HIGH SENSITIVITY OF FRESHWATER ZOOPLANKTON TO LOW CHLORIDE CONCENTRATIONS IS UNALTERED BY NUTRIENT LEVEL

by

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Abstract

Driven by decades of road salt use, freshwater salinization is prevalent across North America. Increasing chloride concentrations threaten freshwater species, and zooplankton are particularly sensitive. In Canada, the Canadian Water Quality Guidelines for the Protection of Aquatic Life (CWQG) set the chronic limit for chloride in freshwater at 120 mg/L. The ability of this guideline to protect zooplankton is questionable, given that: it is based on only five species of zooplankton, it relies on controlled laboratory studies that can miss indirect effects of contaminants on complex species interactions, and it does not account for factors that modify response to chloride. Increased food availability is one factor that has been shown to increase chloride tolerance in zooplankton species *Daphnia pulex/pulicaria*. It follows that differences in nutrient concentration, via their influence on phytoplankton biomass and availability of prey, could drive variation in response to chloride. Our main objectives were to understand how freshwater zooplankton respond to chloride, how nutrients could potentially modify response to chloride, and to determine if the CWQG chronic limit of 120 mg Cl^-/L adequately protects zooplankton. We exposed a freshwater zooplankton community to thirty increasing increments of chloride concentrations (ambient to 1500 mg/L) for six weeks, crossed with either ambient or high nutrient treatments. Total biomass, richness and abundance of all zooplankton taxa, genera and species declined with increasing chloride, except for two rotifer genera, *Keratella spp.* and *Lecane spp.* Higher nutrients did not reduce the impact of chloride in any group, genus or species. Additionally, we observed 27.7–70.9% loss in abundance by the CWQG of 120 mg Cl^-/L, suggesting that these guidelines should be revisited.

Co-Authorship

This thesis conforms to the manuscript format as outlined by the School of Graduate Studies and Research. Phytoplankton samples were collected from this experiment and analyzed by Isabelle Fournier, a Ph.D. student at Université Laval. These data will be incorporated into the manuscript resulting from this thesis. Its co-authors are:

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AICc – Akaike's Information Criterion corrected for small sample sizes
ANOVA – Analysis of variance
ATPase – Adenosine triphosphatase
CCME – Canadian Council of Ministers of the Environment
Cl^- – Chloride
CWQG – Canadian Water Quality Guidelines for the Protection of Aquatic Life
dbRDA – Distance-based redundancy analysis
dbRDA1 – Distance-based redundancy analysis axis 1
DOC – Dissolved organic carbon
EC_{25} – Effective concentration, 25%
EC_{50} – Effective concentration, 50%
EPA – Environmental Protection Agency
GAM – Generalized additive model
GLM – Generalized linear model
IC_{10} – Inhibition concentration, 10%
IC_{25} – Inhibition concentration, 25%
K^+ – Potassium
KH_2PO_4 – Potassium phosphate
LC_{50} – Lethal concentration, 50%
LM – linear model
LOEC – Lowest observed effect concentration
MATC – Maximum acceptable toxicant concentration
N_2 - Nitrogen
Na^+ – Sodium
NaCl – Sodium chloride
NH_4NO_3 – Ammonium nitrate
NO_3 - Nitrate
NOEC – No observed effect concentration
QUBS – Queen's University Biological Station
RDA – Redundancy analysis
TN – Total nitrogen
TP – Total phosphorus
ZINB – Zero-inflated negative binomial generalized linear model

Chapter 1

General Introduction

Potential impacts of multiple stressors

Freshwater bodies are increasingly affected by anthropogenic activities, because they tend to occupy low points in the landscape and therefore integrate stressors from throughout the local catchment and regional atmospheric processes (Williamson et al. 2009). Aquatic ecosystems are facing numerous anthropogenic threats, including warming (e.g., Sharma et al. 2019), shoreline development (e.g., Rosenberger et al. 2008), species invasion (Dextrase and Mandrak 2006), contamination (Pal et al. 2010) and eutrophication (Smith and Schindler 2009), among others (Reid et al. 2019). Worse still, stressors frequently co-occur, and are potentially interactive, modifying the effects of the other stressor and making it difficult to predict overall outcomes (Jackson et al. 2016, Côté et al. 2016).

There is still some debate over how to best define thresholds for interactions, but generally, two stressors are synergistic if their combined effects are more than the sum of each stressor individually and antagonistic if their combined effects are less than the sum of each stressor individually (Folt et al. 1999). Côté et al. (2016) include further classifications and modifications to better describe potential relationships. Use of a multiplicative rule to lower the threshold for interaction may be beneficial in situations where mortality is used as a response variable, with the logic that an organism that succumbs to one stressor cannot be affected by another. Furthermore, one stressor may be dominant to the other, in that it is more lethal and faster acting on vulnerable individuals, and the sum of both appear to be the same as if only the dominant stressor were occurring. These relationships are more difficult to define when two stressors inflict opposing effects. Côté et al. (2016) suggest comparing total effect to either effect individually. If the sum of the combined stressors is less than the effect of either alone, this should be defined as an antagonism; if the sum is more than either alone, it is a synergism. Identifying synergisms between stressors is crucial to prevent greater ecological damage than was initially expected. Jackson et al. (2016) found that most multiple stressor relationships found in aquatic ecosystems were not synergistic, but in most cases, one stressor was dominant. In these situations, understanding the relative effect of each stressor is still beneficial, as we can then prioritize curtailing the most dominant stressor. In this thesis, I will be examining the interactive effects of two stressors that are common in lake ecosystems; increasing salinity and nutrients.

Trends in freshwater salinization

Freshwater salinization is increasing, fueled by anthropogenic influences such as agricultural practices, resource extraction and road salt application (Dugan et al. 2017; Kaushal et al. 2018). In Canada and the northeastern United States, the single greatest contributor to rising salinity is road salt runoff (Kaushal et al. 2018). Use of sodium chloride to deice winter roads began in the 1940s. By 1955, 1 million tons of salt were being applied in the US (TRB 1991); in 2018, approximately 24 million tons were applied (Bolen 2019). In Canada, Environment Canada tracks road salt sales and reported use, with sales increasing from near five million tonnes in 1995 to approximately seven million tonnes in 2009 (Environment Canada 2012). Reported salt use is generally lower, because the Province of Quebec, small municipalities and private institutions, are not required to report salt application (Environment Canada 2012), so actual salt use is likely close to salt sales.

Chloride concentration is most often used to assess salt loading in freshwater. It is common to all salts used for de-icing and can therefore be linked to all salting practices. Chloride is not normally dominant in aquatic systems except under rare circumstances where local lithology and pollution permit chloride accumulation, so elevated chloride can be reliably traced to local salting practices (Mayer et al. 1999). It is generally a conservative ion in that it does not frequently undergo biological or chemical reactions in aquatic systems. Therefore, it rarely bioaccumulates or moves out of the system; concentrations will only change with evaporation or addition of water (Mayer et al. 1999). Additionally, chloride has been identified as the main toxicant in sodium chloride and calcium chloride salts, with negligible effects of sodium and calcium (Mount et al. 1997).

Freshwater chloride concentrations have been tied to the amount of nearby impervious surface (Dugan et al. 2017). Streams in the Adirondack Mountains in northern New York increased significantly in chloride levels from April to July 1987 and again from December 1987 to September 1988, but only in places downstream from a roadway (Demers and Sage 1990). Similarly, in Connecticut, groundwater chloride concentrations increase with greater proximity to highways (Cassanelli and Robbins 2013). Dugan et al. (2017) analyzed long-term monitoring data of lakes across North America and found that chloride concentrations were best predicted by percentage of nearby impervious land cover. However, it has also been suggested that salt use is now outpacing the rate of road expansion in the US, likely due to a combination of more vigorous attempts to stay ice-free, an increase in impervious surface density per unit area, and in some areas only, increases in snowfall (Corsi et al. 2015).

Consequently, it follows that urban lakes and streams typically have high chloride concentrations. In the US, freshwater bodies in partially urban areas frequently exceed the EPA guideline of 230 mg Cl^-/L (Corsi et al. 2015; USEPA 1988). Third Sister Lake, located just outside the city of Ann Arbor, MI, rose from ~20 mg Cl^-/L in 1965 to ~296 mg Cl^-/L by 2002

(Benbow and Merritt 2004). After large snowfalls, urban streams can experience dramatic increases in chloride levels. In Baltimore, streams can exceed 5000 mg Cl^-/L (Kaushal et al. 2018). In Toronto, stream values have been recorded as high as 14 000 mg Cl^-/L (Howard and Haynes 1993). Increasing trends in chloride (though not as extreme) are not limited to urban areas only. Rural streams in the White Mountains of New Hampshire have increased from near zero to over 100 mg Cl^-/L since the construction of the interstate highway (Kaushal et al. 2005). Even the vast Laurentian Great Lakes have been experiencing recent elevations in chloride attributed to increasing road salt use (Chapra et al. 2009).

Trends of increasing chloride concentrations are not likely to reverse course anytime soon. Once applied, sodium chloride moves slowly through the watershed. Sodium and chloride can be retained conservatively in groundwater (retained purely because the porewater is retained) and non-conservatively in soil (bound to organic matter, plants and/or soil microbes; Robinson et al. 2017). Sodium and chloride also differ in their capacity to be retained by soil. Sodium is more reactive and is removed from porewater more easily, undergoing cation exchange in soil organic matter. Though there is evidence some chloride can be sequestered by organic matter, this occurs much less frequently, and chloride is released in greater volumes than sodium (Robinson et al. 2017). Based on soil retention tests performed in the lab, Robinson et al. (2017) predict a two and a half to five-month retention time for NaCl in groundwater and soils. However, they present this timeline with the caveat that soils in nature have much more complex flowpaths and will be subject to more variation in moisture and periods of higher salt application. Given this, salt retention time is likely much longer in reality, and salting usually begins again before NaCl from the previous winter season is released from the watershed (Corsi et al. 2015). Oswald et al. (2019) found that eleven watersheds (a mix of urban and rural sites) across the Greater Toronto

Area, Hamilton and Lake Simcoe areas had mean annual chloride retention rates ranging from 40-90% from 2007-2011. Though they were unable to identify which factors were likely responsible for this variation, they believe it was likely due to underlying hydrological, geological and biochemical processes. Net watershed salt retention has been implicated in elevated chloride in Wappinger Creek, NY, where local road salt application has remained stagnant (Kelly et al. 2008). Long-term measurement and modelling showed a lag effect; increasing chloride concentrations were due to surges in salt export from the watershed (Kelly et al. 2008). Taken together, this evidence suggests that surface water chloride concentrations are likely to increase into the future, even if road salt application is significantly reduced.

Response of freshwater organisms to chloride

Freshwaters, by definition, have naturally low salt concentrations, and habitant species must be able to maintain their internal ion concentration higher than that of the surrounding water environment (Hart et al. 1991). For example, *Daphnia magna*, a freshwater cladoceran species, demonstrates frequent, active salt uptake to counteract ion loss to the hypo-osmotic environment (Bianchini and Wood 2008). Furthermore, permeability to water and ions is much lower in freshwater species (Aladin and Potts 1995), allowing them to slow ion loss across the gradient and prevent swelling via osmosis.

If external salt concentrations increase, freshwater species are unable to efficiently reverse ion flow, and suffer as a result (Hart et al. 1991). Salt affects freshwater species in two major ways: inducing osmotic stress and ion cytotoxicity (Latta et al. 2012). Under osmotic stress, cells lose water via osmosis. Because ions are more available and readily move along a gradient into the cell, an increase in uptake occurs, resulting in ion cytotoxicity, disrupting membrane stability and enzymatic function (Wyn Jones and Pollard 1983).

For organisms to adapt, they need to increase production of osmoprotectants, increasing internal osmotic pressure to prevent water loss (Zhu 2002), and stabilizing membrane and enzymatic structures (Yancey et al. 1982). Furthermore, they need to alter their ion exchange processes to increase ion release. In crustaceans and insects, Na^+/K^+-ATPase actively pumps Na^+ out of the cell and K^+ into the cell (Bianchini and Wood 2008). K^+ then is able to passively flow back out of the cell via K^+ channels, and in doing so, creates an electrochemical gradient that drives Cl^- extracellularly along with it. To rid themselves of excess ions, ion transporters, particularly Na^+/K^+-ATPase, must be upregulated (Latta et al. 2012). These processes, though necessary, are energetically costly, and pose their own problems in terms of increased energy requirements.

Freshwater zooplankton are one of the most sensitive groups to chloride (Elphick et al. 2011). Individual cladoceran species are adapted to narrow ranges in salinity and, as such, salinity is a major factor in limiting their distribution (Dodson et al. 2010). Zooplankton taxa have lifespans ranging from a few days (Wallace and Snell 2010) to several months (Reid and Williamson 2010), and after maturation, generally reproduce every few days under favourable conditions (Dodson et al. 2010; Reid and Williamson 2010; Wallace and Snell 2010). Declines in abundance due to chloride could reflect both direct mortality and declining reproductive rate. Zooplankton also form a dominant component in freshwater habits and are an important link between primary producing algae and planktivorous fish (Carpenter et al. 1987; Lampert et al. 1986). Consequently, effects on zooplankton will have a disproportionate effect on ecosystem functioning relative to other groups. Loss of zooplankton could lead to an increase in algal blooms and affect planktivores at values lower than known lethal concentrations of salt due to a reduction in prey.

Current guidelines concerning chloride levels in Canada

Currently in Canada, the Canadian Water Quality Guidelines for the Protection of Aquatic Life (CWQG) set limits for chloride in freshwater, implemented in 2011 by the Canadian Council of Ministers of the Environment (CCME). These guidelines put forth a short-term acute limit and a long-term chronic limit for freshwater. To calculate the acute limit, the 24-96h lethal concentrations, 50% (LC_{50}) or effective concentrations, 50% (EC_{50}) for chloride in 51 species, representing invertebrates (including five cladoceran species, two rotifer species, two copepod species), fish, aquatic plants and algae, were considered from various laboratory studies and ranked from least to most sensitive (CCME 2011). The acute limit was then set at 640 mg Cl^-/L, a value that protects 95% of these species under short-term exposure. Similarly, the chronic limit was calculated from ≥7d inhibition concentrations, 10% (IC_{10}), EC_{10}, IC_{25}, EC_{25}, no observed effect concentrations (NOEC), lowest observed effect concentrations (LOEC), or maximum acceptable toxicant concentrations (MATC) for fish and invertebrates and ≥96h MATC for aquatic plants and algae (CCME 2011). The chronic limit is meant to be a conservative value, and so studies chosen for inclusion in the calculation focused mainly on sublethal effects, i.e., inhibition of growth, reproduction, development, juvenile survival, though some using mortality as an endpoint were included. This calculation used a total of 28 species, including the same groups as above (including four cladoceran species, one rotifer species and no copepod species). Species were ranked by sensitivity, and a chronic exposure limit of 120 mg Cl^-/L was chosen, protecting 95% of these species.

The guidelines are not meant to spur immediate action when chloride limits are exceeded but hope to inform regulation and policy. Even with them in place, chloride concentrations in waterways in Ontario still regularly rise above both chronic and acute values (TRCA 2018).

While CCME (2011) attempts to be overprotective by accounting for sublethal effects in the chronic exposure limit, they acknowledge that the limits are not corrected for potential toxicity modifying factors such as water hardness, which will vary across sites, and make the guidelines difficult to apply broadly.

Variation in zooplankton response to chloride and potential modifying factors

As the laboratory tests included in determining the CWQG put the chronic IC_{10} and IC_{25} for tested zooplankton species safely over the chosen 120 mg Cl^-/L, zooplankton should be protected under these guidelines. However, these guidelines include just a few species to represent diverse groups, likely because most studies which examine chloride toxicity in zooplankton focus on daphniids. This is problematic, as species show wide variation in response to chloride, even among daphniids. For example, Hall and Burns (2002) found that the LC_{50} for *Daphnia carianata* was 667 mg Cl^-/L, while the LC_{50} for *Daphnia magna* was calculated as 4732 mg Cl^-/L (Schuytema et al. 1997). Furthermore, a fair amount of variation also exists between tests within species. The LC_{50} for *Daphnia pulex* has been found to be as low as 891 mg Cl^-/L (Birge et al. 1986) and as high as 2529 mg Cl^-/L (Bezirci et al. 2012).

Less information is available about how rotifer and copepods respond to chloride. Sarma et al. (2006) have found reduced abundance of *Anuraeopsis fissa*, *Brachionus calyciflorus* and *Brachionus havanaensis* at 910 mg Cl^-/L, while reductions of *Brachionus patulus* and *Brachionus rubens* were not observed until concentrations doubled to 1820 mg Cl^-/L. Studies that look at effects of chloride on copepods are decades old, and highly variable in their results. *Cyclops serrulatus* was found to have a maximum tolerance of only 239 mg Cl^-/L (Kanygina and Lebedeva 1957, reviewed in Evans and Frick 2001). *Diaptomus oregonensis* and *Cyclops vernalis* were found to be immobilized at 1844 mg Cl^-/L and 3688 mg Cl^-/L respectively

(Anderson et al. 1948, reviewed in Evans and Frick 2001). Finally, the 48h LC_{50} of *Eupdiaptomus padanus padanus* was 7077 mg Cl^-/L, while *Cyclops abyssorum prealpinus* was much higher at 12 385 mg Cl^-/L (Baudoin and Scoppa 1974).

Few laboratory studies have been done that look at chronic toxicity. Of those, reductions in reproduction and survival were recorded between 368-454 mg Cl^-/L for cladocerans (Elphick et al. 2011; Bezirci et al. 2012), and *Brachionus calyciforus*, a rotifer, had an IC_{10} of 1241 mg Cl^-/L (Elphick et al. 2011). No studies on the long-term effects of chloride on copepods exist, and none were included in the calculation of the chronic limit of the CWQG. However, several field studies have looked at effects of chloride on zooplankton over a period of weeks. The results of these are even more widely variable. Some suggest lower sensitivities than previously thought; Thompson and Shurin (2012) found a reduction in zooplankton biomass at 162 mg Cl^-/L, and Petranka and Francis (2012) found that cladocerans were absent or very uncommon once chloride concentrations met and exceeded 255 mg/L. Copepods showed a weak tendency to decrease with chloride in this same study, and rotifers were not measured. In contrast, Stoler et al. (2017b) did not find any differences in abundance in cladocerans, copepods nor rotifers, nor did Hintz et al. (2017) find a reduction in total zooplankton abundance when chloride concentrations varied from 114-867 mg/L and 15-1000 mg/L, respectively. Taken together, this variation in response calls the applicability of the current guidelines into question.

Other factors may affect how zooplankton respond to chloride. Stoler et al. (2017a) did not find any differences in copepod abundance in chloride-only treatments, they did find that abundance was reduced with chloride and carbaryl insecticide. Likewise, Hintz et al. (2017) saw a 56% drop in zooplankton abundance at 500 mg Cl^-/L and a 78% drop at 1000 mg Cl^-/L when bridle shiners (*Notropis bifrenatus)* were added to mesocosms, but had no detectable effects of chloride in the absence of fish. Thompson and Shurin (2012) found that increased warming and salinity synergistically reduced zooplankton biomass. Furthermore, food availability is an important consideration when looking at the effects of toxins on zooplankton. Ability to withstand toxins is reliant upon having enough energy to counteract their effects (Heugens et al. 2001). Under elevated chloride, greater access to prey should provide the energy required to boost osmoregulation in response to external ion increases. Indeed, in a laboratory-controlled experiment, the LC_{50} of *Daphnia pulex/ pulicaria* was higher when they were given greater amounts of their *Pseudokirchneriella subcapitata* prey (Brown and Yan 2015). In contrast, in a mesocosm setting, Lind et al. (2018) found that nutrient addition did not affect response to chloride, though it did increase zooplankton abundance overall. In natural settings, food availability will depend on lake nutrient level, through its influence on phytoplankton composition (Leibold 1999).

Effects of nutrients on phytoplankton and zooplankton

Nutrient levels, primarily phosphorus and nitrogen, can have widely variable effects on freshwater communities across their range. In oligotrophic settings, nutrient addition can increase phytoplankton abundance, leading to a subsequent bottom up increase in abundance across trophic levels, including zooplankton (Leibold 1999). Under these circumstances, elevated nutrients will be beneficial in aiding zooplankton under chloride stress by increasing prey and therefore, sources of energy. As total nutrient concentrations increase toward eutrophic conditions, however, cyanobacteria become a dominate component of phytoplankton communities (Chow-Fraser 1994; Leibold 1999). How this dominance shift affects zooplankton has been subject for debate. Though *Daphnia* graze most efficiently on prey <50 um (Burns 1968), ingesting and digesting anything larger, such as cyanobacteria, may be difficult and less nutritious but still support some level of *Daphnia* growth (Arnold 1973). On the other hand, cyanobacteria tend to congregate into large colonies, which can reduce predation (Paerl and Otten 2013), and some gelatinous species can resist digestion by grazers and absorb nutrients while passing through the gut (Porter 1973). As well, many cyanobacterial species produce toxins that harm zooplankton and lead to their reduction (Haney 1987; Olvera-Ramirez et al. 2010). Regardless of whether cyanobacteria consumption is overall neutral or outright harmful to zooplankton, they are less than ideal prey during times of salt stress, given that at best they are difficult to consume and provide a less nutritious energy source.

Effects of chloride on phytoplankton

Changes in phytoplankton composition could potentially be driven by chloride levels as well as nutrient concentrations. Phytoplankton generally have high tolerances to salinity. Optimal chloride range for freshwater diatoms, the most sensitive group, is up to 1800 mg Cl^-/L (Wilson et al. 1994), near concentrations considered 'saline' (~1820 mg Cl^-/L; CCME 2011). Chlorophyte *Chlorella vulgaris* can grow in salinities up to 4200 mg Cl^-/L, and *Chlorela minutissimo*, *Chlorella zofingiensis* and *Chlorella emersonii* have MATC of 6066, 6066 and 6824 mg Cl^-/L respectively. *Scenedesmus obliquus* can grow in concentrations up to 3500 mg Cl^-/L (Pandit et al. 2017) and 4200 mg Cl^-/L (Mohammed and Shaffea 1992). Cyanobacteria demonstrate the highest chloride tolerance. *Cylindrospermopsis sp.* sustained growth up to 1200 mg Cl^-/L and was tolerant up to 3600 mg Cl^-/L, and *Anabaena aphanizomenoides* sustained growth up to 6000 mg Cl^-/L with tolerance up to 12 000 mg Cl^-/L (Moisander et al. 2002). *Anabaenopsis sp.* and *Nodularia sp.* continued to grow up to 9100 mg Cl^-/L. *Microcystis aeruginosa* showed constant growth up to 6000 mg Cl^-/L (Tonk et al. 2007).

