# Exploring the impacts of increased salinity on zooplankton communities in Sturgeon Lake

A Thesis Submitted to Wilfrid Laurier University

by

Jaclyn Franceschini

In partial fulfilment of the requirements for the Bachelor of Science in Biology

April 2019

# Contents

# Abstract

Every winter, salt deicers are used on Ontario roadways and sidewalks to increase traction and reduce accidents. However, road salt can leach into freshwater ecosystems and expose aquatic organisms to elevated salt concentrations. This study investigated the impacts of increased salinity from road salt application on the community structure and diversity of freshwater zooplankton, a group known to be sensitive to changes in salinity. I hypothesized that increased salinities would result in a shift towards halotolerant species in the community and would lead to a reduction in zooplankton species richness, abundance, and diversity. I ran a six week field mesocosm experiment on the shore of Sturgeon Lake to test the effects of elevated sodium chloride (NaCl) levels. Elevated salinity levels did not significantly affect diversity, and there were no clear shifts in species composition associated with salinity. However, elevated salinity levels resulted in a significant decrease in zooplankton species richness and abundance. When the relationships between salinity, richness, and abundance are put into the context of current chloride trends, my results suggest that salinity increases are unlikely to cause any significant changes in Sturgeon Lake zooplankton communities over the next century. Further studies will need to examine how other types of freshwater organisms (e.g. phytoplankton) respond to increased salinity, but my results suggest that the risks of road salt contamination for Ontario lakes may have been exaggerated in recent media coverage.

## Introduction

Salinization can be caused artificially from human activities such as agricultural practices and resource extraction (Cañedo-Argüelles et al., 2016). In Canada, a common cause of salinization of freshwater systems is road deicing salt. According to Environment Canada, an average of 2.75 million tonnes of salt were used every year between 2004 and 2015. Moreover, road salt usage has been increasing to prioritize winter safety under changing climate conditions (i.e. increased precipitation in colder regions) (Coldsnow et al. 2017). Increased salinities could be harmful to lakes in southern Ontario, which have been historically freshwater with salinities ranging between 0.002-0.013 parts per thousand (‰) (Evans and Frick 2001). While the immediate safety benefits of road salt use are obvious for drivers and pedestrians, less is known about the long-term ecological harm caused to river, lake and stream ecosystems. Past studies have indicated that salinization can produce large ecological changes, including bottom-up trophic cascades that change the structure of aquatic communities (Coldsnow et al. 2017, Hintz et al. 2017). As a result, investigating road salt impacts at lower trophic levels is necessary in order to understand how salt runoff could impact our freshwater ecosystems.

Zooplankton play an integral role in freshwater communities, linking primary producers and higher trophic animals (Sarma et al. 2006). They are also a group that has shown particular vulnerability to salinization, undergoing drastic reductions in biodiversity, abundance and community structure in salinity-stressed waters (Schallenberg et al. 2003, Hintz et al. 2017). For example, one study concluded that higher than average salinities resulted in a decrease in calanoid copepods and an overall decrease in the biomass of the zooplankton community. There are also single species studies that show cladocerans are highly sensitive to salinity changes (Sarma et al. 2006, Liu & Steiner 2017, and Schallenberg et al. 2003), especially at early life stages (Hall and Burns 2002). These responses to salinity result in an inverse relationship between salinity and diversity of zooplankton in freshwater communities (Evans and Frick 2001). However, patterns of community change and salinity tolerance within species are highly variable among studies and regions, making it difficult to reach firm conclusions about the effects of salinization in any particular circumstance. Therefore, studies need to be conducted to further elucidate patterns and determine what factors are driving the variability among studies.

Past studies provide some clues as to the salinity levels that can be tolerated by freshwater zooplankton. Hintz et al. (2017) found that if salinity rises above 1.8 ‰ in experimental mesocosms the abundance of cladocerans and copepods significantly declined (Hintz et al. 2017). These results are similar to those found by Schallenberg et al. (2003), where the zooplankton community shifted from one represented by a variety of species at low salinities, to one mainly composed of rotifers at higher salinities (> 2.7 ‰). Laboratory experiments conducted with cladoceran species also show salinities exceeding 2-3 ‰ can be lethal. For example, Hall and Burns (2002) found the 96h $LC_{50}$ values of salinity tolerance for adult *Daphnia carinata* were 2.5 ‰ at 10°C and 1.9 ‰ at 20°C. Individuals of some species can survive at salinities exceeding 3 ‰, but Corsi et al. (2010) showed that reproduction could not occur for *Ceriodaphnia dubia* when chloride concentration exceeded 3.20 ‰.

The loss of sensitive species as salinity levels increase may cause changes in the structure of zooplankton communities. To describe differences among zooplankton communities, ecologists have developed both univariate and multivariate methods. Univariate methods include species richness, abundance, evenness and diversity. Species richness is simply the number of species in a community, whereas abundance is measured by the total number of individuals in the community. Evenness is a measure of consistency in abundances among the different species, meaning whether their abundance is approximately the same, or if one or two species are dominant. Finally, diversity takes both evenness and richness into account. For this study, I will use the Shannon Index to measure diversity. Shannon Diversity is calculated according to:

$$H' = -\sum_{i=1}^{S} p_i ln p_i$$

Where $S$ is total number of species in the community (richness), and $p_i$ is the proportion of $S$ made up of the $i$th species. Multivariate methods include Principle Component Analysis (PCA), a data exploration and visualization technique that allows the investigator to visually assess differences in the abundance and taxonomy of species among communities. Past studies have shown that multivariate techniques are often more sensitive than univariate measures when examining change. In this study, I will use both univariate and multivariate measures of zooplankton community change in response to salinization.

For this project, I investigated the impact of increased NaCl concentrations on freshwater zooplankton communities in Sturgeon Lake Ontario. My mesocosm experiment was carried out to examine how zooplankton communities exposed to a range of NaCl concentrations would change over a six week period. Based on the results of previous studies, I predicted that increased salinity would result in lower species richness, diversity and an overall lower abundance of zooplankton. I also expected increased salinity would cause a shift in community structure such that high salinity treatments would be dominated by salinity tolerant species such as copepods. I found that salinity significantly decreased both zooplankton species richness and abundance, but not diversity or the composition of species. However, given current trends in chloride concentrations for North American lakes, along with the regression models from my study, my calculations suggest it would take almost 2000 years for the ambient salinity of Sturgeon Lake to reach a level that would cause a significant decrease in zooplankton richness and abundance. These results suggest that the salinization of Sturgeon Lake may not be as immediate of a concern as I originally thought.

## Methods

### *Field experiments*

My field experiment was conducted on the shoreline of Sturgeon Lake (44.46,-78.71), a 44 $km^2$ lake with a maximum depth of 7.6 m. Sturgeon Lake is a part of a large system of lakes called the Trent-Severn waterway system, which connects Lake Huron and Lake Ontario. The land directly surrounding the lake consists of residential homes and cottages, however the majority of the watershed consists of agricultural land. The lack of major roadways within 500 m of the shoreline suggests that road salt contamination is unlikely. At the start of the experiment, the ambient salinity of Sturgeon Lake was 0.01 ‰, which is on the lower end of salinity for what is considered average in southern Ontario Lakes (Evans and Frick 2001). Therefore, this site likely contains zooplankton that are naïve to elevated salinity levels.

