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Benthic Responses to Nitrogen and Phosphorus Deposition on Alpine Ponds in Banff National Park: A Replicated Whole-Ecosystem Experiment

by

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ABSTRACT

Nitrogen (N) and Phosphorous (P) deposition at high elevations has increased by 40% over the last fifteen years, causing concern for the 3000^+ alpine ponds in Banff National Park. A novel whole-ecosystem experiment was used to test for the effects of elevated N and P deposition on benthic communities in 16 ponds using a two-factor (N × P) experimental design. The findings showed periphyton was N-limited, as total chlorophyll was significantly higher in N- and NP-amended ponds, reflecting the positive response by algal groups. Periphyton appeared co-limited by N and P towards the end of the experiment, with strong grazing pressure by abundant populations of fairy shrimp (*Branchinecta paludosa*). The benthic consumer community consisted mainly of small omnivores (family Chironomidae) and large algal grazers (family Limnephilidae). These findings highlight the strong potential for eutrophication in the pristine high-elevation environments of the Canadian Rockies.

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General Ecology of Alpine Ponds

In limnological research, there has been a historically strong emphasis towards the study of large lakes (Downing et al., 2006; Downing and Duarte, 2009; Downing, 2010). While these large lakes do represent more water in volume, they are not representative of the total surface area or number of fresh water bodies in North America. Approximately 190,000 km^2 of the Earth's surface area is composed of small ponds, with over 3000 alpine ponds in Banff National Park, AB, Canada alone (Holland and Coen, 1982; McMaster and Schindler, 2005). When compared to the 480 alpine lakes in Banff National Park (McMaster and Schindler, 2005), it is apparent that these alpine ponds represent an important part of the freshwater resources within the park. Despite this abundance of alpine ponds, only six studies to date have been published on the limnology of alpine ponds in the Canadian Rockies (Anderson, 1971; Anderson 1974; Strecker et al., 2004; McMaster and Schindler, 2005; Thompson et al., 2008; Murphy et al. 2010), and only one of which addresses the benthic communities (McMaster and Schindler, 2005).

Formation

Alpine ponds may be distinguished from alpine lakes in that they they are shallow water bodies (max 2 m depth) that freeze completely in the winter months, whereas lakes maintain a layer of liquid water below the ice (Welch, 1985; Douglas and Smol, 2010). Alpine ponds are defined by their high elevation, which in the Central Canadian Rockies is $\geq$ 2200 m a.s.l, depending on aspect, slope angle, and substrate type. Alpine ponds in the Snow Pass area of Banff National Park are commonly known as kettle ponds, which are fluvioglacial landforms commonly associated with alpine meadows and typically lack large inflows or outflows (Vincent and Laybourn-Parry, 2008; McMaster and Schindler, 2005). Pond shapes are usually circular or oval with steep sided, U-shaped basins. Kettle ponds are fed by precipitation or a groundwater table, not by rivers or streams. These ponds are commonly found in small groups that are sometimes interconnected. The hydroperiod of alpine lakes in the area is short, with less than 30-day renewal rates (Vinebrooke and Leavitt, 1999a). Pond bottom substrate type ranges from dark, unconsolidated organic sediment, to rocky with very low organic content (McMaster and Schindler, 2005).

Temperature

Diurnal temperature fluctuations in alpine ponds in Banff National Park (BNP) during the ice-free season are extremely variable, ranging from 0 to 24º C, with a mean growing season (ice-free) temperature of 11 ± 6 º C (McMaster and Schindler, 2005). Cloud cover, wind, and air temperatures greatly influence water temperatures at this elevation (Linacre, 1982; Wilhelm and Schindler, 2000). Due to their shallow depths, thermal stratification is either weak or does not occur due to mixing or full light penetration, especially in the case of ponds with lower dissolved organic carbon (DOC) values (Rautio et al., 2011). Arctic and alpine lakes are similar in that thermal stratification is often non-existent, and often freeze entirely in the winter months (Douglas and Smol, 1994). Temperature also affects chlorophyll *a* concentrations contained within the benthic algal community of an alpine pond, with warmer temperatures increasing chlorophyll concentration (Thompson et al., 1992; Baulch et al., 2009). Water temperature can also be a predictor of nutrient ratios in alpine ponds (McMaster and Schindler, 2005), and is a significant predictor of epipelic total chlorophyll concentrations (Vinebrooke et al., 2010; Hobbs et al., 2011).

Light and UV Radiation

Known to be ecologically different from larger water bodies, ponds can be distinguished from lakes in that no deep, aphotic zone exists in the former (Forel, 1904). Due to the shallow nature of alpine ponds, there is no thermal stratification and aquatic plants are potentially able to colonize the entire floor of a pond (Oertli et al., 2000). In the alpine, ponds are subject to relatively high levels of ultraviolet radiation (UV) because of extremely low concentrations of chromophoric DOC (Laurion et al., 2000). Algal and invertebrate responses to ultraviolet (UV; 280 – 400 nm wavelength band) radiation are habitat and taxon specific, with epilithic algal growth suppressed with UV-B (300 – 320 nm wavelength band) radiation, and epipelic algal growth enhanced with UV-A radiation (Vinebrooke and Leavitt, 1999b). Benthic algal exposure to UV-B radiation can also lead to pigment degradation (Xue et al., 2005) which in turn can make biomass estimates using chlorophyll *a* artificially low (Baulch et al., 2009). UV irradiance when severe, has indirect effects on food web processes within the pond, mainly by means of altering community composition of algal groups (Vinebrooke and Leavitt, 1999b).

Similarities of Alpine and Arctic Ponds

With the small amount of literature available on alpine ponds in remote, mountain regions, noting the similarities of the ponds in the Canadian Rockies and the Canadian Arctic is useful. These ponds share many fundamentally important attributes including growing season, biological communities, catchment type and area, and formation features (Orbaek et al., 2007). Both alpine and arctic ponds have very short growing seasons, with less than 100 days of the year in ice-free conditions (Douglas and Smol, 1994; Neldner and Pennak, 1955; Schmitz, 1959). The ponds freeze completely over the winter months, allowing little or no growth during that time (McMaster and Schindler, 2005). Diurnal temperature fluctuations are extremely variable in alpine ponds of BNP (0.2 to 28 º C in one summer day), similar to the closely studied sub-arctic and arctic ponds of Canada (McMaster, 2002; Rautio et al., 2011). Many arctic and alpine ponds contain no higher aquatic macrophytes or fish, as many are actually ephemeral and freeze completely in the winter (Neldner and Pennak, 1955; Schmitz, 1959; Rautio et al., 2011). The landscape surrounding the ponds is often devoid of higher plants, and characterized as a barren, sparsely vegetated landscape (Rautio et al., 2011).

Atmospheric Nitrogen and Phosphorous Deposition

Human activities have modified and accelerated the global nitrogen (N) and phosphorus (P) cycles, more than doubling the amount on N circulating in the atmosphere (Burns, 2004; Elser et al., 2009). Elevated deposition of N can be attributed to energy production, fertilizer use, and crop cultivation (Galloway et al., 1995), while changes in land use and desertification account for increases in atmospheric P deposition (Morales-Baquero et al., 2006). Particulates laden with N and P are emitted into the atmosphere, transported, and then deposited on land as dry or wet deposition (Anderson and Downing, 2006). Nitrogenous gases, such as nitrous oxides (NO_x) and ammonia (NH_3) are emitted into the atmosphere from agriculture and the combustion of fossil fuels, and fall as wet or dry deposition in the form of nitrate (NO_3^-) and ammonium (NH_4^+) (Vitousek et al., 1997). There is much less P deposition in western Canada due to a lack of local anthropogenic inputs or desert ecosystems, in contrast to the Eastern hemisphere where aeolian P deposition greatly impacts ecosystem productivity (Okin et al., 2004). The rates at which these elements are being deposited on land has recently dramatically increased, with N and P cycles 100% and 400% respectively, greater than pre-industrial levels (Falkowski et al., 2000).

In Alberta, the production and refinement of oil and natural gas, coal and natural gas fired utility generation, transportation, and agriculture are the main contributors to the emission of N compounds (Foster et al., 2001). The same is true for mountainous regions in the US and Europe, where N enrichment in alpine lakes is well documented (Saros et al., 2011; Fenn et al., 2003). In the eastern front ranges of the Canadian Rockies, N deposition ranges from 5 to 10 kg ha^{-1} y^{-1}, with much lower rates of P at 0.3 kg ha^{-1} y^{-1} (Alberta Environment, 2011; Anderson and Downing, 2006). Current N deposition rates in the Rocky Mountains of Colorado and Wyoming range from 2 – 7 kg ha^{-1} y^{-1} , which is 20X higher than pre-industrial levels of 0.2 kg ha^{-1} y^{-1} (Burns, 2003; Baron, 2006). In the Colorado Rockies, wet deposition of NO_3^- (which accounts for about ¾ of total N deposition) increased from 1.8 kg ha^{-1} y^{-1} to 4.7 kg ha^{-1} y^{-1} between the late 1980's and early 1990's (Burns, 2003). Similarly in Alberta, N deposition rates have increased by 40% over the last 15 years (Alberta Environment, 2011). All of the currently reported values in the literature are quite high compared to background levels observed in highly remote regions worldwide, which range from 0.1 kg ha^{-1} y^{-1} to 1.0 kg ha^{-1} y^{-1} (Galloway et al., 1982). Nutrient enrichment effects in alpine lakes due to atmospheric deposition of N has helped determine critical loads of N using changes observed in diatom communities. For alpine lake ecosystems in the western US, this is currently between 1.4-1.5 kg ha^{-1} y^{-1}, which has been exceeded in the front ranges of the Canadian Rockies (Baron, 2006; Saros et al., 2011).

Weather patterns in the mountains result in greater atmospheric deposition at high elevations due to orographic lift, which is when an air mass is lifted by the topography (Grennfelt and Hultberg, 1986). The front ranges of the Rocky Mountains intercept the pollutant-laden clouds that release moisture due to cooling, and subsequently have higher levels of deposition than the interior ranges. Consequently, researchers have observed alpine lakes in both North American and European mountain ranges with elevated levels of nitrate deposition (Kopacek et al., 2005; Baron et al., 2000). In the Canadian Rockies, atmospheric deposits are stored in the snow pack over seven months of winter snow accumulation. A large discharge is released into alpine streams, lakes and ponds during snowmelt. The terrain surrounding the ponds is steep and sparsely vegetated providing little buffering capacity, which makes them highly susceptible to the sudden input of nutrients stored in the snow pack. The effect is similar with rain events during the summer. The input of nutrients can shock the relatively nutrient poor alpine pond, potentially decreasing the pH of the water, increasing primary production, and altering species diversity (Goldman et al., 1993; Wolfe et al., 2001; Nydick et al., 2004).

Nutrient Limitation

One of the drivers of nutrient limitation may in fact be the atmospheric deposition of nutrients, particularly N and P. How aquatic ecosystems respond to these increases in nutrient levels, is dependent upon which nutrient is limiting primary production in the system (Elser et al., 2009). Freshwater systems are typically limited by the availability of both N and P (Elser et al., 2007). However, in many alpine ponds in the Canadian Rockies, the water chemistry suggests that they are nitrogen-limited ecosystems (McMaster and Schindler, 2005; Murphy et al., 2010). Nutrient limitation in fresh water is influenced by several physical and chemical variables. One of the chemical variables that is commonly used as an indicator for nutrient limitation is the ratio of Dissolved Inorganic Nitrogen (DIN) to Total Phosphorous (TP) concentration, or DIN:TP. Ratios of less than one have been used to infer N limitation in alpine lakes and ponds (Morris and Lewis, 1988; Sickman et al., 2003; McMaster and Schindler, 2005, Murphy et al., 2010), and more than four to infer P limitation. This chemical index is preferred over TN:TP when interested in the biological response to N and P ratios, since DIN and TP are the most biologically available forms for uptake in aquatic communities (Morris and Lewis, 1988).