Unsurprising due to their ability to tolerate high chloride concentrations, cyanobacteria in particular are well adapted to combat salt stress. They produce osmoprotectants to prevent water loss and stabilize protein and membrane structures (Reed et al. 1985). Additionally, cyanobacteria are able to regulate intake and discharge of Na^+ (Molitor et al. 1986). Some evidence suggests that Na^+ might outright enhance cyanobacterial growth. Through use of an Na^+/NO_3 symport, cyanobacteria can import greater quantities of NO_3 and save energy that would normally be spent on N_2 fixation (Rai and Tiwari 1999).

Based on known algal tolerances, which most frequently fall within high 'sub-saline' (300-1820 mg Cl^-/L) and 'saline' ranges (>1820 mg Cl^-/L; CCME 2011), phytoplankton should be relatively unaffected by freshwater salinization. However, these values are based on species in isolation and in ideal conditions. Nutrient availability has the potential to modify algal response to chloride. Mohapatra et al. (1998) demonstrated that *Scenedesmus bijugatus* had increased tolerance to chloride from 500 mg/L to 2000 mg/L when media was enriched with nutrients. Similarly, *Anabaena sp*. experienced higher growth rates up to 8800 mg Cl^-/L in NO_3-containing media (Rai and Tiwari 1999). Periphytic diatom assemblages in the Muskoka River Watershed have been found to vary with distance to nearest roadway (used as a proxy for salt loading due to road de-icing) even though mean chloride concentration in this region is only 4.35 mg/L (MacDougall et al. 2016). Phytoplankton compositional changes in response to increases in chloride will depend on relative ability of each group to respond to chloride, which may be altered by the presence of abiotic (e.g., nutrient enrichment) and/or biotic factors (e.g., competitive interactions).

Community-level effects of chloride

To fully understand how the effects of a toxin will manifest in nature, it is crucial to not only investigate the direct effects of that toxin, but also how it could alter community interactions. Rohr et al. (2006) have likened the effects of contaminants to those of predators. Contaminants can disproportionately affect dominant species, indirectly increasing species diversity, compound the effects of other stressors, or cause multitrophic effects. In this final case, the contaminant can decrease primary producers resulting in a food shortage for primary consumers, or decrease higher trophic level organisms, resulting in a release from grazing pressure and altering species composition.

Chloride has the potential to modify aquatic communities in a number of ways. If chloride addition results in conditions that favour certain less sensitive species or groups over others, this could lead to a shift in dominance and further affect species across trophic levels. For example, if salinity increases favour cyanobacteria, which are better equipped to handle ion increase (as discussed in the previous section), this shift in dominance will result in less nutritious and potentially harmful prey. This reduction in available energy for zooplankton will reduce their ability to combat salt stress (Latta et al. 2012) and likely increase mortality. Anti-predator defences likewise require large amounts of energy (Hanazato 1991; Pauwels et al. 2010). Zooplankton may be able to fulfil the energy requirements needed to deal with chloride on its own. However, if both stressors are encountered simultaneously, the energy demands may be too high to meet, leading to an overall loss in zooplankton much greater than the sum of both stressors independently. A similar result was seen in leopard frogs (*Rana pipiens*), where treatments including their newt predators (*Notophthalmus viridescens*) and Roundup pesticide resulted in a much higher mortality rate than those attributed to either stressor alone (Relyea et al. 2005).

Additionally, increases in chloride could lead to a trophic cascade, with indirect effects on species occurring at concentrations much lower than predicted by toxicity tests. Generally, higher trophic level organisms have greater tolerance to chloride than zooplankton (CCME 2011). However, they can be affected at lower concentrations when their prey decline. Petranka and Francis (2013), for example, found that spotted salamander larvae (*Ambystoma maculatum*) were not directly affected by chloride, but did experience declines in growth rate due to the decline of their zooplankton prey. Furthermore, declines in zooplankton can release algae from grazing pressure, leading to an increase in phytoplankton biomass and primary production (Hintz et al. 2017). These effects would not be apparent based on laboratory experiments alone.

Thesis objectives

As the impacts of decadal road salt use come to light, we wish to understand the effects of the resultant salinization on freshwater zooplankton communities. Little is known about how lake nutrient level may or may not alter how zooplankton respond to elevations in chloride. Previous evidence suggests that an increase in food availability lessens the severity of chloride toxicity. Additionally, the Canadian Water Quality Guidelines for the Protection of Aquatic Life chloride limit may not sufficiently protect freshwater zooplankton across their range. The primary objectives of this thesis are:

1. To determine how a freshwater zooplankton community responds to increasing chloride concentrations.

2. To determine how nutrients influence the response of zooplankton to chloride. Specifically, is the impact of chloride on a zooplankton community reduced at high nutrients compared to ambient nutrient concentrations?
3. To determine if the current Canadian Water Quality Guidelines limit for long-term exposure of 120 mg Cl^{-}/L is sufficient to protect freshwater zooplankton.

Chapter 2

High sensitivity of freshwater zooplankton to low chloride concentrations is unaltered by nutrient level

Introduction

After nearly eighty years of road salt use, we are beginning to realize the effects of salt accumulation in freshwater systems. Approximately 44% of North American lakes with long-term monitoring data show evidence of salinization (Dugan et al. 2017). This steady increase of chloride threatens many species, including plants (e.g., Bryson and Barker 2002), insects (e.g., Clements and Kotalik 2016), amphibians (e.g., Karraker et al. 2008) and fish (e.g., Corsi et al. 2010). Loss of sensitive species and potential shifts in community composition could result in a loss of ecosystem functioning and changes in food web dynamics (Hintz and Relyea 2019). Increased salinization can also result in physical changes that can cause further indirect effects in aquatic ecosystems, such as cultural meromixis (Sibert et al. 2015), increasing oxygen depletion in the hypolimnion and phosphorus release from sediments (Novotny et al. 2008).

Zooplankton are particularly vulnerable to chloride increases, as they typically display some of the lowest tolerances to chloride (CCME 2011). They form an important component in aquatic food webs, as they are efficient grazers and an important food source for planktivorous fish and invertebrates (Lampert et al. 1986; Carpenter et al. 1987), and as such, any direct effect on zooplankton will also have consequences throughout the aquatic community. Additionally, we are lacking in our understanding of how they may respond; chloride toxicity tests generally focus on *Daphnia spp.* (e.g., Birge et al. 1988; USEPA 1988; Schuytema et al. 1997; Hall and Burns 2002; Martínez-Jerónimo and Martínez-Jerónimo 2007; CMEE 2011; Bezirci et al. 2012).

Community-level studies can further our understanding of how stressor effects will manifest in nature (Relyea and Hoverman 2006; Ruhr et al. 2006), but few have been done on chloride. Those which have been done often have varying results. For example, Petranka and Francis (2013) observed a near loss of cladocerans at a chloride concentration of 250 mg Cl^-/L, while Stoler et al. (2017a) did not see any difference in abundance even in concentrations as high as 867 mg Cl^-/L.

In Canada, the chronic chloride limit for freshwater is set by the Canadian Water Quality Guidelines for the Protection of Aquatic Life (CWQG) at 120 mg/L (CMEE 2011). This limit is based on a series of laboratory toxicity tests and includes four cladoceran species and one rotifer species. All zooplankton included had sensitivities greater than 259 mg Cl^-/L and thus should be protected under the guidelines. These guidelines, however, are lacking in a few critical areas that may complicate their broad implementation. First, these five species are not necessarily reflective of the sensitivities of all zooplankton species. Interspecific differences in response have already been demonstrated; LC_{50} values vary between *Daphnia carianata* and *Daphnia magna*, for example, ranging from 667 mg Cl^-/L for the former (Hall and Burns 2002) and 4732 mg Cl^-/L for the latter (Schuytema et al. 1997). Additionally, copepods are noticeably absent in the determination of these limits, likely because they are understudied. Previous work on copepod species is decades old, and tolerances range from 239 mg Cl^-/L to 12 385 mg Cl^-/L (Kanygina and Lebedeva 1957, reviewed in Evans and Frick 2001; Baudoin and Scoppa 1974). Second, these guidelines rely entirely on laboratory-based studies, which can miss indirect, community-level effects of contaminants. Increases in chloride concentration could result in changes in species or group dominance, if some species or groups are more sensitive than others. Van Meter et al. (2011) observed significant declines in copepods and cladocerans, while the number of rotifers increased (albeit insignificantly) under elevated chloride conditions. Third, and finally, the implementation of these guidelines does not take into account the presence of other stressors or factors that can modify response to chloride.

Food availability is one such factor that may affect how zooplankton respond to chloride. In freshwater organisms like zooplankton, an increase in external salt content can cause osmotic stress and ion cytotoxicity. Organisms that successfully combat salt stress are those that are able to upregulate production of osmoprotectants and ion transporters, energetically costly processes (Latta et al. 2012). Brown and Yan (2015) observed this effect with *Daphnia pulex/pulicaria* in a laboratory study. *D. pulex/pulicaria* was able to withstand higher chloride concentrations when given greater amounts of *Pseudokirchneriella subcapitata* prey, measured by carbon content. In a natural setting, food availability will be dependent on phytoplankton composition. This, in turn, is driven by lake nutrient concentration; as phosphorus increases, phytoplankton become more readily available (Leibold 1999). If chloride sensitivity can be modified by food availability, zooplankton in high nutrient lakes are likely less susceptible to chloride increases. However, it is currently unknown what indirect effects chloride may have on phytoplankton and zooplankton communities. While known algal tolerances to chloride are relatively high (Wilson et al. 1994 Moisander et al. 2002; Pandit et al. 2017), cyanobacteria are better equipped to combat salt stress (e.g., Reed et al. 1985). A dominance shift to cyanobacteria would negate the benefits of higher nutrient concentrations, as they are difficult to consume (Paerl and Otten 2013) and digest (Porter 1973), less nutritious (Arnold 1973) and may produce toxins (Haney 1987; Olvera-Ramirez et al. 2010).

To assess the potential impact salinization will have on lake ecosystems, we investigated the effects of increasing chloride concentration and the potential modifying influence of nutrient level on freshwater zooplankton communities. Additionally, we quantified the effects of chloride on species, zooplankton groups, and community metrics at 120 mg/L to assess the broad applicability of the Canadian Water Quality Guidelines.

Methods

Experimental design

To understand how nutrient level modifies the effects of chloride, we performed a mesocosm experiment in Long Lake (44°31'51.4"N 76°23'58.8"W) near the Queen's University Biological Station (QUBS) from June 8 to August 16, 2018 (see timeline, Table 1). Long Lake is situated on the Pangman Conservation Reserve, approximately 1.5 km from the nearest road and is only accessible by hiking trail. Consequently, Long Lake has maintained low chloride levels ($\mu = 0.43 \pm 0.08$ mg/L from 2013-2016, Arnott unpub.) and generally low phosphorus levels ($\mu = 6.2 \pm 0.73$ µg/L from 2013-2016, Arnott unpub.). However, initial water chemistry, collected near the beginning of the experiment on June 10, revealed the baseline phosphorus level at the start of the experiment to be 13.6 µg/L.

This experiment used a regression design in which a thirty-increment chloride gradient (0.41, 10, 20, 30, 40, 50, 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 300, 350, 400, 450, 500, 550, 600, 700, 800, 900, 1000, 1100, 1300, 1500 mg Cl^-/L) was crossed with ambient (13.6 µg P/L) and high (31.4 µg P/L) nutrient levels. This gradient design allowed us to examine possible non-linear effects of chloride by viewing response at a finer scale (Kreyling et al. 2018). We focused especially on chloride values below the Canadian Water Quality Guidelines (CWQG) limit of 120 mg Cl^-/L. The ambient total phosphorus concentration of Long Lake, 13.6 µg P/L, falls within the lower range of mesotrophic (10-20 µg P/L) as defined by the CWQG. The high nutrient level, 31.4 µg P/L, falls within the meso-eutrophic range (20-35 µg P/L, CCME 2004).

We suspended 1 570 L polyethylene bags (1 m diameter × 2.5 m long, Filmtech Plastics, Brampton, Canada) from five floating wooden rafts anchored in Long Lake. Each raft contained twelve 1 m × 1 m open square sections to hold bags, for a total of sixty mesocosms. On June 8, we filled the bags with water from Long Lake from a depth of 2 m, pumping it through 80 µm mesh to filter out crustacean zooplankton but allowing most phytoplankton and small rotifer zooplankton to pass. To prevent insect colonization, we covered each mesocosm with 1 mm mesh. Additionally, nine HOBO 64K pendant data loggers (Onset, Bourne, Massachusetts) were placed 1 m below surface in bags, distributed across all rafts; two loggers per raft except one raft had only one logger. Temperatures were recorded every 15 minutes for the duration of the experiment.

Treatments were randomly assigned among mesocosms using the sample function from R 'base'. On June 10, we added phosphorus (as KH_2PO_4, Fisher Scientific, Pittsburgh, Pennsylvania) and nitrogen (as NH_4NO_3, Sigma-Aldrich, St. Louis, Missouri) to the thirty high nutrient treatment mesocosms. Our goal was to add nitrogen to keep the N:P ratio similar between nutrient treatments. Because initial phosphorus concentrations were higher than anticipated, based on previous years' data, N:P ratios differed between treatments, with 24:1 N:P in the ambient treatment and 41:1 N:P in the high treatment. Nitrogen and phosphorus were added weekly to replenish amounts lost to periphyton growth, assuming a loss of 5% per day (Downing 2005). Nine or ten days after our nutrient addition, on June 19 and 20, we added zooplankton collected from five 4 m deep tows to each mesocosm, representing 1 924 L of filtered lake water. Zooplankton were captured using 35 cm diameter nets with 80 µm mesh. Two days later, on June 22 (day 1 of our experiment), we added reagent grade sodium chloride (NaCl; >99% pure, Fisher Scientific, Pittsburgh, Pennsylvania) to establish the chloride gradient within each nutrient treatment. Sodium chloride was dissolved in 2 L containers of water from each mesocosm. We stirred each mesocosm with a canoe paddle after NaCl addition.

Sampling protocol

Prior to nutrient addition, we collected initial water chemistry samples from each mesocosm on June 10 to obtain baseline chloride and total phosphorus (TP) levels, as well as total nitrogen (TN), calcium, dissolved organic carbon (DOC), pH, conductivity, and alkalinity (Table 2). 250 ml samples were collected from each mesocosm at 10 cm below the surface and mixed. Additionally, we collected chloride samples on June 24, two days after salt was added, and on July 30, four days prior to final sampling on August 2. We measured conductivity weekly using a YSI 600R multiparameter probe (YSI Inc., Yellow Springs, Ohio).

On June 22, two days after stocking zooplankton, we collected initial zooplankton, chlorophyll *a* and seston carbon samples prior to the addition of salt. We collected zooplankton samples using a 15 cm diameter zooplankton net with 50 µm mesh. For the initial sample, two tows were taken from the center of each mesocosm at a depth of 1.5 m for a total of 70.7 L water filtered for each sample. At the end of the experiment (August 2, week 6), five tows were taken at 2 m depths from each mesocosm, one from the center and four around the perimeter, for a total of 176.7 L water filtered for each sample. We preserved zooplankton using 70% ethanol. Zooplankton identification and abundance calculations were performed for initial (week 0) and final samples (week 6) samples using a Leica MZ16 dissecting scope and a Leica DM E compound microscope. Multiple sub-samples were processed until at least 250 individuals were counted, and three consecutive subsamples did not contain any new species. Individual species were not counted in subsequent subsamples after their count reached 50 individuals. If there were fewer than 250 individuals in a sample, the full sample was counted. Crustacean zooplankton were identified to species, except *Bosmina freyi/liederi* and *Daphnia pulex/pulicaria* were grouped by genus and all chydorid species were grouped together under the family Chydoridae. Cladocera and Copepoda were identified to species using Witty (2004) and Smith and Fernando (1978) respectively, while Rotifera were identified to genus using Stemberger (1979). Additionally, Haney (2013) was used to aid in identification of all taxa. Zooplankton body size was measured using the Plankton Counting Tool (Wong 2018). Biomass was then calculated using biomass-length associations (Table A1.1).

We collected water for chlorophyll *a* from each mesocosm on weeks 0 and 6 by taking 250 ml grab samples collected in brown Nalgene bottles from a depth of 10 cm. Samples were kept cool during transport using ice packs then stored in a refrigerator overnight. The next day, each sample was filtered through a G4 glass fiber filter (1.2 μm pore size; Fisher Scientific, Pittsburgh, Pennsylvania) and frozen at -80 °C. Chlorophyll *a* was later extracted in methanol for 24 hours then analysed using a TD-700 fluorometer (Turner Designs, Sunnyvale, California) and the methods of Welschmeyer (1994).

To quantify seston carbon, on weeks 0 and 6, we collected 250 ml of water from each mesocosm, then filtered it using GF/F glass fiber circles (precombusted at 400 °C for 4 h, 0.7 μm pore size; Whatman plc, Maidstone, United Kingdom), which were frozen at -80 °C. Samples were subsequently thawed, wrapped in tin foil and combusted using the Flash 2000 Organic Elemental Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts), following methods from the FlashEA 1112 Series Operating Manual (Thermo Fisher Scientific 2007).

Statistical analyses

To assess the effect of chloride, nutrients, and their interaction on response variables in week 0 and 6, we fit linear models (LM) and generalized additive models (GAM) with chloride concentration (continuous), nutrient level (categorical), and an interaction between chloride and nutrient levels as predictors. These analyses were performed in R.3.6.0 (R Core Team 2019), using the packages ‘car’ v.3.0-4 (Fox et al. 2019), ‘pscl’ v.1.5.2 (Jackman et al. 2017), and ‘mgcv’ v.1.8-30 (Wood 2019). Models were fit with all possible combinations of predictors (chloride, nutrient level, chloride × nutrient level) for each response variable and then compared using Akaike Information Criterion corrected for small sample sizes (AICc). The best model was the model with the lowest AICc. When multiple models were within 2 AICc units of the top model (occurring in 15 of 25 cases), the most scientifically conservative model containing only predictors found within all top models was chosen. The dropped predictors did not significantly contribute to observed effects in the response variable.

Response variables included: the abundance of major taxa (Cladocera, Copepoda, Rotifera); Chydoridae; common crustacean species (present in >20 mesocosms; except for *Bosmina freyi/liederi*) and common rotifer genera; as well as richness; chlorophyll *a* concentration; and total zooplankton dry weight. Chloride concentrations fluctuated between week 0 and 6 due to evaporation and rainfall, and so mean chloride concentrations between these weeks were used in all analyses (Table 3). One mesocosm bag was punctured during week 0 (ambient nutrients, 400 mg Cl^-/L) and another was not given the proper nutrient treatment (high nutrients, 800 mg Cl^-/L). Both were left out of all analyses. By week 6, an additional mesocosm bag was punctured (high nutrients, 900 mg Cl^-/L) and left out of week 6 analyses. Statistical assumptions for LMs were checked using residual vs. fitted plots, normal quantile-quantile plots, scale location plots and constant leverage using the ‘autoplot’ function from the ggfortify v.0.4.7 package (Tang 2019). If assumptions were violated, the best transformation to improve fit was determined using the ‘symbox’ function from the ‘car’ v.3.0-4 package (Fox et al. 2019). For initial samples, both $\log_{10}$ transformations and square root transformations were used; for week 6 samples, only $\log_{10}$ transformations were used.

Many species and groups were not found in all samples; however, we cannot determine if these zeroes were due to a complete absence from the mesocosm, or if they occurred at low levels and we were unable to detect them. To account for the possibility that these species and groups were present, but below detection, the equivalent of one individual per sample (0.0283/L for initial samples, 0.00566/L for final samples) was added to all mesocosms prior to transformation.

LMs provided the best fit for all week 0 response variables, and week 6 total rotifers and *Monostyla spp. Bosmina freyi/liederi* abundance contained many zeroes, so this taxa was analyzed using a zero-inflated negative binomial generalized linear model (ZINB) with chloride concentration and nutrient level as predictors, along with an interaction term. This model allowed us to determine whether the abundance as well as overall presence of *B. freyi/*liederi in mesocosms was influenced by chloride and/or nutrient level.

Generalized additive models were fit for most week 6 response variables, which tended to be non-linear. Statistical assumptions for GAMs were checked using enhanced residual quantile-quantile plots, residual vs. linear predictor plots, response vs. fitted plots, and the k-index via use of the 'gam.check' function from the 'pscl'package (Fox et al. 2019). GAMs provided the best fit for week 6 total abundance, total biomass, richness, copepods, cladocerans, nauplii copepods, cyclopoid copepodids, *Tropocyclops extensus*, *Scapholeberis mucronata*, Chydoridae, *Sida crystallina*, *Lecane spp.* and chlorophyll *a* concentrations.

Additionally, we examined the effect of nutrients, chloride and their possible interaction on seston carbon concentrations across weeks. Week 0 seston carbon concentration was modeled using an LM with nutrient level, chloride concentration and an interaction term as predictors.

Week 4 and week 6 carbon concentrations were $\log_{10}$ transformed and fit with GAMs using nutrient level, chloride concentration and an interaction term as predictors. Assumptions were verified using methods described above.

To assess zooplankton community response to chloride and nutrient level, we performed a distance-based redundancy analysis (dbRDA). We $\log_{10}$ transformed abundances of each species and genera, after adding the equivalent of one individual per sample to all counts ($\log_{10}(x + 0.00566)$) and calculated Bray-Curtis dissimilarities. Bray-Curtis was chosen as it provided the most gradient separation, assessed via the 'rankindex' function from the package 'vegan' v.2.5-6 (Oksanen 2019). RDA with all possible combinations of chloride, nutrient level and chloride × nutrient level as predictors were conducted, using the 'capscale' function from 'vegan' v.2.5-6 (Oksanen 2019). To confirm linearity of responses, we used the 'decorana' function from 'vegan' v.2.5-6 (Oksanen 2019). Models were compared using the AICc scores, with the lowest chosen as the best model.