Thirty mesocosms were set up on land approximately 15-20 m from the shoreline of Sturgeon Lake on June 26th, 2018. Each mesocosm was filled with 200 L of filtered lake water using a 50 μm net to exclude plankton and debris. I stocked the mesocosms with zooplankton on June 29th at natural densities by collecting a calculated volume of animals and equally distributing them among enclosures. The mesocosms were left overnight so the zooplankton could adjust to their new environment. A sample from each mesocosm was then taken on June 30th, by taking a vertical tow in each mesocosm using a 15 cm diameter 50 μm zooplankton net. These samples were brought back to the laboratory for later species identification and counting.

Sodium chloride (NaCl) was added to each of the mesocosms on June 30th to reach the concentrations listed in Table 1. The assignment of a salt concentration to each mesocosm was conducted using a random number generator. Concentrations at the low range were chosen based on ambient salinity levels of southern Ontario lakes residing around 0-0.4 ‰. Higher concentrations were used to determine how zooplankton would respond to elevated salinity levels that could be reached in the future due to processes such as road salt usage. While adding the NaCl, the water in each mesocosm was gently stirred until the salt was completely dissolved. NaCl was chosen for this experiment as it is the most commonly used road deicing salt (Coldsnow 2017). Immediately after the salt was dissolved, I measured the conductivity (μS), the pH and the dissolved oxygen (%) of each mesocosm. A mesh screen was used to cover each mesocosm to limit the input of organic matter and other organisms (e.g. mosquitoes). For the next 6 weeks, the pH and conductivity were recorded weekly and the dissolved oxygen was recorded at weeks 3 and 6. Temperature loggers were placed in two of the mesocosms and in the nearshore area of the lake at approximately 0.5 m depth. These loggers recorded temperature (°C) of the water every 1 hour for the duration of the 6 weeks. Filtered samples were later analyzed for the concentration of chlorophyll-a using a spectrophotometer.

*Table 1. Salinity of each mesocosm used for the experiment.*

| Mesocosm | Salinity (‰) |
|---|---|
| 9 | 2.47 |
| 28 | 2.14 |
| 23 | 1.98 |
| 26 | 1.81 |
| 21 | 1.65 |
| 17 | 1.48 |
| 14 | 1.40 |
| 15 | 1.32 |
| 7 | 1.24 |
| 29 | 1.15 |
| 2 | 1.07 |
| 5 | 0.99 |
| 24 | 0.91 |
| 6 | 0.82 |
| 4 | 0.74 |
| 27 | 0.66 |
| 1 | 0.58 |
| 13 | 0.49 |
| 25 | 0.41 |
| 16 | 0.33 |
| 10 | 0.26 |
| 18 | 0.23 |
| 19 | 0.20 |
| 30 | 0.16 |
| 12 | 0.13 |
| 22 | 0.10 |
| 8 | 0.07 |
| 11 | 0.03 |
| 3 | 0.02 |
| 20 | 0.01 |

*Laboratory work*

The plankton samples collected at week 0 and week 6 were counted by enumerating individuals in a minimum of three subsamples per sample such that a minimum of 100 individuals were counted in each subsample. Subsamples were usually 5 mL of the 100 mL diluted samples; however, depending on the relative abundance of individuals in a sample the volume of the subsample occasionally varied between 1.5 to 10 mL. Zooplankton were counted and identified under a dissecting microscope at 10-40x magnification using the taxonomic key by Haney et al. (2013). The zooplankton were identified to the lowest taxonomic resolution possible (usually species level). Occasionally, a compound microscope at 40x- 160x was used for aiding in the visualization of small structures. A total of 180 subsamples were counted overall for this experiment.

*Statistical analyses*

To examine relationships between salinity and richness, diversity, and evenness, I used linear regression in the R programming language (R Development Core Team 2019). To test that the data met the assumptions required for the regression, I used the gvlma function in R, which tests for a linear relationship, homoscedasticity, normality of residuals, and independence of samples (Pena and Slate 2014). Richness is associated with sample size, or number of individuals identified. To ensure that richness was based on equal sampling effort, I used rarefaction. This was carried out using the rarefy function in the vegan package for R (Oksanen et al. 2018). Evenness is used to describe the equality of the relative abundance between species in a community. The scale ranges from 0 (lack of evenness) to 1 (complete evenness). The closer the value of evenness is to 0, the more likely it is that the community is dominated by a small number of species, with the remaining species being rare. For this study, I decided to use Pielou's evenness (J') as it is the most common index for measuring evenness in ecology, and the formula is as follows:

$$E_H = \frac{H}{H_{max}} = \frac{H}{\ln S}$$

Where *H* represents Shannon's entropy, *Hmax* represents the maximum entropy and *S* represents the number of species (Alatalo 2016).

To examine the change in zooplankton community structure in response to salinity, I used a Principal Component Analysis in the R programming language (R Development Core Team 2019). First, I performed a Hellinger transformation on the raw species data which is defined as;

$$y'_{ij} = \sqrt{\frac{y_{ij}}{y_{i^*}}}$$

Where *j* represents the list of species, *i* represents the sample and *i** represents the row sum in the *i*th sample. The purpose of the Hellinger transformation is to reduce the bias caused by zero counts which often occur in community data (Legendre & Gallagher 2001). Next, I arranged each community by salinity level and removed rare species that were present in less than 20 % of the mesocosms. The removal of rare species is suggested by Lepš J. & Šmilauer (2003), since they can distort results of the PCA.

Finally, I examined the relevance of my results given the actual chloride concentration trends in North America according to the dataset published by Dugan et al. (2017b). I did this by using the chloride trend data and applying it to both of my significant results – species richness and abundance. To do this, I used linear regression to find the mean trend for chloride concentration over time using the 354 North American Lakes found in Dugan et al. (2017b). The mean trend for North American lakes was 0.00051 ‰ per year. Using this trend, along with the equation of the fitted line from my regressions, I estimated the percentage decrease in species richness and abundance over the next 10 and 100 years (corresponding to a chloride concentration increase of 0.0051and 0.051 ‰, respectively). The change in richness and abundance was calculated assuming lakes started at a range of initial salinity levels from 0 to 2.0‰.

# Results

## *Conditions within the mesocosms*

Over the course of my 6-week experiment, conductivity in the mesocosms gradually declined through time due to precipitation, but the relative differences in conductivity among treatments were maintained (Figure 1). For my highest salinity mesocosm (#23), I added NaCl on August 8th (within the 5th week of the experiment) to bring salinity back to its original level. Water temperatures in the instrumented mesocosms varied between 23-28 degrees Celsius (°C) over the course of the experiment. There was ~3°C difference on most days between the water in the "shaded" mesocosm and that in the "sunny" mesocosm (Figure 2). Surprisingly, the third temperature logger located in a near shore area of Sturgeon Lake showed that the lake typically had a higher temperature than the two instrumented mesocosms (Figure 2). Chlorophyll levels were found to vary slightly, but this variation did not appear to be related to salinity level of the enclosures (Figure 3).

