ASSESSING THE ROLE OF DECLINING CALCIUM IN BIOLOGICAL RECOVERY ON ZOOPLANKTON IN HISTORICALLY ACIDIFIED LAKES

by

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Abstract

Throughout much of the 20th century, industrial emissions caused the widespread acidification of thousands of lakes in North America and Europe. Acidification has led to unprecedented damage on zooplankton communities, with reductions in species richness, and in extreme cases, local extirpations. Although lake pH is recovering due to emission control policies, many acid-sensitive species (e.g. large *Daphnia*) have failed to re-establish in lakes, despite their occasional detection in plankton records. Coincidently, calcium, a critical component of cladoceran exoskeletons, has declined precipitously in many historically acidified lakes over the last few decades, and may be approaching ecologically concerning concentrations. Studies suggest that crustaceous zooplankton are likely vulnerable to calcium decline because of their fully aquatic lifecycle, calcified exoskeleton, and inherent reliance on available aqueous calcium. However, disentangling biological impacts of low calcium in acidic, and chemically recovered (pH>6.0) lakes is difficult because of a strong correlation between pH and calcium concentration.

To investigate the impact of low calcium concentration on zooplankton recovery in acid-damaged lakes, lake mesocosms were stocked with zooplankton from the regional species pool and four treatments were applied in a factorial design: pH (5.7 and 6.3) and calcium (0.9mg L^{-1} and 2.3mg L^{-1}). A comparison between the effects of calcium and pH indicates that impacts from declining calcium are likely to be similar to those of acidification. Total community metrics (i.e. abundance, richness, evenness, size-structure) remained similar across treatments; however, low calcium and low pH each resulted in lower abundance of *Daphnia pulex/pulicaria* – a large acid-sensitive, and heavily calcified cladoceran. In contrast, the abundance of small cladocerans, including smaller, acid-tolerant daphniids (*Daphnia catawba, Daphnia ambigua*), was higher in low calcium treatments. When both stressors were combined, changes largely reflected the effect of the most severe independent stressor. By prohibiting recovery of acid-sensitive species, and promoting establishment of zooplankton already common to acid-structured

communities, our results suggest that declining calcium is likely a barrier to biological recovery in historically acidified lakes.

Co-Authorship

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List of Abbreviations

Al – Total Aluminum

ANOVA – Analysis of variance

Ca – Calcium (Ca^{2+})

DOC – Dissolved organic carbon

DW – Dry weight

E_{var} = Species evenness

GLM – Generalized linear model

GLMM – Generalized linear mixed-effect model

Killarney PP – Killarney Provincial Park

LMM – Linear mixed-effect model

MAM – Minimum adequate model

NO_x – Nitrogen oxides

PCA – Principle components analysis

SO_2 – Sulphur dioxide

SO_4 - Sulfate

sd – Standard deviation

TP – Total Phosphorus

Chapter 1

General Introduction

Thousands of lakes in North America and Europe experienced significant damage from acid rain, and while general improvements to lake pH have been witnessed (Stoddard et al. 1999), biological recovery of acid sensitive species has been limited (Valois et al. 2010, Gray et al. 2012). Complicating our understanding of biological recovery is the presence of other regional and local stressors. Acidified lakes are now experiencing other novel impacts including, but not limited to; global climate change influencing seasonal zooplankton species dynamics (Rusak et al. 2008); introductions of invasive species causing reductions in abundance, richness, and diversity of cladocerans (Strecker et al. 2006); alterations of predator communities influencing zooplankton community composition (Yan et al. 2001); drought-induced and persistent acidification (Arnott et al. 2001, Gray et al. 2012), and calcium decline (Keller et al. 2001). Historically, impacts of environmental stressors have been looked at in isolation to understand the direct effect of a given stressor. However, it is becoming increasingly clear that various stressors are acting in concert with one another, producing complex, and sometimes unexpected effects (Schindler et al. 1996, Yan et al. 2008, Palmer and Yan 2013). Keller (2009) identified that any combination of persistently acidic conditions, decreased UV-B attenuation, climate change, chronic heavy metal toxicity, and declining aqueous calcium concentrations are likely influencing biological recovery from acidification. Thus, to understand the overall effect a stressor has on an ecosystem, it is necessary to not only understand its independent effect, but also possible interactive effects with other co-occurring stressors (Folt et al. 1999, Vinebrooke et al. 2004).

Investigating potential stressor interactions will inform future research direction and improve our understanding of recovery pathways in historically acidified communities.

Long-range and regional point-source acidification events occurred across North America (Adirondacks, Quebec, Nova Scotia), and many northern European countries (Stoddard et al. 1999, Jeffries et al. 2003, Menz and Seip 2004, Skjelkvale et al. 2005). However, for the purpose of this review I will focus solely on acidification and its subsequent legacy effects for the Sudbury, Canada region.

Sudbury – A history of anthropogenic influence

Extensive damage to the Sudbury, Ontario, Canada landscape has been a result of industrial activities occurring in the region for over the last century and a half. A timber industry established in the Sudbury region near the mid-19th century caused widespread deforestation, and destruction to landscapes (Gunn 1996). Subsequently, in the mid- to late-19th century large concentrations of nickel, copper and iron ores were discovered within an area known as the Sudbury Structure (Boerner et al. 2000). Mining activity began in 1883, and has continued ever since (Gunn 1996). Extraction techniques between 1888 and 1929 involved the smelting of sulphur-rich nickel ore in open roast beds. This crude smelting technique released fumes of toxic sulfur dioxide (SO_2), and elevated concentrations of particulate metal in the immediate environment (Gunn 1996). Techniques changed over time with smelting occurring at larger and more sophisticated facilities, however elevated industrial emissions persisted. At its peak in the 1960s, Sudbury was the largest point source emitter of industrial emissions in the world, with SO_2 emissions reaching 7034 tonne^{-1}day^{-1} (Keller and Pitblado 1986). After more than a half-century of intensive industrial activity, impacts to terrestrial (Gorham and Gordon 1960) and aquatic ecosystems (Beamish and Harvey 1972) were becoming increasingly evident, and the Sudbury

region became internationally recognized for its anthropogenically-degraded environment (Keller 1992). Approximately 7,000 lakes within an area of 17,000km^2 surrounding Sudbury acidified below a pH of 6.0 due to elevated concentrations of SO_2 from industrial emissions (Neary et al. 1990). High levels of acid deposition altered catchment and lake chemistry due to changes in base cations, alkalinity, and lake pH (Beamish and Harvey 1972, Dillon et al. 1977). In turn, the Sudbury area experienced widespread biological damage to terrestrial vegetation, fish, invertebrate, and phytoplankton communities (Gorham and Gordon 1960, Beamish and Harvey 1972, Sprules 1975, Kwiatkowski and Roff 1976).

In an effort to reduce impacts from acidification, national and international policies such as the Eastern Canada Acid Rain Program (1985), the transboundary Canada-US Air Quality Agreement, the Clean Air Act (1970) and its subsequent amendment, the Acid Deposition Control Program (1990) were put in place to reduce SO_2 emissions (Menz and Seip 2004). Emission abatement programs have been successful in reducing industrial emissions from Sudbury smelting operations by roughly 90% since peak output (Keller et al. 2007).

Chemical and biological effects of acid deposition

Chemical effects

Lakes in the Sudbury area were susceptible to acidification because of two main factors: (1) the underlying geology of the region; and (2) proximity to smelter facilities. Sedimentary bedrock, such as calcareous limestone, is prone to weathering and has high base cation concentrations that are able to buffer against acidic deposition (Debicki 1982). In contrast, catchments containing non-calcareous bedrock, such as granite, are susceptible to acid deposition because of characteristically weathering- and erosion-resistant bedrock and low natural buffering capacities (Nixdorf et al. 2003). Killarney Provincial Park, located approximately 60km

southwest of Sudbury, Ontario was one of the first locations in North America where impacts of acid deposition were described (Beamish and Harvey 1972). Killarney PP is located on the Canadian Shield and has a range in geology from calcareous to non-calcareous bedrock (Debicki 1982, Snucins and Gunn 1999). Lakes that were minimally affected by intensive acidification are in regions where calcareous deposits of limestone, sandstone and diabase are common (Debicki 1982, Snucins et al. 2001). However, the majority of Killarney PP lakes are surrounded by non-calcareous orthoquartzite and granite bedrock of the LaCloche Mountain range (Sprules 1975). Many of these lakes were susceptible to acidification because of low dissolved organic carbon (DOC), exchangeable base cations, and naturally low buffering capacity (Dillon et al. 1977, Snucins et al. 2001). Impacts of acidification in Killarney PP were severe and well documented, with many lakes in Killarney PP falling below pH 5.0, and one even below pH 4.0 during the 1970s (Beamish and Harvey 1972).

Sudbury area lakes also experienced elevated concentrations and bioavailability of toxic metals. Severe accumulation of heavy metals in lakes and lake sediments occurred within 40km of smelting facilities (Keller and Pitblado 1986, Neary et al. 1990, Keller and Yan 1991), with concentrations that exceeded environmental guidelines (Valois et al. 2010). Additionally, at low pH ($pH<5.5$), aluminum (Al) was leached from soils because of its high affinity to complex with hydroxide and sulphate ligands (Driscoll et al. 1980). Al toxicity varied spatially and temporally, as Al concentrations are correlated with temporal pulses of decreased lake pH, particularly in catchments with high amounts of total organic carbon (Driscoll et al. 1980). Despite this spatio-temporal variability, many Sudbury area lakes experienced significant increases in the mobilization and bioavailability of Al during peak acidification (Dixit et al. 1992, Keller et al. 2003).

Biological Effects

Biological impacts of acid deposition in the Sudbury region were severe and biodiversity in multiple trophic levels declined. Fish species declined in population size, species richness, and in some cases were extirpated from acidic lakes (Beamish and Harvey 1972). Zooplankton species sensitive to low pH (e.g. large daphniids, *Epischura lacustris*) were extirpated in lakes pH<6.0 (Sprules 1975, Havens et al. 1993), and lakes of pH<5.0 were often dominated by a single, or few acid-tolerant species (e.g. *Leptodiaptomus minutus*, *Bosmina* spp.; Sprules 1975, Keller and Pitblado 1984). Rotifer communities declined in diversity and were dominated by *Keratella taurocephala* (MacIsaac et al. 1986), and phytoplankton assemblages declined in standing crop, were less diverse, and shifted from dominance of Chlorophyta to dominance of Cyanobacteria in low pH lakes (Kwiatkowski and Roff 1976). In light of the observed effects to aquatic biota in acidified lakes, a general threshold for community change for invertebrates was proposed at a pH of 6.0 (as summarized Schindler 1988), and subsequent analysis by Holt et al. (2003) confirmed the appropriateness of this threshold for crustacean zooplankton.

Biota were impacted both directly (e.g. physiological tolerance) and indirectly (e.g. altered biotic interactions) through acidification. Physiological tolerance among zooplankton species was highly variable. As summarized by Brett (1989), low pH both acutely (e.g. reduced filtering rates, sodium ion imbalance, gill tissue damage), and chronically affected zooplankton populations (e.g. impaired life history traits: reduced survivorship, reduced brood size, delayed onset of maturity). Lake surveys suggest that large herbivores such as *Daphnia* are sensitive to acidic conditions (Sprules 1975, Keller and Pitblado 1984, Havens et al. 1993). Subsequently, laboratory studies identified strong interspecific variation in physiological tolerance to acid-stress between daphniids and non-daphniid zooplankton (Bulkowski et al. 1985, Price and Swift 1985).

In general, smaller cladocerans such as bosminids, the large jelly-clad *Holopedium*, and most copepods were tolerant to acidic conditions (Sprules 1975, 1977; Havens et al. 1993). Of these, the copepod *Leptodiaptomus minutus* is likely the most tolerant, as it was often the only zooplankton present in severely acidified lakes (Sprules 1977, Keller and Pitblado 1984, as summarized Brett 1987).

Indirect effects of acidification also influenced zooplankton communities. Decreased abundance of large dominant grazers (i.e. daphniids) allowed acid-tolerant species to be released from competition and proliferate in acidic conditions (Havens and Carlson 1998, Fischer et al. 2001, Frost et al. 2006). Additionally, alterations to predator communities, namely the extirpation of planktivorous fish, modified zooplankton communities in acidic lakes (as reviewed Brett 1989). In fishless lakes, the role of top predator often shifted to macroinvertebrates such as *Chaoborus*, which have been found to suppress densities of small cladocerans (Arnott and Vanni 1993), including small daphniids (Vanni 1988).

Chemical and biological recovery of acidified lakes

Chemical Recovery

As a consequence of national and international agreements targeted at reducing acid-producing industrial emissions, there has been marked recovery in pH of formerly acidified lakes. In the Sudbury region, widespread increases of lake pH (Keller and Pitblado 1986, Keller et al. 1999, Keller et al. 2003, Gray et al. 2012) and reductions of heavy metal concentrations (Yan et al. 1996, Keller et al. 1999, Valois et al. 2010) have been documented in response to emission abatement programs. Despite these overall improvements in water chemistry, many lakes remain acidic (Gray et al. 2012) and metal contaminated (Valois et al. 2010). Additionally, a legacy

effect from past acidification is that calcium concentrations are declining regionally (Keller et al. 2001, Keller 2009).

Biological Recovery

Biological recovery of zooplankton from historically acidified lakes is much less clear. Species richness has increased in many formerly acidified lakes (Yan et al. 1996, Snucins et al. 2001, Keller et al. 2002, Gray et al. 2012), however multivariate analyses suggest that despite subtle improvements in community composition, many zooplankton communities from acidic and formerly acidic lakes remain dissimilar to regional non-impacted reference lakes (Holt and Yan 2003, Gray et al. 2012, Palmer and Yan 2013). Biological recovery is still incomplete, most notably due to the low abundances of large *Daphnia* spp. (Yan et al. 2004, Palmer et al. 2013).

To date, three main barriers have been attributed to the lag in biological recovery of regional zooplankton: (1) local abiotic conditions of chronic lake acidity (Locke et al. 1994, Keller et al. 2002, Yan et al. 2004), and persistent heavy metal toxicity (Labaj et al. 2015) are prohibiting re-establishment of acid- and metal-sensitive species; (2) biological resistance from local, acid-structured zooplankton communities (Binks et al. 2005) and predator communities (Yan et al. 1991, Arnott et al. 2006, Valois et al. 2010) are preventing re-establishment of sensitive species despite improvements to water quality; and, (3) dispersal limitation inhibiting the establishment of potential zooplankton colonists from the regional species pool (Gray and Arnott 2009). While these processes surely influence recovery, a novel stressor in historically acidified lakes, regional calcium decline, has yet to be experimentally manipulated to investigate its relative importance when considering recovery pathways.

Calcium decline – a legacy effect

Displacement of calcium within catchments experiencing acid deposition is part of a well-known geochemical relationship (Likens et al. 1996, Dillon et al. 1987); however, the strength and speed at which concentrations are declining in historically acidified lakes is ecological concerning. Anthropogenically-elevated concentrations of SO_4 and NO_x leached base cations (Ca is primary component of base cations) from soil complexes, where they were then washed out of solution and lost to the environment (Likens et al. 1996). In lakes with a low buffering capacity, such as those in Killarney PP and the Sudbury area, an initial pulse of calcium occurred, artificially raising concentrations during peak acidification (Watmough and Dillon 2003, Molot and Dillon 2008). Recent reductions to industrial-emissions has slowed cation export rates from catchment soils (Likens et al. 1996), however the legacy of over a half-century of intensive acidification has led to calcium being lost at a higher rate than it can be replenished (Watmough et al. 2005). In addition, past and present forest management practices have influenced, and will continue to influence rates of calcium decline. Large-scale forest harvesting has resulted in declining calcium budgets for catchments through the removal of calcareous laden biomass (i.e. trees) (Watmough and Aherne 2008). Calcium decline in historically acidified lakes is not limited to the Sudbury region. Stoddard et al. (1999) found that large parts of Europe and North America have also witnessed substantial calcium decline over the last few decades.

Biological implications of calcium decline

Calcium is a vital nutrient for all biota, however there is concern that in lakes where calcium is declining that freshwater crustaceans may be disproportionally affected (Cairns and Yan 2009). Crustacea acquire the vast majority of their necessary calcium via bronchial uptake from the ambient environment rather than through dietary sources (Wheatly 1999), so a decrease

in available calcium may increase vulnerability to physiological stress. As primary consumers, many zooplankton species are grazers, regulating phytoplankton biomass in lakes, and as secondary producers zooplankton provide energy to higher trophic levels (as prey). As such, if declining calcium concentrations affect zooplankton communities the resulting bottom-up, or top-down impacts might have wide-ranging implications on aquatic ecosystems (Carpenter et al. 1985).

Studies suggest that vulnerability of freshwater crustaceans increase in low calcium environments (as reviewed Cairns and Yan 2009). Crustaceous zooplankton rely almost entirely on the availability of ambient calcium from their external environment for many physiological and morphological processes (Greenaway 1985), including the requirement to continually re-mineralize a calcified carapace throughout moult cycles (Cowgill et al. 1986, Alstad et al. 1999). Calcium requirements vary amongst zooplankton species. Daphniids, with the presence of a heavily calcified carapace (74-92% of total body calcium; Muyssen et al. 2009), are calcium-rich (percent body calcium: 3-8% Ca/dry weight (DW)), compared to non-daphniid cladocerans and copepods (0.2-0.5 %Ca/DW) (Yan et al. 1989, Waervagen et al. 2002, Jeziorski and Yan 2006). Studies investigating regulation of calcium on *Daphnia magna* in low and high calcium treatments suggest that calcium content of individuals is reduced in low calcium environments (Alstad et al. 1999, Tan and Wang 2009). Furthermore, reductions in calcium uptake correspond to decreased intrinsic population growth rates for daphniids, relative to *Ceriodaphnia dubia* and *Moina macrocopa*, two non-daphniid cladocerans (Tan and Wang 2010). In light of these interspecific differences, laboratory studies investigating the impacts of calcium decline on zooplankton have primarily focused on *Daphnia* species. Direct effects of low calcium on *Daphnia magna* survival have been observed with a survival threshold between 0.1-0.5mg/L Ca, and limited reproduction in trials between 1.0-5.0mg/L Ca (Hessen et al. 2000). Similar effects

were seen in *Daphnia pulex* with survival thresholds observed between 0.1-0.5mg/L Ca, and a reproductive threshold of 1.5mg/L Ca (Ashforth and Yan 2008). Additionally, indirect effects of low calcium were seen in *D. pulex* individuals that are unable to respond to predators and induce suitable anti-predator defenses (e.g. formation of neck spines, larger body-size, strengthening of carapace) at calcium concentrations <1.5mg/L (Riessen et al. 2012).

To date, literature on community-level impacts of declining calcium is primarily from synoptic lake surveys and paleolimnological evidence. A survey of 146 lakes in eastern United States suggested that zooplankton communities shift from dominance of large *Daphnia* (primarily *Daphnia pulex/pulicaria*, *Daphnia galeata mendotae*, *Daphnia schodleri*) in high calcium lakes, to communities dominated by smaller zooplankton species (primarily *Diaptomus* copepods, rotifers, *Daphnia catawba*, *Daphnia ambigua*, *Daphnia retrocurva*, *Daphnia parvula*) in lower calcium lakes (Tessier and Horwitz 1990). Additionally, a survey of 346 Norwegian lakes demonstrated a positive relationship between calcium concentrations and the presence of calcium-rich *Daphnia* in pelagic zooplankton communities (Hessen et al. 1995). Paleolimnological evidence provides additional support that daphniids are susceptible to population declines in low calcium lakes. Low calcium lakes and sedimentary layers of lakes representing periods of recent calcium decline reveal impoverished *Daphnia* densities relative to high calcium reference lakes and time periods (Jeziorski et al. 2008, DeSelles 2011, Korosi et al. 2012). However, a lack of taxonomic resolution (only two distinguishable daphniid complexes; *longispina* and *pulex*, both contain presumed calcium-sensitive/tolerant species) limits our ability to determine species-specific sensitivities. The paleoecological record also suggests that associated with declines in relative abundance of daphniids are increases in calcium-poor taxa, such as bosminids (Korosi et al. 2012) and *Holopedium glacialis,* which is an efficient competitor at low calcium (Hessen et al. 1995, Jeziorski et al. 2015). In both lake surveys and

paleolimnological studies it is difficult to disentangle the direct and indirect effects of low calcium from other confounding and unmeasured factors. Consequently, our ability to understand the mechanisms driving community shifts is limited.

Thesis objectives

The overall objective of this thesis is to investigate how a legacy effect from acid deposition – regional calcium decline – is influencing biological recovery on zooplankton from historically acidified lakes. By experimentally manipulating pH and calcium above literature-defined thresholds we attempt to uncouple the strong correlation that exists between population- and community-level effects from pH and calcium. By observing zooplankton response at both low and high concentrations for calcium and pH we seek to understand not only the independent effects of these stressors, but also their combined effect on zooplankton in chemically recovering environments.

