

**Assessing the Influence of Geological and Land-Use Gradients on
Zooplankton and Phytoplankton Biodiversity in The Land Between
Ecotone**

by

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A thesis submitted to the
School of Graduate and Postdoctoral Studies in partial
fulfillment of the requirements for the degree of

Master of Science in Applied Bioscience

Faculty of Science

University of Ontario Institute of Technology (Ontario Tech University)

Oshawa, Ontario, Canada

November 2021

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THESIS EXAMINATION INFORMATION

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Master of Science in Applied Bioscience

Thesis title: Assessing the Influence of Geological and Land-Use Gradients on Zooplankton and Phytoplankton Biodiversity in The Land Between Ecotone
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An oral defense of this thesis took place on November 4th 2021 in front of the following examining committee:

Examining Committee:

Chair of Examining Committee	Dr. Janice Strap
Research Supervisor	Dr. Andrea Kirkwood
Examining Committee Member	Dr. Denina Simmons
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The above committee determined that the thesis is acceptable in form and content and that a satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate during an oral examination. A signed copy of the Certificate of Approval is available from the School of Graduate and Postdoctoral Studies.

ABSTRACT

The Land Between (TLB) is an ecotone in south-central Ontario that represents a transition from limestone-dominated bedrock to granite-dominated bedrock, creating notable calcium and land-use gradients across this unique geographic region. The goal of this study was to investigate water quality and plankton community patterns in lakes across TLB as a function of geological and spatial drivers. Lake water quality profiles were differentiated based on calcium concentrations, and increased watershed land-use was related to increased lake nutrient levels. Plankton communities were not spatially structured. Phytoplankton communities were influenced by environmental drivers including nutrients and water temperature, as well as the inferred effect of zooplankton grazing. Zooplankton communities were influenced by environmental drivers including calcium concentration. Additionally, plankton communities appeared to be influenced by species-specific and trophic interactions. Overall, my research has provided insight into the important drivers of lake water quality and plankton communities in TLB.

Keywords: Plankton communities; Water quality; Land-use; Calcium; Ontario lakes

AUTHOR'S DECLARATION

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STATEMENT OF CONTRIBUTIONS

I hereby certify that I am the sole author of this thesis and that no part of this thesis has been published or submitted for publication. I have used standard referencing practices to acknowledge ideas, research techniques, or other materials that belong to others. Furthermore, I hereby certify that I am the sole source of the creative works and/or inventive knowledge described in this thesis.

ACKNOWLEDGEMENTS

I would first like to thank Dr. Andrea Kirkwood for providing me with the opportunity to perform this research, and for all of the guidance and support she has provided me throughout the last 3 years. I am extremely lucky to have her as a supervisor and mentor. I would also like to thank my thesis committee members, Dr. Denina Simmons and Dr. H  l  ne LeBlanc for their valuable feedback throughout my project.

Next, I would like to thank NSERC for funding this research. This project could not have been completed without their support.

I would also like to thank the members of the Kirkwood Lab: Tyler Harrow-Lyle, Erin Smith, Eric Anderson, Alex Johnston, as well as our summer lab and field assistants Claire Gibbs, Denin Gray, and Jesse Killoran, who provided so much help and support throughout the completion of my project. I could never have completed this project without them, and they also provided me with an abundance of positive experiences that made my graduate studies enjoyable and memorable.

Finally, I would like to thank my family: Mom, Dad, Steph, Max, Grace, and Morley, as well as my partner, Tristen, and my friends for providing me with endless support and encouragement throughout my academic career.

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LIST OF ABBREVIATIONS

Ca	Calcium
CHLa	Chlorophyll a
DO	Dissolved oxygen
Fe	Iron
GIS	Geographic information systems
GLLVM	General linear latent variable model
K	Potassium
Mg	Magnesium
Mn	Manganese
Na	Sodium
PCA	Principal components analysis
Permanova	Permutation analysis of variance
RDA	Redundancy analysis
Temp	Temperature
TLB	The Land Between
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorous
Tur	Turbidity

Chapter 1: General Introduction

Freshwater lakes are important ecosystems that provide habitat to a wide variety of aquatic organisms, play a key role in nutrient cycling, and offer ecosystem services to people such as drinking water and recreational activities (Balian et al., 2008). Despite their importance, freshwater lakes are being increasingly impacted by anthropogenic stressors, which can negatively affect their water quality and aquatic communities (Søndergaard & Jeppesen, 2007). Land-use stressors can lead to the eutrophication of lakes and have negative outcomes such as sensitive species declines, harmful algal blooms, and anoxic events (Brauns et al., 2011; Gildow et al., 2016). Due to the complexity of these ecosystems, the underlying mechanisms behind degradation of lake water quality are not fully understood, and this continues to impede efforts to protect and preserve lake health.

Lakes are not all equal in their resistance and resilience to anthropogenic stressors. Lakes can have different underlying geological components which can contribute to a lake's water properties and consequently impact the way a lake will respond to these stressors. For example, a lake located on limestone-dominated bedrock will have high inputs of calcium carbonate to its water column, causing it to have increased water hardness and pH buffering capacity (Schindler et al., 1980). In contrast, a lake located on a granite-dominated bedrock will have much less calcium carbonate and will therefore have decreased water hardness and pH buffering capacity (Schindler et al., 1980). Differences in water hardness and buffering capacity influence how a lake responds to acid rain, as well as invasive species. For example, hardwater lakes may be

more susceptible to invasion by the invasive zebra mussel because of the abundant calcium carbonate available for their growth requirements (Whittier et al., 2008).

Despite these well-known differences among softwater and hardwater lakes, very few studies have compared the responses of hardwater and softwater lakes and their communities to multiple anthropogenic stressors. With expanded land development from a growing human population increasing the intensity and variety of stressors impacting freshwater lakes, it is critical to better understand how specific lakes will respond to enhance our ability to protect these vital ecosystems.

1.1 Plankton Community Response to Environmental Factors

Phytoplankton are microscopic photosynthesizing organisms which act as primary producers in lakes. Zooplankton are small, often microscopic invertebrate animals found throughout the water column in lakes. Studying phytoplankton and zooplankton communities is an effective way to gain an understanding of lake health, because these plankton communities are key components of the aquatic food web and they are very sensitive to changes in water quality (Keller & Yan, 1991). Additionally, their biodiversity is positively correlated with lake health (Keller & Yan, 1991). As such, plankton communities can be utilized to study lake health or to understand how lakes respond to various stressors. Below is an overview of how plankton communities respond to various water quality changes which occur as the result of anthropogenic stressors.

Nutrients

In most freshwater lakes in Ontario, phosphorus is a limiting nutrient, which means that phytoplankton and zooplankton populations are limited by nutrient

availability in the system (Schindler, 1977). However, manure and fertilizer runoff from agriculture and septic or sewage pollution from developed areas can introduce significant loadings of phosphorus and nitrogen into aquatic ecosystems, which leads to increased phytoplankton abundance and production (Schindler, 1977; Xu et al., 2010). While phosphorus has been found to be the primary limiting nutrient in lakes (Schindler, 1977), simultaneous additions of phosphorus and nitrogen have been found to produce higher responses than those recorded individually (Elser et al., 2007; Xu et al., 2010). Increases in phytoplankton abundance can alter light penetration in the system by increasing water turbidity (Flöder et al., 2002), which can lead to competitive exclusion of certain light-sensitive species. High nutrient conditions tend to favour larger-bodied species due to their ability to store more nutrients than small-bodied species (Duarte et al., 2000), whereas low nutrient conditions favor small-bodied species which have shorter diffusion distances for nutrients (Litchman et al., 2007). For this reason, high loadings of nutrients can alter community dynamics in historically low-nutrient systems. Additionally, bloom-forming cyanobacterial species thrive under high-nutrient conditions and can outcompete other species (Schindler, 1977), resulting in potentially toxic blooms which can be harmful to the aquatic community, surrounding wildlife, and even humans (Willén et al., 2000). These blooms can decrease species richness and result in negative effects such as fish kills, surface scums, and polluted drinking water (Barica, 1975; Paerl, 1988; Smith et al., 2002; Willén et al., 2000).

Zooplankton communities are indirectly affected by nutrient pollution via their response to the changes in phytoplankton communities. As phytoplankton are an important food source for zooplankton, an increase in their abundance due to increased

nutrients can lead to an increase in zooplankton abundance (Jeppesen et al., 2001). High-nutrient inputs can lead to eutrophication of lakes, and eutrophic conditions favor smaller-bodied zooplankton that can tolerate bloom-forming cyanobacteria and the higher dissolved oxygen levels produced from photosynthesis. Increased abundance of filamentous and colonial algae in eutrophic systems are also more difficult for large bodied zooplankton to graze (Forrest & Arnott, 2006; Jeppesen et al., 2001). Overall, species richness of zooplankton decreases under eutrophic conditions and can even lead to trophic cascades or regional extinction of zooplankton or species higher in the food web (Jeppesen et al. 2001; Gildow et al., 2016).

Water Temperature

While water temperature naturally varies both seasonally and spatially, humans are impacting water temperature through anthropogenic climate change, which is leading to increased mean temperatures during summer (Poesch et al., 2016). Temperature can directly influence phytoplankton by influencing metabolic rates and physiology, as many processes such as respiration, photosynthesis, and growth rates increase with increasing temperature up to a threshold (Atkinson, 1994). As described in the Temperature Size Rule, warmer climates tend to favor smaller species, and colder climates favor larger species (Atkinson, 1994). This relationship has been found to have an even greater effect in aquatic systems than in terrestrial ones (Forster et al., 2012), and as such, community shifts to smaller-bodied species may be observed in response to climate change. The community shift towards smaller species in response to warming has been observed in both mesocosm studies (e.g., Pulina et al. (2016)) and lake surveys (e.g. Winder et al.

(2009)). Additionally, warmer conditions have been found to favor harmful cyanobacteria which outcompete other, less harmful taxa (Elliott et al., 2006; Robarts & Zohary, 1987).

The temperature-size rule has also been found to influence zooplankton (Mallin & Partin, 1989). There are several theories explaining this trend in zooplankton communities: several species may stop growing due to temperatures reaching above their thermal optima (Jamieson & Burns, 1988), but it also may be due to energy deficits resulting from elevated costs for respiration as well as development (Gophen, 2018). Increases in predation at higher temperatures may also play a role (Atkinson, 1994). Climate change is also causing more temperature fluctuations within ecosystems, and as different species have different sensitivities to sudden temperature fluctuation, this can cause a decrease in abundance or even total loss of (Rajakaruna & Lewis, 2018)cies (Rajakaruna & Lewis, 2018).

Acidity

Acid deposition was a large problem for many Canadian lakes in the recent past, and recurring acidification due to anthropogenic stressors continues to affect freshwater lakes (Korosi & Smol, 2012). The pH of a lake influences many chemical properties within the water column. A lake's pH determines the source of carbon available to aquatic biota, with carbon dioxide concentrations decreasing with increasing pH (Moss, 1973). Different taxa vary in their ability to utilize different carbon sources, so some are limited at certain pH levels. When pH levels are very high, several phytoplankton taxa may be unable to utilize carbon for photosynthesis resulting in decreased abundance of certain populations and decreased species richness in the community (Moss, 1973). Lake pH also influences solubility and concentration of nutrients and metals. Acidic conditions

can result in increased metal concentration and toxicity in the water column, which can harm phytoplankton (Campbell & Stokes, 1985). Neutral pH can limit the release of bioavailable phosphorous into the water column due to reduced redox potential (Reynolds, 2006). Many species thrive between a neutral and slightly alkaline pH, and species richness has been found to increase in slightly alkaline conditions when compared to neutral or slightly acidic conditions (Agrawal, 2012; Chen & Durbin, 1994). Because different species have different tolerance levels for acidic conditions or fluctuations in pH, community composition can shift following pH alterations. For example, Korneva (1996) surveyed several lakes with varying acidification and found that cyanobacteria and diatoms were positively correlated with pH while greens, chrysomonads, and cryptomonads had were negatively correlated with pH.

As for zooplankton, low pH has been shown to significantly decrease oxygen uptake rates in crustacean zooplankton by decreasing diffusion of CO₂ from the carapace (Alibone & Fair, 1981). This respiratory stress can cause extinctions in sensitive species if pH is low enough and can also limit growth of populations and communities of zooplankton (Sprules, 1975). Increasing acidity is correlated with the prevalence of smaller-bodied organisms due to the respiratory stress induced by the conditions (Alibone & Fair, 1981).

Water Hardness

Water hardness is determined by the mineral content of a body of water, with calcium and magnesium typically being the dominant elemental contributors. There has been surprisingly little research looking specifically at the effect of water hardness on plankton communities. Water hardness (i.e., as CaCO₃) in most lakes likely does not have

a direct effect on phytoplankton because, despite calcium being an essential nutrient for their survival, calcium nutritional requirements are generally low in phytoplankton (0.01 to 0.95 mg/L) (Gerloff & Fishbeck, 1969). However, water hardness can have a number of indirect effects that impact phytoplankton. Hardwater lakes have a significant buffering capacity due to the presence of calcium carbonate that can bind excess hydrogen ions in water, causing them to have a stable, slightly alkaline pH. Generally, neutral to slightly alkaline conditions exhibit higher species richness: for example, in a study surveying waters which varied from pH 4 to 8.5, species richness was highest at 7.0 – 7.6 (Hörnström, 2002).

Softwater ecosystems are more susceptible to acidification, which can reduce species richness (Stokes, 1986). Additionally, calcium can be a limiting factor for zooplankton populations, especially for cladoceran zooplankton which are efficient grazers of phytoplankton (Ashforth & Yan, 2008; Hessen et al., 2000). As such, phytoplankton grazing may be higher in hardwater lakes where calcium is not a limiting factor for zooplankton populations when compared to softwater lakes where cladoceran populations could be limited from a low calcium supply. Overall, cladoceran and copepod zooplankton have high calcium requirements due to their calcium-rich carapaces, and these groups can therefore be limited in softwater conditions where calcium levels are low (Cowgill, 1976). Large-bodied cladocerans such as daphniids are especially disadvantaged in low calcium conditions because they lose 90% of their body calcium each time they moult (Porcella et al., 1969), and this needs to be recovered from dissolved calcium in the water column (Greenaway, 1985). Unlike cladocerans and copepods, rotifers do not have high calcium requirements, so they may have a

competitive advantage in softwater lakes (Wærvågen et al., 2002). Calcium can also indirectly impact zooplankton communities by affecting lake pH as previously mentioned, which can cause stress for sensitive taxa and result in loss of species richness in the lake (Alibone & Fair, 1981; Sprules, 1975).

Salinity

While freshwater ecosystems do not typically have high salt concentrations, road salt runoff is introducing more regional variability of salinity in lakes near large roadways (Hintz & Relyea, 2019). This can lead to higher overall salt concentrations in lakes, as well as seasonal fluctuations in salinity. While the mechanisms by which phytoplankton are impacted by salinity have not been well studied, it has been recognized that some species are more tolerant than others to salt deposition and fluctuations (Hintz et al., 2017). Judd et al. (2005) reported a community shift towards salt-tolerant species typically found in brackish waters in a freshwater lake experiencing road salt runoff. Influxes of road salt have also been connected to shifts in lake diatom populations (MacDougall et al., 2016), with more sensitive species decreasing in abundance. In contrast, increasing salinity has also been found to increase abundance of phytoplankton (Hintz et al., 2017), though this may be due to the simultaneous effects of eutrophication which can occur concurrently with other anthropogenic stressors (Judd et al., 2005). Salt fluctuations can be harmful to zooplankton (Martínez-Jerónimo & Martínez-Jerónimo, 2007), which can lead to reduced grazing pressure on phytoplankton and in turn increase phytoplankton abundance (Judd et al., 2005).