## *Zooplankton communities*

The abundance, richness, and diversity of zooplankton communities did not differ significantly among enclosures at the start of the study based on week 0 samples (linear regression; $p>0.05$; Figure 4A, B, & C). At the end of the experiment, species richness and species abundance were significantly related to salinity levels in my mesocosms, showing a clear decrease as the salinity increased (linear regressions; $p<0.05$; Figure 5A & D). However, increased salinity seemed to result in increased evenness in communities (linear regression; $p<0.05$; figure 5C), with some communities approaching a value of 1 (complete evenness). Shannon Diversity was not significantly related to salinity level of the mesocosms (linear regression; $p>0.05$; figure 5B).

My regression models based on the relationships between species richness versus salinity and abundance versus salinity, showed that assuming lake chloride concentrations continue along their current trajectory (based on the North American average; Dugan et al. 2017b), there will be a relatively minor impact on zooplankton communities over the next decade (Figure 6). However, if the trends continue over the next century, my models predict a significant decline in zooplankton abundance for affected lakes (Figure 7). In both the 10- and 100-year scenarios, the impact of rising salinities is predicted to be worse for lakes higher original ambient salinities (Figures 6, 7).

Zooplankton communities in the mesocosms consisted of a mix of cladocerans and copepods. The principal component analysis did not show a clear difference in structure among mesocosm that was related to salinity (Figure 8). Communities tended to be dominated by either the cladoceran *Bosmina longirostris* or by the copepods *Acanthocyclops vernalis*, *Microcyclops rubellus*, or *Microcylops varicans*.

## Figures

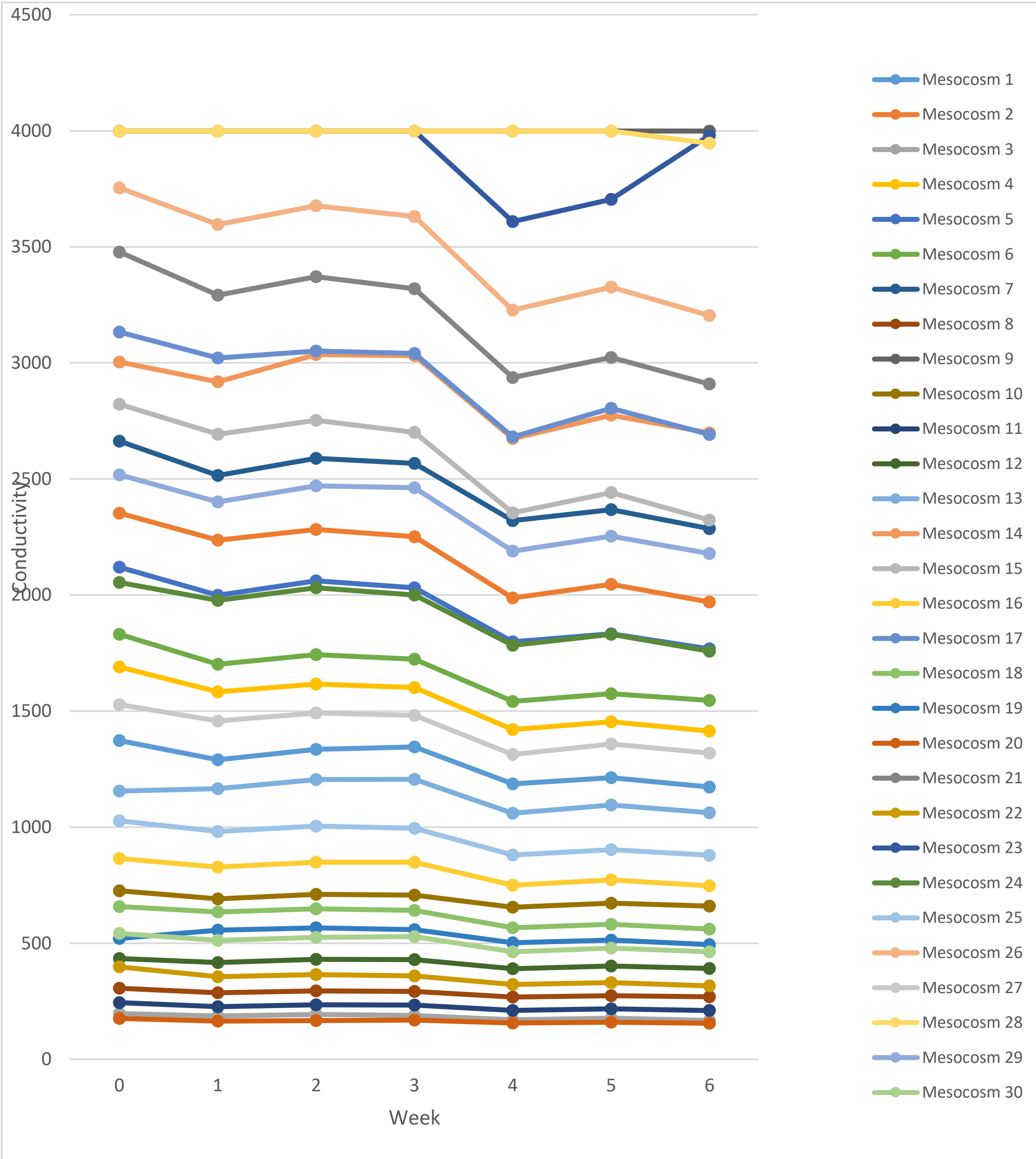


*Figure 1. A scatterplot of the recorded conductivity in 30 mesocosms during my six week experiment.*