Chapter 2

The effect of declining calcium on biological recovery of zooplankton in historically acidified lakes

Abstract

Throughout much of the 20th century, industrial-emissions caused widespread acidification to thousands of lakes in North America and Europe, which resulted in unprecedented damage to zooplankton communities. Although lake pH is recovering due to emission control policies, many acid-sensitive species (e.g. large *Daphnia*) have not re-established. Coincidently, calcium, a critical component of crustacean exoskeletons has declined precipitously in many historically acidified lakes over the last few decades and is now approaching ecologically concerning concentrations. The goal of this study was to investigate the role of low calcium on recovery of zooplankton from acid-damaged lakes. Lake mesocosms were stocked with zooplankton from the regional species pool and four treatments were applied in a factorial design: pH (5.7 and 6.3) and calcium (0.9mg L^{-1} and 2.3mg L^{-1}). A comparison between the effects of calcium and pH indicates that impacts from declining calcium may be similar to those of acidification. Total community metrics (i.e. abundance, richness, evenness, mean body size) remained similar across treatments; however, low calcium and low pH each resulted in reductions of *D. pulex/pulicaria* abundance – a large acid sensitive, and heavily calcified cladoceran. In contrast, the abundance of small cladocerans, including smaller acid-tolerant daphniids (*D. catawba, D. ambigua*), increased in low calcium treatments. When both stressors were combined, changes largely reflected the effect of the most severe independent stressor. Despite improvements to lake pH, our results suggest that low calcium is a factor that is influencing biological recovery of zooplankton from historically acidified lakes.

Introduction

Despite powerful legislation to reduce the emission of acid-producing compounds, recent declines of aqueous calcium – a legacy of regional acid deposition – has resulted in a potential new threat to thousands of lakes recovering from acidification across Europe and North America. Decades of SO_2 and NO_x deposition on poorly-buffered catchments resulted in widespread acidification of soils and waterbodies, and subsequent releases of large quantities of base cations (i.e., calcium) (Likens et al. 1996, Watmough and Dillon 2003). In severely acidified regions, such as Sudbury, Canada, acidic deposition killed vegetation through acute acid exposure, and bedrock was blackened by particulates containing high concentrations of toxic heavy metals (Gorham and Gordon 1960, Gunn 1996). However, the most pronounced effects of acidic deposition occurred in aquatic ecosystems (Menz and Seip 2004). In Sudbury, local acid deposition and elevated concentrations of particulate metals severely acidified and contaminated lakes within 40km of smelter facilities (Keller and Pitblado 1986). Additionally, long-range transport of pollutants caused the acidification of over 7000 lakes in a 17,000km^2 area surrounding Sudbury (Neary et al. 1990). Aquatic biota was severely impacted by acidification. Certain crustaceous zooplankton species were extirpated in lakes pH<6.0 (e.g. large daphniids, *Epischura lacustris*) (Sprules 1975, Havens et al. 1993). At increasingly lower lake pH zooplankton richness declined, and at pH<5.0 communities were often dominated by one or few acidophilic species (e.g. *Leptodiaptomus minutus*, small cladocerans) (Sprules 1977, Keller and Pitblado 1984, Brett 1989). Globally, strong national and international policies targeted at reducing industrial-emissions have been successful in improving various aspects of water quality in acidified regions (e.g. lake pH, metals, aqueous SO_4 concentrations) (Stoddard et al. 1999,

Keller et al. 2003, Skjelkvale et al. 2005). However, recovery of sensitive biota has lagged behind chemical improvements, indicating that other factors also are influencing biological recovery.

Lake pH has recovered above aquatic thresholds in numerous historically acidified lakes (Stoddard et al. 1999), however many acid-sensitive zooplankton have yet to re-colonize. Although copepod assemblages have shown promising recovery (Yan et al. 2004), biological recovery is incomplete because of the failure of various acid-sensitive cladocerans to re-establish and attain densities observed in reference lakes (Yan et al. 2004, Palmer et al. 2013). Intriguingly, acid-sensitive cladoceran taxa exist in the regional species pool, and have sporadically appeared in the monitoring record yet have failed to establish or attain detectable population sizes (Keller and Yan 1991, Yan et al. 2004, Palmer et al. 2013) To date, three main barriers have been attributed to this lag in biological recovery: (1) local abiotic conditions of chronic lake acidity (Keller et al. 2002, Yan et al. 2004, Gray et al. 2012) and persistent heavy metal toxicity (Labaj et al. 2015); (2) biological resistance from local, acid-structured zooplankton communities (Binks et al. 2005) and predator communities (Yan et al. 1991, Arnott et al. 2006, Valois et al. 2010); and (3) dispersal limitation inhibiting the establishment of potential zooplankton colonists (Gray and Arnott 2009). While these processes surely influence recovery, a novel stressor in formerly acidified lakes, regional calcium decline, has yet to be experimentally manipulated to understand its role in recovery of zooplankton from historic acidification.

In aquatic ecosystems calcium and pH are highly correlated. As a result of past acidification, calcium has been declining in historically acidified regions over the last few decades (Stoddard et al. 1999, Jeziorski et al. 2008). During periods of elevated acid deposition, high SO_4 and NO_x resulted in base cations being leached out of soil complexes (Likens et al. 1996), causing an initial pulse of calcium (Watmough and Dillon 2003). However, the legacy of more than a half-century of intensive acidification has caused calcium to be lost at a higher rate

than it can be replenished via soil weathering and atmospheric calcium deposition (Watmough and Dillon 2003). Additionally, timber harvesting has exacerbated calcium loss within catchments through the removal of calcareous laden biomass (i.e. trees) (Watmough and Aherne 2008). With elevated rates of calcium export and minimal sources of calcium inputs, ubiquitous declines of aqueous calcium have been documented in eastern North America and parts of Northern Europe (Stoddard et al. 1999, Clair et al. 2002, Jeziorski et al. 2008). At the time of publication, Jeziorski et al. (2008) show that for 770 lakes from western to eastern Ontario, of which many experienced acidification, 35% have calcium concentrations below 1.5mg/L, and 62% of these same 770 lakes have less than 2.0mg/L calcium. As a vital element for all organisms, such low concentrations of aqueous calcium are ecologically concerning.

Laboratory studies suggest that freshwater crustaceans may be disproportionately affected by calcium decline. Crustaceous zooplankton rely on the availability of sufficient ambient calcium from their external environment for many physiological and morphological processes (Greenaway 1985). Regardless of ambient calcium concentrations, moult cycles for *Daphnia* are fixed (Hessen et al. 2000) and the complete carapace including up to 90% of an individual's total body calcium is shed (Alstad et al. 1999), and subsequently must be remineralized (Greenaway 1985, Cowgill et al. 1986). Thus, decreased availability of calcium is likely to increase vulnerability of these organisms in low calcium environments. Because of strong interspecific variation in calcium content between daphniids (percent body calcium: ~3-8% Ca/dry weight (DW)) and non-daphniid zooplankton (~0.2-0.5 %Ca/DW; Yan et al. 1989, Waervagen et al. 2002, Jeziorski and Yan 2006), laboratory studies investigating the influence of calcium have primarily focused on *Daphnia*. Direct effects of low calcium for *Daphnia magna* include a 4-fold reduction in body calcification in low compared to high calcium environments (Alstad et al. 1999), limited survival between 0.1-0.5mg/L Ca, and limited reproduction in trials

between 1.0-5.0mg/L Ca (Hessen et al. 2000). Similar effects are seen in *Daphnia pulex* where survival is limited between 0.1-0.5mg/L Ca, and reproduction is significantly lower below 1.5mg/L Ca (Ashforth and Yan 2008). Conversely, indirect effects of low calcium are seen in *D. pulex* at calcium concentrations less than 1.5mg/L, where individuals are not able to effectively respond to predators and induce suitable anti-predator defenses (e.g. formation of neck spines, larger body-size, strengthening of carapace) (Riessen et al. 2012). Despite many other non-daphniid species that are known to inhabit softwater lakes, little is known about their tolerance to low calcium environments.

To date, studies examining community-level impacts of declining calcium have relied on correlative information from synoptic lake surveys and paleolimnological studies. Lake surveys in the eastern United States (n=146), and Norway (n=346) both suggest that calcium is a primary driver behind declines of large daphniids, and increases of smaller cladocerans and small daphniids in low calcium environments (Tessier and Horwitz 1990, Hessen et al. 1995). Paleolimnological evidence provides additional support that daphniids are susceptible to population declines in low calcium lakes. Low calcium lakes and sedimentary layers representing periods of recent calcium decline indicate lower relative abundance of *Daphnia* remains compared to high calcium environments (Jeziorski et al. 2008, DeSelles 2011, Korosi et al. 2012). However, a lack of taxonomic resolution among chitinous *Daphnia* remains in lake sediments (only two distinguishable daphniid complexes; *longispina* and *pulex*, both contain presumed calcium-sensitive/tolerant species) limits our ability to determine species-specific sensitivities. Associated with declines in relative daphniid abundance within the paleoecological record are increases in chitinous remains of calcium-poor taxa, such as bosminids (Korosi et al. 2012) and *Holopedium glacialis,* which has been shown to be an efficient competitor at low calcium (Hessen et al. 1995, Jeziorski et al. 2015). Despite the strong evidence provided by these lake

surveys and paleolimnological studies, uncoupling the direct and indirect effects of low calcium from external factors such as predation, competition, and the correlation between pH and calcium limits our ability to determine true mechanisms driving community shifts in low calcium lakes. Experimental manipulations are necessary to understand the relative role of pH and calcium on zooplankton.

Killarney Provincial Park (PP) in Ontario, Canada is an ideal location to investigate the role of calcium decline on biological recovery of acidified regions. Many Killarney PP lakes were chemically and biologically damaged by acid deposition due to an unfortunate combination of highly weathering-resistant bedrock and thin soils (Snucins and Gunn 1999) and geographical location only 60km southwest from what at one point was the largest point-source emitter of SO_2 in the world – Sudbury, Ontario (Gunn et al. 1995). In 1971, a survey of 150 lakes within approximately 65km of Sudbury, which included many Killarney PP lakes, indicated that 70 lakes had pH<5.5, and of these 33 were critically acidic at pH<4.5 (Beamish and Harvey 1972). Biological impacts of acidification at this magnitude were severe, evidenced by widespread extirpations of fish species (Beamish and Harvey 1972), local extirpations of regional zooplankton species (Sprules 1975, Sprules 1977), reductions in rotifer species richness (MacIsaac et al. 1986), and shifts in dominant phytoplankton assemblages (Kwiatkowski and Roff 1976). Long-term monitoring data for eight Killarney PP lakes demonstrate that lake acidity has recovered appreciably in many lakes over the last three decades; however, over the same time period aqueous calcium concentrations have declined precipitously (Figure 1). Despite improvements to lake pH and the presence of potential colonists within the regional species pool, community composition in acidic and many recovering Killarney PP lakes has not recovered relative to non-acidic reference conditions (Figure 2) (Gray et al. 2012, Palmer et al. 2013). Declining calcium concentrations present a novel stressor in these recovering lakes, and as such,

our study aimed to experimentally uncouple and investigate the roles of pH and calcium on biological recovery on zooplankton.

We stocked a diverse assemblage of regional zooplankton in a lake mesocosm to investigate community response of species being introduced to unfavourable conditions of low calcium (0.9mg/L), and low pH (5.7). We hypothesized that: (1) low pH treatments (pH 5.7) would have low abundance of acid-sensitive taxa; (2) low calcium treatments (0.9 mg/L Ca) would have low abundance of large calcium-rich *Daphnia spp.*, high abundance of smaller calcium-poor zooplankton, and reductions in community mean body size and individual body size; and lastly (3) in combination, the impacts of low pH and low calcium would be comparable to the strength of the most severe independent effect because of similarities in predicted individual effects.

Materials and Methods

Site description

Lumsden Lake (46°01' N/81°25' W) is a small oligotrophic lake in Killarney Provincial Park, approximately 65km southwest of Sudbury, Ontario, Canada. Located within the La Cloche Mountain Range, the surrounding bedrock is primarily quartzite (Beamish and Harvey 1972), and as such the lake has characteristically low alkalinity, nutrients and calcium (Beamish and Harvey 1972). Lumsden Lake occurs at the bottom of a 6-lake chain, however a series of waterfalls above the lake inhibits movement of fish (Beamish and Harvey 1972). The lake was severely acidified in the 1960s, evidenced by readings of pH of 6.8 in 1961 and pH of 4.4 in 1971 (Beamish and Harvey 1972). This dramatic decrease in pH resulted in the extirpation of all but one fish species (although it was rare, and was subsequently extirpated) (Beamish and Harvey 1972), and zooplankton communities were dominated by the copepod *Leptodiaptomus minutus* and

bosminids (0.56 and 0.40 relative abundance, respectively) (Sprules 1975). Other zooplankton species present but in low abundance were *Daphnia catawba*, *Holopedium*, *Diacyclops thomasi*, and *Polyphemus pediculus* (Sprules 1975).

Lumsden Lake pH has increased to a pH of 5.7 since peak acidification, however calcium concentrations have declined considerably to 0.9mg/L (pre-acidification Ca – 4mg/L; Beamish and Harvey 1972, 2013 Ca; Arnott unpublished data 2013). Lumsden Lake has remained fishless since acidification (Snucins and Gunn 1999), and the present-day zooplankton community is depauperate and dominated almost exclusively by *L. minutus* (Figure 2, Figure 3). Other species present but in low densities, include *Skistodiaptomus oregonensis*, *D. thomasi*, *Chaoborus* spp., *Diaphanasoma* spp., *P. pediculus*, *Eubosmina tubicen*, and *Mesocyclops edax* (Figure 3). Paleoecological analysis of Lumsden Lake found 1 *Daphnia pulex* complex individual (presumably *D. catawba*) in present-day sediments, and occasional appearances (no >4 individuals) dating back to ca. ~1990 (Labaj et al. 2015). Additionally, a pelagic survey in 2011 found that *D. pulex* complex comprised 2.5% of the total sampled community (Arnott unpublished data). Thus, it appears that *D. pulex* complex has either experienced failed colonization attempts on multiple occasions, or is simply persisting in such low densities that it was missed at the time of our sampling.

Experimental design

To assess the influence of pH and calcium concentration on biological recovery of zooplankton from historically acidified lakes, a lake mesocosm experiment was established in Lumsden Lake (46°01' N/81°25' W), Killarney Provincial Park, Ontario, Canada from June 9th, 2013 - August 5th, 2013. We used a fully factorial design with calcium (low and high) and pH (low and high) as main factors. The ambient water chemistry of Lumsden Lake represented our

baseline low calcium (mean concentration, 0.93 mg/L, sd=0.065), low pH (mean pH, 5.72, sd=0.094) environment. Our low calcium/low pH treatments represent concentrations that are likely to limit zooplankton based on previously determined thresholds (1.5mg/L Ca^{2+} reproduction threshold, Ashforth and Yan 2008; pH 6 community composition threshold, Holt et al. 2003). High concentrations of calcium (mean concentration, 2.36 mg Ca/L, sd=0.31) and pH (mean, 6.33, sd =0.11) represent levels that should not impose biological limitations on regional zooplankton species, and that are in the range of pH and calcium observed in Killarney Provincial Park lakes (Arnott unpublished data, 2011). Each treatment was replicated 5 times, totaling 20 individual enclosures.

Individual enclosures were suspended from floating wooden frames that were oriented in a north-south direction and anchored in 16-20m of water. Enclosures were 1m in diameter, approximately 3.2m deep and constructed using clear, 4mm thick polyethylene plastic tubes (Filmtech Plastics, Brampton, Ontario) that were sealed at the bottom. To ensure minimal transfer of water and organisms between enclosures and the lake, enclosures were raised ~30cm from the lake surface by foam floats. Each enclosure was covered in black window screen to minimize accumulation of debris and aerial colonization of organisms. Enclosures were filled with Lumsden Lake water on June 6th, 2013 with a gas-powered water pump. Water was filtered through 80μm mesh to remove zooplankton and macroinvertebrates, but allow most phytoplankton to pass through. Treatments were assigned to enclosures in a haphazard manner to ensure treatments were interspersed.

Treatments were established on June 8th, 2014. High pH treatments were achieved by adding 92ml 0.5M NaOH to each enclosure. High calcium treatments received a dry mass addition of 15.73g of $CaSO_4\,2H_2O$. After the addition of chemicals, a Secchi disk was lowered to

the bottom of the enclosure, and returned to the enclosure surface a total of 10 times to thoroughly mix the entire water column.

Each enclosure was stocked with zooplankton that were collected on June 9th, 2013 from three chemically recovered lakes in Killarney Provincial Park (Table D.1). The three lakes were chosen for (1) their proximity to Lumsden Lake as a realistic source for potential dispersers; (2) a history of acidification (pH<6.0), but now having a pH>6.0; (3) containing a diverse assemblage of zooplankton – specifically a diverse assemblage of *Daphnia* species; and (4) having the absence of the regional aquatic invader, *Bythotrephes longimanus*. For each enclosure, a total of 840L of water, representing 1/3 of enclosure total volume, was filtered from each of the three lakes by passing a conical tow net (80μm mesh, 0.25m diameter) vertically from 2m above the sediment-water interface to the top of the water column. For lakes that were too shallow to filter this amount of water in one haul, composite hauls of lesser volumes were taken. Samples for each enclosure were placed in separate 1L opaque Nalgene bottles at each source lake. Samples were transported in coolers to Lumsden Lake after hiking for approximately 1 hour, and inoculated into their respective enclosures.

Sampling

Enclosures were sampled weekly for zooplankton, total/edible chlorophyll-*a*, pH, conductivity, and temperature (Table D.2).

Initial zooplankton communities were assessed June 10th, 2013, and sampled every two weeks until August 5th, 2013. Zooplankton were sampled using a conical tow-net (80μm mesh, 0.15m diameter), with two, 3m vertical hauls near the center of each enclosure. The two hauls (representing ~7% of the total enclosure volume) were pooled, and zooplankton were preserved in 70% ethanol for future analysis. Additionally, two 19.6m hauls zooplankton hauls (conical

tow-net 80μm mesh, 0.15m diameter) were pooled and preserved to assess the resident Lumsden Lake zooplankton community.

Zooplankton enumeration was completed at Queen's University, using a Leica MZ16 (Leica Microsystems; Wetzlar, Germany) dissecting microscope. Samples were re-suspended in 100mL of water, and then subsampled in 5mL increments. Sequential subsamples were processed until no new species were detected in three consecutive subsamples. Cladocerans and copepods were identified to species using a combination of resources including Ward and Wipple (1966), Smith and Fernando (1978), Witty (2004), and Aliberti et al. (2013). Owing to taxonomic similarity, *Bosmina freyi*, and *Bosmina liederi* were combined as *Bosmina* spp., and *Daphnia pulex* and *Daphnia pulicaria* as *Daphnia pulex/pulicaria*. Additionally, body length was measured for samples taken on the final sampling date to assess whether treatment effects influenced mean individual size and community size-structure. The first 30 individuals encountered of each species were measured using a calibrated eyepiece micrometer. If 30 individuals were not present following the subsampling protocol outlined above, only those found present were sized.

Water samples were collected with a 3m Arnott Tube Sampler for pH, conductivity, and total/edible chlorophyll-*a*. pH was analyzed with a desktop pHm220 lab pH meter (Radiometer Analytical; Lyon, France). Conductivity was measured using a WTW TetraCon 325 conductivity probe (Loligo Systems; Tjele, Denmark). Total chlorophyll-*a* was measured using 300mL of treatment water filtered through a G4 glass fibre filter with a 1.2μm pore size (Fisher Scientific; Pittsburg, Pennsylvania, United States). Edible chlorophyll-*a* was measured using 300mL of water, passed through a 30μm mesh, followed by filtration through a glass fibre filter. Chlorophyll-*a* samples were frozen for a minimum of 24 hours then extracted with methanol for analysis using a TD-700 fluorometer (Turner Designs; Sunnyvale, California, United States)

following Welschmeyer (1994). Calcium concentrations were measured on June 10th, July 8th, and August 5th. Water samples for calcium were taken as a 3m integrated tube sample, and analyzed by atomic absorption at the Dorset Environmental Science Centre (1026 Bellwood Acres Rd., Dorset, ON, P0A 1E0).

Statistical Analysis

Linear mixed-effect models and generalized linear mixed-effect models were used to determine the effect of calcium and pH on species abundance, species richness, species evenness (E_{var}), and total/edible chlorophyll-*a*. Calcium and pH were fixed categorical factors, while sample date was a continuous factor with four levels starting at Day 14 and ending at Day 56. Models contained a random factor of individual enclosure to satisfy our repeated-measures design and to account for temporal pseudoreplication. Standard mixed-effect models used the 'lmer' function from the 'lme4' package (Bates et al. 2014). To determine a minimum adequate model (MAM) that best explained treatment effects, full models with interactions of all individual factors were used for model selection using the 'dredge' function in the 'MuMIn' package (Barton 2014). Once the MAM was determined for a given model, model assumptions were tested by histograms of residual values, residual vs. fitted plots, correlations tests between residual vs. fitted values, and scale-location plots. P-values for mixed-effect MAMs were interpreted for the fixed-effects of the model using 'anova' in the 'lmerTest' package (Kuznetsova et al. 2014). When model assumptions were violated, data were log-transformed to improve normality of residuals and to reduce unequal variance among treatments. If log-transformed data did not satisfy model assumptions, generalized linear mixed-effect models with a negative binomial distribution were employed using the 'glmmadmb' function from package 'glmmADMB' (Bolker et al. 2012). MAMs were determined the same way as outlined above for

mixed-effect models. Model diagnostics of generalized linear mixed models were assessed using scale-location plots, residual vs. fitted plots, and an estimate of model dispersion (theta). Significance of generalized linear mixed-effect models was determined using log-likelihood ratio tests. Final model structure for each response variable is included in Table D.3. Figures are shown as grand means of Day 14-Day 56 to highlight treatment effects. Table D.4 demonstrates that species abundances on the final day of the experiment show consistent direction and strength of effect when looking at species response over time compared to the final sampling day.

Non-metric multidimensional scaling (NMDS) was used to assess changes in community composition over the course of the experiment with the 'metaMDS' function from R package 'vegan' (Oksanen et al. 2013). Rare species (<10% occurrence) were removed, and the community plot was created with Bray-Curtis dissimilarity distances on untransformed abundance data. Treatment centroids were plotted to avoid clutter.

