

**University of Alberta**

**PLANKTONIC RESPONSES TO ELEVATED NITROGEN AND PHOSPHORUS  
DEPOSITION - A REPLICATED NATURAL ALPINE POND EXPERIMENT**

by

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## **Abstract**

Several lines of evidence suggest small alpine lakes and ponds are sensitive to nitrogen deposition. Paleolimnological studies, nutrient bioassays, and mesocosm experiments show the positive effects of nitrogen on aquatic alpine primary producers. In particular, alpine pond ecosystems have been inferred to be nitrogen-limited based on low availability of dissolved inorganic nitrogen relative to total phosphorus. However, nitrogen-limitation of alpine ponds has never been tested at the whole-ecosystem level. I performed a replicated *in situ* whole-pond experiment, consisting of two crossed treatments (2 nitrogen x 2 phosphorus levels) applied across 16 natural alpine ponds (n = 4) immediately following ice-out in 2008. Surprisingly, neither nutrient amendment stimulated phytoplankton or zooplankton abundance although subtle shifts in community composition were detected over a two-month period. Intensive grazing pressure exerted by high densities (> 100 individuals/ m<sup>2</sup>) of herbivorous *Branchinecta paludosa* (fairy shrimp) may have suppressed planktonic responses to nutrient additions. Another ecological explanation for the lack of a positive effect of nutrients on phytoplankton abundance was competition from periphyton, which are comparatively more abundant in most shallow ponds on an areal basis. Therefore, density-dependent ecological interactions (competition and predation) may mediate the responses of phytoplankton to nitrogen deposition over ponds situated in extreme environments.

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# **Chapter 1 Introduction to alpine and arctic pond ecosystems**

## ***Introduction***

A pond can be defined as a shallow waterbody of permanent or seasonal nature between 1 m<sup>2</sup> and 2 ha in area that may be of man-made or natural form (Biggs et al. 2005). A deep waterbody small in area could also be considered a pond. Alpine and arctic pond systems show many similar physical, chemical, and biological characteristics (Orbaek et al. 2007; Vincent and Laybourn-Parry 2008). These extreme environments are often remote and poorly characterized (Orbaek et al. 2007). Cloud-cover, wind turbulence and extreme daily fluctuations in temperature are common at high elevation sites (Linacre 1982). Polar regions receive pronounced seasonal oscillations in solar radiation and the light regime changes dramatically due to their position on the globe (Orbaek et al. 2007).

## ***Physical Characteristics***

### **Distribution**

Global surveys have shown that inland waters are predominately composed of small (<10 ha surface area), shallow (<10 m mean depth) lakes (Downing et al. 2006). Ponds located in alpine and polar regions are more abundant than lakes that receive greater scientific attention (Orbaek et al. 2007; Vincent and Laybourn-Parry 2008). In comparison to lakes, ponds are considered biological “hotspots” that often contain greater microbial and invertebrate biodiversity (Williams et al., 2004; Biggs et al., 2005; Smol and Douglas 2007). Overall, the

globe is covered with a much greater proportion of small waterbodies and shallow lakes compared to large deep lakes, which have received greater scientific attention (Oertli et al. 2005; Downing et al. 2006; Moss 2010). For example, over 3000 small waterbodies can be found in Banff National Park relative to 480 lakes over the same region (McMaster and Schindler 2005). Alpine ponds are potentially sensitive to environmental inputs and changes in part due to factors such as high water renewal rates, thin soils often with poor buffering capacity, and small catchments (Hinden et al. 2005). Despite their high abundance and ecological importance, research on pond ecosystems is limited, especially in remote alpine and polar environments.

## **Formation**

Thermokarst or thrust ponds are common in the alpine and polar regions across much of the northern hemisphere, including Alaska, Canada, and Siberia (Cote and Burn 2002; Hinkel et al. 2005; Walter et al. 2006). These small waterbodies are formed by physical depressions from melting ice and permafrost. They are typically found in alpine meadows lacking inflows/outflows, surrounded by steep banks, less than 1 m in depth and characterized by fine flocculent organic silt sediments (Vincent and Laybourn-Parry 2008).

Ponds of alpine and polar regions share many similar physical features (Neldner and Pennak 1951; Hobbie 1980; Douglas and Smol 1994). Water budgets of many ponds depend mainly on precipitation, evaporation and seepage through the sediment (Neldner and Pennak 1951). Surface drainage supplies much of the water in the early growing season from snowmelt in the surrounding catchment, while the ground is usually frozen (McMaster 2003). By July, showers and

seepage are the main sources of water. During warm-dry weather periods, pond volumes decrease most rapidly, but may rebound after major precipitation events (McMaster 2003). Therefore, many ponds may disappear because of climate change effects and decreases in precipitation/evaporation ratios (Douglas and Smol, 1994; Schindler and Smol, 2006). In early fall, alpine ponds freeze to the bottom and thaw in late spring from the top downwards (Daborn and Clifford 1974; McMaster 2003). Pools of water form overlying thick masses of ice bound to sand and gravel particles. Ponds are often subject to a short hydroperiod of less than 2 months (see Chapter 2) and short growing seasons with less than 100 ice-free days (Neldner and Pennak 1955; Schmitz 1959).

## **Temperature**

Alpine ponds have highly variable thermal environments because of a limited capacity for heat storage during the ice-free period, often closely tracking daily changes in air temperatures ranging from below freezing to  $>20^{\circ}\text{C}$  (Strecker et al. 2004; McMaster 2003). Most polar aquatic habitats experience temperatures close to  $0^{\circ}\text{C}$  most of the year, however, some shallow waterbodies high in light absorbing dissolved inorganic carbon (DOC) may reach temperatures above  $15^{\circ}\text{C}$  (Vincent and Laybourn-Parry 2008). The water column is often well-mixed, driven by high wind velocities. During the winter when air temperatures remain below zero for several months, the water surface of ponds freeze to the sediments in high elevation (Neldner and Pennak 1955) and high latitude regions (Daborn and Clifford 1974). Pond particulate matter and dissolved solids become increasingly more concentrated in the water column as overlying ice thickens (Daborn and Clifford 1974). The remaining solutes are forced into the sediment

when the pond freezes completely (Prentki 1980). Aquatic organisms must have the ability to migrate to a more suitable habitat, or mechanisms to avoid freezing (Danks 2004).

## **Light**

Alpine and arctic ponds are exposed to relatively high levels of ultraviolet radiation (UV: 290 – 400 nm wavelength band) following ice-out during early summer (Laurion et al. 2000). For example, under clear sky conditions, solar UV- B (290-320 nm) radiation increased by almost 20% while UVA increased by approximately 10% per 1000 m gain in elevation (Blumthaler et al. 1997). Native populations of amphibians (Palen et al. 2000) heterotrophic protists, bacteria, and viruses are particularly sensitive to UVR (Sommaraga 2001) . In comparison, several primary producers and zooplankton appear tolerant of high solar irradiance (Sommaraga 2001), exhibiting high concentrations of photoprotective pigments (Quesada et al. 1999; Vincent and Roy 1993). Photodegradation changes the composition and structure of dissolved organic carbon producing less aromatic products (Bertilsson and Tranvik 2000), liberating phosphorus (Franko and Heath 1982; Cotner and Heath 1990) and nitrogen (Bushaw-Newton and Moran 1999; Kieber 2000).

## ***Chemical composition***

### **Dissolved organic carbon**

DOC often regulates the underwater light environment of alpine and polar ponds by attenuating shorter wavelengths of the visible spectrum (400 – 700 nm) and ultraviolet (300 – 400 nm) band (Vincent and Roy 1993; Laurion et

al. 2000). In alpine and polar regions, ponds surrounded by terrestrial vegetation in their catchment generally appear more brown than lakes owing to high levels of DOC (Graneli 2004; Vinebrooke and Leavitt 1999). DOC concentrations in these regions can range from less than 1 mg C/L to higher than 20 mg C/L, owing to potentially pronounced terrestrial (allochthonous) inputs of humic substances and internal (autochthonous) production of organic exudates by benthic biofilms (McKnight et al. 1994; McMaster 2003; Lim et al 2005).

### **Alkalinity, pH, conductivity and major ions**

The chemistry of alpine and arctic ponds may vary depending on the water budget characteristics, surrounding geology, and atmospheric inputs. Average conductivity measurements have ranged from as low as  $9.7 \mu\text{S cm}^{-1}$  in alpine ponds of Switzerland (Oertli et al. 2008; Hinden et al. 2005) to  $61 \mu\text{S cm}^{-1}$  in the Canadian Rocky Mountains (McMaster and Schindler 2005). Ponds are often weakly buffered (mean alkalinity  $0.55 \text{ meq L}^{-1}$ ) and close to neutral in pH (7.2; McMaster and Schindler 2005; Hinden et al. 2005). The most common ions in solution consist of bicarbonate, magnesium and calcium (McMaster 2003). Seepage losses may be more important than evaporation in some alpine ponds, shown by relatively stable concentrations in alkalinity, pH, conductivity, bicarbonate, magnesium, and calcium (McMaster 2003). In contrast, arctic ponds have shown higher pH, ion concentrations, mean alkalinity, and conductivity presumably due differences in geology (Prentki et al. 1980; Douglas and Smol 1994; Lim et al. 2005).

Dissolved reactive silica concentrations are frequently very low in arctic (Prentki et al. 1980) and alpine ponds (McMaster 2003). Primary producers such as diatoms and other siliceous algae are often limited by this element at low concentrations ( $<0.5 \text{ mg L}^{-1}$ ; Conley et al. 1993). Phycological analyses of alpine ponds in the Canadian Rocky Mountains showed diatoms were scarce in the alpine pond phytoplankton (McMaster 2003; Zettel and Vinebrooke, unpublished data). Epipellic diatoms were more abundant than pelagic diatoms but were low in concentration owing to their sensitivity to desiccation (Mosisch 2001), grazing (McMaster 2003) and low concentrations of silica (Douglas and Smol 1993; Lim et al. 2005).