Research on the effects of road salt on freshwater zooplankton has typically focused on its toxicity to cladocerans, with few focused on its effects on copepods or

rotifers (Hintz & Relyea, 2019). Cladoceran zooplankton are very sensitive to salinity due to their inability to regulate high influxes of chloride, so sporadic increases in salinity can have negative effects on survival of many species, potentially leading to local extinction of sensitive species (Hintz et al., 2016), which can also have grave effects on the food web. Increases in salinity also negatively affect growth rate and reproduction capabilities of cladocerans (Arnér & Koivisto, 1993; Martínez-Jerónimo & Martínez-Jerónimo, 2007). The species-level effects of chloride on either copepods or rotifers have not been heavily studied, but it has been shown that different species within each group vary in their tolerance to chloride (Evans & Frick, 2001; Sarma et al., 2006). Overall, increased salinity is harmful for zooplankton communities.

Light Penetration

Light is a very important resource for phytoplankton as it is necessary for photosynthesis. Fluctuations in light intensity as the result of seasonal changes leads to shifts in plankton community composition as each species has a different critical light intensity which influence their ability to compete with other taxa (Huisman et al., 1999). Anthropogenic stressors from recreational activity and clear-cut shorelines can lead to increased sediment runoff into lakes, which can diminish water clarity and light penetration (Baustian et al., 2018), decreasing the light available to the phytoplankton community and consequently limiting biomass (Knowlton & Jones, 1996). Competitive exclusion can occur in low-light conditions because species with lower critical light intensities will outcompete other species which cannot reach maximal photosynthetic output (Litchman, 1998). This can lead to community shifts, and in some cases, harmful outcomes, as many toxin-producing cyanobacterial species such as *Microcystis sp.* have

low critical light intensities and can thus outcompete other taxa and form harmful algal blooms (Flöder et al., 2002). In addition, light penetration can be limited by phytoplankton density itself as high phytoplankton density can lead to self-shading effects (Flöder et al., 2002).

Zooplankton typically respond to light by altering their position in the water column through a strategy known as diel migration where they migrate to deep, low-light conditions during the day and come up to shallower areas at night to feed (Hays, 2003). This is a behavioral trade-off mainly thought to reduce predation (Lampert, 1993). This behavior is triggered by a variety of factors including UV penetration, meaning that decreased transparency causing low-light conditions in lakes can alter zooplankton behavioral patterns (Leach et al., 2015). Low-light conditions can also harm zooplankton indirectly by decreasing algal abundance (Knowlton & Jones, 1996), and therefore decreasing their food source. Decreases in water clarity have also been found to have a negative correlation with zooplankton biodiversity in general (Gayosso-Morales et al., 2017), especially in benthic zooplankton (Lyons et al., 2012).

1.2 Overview of the Study Region

The Land Between (TLB) is an ecotone in south-central Ontario that represents a transition from limestone-dominated bedrock to granite-dominated bedrock. As the underlying bedrock shifts in TLB, there is a geological mosaic formed throughout the geological transition zone. Like other transition zones known as “ecotones”, TLB is a biodiversity hotspot for plants and wildlife (Risser, 1995). TLB is home to many lakes that experience a notable shift in water chemistry across the transition zone. Due to the geological mosaic in TLB, it is possible to have lakes situated within kilometers of each

other but differ in water hardness. Like many other freshwater ecosystems worldwide, lakes in TLB currently face a variety of anthropogenic stressors such as nutrient pollution, road-salt runoff, and shoreline development, which can negatively affect their water quality and biological communities (Brauns et al., 2011; Gildow et al., 2016).

In this study, 60 lakes along TLB's calcium gradient were selected and surveyed for their water quality and biological communities (Figure 1). These lakes were selected based on historical calcium concentrations in an effort to capture a wide range of calcium concentrations. When surveyed for this project, these lakes ranged from 0.43 – 83.7 mg/L in their calcium concentrations (Figure 2) and should therefore represent a wide gradient in water hardness. Additionally, as seen in Figure 1, land-cover also varies across the TLB, with higher developed and agricultural land in the southern watersheds than in the northern watersheds.

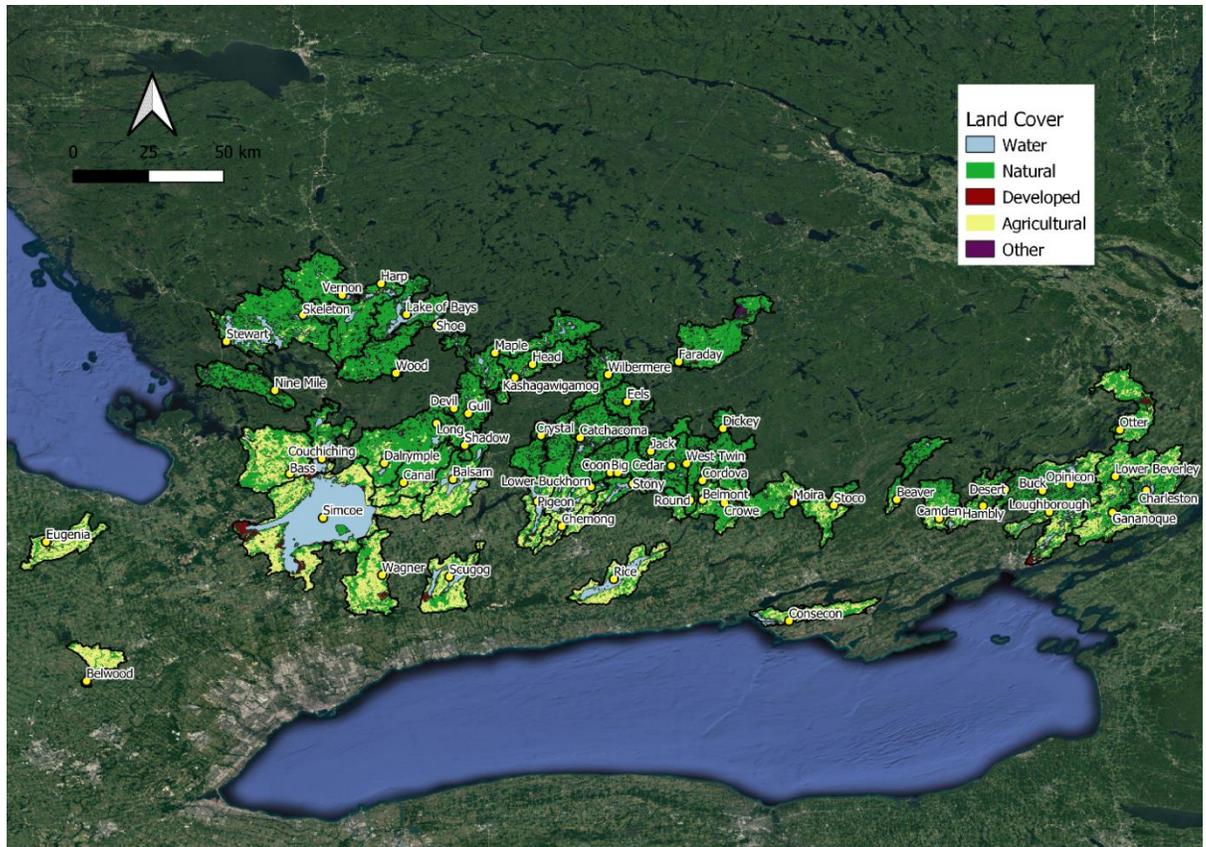


Figure 1.1 Map of 60 study lakes and their watersheds with land-cover data.

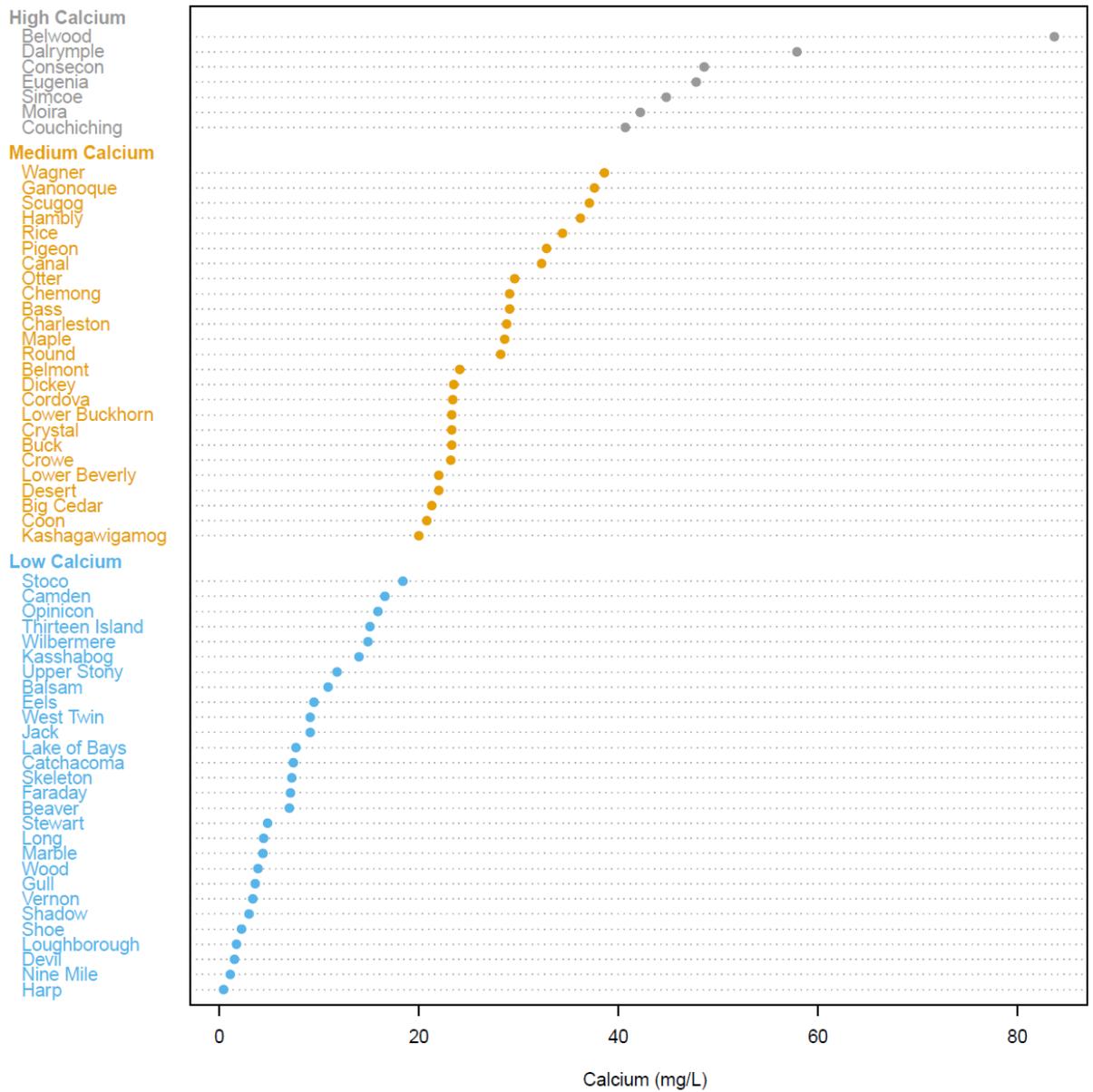


Figure 1.2 Study lakes ordered by their calcium concentrations.

1.3 Research Objectives

The geological and land-use gradients across TLB provide a unique opportunity to study how these environmental factors influence whole plankton communities in situ across a heterogeneous landscape. While many studies have focused on the responses of individual taxa or whole communities to various environmental gradients in lab or

mesocosm studies, there are rarely opportunities to study their multivariate responses to regional-scale environmental gradients in lakes. In addition to its ecological value, TLB represents a large portion of Ontario's cottage country, and many local areas are economically dependent on seasonal cottagers, tourism, and fishing that its lakes provide. As anthropogenic stressors such as climate change and land-use increase their influence in TLB, it is important to establish baseline information about the current water quality and of its lakes to assist in preserving and maintaining their ecological integrity.

The goal of this study was to investigate water quality and plankton community patterns in lakes across TLB for the first time. This research investigates how plankton communities vary along water hardness, water quality, and land-use gradients in order to not only better understand plankton ecology in this region, but to also provide valuable information to lake managers. To achieve this goal, several research objectives were investigated:

1. Document and characterize water quality profiles as well as zooplankton and phytoplankton communities across The Land Between;
2. Investigate how water quality profiles and land-use in softwater and hardwater lakes influence community structure; and
3. Determine if spatial differences are drivers of community structure

In Chapter 2, I characterized the lakes in TLB based on a suite of water quality parameters and land-use characteristics. In lieu of having water hardness data, I classified lakes based on calcium concentration categories: low-calcium lakes (< 20 mg/L of calcium), medium-calcium lakes (20-40 mg/L of calcium), and high-calcium lakes (> 40 mg/L of calcium). I predicted that lakes would vary based on calcium categorical

classification and land-use, as these environmental parameters are likely impacting lake water quality either directly (e.g., water hardness, macronutrient availability) or indirectly (e.g., nutrients sourced from agriculture). I expected low-calcium lakes to have fewer nutrients and lower pH levels, and high-calcium lakes to have more nutrients and higher pH levels as a result of their calcium and land-use profiles.

In Chapter 3, I examined both the bottom-up and top-down drivers of phytoplankton community structure and abundance in TLB. I predicted that calcium, land-use, and pH would emerge as important drivers of phytoplankton biomass and diversity due to their known effects on phytoplankton from previous studies. I expected that the nutrient-rich hardwater lakes in the south of TLB would have higher phytoplankton biomass as a result of increased nutrient availability compared to the softwater lakes located in the north of TLB, which are surrounded by more natural land-cover. I also expected zooplankton biomass to emerge as an important top-down driver of phytoplankton biomass as a result of their grazing of phytoplankton.

In Chapter 4, I examined the multivariate drivers of zooplankton community diversity and composition across TLB. I predicted that calcium levels, land-use, and pH will emerge as important controlling variables of zooplankton community composition based on their established effects on zooplankton in other lakes. I hypothesized that higher calcium lakes would favor high-calcium requiring large-bodied cladocerans, while low-calcium conditions would favor low-calcium requiring rotifers. I also hypothesized that small-bodied zooplankton would be more abundant in nutrient-rich lakes due to their tolerance of eutrophic conditions, while large-bodied zooplankton would be more abundant in nutrient-poor lakes due to their competitive feeding strategies.

In Chapter 5, I explored the role of taxa-specific and trophic interactions on plankton communities in TLB using a novel multivariate modelling technique called Generalized Linear Latent Variables Model (GLLVM). I expected that while environmental variables would continue to play an important role in plankton community structure, taxa-specific interactions would also emerge as having a significant role in driving community structure based on positive and negative co-occurrence patterns revealed in the analysis.

In the final chapter, I summarized my key findings regarding the characterization and drivers of water quality and plankton communities across TLB ecotone. I also discussed the limitations of my study design and provide insight into the future directions which could be taken with this research to both address the limitations and expand on the results of my research. Finally, I discussed the significance of my results and how they can be used by lake managers to maintain the lakes of TLB in the future.

Chapter 2: Characterization of Water Quality and Land-Use Profiles Across The Land Between

2.1 Introduction

When studying lake ecosystems, it is important to consider their physical and chemical properties. Inherent physical and chemical differences between lakes like lake depth or underlying geology can result in major differences in water quality and lake productivity that affect lake ecosystem functions. For example, a shallow lake may have more concentrated nutrients in its water column than a deep lake, resulting in more eutrophic conditions and a susceptibility to harmful algal blooms which can harm biological communities within and around the lake (Xu et al., 2010). Additionally, lake water quality is closely tied to surrounding land-use (Soranno et al., 1996), so development in a lake's watershed can have significant negative effects on a lake's overall water quality. Developed and agricultural land in a lake's watershed can lead to nutrient pollution from fertilizers and sewage, and nearby roads can pollute lakes with road salt runoff which can lead to toxic conditions for organisms in the lake (Novotny & Stefan, 2012; Taranu & Gregory-Eaves, 2008). Therefore, lakes surrounded by mostly natural landcover often represent "pristine" ecosystems compared to lakes surrounded by developed and agricultural land. A pristine lake is not necessarily a low nutrient or clear-water lake, but rather an ecosystem that supports aquatic communities that have co-evolved and adapted to local lake conditions post glaciation, without pronounced human impacts.

