AN EVALUATION OF ECOLOGICAL STOICHIOMETRY IN PELAGIC SYSTEMS

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ABSTRACT

Ecological stoichiometry is the study of the balance of chemical substances in ecosystems. In freshwaters, research has focused on how the ratios of carbon, nitrogen and phosphorus in organisms and their environment affect ecosystem processes. Because autotrophs have variable stoichiometry, particulate C:N:P ratios are used to assess nutrient availability in lakes. Zooplankton have relatively fixed stoichiometry and so differences between their body stoichiometry and the stoichiometry of their food can constrain their growth. Ecological stoichiometry predicts that zooplankton with low C:P body ratios (e.g., *Daphnia*) will be limited by the P content of their food in lakes where seston C:P is high. The stoichiometric theory of consumer-driven nutrient recycling (CNR) predicts that the stoichiometry of a consumer will influence the stoichiometry of the nutrients they regenerate through such processes as egestion and excretion. In lakes, zooplankton with a low body N:P are expected to regenerate nutrients in a high N:P ratio, potentially shifting nutrient limitation of the food web from N to P limitation. I used data from 99 Canadian lakes to test the following:

a. Are particulate C:P and N:P ratios consistent with other P deficiency indicators?

b. Do seston C:P and N:P ratios affect zooplankton community composition?

c. Does zooplankton community composition affect plankton P limitation as predicted by CNR?

Particulate C:P and N:P ratios generally agreed with other P deficiency indicators, except dissolved phosphate turnover times (TT_{PO4}). C:P and N:P suggested P sufficiency more often than TT_{PO4}, possibly because these two indicators respond to P deficiency over different time scales. Most zooplankton biomass parameters were negatively related to seston C:P ratios consistent with improved food quality at lower seston C:P. There was, however, no evidence that

Daphnia were more strongly affected than any other zooplankton. Turnover times of particulate P in the whole plankton assemblage were not related to zooplankton community structure parameters. However, particulate P turnover in the >200 µm size fraction increased with increasing zooplankton biomass. There was no evidence for a particular effect of *Daphnia* on particulate P turnover. Phosphorus deficiency indicators showed a trend of relaxing P deficiency as zooplankton biomass and the proportion of *Daphnia* increased. This contradicts the predictions of CNR which suggest that *Daphnia* should cause greater P deficiency in lakes.

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CHAPTER 1 – GENERAL INTRODUCTION

1.1 Review of Ecological Stoichiometry Literature

1.1.1 Introduction

Ecological stoichiometry is the study of the balance of chemical substances in ecosystems (Sterner and Elser 2002). Research in the field of ecological stoichiometry centers around investigating how mismatches in elemental ratios at various ecological interfaces affect ecosystem processes. Considerable work has looked at how ratios of available nutrients (especially N and P) in the environment affect primary producers, and how elemental ratios in producers affect the nutrition of consumers (Sterner and Elser 2002). Because organisms are constrained by the rules of mass balance, predictions about how ecosystems function can be made based on the ratios of available resources (Moe et al. 2005, Hall 2009). Elemental stoichiometry has been used in freshwater systems to assess phytoplankton nutrient limitation (Healey and Hendzel 1980). Recent work continues to investigate what influences phytoplankton stoichiometry (Klausmeier et al. 2008). The role of stoichiometry in determining the quality of phytoplankton as food for zooplankton has also been an area of research. This research has focused in particular on *Daphnia*, which is a relatively P-rich zooplankton that might be subject to dietary P limitation rather than food quantity limitation (Sterner and Elser 2002). Finally, research has also focused on the role of zooplankton in regenerating nutrients, particularly the effect of zooplankton body stoichiometry on the stoichiometry of their regenerated nutrients. It is my aim here to review recent research in ecological stoichiometry in the following areas: the use of C:N:P ratios as indicators of phytoplankton nutrient deficiency, the role of phytoplankton stoichiometry in determining food quality for zooplankton, and the role of stoichiometry in consumer-mediated nutrient recycling.

1.1.2. C:N:P ratios as indicators of nutrient deficiency

Some of the earliest work in the field of phytoplankton stoichiometry was conducted by Redfield (1958), who found that the elemental ratios of the ocean's plankton were relatively constant. The "Redfield ratio", 106C: 16N:1P (in moles), is still often used as a benchmark for comparing measurements of phytoplankton stoichiometry. However, it is now known that phytoplankton stoichiometry is quite variable, particularly in lakes, where C:P ratios are often much greater than the Redfield ratio (Hecky et al. 1993). Early work with algal cultures demonstrated that phytoplankton stoichiometry reflected the N:P ratio of the growth medium (Rhee 1978) and specific growth rate of the algae (Goldman et al. 1979). Many of the predictions of ecological stoichiometry are based on the idea that autotroph stoichiometry is variable and regulated by the environmental conditions during growth (Sterner and Elser 2002).

The light-nutrient hypothesis is an important part of stoichiometric theory and suggests that algal C:N:P is regulated by the relative availability of N, P and light (Sterner and Elser 2002). When nutrient availability is high, algae can take up and store excess N and P. This "luxury consumption" can be responsible for the wide range of N:P ratios observed in phytoplankton. The rate at which algae incorporate organic C into their biomass depends on their photosynthetic rate, which depends on light availability. When nutrients are plentiful, but light levels are low, luxury consumption and reduced photosynthetic rates would be expected to cause high cellular N and P concentrations relative to cellular C concentrations (Healey 1985). Conversely, when nutrient availability is low, algae are likely to have high C:nutrient ratios, especially when light availability is high. The interacting effects of light and nutrient limitation in determining algal stoichiometry has been demonstrated in laboratory and field studies (Healey 1985, Urabe and Sterner 1996, Sterner et al. 1997, Hessen et al. 2002).

Seston C:N:P ratios are currently used extensively to assess the nutrient deficiency of primary producers in lakes. Often they are used together with several other nutrient deficiency indicators to determine the identity of the limiting nutrient and the severity of limitation (e.g., Vrba et al. 1995, Jarvinen et al. 1999, Guildford et al. 2005). Stoichiometric ratios are also useful for assessing how anthropogenic nutrient inputs are affecting nutrient limitation over time and for evaluating the success of nutrient reduction programs (Kilham 1990). C:N:P ratios are generally understood to be integrated measures of nutrient deficiency over longer time scales than physiological bioassays (Davies et al. 2004). C:N:P ratios are known to be influenced by multiple factors including N:P loading ratios (Healey and Hendzel 1980), lake size, water residence time (Hecky et al. 1993), mixing depth (Berger et al. 2006), and seasonal changes in phytoplankton growth rates (Hessen et al. 2005). In a survey of Norwegian lakes, total phosphorus (TP) was found to be the major determinant of seston C:P, with the fraction of detritus in seston, chlorophyll concentrations or Secchi depth, and lake colour as secondary contributors (Hessen 2006).

Seston stoichiometric ratios have several potential shortcomings as indicators of nutrient deficiency. Seston is composed of a variety of heterotrophic, autotrophic and mixotrophic organisms combined with both allochthonous and autochthonous detritus. Each of these seston components will contribute to the overall C:N:P, but may not respond to changes in nutrient limitation in the same way. For instance, bacteria are known to have elemental ratios that vary with nutrient availability (Vrede et al. 2002, Makino and Cotner 2004), but zooplankton often have relatively fixed elemental compositions (Andersen and Hessen 1991). Furthermore different seston components will have different average elemental ratios. Bacteria (Vadstein et al. 1988, Vrede et al. 2002) and some zooplankton species (Andersen and Hessen 1991) generally have

higher P content than phytoplankton and therefore changes in the relative abundance of these organisms could change seston C:N:P without reflecting an actual change in plankton nutrient deficiency. Finally, detritus is often a major portion of seston carbon (Hessen et al. 2003), but may not have C:P and N:P ratios reflecting the nutrient status of currently living plankton. Despite these potential confounding factors, seston stoichiometry is generally consistent with nutrient deficiency assays (Hecky et al. 1993).

1.1.3. Seston C:N:P and food quality constraints on zooplankton

Consumers obtain both energy and essential nutrients from their food. A trophic-dynamic understanding of ecosystems emphasizes the role of energy (closely tied to organic C) transfer between trophic levels (Lindeman 1942). Ecological stoichiometry focuses on the balance of elements and the potential for elemental imbalances to constrain ecosystem processes (Sterner and Elser 2002). A consumer can be limited by the content of specific elements in their food, rather than by food quantity or C content.

In freshwaters, research into the role of stoichiometry in determining food quality has focused on the phytoplankton-zooplankton interaction. Because phytoplankton have C:N:P stoichiometry that varies widely based on environmental factors, herbivores experience a broad range of elemental content in their food. Unlike phytoplankton, zooplankton maintain a relatively constant body C:N:P which differs between zooplankton species (Andersen and Hessen 1991, Hessen and Lyche 1991). Threshold elemental ratios (TERs) have been developed by several authors for food P content for the genus *Daphnia*, which has been the model group for most of this research. When food C:P is higher than the TER, *Daphnia* are expected to be limited by P rather than C in their food. TERs for *Daphnia* are often cited at a C:P of approximately 300 (Sterner 1993, Urabe et al. 1997), but lower values [e.g., 190-200 (Anderson and Hessen 2005), 90 (Demott et al. 1998)] have been reported. The actual TER will be higher when food

availability is low, because when food is scarce, animals will devote most of it to meeting basic metabolic demands rather than growth and reproduction which require more P (Anderson et al. 2005). Seston C:P values in lakes are often higher than these predicted TER values (Hecky et al. 1993), suggesting that P limitation of *Daphnia* growth is a real possibility. Using model simulations based on several literature datasets, Brett et al. (2000) found support for a reduction in food quality for daphnids when algal C:P >300, but also found that changes in algal species composition accounted for four times more of the variability in daphnid growth rates than C:P ratios.

Numerous lab studies have found a reduction in growth rate when *Daphnia* fed on P limited algae (Sommer 1992, Sterner 1993, Sterner et al. 1993, DeMott 1998, Demott et al. 1998, Ferrao-Filho et al. 2007). An alternate hypothesis has suggested that *Daphnia* are more likely to be limited by highly unsaturated fatty acids in their diet than simply N or P (Muller-Navarra 1995). However, addition of inorganic P to *Daphnia* growth experiments and the subsequent decrease in food C:P has resulted in increases in *Daphnia* growth rates (Urabe et al. 1997, DeMott 1998, Elser et al. 2001). In an experiment in field enclosures Urabe et al. (2002) found that when incident light was reduced, phytoplankton C:P decreased and led to increased *Daphnia* growth despite reductions to total seston C caused by shading. Overall, studies suggest that *Daphnia* can be limited by P in nature.

At the community level, stoichiometric theory predicts that competition between zooplankton species will be affected by their elemental requirements. For example, *Daphnia*, a genus with low C:P is expected to be a poor competitor against species with a high C:P when food is P deficient (Sterner and Elser 2002). Sterner (1998) found little evidence for reduced *Daphnia* recruitment in a lake with very high seston C:P ratios. In contrast, DeMott and Gulati (1999)

found that increasing seston C:P ratios caused reductions in *Daphnia* biomass but did not affect other zooplankton with lower P requirements in 2 of 3 hypereutrophic study lakes. Subsequent experiments confirmed that *Daphnia* populations in these lakes were limited by P (DeMott et al. 2001). Hassett et al. (1997) found a negative relationship between the percent of *Daphnia* in zooplankton biomass and seston C:P and N:P in a 31 lake survey and also found a positive relationship between the percentage of calanoids (typically N-rich) and seston C:P and N:P. These findings support a stoichiometric role in structuring zooplankton communities. Hessen (2006) found a negative correlation between both total zooplankton and *Daphnia* biomass and seston C:P in a survey of Norwegian lakes. McCarthy and Irvine (2010) found a weak positive correlation between zooplankton C:P and seston C:P in six Irish lakes but a negative correlation between *Daphnia* abundance and seston C:P and N:P, supporting the idea of stoichiometric food quality limitation. Overall, there is considerable evidence that zooplankton can be limited by food stoichiometry. However, the importance of food stoichiometry in relation to other factors structuring zooplankton communities is not well understood.

1.1.4. Consumer-mediated nutrient recycling

Consumers have the potential to impact nutrient cycling in several ways. As they consume, they sequester nutrients in their biomass, but also release nutrients via excretion, egestion and sloppy feeding. Consumers also affect nutrient cycling indirectly by changing the abundance and species composition of other organisms in the ecosystem (Vanni 2002). The importance of consumers as sources and sinks of nutrients has been a matter of debate. Experiments where phytoplankton are separated from consumers by nutrient permeable barriers have shown increased phytoplankton growth due to regeneration by zooplankton (Sterner 1986) and fish (Vanni and Layne 1997, Attayde and Hansson 2001). Other studies suggest that consumers act as sinks of nutrients by sequestering them in their biomass (Sommer et al. 2003, Sereda et al. 2008).

Consideration of ecological stoichiometry allows a greater understanding of how consumers can affect nutrient availability for producers.

The stoichiometric theory of consumer-mediated nutrient recycling (CNR) allows predictions of how consumers will affect nutrient availability for producers based on the stoichiometry of the consumers and their food (Sterner and Elser 2002). In lakes, zooplankton have relatively fixed elemental compositions (Andersen and Hessen 1991), and therefore cannot change their body stoichiometry to match that of their food. Ecological stoichiometry predicts then that the relative amount of P regenerated by zooplankton will depend on body C:P and food C:P (Olsen et al. 1986). Likewise, the ratio of N:P regenerated by zooplankton will depend on zooplankton N:P and food N:P (Sterner 1990). When N:P ratios in algae match N:P ratios in zooplankton, the N:P ratio regenerated by zooplankton should match that of their food. When algae have a higher N:P than zooplankton, zooplankton will retain relatively more P than N and their regenerated material will have a higher N:P than their food. Similarly, when algal N:P is lower than zooplankton N:P, zooplankton will retain relatively more N and will regenerate nutrients with a lower N:P ratio than their food. Because zooplankton generally have lower C:nutrient ratios than their food and also often have lower N:P ratios (Urabe et al. 1995), zooplankton might be expected to generally reduce the relative amount of nutrients, particularly P, that are resupplied to phytoplankton.

Zooplankton have low intraspecific but relatively high interspecific variation in C:N:P (Andersen and Hessen 1991). This variation means that zooplankton communities with different species compositions will have different community C:N:P ratios and are expected to recycle nutrients in different ratios (Sterner 1990, Elser and Urabe 1999). *Daphnia* in particular have low C:P and N:P ratios relative to other zooplankton (Hessen and Lyche 1991). Because of their relatively high P content, *Daphnia* are expected to regenerate relatively little P and sequester

more of it in their biomass. Communities dominated by *Daphnia* are expected to regenerate relatively less P than communities dominated by other zooplankton, potentially leading to increased P limitation for phytoplankton when *Daphnia* are a dominant group in the zooplankton community (Sterner and Elser 2002).

Most research conducted on consumer-driven nutrient recycling has been based on models (e.g., Sterner 1990, Elser and Urabe 1999) including a recent modeling study of how CNR might regulate primary production in the ocean (Nugraha et al. 2010). Models studying CNR are often complex and make various predictions about how grazers can affect nutrient limitation for producers and which circumstances are required for grazers to cause a shift in the limiting nutrient (Andersen 1997, Daufresne and Loreau 2001). They also predict a stoichiometric effect of decomposers on autotroph nutrient limitation, which is affected by the extent to which decomposers are preyed upon by other consumers (Cherif and Loreau 2009). Stoichiometric models of food web dynamics are reviewed by Hall (2009) and further discussion of these models is beyond the scope of this study.

Differences in regenerated nutrient ratios between zooplankton taxa have been demonstrated in laboratory cultures (Rothhaupt 1997) and in controlled experiments using lake water plankton communities (Brett et al. 1994). Enclosure studies where natural zooplankton are removed and replaced with *Daphnia* have found increased dissolved N:P in *Daphnia* treatments relative to controls (Mackay and Elser 1998, Paterson et al. 2002). Elser et al. (1988) studied the effects of zooplankton on algal nutrient limitation in 3 lakes and in experimental mesocosms. They found that as the zooplankton community shifted to larger taxa, particularly *Daphnia*, algal P-limitation increased while N-limitation decreased. Sterner et al. (1992) later interpreted these results in a

stoichiometric context, suggesting that as communities shifted to *Daphnia* dominance, P became limiting because *Daphnia* recycle relatively more N than P.

Elser et al. (2000) reported large changes in dissolved nutrients and TIN:TDP ratios in a lake after a piscivore introduction experiment changed the structure of the zooplankton community. They observed increased zooplankton biomass dominated by *Daphnia*, greatly increased dissolved nitrogen (sevenfold) and significantly increased dissolved phosphorus (twofold). They attributed these changes in nutrient concentrations to changes in nutrient recycling by zooplankton. They suggested that changes in the TIN:TDP ratio made nitrogen relatively more available than phosphorus. However, given the lower phytoplankton biomass they observed, and the increased supply of both nutrients, it is likely that nutrient limitation was relaxed for both N and P. This finding suggests that zooplankton may make nutrients more available to phytoplankton, rather than increasing nutrient limitation through differential recycling of N and P. This is consistent with the seston C:P and N:P ratios they observed in the study year with high *Daphnia* biomass, which indicated reduced P limitation despite the increase in the TIN:TDP ratio. McCarthy et al. (2006) similarly found DIN: TP ratios to increase when *Daphnia* dominated zooplankton biomass, while DIN:TP decreased with the dominance of calanoid copepods in Lough Carra. While these studies provide evidence that zooplankton can differentially recycle nutrients in lakes, the importance of CNR relative to other processes has not been established. Furthermore, the importance of CNR on a multi-lake scale has not been assessed.

1.2. Research Introduction

1.2.1. Approach

My research uses radiophosphate uptake and regeneration dynamics to test hypotheses of ecological stoichiometry in whole plankton communities across multiple lakes. I compared seston C:P and N:P ratios with other measurements of P deficiency in order to assess how these measures relate to each other and how well they indicate P deficiency. I also investigated how seston C:P and N:P ratios are related to zooplankton community structure to test the hypothesis of stoichiometric nutrient limitation for herbivores in lakes. Finally, I assessed the importance of consumer-driven nutrient recycling by examining the effect of different zooplankton assemblages (e.g., *Daphnia* dominated vs. copepod dominated) on phosphate limitation and regeneration.