## **Nitrogen**

Ponds of alpine and polar regions contain total nitrogen (TN) concentrations that vary regionally (Table 1; Lim et al. 2005; Oertli et al. 2008; Murphy et al 2010; Zettel and Vinebrooke, unpublished data). TN consists mainly of total dissolved nitrogen (TDN). Particulate nitrogen (PN) accounts for only ~10% of the TN pool (Lim et al. 2005; Murphy et al 2010; Zettel and Vinebrooke, unpublished data). In certain small mountain lakes, TDN is primarily composed of dissolved organic nitrogen (DON). Low concentrations of bioavailable dissolved inorganic nitrogen (DIN), which is comprised of ammonia, nitrites, and nitrates (Bunting et al. 2010), suggests the potential for nitrogen-limitation in these ecosystems.



## Phosphorus

Average total phosphorus (TP) concentrations reported in ponds of the Canadian Arctic and Rocky Mountains are reported in Table 1 and many ponds could be classified as meso- to eutrophic ecosystems (Lim et al. 2005; Murphy et al 2010; McMaster and Schindler 2005; Zettel and Vinebrooke, unpublished). Here, TP can contain approximately equal fractions of total dissolved phosphorus (TDP) and particulate organic phosphorus (PP), while soluble reactive phosphorus (SRP) is low, averaging approximately 1-2  $\mu\text{g/L}$  (Lim et al. 2005; McMaster and Schindler 2005; Van Geest 2007; Murphy et al. 2010). Organically bound phosphorus may however be made bioavailable as orthophosphate through the hydrolytic activity of microbial enzymes, such as phosphatases (Chrost and Overbeck 1987).

### Chemical Index of Nutrient Limitation (DIN:TP)

Nutrient limitation status is often inferred using a dissolved inorganic nitrogen (DIN) to total phosphorous (TP) ratio (Morris & Lewis 1988). The DIN:TP index is favored over others because it most accurately represents N and P fractions truly available to phytoplankton communities from cellular reserves and surrounding water column (Morris and Lewis 1988). Short-term bioassays of mountain lakes in Colorado showed that nitrogen either alone or in combination with P limited phytoplankton in almost 80% of all cases (Morris and Lewis 1988). Similarly, positive bioassay responses to N amendment were matched by low DIN:TP values (Murphy et al. 2010). Historically, many lakes in the Sierra Nevada showed phosphorus (P)

limitation but as a result of increased P loading, N limitation is progressively more common (Sickman et al. 2003).

Phytoplankton may readily assimilate DIN while DON composed of high molecular weight compounds (peptides, humic acids, fulvic acids) is too large for cellular uptake (Morris and Lewis 1988). Although low molecular weight organic compounds such as urea and amino acids are also accessible to phytoplankton, they are usually found in low concentrations in inland waters (Bonin and Maestrini 1981; Styrett 1981). Therefore, DIN most closely approximates the amount of nitrogen available to the algal community.

TP is also a good predictor of nutrient limitation because numerous forms (SRP, TDP, PP) are available both extra- and intracellularly to primary producers. For example, SRP may be directly assimilated whereas dissolved organic phosphorus may be chemically broken down by extracellular phosphatase excreted by bacteria and algae (Chrost and Overbeck 1987). Algae also store excess P as particulate phosphorus (PP) and therefore it is an important component to measure when investigating nutrient status (Morris and Lewis 1988).

Empirical evidence of nutrient-limitation in alpine and polar pond ecosystems varies by region, type of primary producer (periphyton/phytoplankton), and scale of the experiment. A field-based study (McMaster and Schindler 2005) examining the correlation between environmental parameters and sediment-dwelling algae (epipelton) abundance found that nitrate and nitrite were the only significant predictor of epipelton abundance. However, phosphorus was a significant predictor of phytoplankton abundance. Benthic biofilms may

rapidly assimilate DIN in the water column and outcompete phytoplankton (Axler and Reuter 1996; Mcknight et al. 2004; Nydick et al. 2004). Laboratory bioassays suggested alpine ponds were most often co-limited (Vinebrooke, unpublished) and very few showed nitrogen (N) limitation (Murphy et al. 2010). Overall, increased N deposition in the Rocky Mountains was not suspected to result in widespread eutrophication of alpine waterbodies because the surrounding lakes of the region are primarily phosphorus-limited (Gardner et al. 2008; Murphy et al. 2010). In contrast, a 24 hour bioassay experiment conducted by Levine and Whalen (2001) proposed that N more commonly stimulated phytoplankton production in arctic ponds rather than phosphorus. However, bottle and mesocosm nutrient bioassays may not be ecologically realistic therefore there is a need for experiments at the whole ecosystem scale (Carpenter 1996; Schindler 1998).

## ***Biological Communities***

### **Pelagic**

#### **Primary producers (phytoplankton)**

Ponds in arctic and alpine regions contain chlorophyll concentrations ranging from 1-27  $\mu\text{g/L}$  (Lim et al. 2005; McMaster and Schindler 2005; Murphy et al. 2010). Seasonal fluctuations in primary producer biomass in these systems have been attributed to variation in resources and predation (i.e., grazing pressure; Van Geest et al. 2007). Phytoplankton primary production was measured in arctic ponds between 2.1 – 3.8  $\mu\text{mol C/L/day}$  (Whalen et al. 2008). Low temperatures and supply of nutrients in the alpine limit autotrophic production (Ward 1985; Hinden et al. 2005).

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 *Gloeocapsa*, *Oscillatoria*, *Scenedesmus*, *Crucigenia*, *Synedra*, *Anabaena*, and *Tabellaria* (Strecker et al. 2004; Zettel and Vinebrooke, unpublished data). Phytoplankton from the genera such as *Chlamydomonas* and *Ochromonas* are found in many polar and alpine ponds (Laybourn 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; Hozapfel 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).

## **Bacteria**

Bacterial production and biomass are more important than algae as food sources to higher trophic levels in some arctic ecosystems. For example, Rautio and Vincent (2006) showed that lakes and ponds had high zooplankton concentrations (up to 17 000 crustaceans per m<sup>3</sup>) despite clear waters low in phytoplankton and primary productivity. The planktonic resource pool was primarily composed of detritus (89–98%) instead of and bacteria biomass (up to 29 mg / m<sup>3</sup>) - the most important food item to zooplankton in this food web (Rautio and Vincent 2006). Graneli (2004) found that bacterial production and abundance were over 10 times higher in

the ponds than in lakes despite equally low P levels across both types of waterbodies. Bacterioplanktonic growth is well correlated with temperature, especially at low temperatures (<10°C; Scavia and Laird 1987; Morris and Lewis 1992; Coveney and Wetzel 1995). Therefore, in northern shallow lakes and ponds the classical foodweb where phytoplankton provides the sole food base for higher trophic levels does not occur. Instead, microbial mats and bacterioplankton are the primary food source to zooplankton.

There are many similarities in the bacterioplankton communities of polar regions. Pearce et al (2007) showed that freshwater ecosystems of the Arctic and Antarctic share many of the same bacterioplankton by employing RNA-sequencing techniques. Differences in bacterial community composition were evident between some lakes (Lindstrom & Leskinen 2002). Many bacterial species and protists found in polar regions resembled those found in temperate waters (Vincent and Laybourn-Parry 2008).

## **Zooplankton**

Many alpine and arctic ponds have food webs that differ from nearby lakes because to they lack fish and have an abundance of basal resources. Dense populations of pigmented zooplankton (>17 000 crustaceans / m<sup>3</sup>) including cladocerans, anostracans (fairy shrimp) and copepods inhabit ponds but are less abundant in lakes of these regions (Bertilsson et al. 2003; Rautio and Vincent 2006). Large zooplankton are often present in these ponds because they lack predation pressure by fish (Parker et al. 2001).

Food is the most important environmental factor explaining zooplankton size distribution in polar waterbodies (Vincent and Laybourn-Parry 2008). Benthic microbial mats sustain large zooplankton in many highly productive ponds (Hansson and Tranvik 2003; Rautio and Vincent 2006). Therefore, grazing pressure exerted by zooplankton in ponds may be much stronger than in lakes (Granelli 2004). Zooplankton species richness typically decreases with elevation (Rautio 1998; Hessen et al. 2006).

Cladocerans such as *Daphnia pulex* are common to many alpine ponds and widely distributed (Schmitz 1959). During favourable environmental conditions cladocerans reproduce through parthenogenesis. Changes in food availability, water quality, photoperiod, population density can produce ephippia well in advance of the early winter freeze, which usually occurs in middle to late October on the alpine tundra (Schmitz 1959).

Several species of predatory copepods and anostracans that reproduce sexually may be distributed across many arctic and alpine ponds (Dodson 1974).

Planktonic rotifers such as *Keratella*, *Notholca*, and *Filinia* are generally uncommon in polar and alpine ponds, owing possibly to strong competitive interference by larger cladocerans and predation by copepods (Strecker et al. 2004; Vincent and Laybourn-Parry 2008).

## **Anostraca (Fairy Shrimp)**

Fairy shrimp are very abundant in many ponds and may even be regarded as a dominant life form (i.e. keystone species) linking pelagic and benthic habitats. In an Antarctic study, more than 40000 juvenile *Branchinecta gaini* were reported in a cubic meter, feeding on 8  $\mu\text{g}$  of chlorophyll *a* per hour per individual (Hawes 1985). Fairy shrimp are ubiquitous and one in particular, *Branchinecta paludosa* covers many regions of the globe, inhabiting alpine waterbodies and latitudes above 60°N in Europe, Russia and North America (Saunders et al. 1993). They frequently inhabit fishless ponds that have a short hydroperiod, and freeze to the bottom during winter months. On occasion, fairy shrimp do not hatch in a particular pond each year, possibly due to extirpation and recolonization, or specific physiological requirements that are not always met (Neldner and Pennak 1955, Donald 1983).