The alpine ponds in BNP are characterized by low levels of DIN, and relatively elevated levels of P due to internal loading from the sediments (McMaster and Schindler, 2005). Despite low levels of DIN in alpine ponds of this region, a mean DIN:TP ratio of 3.7 was reported in the alpine ponds in Banff National Park, and indicates the potential for co-limited by N as well as P (Murphy et al., 2010). Phosphorous limitation is also common in aquatic systems due to the rapid biological uptake of usable forms, commonly by the plankton (Schindler, 1977).

Shifts in nutrient limitation status are known to occur due to increases in P loading, such as in the previously P-limited alpine lakes of the Sierra Nevada mountains, where N limitation is now more commonly observed (Sickman et al., 2003). Nutrient ratios are critical for understanding ecosystem response to atmospheric deposition, as nutrient status is often the single most important factor in regulating freshwater communities (Borchardt, 1996).

Dissolved Organic Carbon

Nutrient limitation is also influenced by other chemical variables, particularly the content of DOC in the water column. In freshwater systems, DOC is largely responsible for the attenuation of light wavelengths in the visible and UV spectrums (Laurion et al., 2000). It also organically binds P and N particulates, making these nutrients temporarily unusable to organisms in the ponds. Reports on DOC concentrations in alpine ponds of BNP are highly variable, with Murphy et al. (2010) reporting relatively low DOC (generally <2 mg L^{-1}) compared to means of 5.4-8.6 mg L^{-1} (McMaster and Schindler, 2005). In the alpine lakes of the Alps and Pyrenees, DOC ranges from 0.2-3.5 mg L^{-1} (Sommaruga et al., 1999; Laurion et al., 2000). In alpine environments, glacially fed lakes have lower DOC concentrations than non-glacially fed, and DOC may be negatively correlated to elevation due to a decrease in allochthonous inputs (Sommaruga et al., 1999; Nelson, 1991).

Benthic Communities- Periphyton

Due to the littoral nature of ponds, periphyton dominates their responses to changes in nutrient status (Vadeboncoeur et al., 2002). Diatoms are siliceous algae that often preserve well in aquatic sediments, making them excellent paleoindicators of environmental change in alpine and arctic lakes and ponds (Drake & Naiman, 2000; Wolfe et al., 2001; Saros et al., 2003). As nutrient ratios shift, these diatoms are often replaced with non-siliceous algal groups (Das et al., 2005). This is well documented in the Colorado front ranges, where enhanced atmospheric N deposition was the primary factor behind shifts in the algal community, with diatom abundance negatively correlated with nutrient deposition (Saros et al., 2005). Often, paleolimnolgical evidence is used to reveal shifts in algal community composition, as it easier to understand what changes are occurring due to recent increases in atmospheric deposition of nutrients when the community structure can be compared to that of the past (Wolfe et al., 2003).

The rate of development and biomass of periphyton is dependent upon several abiotic factors including light, temperature, suspended solids, and substrate type. There are several methods of accounting for different algal groups in ponds, with the use of naturally occurring biochemicals (e.g., carotenoids) in photosynthetic algae an accepted practice (Vinebrooke et al., 1999a). Total chlorophyll is the most widely used biological variable to estimate periphyton abundance. Chlorophyll concentrations of algal communities in alpine ponds of this region can range from 0.5 to 20 μg L^{-1} (McMaster and Schindler, 2005; Murphy et al., 2010). The periphyton community in the alpine ponds of BNP is mainly comprised of chlorophytes, chrysophytes, and cyanobacteria (McMaster and Schindler, 2005).

Algae, bacteria, and fungi associated with submerged surfaces, termed "periphyton," represent the most functionally dominant component of most shallow ponds (Wetzel, 2001). Benthic primary production (BPP) can be up to seven times greater than that of its pelagic counterpart, phytoplankton, at rates of 8.4 g C/m^2/yr in shallow arctic ponds (Hobbie, 1980), which indicates high functional importance in pond food webs. Epilithic (rock-attached) algae are considered to be the best indicators of variations in the abiotic environment of shallow mountain lakes and ponds (Vinebrooke and Leavitt, 1999b). Epipelon (sediment-dwelling algae), can form cyanobacterial dominated mats on the sediment-water column interface of ponds, which is often indistinct due to constant mixing and resuspension (McMaster, 2003). Epipelon abundance is best explained by water column DOC and nitrate+nitrite concentrations, while community composition is best explained by Total Nitrogen (TN), TP and DOC (Vinebrooke and Leavitt, 1999; Lafrancois et al., 2003; McMaster and Schindler, 2005).

Benthic Communities- Zoobenthos

The largest and most thoroughly documented consumer in aquatic systems are fish. However, few fish species are actually native to alpine systems, and when the water body is shallow enough to freeze throughout in the winter, there is a complete absence of fish (Rautio et al., 2011; Schindler and Pacas, 1996). With no fish in alpine ponds, the role of top trophic level consumer is now taken over by predacious zooplankton or benthic macroinvertebrates (McNaught et al., 1999). Due to the lack of predators, benthic invertebrate density is mainly dependent upon cold tolerance and food supply (Vincent and Laybourn-Parry, 2008). In the alpine lakes and ponds of the Canadian Rockies, the prominent members of the zoobenthic community include the Limnephilidae family (northern caddisfly, a large algal grazer and omnivore), Chironomidae family (true flies/midges, mainly omnivorous), and Dysticidae family (predacious diving beetle). The dominant grazers in alpine lakes of this region are the caddisfly larvae, and as such herbivory rather than predation by the benthic invertebrates may be functionally more dominant. Ponds in extreme environments (i.e. alpine and arctic) are typically considered to contain sparse populations of benthic invertebrates, and diversity is reduced by the environmental harshness of the climate (Donohue et al., 2009).

In shallow alpine pond ecosystems, secondary production is limited by several factors, including hydroperiod of the ponds, cold temperatures due to the high elevation, and the availability of resources (i.e. periphyton). Periphytic primary production can be regulated by nutrient availability as well as benthic grazing (Nydick et al., 2004; Peterson, 1999). Grazing pressure in alpine ponds is not well studied; however, Smol and Douglas (2007) show large potential impacts of fairy shrimp grazing on smaller members of the community in arctic ponds. Grazing by aquatic insects such as the caddisfly larvae, can have an overriding influence on periphytic community in the form of decreased biomass, changes in rate of primary production, taxonomic composition, and finally community structure (Walton et al., 1995).

Pelagic Communities- Phytoplankton

Phytoplankton responses to nutrient enrichment have been well documented in alpine ponds compared to periphyton (Murphy et al., 2010), as pelagic processes tend to be the main focus in limnological studies (Vadeboncouer et al., 2002). However, the shallow, oligotrophic nature of alpine ponds allows most production to occur at the water-sediment interface. As such, the planktonic community plays a lesser role in terms of ecosystem functioning and contribution to biodiversity (Vadeboncouer et al., 2002). The importance of benthic vs. pelagic communities is a function of lake size, with phytoplankton abundance (measured using chlorophyll a concentrations) being negatively correlated with surface area (McMaster and Schindler, 2005). Phytoplankton abundance in alpine ponds of BNP average 0.4 μg L^{-1}, which is 4X higher than lakes of this region (Murphy et al., 2010). However, phytoplankton abundance can range from 1 to 27 μg L^{-1} in arctic and alpine regions, which is best explained by TP, pH, and conductivity (Lim et al., 2005; McMaster and Schindler, 2005). Low temperatures and a limited supply of nutrients in the alpine can lower primary production (Ward, 1985; Hinden et al., 2005). This may be reflected in the rate of phytoplankton production, which in arctic ponds is between 2.1 – 3.8 µmol C/L/day (Whalen et al., 2008).

Alpine and arctic ponds support a wide range of phytoplankton species that belong to the Bacillariophyta (diatoms), Chrysophyta (chrysophytes), Cryptophyta (cryptophytes), Chlorophyta (green algae), and Cyanophyta (cyanobacteria or "blue-green algae") (Vincent and Laybourn-Parry, 2008). Common genera in alpine ponds include cyanobacteria (*Gloeocapsa, Oscillatoria, Anabaena),* chlorophytes (*Scenedesmus, Crucigenia),* and diatoms (*Synedra,and Tabellaria)* (Strecker et al., 2004; Zettel, 2010). Phytoplankton from the genera such as *Chlamydomonas* and *Ochromonas* are found in many polar and alpine ponds (Laybourn-Parry et al., 1991). Shifts in alpine phytoplankton community composition have been documented in response to various environmental stressors, including environmental warming (Strecker et al., 2004; Holzapfel and Vinebrooke, 2005; Flanagan et al., 2009; Parker et al., 2008) and nitrogen deposition (Baron et al., 2000; Lafrancois et al., 2003; Nydick et al., 2004; Thompson et al., 2008). Due to the low concentration (or absence) of aquatic macrophytes in these pond ecosystems, grazing pressure is centered on the phytoplankton and periphyton (Wetzel, 2001).

Pelagic Communities- Zooplankton

Many zooplankton species commonly found below treeline are not found in the alpine. This is due to limiting physical factors including temperature and morphometry, and the lack of large predators (Anderson, 1971; Parker et al., 2001). Large, abundant populations of zooplankton (>17 000 crustaceans/ m^2) are found in alpine ponds due to the lack of fish (Parker et al., 2001). Benthic algae are a common food source for zooplankton, with large-bodied consumers exerting a stronger grazing pressure than smaller species (Diehl, 1992). In alpine ponds of Western Canada, crustaceans (cladocerans, copepods, anostracans) dominate the zooplankton community with a mean of almost 3 species per community (Anderson, 1971; Rautio and Vincent, 2006). *Diaptomus articus* is the most commonly found species of zooplankton, occurring in 60 % of the 146 surveyed alpine lakes and ponds in Western Canada (Anderson, 1971). Zooplankton community composition changes little from year to year, with more copepods than cladoceran species in the community. Anostracans, or fairy shrimp, are one of the few zooplankton species that are only found in alpine ponds of western Canadian Mountain National Parks (Anderson, 1971).

Rationale For The Study

In the literature, there is a growing body of evidence demonstrating the physical and biological changes in mid-continental alpine lakes and ponds in response to to increased nutrient deposition (Baron, 2006; Burns, 2003; Wolfe et al., 2001). These high elevation ecosystems are ideal sentinels as detectors of environmental change due to their remote, simple, and pristine characteristics (Oertli et al., 2008; Vinebrooke and Leavitt, 1999a; Battarbee et al., 2002). However, there are conflicting reports on the nutrient status of alpine ponds in the Canadian Rockies (Murphy et al., 2010; McMaster and Shindler, 2005), which is the first step to investigating the potentially large changes due to atmospheric N deposition (Lepori and Keck, 2012). Recent studies show that shallow alpine ponds are more likely to be nitrogen limited than alpine lakes, with low DIN:TP ratios and evidence of internal loading of P from the sediments to support this (Murphy et al., 2010; McMaster and Schindler, 2005). The littoral nature of ponds, along with known low phytoplankton and zooplankton abundances in the region, suggests that benthic communities play a larger role in ecosystem functioning in the alpine ponds of BNP. With simulated nutrient deposition, it is suspected that the response will be primarily seen in, and driven by the benthic community. To investigate the benthic responses to N and P loading in the alpine ponds of BNP, a replicated, whole ecosystem approach was used. It is suspected that changes in the benthic communities in alpine ponds will correspond to, and provide insights into changes in alpine lakes and larger lakes at lower elevations in mountainous environments worldwide (Vitousek et al., 1997).