The lakes in TLB are subject to gradients in calcium concentration (i.e., water hardness) and varying land-use, where both increase in concentration and intensity from

north to south, respectively. Both of these environmental factors originate in the watershed and are likely impacting lake water quality either directly (e.g., water hardness, macronutrient availability) or indirectly (e.g., nutrients sourced from agriculture). As such, the goal of this chapter was to characterize the lakes in TLB based on a suite of water quality parameters and land-use characteristics. Examining the role of land-use as a driving factor in TLB's lakes water quality will provide insight into the habitat condition of plankton communities, which is explored in subsequent chapters.

2.2. Methods

2.2.1 Study site and lake selection criteria

A total of 60 lakes were selected for this study based on their known or estimated calcium concentrations. These lakes are located throughout TLB (see Figure 1.1 from Chapter 1) and represent a linear calcium gradient varying from <0.5 to 80 mg/L (see Figure 1.2 from Chapter 1). The selected lakes were required to have public boat launches. Additionally, none of the study lakes were directly connected by water inflow or outflow to ensure that each lake could be treated as an independent sample for statistical analyses. Sample collection for this study was done alongside another project conducted by PhD candidate Tyler Harrow-Lyle, for which four sites with varying proximity from the lake's boat launch were sampled. For the present study, the deepest of the four sites was sampled in order to collect integrated composite samples of water and plankton communities reflecting the entire water column. Site depth ranged from 0.85m to 19m. Although the sampling sites do not reflect the deepest, open-water areas in each lake, the study design ensured comparable sampling locations across the study lakes.

2.2.2 Data Collection

Samples were collected once from all 60 lakes within a narrow timeframe (3 weeks) in August 2019 to minimize seasonal effects. On-site measurements of temperature, pH, conductivity, and dissolved oxygen were obtained using a YSI multi-meter probe. Readings were collected at 1m intervals up to 10m deep in order to incorporate the entire water column. In cases where sites were less than 1m deep, readings were collected below the water's surface level. This was only necessary at Lake Opinicon (0.85-m) and Lake Simcoe (1-m).

Cation samples were collected from below the surface of each site in acid-washed falcon tubes and frozen within 24 hours. These samples were sent to SGS Canada, an accredited lab in Lakefield, Ontario, for cation analysis (calcium, magnesium, potassium, sodium, manganese, and iron) using inductively coupled plasma (ICP) spectrometry.

Water samples were taken at each lake using a 10-m long integrated tube which was lowered to at least 1-m above the bottom of the water column (in order to prevent the collection of sediment). This sample was then poured into a large, clean plastic container and mixed to form a composite sample of the whole water column. Total nitrogen and total phosphorous samples were collected from the composite sample using acid-washed 50-mL falcon tubes, which were then frozen until further analysis. One-liter composite samples were collected in acid-washed Nalgene bottles for chlorophyll a analysis. Back at the lab, 300-mL of composite lake water was filtered using glass-fiber filters (0.7 μm , 47 mm), and then wrapped in aluminum foil and frozen until further analysis using a 90% ethanol extraction method developed by Ritchie (2008). All water samples were kept in coolers after being collected and were frozen within 24 hours. To process total

phosphorous samples, a modified ascorbic acid method developed by Murphy & Riley (1962) and the Ontario Ministry of Environment (1983) was used. Total nitrogen samples were processed at York University using combustion catalytic oxidation methods.

Land-use data was determined for each lake's watershed in QGIS (QGIS Development Team, 2020) using land-cover data from Natural Resources Canada (2015) as well as waterbody and watershed data obtained from Ontario Ministry of Natural Resources and Forestry (2018 and 2020).

2.2.3 Data Analysis

Statistical analyses were performed using R version 4.0.4 (R Core Team, 2021). In order to visualize how land-use and land-cover in the watersheds varied between lakes, a bar plot was constructed in R using proportional agricultural, developed, and natural land-cover of each lake's quaternary watershed with the package ggplot2 (Wickham, 2016).

For subsequent analyses, lakes were sorted into three groups based on calcium concentrations: low calcium (<20-mg/L of calcium), medium calcium (20-40-mg/L of calcium), and high calcium (>40-mg/L of calcium). To investigate the trophic status of the study lakes, a scatterplot was produced in R to show the proportion of oligotrophic, mesotrophic, and eutrophic conditions in each calcium group. Trophic state was inferred using total phosphorous data (Carlson, 1977).

A PCA biplot was constructed with center-standardized water quality and land-use data using R packages FactoMineR, factoextra, and ggplot2 (Kassambara et al., 2017; Lê et al., 2008; Wickham, 2016). Lakes were sorted into low, medium, and high calcium

groups as previously described and ellipses showing clusters of each group are shown. A permutational analysis of variance (permanova) was used to determine whether there was a significant difference between lakes based on calcium categorical concentration by comparing the centroids and dispersion of each ellipse.

2.3. Results

Average water quality variables and standard deviations for each of the 60 study lakes are shown in Table 2.1. Sampled lakes varied widely in depth (0.85m to 19m) and in pH (6.57 to 9.51), though most lakes were slightly alkaline as the average pH was 8.3 ± 0.67 . There was a general trend of increasing nutrients (TP and TN) with increasing calcium, but several exceptions to this were found (e.g., Nine Mile Lake which had 1.1-mg/L of Calcium and 17- μ g/L of TP).

Table 2.1 Average water quality data of whole water column at each site (each lake was sampled once, within-lake replicates depended on sample depth and ranged from 1-10). Lakes are ordered based on calcium concentrations. Temp = Temperature, DO = dissolved oxygen, Con = Conductivity, Secchi = Secchi depth, TOC = total organic carbon, TN = total nitrogen, TP = total phosphorus, Ca = calcium, Fe = iron, K = potassium, Mg= magnesium, Mn = manganese, Na = sodium. Ca, Fe, K, Mg, Mn, Na do not have corresponding standard deviations as one sample per lake, from the deepest spot, was sent away for analysis. Balsam, Bass, Camden, Canal, Head, Long, Lower Buckhorn, Opinicon, Rice, Scugog, and Simcoe do not have standard deviations for Temp, DO, pH, and Con because they had a sample size of 1.

Calcium Group	Lake Name	Temp (°C)	DO (mg · L ⁻¹)	pH	Con (µS cm ⁻¹)	Depth (m)	Secchi (m)	TOC (mg · L ⁻¹)	TN (µg · L ⁻¹)	TP (µg · L ⁻¹)	Ca (mg · L ⁻¹)	Fe (mg · L ⁻¹)	K (mg · L ⁻¹)	Mg (mg · L ⁻¹)	Mn (mg · L ⁻¹)	Na (mg · L ⁻¹)
Low	Harp	16.1(7.1)	9.2(1.4)	7.4(0.6)	5206.4(496.4)	10.94	3	4.795	118.1	6.833333	0.43	0.025	0.118	0.128	0.00343	0.39
	Nine mile	20.4(6.4)	9.0(1.9)	7.3(0.4)	2616.8(167.7)	7.5	2.7	7.864	184.7	17.01852	1.1	0.058	0.533	0.289	0.0078	1.12
	Devil	21.4(1.7)	8.2(1.7)	7.8(0.6)	3532.5(1222.4)	6.4	3	5.58	67.777	10.35185	1.52	0.113	0.221	0.455	0.00698	0.53
	Long	23	7.8	7.1	459.1	6.7	2.5	7.562	340.6	11.64815	1.72	0.109	0.312	0.525	0.00689	0.68
	Shoe	22.1(1.9)	9.4(1.9)	7.4(0.3)	4813.5(237.8)	6	6	4.965	118.695	13.68519	2.23	0.013	0.469	0.664	0.00376	1.15
	Shadow	22.3(0.3)	8.0(0.7)	8.1(0.1)	25817.8(609.1)	9.8	5.5	3.976	142.4	38.68519	2.98	0.022	0.182	0.326	0.00291	0.93
	Vernon	21.9(0.8)	8.3(2.4)	7.6(0.1)	8835.3(81.6)	14.8	4	5.261	74.963	4.981481	3.36	0.161	0.632	0.999	0.00636	3.99
	Gull	18.8(6.4)	9.2(0.7)	8.2(0.1)	20781.3(7983)	8.5	6.5	3.474	118.5	2.018519	3.6	0.011	0.353	0.805	0.0014	2.09
	Wood	23.5(0.2)	9.7(0.1)	7.7(0)	73.1(0.5)	3.2	1	6.015	266.608	7.018519	3.88	0.007	0.148	1.69	0.00125	0.58
	Maple	23.6(2.3)	10.1(1.0)	8.3(0.1)	12249.4(157.1)	7.6	2.5	4.407	58.805	17.75926	4.37	0.059	0.802	0.939	0.00737	1.76
	Lake of Bays	15.3(8.3)	11.0(0.6)	8(0.5)	7132.7(533.5)	16	4	4.335	275.2	10.16667	4.44	0.054	2.39	1.25	0.00526	5.99
	Stewart	22.8(0.6)	21.5(35.3)	8.3(0.1)	9636.1(6427.5)	5.88	3	4.263	312.5	5.722222	4.84	0.026	0.374	0.38	0.00379	5.52
	Beaver	23(0)	8.4(0.2)	6.6(4.2)	245.9(0.1)	5.2	2.5	6.676	249.81	18.31481	7.02	0.019	0.264	1.21	0.0206	1.77
	Faraday	22.3(0.6)	9.2(0.2)	8.1(0.1)	15777(103.8)	7.25	3	3.692	58.692	6.462963	7.13	0.067	0.855	1.16	0.00346	2.38
	Skeleton	22.5(0.7)	32.0(46.7)	8.2(0.1)	14530.6(3905)	7.46	3	2.421	163	7.388889	7.26	0.029	1.08	1.1	0.00315	8
	Catchacoma	24.1(0)	8.9(0.1)	8.4(0.1)	85.9(0.1)	3.5	1	7.5727	194.7	4.055556	7.42	0.043	0.475	0.891	0.00243	1.83
	Kashagawigamog	21.0(5.0)	17.1(35.0)	7.9(0.4)	131.7(18.6)	19	8	2.991	69.631	39.24074	7.68	0.0403	0.842	0.895	0.00675	5.28
	Head	23.5	9	8.2	2629	2.16	2.16	4.706	711.1	27	9.11	0.037	0.732	2.21	0.005	3.16
	West Twin	24.3(0.2)	9.2(0.2)	8(0)	147.3(0.1)	4.23	4.23	5.873	340.4	4.796296	9.11	0.06	0.768	0.894	0.00405	11.6
	Eels	21.2(1.5)	8.5(0.7)	8(0.1)	16606.3(873.1)	8.6	4.5	4.753	461.5	8.5	9.49	0.022	0.532	0.895	0.00489	1.36
	Balsam	24.8	8.9	7.4	276.5	5.9	2.5	25.39	143.202	9.055556	10.9	0.021	0.521	1.15	0.00653	3.34
	Upper Stoney	16.7(8.1)	8.2(2.7)	7.3(0.5)	382.4(132.5)	2.5	2.5	5.681	117.072	25.72222	11.8	0.012	0.329	0.436	0.0109	1.09
	Kashabog	23.6(0.0)	8.0(1.6)	8(0)	36529(226.3)	1.95	1.95	6.603	151.7	6.462963	14	0.066	2.26	2.89	0.0112	7.37
	Wilbermere	19.3(2.4)	8.7(0.2)	7.5(0.1)	32992.7(6880)	18	5	4.4	52.93	2.018519	14.9	0.037	0.905	2.12	0.00207	4.28
	Thirteen Island	25.0(0.0)	9.6(0.0)	7.3(0.0)	444.2(0.7)	6.6	2.5	4.796	243.8	8.12963	15.1	0.018	1.57	1.87	0.00449	2.36
	Opinicon	27.5	13	8.7	2998	0.85	0.85	9.405	113.91	19.98148	15.9	0.012	0.543	4.08	0.00335	3.3
	Camden	23.1	9.5	9.5	403.7	1.1	1.1	40.14	1759	17	16.6	0.021	0.672	4.91	0.0118	2.78
	Stoco	22.9(0.2)	8.4(0.7)	8.8(0.1)	366.3(1.4)	3.77	1.5	7.604	329.8	13.5	18.4	0.037	0.384	2.27	0.052	2.27
Medium	Jack	16.9(7.0)	5.2(4.6)	8.2(0.4)	173(33.8)	12.5	3	14	291.7	10.90741	20	0.029	1.72	1.63	0.00618	4.82
	Coon	19.9(5.7)	6.3(3.6)	8.3(0.4)	181.3(19.8)	8.2	1.5	6.078	221.2	9.796296	20.8	0.011	0.548	2.73	0.0056	1.66
	Big Cedar	23.1(0.1)	6.5(0.4)	8.8(0)	85440.0(3234)	4.9	3	4.687	345.5	20.33333	21.3	0.074	0.672	2.78	0.014	7.78
	Desert	20.4(7.6)	11.5(2.3)	7.7(0.5)	332.8(52.9)	11.73	4.1	4.531	74.67	6.648148	22	0.009	0.991	6.97	0.00163	4.73
	Loughborough	25.7(0.1)	9.5(0.2)	8.1(0)	606.8(20.5)	6.4	2.5	6.15	357.5	14.7963	22	0.032	1.48	4.6	0.00338	6.43
	Crowe	21.6(3.6)	5.4(4.2)	8.9(0.3)	4880.1(2258.8)	8.13	3	7.864	183.042	11.27778	23.2	0.03	0.389	1.84	0.00875	3.19
	Buck	25.7(0.1)	9.9(0.2)	8.4(0)	375.8(0.1)	5.27	4.5	5.048	175.2	0.351852	23.3	0.011	1.29	6.93	0.00258	6.29
	Crystal	23.3(0.2)	7.3(0.1)	8.8(0)	81763(844.3)	12.6	5	5.454	286.9	10.35185	23.3	0.007	0.778	2.05	0.00095	1.66
	Lower Beverly	26.4(0.1)	7.5(5.0)	8.5(0)	4785(11.6)	8.23	2.75	9.073	155.7	34	23.3	0.05	0.831	5.09	0.0188	4.36
	Cordova	22.3(2.4)	6.9(3.1)	8.8(0.3)	4946(299.6)	9.42	4.3	7.315	338.656	11.64815	23.4	0.021	0.865	2.45	0.00688	4.67
	Dickey	22.3(1.1)	8.6(0.6)	8.7(0.2)	46535.4(2053)	14.87	4	6.011	73.608	2.759259	23.5	0.051	1.06	1.52	0.00408	2.97
	Belmont	21.5(3)	7.8(2.9)	8.5(0.4)	255.6(247.7)	9.7	3	7.764	200	5.351852	24.1	0.008	0.995	2.82	0.00526	4.87