Although limited research has been conducted on their feeding habits and life history, most fairy shrimp are described as non-selective filter feeders that collect algal and detrital material by particle filtration using thoracic appendages (Brendonck 1993). They target a variety of food items that include phytoplankton (Mitchell 1991), benthic microbial mats (Rautio and Vincent 2006), rotifers and cladocerans (Sarma 2002; Hawes 2008). Fairy shrimp are described as generalist feeders that are also capable of feeding directly on dead conspecifics (Hawes 2008).

## ***Benthic Communities***

### **Periphyton**

Benthic primary production (BPP; 8.4 g C / m<sup>2</sup>/year) was seven times greater than that of phytoplankton in some polar ponds, suggesting its importance to the food webs of shallow systems (Hobbie 1980). BPP (53 g C / m<sup>2</sup>/year) comprised more than 66% of the total primary production in shallow systems (Whalen et al. 2008) while abundances may range from 18 – 90 mg chlorophyll *a*/m<sup>2</sup> (Vincent and Laybourn-Parry 2008). Therefore, dense microbial mats may be more ecologically significant than the overlying dilute concentrations of phytoplankton.

The periphyton community of ponds located in the alpine and polar regions are often dominated by cyanobacterial mats (McMaster 2003, Vincent and Castenholz 1993; Quesada et al. 1999), which may be several centimeters thick and completely cover the bottom substrate. Genera that are common to the periphytic community of ephemeral ponds include: *Anabaena*, *Oscillatoria*, *Phormidium*, *Lyngbya*, *Microcoleus*, *Nodularia*, *Nostoc*, *Schizothrix*, *Synechococcus*, *Scytonema*, *Calothrix*, *Tolypothrix*, and *Scytonema* (Vincent and Castenholz 1993) while diatoms and chlorophytes are also present in small proportions (Quesada et al. 1999). Mats are bound by a cohesive mucilaginous layer, often overlying silt and sand particles. Tolerant of extreme environmental conditions (i.e., pH, temperature, salinity), these microbial mats are most abundant where macroinvertebrate grazing is limited (Palmisano et al. 1989). Benthic cyanobacterial mats quickly assimilate SRP and DIN, ultimately affecting the nutrient limitation status of smaller waterbodies (McKnight et al. 2004).



## **Zoobenthos**

Benthic invertebrates of polar and alpine ponds often form the highest trophic level in the aquatic food web. Their diversity typically declines with elevation (Oertli et al. 2008) and latitude (Gibson and Bayly 2007) and is comprised of taxa belonging to Chironimidae, Coleoptera, Oligochaeta, Tricoptera, Heteroptera, Gastropoda, Odonata, and Ephemeroptera (Oertli et al. 2008). Top-down control on macroinvertebrates is usually weak as the majority of ponds are fishless therefore, invertebrate density is dependent on cold tolerance and food supply (Vincent and Laybourn-Parry 2008). Oertli et al. (2008) documented a weak connection of macroinvertebrate assemblage (composition, richness) to environmental parameters. Instead, regional processes were more important such as physical connection to other ponds and tributaries were better predictors of invertebrate communities.

## ***Study Rational***

Dramatic increases in wet deposition of nitrogen (N) over North America and Europe due to anthropogenic emissions are well known (Galloway and Cowling 2002; Fenn et al 2003). Decade-long impacts of increased N deposition on mountain lakes have been inferred paleolimnologically based on fossilized diatom assemblages (Wolfe et al. 2001; Saros et al. 2003). Experimental evidence has also shown the positive effects of nitrogen on alpine waters (Nydick et al 2004; Thompson et al 2008; Murphy et al. 2010). In particular, alpine ponds are more likely N limited compared to lakes based on lower DIN:TP ratios (Strecker et al. 2004; McMaster and Schindler 2005; Murphy et al. 2010). Increasingly, freshwater ecosystems are reported as

being co-limited by N and P (Vinebrooke, unpublished; Elser et al 1990, 2007; Ogbebo et al 2009). Consequently, the distinct effect of either nutrient N or P on primary producers is amplified by addition of the other, resulting in a synergistic interaction that denotes co-limitation.

I designed a replicated whole-pond experiment to determine the effects of N and P on phytoplankton and zooplankton communities using 16 alpine ponds located along the Eastern Front ranges of the Canadian Rocky Mountains. I proposed that the degree of the positive ecological effects of N would depend on P availability (i.e. nutrient co-limitation driven by phosphorus). The rationale for this hypothesis came from findings from earlier in vitro nutrient bioassays, which indicated that many of these ponds were co-limited by N and P (Vinebrooke, University of Alberta, unpublished data). Also, I expected the nutrient additions to stimulate the proliferation of large inedible phytoplankton, suppressing higher trophic levels by impairing energy transfer to herbivores (Beisner et al. 1997).

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## **Chapter 2 Planktonic responses to elevated nitrogen and phosphorus deposition-a replicated natural alpine pond experiment**

### ***Introduction***

Anthropogenic emissions have led to dramatic increases in wet deposition of nitrogen (N) globally (Galloway and Cowling 2002; Galloway et al. 2008). The present global rate of anthropogenic N deposition is over 10 times higher than a century ago (Steffan et al. 2004). Nitrogen deposition in western North America is highest downwind of urban areas or agricultural sources, ranging in concentrations from 30 – 90 kg per hectare per year (Fenn et al. 2003). In western Canada, deposition rates currently range from 5 to 10 kg/ha/yr (Murphy et al. 2010), having more than doubled over the past 20 years (Schindler et al. 2006). The severe, long-term ecological consequences of N deposition on ecosystem functioning have been documented mainly for terrestrial, but not aquatic, environments at a global scale (Vitousek 1997).

Several lines of evidence suggest that thousands of small lake and pond ecosystems of the North American Rockies are especially sensitive to increases in N deposition because this element limits their productivity (Baron et al. 2000; McMaster and Schindler 2005; Murphy et al. 2010). Decade-long impacts of increased N deposition on mountain lakes have been inferred paleolimnologically based on fossilized diatom assemblages (Wolfe et al. 2001; Saros et al. 2003). Nydick et al. (2004) reported that experimental addition of nitrogen to small enclosures (500 L) in an alpine lake altered phytoplankton community

composition and stimulated primary production. However, zooplankton did not respond significantly to the N amendments (Nydick et al. 2004), possibly because of the relatively small spatial and temporal scale of their experiment. Nutrient bioassays showed the positive effects of N on primary producers of alpine ponds and lakes in the Canadian Rockies (Murphy et al. 2010). Similarly, experimental N amendment did stimulate alpine primary producers and herbivorous zooplankton when cultured in warmed growth chambers (Thompson et al. 2008). In comparison, solid empirical evidence of the consequences of elevated N deposition on trophic energy transfer (e.g., grazing and predation rates) and higher trophic levels (e.g., fish) at the whole-ecosystem level is not yet available. Nevertheless, small alpine waterbodies are considered susceptible to increased N deposition because they are typically found in steep watersheds with sparse vegetation that retain only small amounts of deposited nutrients (Baron et al. 2000).

The widespread ecosystem effects of airborne (aeolian) phosphorus (P) deposition have also been recently reported for high-elevation lakes. Common sources of aeolian P include plowing, unpaved roads, biomass burning, fossil fuel combustion and deserts. Psenner (1999) found that Saharan dust inputs were important sources of nutrients for remote mountain lakes of the European Alps and Pyrenees. Similarly, anthropogenic sources of P deposition of the high Sierra Nevada (e.g., Emerald Lake) have resulted in the switch from phosphorus to nitrogen limitation in several remote mountain lakes (Sickman et al. 2003).

There is increasing evidence of freshwater ecosystems being co-limited by N and P (Elser et al. 1990, 2007; Ogbebo et al. 2009). Here, the single effect of either nutrient on primary production is amplified by addition of the other, resulting in a

synergistic interaction that denotes co-limitation. Further, nutrient co-limitation is defined by the combined net impact of both nutrients exceeding the sum of their individual effects (i.e. a non-additive effect), which can be detected statistically as a significant interaction using a two-way analysis of variance (ANOVA; Morris and Lewis 1988). *In vitro* fertilization bioassays also suggest that nutrient co-limitation is common among several small lakes and ponds of the Canadian Rocky Mountains (R.D. Vinebrooke, University of Alberta, unpublished data) and the Californian Sierra Nevada (Sickman 2001). Further, low dissolved inorganic nitrogen: total phosphorus (DIN:TP) mass ratios ranging between 2 - 8, of these and other high-elevation aquatic ecosystems highlight their sensitivity to the combined effect of elevated P and N deposition (Michel et al. 2006; Murphy et al. 2010).

Traditionally, ecological theory has predicted that abiotic or bottom-up forces (e.g., nutrient availability) are more important than biotic interactions in determining ecosystem functioning in extreme environments (Menge and Sutherland 1987). However, unexpectedly high abundances of consumers in extreme arctic environments suggest that herbivory may be an important ecological factor structuring primary producers. A study of arctic ponds across a nutrient gradient of N and P showed that zooplankton grazing suppressed primary producers (Van Geest et al. 2007) negating any observable nutrient effect. In addition, mountain lakes have shown strong top down cascading trophic interactions (Parker and Schindler 2006).

Numerous studies have addressed the impacts of nutrients on primary producers through small-scale experiments (Elser et al 2009, Murphy et al. 2010). Unlike such experiments (e.g., bottle bioassays and mesocosms),

ecosystem-level experiments do not suffer from a lack of ecological realism (Carpenter 1996; Schindler 1998). For example, heavy zooplankton grazing, predation, nutrient recycling, benthic microbial biofilms, realistic scale of space and time, temperature fluctuations, wind, and biogeochemical reactions may all play important roles in alpine pond ecosystems (Wilbur 1995) but short term bottle experiments would not capture these processes.

Therefore, I performed a whole-ecosystem experiment to test for the effects of N and P on phytoplankton and zooplankton communities in 16 alpine ponds located along the Eastern Front ranges of the Canadian Rocky Mountains. The driving hypothesis was that phosphorus amendment would amplify the positive effect of nitrogen on planktonic communities (i.e. a synergistic nutrient interaction). The basis for this hypothesis came from an earlier demonstration of nutrient co-limitation of phytoplankton from chemically inferred nitrogen-limited alpine pond ecosystems being driven by phosphorus (R.D. Vinebrooke, University of Alberta, unpublished data).