The main objective of this research was to experimentally determine the nature of nutrient limitation, and the reaction of the benthic communities, to increased nutrient loading in the alpine ponds of BNP along the Eastern Front Ranges of the Canadian Rocky Mountains. While the purpose of Chapter 1 was to provide a general background to the ecology of alpine pond ecosystems, Chapter 2 contains a report of benthic responses to experimental nutrient amendments, which were conducted during the ice-free season in 2008. Chapter 3 provides a summary of the findings, as well as a synthesis of the implications that increasing nutrient levels will have on the benthic communities in the alpine lakes and ponds here in the Canadian Rocky Mountains. Future directions for research on alpine ponds and atmospheric deposition in the Canadian Rockies are proposed, as well as a discussion of management implications of monitoring the deposition as well as the benthic communities in these alpine ponds.

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CHAPTER TWO: BENTHIC RESPONSES TO ELEVATED NITROGEN AND PHOSPHOROUS DEPOSITION- A REPLICATED ALPINE POND EXPERIMENT

INTRODUCTION

Mountain ecosystems are particularly sensitive to increases in atmospheric nitrogen (N) deposition (Hauer et al., 1997; Bowman, 2000; McMaster, 2003). Anthropogenic inputs of N into the atmosphere from fertilizer use, combustion in industrial processes, as well as transportation have exceeded natural inputs at approximately 140 Tg/year (Galloway and Cowling, 2002). These atmospheric inputs are important sources of nutrients, and thus can strongly affect the biogeochemistry of nutrient poor oligotrophic systems. Nitrogen deposition to a high elevation site in Rocky Mountain National Park, CO, USA has been increasing at a rate of 2% per year over the last two decades. Ecosystem health began to decline at high elevation lakes on the East side of the Continental Divide between 1950-1964, at which time wet N deposition levels are estimated to have been above natural background levels (0.2 kg ha^{-1} y^{-1}) at 1.5 kg ha^{-1} y^{-1} (Baron, 2006). The major ecological effects of this elevated N deposition in high elevation lakes include nutrient enrichment and acidification (Baron et al., 2011). However, the ecological responses to nitrogen saturation of surface waters are variable. Responses are reflected in the level of nutrient enrichment from anthropogenic sources, the availability of nutrients, and the dominant species in the system (Gardener et al., 2008).

N-limited Colorado lakes have responded to the almost century long use of nitrogen fertilizers with shifts in diatom composition from pelagic,to benthic dominated species (Waters, 1999). Rapid taxonomic shifts in diatom community structure have also been reported, and can be attributed mainly to anthropogenic N deposition and climate change (Saros et al., 2003). Productivity increases in phytoplankton have also been observed due to additions of N, P, or both (Morris and Lewis, 1988; Nydick et al., 2004; Lafrancois et al., 2003). A recent study of nutrient enrichment in Colorado alpine lakes revealed increases in cyanobacteria and chlorophytes when treated with N and N + P (Nydick et al., 2004), although cyanobacteria may already have an advantage in low N environments. Atmospheric deposition can cause a shift from N-limitation to co-limitation for algal production possibly due to N saturation (Gardener et al., 2008; Jassby et al., 1994). In weakly buffered alpine lakes with low P availability, eutrophication events can actually decline (Lewis, 1982; McKnight et al., 1990). These responses are a reflection of nutrient availability (Gardener et al., 2008), which is in part regulated by catchment characteristics in a complex, snow melt dominated landscape (Baron et al., 2011; Kamenik et al., 2001).

Atmospheric deposition of N and P affects the chemical and biological operations of aquatic systems (Burns, 2003). However, little is known about alpine pond ecosystems, and the effects of atmospheric deposition on them. Even less is known about how the benthic communities in these ponds will respond to these changes. To investigate the ecological effects of nitrogen deposition, studies have been done to simulate deposition in aquatic systems. In the Rocky Mountains, USA, deposition was simulated using 1000 μg N/L, which is now a consistent and comparable nutrient amendment concentration (Nydick et al., 2004; Thompson et al., 2008). Due to the littoral nature of ponds, the benthic periphyton is often the most responsive community to changes in nutrient status (Vadeboncoeur et al., 2002).

I conducted a replicated whole-ecosystem fertilization experiment to determine the direct and interactive effects of simulated N and P deposition on benthic communities in alpine ponds located along Snow Pass in Banff National Park, AB. To do this I used a two-factor (N × P) experimental design replicated four times, with four applications performed over the ice-free season. Based on earlier chemical and bioassay evidence (McMaster, 2003; Murphy et al. 2010), I hypothesized that periphyton production and community composition would respond more strongly to N than P amendments (i.e. nitrogen limitation). I also expected that benthic invertebrate biomass would be positively stimulated in N-fertilized ponds owing to increased algal food supply.

METHODS

Study Site

Sixteen alpine ponds located along Snow Pass in the Cascade Valley of Banff National Park, AB, Canada were used in this study (51' 27'-51’ 44'N, 115' 39'-115' 56'W (Figure 1). The ponds are located along the eastern Front Ranges of the Canadian Rockies in the alpine eco-region. These small, glacial kettle ponds are ice-free for approximately five months of the year (June to October), and have rapid flushing rates (<30 days) (McMaster and Schindler, 2005). The surrounding landscape is steep and sparsely vegetated, with exposed sedimentary rock (mainly limestone and shale) and patches of vegetated orthic eutric brunisolic soil (Holland and Coen, 1982). White heather *(Cassiope mertensiana)*, arctic willow *(Salix arctica)*, lichens, krummholz alpine fir *(Abies lasiocarpa)*, krummholz Engelmann Spruce *(Picea engelmannii),* white mountain avens *(Dryas octopetala)*, bladder locoweed *(Oxytropis podocarpa)*, and sedges dominated the vegetation.

The coordinates of the ponds were taken using a Global Positioning System (GPS) and 1:50,000 maps. Maximum pond depth was recorded using a weighted measuring stick tied into the middle a rope, with the rope held tight on either end from opposite shores of the ponds. This method ensured the pond sediment was not disturbed. Pond basin shape was categorized as rectangular, elliptical, or round. Maximum length and width of the ponds were also measured. Maximum depth, basin shape, and surface area were used together to determine the volume of each pond.

Elevation of the ponds from this study ranged from 2250 to 2570 m a.s.l. Catchment type across the ponds varied from well-drained high alpine meadows at the upper elevation range, to slower draining ponds in slight depressions at 2250 m. Substrate types differed among ponds, with most (70%) having rocky bottoms containing little organic matter except for small pieces of wood, pine needles, and small bunches of root material. The remaining 30% were characterized as silty with fine unconsolidated decomposing organic matter, and often very grassy bottoms. Pond depths ranged from 15 to 150 cm, and pond volume ranged from 236,559 L in the larger ponds after ice-out, to only 6040 L in smaller ponds during late summer, before desiccation. Pond desiccation occurred in six of the 16 ponds, from mid-July onwards. This was inclusive of all the North Ridge Ponds, which are characterized by having small surface areas, shallow depths, and lacking any substantial inflows.

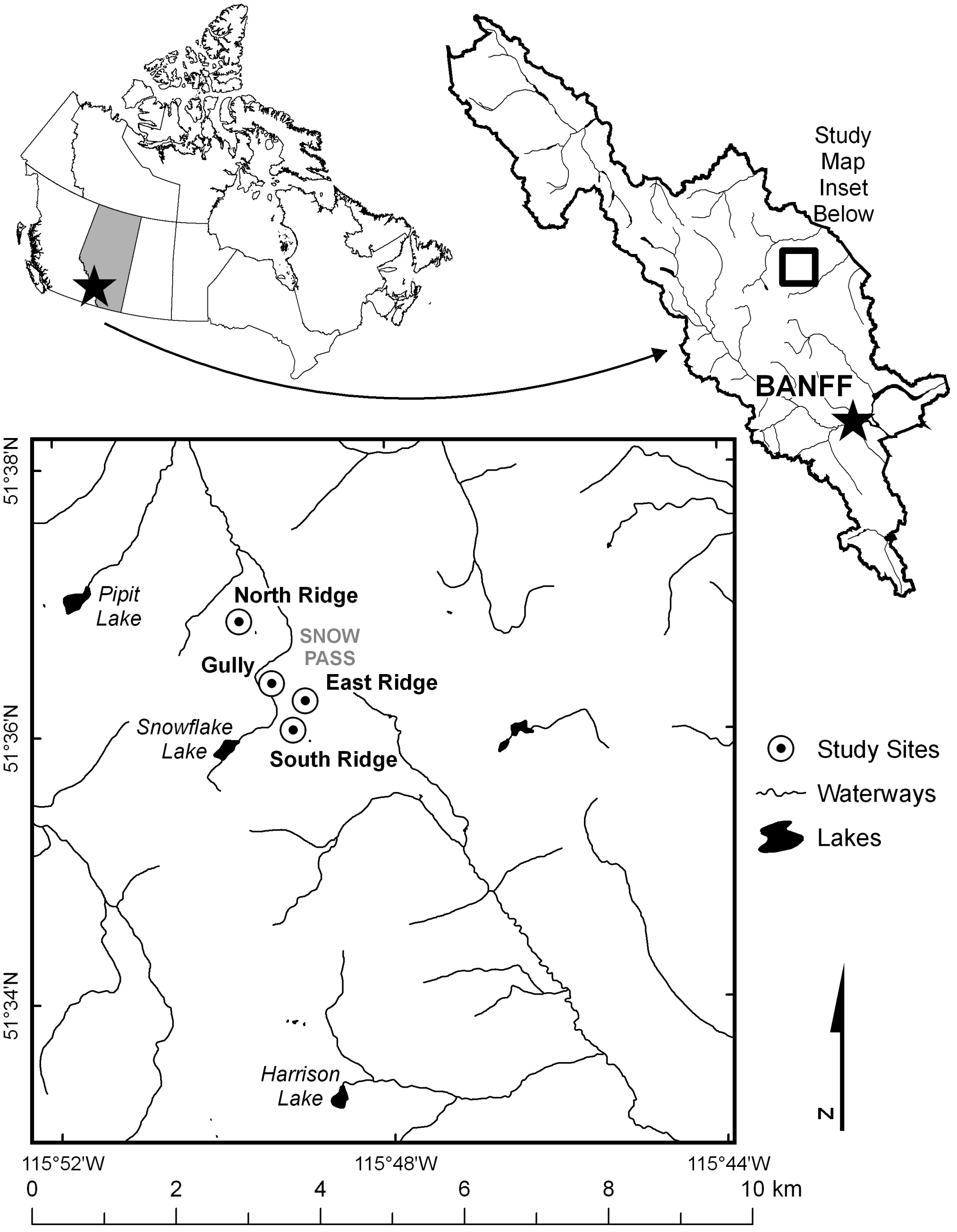

Figure 2.1. The sixteen study ponds are located in the Cascade Valley in Banff National Park, Alberta, Canada (51° 36'N 115° 49'W). The study ponds were in four areas on Snow Pass (2500 m), as denoted by the four study sites (North Ridge, South Ridge, East Ridge, and Gully). The four study site elevations ranged from 2250-2570 m. Map by L. Porter and C. Nielsen.

The ponds were chosen for their close proximity to each other (2 km^2), and their similarity in size (< 1 ha) and depth (< 2 m). DIN:TP ratios are below 4, indicating potential biological nitrogen limitation (McMaster and Schindler, 2005; Murphy et al., 2010). Before the experiment began, TN levels across all the ponds ranged from 226 µg/L to 673 µg/L, and TP levels ranged from 14 ug/L to 1140 µg/L (Table 2.3).