Results

Seston carbon concentrations

With nutrient addition, we achieved higher seston carbon across all weeks of the experiment (Fig. 1), though it was more variable than in the ambient treatment. One week after adding nutrients, and prior to salt addition, nutrient addition significantly increased seston carbon concentration (LM, $p<0.001$, $r^2=0.62$, Fig. 1); mean ambient concentration was 0.38 ± 0.09 mg C/L, and mean high concentration was 0.66 ± 0.11 mg C/L. By the end of the experiment (week 6), mean ambient carbon concentration was 0.81 ± 0.46 mg/L and mean high concentration was 2.83 ± 1.4 mg/L. Chloride and nutrients were significant contributing factors to differences in seston carbon among mesocosms on week 6 (GAM, $p<0.001$, $r^2=0.65$, Fig. 1) with seston carbon increasing as both chloride and nutrients increased.

Initial abundance (week 0)

Initial zooplankton samples were collected eleven days after nutrient treatments had been established, two to three days after stocking, and a few hours before salt addition. Although mesocosms were stocked at ambient lake density, cladoceran abundance ($\mu=0.062$ /L ± 0.09) and copepod abundance ($\mu=0.012$ /L ± 0.02) in the initial week tended to be low. Rotifer abundance was higher ($\mu=121.9$ /L ± 109.3), driven by the high abundances of *Keratella spp.*, *Polyarthra spp.*, and *Trichocerca spp.* Only taxa, genera and species that were found in week 0 and week 6 were used in week 0 analyses.

Total zooplankton abundance (LM, $p<0.001$, $r^2=0.26$, Fig. 2A), rotifer abundance (LM, $p<0.001$, $r^2=0.26$, Fig. 2B) and *Keratella spp.* abundance (LM, $p<0.001$, $r^2=0.26$, Fig. 2C) were significantly higher under high nutrient treatment than in mesocosms at the ambient nutrient level (driven by nutrient application eleven days prior) but were not related to our proposed chloride gradient.

Nutrient addition did not affect the initial abundance of cladocerans (Fig A2.1), *Scapholeberis mucronata* (Fig. A2.2), *Bosmina freyi/liederi* (Fig. A2.3) nor cyclopoid copepodids (Fig. A2.4), and there was no significant trend in abundance associated with chloride concentration.

Final abundance (week 6)

Community response

Total abundance significantly decreased as chloride concentration increased, and was unaffected by nutrient addition (LM, $p<0.001$, $r^2=0.53$, Fig. 3A). At chloride concentrations equal to the Canadian Water Quality Guidelines (CWQG) limit of 120 mg/L, total abundance was reduced by 34.8% compared to the ambient chloride concentration. Similarly, there was a significant decrease in total zooplankton biomass with increasing chloride concentrations (GAM, $p<0.001$, $r^2=0.53$, Fig. 3B), but no difference in total biomass between the ambient and high nutrient levels. At 120 mg Cl^-/L, total biomass was reduced by 68.7%.

Taxonomic richness also significantly decreased with increasing chloride, with no effect of nutrient addition (GAM, $p<0.001$, $r^2=0.35$, Fig. 3C). Taxonomic richness decreased by 8.5% by 120 mg Cl^-/L.

Zooplankton community composition changed along the chloride gradient, but was unaffected by nutrients; only chloride was retained as an axis after model selection in the distance-based redundancy analysis (dbRDA) ordination (dbRDA1, $p<0.001$,3 pseudo-$F_{1,55}=12.2$, Fig. 4). Chloride explained 18.2% of the total variation. All species and genera had higher abundances with low chloride concentrations based on negative loadings for all taxa on the chloride axis.

Copepod response

No calanoid copepods were found in mesocosms by week 6. Total adult cyclopoid copepod abundance significantly decreased with elevated chloride (GAM, $p<0.001$, $r^2=0.48$, Fig. 5A). Nutrient level did not have any significant effect on abundance nor their response to chloride in any copepod group or taxa. At chloride concentrations equal to the CWQG concentration of 120 mg/L, adult cyclopoid copepods declined by 70.9%. Additionally, adult cyclopoid copepods were completely absent from mesocosms when concentrations exceeded 350 mg Cl^-/L (Fig. 5A).

Tropocyclops extensus was the only copepod present in sufficient numbers for statistical modeling. *T. extensus* was significantly affected by elevated chloride, experiencing a decline in abundance with increasing chloride (GAM, $p<0.001$, $r^2=0.45$, Fig. 5B). By the 120 mg Cl^-/L limit, abundance was reduced by 72.5% compared to ambient chloride concentration. Likewise, abundance of cyclopoid copepodids significantly decreased with increasing chloride concentration (GAM, $p<0.001$, $r^2=0.50$, Fig. 5C). Abundance was 81.8% lower at 120 mg Cl^-/L compared to ambient chloride concentration. Copepod nauplii were the most severely affected by chloride addition, with overall declines in abundance as chloride concentrations increased (GAM, $p<0.001$, $r^2=0.73$, Fig. 5D). At 120 mg Cl^-/L, nauplii abundance declined by 89.9%.

Cladoceran response

Cladocerans were less affected than copepods but still declined across the chloride gradient. Abundance significantly decreased along the increasing chloride gradient (GAM, $p<0.001$, $r^2=0.69$, Fig. 6A). Changes in nutrient level did not affect abundance nor response to chloride. Abundance dropped by 61.8% once chloride concentrations reached the CWQG of 120 mg/L.

The abundance of most cladocerans, including the family Chydoridae, *Scapholeberis mucronata,* and *Bosmina freyi/liederi*, were unaffected by nutrient addition but significantly reduced by chloride increases. At 120 mg Cl^-/L, Chydoridae abundance declined by 67.4% (GAM, $p<0.001$, $r^2=0.50$, Fig. 6B), *S. mucronata* by 32.8% (GAM, $p<0.001$, $r^2=0.41$, Fig. 6C), and *B. freyi/liederi* by 12.4% (ZINB, $p=0.004$, $\theta=3.26$, Fig. 6D). *Bosmina freyi/liederi* was not detected in 61.6% of the mesocosms on week 6, but their occurrence was not influenced by the chloride gradient (ZINB, $p=0.16$, Fig. 6D).

Sida crystallina was the only zooplankton species where its response to chloride was dependent upon nutrient level. At the ambient nutrient level, *S. crystallina* abundance significantly declined as chloride concentrations increased, resulting in an 89% drop in abundance by 120 mg Cl^-/L (GAM, p<0.001, r^2=0.47, Fig. 6E). At the high nutrient level, chloride did not affect abundance (GAM, p=0.18, r^2= 0.033).

Rotifer response

Rotifers were generally the most resistant to chloride, but also more variable in response than the other taxa. As with most other taxa, nutrient addition did not affect abundance nor response to chloride. There was an overall decline in total rotifer abundance as chloride increased (LM, p<0.001, r^2=0.31, Fig. 7A). At chloride concentration equal to the CWQG limit of 120 mg/L, there was a decrease in total rotifer abundance of 27.7%.

Monostyla spp. response was similar to the overall rotifer response; abundance declined with increasing chloride concentrations, falling by 28.6% by 120 mg Cl^-/L (LM, p<0.001, r^2=0.31, Fig. 7B). *Lecane spp.* was the only taxon that had a positive response to chloride concentration. Abundance peaked as chloride concentrations rose to 384 mg/L. Increases in chloride after this point led to a decline in *Lecane spp.* abundance (GAM, p=0.007, r^2=0.19, Fig. 7C). At the CWQG limit of 120 mg Cl^-/L, abundance increased by 107%. Lastly, *Keratella spp.* abundance did not significantly differ along the chloride gradient (Fig 7D).

Phytoplankton response

Phytoplankton response to chloride differed between nutrient treatments. At the ambient nutrient level, chlorophyll *a* concentration increased as chloride concentration rose up to 500 mg/L (GAM, $p<0.001$, $r^2=0.53$, Fig. 8). Chlorophyll *a* concentration increased by 223% at the CWQG limit of 120 mg Cl^-/L. At chloride concentration greater than 500 mg/L, chlorophyll *a* concentration leveled off. At the high nutrient level, chlorophyll *a* tended to increase but was not significantly affected by increased chloride concentration (GAM, $p=0.08$, $r^2=0.08$, Fig. 8).

Discussion

Our six-week field mesocosm experiment demonstrated that zooplankton communities are susceptible to increasing chloride concentrations, even at concentrations below the current Canadian Water Quality Guidelines. As chloride increased along a gradient from ambient concentrations (0.41 mg Cl^-/L) to 1500 mg Cl^-/L, we observed changes in community composition driven by declines in all three zooplankton groups: copepods, cladocerans and rotifers.

Community and taxa-level response to chloride

The zooplankton community altered with increasing chloride, resulting in changes in community composition (as indicated by the dbRDA), loss of richness, and an overall decline in biomass. All zooplankton groups, crustacean species, and rotifer genera were negatively affected by chloride except for *Lecane spp.*, which initially benefitted but ultimately declined above 384 mg Cl^-/L, and *Keratella spp.*, which was unaffected by chloride. Our experiment revealed that zooplankton taxa were more sensitive to chloride than found in previous studies, with some exceptions. In some studies zooplankton did not decline in abundance until chloride concentrations were 727 mg/L or higher (Jones et al. 2017; Stoler et al. 2017b; Lind et al. 2018) or no effects of chloride on abundance were observed (Stoler et al. 2017a). Our results were more similar to those of Thompson and Shurin (2012), who observed a decline in total zooplankton biomass at 162 mg Cl^-/L, and Petranka and Francis (2013), who detected few or no cladocerans in chloride concentrations >255 mg/L. Petranka and Francis (2013), however, detected a weak effect of chloride on copepod abundance whereas copepods in our study were undetected in chloride concentrations >350 mg/L.

The source of variation in chloride sensitivity among studies is unclear. Differences in water chemistry could be a contributor. Increased water hardness has been proven to elevate tolerance in *Ceriodaphnia dubia* (Elphick et al. 2011), as well as greater food availability in *Daphnia pulex/pulicaria* (Brown and Yan 2015).

Previous exposure to chloride may also be an important factor, as zooplankton have demonstrated some ability to adapt to chloride (Coldsnow et al. 2017; Hintz et al. 2019). Zooplankton in our experiment may be particularly sensitive to chloride owing to their lack of previous exposure (mean [Cl-] in Long Lake = 0.43 compared to 15-60 mg/L for source lakes in other published studies). Sinclair and Arnott (2018) compared response to chloride for zooplankton from Long Lake and nearby Loughborough Lake ([Cl-]=19 mg/L) and found that species common to both lakes tended to respond to salt in opposite ways, or the response differed by an order of magnitude. It is also possible that differences in species composition drive variation in chloride sensitivity. Most studies only report response for total zooplankton, cladocerans, copepods or rotifers, so we do not know the degree to which our community composition differs. For many species, we lack information on their sensitivity to chloride. Most toxicity studies for zooplankton focus on daphniids and *Brachionus spp.* (e.g., USEPA 1988; CMEE 2011), while none of these species were common in our mesocosms.

Finally, differences in detected responses among studies may be due to variation in study design. Most previous field studies examining zooplankton response to salt have employed an ANOVA design, in contrast to our study, and Petranka and Francis (2013) which employed a regression design. Regression designs provide a more powerful statistical approach (Cottingham et al. 2005; Kreyling et al. 2018) and allowed us to detect non-linear relationships between zooplankton responses and chloride concentrations that ranged from <1 to 1500 mg/L. This study design also revealed high sensitivity to chloride across multiple sites in Canada, USA, and Europe (Hintz, Arnott et al. in prep). Eleven out of 16 lakes (including Long Lake) had cladoceran LC_{50} values lower than the EPA chloride limit of 230 mg Cl^-/L (USEPA 1988).

In our field experiment, zooplankton taxonomic groups differed in the relative severity of their response to chloride. Cyclopoid copepods experienced the greatest declines and were completely absent in mesocosms with chloride concentrations > 350 mg Cl^-/L. Cladoceran abundance also declined with increasing chloride, although they were more tolerant than copepods and were found throughout the chloride gradient. Rotifers were the most tolerant of the three taxa, but most declined as a result of increasing chloride. In previous studies, cladocerans tended to be the most sensitive (Van Meter et al. 2011; Petranka and Francis 2013; Stoler et al. 2017b), except in Sinclair and Arnott (2018) where increases in littoral cladoceran abundance in the high salt (250 mg Cl^-/L) drove the overall response. Rotifers are generally the most tolerant, only showing declines with chloride in one study, and only on day 4 (Stoler et al. 2017b). Copepods are more variable in their response to chloride, sometimes declining (Van Meter et al. 2011; Petranka and Francis 2013; Lind et al. 2018) or not responding to chloride (Stoler et al. 2017a, b, Sinclair and Arnott 2018).

The above differences in relative sensitivity to chloride between taxa carry important implications for freshwater ecosystems as chloride concentrations continue to rise. Zooplankton are essential to ecosystem function, contributing to respiration, moderation of algal biomass, moderation of predator biomass and composition, and nutrient recycling via egestion and excretion (Hébert et al. 2017). They can be divided into functional groups with diverse traits including feeding mode, differences in growth rate, excretion rate and respiration rate (Barnett et al. 2007). With changes in species composition, replacement of some species with others will not necessarily maintain ecosystem function because they may not have the same functional capacity. In particular, growth rate, excretion rate and respiration rate increase with body size, in turn increasing ecosystem respiration, predator biomass and nutrient recycling, and decreasing algal biomass. As relative abundances shift from large crustaceans to small rotifers, total biomass will drop (as observed in this study; Fig. 3B), and these processes will decline.

Our observed shift in dominance from cladocerans to rotifers at higher salt concentrations was associated with a trophic cascade, where phytoplankton abundance increased. As total zooplankton biomass declined along the chloride gradient, phytoplankton was likely released from grazing pressure, resulting in increasing chlorophyll *a*, an indication of phytoplankton biomass (Fig. 8). A similar trend was observed at the high nutrient level, but chlorophyll *a* concentrations in this treatment were more variable and this trend was not significant. Although we only included two trophic levels in our experiment, Hintz et al. (2017) observed a trophic cascade induced by salinity and the addition of fish predators, leading to a decline in total zooplankton and a subsequent increase in phytoplankton. Furthermore, though we did not include planktivores in our study, salt-induced declines in zooplankton biomass can have negative consequences on higher trophic level organisms as well. This was seen by Petranka and Francis (2013), who found that spotted salamander larvae growth was unaffected by salt but limited by declines in their salt-affected zooplankton prey.

Impact of nutrients on response to chloride

Added nutrients increased the available carbon within the high nutrient level treatment, and this increase was consistent across weeks (Fig. 1). Despite this increase in carbon, suggesting an increase in food availability, nutrient addition did not influence the response to chloride, except in *Sida crystallina* (Fig. 6E). This species seemingly benefited from lower nutrients, as it was more common at the ambient nutrient level.

Contrary to our expectations, increases in nutrients did not reduce the impact of chloride in any group or species. Lind et al. (2018) found a similar result with phosphorus treatments of 4.36 µg/L and 100 µg/L; nutrient addition did not affect response to chloride, although they did find that zooplankton abundance was higher with increased nutrients. Our lack of response, in contrast to Brown and Yan's (2015) finding that tolerance to chloride was positively associated with food concentrations, may be associated with high seston carbon concentrations in our experiment. The mean seston carbon concentration in our ambient nutrient treatment, 0.81 ± 0.46 mg/L was at the high end of the carbon range tested by Brown and Yan (2015), 0.2 to 1 mg/L (Fig. 1). Our mean high nutrient treatment carbon concentration was 2.83 ± 1.4 mg/L (Fig. 1). It is possible that carbon wasn't limiting at our ambient levels, and any further increase did not lead to any measurable difference. Finally, the discrepancy between this study and Brown and Yan (2015) may be due to differences between controlled laboratory studies and field-based studies. They used the green microalga *Pseudokirchneriella subcapitata* as their only food source. We did not control phytoplankton composition in our study, and it is possible that salinity shifted phytoplankton composition to become dominated by inedible species. This shift has been documented in an estuary (Pilkaitytë et al. 2004), but no studies have been done on effects of salinity on freshwater phytoplankton composition. Proliferation of inedible and non-nutritious cyanobacteria due to salt increase within our experiment is possible, but would not be detected in controlled laboratory settings and could negate any potential benefits to zooplankton brought on by nutrient addition.

Applicability of the Canadian Water Quality Guidelines for the Protection of Aquatic Life

We observed declines in zooplankton biomass and abundance across most zooplankton species and genera with chloride addition at relatively low concentrations. Only *Keratella spp.*, which was unaffected by chloride at any concentration, and *Lecane spp.*, which initially benefitted from chloride addition, were protected at 120 mg Cl^-/L, the current chronic limit for chloride as defined by the Canadian Water Quality Guidelines for the Protection of Aquatic Life (CWQG; CCME 2011). Alarmingly, total biomass, copepod and cladoceran abundance declined by 61.8-70.9% once chloride concentrations reached this reputedly safe limit, which would likely lead to a decline in ecosystem services. The CWQG should be re-evaluated to ensure zooplankton are fully protected across their range.

Conclusions

Controlled laboratory studies are essential to understand the magnitude of a stressor’s effect and confirm direct cause and effect relationships outside of the influence of confounding variables. However, these studies rely on idealistic conditions, which can make the results challenging to apply to natural environments where multiple species are interacting. This work highlights the importance of field studies and regression designs in further understanding the impact of stressors across their range on whole communities in natural settings.

Use of mesocosms allowed us to observe the effects of chloride under more realistic conditions than studies carried out in the laboratory, including natural fluctuations in food supply, temperature, and light conditions. Our experiment also permitted the inclusion of multiple species and trophic levels, which allowed for the occurrence of indirect effects of stressors on species interactions, which often result in different outcomes from what laboratory results predict (Relyea and Hoverman 2006). Mesocosms studies, however, are not without their limitations. Higher trophic levels, including vertebrate predators were not included in our assemblage. Enclosure environments also tend to promote periphyton buildup on wall which results in zooplankton assemblage shifts from pelagic to littoral species (Arnott et al. 2017). These effects tend to be stronger after six weeks, so our experiment length should have minimized the impact of these effects, while allowing for the production of several generations of many zooplankton species. Overall, mesocosm studies offer an expanded view of how stressors may impact whole communities in natural settings.

In our experiment, chloride affected freshwater zooplankton communities at concentrations well below values obtained in laboratory toxicity tests. This approach additionally allowed us to understand how the community shifts with chloride, which is difficult to garner from individual toxicity tests. With the application of chloride, the community as a whole declined. However, due to differences in sensitivity, the relative abundance of rotifers increased in comparison to copepods and cladocerans. This shift will potentially contribute to further changes in ecosystems, including increases in algal blooms and decreases in secondary consumer biomass (Hintz and Relyea 2019). We detected evidence of possible ecosystem changes in our experiment; increased chlorophyll *a* concentrations indicated that phytoplankton biomass increased (Fig. 8).

We tested the potential mitigating effect of elevated nutrient level on zooplankton response to chloride. Previous evidence suggests increased food availability can protect against rising chloride by providing adequate energy to expel the increase in ions (Brown and Yan 2015). Lake nutrient content drives changes in phytoplankton composition and biomass (Barnett and

Beisner 2007) and may be a factor driving differences in response to chloride across landscapes. However, we did not detect any evidence that chloride is less toxic under higher nutrient conditions. Rising salinity may promote cyanobacteria, which can more efficiently counter osmotic stress than other phytoplankton groups (Pilkaitytë et al. 2004), potentially reversing any increase in edible, nutritious prey.

Finally, the broad application of the current Canadian Water Quality Guidelines chronic chloride limit of 120 mg/L should be done with caution. It is clear that more research is needed to fully understand how multiple species and communities respond to chloride across heterogeneous landscapes.

Summary

1. Our main objectives were to determine how freshwater zooplankton respond to chloride, how nutrients could potentially modify response to chloride, and if the CWQG of 120 mg Cl^-/L adequately protects zooplankton.
2. We exposed a freshwater zooplankton community to a chloride gradient crossed with a nutrient treatment for six weeks.
3. We observed significant declines in all taxa, genera and species except for a rotifer genus, *Keratella spp.*
4. Taxa differed in their sensitivity; copepods were the most sensitive, followed by cladocerans, then rotifers.
5. Higher nutrients did not reduce the negative impact of chloride.
6. By the CWQG limit of 120 mg Cl^-/L, we observed significant declines in all taxa, genera and species except for rotifer genera *Keratella spp.* and *Lecane spp.*, suggesting that this limit does not sufficiently protect freshwater zooplankton.

Table 1 Timeline of experimental procedures during of the mesocosm experiment, from June 8 to August 2, 2018.

Date	Experimental procedure
June 8	Filled mesocosms with filtered water from Long Lake
June 10	Collected water chemistry samples
	Added KH_2PO_4 and NH_4NO_3 to mesocosms
June 19-20	Added zooplankton (collected from Long Lake) to mesocosms
June 22	Collected week 0 (initial) zooplankton samples
	Collected week 0 (initial) chlorophyll *a* samples
	Collected week 0 (initial) seston carbon samples
	Added sodium chloride to mesocosms
June 24	Collected week 0 (initial) chloride samples
July 30	Collected week 6 (final) chloride samples
July 31	Collected week 6 (final) chlorophyll *a* samples
	Collected week 6 (final) seston carbon samples
August 2	Collected week 6 (final) zooplankton samples

Table 2 Results from initial water chemistry samples collected from mesosms in Long Lake on June 10, 2018.