## ***Materials and Methods***

### **Study Site**

The ponds are located near treeline (~ 2200 m above sea level) along Snow Pass at the top of the Cascade Valley catchment (Fig. 1), in the northeastern sector of Banff National Park, Canada (51° 36'N 115° 49'W). They ranged from 0.3 – 1.0 m in depth and were less than 2000 m<sup>2</sup> in area during the summer of 2008. Physical and chemical parameters of each of the ponds are shown in Table 2. Local geology consists of orthic eutric brunisol soil covered by sparse vegetation and exposed sedimentary rock such as

limestone, shale, and sandstone (Holland and Coen 1982). The surrounding vegetation includes alpine fir (*Abies lasiocarpa*), arctic willow (*Salix arctica*), bladder locoweed (*Oxytropis podocarpa*), Engelmann spruce (*Picea engelmannii*), lichens, sedges (*Carex* spp.), white heather (*Cassiope tetragona*), yellow heather (*Phyllodoce glanduliflora*) and white mountain avens (*Dryas hookeriana*). The nearest available historical climate records are from the townsite of Banff (1383 m a.s.l.; 51° 11' N, 115° 34' W), located approximately 50 km south of the study site, showing a mean summer (June – September) temperature of 12.1°C, and a daily temperature range of -1.4°C to 26.6°C. Small alpine ponds in the region typically freeze in September/October. Average annual precipitation of Banff was 467 mm with 267 mm as rain and 200 mm as snow (McMaster and Schindler 2005).



## Experimental Design

A two-factor experiment with random blocking design was replicated four times for a total of 16 ponds. Blocking was based on the proximity of ponds to each other and shared landscape features (ridge vs gully setting; Figure 1). Three blocks of ponds were located atop corresponding ridges along the North Ridge, East Ridge and South Ridge of the study site. A Gully block was selected that contained ponds with a lower elevation and exhibited more willow along the bank than other blocks. The North Ridge block (N=4) was not included in the analysis owing to a brief hydroperiod.

Total volume for each pond was first estimated by measuring the maximum width, length, and depth, and then applying the volumetric formula for a best-fitting geometric shape. The required amounts of added nutrients were then calculated for each pond, ensuring volume-corrected equal dosages were applied across replicate ponds. Nutrients were dissolved in a 4-L fertilizer sprayer containing water, and broadcast over each pond on day 0, 15, and 30 of the experiment. Ammonium nitrate was added to achieve a target concentration of 1000  $\mu\text{g N/L}$  in N-amended ponds while phosphoric acid was added to achieve a final concentration of 30  $\mu\text{g/L}$  in P-amended ponds. These amendments were added simultaneously to all NP-amended ponds. No nutrients were added to a set of control ponds.

These nutrient amendments represented reported worst-case scenarios of anthropogenically elevated deposition over high-elevation sites (Fenn et al. 2003). For example, a target concentration of 1000  $\mu\text{g N/L}$  in the N-amended ponds was based on a deposition rate of 20 kg/ha/yr with the assumption of

no runoff and complete retention of N deposited on the surrounding catchment. In comparison, Fenn et al. (2003) reported annual nitrogen deposition rates in the western United States as high as 90 kg/ha/yr downwind of major urban and agricultural regions. Furthermore, numerous studies have shown that nutrient-rich dust high in phosphorus contribute to atmospheric P deposition in remote alpine environments (Ahl 1988, Psenner 1999, Sickman et al 2003).

### **Sample Collection**

All ponds were sampled on day 0, 15, 30, and 45 of the experiment before and following nutrient amendments. Water samples for chemical and phytoplankton analyses were collected using a two-litre Van Dorn water bottle and acid-washed Nalgene sample containers. Samples for chemical analysis were stored in ice and returned to the laboratory within five days. Chemical analyses were performed by the University of Alberta Biogeochemical Analytical Facility (URL link:<http://www.biology.ualberta.ca/facilities/limnology>). Unfiltered water samples were used for determination of total nutrient concentrations while filtrate using a 0.45- $\mu\text{m}$  filter (pre-ashed Whatman GF/F filters) was used for analysis of dissolved fractions. Total nitrogen (TN), total dissolved nitrogen (TDN), ammonium ( $\text{NH}_4$ ), and nitrate/nitrite ( $\text{NO}_3 + \text{NO}_2$ ) were measured colorimetrically using a Lachat Quikchem 8500 FIA automated ion analyzer with detection limits of 7, 7, 2 and 1  $\mu\text{g/L}$ , respectively. Total phosphorus (TP), total dissolved phosphorus (TDP) were quantified by automated Ascorbic Acid Reduction Method using a Lachat QuickChem 8500 multi-channel flow analyzer with a detection limit of 1  $\mu\text{g/L}$ . Dissolved organic carbon (DOC) was measured

using a Shimadzu 5000A TOC Analyzer with a detection limit of 0.1 mg/L.

Phytoplankton were concentrated on a Whatman GF/F filter by filtering one litre of pond water. Filters were wrapped in foil, placed in petri dishes, and stored on ice. Algal community structure was quantified using high performance liquid chromatographic analysis of taxonomically diagnostic chlorophylls and carotenoids (Vinebrooke et al. 2002). Total phytoplankton biomass was inferred based on concentrations of chlorophyll *a* (all algae), while community composition was estimated using chlorophyll *b* (chlorophytes), fucoxanthin (chrysophytes, diatoms, some dinoflagellates), lutein (chlorophytes and euglenoids), and zeaxanthin (cyanobacteria). Qualitative analysis of the preserved phytoplankton samples using light microscopy was conducted to determine taxonomic composition.

Zooplankton were collected using a 64-micron mesh-sized plankton net, which was hauled at a rate of 1 m/s horizontally across each pond basin. Samples were immediately preserved in 70% ethanol. A minimum of 300 individuals per sample were measured and identified to species using a stereomicroscope. Standard length-weight regression equations were applied to calculate invertebrate biomass (McCauley 1971). During the first sample period, juvenile fairy shrimp (*Branchinecta paludosa*) were effectively captured with the plankton net. However, capture efficiency appeared to decrease during the experiment as adult fairy shrimp were observed effectively evading net hauls.

## Statistical Analysis

Fertilization effects on total chlorophyll-inferred algal abundance and total zooplankton biomass were tested statistically using a one factor Repeated Measure Analysis of Variance (RM-ANOVA) while Repeated Measure Multivariate Analysis of Variance (RM-MANOVA) of phytoplankton pigments (fucoxanthin, chlorophyll *b*, and lutein/zeaxanthin) was used to test for treatment effects on community composition. East Ridge Block P Treatment (ERP), South Ridge Block Nitrogen Treatment (SRN) and all ponds in the North Ridge Block with the exception of the NP treatment were dry by day 30 of the experiment (July). Gully Block phosphorus was not included in the analysis as it had abnormally high concentrations of total chlorophyll at the beginning of the experiment. Therefore a total of six ponds were not included in the analysis, resulting in an uneven loss of replicates across treatment combinations. Consequently, it was not possible to conduct a two-factor ANOVA of the zooplankton/phytoplankton data for the entire experiment

Canonical Correspondence Analysis (CCA) was performed using CANOCO version 4.5 (ter Braak 1998) to determine the environmental variables that best explained changes in zooplankton community composition and phytoplankton presence/absence data. CCA is a multivariate technique that tests differences in community structure between samples and compares the maximum amount of variation in the taxonomic data set to environmental variables. Each environmental variable (log-transformed) is represented by a CCA axis and its eigenvalue ( $\lambda$ ), which is a measure of the explained species variance. The length and orientation of each environmental vector symbolizes

its importance and direction of maximum change in ordination space. The proximity and alignment of a species with a particular vector shows how closely the variation in its abundance is related to that environmental variable. Similarly, site score differences in ordination space suggest taxonomic dissimilarity of invertebrate assemblages between different ponds, or across time. Time, DOC, TP, TN PN, PP, and chlorophyll *a* were included in the model.

## ***Results***

### **Water chemistry**

Chemical conditions in all of the experimental ponds were similar prior to the start of the experiment (Table 2). Most importantly, TN, TDN, TP, TDP and DOC concentrations did not differ among the ponds on day 0. All ponds initially contained 14 – 33  $\mu\text{g TP/L}$ , making them naturally mesotrophic. Further, ponds were noticeably stained in appearance, containing high concentrations of brownish DOC.

Immediately following fertilization, TP and TN concentrations exceeded target levels of 1000  $\mu\text{g N/L}$  and 30  $\mu\text{g P/L}$  (Figs. 2a, b) on day 0 through day 30 of the experiment. Nutrient target concentrations were exceeded during a few occasions probably due to over estimates of pond volumes. Upon returning to the ponds following a two-week period, TN and TP in the water column significantly declined in concentration (Figs. 3a, b). The majority of the added nutrients did not persist in the water column in a dissolved or particulate form two weeks following fertilization.

Low concentrations of particulate fractions of TN and TP did not differ significantly among the nutrient-amended ponds (Figs. 4a, b). TN consisted mainly of TDN relative to PN, which accounted for only ~10% of the total nitrogen pool. Similarly, TP was comprised primarily of TDP while PP represented only ~30% of the total phosphorus pool.

## Phytoplankton

N and P did not significantly (fertilization effect,  $F_{3,40} = 0.17$ ,  $P = 0.91$ ) increase phytoplankton chlorophyll *a* concentrations despite three additions of nutrients over a 45-day period (Fig. 5). Instead, chlorophyll *a* concentrations remained low ( $< 2 \mu\text{g/L}$ ), showing high variance among the ponds during the experiment. Chlorophyll *a* did not vary significantly over time (time effect,  $F_{3,40} = 8.70$ ,  $P = 0.10$ ), but steadily increased from day 0 to 30 before declining thereafter by day 45.