To investigate the effect of Ca and pH on mean community size and individual size, weighted species averages and average size, respectively, were calculated. General linear models were used with categorical factors of calcium (Low/High), and pH (Low/High). Model selection used 'dredge' function in the "MuMIn" package (Barton 2014) to determine MAMs. Model assumptions were verified using residual vs. fitted plots, normal quantile-quantile plots, standardized residual vs. fitted plots (scale-location), and residual vs. leverage plots (constant leverage). Significance of treatments was determined using analysis of variance (ANOVA) on the MAM.

To compare our experimental results with *Daphnia* across the Killarney Provincial Park landscape, survey data collected in 2011 from 45 Killarney Provincial Park lakes (Arnott, unpublished data) were examined. Lakes were separated into four separate categories based on calcium concentration (<1.5mg/L Ca^{2+} and >1.5mg/L Ca^{2+}) and pH (<6.0 and >6.0). For each of

the four lake groups, daphniids were split into two species groups determined by relative calcium content – calcium-rich daphniids (*D. dubia, D. longiremis, D. mendotae, D. pulex/pulicaria, D. retrocurva*), and calcium-poor daphniids (*D. ambigua, D. catawba*) (Jeziorski *et al.* 2015). Generalized linear models with a negative binomial distribution were applied to both calcium-rich and calcium-poor daphniid abundance, with explanatory factors of calcium concentration (low vs. high), and pH (low vs. high). Model selection, and p-values were obtained in the same manner as described above. Additionally, to observe recent community composition of the 45 lakes, survey data were used to perform a Principal Component Analysis (PCA) using the 'vegan' package (Oksanen et al. 2013). Rare species were removed (<10% occurrence), and species abundances were Hellinger transformed to reduce the weight of zeros and low abundances (Legendre and Gallagher 2001).

Results

Chlorophyll-a

There were no differences in food resources measured as total or edible chlorophyll-*a* at the beginning of the experiment (Total: null model, Table C.1; Edible: null model, Table C.2). Furthermore, total and edible chlorophyll-*a* remained similar across treatments over the duration of the experiment (Total: null model, Table C.3; Edible: null model, Table C.4).

Response to calcium and pH manipulations

We stocked 18 zooplankton species, including 10 cladoceran and 8 copepod species into our experimental enclosures (Table D.5). No differences in zooplankton abundance, richness or evenness were observed at the beginning of the experiment (Table 1). At the end of the experiment we detected 14 of the original 18 species that were stocked into the enclosures, with

Daphnia mendotae, *Daphnia retrocurva*, *Epischura lacustris* and *Skistodiaptomus oregonesis* absent in all enclosures by the final day.

Of the 18 species stocked at the beginning of our experiment, only 7 responded to calcium or pH. In low Ca treatments the abundance of the acid-sensitive *Daphnia pulex/pulicaria* was 38% lower compared to high Ca treatments (ANOVA, $F_{(1,77)} = 5.34$, $p = 0.023$), and 40% lower in low pH compared to high pH treatments (ANOVA, $F_{(1,77)} = 5.34$, $p = 0.02$; Figure 4.a, Table A.6). In contrast, abundance of *Daphnia ambigua*, a smaller, and generally acid-tolerant species was 57% higher in low Ca treatments (ANOVA, $F_{(1,76)} = 4.60$, $p = 0.035$; Figure 4.c, Table A.8) and 35% lower in low pH treatments (ANOVA, $F_{(1,76)} = 4.12$, $p = 0.046$; Figure 4.c, Table A.8). Abundance of another acid-tolerant daphniid, *Daphnia catawba*, did not respond to our pH treatment but was 34% higher in low Ca compared to high Ca treatments, however this increase was marginally non-significant (ANOVA, $F_{(1,18)} = 2.94$, $p = 0.10$; Figure 4.b, Table A.7). Individual body sizes of *D. pulex/pulicaria*, *D. catawba*, and *D. ambigua* were not influenced by pH or calcium (Figure 5.a, 5.b, 5.c; Table A.21, A.22, A.23, respectively).

Other cladocerans, including small non-daphniid cladocerans and *Holopedium glacialis* (hereafter referred to as *Holopedium*), tolerated, and in some cases, thrived in low Ca and low pH treatments. *Eubosmina tubicen* abundance was 92% higher in low compared to high Ca treatments (ANOVA, $F_{(1,77)} = 6.66$, $p = 0.012$; Figure 4.d, Table A.4) and *Eubosmina longispina* abundance was also higher in low Ca treatments, albeit not significantly (ANOVA, $F_{(1,18)} = 1.91$, $p = 0.18$; Figure 4.e, Table A.5). *Holopedium* abundance decreased in all treatments over the duration of the experiment (ANOVA, $F_{(1,76)} = 121.88$, $p = <0.0001$; Table A.9), however *Holopedium* abundance in both low Ca and low pH treatments was significantly higher on the final day of the experiment (Log Ratio Test - Calcium: $Deviance_{(1,17)} = 5.10$, $Theta = 0.77$, $p =$

0.024; pH: $Deviance_{(1,18)}$ = 15.26, Theta = 0.77, p= <0.0001; Figure 4.f, Table A.11). Individual body sizes of *E. tubicen*, *E. longispina*, and *Holopedium* were not influenced by pH or calcium.

High abundance of small cladocerans and small *Daphnia* in low Ca, resulted in total cladoceran abundance being 57% higher in low Ca compared to high Ca treatments (ANOVA, $F_{(1,17)}$ = 14.69, p = 0.003; Figure 6.b, Table A.3). Cladoceran richness was similar between treatments (null model; Figure 6.e, Table A.16), however cladoceran evenness (E_{var}) was lower in low Ca treatments (Log ratio test, Deviance = 7.57, Theta = 1.00, p = 0.006; Figure 6.h, Table A.17) due to the dominance of small cladocerans in low Ca. Neither pH nor Ca treatments affected mean weighted cladoceran body size (Figure 7, Table A.19). However, mean weighted *Daphnia* body size declined 22% in low Ca treatments (ANOVA, $F_{(1,18)}$ = 3.70, p = 0.071, R^2 = 0.17; Figure 8, Table A1.20).

Most copepod species were not sensitive to our Ca and pH treatments. One exception was *Mesocyclops edax*, an omnivorous copepod, that had higher abundance in low pH treatments (ANOVA, $F_{(1,77)}$ = 9.30, p = 0.0031; Table A1.21). The remaining copepod species displayed either no preference in experimental environments, or were found at such low densities among all treatments that relative effects could not be calculated.

As most individual copepod species did not respond to our pH and Ca treatments, total copepod metrics were also largely unaffected by Ca and pH treatments. Copepod abundance was not influenced by Ca or pH treatments (Ca: ANOVA, $F_{(1,18)}$ = 2.69, p = 0.12; pH: null; Figure 6.c. and Table A.2). However, copepod richness was lower in low Ca treatments (ANOVA, $F_{(1,77)}$ = 4.94, p = 0.029; Figure 6.f, Table A.14), although this significant difference was only a change in richness from 4.25 species in high Ca and 3.75 species in low Ca treatments. Copepod evenness (E_{var}) was marginally higher in our low pH treatments (Log ratio test, Deviance = 3.93, Theta = 1.34, p = 0.047; Figure 6.i, Table A.15). Neither Ca nor pH influenced average copepod size.

Overall, calcium and pH did not influence the trajectory of community composition over the duration of our experiment (Figure 9). Total crustacean zooplankton abundance and species richness remained similar across all treatments (Figure 6.a, Table A.1; Figure 6.d, Table A.12), however community evenness (E_{var}) was lower, albeit non-significantly, in our low Ca treatments (ANOVA, $F_{(1,77)}$=3.03, p=0.086; Figure 6.g, Table A.13). Ca was the only variable retained in the MAM for crustacean zooplankton mean body size (Table A.18), however its inclusion was non-significant (ANOVA, $F_{(1,18)}$ = 2.88, p = 0.11, R^2 = 0.14; Figure 10).

Landscape Comparison

Overall, *Daphnia* spp. abundance was significantly lower in lakes with pH<6.0 than lakes with pH>6.0 (Log ratio test, deviance = 16.04, theta = 0.79, p = <0.0001; Figure 11, Table A2.1). However, the average abundance of Ca-poor *Daphnia* (*D. catawba*, *D. ambigua*; Jeziorski et al. 2015) was marginally higher in low Ca lakes (Log ratio test, deviance = 2.3, theta=0.89, p = 0.13; Figure 11), whereas Ca-rich *Daphnia* (*D. pulex/pulicaria*, *D. mendotae*, *D. retrocurva*, *D. longispina*, *D. dubia*; Jeziorski et al. 2015) abundance was significantly lower in low Ca lakes (Log ratio test, deviance = 24.6, theta=0.69, p = <0.001; Figure 11).

Discussion

To assess the role of declining calcium concentrations on biological recovery in formerly acidified lakes we inoculated regional zooplankton into low and high pH (pH 5.7-6.3) and calcium enclosures (Ca 0.9-2.3mg/L). Of the 18 initial species added only 7 species (*D. pulex*/*pulicaria*, *D. catawba*, *D. ambigua*, *Eubosmina tubicen*, *Eubosmina longispina*, *Holopedium*, *Mesocylcops edax*) responded to our calcium and pH treatments, and of these only

D. pulex/*pulicaria* was negatively affected by low calcium and low pH. In general, the independent effect of low calcium was similar to that of taxa historically tolerant to low pH (Brett 1989), evidenced by the acid-tolerant *D. catawba*, *D. ambigua*, and *Holopedium* achieving higher abundance in low compared to high calcium treatments. Additionally, small cladocerans (*Eubosmina tubicen* and *Eubosmina longispina*) were higher in low compared to high calcium treatments indicating a potential release from competition in the absence of the large grazer, *D. pulex/pulicaria.* As 11 of the species initially stocked in our enclosures did not respond to calcium or pH, few effects were detected at the functional group or community-level. We show that calcium and pH influence population success for certain zooplankton, however the relatively depauperate zooplankton community of Lumsden Lake indicates that other biotic factors are also important for zooplankton recovery in formerly acidified lakes.

Species-Specific Response

D. pulex/*pulicaria* abundance was 38% lower in low compared to high calcium treatments, and 40% lower in low compared to high pH treatments (Figure 4.a). There is extensive support that pH<6.0 is a threshold for large daphniids, including *D. pulex*/*pulicaria* (Keller and Pitlbado 1984, Tessier and Horwitz 1990, Holt et al. 2003, Palmer et al. 2013), however our results provide field evidence that also suggests *D. pulex*/*pulicaria* is limited in low calcium environments. A reduction of *D. pulex/pulicaria* in low calcium is mostly consistent with current speculation that larger and more heavily calcified cladocerans are vulnerable to low calcium environments (Tessier and Horwitz 1990, Hessen et al. 1995, Jeziorski et al. 2008). Abundance of *D. pulex*/*pulicaria* in our low calcium treatment (0.9mg/L) reveals analogous effects to results from Ashforth and Yan (2008), particularly in their low food treatment (6µg chlorophyll-*a*/L) where survivorship, reproduction, and intrinsic rate of natural increase were

significantly reduced below 1.5mg/L Ca. While our results cannot address reproduction or population growth rates, declines of *D. pulex*/*pulicaria* in our low calcium and low food environment (experiment mean total chl-*a*: 1.07µg chlorophyll-*a*/L sd=0.74) are likely indicative of negative impacts to life history traits.

In contrast, abundance of the acid-tolerant *D. catawba* and *D. ambigua* (Brett 1989), was higher in low calcium compared to high calcium treatments (57% and 34%, respectively) (Figure 4.c, 4.b). *Daphnia ambigua* is the smallest daphniid we detected and has low calcium content relative to *D. pulex/pulicaria* and *D. catawba* (Jeziorski and Yan 2006). Currently no studies have investigated direct or indirect effects of low calcium on *D. ambigua*; however, it could be reasonably assumed that *D. ambigua* is better suited to low calcium environments than other daphniids because of their relatively low calcium content. Nonetheless, it is apparent that the relationship between body size, calcium content and presumed daphniid vulnerability to low calcium is not universal. *D. catawba* has similar calcium content to *D. pulex*/*pulicaria* (Jeziorksi and Yan 2006), yet their difference in tolerance to low calcium suggests that *D. catawba* possess a physiological advantage in low calcium. A potential explanation for tolerance of *D. catawba* in low calcium is interspecific variation in calcium uptake rates. For example, Tan and Wang (2010) found that *Daphnia caranita* has a higher calcium influx rate and assimilation efficiency (ability to retain dietary calcium) than *Daphnia galeata*, which resulted in higher population growth rates for *D. caranita* populations at 0.5mg/L Ca. While similar biokinetic studies for *D. catawba* and other *Daphnia* common to our study area do not exist, it is possible that *D. catawba* are more efficient at uptaking, regulating, and retaining calcium than other heavily calcified daphniids in low-calcium environments.

The combined and independent effects of low calcium and low pH on daphniids are quite similar, which suggests that a common physiological mechanism may influence *Daphnia*

sensitivity to both calcium and pH. Past acidification research has identified that elevated hydrogen ion concentrations cause mortality to daphniids by disrupting sodium ion homeostasis (Havas et al. 1984). However, availability of aqueous calcium may modulate the effects of increased hydrogen ions in sodium regulation of daphniids. For example, Havas et al. (1984) show that only sodium efflux rate for daphniids reared in hardwater medium (Ca^{2+}: >50mg/L) was impaired at pH<4.5. In contrast, sodium efflux rate was impaired at pH 5.5 in softwater medium (Ca^{2+}: 2.3 mg/L), and sodium influx rate was inhibited by 23% at pH 5.0 (Havas et al. 1984). While the authors did not implicitly investigate the mechanism behind calcium's role in reducing hydrogen ion toxicity, they argue that augmented calcium concentrations reduce branchial membrane permeability to hydrogen ions (Havas et al. 1984). Unfortunately, the mechanisms behind the role of calcium in sodium regulation are still not well understood, but this potential feedback mechanism may explain the weak additive effect on *D. pulex*/*pulicaria* in treatments where low calcium and low pH co-occurred. When low calcium and low pH occurred individually population abundance declined by 38% and 40%, respectively; however, when the two stressors co-occurred population abundance declined 60% relative to high calcium/high pH treatments (Figure 4.a). With a reduction in both calcium and pH one could assume that internal regulation of sodium may be further impaired. In any case, declining calcium in historically acidified lakes may be tempering improvements of sodium regulation for *Daphnia* that would otherwise be expected with increasing pH.

Survey data from 45 Killarney PP lakes indicates that abundance of all *Daphnia* is lower at low pH, and that low calcium causes a compositional shift from Ca-rich daphniids (*D. dubia, D. longiremis, D. mendotae, D. pulex/pulicaria, D. retrocurva*) to Ca-poor daphniids (*D. catawba*, *D. ambigua*) (Figure 11). As such, landscape survey results are analogous to experimental results for low calcium environments. Species that failed to establish, or that were

present but in low densities in our low calcium treatments (i.e. *D. mendotae*, *D. retrocurva, D. pulex*/*pulicaria*) were rarely found in lakes with calcium concentration <1.5mg/L, whereas species commonly found in low calcium treatments (*D. catawba*, *D. ambigua*) were present in relatively high densities in lakes with calcium concentration <1.5mg/L. Where experimental and survey results disagree is that we did not detect an overall reduction on *Daphnia* abundance in our experimental low pH treatments. *D. pulex/pulicaria* abundance declined in low pH treatments, however it was the only acid-sensitive daphniid (Brett 1989) that persisted throughout the experiment. In contrast, *D. catawba* and *D. ambigua* were the most common daphniids across all treatments, so the relatively high abundance of non-responding acid-tolerant species (Brett 1989) may have negated an overall *Daphnia* response in low pH treatments. The general complementarity of our experimental results with landscape observations, particularly in regards to low calcium environments, provides additional support that biological recovery of daphniid species is likely to be influenced by both low calcium and low pH.

While most small cladocerans and copepods did not respond to calcium or pH, the abundance of *Eubosmina tubicen* and *Eubsomina longispina* was higher in low compared to high calcium treatments (92% and 87%, respectively) (Figure 4.d, 4.e). High *Eubosmina* spp. abundance in our low Ca treatments was likely due to a release from competition associated with decreased abundance of large grazers such as *D. pulex/pulicaria*. Bosminid densities are often suppressed when large herbivore (e.g. *D. pulex*/*pulicaria*) densities are high, but flourish in the absence of large herbivores (Vanni 1986). While no studies have investigated calcium content of *Eubosmina* spp., *Bosmina* spp., a related species has significantly lower calcium content than daphniids (Jeziorski and Yan 2006). Other non-daphniid cladocerans with low calcium content have been found to require less calcium to maintain positive population growth rates, and can therefore better survive in low calcium environments relative to *Daphnia* (Tan and Wang 2010).

Thus, with a lower calcium content and presumably low calcium requirements, it is likely that *Eubosmina spp.* are more competitive than *D. pulex/pulicaria* in low calcium environments and increase in abundance due to a release from competition.

Although *Holopedium* abundance declined through time in all treatments, on the last day of the experiment abundance was higher in low calcium and low pH treatments (Figure 4.f). *Holopedium* are common in acid-structured communities (Locke and Sprules 1994, Waervagen et al. 2002), and synoptic lake surveys suggest a strong negative correlation between calcium concentration and *Holopedium* biomass and frequency (Hessen et al. 2005, Jeziorski et al. 2015). Our results demonstrate that when other variables known to influence *Holopedium* are held constant (TP, predation, food; Hessen et al. 1995, Jeziorski et al. 2015) that both calcium and pH can independently influence *Holopedium* abundance. The increase in abundance of *Holopedium* may not be a direct effect of low calcium and low pH but rather an indirect effect associated with reduced competition with other large *Daphnia* (Hessen et al. 1995, Jeziorski et al. 2015).

Community and Functional Group

Total cladoceran abundance was 57% higher in low calcium compared to high calcium treatments (Figure 6.b). This significant increase was due to higher abundances of both small cladocerans (*Eubosmina* spp.) and small *Daphnia* (*D. catawba*, *D. ambigua*) in low calcium treatments. In contrast, treatment effects did not influence total copepod abundance (Figure 6.c) due to a lack of responding species. When combined, the more abundant and non-responding copepod group reduced the influence of calcium and pH at the community level (Figure 6.a).

Coarse univariate metrics (species richness, community evenness (E_{var})) were largely unresponsive to experimental treatments, except for cladoceran evenness. In low calcium treatments, we found that cladoceran evenness was lower due to the dominance of small

cladocerans (Figure 6.h). Surprisingly, total community and cladoceran richness (Figure 6.d and 5.e, respectively) did not respond to our treatments. While our experimental gradients for calcium and pH spanned defined thresholds (pH: acid sensitive zooplankton, pH 6.0; Ca: *D. pulex* reproduction, 1.5mg/L Ca), no response in species richness may in part be due to our relatively short gradient of pH (5.7 – 6.3), and calcium concentrations (0.9mg/L – 2.3mg/L). The pH 6.0 threshold for crustaceous zooplankton was established objectively with a step-function model from 47 lakes in the Muskoka region, however when the same method was used on a smaller 15-lake dataset in Killarney PP the step (i.e. threshold) for species richness was placed at pH 5.8 (Holt et al. 2003). Therefore, it is possible that small populations of sensitive species (such as *D. pulex*/*pulicaria*) were able to persist on the edge of this pH range, and avoid extirpation. Low calcium reduced the abundance of *D. pulex*/*pulicaria*, although its persistence, and therefore lack of effect on species richness, is likely due to our low calcium treatment occurring above the recognized survival thresholds for calcium-rich daphniids (0.1-0.5mg/L; Hessen et al. 2000, Ashforth and Yan 2008). Additionally, our most pronounced effect of low calcium was not an exclusion of species, but rather increased abundance of *Eubosmina* spp., *D. catawba* and *D. ambigua*. This suggests that species richness may under-estimate the influence, particularly of low calcium, on zooplankton communities. Accordingly, a diversity metric that takes into account species relative densities, such as evenness (E_{var}), may be a more informative option than a simple presence/absence index.

Community Size Structure

Our hypothesis that low calcium would decrease mean community body size was not supported (Figure 10). However, *Daphnia* mean body size was higher (albeit not significantly) in high calcium treatments (Figure 8). The differences in *Daphnia* mean body size between Ca

treatments are driven by changes in relative species abundance, rather than changes in mean body size of *D. catawba* and *D. ambigua* (Figure 5.b; Figure 5.c). Unfortunately, small sample size of *D. pulex/pulicaria* prevents us from investigating treatment effects on individual body size (Figure 5.a). Despite differences in daphniid body size among Ca treatments, low relative abundances of these species relative to the remaining community resulted in no detectable differences in total community body size. For example, *Daphnia* spp. represented a small percentage of the overall community in low calcium and high calcium treatments (low Ca, 4.4%±1.7; high Ca, 24.89%±27.38). With at minimum 75% of the community being dominated by smaller, non-responsive species a change in mean community body size was not detected.