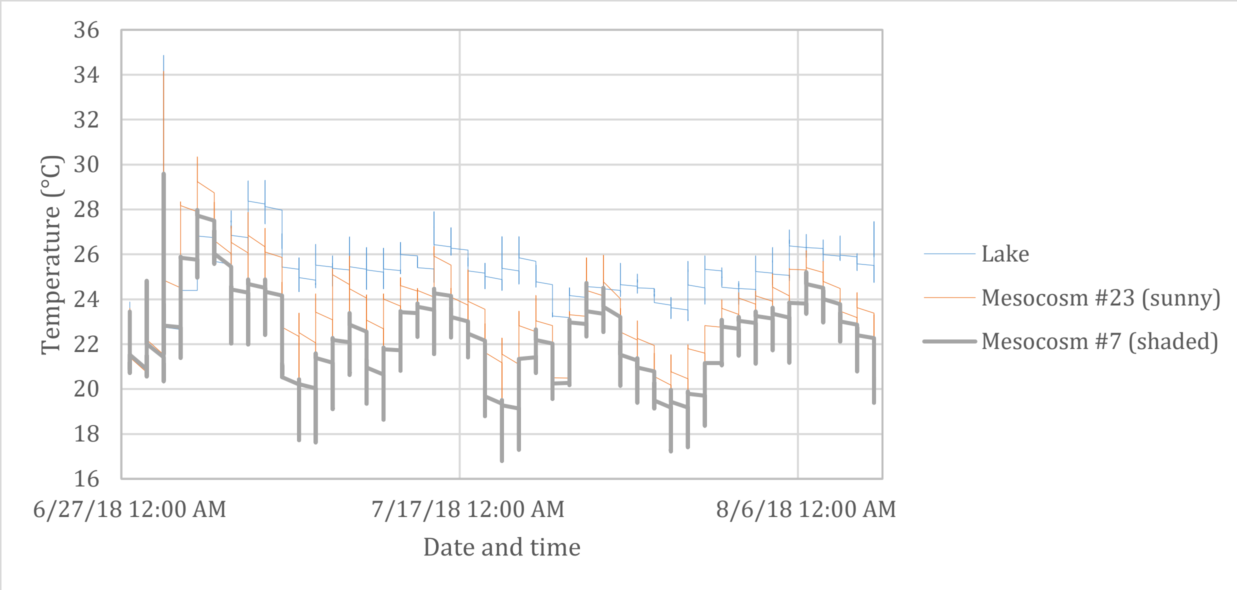


*Figure 2. A scatterplot of the recorded temperature from two temperature loggers in mesocosms and a third off a nearby dock in Sturgeon Lake from June 27th 2018 till August 10th 2018*

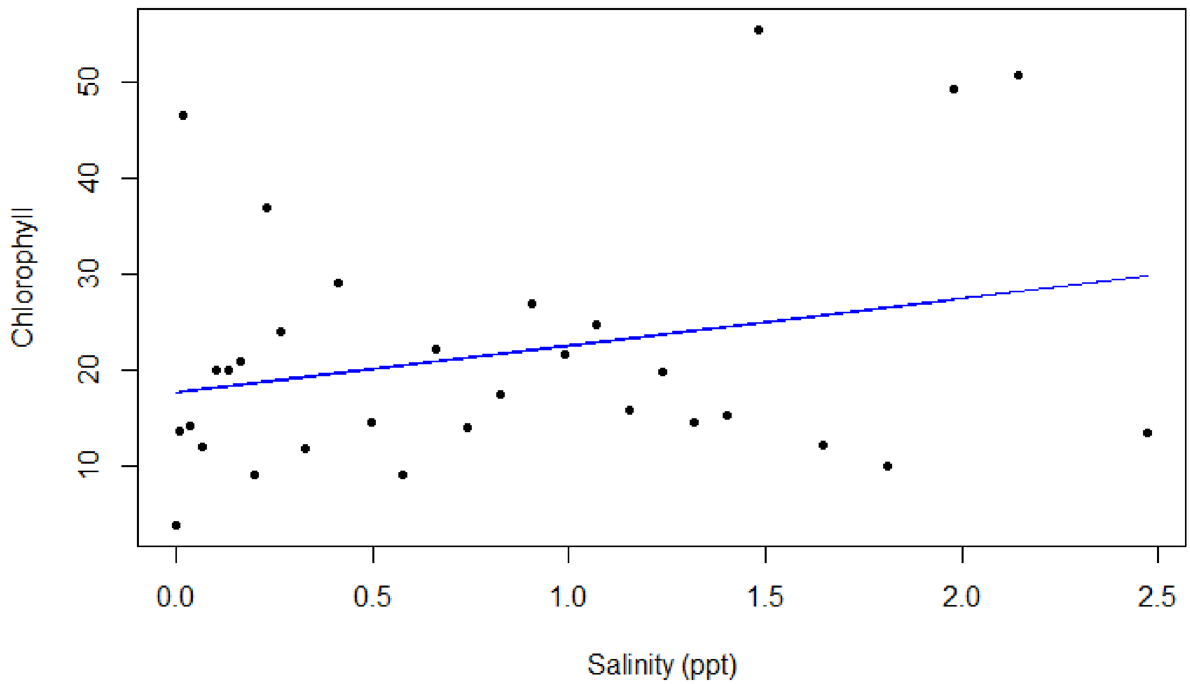


*Figure 3. A scatterplot of the recorded chlorophyll a levels in 30 mesocosms on the shoreline of Sturgeon Lake at the beginning of the 6 week experiment period (linear regression, t=1.467, p>0.05,* $R^2$*=0.03695).*