Fertilization also did not significantly ( $F_{9,40} = 0.20$ ,  $P = 0.49$ ) alter carotenoid-inferred phytoplankton community composition (Fig. 6). However, RM – MANOVA using three pigments common to all ponds (fucoxanthin, lutein/zeaxanthin, chlorophyll *b*) showed that community composition changed significantly over time (RM MANOVA;  $F_{9,40} = 2.261$ ,  $P = 0.038$ ). Specifically, three carotenoid concentrations increased during the first half of the experiment, before declining by day 45 (Fucoxanthin,  $F_{3,40} = 3.09$ ,  $P = 0.053$ ; Chlorophyll *b*,  $F_{3,40} = 5.17$ ,  $P = 0.009$ ; Lutein/zeaxanthin,  $F_{3,40} = 5.52$ ,  $P = 0.007$ ).

CCA using automatic backward selection showed that fertilization variables of (TN; $F = 0.97$ ,  $P = 0.44$ , TP; $F = 0.78$ ,  $P = 0.71$ ), and DOC ( $F = 0.84$ ,  $P = 0.6$ ) were not significant predictors of phytoplankton community composition after 45 days (Fig. 7). The first axis was best explained by TN accounting for only 13% of the variance in the taxonomic presence/absence data. The second axis was best explained by TP accounting for only 8% of the variance in the taxonomic data.

## Zooplankton

Fertilization also did not significantly affect total zooplankton biomass during the experiment (Fig. 8; time-fertilization effect,  $F_{3,40} = 0.36$ ,  $P = 0.78$ ). Total zooplankton biomass did not change significantly over time (time effect;  $F_{3,40} = 0.45$ ,  $P = 0.55$ ). However, two phosphorus-amended ponds did contain substantially greater, but also highly variable zooplankton biomass by the end of the experiment, consisting mainly of fairy shrimp.

CCA using automatic backward selection showed that time ( $F = 10.49$ ,  $P = 0.002$ ), TP ( $F = 3.70$ ,  $P = 0.008$ ) and TN ( $F = 3.96$ ,  $P = 0.03$ ) were significant predictors of zooplankton community composition across the experimental ponds (Fig. 9; App 1). The first axis was best explained by time accounting for 21% of the variance in the taxonomic abundance data. Ponds contained juvenile zooplankton (nauplii and copepodites, small fairy shrimp) early in the summer and contained more adults later on in the season. The second axis captured 12% of the variance in the taxonomic data, being best represented by opposing gradients of

N and P. Here, diverse assemblages of cladocerans and copepods were associated with higher concentrations of TN while adult fairy shrimp were more closely related to TP. Adult fairy shrimp were detected in all ponds at one point in time during the experiment but fertilization did affect fairy shrimp growth rate.

## ***Discussion***

My experimental findings did not support the co-limitation hypothesis, namely that phosphorus additions would amplify the positive effects of nitrogen on these chemically inferred nitrogen-limited alpine pond ecosystems. Despite low DIN:TP ratios across all ponds, neither nitrogen nor phosphorus amendments significantly stimulated phytoplankton or zooplankton abundance. Although aggregate community properties did not respond significantly to fertilization, elevated N and P levels were significant explanatory variables of species-level shifts in the zooplankton communities. Several potential ecological and methodological explanations could account for this surprising lack of statistically detectable effects of N and P.

One possible ecological explanation for the lack of a phytoplankton response to nutrient fertilization involves suppression by intensive grazing pressure (Schindler and Comita 1972; Elser et al. 2000). Two observations lend support to this explanation: very low chlorophyll concentrations relative to dissolved nutrient concentrations, and observed high densities of filter-feeding fairy shrimp. For instance, Van Geest et al. (2007) found strong top-down regulation of phytoplankton by cladocerans precluded any significant relationships between chlorophyll and measured nutrients in a series of arctic



ponds. Parker and Schindler (2006) also showed pronounced grazing pressure and a strong grazer-algal interaction in an alpine lake located near my study area.

Fairy shrimp were very abundant in the study ponds and may even be regarded as a keystone species. In an Antarctic study, more than 40000 juvenile *Branchinecta gaini* were reported in a cubic meter, feeding on 8  $\mu\text{g}$  of chlorophyll *a* per hour per individual (Hawes 1985). However, limited research has been conducted on their feeding habits and life history. Hawes (1985) suggested that immature *Branchinecta gaini* feed in the water column but adopt a benthic habitat when mature. Although many fast-swimming fairy shrimp likely evaded horizontal net hauls with a plankton net during the sampling of my experimental ponds, observation of high densities of this species on surface sediments attest to its functional importance in these systems. Also, ponds drying down may have concentrated fairy shrimp and over-inflated biomass estimates.

Competition with periphyton may have also contributed to suppression of a phytoplankton response to added nutrients. Importantly, periphyton can be an order of magnitude more abundant than phytoplankton in shallow (~1-m depth) habitats, providing them with a competitive advantage over phytoplankton for resources (Hansson 1988; Vinebrooke & Leavitt 1999; Vadeboncoeur et al. 2003). A mesocosm experiment by Confer (1972) showed periphyton immediately sequestered large amounts of phosphorus and decreased soluble phosphorus as well as phytoplankton standing stock. Hansson (1990) also found that mesocosms without periphyton contained 32-44% more phosphorus in the water column compared to controls including

periphyton. Furthermore, a bioassay of shallow oligotrophic mountain lakes in the Colorado Front Range showed that N uptake by the benthic processes may be more important than in the pelagic (Nydick et al. 2004). Periphyton responded significantly to the addition of N in the ponds of this study (L Porter, University of Alberta, unpublished data).

Bacteria may also have taken up the added nutrients before an algal response was possible. Bacteria may represent a substantial fraction of the TPP given high concentrations of DOC likely fueling heterotrophy in these ponds (Siuda and Chrost 2002). Heterotrophic bacteria may be responsible for low chlorophyll concentrations relative to ambient nutrient levels in the study ponds. Similarly, laboratory and field based studies have shown that bacterial production in humic lakes is more important than algal primary production (Tranvik and Hofle 1987, Arvola and Kankaala 1989, Tranvik 1989, Jones 1990). However, significant bacterial biomass responses to the added nutrients would have been reflected in elevated particulate N and P fractions, which was not the case in my experiment.

Another possible explanation involves high concentrations of DOC in these ponds resulting in organic binding of amended nutrients, thereby reducing their bioavailability. DOC may inhibit microbial growth by binding with inorganic nutrients such as iron (Jackson and Hecky 1980; Guildford et al 1987) and phosphorus (Stewart and Wetzel 1981) as well as deactivating phosphatases (Wetzel 1992; Boavida and Wetzel 1998). Experimentally added orthophosphate may bind with DOC but may be less of a consequence than nitrogen-binding because of the likely presence of phosphatases (Jones et al. 1988). Therefore, chemical complexation of the added inorganic

nutrients may have precluded algal uptake, thereby dampening the expected positive effect of fertilization as measured by chlorophyll *a*-inferred phytoplankton biomass.

The observed general lack of detectable statistically significant responses of planktonic communities to nutrient amendments may also have been a consequence of poor study power owing to insufficient replication. Power analysis suggests more than sixty ponds would have been required to detect a significant difference between treatment groups based on an effect size of 0.26. There was a high degree of variation between study ponds with similar treatment. For instance, in the control treatment the coefficient of variation was 0.65 on day 30 of the experiment. Pond permanence is another important factor in determining zooplankton community structure (Wellborn et al. 1996; Brooks 2000; Rundle et al. 2002; Urban, 2004; Jocque et al., 2007). Ponds that dry out quickly cannot support long living species that require time to reproduce before desiccation.

Although the spatial scale of the experiment captured whole-ecosystem responses to nutrient amendments, its temporal scale was limited to a part of single growing season. I believe that if the experiment had run a month or two longer treatment effects may have been more evident. Large filamentous blooms of green algae were observed in two of the NP treatments in support of our hypothesis and another had turned dark brown. Therefore, had the experiment lasted a month longer we may have observed more pronounced effects between treatment groups, most notably in the NP treatment. The moment of ice out may have been unique to each pond studied corresponding to a phytoplankton bloom that we may have missed. Enrichment of ponds

during ice out may be critical and the seasonality influences of ponds may make them incomparable.

Though I have little empirical evidence, field observations suggest that unexpected swarms of fairy shrimp were in all likelihood the primary reason why added nutrients did not stimulate either phytoplankton or zooplankton abundance in these alpine ponds. Further research including the grazing effects on periphyton and phytoplankton communities may provide a greater understanding of the nutrient status of alpine ponds. Overall despite the high degree of variance found within treatment groups and the lack of replication, whole ecosystem projects are an invaluable component to understanding ecosystem processes and community interactions (Schindler 1998).

Biogeochemical interactions within sediment, presence of periphyton and multiple trophic levels, and longer experimental time periods are all factors left unconsidered in previous bottle experiments (Murphy et al. 2010).

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Table 1 Average Total Phosphorus (TP) and Total Nitrogen (TN) concentrations and standard error (SE) of alpine and arctic ponds reported by various studies. Hyphenated sections represent data unavailable.

	TP ( $\mu\text{g/L}$ )	SE	TN ( $\mu\text{g/L}$ )	SE	n
Lim et al. 2005	21.8	6.3	631.3	80.6	29
McMaster and Schindler 2005	51.3	11.8	-	-	28
Oertli et al. 2008	-	-	240	22.5	16
Murphy et al. 2010	19.8	4.0	382.2	61.4	23
Zettel and Vinebrooke, unpubl.	23.5	1.8	350.0	28.7	12

Table 2 Physical and chemical parameters of studied alpine ponds in the Cascade Valley, Banff National Park, Canada.