Calcium Group	Lake Name	Temp (°C)	DO (mg · L ⁻¹)	pH	Con (µS cm ⁻¹)	Depth (m)	Secchi (m)	TOC (mg · L ⁻¹)	TN (µg · L ⁻¹)	TP (µg · L ⁻¹)	Ca (mg · L ⁻¹)	Fe (mg · L ⁻¹)	K (mg · L ⁻¹)	Mg (mg · L ⁻¹)	Mn (mg · L ⁻¹)	Na (mg · L ⁻¹)
Medium	Round	23.9(0.5)	9.5(0.5)	8.8(0.1)	356(0.8)	4.5	2.5	5.281	247.1	10.72222	28.2	0.036	0.562	2.33	0.0121	4.49
	Lower Buckhorn	23.4	10.4	9	331.5	2	2	11.77	978.4	7.388889	28.6	0.013	0.711	3.99	0.00791	8.81
	Charleston	27.4(0.3)	308.7(594.9)	7.9(0)	29.9(0.2)	9	1.5	9.26	139.564	10.35185	28.8	0.008	1.26	11.4	0.00913	5.1
	Bass	23.1	10.2	9	467.8	2	2	3.378	80.663	35.66667	29.1	0.008	1.26	11.5	0.00914	5.12
	Chemong	24.1(0.6)	131.7(12.1)	9.2(0)	25528.3(166.2)	5.5	1.5	12.37	269.2	42.38889	29.1	0.049	2.28	2.85	0.029	9.04
	Otter	25.7(0.2)	10.1(0.4)	8.8(0.8)	4802.3(4.3)	10.7	4.25	7.562	152.288	8.12963	29.6	0.007	1.5	14.7	0.00257	9.48
	Canal	22.9	8.9	9.5	70250	2.5	2.5	7.449	504.1	266.463	32.3	0.061	1.07	4.86	0.0354	13.3
	Pigeon	24.5(0.1)	9.3(0.1)	7.6(0)	402.6(150.8)	6.4	3	5.684	92.159	21.09259	32.8	0.018	1.09	3.94	0.00842	8.28
	Rice	24.5	10.2	9.1	446.2	1.7	1.7	6.171	358.9	17.33333	34.4	0.046	0.605	2.23	0.0312	4.69
	Hambly	22.1(5.4)	5.7(4.2)	7.2(0.6)	729.7(33.1)	7.92	4.5	7.258	413.2	32.38889	36.2	0.007	1.83	11.4	0.004	12.4
	Scugog	20.9	12	9.4	589	1.5	1	7.928	462	41.46296	37.1	0.014	2.96	9.27	0.0297	23.2
	Gananoque	26.1(0.8)	8.8(1.4)	7.9(0.2)	4615(39)	9.07	2.5	14.96	408.8	20.35185	37.6	0.015	2.51	12.1	0.0185	7.77
	Wagner	20.7(2.5)	7.3(6.8)	8.7(0.3)	966.5(12.6)	6.3	1.25	11.36	111.229	38.68519	38.6	0.008	2.01	10.5	0.00322	34.3
High	Couchiching	23.1(0.1)	6.2(0.0)	9.2(0)	152769(1402)	2.8	2.8	5.825	338.7	28.68519	40.7	0.009	3.8	12.4	0.00311	46.4
	Moira	23.1(0.1)	8.5(0.2)	8.8(0)	479.7(0.4)	5.88	3	10.8	79.181	15.35185	42.2	0.022	1.3	6.15	0.029	7.31
	Simcoe	23.6	8.2	9.3	1507	1	1	4.45	224.6	16.83333	44.8	0.007	3.3	10.5	0.00206	40.7
	Eugenia	22.8(0.1)	9.8(0.1)	8.9(0)	661.5(1.9)	4.84	4	10.44	497.2	9.981481	47.8	0.028	1.04	33	0.0025	46.4
	Consecon	27(0.1)	9.4(0.1)	7.9(0)	752(1.7)	5.97	2	7.687	86.751	23.31481	48.6	0.016	0.982	2.36	0.00457	3.9
	Dalrymple	22.9(0.0)	7.8(2.0)	8.7(0)	472.5(0.6)	7.9	1.5	6.868	104.726	53.87037	57.9	0.043	2.86	7.21	0.0479	9.39
	Belwood	22.8(0)	8.6	9	742.5	13	1.5	5.284	1153	18.31481	83.7	0.033	3.4	24.8	0.00473	13.8

Lake trophic status is displayed in Figure 2.1. Low-calcium lakes had the greatest proportion of oligotrophic lakes (17 out of 28) and the lowest proportion of eutrophic lakes (3 out of 28). High-calcium lakes had the lowest proportion of oligotrophic lakes (1 out of 8) and the highest proportion of eutrophic lakes (2 out of 8).

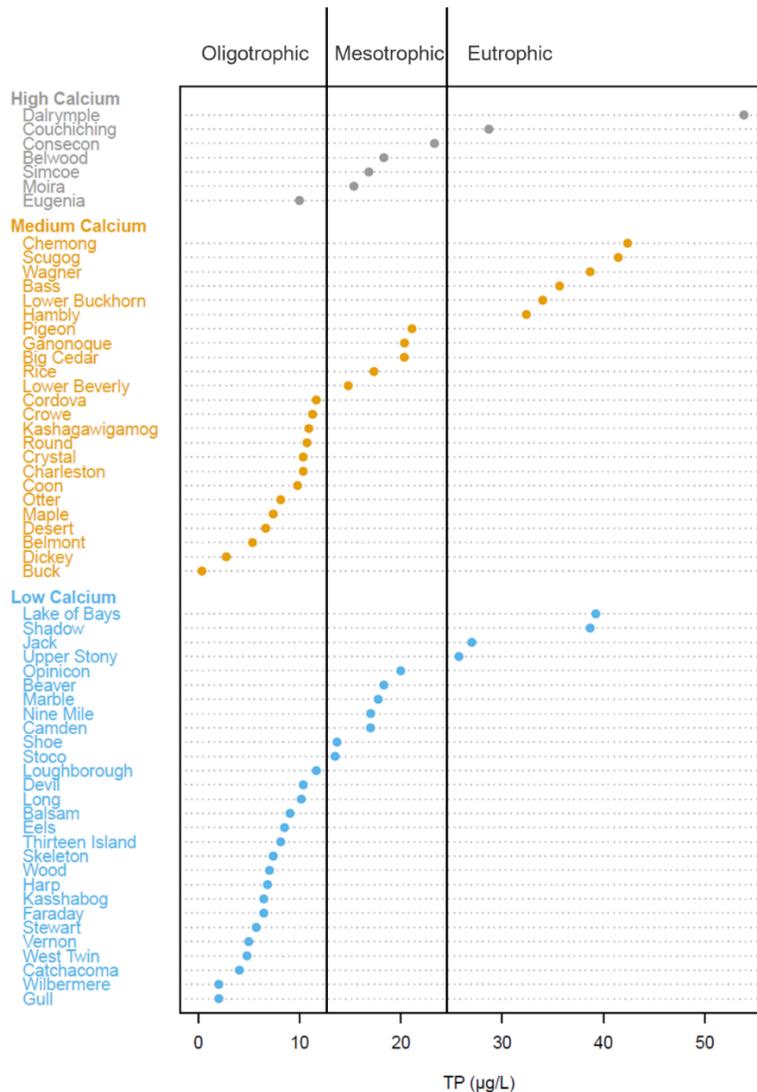


Figure 2.1 Scatter plot depicting trophic status of lakes based on TP concentrations. Lakes are grouped based on calcium levels with low calcium (0-20 mg/L), medium calcium (20-40 mg/L), and high calcium (>40 mg/L). Lakes below 12 $\mu\text{g/L}$ of TP are classified as oligotrophic, lakes between 12 and 24 $\mu\text{g/L}$ of TP are mesotrophic, and lakes above 24 $\mu\text{g/L}$ of TP are eutrophic.

Figure 2.2 shows how land-use varies among each of the 60 study lakes. Natural land-cover dominates land-use in the watersheds of most of the study lakes, but several lakes had high agricultural land-cover as well. There is very little developed land in these watersheds with the exception of Lake Couchiching, whose watershed is 37% developed. There is a trend of decreasing land-use moving north in TLB, as can be seen in Figure 1.1. Watershed size varied among the lakes, with the smallest being 109.9 km² (Lake Couchiching) and the largest being 329.7km² (Lake Simcoe).

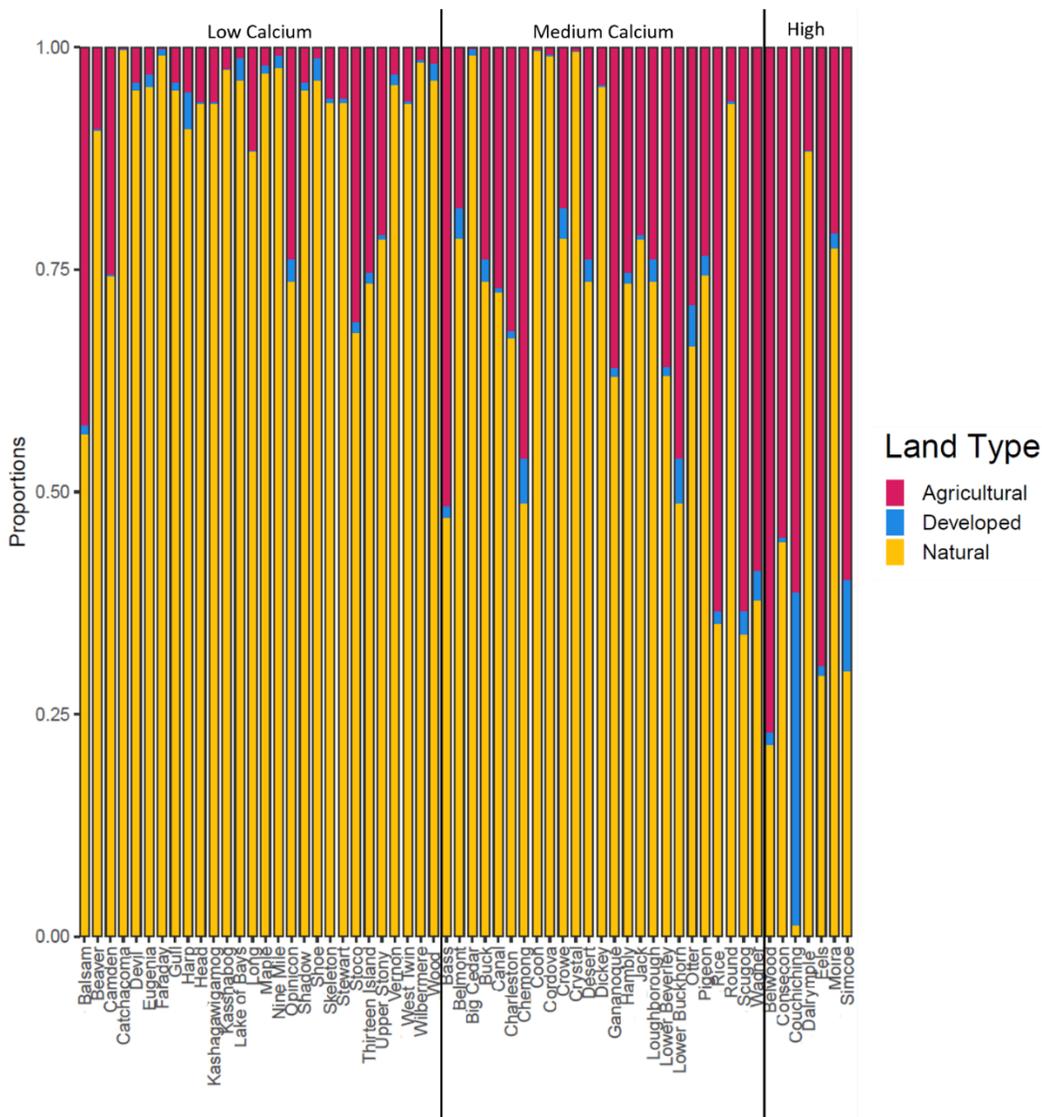


Figure 2.2 Bar plot showing proportions of agricultural, developed, and natural land-cover in the watersheds of the study lakes. Lakes are grouped based on their calcium levels with low-calcium lakes being < 20 mg/L of calcium, medium-calcium lakes being 20-40 mg/L of calcium, high-calcium lakes being > 40 mg/L of calcium). Land-use area was calculated in QGIS using data from Natural Resources Canada (2015).

A principal component analysis (PCA) biplot was constructed using water quality and land-use parameters to compare the study lakes (Figure 2.3). This PCA explains 42.9% of the variance observed between lakes. Lakes somewhat cluster based on calcium concentration, and PERMANOVA results revealed significant differences between the

water quality variables based on calcium categorical classifications ($p < 0.05$).

Agricultural and developed land are positively associated with each other and are negatively associated with Secchi depth. Natural land-cover is negatively associated with nutrients (TP and TN), TOC, and pH. Calcium is somewhat positively associated with pH, and negatively associated with Secchi depth.

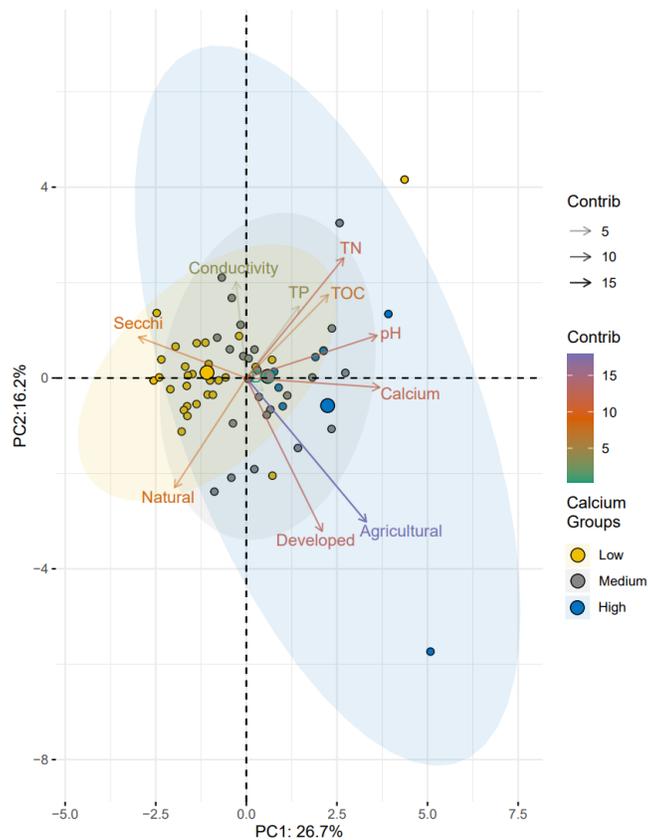


Figure 2.3 Principal components analysis biplot depicting water quality and land-use variables as vectors and lakes as points. Lakes are grouped based on their calcium levels, with low-calcium lakes (< 20 mg/L of calcium) being yellow, medium-calcium lakes (20-40 mg/L of calcium) being gray and high-calcium lakes (> 40 mg/L of calcium) being blue. Ellipses help to visualize the lake clustering of each calcium group. Relative contribution of each environmental parameter are visualized along a color gradient and vector transparency.

2.4 Discussion

The summary results presented in Table 2.1 show the high degree of variability among the 60 study lakes, as they vary widely in depth, pH, nutrient levels, and productivity. Even though these lakes are located within one geographical region in south-central Ontario, the wide variation in water quality profiles is not that surprising given several factors such as differences in morphometry (including depth and size), notable shifts in bedrock geology across TLB, as well as the land-use type and amount. As can be seen in Figure 2.2, there is a general pattern of increasing land-use in lake watersheds where there is also increasing calcium. High-calcium lakes, located in the south of TLB, tend to have higher land-use in their watersheds. This is due to several factors such as the arable soil that supports agriculture in southern Ontario, and proximity to expanding urban regions like the Greater Toronto Area. This is in contrast to the low-calcium lakes in the northern part of TLB, where the granite dominated landscape and shallow soils do not support agricultural activities.

Notable differences between trophic states of low, medium, and high-calcium lakes are presented in Figure 2.1, where low-calcium lakes are mostly oligotrophic and high-calcium lakes are mostly mesotrophic/eutrophic. This pattern is likely related to the land-use gradient presented in Figure 2.2, as land-use typically results in increased nutrient input into lakes (Howarth et al., 2002), and loss of natural land-cover can lead to reduced protection of lakes from nutrient deposition (Norris, 1993). These relationships can be better explored using the results of the PCA biplot in Figure 2.3.

The environmental parameters in the PCA biplot had some notable relationships. Nutrients (total nitrogen, and total phosphorous) co-varied with total organic carbon,

which was expected because more nutrients typically result in higher levels of primary production in the water column. Nutrients were also expected to be strongly associated with developed and agricultural land-use due to these typically resulting in nutrient pollution into lakes (i.e., fertilizer runoff, wastewater) (Sharpley & Smith, 1994), but this was not the case. However, nutrients were negatively associated with natural land-cover, indicating that natural cover is not a major source of nutrients, but also may help to mitigate nutrient runoff into TLB lakes. Forests and wetlands around lakes typically have low labile sources of nutrients and are effective at nutrient retention. If there is a high degree of natural landcover surrounding the shoreline of lakes, this can slow the movement of nutrients into lakes by slowing runoff from the watershed (Norris, 1993). The lack of co-variation between nutrients and land-use categories may be an artifact of not capturing a large enough gradient in agriculture and developed land-use.