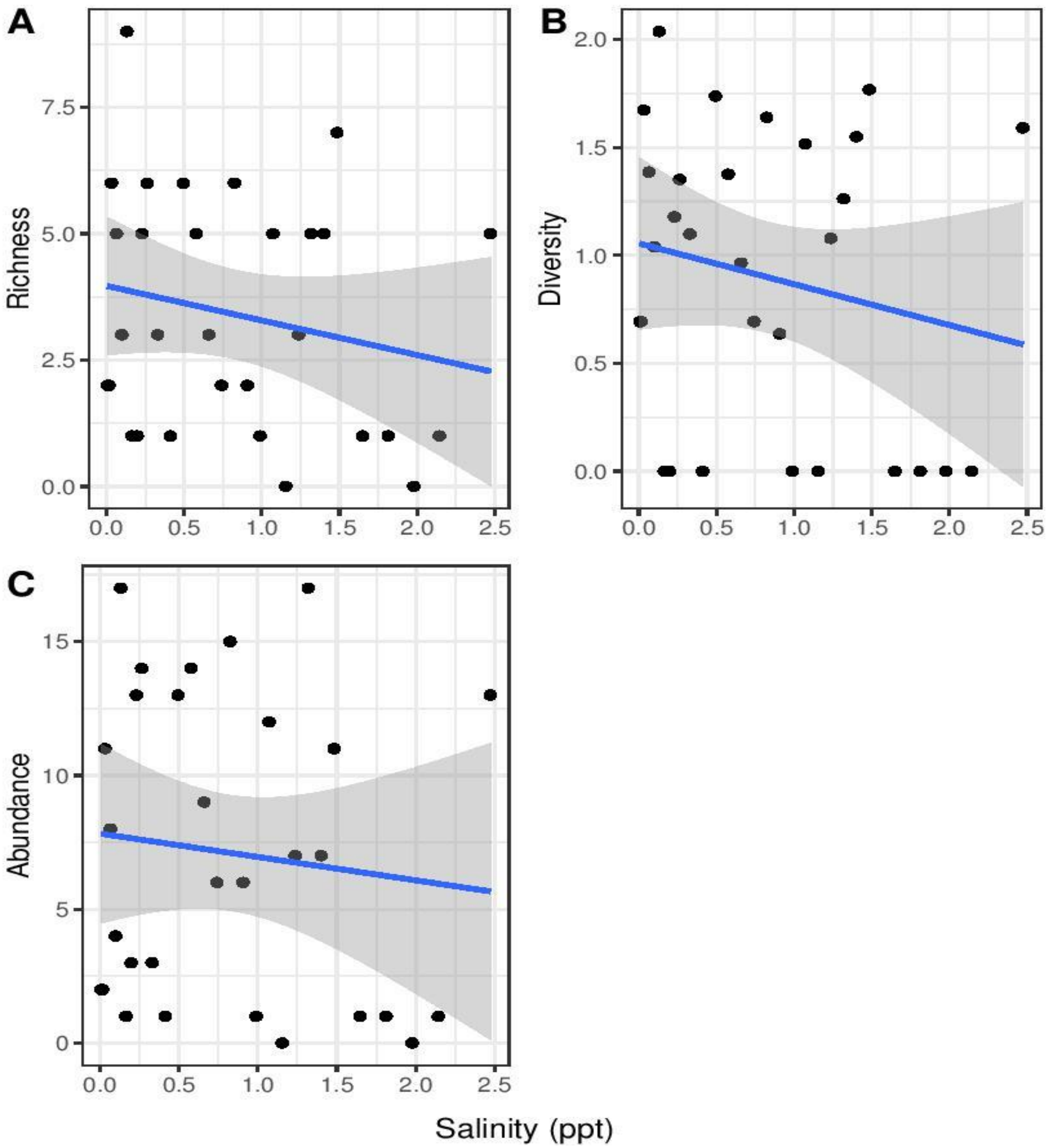


*Figure 4. Three plots displaying the initial zooplankton species richness (A. linear regression, t-1.109, p>0.05, $R^2$=0.0078), diversity (B. linear regression, t=-1.053, p>0.05, $R^2$=0.0038) and abundance (C. linear regression, t=-0.577, p>0.05, $R^2$=-0.02354) based on zooplankton samples taken from the thirty experimental mesocosms at week 0.*

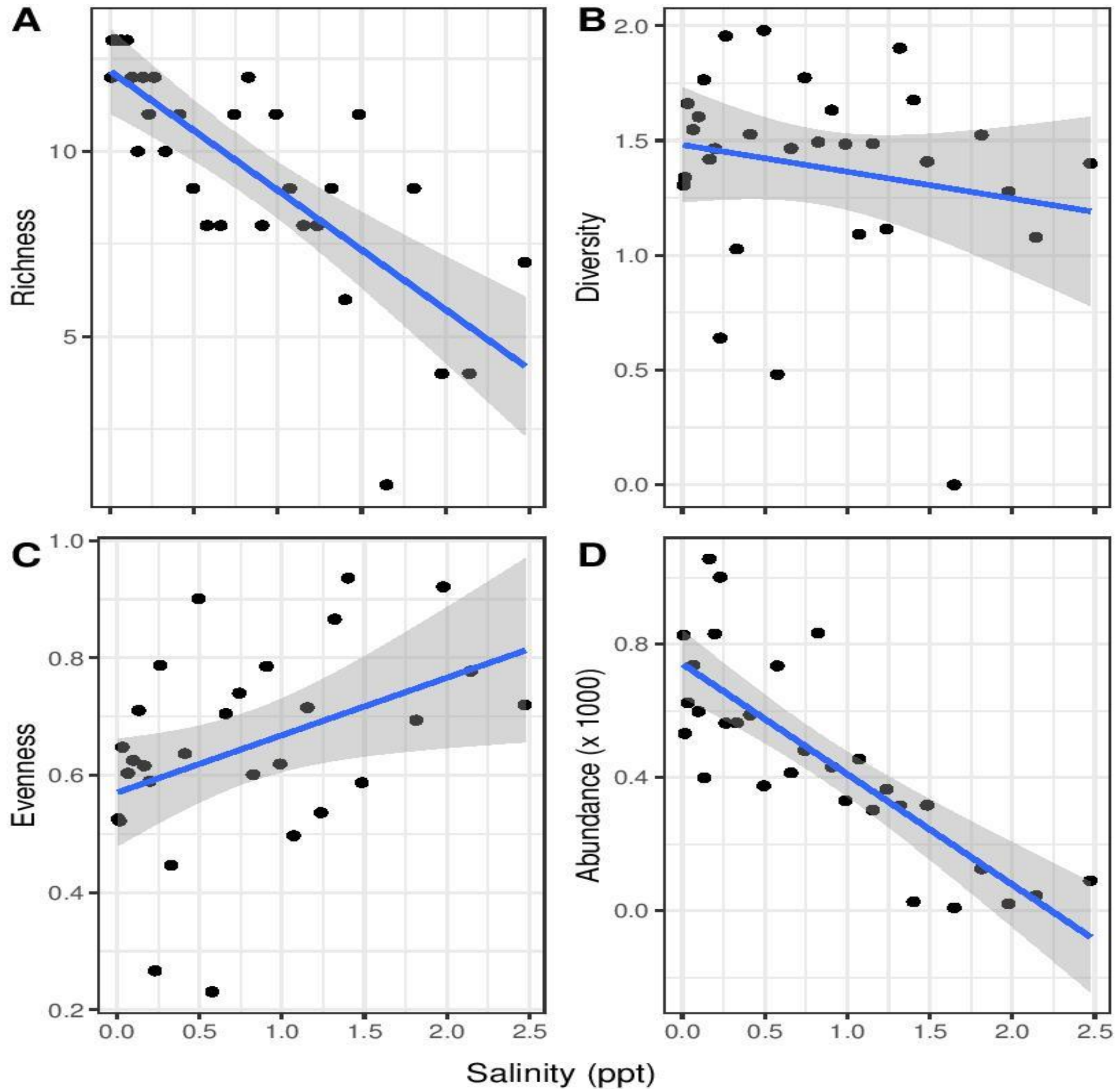


*Figure 5. Four plots the linear regression of species richness (A. linear regression, t=-6.303, p<0.05, R2=0.5718), diversity (B. linear regression, t=-1.042, p>0.05, R2= 0.0029), evenness (C. linear regression, t=2.329, p<0.05, R2= 0.1364) and abundance (D. linear regression, t= -7.368, p<0.05, R2=0.6746) versus salinity (‰) based on zooplankton samples taken from the thirty experimental mesocosms at week 6. From the richness and abundance regressions, the equations of line were found to be: Richness = 12.1679 - 3.2186*Salinity; Abundance = 741.22 – 331.54*Salinity*