Parameter	Measurement
Chloride (mg/L)	0.41
Mean total phosphorus (μg/L)	13.6
Total nitrogen (mg/L)	0.33
Calcium (mg/L)	34.7
Dissolved organic carbon (mg/L)	4
pH	8.1
Conductivity (μS/cm)	191
Alkalinity (mg/L $CaCO_3$)	92.6

Table 3 Target nominal chloride concentrations vs. actual measured values in week 0 and week 6 of the experiment under ambient (13.6 µg P/L) and high (31.4 µg P/L) nutrients. Measured chloride concentrations differed from nominal concentrations, so mean chloride concentrations were calculated from week 0 and week 6 measurements and used in all analyses. Missing values occur where a mesocosm was punctured (ambient nutrients, 400 mg Cl^-/L; high nutrients, 900 mg Cl^-/L) or received the wrong nutrient treatment (high nutrients; 800 mg Cl^-/L) and were not used. Additionally, data for one mesocosm from week 6 was missing (ambient nutrients; 1500 mg Cl^-/L).

Nutrient treatment	Nominal chloride (mg/L)	Measured chloride, week 0 (mg/L)	Measured chloride, week 6 (mg/L)	Mean measured chloride (mg/L)
Ambient	0.41	0.72	1.46	1.09
High	0.41	0.47	0.43	0.45
Ambient	10	9.3	9.73	9.515
High	10	8.98	9.85	9.415
Ambient	20	17.2	17.7	17.45
High	20	17.8	18.7	18.25
Ambient	30	25.4	27.3	26.35
High	30	25.5	26.5	26
Ambient	40	36.3	37.2	36.75
High	40	35.4	36.3	35.85
Ambient	50	43.9	45.6	44.75
High	50	43.1	45.6	44.35
Ambient	60	56.1	56.3	56.2
High	60	55.2	55.5	55.35
Ambient	80	67.9	67.9	67.9
High	80	75.1	74	74.55
Ambient	100	89.8	89.4	89.6
High	100	88.6	77.6	83.1
Ambient	120	101	102	101.5
High	120	103	107	105
Ambient	140	75.2	122	98.6
High	140	92.5	114	103.25
Ambient	160	136	136	136
High	160	140	138	139
Ambient	180	213	224	218.5
High	180	153	150	151.5
Ambient	200	171	170	170.5
High	200	241	182	211.5
Ambient	225	194	195	194.5
High	225	203	203	203
Ambient	250	224	224	224
High	250	223	233	228
Ambient	300	255	262	258.5

High	300	241	263	252
Ambient	350	324	329	326.5
High	350	310	311	310.5
Ambient	400			
High	400	362	342	352
Ambient	450	389	387	388
High	450	381	387	384
Ambient	500	427	432	429.5
High	500	441	431	436
Ambient	550	463	471	467
High	550	505	504	504.5
Ambient	600	495	487	491
High	600	610	636	623
Ambient	700	566	591	578.5
High	700	606	603	604.5
Ambient	800	688	699	693.5
High	800			
Ambient	900	796	793	794.5
High	900	887		
Ambient	1000	843	884	863.5
High	1000	877	860	868.5
Ambient	1100	1060	1010	1035
High	1100	969	965	967
Ambient	1300	1100	1150	1125
High	1300	1170	1170	1170
Ambient	1500	1270		1270
High	1500	1380	1370	1375

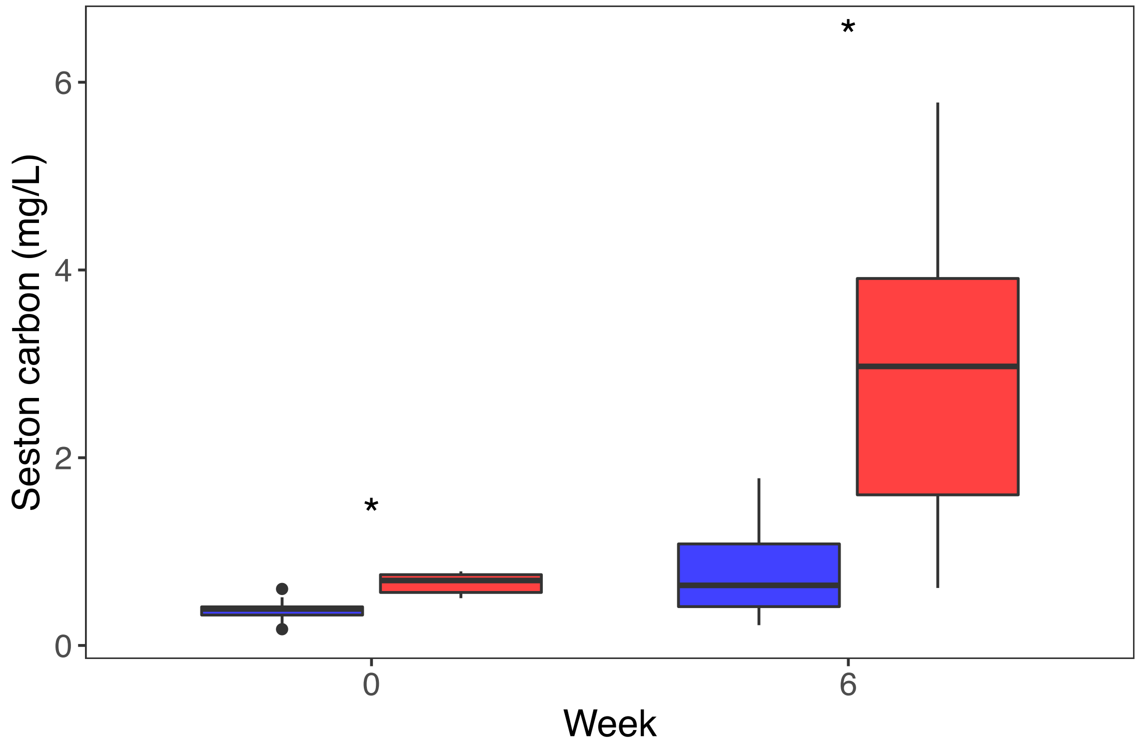

Figure 1 Seston carbon concentrations between water samples from the ambient nutrient treatment (13.6 µg P/L; blue) and the high nutrient treatment (31.4 µg P/L; red) in weeks 0 (initial; ambient n=28, high n=8) and 6 (final; ambient n=29, high n=27). The bottom and top edges of the boxes represent the first and third quartiles, the thick horizontal line within the boxes represents the medians, and the whiskers represent data points within 1.5 times the interquartile range. Separate points signify data lying outside the interquartile range. Weeks marked with an asterisk (*) had significant differences in seston carbon concentrations between the two nutrient treatments ($p<0.05$).

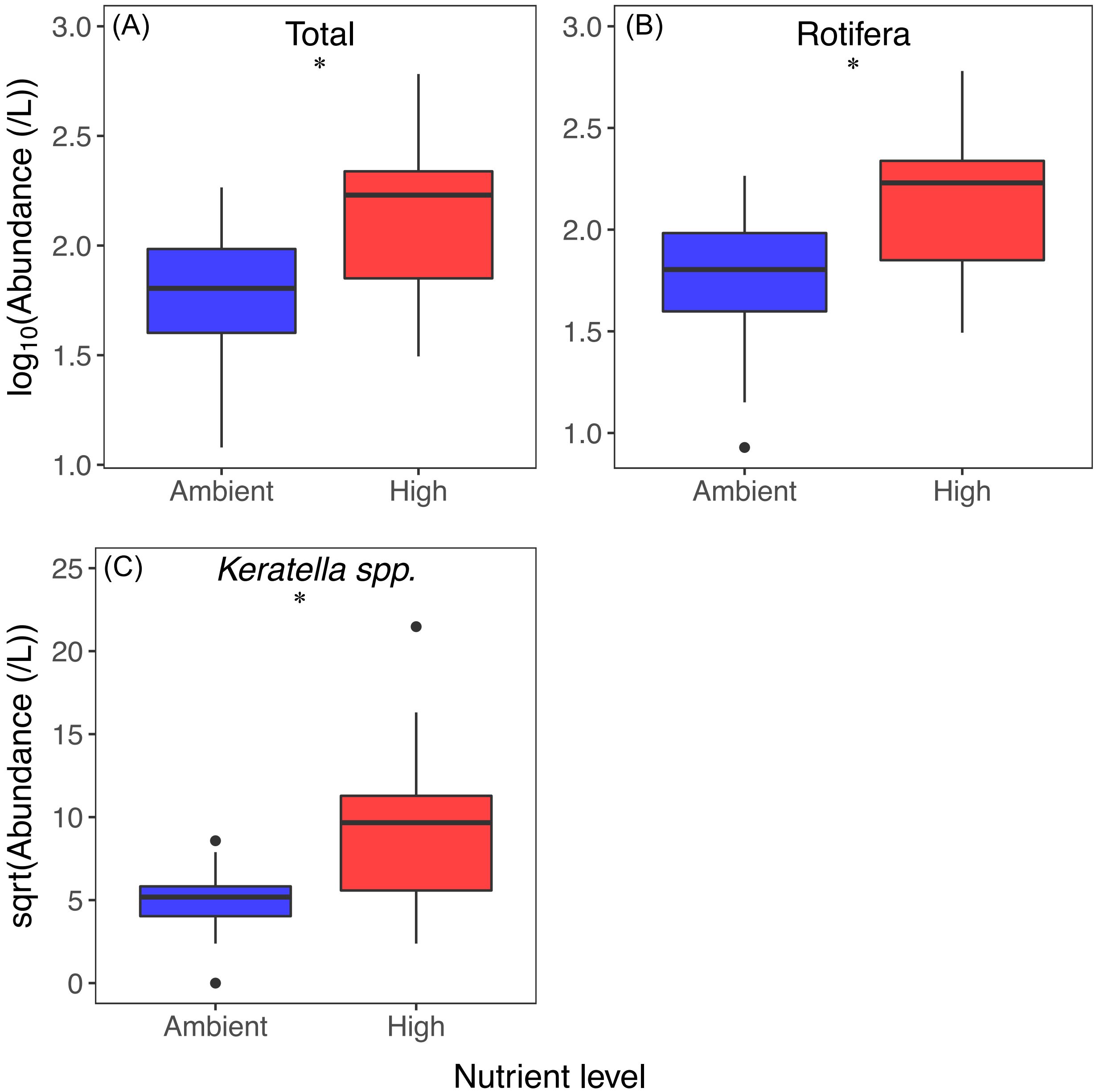

Figure 2 Week 0 (initial) abundance of total zooplankton (A), total rotifers (B), and *Keratella spp.* (C) at ambient (13.6 µg P/L; blue; n=28) and high (31.4 µg P/L; red; n=29) nutrient levels. Total zooplankton and rotifer abundance were $\log_{10}$ transformed while *Keratella spp.* abundance was square root transformed. The bottom and top edges of the boxes represent the first and third quartiles, the thick horizontal line within the boxes represents the medians, and the whiskers represent data points within 1.5 times the interquartile range. Separate points signify data lying outside the interquartile range. Taxa marked with an asterisk (*) had significant differences in abundance between the two nutrient treatments ($p<0.05$).

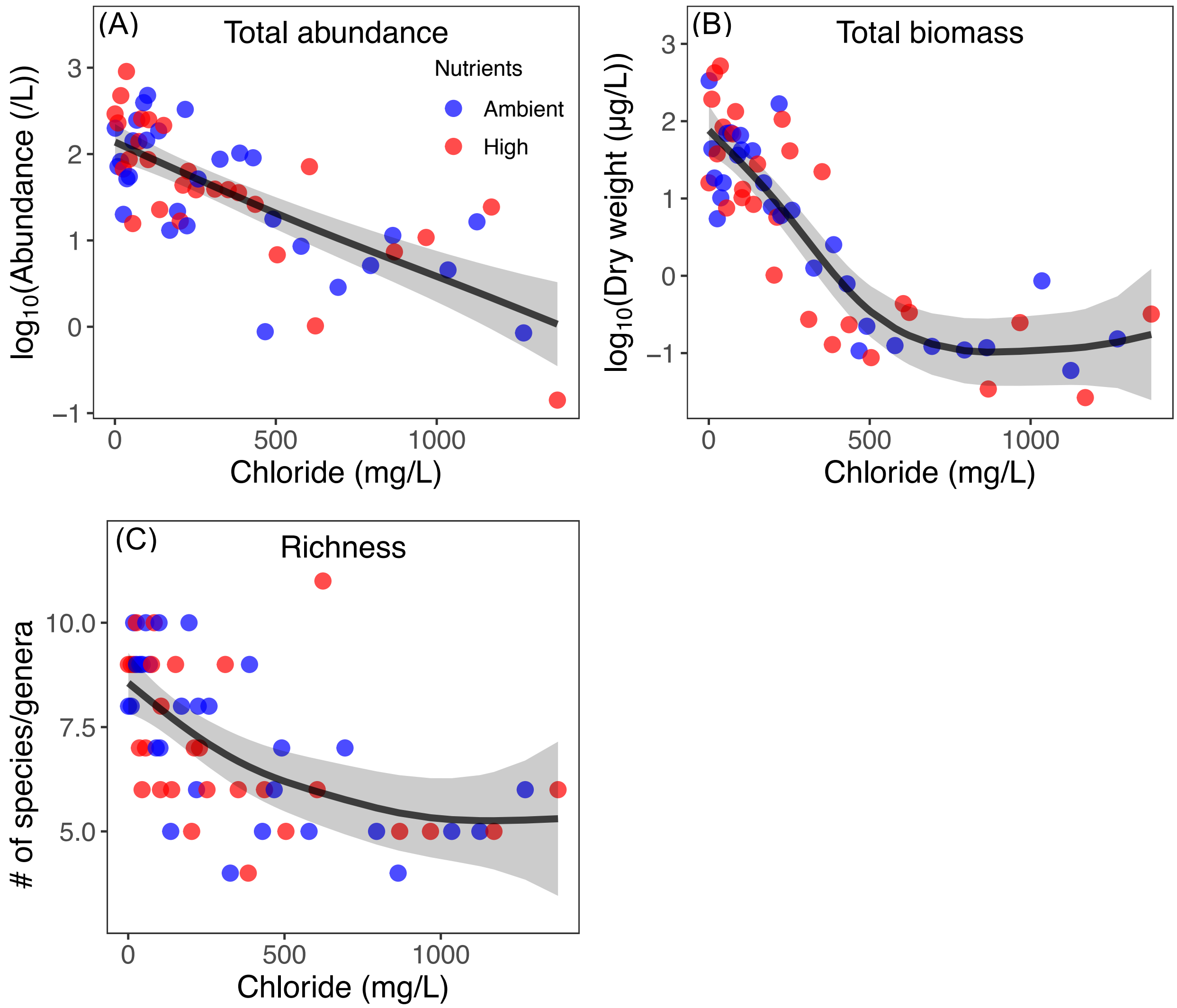

Figure 3 Week 6 (final) total abundance (A) and total biomass (B) of zooplankton, and richness (C; as the sum of crustacean species and rotifer genera) under increasing chloride concentrations (0.41 – 1500 mg/L; n=57). Total abundance was log_{10} transformed and fit with a linear model. Total biomass was log_{10} transformed. Both total biomass and richness were fit with generalized additive models. Total abundance (A, $p<0.001$, $r^2=0.53$), biomass (B, $p<0.001$, $r^2=0.77$) and richness (C, $p<0.001$, $r^2=0.38$) declined significantly with increasing chloride. Shaded areas represent 2x the standard error. As there was no effect of nutrient level on abundance, biomass or richness, both treatments were combined into one line of best fit.

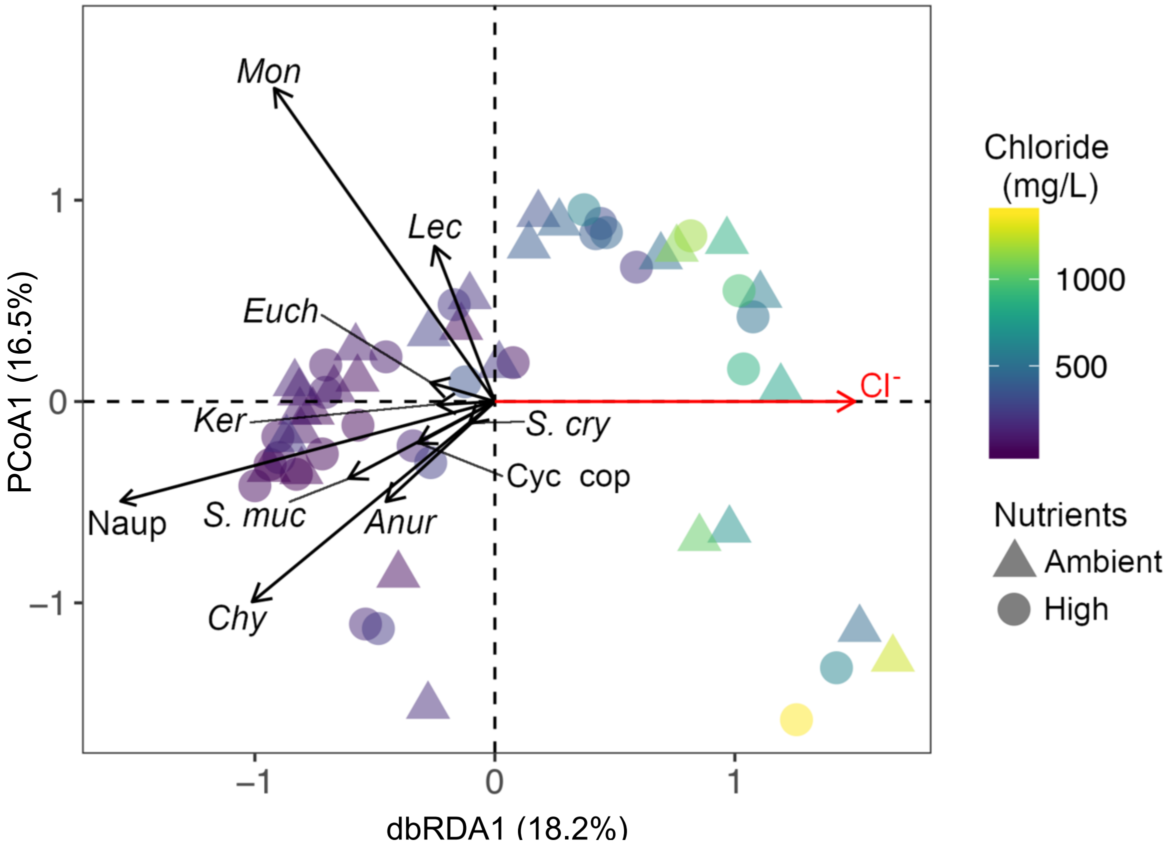

Figure 4 Distance-based redundancy analysis (dbRDA) ordination using Bray-Curtis dissimilarities among zooplankton communities with chloride concentration as a constrained axis (dbRDA1, pseudo-$F_{1,55}$=12.2, $p<0.001$). Each point represents a mesocosm community. Chloride level (red arrow) explained 18.2% of the total variation in zooplankton community. Nutrient level was not a significant source of variation. Family, genera and species abbreviations are defined in Table A3.1.

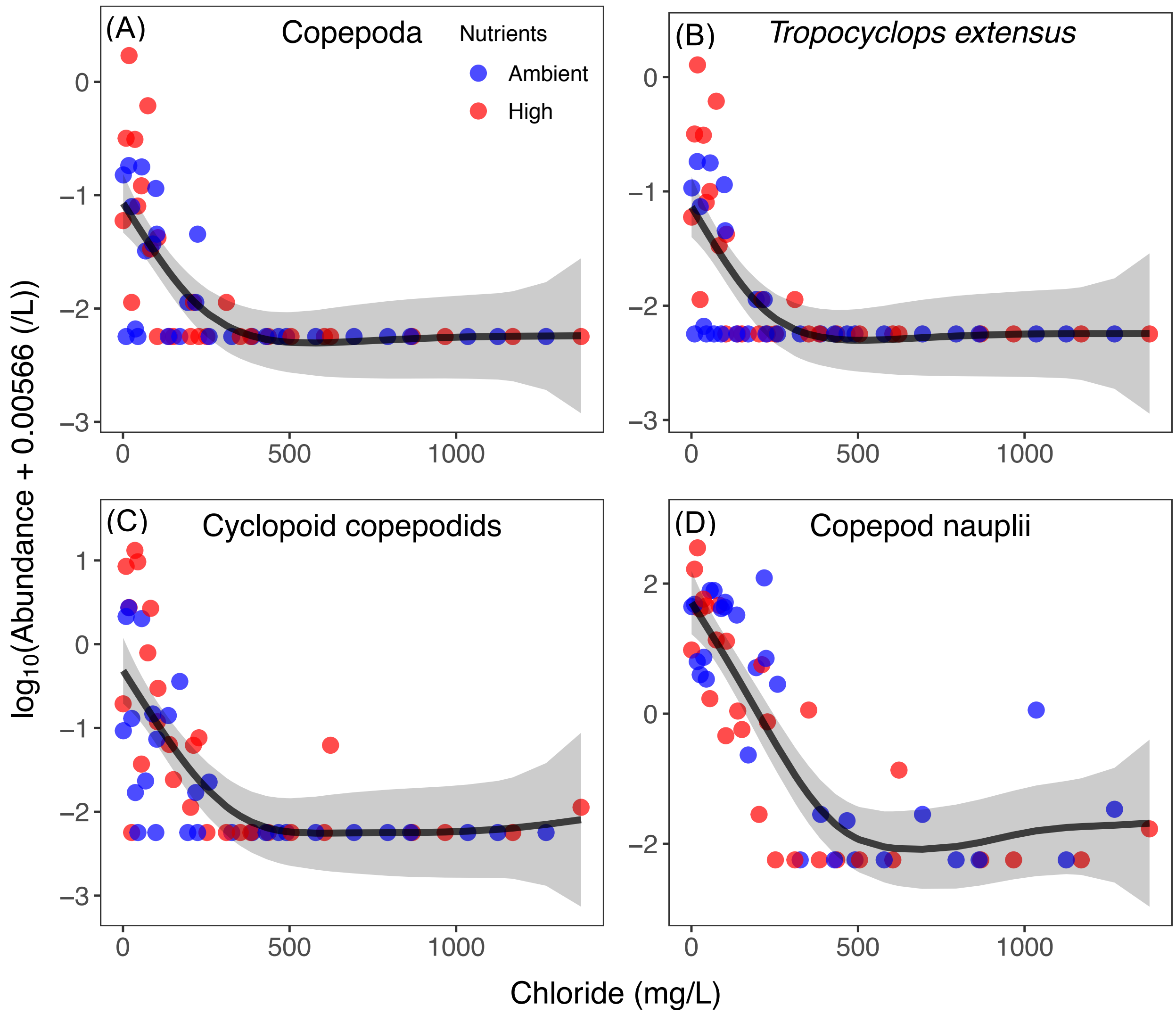

Figure 5 Week 6 (final) abundance of total and common copepods under increasing chloride concentrations (0 – 1500 mg/L; n=57). All were $\log_{10}$ transformed and fit to generalized additive models. Total copepods (A, $p<0.001$, $r^2=0.48$), *Tropocyclops extensus* (B, $p<0.001$, $r^2=0.45$), cyclopoid copepodids (C, $p<0.001$, $r^2=0.50$) and copepod nauplii (D, $p<0.001$, $r^2=0.73$) abundance declined significantly with chloride increases. Shaded areas represent 2x the standard error. As there was no effect of nutrient level on abundance of any copepods, both treatments were combined into one line of best fit for each panel (A – D).