This is a significant step in testing the predictions of ecological stoichiometry because, unlike other studies, I will be using unmanipulated natural plankton communities. Many studies have devoted significant time to the modeling of CNR, yet empirical tests of its importance have been few. Additionally, measures of nutrient flux have rarely been applied to test stoichiometric theory. My approach will provide a more holistic (natural zooplankton communities) and direct (flux measurements) test of the predictions of ecological stoichiometry which are derived mostly from models and theoretical considerations.

1.2.2. Study Lakes

A total of 99 lakes were sampled once in summer between 1997 and 2010. These form a diverse set of freshwater lakes that span a large gradient in size and depth, and include both stratified and isothermal systems. The lakes are located in Ontario, Saskatchewan, Manitoba and Alberta, Canada (Fig. 1.1). Fifty seven of the study lakes are located on the Great Plains 35 are

on the Canadian Shield and 7 are in the Rocky Mountains. All of the Alberta lakes, the ELA

lakes and 3 lakes from the Saskatchewan plains were sampled in 1997 or 1998. Fifteen of the

Saskatchewan shield lakes were sampled between 2003 and 2005. The remainder of the

Saskatchewan lakes were sampled between 2008 and 2010. The names, locations and sampling

dates of my study lakes are presented in Appendix I.

Lakes on the Great Plains tend to be shallow, with relatively high nitrogen, phosphorus, and

major ion concentrations (Pham et al. 2008). These lakes often have low dissolved N:P ratios

(Leavitt et al. 2006), and experience blooms of N_2 fixing cyanobacteria (Patoine et al. 2006).

Nutrient limitation of lakes on the Great Plains varies, with some often limited by N (Leavitt et

al. 2006) while others are more often limited by P (Prepas and Trimbee 1988, Prepas and Trew

1983). General information on many Alberta lakes is available in Mitchell and Prepas (1990).

Further information on the montane lakes in my study can be found in Anderson (1974).

Lakes located on the Canadian Shield in northern Saskatchewan are generally oligotrophic,

with low dissolved ion concentrations and circumneutral to slightly alkaline pH (Rawson 1960,

Jeffries et al. 2010). Basic limnological data for most of the Saskatchewan shield lakes in my

study are presented by Helps (2009). Lakes located in the Experimental Lakes Area are also

located on the Canadian Shield. These lakes have been well studied and similarly have low

nutrient concentrations and low dissolved ions concentrations (Armstrong and Schindler 1971).

Particulate C:P ratios tend to be relatively high in these lakes and lakes are generally P limited

(Hecky et al. 1993).

A regional summary of basic limnological parameters is given in Figure 1.2. In general, lakes

spanned a wide gradient of TP, Chl *a*, Secchi depth and zooplankton biomass. Lakes located on

the Great Plains tended to be more productive than lakes from the other regions, having greater

TP, Chl *a* and zooplankton biomass, but lower Secchi depths. The Great Plains also had the

widest range in TP, Chl *a* and zooplankton biomass, but had a narrower range for Secchi depth.
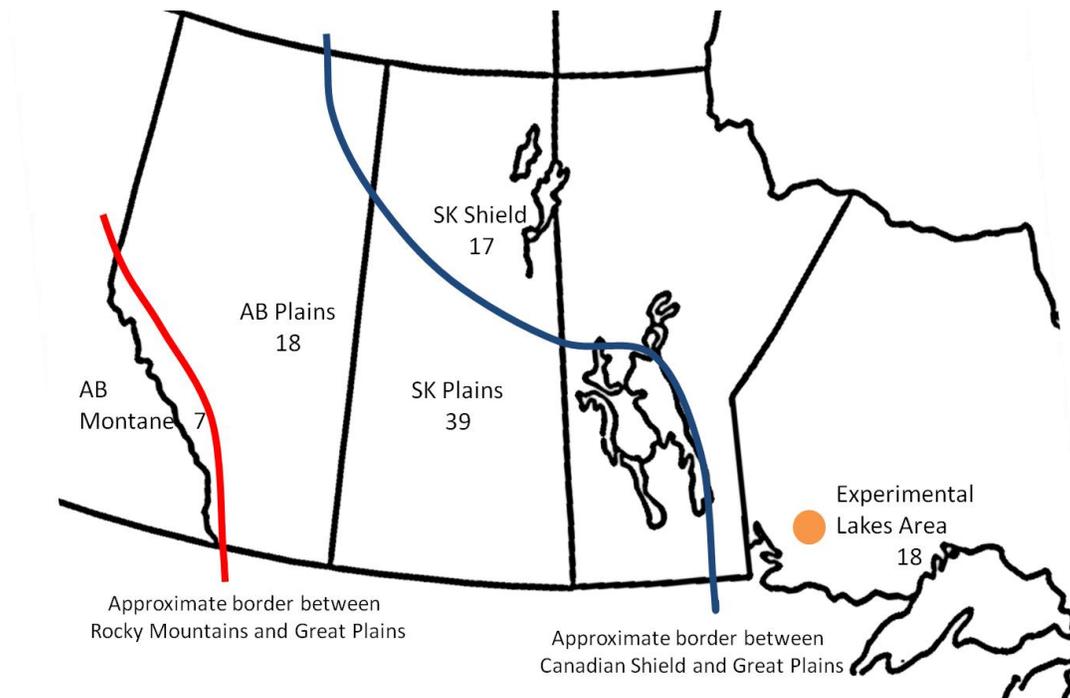
Figure 1.1. Map of western Canada showing the location of the study lakes. The number of study lakes from each region is indicated. The shaded circle represents the location of the experimental lakes area. AB, Alberta; SK, Saskatchewan.
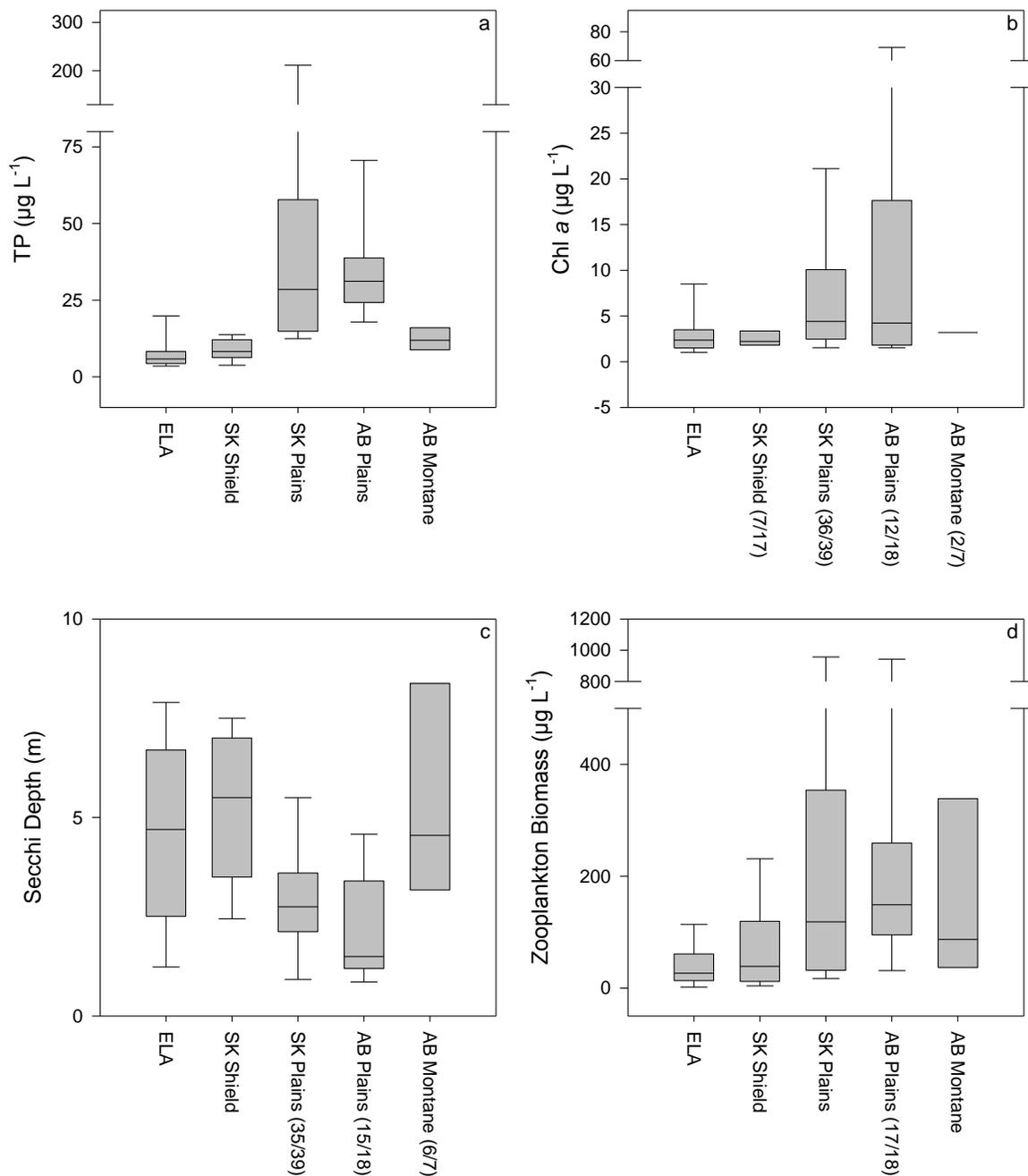
Figure 1.2. Box and whisker plots of basic limnological variables from the different lake regions. Where data is not available for all lakes from a region, the number of lakes for which data is plotted is indicated in parentheses after the region name. Whiskers indicate the 10th and 90th percentiles, while boxes cover the range between the 25th and 75th percentile. The median is indicated by a horizontal line. Where whiskers and boxes are absent (AB Montane) the corresponding percentiles could not be calculated because the sample size was too small. Zooplankton biomass (> 200 μm) is in dry weight. AB, Alberta; SK, Saskatchewan; ELA, Experimental Lakes Area.

CHAPTER 2 – SESTON C:N:P RATIOS AS INDICATORS OF PLANKTONIC NUTRIENT DEFICIENCY AND FOOD QUALITY FOR ZOOPLANKTON

2.1. Introduction

Elemental ratios in lake plankton have been used to assess several aspects of ecosystem function. Redfield (1958) interpreted the similarity between seston N:P and the ratios of dissolved inorganic N and P in the oceans as evidence for biotic control of ocean nutrient concentrations. Molar ratios of C:P, C:N and N:P are commonly used as phytoplankton nutrient deficiency indicators in lakes (Healey and Hendzel 1980) and may also be useful to evaluate how changes in nutrient loading affect resource supply to phytoplankton (Kilham 1990). C:P and C:N ratios are also informative for assessing the relative availability of light and nutrients, because seston organic C increases when photosynthetic rates increase. More recently, elemental ratios have also been recognized as important determinants of the nutritional quality of phytoplankton for herbivores (Sterner and Elser 2002). Seston C:N:P ratios are important ecosystem parameters that are understood to affect aquatic food webs and biogeochemical processes.

2.1.1. Factors affecting seston C:N:P ratios

Chemostat studies with algal cultures have shown that phytoplankton stoichiometry depends strongly on nutrient supply ratios (Rhee 1978) and algal growth rate (Goldman et al. 1979). Algal cells are able to take up nutrients in excess of their immediate requirements. Therefore, cells may have relatively high concentrations of non-limiting nutrients but low concentrations of limiting nutrients. When growth rates are rapid (i.e., nutrient supply and other environmental conditions are favourable for rapid growth) algal N:P reflects species specific optimum N:P ratios instead of nutrient supply ratios (Klausmeier et al. 2004). This N:P ratio is interpreted to be the N:P ratio actually required by the algae for rapid growth. While supply N:P ratios are thought

to influence algal N:P ratios, Hall et al. (2005) found little effect of supply N:P on seston N:P in a pond survey and in mesocosm experiments.

Light is also known to influence phytoplankton stoichiometry because of its influence on photosynthetic carbon fixation. The balance of light availability and nutrient availability is therefore expected to regulate C:nutrient ratios, in accordance with the light-nutrient hypothesis (LNH) (Sterner et al. 1997, Sterner and Elser 2002). The LNH predicts that lakes with low light and high nutrient conditions will have low seston C:nutrient ratios while lakes with high light and low nutrients will have high C:nutrient ratios. In lakes, light availability for algae is related to water transparency and mixing depth. Lakes with low water transparency and a deep mixing depth will provide the least amount of light because algae will likely spend a greater amount of time in the aphotic zone. The light-nutrient hypothesis is supported by both lab (Urabe and Sterner 1996, Hessen et al. 2002, Hessen et al. 2008) and field studies (Sterner et al. 1997).

Many studies interpret seston C:N:P to be reflective of phytoplankton elemental composition. However, phytoplankton rarely make up the majority of the seston (Hessen et al. 2003). Much of seston C comes from autochthonous and allochthonous detritus, bacteria, protozoa and zooplankton. Autochthonous detritus may have similar elemental stoichiometry to live phytoplankton (Hessen et al. 2003), but allochthonous detritus may not, and would not be expected to vary with nutrient conditions in the lake. Bacteria are expected to have stoichiometry that varies with nutrient availability (Vrede et al. 2002, Makino and Cotner 2004), but zooplankton are not (Hessen and Lyche 1991). Bacteria (Vrede et al. 2002) and zooplankton (Hessen and Lyche 1991) also generally have higher P content than phytoplankton. Seston stoichiometry could therefore change due to changes in the relative abundance of different

organisms in the food web and such changes to seston stoichiometry may not reflect a change in phytoplankton stoichiometry or nutrient status.

2.1.2. P deficiency indicators

Eutrophication of lakes due to anthropogenic nutrient input is a global concern (Cooke et al. 2005). Nutrient inputs from industry, agriculture and municipalities can cause increases in plankton biomass and shifts in plankton species composition. Such changes can have adverse effects on lakes, including reduction of oxygen in the water column, increased presence of toxin-producing Cyanobacteria and reduction of water quality for recreational and drinking water purposes (Schindler et al. 2008). Because of the key role nutrients play in the function of lake ecosystems, an understanding of the type (e.g., N or P limitation) and the degree of limitation is fundamental for proper lake management.

Phosphorus is a limiting nutrient in many lakes (Sterner 2008) and a variety of techniques have been developed to assess the degree of P limitation of plankton (Beardall et al. 2001). Interpretation of P deficiency measurements should distinguish between proximate deficiency, which reflects the physiological needs of plankton, and ultimate deficiency, which determines which factors control productivity over longer time scales (Davies et al. 2004, Davies et al. 2010). In general, total and particulate nutrient ratios are expected to reflect longer term limitation, while physiological assays indicate proximate deficiency. P deficiency indicators are often used to exclusively assess phytoplankton nutrient deficiency, despite the fact that many indicators are also affected by other organisms (e.g., zooplankton and bacteria). Using multiple P deficiency indicators reduces the likelihood of incorrect interpretations due to differences in time scales or sensitivity to nutrient deficiency in organisms other than phytoplankton.

The measurement of alkaline phosphatase activity (APA) in lake water has been used extensively to determine phosphorus limitation in plankton (Pettersson 1980, Rose and Axler

1998, Guildford and Hecky 2000). Alkaline phosphatases are a group of enzymes produced by plankton that catalyze the hydrolysis of phosphoester linkages, enabling plankton to obtain PO_4^{3-} from dissolved organic matter. Two properties of alkaline phosphatases make them useful for measuring P limitation: their synthesis is repressed and their activity is inhibited by elevated PO_4^{3-} concentrations (Jansson et al. 1988). Under low PO_4^{3-} concentrations, cells are expected to increase production of alkaline phosphatases in order to access organically bound P from their environment. Alkaline phosphatase activity (normalized to plankton biomass) has been found to have an inverse relationship with cellular P (Gage and Gorham 1985), algal surplus P (Pettersson 1980, Chrost and Overbeck 1987), and water column SRP (Nedoma et al. 2006) in lakes. However, other studies have found no relationship between APA and SRP (Pettersson 1980, Jamet et al. 1997, 2001, Cao et al. 2010). Pick (1987) found that APA was relatively insensitive to phosphate additions in Lake Ontario, where 1 μM phosphate additions caused only 50% reductions in APA after 18 hours. Alkaline phosphatase activity may respond more slowly than other physiological assays and indicate P deficiency over a slightly longer timescale than more proximate measures (e.g., radiophosphate turnover times).

Measuring radiophosphate uptake kinetics may be a more direct approach to determining plankton P demand (Lean and Nalewajko 1979, Lean 1984). Phosphate uptake by bacteria and algae is known to increase during P starvation (Jansson 1988), which should cause a shortening in the turnover time of the dissolved phosphate pool (TT_{PO4}). TT_{PO4} should be influenced by the size of the PO_4^{3-} pool, as well as the demand for PO_4^{3-} by the entire plankton assemblage. Phosphate demand is expected to be a function of the P demand of individual organisms and total plankton biomass. Short turnover times (<20 minutes) may be indicative of P limitation (Lean and Pick 1981). However, PO_4^{3-} uptake is often dominated by bacteria (Currie and Kalff 1984b)

and may therefore be most indicative of P deficiency in these organisms. Radiophosphate uptake assays have been considered reliable, but limited in use because of the logistics of using radioisotopes.

Hudson et al. (2000) and Hudson and Taylor (2005) developed a steady state radiobioassay to estimate PO_4^{3-} concentrations in lakes ($ssPO_4^{3-}$). This technique typically reports PO_4^{3-} concentrations in the picomolar range in P limited systems, which is 2-3 orders of magnitude lower than typical SRP concentration in these lakes (Hudson et al. 2000). These estimates are consistent with other advanced techniques for measuring PO_4^{3-} (Taylor and Lean 1991, Dodds 1993, Gillor et al. 2010). Steady state phosphate has not yet been compared with other P deficiency measurements, but low PO_4^{3-} concentrations are generally considered to indicate P deficiency in freshwater and marine environments.