Additional Factors influencing zooplankton establishment

The resident Lumsden Lake zooplankton community indicates that factors in addition to low calcium and low pH are influencing zooplankton recovery. Lumsden Lake was dominated by *Leptodiaptomus minutus* (Figure 3), and contained only rare occurrences of *Skistodiaptomus oregonensis*, *Diacyclops thomasi*, *Eubosmina tubicen*, and *Mesocyclops edax* at the time of our experiment. In contrast, species common to low calcium and low pH treatments (e.g. *D. catawba*, *D. ambigua*, *E. longispina*, *E. tubicen*, *Holopedium, M. edax*, *Orthocyclops modestus*) were notably absent, or in low densities in Lumsden Lake at the time of our experiment. Extirpation of fish species from Lumsden Lake (Beamish and Harvey 1972, Snucins and Gunn 1999), has led to predator communities dominated by the macroinvertebrate *Chaoborus*, and the predatory cladoceran *Polyphemus pediculus* (Figure 3). *Polyphemus pediculus* generally select small prey (e.g. rotifers) over larger items (e.g. copepod nauplii, small cladocerans) (Packard 2001), however when fish are absent *Chaoborus* can regulate cladoceran assemblages through direct predation on small daphniids (Vanni 1988), small cladocerans such as bosminids, and copepods such as *O.*

modestus, and *Diaptomus oregonensis* (Yan et al. 1991, Arnott and Vanni 1993). Therefore, the importance of low calcium and low pH in Lumsden Lake may be seceded by biotic factors, with macroinvertebrate predation suppressing recolonization attempts by calcium- and pH tolerant species.

Additionally, calcium and pH cannot explain the loss of four species (*Daphnia mendotae*, *Daphnia retrocurva*, *Epischura lacustris*, and *Skistodiaptomus oregonensis*) that were stocked from the regional species pool. Mesocosm experiments deployed in Carlyle Lake, Killarney PP, observed successful establishment of *D. retrocurva*, *E. lacustris*, and *S. oregonensis* over a 10-week period (Gray and Arnott 2012), and further, the establishment of *D. mendotae* and *E. lacustris* populations over an 8-week period (Gray and Arnott 2011). Past success in culturing these species in mesocosms indicates that extirpation was not a result of historically poor performance in experimental mesocosms. It is possible that introduction to a highly oligotrophic, low pH and poorly buffered lake was not a suitable environment for *D. mendotae* and *D. retrocurva*, as a survey of 249 Sudbury area lakes found that *D. mendotae*, and *D. retrocurva* are most common in neutral/near-neutral, well buffered, and relatively productive lakes (Keller and Pitblado 1984). Mean stocking density of *E. lacustris* (100 individuals/m^{-3}) was far above critical mate finding densities outlined by Gray and Arnott (2012), suggesting that the most likely cause for extirpation was a change in food source. A field study demonstrated that reproduction of *E. lacustris* is dependent on abundance of large, and non-evasive zooplankton (Schulze and Folt 1990). *Epischura lacustris* was last detected on Day 28, so it is possible that shifts to smaller zooplankton up to this point resulted in its disappearance. *Skistodiaptomus oregonensis* was only detected in 3 of the 20 enclosures at the beginning of the experiment and was extirpated by Day 14. Stocked at a mean density of 7 individual/m^{-3}, initial *S. oregonensis* densities are within the critical range for mate finding (3-9 individuals/m^{-3}; Gray and Arnott 2012), therefore any

stochastic event resulting in mortality for *S. oregonensis* may have resulted in an Allee effect, resulting in population extirpation.

Conclusions

Our results demonstrate that declining calcium is another factor that must be considered when investigating biological recovery of chronically and historically acidified lakes. We found that *D. pulex*/*pulicaria* populations were lower in not just low pH environments, but additionally in low calcium environments. This suggests that lakes approaching or that have surpassed a pH of 6.0 may still not experience recovery of *D. pulex*/*pulicaria* if calcium concentrations are low. Additionally, we found that small cladocerans (*E. tubicen*, *E. longispina*) and small *Daphnia* (*D. catawba*, *D. ambigua*) that were tolerant to acidic conditions are likely to persist in high densities of low calcium lakes. While the effects of calcium on cladocerans show strong experimental effects, we acknowledge that other factors are also influencing biotic recovery in the lakes. The depauperate Lumsden Lake zooplankton community clearly indicates that other local processes such as biotic interactions with predators influence biological recovery.

Scaling-up our experimental findings to historically acidified, softwater lakes around the world should be done with caution. However, the complementarity of our experimental results on *Daphnia* with those from the 45-lake Killarney PP survey suggests that calcium will predictably influence daphniid composition in historically acidified lakes. Thousands of lakes across North America and Europe have seen improvements to pH; however, many of these same lakes are now experiencing precipitous declines in calcium (Jeziorski et al. 2008, Skjelkvale et al. 2005). Identifying how declining calcium may be interacting with other stressors that influence biological recovery will be crucial for identifying realistic recovery targets for these thousands of historically acidified lakes.

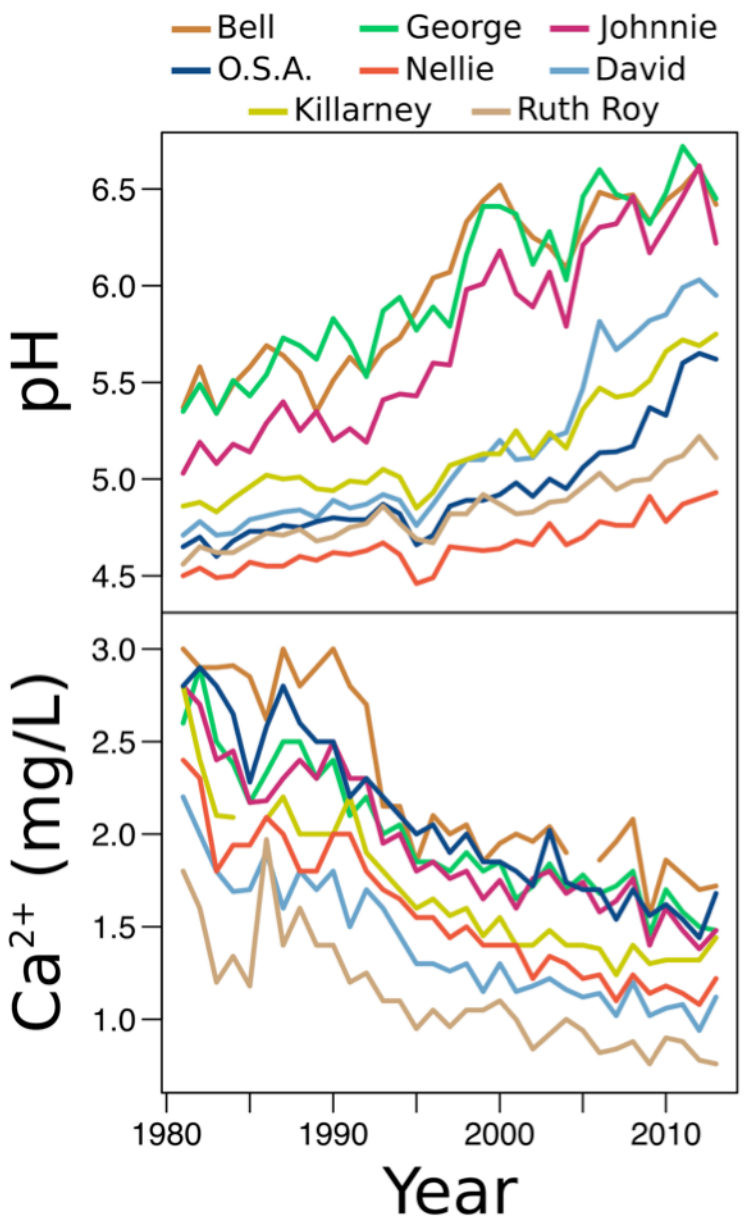

Figure 1 Long-term pH and calcium trends for 8 Killarney PP lakes monitored annually between 1981-2013 (Co-operative Freshwater Ecology Unit, Ontario Ministry of Environment, unpublished data).

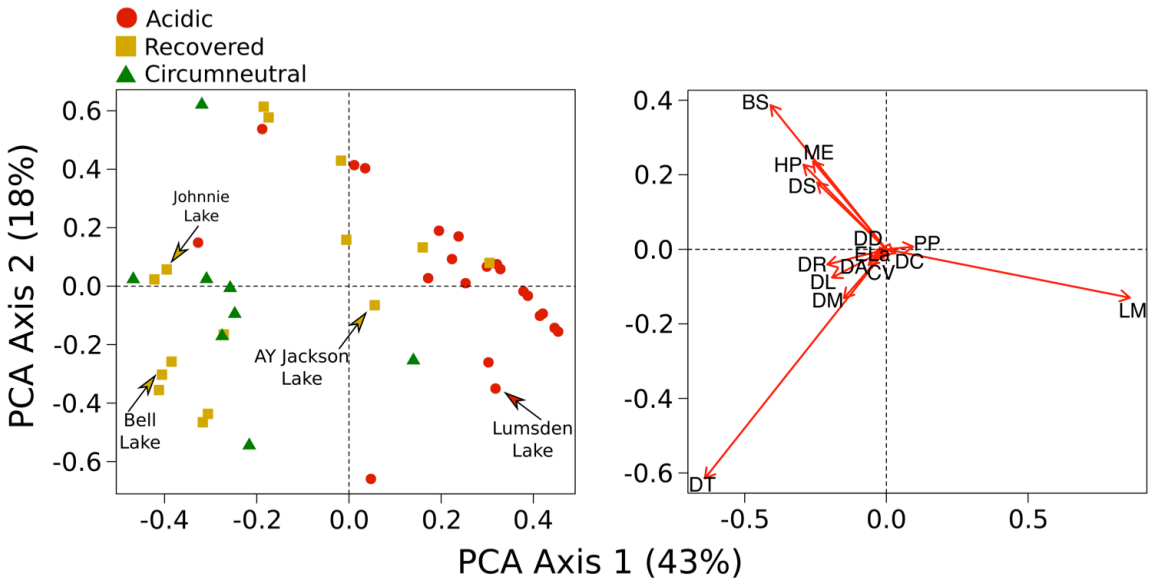

Figure 2 Principal component analysis (PCA) of zooplankton communities from Killarney Provincial Park lakes (n=45) surveyed in 2011. For ease of interpretation, results are presented as a bi-plot, where lake scores are plotted on the left panel, and species scores plotted on the right panel. Red circles = acidic lake (pH<6.0), yellow squares = recovered lakes (acidified to pH<6.0, current pH>6.0), green triangles = circumneutral lakes (always >6.0). Our three source lakes (AY Jackson, Bell, Johnnie Lake) and our experimental lake, Lumsden Lake, are identified by title and arrow specifying their location in multivariate space. Species scores clustered around 0 for PCA axis 1 and 2 were removed from the plot to avoid clutter. Species names are *Acanthocyclops vernalis* (CV), *Bosmina* spp. - including *Eubosmina* spp. (BS), *Daphnia ambigua* (DA), *Daphnia catawba* (DC), *Daphnia dubia* (DD), *Daphnia longiremis* (DL), *Daphnia mendotae* (DM), *Daphnia retrocurva* (DR), *Diacyclops thomasi* (DT), *Diaphanosoma spp.* (DS), *Epischura lacustris* (ELa), *Holopedium* (HP), *Leptodiaptomus minutus* (LM), *Mesocyclops edax* (ME), *Polyphemus pediculus* (PP). (Arnott, unpublished data)

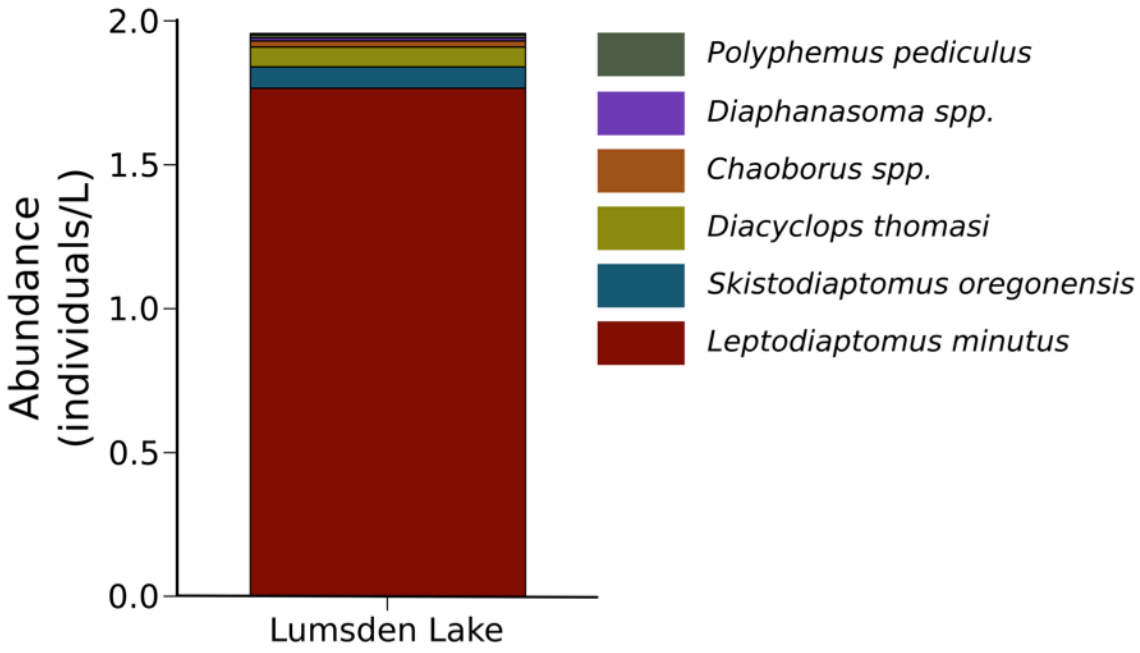

Figure 3 Mid-summer zooplankton abundance (individuals/L) of Lumsden Lake. *Eubosmina tubicen* and *Mesocyclops edax* were both present at the time of sampling, but occurred at extremely low densities, so are not visible on the plot. (Ross, unpublished data)

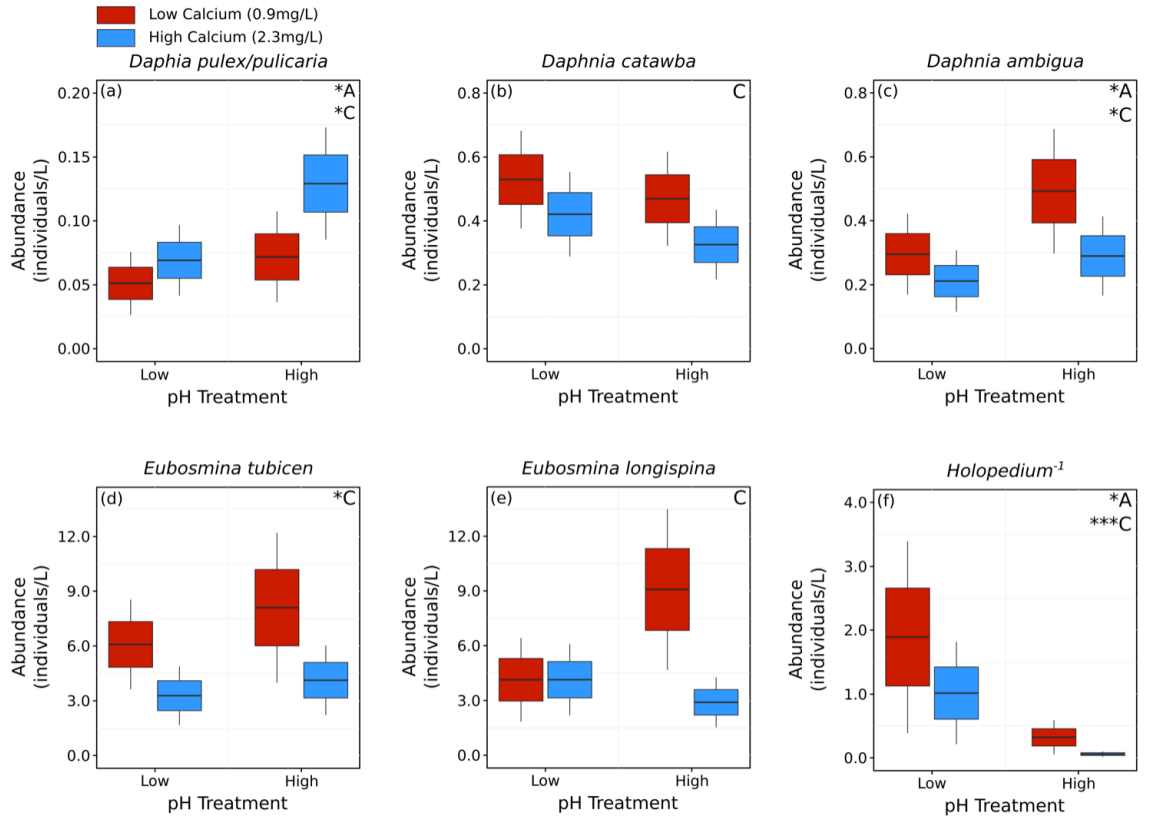

Figure 4 Mean abundance from Day 14 to Day 56 of (a) *Daphnia pulex/pulicaria*, (b) *Daphnia catawba*, (c) *Daphnia ambigua*, (d) *Eubosmina tubicen*, (e) *Eubosmina longispina*, and (f) *Holopedium* for low Ca (red; 0.9mg/L) and high Ca (blue; 2.3mg/L) treatments with low pH (5.9) and high pH (6.3) (n=5, respectively). The thick, horizontal black line represents the treatment sample mean, with the bottom and top edges of the box representing the standard error of the mean. Whiskers extending from the box represent the 95% confidence intervals of the mean. Letters in the top right of each panel represent components retained in the best model (C=Calcium, A= pH), along with associated significance levels (*=p<0.05, **=p<0.01, ***=p<0.001, no symbol=p>0.05). Absence of letters in the top right corner indicates the absence of that variable within the best model.

[-1] *Holopedium* presented as final day abundance (Day 56)

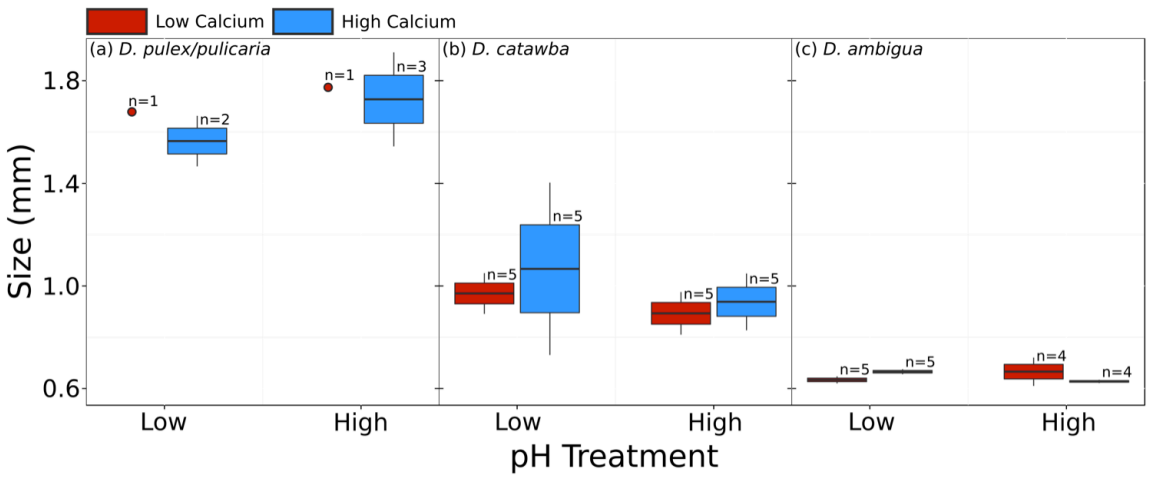

Figure 5 Mean body size of (a) *Daphnia pulex*/*pulicaria*, (b) *Daphnia catawba*, and (c) *Daphnia ambigua* for low Ca (red; 0.9mg/L) and high Ca (blue; 2.3mg/L) treatments with low pH (5.9) and high pH (6.3) on the final day of the experiment. Sample size is indicated for each treatment. The thick, horizontal black line represents the mean weighted size of *Daphnia* within a treatment, with the bottom and top edges of the box representing the standard error of the mean. Whiskers extending from the box represent the 95% confidence intervals of the mean. Letters in the top right of each panel represent factors retained in the best model (C=Calcium, A= pH), along with associated significance levels (• =p<0.1, *=p<0.05, **=p<0.01, ***=p<0.001, no symbol=p>0.1). Absence of letters in the top right corner indicates the absence of that factor within the best model.