Block	Treatment	Latitude (dd mm ss)	Longitude (dd mm ss)	Area (m <sup>2</sup> )	Depth (m)	TN ( $\mu\text{g L}^{-1}$ )	TDN ( $\mu\text{g L}^{-1}$ )	PN ( $\mu\text{g L}^{-1}$ )	TP ( $\mu\text{g L}^{-1}$ )	TDP ( $\mu\text{g L}^{-1}$ )	PP ( $\mu\text{g L}^{-1}$ )	DOC (mg L <sup>-1</sup> )	Chl a ( $\mu\text{g L}^{-1}$ )
1	C	51° 36' 16 N	115° 49' 04 W	390	0.8	226	209	17	14	7	7	5.8	1.02
1	N	51° 36' 12 N	115° 49' 04 W	240	0.3	427	389	38	18	10	8	9.6	0.05
1	P	51° 36' 03 N	115° 49' 10 W	180	0.4	406	356	50	30	18	12	8.7	0.87
1	NP	51° 36' 15 N	115° 49' 00 W	120	0.3	353	349	4	27	12	15	6.9	0.13
2	C	51° 36' 01 N	115° 49' 11 W	176	0.6	299	266	33	14	9	4	8.6	0.52
2	N	51° 36' 45 N	115° 49' 46 W	308	0.3	385	316	31	22	21	1	8.9	0.19
2	P	51° 36' 44 N	115° 49' 48 W	1000	0.5	310	264	46	25	17	8	6.6	0.72
2	NP	51° 36' 02 N	115° 49' 09 W	286	0.4	549	525	24	30	21	9	11.5	0.57
3	C	51° 36' 23 N	115° 49' 24 W	862	1.0	487	472	15	*	*	0	8.2	1.07
3	N	51° 36' 45 N	115° 49' 36 W	690	0.6	290	266	24	28	20	8	7.2	0.30
3	P	51° 36' 17 N	115° 49' 08 W	324	0.3	673	638	217	129	16	9	10.2	8.85
3	NP	51° 36' 46 N	115° 49' 46 W	495	0.6	236	216	20	33	21	12	5.4	1.15
4	C	51° 36' 50 N	115° 49' 51 W	450	0.3	364	318	20	40	30	10	9.9	0.29
4	N	51° 36' 51 N	115° 49' 51 W	90	0.3	545	503	32	35	28	12	13.8	0.37
4	P	51° 36' 43 N	115° 49' 47 W	1000	0.5	325	284	32	28	20	27	8.7	0.82
4	NP	51° 36' 51 N	115° 49' 47 W	1512	0.5	233	203	30	19	12	7	6.2	0.44

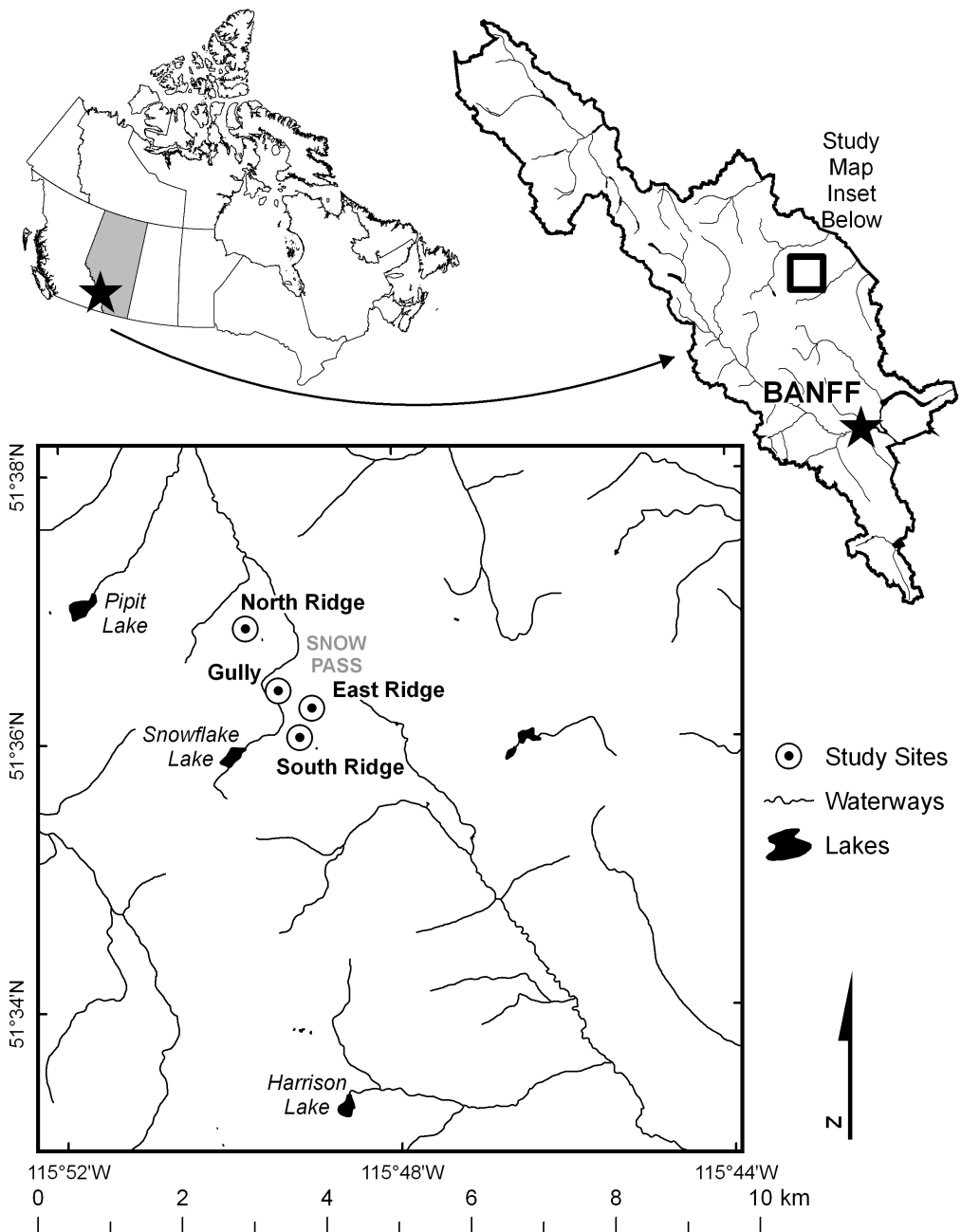


Figure 1 Study site location of surveyed alpine waterbodies in Banff National Park, Canada

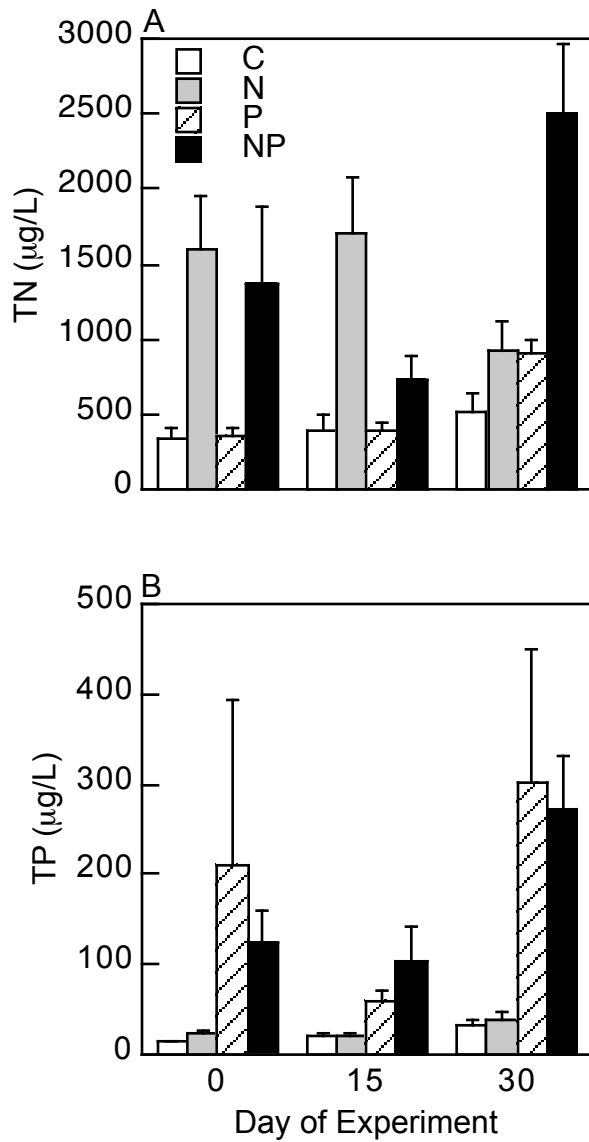


Figure 2 (A) Total Nitrogen (TN) and (B) Total Phosphorus (TP) concentrations in the water column. C (Control), N (Nitrogen), P (Phosphorus), and NP (Nitrogen +Phosphorus) treatment concentrations were measured immediately following nutrient amendment. Error bars represent one standard-error unit based on n = 3.

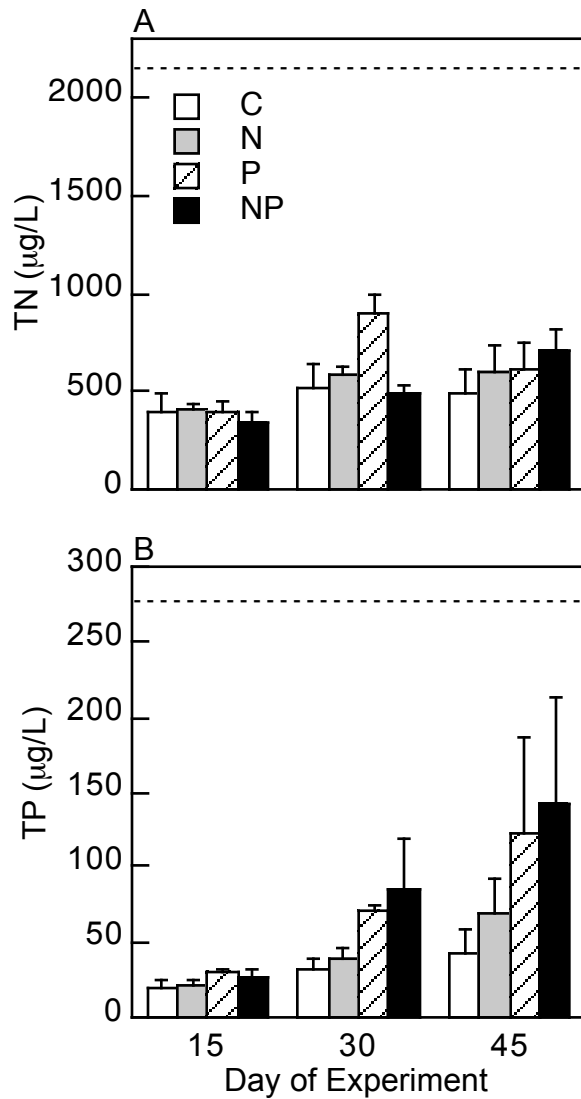


Figure 3 (A) Total nitrogen (TN) and (B) total phosphorus (TP) concentrations in the water column 15 days following nutrient amendments: N, P, and NP treatments. Dashed line depicts the average concentration of each nutrient immediately following amendment (see Fig. 1). Error bars represent one standard-error unit based on  $n = 3$ .