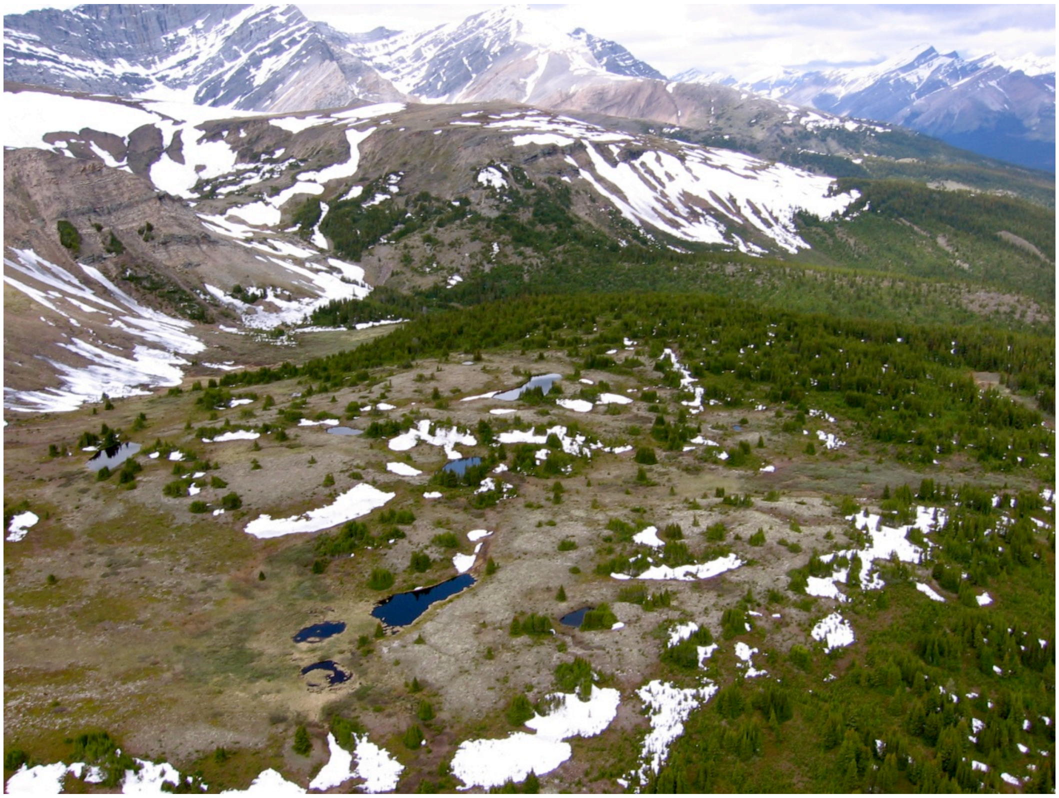

Figure 2.2. Aerial photo of the North Ridge ponds, taken looking NNE from above the gully ponds. Photo by L. Porter.

Climate data collected from the study site between 1999 and 2001 indicate an average annual air temperature of 2.5^{o}C, with a mean summer average of 12.1 ^{o}C and daily temperature fluxing from -1.4 ^{o}C to 26.6 ^{o}C. Over the summer months

(June 15 to September 1) rainfall averages 210 mm (McMaster and Schindler, 2005). Wet nitrate deposition in Kananaskis Country (70 km South-West, 1500 m a.s.l.) is 2.8 kg ha^{-1} y^{-1} (Alberta Environment, 2011). There are no estimates of P deposition for this area.

N and P deposition was measured every two weeks at three different study sites along Snow Pass. Three Stratus RG202 Long Term Professional Rain and Snow Gauge (4.25" x 14"), accurate to 1 mm were mounted 1 m above the ground with steel rebar and a wood back mount. The gauges were placed along an elevation gradient as follows: a high elevation site (South Ridge, 2400 m), mid-elevation site (East Ridge, 2300 m), and the low elevation site (Gully, 2250 m) (Figure 2.1 and Table 2.2). N deposition was measured as Total Dissolved Nitrogen (TDN) and TN, and P deposition as Total Dissolved Phosphorous (TDP) and TP. Samples were kept in 1L acid-washed Nalgene containers and stored on ice and brought back to the Alberta Biogeochemical Analytical Facility at the University of Alberta for analysis within seven days of collection. Collection procedures can be found in Murphy et al. (2010).

Experimental Design

A randomized, two-factor experimental design was used, crossing nitrogen and phosphorous nutrient amendments. The four treatment combinations (+N,+ P, +NP, Control) were replicated four times in a total of sixteen alpine ponds. To simulate P deposition, 30 µg P/L in the form of phosphoric acid (H_3PO_4) was added to each pond, as well as 1000 µg/L of ammonium nitrate (NH_4NO_3) to simulate N deposition. N amendments equated to a deposition rate of approximately 25 kg ha^{-1} y^{-1}, and P amendments to 5 kg ha^{-1} y^{-1}. These nutrient concentrations were chosen based on deposition levels observed in other mountainous regions which are already experiencing the adverse effects of increased nutrient deposition (Baron et al., 2006; Fenn et al., 2003). The nutrients were added to the ponds every fourteen days, over a three-month period beginning immediately after ice-out on June 5th, 2008. Total N deposition is naturally highest in the summer months (June, July & August) (Baron et al., 2006). A 5-L fertilizer sprayer was used to mix the phosphoric acid and ammonium nitrate with pond water, and to simulate natural atmospheric deposition by broadcasting the nutrients evenly across the surface of the pond.

SAMPLING PROTOCOL AND ANALYSES

Pond Water Chemistry

Before and after nutrient additions, samples were collected in 2-L Van Dorn water bottles, and subsequently divided into acid-washed Nalgene sample containers. The water was stored on ice and brought back to the University of Alberta for analysis within seven days of collection. Collection procedures which I followed can be found in Murphy et al. (2010). TN, TP, TDN, TDP, ammonia (NH_4), nitrate and nitrite (NO_3 + NO_2) and DOC were measured. These chemical analyses were performed at the University of Alberta Biogeochemical Analytical Facility (URL: http://www.biology.ualberta.ca/facilities/limnology). Total nutrient concentrations were determined using unfiltered water, while dissolved concentrations were determined using filtered water (0.45 μm Whatman GF/F filters).

TN, TDN, and $NO_3 + NO_2$ were quantified colorimetrically at 520 nm. TP and TDP were quantified using proportional absorbance at 880 nm. Ammonia (NH_4), was also quantified using proportional absorbance at 630 nm. All of these measurements were taken with a Lachat QuikChem 8500 FIA automated ion analyzer with a detection limit of 1 μg/L. Standard methods for the procedures followed can be found in American Water Works Association (1999). DOC was measured by infrared detection of CO_2 from combustion at 680^oC, using a Shimadzu 500A TOC analyzer with a 0.1 mg/L detection limit (EPA methods 415.1).

Periphyton Sampling

On day 0 of the experiment, I harvested epilithic periphyton from five flat rocks, each having a total surface area of approximately 5 cm^2. The rocks were taken from a depth of 10-30 cm along the pond perimeter at 5 m intervals. During subsequent sampling periods, periphyton was sampled using small 5 cm^2 unglazed beige ceramic tiles, which simulated the natural rock surface in the ponds. The tiles were arranged in sets of 10, and were enclosed in 1/8” galvanized steel wire mesh. Tile sets were elevated approximately 10 cm above the substrate using stainless steel tent pegs in an effort to limit grazing pressure from the larger benthic invertebrates. Tile sets were distributed evenly within the ponds. Five tiles (one from each set) were removed from the mesh enclosures in each pond every 14 days.

Periphyton was scraped off the rock and tiles using a flat toothbrush. The toothbrush and rocks/tiles were then rinsed with 250 mL of filtered (Whatman GF/C filters, 0.7μm mesh size) pond water. The periphyton from the five rocks/ tiles was pooled into one sample. Twenty milliliters of the sample was filtered through Whatman GF/C filters, stored in Petri dishes, wrapped in aluminum foil, and then put on ice. The samples were stored at -80°C until they were freeze-dried and extracted. The remainder of the sample was stored in 250 mL wide-mouth Nalgene containers and preserved in Lugol’s solution (approximately 3 drops).

High performance liquid chromatography (HPLC) was used to quantify algal abundance and composition from concentrations of taxonomically diagnostic pigments. An Agilent 1100 Series HPLC unit equipped with a Varian Microsorb - 100Å C18 column was used to perform these algal analyses. Extraction and separation techniques were performed as reported in Vinebrooke and Leavitt

(1999a). The pigments and major algal groups identified included chlorophyll *a* (all algae), chlorophyll *b* (chlorophytes, euglenoids), chlorophyll *c* (chrysophytes, diatoms, dinoflagellates), alloxanthin (cryptophytes), canthaxanthin (filamentous cyanobacteria), diadinoxanthin (chrysophytes, diatoms, dinoflagelates, euglenoids), diatoxanthin (diatoms), fucoxanthin (chrysophytes, diatoms, dinoflagellates), lutein (chlorphytes), zeaxanthin (cyanobacteria), and violaxanthin (chlorophytes) (Table 2.1). Chlorophylls were identified using in-line diode array and fluorescence detectors with detection limits of 0.2 and 0.1 μg/L, respectively. Total chlorophyll concentrations were quantified using calibration equations and an electronic spectral library constructed using standards of chlorophylls *a*, *b*, and *c* purchased from DHI Water and Environment (Agern Alle 5, DK-2970 Hoersholm, Denmark).

Table 2.1 Taxonomically diagnostic pigments (carotenoids and chlorophylls (Chl)), with their representative algal groups (adapted from Vinebrooke and Leavitt, 1999a).

Pigment	**Algal Group**
chl *a*	all algae
chl *b*	chlorophytes, euglenoids
chl *c*	chrysophytes, diatoms, dinoflagellates
alloxanthin	cryptophytes
canthaxanthin	filamentous cyanobacteria
diadinoxanthin	chrysophytes, diatoms, dinoflagelates, euglenoids
diatoxanthin	diatoms
fucoxanthin	chrysophytes, diatoms, dinoflagellates
lutein	chlorophytes
zeaxanthin	cyanobacteria
violaxanthin	chlorophytes

Zoobenthos Sampling

I sampled the benthic invertebrates using a 28-L stainless steel stovepipe (33 cm diameter, 61 cm in height). Samples were taken at 5 m intervals along the shorelines of the ponds at a water depth of approximately 20 cm. The stovepipe was pushed 10 cm into the sediment, and 20 cm above the surface of the water. All of the water within the stovepipe and the top five centimeters of sediment were removed from the stovepipe with a hand pump. All sediment and water from the stovepipe was sieved through a 250 µm mesh-sized sieve bucket. Two stovepipe samples were taken from each pond for each sampling period. The samples were stored in 1-L wide-mouth Nalgene containers and preserved in 80% ethanol.

In the laboratory, I rinsed the samples in a 500 µm mesh-sized sieve, and then placed in a 20 x 30 cm white sorting dish with water. Invertebrates were picked out with insect tweezers and placed in 20 mL vials with 80% ethanol for preservation. Under a dissecting microscope, the invertebrates were identified to family level using keys by Merritt and Cummins (1996) and Clifford (1991). Following identification, all full specimens from the Limnephilidae and Chironomidae families were measured to the nearest 0.1 mm under a dissecting microscope for biomass calculations. The length-mass regressions used to calculate biomass can be found in Benke et al. (1999).

Lab Grazing Experiment

I also wanted to determine if fairy shrimp use periphyton as a food source, and if so, was there a significant effect on total chlorophyll concentration (used as an indicator of periphyton abundance). Therefore, the whole-ecosystem pond experiment was also replicated back in the laboratory, with an additional treatment consisting of two levels of grazers (no grazers versus ambient grazer density) added to the design. This made for a three-way factorial design with randomized blocking (ponds). I used 10 L aquaria containing 8 L of pond water, and biota collected from each of the four control ponds which were pooled together before being poured into the aquaria. The water was filtered with a 64 µm mesh screen to remove any zooplankton or large pieces of organic matter. Once the aquaria were each filled with pond water, the unglazed ceramic tiles were lowered inside and allowed to be conditioned by periphyton for 10 days at 12 °C with a 12h light-dark photoperiod. The nutrient amendments were made at the beginning of the experiment, to allow periphyton conditioning to the nutrient addition before any grazing occurred. After 10 days (T= 0h), naturally occurring densities of fairy shrimp (approximately 1/L or 8 individuals) were added to each aquarium, and randomly assigned the +grazer treatment level. Water and tiles were then harvested from each aquarium at T= 0h (just before grazers were added), after 3 hrs of grazing treatment (T= 3h), after 18 hrs of grazing treatment (T= 18h), and finally at 48 hours (T= Final). Periphyton were scraped from tiles as described in the alpine pond experimental protocol, then and analyzed for total chlorophyll content at the University of Alberta Biogeochemical Analytical Facility (URL: http://www.biology.ualberta.ca/facilities/limnology).