Because there is no definitive measure of P limitation in freshwater systems, P deficiency indicators may be best evaluated by comparison with several other indicators. My first objective in this chapter is to examine how seston C:N:P ratios compare with other P deficiency measures. I compare C:N:P ratios with APA, TT_{PO4} and $ssPO_4^{3-}$. I also compare C:N:P ratios with total phosphorus (TP) and total dissolved phosphorus (TDP) concentrations. TP and TDP are often used as measures of lake trophic status, affect several aspects of ecosystem function, and have been found to be correlated with seston C:P ratios (Hessen 2006).

2.1.3. Role of seston stoichiometry in determining zooplankton community structure

Zooplankton community structure is influenced by several factors including both top-down and bottom-up interactions. Fish predation has long been known to be a major factor, with strong predation associated with smaller zooplankton size distributions and changes in species composition (Brooks and Dodson 1965). Food availability (Vanni 1987), food digestibility (Van-Donk and Hessen 1993), interspecific competition (Demott and Kerfoot 1982) and seasonal

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dynamics (Hairston et al. 2000) are also important factors structuring zooplankton communities. More recently, considerable attention has focused on the influence of food quality on zooplankton growth and community composition. Specifically, research has focused on the influence of biochemical nutrient limitation (Muller-Navarra 1995) and stoichiometric element limitation (Sterner and Elser 2002).

Nutrient limitation of zooplankton is expected to occur in lakes when there is a mismatch between phytoplankton C:N:P ratios and zooplankton demand for these nutrients. Zooplankton generally have lower C:N and C:P ratios than phytoplankton. Because phytoplankton have variable stoichiometry, but zooplankton have fixed stoichiometry, there may come a point at which herbivorous zooplankton are limited by the nutrient content of their food, rather than its energy content (i.e., carbon). Several studies have aimed to determine threshold elemental ratios (TERs) for food, above which different consumers will become nutrient rather than C limited (Olsen et al. 1986, Urabe and Watanabe 1992, Anderson and Hessen 2005, Anderson et al. 2005, Frost et al. 2006). In lakes, *Daphnia* are known to have a low body C:P and a low TER, which is often cited as a C:P of ~ 300 (Sterner 1993, Urabe et al. 1997). In general, herbivorous cladocerans have lower C:P ratios, while copepods and predatory cladocerans have higher C:P ratios (Hessen and Lyche 1991, Sterner and Elser 2002). Because of its relatively high P requirements, *Daphnia* is expected to be P limited in lakes with high seston C:P and should be at a competitive disadvantage compared to other zooplankton with lower P requirements (Sterner and Elser 2002).

Evidence that the stoichiometry of food has a pronounced effect on zooplankton has been found in many lab culture studies, where *Daphnia* experienced reduced growth when fed P deficient algae (Sommer 1992, Sterner 1993, Sterner et al. 1993, DeMott 1998, Demott et al.

1998, Ferrao-Filho et al. 2007). Field evidence that food stoichiometry influences zooplankton community structure exists, but is not as conclusive. Hassett et al. (1997) found that low N:P zooplankton tended to dominate in lakes with low N:P seston while high N:P zooplankton were favoured in lakes with high N:P seston in a 31 lake survey. DeMott and Gulati (1999) observed declines of P-rich *Daphnia* as seston C:P increased in 3 Dutch lakes, but no change in populations of lower-P zooplankton including *Bosmina* and cyclopoid copepods. Using model simulations based on several literature datasets, Brett et al. (2000) found support for a reduction in food quality for daphnids when algal C:P >300, but also found that changes in algal species composition accounted for four times more of the variability in daphnid growth rates than C:P ratios. More recently, McCarthy and Irvine (2010) found weak support for reduction in *Daphnia* abundance with increasing seston C:P in a set of Irish lakes. While field evidence for a stoichiometric effect of food quality on zooplankton is building, the role of dietary stoichiometric constraints in structuring zooplankton communities is poorly understood and merits further study. My second objective in this chapter is therefore to examine how seston C:P and N:P ratios relate to zooplankton community structure at the multi-lake scale. If *Daphnia* are limited by P as predicted by stoichiometric theory, they should be a smaller proportion of the zooplankton in lakes where seston C:P is high. I would therefore expect to find decreasing relationships between the percentage of zooplankton biomass as *Daphnia* and seston C:P and N:P ratios.

2.2. Methods

2.2.1. Field Sampling

Analyses in this chapter are restricted to 59 study lakes where C:N:P measurements were taken. These lakes were sampled during the summer months (July – September) 1997-2003 and 2010. Lake water samples for laboratory analyses (~20 L) were collected from a central location

with a van Dorn sampler at mid-epilimnetic depth in stratified lakes and at 1 m in isothermal lakes. Water was gently decanted into a collapsible polyethylene bag that had been washed (0.1% Liqui-Nox P-free detergent), leached (dilute HCl) and rinsed with lake water. Water samples were transported to the laboratory in a cooler.

2.2.2. Zooplankton Sampling and Counting

Crustacean zooplankton were sampled from the same depth as water samples, using a van Dorn sampler from 1997 to 2003 and using a 30 L Schindler-Patalas trap in 2010. Water was passed through a 200 μm mesh and animals were preserved in a 4% sucrose-formalin solution. Zooplankton were identified and photographed using a stereomicroscope fitted with a digital camera (Leica MZ16A microscope with Leica DFC 480 camera, Leica Microsystems (Canada) Inc.). Zooplankton biomass (dry weight) was estimated from body lengths measured electronically using ZEBRA 3, a semi-automated zooplankton counting program updated from Allen et al. (1994). Length-weight relationships are from Girard and Reid (1990) and with the exception of *Holopedium* were not corrected for shrinkage in formalin (Campbell and Chow-Fraser 1995, Yan et al. 2001). Zooplankton samples were subdivided and a minimum of 250 individuals were counted where possible. Samples were subdivided by making the sample up to a known volume, thoroughly mixing the sample and subsampling a known volume with a wide-bore plastic pipette. Zooplankton samples were not available from one of the lakes, and so comparisons with zooplankton community parameters are restricted to 58 lakes. Of the 58 lakes, 28 had fewer than 250 individuals per sample and in these cases the entire sample was counted.. Of the zooplankton samples with less than 250 individuals, 8 lakes had fewer than 50, 8 lakes had between 50 and 100 and 12 lakes had between 100 and 250 individuals. The zooplankton sample with the fewest individuals had 17 individuals.

2.2.3. Chemical analyses

TP, and TDP were measured according to (Parsons et al. 1984). Samples for TDP were obtained through syringe filtration (0.2 μm polycarbonate filters). Particulate phosphorus (PP) was calculated by subtracting TDP from TP. Chlorophyll *a* (Chl *a*) samples were collected on 47 mm glass fiber filters GF/F filters (Whatman GF/F or Advantec GF75, vacuum filtration 10 psi) and frozen until analysis. Chlorophyll pigments were extracted and analyzed according to Bergmann and Peters (1980) except that absorbance was read at 665 rather than 655 (Arvola 1981, Dessouki et al. 2005).

Particulate C and N samples (n = 3) were collected by vacuum filtration on precombusted (450 ℃ for 3 hours) glass fiber filters (Whatman GF/F or Advantec GF75). Filtered samples were dried for 3 hours at 60 °C and stored at room temperature until analysis. Samples were not acidified. Some of the measured C may therefore have been particulate inorganic C. It is relatively common for researchers not to acidify samples when determining seston C and N [e.g., Hecky et al. (1993) Hassett et al. (1997)]. It is possible that some of our C estimates are artificially high, however, our highest C:P ratios are from lakes in the ELA, where waters are dilute and particulate inorganic carbon is unlikely to be a major concern (Hecky et al. 1993). It is unlikely that these high C:P ratios are artificially high because of inorganic C contributions. C and N were analyzed on an elemental analyzer (model 440, Control Equipment Corporation) for samples collected prior to 2003. The remainder of C and N analysis was completed using an ANCA-GSL sample preparation unit coupled to a Tracer 20 mass spectrometer (Europa Scientific). Dissolved P was measured in the filtrate that was collected while filtering for particulate C and N. This dissolved P concentration was subtracted from TP to determine seston P content. In order to assess the analytical variability of PP estimates, I computed the coefficient of variation for particulate P determinations (n=3) for the 12 lakes with particulate C:N:P

measurements in 2010. Coefficients of variation ranged from 0.037 to 0.26 and had an average of 0.10.

2.2.4. Alkaline Phosphatase Activity

Alkaline phosphate assays were conducted within 48 hours of sampling, usually the day after sampling. Alkaline phosphatase activity was determined fluorometrically (Pettersson 1980) in whole lake water. Autoclaved lake water was used as a blank. First, 2 mL of each sample were placed into individual wells on a multi-well plate (Falcon). Next, 0.5 mL of 4 mM 4-methylumbelliferyl phosphate (MU-P) (MP Biomedicals) in Tris buffer adjusted to pH 8 with HCl was added. The hydrolysis of MU-P to methylumbelliferone (MU) was monitored at regular time intervals with a Varioskan® Flash spectral scanning multimode reader (Thermo Electron Corporation). Assays were conducted at 30 ºC. Standard curves were constructed in whole lake water using a 5 µM stock solution of MU in Tris buffer, which was diluted with appropriate volumes of lake water to give a final volume of 2.5 mL in each well. Enzyme activities are expressed as a rate of P release standardized to Chl *a* concentration. APA was measured at only 12 of the 59 lakes where C:N:P measurements were taken, so analyses with APA are restricted to these 12 lakes. These lakes are all located in Saskatchewan, with 2 located on the Canadian Shield and 10 in the Great Plains region.

2.2.5. Radiophosphate Bioassays

Radiophosphate uptake bioassays were begun within 24 hours of sampling in acid-washed polyethylene containers. Carrier-free radiophosphate ($^{33}PO_4$, final activity ~50 000 cpm ml^{-1}) was added into 4 L of lake water. Planktonic uptake of radiophosphate was monitored by sub-sampling the dissolved pool at approximately 1, 2, 5, 8, and 12 min after radiophosphate addition and less frequently over the next 2 hours (syringe filtration, 3 to 10 ml sub-samples, 25 mm diameter polysulphone, 0.2 µm pore size). Lakes with very slow ^{33}P uptake were sub-sampled

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over a longer time, up to 48 hours. Radioactivity was measured by liquid scintillation counting and corrected for background radioactivity. Quenching of samples was not detected.

Radioactivity remaining in the dissolved fraction (i.e., total disintegrations per minute) over time was fitted to a polynomial function (Currie and Kalff 1984a, Bentzen and Taylor 1991). The polynomial of best fit to the initial time series points (e.g., at times equal to 0, 1, 2, and 5 minutes) was used. The uptake constant (k) was determined by taking the derivative of the polynomial at time zero and dividing by the total radioactivity (Currie and Kalff 1984a, Bentzen and Taylor 1991). The reciprocal of this uptake constant is equal to TT_{PO4}.

The remaining water was incubated in an environmental chamber at ambient lake temperature and photoperiod for an additional 12-24 h to label the plankton assemblage. Then these incubations were terminated with the addition of unlabelled $^{31}PO_4^{3-}$ as a competitive inhibitor (final concentration 1 mg L^{-1}) (Hudson and Taylor 1996) to prevent further uptake of radiophosphate. Occasionally regeneration experiments were started up to 2 days later if ^{33}P uptake was very slow. Assaying of the accumulation of radiophosphate in the dissolved pool (syringe filtration, 25 mm diameter polysulphone 0.2 μm pore size filters) started approximately one hour after addition of the competitive inhibitor. The slope resulting from the accumulation of dissolved radiophosphate over time provided an estimate of the rate of release of dissolved phosphorus which was used to calculate the rate of planktonic P regeneration (Hudson and Taylor 1996). Regeneration rates were used to calculate $ssPO_4^{3-}$ concentrations: $ssPO_4^{-3} = R$ (ng L^{-1} min^{-1}) $\div$ k (min^{-1}), where R is the measured regeneration rate and k is the uptake constant (Hudson et al. 2000).

2.2.6. Statistical Analyses

The relationships between P deficiency measures and seston stoichiometry were examined (correlation analysis). Relationships were examined further with reduced major axis linear

regression (Model II). Model II regression was used in favour of Model I because substantial error is likely in all P deficiency measurements. Zooplankton community structure was compared to seston stoichiometry using Model I linear regression analysis. Model I regression was used for these comparisons because they test specific predictions of ecological stoichiometry and the error in the independent variable is assumed to be much smaller than the error in the dependent variable. For all analyses, data were transformed to meet the assumptions of parametric statistics. The Box-Cox method was used to suggest an appropriate variance stabilizing transformation. I analyzed zooplankton community structure by considering the importance of cladocerans, copepods and the genus *Daphnia*. For each group, I considered biomass, proportion of total zooplankton biomass (% Cladocera, % Daphnia), and biomass standardized to particulate phosphorus concentrations (Zooplankton/PP, Cladocera/PP, Copepoda/PP, *Daphnia*/PP). For analyses involving measures of *Daphnia* biomass, lakes with no *Daphnia* were excluded (22 of 58 lakes) to avoid the disproportionate influence these lakes would have on relationships. The exclusion of samples where *Daphnia* is absent has precedent in the ecological stoichiometry literature (Hessen 2006, McCarthy and Irvine 2010).

2.3. Results

2.3.1. Variation in trophic state and P deficiency indicators

Summary statistics for the measured P deficiency indicators are presented in Table 2.1. The study lakes span a wide gradient of lake productivity as indicated by their range in total P concentrations (1.79 - 140 μg L^{-1}). The P deficiency indicators also had wide ranges (Table 2.1). Phosphate turnover times were generally rapid (55 of 59 lakes had TT_{PO4} <15 min), suggesting P deficiency in most lakes. Steady state PO_4^{3-} concentrations were generally in the low ng L^{-1} range (median 3.92 ng L^{-1}). Bradwell Reservoir and Cowan Lake were exceptions, with $ssPO_4^{3-}$

concentrations of 1.6 and 2 µg L^{-1} respectively. Alkaline phosphatase activities spanned one and

a half orders of magnitude but were only measured for 12 of the 59 lakes.

Table 2.1. Characteristics of the measured P deficiency indicators. C:P and N:P are particulate ratios in moles. TT$_{PO4}$, turnover time of dissolved phosphate (min); ssPO$_4^{3-}$, steady state phosphate (ng L^{-1}); APA, alkaline phosphatase activity (nmol P µg Chl a^{-1} L^{-1}); TP, total phosphorus (µg L^{-1}); TDP, total dissolved phosphorus (µg L^{-1}).

	C:P	N:P	TT$_{PO4}$	ssPO$_4^{3-}$	APA	TP	TDP
maximum	1208	93.1	276	2004	1.10	140	68.9
minimum	48.1	6.55	0.92	0.84	0.054	1.79	0.30
median	187	21.1	3.57	3.92	0.36	13.4	4.56
mean	252	25.1	10.5	50.3	0.49	20.5	7.75
n	59	59	59	59	12	59	59

2.3.2. Particulate C:N:P

Particulate C:P and N:P ratios were tightly correlated and agreed in their assessment of P

deficiency (Fig. 2.1a). Based on C:P deficiency thresholds (Healey and Hendzel 1980) 14 of 59

lakes had P sufficient plankton, 24 were moderately deficient and 21 showed severe P

deficiency. Based on N:P deficiency criteria, 31 lakes were P sufficient and 28 were P deficient.

The relationships between C:P and N:P and P deficiency indicators are presented in Table 2.2.

C:P and N:P ratios were negatively correlated with TP (Fig. 2.1b), TDP (Fig. 2.1c) and ssPO$_4^{3-}$

(Fig. 2.2a). The relationships of C:P and N:P with TP, TDP and ssPO$_4^{3-}$ are such that P sufficient

and moderately P deficient plankton occur across the entire range of TP, TDP and ssPO$_4^{3-}$, while

extremely P deficient plankton occur at moderate to low concentrations of TP, TDP and ssPO$_4^{3-}$.
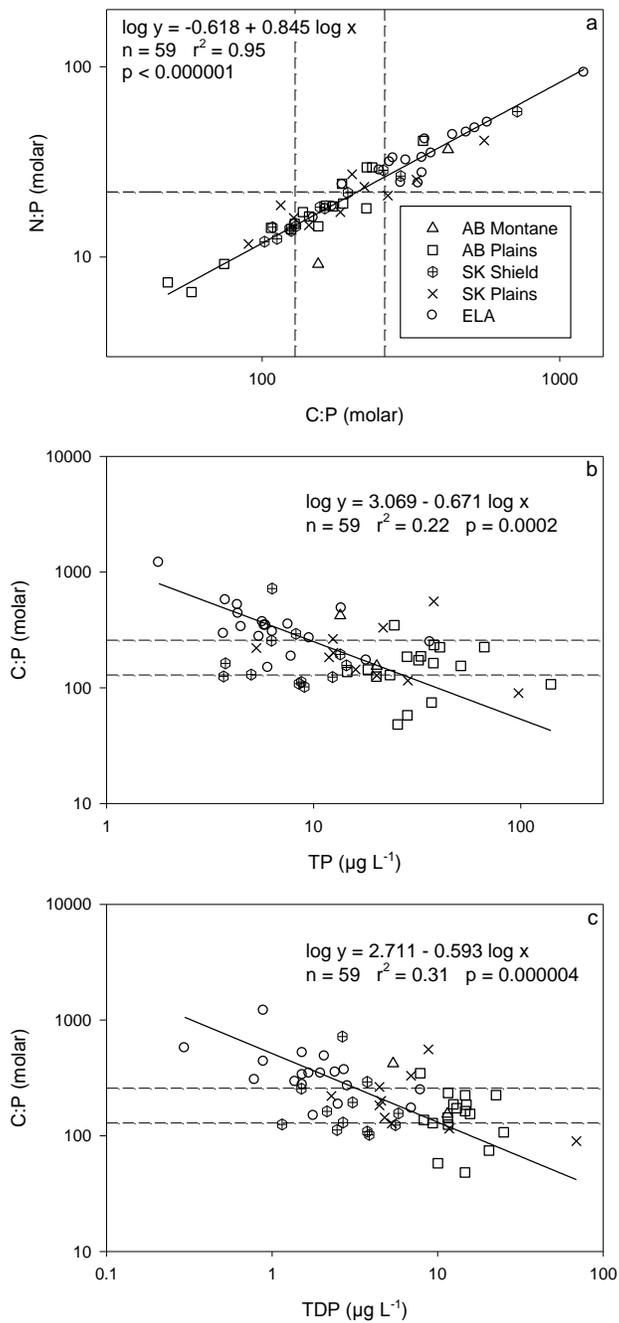
Figure 2.1. Relationships between N:P and C:P (a), C:P and TP (b) and C:P and TDP (c). Symbol type indicates the region where each lake is located. Trend lines are based on reduced major axis regression. Dashed lines associated with C:P ratios are thresholds between P sufficiency and moderate deficiency (lower line) and between moderate deficiency and severe deficiency (upper line). The dashed line associated with N:P ratios in a is the threshold between P deficiency and sufficiency (Healey and Hendzel 1980).