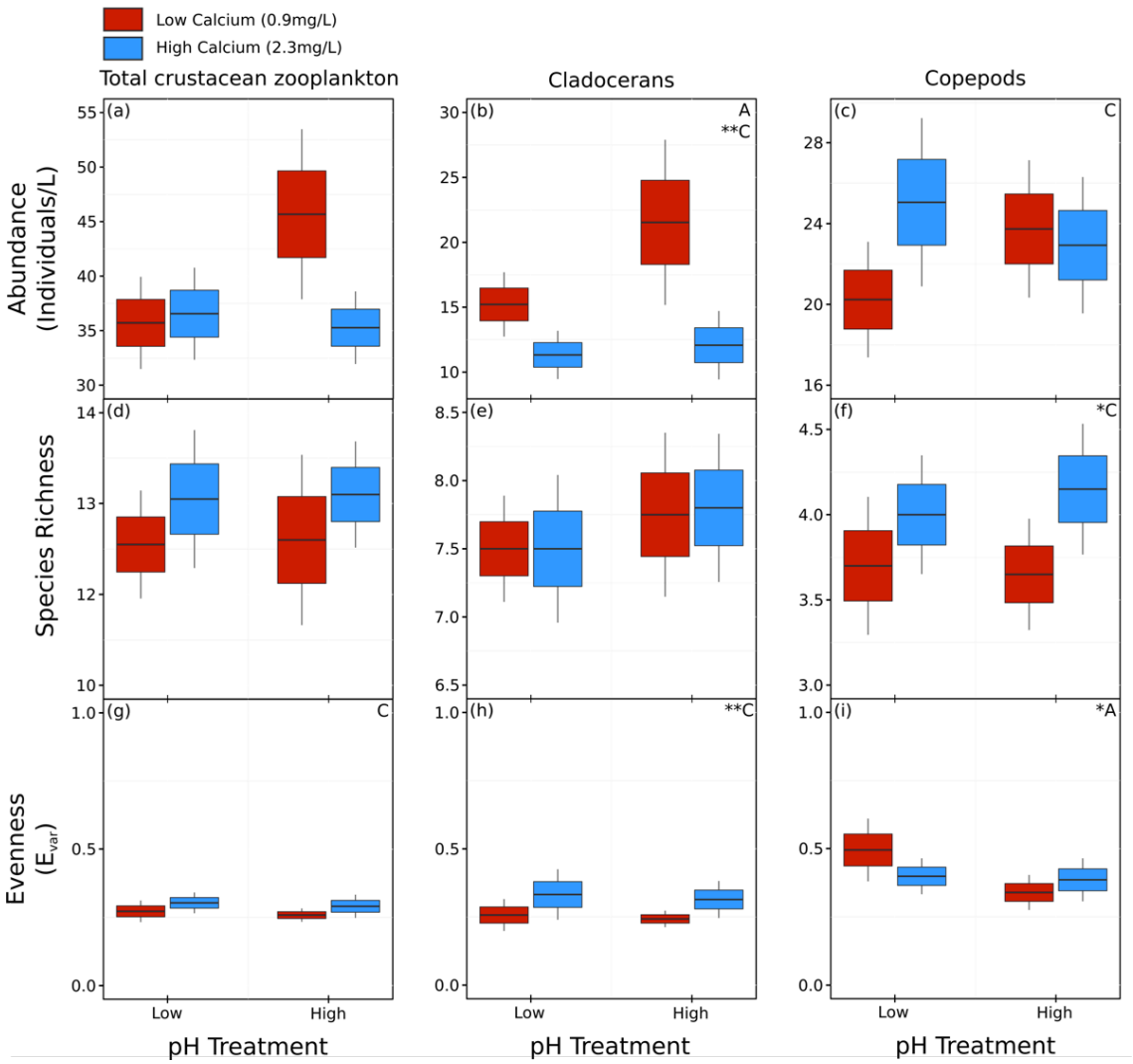

Figure 6 Mean abundance from Day 14 to Day 56 of (a) total crustacean zooplankton, (b) cladocerans, and (c) copepods, species richness of (d) total crustacean zooplankton, (e) cladocerans, and (f) copepods, and evenness (expressed as E_{var}) of (g) total crustacean zooplankton, (h) cladocerans, and (i) copepods for low Ca (red; 0.9mg/L) and high Ca (blue; 2.3mg/L) treatments with low pH (5.9) and high pH (6.3) (n=5, respectively). The thick, horizontal black line represents the treatment sample mean, with the bottom and top edges of the box representing the standard error of the mean. Whiskers extending from the box represent the 95% confidence intervals of the mean. Letters in the top right of each panel represent factors retained in the best model (C=Calcium, A= pH), along with associated significance levels (*=p<0.05, **=p<0.01, ***=p<0.001, no symbol=p>0.05). Absence of letters in the top right corner indicates the absence of that factor within the best model.

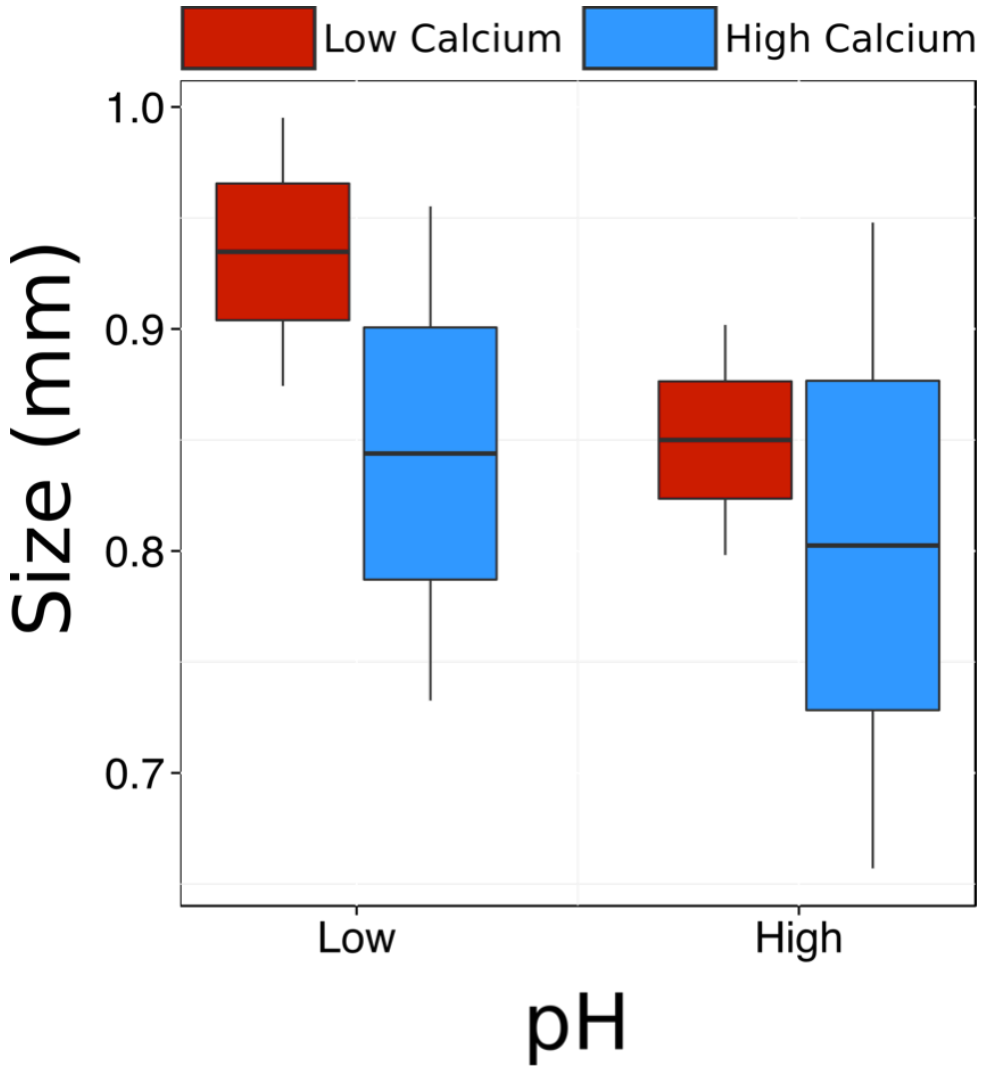

Figure 7 Cladoceran mean body size for low Ca (red; 0.9mg/L) and high Ca (blue; 2.3mg/L) treatments with low pH (5.9) and high pH (6.3) (n=5) on the final day. The thick, horizontal black line represents the mean weighted size of cladocerans within a treatment, with the bottom and top edges of the box representing the standard error of the mean. Whiskers extending from the box represent the 95% confidence intervals of the mean. Letters in the top right of each panel represent factors retained in the best model (C=Calcium, A= pH), along with associated significance levels (*=p<0.05, **=p<0.01, ***=p<0.001, no symbol=p>0.05). Absence of letters in the top right corner indicates the absence of that factor within the best model.

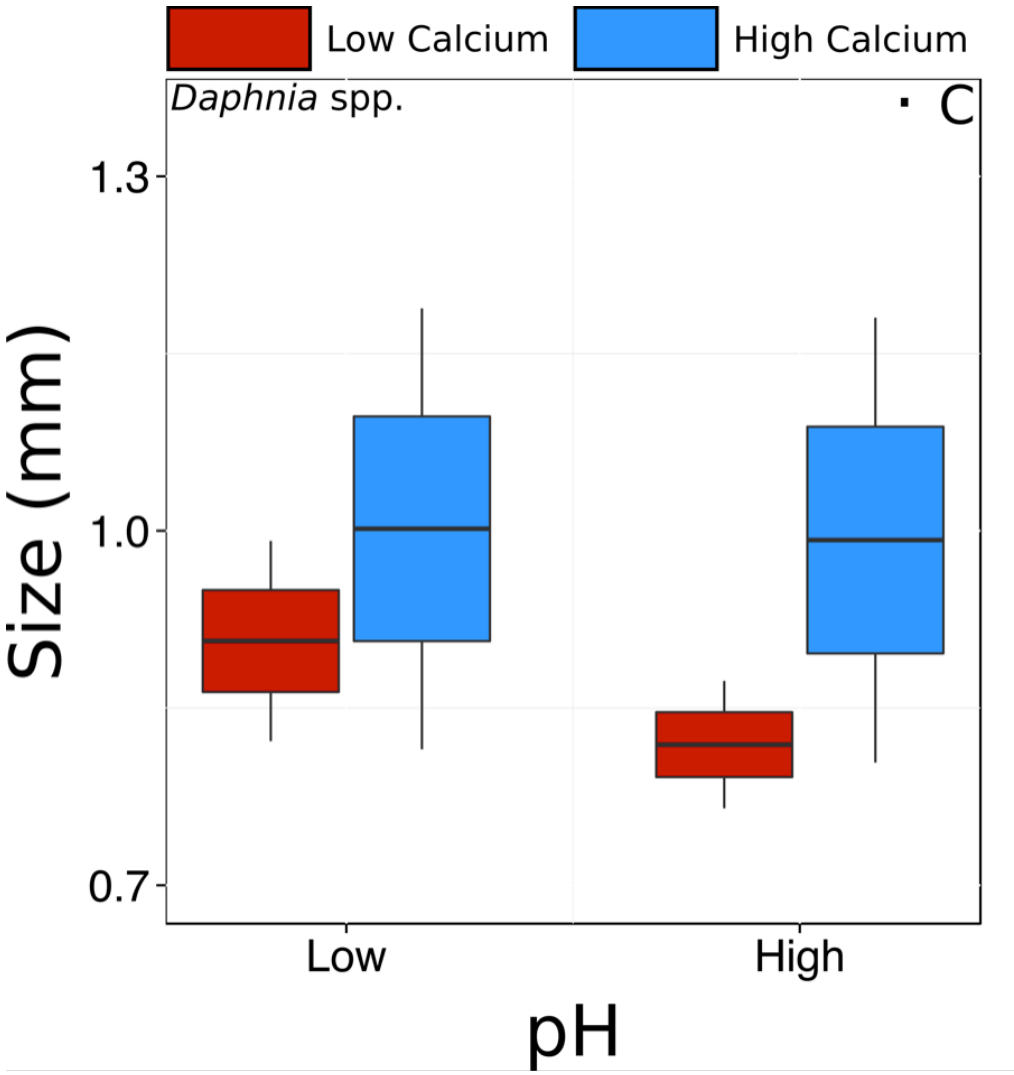

Figure 8 *Daphnia* spp. mean body size for low Ca (red; 0.9mg/L) and high Ca (blue; 2.3mg/L) treatments with low pH (5.9) and high pH (6.3) (n=5) on the final day. The thick, horizontal black line represents the mean weighted size of *Daphnia* within a treatment, with the bottom and top edges of the box representing the standard error of the mean. Whiskers extending from the box represent the 95% confidence intervals of the mean. Letters in the top right of each panel represent factors retained in the best model (C=Calcium, A= pH), along with associated significance levels (• =p<0.1, *=p<0.05, **=p<0.01, ***=p<0.001, no symbol=p>0.1). Absence of letters in the top right corner indicates the absence of that factor within the best model.

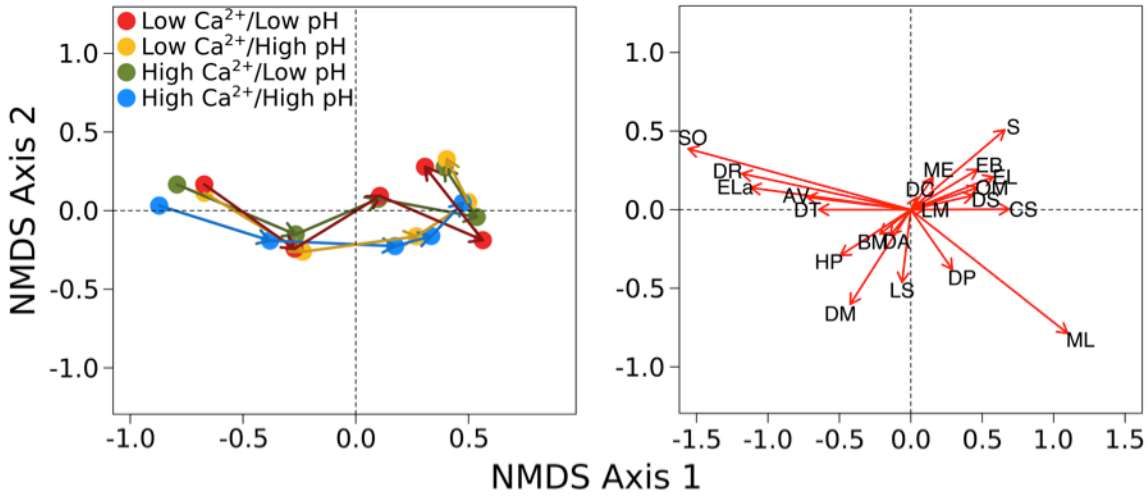

Figure 9 Non-metric Dimensional Scaling (NMDS) ordination of zooplankton assemblages in experimental treatments over the course of 5 time points (Day 0, Day 14, Day 28, Day 42, Day 56, respectively). For ease of interpretation, results are presented as a bi-plot, where treatment trajectories over time (arrows joining consecutive points) are plotted on the left panel, and species scores are plotted on the right panel. Species scores clustered around 0 for NMDS axis 1 and 2 were removed from the plot to avoid clutter. Species names are *Acanthocyclops vernalis* (AV), *Bosmina spp.* (BS), *Chydorus sphaericus* (CS), *Daphnia ambigua* (DA), *Daphnia catawba* (DC), *Daphnia mendotae* (DM), *Daphnia pulex* (DP), *Daphnia retrocurva* (DR), *Diacyclops thomasi* (DT), *Diaphanosoma spp.* (DS), *Epischura lacustris* (ELa), *Eubosmina longispina* (EL), *Eubosmina tubicen* (EB), *Holopedium* (HP), *Leptodiaptomus minutus* (LM), *Leptodiaptomus siciloides* (LS), *Mesocyclops edax* (ME), *Megacyclops latipes* (ML), *Orthocyclops modestus* (OM), *Sida crystallina* (S), *Skistodiaptomus oregonensis* (SO).

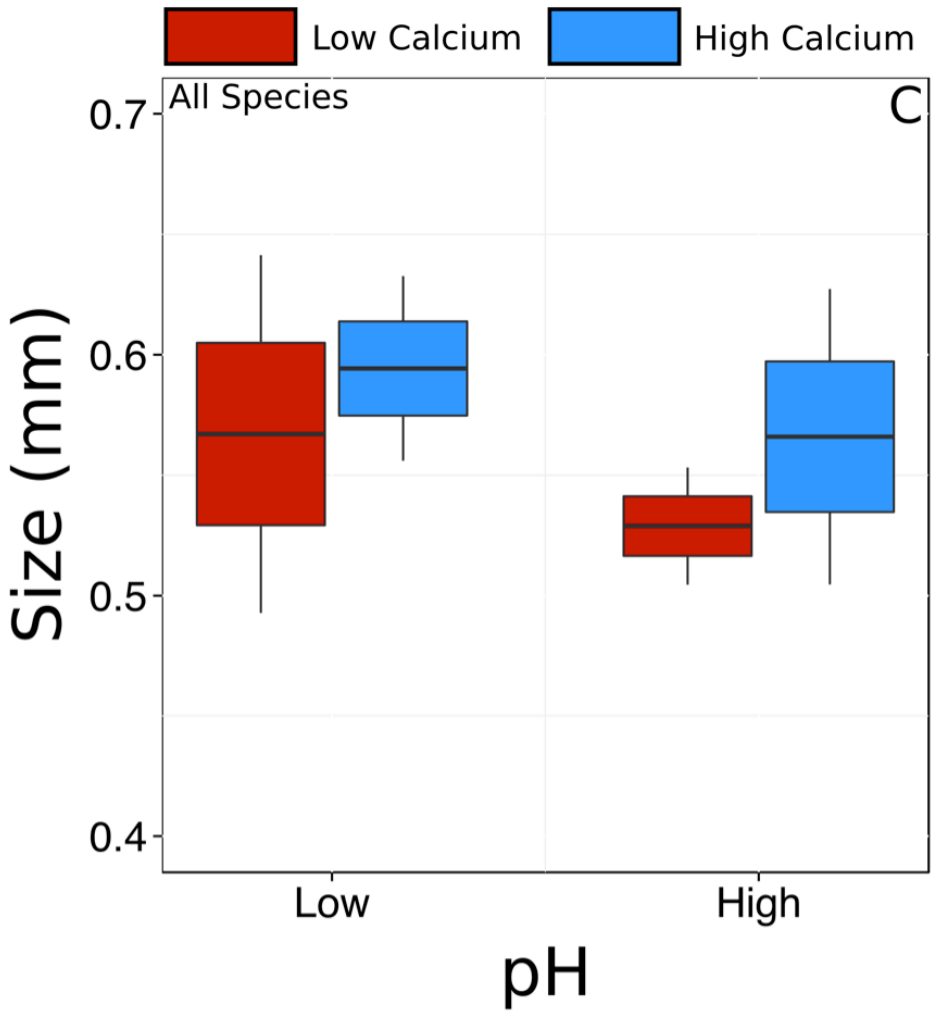

Figure 10 Total crustacean zooplankton mean body size for low Ca (red; 0.9mg/L) and high Ca (blue; 2.3mg/L) treatments with low pH (5.9) and high pH (6.3) (n=5). The thick, horizontal black line represents the mean weighted size of zooplankton within a treatment, with the bottom and top edges of the box representing the standard error of the mean. Whiskers extending from the box represent the 95% confidence intervals of the mean. Letters in the top right of each panel represent factors retained in the best model (C=Calcium, A= pH), along with associated significance levels (*=p<0.05, **=p<0.01, ***=p<0.001, no symbol=p>0.05). Absence of letters in the top right corner indicates the absence of that factor within the best model.

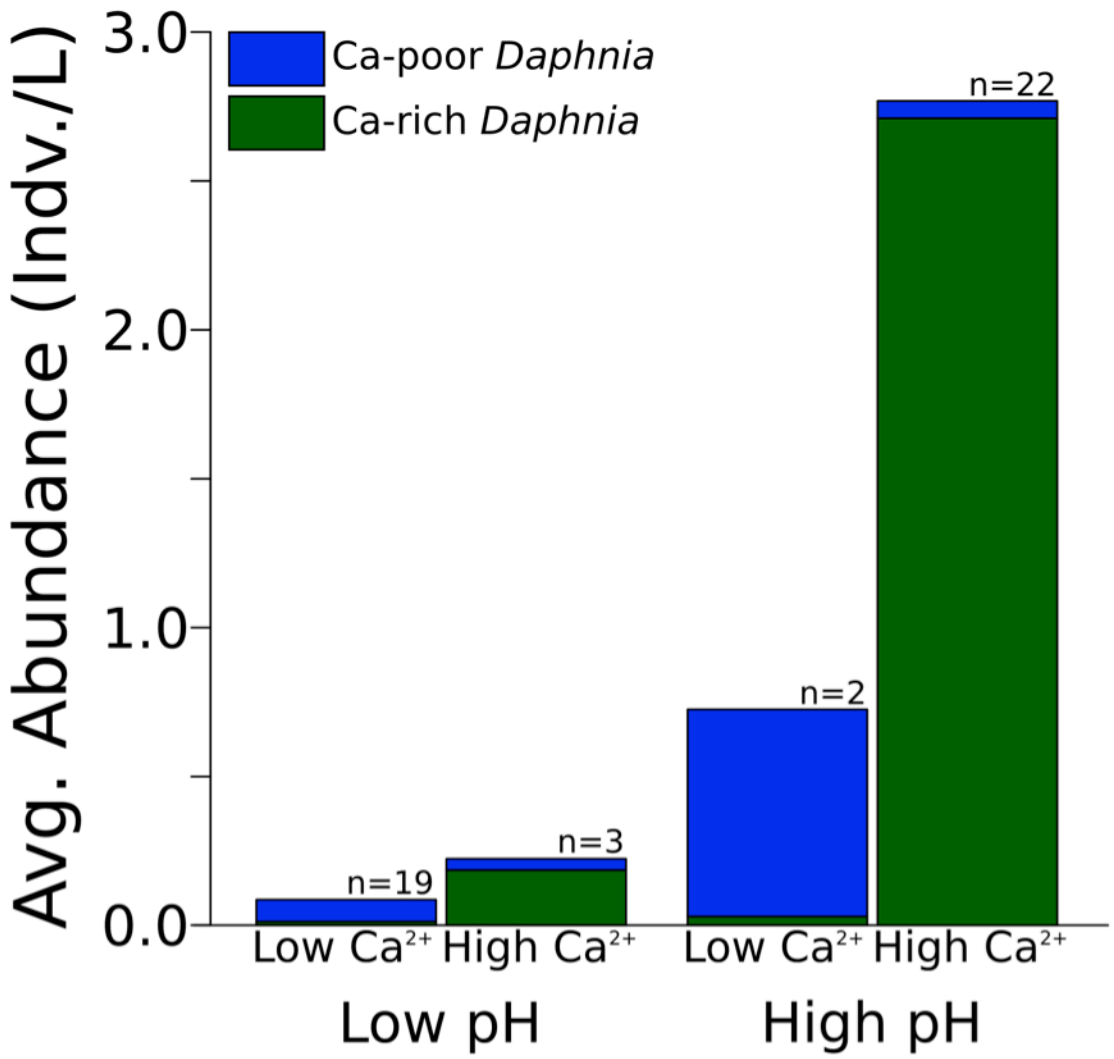

Figure 11 Average abundance of calcium-poor daphniids (blue bars; *D. catawba, D. ambigua*), and calcium-rich daphniids (green bars; *D. pulex/pulicaria, D. mendotae, D. retrocurva, D. longiremis, D. dubia*) for a set of 45 lakes in Killarney Provincial Park sampled in 2011. Lakes are grouped by 2011 water chemistry; low calcium <1.5 mg/L, high calcium >1.5mg/L; low pH <6, high pH >6. (Arnott unpublished data)

Table 1 Results for ANOVA comparing the effect of Ca (low/high), pH (low/high), and their interaction (low Ca:low pH/high Ca:high pH) on zooplankton abundance (individuals/L), species richness (# species present), and species evenness (E_{var}) for initial Day 0 communities.