Statistical Analysis

Effects of nutrient additions and time on water chemistry, nutrient status, periphyton abundance, periphyton community composition, and benthic invertebrate density and biomass (using two major families Limnephilidae and Chironomidae) were tested using a Repeated Measures Analyses of Variance (RM-ANOVA). Due to an unavoidable shrinkage of some ponds owing to dry conditions during the experiment and the ephemeral nature of some of the ponds, some pond replicates were lost during the latter half of the experiment (beginning mid-July). All statistics were performed using SPSS Statistics 18.0 for Macintosh. Tukey's post hoc test was used to determine which treatments and dates were significantly different.

RESULTS

Nitrogen and Phosphorous Deposition

Average dry and wet N deposition as quantified using rain gauges at Snow Pass, Banff National Park, AB throughout the summer months ranged from 9.5 to 69 μg N/L/wk respectively. Total wet deposition of TDN across all three of these sites was 370 μg/L over the course of the 60-day experiment. The respective total wet deposition of TDP was 343 μg/L. However, TN and TP amounts were undetectable until the final sampling period (late summer). The maxima of atmospheric N deposition occurred at 92.5 µg/L/wk during the first 2 weeks of July and the minima of 4 µg/L/wk during the last 2 weeks of July (Table 2.2). The maxima of atmospheric P deposition occurred at 96.5 µg/L/wk during the last 2 weeks of July and the minima of 13 µg/L/wk during the first 2 weeks of August.

Table 2.2. Average wet deposition of N and P in µg/L/2 wks at three different sites on Snow Pass, representing three different elevations (High (South Ridge)= 2400m, Mid (East Ridge)= 2300m, and Low (Gully)= 2250m). Level of detection (LOD) was 0.1 µg/L.

	2 Week Period	**High**	**Mid**	**Low**
TDN	Early July	185	197	<LOD
	Late July	90	66	8
	Early August	10	36	12
TDP	Early July	90	61	42
	Late July	193	85	136
	Early August	63	26	80

Water Chemistry

Prior to the onset of the experiment, all ponds appeared N-limited as inferred from a very low average DIN:TP ratio of 1.0 (0.68 SD). Average TN and TP across all the ponds, before the experiment began was 361 and 25 µg/L, respectively. Initial TP concentrations revealed mesotrophic pond conditions, however initial total chlorophyll concentrations in the periphyton of < 0.2 µg/L in all ponds suggested oligotrophy. DOC concentrations ranged from 5.4 - 13.8 mg/L, which is within the known range of these ponds, and is in line with the observed stained humic appearance of some ponds (Table 2.3). Ponds with low DOC had rocky bottoms, while ponds with higher DOC had dark, highly organic unconsolidated sediment and were generally found at lower elevations.

Table 2.3. Physical and chemical variables for all 16 alpine ponds sampled in 2008 prior to nutrient amendments.

Treatment	Latitude (dd mm ss)	Longitude (dd mm ss)	Area (m^2)	Depth (m)	TN (µg L^{-1})	TDN (µg L-^{1})	PN (µg L^{-1})	TP (µg L^{-1})	TDP (µg L^{-1})	PP (µg L^{-1})	DOC (mg L^{-1})
C	51° 36' 16 N	115° 49' 04 W	390	0.8	226	209	17	14	7	7	5.8
N	51° 36' 12 N	115° 49' 04 W	240	0.3	427	389	38	18	10	8	9.6
P	51° 36' 03 N	115° 49' 10 W	180	0.4	406	356	50	30	18	12	8.7
NP	51° 36' 15 N	115° 49' 00 W	120	0.3	353	349	4	27	12	15	6.9
C	51° 36' 01 N	115° 49' 11 W	176	0.6	299	266	33	14	9	4	8.6
N	51° 36' 45 N	115° 49' 46 W	308	0.3	385	316	31	22	21	1	6.9
P	51° 36' 44 N	115° 49' 48 W	1000	0.5	310	264	46	25	17	8	6.6
NP	51° 36' 02 N	115° 49' 09 W	266	0.4	549	525	24	30	21	9	11.5
C	51° 36' 23 N	115° 49' 24 W	862	1.0	487	472	15	*	*	0	8.2
N	51° 36' 45 N	115° 49' 36 W	690	0.6	290	266	24	28	20	8	7.2
P	51° 36' 17 N	115° 49' 08 W	324	0.3	673	638	217	129	16	9	10.2
NP	51° 36' 46 N	115° 49' 46 W	495	0.6	236	216	20	33	21	12	5.4
C	51° 36' 50 N	115° 49' 51 W	450	0.3	364	318	20	40	30	10	9.9
N	51° 36' 51 N	115° 49' 51 W	90	0.3	545	503	32	35	28	12	13.8
P	51° 36' 43 N	115° 49' 47 W	1000	0.5	325	284	32	28	20	27	8.7
NP	51° 36' 51 N	115° 49' 47 W	1512	0.5	233	203	30	19	12	7	6.2

Water Chemistry - Control Ponds

Mean TN and TDN values for all control ponds increased over time (time effect, $F_{3,33} = 3.27$, $P = 0.045$ and $F_{3,33} = 4.18$, $P = 0.021$), while TP (marginally significant) and TDP values decreased (time effect, $F_{3,33} = 2.43$, $P = 0.041$). On Day 0, the concentration of phosphorous was the highest observed over the experiment at 300 µg/L possibly due to large influx of nutrients from snow melt, or mixing of the sediments during the first wind event of the ice-free season. The control ponds DIN:TP remained extremely low (close to zero) throughout the summer with an average of 0.82, suggesting N-limitation was prevalent throughout the experiment.

Water Chemistry- Nutrient Amendments

Immediately after initial nutrient amendments, average TN and TP in all the amended ponds increased almost three-fold to means of 1011 µg N/L and 89 µg P/L, exceeding targeted amended concentrations of 1000 µg N/L and 30 µg P/L (Figure 2.4). Two weeks following the nutrient amendments, there was a significant decline in TN and TP concentrations (Figure 2.4). DOC was significantly higher in the +NP amended ponds (treatment effect, $F_{3,33} = 5.75$, $P = 0.006$). TN and TP measurements after nutrient amendments stop at day 45, as no amendments were made on day 60 (Figure 2.4).

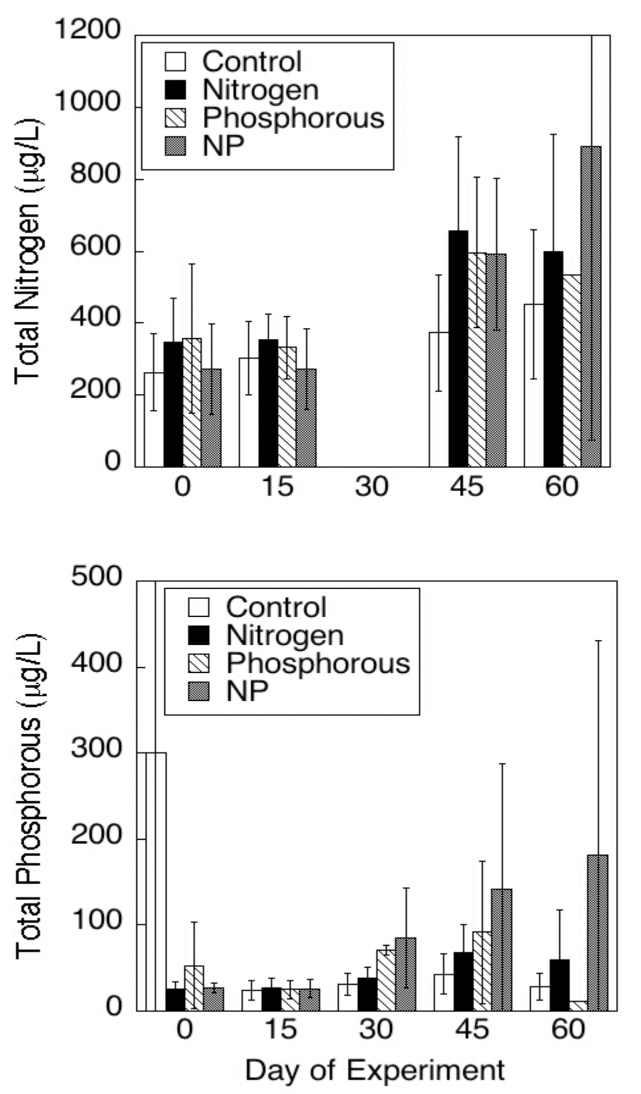

Figure 2.3. TN and TP concentrations in the water column immediately prior to each nutrient amendment. Error bars represent ±1 SE (n= 1-4 pond replicates).

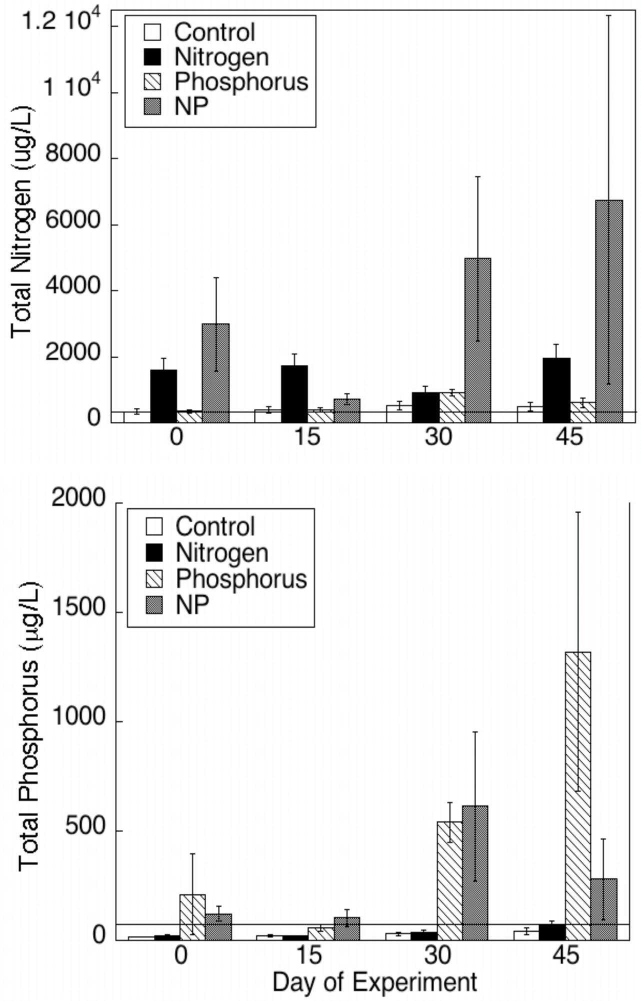

Figure 2.4. TN and TP concentrations in the water column of each pond immediately following each nutrient amendment. The line represents the mean pre-treatment level of nitrogen and phosphorous across all the treatments. Error bars represent ±1 SE (n= 2-4 pond replicates).

Effect of Nutrient Amendments on Periphyton

Periphyton chlorophyll concentrations remained low throughout the experiment (< 2 µg/L), however they did significantly vary over time (time effect, $F_{3,33}$ = 4.52, P = 0.012). There was also a significant treatment effect on total chlorophyll involving higher concentrations in both the N- and NP-amended ponds (treatment effect, $F_{3,33} = 6.11$, $P = 0.005$) (Figure 2.5).