Calcium and pH vectors were somewhat associated, which is likely due to calcium carbonate, a strong buffering agent from limestone bedrock, influencing lake pH. Calcium was also negatively associated with Secchi depth, which may be the result of the increased proportion of oligotrophic conditions seen in low-calcium lakes compared to the mesotrophic and eutrophic conditions observed in high-calcium lakes (see Figure 2.2). In effect, the lower nutrients in low calcium, oligotrophic lakes supported lower phytoplankton growth, thus increasing water clarity and allowing Secchi depth to be deeper (Carlson, 1977).

The study lakes generally cluster according to their calcium-level category, with low-calcium lakes clustering opposite the calcium vector on the left of the plot, medium-calcium lakes somewhat clustering in the center of the plot, and high-calcium lakes

somewhat clustering along the calcium vector on the right of the plot. However, there is a lot of overlap between the lakes, as well as some outliers, indicating that the variation observed between lakes is being driven by other local abiotic factors as well.

Low-calcium lakes appear to be positively associated with natural land-cover, while being negatively associated with TP, TN, TOC, as well as pH. So, the low-calcium lakes appear to be generally low-nutrient, circumneutral pH systems with high water clarity when compared to the other lakes. Their negative association with nutrients and positive association to natural land-cover is likely related as previously mentioned, with natural land-cover potentially mitigating the deposition of nutrients into these lakes. As shown in Figure 2.2, low-calcium lakes have the most natural land-cover out of all three categories.

High-calcium lakes appear to be positively associated with pH and both developed and agricultural land-cover, while being negatively associated with Secchi depth and natural land-cover. The high pH of many of these lakes is likely the result of high mineral contents from their limestone bedrock acting as a buffer to neutralize dissolved carbon dioxide and any acid deposition (e.g., NO_x and SO_x) (Schindler et al., 1980). Their negative association with natural land-cover is likely due to their watershed's land-use profiles reflecting increased agriculture and urban development as previously discussed. Additionally, 6 out of 7 of the high-calcium lakes were mesotrophic or eutrophic, which typically have a lower Secchi depth than oligotrophic lakes due to increased production

Medium-calcium lakes are highly variable in their position on the PCA biplot. The variability among these lakes is likely owed to their spatial location in TLB's

geological mosaic, as well as watershed suitability for agriculture and proximity to urban regions. Many medium-calcium lakes (i.e., moderately hardwater) appear to coincide with the developed and agricultural land-use vectors, with some being positively associated with them and some being negatively associated with them. As shown in Figure 2.2, medium-calcium lakes vary in their land-use distributions with some having higher amounts of non-natural land than others, and this appears to account for some of the variation observed between them in the PCA. Some medium-calcium lakes are also positively associated with conductivity, some with pH, and some are negatively associated with Secchi depth, with all of these variations highlighting how different these ecosystems can be along the geological mosaic in TLB. Overall, the PCA biplot demonstrates that while calcium is an important driver of the water quality profiles of these lakes, other local factors such as land-use and land-cover in the watersheds play a role as well, particularly for nutrients.

Chapter 3: Exploring Drivers of Phytoplankton Community Structure across The Land Between Ecotone

3.1 Introduction

Phytoplankton are a very diverse community of microalgal organisms representing thousands of species varying in size, morphology, and physiology. The structure of lake phytoplankton communities is heavily influenced by environmental conditions (e.g., nutrient levels, light penetration, temperature, pH, etc.), thus lakes with differing physico-chemical profiles tend to have contrasting phytoplankton communities. As such, phytoplankton community structure and composition can be an informative indicator of lake health. The seven major groups of phytoplankton relevant to freshwater ecosystems are cyanobacteria, chlorophytes, chrysophytes, diatoms, euglenophytes, dinoflagellates, and cryptophytes, and a brief overview of the ecological distributions of each of these groups is provided below.

Cyanobacteria are very common in freshwater systems of all kinds, and some forms can produce nuisance blooms in eutrophic conditions, sometimes producing toxins which harm aquatic and surrounding wildlife (Paerl, 1988; Zohary & Robarts, 1990). The small cell-size of non-blooming cyanobacteria makes them efficient in oligotrophic systems where nutrients are limited (Litchman et al., 2007). Some taxa are also capable of nitrogen fixation and can therefore be dominant in nitrogen-limited systems (Winkenbach & Wolk, 1973). Chlorophytes are also common in all freshwater systems, but they vary in their distributions: small unicellular chlorophytes have an advantage in oligotrophic systems due to their efficient nutrient uptake while larger taxa are more common in shallow eutrophic systems (Wehr et al., 2015). Chrysophytes are most commonly found

in oligotrophic or mesotrophic soft waters, as they are often outcompeted in high nutrient conditions (Cottingham et al., 1998; Wiedner & Nixdorf, 1998).

Diatoms are a cosmopolitan group of algae where species can vary by orders of magnitude in cell size. Diatoms are also known to be able to grow in a wide range of environmental conditions, with individual species having advantages in specific conditions over others (Wehr et al., 2015). They have been noted to prefer mesotrophic to eutrophic conditions (Willén, 1991), but can be found in oligotrophic systems as well. Euglenophytes are common in nutrient and organic rich environments and are typically rare in oligotrophic or mesotrophic conditions. Some euglenophytes are able to tolerate acidic conditions that restrict most other phytoplankton (Wehr et al., 2015).

Dinoflagellates are uncommon compared to other algal groups in freshwater ecosystems, and different dinoflagellate taxa are adapted to a wide range of environmental conditions such as soft or hard water (Wehr et al., 2015). Finally, cryptophytes are found in all kinds of freshwater conditions but are most common in deep oligotrophic and mixotrophic lakes as they are adapted to low-light conditions (Henderson & Mackinlay, 1989). However, cryptophytes are often preferentially targeted by predators, making them less common in the presence of grazing predators (Knisely & Geller, 1986).

Phytoplankton communities are very responsive to changes in environmental conditions because their small size and unicellular architecture ensures that they are directly exposed to external temperature, pH, light, and dissolved nutrients in their immediate habitat space. Different phytoplankton taxa have different optimal conditions for growth, making them more or less competitive in a given environment. The relatively quick growth rates of phytoplankton, compared to larger organisms, means that a

community can respond to big changes in environmental conditions by shifting in species composition over a matter of days to weeks. For this reason, phytoplankton community assemblage varies in different ecosystems, and thus monitoring of community composition and structure can be a useful tool in aquatic ecosystem health assessment.

Across TLB, there is a shift from limestone-dominated bedrock in the south to granite-dominated bedrock in the north. This shifting geology drives changes in surface water chemistry, such as decreases in major cations like calcium, pH, alkalinity, and water hardness. The shift from limestone-dominated bedrock to granite-dominated bedrock affects lake pH due to limestone's contribution of sedimentary carbonates. Lakes influenced by limestone bedrock will have higher concentrations of carbonate salts, such as calcium and magnesium carbonates, than those on granite-dominated landscapes. Increasing concentrations of carbonates not only causes an increase in pH, but also alkalinity. Increasing alkalinity helps to stabilize a lake's pH by adding buffering capacity. Although there is surprisingly little research on the effect of alkalinity or water hardness on phytoplankton, there are studies that focused on the effects of pH. Several studies have shown that phytoplankton species richness increases with increasing pH (Agrawal, 2012; Chen & Durbin, 1994). The pH of a lake can also indirectly affect phytoplankton by influencing ion speciation. Forms of dissolved inorganic carbon shift in response to changing pH. As pH increases, dissolved inorganic carbon shifts to the bicarbonate and carbonate forms, whereas decreasing pH causes a shift to more carbon dioxide. All phytoplankton species can utilize carbon dioxide for photosynthetic growth, but only a select group of taxa can utilize bicarbonate or carbonate. For example, species that can only use carbon dioxide for growth are incapable of survival when high pH

reduces carbon dioxide availability in the water column (Moss, 1973). So, as pH changes across TLB, phytoplankton communities may shift to reflect taxa that are better suited for a lake's dominant carbon source.

In addition to influencing water hardness in lakes, calcium is an essential nutrient for phytoplankton. However, the minimum concentrations required for growth are low, ranging from 0.01 to 0.95 mg/L (Gerloff & Fishbeck, 1969). Considering that the calcium concentrations in TLB lakes are all well above this minimum threshold, it is assumed that calcium levels would not be a growth-limiting factor for phytoplankton. Even though calcium concentrations are not low enough to impede phytoplankton growth, it remains an important limiting growth factor for crustacean zooplankton, which are important phytoplankton grazers. Therefore, calcium concentration may have indirect effects on phytoplankton communities by way of its role in supporting the growth of zooplankton grazers.

Cladoceran zooplankton, which have high calcium requirements, are very effective grazers and have been found to limit phytoplankton biomass when present in a lake (Søndergaard et al., 1990). Additionally, they preferentially feed on small-to-moderately sized unicellular phytoplankton, and often will reject larger filamentous and colonial algae, particularly colonial cyanobacteria, as food (Brett & Müller-Navarra, 1997; Webster & Peters, 1978). Thus, this selective feeding strategy can affect phytoplankton community composition and diversity when large cladocerans are abundant. So, where calcium is low, there may be less grazing pressure on phytoplankton, but as calcium increases, there may be a decrease in phytoplankton abundance and diversity due to grazing pressure.

Coinciding with the shift in underlying bedrock geology, land-use changes across TLB from predominantly agricultural in the south, to predominantly natural land-cover in the north. This is largely because the granitic and shallow soils in the northern part of TLB are not conducive to growing crops. As such, it is not surprising that lakes here are oligotrophic (i.e., low nutrient) due to decreased land-use activities that would normally be an important source of nutrients.

The overall gradient in water chemistry driven by both underlying geology and land-use activities may have variable effects on phytoplankton communities. Lakes located in the south of TLB are situated in predominantly agricultural watersheds, which can be the source of high nutrient input into lakes from fertilizer application and manure runoff. Increased nutrient loadings from land-use activities can causing a shift toward eutrophic conditions, with increased phytoplankton biomass and potentially decreased biodiversity (Proulx et al., 1996). Higher nutrient conditions can also promote algal blooms, which in turn can reduce a lake's diversity and negatively impact other lake organisms (Schindler, 1977; Xu et al., 2010). Additionally, developed (i.e., urban) land-use can cause increases in salinity from road salt runoff, which can affect phytoplankton communities in several ways. When input is high, phytoplankton communities may experience shifts towards salt-tolerant species (Hintz et al., 2017), and there is evidence to suggest that increased cations in the water column from road salt can stimulate phytoplankton growth (Fay & Shi, 2012). Additionally, because zooplankton are sensitive to salinity, road salt can actually relieve phytoplankton from grazing pressure, resulting in increased biomass and changes in diversity (Hintz et al., 2017). The lakes located further north in TLB are mostly surrounded by natural land-cover, and are

therefore expected to have lower nutrient and chloride levels, which likely results in lower algal biomass overall compared to lakes located in the south of TLB.

Another important driver of freshwater phytoplankton community structure is regional connectivity. While TLB spans a broad geological space, and represents multiple ecological gradients, there is also lots of connectivity between its lakes, rivers, streams, and groundwater. Lakes are also connected by passive vectors such as birds or boats which can transport phytoplankton cells from one lake to another. In this way, TLB can be considered a metacommunity, or an assembly of local communities connected through means of dispersal of species (Wilson, 1992).

Because of the high level of variation in water quality parameters across TLB's environmental gradients and geological mosaic, the phytoplankton communities may be very different from one lake to the next. The goal of this chapter in my thesis research was to investigate the multivariate drivers of phytoplankton community biomass and diversity across the heterogeneous landscape of TLB. In addition to water quality variables, I determined land-use composition in all subwatersheds of 60 lakes to assess their role in affecting phytoplankton community structure. Therefore, my research objectives for this chapter were to:

1. Characterize phytoplankton community composition and diversity in the 60 study lakes across TLB.
2. Assess spatial and land-use drivers of phytoplankton community structure across TLB; and
3. Investigate the roles of abiotic (water quality) and biotic (zooplankton grazing) drivers of phytoplankton community structure across TLB.

I expected that calcium, land-use (i.e., source of nutrients), and pH would emerge as important drivers of phytoplankton biomass and diversity due to their known effects on phytoplankton from previous studies. In southern lakes in TLB, which are high in calcium and surrounded by predominantly agricultural land, I expected that there would be larger-bodied phytoplankton due to increased nutrients, but also due to increased grazing pressure by calcium-loving cladoceran zooplankton that selectively feed on smaller, edible taxa. In lakes in the north of TLB, which are low calcium and surrounded by mostly natural land-cover, I expected less grazing pressure due to potential calcium limitation of large-bodied zooplankton grazers, but also expected that limited nutrients in these oligotrophic lakes would support lower phytoplankton biomass and cell size. The lakes located throughout the transitional zone of TLB will likely have highly variable communities dependent on local environmental conditions influenced by the geological mosaic.

3.2 Methods

3.2.1 Data Collection

Water quality and land-use data were collected according to the methods described in Chapter 2.

To capture phytoplankton samples, 100-mL of the composite water sample collected at each site was decanted into a glass Qorpak™ bottle and preserved in Lugol's solution until analysis. Phytoplankton samples were settled using a 100-mL graduated cylinder to concentrate the sample to 5-mL. A subsample from that 5-mL was transferred to a 0.98-mL nanoplankton chamber and placed under a phase contrast microscope where phytoplankton taxa were identified to genus level along one transect (i.e., 40 fields of

view) or until a minimum of 300 individuals were counted and identified. Phytoplankton are identified using keys by Baker (2012) and Wehr et al. (2015).

3.2.2 Data Analysis

Statistical analyses were performed using R version 4.0.4 (R Core Team, 2021) and SigmaPlot version 14.0. In order to visualize community composition across study lakes, relative abundance plots using genus biomass data were constructed in R using packages ggplot2 and cowplot (Wickham, 2016; Wilke, 2020). A bar plot depicting phytoplankton biomass was created using R package ggplot2 ((Wickham, 2016).

Simpson's diversity index (1-D) was calculated for each site in order to account for not only the number of taxa found at each site, but also their relative abundances (Simpson, 1949).

A detrended correspondence analysis was run to determine whether the relationship between phytoplankton communities and the water quality and land-use parameters was linear or unimodal. This step evaluated if the data was more suited to a canonical correspondence analysis (CCA) or a redundancy analysis (RDA). Because the resulting longest axis-gradient length was smaller than 3, an RDA was used (Šmilauer & Lepš, 2014). A Hellinger transformation was applied to phytoplankton biomass data in order to give low weights to rare taxa in the RDAs, and RDAs were constructed using R packages ggord, stringr, dplyr, tidyr, vegan, and ggplot2 (Beck, 2016; Oksanen et al., 2019; Wickham, 2010, 2016; Wickham et al., 2019; Wickham & Henry, 2018). The genus-level RDA was made using the 30 most common taxa across all 60 study lakes based on number of sites at which they were present. A permutational analysis of variance (permanova) was used to determine whether there was a significant difference

between the phytoplankton communities of low, medium, and high-calcium lakes by comparing the centroids and dispersion of each ellipse.

Spatial scale is an important component in species distribution models as well as establishment. As the study area covered a large region of central Ontario, implicit spatial structure within my data was investigated. To determine whether there was spatial autocorrelation within an environmental parameter, Moran's positive and negative coefficients were calculated, and those that were identified to autocorrelate spatially were removed from subsequent analyses. Moran's eigenvector mapping was conducted in the R packages *ade4*, *adespatial*, *spdep*, *adegraphics*, and *maptools* (Bivand et al., 2016; Bivand & Wong, 2018; Dray et al., 2019; Dray & Dufour, 2007; Siberchicot et al., 2017). The spatial model was built with irregular sampling excluding distance-based methods to account for unbalanced study design, and neighbourhood criteria was based on nearest neighbours due to the clustering of the study lakes. Moran's eigenvector mapping was conducted to investigate whether there was an implicit spatial structure present which influenced the species distributions of phytoplankton communities across central Ontario lakes. Due to the underlying study design, which had a strong emphasis on exploiting the natural gradient of cations present due to parent geology, no spatially implicit structures were identified to influence species distributions ($p > 0.05$) and therefore I was able to exclude spatial parameters from further analysis.