Table 2.2. Relationships between C:P and N:P and indicators of P deficiency. Slopes and intercepts are from reduced major axis regression. C:P and N:P are particulate ratios in moles. TP, total phosphorus (μg L^{-1}); TT$_{PO4}$, turnover time of dissolved phosphate (min); ssPO$_4^{3-}$, steady state phosphate (ng L^{-1}); TDP, total dissolved phosphorus (μg L^{-1}); APA, alkaline phosphatase activity (nmol P μg Chl a^{-1} L^{-1}).

Parameters (x,y)	Transforms (x,y)	n	r	p	Slope	Intercept
TP, C:P	log, log	59	-0.47	0.0002	-0.671	3.069
TP, N:P	log, log	59	-0.43	0.0007	-0.567	1.977
TT$_{PO4}$, C:P	$x^{-1/2}$, log	59	0.12	0.36	1.447	1.542
TT$_{PO4}$, N:P	$x^{-1/2}$, log	59	0.19	0.14	0.237	1.213
ssPO$_4^{3-}$, C:P	$x^{-1/3}$, log	59	0.57	0.000002	0.701	1.867
ssPO$_4^{3-}$, N:P	$x^{-1/3}$, log	59	0.60	0.000001	0.615	0.947
TDP, C:P	log, log	59	-0.56	0.000004	-0.593	2.711
TDP, N:P	log, log	59	-0.54	0.00001	-0.502	1.674
C:P, N:P	log, log	59	0.95	<0.000001	0.845	-0.618
APA, C:P	log, log	12	0.78	0.003	0.577	2.525
APA, N:P	log, log	12	0.88	0.0001	0.389	1.469

Seston stoichiometric ratios were not correlated with TT$_{PO4}$ (Fig. 2.2b), but both C:P and N:P were positively correlated with APA (Fig. 2.2c).

2.3.3. Zooplankton community parameters and seston C:N:P

Zooplankton biomass ranged from 0.91 to 1175 μg L^{-1} and was variable in the percentage of biomass as cladocerans or copepods. *Daphnia* was absent in 22 of the 58 lakes but in the remaining lakes the percentage of *Daphnia* in the total zooplankton biomass was variable, with 3 lakes having over 80% of the zooplankton biomass as *Daphnia*. Relationships between zooplankton community parameters and C:P and N:P ratios are presented in Table 2.3.

a

$$\log y = 1.534 + 1.222\,x^{-1/3}$$
$$n = 59 \quad r^2 = 0.33 \quad p = 0.000002$$

C:P (molar ratio)

10000

1000

100

10

0.1   1   10   100   1000   10000

$ssPO_4^{3-}$ (ng L^{-1})

b

	△	AB Montane
	□	AB Plains
	⊕	SK Shield
	×	SK Plains
	○	ELA

C:P (molar ratio)

10000

1000

100

10

0.1   1   10   100   1000

TT_{PO4} (min)

c

$$\log y = 1.469 + 0.389\,\log x$$
$$n = 59 \quad r^2 = 0.61 \quad p = 0.003$$

C:P (molar ratio)

10000

1000

100

10

0.01   0.1   1   10

APA (nmol P µg Chl a^{-1} min^{-1})
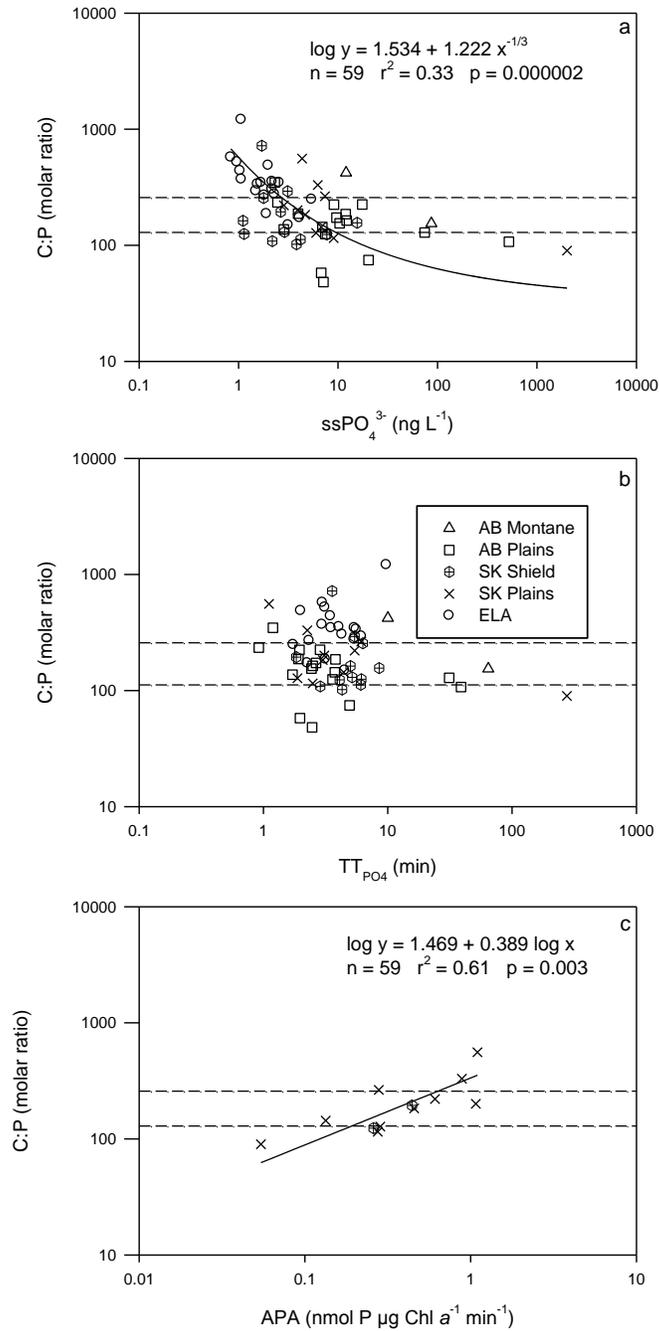
Figure 2.2. Relationships between seston C:P and $ssPO_4^{3-}$ (a), TT_{PO4} (b) and APA (c). Symbol type indicates the region where each lake is located. Trend lines are based on reduced major axis regression. Dashed lines indicate threshold C:P ratios between P sufficiency and moderate deficiency (lower line) and between moderate deficiency and severe deficiency (upper line) (Healey and Hendzel 1980).

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Total zooplankton biomass was negatively correlated with C:P (Fig. 2.3a) and N:P ratios as were

cladoceran, copepod and *Daphnia* biomasses (Fig. 2.3b). Percent Cladocera and % *Daphnia*

were not correlated with C:P or N:P. Zooplankton biomass standardized to particulate P

concentrations was negatively correlated with both C:P (Fig. 2.3c) and N:P. The remaining

zooplankton biomass parameters standardized to PP were not correlated with C:P or N:P ratios.

2.4. Discussion

2.4.1. Do C:N:P ratios agree with other P deficiency indicators?

C:P and N:P ratios were broadly consistent with the other P deficiency measurements, except

TT_{PO4}. C:P and N:P were strongly correlated with each other and agreed very closely with each

other in their assessment of deficiency based on commonly used deficiency criteria (Healey and

Hendzel 1980). Lake plankton are often considered to be either N or P limited and it is expected

that the N:P ratio will reflect whether N or P limits plankton biomass. The C:P ratio should be

affected by P limitation, but also by the availability of light for photosynthesis, which is largely

determined by water transparency and mixing depth (Sterner et al. 1997). It is significant that

C:P and N:P ratios agree so closely with each other because C:P ratios are also influenced by

physical factors and not just nutrient availability. Both C:P and N:P were negatively correlated

with TP and TDP (Figs. 2.1b,c, Table 2.2). These negative relationships suggest that plankton are

generally less P deficient in more productive lakes and are consistent with patterns of C:P and

N:P variation with TP in many of the world's lakes and oceans (Guildford and Hecky 2000).

They agree with the results of Hessen (2006) who found TP and to a lesser extent TDP to be the

major determinants of lake seston C:P in Norwegian lakes. C:P ratios have also been shown to

decrease with increasing seston abundance (Sterner et al. 2008) which is consistent with our data

given that seston abundance tends to increase with TP.

Table 2.3. Relationships between C:P and N:P ratios and zooplankton community parameters. C:P and N:P are particulate ratios in moles. Biomasses and particulate P concentrations (PP) are in $\mu g \ L^{-1}$.

Parameters (x,y)	Transforms (x,y)	n	r	p
C:P, Zooplankton Biomass	log, log	58	-0.44	0.0006
C:P, Cladoceran Biomass	log, log	58	-0.29	0.030
C:P, Copepoda Biomass	log, $y^{1/4}$	58	-0.34	0.009
C:P, *Daphnia* Biomass	log, log	36	-0.36	0.033
C:P, % Cladocera	log, $y^{1/2}$	58	0.004	0.98
C:P, % *Daphnia*	log, $y^{1/4}$	36	-0.23	0.17
C:P, Zooplankton/PP	log, $y^{1/4}$	58	-0.30	0.020
C:P, Cladocera/PP	log, log	58	-0.12	0.37
C:P, Copepoda/PP	log, $y^{1/3}$	58	-0.22	0.095
C:P, *Daphnia*/PP	log, log	36	-0.25	0.062
N:P, Zooplankton Biomass	log, log	58	-0.44	0.0005
N:P, Cladoceran Biomass	log, log	58	-0.31	0.016
N:P, Copepoda Biomass	log, $y^{1/4}$	58	-0.27	0.041
N:P, *Daphnia* Biomass	log, log	36	-0.42	0.010
N:P, % Cladocera	log, $y^{1/2}$	58	-0.05	0.73
N:P, % *Daphnia*	log, $y^{1/4}$	36	-0.33	0.051
N:P, Zooplankton/PP	log, $y^{1/4}$	58	-0.33	0.012
N:P, Cladocera/PP	log, log	58	-0.16	0.23
N:P, Copepoda/PP	log, $y^{1/3}$	58	-0.16	0.23
N:P, *Daphnia*/PP	log, log	36	-0.19	0.14
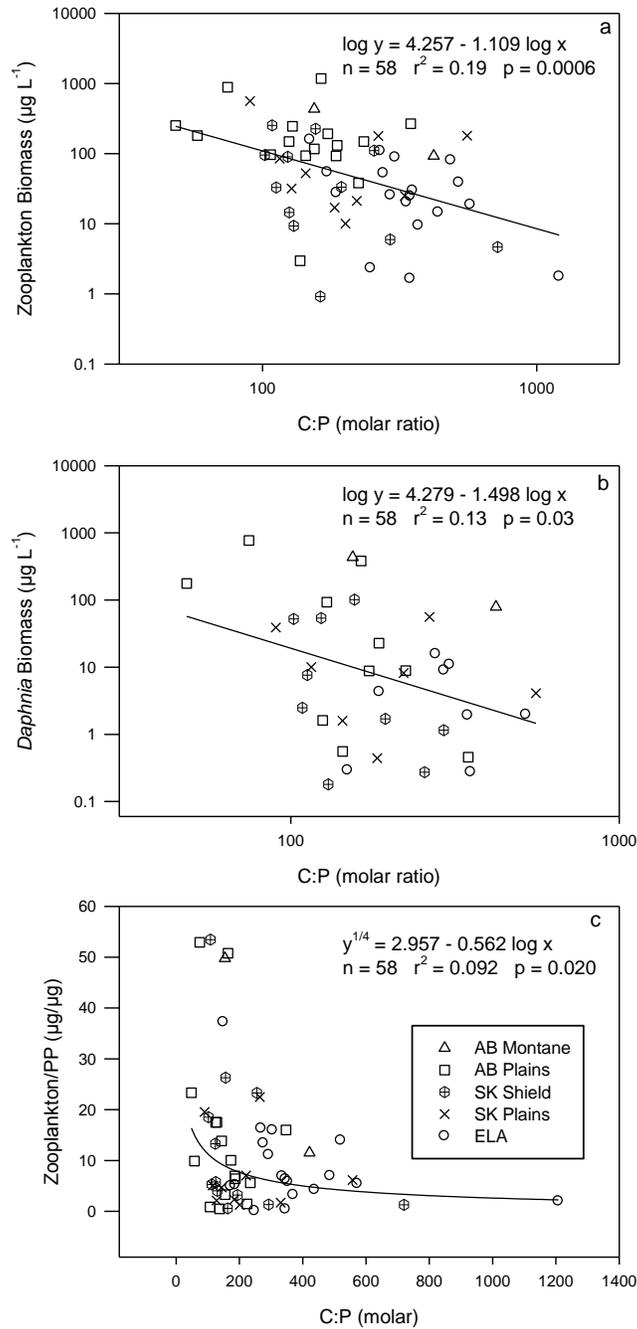
Figure 2.3. Zooplankton biomass as a function of seston C:P (a), *Daphnia* biomass as a function of seston C:P (b) and Zooplankton/PP as a function of seston C:P (c). Symbol type indicates the region where each lake is located. Trend lines are based model I least squares regression.

The relationships between seston C:P and N:P ratios and TP are not statistically independent, which could be a major reason for the observed relationships. Particulate phosphorus is a major component of TP and the two are strongly correlated ($r^2 = 0.85$, $p < 0.000001$, $n = 59$). Even if a lack of statistical independence is driving the relationship, it is worth knowing that C:P and N:P ratios tend to decrease as lake TP concentrations increase. This is particularly true because many other lake parameters are also correlated with TP (e.g. Chl *a*, zooplankton biomass, various P deficiency measures). It is important to be aware of these correlations when comparing C:P and N:P ratios with many of these other measurements because of the way they all have a tendency to vary with TP.

Sestonic C:P and N:P ratios were consistent with $ssPO_4^{3-}$ and APA in their assessment of P deficiency. Plankton stoichiometry and $ssPO_4^{3-}$ have not previously been compared as P deficiency indicators and the negative relationship we found (Fig. 2.2a) is consistent with expectations (i.e., when phosphate concentrations are high, plankton are less P deficient). However, both C:P ratios and $ssPO_4^{3-}$ concentrations are correlated with TP. It is possible that this mutual correlation with TP is responsible for the relationship between $ssPO_4^{3-}$ and C:P. The negative relationship between C:P and $ssPO_4^{3-}$ is also scattered. For example, for lakes with $ssPO_4^{3-}$ in the range of 1-10 ng L^{-1}, C:P ratios span the full range of measured values, indicating severe, moderate and no P deficiency within the same range of $ssPO_4^{3-}$. APA was positively correlated with particulate C:P (Fig. 2.2c), and N:P ratios suggesting agreement between these P deficiency indicators. While our dataset is limited to 12 lakes it is consistent with other studies showing agreement between these measurements (Hecky et al. 1993, Steinhart et al. 2002, Rattan 2009).

Dissolved PO_4^{3-} turnover times were not related to seston C:P (Fig. 2.2b) or N:P ratios. Of the 59 lakes, only 5 lakes had TT_{PO4} >10 minutes, suggesting strong P demand in most lakes. In contrast, C:P ratios suggest that 14 lakes had P sufficient seston, 24 showed moderate P deficiency and 21 had severely P deficient seston. While Istvanovics et al. (1992) also found no correlation between TT_{PO4} and particulate C:P and N:P in a study of phytoplankton P deficiency in Lake Erken, Nedoma et al. (1993) found strong agreement between the phosphate uptake constant and both C:P and N:P ratios over the course of the growing season in the eutrophic Římov reservoir. Bacteria are known to be responsible for most P uptake when turnover times are short (Currie and Kalff 1984b). It may be that TT_{PO4} is more sensitive to P deficiency in bacteria while C:N:P ratios reflect the stoichiometry of the whole plankton food web. Another possible explanation for the disagreement between these two measures is the timescale over which they are sensitive to P deficiency. Because TT_{PO4} is generally very short, small changes in PO_4^{3-} availability should cause rapid changes in TT_{PO4}. In contrast, particulate stoichiometry is likely an integrated measure of nutrient availability over the course of organism growth.

2.4.2. Is there evidence that seston C:N:P influences zooplankton community composition?

This study provides weak evidence that zooplankton community composition is affected by seston stoichiometry. Stoichiometric theory predicts that nutrient rich zooplankton can be limited by N or P when their food has high C:nutrient ratios. In particular, *Daphnia*, a P-rich species with a low C:P (Andersen and Hessen 1991) is predicted to be more strongly affected by P deficient food than other zooplankton with lower P requirements. *Daphnia* should be relatively less abundant in lakes with P-deficient phytoplankton because they will be more affected by P limitation in their food. I found a negative relationship between *Daphnia* biomass and seston C:P ($r^2 = 0.13$, p = 0.03, Fig. 2.3b) consistent with stoichiometric predictions. Negative correlations

between *Daphnia* biomass and seston C:P have been found in a survey of Norwegian lakes (Hessen 2006) and in 6 Irish lakes (McCarthy and Irvine 2010). Studies predict a range of critical threshold elemental ratios above which *Daphnia* become limited by P rather than C in their food. The TER should depend on feeding rates and a wide range of values have been predicted, for example: 90, (Demott et al. 1998) 181 (Anderson et al. 2005), and ~ 300 (Olsen et al. 1986, Urabe and Watanabe 1992). The weak relationship I found between *Daphnia* biomass and seston C:P does not provide evidence for a distinct threshold in determining *Daphnia* biomass.