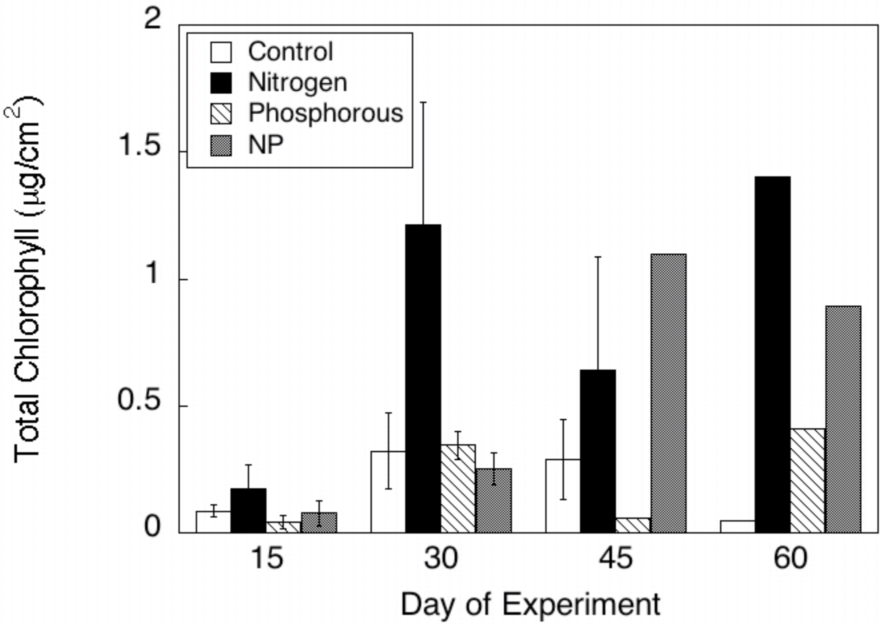

Figure 2.5. Periphytic total chlorophyll concentration in the alpine ponds after nutrient amendments. Error bars represent ±1 SE (n= 1-4 pond replicates).

Diatom abundance as measured by the sum of diadinoxanthin and fucoxanthin pigment concentrations was not significantly affected by the nutrient amendments. Green algal abundance and cyanobacterial abundance as measured by violaxanthin and canthaxathin/zeaxanthin pigments, respectively were also not significantly affected by the nutrient amendments. Nevertheless, all measured algal groups were consistently most abundant in the N-amended ponds. There was also no significant effect of time, likely due to high variance among replicates. On Days 45 and 60 of the experiment, several pond replicates were lost (Figures 2.3 through 2.7).

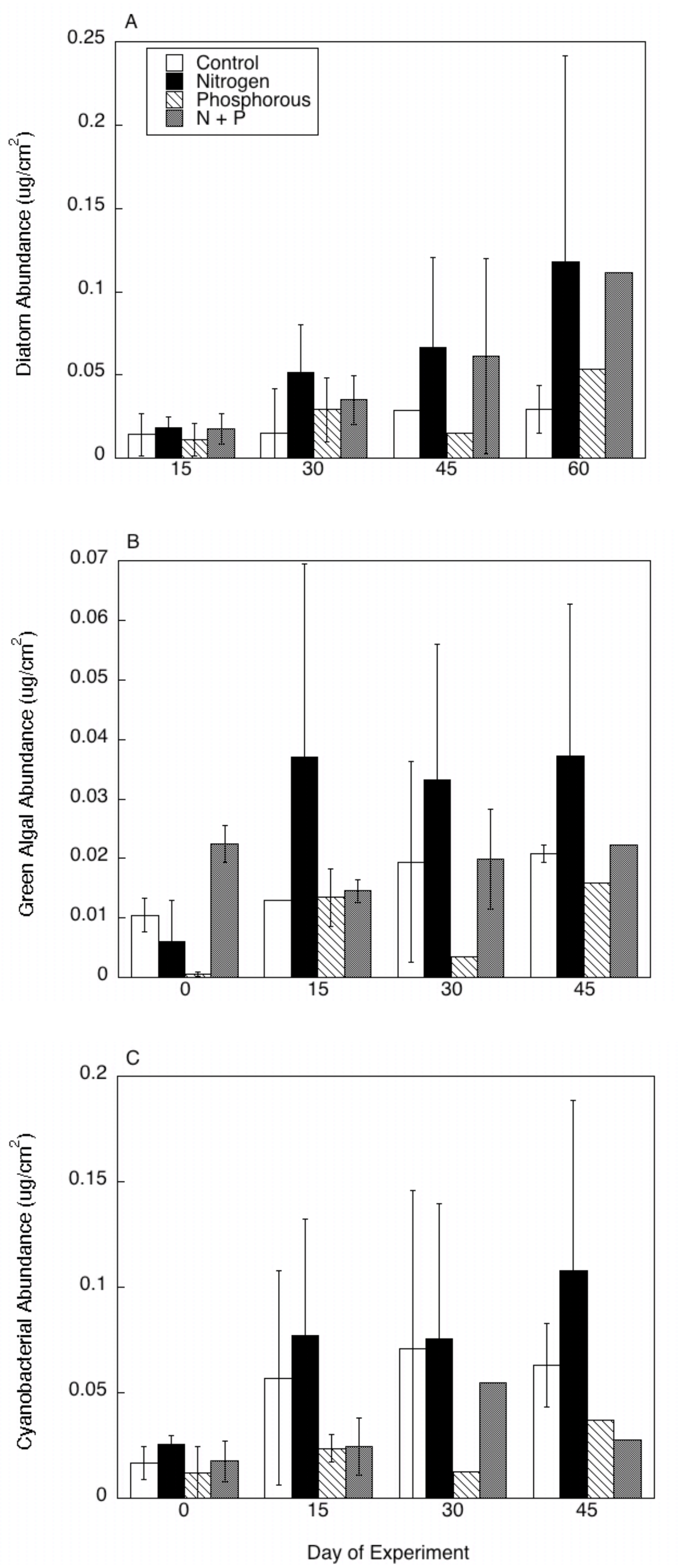

Figure 2.6. Pigment inferred diatom abundance (fucoxanthin and diadinoxanthin pigments) (A), green algal abundance (violaxanthin pigment) (B) and

cyanobacterial abundance (canthaxanthin and zeaxanthin pigments) (C) in µg/cm^2 from tiles taken from the alpine ponds from June to August 2008. Error bars represent ±1 SE (n= 1-4 pond replicates).

Zoobenthos

The most abundant benthic invertebrate families in the ponds were Chironomidae (midges) and Limnephilidae (northern caddisfly). Other families often found in the ponds were Dytiscidae (predacious diving beetles), Culicidae (mosquito larvae), Spaeriidae (fingernail clams) and Lymnaeidae (freshwater snails). Chironomids were the most widely distributed benthic invertebrate, and were found in every pond. In some of the ponds, there were over 1000 chironomids found in one stovepipe sample (max=1256, min=0). There was no significant nutrient effect on the biomass of Chironomidae (Figure 2.7; treatment effect, F = 1.85, P = 0.234). In contrast, Limnephilidae biomass in the control ponds was significantly different from the +N pond, but only prior to the experiment (Figure 2.7; t = 2.59, P = 0.018). Average zoobenthos richness across all the ponds was 8.2.

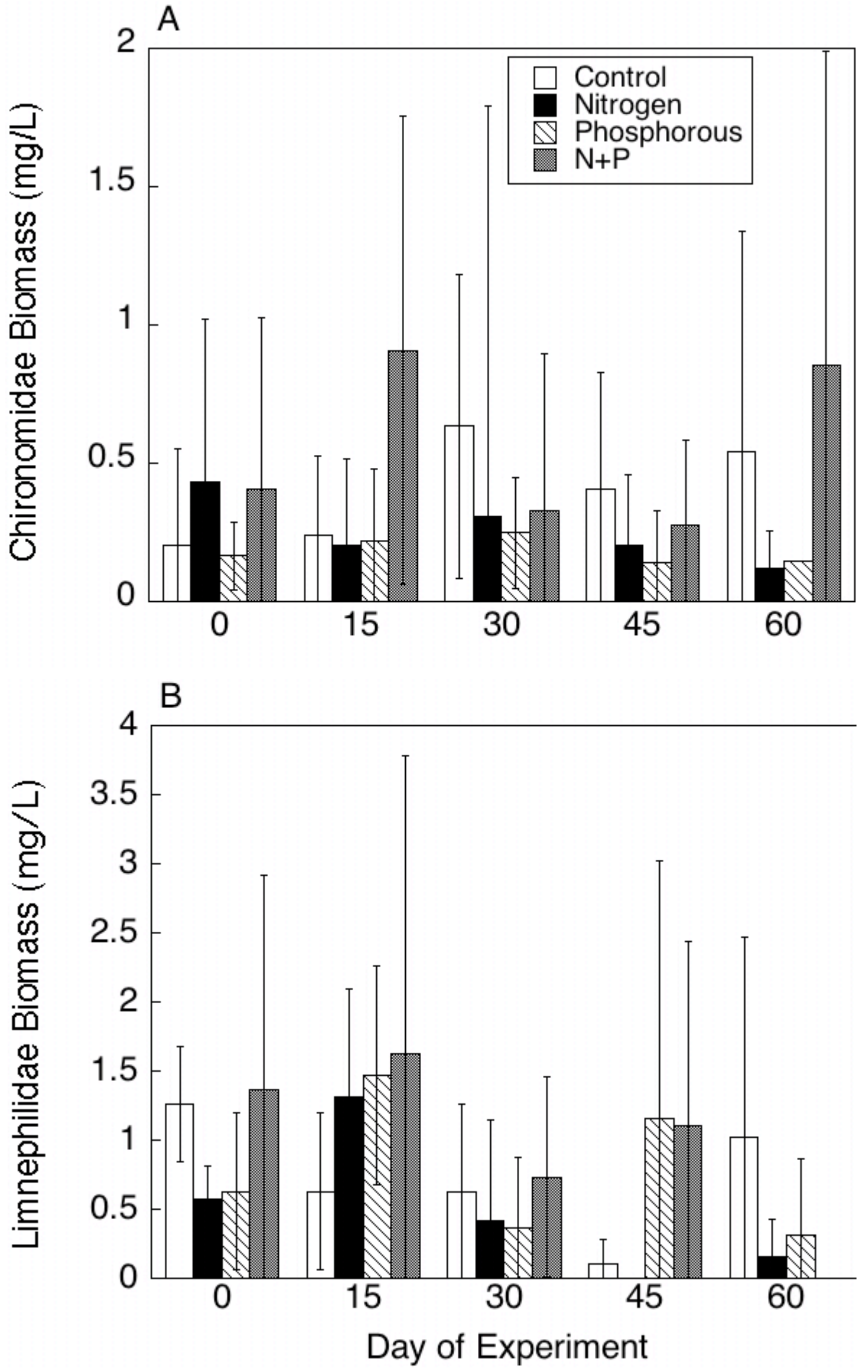

Figure 2.7. Biomass of two main benthic invertebrate families found in the alpine ponds of BNP: Chironomidae (A) and Limnephilidae (B). Error bars represent ±1 SE (n= 1-3 pond replicates).

Zoobenthos Grazing on Periphyton

There was a strong nutrient effect on periphyton abundance, with the +NP treatment having significant effect on periphyton growth during the laboratory simulation of the field experiment (Figure 2.8). I also found a significant effect of grazers after 48 hours in the +NP treatment. In the +N treatment, there was a significant grazing effect at each time interval. Time was not a significant factor in the experiment. Branchinectidae (*Brancinecta paludosa* or fairy shrimp) were observed clearly grazing on periphyton during the lab mesocosm experiment, but did not exert a significant effect on total chlorophyll. Nevertheless, total chlorophyll content of NP-amended periphyton was substantially suppressed in the presence of grazers on the final date.