To explore relationships between zooplankton genera and environmental parameters including water quality and land-use, Kendall correlation analysis was conducted using R packages *ggplot2*, *corrplot*, and *Hmisc* (Harrell & Dupont, 2016; Wei & Simko, 2017; Wickham, 2016). Kendall correlation was used due to the non-normal

distribution of the environmental data, and Kendall's coefficient produces narrower confidence intervals than Spearman's coefficient (Puth et al., 2015).

Multiple linear regression was used to determine if certain environmental parameters could account for the variation in phytoplankton biomass and diversity. Simpson's Diversity (1-X) was calculated using R package *vegan* (Oksanen et al., 2019). Multiple linear regressions were run between phytoplankton data and environmental parameters including zooplankton biomass (μg) using R package *vegan* (Oksanen et al., 2019). All environmental and biomass data was $\log(X+1)$ transformed. Variance inflation factors (VIF) were calculated and used to assess whether any of the variables included in the models exhibited collinearity, but because none of the values had a VIF value above 4, none were omitted (James et al., 2013).

3.3 Results

3.3.1 Characterizing phytoplankton communities across TLB

Relative abundance of phytoplankton genera is presented in Figure 3.1. Community composition was highly variable across all 60 lakes, and no distinct patterns emerged within low (<20-mg/L Ca), medium (20-40-mg/L Ca), or high calcium (>40-mg/L Ca) lakes. The diatom *Fragilaria* had the highest relative abundance of all taxa, composing 17.6% of the total biomass across all 60 study lakes. Cyanobacteria appeared in most of the study lakes but were rarely dominant. The only lakes where cyanobacteria were the dominant taxa were Skeleton, Loughborough, Crowe, West Twin, and Wilbermere where cyanobacteria made up 50% of total site biomass (West Twin and Wilbermere are included because cyanobacteria made up over 49% of total site biomass). However, in all of these lakes, there was a mix of cyanobacterial taxa making up this

biomass, and no lake was dominated by any one cyanobacterial taxon. Otherwise, most study lakes each had unique phytoplankton community profiles. Heterocysts, which are cyanobacterial cells capable of nitrogen fixation, were present at 9 out of 60 lakes.

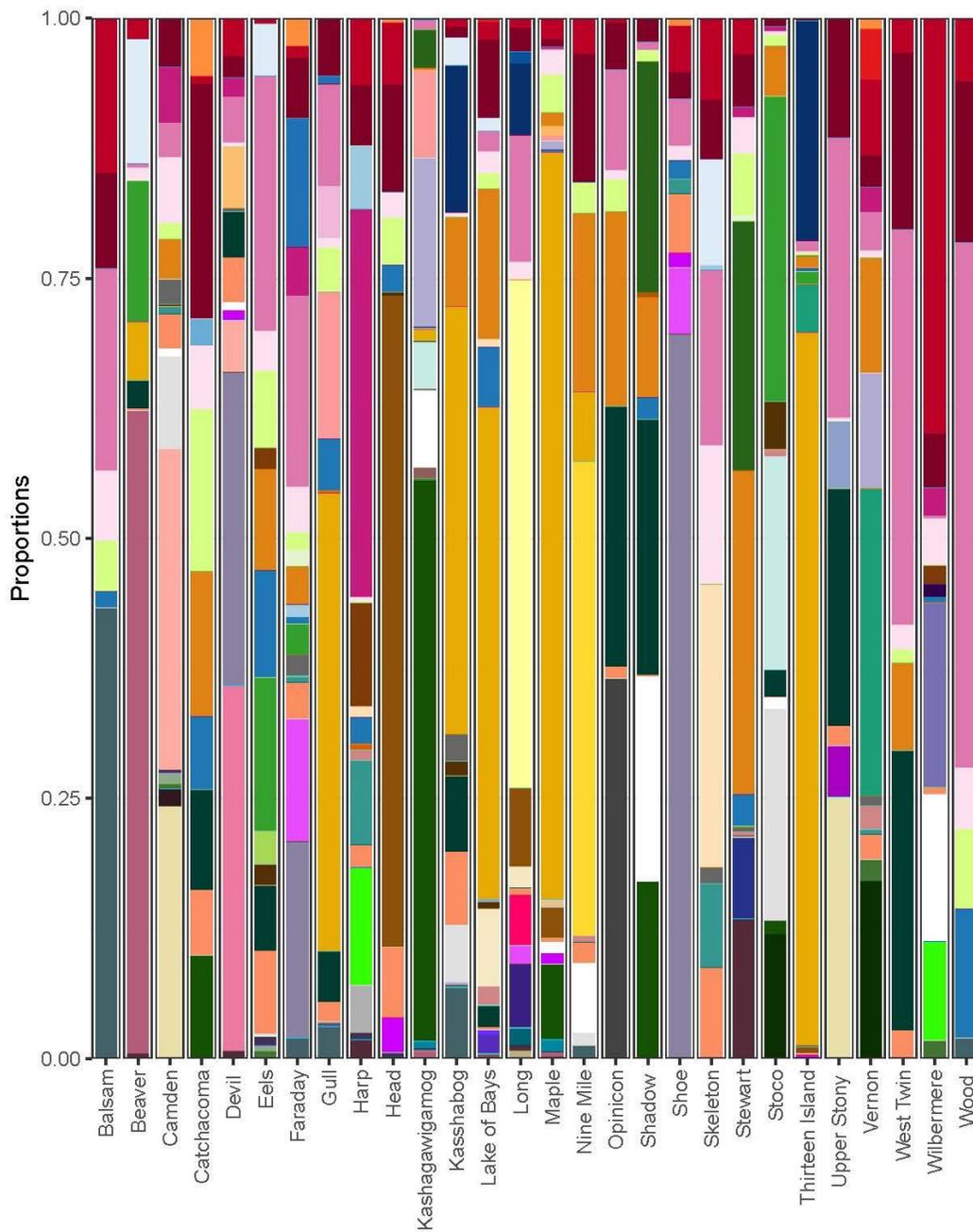


Figure 3.1a Relative abundance plot for phytoplankton at the genus level in low-calcium lakes (<20mg/L) based on biomass.

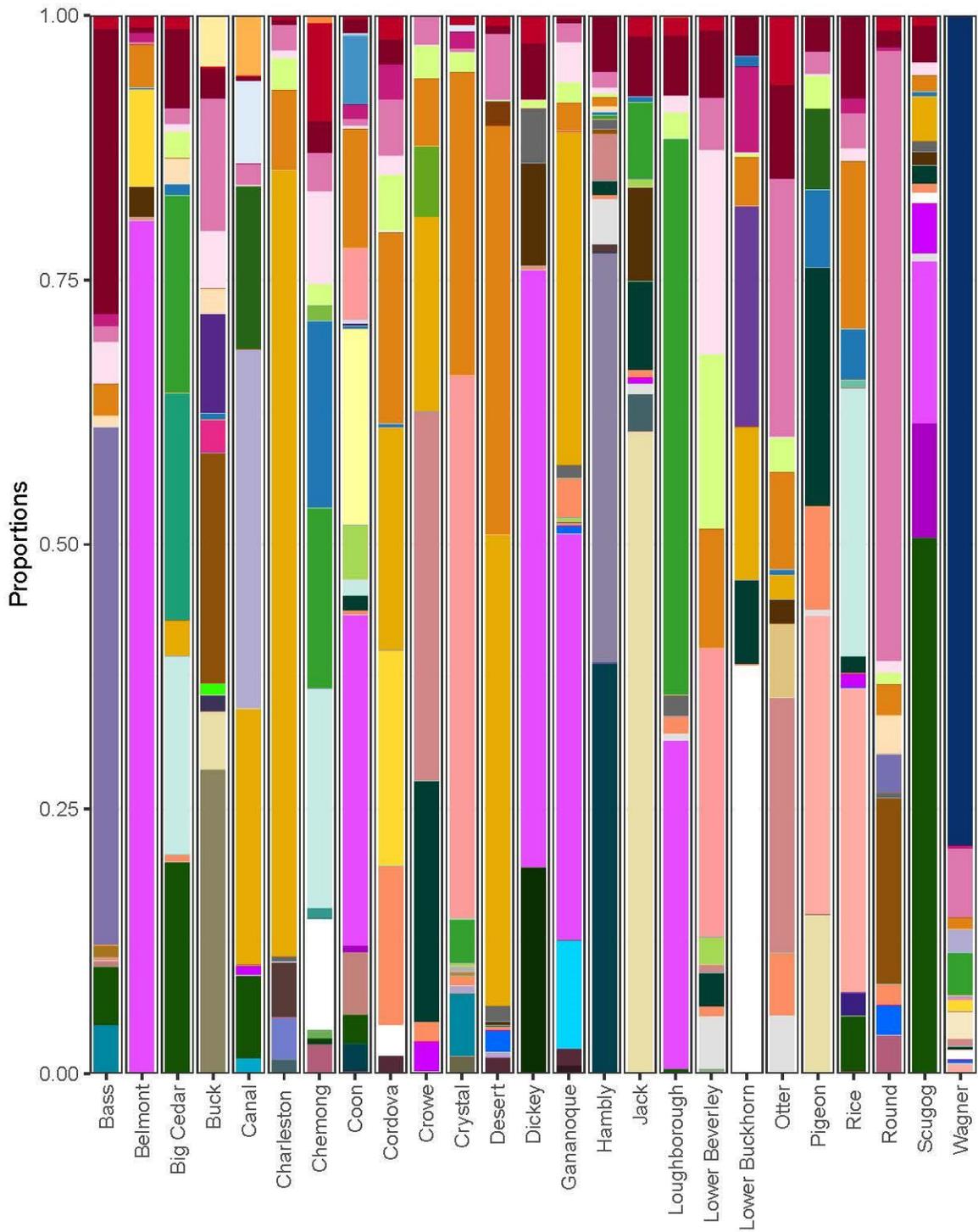


Figure 3.1b Relative abundance plot for phytoplankton at the genus level in medium-calcium lakes (20 – 40 mg/L) based on biomass.

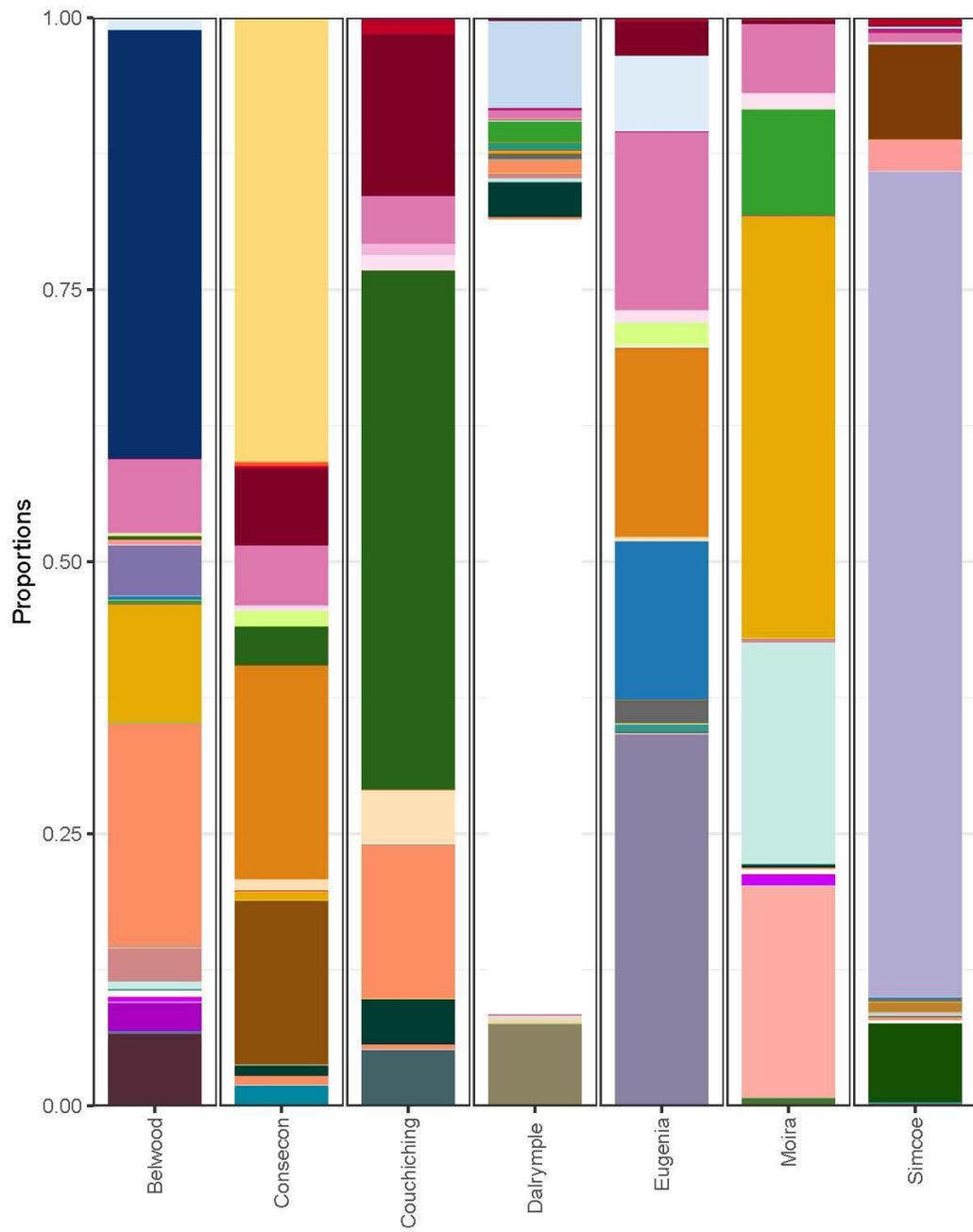


Figure 3.1c Relative abundance plot for phytoplankton at the genus level in high-calcium lakes (> 40 mg/L) based on biomass.



Figure 3.1d. Legend for relative abundance plots.

3.3.2 Investigating drivers of phytoplankton diversity and abundance across TLB

Moran's eigenvector mapping determined that phytoplankton communities across the 60 study lakes were not found to be driven by underlying spatial structure (i.e., lake proximity to each other), so local water quality conditions and watershed land-use were examined as drivers of phytoplankton community structure across TLB. Phytoplankton biomass varied from site to site in each calcium category (Figure 3.2), with Thirteen Island and Otter Lakes having the highest recorded biomasses in all 60 lakes.

