystems. Initially research on

CNR focused on microbes (Vadstein et al. 1993) and zooplankton (Lehman 1988), but several studies have shown that recycling rates of N and P by fish can be relatively large compared phytoplankton nutrient demand (Schindler et al. 1993), and to watershed loading (Brabrand et al. 1990). In contrast, direct excretion of N and P by fish has also been shown to be relatively small contribution to lake nutrient budgets compared to lower trophic levels (Sarnelle and Knapp 2005), and indirect effects of fish on zooplankton communities (Vanni and Layne 1997; Vanni et al. 1997) may be a more important interaction between fish and lake CNR.

In arctic Alaskan lakes CNR has the potential to be particularly important in lake nutrient budgets. Arctic Alaskan lakes are typically ultra-oligotrophic, and primary production is ~ 7 fold lower than oligotrophic lakes at lower latitudes (McDonald et al. 1996). In addition, watershed N and P loading to lakes in this region occurs predominantly during snowmelt runoff in early spring (Whalen and Cornwell 1985), making the potential for internal nutrient cycling to be particularly important for sustaining primary production during the summer months when watershed loading is minimal. Finally, top-down vs. bottom-up control over lake production has been intensively studied in this region (O'Brien et al. 1979; Hershey 1985; Levine and Whalen 2001; O'Brien et al. 2005;), but CNR as a potential resource of N and P for phytoplankton production has not yet been explored in these lakes. As such, the aim of my research was to quantify N and P recycling from fish and meso-zooplankton consumer groups, and determine: 1) if consumer nutrient recycling (CNR) was a significant source of N and P for phytoplankton production in arctic lakes; 2) if CNR increased the mean residence

time of N in the upper mixed layer of lakes; and 3) if differential recycling of N versus P by meso-zooplankton communities with contrasting proportions of copepods and cladocerans can affect the availability of N and P for phytoplankton production.

To address my first research question (chapter 2) I quantified excretion rates of N and P from fish and meso-zooplankton communities in six arctic Alaska lakes, three with fish and three without fish. I compared the rates of N and P recycling by fish and meso-zooplankton communities to N and P required to support measured rates of phytoplankton productivity. From this comparison I calculated a proportion of phytoplankton N and P demand that could be attributed to fish and meso-zooplankton N and P recycling.

Rates of N and P recycling by fish were relatively small in research lakes, ranged from $0.014 - 0.052 \mu\text{g N L}^{-1} \text{d}^{-1}$ and $0.002 - 0.006 \mu\text{g P L}^{-1} \text{d}^{-1}$, and accounted for $<1 - 3$ % of phytoplankton N and P demand. The rates of N and P recycling by fish I measured in my study were approximately an order of magnitude lower than rates found by (Sereda et al. 2008) during a similar study in oligotrophic Canadian Shield lakes. Verant et al. (2007) found the most important factor for predicting N and P excretion rates from fish communities was fish density. Fish population sizes in arctic Alaskan lakes are relatively small due to poor food availability (McDonald et al. 1996), and a very short summer growing season, and low density is likely the primary driver of low N and P recycling rates by fish in these lakes.

Zooplankton, however, had rates of N and P recycling ~ 100 fold greater than fish in lakes under investigation, and had the potential to supply ~ 100 % of the N and P

requirements for phytoplankton production. N and P recycling rates, and proportion of phytoplankton nutrient demand supplied by zooplankton recycling was variable between lakes in our study, and although fish had a relatively insignificant direct impact on lake N and P budgets, fish indirect controls (*sensu* Vanni 2002) over zooplankton N and P recycling were observed.

Mass-specific rates of N and P recycling increase inversely with organism body size (Peters 1983), and we found the mean individual body size of meso-zooplankton was 40 % lower in lakes where fish were present due to size-selective predation. As a result average mass-specific excretion rates by meso-zooplankton were higher in lakes where fish were present. Fish were also found to have a negative impact on total meso-zooplankton biomass in our study lakes. Because N and P excretion rates at the community level are a function of mass-specific excretion rates and total community biomass, lower meso-zooplankton biomass in the presence of fish would have reduced rates of N and P recycling. Our study showed that rates of N and P recycling were similar between lakes with and without fish, and this suggests that fish have compensatory indirect effects on meso-zooplankton CNR. Both of these indirect effects of fish on meso-zooplankton recycling rates have been observed in other studies (Vanni and Layne 1997; Vanni et al. 1997), but have typically been shown using enclosure experiments. Our research shows evidence of these two mechanisms occurring simultaneously at the lake level.

The large variation in proportion of phytoplankton N and P demand supplied by CNR, compared with the relatively similar rates of N and P excretion by zooplankton in

our study lakes may be due, in part, to the fact that N and P uptake rates were taken from published values and not measured concurrently with estimations of consumer community composition and biomass. In addition, our estimates of consumer N and P recycling rates provide a mid-summer snapshot of potential rates in arctic lakes. The importance of CNR to phytoplankton production in these lakes may have a strong temporal component, and future research should include simultaneous measurements of consumer biomass, mass-specific excretion rates, and N and P uptake at multiple intervals over the course of the summer growing season.

My second chapter showed the potential importance of meso-zooplankton nutrient recycling as a source of N and P for phytoplankton production. Direct excretion by fish, however, was found to be a relatively insignificant source of N and P. As a result of these findings, I focused chapters three and four specifically on developing a greater understanding of the role N and P recycling by meso-zooplankton communities plays in lake ecosystems.