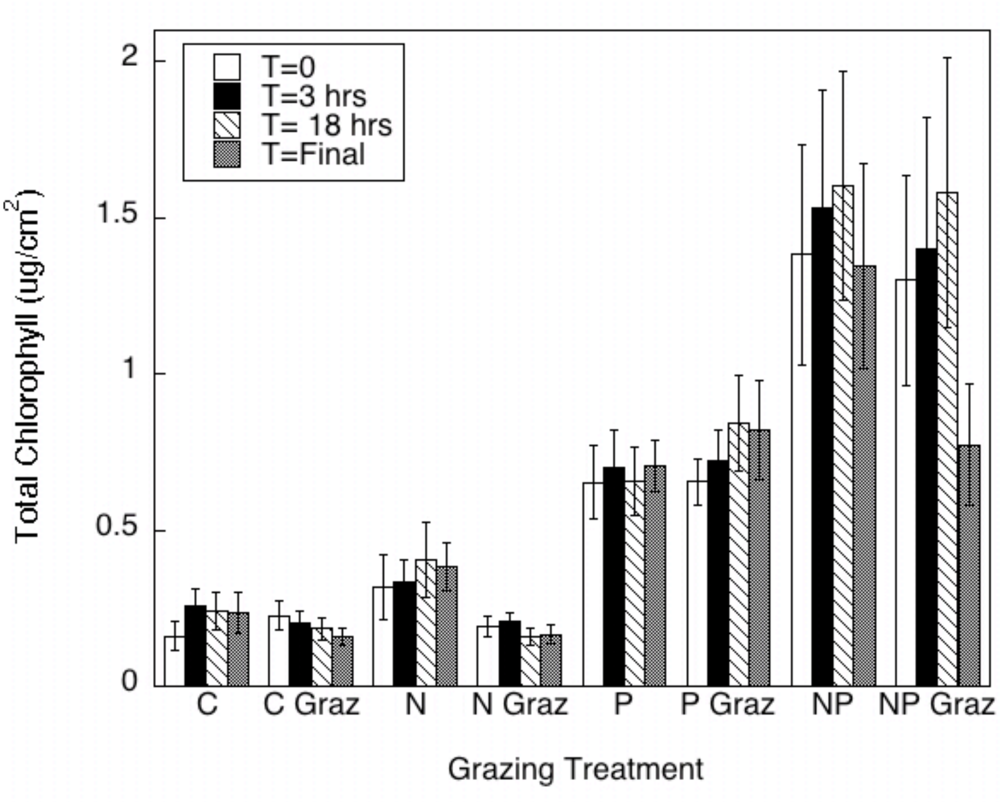

Figure 2.8. Periphyton chlorophyll concentration (ug/cm^2) across all treatments over the 48-hour grazer experiment period. Error bars represent ±1 SE (n= 4 per treatment).

DISCUSSION

I discovered observational and experimental evidence of periphytic algae being either N- or co-limited in the alpine study ponds. The common chemical index for nutrient limitation (DIN:TP) suggested N-limitation was prevalent in the study ponds throughout the summer. This chemically-based inference of N-limited periphyton supports similar evidence of the phytoplankton in these ponds being more limited by N than P, while those in nearby lakes appeared P-limited (see Murphy et al. (2010). However, the mean DIN:TP ratio was extremely low (0.82) compared to the ratio of 7.4 found in some of the same ponds at Snow Pass during the Murphy et al (2010) survey, which occurred in the summer of 2007. Low DIN:TP ratios coupled with significant periphyton chlorophyll increases in response to the experimental nutrient amendments suggests these alpine ponds could be important indicators of increases in atmospheric nitrogen deposition. Below, I discuss potential explanations for my key findings.

Nutrient concentrations rose steadily in the amended ponds over the course of the experiment, which likely involved several mechanisms. Throughout the summer, the water levels of most ponds visibly dropped, and in some cases the ponds had dried up completely during the course of the summer. The increases in nutrient concentrations over time may have been partially attributed to evaporation of the pond water. With less water, the concentration of nutrients will increase as long as it is due to evaporation, rather than leaching. This would have been dependent upon the substrate of the pond, which varied widely from large cobble to super-fine organic sediment. Further, P levels rose more dramatically than N in the amended ponds, likely reflecting the contrasting sedimentary versus gaseous nature of the two elements. On a related point, some of the added N may be have been undetectable in the water column due to rapid assimilation by benthic biofilms or lost completely as N_2 owing to microbial activity. High DOC concentrations in the ponds may have masked some of the nutrient amendment effect by organically binding the inorganic nutrients, and rendering them unavailable to biological communities. Large differences between TDN and DIN levels within the ponds suggest much of the dissolved nitrogen was organically bound and likely not available for biological uptake (Antia et al., 1991).

The strong chlorophyll-inferred periphytic response to the +N amendment suggests P was not the limiting nutrient in benthic algal production. The immediate proximity of the sampling substrates to the sediment likely provided the algal communities with ample supply of P (Nydick et al., 2004), which can be released from sediments during wind events and anoxic conditions (Schallenberg and Burns, 2004). In comparison, the boundary layer overlying the periphyton may impede gas exchange, thereby causing N supply to periphyton to be lacking during metabolically active periods.

The significant drop in total periphyton chlorophyll in the +NP grazing treatment of the lab mesocosm experiment on the last sampling date demonstrates the potential effects of grazing in the ponds. The littoral nature of the ponds allows for heightened interactions between benthic algae and planktivorous grazers. It also may explain the lack of +NP effect despite some of the ponds actually being co-limited by N and P. Fairy shrimp (*Branchinecta paludosa*) were very abundant in the study ponds and have extremely high feeding rates (up to 8 μg of chlorophyll *a* per hour per individual) (Hawes, 1985). They may be considered benthic once mature (Hawes, 1985), and could have shifted from grazing on phytoplankton to periphyton as the latter became more abundant (Rautio et al., 2009). Further, as pond volume decreased throughout the summer, this may have concentrated fairy shrimp and intensified the grazing pressure on the periphyton. This may explain why Thompson et al. (2008) did not find grazing pressure to be a significant factor in phytoplankton abundance in a mesocosm experiment in late summer with water and organisms collected from some of the same alpine ponds as my study.

All major periphytic taxonomic groups responded positively to the +N amendments. No major shifts in community structure from diatoms to cyanobacteria demonstrate potential resilience within the benthic communities of these alpine ponds to eutrophication. Shifts in diatom community composition as a result of +N amendment have previously been documented in the American Rockies (Burns, 2003), when high elevation alpine lakes were subject to elevated N deposition. It is important to note that there can be an increase in the concentration of chlorophyll per periphytic cell, however the number of cells can stay the same, making chlorophyll a levels artificially low (Baulch et al., 2009). Thus, in future studies it would be essential to look at the chlorophyll concentration per unit biomass over time to document the changes occurring in the primary producers.

Average pond zoobenthos richness of 8.2 taxa was comparably low to the previously reported low average of 11.3 taxa in a survey of 25 high alpine ponds in the Swiss Alps (Oertli et. al. 2008). All of the zoobenthic families found in the ponds are reported to also be present in nearby alpine lakes (Pipit and Snowflake lakes), which are located within 5 km of this region (Snow Pass). These nearby lakes may play an important role in mitigating the effects of N deposition, by providing a local species bank, assuming distribution mechanisms are sufficient. However, it is this relatively simple community structure that makes the ponds ideal sentinels of change (Hinden et al., 2005). The control ponds having higher densities of Limnephilidae than the +N ponds before nutrient amendments may be an example of notoriously patchy distributions of benthic organisms (Baumgartner et al., 2008). Further, the single growing season experiment may not have been long enough to detect a nutrient response in the zoobenthos considering their longer life cycles.

Phytoplankton were suspected to show a less significant response than the periphyton, as indicated by the nonsignificant growth response to N during Murphy et al. (2010)'s bioassay. Phytoplankton community shifts to less edible species in response to N deposition have been previously documented (Nydick et al., 2004; Zettel, 2010) and may suppress the zooplankton response. The lack of a planktivorous response to the nutrient amendments in these ponds during this experiment (Zettel, 2010) and to Thompson et al. (2008)'s mesocosm experiment in the absence of warming may be explained by rapid biological uptake by periphyton.

The findings presented here refute the recent findings by Murphy et al. (2010), which stated that increased N deposition should not result in immediate widespread eutrophication in the Canadian Rockies. However, my results support Murphy et al. (2010)'s suggestions that smaller water bodies (ponds) in BNP are likely to be N-limited, and therefore susceptible to increased N deposition. More research is needed to determine the discrepancies found in the nutrient limitation status between alpine lakes and ponds in BNP. Finally, benthic responses may be easier to detect and more pronounced than their pelagic counterparts in alpine ponds due to their littoral nature. These ponds should be considered special cases for early indicators of the effects of N deposition in alpine lakes and ponds in Banff National Park, AB, Canada.

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CHAPTER THREE: GENERAL DISCUSSION AND SYNTHESIS

The findings of this whole-ecosystem experiment blend together the lines of previous experimental evidence by suggesting that resources, as well as density dependent species interactions, may both be important regulators of benthic communities in alpine ponds (Vinebrooke and Leavitt, 1998; Vinebrooke and Leavitt, 1999; Zettel, 2010). N-deposition will likely be a key stressor that defines the impacts of environmental change on the aquatic ecosystems of shallow-water alpine environments in Banff National Park (Sala et al., 2000; Bergstrom and Jansson, 2006). Further, grazing by fairy shrimp may play a previously unappreciated important role in dampening the response of primary producers to increased nutrient deposition (Zettel, 2010).

Often, there is more than one environmental stressor playing a role in the functionality of an ecosystem. In the case of these alpine ponds, it has been shown that climate change is a significant concern (Holzapfel and Vinebrooke, 2005), especially when coupled with N-deposition (Thompson et al., 2008). Cold water lakes are predicted to warm 7°C by 2100 (Magnuson et al., 1997), however, over a recent 20 year period, the average temperature of cold water lakes in central Canada increased by 2°C (Schindler et al., 1990). Holzapfel and Vinebrooke (2005) found that warming of these alpine ponds significantly reduces the consumer biomass, which results in a greater abundance of photosynthetic autotrophs. It remains relatively uncertain whether the combination of these stressors are additive, but given the low biodiversity of zoobenthos (Oertli et al., 2008) and dispersal limitations (Holzapfel and Vinebrooke, 2005), the ponds may have difficulty adapting to even a single environmental stressor.

In future studies, consideration must be given to the strength of nutrient deposition simulations. The approach in this study may be considered aggressive, with simulation targets of 25 kg/ha/yr N deposition, when current levels are around 6 kg/ha/yr throughout the province of Alberta. The result of this was reflected in very high levels of N and P in the water column immediately after nutrient additions. However, due to high flushing rates in the ponds (some have inflows and/or outflows), and suspected high leaching rates in ponds with sandy or rocky bottoms, this issue may have been mitigated.

The nutrient enrichment critical load (or rate of atmospheric deposition) at which ecological degradation occurs in the alpine ponds of Banff National Park is unknown. However, the current estimates of N deposition in the Canadian Rockies are currently higher than the critical load of 1.5 kg N/ha/yr determined for alpine ecosystems in the Eastern front ranges of Rocky Mountain National Park (US) (Saros et al., 2011). High elevation lakes on the East side of the Continental divide were the first ecosystems whose health began to decline, beginning in the 1950's coinciding with the industrialization era (Nanus et al., 2012). The experiment presented in Chapter 2 of this study had only one nutrient amendment level of each +N, +P, and +NP, thereby only simulating high deposition levels. Therefore, it is also unknown if the changes observed here will occur at lower rates of deposition, as they have in other alpine ecosystems.