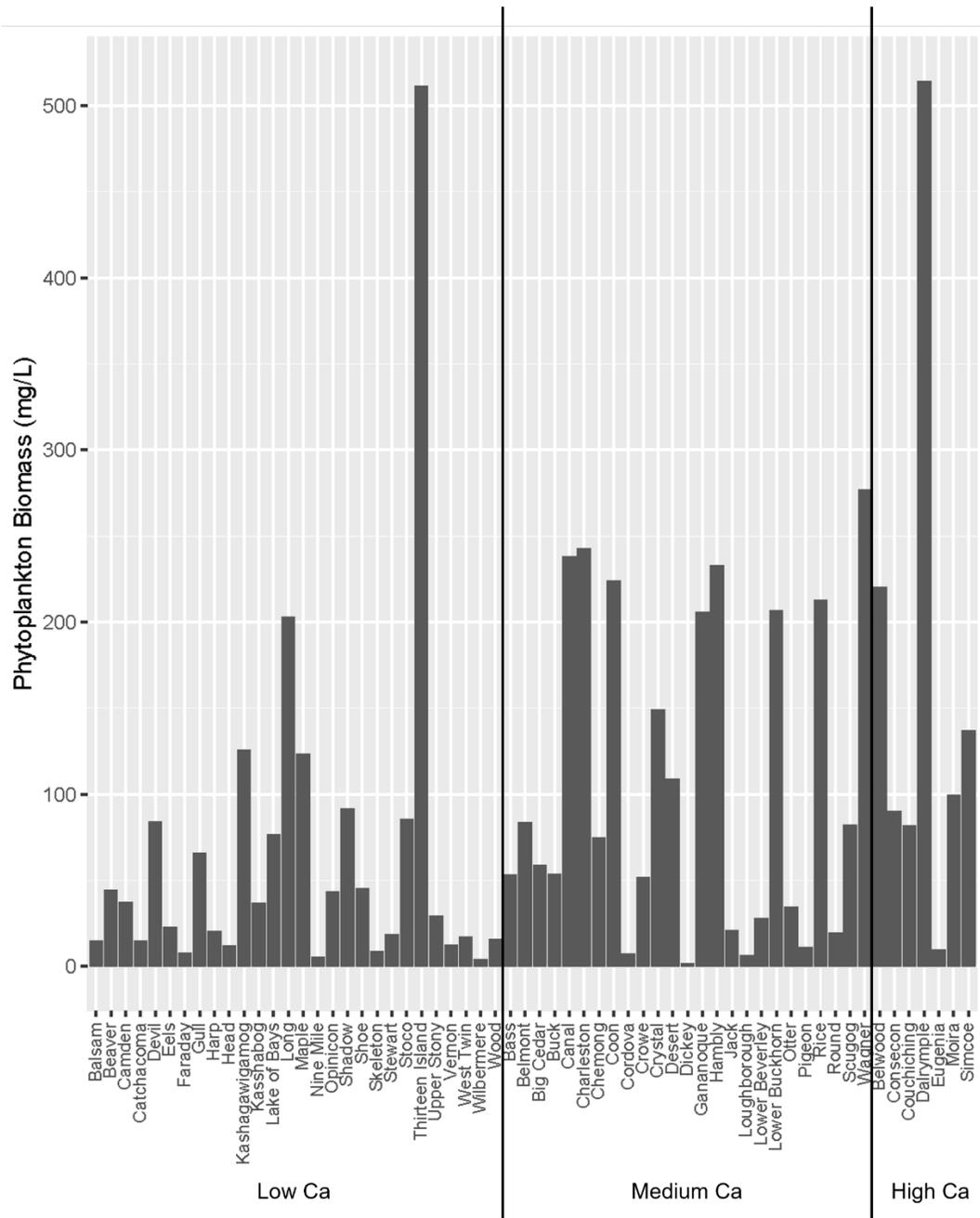


Figure 3.2 Bar plot depicting phytoplankton biomass at each of the 60 study lakes.

The redundancy analysis between phytoplankton biomass at the group level and environmental parameters including water quality and land-use is shown in Figure 3.3, and the first two axes explain 74.02% of the variance observed in the data. Permanova results revealed that phytoplankton communities were not significantly different among low, medium, and high-calcium lakes ($p > 0.05$). Based on vector length, TP, calcium, and conductivity appear to be the biggest drivers of variance in the data. The phytoplankton groups vary in their placement in the plot, with most groups being fairly distinct from one another with the exception of chrysophytes and cyanobacteria, which are somewhat close to each other in the biplot. Chrysophytes and cyanobacteria are positively associated with natural land-cover and Secchi depth and are negatively associated with agricultural land-use and calcium. Chlorophytes are somewhat positively associated with natural land-cover and Secchi depth, and negatively associated with pH. Diatoms are positively associated with TP and developed land-cover, and negatively associated with water temperature. Dinoflagellates are positively associated with TN. Euglenophytes are positively associated with TN and negatively associated with conductivity. Cryptophytes are positively associated with temperature and are negatively associated with TP, agricultural land, and conductivity.

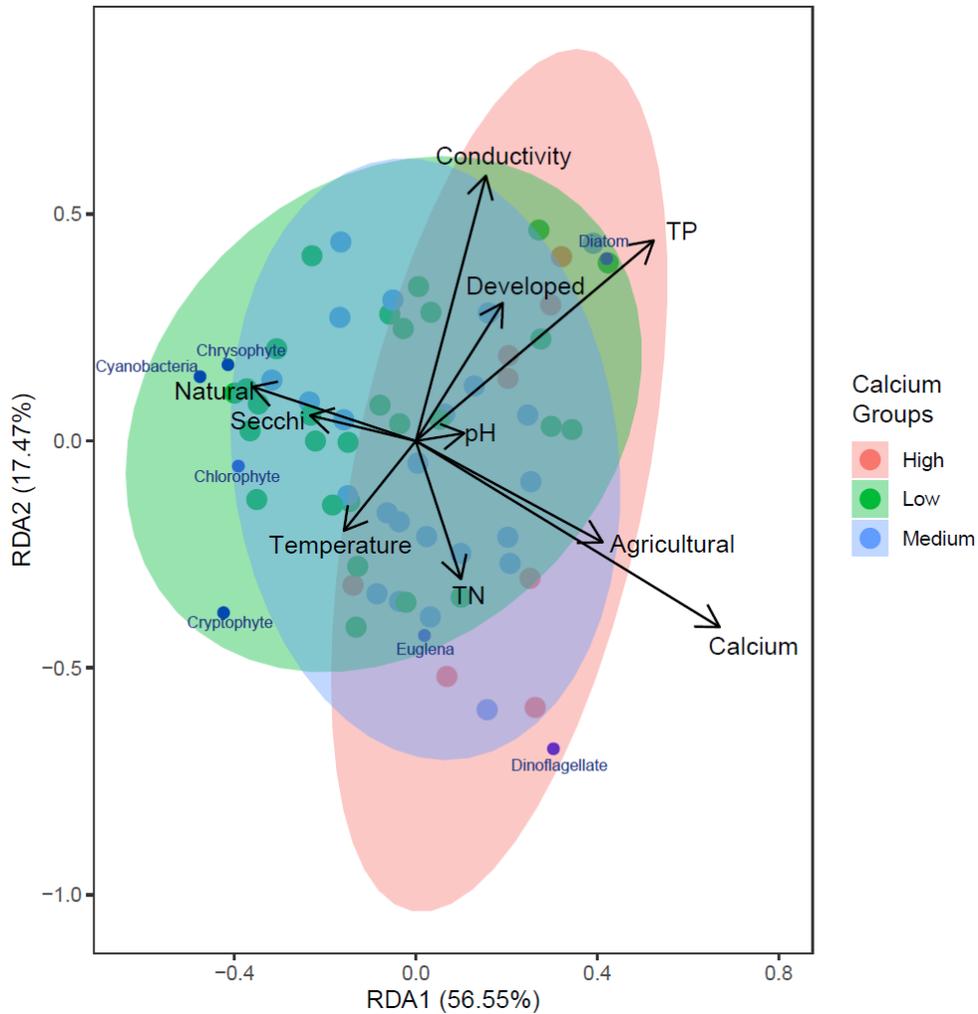
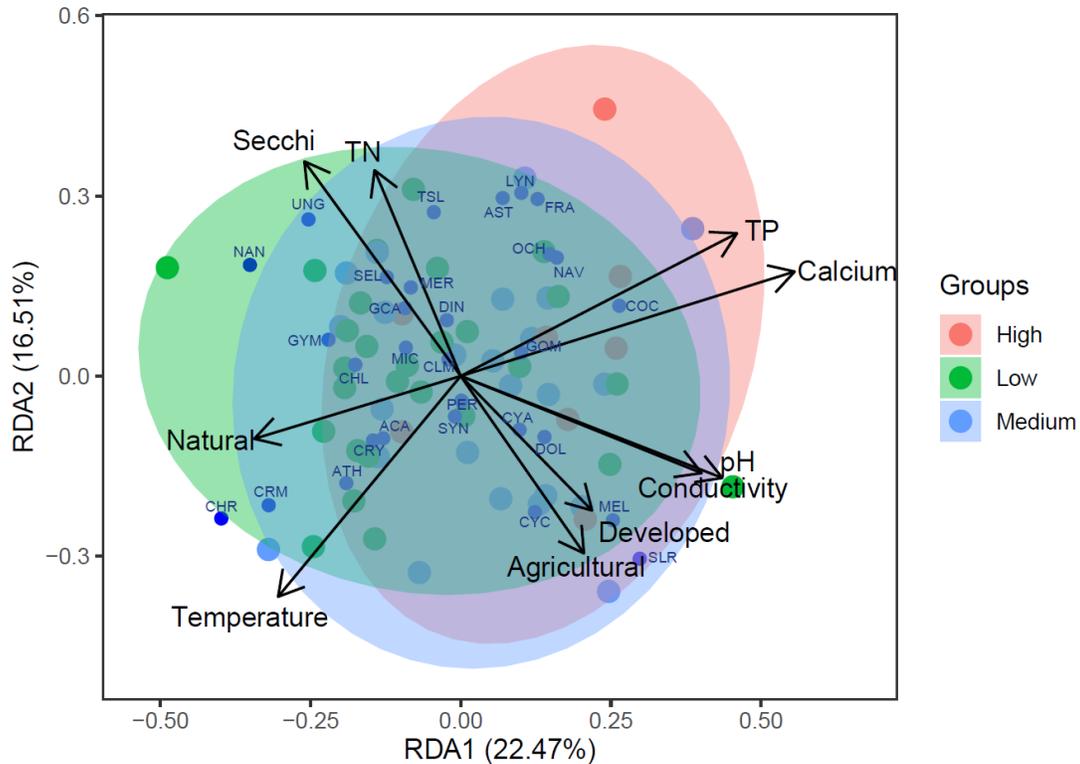


Figure 3.3 Redundancy analysis of phytoplankton groups with water quality and land-use overlaid with ellipses grouping low, medium, and high-calcium lakes. Phytoplankton biomass data was transformed using Hellinger transformation to give low weights to rare species.

Another redundancy analysis between the 30 most common phytoplankton at the genus level and environmental parameters is shown in Figure 3.4, and the first two axes explain 38.98% of the variation observed in the data. Permanova results determined that the phytoplankton communities of lakes classified by calcium category were not significantly different ($p > 0.05$). Interestingly, genera from the same algal group differed in their associated drivers, but many still conformed to the observed patterns in the group-

level RDA. All environmental parameters used in Figure 3.4 appear to drive the variance in the data, with calcium, TP, and temperature having the greatest influence based on vector length. A Kendall correlation matrix was produced which presents correlations between phytoplankton genera and environmental parameters (Figure A1).



ACA = Aphanocapsa, AST = Asterionella, ATH = Aphanothece, CHL = Chlorella, CHR = Chroococcus, CLM = Chlamydomonas, COC = Cocconeis, CRM = Chroomonas, CRY = Cryptomonas, CYA = Cyanodictyon, CYC = Cyclotella, DIN = Dinobryon, DOL = Dolichospermum, FRA = Fragillaria, GCA = Gleocapsa, GOM = Gomphosphaeria, GYM = Gymnodinium, LYN = Lyngbya, MEL = Melosira, MER = Merismopedia, MIC = Microcystis, NAN = Nanoflagellate, NAV = Navicula, OCH = Ochromonas, PER = Peridinium, SEL = Selenastrum, SLR = Sellaphora, SYN = Synedra, TSL = Tetraselmis, UNG = Unicell Green

Figure 3.4 Redundancy analysis of top 30 most common phytoplankton genera with water quality and land-use overlaid with ellipses grouping low, medium, and high-calcium lakes. Phytoplankton biomass data was transformed using Hellinger transformation to give low weights to rare species.

The MLR results displayed in Table 3.1 show predictor variables for phytoplankton Simpson diversity index (1-D). This model was found to explain 13.5% of the variation observed in diversity across TLB ($p < 0.05$). TN was found to be a significant positive driver of phytoplankton diversity, while temperature was found to be a marginally significant negative driver of phytoplankton diversity ($p = 0.071$).

Table 3.1 Summary Statistics for multiple linear regression of environmental parameters predicting phytoplankton diversity (Simpson Diversity Index 1-D) in TLB.

	Estimate	Standard Error	t value	p-value (t-test)	Model Adjusted R ²	Model p-value
Zooplankton Biomass	0.041	0.037	1.104	0.275	0.135	0.031
TN	0.775	0.283	2.740	0.008		
TP	-0.091	0.058	-1.582	0.120		
Temperature	-0.788	0.428	-1.840	0.071		
pH	-0.672	0.806	-0.834	0.408		
Calcium	0.071	0.064	1.102	0.275		

The MLR results in Table 3.2 show predictor variables of phytoplankton biomass. This model explains 22.4% of the variation observed in phytoplankton biomass across TLB ($p < 0.05$). TP and calcium were found to be significant positive drivers of phytoplankton biomass, while zooplankton biomass was found to be a significant negative driver of phytoplankton biomass. Water temperature was a marginally significant negative predictor for zooplankton biomass.

Table 3.2 Summary Statistics for multiple linear regression of environmental parameters predicting phytoplankton biomass in TLB.

	Estimate	Standard Error	t value	p-value (t-test)	Model Adjusted R ²	Model p-value
Zooplankton Biomass	-0.2346	0.1147	-	0.0457	0.224	0.003
TN	-0.4697	0.8645	-	0.5892		
TP	0.4209	0.1823	2.309	0.0249		
Temperature	-2.516	1.3524	-1.86	0.0684		
Secchi	-0.6152	0.4321	-	0.1603		
Calcium	0.4427	0.179	2.472	0.0167		

3.4 Discussion

Environmental drivers of phytoplankton community diversity and composition across The Land Between

Phytoplankton communities across TLB did not appear to be spatially structured. This is an important finding because it indicates that habitat proximity (i.e., distance between lakes) did not play a role in phytoplankton community structure, including composition, biomass, and diversity. This is not surprising as phytoplankton are able to be passively dispersed over wide geographical areas through a number of vectors (e.g., rivers/streams, wind, animals, humans) (Padisák et al., 2016; Shurin et al., 2009), and so they would not be expected to be limited by spatial structure in a geographical region such as TLB. Instead, phytoplankton communities in TLB appeared to be mainly structured by differences in local environmental conditions such as water quality, temperature, and water clarity as can be seen in Figures 3.3 and 3.4. Calcium, nutrient levels (TP and TN), land-use, and temperature appeared to be the biggest influences on phytoplankton community composition and diversity based on these results.

In the multiple linear regression (MLR) in Table 3.1., calcium did not have a strong direct effect on overall phytoplankton diversity, having only a slightly positive effect that was not statistically significant ($p > 0.05$). This was somewhat expected, because despite calcium's status as an essential nutrient for phytoplankton, the concentrations of calcium required for most phytoplankton to survive and reproduce is low (0.01 – 0.95 ml/L) (Gerloff & Fishbeck, 1969). The only lake below this requirement was Harp Lake (0.43 mg/L of calcium), meaning 59 out of 60 lakes would not have had limiting levels of calcium. Prior studies have shown that calcium can directly impact zooplankton communities (Ashforth & Yan, 2008), and thus potentially indirectly affect phytoplankton community composition, but no strong calcium effects on phytoplankton diversity were detected.

Despite the MLR showing that calcium has little direct effect as an explanatory variable of phytoplankton diversity, it still appears as a sizeable vector in both the group-level and genus-level RDAs. This does not contradict the MLR findings, but instead reveals the notable covariation of calcium with land-use and underlying geology. It makes sense that calcium would have a negative association with chrysophytes and cyanobacteria (Figure 3.3) since these algal groups were most prevalent in softwater, oligotrophic lakes in my study. Thus, overall diversity may not be influenced by calcium, but calcium's association with water hardness seems to have an effect on certain algal groups and genera as can be seen by the negative association between calcium with cyanobacteria and chrysophytes.