While the negative relationship between *Daphnia* biomass and seston C:P and N:P is consistent with other studies and stoichiometric theory, my data provides little evidence that a stoichiometric food quality effect is responsible for the correlation. First, total zooplankton biomass was also correlated with seston C:P (Fig. 2.3a). It may be that zooplankton in general are negatively affected by high C:P ratios in their food because zooplankton usually have greater C:P ratios than the seston (Hessen and Lyche 1991, Urabe et al. 1995). Zooplankton biomass, cladoceran biomass, *Daphnia* biomass and copepod biomass were all negatively correlated with C:P ratios, suggesting that *Daphnia* are not more severely affected by high seston C:P than other zooplankton as is predicted by ecological stoichiometry. Second, C:P and N:P ratios decreased with TP concentrations (Table 2.2). Given that zooplankton biomass and *Daphnia* biomass increase with TP, the correlations between zooplankton biomass and C:P may simply result from a mutual correlation with TP. More productive lakes (which have higher TP) would then tend to have both greater zooplankton biomass and smaller C:P and N:P ratios.

The percentage of cladocerans and *Daphnia* were not correlated with C:P or N:P. This strongly suggests that *Daphnia* are not more severely affected by high C:P and N:P than other zooplankton. Furthermore, *Daphnia*/PP was not correlated with C:P or N:P, suggesting that

Daphnia biomass as a proportion of the food web is not negatively affected by low seston P concentrations. Sterner (1998) studied a *Daphnia* population in a lake with high seston C:P and found that effects of stoichiometric food quality were difficult to detect in the population. It may be that *Daphnia* are able to compensate for P limitation by consuming more food, or that other factors are much more important in determining zooplankton community structure. Brett et al. (2000) found that algal taxonomy explained 4 times more of the variability in algal food quality than elemental stoichiometry. In a series of growth assays DeMott and Tessier (2002) found that *Daphnia* growth was negatively correlated with food C:P, but also found that growth limitation was due to algal digestion resistance rather than its C:P ratio. A recent modelling study suggests that *Daphnia* are most likely to be limited by food quantity in oligotrophic lakes and by essential fatty acid content in eutrophic lakes (Persson et al. 2007). If this is true, it suggests that food stoichiometry has a minor effect on *Daphnia* in lakes and may explain why P limitation of *Daphnia* is not evident across a gradient of lake productivity. In addition to limitation by dietary P and fatty acids, *Daphnia* growth can also be limited by dissolved calcium concentrations (Hessen et al. 2000). Calcium limitation of *Daphnia* is expected to be fairly widespread in nature, with some degree of metabolic stress expected when concentrations are below 10 mg L^{-1} (Cairns and Yan 2009). While some instances of P limitation for *Daphnia* have been demonstrated in the field (DeMott and Gulati 1999, DeMott et al. 2001) it appears to be a minor influence on zooplankton communities in most cases.

2.4.3. Summary

C:P and N:P ratios broadly agreed with most indicators of P deficiency, consistent with current understanding and the common use of these ratios as indicators of nutrient deficiency. C:P and N:P ratios were not correlated with TT_{PO4} and particulate stoichiometry suggested P sufficiency more often than TT_{PO4}. Elemental ratios and TT_{PO4} may be sensitive to different food

web components or may respond to plankton P deficiency at different time scales. I found negative relationships between most zooplankton biomass parameters and C:P ratios, consistent with reduced seston food quality at high C:P. The percent contribution of *Daphnia* to zooplankton biomass was not related to C:P, suggesting that P-deficient food did not affect *Daphnia* more severely than other taxa. This is contrary to stoichiometric predictions regarding food quality for herbivores, which suggest that *Daphnia* should be more severely affected by high seston C:P ratios because they have lower C:P ratios than other zooplankton.

CHAPTER 3 – CONSUMER-MEDIATED NUTRIENT RECYCLING

3.1 Introduction

Phosphorus is often the main nutrient limiting plankton growth in aquatic systems (Sterner 2008). It has long been understood to limit algal and bacterial growth, and more recently has been shown to potentially limit consumer growth (Sterner and Elser 2002). Understanding the cycling of phosphorus in aquatic food webs has therefore been of considerable interest. One area of focus has been on the role of specific groups of organisms in determining overall P availability. For instance, the role of fish as either sources or sinks of P in lakes has been a contentious topic (Chidami and Amyot 2008, Sereda et al. 2008, Amyot et al. 2010, Sereda and Hudson 2010). In recent decades, the role of zooplankton in P cycling has also been a matter of considerable debate.

Herbivorous zooplankton can influence algal biomass through direct grazing and indirectly through nutrient regeneration (Vanni 2002). Changes in zooplankton abundance, size structure and species composition are known to affect primary production, phytoplankton species composition and phytoplankton biomass. Dominance of the zooplankton community by large cladocerans, particularly *Daphnia*, has been associated with reduced algal standing stock, and also a shift to larger filamentous algae (Sterner 1989). Less clear though, is the impact of changing zooplankton community structure on P regeneration and P limitation of the food web.

3.1.1. Influence of zooplankton species composition on ratios of regenerated nutrients

In the last two decades, considerable research has focused on the role of consumer stoichiometry in determining consumer nutrient release (Sterner and Elser 2002). The stoichiometric theory of consumer-mediated nutrient recycling (CNR) predicts that the N:P regenerated by zooplankton will depend on their food N:P and their body N:P (Sterner 1990).

Algal N:P varies with nutrient supply (Rhee 1978). Zooplankton N:P on the other hand shows little intraspecific variability but is variable between species (Hessen and Lyche 1991). Much of the research into ecological stoichiometry has focused on the role of *Daphnia*, which has relatively low body C:P and N:P ratios. Because they have a relatively high proportion of P compared to C and N, *Daphnia* are expected to act as P sinks, while regenerating relatively more N than other zooplankton with lower P content (Elser and Urabe 1999). In contrast, copepods have relatively high body N:P ratios and are expected to retain more N and recycle more P. The relative importance of copepods and *Daphnia* in zooplankton assemblages is therefore predicted to alter resupply ratios of N and P.

Studies of CNR in lakes have been primarily based on models (Sterner 1990, Sterner et al. 1992, Elser and Urabe 1999). Others have studied CNR in small bottles or field enclosures with manipulated zooplankton communities (Brett et al. 1994, Mackay and Elser 1998, Paterson et al. 2002). Such tests have generally found increases in dissolved N:P ratios (i.e., DIN:SRP) associated with treatments where *Daphnia* has been experimentally added. Field studies of CNR have primarily examined shifting zooplankton communities in the context of biomanipulation experiments (Elser et al. 1988, Elser et al. 2000). Urabe et al. (1995) determined N and P regeneration by zooplankton in Lake Biwa and found that the N:P released was negatively correlated with zooplankton N:P, in agreement with CNR theory. Overall there is evidence for differential nutrient recycling by zooplankton, but the importance of this effect has not been put into context with regeneration from other sources. Furthermore, research has focused on how differential recycling influences N:P ratios, but has not considered how zooplankton community composition impacts the total regeneration of N and P.

3.1.2. Influence of zooplankton species composition on plankton N vs. P limitation

Several potentially contradictory lines of reasoning have been put forward to predict how zooplankton composition could affect P limitation. Trophic cascade research has associated large grazers, particularly *Daphnia*, with reductions in algal biomass (Shapiro and Wright 1984, Carpenter et al. 1996, Carpenter et al. 2001) and transitions to zooplankton communities dominated by *Daphnia* have been associated with relaxed P limitation (Sarnelle and Knapp 2005). On the other hand, allometric theory predicts that mass specific grazing rates and nutrient regeneration should decrease with increasing organism size (Peters and Downing 1984, Wen and Peters 1994). However, studies have found no influence of zooplankton size distribution (Cyr and Pace 1992), or the dominance of cladocerans vs. copepods (Cyr 1998, Sommer et al. 2001) on zooplankton community grazing rates.

Ecological stoichiometry offers an alternate prediction based on resupply ratios of N and P. In addition to grazing effects on algal biomass and the overall regeneration of nutrients, zooplankton species composition could affect nutrient limitation through differential recycling of N and P (Sterner and Elser 2002). This is the explanation given by Sterner et al. (1992) for the results of Elser et al. (1988), which showed increasing P limitation (evaluated using alkaline phosphatase activities) of the algae when zooplankton communities shifted to dominance by larger zooplankton, particularly *Daphnia*, in association with lake biomanipulation experiments. The extent to which CNR influences nutrient limitation will likely depend on how large of a stoichiometric mismatch there is between zooplankton and their food (Urabe et al. 1995), the severity of nutrient limitation and the importance of other zooplankton grazing effects, such as shifts in algal species composition and reduction in overall algal biomass.

In this chapter I examine the effects of zooplankton community structure on P regeneration and P limitation in a diverse set of Canadian lakes. To evaluate effects of zooplankton on P

regeneration, I compare zooplankton community parameters with the turnover time of the particulate P pool (TT_{PP}) and the turnover time of the >200 μm particulate P pool ($TT_{>200}$). Both of these parameters are measured using radioisotope techniques and provide a measure of P release relative to the size of the particulate P pool. Measuring $TT_{>200}$ allows a measurement of particulate P turnover for the >200 μm size fraction (largely zooplankton), while TT_{PP} measures particulate P turnover in the whole planktonic assemblage. To evaluate effects of zooplankton on P limitation, I use phosphate turnover times (TT_{PO4}), steady state phosphate concentrations ($ssPO_4^{3-}$) and alkaline phosphatase activity (APA) as indicators of P deficiency. These indicators are based on short-term bioassays and should respond to short term changes in P deficiency. Using shorter-term indicators of deficiency (as opposed to particulate or total stoichiometry) is more relevant for assessing how the current zooplankton community is affecting P limitation for the rest of the plankton. I also compare both zooplankton community structure and P turnover and deficiency measurements to total phosphorus concentrations (TP) in order to understand how these measures vary across a trophic gradient. In particular, I look for evidence of a stoichiometric effect on P availability associated with dominance of P-rich *Daphnia* or N-rich copepods. By looking for relationships in natural plankton communities across multiple lakes I can assess whether CNR is an important process relative to the many other factors influencing nutrient limitation in lakes. If *Daphnia* are differentially recycling N and P in accordance with the stoichiometric predictions of CNR, P turnover should be slower as *Daphnia* become a larger proportion of the zooplankton. The plankton community should also be more P deficient in lakes where *Daphnia* are a larger proportion of the zooplankton.

3.2 Methods

3.2.1. Field Sampling

I use 98 of the 99 study lakes previously noted to test CNR. The zooplankton sample from one lake decomposed and the zooplankton biomass could not be determined for this lake. Sampling was conducted during the summer months (July - September) between 1997 and 2010. Lake water samples for laboratory analyses were collected from a central location with a Van Dorn sampler at mid-epilimnetic depth in stratified lakes and at 1 m depth in isothermal lakes. Water was gently decanted into a 20 L collapsible polyethylene bag that had been washed (0.1% Liqui-Nox P-free detergent), leached (dilute HCl) and rinsed with lake water. Water samples were transported to the laboratory in a cooler.

3.2.2. Zooplankton Sampling and Counting

Crustacean zooplankton were sampled at the same depth using a Van Dorn sampler from 1997 to 2008 and using a 30 L Schindler-Patalas trap in 2009 and 2010. Water was passed through a 200 μm mesh and animals were preserved in a 4% sucrose-formalin solution. Zooplankton were identified and photographed using a stereomicroscope fitted with a digital camera (Leica MZ16A microscope with Leica DFC 480 camera, Leica Microsystems (Canada) Inc.). Zooplankton biomass (dry weight) was estimated from body lengths measured electronically using ZEBRA 3, a semi-automated zooplankton counting program updated from Allen et al. (1994). Length-weight relationships are from Girard and Reid (1990) and with the exception of *Holopedium* were not corrected for shrinkage in formalin (Campbell and Chow-Fraser 1995, Yan et al. 2001). Zooplankton samples were subsampled for counting in order to count a minimum of 250 individuals where possible. Subsamples were taken by making the sample up to a known volume, thoroughly mixing the sample and subsampling a known volume

with a wide-bore plastic pipette. Of the 98 lakes with zooplankton samples, 41 had fewer than

250 individuals per sample and in these cases the entire sample was counted. Of the zooplankton

samples with less than 250 individuals, 9 lakes had fewer than 50, 13 lakes had between 50 and

100 and 19 lakes had between 100 and 250 individuals. The zooplankton sample with the fewest

individuals had 17 individuals.

3.2.3. Chemical analyses

Analyses of TP, TDP, Chl *a*, particulate C and particulate N were performed as described in

section 2.2.3. Particulate P (PP) in the >200 μm size fraction was determined by passing water

(0.5 – 10 L) through a 200 μm nitex screen and backwashing the material collected on the screen

into a sample bottle with deionized water. The samples were then analyzed in the same manner

as TP. PP was only measured for 70 of the 99 lakes, so analyses requiring PP concentrations are

restricted to these lakes.

3.2.4. Alkaline Phosphatase Activity

Alkaline phosphatase activity was measured as described in section 2.2.4. Activities are

expressed as rates standardized to Chl *a* concentrations. APA was measured in 38 of the study

lakes, so analyses with APA are restricted to these lakes.

3.2.5. Radiophosphate Bioassays

Methods for determining TT_{PO4}, phosphorus regeneration rates, and $ssPO_4^{3-}$ concentrations

are given in section 2.2.5. Regeneration rates for the >200 μm size fraction were determined

concurrently with whole water regeneration rates. Approximately 100 mL of ^{33}P-labelled lake

water was passed through a 200 μm screen using syringe filtration just prior to the addition of

competitive inhibitor. Competitive inhibitor ($^{31}PO_4^{3-}$, 1 mg L^{-1} final concentration) was added to

this filtrate and the accumulation of radiophosphate in the dissolved pool was monitored in the

same manner as for whole water regeneration. Phosphorus regeneration rates in the >200 μm size

fraction were calculated by subtracting the <200 μm rate from the whole water regeneration rate. TT_{PP} was determined by dividing the particulate phosphorus concentration by the whole water regeneration rate. $TT_{>200}$ was calculated by dividing the particulate P concentration in the >200 μm size fraction by the regeneration rate in the >200 μm size fraction.

3.2.6. Statistical Analyses

Model I linear regression analysis was used to compare TT_{PP}, $TT_{>200}$ and P deficiency indicators with zooplankton community structure parameters and TP concentrations. When necessary, data were transformed to meet the assumptions of parametric statistics. The Box-Cox method was used to suggest an appropriate variance stabilizing transformation. I analyzed zooplankton community structure by considering the importance of cladocerans, copepods and the genus *Daphnia*. For each group, I considered their absolute biomass, their percentage of total zooplankton biomass (% Cladocera, % Daphnia) and their biomass standardized to particulate phosphorus concentrations (Zooplankton/PP, Cladocera/PP, Copepoda/PP, *Daphnia*/PP). For analyses involving measures of *Daphnia* biomass I excluded lakes with no *Daphnia* (25 of 98 lakes) to avoid the disproportionate influence these lakes would have in these relationships. The exclusion of samples where *Daphnia* is absent has precedent in the ecological stoichiometry literature (Hessen 2006, McCarthy and Irvine 2010).

3.3. Results

3.3.1. Basic Limnological Parameters

A summary of basic limnological parameters for the study lakes is given in Table 3.1. Total phosphorus concentrations ranged from 1.78 to 298 μg L^{-1} representing a wide range in lake trophic state. Total zooplankton biomass ranged from 0.92 to 1633 μg L^{-1} and was positively correlated with TP (Fig. 3.1a). The biomass of cladocerans, copepods and *Daphnia* were also all

positively correlated with TP. The percentage of zooplankton biomass as cladocerans was not related to TP, and neither were Zooplankton/PP, Cladocera/PP, Copepoda/PP or *Daphnia*/PP. There was a positive correlation between % *Daphnia* and TP (Fig. 3.1b), but this relationship was influenced by 3 high TP lakes. When these lakes are removed from the analysis, there is no relationship between % *Daphnia* and TP.

Table 3.1. Basic limnological characteristics of the study lakes. TP, total phosphorus; Chl a, chlorophyll a.