Variable	**Calcium**	**pH**	**Calcium*pH**
	p	*p*	*p*
Abundance	0.76	0.27	0.11
Richness	0.39	0.54	0.22
Evenness	0.64	0.6	0.11

Chapter 3

General Discussion

Lakes in Killarney Provincial Park experienced significant acidification from nearby copper and nickel smelting operations in Sudbury, Ontario. Within a 65km radius of Sudbury nearly half of 150 lakes sampled in 1971 had lake pH<5.5, and of these 33 were critically acidified below pH 4.5 (Beamish and Harvey 1972). The result was widespread biological damage across multiple aquatic trophic levels (Beamish and Harvey 1972, Sprules 1975, Kwiatkowski and Roff 1976). With the implementation of legislative controls, acid-producing emissions have been reduced, and there has been promising recovery in lake pH (Keller et al. 1999, Gray et al. 2012). Despite increases of lake pH, studies in the Killarney area suggest that biological recovery is lagging behind chemical improvements (Gray et al. 2012, Valois 2011). To date, studies have acknowledged that biotic interactions within acid-structured zooplankton and predator communities (Binks et al. 2005, Arnott et al. 2006, Valois et al. 2010), and dispersal-limitation of potential colonists are among the factors limiting biological recovery. However, coinciding with recovery in lake pH since peak acidification, calcium concentrations have been in steady decline, and there is worry that concentrations are now approaching, or have surpassed critical biological thresholds. Disentangling the effects of calcium and pH are difficult because of strong environmental correlations, and to date no study has attempted to experimentally separate their relative effects. Understanding the relative role of pH and calcium in the biological recovery of zooplankton communities is critical for our understanding of recovery pathways in historically acidified lakes.

The primary objective of this study was to determine whether declining calcium concentrations are limiting biological recovery, and acting not only as a chemical legacy effect from acid deposition, but also as a biological legacy effect of historic acidification. Our experimental results, corroborated with a landscape survey of 45 Killarney Provincial Park lakes indicate that declining calcium does have an influence on recovering environments. Species-specific responses from the independent stressors of low calcium and low pH are quite similar in terms of species sensitive to low calcium and low pH (*Daphnia pulex*/*pulicaria*), and species tolerant to low calcium and low pH treatments (*Daphnia catawba*, *Daphnia ambigua*, *Eubosmina longispina*, *Eubosmina tubicen*, *Holopedium*, copepod species). When both stressors co-occur, effects are similar to the strength of the most severe independent effect, except for the sensitive *D. pulex*/*pulicaria*, where there is a weakly additive effect of the two stressors on populations inhabiting low calcium/low pH treatments. Our results suggest that in lakes where pH has increased above established thresholds for acid-sensitive zooplankton species (pH 6.0) that acid-structured zooplankton communities may persist should aqueous calcium concentrations decline to concentrations around 0.9mg/L.

Implications of these effects are that community size-structure in low calcium lakes may be reduced. A decline in community size structure could have ecological consequences resulting from potential bottom-up effects on predator communities, or potential top-down effects on phytoplankton communities. Qualifying this, we did not detect a change in community mean body size as declines in calcium-sensitive species were likely negated by relatively high abundances of non-responding species. However, in other regions where large daphniids are dominant in zooplankton communities, the reduction of large calcium-sensitive species may have worrisome effects. This has already been suggested in historically acidified, and declining calcium lakes in Nova Scotia where rising algal biomass has been linked to reductions of

daphniid biomass due to declining calcium (Korosi et al. 2012) – a peculiar legacy effect from acid rain to say the least.

The results of this study may have broad application to other historically acidified regions around the world. Stoddard et al. (1999) compiled a review of water chemistry from historically acidified regions, and have identified that lakes in Germany, Netherlands, Poland, Norway, Sweden, and large areas of eastern North America (Canada and United States) are all experiencing trends in declining base cations. With trends likely to persist over at least the next few decades (Watmough and Aherne 2008), the legacy effect of calcium decline may have severe global consequences on aquatic ecosystems. To date, liming programs to manipulatively augment calcium concentrations have been used in Northern Europe, but have rarely been implemented in North America. It is possible that this decision may need to be revisited by North American lake managers, despite the nearly prohibitive costs and short-term improvements associated with liming watersheds at the scale necessary (Keller et al. 1990).

Future Directions

This study provides evidence that declining calcium concentrations are likely to influence zooplankton communities, and biological recovery in historically acidified regions. However, further work needs to be completed to understand the physiological tolerances of various Canadian Shield zooplankton to low calcium. To date, much of the work on physiological tolerance to low calcium has been done using laboratory-isolated clones, and furthermore, work has focused on just two *Daphnia* species – one of which that is rarely found in softwater (*Daphnia magna*). Specifically, studies on calcium regulation (e.g. uptake, efflux) will help our understanding of why certain daphniids are more tolerant to low calcium than others. Additionally, understanding calcium regulation will be important to determine the underlying

mechanisms of sodium regulation in low pH and low calcium environments. By improving our knowledge on physiological constraints of zooplankton to low calcium, we can improve our understanding of interspecific competition in low calcium environments. This would be especially useful for determining whether species have higher physiological tolerance, or are simply better competitors at low calcium.

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Appendix A

Model Selection for Species Abundance, Richness, Evenness (E_{var}), and Size Structure – Dredge Tables

Table A.1: Dredge result for total community abundance (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(TA ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
5	**3.516**			**0.003265**					**4**	**-8.309**	**25.2**	**0**	**0.114**
16	3.457	+	+	0.003265	+				7	-4.897	25.3	0.2	0.104
7	3.565		+	0.003265					5	-7.442	25.7	0.54	0.087
6	3.467	+		0.003265					5	-7.445	25.7	0.55	0.087
8	3.516	+	+	0.003265					6	-6.496	26.1	0.99	0.07
1	3.63								3	-9.955	26.2	1.08	0.067
12	3.571	+	+		+				6	-6.544	26.2	1.09	0.066
48	3.388	+	+	0.005229	+		+		8	-4.278	26.6	1.43	0.056
3	3.679		+						4	-9.088	26.7	1.56	0.052
2	3.582	+							4	-9.091	26.7	1.57	0.052
39	3.496		+	0.005229			+		6	-6.823	26.8	1.65	0.05
4	3.631	+	+						5	-8.143	27.1	1.95	0.043
40	3.448	+	+	0.005229			+		7	-5.877	27.3	2.16	0.039
32	3.47	+	+	0.002892	+	+			8	-4.875	27.8	2.63	0.031
22	3.481	+		0.002892		+			6	-7.423	28	2.85	0.028
24	3.529	+	+	0.002892		+			7	-6.474	28.5	3.35	0.021
64	3.401	+	+	0.004857	+	+	+		9	-4.255	29.1	3.93	0.016
56	3.461	+	+	0.004857		+	+		8	-5.855	29.7	4.59	0.012
128	3.387	+	+	0.005257	+	+	+	+	10	-4.23	31.6	6.5	0.004

Random terms (all models)
'1|ENCL'

Table A.2: Dredge result for copepod abundance (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(TA ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
3	**1.145**		**+**						**4**	**-6.48**	**21.5**	**0**	**0.258**
1	1.204								3	-7.872	22.1	0.57	0.194
4	1.117	+	+						5	-6.139	23.1	1.6	0.116
2	1.176	+							4	-7.577	23.7	2.19	0.086
7	1.141		+	9.56E-05					5	-6.478	23.8	2.27	0.083
5	1.2			9.56E-05					4	-7.871	24.3	2.78	0.064
12	1.095	+	+		+				6	-5.932	25	3.52	0.044
8	1.113	+	+	9.56E-05					6	-6.137	25.4	3.93	0.036
6	1.172	+		9.56E-05					5	-7.575	26	4.47	0.028
39	1.16		+	-4.49E-04			+		6	-6.431	26	4.52	0.027
16	1.092	+	+	9.56E-05	+				7	-5.931	27.4	5.92	0.013
24	1.152	+	+	-1.01E-03		+			7	-5.944	27.4	5.95	0.013
40	1.132	+	+	-4.49E-04			+		7	-6.091	27.7	6.24	0.011
22	1.211	+		-1.01E-03		+			6	-7.382	27.9	6.42	0.01
32	1.13	+	+	-1.01E-03	+	+			8	-5.738	29.5	8.01	0.005
48	1.111	+	+	-4.49E-04	+		+		8	-5.884	29.8	8.3	0.004
56	1.171	+	+	-1.55E-03		+	+		8	-5.897	29.8	8.33	0.004
64	1.149	+	+	-1.55E-03	+	+	+		9	-5.691	32	10.46	0.001
128	1.187	+	+	-2.63E-03	+	+	+	+	10	-5.506	34.2	12.71	0

Random terms (all models)
'1|ENCL'

Table A.3: Dredge result for cladoceran abundance (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(TA ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
3	**2.855**		**+**						**4**	**-46.294**	**101.1**	**0**	**0.163**
4	2.79	+	+						5	-45.384	101.6	0.46	0.13
7	2.715		+	0.003992					5	-45.432	101.7	0.55	0.124
8	2.65	+	+	0.003992					6	-44.517	102.2	1.06	0.096
12	2.728	+	+		+				6	-44.518	102.2	1.06	0.096
39	2.574		+	0.008023			+		6	-44.528	102.2	1.08	0.095
40	2.509	+	+	0.008023			+		7	-43.612	102.8	1.66	0.071
16	2.588	+	+	0.003992	+				7	-43.631	102.8	1.7	0.07
48	2.447	+	+	0.008023	+		+		8	-42.707	103.4	2.32	0.051
24	2.701	+	+	0.002541		+			7	-44.401	104.4	3.24	0.032
56	2.56	+	+	0.006571		+	+		8	-43.492	105	3.89	0.023
32	2.639	+	+	0.002541	+	+			8	-43.513	105.1	3.93	0.023
64	2.498	+	+	0.006571	+	+	+		9	-42.586	105.7	4.62	0.016
128	2.504	+	+	0.006398	+	+	+	+	10	-42.584	108.4	7.24	0.004
1	2.656								3	-52.262	110.8	9.72	0.001
5	2.517			0.003992					4	-51.4	111.3	10.21	0.001
2	2.591	+							4	-51.768	112.1	10.95	0.001
6	2.451	+		0.003992					5	-50.906	112.6	11.5	0.001
22	2.502	+		0.002541		+			6	-50.791	114.7	13.61	0

Random terms (all models)
'1|ENCL'

Table A.4: Dredge result for *Eubosmina tubicen* abundance (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(TA ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
7	**0.3821**		**+**	**0.036**					**5**	**-94.86**	**200.5**	**0**	**0.214**
24	0.6522	+	+	0.025		+			7	-92.60	200.8	0.23	0.19
39	0.175		+	0.042			+		6	-94.31	201.8	1.24	0.115
8	0.2909	+	+	0.036					6	-94.33	201.8	1.27	0.113
56	0.4451	+	+	0.031		+	+		8	-92.02	202.1	1.54	0.099
40	0.08382	+	+	0.042			+		7	-93.77	203.1	2.56	0.059
32	0.6458	+	+	0.025	+	+			8	-92.60	203.2	2.7	0.055
16	0.2845	+	+	0.036	+				7	-94.32	204.2	3.67	0.034
64	0.4387	+	+	0.031	+	+	+		9	-92.02	204.6	4.07	0.028
5	0.1492			0.036					4	-98.18	204.9	4.36	0.024
128	0.1786	+	+	0.039	+	+	+	+	10	-91.08	205.3	4.82	0.019
22	0.4194	+		0.025		+			6	-96.10	205.4	4.83	0.019
48	0.07739	+	+	0.042	+		+		8	-93.77	205.6	5.03	0.017
6	0.05807	+		0.036					5	-97.69	206.2	5.65	0.013
3	1.632		+						4	-111.02	230.6	30.05	0
4	1.541	+	+						5	-110.67	232.1	31.62	0
1	1.399								3	-113.27	232.9	32.32	0
2	1.308	+							4	-112.93	234.4	33.87	0
12	1.534	+	+		+				6	-110.67	234.5	33.95	0

Random terms (all models)
'1|ENCL'

Table A.5: Dredge result for *Eubosmina longispina* abundance (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(TA ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
5	0.008322			0.03671					4	-98.74	206	0	0.202
16	-0.1248	+	+	0.03671	+				7	-95.344	206.2	0.23	0.18
7	**0.1619**		**+**	**0.03671**					**5**	**-97.732**	**206.3**	**0.26**	**0.177**
6	-0.07972	+		0.03671					5	-98.42	207.6	1.64	0.089
8	0.07382	+	+	0.03671					6	-97.376	207.9	1.89	0.079
32	-0.05597	+	+	0.03471	+	+			8	-95.282	208.6	2.58	0.056
39	0.1463		+	0.03715			+		6	-97.728	208.6	2.59	0.055
48	-0.1403	+	+	0.03721	+		+		8	-95.341	208.7	2.7	0.053
22	-0.01091	+		0.03471		+			6	-98.358	209.9	3.85	0.029
24	0.1426	+	+	0.03471		+			7	-97.315	210.2	4.17	0.025
40	0.05826	+	+	0.03721			+		7	-97.373	210.3	4.29	0.024
64	-0.07152	+	+	0.03522	+	+	+		9	-95.279	211.1	5.12	0.016
128	0.1412	+	+	0.02910	+	+	+	+	10	-94.683	212.6	6.54	0.008
56	0.1271	+	+	0.03522		+	+		8	-97.312	212.7	6.64	0.007
1	1.293								3	-114.962	236.2	30.23	0
3	1.447		+						4	-114.042	236.6	30.6	0
12	1.16	+	+		+				6	-112.134	237.4	31.41	0
2	1.205	+							4	-114.662	237.9	31.84	0
4	1.359	+	+						5	-113.735	238.3	32.27	0

Random terms (all models)
'1|ENCL'

Table A.6: Dredge result for *Daphnia pulex/pulicaria* abundance (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(TA ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
4	**0.03991**	+	+						**5**	**102.377**	**-193.9**	**0**	**0.23**
12	0.04852	+	+		+				6	103.037	-192.9	1.02	0.138
8	0.05871	+	+	-0.00053					6	103.007	-192.9	1.08	0.134
16	0.06732	+	+	-0.00053	+				7	103.677	-191.8	2.15	0.079
2	0.05687	+							4	99.927	-191.3	2.62	0.062
40	0.07186	+	+	-0.00091			+		7	103.318	-191.1	2.86	0.055
3	0.05764		+						4	99.718	-190.9	3.04	0.05
24	0.0652	+	+	-0.00072		+			7	103.082	-190.6	3.33	0.043
6	0.07568	+		-0.00054					5	100.53	-190.2	3.69	0.036
48	0.08046	+	+	-0.00091	+		+		8	103.994	-190	3.98	0.031
7	0.07644		+	-0.00054					5	100.321	-189.8	4.11	0.029
32	0.0738	+	+	-0.00072	+	+			8	103.754	-189.5	4.46	0.025
1	0.0746								3	97.699	-189.1	4.86	0.02
56	0.07834	+	+	-0.0011		+	+		8	103.394	-188.8	5.18	0.017
39	0.08959		+	-0.00091			+		6	100.62	-188.1	5.86	0.012
5	0.09341			-0.00054					4	98.302	-188.1	5.87	0.012
22	0.08216	+		-0.00072		+			6	100.602	-188.1	5.89	0.012
64	0.08695	+	+	-0.0011	+	+	+		9	104.071	-187.6	6.37	0.01
128	0.07741	+	+	-0.00083	+	+	+	+	10	104.239	-185.3	8.65	0.003

Random terms (all models)
'1|ENCL'

Table A.7: Dredge result for *Daphnia catawba* abundance (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(TA ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
3	**0.381**		**+**						**4**	**13.392**	**-18.3**	**0**	**0.209**
1	0.3398								3	11.878	-17.4	0.81	0.139
4	0.4069	+	+						5	14.038	-17.3	0.99	0.128
7	0.3371		+	0.001255					5	13.762	-16.7	1.54	0.097
2	0.3657	+							4	12.45	-16.4	1.88	0.082
5	0.2959			0.001255					4	12.247	-16	2.29	0.067
8	0.363	+	+	0.001255					6	14.414	-15.7	2.57	0.058
12	0.4006	+	+		+				6	14.078	-15	3.25	0.041
39	0.2995		+	0.00233			+		6	14.036	-14.9	3.33	0.04
6	0.3218	+		0.001255					5	12.819	-14.8	3.42	0.038
40	0.3254	+	+	0.00233			+		7	14.693	-13.8	4.42	0.023
24	0.3291	+	+	0.002222		+			7	14.639	-13.7	4.53	0.022
16	0.3566	+	+	0.001255	+				7	14.454	-13.4	4.9	0.018
22	0.288	+		0.002222		+			6	13.041	-12.9	5.32	0.015
56	0.2915	+	+	0.003297		+	+		8	14.919	-11.8	6.44	0.008
48	0.319	+	+	0.00233	+		+		8	14.733	-11.4	6.81	0.007
32	0.3228	+	+	0.002222	+	+			8	14.68	-11.3	6.92	0.007
64	0.2851	+	+	0.003297	+	+	+		9	14.96	-9.3	8.9	0.002
128	0.296	+	+	0.002988	+	+	+	+	10	14.983	-6.8	11.47	0.001

Random terms (all models)
'1|ENCL'

Table A.8: Dredge result for *Daphnia ambigua* abundance (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(TA ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
8	**0.4459**	**+**	**+**	**-0.005435**					**6**	**17.951**	**-22.8**	**0**	**0.246**
40	0.4988	+	+	-0.006948			+		7	18.555	-21.6	1.2	0.135
16	0.4291	+	+	-0.005435	+				7	18.254	-21	1.8	0.1
7	0.4909		+	-0.005435					5	15.837	-20.9	1.89	0.096
24	0.4238	+	+	-0.004805		+			7	18.055	-20.6	2.2	0.082
6	0.3984	+		-0.005435					5	15.601	-20.4	2.36	0.076
48	0.482	+	+	-0.006948	+		+		8	18.863	-19.7	3.05	0.053
39	0.5439		+	-0.006948			+		6	16.41	-19.7	3.08	0.053
56	0.4768	+	+	-0.006317		+	+		8	18.661	-19.3	3.46	0.044
5	0.4434			-0.005435					4	13.649	-18.8	3.99	0.034
32	0.407	+	+	-0.004805	+	+			8	18.359	-18.7	4.06	0.032
22	0.3763	+		-0.004805		+			6	15.7	-18.2	4.5	0.026
64	0.46	+	+	-0.006317	+	+	+		9	18.97	-17.4	5.38	0.017
128	0.4611	+	+	-0.006349	+	+	+	+	10	18.97	-14.8	8	0.005
4	0.2557	+	+						5	10.871	-10.9	11.82	0.001
3	0.3007		+						4	9.093	-9.7	13.1	0
2	0.2081	+							4	8.893	-9.3	13.5	0
12	0.2389	+	+		+				6	11.125	-9.1	13.65	0
1	0.2532								3	7.199	-8.1	14.67	0

Random terms (all models)
'1|ENCL'

Table A.9: Dredge result for *Holopedium* abundance (Day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(TA ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
6	2.77E+00	+		-4.58E-02					5	-69.145	149.1	0	0.204
22	**2.55E+00**	**+**		**-3.97E-02**		**+**			**6**	**-68.031**	**149.2**	**0.11**	**0.193**
8	2.85E+00	+	+	-4.58E-02					6	-68.191	149.5	0.43	0.164
24	2.64E+00	+	+	-3.97E-02		+			7	-67.051	149.7	0.56	0.154
16	2.89E+00	+	+	-4.58E-02	+				7	-68.06	151.7	2.58	0.056
40	2.80E+00	+	+	-4.44E-02			+		7	-68.129	151.8	2.71	0.053
32	2.67E+00	+	+	-3.97E-02	+	+			8	-66.916	151.9	2.76	0.051
56	2.59E+00	+	+	-3.83E-02		+	+		8	-66.986	152	2.9	0.048
5	2.59E+00			-4.58E-02					4	-72.615	153.8	4.66	0.02
48	2.84E+00	+	+	-4.44E-02	+		+		8	-67.997	154	4.92	0.017
64	2.62E+00	+	+	-3.83E-02	+	+	+		9	-66.851	154.3	5.17	0.015
7	2.68E+00		+	-0.04582					5	-71.741	154.3	5.19	0.015
39	2.63E+00		+	-4.44E-02			+		6	-71.684	156.5	7.42	0.005
128	2.64E+00	+	+	-3.89E-02	+	+	+	+	10	-66.839	156.9	7.77	0.004
2	1.16E+00	+							4	-106.74	222	72.91	0
1	9.88E-01								3	-108.131	222.6	73.48	0
4	1.25E+00	+	+						5	-106.37	223.6	74.45	0
3	1.08E+00		+						4	-107.774	224.1	74.98	0
12	1.28E+00	+	+		+				6	-106.32	225.8	76.69	0