The ephemeral nature of these alpine ponds does not allow for an investigation of the effects of natural, continual atmospheric deposition of nutrients. Since almost half of the ponds used in this study dried up either partially or completely by late July, it was impossible to account for all the responses which might have occurred after sampling stopped. A split-basin experiment in a shallow alpine lake or permanent alpine pond would provide a more meaningful tool to evaluate the effects of deposition by eliminating much of the variation between ponds. There was large variability in sediment size and type between the ponds, with sediment potentially being a significant P source for benthic algae (Nydick et al, 2004). As such, any further studies using artificial enclosures must be able to account for sediment algae biomass being the dominant source of total algal biomass (Nydick et al., 2004).

The proximity to major cities and highways is an important factor when evaluating future N-deposition concerns, as N-deposition is projected to increase with human population growth and increasing energy use (Burns, 2003). Calgary (population 1,042,892), 100 km to the SE of Banff National Park, is a possible major contributor of N and P deposition in BNP, as the fastest growing metropolitan in Canada (Canadian Census, 2011). In Alberta, peak rates of deposition occur in conjunction with agricultural planting and the use of fertilizers (of which ammonium is a main component). The mechanisms of transport for these nutrients is not well documented, however, Anderson and Downing (2006) observed dry deposition of N to wet surfaces (ponds) to be several times greater than to dry surfaces. This suggests ammonia (NH_4) gas absorption by water is an important N transport mechanism, and would be an interesting measure in future studies.

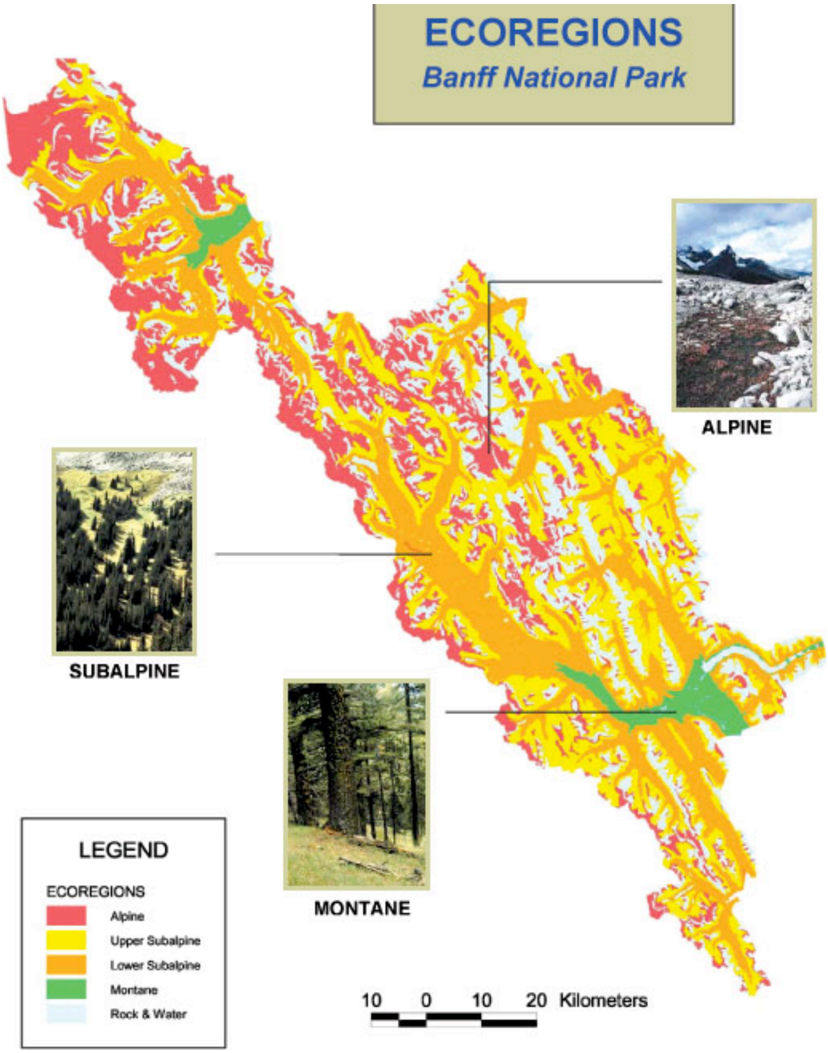

Figure 3.1 Ecoregion map of Banff National Park, AB, Canada (adapted from Parks Canada (http://www.pc.gc.ca/pn-np/ab/banff/plan/gestion-management/plan8a.aspx)).

Within Banff National Park there are 3000$^+$ alpine ponds alone (Parks Canada, 2006), and worldwide ponds represent the largest number of water bodies at 7,200,000 (Downing et al., 2006). Banff National Park's Assessment of Ecological Integrity states that the amounts and effects of long-range pollutants here is unknown (Parks Canada, 2006). Most of the international research to date on N deposition has focused on regions of high deposition (usually densely populated areas) and are small scale (Magill et al., 1997; Vitousek et al., 1997). The alpine lakes and ponds in Banff National Park represent some of Canada's most pristine water bodies, systems once thought to be far from the affects of human disturbance. However, there have been drastic increases in the amount of N that is being deposited across the global landscape (2% increase per year to high elevation sites in the US), with known ecosystem degradation effects (Rabalais, 2002; Baron, 2006). For some time, eutrophication and loss of biodiversity have been pressing issues in Rocky Mountain National Park, U.S. and in the Sierra Nevada Mountains, Spain (Morales-Baquero, 2006). Despite this, the alpine lakes and ponds in Banff National Park have received little attention in terms of current biodiversity status and sustainability of ecosystem functioning. The lack of monitoring of alpine ponds in terms of water quality and biodiversity within the lakes and ponds is concerning, and the inclusion of alpine ponds in limnological assessments will help provide early insights into ecological changes in the park. Further, this alteration of nutrient supply may have many complex interactions with previously known issues such as climate warming (Thompson et al., 2008; Holzapfel and Vinebrooke, 2005).

The current Assessment of State of Ecological Integrity for Banff National Park (Parks Canada, 2006) has management goals applicable to the alpine ponds in this study. Goal 3.6.2 is to acquire a better understanding of the effects of long-range pollution and climate change on the health of ecosystems and humans, and goal

3.9.1 is to maintain biodiversity of aquatic ecosystems. To address these highly relevant management goals, an atmospheric deposition monitoring station should be set up >2200 m a.s.l. to represent the dominant Alpine Ecoregion within the park (Figure 3.1). Ensuing the determination of current N deposition levels in Banff National Park, point sources of N in Alberta should be clearly identified. In Colorado, the top ten point sources identified were responsible for 84% of ammonia emissions (Baron et al., 2004). Identification of critical N deposition loads, and mitigation if these loads are surpassed should be a major future research topic of high interest to land managers and ecosystem scientists alike in Canadian Mountain National Parks (Schindler, 2000), as even slight increases in atmospheric deposition can lead to measurable changes in ecosystem properties (Baron et al., 2000).

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APPENDIX

Appendix A: Benthic invertebrate densities (number/L) for all nutrient treatments, shown for individual ponds from three of the ponds sites (East Ridge, Gully, and South Ridge).

	FAMILY	SPHAERIIDAE					CHIRONOMIDAE				
	DAY OF EXPERIMENT	0	15	30	45	60	0	15	30	45	60
POND SITE	TREATMENT										
EAST RIDGE	C	0.00	0.00	0.11	0.06	0.03	0.03	2.08	18.53	4.25	1.81
EAST RIDGE	N	0.03	0.06	0.08	0.11	0.14	18.17	0.94	0.36	0.39	0.36
EAST RIDGE	P	0.00	0.00	0.00	0.00	0.00	3.72	0.72	1.78	0.00	0.00
EAST RIDGE	NP	0.06	0.06	0.86	0.67	0.39	18.47	17.39	16.17	8.14	27.31
GULLY	C	0.47	0.03	0.56	0.19	0.83	10.00	9.36	12.19	14.56	0.83
GULLY	N	6.11	0.58	3.28	4.31	1.89	3.31	9.22	34.89	6.36	3.58
GULLY	P	0.03	0.00	0.00	0.03	0.03	4.08	8.50	0.00	4.53	2.42
GULLY	NP	0.03	0.00	0.00	0.00	0.03	1.25	27.53	0.08	0.92	0.89
SOUTH RIGDGE	C	0.00	0.00	0.00	0.00	0.00	0.08	0.53	0.64	1.33	24.08
SOUTH RIGDGE	N	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
SOUTH RIGDGE	P	0.00	0.00	0.00	0.00	0.00	0.39	1.69	6.44	0.19	0.00
SOUTH RIGDGE	NP	0.00	0.00	0.00	0.00	0.00	0.44	0.00	0.00	0.00	0.00

	FAMILY	LIMNEPHILIDAE					CULICIDAE				
	DAY OF EXPERIMENT	0	15	30	45	60	0	15	30	45	60
POND SITE	TREATMENT										
EAST RIDGE	C	0.31	0.14	0.22	0.00	0.36	0.00	0.00	0.06	0.00	0.36
EAST RIDGE	N	0.11	0.39	0.00	0.00	0.00	0.39	0.14	0.78	0.00	0.00
EAST RIDGE	P	0.00	0.11	0.17	0.00	0.00	0.36	0.75	0.17	0.00	0.00
EAST RIDGE	NP	0.11	0.11	0.28	0.36	0.00	0.00	0.00	0.25	0.00	1.33
GULLY	C	0.17	0.19	0.11	0.06	4.03	0.33	0.19	0.00	0.17	0.28
GULLY	N	0.14	0.14	0.22	0.00	0.08	0.00	0.08	0.00	0.00	0.72
GULLY	P	0.19	0.39	0.00	0.58	0.17	0.08	0.97	0.00	0.36	0.69
GULLY	NP	0.56	0.72	0.06	0.03	0.00	0.00	1.31	0.44	0.00	0.00
SOUTH RIGDGE	C	0.19	0.00	0.00	0.00	0.00	0.03	0.06	0.00	0.00	0.69
SOUTH RIGDGE	N	0.06	0.17	0.00	0.00	0.00	0.00	0.83	0.00	0.00	0.00
SOUTH RIGDGE	P	0.14	0.28	0.03	0.03	0.00	1.64	5.19	1.78	0.00	0.00
SOUTH RIGDGE	NP	0.06	0.03	0.06	0.00	0.00	0.03	0.03	0.06	0.00	0.00

	FAMILY	BRANCHINECTIDAE					DYTISCIDAE				
	DAY OF EXPERIMENT	0	15	30	45	60	0	15	30	45	60
POND SITE	TREATMENT										
EAST RIDGE	C	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.06	0.25	0.53
EAST RIDGE	N	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00
EAST RIDGE	P	0.00	0.78	0.69	0.00	0.00	0.00	0.00	0.00	0.00	0.00
EAST RIDGE	NP	0.00	0.00	0.00	0.00	0.19	0.03	0.03	0.11	0.00	0.06
GULLY	C	0.00	0.03	0.03	0.00	0.00	0.06	0.06	0.00	0.06	0.03
GULLY	N	0.00	0.00	0.00	0.00	0.06	0.08	0.06	0.06	0.03	0.03
GULLY	P	0.00	0.00	0.00	0.00	0.00	0.11	0.06	0.00	0.08	0.00
GULLY	NP	0.00	0.11	2.39	0.75	7.61	0.06	0.00	0.00	0.03	0.33
SOUTH RIGDGE	C	0.00	0.58	0.06	0.00	0.03	0.03	0.00	0.00	0.03	0.00
SOUTH RIGDGE	N	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SOUTH RIGDGE	P	0.17	0.28	0.00	0.33	0.00	0.00	0.03	0.06	0.03	0.00
SOUTH RIGDGE	NP	0.03	0.03	2.39	0.00	0.00	0.06	0.00	0.00	0.00	0.00