	TP ($\mu g\ L^{-1}$)	Chl *a* ($\mu g\ L^{-1}$)	Secchi Depth (m)	Zooplankton Biomass ($\mu g\ L^{-1}$)
maximum	298	89	11.5	1633
minimum	1.78	0.76	0.5	0.92
median	15.4	3.4	3.4	87.7
mean	32.2	7.4	3.9	196
n	98	74	90	98

3.3.2. Relationships with TT_{PP} and $TT_{>200}$

Particulate P turnover times ranged from 2.50 to 12.3 days with an average of 5.17 days. Relationships between TT_{PP} and zooplankton community parameters are presented in Table 3.2. Particulate phosphorus turnover times were negatively correlated with TP, but were not correlated with any of the zooplankton community parameters (Fig. 3.2). Phosphorus regeneration in the >200 μm size fraction ranged from $0-49\%$ of whole water regeneration with a mean of 19%. $TT_{>200}$ ranged from $0.25-19.8$ days with a mean of 4.59 days. Relationships between $TT_{>200}$ and zooplankton community parameters are presented in Table 3.3. $TT_{>200}$ was
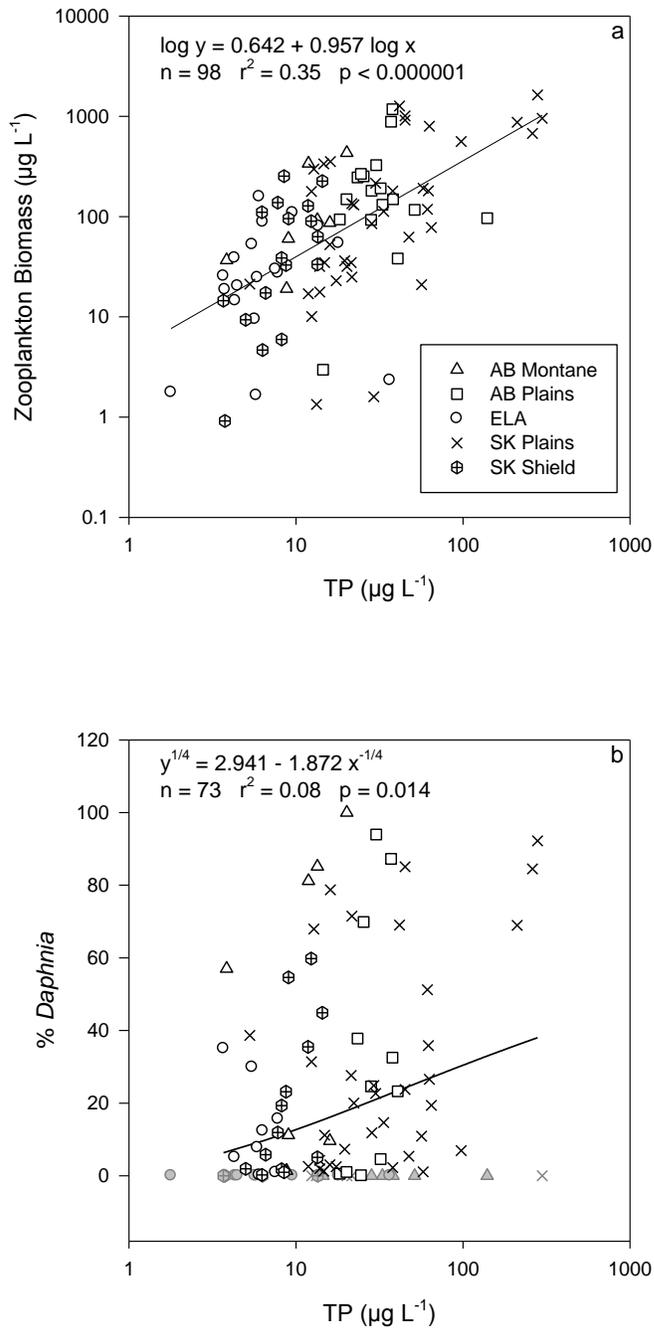
Figure 3.1. Zooplankton biomass as a function of TP (a) and % *Daphnia* as a function of TP (b). Symbol type indicates the region where each lake is located. Grey symbols in b represent lakes where *Daphnia* was not found and were excluded from statistical analyses.

Table 3.2. Relationships between TT_{PP} and zooplankton community parameters. TP, total phosphorus; TT_{PP}, turnover time of particulate phosphorus.

Parameters (x,y)	Transforms (x,y)	n	r	p
TP, TT_{PP}	log, log	70	-0.39	0.0007
Zooplankton Biomass, TT_{PP}	log, log	70	-0.21	0.077
Cladoceran Biomass, TT_{PP}	log, log	70	-0.21	0.088
Copepod Biomass, TT_{PP}	$x^{1/4}$, log	70	-0.19	0.11
Daphnia Biomass, TT_{PP}	log, $y^{-1/2}$	47	-0.23	0.12
% Cladocera, TT_{PP}	$x^{1/2}$, log	70	-0.048	0.69
% *Daphnia*, TT_{PP}	$x^{1/4}$, $y^{-1/2}$	47	-0.15	0.33
Zooplankton/PP, TT_{PP}	$x^{1/4}$, log	70	-0.11	0.36
Cladocera/PP, TT_{PP}	log, log	70	-0.12	0.34
Copepoda/PP, TT_{PP}	$x^{1/4}$, log	70	-0.11	0.36
Daphnia/PP, TT_{PP}	log, $y^{-1/2}$	47	-0.16	0.28

positively correlated with total zooplankton biomass, cladoceran biomass, *Daphnia* biomass and Zooplankton/PP, Cladocera/PP and *Daphnia*/PP (Fig. 3.3). $TT_{>200}$ was not correlated with TP, % Cladocera, % *Daphnia*, copepod biomass, or Copepoda/PP.

3.3.3. Relationships with TT_{PO4}

Phosphate turnover times ranged from 0.92 to 35174 min, but 87 of 98 lakes had $TT_{PO4} <15$ min. Relationships between TT_{PO4} and zooplankton community parameters are presented in Table 3.4. For the relationships between TT_{PO4} and TP, and TT_{PO4} and zooplankton biomass an appropriate statistical transform could not be found. Using the transforms suggested by the Box-Cox method, TT_{PO4} increased with increasing TP and zooplankton biomass ($p = 0.023$ and $p = 0.009$, respectively). However, these relationships were non-linear and violated the assumption
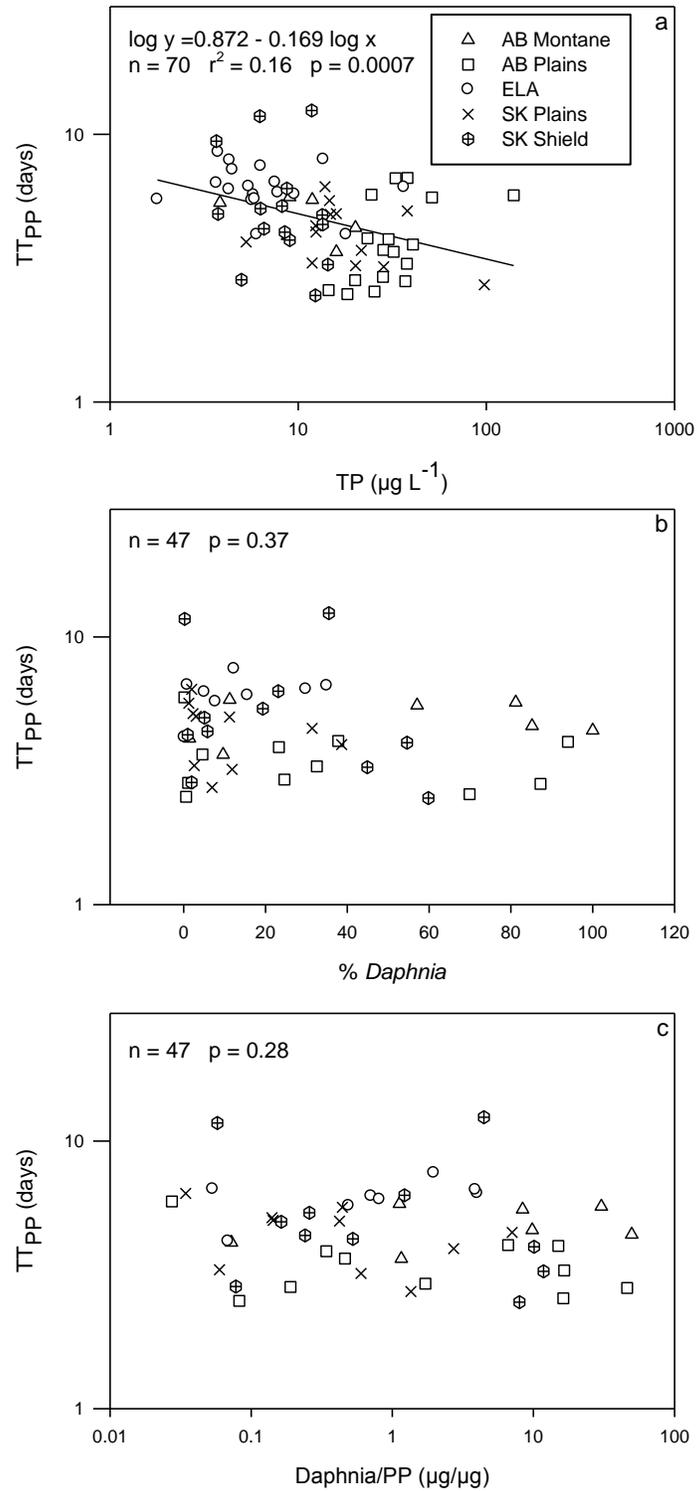
Figure 3.2. TT_{PP} as a function of TP (a) % *Daphnia* (b) and *Daphnia*/PP (c). Symbol type indicates the region where each lake is located. The trend line in a is the model I least squared regression line.

Table 3.3. Relationships between $TT_{>200}$ and zooplankton community parameters. TP, total phosphorus; $TT_{>200}$, turnover time in the >200 μm size fraction.

Parameters (x,y)	Transforms (x,y)	n	r	p
TP, $TT_{>200}$	log, log	35	0.20	0.25
Zooplankton Biomass, $TT_{>200}$	log, log	35	0.55	0.0007
Cladoceran Biomass, $TT_{>200}$	log, log	35	0.45	0.007
Copepod Biomass, $TT_{>200}$	$x^{1/4}$, log	35	0.30	0.080
Daphnia Biomass, $TT_{>200}$	log, log	27	0.49	0.001
% Cladocera, $TT_{>200}$	$x^{1/2}$, log	35	0.12	0.49
% *Daphnia*, $TT_{>200}$	log, log	27	0.28	0.15
Zooplankton/PP, $TT_{>200}$	log, log	35	0.63	0.00005
Cladocera/PP, $TT_{>200}$	log, log	35	0.43	0.009
Copepoda/PP, $TT_{>200}$	$x^{1/4}$, log	35	0.29	0.091
Daphnia/PP, $TT_{>200}$	log, log	27	0.48	0.012

of homogeneity of variance. TT_{PO4} was not correlated with TP or zooplankton biomass using Spearman's rank correlation. When lakes with TT_{PO4} >15 min were removed as outliers, TT_{PO4} was negatively correlated with TP (Pearson's r = -0.41, p = 0.00009, both variables log transformed). TT_{PO4} was not correlated with zooplankton biomass in lakes with TT_{PO4} <15 min (Fig. 3.4a). Phosphate turnover times were positively correlated with cladoceran and Daphnia biomass, but not with copepod biomass. Positive correlations were also found between TT_{PO4} and % Cladocera, % Daphnia (Fig. 3.4b), Zooplankton/PP, Cladocera/PP and Daphnia/PP (Fig. 3.4c). Phosphate turnover times were not correlated with Copepoda/PP. In general zooplankton community structure explained little of the variation in TT_{PO4}. However, relationships with cladoceran and *Daphnia* biomass, % biomass and biomass/PP tended to be

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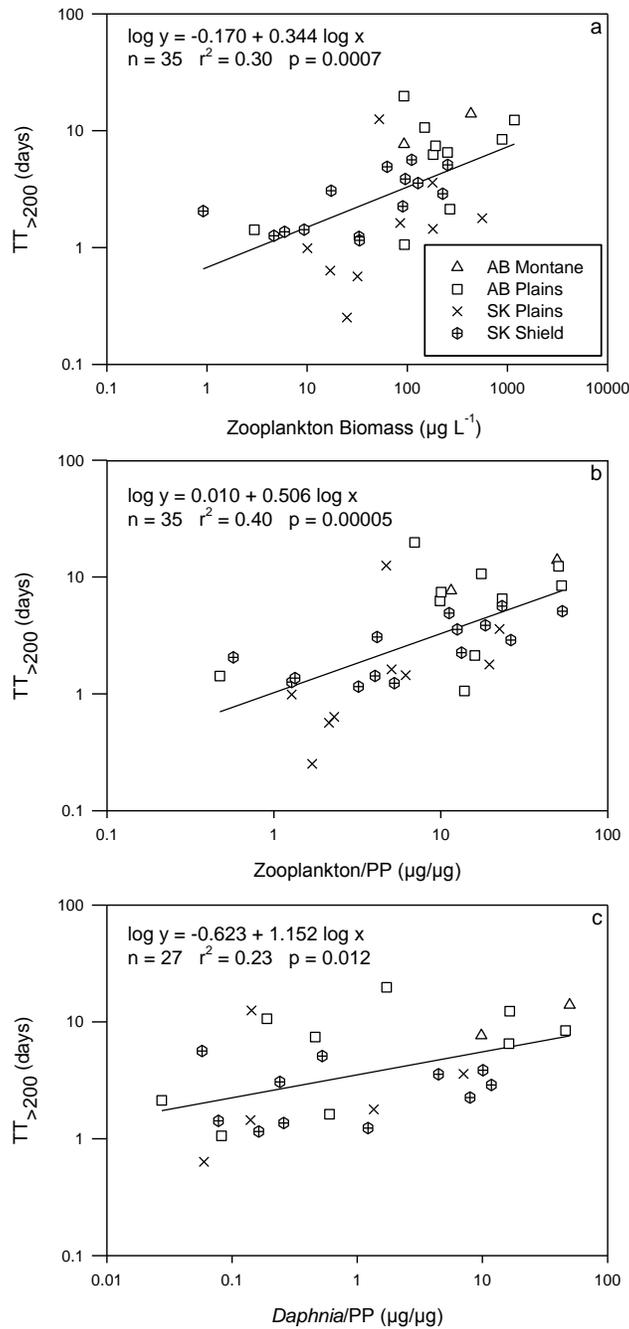
Figure 3.3. $TT_{>200}$ as a function of zooplankton biomass (a), zooplankton/PP (b) and *Daphnia*/PP (c). Symbol type indicates the region where each lake is located. Trend lines are based on model I least squares regression.

Table 3.4. Relationships between TT_{PO4} and zooplankton community parameters. TP, total phosphorus; TT_{PO4}, turnover time of dissolved phosphate.

Parameters (x,y)	Transforms (x,y)	n	r	p
TP, TT_{PO4}*	log, log	87	-0.41	0.00009
Zooplankton Biomass, TT_{PO4}*	log, log	87	-0.08	0.48
Cladoceran Biomass, TT_{PO4}	log, $y^{-1/2}$	98	-0.41	0.00002
Copepod Biomass, TT_{PO4}	$x^{1/4}$, $y^{-1/2}$	98	-0.10	0.33
Daphnia Biomass, TT_{PO4}	log, $y^{-1/2}$	73	-0.48	0.00002
% Cladocera, TT_{PO4}	$x^{1/2}$, $y^{-1/2}$	98	-0.36	0.0003
% *Daphnia*, TT_{PO4}	$x^{1/4}$, $y^{-1/2}$	73	-0.48	0.00002
Zooplankton/PP, TT_{PO4}	$x^{1/4}$, $y^{-1/2}$	70	-0.29	0.016
Cladocera/PP, TT_{PO4}	log, $y^{-1/2}$	70	-0.45	0.00009
Copepoda/PP, TT_{PO4}	$x^{1/4}$, $y^{-1/2}$	70	0.04	0.73
Daphnia/PP, TT_{PO4}	log, $y^{-1/2}$	47	-0.45	0.002

* Lakes with TT >15 min were removed from the analysis. See text for further details.

stronger than with other zooplankton parameters and consistently showed increasing TT_{PO4} with increasing cladocerans and *Daphnia*.

3.3.4. Relationships with $ssPO_4^{3-}$

Steady state PO_4^{3-} was positively correlated with TP (Table 3.5). It was similarly positively correlated with total zooplankton biomass, cladoceran biomass, copepod biomass, and *Daphnia* biomass. Steady state PO_4^{3-} was positively correlated with % *Daphnia* and *Daphnia*/PP (Fig. 3.5b, c) and Zooplankton/PP, but not with % Cladocera, Cladocera/PP or Copepoda/PP (Table 3.5).

Figure 3.4. TT$_{PO4}$ as a function of zooplankton biomass (a), % *Daphnia* (b) and *Daphnia*/PP (c). Symbol type indicates the region where each lake is located. Grey symbols in a represent lakes with TT$_{PO4}$ greater than 15 min which were considered outliers (see text for further details).

Table 3.5. Relationships between $ssPO_4^{3-}$ and zooplankton community parameters. TP, total phosphorus; $ssPO_4^{3-}$, steady state phosphate concentration.

Parameters (x,y)	Transforms (x,y)	n	r	p
TP, $ssPO_4^{3-}$	$\log, y^{-1/3}$	94	-0.82	<0.000001
Zooplankton Biomass, $ssPO_4^{3-}$	$\log, y^{-1/3}$	94	-0.53	<0.000001
Cladoceran Biomass, $ssPO_4^{3-}$	$\log, y^{-1/3}$	94	-0.48	0.000001
Copepod Biomass, $ssPO_4^{3-}$	$x^{1/4}, y^{-1/3}$	94	-0.43	0.00002
Daphnia Biomass, $ssPO_4^{3-}$	$\log, y^{-1/3}$	70	-0.59	<0.000001
% Cladocera, $ssPO_4^{3-}$	$x^{1/2}, y^{-1/3}$	94	-0.14	0.19
% *Daphnia*, $ssPO_4^{3-}$	$x^{1/4}, y^{-1/3}$	70	-0.45	0.00009
Zooplankton/PP, $ssPO_4^{3-}$	$x^{1/4}, y^{-1/2}$	70	-0.29	0.017
Cladocera/PP, $ssPO_4^{3-}$	$\log, y^{-1/2}$	70	-0.18	0.15
Copepoda/PP, $ssPO_4^{3-}$	$x^{1/4}, y^{-1/2}$	70	-0.10	0.41
Daphnia/PP, $ssPO_4^{3-}$	$\log, y^{-1/2}$	47	-0.43	0.002

3.3.5. Relationships with APA

Alkaline phosphatase activity was negatively correlated with TP (Table 3.6). It was also negatively correlated with zooplankton biomass (Fig. 3.6a), cladoceran biomass, copepod biomass and *Daphnia* biomass. There was no correlation between APA and % Cladocera, but there was a negative correlation with % *Daphnia* (Fig. 6b). There was also a negative correlation between APA and both Zooplankton/PP and Copepoda/PP. A relationship between APA and Cladocera/PP or *Daphnia*/PP was not observed.