Random terms (all models)
'1|ENCL'

Table A.10: Dredge result for *Mesocyclops edax* abundance (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(TA ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
6	**6.07E-02**	**+**		**3.52E-03**					**5**	**61.101**	**-111.4**	**0**	**0.26**
40	9.46E-02	+	+	2.21E-03			+		7	62.939	-110.3	1.07	0.152
22	2.98E-02	+		4.40E-03		+			6	61.708	-110.3	1.12	0.148
8	4.86E-02	+	+	3.52E-03					6	61.568	-110	1.41	0.129
56	6.37E-02	+	+	3.09E-03		+	+		8	63.576	-109.1	2.27	0.084
24	1.76E-02	+	+	4.40E-03		+			7	62.182	-108.8	2.58	0.072
48	9.33E-02	+	+	2.21E-03	+		+		8	62.945	-107.9	3.53	0.045
16	4.72E-02	+	+	3.52E-03	+				7	61.573	-107.6	3.8	0.039
64	6.23E-02	+	+	3.09E-03	+	+	+		9	63.582	-106.6	4.8	0.024
32	1.63E-02	+	+	4.40E-03	+	+			8	62.188	-106.3	5.04	0.021
5	2.16E-02			3.52E-03					4	56.707	-104.9	6.51	0.01
128	7.80E-02	+	+	0.002642	+	+	+	+	10	63.746	-104.3	7.09	0.008
39	5.54E-02		+	2.21E-03			+		6	58.354	-103.6	7.83	0.005
7	9.40E-03		+	3.52E-03					5	57.069	-103.3	8.06	0.005
2	1.84E-01	+							4	52.537	-96.5	14.85	0
4	1.72E-01	+	+						5	52.913	-95	16.37	0
12	1.70E-01	+	+		+				6	52.918	-92.7	18.71	0
1	1.45E-01								3	48.815	-91.3	20.08	0
3	1.33E-01		+						4	49.157	-89.8	21.61	0

Random terms (all models)
'1|ENCL'

Table A.11: Dredge result for *Holopedium* abundance on Day 56. Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – glm.nb(Abundance ~ Calcium*Acid)

Model	Intercept	Acid	Calcium	Acid:Calcium	df	logLik	AICc	Delta	Weight
4	**7.847**	+	+		**4**	**-142.228**	**295.1**	**0**	**0.555**
2	7.281	+			3	-144.543	296.6	1.46	0.267
8	7.545	+	+	+	5	-141.607	297.5	2.38	0.169
1	6.711				2	-149.666	304	8.92	0.006
3	7.009		+		3	-149.042	305.6	10.46	0.003

Table A.12: Dredge result for species richness of total crustacean zooplankton (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(SpeciesRichness ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
1	2.5430								3	49.122	-91.9	0	0.266
3	**2.5220**		**+**						**4**	**50.176**	**-91.8**	**0.11**	**0.251**
5	2.5460			-0.0001					4	49.126	-89.7	2.21	0.088
2	2.5420	+							4	49.123	-89.7	2.22	0.088
7	2.5250		+	-0.0001					5	50.18	-89.5	2.38	0.081
4	2.5210	+	+						5	50.177	-89.5	2.39	0.081
6	2.5450	+		-0.0001					5	49.127	-87.4	4.49	0.028
12	2.5240	+	+		+				6	50.196	-87.2	4.69	0.026
39	2.5190		+	0.0001			+		6	50.193	-87.2	4.69	0.025
8	2.5240	+	+	-0.0001					6	50.181	-87.2	4.72	0.025
22	2.5300	+		0.0004		+			6	49.234	-85.3	6.61	0.01
24	2.5090	+	+	0.0004		+			7	50.291	-85	6.9	0.008
16	2.5270	+	+	-0.0001	+				7	50.2	-84.8	7.08	0.008
40	2.5190	+	+	0.0001			+		7	50.194	-84.8	7.1	0.008
32	2.5120	+	+	0.0004	+	+			8	50.31	-82.6	9.34	0.002
56	2.5030	+	+	0.0005		+	+		8	50.305	-82.6	9.35	0.002
48	2.5210	+	+	0.0001	+		+		8	50.214	-82.4	9.53	0.002
64	2.5060	+	+	0.0005	+	+	+		9	50.324	-80.1	11.85	0.001
128	2.5250	+	+	0.0000	+	+	+	+	10	50.492	-77.8	14.13	0

Random terms (all models)
‘1|ENCL'

Table A.13: Dredge result for community evenness of total crustacean zooplankton (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(E_{var}) ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
7	**0.2041**		**+**	**0.0009**					**5**	**111.095**	**-211.4**	**0**	**0.203**
5	0.2161			0.0009					4	109.552	-210.6	0.81	0.135
3	0.2339		+						4	109.184	-209.8	1.55	0.094
39	0.2166		+	0.0005			+		6	111.439	-209.7	1.65	0.089
8	0.2091	+	+	0.0009					6	111.368	-209.6	1.79	0.083
1	0.2459								3	107.711	-209.1	2.27	0.065
6	0.2210	+		0.0009					5	109.814	-208.8	2.56	0.056
24	0.1917	+	+	0.0013		+			7	112.04	-208.5	2.86	0.049
4	0.2389	+	+						5	109.444	-208.1	3.3	0.039
40	0.2215	+	+	0.0005			+		7	111.714	-207.9	3.51	0.035
22	0.2037	+		0.0013		+			6	110.46	-207.8	3.61	0.033
2	0.2509	+							4	107.961	-207.4	3.99	0.028
16	0.2089	+	+	0.0009	+				7	111.368	-207.2	4.2	0.025
56	0.2042	+	+	0.0010		+	+		8	112.391	-206.8	4.63	0.02
32	0.1916	+	+	0.0013	+	+			8	112.04	-206.1	5.33	0.014
12	0.2388	+	+		+				6	109.444	-205.7	5.64	0.012
48	0.2214	+	+	0.0005	+		+		8	111.714	-205.4	5.98	0.01
64	0.2040	+	+	0.0010	+	+	+		9	112.392	-204.2	7.17	0.006
128	0.2229	+	+	0.0005	+	+	+	+	10	113.21	-203.2	8.15	0.003

Random terms (all models)
'1|ENCL'

Table A.14: Dredge result for copepod species richness (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(SpeciesRichness ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
7	**1.6260**		**+**	**-0.0028**					**5**	**29.882**	**-49**	**0**	**0.345**
39	1.5980		+	-0.0021			+		6	30.107	-47.1	1.89	0.134
8	1.6200	+	+	-0.0028					6	29.932	-46.7	2.24	0.112
5	1.6680			-0.0028					4	27.395	-46.3	2.7	0.09
3	1.5270		+						4	27.113	-45.7	3.26	0.067
40	1.5930	+	+	-0.0021			+		7	30.157	-44.8	4.19	0.042
16	1.6290	+	+	-0.0028	+				7	30.034	-44.5	4.44	0.037
24	1.6240	+	+	-0.0030		+			7	29.936	-44.3	4.64	0.034
6	1.6630	+		-0.0028					5	27.442	-44.1	4.88	0.03
4	1.5210	+	+						5	27.159	-43.5	5.45	0.023
1	1.5690								3	24.788	-43.3	5.69	0.02
48	1.6010	+	+	-0.0021	+		+		8	30.259	-42.5	6.46	0.014
56	1.5960	+	+	-0.0022		+	+		8	30.161	-42.3	6.66	0.012
32	1.6330	+	+	-0.0030	+	+			8	30.038	-42	6.91	0.011
22	1.6660	+		-0.0030		+			6	27.446	-41.7	7.21	0.009
12	1.5290	+	+		+				6	27.254	-41.4	7.6	0.008
2	1.5630	+							4	24.831	-41.1	7.82	0.007
64	1.6050	+	+	-0.0022	+	+	+		9	30.264	-40	9	0.004
128	1.6150	+	+	-0.0025	+	+	+	+	10	30.297	-37.4	11.55	0.001

Random terms (all models)
‘1|ENCL'

Table A.15: Dredge result for copepod evenness (E_{var}) (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – GLMM(E_{var} ~ Calcium*Acid*Day +(1|ENCL)): negative binomial distribution

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
6	**0.2461**	**+**		**0.003241**					**5**	**58.463**	**-106.1**	**0**	**0.268**
5	0.2178			0.003241					4	56.766	-105	1.12	0.153
22	0.2284	+		0.003745		+			6	58.663	-104.2	1.94	0.101
16	0.2751	+	+	0.003241	+				7	59.824	-104.1	2.02	0.097
8	0.2526	+	+	0.003241					6	58.562	-104	2.14	0.092
7	0.2243		+	0.003241					5	56.849	-102.9	3.23	0.053
48	0.3032	+	+	0.00244	+		+		8	60.33	-102.6	3.48	0.047
40	0.2806	+	+	0.00244			+		7	59.068	-102.6	3.53	0.046
32	0.2575	+	+	0.003745	+	+			8	60.024	-102	4.1	0.035
24	0.2349	+	+	0.003745		+			7	58.762	-102	4.15	0.034
39	0.2524		+	0.00244			+		6	57.356	-101.6	4.55	0.027
56	0.263	+	+	0.002944		+	+		8	59.271	-100.5	5.6	0.016
64	0.2855	+	+	0.002944	+	+	+		9	60.533	-100.5	5.62	0.016
128	0.2445	+	+	0.004116	+	+	+	+	10	61.654	-100.1	6	0.013
2	0.3595	+							4	51.202	-93.9	12.24	0.001
1	0.3312								3	49.505	-92.7	13.42	0
12	0.3885	+	+		+				6	52.551	-92	14.16	0
4	0.366	+	+						5	51.301	-91.8	14.32	0
3	0.3378		+						4	49.589	-90.6	15.47	0

Random terms (all models)
'1|ENCL'

Table A.16: Dredge result for cladoceran species richness (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(SpeciesRichness ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
1	**2.1460**								**3**	**42.241**	**-78.2**	**0**	**0.352**
2	2.1320	+							4	42.634	-76.7	1.43	0.172
5	2.1370			0.0003					4	42.275	-76	2.15	0.12
3	2.1460		+						4	42.242	-75.9	2.22	0.116
6	2.1230	+		0.0003					5	42.668	-74.5	3.64	0.057
4	2.1320	+	+						5	42.634	-74.5	3.71	0.055
7	2.1370		+	0.0003					5	42.275	-73.7	4.43	0.038
22	2.1080	+		0.0007		+			6	42.753	-72.4	5.81	0.019
8	2.1230	+	+	0.0003					6	42.668	-72.2	5.98	0.018
12	2.1350	+	+		+				6	42.65	-72.1	6.02	0.017
39	2.1330		+	0.0004			+		6	42.28	-71.4	6.76	0.012
24	2.1080	+	+	0.0007		+			7	42.753	-70	8.22	0.006
16	2.1260	+	+	0.0003	+				7	42.683	-69.8	8.36	0.005
40	2.1190	+	+	0.0004			+		7	42.673	-69.8	8.38	0.005
32	2.1110	+	+	0.0007	+	+			8	42.769	-67.5	10.66	0.002
56	2.1040	+	+	0.0008		+	+		8	42.759	-67.5	10.68	0.002
48	2.1220	+	+	0.0004	+		+		8	42.689	-67.3	10.82	0.002
64	2.1070	+	+	0.0008	+	+	+		9	42.774	-65	13.19	0
128	2.1060	+	+	0.0008	+	+	+	+	10	42.775	-62.4	15.81	0

Random terms (all models)
‘1|ENCL'

Table A.17: Dredge result for cladoceran evenness (E_{var}) (day 0 removed). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – GLMM(E_{var} ~ Calcium*Acid*Day +(1|ENCL)): negative binomial distribution

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
7	**0.168**		**+**	**0.001492**					**5**	**69.541**	**-128.3**	**0**	**0.257**
24	0.1303	+	+	0.002694		+			7	71.017	-126.5	1.79	0.105
3	0.2202		+						4	67.474	-126.4	1.86	0.102
39	0.1828		+	0.001068			+		6	69.712	-126.3	2	0.095
8	0.1723	+	+	0.001492					6	69.615	-126.1	2.19	0.086
5	0.1938			0.001492					4	67.032	-125.5	2.74	0.065
56	0.1451	+	+	0.00227		+	+		8	71.195	-124.4	3.91	0.036
4	0.2246	+	+						5	67.545	-124.3	3.99	0.035
40	0.1872	+	+	0.001068			+		7	69.787	-124	4.25	0.031
32	0.13	+	+	0.002694	+	+			8	71.017	-124	4.26	0.03
1	0.246								3	65.088	-123.9	4.41	0.028
22	0.1561	+		0.002694		+			6	68.417	-123.7	4.59	0.026
16	0.1721	+	+	0.001492	+				7	69.615	-123.7	4.6	0.026
6	0.1982	+		0.001492					5	67.102	-123.4	4.88	0.022
128	0.1913	+	+	0.0009416	+	+	+	+	10	72.985	-122.8	5.49	0.017
12	0.2243	+	+		+				6	67.545	-121.9	6.33	0.011
64	0.1449	+	+	0.00227	+	+	+		9	71.195	-121.8	6.45	0.01
2	0.2504	+							4	65.155	-121.8	6.49	0.01
48	0.1869	+	+	0.001068	+		+		8	69.787	-121.5	6.72	0.009

Random terms (all models)
'1|ENCL'

Table A.18: Dredge result for total zooplankton mean body size on Day 56. Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – glm(Size ~ Calcium*Acid)

Model	Intercept	Acid	Calcium	Acid:Calcium	df	logLik	AICc	Delta	Weight
3	**0.4178**		**+**		**3**	**29.593**	**-51.7**	**0**	**0.406**
1	0.3957				2	28.107	-51.5	0.18	0.372
2	0.4029	+			3	28.254	-49	2.68	0.107
4	0.425	+	+		4	29.764	-48.9	2.82	0.099
8	0.4272	+	+	+	5	29.78	-45.3	6.41	0.016

Table A.19: Dredge result for cladoceran size-structure on Day 56. Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – glm(Size ~ Calcium*Acid)

Model	Intercept	Acid	Calcium	Acid:Calcium	df	logLik	AICc	Delta	Weight
1	**0.8578**				**2**	**15.293**	**-25.9**	**0**	**0.381**
3	0.8924		+		3	16.283	-25.1	0.81	0.254
2	0.8893	+			3	16.11	-24.7	1.16	0.213
4	0.9239	+	+		4	17.189	-23.7	2.17	0.129
8	0.9347	+	+	+	5	17.302	-20.3	5.56	0.024

Table A.20: Dredge result for *Daphnia spp.* size-structure on Day 56. Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – lm(Size ~ Calcium*Acid)

Model	Intercept	Acid	Calcium	Acid:Calcium	df	logLik	AICc	Delta	Weight
3	**0.8628**		+		**3**	**9.833**	**-12.2**	**0**	**0.467**
1	0.9299				2	7.966	-11.2	0.94	0.292
4	0.8871	+	+		4	10.107	-9.5	2.62	0.126
2	0.9542	+			3	8.192	-8.9	3.28	0.091
8	0.9067	+	+	+	5	10.288	-6.3	5.88	0.025

Table A.21: Dredge result for *Daphnia pulex* mean body size on Day 56. Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – lm(Size ~ Calcium*Acid)

Model	Intercept	Ad	Ca	Ad:Ca	df	logLik	AICc	Delta	Weight
1	**1.681**				**2**	**4.952**	**-2.9**	**0**	**0.867**
2	1.603	+			3	6.3	1.4	4.3	0.101
3	1.726		+		3	5.162	3.7	6.58	0.032
4	1.655	+	+		4	6.777	14.4	17.35	0
8	1.679	+	+	+	5	6.875	56.3	59.15	0

Table A.22: Dredge result for *Daphnia catawba* mean body size on Day 56. Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – lm(Size ~ Calcium*Acid)

Model	Intercept	Ad	Ca	Ad:Ca	df	logLik	AICc	Delta	Weight
1	**0.9674**				**2**	**3.785**	**-2.9**	**0**	**0.501**
2	1.019	+			3	4.471	-1.4	1.42	0.246
3	0.932		+		3	4.101	-0.7	2.16	0.17
4	0.9836	+	+		4	4.811	1	3.91	0.071
8	0.9708	+	+	+	5	4.856	4.6	7.44	0.012

Table A.23: Dredge result for *Daphnia ambigua* mean body size on Day 56. Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – lm(Size ~ Calcium*Acid)

Model	Intercept	Ad	Ca	Ad:Ca	df	logLik	AICc	Delta	Weight
1	**0.9674**				**2**	**3.785**	**-2.9**	**0**	**0.501**
2	1.019	+			3	4.471	-1.4	1.42	0.246
3	0.932		+		3	4.101	-0.7	2.16	0.17
4	0.9836	+	+		4	4.811	1	3.91	0.071
8	0.9708	+	+	+	5	4.856	4.6	7.44	0.012

Appendix B

Model Selection for Landscape Comparison – Dredge Tables

Table B.1: Dredge result for *Daphnia spp.* abundance in a 2011 landscape survey of 45 Killarney Provincial Park lakes. Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – glm.nb(DaphAbun ~ Calcium*Acid) – general linear model; negative binomial distribution

Model	Intercept	Ad	Ca	Ad:Ca	df	logLik	AICc	Delta	Weight
2	**7.857**	**+**			**3**	**-276.81**	**560.2**	**0**	**0.548**
4	7.907	+	+		4	-276.23	561.5	1.27	0.291
8	7.926	+	+	+	5	-276.213	564	3.78	0.083
3	7.798		+		3	-278.756	564.1	3.89	0.078
1	7.203				2	-284.832	574	13.74	0.001

Table B.2: Dredge result for calcium-poor *Daphnia spp.* abundance in a 2011 landscape survey of 45 Killarney Provincial Park lakes. Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – glm.nb(DaphAbun ~ Calcium*Acid) – general linear model; negative binomial distribution

Model	Intercept	Ad	Ca	Ad:Ca	df	logLik	AICc	Delta	Weight
1	5.027				3	-159.497	325.6	0	0.352
3	**4.48**		**+**		**4**	**-158.332**	**325.7**	**0.1**	**0.335**
4	4.632	+	+		5	-157.569	326.7	1.12	0.2
2	5.142	+			4	-159.42	327.9	2.27	0.113
8	4.49	+	+	+	6	-165.21	344.7	19.1	0

Table B.3: Dredge result for calcium-rich *Daphnia spp.* abundance in a 2011 landscape survey of 45 Killarney Provincial Park lakes. Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – glm.nb(DaphAbun ~ Calcium*Acid) – general linear model; negative binomial distribution

Model	Intercept	Ad	Ca	Ad:Ca	df	logLik	AICc	Delta	Weight
3	**7.775**		**+**		**3**	**-234.734**	**476.1**	**0**	**0.477**
4	7.841	+	+		4	-233.784	476.6	0.53	0.367
8	7.905	+	+	+	5	-233.505	478.6	2.52	0.135
2	7.811	+			3	-237.858	482.3	6.25	0.021
1	7.132				2	-246.85	498	21.92	0

Appendix C

Model Selection for Total/Edible Chlorophyll-*a* – Dredge Tables

Table C.1: Dredge result for total chlorophyll-*a* on the first day of the experiment (Day 0). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – glm(TotChl~Calcium*Acid)

Model	Intercept	Ad	Ca	Ad:Ca	df	logLik	AICc	Delta	Weight
1	**1.216**				**2**	**10.601**	**-16.5**	**0**	**0.392**
3	1.265		+		3	11.829	-16.2	0.34	0.331
2	1.188	+			3	11.003	-14.5	1.99	0.145
4	1.237	+	+		4	12.284	-13.9	2.59	0.107
8	1.214	+	+	+	5	12.593	-10.9	5.6	0.024

Table C.2: Dredge result for edible chlorophyll-*a* on the first day of the experiment (Day 0). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – glm(EdChl~Calcium*Acid)

Model	Intercept	Ad	Ca	Ad:Ca	df	logLik	AICc	Delta	Weight
1	**0.7478**				**2**	**38.513**	**-72.3**	**0**	**0.408**
2	0.76	+			3	39.796	-72.1	0.23	0.364
3	0.7458		+		3	38.543	-69.6	2.73	0.104
4	0.7581	+	+		4	39.831	-69	3.32	0.077
8	0.7696	+	+	+	5	41.128	-68	4.35	0.046