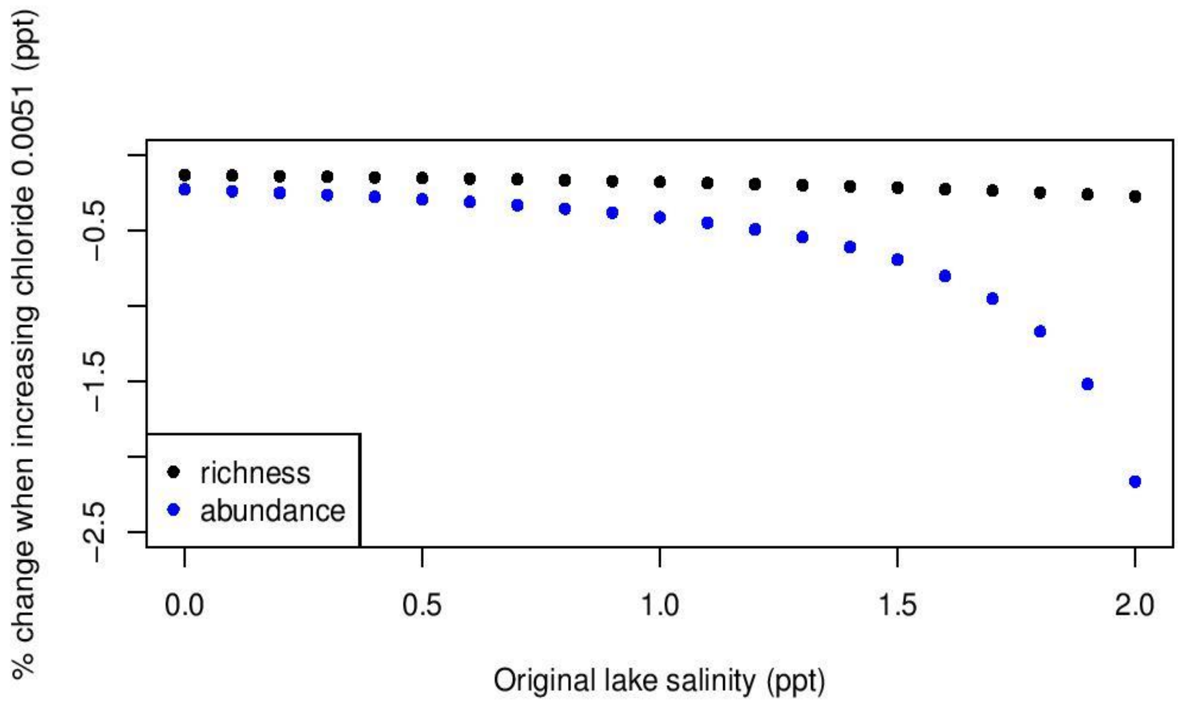


*Figure 6. A scatterplot of the projections for zooplankton species richness and abundance over the next 10 years based on the data collected from Dugan et al. 2017b*

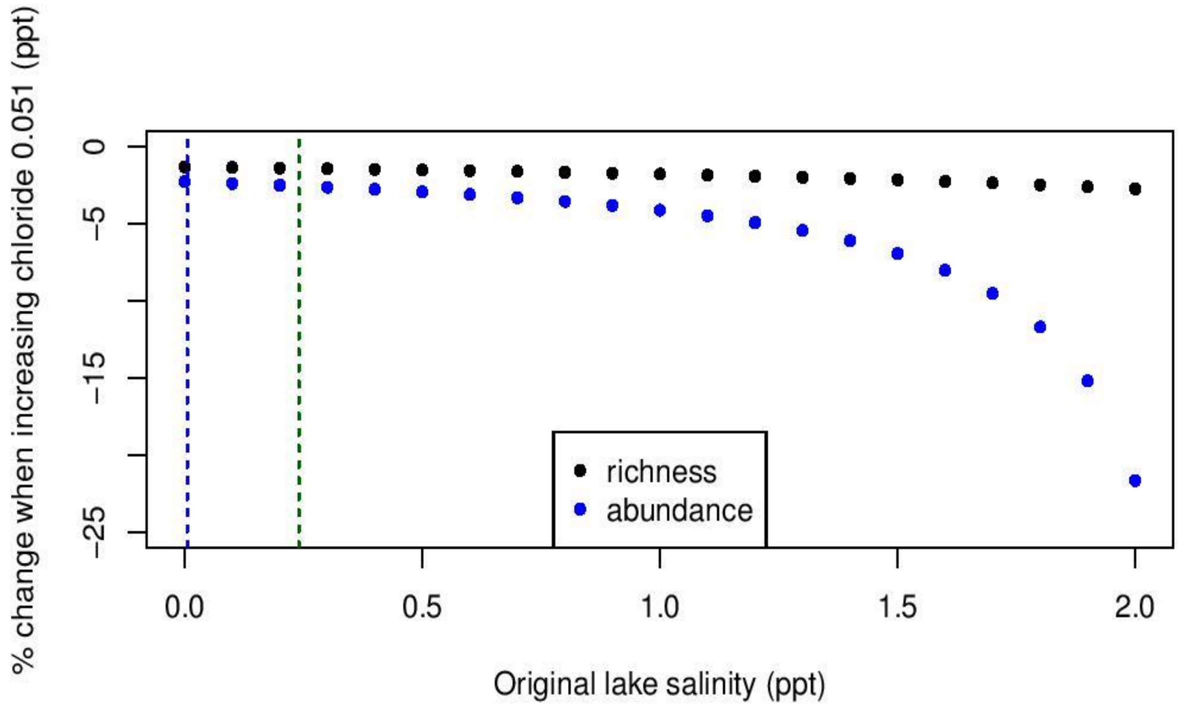


*Figure 7. A scatterplot of the projections for zooplankton species richness and abundance over the next 100 years based on the data collected from Dugan et al. 2017b. The dashed blue line represents the median chloride concentration in freshwater North American lakes (Dugan et al. 2017b), while the green line represents the maximum.*