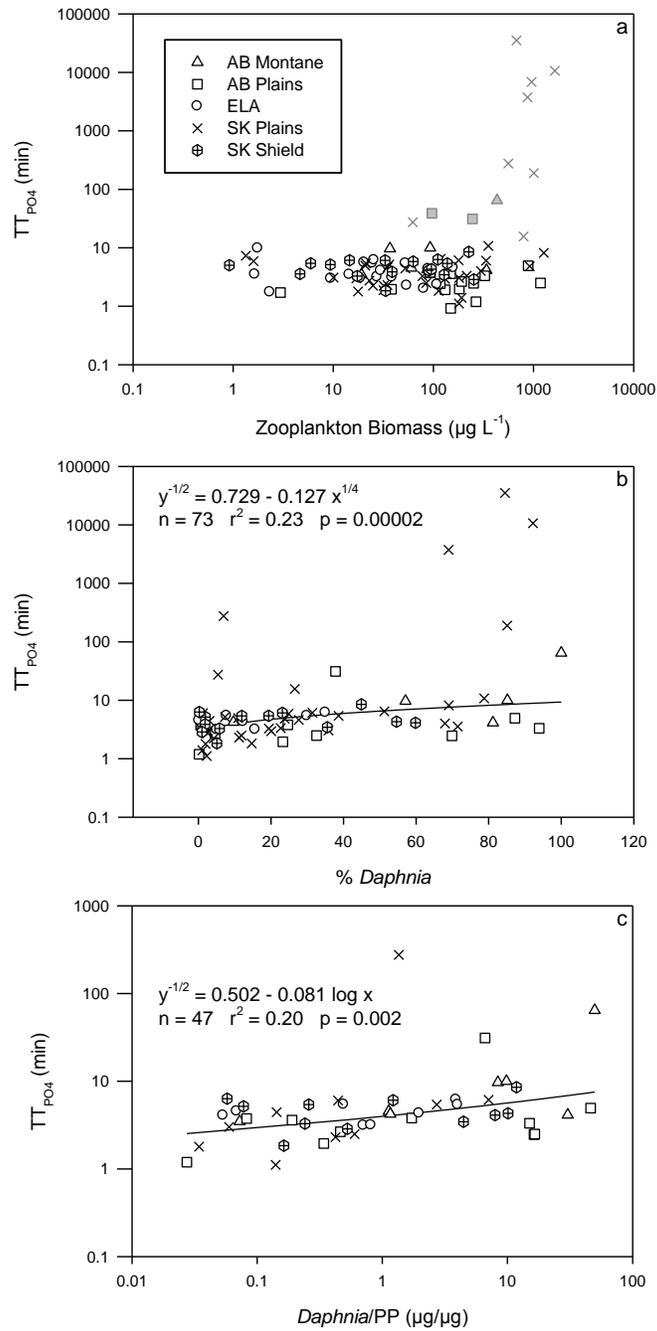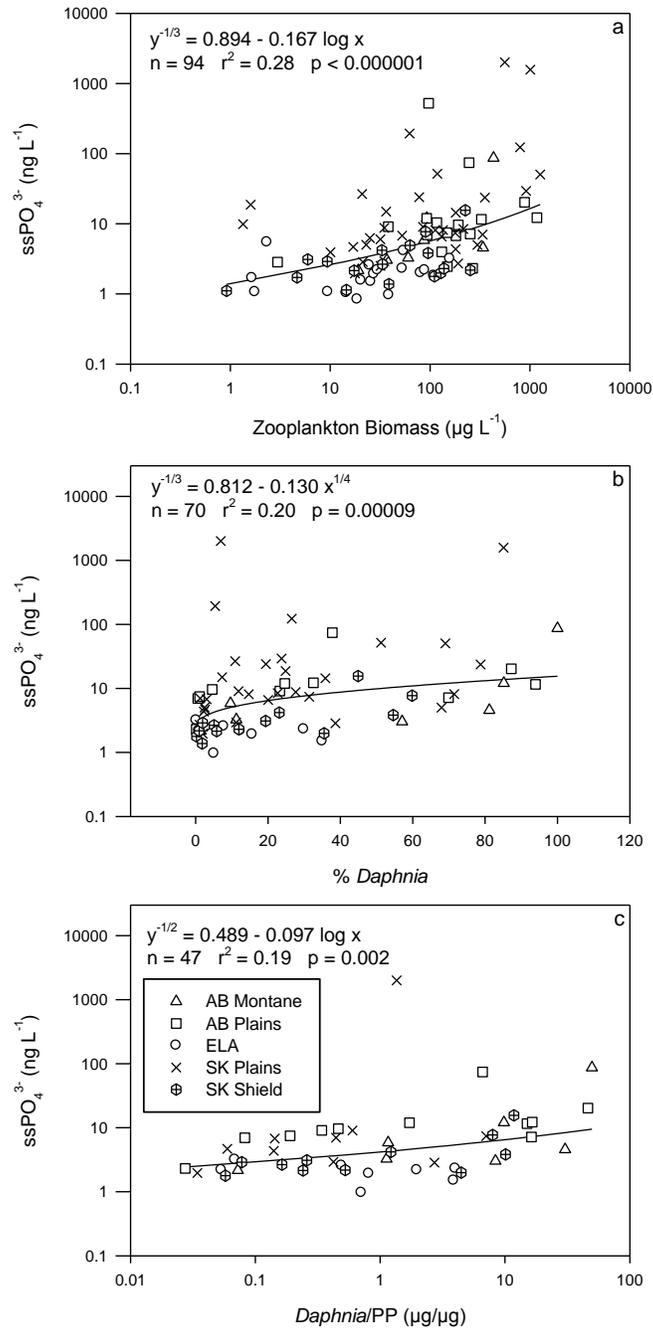**Figure 3.5.** Steady state phosphate as a function of zooplankton biomass (a), % *Daphnia* (b) and *Daphnia*/PP (c). Symbol type indicates the region where each lake is located. Trend lines are based on model I least squares regression.

Table 3.6. Relationship between APA and zooplankton community parameters. TP, total phosphorus; APA, alkaline phosphatase activity.

Parameters (x,y)	Transforms (x,y)	n	r	p
TP, APA	$x^{-1/3}$, $y^{1/3}$	38	0.54	0.0005
Zooplankton Biomass, APA	log, $y^{1/3}$	38	-0.54	0.0005
Cladoceran Biomass, APA	log, $y^{1/3}$	38	-0.48	0.002
Copepod Biomass, APA	log, $y^{1/3}$	38	-0.45	0.005
Daphnia Biomass, APA	log, $y^{1/3}$	33	-0.55	0.0008
% Cladocera, APA	$x^{1/2}$, $y^{1/3}$	38	-0.16	0.35
% *Daphnia*, APA	$x^{1/4}$, $y^{1/3}$	33	-0.46	0.007
Zooplankton/PP, APA	log, $y^{1/3}$	12	-0.58	0.049
Cladocera/PP, APA	log, $y^{1/3}$	12	-0.22	0.50
Copepoda/PP, APA	log, $y^{1/3}$	12	-0.65	0.022
Daphnia/PP, APA	log, $y^{1/4}$	9	-0.27	0.49

3.4. Discussion

3.4.1. Is zooplankton species composition related to P regeneration?

Ecological stoichiometry predicts that zooplankton communities dominated by *Daphnia*

should regenerate relatively more N and less P compared to communities dominated by

zooplankton with higher C:P and N:P ratios (Sterner and Elser 2002). The extension of this idea

is that communities dominated by *Daphnia* will have greater P limitation because P will be

sequestered in *Daphnia* biomass. I examined the effect of zooplankton species composition on P

recycling by directly measuring particulate P turnover rates in whole plankton communities and
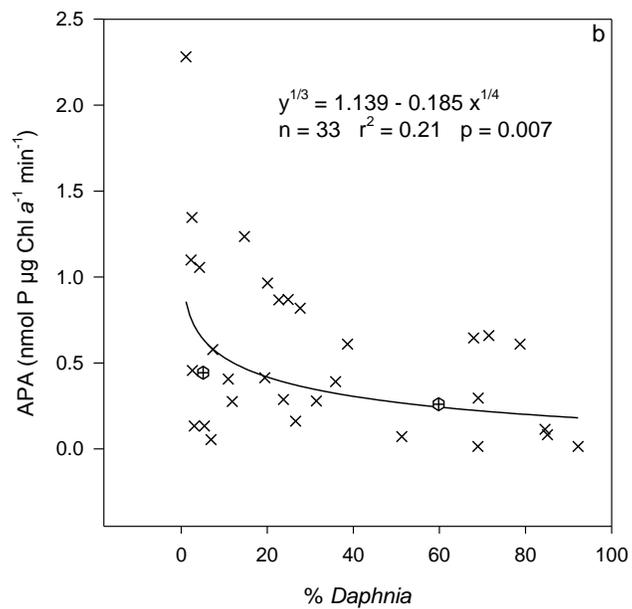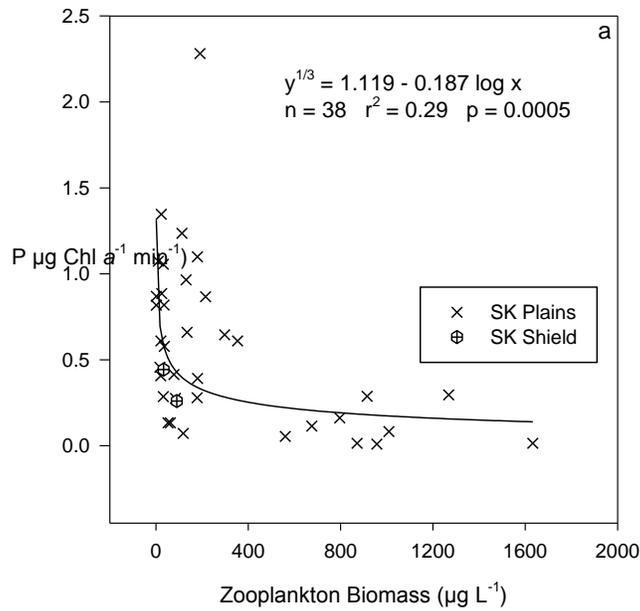
Figure 3.6. Alkaline phosphatase activity as a function of zooplankton biomass (a) and % *Daphnia* (b). Symbol type indicates the region where each lake is located. Trend lines are based on model I least squares regression.

in the >200 μm size fraction and comparing these rates with zooplankton community composition.

TT$_{PP}$ was negatively correlated with TP (Fig. 3.2a, Table 3.2). The negative relationship suggests that P cycling becomes more rapid in more productive lakes. This finding is consistent with a recent modelling study of P cycling (Chen and Taylor 2011) and contradicts the previous view that P turnover should become slower with increasing trophic state (Harris 1986, Capblancq 1990). Nowlin et al. (2007) also found a negative relationship between TT$_{PP}$ and TP in a survey of 8 British Columbia lakes, while Hudson et al. (1999) did not find a relationship between TT$_{PP}$ and TP in a 20 lake survey.

TT$_{PP}$ was not related to any of the zooplankton community composition parameters (Table 3.2). Ecological stoichiometry suggests that P should cycle more slowly when *Daphnia* dominate the zooplankton, because P will be retained by these organisms. Instead I found that zooplankton community structure is not having a major influence on P turnover of the whole plankton assemblage. TT$_{>200}$ increased with increasing zooplankton biomass, cladoceran biomass, *Daphnia* biomass and with the biomasses of these groups standardized to PP (Table 3.3, Fig. 3.3). This suggests that as zooplankton become a greater part of the food web, the zooplankton assemblage regenerates proportionately less phosphorus. Longer TT$_{>200}$ at higher zooplankton densities could be affected by the relative proportion of zooplankton to their food. At high zooplankton densities, prey organisms may be relatively fewer, leading to lower specific feeding rates and lower P regeneration rates by zooplankton. TT$_{>200}$ was positively correlated with *Daphnia*/PP, which is consistent with the predictions of ecological stoichiometry (less P regeneration when *Daphnia* are dominant). However, TT$_{>200}$ was more strongly correlated with total zooplankton biomass standardized to PP. Furthermore, TT$_{>200}$ was not correlated to %

Daphnia, suggesting that *Daphnia* is not having the predicted stoichiometric effect on zooplankton regeneration. Finally, even if there is a stoichiometric effect on $TT_{>200}$, it is not affecting the turnover time of the whole planktonic assemblage.

Overall, my data suggest that the proportion of *Daphnia* in the food web is not having a major influence on P regeneration. The lack of relationship between TT_{PP} and any measures of *Daphnia* abundance suggests that *Daphnia* has little effect on P regeneration in the whole plankton assemblage. The $TT_{>200}$ data do not suggest an effect of *Daphnia* in particular on P regeneration in the >200 µm size fraction. Ecological stoichiometry predicts that the dominance of P-rich grazers like *Daphnia* should cause the N:P ratio in regenerated material to increase (Sterner 1990). I have not directly tested this prediction because I did not measure N regeneration concurrently with P regeneration. However, my data shows that if *Daphnia* do cause a stoichiometric shift in N and P availability through changes in zooplankton nutrient recycling, this shift does not result from a decrease in P regeneration.

3.4.2. Is zooplankton species composition related to P deficiency in plankton

One of the main predictions of the stoichiometric theory of CNR is that differential recycling of nutrients by consumers can affect the severity of nutrient limitation for producers (Sterner and Elser 2002). In particular, *Daphnia* are expected to increase the severity of P limitation in lakes. I tested this hypothesis using the P deficiency indicators TT_{PO4}, $ssPO_4^{3-}$ and APA. The three indicators had weak relationships with several zooplankton community composition parameters. In general, the results suggest that zooplankton are not strongly affecting P deficiency for phytoplankton and bacteria in lakes. However, all three indicators suggest that as *Daphnia* become a larger component of the zooplankton, P becomes less limiting. This is contrary to the stoichiometric prediction that *Daphnia* should increase P limitation in lakes.

Total zooplankton biomass was not related to TT_{PO4} (Fig. 3.4a). Instead, most lakes had short TT_{PO4}, with a few lakes having very long TT_{PO4}. Lakes with long TT_{PO4} most often also had high zooplankton biomass. The relationship between zooplankton biomass and TT_{PO4} is very similar to the relationship between TT_{PO4} and TP in these lakes. Most lakes had short TT_{PO4} across a TP gradient, but lakes with high TT_{PO4} occurred most frequently at high TP. In lakes with TT_{PO4} <15 minutes (89 % of lakes), TT_{PO4} was negatively correlated with TP (Table 3.4). This negative relationship suggests that in more productive lakes, phosphate deficiency tends to be more severe than in more oligotrophic lakes. However, lack of P deficiency (long TT_{PO4}) tended to occur more often in higher TP lakes. Both outcomes suggest that P limitation, as measured with TT_{PO4}, becomes less predictable from oligotrophic to hypereutrophic lakes.

Phosphate turnover times were positively correlated with % Cladocera and % *Daphnia* (Table 3.4, Fig. 3.4b), which suggests greater P availability when cladocerans and *Daphnia* dominate the zooplankton. TT_{PO4} was also positively correlated with all zooplankton biomass parameters standardized to PP, except Copepoda/PP (Table 3.4). The correlation between TT_{PO4} and Zooplankton/PP was relatively weak compared to the correlations between TT_{PO4} and Cladocera/PP and *Daphnia*/PP. These correlations suggest that P becomes less limiting when cladocerans and *Daphnia* occupy a greater proportion of the food web. This is a contradiction of ecological stoichiometry, particularly for *Daphnia*, which should be associated with increased P limitation. Percent *Daphnia* was also positively correlated with $ssPO_4^{3-}$ concentrations (Fig. 3.5b, Table 3.5) and negatively correlated with APA (Fig. 3.6b, Table 3.6). The three measures of P limitation I used (TT_{PO4}, $ssPO_4^{3-}$ and APA) support each other and contradict the idea that P should become more limiting when *Daphnia* dominate the zooplankton community.

My observation that P becomes less limiting when *Daphnia* biomass is proportionally greater suggests that *Daphnia* have a stronger role as grazers than as P sinks. Despite their large size and the expectation that smaller zooplankton should have higher grazing rates (Peters and Downing 1984), *Daphnia* are often associated with strong reductions in algal biomass (e.g., clear water events) and increases in dissolved nutrient pools (Shapiro and Wright 1984, Elser et al. 2000, Carpenter et al. 2001). Phytoplankton likely experience increased nutrient availability because reductions in algal biomass have made nutrients relatively more available. Sarnelle and Knapp (2005) found reduced Chl *a* and relaxed P limitation in a lake where *Daphnia* became dominant after a fish removal experiment. Similarly, Elser et al. (2000) found an increase of both dissolved N and P and a decrease in seston C:P and N:P after increases in a *Daphnia* population following a piscivorous fish introduction. The authors emphasize that differential recycling of N and P by *Daphnia* caused the DIN:TDP ratio to increase, in accordance with stoichiometric theory. While this may be true, the differential recycling did not make P more limiting as stoichiometric theory has often predicted (Sterner 1990, Elser and Urabe 1999). My data does not refute the differential recycling of nutrients by zooplankton, rather, it suggests that *Daphnia* are not associated with increased P deficiency in lakes. It may be that *Daphnia* do regenerate relatively more N and less P than other zooplankton with lower P content, as lab studies and models predict (Sterner 1990, Rothhaupt 1997, Elser and Urabe 1999) however, this is not causing more severe P limitation when *Daphnia* are the dominant zooplankton.

Multi-lake field studies relating zooplankton community structure and P limitation are lacking. Most studies have instead been in single lakes. These have often either been in the context of biomanipulation experiments (e.g., Elser et al. 2000) or experimental additions of zooplankton to small vessels of lake water where ambient zooplankton have been removed by

passing the water through a screen (e.g., Brett et al. 1994, Mackay and Elser 1998). More recently, McCarthy et al. (2006) found a positive correlation between *Daphnia* biomass and DIN:TP in a 2 year study in Lough Carra. Their findings were consistent with stoichiometric theory, but they also found positive correlations with other zooplankton groups and DIN:TP. Unfortunately they did not make comparisons with zooplankton groups standardized as percent biomass or as a proportion of the total food web. Consequently, while their results are consistent with the stoichiometric theory of CNR, they do not provide strong evidence for a stoichiometric effect on nutrient availability. In contrast to the above studies, I examined the relationship between zooplankton community structure across many diverse and unmanipulated lakes.