When resources availability limits productivity, retention of potentially limiting nutrients within an ecosystem can be important for sustaining productivity during periods when nutrient inputs are relatively low. In my third chapter I tested the hypothesis that N recycling by zooplankton would increase the mean residence time of N in the upper mixed layer of a lake. I used meso-zooplankton that had been experimentally enriched with ^{15}N as a source pool for a stable isotope tracer experiment. Using a stable isotope tracer approach, set up in a mesocosm experiment, I was able to empirically measure the ^{15}N enrichment of seston as isotopically enriched N excreted by meso-zooplankton was

assimilated into this pool. Using the measured ^{15}N enrichment of the seston pool I was able to calculate rates of zooplankton N excretion and subsequent assimilation rates of N into seston biomass.

I use the excretion and assimilation rates calculated from the isotope tracer experiment to construct an ecosystem model for the upper mixed layer of a lake. I used this model to run two simulations, one where N recycling by meso-zooplankton was present, and another where meso-zooplankton N recycling was removed. I parameterized both model simulations with observed data from Lake NE-12 in northern Alaska, and compared both simulations to observed concentrations of ^{15}N in the upper mixed layer of the same lake. Mean residence time of N in the upper mixed layer was 14 days with recycling of N by meso-zooplankton, compared to 8 days when recycling was absent. The observed mean residence time of ^{15}N calculated in Lake NE-12 was 16 days, and closely resembled the model simulation with recycling of N by meso-zooplankton.

Although there has been considerable research investigating the importance of zooplankton N excretion for lake nutrient budgets (Lehman 1980; Sarnelle and Knapp 2005), to my knowledge this is the first study to utilize an isotopic tracer approach to model the influence of zooplankton nutrient recycling on the mean residence time of N in the surface waters of a lake. The model prediction from this research showed an increase in N mean residence time of 6 days in the presence of zooplankton recycling. However, it should be noted that this was a very basic model, and a more sophisticated model which considers microbial mineralization, translocation of N by fish, loss to lake outflow, and resuspension of N from the sediments would be required to fully appreciate the

complexity of N retention in the upper mixed layer of a lake. Nonetheless, the simulation from my relatively simple model was similar to observed N retention from a whole-lake isotope tracer study, and supports the hypothesis that zooplankton N recycling is an important mechanism for N retention in lake nutrient budgets.

In my fourth chapter, I investigated whether N and P recycling by meso-zooplankton could affect the availability of these two potentially limiting nutrients at the landscape level. I compared N and P deficiencies in phytoplankton communities in lakes where meso-zooplankton communities were dominated by copepods or cladocerans, two broad taxonomic groups of meso-zooplankton. In addition, I made these comparisons on four separate occasions with variable amounts of precipitation during the 10 days prior to sampling. I found a negative relationship between precipitation and both N and P deficiency in the lakes sampled, indicating that N and P loading to lakes during high runoff events can alleviate nutrient deficiency in phytoplankton communities. I also found a positive relationship between lakes where the meso-zooplankton community had a higher proportion of copepods relative to cladocerans and N deficiency. Results from this research indicate that not only can recycling of N and P by meso-zooplankton be an important nutrient source for phytoplankton production, but due to stoichiometric differences in the N:P ratio of recycled nutrients, meso-zooplankton community composition can affect N versus P limitation of phytoplankton production.

Results from my research showed a stronger influence of zooplankton stoichiometry on N availability, and less of a relationship with P. (Elser et al. 1988), found a stronger relationship between P availability and meso-zooplankton community

composition in a similar study on temperate, lower-latitude lakes. Soils and lake sediments in arctic Alaska have high concentrations of reduced iron that can sequester bioavailable P through chemical sorption. Geochemical processes may have a larger influence on the bioavailability of P in this region. In addition, the relatively low number of lakes in this study resulted in considerable unexplained variability. In order to further expand our understanding of the role meso-zooplankton community composition has in N versus P availability for phytoplankton production this research would need to be expanded to a larger number of lakes across the region.

The body of my dissertation research has shown that internal nutrient recycling by consumers in arctic lakes has the potential to be a substantial source of N and P required for phytoplankton production. CNR has not previously been explored as a source of lake N and P in this region, and my research adds to our understanding of the drivers of phytoplankton production in arctic lakes. In other systems internal nutrient recycling can be greater than watershed loading as a source of N and P (Hudson et al. 1999), but arctic lakes receive the majority of their N and P from snowmelt runoff in early spring (Whalen and Cornwell 1985). Following snowmelt runoff, watershed N and P loading to lakes can be very low as nutrient uptake by vegetation increases. My research has shown that CNR increases the residence time of nutrients within lake ecosystems, and would be important for retaining an early season pulse of N and P in lake ecosystems.

Although my research has shown the importance of CNR in arctic lakes, more research is required to fully appreciate the roll of consumers in lake nutrient budgets. Concurrent measurements of phytoplankton nutrient uptake and N and P excretion would

elucidate the connections between CNR and lake productivity. In addition, more analysis of the stoichiometric relationship between consumers, food resources, and excreted nutrients would further our understanding of the potential for consumers to be both nutrient sources and sinks in these lakes.

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APPENDIX

18-June-2009

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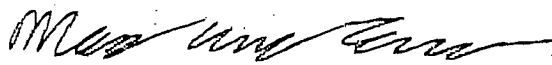
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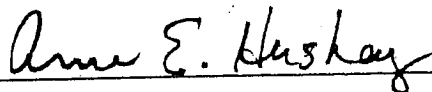
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