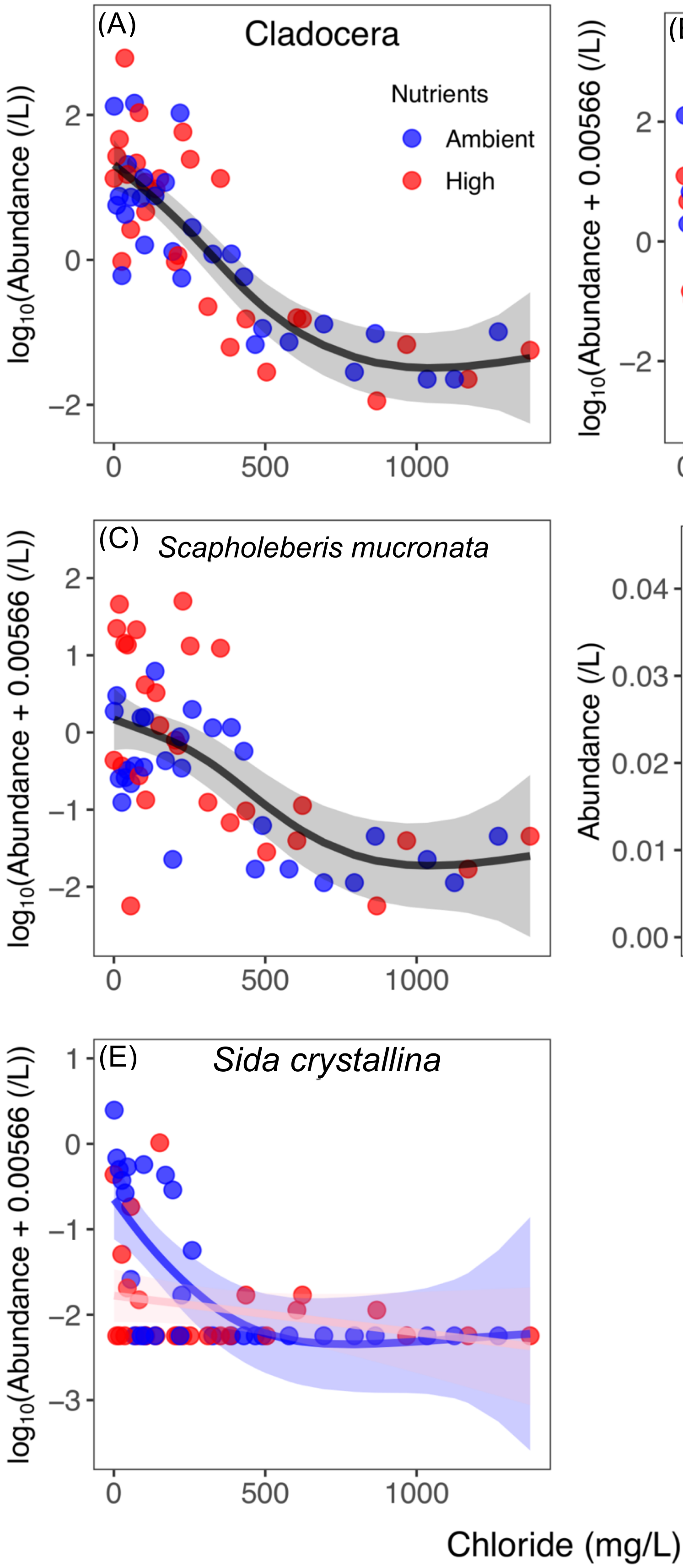
(A) Cladocera
Nutrients
Ambient
High
log10(Abundance (/L))
(B) Chydoridae
log10(Abundance + 0.00566 (/L))
(C) Scapholeberis mucronata
log10(Abundance + 0.00566 (/L))
(D) Bosmina freyi/liederi
Abundance (/L)
(E) Sida crystallina
log10(Abundance + 0.00566 (/L))
Chloride (mg/L)

Figure 6 Week 6 (final) abundance of total and common cladocerans under increasing chloride concentrations (0 – 1500 mg/L; n=57). All abundances were $\log_{10}$ transformed, except for *Bosmina liederi.freyi*. Total cladocerans, Chydoridae, *Scapholeberis mucronata* and *Sida crystallina* abundance were fit with generalized additive models, while *Bosmina freyi/liederi* abundance was fit with a zero inflated-negative binomial generalized linear model. Total cladoceran (A, $p<0.001$, $r^2=0.69$), Chydoridae (B, $p<0.001$, $r^2 = 0.50$) and *Scapholeberis mucronata* abundance (C, $p<0.001$, $r^2=0.41$) declined significantly with increasing chloride. *Bosmina freyi/liederi* abundance significantly declined with increasing chloride, but its occurrence was not affected by chloride (D, $p=0.004$, $\theta=3.26$). There was an interaction between chloride and nutrients on *Sida crystallina* abundance. At ambient nutrients, abundance significantly decreased with chloride (E, $p<0.001$, $r^2=0.47$). At high nutrients, *S. crystallina* was unaffected by chloride (E, $p=0.19$, $r^2=0.03$). Shaded areas represent 2x the standard error. There was no effect of nutrient level on abundance of total cladocerans, Chydoridae, *S. mucronata* or *Bosmina freyi/liederi*, so both nutrient treatments were combined into one line of best fit for each panel (A – D).

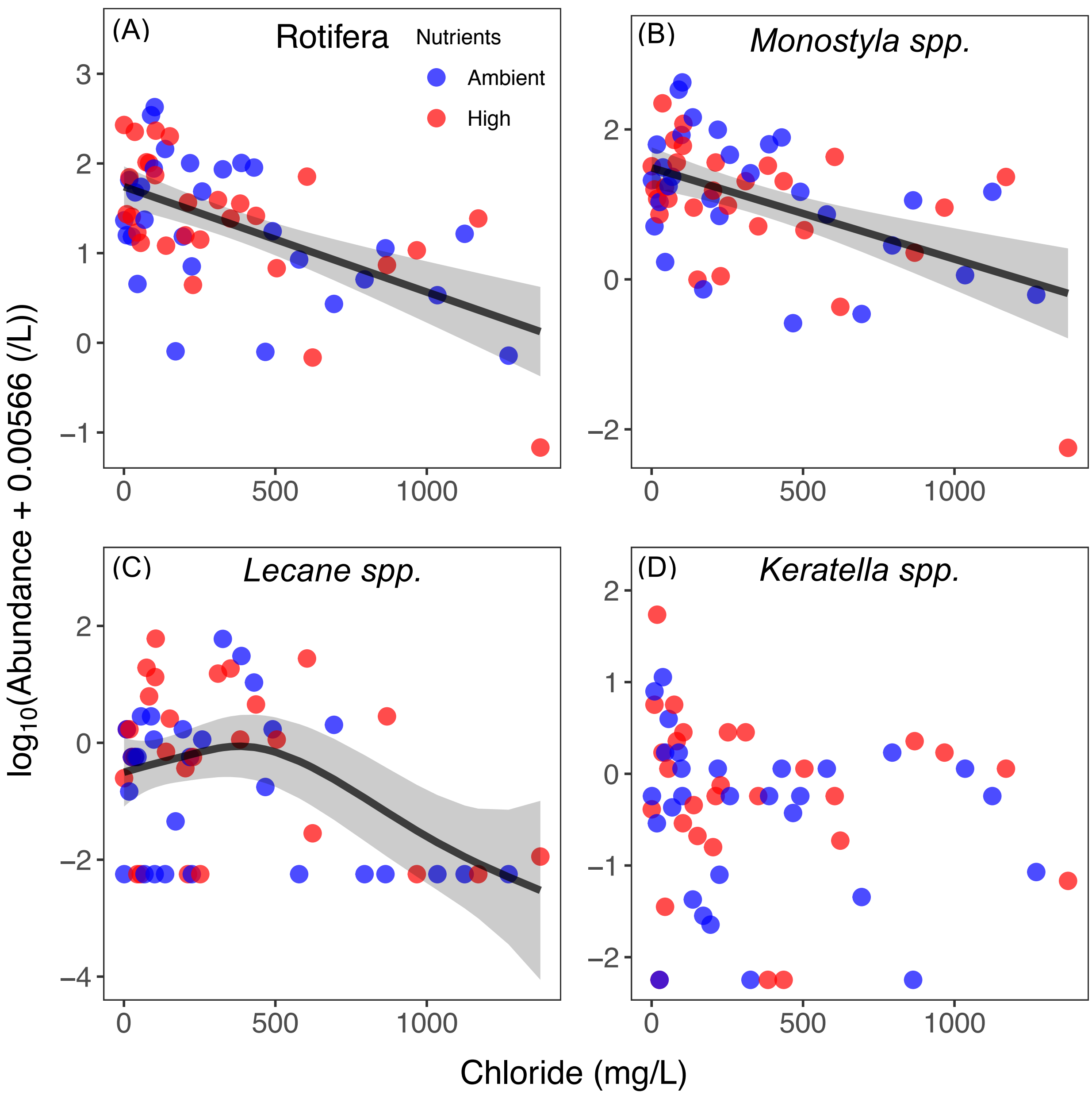

Figure 7 Week 6 (final) abundance of total and common rotifers under increasing chloride concentrations (0 – 1500 mg/L; n=57). All abundances were $\log_{10}$ transformed. Total rotifers and *Monostyla spp.* abundance were fit with linear models, while *Lecane spp.* abundance was fit with a generalized additive model. Total rotifer (A*,* $p<0.001$, $r^2=0.31$) and *Monostyla spp.* abundance (B, $p<0.001$, $r^2 = 0.25$) declined significantly with increasing chloride. Initial increases in *Lecane spp.* were followed by declines in abundance as chloride concentrations increased (C*,* $p=0.007$, $r^2=0.19$). *Keratella spp.* abundance was significantly not affected by chloride (D). Shaded areas represent 2x the standard error. There was no effect of nutrient level on abundance of any taxa, so both nutrient treatments were combined into one line of best fit for each panel (A – C).

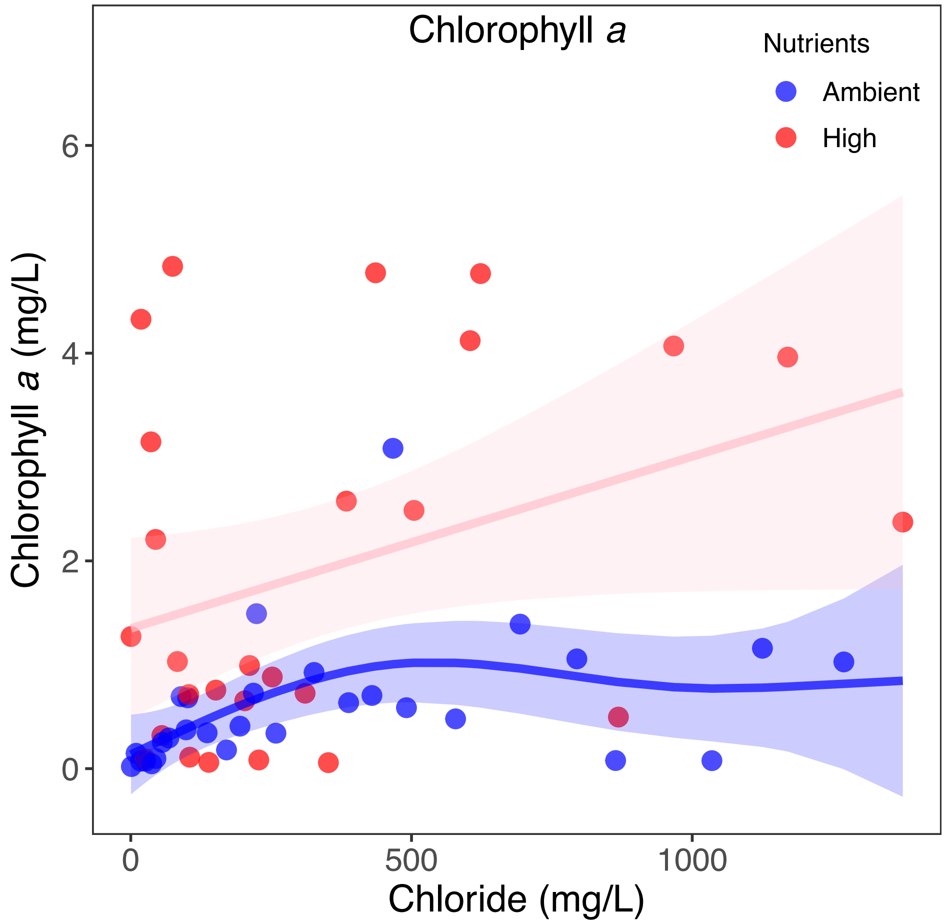

Figure 8 Chlorophyll *a* concentration under increasing chloride concentrations across nutrient levels. Chlorophyll *a* concentration was fit with a generalized additive model. Shaded areas represent 2x the standard error. Faded line indicates a non-significant effect of chloride, solid line indicates a significant effect of chloride. Effects of chloride differed between nutrient levels. Under ambient nutrients, chlorophyll *a* concentration significantly increased up to chloride concentrations of 500 mg/L before leveling off (n=29, p<0.001, r^2=0.53). Under high nutrients, there was no significant effect of chloride (n=27, p=0.08, r^2=0.08).

References

Aladin, N. V, and W. T. W. Potts. 1995. The osmoregulatory capacity of the Cladocera. J. Comp. Physiol. B **164**: 671–683.

Anderson, B.G., D.C. Chandler, T.F. Andrews. and W.J. Jahoda. 1948. The evaluation of aquatic invertebrates as assay organisms for the determination of the toxicity of industrial wastes. Amer. Petroleum Inst. Project Final Report 51. (In Evans and Frick 2001).

Arnold, D. E. 1971. Ingestion, assimilation, survival, and reproduction by *Daphnia pulex* fed seven species of blue-green algae. Limnol. Oceanogr. **16**: 906–920. doi:10.2307/2834296

Arnott, S. E., S. S. E. Azan, and A. J. Ross. 2017. Calcium decline reduces population growth rates of zooplankton in field mesocosms. Can. J. Zool. **333**: 323–333.

Barnett, A., and B. E. Beisner. 2007. Zooplankton biodiversity and lake trophic state: Explanations invoking resource abundance and distribution. Ecology **88**: 1675–1686. doi:10.1890/06-1056.1

Barnett, A. J., K. Finlay, and B. E. Beisner. 2007. Functional diversity of crustacean zooplankton communities: Towards a trait-based classification. Freshw. Biol. **52**: 796–813. doi:10.1111/j.1365-2427.2007.01733.x

Baudouin, M. F., and P. Scoppa. 1974. Acute toxicity of various metals to freshwater zooplankton. B. Environ. Contam. Tox. **12**: 745–751.

Benbow, M. E., and R. W. Merritt. 2006. Road-salt toxicity of select Michigan wetland macroinvertebrates under different testing conditions. Wetlands **24**: 68–76. doi:10.1672/0277-5212(2004)024[0068:rtosmw]2.0.co;2

Bezirci, G., S. B. Akkas, K. Rinke, F. Yildirim, Z. Kalaylioglu, F. Severcan, and M. Beklioglu. 2012. Impacts of salinity and fish-exuded kairomone on the survival and macromolecular profile of *Daphnia pulex*. Ecotoxicology **21**: 601–614. doi:10.1007/s10646-011-0820-0

Bianchini, A., and C. M. Wood. 2008. Sodium uptake in different life stages of crustaceans: The water flea *Daphnia magna* Strauss. J. Exp. Biol. **211**: 539–547. doi:10.1242/jeb.009175

Birge, W.J., J.A. Black, A.G. Westerman, T.M. Short, S.B. Taylor, D.M. Bruser, and E.D. Wallingford. 1985. Recommendations on numerical values for regulating iron and chloride concentrations for the purpose of protecting warmwater species of aquatic life in the Commonwealth of Kentucky. Memorandum of Agreement No. 5429. Kentucky Natural Resources and Environmental Protection Cabinet

Bolen, W.P. 2019. US Geological Survey Minerals Yearbook 2019: Salt. https://minerals.usgs.gov/minerals/pubs/commodity/salt/myb1-2014-salt.pdf

Brown, A. H., and N. D. Yan. 2015. Food quantity affects the sensitivity of *Daphnia* to road salt. Environ. Sci. Technol. **49**: 4673-4680. doi:10.1021/es5061534

Bryson, G. M., and A. V. Barker. 2002. Sodium accumulation in soils and plants along Massachusetts roadsides. Commun. Soil Sci. Plant Anal. **33**: 67–78. doi:10.1081/CSS-120002378

Burns, C. W. 1968. Direct observation of mechanisms regulating feeding behavior of *Daphnia* in lake water. Int. Revue ges. Hydrobiol. **53**: 83-100.

Carpenter, A. S. R., J. F. Kitchell, J. R. Hodgson, and others. 1987. Regulation of lake primary productivity by food web structure. Ecology **68**: 1863–1876.

Cassanelli, J. P., and G. A. Robbins. 2013. Effects of road salt on Connecticut's groundwater: A statewide centennial perspective. J. Environ. Qual. **42**: 737. doi:10.2134/jeq2012.0319

CCME. 2004. Canadian Environmental Quality Guidelines for the Protection of Aquatic life. Quality 1–5.

CCME. 2011. Canadian Water Quality Guidelines for the Protection of Aquatic Life. Can. Environ. Qual. Guidel. 1–9.

Chapra, S. C., A. Dove, and D. C. Rockwell. 2009. Great Lakes chloride trends: Long-term mass balance and loading analysis. J. Great Lakes Res. **35**: 272–284. doi:10.1016/j.jglr.2008.11.013

Chow-Fraser, P., D. O. Trew, D. Findlay, and M. Stainton. 1994. A test of hypotheses to explain the sigmoidal relationship between total phosphorus and chlorophyll *a* concentrations in Canadian Lakes. Can. J. Fish. Aquat. Sci. **51**: 2052–2065. doi:10.1139/f94-208

Clements, W. H., and C. Kotalik. 2016. Effects of major ions on natural benthic communities: An experimental assessment of the US Environmental Protection Agency aquatic life benchmark for conductivity. Freshw. Sci. **35**: 126–138. doi:10.1086/685085

Coldsnow, K. D., B. M. Mattes, W. D. Hintz, and R. A. Relyea. 2017. Rapid evolution of tolerance to road salt in zooplankton. Environ. Pollut. **222**: 367–373. doi:10.1016/j.envpol.2016.12.024

Corsi, S. R., D. J. Graczyk, S. W. Geis, N. L. Booth, and K. D. Richards. 2010. A fresh look at road salt: Aquatic toxicity and water-quality impacts on local, regional, and national scales. Environ. Sci. Technol. **44**: 7376–7382. doi:10.1021/es101333u

Corsi, S. R., L. A. De Cicco, M. A. Lutz, and R. M. Hirsch. 2015. River chloride trends in snow-affected urban watersheds: Increasing concentrations outpace urban growth rate and are common among all seasons. Sci. Total Environ. **508**: 488–497. doi:10.1016/j.scitotenv.2014.12.012

Côté, I. M., E. S. Darling, and C. J. Brown. 2016. Interactions among ecosystem stressors and their importance in conservation. Proc. R. Soc. B Biol. Sci. **283**: 20152592. doi:10.1098/rspb.2015.2592

Cottingham, K. L., J. T. Lennon, and B. L. Brown. 2005. Knowing when to draw the line: Designing more informative ecological experiments. Front. Ecol. Environ. **3**: 145–152. doi:10.1890/1540-9295(2005)003[0145:KWTDTL]2.0.CO;2

Demers, C.L., and R.W. Sage. 1990. Effects of road deicing salt on chloride levels in four Adirondack streams. Water Air Soil Poll. **49**:369-373

Dextrase, A. J., and N. E. Mandrak. 2006. Impacts of alien invasive species on freshwater fauna at risk in Canada. Biol. Invasions **8**: 13–24. doi:10.1007/s10530-005-0232-2

Dodson, S. L., C. E. Cáceres, and D. C. Rogers. 2010. Cladocera and other Branchiopoda, p. 773-827. In J. H. Thorpe and A. P. Covich [eds.], Ecology and classification of North American freshwater invertebrates. Academic Press.

Downing, A. L. 2005. Relative effects of species composition and richness on ecosystem properties in ponds. Ecology **86**: 701–715.

Dugan, H. A., S. L. Bartlett, S. M. Burke, and others. 2017. Salting our freshwater lakes. Proc. Natl. Acad. Sci. **114**: 4453–4458. doi:10.1073/pnas.1620211114

Elphick, J. R. F., K. D. Bergh, and H. C. Bailey. 2011. Chronic toxicity of chloride to freshwater species: Effects of hardness and implications for water quality guidelines. Environ. Toxicol. Chem. **30**: 239–246. doi:10.1002/etc.365

Environment Canada. 2012. Five-Year Review of Progress: Code of Practice for the Environmental Management of Road Salts. Environment Canada, Ottawa.

Evans, M., and C. Frick. 2001. The Effects of Road Salts on Aquatic Ecosystems. WSTD Contrib. No. 02-308 298.

Folt, C.L., C.Y. Chen, M.V. Moore, and J. Burnaford. 1999. Synergism and antagonism among multiple stressors. Limnol. Oceanogr. **44**:864-877.

Fox J, and S. Weisberg. 2019. An R Companion to Applied Regression, Third edition. Sage, Thousand Oaks CA.