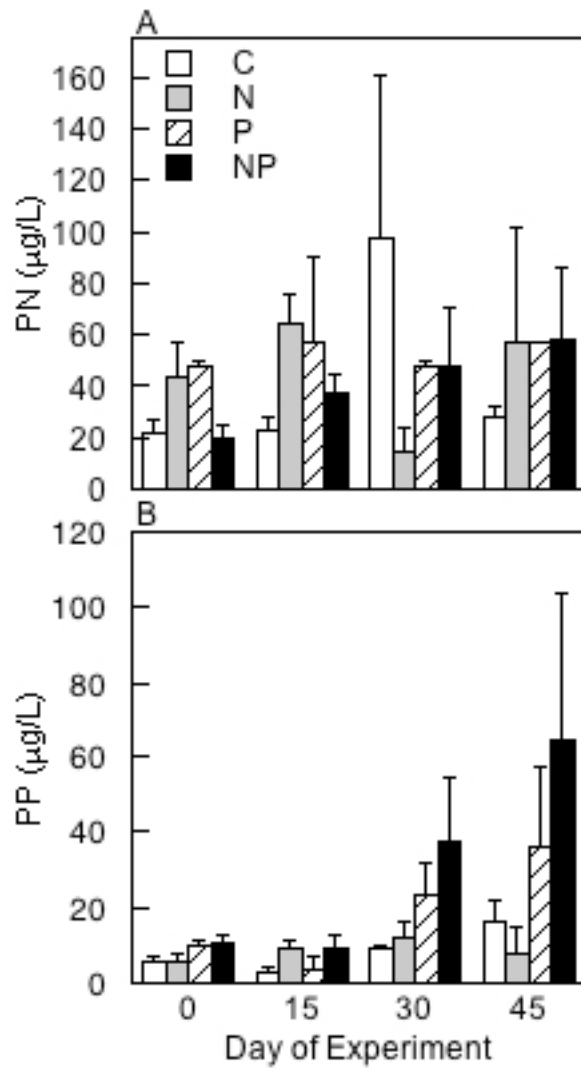


Figure 4(A) Particulate nitrogen (PN) and (B) particulate phosphorus (PP) prior to each nutrient amendment during the 45-d experiment: C (Control), N (Nitrogen), P (Phosphorus), and NP (Nitrogen + Phosphorus). Error bars represent one standard-error unit based on n = 3.



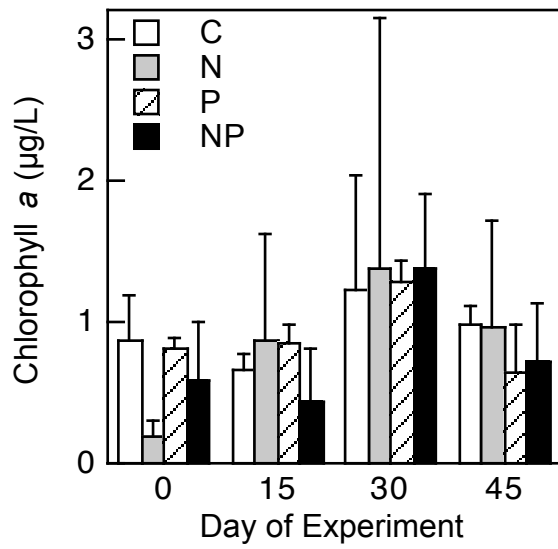


Figure 5 Phytoplankton chlorophyll a concentration prior to each nutrient amendment during the 45-d experiment: C (Control), N (Nitrogen), P (Phosphorus), and NP (Nitrogen +Phosphorus). Error bars represent one standard-error unit based on n = 3.

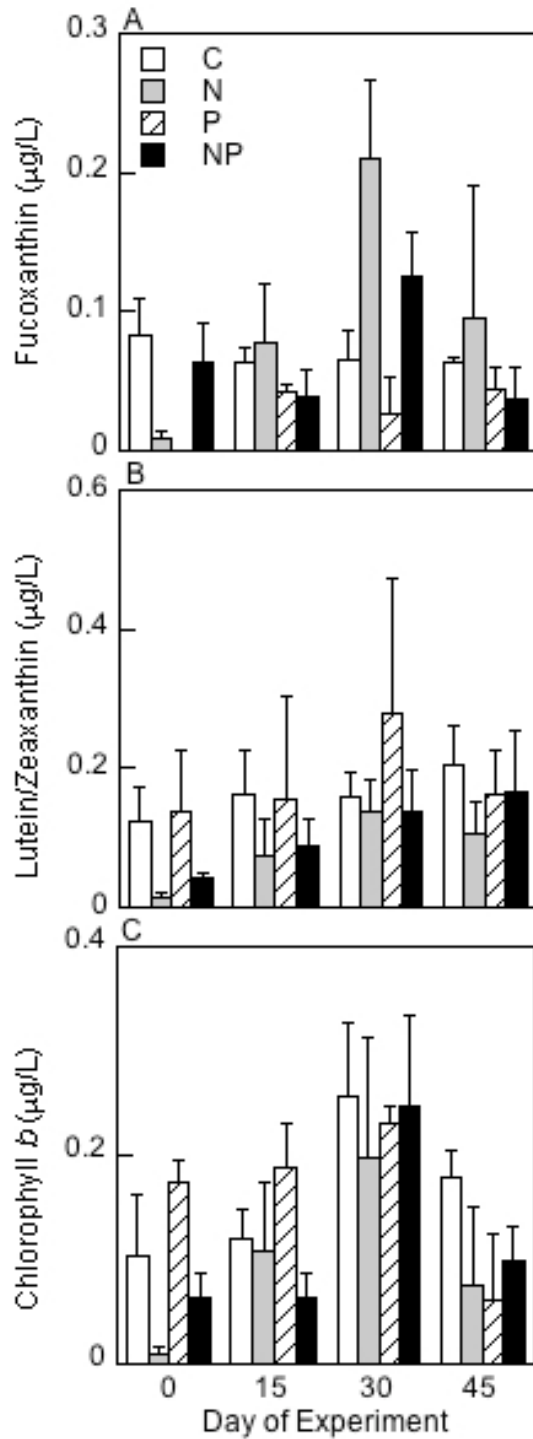


Figure 6 Pigment-inferred abundance of (A) Chromophytes (Fucoxanthin) (B) Chlorophytes/Cyanobacteria (Lutein/Zeaxanthin) (C) Chlorophytes and Euglenophytes (Chlorophyll *b*) prior to each nutrient amendment during the 45-d experiment: C (Control), N (Nitrogen), P (Phosphorus), and NP (Nitrogen +Phosphorus). Error bars represent one standard-error unit based on n = 3.

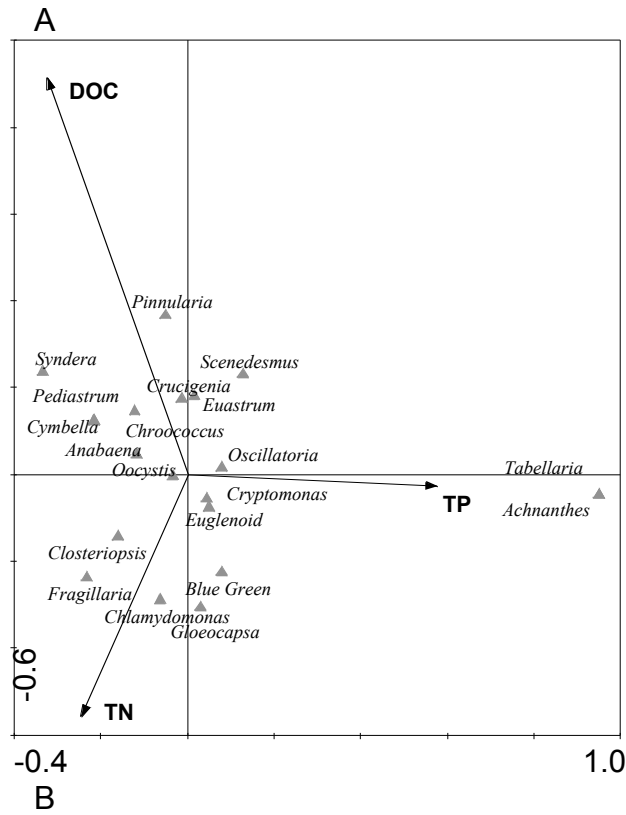


Figure 7 (A) Ordination biplot of phytoplankton taxa and explanatory environmental variables during the 45-d experiment based on canonical correspondence analysis. Total phosphorus (TP), total nitrogen (TN) and dissolved organic carbon (DOC) are shown as passive, statistically non-significant predictors of taxonomic composition.