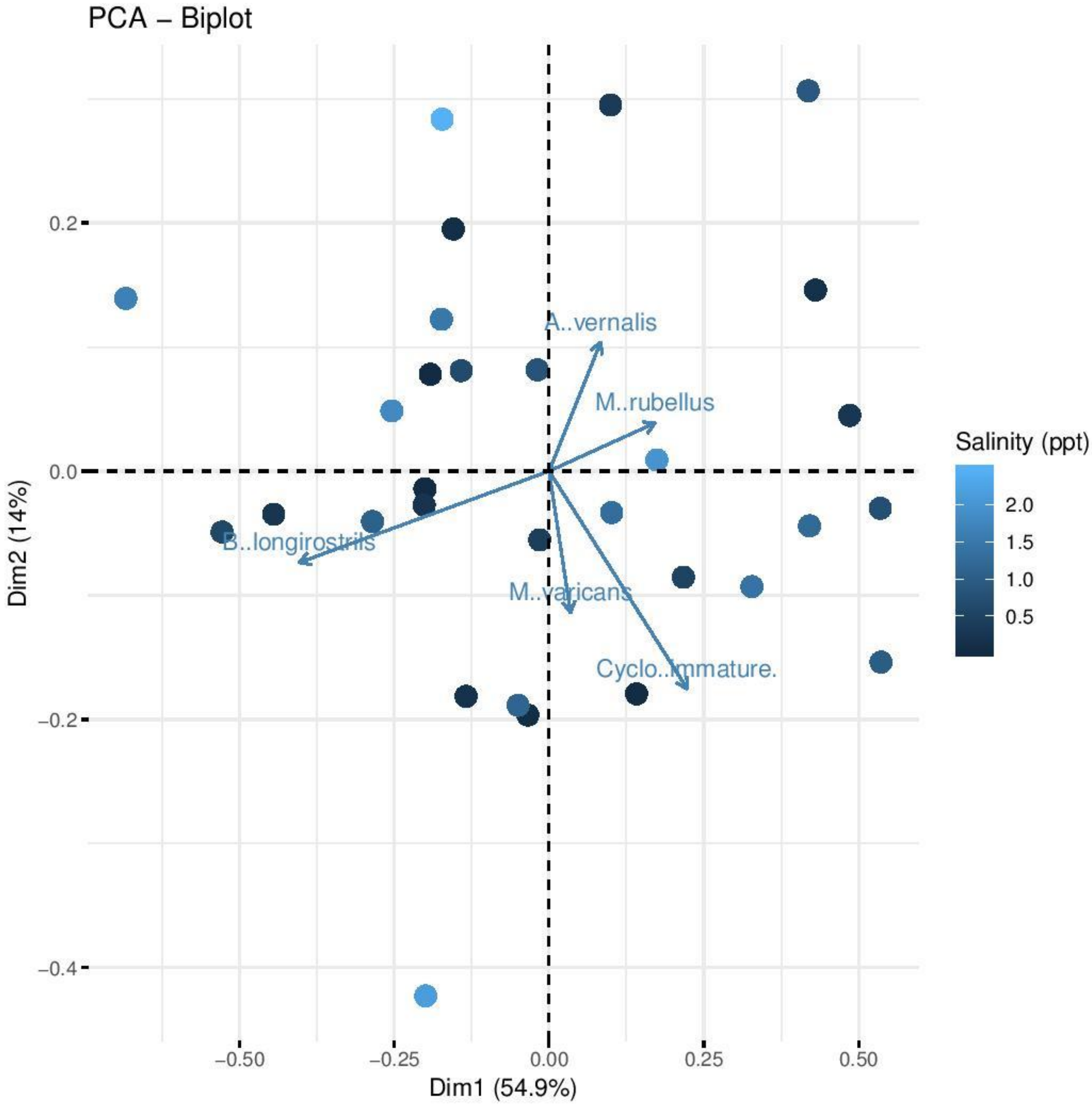


*Figure 8. A principle component analysis of the week 6 zooplankton samples, displaying the salinity range in a gradient blue colour and the dominant species in these mesocosms as the blue arrows.*

## Discussion

Increased salinity resulted in a significant decrease in species richness and abundance. The decline in both richness and abundance was gradual, with no breakpoint evident in the relationship. This suggests that zooplankton communities may respond to small increases in salinity, rather than experiencing change after a threshold has been reached. These results are similar to those found by Schallenberg et al. (2003) where they tested the relationship between salinity and community structure for 3 sites. They found that zooplankton abundance decreased exponentially with increasing salinity and species richness decreased linearly (Schallenberg et al. 2003,). Other studies have found results that support a similar trend where zooplankton abundance and richness decreased with increasing salt concentration (Dananay et al. 2015, Corsi et al. 2010, Hintz 2017). Increased salinity seemed to increase community evenness significantly, with some communities approaching a value of 1 (complete evenness). This was most likely because in the higher salinities there was a low number of salinity-tolerant species that were equally abundant.

My results clearly show that zooplankton communities can be impacted by rising salinities. However, given the relationships I found between richness, abundance, and salinity, my projections suggest very little change in species richness and modest decreases in abundance if current trends in chloride levels continue for the next century. My analysis of North American chloride trends showed that mean chloride levels in North American freshwater lakes are increasing by approximately 0.0051 ‰ per decade, or 0.051 ‰ over a century. Given that most freshwater lakes in North American range from 0.00018-0.24080 ‰ with a median value of 0.006 ‰ (Dugan et al. 2017a), these increases will not cause most lakes to surpass the US Environmental Protection Agency's Aquatic Life Criteria of 0.23 ‰. It is possible that other aquatic species such as phytoplankton may be more sensitive to chloride levels (Quinlan & Phlips 2007, Stefanidou et al. 2018), but for zooplankton, salinity-related changes over the next century should be minor.

My results suggest that rising salinity levels are probably of minor importance for Sturgeon Lake. The ambient salinity level in Sturgeon Lake at the beginning of the field experiment was measured at approximately 0.01‰, so an increase of 0.051 ‰ over the century will not push the salinity into a level where my results indicate significant changes in zooplankton communities. The starting salinity of the lake along with the rate of change in chloride through time also means that the salinity range I selected for the experiment (ambient-2.4 ‰) was probably unrealistic. The salinity range used for this experiment was selected based on previous studies that showed zooplankton responding to values above 2 ‰ (Dananay et al. 2015, Hall & Burns 2002, Hintz et al. 2017, Schallenberg et al. 2003). The goal was to select a salinity high enough to ensure detectable change in the zooplankton population. However, even under a worst-case scenario, it is hard to imagine road salt-driven increases in salinity allowing Sturgeon Lake to reach levels above 1‰.

The dominant species in the enclosures were *Acanthocyclops vernalis*, *Microcyclops rubellus*, and *Bosmina longirostris*. However, there was no compelling pattern in community structure defined in the principal component analysis related to salinity. One exception was *B. longirostris*, which did not persist in mesocosms with higher salinities (> 2 ‰). A study done Bailey et al. (2004) found similar results when examining the diapausing eggs of a different *Bosmina* species. Their results showed that none of the eggs from the species would hatch when exposed to elevated salinity levels (Bailey et al. 2004). Typically, cladoceran species have especially low tolerance for increased salinity. This is supported by the results found by Sarma et al. (2006), where almost all the cladoceran species were unable to reproduce after two weeks when exposed to a salinity above 4.5 ‰. Another interesting results from the principal component analysis was the low tolerance of copepod nauplii to increased salinity. This is consistent with previous research done by Hintz et al. (2017), where they found the threshold salinity for the nauplii abundance was approximately 0.9 ‰. Van Meter & Swan (2014) also found similar results which showed nauplii were at a much higher abundance in their lower salinity treatment of 0.32 ‰ than their higher salinity treatment of 1.93 ‰.

Although the field experiment was executed mostly to plan, there were some minor issues that could affect the interpretation of my results. I monitored conductivity throughout the experiment to ensure that salinity remained constant in each mesocosm. However, the conductivity did slightly decrease throughout the six-week experimental period due to some heavy rain periods during weeks three and four (figure 1). I added NaCl to my highest salinity mesocosm as it decreased the most dramatically but did not further manipulate the salinity of the remaining mesocosms. Therefore, although differences in salinity among my mesocosms were maintained, the absolute salinity level by the end of the experiment was approximately 10-15% lower than at the beginning. Temperature of the mesocosms could also be a consideration. Temperature over the six weeks was found to be highest in Sturgeon Lake itself, fluctuating between 23-28 degrees Celsius (°C) (figure 2). It was approximately 2 °C lower in the instrumented mesocosm in the sun and then 5 °C lower in the mesocosm in the shade. The temperatures of the mesocosms also seemed to fluctuate more than that of the lake, which is to be expected as the lake is a larger body of water than the 200 L mesocosms on land. However, when the lake's temperature did fluctuate, the changes in temperature of the mesocosms were similar. This is important for my results as the temperature plays a role in the tolerance of salinity of zooplankton. For example, Hall and Burns (2002) found that the salinity tolerance of *Daphnia carinata* declined when temperature increased and Thompson and Shurin (2012) found that salinity combined with the addition of higher than average temperatures further induced negative impacts on zooplankton species richness and decreased biomass. As a result, we might expect that the impacts of increasing salinity levels might cause more dramatic changes in zooplankton communities found in a warmer lake than in the artificially cool mesocosm environment.