Hanazato, T. 1991. Influence of food density on the effects of a *Chaoborus*-released chemical on *Daphnia ambigua*. Freshw. Biol. **25**: 477–483. doi:10.1111/j.1365-2427.1991.tb01390.x

Haney, J. F. 1987. Field studies on zooplankton-cyanobacteria interactions. New Zeal. J. Mar. Freshw. Res. **21**: 467–475. doi:10.1080/00288330.1987.9516242

Haney, J.F. 2013. An-Image-based Key to the Zooplankton of North America version 5.0. University of New Hampshire Center for Freshwater Biology. http://cfb.unh.edu

Hart, B. T., P. Bailey, R. Edwards, K. Hortle, K. James, A. McMahon, C. Meredith, and K. Swadling. 1991. A review of the salt sensitivity of the Australian freshwater biota. Hydrobiologia **210**: 105–144. doi:10.1007/BF00014327

Hébert, M. P., B. E. Beisner, and R. Maranger. 2017. Linking zooplankton communities to ecosystem functioning: Toward an effect-Trait framework. J. Plankton Res. **39**: 3–12. doi:10.1093/plankt/fbw068

Heugens, E. H. W., A. J. Hendriks, T. Dekker, N. M. Van Straalen, and W. Admiraal. 2001. A review of the effects of multiple stressors on aquatic organisms and analysis of uncertainty factors for use in risk assessment. Crit. Rev. Toxicol. **31**: 247–284. doi:10.1080/20014091111695

Hintz, W. D., B. M. Mattes, M. S. Schuler, D. K. Jones, A. B. Stoler, L. Lind, and R. A. Relyea. 2017. Salinization triggers a trophic cascade in experimental freshwater communities with varying food-chain length. Ecol. Appl. **27**: 833–844. doi:10.1002/eap.1487

Hintz, W. D., D. K. Jones, and R. A. Relyea. 2019. Evolved tolerance to freshwater salinization in zooplankton: Life-history trade-offs, cross-tolerance and reducing cascading effects. Philos. Trans. R. Soc. B Biol. Sci. **374**. doi:10.1098/rstb.2018.0012

Hintz, W. D., and R. A. Relyea. 2019. A review of the species, community, and ecosystem impacts of road salt salinisation in fresh waters. Freshw. Biol. **64**: 1081–1097. doi:10.1111/fwb.13286

Howard, K.W.F., and Haynes, J. 1993. Groundwater contamination due to road de-icing chemicals—salt balance implications. Geosci. Can. **20**: 1–8.

Jackman, S. 2017. pscl: Classes and Methods for R Developed in the Political Science Computational Laboratory. United States Studies Centre, University of Sydney. Sydney, New South Wales, Australia. R package version 1.5.2. https://github.com/atahk/pscl/

Jackson, M. C., C. J. G. Loewen, R. D. Vinebrooke, and C. T. Chimimba. 2016. Net effects of multiple stressors in freshwater ecosystems: A meta-analysis. Glob. Change Biol. **22**: 180–189. doi:10.1111/gcb.13028

Jones, D. K., B. M. Mattes, W. D. Hintz, M. S. Schuler, A. B. Stoler, L. A. Lind, R. O. Cooper, and R. A. Relyea. 2017. Investigation of road salts and biotic stressors on freshwater wetland communities. Environ. Pollut. **221**: 159–167. doi:10.1016/j.envpol.2016.11.060

Kanygina, A.V., and M.P. Lebedeva. 1957. The influence of industrial wastes containing sodium chloride on a (river) water system. Chem Abs. 51: 9045 (1957) Pub. Health Engineering Abs **37**: 21 (In Evans and Frick 2001).

Karraker, N. E., J. P. Gibbs, and J. R. Vonesh. 2008. Impacts of road deicing salt on the demography of vernal pool-breeding amphibians. Ecol. Appl. **18**: 724–734. doi:10.1890/07-1644.1

Kaushal, S. S., P. M. Groffman, G. E. Likens, K. T. Belt, W. P. Stack, V. R. Kelly, L. E. Band, and G. T. Fisher. 2005. Increased salinization of fresh water in the northeastern United States. Proc. Natl. Acad. Sci. **102**: 13517–13520. doi:10.1073/pnas.0506414102

Kaushal, S. S., G. E. Likens, M. L. Pace, R. M. Utz, S. Haq, J. Gorman, and M. Grese. 2018. Freshwater salinization syndrome on a continental scale. Proc. Natl. Acad. Sci. **115**: E574–E583. doi:10.1073/pnas.1711234115

Kelly, V. R., G. M. Lovett, K. C. Weathers, S. E. G. Findlay, D. L. Strayer, D. J. Burns, and G. E. Likens. 2008. Long-term sodium chloride retention in a rural watershed: Legacy effects of road salt on streamwater concentration. Environ. Sci. Technol. **42**: 410–415. doi:10.1021/es071391l

Kreyling, J., A. H. Schweiger, M. Bahn, and others. 2018. To replicate, or not to replicate – that is the question: how to tackle nonlinear responses in ecological experiments. Ecol. Lett. **21**: 1629–1638. doi:10.1111/ele.13134

Lampert, W., W. Fleckner, H. Rai, and B. E. Taylor. 1986. Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase. Limnol. Oceanogr. **31**: 478–490. doi:10.4319/lo.1986.31.3.0478

Latta, L. C., L. J. Weider, J. K. Colbourne, and M. E. Pfrender. 2012. The evolution of salinity tolerance in *Daphnia*: A functional genomics approach. Ecol. Lett. **15**: 794–802. doi:10.1111/j.1461-0248.2012.01799.x

Leibold, M. A. 1999. Biodiversity and nutrient enrichment in pond plankton communities. Evol. Ecol. Res. **1**: 73–95.

Lind, L., M. S. Schuler, W. D. Hintz, A. B. Stoler, D. K. Jones, B. M. Mattes, and R. A. Relyea. 2018. Salty fertile lakes: how salinization and eutrophication alter the structure of freshwater communities. Ecosphere **9**. doi:10.1002/ecs2.2383

MacDougall, M. J., A. M. Paterson, J. G. Winter, F. C. Jones, L. A. Knopf, and R. I. Hall. 2016. Response of periphytic diatom communities to multiple stressors influencing lakes in the Muskoka River Watershed, Ontario, Canada. Freshw. Sci. **36**: 77–89. doi:10.1086/690144

Martínez-Jerónimo, F., and L. Martínez-Jerónimo. 2007. Chronic effect of NaCl salinity on a freshwater strain of *Daphnia magna* Straus (Crustacea: Cladocera): A demographic study. Ecotoxicol. Environ. Safe. **67**: 411–416. doi:10.1016/j.ecoenv.2006.08.009

Mayer, T., W. J. Snodgrass, and D. Morin. 1999. Spatial characterization of the occurrence of road salts and their environmental concentrations as chlorides in Canadian surface waters and benthic sediments. Water Qual. Res. Canada **34:**545-575.

Mohammed, A. A., and A. A. Shafea. 1992. Growth and some metabolic activities of *Scenedesmus obliquus* cultivated under different NaCl concentrations. Biol. Plant. **34**: 423–430.

Mohapatra, P. K., R. C. Dash, S. S. Panda, R. K. Mishra, and R. C. Mohanty. 1998. Effects of nutrients at different salinities on growth of the freshwater green alga *Scenedesmus bijugatus* in water of Narendra Pond, Puri, Orissa. Int. Rev. Hydrobiol. **83**: 297–304. doi:10.1002/iroh.19980830405

Moisander, P. H., E. McClinton, and H. W. Paerl. 2002. Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. Microb. Ecol. **43**: 432–442. doi:10.1007/s00248-001-1044-2

Molitor, V. W. Erber, and G. A. Peschek. 1986. Increased levels of cytochrome oxidase and sodium-proton antiporter in the plasma membrane of *Anacystis nidulans* after growth in sodium-enriched media. FEBS Lett. **204**: 251–256. doi:10.1016/0014-5793(86)80822-5

Mount, D. R., D. D. Gulley, J. R. Hockett, T. D. Garrison, and J. M. Evans. 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna* and *Pimephales promelas* (fathead minnows). Environ. Toxicol. **16**: 2009–2019

Novotny, E. V., D. Murphy, and H. G. Stefan. 2008. Increase of urban lake salinity by road deicing salt. Sci. Total Environ. **406**: 131–144. doi:10.1016/j.scitotenv.2008.07.037

Oksanen, J. F., G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs and H. Wagner. 2019. vegan: Community Ecology Package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Olvera-Ramírez, R., C. Centeno-Ramos, and F. Martínez-Jerónimo. 2010. Toxic effects of *Pseudanabaena tenuis* (cyanobacteria) on the cladocerans *Daphnia magna* and *Ceriodaphnia dubia*. Hidrobiolog. **20**: 203–212.

Oswald, C. J., G. Giberson, E. Nicholls, C. Wellen, and S. Oni. 2019. Spatial distribution and extent of urban land cover control watershed-scale chloride retention. Sci. Total Environ. **652**: 278–288. doi:10.1016/j.scitotenv.2018.10.242

Paerl, H. W., and T. G. Otten. 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. Microb. Ecol. **65**: 995–1010. doi:10.1007/s00248-012-0159-y

Pal, A., K. Y. H. Gin, A. Y. C. Lin, and M. Reinhard. 2010. Impacts of emerging organic contaminants on freshwater resources: Review of recent occurrences, sources, fate and effects. Sci. Total Environ. **408**: 6062–6069. doi:10.1016/j.scitotenv.2010.09.026

Pandit, P. R., M. H. Fulekar, and M. S. L. Karuna. 2017. Effect of salinity stress on growth, lipid productivity, fatty acid composition, and biodiesel properties in *Acutodesmus obliquus* and *Chlorella vulgaris*. Environ. Sci. Pollut. Res. **24**: 13437–13451. doi:10.1007/s11356-017-8875-y

Pauwels, K., R. Stoks, and L. De Meester. 2010. Enhanced anti-predator defence in the presence of food stress in the water flea *Daphnia magna*. Funct. Ecol. **24**: 322–329. doi:10.1111/j.1365-2435.2009.01641.x

Petranka, J. W., and R. A. Francis. 2013. Effects of road salts on seasonal wetlands: Poor prey performance may compromise growth of predatory salamanders. Wetlands **33**: 707–715. doi:10.1007/s13157-013-0428-7

Pilkaitytë, R., A. Schoor, and H. Schubert. 2004. Response of phytoplankton communities to salinity changes - A mesocosm approach. Hydrobiologia **513**: 27–38. doi:10.1023/B:hydr.0000018162.50270.54

Porter, K G. 1973. Selective grazing and differential digestion of algae by zooplankton. Nature **244**: 19–180.

Rai, A. K., and S. P. Tiwari. 1999. NO_3 - nutrition and salt tolerance in the cyanobacterium *Anabaena sp.* PCC 7120 and mutant strains. J. Appl. Microbiol. **86**: 991–998. doi:10.1046/j.1365-2672.1999.00788.x

Reed, R. H., D. L. Richardson, and W. D. P. Stewart. 1985. Na + Uptake and extrusion in the cyanobacterium *synechocystis*PCC6714 in response to hypersaline treatment. Evidence for transient changes in plasmalemma Na^+ permeability. BBA - Biomembranes **814**: 347–355. doi:10.1016/0005-2736(85)90455-9

Reid, J. W., and C. E. Williamson. 2010. Copepoda. p. 829-899. In J. H. Thorpe and A. P. Covich [eds.], Ecology and classification of North American freshwater invertebrates. Academic Press.

Relyea, R. A., N. M. Schoeppner, and J. T. Hoverman. 2005. Pesticides and amphibians: The importance of community context. Ecol. Appl. **15**: 1125–1134. doi:10.1890/04-0559

Relyea, R., and J. Hoverman. 2006. Assessing the ecology in ecotoxicology: A review and synthesis in freshwater systems. Ecol. Lett. **9**: 1157–1171. doi:10.1111/j.1461-0248.2006.00966.x

Robinson, H. K., E. A. Hasenmueller, and L. G. Chambers. 2017. Soil as a reservoir for road salt retention leading to its gradual release to groundwater. Appl. Geochemistry **83**: 72–85. doi:10.1016/j.apgeochem.2017.01.018

Rohr, J. R., J. L. Kerby, and A. Sih. 2006. Community ecology as a framework for predicting contaminant effects. Trends Ecol. Evol. **21**: 606–613. doi:10.1016/j.tree.2006.07.002

Rosenberger, E. E., S. E. Hampton, S. C. Fradkin, and B. P. Kennedy. 2008. Effects of shoreline development on the nearshore environment in large deep oligotrophic lakes. Freshw. Biol. **53**: 1673–1691. doi:10.1111/j.1365-2427.2008.01990.x

Tonk, L., K. Bosch, P. M. Visser, and J. Huisman. 2007. Salt tolerance of the harmful cyanobacterium *Microcystis aeruginosa*. Aquat. Microb. Ecol. **46**: 117–123. doi:10.3354/ame046117

Sarma, S. S. S., S. Nandini, J. Morales-Ventura, I. Delgado-Martínez, and L. González-Valverde. 2006. Effects of NaCl salinity on the population dynamics of freshwater zooplankton (rotifers and cladocerans). Aquat. Ecol. **40**: 349–360. doi:10.1007/s10452-006-9039-1

Schuytema, G.S., A.V. Nebeker, and T.W. Stutzman. 1996. Salinity tolerance of *Daphnia magna* and potential use of estuarine sediment toxicity tests. Arch. Environ. Contam. Toxicol. **33:**194-198.

Sharma, S., K. Blagrave, J. J. Magnuson, and others. 2019. Widespread loss of lake ice around the Northern Hemisphere in a warming world. Nat. Clim. Chang. **9**: 227–231. doi:10.1038/s41558-018-0393-5

Sibert, R. J., C. M. Koretsky, and D. A. Wyman. 2015. Cultural meromixis: Effects of road salt on the chemical stratification of an urban kettle lake. Chem. Geol. **395**: 126–137. doi:10.1016/j.chemgeo.2014.12.010

Sinclair, J. S., and S. E. Arnott. 2018. Local context and connectivity determine the response of zooplankton communities to salt contamination. Freshw. Biol. **63**: 1273–1286. doi:10.1111/fwb.13132

Smith, K., and C.H. Fernando. 1978. A guide to the freshwater calanoid and cyclopoid copepod Crustacea of Ontario. Department of Biology, Waterloo, Ontario, Canada.

Smith, V. H., and D. W. Schindler. 2009. Eutrophication science: where do we go from here? Trends Ecol. Evol. **24**: 201–207. doi:10.1016/j.tree.2008.11.009

Stemberger, R. S. 1979. A guide to rotifers of the Laurentian Great Lakes. EPA-600/4-79-021. U.S. Environmental Protection Agency, Cincinnati, Ohio, USA.

Stoler, A. B., W. D. Hintz, D. K. Jones, L. Lind, B. M. Mattes, M. S. Schuler, and R. A. Relyea. 2017a. Leaf litter mediates the negative effect of road salt on forested wetland communities. Freshw. Sci. **36**: 415–426. doi:10.1086/692139

Stoler, A. B., B. M. Walker, W. D. Hintz, D. K. Jones, L. Lind, B. M. Mattes, M. S. Schuler, and R. A. Relyea. 2017b. Combined effects of road salt and an insecticide on wetland communities. Environ. Toxicol. Chem. **36**: 771–779. doi:10.1002/etc.3639

Tang, Y., A. Dickey, M. Grenié, M. Horikoshi, L. Selzer, D. Strbenac, R. Thompson, and K. Voronin. 2019. ggfortify: Data visualization tools for statistical analysis results. R package version 0.4.8. https://cran.r-project.org/web/packages/ggfortify

Thermo Fisher Scientific. 2007. FlashEA 1112 Elemental Analyzer Operating Manual. Delft, Netherlands.

Thompson, P. L., and J. B. Shurin. 2012. Regional zooplankton biodiversity provides limited buffering of pond ecosystems against climate change. J. Anim. Ecol. **81**: 251–259. doi:10.1111/j.1365-2656.2011.01908.x

TRB. 1991. Highway deicing: Comparing salt and calcium magnesium acetate. Special Report 235, National Research Council, Washington, DC.

TRCA. 2018. 2017 Surface Water Quality Summary –Regional Watershed Monitoring Program.

USEPA. 1988. Ambient water quality criteria for chloride–1988. Washington, DC: U.S. Environmental Protection Agency.

Van Meter, R. J., C. M. Swan, J. Leips, and J. W. Snodgrass. 2011. Road salt stress induces novel food web structure and interactions. Wetlands **31**: 843–851. doi:10.1007/s13157-011-0199-y

Wallace, R. L., and T. W. Snell. 2010. Rotifera, p. 173-235. In J. H. Thorpe and A. P. Covich [eds.], Ecology and classification of North American freshwater invertebrates. Academic Press.

Welschmeyer, N.A. 1994. Fluorometric analysis of chlorophyll-a in the presence of chlorophyll-b and pheopigments. Limnol. Oceanogr. **39**:1985-1992.

Williamson, C. E., J. E. Saros, and D. W. Schindler. 2009. Sentinels of Change. Science **323**: 887–888.

Wilson, S. E., B. F. Cumming, and J. P. Smol. 1994. Diatom-salinity relationships in 111 lakes from the Interior Plateau of British Columbia, Canada: the development of diatom-based models for paleosalinity reconstructions. J. Paleolimnol. **12**: 197–221. doi:10.1007/BF00678021

Witty, L.M. 2004. Practical guide to identifying freshwater crustacean zooplankton. Cooperative Freshwater Ecology Unit, Sudbury, Ontario, Canada.

Wong, N. 2018. Plankton counting tool. https://arnottlab.weebly.com/counting-tool.html

Wood, S. N. 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J. R. Stat. Soc. B. **73**: 3–36.

Wyn Jones, R.G., and A. Pollard. 1983. Proteins, enzymes and inorganic ions, p. 528-562. In A. Lauchli and A. Pirson [eds.], Encyclopedia of Plant Physiology: New Series Vol 15B. Springer.

Yancey, P.H., W.E. Clark, S.C. Hand, R.D. Bowlus, G.N. Somero. 1982. Living with water stress: evolution of osmolyte systems. Science **217:**1214-1222.

Zhu, J.K. 2002. Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol. **53**:247-273.

Appendix A
Biomass Formulae

Table A1.1 Formulae used to calculate biomass for each family, genus or species of zooplankton. W = dry weight (µg); L = length (mm); V = volume (mm^3)

Species/Genus	Formula	Source
Bosmina freyi/liederi	lnW = 4.9344+4.849×lnL	Rosen (1981)
Ceriodaphnia lacustris	lnW = 1.915+2.02×lnL	Dumont et al. (1975)
Chydoridae[a]	lnW = 4.49+3.93×lnL	Dumont et al. (1975)
Cyclopoid copepodids[b]	lnW = 1.3135+2.9005×lnL	Malley et al. (1989)
Copepod nauplii	lnW = 0.6977+0.569×lnL	Rosen (1981)
Daphnia mendotae	lnW = 2.64+2.54×lnL	Bottrell et al. (1976)
Daphnia pulicaria	lnW = 1.4663+3.1932×lnL	Bottrell et al. (1976)
Diaphansoma brachyurum	lnW = 1.6242+3.0468×lnL	Bottrell et al. (1976)
Holopedium gibberum	lnW = 5.3976+2.056×lnL	Bottrell et al. (1976)
Ilyocryptus spinifer[c]	lnW = 5.9913+7.942×lnL	McCauley (1984)
Mesocyclops edax	lnW = 1.3472+3.0087×lnL	Lawrence et al. (1987)
Ophryoxus gracillis[d]	lnW = 2.64+2.54×lnL	Bottrell et al. (1976)
Scapholeberis mucronata	lnW = 2.8713+3.079×lnL	McCauley (1984)
Sida crystallina	lnW = 2.0539+2.189×lnL	McCauley (1984)
Tropocyclops extensus	lnW = 1.7116+3.6663×lnL	Lawrence et al. (1987)
Anuraeopsis spp.	$V = 0.03 \times L^3$	Ruttner-Kolisko (1977)
Asplancha spp.	$V = 0.23 \times L^3$	Ruttner-Kolisko (1977)
Collotheca spp.	$V = 1.44 \times L^3$	Ruttner-Kolisko (1977)
Euchlanis spp.	$V = 0.1 \times L^3$	Ruttner-Kolisko (1977)
Kellicottia spp.	0.05 µg	Cajander (1983)
Keratella spp.	$V = 0.02L^3$	Ruttner-Kolisko (1977)
Lecane spp.	$V = 0.1L^3$	Andersson et al. (2003)
Monostyla spp.	0.12 µg	Dickerson and Robinson (1984)
Trichocerca spp.	0.1 µg	Dumont et al. (1975)

[a]formula based on *Chydorus sphaericus*

[b]formula based on *Diacyclops bicuspidatus thomasi* and *Mesocyclops edax* copepodids

[c]formula based on *Ilyocryptus sordidus*

[d]formula based on *Daphnia mendotae*

References

Andersson, E., M-M. Tudorancea, C. Tudorancea, A-K. Brunberg, and P. Blomqvist. 2003. Water chemistry, biomass and production of biota in lake Eckarfjärden during 2002. Report to Svensk Kärnbränslehantering AB.

Bottrell, H. H., A. Duncan, Z. M. Gliwicz, E. Grygierek, A. Herzig, A. Hillbricht-Ilkowska, H. Kurasawa, P. Larsson and T. Weglenska. 1976. A review of some problems in zooplankton production studies. Norw. J. Zool. **24**: 419-456.

Cajander, V-R. 1983. Production of planktonic Rotatoria in Ormajärvi, an eutrophicated lake in southern Finland. Hydrobiologia **104**: 329-333.

Dickerson, J. E. Jr., and J. V. Robinson. 1984. The assembly of microscopic communities: patterns of species importance. Trans. Am. Microsc. Soc. **103**: 164-171.

Dumont, H. J., I. Van de Velde, and S. Dumont. 1975. Dry weight estimate of biomass in a selection of Cladocera, Copepoda, and Rotifera from the plankton, periphyton, and benthos of continental waters. Oecologia **19**: 75-97.