Table C.3: Dredge result for total chlorophyll-*a* over the duration of the experiment (excluding Day 0). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. The Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(log(TotChl+1) ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
5	**8.13E-01**			**9.28E-03**					**4**	**-196.095**	**400.4**	**0**	**0.397**
7	7.80E-01		+	9.28E-03					5	-195.909	402.2	1.74	0.166
6	7.98E-01	+		9.28E-03					5	-196.055	402.5	2.04	0.143
22	7.41E-01	+		1.13E-02		+			6	-195.82	404.1	3.71	0.062
8	7.65E-01	+	+	9.28E-03					6	-195.869	404.2	3.81	0.059
39	7.95E-01		+	8.75E-03			+		6	-195.893	404.3	3.85	0.058
24	7.08E-01	+	+	1.13E-02		+			7	-195.634	405.9	5.5	0.025
16	7.45E-01	+	+	9.28E-03	+				7	-195.798	406.2	5.83	0.022
40	7.80E-01	+	+	8.75E-03			+		7	-195.853	406.4	5.94	0.02
1	1.07E+00								3	-200.861	407.9	7.44	0.01
32	6.88E-01	+	+	1.13E-02	+	+			8	-195.562	408	7.55	0.009
56	7.23E-01	+	+	0.01078		+	+		8	-195.618	408.1	7.66	0.009
48	7.59E-01	+	+	8.75E-03	+		+		8	-195.782	408.4	7.99	0.007
3	1.04E+00		+						4	-200.685	409.6	9.18	0.004
2	1.06E+00	+							4	-200.824	409.9	9.46	0.004
64	7.03E-01	+	+	0.01078	+	+	+		9	-195.546	410.2	9.73	0.003
4	1.03E+00	+	+						5	-200.647	411.6	11.22	0.001
128	7.35E-01	+	+	0.009618	+	+	+	+	10	-195.469	412.2	11.82	0.001
12	1.00E+00	+	+		+				6	-200.58	413.6	13.23	0.001

Random terms (all models)
‘1|ENCL'

Table C.4: Dredge result for edible chlorophyll-*a* over the duration of the experiment (excluding Day 0). Retention of categorical variables (Ad=pH, Ca=Calcium, subsequent interactions) is represented by (+) symbol; retention of continuous variable (Day) is represented by inclusion of a number for the model coefficient. The top models where chosen from models with Delta <2. the Minimum Adequate Model (MAM) is shown in bold.
Full Model – LMM(log(TotChl+1) ~ Calcium*Acid*Day +(1|ENCL))

Model	Intercept	Ad	Ca	Day	Ad:Ca	Ad:Day	Ca:Day	Ad:Ca:Day	df	logLik	AICc	Delta	Weight
5	7.68E-01			3.35E-03					4	-138.175	284.6	0	0.203
1	**8.62E-01**								**3**	**-139.377**	**284.9**	**0.31**	**0.173**
6	7.30E-01	+		3.35E-03					5	-137.697	285.7	1.16	0.114
2	8.24E-01	+							4	-138.906	286	1.46	0.098
7	7.45E-01		+	3.35E-03					5	-137.993	286.3	1.75	0.084
3	8.38E-01		+						4	-139.198	286.6	2.05	0.073
8	7.07E-01	+	+	3.35E-03					6	-137.514	287.5	2.94	0.047
22	7.55E-01	+		2.47E-03		+			6	-137.614	287.7	3.14	0.042
4	8.00E-01	+	+						5	-138.726	287.8	3.22	0.041
39	7.28E-01		+	3.95E-03			+		6	-137.954	288.4	3.82	0.03
16	6.80E-01	+	+	3.35E-03	+				7	-137.278	289.2	4.63	0.02
12	7.74E-01	+	+		+				6	-138.492	289.5	4.89	0.018
24	7.31E-01	+	+	2.47E-03		+			7	-137.431	289.5	4.94	0.017
40	6.90E-01	+	+	3.95E-03			+		7	-137.475	289.6	5.02	0.016
32	7.04E-01	+	+	0.002471	+	+			8	-137.194	291.2	6.65	0.007
48	6.63E-01	+	+	0.003945	+		+		8	-137.239	291.3	6.74	0.007
56	7.14E-01	+	+	3.07E-03		+	+		8	-137.392	291.6	7.05	0.006
64	6.88E-01	+	+	0.003071	+	+	+		9	-137.155	293.4	8.79	0.003
128	7.55E-01	+	+	0.0006774	+	+	+	+	10	-136.526	294.4	9.78	0.002

Random terms (all models)

Appendix D

Miscellaneous Tables

Table D.1: Physical, chemical, and biological characteristics for all lakes involved in our experiment. Lumsden, our experimental lake is where water was drawn, and filtered from for enclosures. The remaining three lakes are our regional source lakes, where sampled zooplankton were stocked into mesocosms. Unless otherwise identified, all chemical and biological information was collected in 2011 (Arnott, unpublished data).

Lake	Latitude/ Longitude	Elevation (m)	Area (ha)	Max Depth (m)	pH 1972	pH 2011	Calcium (mg/L)	Conduct.	Species Richness	*Daphnia* Richness
Lumsden	46° 01'N/ 81° 25'W	213	21.5	23.3	4.4	5.71	0.94	12	7	1
AY Jackson	46° 01'N/ 81° 23'W	197	6.1	10.1	5	6.14	1.44	17.4	8	2
Bell	46° 07'N/ 81° 12'W	223	281.3	27.2	5.2	6.3	1.82	20.6	13	3
Johnnie	46° 05'N/ 81° 14'W	200	395.5	32.4	5.1	6.21	1.52	18.4	14	3

Table D.2: Temperature (Temp.), pH, Conductivity (Cond.), and Chlorophyll-*a* measurements for each enclosure over the duration of the experiment. Enclosures are identified by the combination of the first two letters, followed by the specific replicate number for a given treatment. The first letter identifies the relative calcium concentrations (L, "low"; H, "high"), while the second letter identifies the relative pH of an enclosure (L, "low"; H, "high").

Encl. #	Sample Date	Temp. (°C)	pH	Cond. (µS/cm)	Chl-*a* (µg/L)
LL-1	10-Jun	17.1	5.64	14.2	1.15
LL-2	10-Jun	17	6.11	15	1.18
LL-3	10-Jun	17	5.63	14.1	1.24
LL-4	10-Jun	17.1	5.85	14.2	1.12
LL-5	10-Jun	17.1	6.00	15	1.38
LH-1	10-Jun	17.1	6.29	15.2	1.19
LH-2	10-Jun	17.2	6.83	24.6	1.09
LH-3	10-Jun	17.4	6.26	15.2	1.33
LH-4	10-Jun	17.2	6.31	15.4	1.63
LH-5	10-Jun	17.2	6.07	15.4	1.34
HL-1	10-Jun	17.3	5.72	22.8	1.15
HL-2	10-Jun	17.2	5.64	23.6	1.04
HL-3	10-Jun	17.3	5.77	22.8	1.20
HL-4	10-Jun	17.2	5.94	22.8	1.31
HL-5	10-Jun	17.2	5.85	22.6	1.11
HH-1	10-Jun	17.5	6.18	23.4	1.34
HH-2	10-Jun	17.5	6.26	22.4	1.09
HH-3	10-Jun	17.4	6.46	25.1	1.01
HH-4	10-Jun	17.4	6.06	23.6	1.30
HH-5	10-Jun	17.3	6.07	23.4	1.14
LL-1	17-Jun	18.6	5.58	13.5	0.81
LL-2	17-Jun	18.6	5.63	13.5	1.19
LL-3	17-Jun	18.6	5.56	13.5	1.08
LL-4	17-Jun	18.6	5.58	13.5	0.92
LL-5	17-Jun	18.6	5.51	13.5	0.95
LH-1	17-Jun	18.6	6.19	17.5	1.04
LH-2	17-Jun	18.6	6.39	14	0.67
LH-3	17-Jun	18.6	6.20	13.9	0.73
LH-4	17-Jun	18.6	6.25	13.9	0.77
LH-5	17-Jun	18.6	6.30	14	1.02
HL-1	17-Jun	18.6	5.59	22.2	0.94
HL-2	17-Jun	18.6	5.79	22.3	1.14
HL-3	17-Jun	18.6	5.54	22.2	1.08
HL-4	17-Jun	18.6	5.97	22	0.92
HL-5	17-Jun	18.6	5.81	22.1	1.07
HH-1	17-Jun	18.6	6.28	22.5	0.73
HH-2	17-Jun	18.6	6.16	22.2	0.60
HH-3	17-Jun	18.6	6.25	22.2	0.88
HH-4	17-Jun	18.6	6.38	23.2	0.47
HH-5	17-Jun	18.6	6.25	22.9	0.52
LL-1	24-Jun	20.0	5.55	13.6	0.28
LL-2	24-Jun	19.8	5.62	13.6	0.34
LL-3	24-Jun	20.0	5.56	13.6	0.19
LL-4	24-Jun	20.0	5.61	13.6	0.22
LL-5	24-Jun	20.2	5.62	13.6	0.26
LH-1	24-Jun	20.1	6.20	16.8	0.16
LH-2	24-Jun	20.0	6.17	14.1	0.20
LH-3	24-Jun	20.1	6.16	14.2	0.14
LH-4	24-Jun	20.1	6.16	14.1	0.17
LH-5	24-Jun	20.0	6.18	14.1	0.18
HL-1	24-Jun	20.2	5.61	22.3	0.18
HL-2	24-Jun	20.2	5.65	22.2	0.24
HL-3	24-Jun	20.1	5.66	22.2	0.33
HL-4	24-Jun	20.2	5.80	22.1	0.26
HL-5	24-Jun	20.3	5.67	22.3	0.22
HH-1	24-Jun	20.4	6.18	22.8	0.18
HH-2	24-Jun	20.3	6.19	22.5	0.21
HH-3	24-Jun	20.4	6.18	22.4	0.16
HH-4	24-Jun	20.3	6.23	23.4	0.19
HH-5	24-Jun	20.4	6.17	23.2	0.24
LL-1	01-Jul	21.1	5.60	13.7	0.34
LL-2	01-Jul	21.4	5.70	13.7	0.60
LL-3	01-Jul	21.1	5.62	13.7	0.20
LL-4	01-Jul	21.1	5.62	13.7	0.32
LL-5	01-Jul	21.1	5.69	13.7	0.23
LH-1	01-Jul	21.1	6.29	14.6	0.84
LH-2	01-Jul	21.1	6.27	14.1	1.34
LH-3	01-Jul	21.1	6.25	14.1	1.22
LH-4	01-Jul	21.1	6.22	14.1	0.90
LH-5	01-Jul	21.1	6.24	14.2	0.61
HL-1	01-Jul	21.1	5.64	22.6	0.25
HL-2	01-Jul	21.1	5.68	22.5	0.25
HL-3	01-Jul	21.1	5.66	22.6	0.16
HL-4	01-Jul	21.1	5.80	22.3	0.31
HL-5	01-Jul	21.1	5.67	22.4	0.33
HH-1	01-Jul	21.1	6.24	22.8	0.95
HH-2	01-Jul	21.1	6.26	22.5	0.77
HH-3	01-Jul	21.1	6.25	22.5	0.98

Encl. #	Sample Date	Temp. (°C)	pH	Cond. (µS/cm)	Chl-*a* (µg/L)
HH-4	01-Jul	21.1	6.31	23.3	1.92
HH-5	01-Jul	18.9	6.37	23.2	1.43
LL-1	08-Jul	23.9	5.78	13.7	0.74
LL-2	08-Jul	24.1	5.71	13.7	1.00
LL-3	08-Jul	23.9	5.73	13.7	0.91
LL-4	08-Jul	23.9	5.75	13.8	1.18
LL-5	08-Jul	23.9	5.67	13.8	0.67
LH-1	08-Jul	23.9	6.42	14.3	2.57
LH-2	08-Jul	23.8	6.32	14.1	0.50
LH-3	08-Jul	23.8	6.40	14	0.88
LH-4	08-Jul	23.8	6.40	14	1.21
LH-5	08-Jul	23.8	6.38	14	2.17
HL-1	08-Jul	23.8	5.78	22.7	2.09
HL-2	08-Jul	23.8	5.87	22.5	0.76
HL-3	08-Jul	23.8	5.78	22.6	0.82
HL-4	08-Jul	23.8	5.90	22.3	0.94
HL-5	08-Jul	23.8	5.71	22.4	1.08
HH-1	08-Jul	23.9	6.38	22.7	0.86
HH-2	08-Jul	23.8	6.43	22.5	1.42
HH-3	08-Jul	23.9	6.37	22.3	3.06
HH-4	08-Jul	23.8	6.49	23.2	2.59
HH-5	08-Jul	23.9	6.41	23.1	3.15
LL-1	15-Jul	25.7	5.72	13.7	2.24
LL-2	15-Jul	25.6	5.79	13.8	2.49
LL-3	15-Jul	25.7	5.78	13.7	1.53
LL-4	15-Jul	25.6	5.70	13.7	1.19
LL-5	15-Jul	25.6	5.74	13.8	0.55
LH-1	15-Jul	25.8	6.46	14.2	0.30
LH-2	15-Jul	25.9	6.31	14.2	2.19
LH-3	15-Jul	25.9	6.31	14.2	2.72
LH-4	15-Jul	25.8	6.26	14.2	0.74
LH-5	15-Jul	25.9	6.37	14.2	1.47
HL-1	15-Jul	25.9	5.72	22.6	1.45
HL-2	15-Jul	26.0	5.84	22.5	0.51
HL-3	15-Jul	26.1	5.70	22.6	1.05
HL-4	15-Jul	26.0	5.90	22	2.03
HL-5	15-Jul	26.0	5.60	22.5	3.40
HH-1	15-Jul	26.2	6.28	22.8	0.80
HH-2	15-Jul	26.1	6.43	22.3	1.06
HH-3	15-Jul	26.1	6.41	22.3	0.53
HH-4	15-Jul	26.2	6.42	22.3	2.25
HH-5	15-Jul	26.0	6.37	23.1	2.10
LL-1	22-Jul	23.8	5.64	13.7	1.94
LL-2	22-Jul	23.8	5.76	13.6	1.16
LL-3	22-Jul	23.8	5.69	13.7	1.93
LL-4	22-Jul	23.8	5.70	13.6	2.06
LL-5	22-Jul	23.8	5.65	13.8	0.78
LH-1	22-Jul	23.8	6.33	14	1.06
LH-2	22-Jul	23.8	6.41	14.1	0.62
LH-3	22-Jul	23.8	6.42	14.1	0.68
LH-4	22-Jul	23.9	6.27	14.1	1.03
LH-5	22-Jul	23.8	6.48	14.1	0.87
HL-1	22-Jul	23.9	5.66	22.5	2.76
HL-2	22-Jul	23.9	5.81	22.3	2.10
HL-3	22-Jul	23.9	5.63	22.6	0.70
HL-4	22-Jul	23.9	5.91	21.9	1.98
HL-5	22-Jul	23.9	5.78	22.2	4.12
HH-1	22-Jul	23.9	6.28	22.7	0.61
HH-2	22-Jul	23.9	6.36	22.2	1.01
HH-3	22-Jul	23.9	6.32	22.2	0.38
HH-4	22-Jul	23.9	6.44	22.9	1.10
HH-5	22-Jul	23.9	6.47	23.1	1.45
LL-1	29-Jul	21.7	5.82	13.6	0.64
LL-2	29-Jul	21.7	5.84	13.5	1.10
LL-3	29-Jul	21.7	5.70	13.5	0.66
LL-4	29-Jul	21.7	5.68	13.5	0.50
LL-5	29-Jul	21.7	5.85	13.5	0.53
LH-1	29-Jul	21.7	6.41	14.0	1.34
LH-2	29-Jul	21.7	6.43	14.1	0.70
LH-3	29-Jul	21.7	6.38	14.0	0.83
LH-4	29-Jul	21.8	6.27	14.0	1.07
LH-5	29-Jul	21.7	6.41	14.0	0.96
HL-1	29-Jul	21.8	5.70	22.3	0.42
HL-2	29-Jul	21.8	5.91	22.2	1.36
HL-3	29-Jul	21.8	5.69	22.5	0.82
HL-4	29-Jul	21.8	6.00	21.7	1.02
HL-5	29-Jul	21.7	5.81	22.1	1.18
HH-1	29-Jul	21.8	6.36	22.6	0.97

Encl. #	Sample Date	Temp. (°C)	pH	Cond. (µS/cm)	Chl-*a* (µg/L)
HH-2	29-Jul	21.8	6.37	22.1	0.80
HH-3	29-Jul	21.8	6.39	22.0	1.13
HH-4	29-Jul	21.8	6.40	22.8	0.90
HH-5	29-Jul	21.8	6.43	23.0	1.10
LL-1	05-Aug	21.2	5.76	13.5	0.65
LL-2	05-Aug	21.1	5.74	13.6	0.50
LL-3	05-Aug	21.1	5.73	13.6	2.04
LL-4	05-Aug	21.1	5.70	13.5	0.61
LL-5	05-Aug	21.1	5.85	13.6	3.60
LH-1	05-Aug	21.3	6.46	14.1	1.19
LH-2	05-Aug	21.3	6.36	14	0.59
LH-3	05-Aug	21.2	6.38	14	2.56
LH-4	05-Aug	21.2	6.32	13.9	1.88
LH-5	05-Aug	21.2	6.43	14.1	1.68
HL-1	05-Aug	21.3	5.73	22.3	0.90
HL-2	05-Aug	21.3	5.87	22.2	0.43
HL-3	05-Aug	21.3	5.66	22.6	3.13
HL-4	05-Aug	21.3	6.00	21.8	0.39
HL-5	05-Aug	21.3	5.73	22.1	1.06
HH-1	05-Aug	21.4	6.37	22.7	0.72
HH-2	05-Aug	21.3	6.33	22.2	2.65
HH-3	05-Aug	21.4	6.34	22	0.77
HH-4	05-Aug	21.4	6.31	23	0.99
HH-5	05-Aug	21.4	6.38	23	1.01

Table D.3: Structure of minimum adequate model for response variables (Abundance, Richness, Evenness).

Response Variable	Model
Abundance	
Total Crustacean Zooplankton	log transformed LMM
Cladocerans	log transformed LMM
Copepods	log transformed LMM
Daphnia pulex	log transformed LMM
Daphnia catawba	log transformed LMM
Daphnia ambigua	log transformed LMM
Eubosmina tubicen	log transformed LMM
Eubosmina longispina	log transformed LMM
*Holopedium**	negative-binomial GLM
Mesocyclops edax	log transformed LMM
Richness	
Total Crustacean Zooplankton	log transformed LMM
Cladocerans	log transformed LMM
Copepods	log transformed LMM
Evenness	
Total Crustacean Zooplankton	log transformed LMM
Cladocerans	negative-binomial GLMM
Copepods	negative-binomial GLMM

Notes: Model structure is provided as an abbreviation; LMM= linear mixed-effect model, GLM= general linear model, GLMM= generalized linear mixed-effect model

*Holopedium abundance was calculated for only the final day (Day 56)

Table D.4: Comparison of ANOVA results for abundance from Day 14-56 (Over Time) against just the final day (Day 56) for minimum adequate models (MAM). Effect sizes (ES; percent change of treatment means) are included to describe the strength of treatment effects; negative values indicate increased abundance (%) in unfavourable conditions (low calcium, low pH, interaction), and positive values indicate increased abundance in favourable conditions. Predictors not included in the MAM are represented by " – ". Additionally, interactions that did not factor in to any MAM for group/species observed were removed from the table.
Bolded values indicate significant variables ($p<0.05$), italic values ($p<0.10$).

		Ca			**pH**			**Ca:pH**			**Day**			**pH:Day**		
Group/Species	Time	F	P	ES	F	P	ES	F	P	ES	F	P	ES	F	P	ES
Total Community	Over Time	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Day 56	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Cladocerans	Over Time	**14.69**	**0.003**	**-57.00**	–	–	21.02	–	–	–	–	–	–	–	–	–
	Day 56	**14.53**	**0.0013**	**-67.56**	–	–	1.46	–	–	–	–	–	–	–	–	–
Copepods	Over Time	2.69	0.12	13.37	–	–	6.99	–	–	–	–	–	–	–	–	–
	Day 56	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Daphnia pulex	Over Time	**5.34**	**0.023**	**38.02**	**4.89**	**0.03**	**40.16**	–	–	–	–	–	–	–	–	–
	Day 56	**5.04**	**0.038**	**84.75**	–	–	11.11	–	–	–	–	–	–	–	–	–
Daphnia catawba	Over Time	*2.94*	*0.1*	*-33.72*	–	–	-19.46	–	–	–	–	–	–	–	–	–
	Day 56	**5.3**	**0.034**	**-75.89**	*3.36*	*0.084*	*-60.50*	–	–	–	–	–	–	–	–	–
Daphnia ambigua	Over Time	**4.6**	**0.035**	**-57.22**	**4.12**	**0.46**	**35.21**	–	–	–	**14.72**	**0.0002**	–	–	–	–
	Day 56	–	–	-78.45	–	–	-28.97	–	–	–	–	–	–	–	–	–
Eubosmina longispina	Over Time	1.91	0.18	-87.80	–	–	30.94	–	–	–	**42.26**	**<0.0001**	–	–	–	–
	Day 56	–	–	-17.15	–	–	-0.49				–	–	–	–	–	–
Eubosmina tubicen	Over Time	**6.66**	**0.012**	**-91.56**	–	–	23.42	–	–	–	**38.34**	**<0.0001**	–	–	–	–
	Day 56	**8.3**	**0.0099**	**-113.8**	–	–	-18.70	–	–	–	–	–	–	–	–	–
Holopedium	Over Time	–	–	-16.91	0.063	0.8	16.11	–	–	–	**121.88**	**<0.0001**	–	2.15	0.15	–
	Day 56	***5.10**	**0.024**	**-106.1**	***18.2**	**<0.0001**	**-661.9**	–	–	–	–	–	–	–	–	–

Table D.5: Initial zooplankton species that were stocked from regional source lakes

Cladocerans	Copepods
Eubosmina tubicen	*Leptodiaptomus minutus*
Eubosmina longispina	*Epishcura lacustris*
Bosmina spp.	*Skistodiaptomus oregonensis*
Daphnia catawba	*Mesocylops edax*
Daphnia ambigua	*Diacyclops thomasi*
Daphnia pulex	*Orthocyclops modestus*
Daphnia mendotae	*Acanthocyclops vernalis*
Daphnia retrocurva	*Leptodiaptomus siciloides*
Holopedium glacialis	
Diaphanosoma birgei	