Rising salinity levels drove changes in zooplankton communities in my experiment. However, based on chloride trends for North American lakes, it seems likely that the "road salt causing lake salinization" crisis has been overstated – at least for zooplankton. Significant changes in zooplankton species abundance aren't likely to occur until the end of the next century given current trends, and the most significant changes will be confined to lakes that currently have a higher ambient salinity (>1.5 ‰). Based on my results, I would argue that we should continue to focus efforts on using salt efficiently and taking precautions to avoid polluting waterways unnecessarily. Reducing or eliminating the use of road salt could have important public safety consequences, so it is important to consider both the costs and benefits when making these decisions. My study represents only one test of the effects of salt on zooplankton, but fortunately, the validity of my conclusions will be tested many times over as part of a larger project called the Global Salt Experiment organized by Dr. Shelley Arnott at Queen's University. The main goal of that project is to examine differences in zooplankton community response to salinity among different regions, and to determine if there are consistent patterns. When my results are put into the context of this larger project, we should be able to achieve consensus as to the potential impacts of salinization on freshwater zooplankton communities.

## Acknowledgements

I would like to thank Dr. Gray and Mercedes Huynh for mentoring me throughout this project. As well as Dr. Arnott for designing the experimental protocol used for Global Salt Experiment. I'd also like to thank my amazing field team; Evan Chang, Kayla Tasky and Tom Pretty. As well as my parents Dan and Nadine Franceschini for allowing us to use their backyard as the experimental site. I'd like to acknowledge the funding support of NSERC, FOSSA and Wilfrid Laurier University's Department of Biology.

## References

Alatalo R. V. (2016). Problems in the Measurement of Evenness in Ecology. 37: 199-204

Bailey S. A., Duggan I. C., Van Overdijk C. D. A., Johengen T. H., Reid D. F., Macisaac H. J. (2004). Salinity tolerance of diapausing eggs of freshwater zooplankton. Freshwater Biology. 49, 286-295.

Cañedo-Argüelles M., Hawkins C. P., Kefford B. J., Schäfer R. B., Dyack B. J., et al. (2016). Saving freshwater from salts. Science 351(6276):914-916

Coldsnow K., Mattes B., Hintz W., & Relyea R. (2017). Rapid evolution of tolerance to road salt in zooplankton. Environmental Pollution. 222: 367-373.

Corsi S. R., Graczyk D. J., Geis S. W., Booth N. L., Richards K. D. (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.

Dananay K. L., Krynak K. L., Krynak T. J., Benard M. F. (2015). Legacy of Road Salt: Apparent positive larval effects counteracted by negative postmetamorphic effects in wood frogs. Environmental Toxicology and Chemistry. 34(10):2417-2424.

Dugan H. A., Bartlett S. L., Burke S. M., Doubek J. D., Krivak-Tetley F. E., et al. (2017a). Salting our freshwater lakes. PNAS 114(17): 4453-4458.

Dugan H. A., Summers J. C., Skaff N. K., Krivak-Tetley F. E., Doubek J. P., Burke S. M., Bartlett S. L., Arvola L., Jarjanazi H., Korponai J. and Kleeberg A. (2017b). Long-term chloride concentrations in North American and European freshwater lakes. Scientific data, 4, p.170101.

Evans M., & Frick C. (2001). The Effects of Road Salts on Aquatic Ecosystems. NWRI Contribution No. 02-308. Environment Canada, National Water Research Institute, Saskatoon, SK.

Hall C. J., & Burns C. W. (2002). Mortality and growth responses of *Daphnia carinata* to increases in temperature and salinity. Freshwater Biology, 47: 451–458, doi: https://doi.org/10.1046/j.1365-2427.2002.00815.x.

Haney, J. F., M. A. Aliberti, E. Allan, S. Allard, D. J. Bauer, W. Beagen, S. R. Bradt, B. Carlson, S. C. Carlson, U. M. Doan, J. Dufresne, W. T. Godkin, S. Greene, A. Kaplan, E. Maroni, S. Melillo, A. L. Murby, J. L. Smith, B. Ortman, J. E. Quist, S. Reed, T. Rowin, M. Schmuck,

R. S. Stemberger, and B. Travers. (2013): An-Image-based Key to the Zooplankton of North America, version 5.0accessed <http://cfb.unh.edu/cfbkey/html/>.

Hintz W.D., Mattes B. M., Schuler M. S., Jones D. K., Stoler A. B., Lind L., Relyea R. A. (2017). Salinization triggers a trophic cascade in experimental freshwater communities with varying food-chain length. Ecological Applications. 27(3):833-844.

Legendre P., & Gallagher E. D. (2001). Ecologically meaningful transformations for ordination of species data. Oecologia. 129(2):p.271-280.

Lepš J. & Šmilauer P. (2003). Multivariate analysis of ecological data using CANOCO. Cambridge University Press, Cambridge, U.K.

Liu X., & Steiner C. F. (2017). Ecotoxicology of salinity tolerance in *Daphnia pulex:* interactive effects of clonal variation, salinity stress and predation. J. Plankton Res. 39(4): 687-697.

Oksanen J., Blanchet F. G., Friendly M., Kindt R., Legendre P., McGlinn D., Minchin P. R., O'Hara R. B., Simpson G. L., Solymos P.,  Stevens M. H., Szoecs E., & Wagner H. (2018). vegan: Community Ecology Package. R package version 2.5-1. https://CRAN.R-project.org/package=vegan

Pena E. A., & Slate E. H., (2014). gvlma: Global Validation of Linear Models Assumptions. R package version 1.0.0.2.  https://CRAN.R-project.org/package=gvlma

Quinlan E.L., & Phlips E.J., (2007). Phytoplankton assemblages across the marine to low-salinity transition zone in a blackwater dominated estruary. Journal of Plankton Research. 29(5): 401-416.

R Development Core Team. (2019): R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Sarma S., Nandini S., Morales-Ventura J., Delgado-Martinez I., Gonzalez-Valverde L (2006). Effects of NaCl salinity on the population dynamics of freshwater zooplankton (rotifers and cladocerans). Aquatic Ecology. 40:349-360.

Schallenberg M., Hall C. J., Burns C. W. (2003). Consequences if climate-induced salinity increases on zooplankton abundance and diversity in coastal lakes. Marine Ecology Progress Series 251: 181-189.

Stefanidou N., Genitsaris S., Lopez-Bautista J., Sommer U., Moustaka-Gouni M. (2018). Effects of heat shock and salinity changes on coastal Mediterranean phytoplankton in a mesocosm experiment. Marine Biology. 165:154.

Thompson P. L., & Shurin J. B. (2012). Regional zooplankton biodiversity provides limited buffering of pond ecosystems against climate change. Journal of Animal Ecology 81. 251-259, doi: 10.1111/j.1365-2656.2011.01908.x

Van Meter R. J. & Swan C. M. (2014). Road Sakts as Environmental Constraints in Urban Pond Food Webs. PLoS ONE. 9(2):1-12.