Lawrence, S. G., D. F. Malley, W. J. Findlay, M. A. MacIver, and I. L. Delbaere. 1987. Method for estimating dry weight of freshwater planktonic crustaceans from measures of length and shape. Can. J. Fish. Aquat. Sci. **44**: 264-274.

Malley, D. F., S. G. Lawrence, M. A. MacIver, and W. J. Findlay. 1989. Range of variation in estimates of dry weight for planktonic Crustacea and rotifera from temperate North American lakes. Can. Tech. Rep. Fish. Aquat. Sci. No. 1666.

McCauley, E. 1984. The estimation of the abundance and biomass of zooplankton in samples, p. 228-265. In J.A. Downing and F.H. Rigler [eds.], Manual on Methods for the Assessment of Secondary Productivity in Fresh Waters. Blackwell Scientific.

Rosen, R. A. 1981. Length-dry weight relationships of some freshwater zooplankton. J. Freshwater Ecol. **1**: 225-229.

Ruttner-Kolisko, A. 1977. Suggestions for biomass calculations of plankton rotifers. Arch. Hydrobiol. – Beih. Ergebn. Limnol. **8**: 71-76.

Appendix B
Seston Carbon and Chloride Concentrations

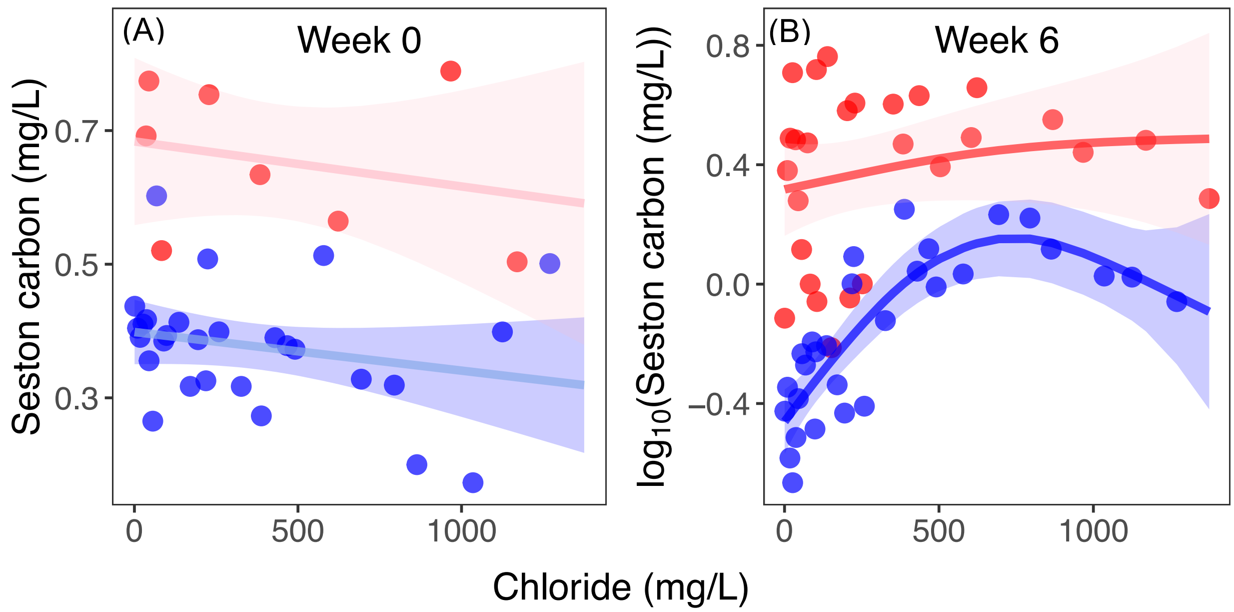

Figure A2.1 Week 0 (initial; A) and week 6 (final; B) seston carbon concentration under increasing chloride concentrations (0.41 – 1500 mg/L). Week 0 seston carbon (ambient, n=8; high, n=28) was fit with a linear model. Week 6 seston carbon (ambient, n=29; high, n=27) was $\log_{10}$ transformed and fit with a generalized additive model. Week 0 carbon significantly differed between nutrient treatments, but was not affected by chloride (A, $p<0.001$, $r^2=0.62$). Week 6 carbon was significantly affected by nutrient addition and by increases in chloride; however, there was no interaction (B, $p<0.001$, $r^2=0.65$). Opaque lines represent a significant chloride effect. Shaded areas represent 2x the standard error.

Appendix C
Week 0 Abundance of Cladocerans, *Scapholeberis mucronata* and *Bosmina freyi/liederi*

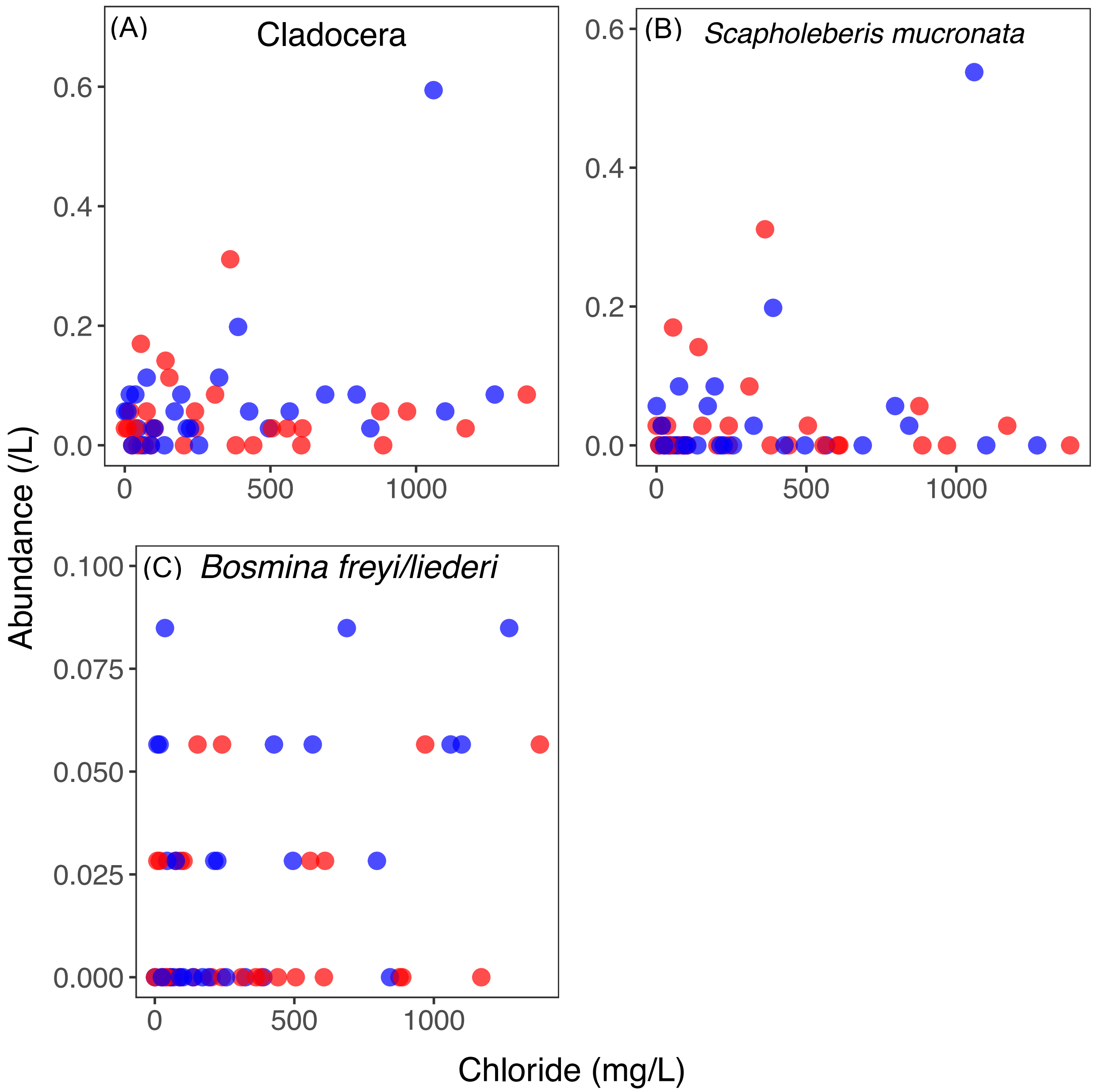

Figure A3.1 Week 0 (initial) abundance of total and common cladocerans under ambient (13.6 µg P/L; blue; n=28) and high (31.4 µg P/L; red; n=29) nutrient levels. There were no effects of nutrients or chloride on cladoceran abundance (A), *Scapholeberis mucronata* abundance (B) or *Bosmina freyi/liederi* abundance (C).

Appendix D
Abbreviations for the Distance Based Redundancy Analysis

Table A4.1 Family, genus or species abbreviations found in the dbRDA (Fig. 4).

Abbreviation	Family/genus/species
Anur	*Anuraeopsis spp.*
Lec	*Lecane spp.*
Mon	*Monostyla spp.*
Euch	*Euchlanis spp.*
Ker	*Keratella spp.*
Naup	Nauplii
S. muc	*Scapholeberis mucronata*
Chy	Chydoridae
S. cry	*Sida crystallina*
Cyc cop	Cyclopoid copepodids

Appendix E
Week 0 Model Selection Parameters

Table A5.1 Model selection parameters for variables predicting log_{10} transformed week 0 total zooplankton abundance. The most scientifically conservative model containing only predictors found within all top models (<2 AICc points), with nutrients only (italicized), was selected.

Predictors	Model type	df	AICc	ΔAICc
nutrients	*LM*	*3*	*31.4*	*0.0*
chloride, nutrients	LM	4	32.3	0.9
chloride, nutrients, chloride × nutrients	LM	5	34.2	2.8
none	LM	2	47.4	16.0
chloride	LM	3	48.8	17.4

Table A5.2 Model selection parameters for variables predicting square root transformed week 0 cyclopoid copepodid abundance. The most scientifically conservative model, with no predictors (italicized), was selected.

Predictors	Model type	df	AICc	ΔAICc
none	*LM*	*2*	*-70.2*	*0.0*
chloride	LM	3	-69.6	0.6
nutrients	LM	3	-68.0	2.2
chloride, nutrients	LM	4	-67.3	2.9
chloride, nutrients, chloride × nutrients	LM	5	-64.9	5.3

Table A5.3 Model selection parameters for variables predicting square root transformed week 0 cladoceran abundance. The most scientifically conservative model, with no predictors (italicized), was selected.

Predictors	Model type	df	AICc	ΔAICc
chloride, nutrients, chloride × nutrients	LM	5	-50.9	0.0
chloride	LM	3	-50.6	0.2
chloride, nutrients	LM	4	-49.5	1.4
none	*LM*	*2*	*-49.3*	*1.5*
nutrients	LM	3	-48.1	2.8

Table A5.4 Model selection parameters for variables predicting log_{10} transformed week 0 *Scapholeberis mucronata* abundance. The model containing no predictors (italicized) provided the best fit.

Predictors	Model type	df	AICc	ΔAICc
none	*LM*	*2*	*32.9*	*0.0*
chloride	LM	3	35.0	2.0
nutrients	LM	3	35.2	2.2
chloride, nutrients	LM	4	37.3	4.4
chloride, nutrients, chloride × nutrients	LM	5	38.1	5.1

Table A5.5 Model selection parameters for variables predicting square-root transformed week 0 *Bosmina freyi/liederi* abundance. The most scientifically conservative model, with no predictors (italicized), was selected.

Predictors	Model type	df	AICc	ΔAICc
none	*NB*	*2*	*365.9*	*0.0*
chloride	NB	3	367.2	1.3
nutrients	NB	3	367.3	1.4
chloride, nutrients	NB	4	368.8	2.9
chloride, nutrients, chloride × nutrients	NB	5	371.1	5.2

Table A5.6 Model selection parameters for variables predicting log_{10} transformed week 0 rotifer abundance. The most scientifically conservative model containing only predictors found within all top models (<2 AICc points), with nutrients only (italicized), was selected.

Predictors	Model type	df	AICc	ΔAICc
nutrients	*LM*	*3*	*35.2*	*0.0*
chloride, nutrients	LM	4	35.8	0.6
chloride, nutrients, chloride × nutrients	LM	5	37.5	2.4
none	LM	2	51.0	15.8
chloride	LM	3	52.2	17.0

Table A5.7 Model selection parameters for variables predicting square root transformed week 0 *Keratella spp.* The most scientifically conservative model containing only predictors found within all top models (<2 AICc points), with nutrients only (italicized), was selected.

Predictors	Model type	df	AICc	ΔAICc
nutrients	*LM*	*3*	*308.9*	*0.0*
chloride, nutrients	LM	4	310.0	1.1
chloride, nutrients, chloride × nutrients	LM	5	312.4	3.5
none	LM	2	324.6	15.7
chloride	LM	3	326.1	17.2

Table A5.8 Model selection parameters for variables predicting week 0 chlorophyll *a* concentration. The model containing chloride, nutrients, chloride × nutrients (italicized) provided the best fit.

Predictors	Model type	df	AICc	ΔAICc
chloride, nutrients, chloride × nutrients	*LM*	*5*	*-103.3*	*0.0*
chloride, nutrients	LM	4	-95.2	8.1
nutrients	LM	3	-90.2	13.1
none	LM	2	63.8	167.1
chloride	LM	3	65.7	169.0

Appendix F
Week 6 Total, Biomass and Richness Model Selection Parameters

Table A6.1 Model selection parameters for variables predicting $\log_{10}$ transformed week 6 total abundance. The model containing chloride only (italicized) provided the best fit.

Predictors	Model type	df	AICc	ΔAICc
chloride	*GAM*	*3.56*	*93.9*	*0*
chloride, nutrients	GAM	4.49	96.1	2.2
chloride, nutrients, chloride × nutrients	GAM	5.47	98.3	4.4
none	GAM	2	135.1	41.2
nutrients	GAM	3	137.2	43.3

Table A6.2 Model selection parameters for variables predicting $\log_{10}$ transformed week 6 biomass. The model containing chloride only (italicized) provided the best fit.

Predictors	Model type	df	AICc	ΔAICc
chloride	*GAM*	*5.99*	*109.9*	*0*
chloride, nutrients	GAM	6.96	112.5	2.6
chloride, nutrients, chloride × nutrients	GAM	7.73	114	4.1
none	GAM	2	186.4	76.5
nutrients	GAM	3	188.6	78.6

Table A6.3 Model selection parameters for variables predicting week 6 richness. The model containing chloride only (italicized) provided the best fit.

Predictors	Model type	df	AICc	ΔAICc
chloride	*GAM*	*4.81*	*217.6*	*0*
chloride, nutrients	GAM	5.81	219.7	2.2
chloride, nutrients, chloride × nutrients	GAM	6.72	221.4	3.9
nutrients	GAM	3	239.9	22.4
none	GAM	2	237.8	20.2

Appendix G
Week 6 Copepod Model Selection Parameters

Table A7.1 Model selection parameters for variables predicting $\log_{10}$ transformed week 6 copepod abundance. The most scientifically conservative model containing only predictors found within all top models (<2 AICc points), with chloride only (italicized), was selected.

Predictors	Model type	Df	AICc	ΔAICc
chloride	GAM	6.14	84.6	0
chloride, nutrients	GAM	7.16	86.4	1.8
chloride, nutrients, chloride × nutrients	GAM	9.71	89.7	5.1
none	GAM	2	116.2	31.6
nutrients	GAM	3	117.9	33.3

Table A7.2 Model selection parameters for variables predicting $\log_{10}$ transformed week 6 *Tropocyclops extensus* abundance. The most scientifically conservative model containing only predictors found within all top models (<2 AICc points), with chloride only (italicized), was selected.

Predictors	Model type	df	AICc	ΔAICc
chloride, nutrients	GAM	7.41	86.1	0
chloride	*GAM*	*6.36*	*86.2*	*0.1*
chloride, nutrients, chloride × nutrients	GAM	9.63	88.5	2.4
none	GAM	2	113.8	27.7
nutrients	GAM	3	114.5	28.4

Table A7.3. Model selection parameters for variables predicting $\log_{10}$ transformed week 6 cyclopoid copepodid abundance. The most scientifically conservative model containing only predictors found within all top models (<2 AICc points), with chloride only (italicized), was selected.

Predictors	Model type	df	AICc	ΔAICc
chloride, nutrients	GAM	6.96	133.4	0
chloride	*GAM*	*5.9*	*134.2*	*0.8*
chloride, nutrients, chloride × nutrients	GAM	7.82	134.8	1.4
nutrients	GAM	3	168.4	35
none	GAM	2	237.8	104.4

Table A7.4. Model selection parameters for variables predicting $\log_{10}$ transformed week 6 copepod nauplii abundance. The most scientifically conservative model containing only predictors found within all top models (<2 AICc points), with chloride only (italicized), was selected.

Predictors	**Model type**	**df**	**AICc**	**ΔAICc**
chloride	*GAM*	*7.15*	*153.6*	*0*
chloride, nutrients	GAM	6.14	153.7	0.1
chloride, nutrients, chloride × nutrients	GAM	11.93	159.2	5.6
none	GAM	2	222.2	68.5
nutrients	GAM	3	224.1	70.4

Appendix H
Week 6 Cladoceran Model Selection Parameters

Table A8.1 Model selection parameters for variables predicting $\log_{10}$ transformed week 6 cladoceran abundance. The model containing chloride only (italicized) provided the best fit.

Predictors	Model type	df	AICc	ΔAICc
chloride	*GAM*	*5.55*	*123.9*	*0*
chloride, nutrients	GAM	6.52	126.5	2.3
chloride, nutrients, chloride × nutrients	GAM	7.32	127.6	3.7
none	GAM	2	185.6	61.7
nutrients	GAM	3	187.6	63.7

Table A8.2 Model selection parameters for variables predicting $\log_{10}$ transformed week 6 chydorid abundance. The model containing chloride only (italicized) provided the best fit.

Predictors	Model type	df	AICc	ΔAICc
chloride	*GAM*	*5.16*	*158.3*	*0*
chloride, nutrients	GAM	6.13	160.8	2.4
chloride, nutrients, chloride × nutrients	GAM	6.86	162.1	3.8
none	GAM	2	193.2	34.9
nutrients	GAM	3	195.3	37

Table A8.3 Model selection parameters for variables predicting $\log_{10}$ transformed week 6 *Scapholeberis mucronata* abundance. The most scientifically conservative model containing only predictors found within all top models (<2 AICc points), with chloride only (italicized), was selected.

Predictors	Model type	df	AICc	ΔAICc
chloride, nutrients	GAM	6.11	145.1	0
chloride	*GAM*	*5.29*	*145.8*	*0.7*
chloride, nutrients, chloride × nutrients	GAM	7.32	146.6	1.5
nutrients	GAM	3	170.5	25.4
none	GAM	2	170.8	25.7

Table A8.4 Model selection parameters for variables predicting $\log_{10}$ transformed week 6 *Sida crystallina* abundance. The model containing chloride, nutrients and chloride × nutrients (italicized) provided the best fit.

Predictors	Model type	df	AICc	ΔAICc
chloride, nutrients, chloride × nutrients	*GAM*	*7.39*	*121.2*	*0*
chloride, nutrients	GAM	6.09	124.7	3.5
chloride	GAM	5.05	127	5.8
nutrients	GAM	3	138.1	16.9
none	GAM	2	138.9	17.7

Table A8.5 Model selection parameters for variables predicting week 6 Bosmina freyi/liederi abundance. The most scientifically conservative model containing only predictors found within all top models (<2 AICc points), with chloride only predicting abundance and no predictors for zeroes (italicized), was selected.

Abundance predictors	Zero predictors	Model type	df	AICc	ΔAICc
chloride	*none*	*ZINB*	*4*	*246.3*	*0*
chloride, nutrients	none	ZINB	5	247.6	1.3
chloride	chloride	ZINB	5	248.2	1.9
chloride	nutrients	ZINB	5	248.2	1.9
chloride, nutrients	chloride	ZINB	6	249.6	3.3
chloride, nutrients	nutrients	ZINB	6	249.6	3.3
chloride	chloride, nutrients, chloride × nutrients	ZINB	7	249.8	3.5
chloride, nutrients, chloride × nutrients	none	ZINB	6	250	3.7
chloride	chloride, nutrients	ZINB	6	250.2	3.9
none	none	ZINB	3	251.3	5
chloride, nutrients	chloride, nutrients, chloride × nutrients	ZINB	8	251.5	5.2
chloride, nutrients	chloride, nutrients	ZINB	7	251.7	5.4
chloride, nutrients, chloride × nutrients	nutrients	ZINB	8	252	5.7
chloride, nutrients, chloride × nutrients	chloride	ZINB	7	252.2	5.9
none	chloride	ZINB	4	253	6.7
none	nutrients	ZINB	4	253.1	6.8
nutrients	none	ZINB	4	253.4	7.1
chloride, nutrients, chloride × nutrients	chloride, nutrients, chloride × nutrients	ZINB	9	254.3	8
chloride, nutrients, chloride × nutrients	chloride, nutrients	ZINB	8	254.3	8
none	chloride, nutrients, chloride × nutrients	ZINB	6	254.4	8.1
none	chloride, nutrients	ZINB	5	254.9	8.6
nutrients	chloride	ZINB	5	255.2	8.9
nutrients	nutrients	ZINB	5	255.3	9
nutrients	chloride, nutrients, chloride × nutrients	ZINB	7	256.9	10.6
nutrients	chloride, nutrients	ZINB	6	257.2	10.9

Appendix I
Week 6 Rotifer Model Selection Parameters

Table A9.1 Model selection parameters for variables predicting $\log_{10}$ transformed week 6 rotifer abundance. The model containing chloride only (italicized) provided the best fit.

Predictors	Model type	df	AICc	ΔAICc
chloride	*LM*	*3*	*111.9*	*0*
chloride, nutrients	LM	4	114.1	2.2
chloride, nutrients, chloride × nutrients	LM	5	116.0	4.1
none	LM	2	132.2	20.2
nutrients	LM	3	134.2	22.3

Table A9.2 Model selection parameters for variables predicting $\log_{10}$ transformed week 6 *Monostyla spp.* abundance. The most scientifically conservative model containing only predictors found within all top models (<2 AICc points), with chloride only (italicized), was selected.