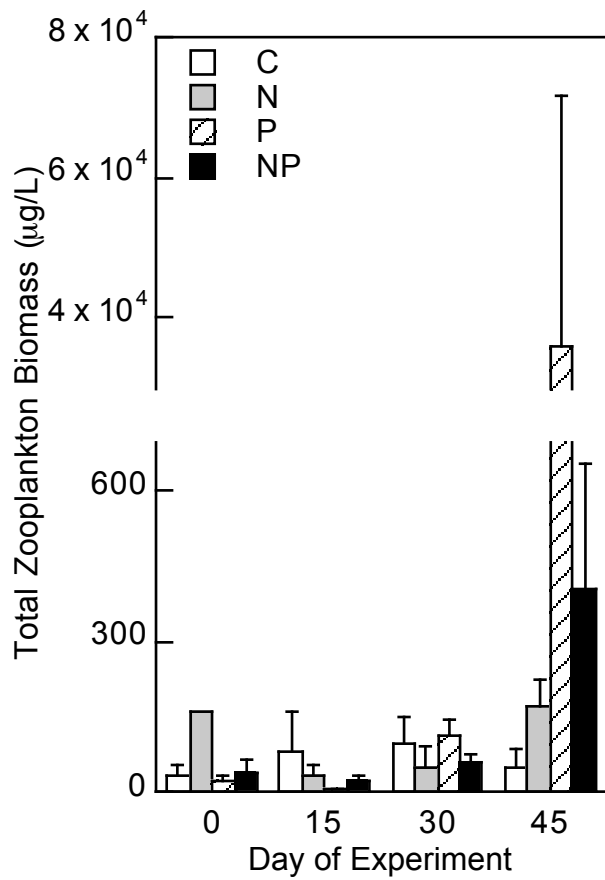
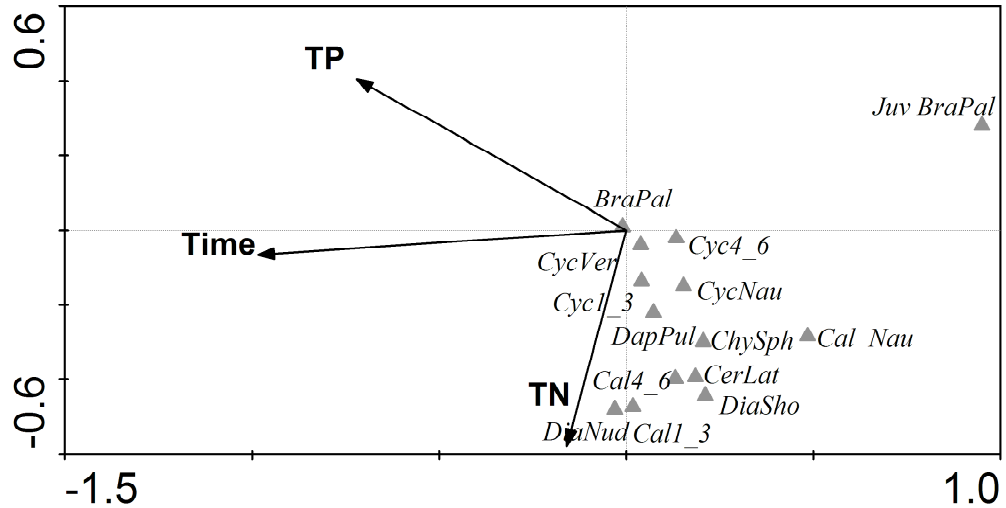


Figure 8 Total zooplankton biomass prior to each nutrient amendment during the 45-d experiment: (C) Control, N (Nitrogen), P (Phosphorus), NP (Nitrogen+Phosphorus). Error bars represent one standard-error unit based on  $n = 3$ .



#### Zooplankton Species Codes

Juvenile <i>Branchinecta paludosa</i>	JuvBraPal
Adult <i>Branchinecta paludosa</i>	BraPal
Cyclopoid Nauplii	CycNau
Cyclopoid instar (1-3)	Cyc1_3
Cyclopoid instar (4-6)	Cyc4_6
Cyclops vernalis	CycVer
Calanoid nauplii	CalNau
Calanoid instar (1-3)	Cal1_3
Calanoid instar (4-6)	Cal4_6
<i>Diaptomus nudus</i>	DiaNud
<i>Diaptomus shoshone</i>	DiaSho
<i>Chydorus sphaericus</i>	ChySph
<i>Daphnia pulex</i>	DapPul
<i>Ceriodaphnia laticaudata</i>	CerLat

Figure 9 Ordination biplot of zooplankton taxa with species codes and explanatory environmental variables during the 45-d experiment. Total phosphorus (TP), total nitrogen (TN) and time are shown as statistically significant predictors of community composition.

## Chapter 3 Conclusions

### *Synthesis*

Higher trophic levels in alpine pond ecosystems such as zooplankton and fairy shrimp may be ecologically important and control the abundance and species composition of primary producers. These findings contradict experimental evidence and surveys that suggest shallow-water communities along the littoral area of alpine lakes are principally regulated by resources and not density-dependent species interactions (Vinebrooke and Leavitt 1998; Vinebrooke and Leavitt 1999). Numerous ( $> 100$  individuals/  $m^2$ ) *Branchinecta paludosa* were observed in the ponds and intensive grazing pressure suppressed the response of primary producers to nutrient additions. Nitrogen and phosphorus amendments did not increase phytoplankton or zooplankton abundance suggesting strong biotic interactions (Dodson 1974). The importance of grazing overshadowing nutrient effects has also been reported in similar pond ecosystems (Van Geest et al. 2007).

Benthic processes that were not reported on in this study likely play very important functional roles (e.g., nutrient cycling, trophic energy transfer) in shallow-water ecosystems. Microbial mats of benthic algae may quickly outcompete phytoplankton and assimilate available DIN in the water column (Axler and Reuter 1996; Mcknight et al. 2004; Nydick et al. 2004). In combination with extensive grazing by zooplankton and fairy shrimp low concentrations of phytoplankton are therefore at a competitive disadvantage for nutrients in these systems.

## ***Future Research Directions***

Environmental monitoring in the remote regions of the mountain parks of Banff, Jasper and Yoho as well as the surrounding area including Kananaskis Country should be improved to better demonstrate changes in nitrogen and phosphorus deposition. High-elevation meteorological field stations are sparse throughout the North American Rockies, especially in Canada (Bradley et al. 2004). Therefore, automated or regular manual monitoring of oxidized forms of N ( $\text{NO}_x$ ) and reduced forms of N ( $\text{NH}_3$  and  $\text{NH}_4$ ) in rainfall, snow and surface waters would be important to detect expected increases in nitrogen deposition in any of these regions (Fenn et al. 2003).

Limited research has been conducted on the physical, chemical, and biological patterns of alpine ponds in North America (McMaster and Schindler 2005). Additional research should include monthly surveys over a number of years throughout the ice free season to document physical, chemical and biological patterns to gain a greater understanding of these ecologically important and abundant shallow systems. A strong sampling emphasis on the periphyton and higher trophic levels could aid in our understanding of trophic dynamics and species interactions.

Mesocosm and split-basin experiments may be a more effective way to empirically demonstrate nutrient limitation in alpine ponds given the observed high degree of heterogeneity or variance that exists among these ecosystems. In other words, one should not assume that the similar physical appearance of these systems reflects habitat homogeneity among ponds.

Instead, a split-basin experiment would enable the investigator to control for site-specific differences in abiotic and biotic factors, which may otherwise regulate the effects of nutrient amendments. Further, factorial experiments could be conducted in each pond to examine the hypothesized mediating influences of fairy shrimp and periphyton on planktonic communities. For example, nutrients could be added directly to surface waters contained in mesocosms where either ambient densities of fairy shrimp are excluded or enhanced relative to controls. Similarly, open- versus closed-bottom mesocosms could be used to examine the interactive effect of benthos and nutrient fertilization on planktonic communities.

Grazing experiments examining the effects of different zooplankton taxonomic groups on primary producers may account for some of the unexplained variation in this study. Ponds were dominated by unequal abundances of a variety of taxonomic groups including copepods, cladocerans, fairy shrimp, as well as higher trophic levels such as *Chaoborus*. The dissimilarity in filtering rates, size selection strategies and natural history of these grazers may ultimately affect primary producer abundance in the benthic and planktonic regimes. Analysis incorporating both planktonic and benthic communities may also aid in a greater understanding of these unique systems.

The ecology of fairy shrimp should be directly examined in these systems as this ubiquitous species appears to play a keystone role in alpine and polar pond ecosystems. The sampling effort of fairy shrimp would have to be improved to generate more accurate and precise estimates of this species' abundance in these ponds. Sampling with a net haul at night may improve



capture efficiency because fairy shrimp may be less likely to see the moving net. Fairy shrimp were quickly able to evade an approaching net and were often observed burrowing under the fine pond sediment. Light traps could be set at night to capture fairy shrimp. Similar to fish stock assessment fairy shrimp abundance could be compared based on a catch per unit effort basis that may be more effective than net hauls.

Alpine ponds are extreme environments that show strong daily temperature fluctuations, lack thermal stratification, and exhibit rapid changes in the physical and chemical parameters over a short ice free season (McMaster 2003). Climate change will likely amplify the effects of these extreme conditions on the biological communities of ponds in polar and alpine regions through increased frequency of drought, pond desiccation and stronger temperature fluctuations (Douglas and Smol 1999). In addition to these stressors alpine pond communities may be particularly sensitive to increased warming, species invasions, nitrogen/phosphorus deposition, persistent organic pollutants and other atmospheric inputs.

Further research is required to understand the potential effects of increased atmospheric deposition on shallow waterbodies in the Canadian Rocky Mountains. Anthropogenic emissions estimates of N in Canada are over 1.4 million tonnes annually and are expected to increase further (Chambers et al. 2001). My limited research does not suggest that widespread eutrophication is likely under current deposition estimates but a multi year study with increased replication should be conducted to compare my findings. Studies have demonstrated that climate change and atmospheric nitrogen deposition alter alpine (Saros et al. 2003) and arctic (Wolfe et al. 2006) phytoplankton communities as

well as higher trophic levels (Thompson et al. 2008). These studies highlight the importance of understanding the effects of multiple stressors on alpine communities. Moreover, the combination of multiple stressors such as atmospheric deposition of pollutants, UV light, and climate change may be more realistic in a dynamic world, ultimately impacting aquatic biodiversity and ecosystem function in these extreme environments.

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