   One of the main weaknesses of my study is that it relies on correlation analysis of zooplankton and P deficiency. It cannot determine whether differential nutrient recycling by zooplankton is actually causing any of the trends found between zooplankton and P deficiency. However, because of its broad, multi-lake approach potential effects of zooplankton are evaluated in the context of the other ecosystem processes that may be affecting P deficiency. Nutrient limitation of plankton is expected to be influenced by nutrient supply from the watershed, chemical and physical conditions within the lake, and internal nutrient cycling pathways. Biotic resupply of nutrients will be affected by bacteria, protozoa, phytoplankton and rotifers in addition to zooplankton. P regeneration in the >200 μm size fraction averaged 19% of total planktonic regeneration. Nowlin et al. (2007) found that P regeneration by the >200 μm fraction averaged 22% of planktonic regeneration in 7 British Columbia lakes and Hudson and Taylor (1996) found an average of 28% for the >40 μm size fraction in Mouse and Ranger lakes. These results suggest that zooplankton regeneration is an important part of overall planktonic regeneration, but that other organisms account for the majority of P regeneration. It would be

expected then that variation in regeneration rates from other organisms would have a larger impact on nutrient supply than potential effects from differential nutrient recycling by zooplankton.

3.4.3 Summary

My results provide strong evidence that zooplankton community composition is not having a major impact on P turnover and P deficiency across lakes. Particulate P turnover times were not correlated with the biomass of any zooplankton groups, the percentage of zooplankton biomass as cladocerans or *Daphnia*, or the biomass of any zooplankton groups standardized to particulate P. The particulate P turnover time in the >200 μm size fraction was positively correlated with the biomass of most zooplankton groups and the biomass of these groups standardized to particulate P concentrations. This result suggests that P is regenerated more slowly as zooplankton become a greater proportion of the food web. It does not suggest that *Daphnia* in particular reduce P recycling rates because of their low C:P ratios as predicted by ecological stoichiometry. Phosphorus deficiency indicators showed trends of relaxing P deficiency as zooplankton biomass increased in the food web and as *Daphnia* became a greater proportion of the zooplankton. Ecological stoichiometry predicts that *Daphnia* should increase P limitation in lakes by regenerating relatively more N and less P than other zooplankton. My results suggest *Daphnia* slightly relaxes P limitation in lakes, rather than increasing P limitation as stoichiometric theory predicts.

CHAPTER 4 – GENERAL CONCLUSIONS

The study of ecological stoichiometry in recent decades has led to the formulation of several theories describing different aspects of ecosystem function. Two of these that have been extensively studied in freshwater concern the elemental limitation of consumer growth and consumer-driven nutrient recycling. Studies in both of these areas have focused on the genus *Daphnia*. The species of this group are recognized as keystone grazers in many lakes. Because *Daphnia* have a low C:P ratio compared to other zooplankton, they are expected to be more strongly affected by P deficient food. *Daphnia* are also expected to recycle nutrients in a high N:P ratio, increasing P limitation in lakes where they are abundant.

Seston C:P and N:P ratios are widely used as P deficiency indicators. They are relatively well researched, having been measured in a wide variety of lakes and compared with many lake and watershed parameters (Hecky et al. 1993, Guildford and Hecky 2000, Hessen 2006). My work adds to the current knowledge about nutrient deficiency indicators. The relationships I found between seston stoichiometry and TP and APA are consistent with current understanding. I present the first comparison between seston stoichiometry and $ssPO_4^{3-}$ and found general agreement between these measures. However, C:P and N:P ratios disagreed with TT_{PO4}. The measures did not correlate with each other and nutrient ratios indicated P sufficiency more often than TT_{PO4}. The differences could be due to the timescales over which the indicators respond to plankton P status (longer term for C:N:P, shorter term for TT_{PO4}). The two measures could also indicate a different type of P deficiency. Ratios of N:P or C:P might indicate an actual shortage of a nutrient in plankton cells, while TT_{PO4} indicates how the supply of PO_4^{3-} is related to plankton demand for the nutrient. C:P and N:P are also likely to be influenced by the detrital component of the seston, which would not respond to changes in nutrient deficiency. Finally,

TT$_{PO4}$ may be more reflective of bacterial P limitation while N:P and C:P reflect algal limitation. The fact that there is disagreement between these two well studied indicators reinforces the need to use multiple P deficiency indicators to get a more robust assessment of P deficiency in lakes.

It is well established that zooplankton growth, particularly in *Daphnia*, can be reduced by a P-deficient diet (Gulati and DeMott 1997). Declines in *Daphnia* populations in response to higher food C:P ratios have been found in the field (DeMott and Gulati 1999). Lab experiments and modelling work have sought to determine when and how zooplankton become limited by food quality (Demott et al. 1998, Brett et al. 2000, Anderson and Hessen 2005), but our understanding of how seston stoichiometry influences zooplankton in nature remains poor. My results suggest that at a very broad, multi-lake scale, *Daphnia* are not more severely affected by high seston C:P ratios than other zooplankton. Hessen (2006) found that *Daphnia* biomass declined with increasing seston C:P in a survey of Norwegian lakes and this has been cited as evidence for stoichiometric food quality limitation of *Daphnia* (Sterner 2009). I also found that *Daphnia* biomass declined with seston C:P, but so did total zooplankton biomass and the biomass of cladocerans and copepods. Furthermore, *Daphnia* biomass as a percentage of zooplankton biomass and *Daphnia* biomass standardized to particulate P concentrations were not related to C:P ratios. I conclude therefore that zooplankton biomass tends to decline with increasing C:P ratios, but that *Daphnia* is not particularly affected in the manner predicted by ecological stoichiometry.

Ecological stoichiometry and the theory of consumer-driven nutrient recycling add another level of understanding to predictions of how consumers can influence nutrient availability for the rest of the food web. According to CNR, zooplankton species composition will influence the stoichiometry of regenerated nutrients because zooplankton require nutrients in species-specific

ratios (Sterner and Elser 2002). In lakes, *Daphnia* are predicted to sequester P but release N, while copepods sequester N and release P. My results suggest that zooplankton species composition is not having an effect on P regeneration of the plankton. I found that P turnover in the >200 µm size fraction is slower as zooplankton biomass increases, but this effect is not specific to *Daphnia* and $TT_{>200}$ was not related to the percentage of *Daphnia* in the zooplankton. I conclude that *Daphnia* are not reducing P regeneration as predicted by ecological stoichiometry. Furthermore, I found that as the proportion of *Daphnia* increased in the zooplankton community and in the whole food web, P limitation tended to be relaxed. Of my results, this finding most strongly contradicts the predictions of ecological stoichiometry.

My study is based largely on previously collected data and is therefore limited by the kinds of data collected in the past. It has also been limited by practical considerations. If given the opportunity to begin the study again there are three main improvements I would make. Firstly I would focus on more thorough sampling of the zooplankton in each lake. My dataset had many samples with very low numbers of zooplankton which make estimates of zooplankton biomass less reliable. The discrete sampling method at a single location also means that I have a weaker understanding of the total zooplankton community in the lake, particularly because zooplankton often migrate vertically in the water column. Secondly I would take C:N:P measurements separately for the >200 µm and <200 µm size fractions. I could then have a measurement of zooplankton community stoichiometry and test the predictions of ecological stoichiometry more directly. My study focused on predictions of theory based on specific taxa (i.e. P-rich *Daphnia* vs. N-rich copepods). A measure of zooplankton stoichiometry would allow comparisons between zooplankton stoichiometry and seston stoichiometry and nutrient limitation of the plankton. For instance I could compare seston stoichiometry to zooplankton stoichiometry to see

if high C:P seston might favour zooplankton communities that also have high C:P ratios. I could also examine, for example, whether high N:P zooplankton communities relax P limitation because of their relatively high N requirements and low P requirements. Finally and most importantly, I would measure N concentrations and N limitation for each lake. Ideally this would include a measure of N release rates and a bioassay of N deficiency. Because stoichiometric theory is based on ratios of nutrients, a thorough test of its predictions really should include an understanding of both N and P limitation. A particularly informative comparison would be to compare the zooplankton community N:P ratio with a ratio of planktonic N regeneration to P regeneration. This would more directly test the predictions of ecological stoichiometry. Specifically, it would test whether the stoichiometry of a consumer community affects the stoichiometry of its regenerated nutrients. I could then also compare zooplankton community N:P with a ratio of N and P deficiency indicators to examine how CNR affects the balance of N vs. P limitation.

At the very centre of ecological stoichiometry is the law of conservation of matter. Elements must be conserved during ecosystem processes. There must also be cases where ecosystem processes are constrained by a shortage of one element while other elements are present in excess. The predictions of ecological stoichiometry are derived from these principles, and there is no serious debate over their validity. Over the past few decades, ecological stoichiometry has generated several predictions about how elemental imbalances can constrain ecosystem processes. The important work now is to understand how relevant these predictions are when all the other factors affecting ecosystem processes are taken into account. In lakes, ecological stoichiometry has emphasized the importance of *Daphnia* as keystone grazers and the importance of dissolved N:P ratios for determining nutrient limitation. My work has looked at

two major predictions of ecological stoichiometry in lakes. Using a diverse dataset of 99

Canadian lakes and a correlative approach, I did not find support for the major predictions

regarding food quality limitation of zooplankton and consumer-driven nutrient recycling.

Daphnia were not specifically disadvantaged as seston C:P ratios increased, and *Daphnia* were

not associated with increased P limitation of the food web. My work does not focus on the

specific stoichiometric mechanisms, but rather on the larger predictions that have been

generated. It may be that *Daphnia* do experience reduced food quality because of high seston

C:P ratios in lakes. If they do though, my work shows that this reduced food quality is not

driving *Daphnia* dynamics in lakes. It may also be that *Daphnia* do recycle more N and retain

more P than other zooplankton. However, if this differential recycling is occurring, it is not

sufficient to drive P deficiency patterns in lakes.

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APPENDIX I – STUDY LAKE LOCATIONS

Table A1.1. Names, date of sampling, region and location of the study lakes.

Lake	Sampling Date (dd/mm/yyyy)	Region	Latitude	Longitude
Jenkins	02/08/1997	AB Plains	54º 45′ N	113º 35′ W
Patricia	23/08/1997	AB Montane	52º 54′ N	118º 06′ W
Trefoil 2	23/08/1997	AB Montane	52º 54′ N	118º 03′ W
Trefoil 1	23/08/1997	AB Montane	52º 54′ N	118º 03′ W
Leach	23/08/1997	AB Montane	52º 42′ N	117º 54′ W
Cabin	23/08/1997	AB Montane	52º 53′ N	118º 08′ W
Heart	03/09/1997	SK Plains	53º 59′ N	106º 02′ W
Trapper	03/09/1997	SK Plains	53º 48′ N	106º 01′ W
Namekus	03/09/1997	SK Plains	53º 50′ N	106º 02′ W
Schumaker	09/09/1997	AB Plains	54º 45′ N	113º 35′ W
Ghost	09/09/1997	AB Plains	54º 45′ N	113º 35′ W
Bilsky	09/09/1997	AB Plains	54º 45′ N	113º 35′ W
Teen	09/09/1997	AB Plains	54º 29′ N	113º 43′ W
Cameron	13/09/1997	AB Plains	53º 38′ N	114º 01′ W
Soldan	03/07/1998	AB Plains	53º 35′ N	114º 08′ W
Glen Mere	09/07/1998	AB Plains	53º 37′ N	114º 05′ W
Byer's	09/07/1998	AB Plains	53º 36′ N	114º 05′ W
Muir, S. basin	09/07/1998	AB Plains	53º 37′ N	114º 05′ W
Roi	09/07/1998	AB Plains	53º 37′ N	114º 05′ W
Little Chickakoo	09/07/1998	AB Plains	53º 37′ N	114º 04′ W

Kettle	09/07/1998	AB Plains	53º 37′ N	114º 03′ W
Brodick	16/07/1998	AB Plains	54º 12′ N	112º 25′ W
Jackfish	16/07/1998	AB Plains	53º 28′ N	114º 14′ W
Amisk, N.	16/07/1998	AB Plains	54º 36′ N	112º 38′ W
Trefoil3	22/07/1998	AB Montane	52º 53′ N	118º 03′ W
Katrine	22/07/1998	AB Montane	52º 55′ N	118º 04′ W
Islet	28/07/1998	AB Plains	53º 27′ N	112º 49′ W
L224	05/08/1998	ELA	49º 41′ N	93º 43′ W
L222	05/08/1998	ELA	49º 41′ N	93º 43′ W
L305	06/08/1998	ELA	49º 41′ N	93º 41′ W
L302 N.	06/08/1998	ELA	49º 40′ N	93º 45′ W
L304	14/08/1998	ELA	49º 39′ N	93º 44′ W
L110	17/08/1998	ELA	49º 44′ N	93º 49′ W
L109	17/08/1998	ELA	49º 44′ N	93º 49′ W
L114	17/08/1998	ELA	49º 40′ N	93º 45′ W
L191	18/08/1998	ELA	49º 34′ N	93º 46′ W
L228	20/08/1998	ELA	49º 41′ N	93º 39′ W
L313	20/08/1998	ELA	49º 38′ N	93º 40′ W
L239	24/08/1998	ELA	49º 39′ N	93º 43′ W
L261	24/08/1998	ELA	49º 42′ N	93º 41′ W
L 442	25/08/1998	ELA	49º 46′ N	93º 49′ W
L 373	25/08/1998	ELA	49º 44′ N	93º 47′ W
L227	26/08/1998	ELA	49º 41′ N	93º 41′ W
L226 S.	26/08/1998	ELA	49º 41′ N	93º 44′ W
L240	27/08/1998	ELA	49º 39′ N	93º 43′ W

Zimmer	30/07/2003	SK Shield	57º 09′ N	105º 45′ W
Little MacDonald	31/07/2003	SK Shield	57º 11′ N	105º 37′ W
Reindeer	12/08/2003	SK Shield	57º 12′ N	102º 23′ W
Lac La Ronge	13/08/2003	SK Shield	55º 06′ N	105º 00′ W
Scoop	16/08/2003	SK Shield	58º 15′ N	103º 38′ W
Wollaston	16/08/2003	SK Shield	58º 16′ N	103º 14′ W
Fulton	16/08/2003	SK Shield	59º 32′ N	108º 25′ W
First	24/08/2003	SK Shield	58º 19′ N	109º 36′ W
Cluff	25/08/2003	SK Shield	58º 20′ N	109º 33′ W
Indigo	10/09/2003	SK Shield	58º 16′ N	103º 58′ W
Ace	27/07/2004	SK Shield	59º 34′ N	108º 26′ W
Mclean	24/08/2004	SK Shield	58º 15′ N	103º 52′ W
Marie	09/09/2004	SK Shield	59º 33′ N	108º 26′ W
Donaldson	09/09/2004	SK Shield	59º 35′ N	108º 24′ W
Fredette	25/07/2005	SK Shield	59º 36′ N	108º 31′ W
Emerald	11/07/2008	SK Plains	53º 11′ N	106º 57′ W
Memorial	13/07/2008	SK Plains	53º 17′ N	107º 03′ W
Pelletier	16/07/2008	SK Plains	49º 59′ N	107º 56′ W
Iroquois	21/07/2008	SK Plains	53º 10′ N	107º 01′ W
Bradwell Reservoir	06/07/2009	SK Plains	51º 55′ N	106º 11′ W
Lower Emma	10/07/2009	SK Plains	53º 34′ N	105º 52′ W
Sandy	10/07/2009	SK Plains	53º 38′ N	106º 05′ W
Edouard	21/07/2009	SK Plains	52º 22′ N	104º 20′ W
Kipabiskau	21/07/2009	SK Plains	52º 34′ N	104º 10′ W
Brightsand	25/07/2009	SK Plains	53º 35′ N	108º 52′ W

Helene	25/07/2009	SK Plains	53º 31′ N	108º 12′ W
Jackfish	29/07/2009	SK Plains	53º 04′ N	108º 24′ W
Murray	29/07/2009	SK Plains	53º 02′ N	108º 17′ W
Diefenbaker	07/08/2009	SK Plains	51º 11′ N	106º 48′ W
Nipekamew	13/08/2009	SK Plains	54º 22′ N	104º 57′ W
East Trout	13/08/2009	SK Plains	54º 21′ N	105º 03′ W
Constance	20/08/2009	SK Plains	53º 10′ N	106º 58′ W
Big Shell	20/08/2009	SK Plains	53º 12′ N	107º 09′ W
Fish	26/08/2009	SK Plains	53º 40′ N	106º 09′ W
Witsukitshak	26/08/2009	SK Plains	53º 39′ N	106º 10′ W
Zelma Reservoir	31/08/2009	SK Plains	51º 49′ N	105º 49′ W
Brightwater Reservoir	02/09/2009	SK Plains	51º 36′ N	106º 31′ W
Anglin	13/09/2009	SK Plains	53º 43′ N	105º 56′ W
Eauclair	19/07/2010	SK Plains	53º 51′ N	107º 41′ W
Shell	19/07/2010	SK Plains	53º 49′ N	107º 36′ W
Chitek	19/07/2010	SK Plains	53º 44′ N	107º 46′ W
Delaronde	26/07/2010	SK Plains	53º 56′ N	106º 57′ W
Cowan	26/07/2010	SK Plains	53º 50′ N	107º 03′ W
Amisk	09/08/2010	SK Shield	54º 35′ N	102º 13′ W
Athapapuskow	09/08/2010	SK Shield	54º 39′ N	101º 39′ W
Fur	23/08/2010	SK Plains	53º 16′ N	106º 53′ W
Little Shell	23/08/2010	SK Plains	53º 15′ N	107º 07′ W
Miko	30/08/2010	SK Plains	53º 52′ N	107º 43′ W
Bug	30/08/2010	SK Plains	53º 54′ N	107º 44′ W
Huard	30/08/2010	SK Plains	53º 45′ N	107º 36′ W

Whiteswan	06/09/2010	SK Plains	54º 02′ N	105º 09′ W
Heritage	06/09/2010	SK Plains	53º 55′ N	105º 09′ W
Candle	06/09/2010	SK Plains	53º 47′ N	105º 14′ W