Predictors	Model type	df	AICc	ΔAICc
chloride	*LM*	*3*	*133.1*	*0.0*
chloride, nutrients	LM	4	135.0	1.9
chloride, nutrients, chloride × nutrients	LM	5	137.2	4.1
none	LM	2	148.3	15.2
nutrients	LM	3	150.3	17.2

Table A9.3 Model selection parameters for variables predicting $\log_{10}$ transformed week 6 *Lecane spp.* abundance. The most scientifically conservative model containing only predictors found within all top models (<2 AICc points), with chloride only (italicized), was selected.

Predictors	Model type	df	AICc	ΔAICc
chloride	*GAM*	*5.16*	*190.6*	*0.0*
chloride, nutrients	GAM	6.15	190.8	0.2
chloride, nutrients, chloride × nutrients	GAM	7.0	193.1	2.4
none	GAM	2	198.0	7.3
nutrients	GAM	3	198.2	7.6

Table A9.4 Model selection parameters for variables predicting $\log_{10}$ transformed week 6 *Keratella spp.* abundance. The most scientifically conservative model containing only predictors found within all top models (<2 AICc points), with no predictors (italicized), was selected.

Predictors	Model type	df	AICc	ΔAICc
none	*LM*	*2*	*155.6*	*0.0*
chloride	LM	3	156.7	1.1
nutrients	LM	3	157.0	1.5
chloride, nutrients	LM	4	158.3	2.7
chloride, nutrients, chloride × nutrients	LM	5	160.7	5.1

Appendix J
Week 6 Chlorophyll *a* Model Selection Parameters

Table A10.1 Model selection parameters for variables predicting week 6 chlorophyll *a* concentration. The model containing chloride, nutrients, chloride × nutrients (italicized) provided the best fit.

Predictors	Model type	df	AICc	ΔAICc
chloride, nutrients, chloride × nutrients	*GAM*	*10.75*	*181.5*	*0.0*
chloride, nutrients	GAM	4.39	187.7	6.2
nutrients	GAM	3	191.0	9.5
chloride	GAM	3.00	200.1	18.6
none	GAM	2	202.4	20.9

Appendix K
Week 6 Raw Data

Mesocosm	Nutrient Level	Nominal Chloride (mg/L)	Actual Chloride (mg/L)	Chlorophyll *a* (mg/L)	Carbon (mg/L)	*Scapholeberis mucronata* (/L)	*Daphnia pulex* (/L)	*Bosmina freyi/liederi* (/L)	*Daphnia mendotae* (/L)
1	Ambient	450	388	0.63525	0.178102094	1.15449915	0	0.00565931	0
2	High	1000	868.5	0.4965	0.355668302	0	0	0	0
3	Ambient	0.39	1.09	0.02025	0.037532891	1.87650791	0	0.01451105	0
4	High	180	151.5	0.756	0.061246518	1.2231411	0	0.00927756	0
5	High	160	139	0.0606	0.57839747	3.26498629	0	0	0
6	High	500	436	4.77375	0.427944858	0.09054895	0	0	0
7	High	30	26	0.092025	0.510854808	0.36219581	0.40747029	0	0
8	Ambient	600	491	0.5865	0.097839446	0.0565931	0	0	0
9	Ambient	200	170.5	0.18	0.045987346	0.42444822	0	0	0.03537069
10	High	140	103.25	0.71325	0.523283666	4.13363533	0	0	0
11	High	700	604.5	4.1205	0.309794584	0.03395586	0	0	0
12	Ambient	900	794.5	1.05675	0.166477621	0.00565931	0	0	0
13	Ambient	400							
14	Ambient	1000	863.5	0.078	0.130924868	0.03961517	0	0.01131862	0
15	Ambient	1100	1035	0.077025	0.106421639	0.01697793	0	0	0
16	High	80	74.55	4.836	0.29727237	21.4183101	0	0	0
17	Ambient	350	326.5	0.92625	0.075464292	1.14318053	0	0	0
18	High	225	203	0.65325	0.381049267	0.79230334	0	0	0
19	High	60	55.35	0.318825	0.130595803	0	0	0.02096041	0
20	Ambient	225	194.5	0.40875	0.036991096	0.01697793	0	0	0
21	Ambient	40	37.2	0.0465		0.25466893	0	0.00565931	0
22	High	350	310.5	0.726		0.1188455	0	0.01131862	0
23	Ambient	80	67.9	0.2955	0.053567483	0.36254952	0.82848	0.02652801	0
24	High	120	105	0.1101	0.087430994	0.12779086	0	0	0
25	Ambient	800	693.5	1.39125	0.170939392	0.00565931	0	0	0
26	Ambient	50	44.75	0.0975	0.041349082	0.31833616	0	0	0
27	Ambient	550	467	3.084	0.131384605	0.01131862	0	0.01131862	0

28	High	200	211.5	0.993	0.089852946	0.67345784	0	0.00565931	0.01131862
29	Ambient	60	56.2	0.25275	0.058475862	0.21222411	0	0	0
30	High	300	252	0.882	0.100214438	13.1781066	0	0	0
31	Ambient	700	578.5	0.47925	0.108130747	0.01131862	0	0.00565931	0
32	High	20	18.25	4.32675	0.308227642	45.8404075	0	0.04716091	0
33	High	1500	1375	2.37375	0.193447579	0.03961517	0	0.00565931	0
34	High	600	623	4.76625	0.454963161	0.10752688	0	0	0
35	High	900							
36	High	1300	1170	3.96225	0.303207625	0.01131862	0	0.00565931	0
37	High	50	44.35	2.2065	0.190270064	13.582343	0	0	0
38	High	100	83.1	1.03125	0.100029974	0.26904914	0	0.00927756	0
39	High	250	228	0.0834	0.404016106	50.2263724	0	0.03537069	0
40	High	800							
41	Ambient	180	218.5	0.72675	0.100253073	0.87209033	0	0	0
42	Ambient	10	9.515	0.14775	0.045142975	2.99134934	0	0.04140958	0
43	Ambient	250	224	1.49175	0.12366715	0.33955857	0	0	0
44	Ambient	1500	1270	1.0275	0.087375199	0.03961517	0	0	0
45	High	400	352	0.05775	0.40082608	12.3475845	0	0.02176658	0
46	Ambient	120	101.5	0.68175	0.059327684	1.57894737	0	0.00565931	0
47	High	0.39	0.45	1.272	0.076886939	0.43118549	0	0	0
48	High	550	504.5	2.48625	0.247201862	0.02263724	0	0	0
49	High	10	9.415		0.240141444	22.21279	0	0	0
50	Ambient	300	258.5	0.34125	0.038994634	1.98075835	0	0.01131862	0
51	High	40	35.85	3.14625	0.304452796	14.2897567	0	0	0
52	Ambient	140	98.6	0.3735	0.03271339	0.3482652	0	0	0
53	Ambient	20	17.45	0.07125	0.026151796	0.24759479	0	0.03537069	0.03537069
54	High	450	384	2.5755	0.294663881	0.06225241	0	0	0
55	Ambient	30	26.35	0.07125	0.021617121	0.1188455	0	0.01131862	0
56	Ambient	500	429.5	0.70425	0.110500048	0.56593096	0	0	0
57	Ambient	100	89.6	0.69375	0.064031824	1.54058983	0	0	0
58	High	1100	967	4.0695	0.276296577	0.03395586	0	0	0
59	Ambient	160	136	0.345	0.062237621	6.20347395	0	0.01230285	0
60	Ambient	1300	1125	1.15875	0.105560061	0.00565931	0	0	0

Mesocosm	***Diaphansoma brachyurum* (/L)**	***Sida crystallina* (/L)**	***Ilyocryptus spinifer* (/L)**	***Holopedium gibberum* (/L)**	***Ophryoxus gracillis* (/L)**	***Ceriodaphnia lacustrus* (/L)**	**Chydoridae (/L)**	***Mesocyclops edax* (/L)**
1	0	0	0	0.02829655	0	0	0.01697793	0
2	0	0.00565931	0	0	0	0	0.00565931	0
3	0	2.4722247	0	0	0	0	127.334465	0
4	0	1.02232689	0	0	0	0	10.9413318	0
5	0	0	0	0	0	0	6.25659773	0
6	0	0.01131862	0	0	0	0	0.05093379	0
7	0.01697793	0.04527448	0	0	0	0.02263724	0.09620826	0
8	0	0	0	0	0	0.05093379	0.00565931	0
9	0	0.42444822	0	0	0	0	10.84701	0
10	0	0	0	0	0	0	7.64439411	0
11	0	0.00565931	0	0	0	0	0.1188455	0
12	0	0	0	0	0	0	0.02263724	0
13								
14	0	0	0	0	0	0	0.04527448	0
15	0	0	0	0	0	0	0.00565931	0
16	0	0	0	0	0	0	0.2611989	0.1741326
17	0	0	0	0	0	0	0.0565931	0
18	0	0	0	0	0	0	0.14714205	0
19	0	0.17816345	0	0	0	0	2.31612484	0.02096041
20	0	0.28296548	0.98471986	0	0	0	0.01697793	0
21	0	0.26032824	0	0	0	0	3.71897486	0
22	0	0	0.00565931	0	0	0	0.09054895	0
23	0	0	0	0	0	0	2.57026976	0.07074137
24	0	0	0	0	0	0	4.47268014	0.01825584
25	0	0	0	0	0	0	0.12450481	0
26	0	0.53056027	0	0.03537069	0	0	11.8331018	0.03537069
27	0	0	0	0	0	0	0.04527448	0

28	0	0	0	0	0	0	0.45840408	0
29	0	0.02021182	0	0	0.05052955	0	7.03876627	0.07074137
30	0	0	0	0	0	0.08084728	11.1569246	0
31	0	0	0	0	0	0	0.0565931	0
32	0	0	0	0	0	0	0.14148274	0.51877004
33	0	0	0	0.00565931	0	0.00565931	0	0
34	0	0.01131862	0.00565931	0	0	0	0.02829655	0
35								
36	0	0	0	0	0	0	0.00565931	0
37	0	0.01489292	0	0	0	0	1.48929199	0
38	0	0.00927756	0	0	0	0	106.960951	0
39	0	0	0	0	0	0	7.78155065	0
40								
41	0	0	0	0	0	0	105.263158	0
42	0	0.67635651	0	0	0	0	1.95337459	0
43	0	0.01131862	0	0	0	0	0.20373514	0.02829655
44	0	0	0	0	0	0	0.06225241	0
45	0	0	0	0	0	0	0.89242959	0
46	0	0	0	0	0	0	0.01697793	0
47	0	0.43118549	0	0	0	0	12.450481	0
48	0	0	0	0	0	0	0.00565931	0
49	0	0	0	0	0	0	4.65321009	0.13660402
50	0	0.05093379	0	0	0	0	0.74702886	0
51	0	0	0	0	0	0	593.661573	0.21766575
52	0	0.56593096	0.02176658	0	0	0	12.6048259	0.02176658
53	0	0.49518959	0	0	0	0	6.72043011	0
54	0	0	0	0	0	0	0	0
55	0	0.36785512	0	0	0	0	0.10752688	0.03961517
56	0	0	0	0	0	0	0.01697793	0
57	0	0	0	0	0	0	5.55319751	0.03144061
58	0	0	0	0.00565931	0	0	0.02829655	0

59	0	0	0	0	0	0	1.41482739	0
60	0	0	0	0	0	0	0.01697793	0

Mesocosm	*Tropocyclops extensus* (/L)	*Keratella spp.* (/L)	*Anuraeopsis spp.* (/L)	*Kellicottia spp.* (/L)	*Euchlanis spp.* (/L)	*Monostyla spp.* (/L)	*Lecane spp.* (/L)	*Collotheca spp.* (/L)
1	0	0.56593096	0.56593096	0	0	63.3842671	30.5602717	0
2	0	2.26372383	0	0	0	2.26372383	2.82965478	0
3	0.10157735	0.56593096	0	0	1.69779287	20.9394454	0	0
4	0	0.20410625	196.943973	0	0	0.98581521	2.58711294	0.00927756
5	0	0.44984256	1.90629375	0	0	9.0548953	0.69653041	0
6	0	0	1.13186191	0	0	20.3735144	4.52744765	0
7	0.00565931	0	17.5438597	0	0	7.35710243	0.56593096	0
8	0	0.56593096	0.56593096	0	0	14.7142049	1.69779287	0
9	0	0.02263724	0.01131862	0	0	0.73005093	0.03961517	0
10	0	0.28312571	0	0	0.05662514	60.3624009	13.3069083	0
11	0	0.56593096	0	0	0	43.0107527	27.7306169	0
12	0	1.69779287	0	0	0.56593096	2.82965478	0	0
13								
14	0	0	0	0	0	11.3186191	0	0
15	0	1.13186191	1.13186191	0	0	1.13186191	0	0
16	0.60946411	5.65930956	3.39558574	0	2.26372383	72.4391624	19.2416525	0
17	0	0	0	0	0	26.032824	59.9886814	0
18	0	0.15280136	0	0	0	15.2801358	0.36219581	0
19	0.09432183	1.13186191	0	0	0	11.8845501	0	0
20	0.00565931	0.01697793	0.00565931	0	1.69779287	11.8845501	1.69779287	0
21	0.00094322	11.3186191	0	0	4.52744765	31.1262026	0.56593096	0
22	0.00565931	2.82965478	0	0	0	20.3735144	15.2801358	0.56593096
23	0	0.42444822	0	0	0.7074137	23.344652	0	0
24	0.03651168	2.82965478	49.8019242	0	0	118.845501	60.5546123	0
25	0	0.03961517	0.29994341	0	0.00565931	0.33955857	2.02603282	0
26	0	1.69779287	0.56593096	0	0	1.69779287	0.56593096	0
27	0	0.36785512	0	0	0	0.25466893	0.16977929	0
28	0.00565931	0.56593096	0	0	0	36.2195812	0	0

29	0.17180047	3.9615167	0	0	30.5602717	17.5438597	2.82965478	0
30	0	2.82965478	1.69779287	0	0	9.62082626	0	0
31	0	1.13186191	0	0	0	7.35710243	0	0
32	1.27334465	54.3293718	2.26372383	0	0	11.8845501	1.69779287	0
33	0	0.06225241	0	0	0	0	0.00565931	0
34	0	0.18109791	0.01697793	0.00565931	0.00565931	0.42444822	0.02263724	0
35								
36	0	1.13186191	0	0	0	23.2031692	0	0
37	0.0744646	0.02978584	0	0	0	16.9779287	0	0
38	0.02783267	2.26372383	55.4612337	0	0.56593096	36.2195812	6.22524052	0
39	0	0.74278438	2.01612903	0	0	1.09649123	0.56593096	0
40								
41	0.00565931	1.13186191	0	0	0	99.0379174	0.56593096	0
42	0	7.92303339	0	0	1.13186191	5.09337861	1.69779287	0
43	0	0.07357102	0	0	0	7.00339559	0	0
44	0	0.07923033	0.01697793	0	0	0.61686474	0	0
45	0	0.56593096	0	0	0	5.09337861	18.6757216	0
46	0.03961517	0.56593096	0.56593096	0	0	423.882286	0	0
47	0.05389819	0.4042364	235.427278	0	0.21559275	32.2580645	0.24254184	0
48	0	1.13186191	0	0	0	4.52744765	1.13186191	0
49	0.31223777	5.65930956	0.56593096	0	3.39558574	15.8460668	1.69779287	0
50	0	0.56593096	1.13186191	0	0	45.8404075	1.13186191	0
51	0.30473205	1.69779287	0	0	0	222.976797	0.56593096	0
52	0.10883288	1.13186191	0	0	1.13186191	84.3237125	1.13186191	0
53	0.17685342	0.28296548	0	0	2.05149972	62.8183362	0.14148274	0
54	0	0	1.69779287	0	0	32.8239955	1.13186191	0
55	0.06791172	0	0	0	3.9615167	10.7526882	0.56593096	0
56	0	1.13186191	0	0	0	78.098472	10.7526882	0
57	0	1.69779287	0	0	1.69779287	338.992643	2.82965478	0
58	0	1.69779287	0	0	0	9.0548953	0	0
59	0	0.03690854	0	0	0	144.878325	0	0
60	0	0.56593096	0	0	1.13186191	14.7142049	0	0

Mesocosm	*Trichocerca spp.* (/L)	Nauplii (/L)	Cyclopoid copepodids (/L)	Total Abundance (/L)	Cladocerans (/L)	Copepods (/L)	Rotifers (/L)	Richness (/L)	Total Biomass (µg/L)
1	0	0.02263724	0	102.529711	1.20543294	0	101.301641	9	2.52441187
2	0	0	0	7.36842105	0.01131862	0	7.35710243	5	0.03431068
3	0	44.1426146	0.0870663	199.27567	131.697709	0.1451105	23.2031692	8	334.23087
4	0	0.56593096	0.01855511	214.520126	13.2053549	0	200.730285	9	27.8846485
5	0	1.09283219	0.0580442	22.7800224	9.52158402	0	12.107562	6	8.39077689
6	0	0	0	26.1856254	0.15280136	0	26.032824	6	0.23312708
7	0	40.7470289	0	67.1703452	0.95076401	0.00565931	25.466893	10	37.7712316
8	0	0	0	17.6570458	0.11318619	0	17.5438597	7	0.22251452
9	0	0.22637238	0.35370685	13.1149783	11.7312771	0	0.80362196	8	15.9174106
10	0	0.45300113	0.11325028	86.3533409	11.7780295	0	74.00906	6	10.2477054
11	0	0	0	71.4657612	0.15846067	0	71.3073005	6	0.43621622
12	0	0	0	5.12167516	0.02829655	0	5.09337861	5	0.10980566
13									
14	0	0	0	11.4148274	0.09620826	0	11.3186191	4	0.11748877
15	0	1.13186191	0	4.55008489	0.02263724	0	3.39558574	5	0.85979143
16	0	13.582343	0.78359671	139.654347	21.679509	0.60946411	102.999434	9	68.489165
17	0	0	0	87.221279	1.19977363	0	86.0215054	4	1.25713391
18	0	0.02263724	0.00565931	16.7628749	0.93944539	0	15.795133	5	1.0203842
19	0	1.69779287	0.03144061	15.6783835	2.63053093	0.11528223	13.016412	7	7.53096825
20	0	5.09337861	0	21.7034522	1.3016412	0.00565931	15.3027731	10	7.78671387
21	0	7.35710243	0.01131862	51.7957528	4.24057455	0.00094322	47.5382003	9	10.2043586
22	0	0	0	39.2812677	0.22637238	0.00565931	39.049236	9	0.27315613
23	0	78.098472	0.01768534	246.639785	144.878325	0.02652801	23.6187748	9	70.6658311

24	0	13.016412	0.2920934	249.97718	4.600471	0.03651168	232.031692	8	13.1348168
25	0	0.02263724	0	2.86361064	0.13016412	0	2.71080928	7	0.12215022
26	0	3.39558574	0	55.008489	20.675773	0	4.52744765	9	15.9418911
27	0	0.01697793	0	0.87719298	0.06791172	0	0.79230334	6	0.1071431
28	0	5.65930956	0.0565931	43.655914	1.14883984	0.00565931	36.7855122	7	5.72702641
29	0	78.098472	2.01612903	142.503436	7.32173175	0.17180047	54.8953028	10	69.5135873
30	0	0	0	38.5641523	24.4158784	0	14.1482739	6	41.2991369
31	0	0	0	8.56253537	0.07357102	0	8.48896435	5	0.12518179
32	0	354.272779	2.73533296	474.910394	46.0290511	1.69779287	70.1754386	9	421.996664
33	0	0.01131862	0.00565931	0.14148274	0.0565931	0	0.06791172	6	0.31979079
34	0.02829655	0.13016412	0.0565931	1.02433503	0.15280136	0	0.68477646	11	0.33597893
35									
36	0	0	0	24.3576684	0.02263724	0	24.3350311	5	0.02654948
37	0	45.2744765	9.62082626	87.0789027	15.0865279	0.0744646	17.0226075	6	83.8051504
38	0	46.4063384	2.67530998	257.093746	107.248555	0.02783267	100.73571	10	133.097478
39	0	0.74278438	0.07074137	63.2781551	58.0432937	0	4.4213356	7	105.888401
40									
41	0	122.241087	0.01131862	329.129023	106.135248	0.00565931	100.73571	6	167.619471
42	0	48.1041313	2.13593296	71.7486211	5.66249002	0	15.8460668	8	43.9114256
43	0	7.07413696	0	14.7849462	0.56027165	0.03961517	7.11092247	8	6.00592764
44	0	0.02829655	0	0.84889644	0.10186757	0	0.71873232	6	0.15274067
45	0	1.13186191	0	38.7286737	13.2617807	0	24.3350311	6	22.1993985
46	0	51.499717	0.06791172	478.222977	1.60158461	0.03961517	425.014148	7	42.4358081
47	0	9.52650443	0.18864365	291.629612	13.312852	0.05389819	268.547713	9	15.9393854
48	0	0	0	6.81946803	0.02829655	0	6.79117148	5	0.08738689

49	0	166.383701	8.48896435	229.215589	26.8660001	0.31223777	27.1646859	9	192.491035
50	0	2.82965478	0.01697793	51.4770798	2.79003962	0	48.6700623	8	7.038511
51	0	57.7249576	13.1578947	904.379435	607.95133	0.30473205	225.240521	7	517.565135
52	0	43.5766836	0	144.945603	13.5407886	0.10883288	87.7192983	10	65.4496177
53	0	6.31956235	2.72354273	82.0481985	7.53395586	0.17685342	65.2942841	10	18.3965358
54	0	0	0	35.7159027	0.06225241	0	35.6536503	4	0.12876825
55	0	3.9615167	0.12450481	20.0452745	0.60554612	0.07357102	15.2801358	9	5.47216102
56	0	0	0	90.565931	0.58290889	0	89.9830221	5	0.78670252
57	0	41.3129598	0.14148274	393.797554	7.09378733	0.03144061	345.217883	7	36.2268241
58	0	0	0	10.8205999	0.06791172	0	10.7526882	5	0.24786368
59	0	32.8239955	0.13533132	185.52977	7.63060418	0	144.939839	5	41.4335804
60	0	0	0	16.434635	0.02263724	0	16.4119977	5	0.05956068