TP emerged as a weak negative explanatory variable for phytoplankton diversity in the MLR ($p > 0.05$). This is somewhat surprising, as diversity typically exhibits a

unimodal response to phosphorous, where it increases until nutrient levels reach eutrophic levels, then decreases (Dodson et al., 2000). Because the study lakes were mainly oligotrophic or mesotrophic, it was expected that phosphorous would have a positive effect on phytoplankton diversity, but this was not observed. There are several possible explanations for the lack of a relationship, the first of which being that confounding factors or latent variables not measured are influencing the effect of TP on diversity. For example, the effect of selective zooplankton grazing was not measured, thus it is possible for diversity to be reduced by selective grazing, while TP remains unchanged. As seen in the RDA biplots (Figures 3.3 and 3.4), TP negatively co-varies with natural land-cover and positively co-varies with agricultural land. Although it is expected that agricultural land-use, a source of TP, would be positively related, there may be confounding factors also caused by agricultural land-use that could negatively impact phytoplankton diversity (e.g., suspended solids, herbicides, etc.). Further investigation would be required to determine the cause of this novel relationship.

TN was found to be the only significant positive predictor of phytoplankton diversity across TLB in the MLR, albeit the effect size is small. While both phosphorous and nitrogen are important macronutrients for phytoplankton, phosphorous is typically the limiting nutrient in aquatic ecosystems (Schindler, 1977), whereas nitrogen is available in abundance and would therefore not be expected to limit most phytoplankton taxa. However, there are cases where nitrogen can be limiting when phosphorous is present in excess (Elser et al., 2007). Nitrogen levels vary across TLB (see Table 2.1), and in 15 study lakes the TN:TP ratio is below 16:1 which can be indicative of nitrogen limitation (Redfield, 1958). These conditions can result in nitrogen becoming an

important driver of phytoplankton diversity and community assemblage (Lafrancois et al., 2003). In cases such as these, species with high nitrogen requirements or inefficient nutrient uptake mechanisms will not survive, whereas taxa with low nitrogen requirements or efficient nutrient uptake systems may thrive, altering community assemblage. For example, as seen in Figure 3.3, euglenophytes and dinoflagellates are strongly positively associated with TN, indicating that these phytoplankton groups may be limited to lakes with higher nitrogen levels. Additionally, when nitrogen levels are low, phytoplankton communities may end up dominated by cyanobacterial taxa that are capable of nitrogen-fixation and thus resulting in low diversity (Smith, 1983). However, this was not observed in the study lakes, as no heterocysts were present in any of the lakes with a TN:TP ratio below 16:1.

However, it is important to note that total nitrogen values do not account for different types of nitrogen which may differ in bioavailability, and so further analysis would be required to discern the true nature of this relationship. Despite this, the apparent nitrogen-limitation of phytoplankton community diversity detected in TLB lakes is critical for lake managers because lake management strategies tend to prioritize minimizing phosphorus inputs into lakes when trying to control algal production, rather than minimizing nitrogen inputs. Instead, as nitrogen appears to be moderately important for phytoplankton diversity in TLB, it would be vital for nitrogen to be managed concurrently with phosphorous inputs.

Temperature was found to be a negative explanatory variable of phytoplankton diversity in the MLR (marginally significant, $p = 0.071$), and was a long vector in the RDAs. All lakes were sampled within three weeks in August, but temperature still varied

widely across the study lakes. Such a broad temperature range suggests that factors other than regional air temperature influence lake temperature, and these can include groundwater influence, dissolved organic carbon (i.e., albedo), and lake morphometry and volume. Many freshwater phytoplankton reach their maximum growth rates at temperatures nearing 20 °C and then slow as temperatures rise above that (Butterwick et al., 2005), but the average temperature across all study lakes was 22.7 °C, and even reached as high as 27.5 °C in Lake Opinicon (see Table 2.1). High temperature conditions such as these can reduce growth rates of temperature-sensitive taxa and thus provide competitive advantages to organisms capable of growing and reproducing at such temperatures.

For example, cyanobacteria are known to grow well at temperatures above 25 °C (Lurling et al., 2013), which gives them a competitive edge in hot summer conditions, particularly if nutrient levels are high enough. While cyanobacterial blooms were not detected in the study lakes, the genus-level RDA reveals a positive relationship between temperature and *Cryptomonas* (a cryptophyte genus), *Aphanocapsa* (a cyanobacterial genus), and *Aphanothece* (a cyanobacterial genus), which are all taxa capable of growing well in warm water conditions (Lurling et al., 2013; Morgan & Kalff, 2008; Yamamoto et al., 2016). While seasonal variation in temperature is common, and lakes were sampled in August when lake temperatures would be peaking, this negative relationship between water temperature and phytoplankton diversity has big implications as climate change continues to increase temperatures globally. With water temperatures rising in response to climate change, phytoplankton communities may become less diverse, which could have severe ramifications for aquatic food webs.

In the MLR, pH emerged as a negative predictor variable for phytoplankton diversity, though this effect was not significant ($p > 0.05$). Despite this, several taxa exhibited either positive or negative associations with pH in the genus-level RDA (Figure 3.4). It is thus possible that pH influences community structure, as seen in the RDA, but does not affect phytoplankton Simpson diversity index which accounts for species richness and evenness, as seen in the MLR.

Zooplankton biomass was used as a proxy for grazing effects, and it did not emerge as a significant predictor variable for phytoplankton diversity, eliciting only a weakly positive response ($p > 0.05$). While zooplankton have been found to alter phytoplankton community structure through selective grazing or avoidance of certain taxa, these relationships can be complex and dependent on the specific community present within an ecosystem. For example, large-bodied zooplankton can feed on larger organisms than small-bodied zooplankton, resulting in different grazing outcomes throughout the season (Levine et al., 1999). It is likely the result of such confounding factors that overall zooplankton biomass did not emerge as a significant predictor of overall phytoplankton biomass.

Environmental drivers of phytoplankton community biomass in TLB

In the MLR for phytoplankton biomass, TP emerged as a statistically significant explanatory variable of phytoplankton biomass ($p < 0.05$). This was expected, as phosphorous is typically the limiting nutrient in freshwater ecosystems (Schindler, 1977), and the positive association detected with phytoplankton biomass indicates that phosphorus is a limiting nutrient in TLB lakes. The effect of TN was negative, though this relationship was not significant ($p > 0.05$), suggesting that TN is not a limiting

nutrient of phytoplankton in TLB and may have a complicated relationship with total phytoplankton biomass. Calcium had a strong, significant positive effect on phytoplankton biomass ($p < 0.05$). One possibility for the positive effect of calcium on phytoplankton biomass was the shift from small-bodied cells to large-bodied cells that occurred in response to the generally increasing nutrients along the calcium gradient.

Similar to its effects on phytoplankton diversity, temperature emerged as a marginally significant negative predictor of phytoplankton biomass ($p = 0.068$). It is likely that this is again the result of temperature-sensitive taxa being limited by the high temperatures experienced during the sample period, as many taxa exhibit reduced growth rates in response to high water temperature (Butterwick et al., 2005). Also, small-celled taxa such as cyanobacteria are generally more tolerant of increased temperature, thus their relative abundance would increase, even though total biomass decreased. This again highlights the potential ramifications of climate change on TLB's lakes, as other members of the aquatic food web rely on phytoplankton for food and oxygen, so a decrease in their biomass could be detrimental to these ecosystems.

While these results demonstrate that phytoplankton biomass is regulated by bottom-up control (i.e., nutrient levels, water temperature, etc.), there is also evidence that they are affected by top-down controls: zooplankton biomass was found to be a significant negative predictor for phytoplankton biomass in TLB ($p < 0.05$). This relationship is not surprising as zooplankton are important grazers of phytoplankton and can have significant roles in limiting algal production (e.g., Hansson, 1992), and grazing effects can become significant in low-nutrient conditions like those observed in many of the lakes in TLB (Carrillo et al., 1995).

Chapter 4: Exploring Drivers of Zooplankton Community Structure across The Land Between Ecotone

4.1 Introduction

Zooplankton communities are made up of three major families of zooplankton: cladocerans, copepods, and rotifers. These groups vary from one another in size, feeding strategies, and predator-prey dynamics, providing them with advantages and disadvantages when faced with varying environmental conditions. Cladocerans and copepods are microcrustaceans typically ranging in size from 0.3 – 3mm (cladocerans) and 0.5 – 5mm (copepods). Both groups are ideal foods for vertebrate predators such as fish, particularly early in their life-cycle (Allan, 1976). Vertebrate predators favour cladocerans over copepods because they move more slowly and may be more nutritious (Baumann & Kitchell, 1974; C.O'Keefe et al., 1998; Strickler & Bal, 1973a), with large species being selectively consumed over small species (Brooks & Dodson, 1965).

Rotifers are the smallest of the three zooplankton groups, ranging in size from 0.2 – 0.6mm, making them a common food for larger invertebrates such as predacious zooplankton (Williamson, 1983). They are suspension feeders that feed on organic matter (e.g., seston or detritus) up to 20 μ m in size (Allan, 1976). Cladocerans are filter feeders that feed on materials up to 50 μ m (Allan, 1976), and their feeding strategy is much more efficient than that of rotifers, making them more competitive in environments where food is limiting or vertebrate predation is low (Brooks & Dodson, 1965; Hurlbert et al., 1972). Copepods include both filter feeders and predators, consuming food up to 100 μ m but have more specialized feeding strategies making them compete less with the other groups when food is limited (Allan, 1976; Strickler & Bal, 1973a). These competitive

interactions between zooplankton families can play a role in determining community composition, in addition to other environmental factors.

As environmental conditions change across natural gradients, so do zooplankton communities. In addition to the interactions mentioned above, individual species have specific requirements related to the physical and chemical characteristics of their habitat. The changing landscape across TLB, from limestone-dominated bedrock to granite-dominated bedrock, results in various environmental gradients including a calcium gradient, a land-use gradient, and a subtle pH gradient resulting from the changing amounts of calcium carbonate in a lake. These gradients offer an important opportunity to evaluate zooplankton community response to changing water quality variables across TLB.

Calcium is an important elemental nutrient for organisms and is necessary in higher quantities for some aquatic organisms than others. Crustaceans like cladocerans and copepods require calcium because it is a major component of their carapaces, and large-bodied cladocerans like *Daphnia sp.* in particular have been noted to require a constant supply of calcium because they lose a majority of their body's calcium content when they molt (Porcella et al., 1969). Crustacean zooplankton get most of their calcium from the water, so low calcium levels can be a limiting factor for them in softwater lakes (Cowgill, 1976). Calcium limitation in daphnids has been found to limit growth, delay or inhibit reproduction, and impact survival (Ashforth & Yan, 2008; Hessen et al., 2000). Typically, low-calcium requiring species such as the cladoceran *Holopedium sp.* will be found in low-calcium lakes while large-bodied high calcium species like several *Daphnia spp.* will be found in high-calcium lakes (Wærvågen et al., 2002). While the effects of

calcium on cladocerans have been well-studied, the effects of calcium levels on rotifers and copepods are not yet known, and so their community-level response to varying calcium levels are not yet established. It has been shown, however, that a transition from large crustaceans to small rotifers has been observed following a decreasing calcium trend (Tessier & Horwitz, 1990).

The land-use gradient in TLB shows a pattern of more developed and agricultural land-use in the south and more natural land-cover in the north. Developed and agricultural land-use is often a source of nutrient pollution from fertilizers and sewage outfalls, which can increase nutrient levels in receiving lakes and promote cultural eutrophication (Smith, 2003). Eutrophic conditions often result in cyanobacterial blooms which can produce harmful toxins (L.-A. Hansson et al., 2007), are poor-quality food for zooplankton (Brett & Müller-Navarra, 1997), and due to their colonial structures, can be difficult or even harmful to ingest by filter-feeding zooplankton (Webster & Peters, 1978). This can result in a shift to smaller-bodied zooplankton incapable of feeding on blooming taxa. For example, Deng et al. (2008) found seasonal shifts in large-bodied cladocerans and copepods to small-bodied cladocerans and copepods in a eutrophic lake in response to cyanobacterial blooms. The specific effects of eutrophic conditions on rotifers, as well as on entire zooplankton communities, are less known due to the focus of most studies on crustacean zooplankton, especially clonal lines of *Daphnia spp.* (Ger et al., 2014).

With limestone being a major source of calcium carbonate, the transition from limestone-dominated bedrock to granite-dominated bedrock, which is calcium-poor, means lakes across TLB will vary significantly in their calcium carbonate levels. Calcium

carbonate acts as a buffer in aquatic ecosystems by essentially protecting a lake from drastic changes in pH in response to events such as acid deposition (Schindler et al., 1980). Acidic conditions can directly affect zooplankton by altering reproduction rates, growth rates, and survival due to respiratory stress and toxic levels of hydrogen ions (Alibone & Fair, 1981; Sprules, 1975), and can also indirectly affect zooplankton by impacting phytoplankton community composition (Yang & Gao, 2003). Fortunately, most of the lakes within this study had a circumneutral or moderately alkaline pH (see Chapter 2), but given that pH is a log-scale, there was more than an order of magnitude difference in pH across the study lakes. The buffering capacity of a lake can also influence the stability of pH within a lake. Lakes with historically stable pH levels have been found to have different community compositions than similar lakes having experienced acidification events in the past (Fischer et al., 2001), so communities may vary widely across TLB in response to their differences in pH and buffering capacity.

All of these environmental gradients presented above occur simultaneously across TLB, and therefore zooplankton communities across TLB face a wide range of environmental conditions that are expected to drive their community composition. The southern part of TLB, has hardwater lakes that are higher in calcium, but also face more pressure from land-use due to arable soils and proximity to expanding urban regions like the Greater Toronto Area. Due to their hardwater status, they tend to be alkaline and have more stable pH levels. In the northern parts of TLB, there are softwater lakes which are low in calcium, have mostly natural land-cover, and are not protected by the buffering capacity of calcium carbonate. In between these extremes, lakes vary widely in water

hardness, land-use, and pH depending on their position along the geological mosaic of limestone and granite.

With such variable water quality conditions across TLB, there are likely a variety of confounding factors that affect zooplankton communities in TLB lakes, making it hard to predict community composition based on single parameters alone. For example, research has shown that in oligotrophic lakes where nutrients are a limiting factor, large-bodied cladocerans have a competitive advantage. However, low calcium levels can limit the growth of large-bodied cladocerans, significantly limiting their survival and populations (Ashforth & Yan, 2008). The unpredictability of the effects of multiple stressors makes the zooplankton community in a lake difficult to predict in theory, and therefore is an important rationale for surveying lakes across TLB to further our understanding of complex drivers of zooplankton community structure in these freshwater lakes.

The primary goal of this research was to investigate the multivariate drivers of zooplankton community diversity and composition across TLB by exploiting the known chemical and land-use gradients across this ecotone. A total of 60 lakes were selected along a known calcium gradient (Figure 1.2), but water quality and land-use data collected during the study also confirmed additional nutrient, pH, and land-use gradients. This study had several objectives to be addressed:

1. Characterize zooplankton community diversity and composition in the 60 study lakes across TLB.
2. Assess spatial and land-use drivers of zooplankton community structure across TLB.

3. Investigate the roles of abiotic (water quality) and biotic (phytoplankton and inter-specific zooplankton co-occurrence) drivers of zooplankton community structure across TLB.

While these objectives are mainly discovery-based because of the unknown latent interactions that may occur among the multiple environmental drivers being investigated at a regional scale, I am expecting that calcium levels, land-use, and pH will emerge as important controlling variables of zooplankton community composition based on their known effects in other lake ecosystems. I hypothesize that the nutrient-rich hardwater lakes in the south of TLB will have proportionally higher amounts of small-bodied zooplankton such as rotifers and small cladocerans due to the lack of calcium limitation and their tolerance of eutrophic conditions. Conversely, zooplankton communities in nutrient-poor softwater lakes will favour large-bodied taxa tolerant to low-Ca conditions such as *Holopedium sp.* and copepods. The lakes located throughout the transitional area of TLB will likely have variable zooplankton communities and biomass reflecting the large variation in environmental parameters, especially calcium and nutrients.

4.2 Methods

4.2.1 Data Collection

Water quality and land-use data were collected according to the methods described in Chapter 2.

Zooplankton samples were collected at each site using a Wisconsin tow-net that was lowered to the same depth as the integrated tube (1m above the bottom of the water column). Zooplankton were rinsed from the tow net into a glass Qorpak™ jar using 95% ethanol to euthanize the zooplankton, and the jars were filled using 70% ethanol to

preserve them to be analyzed at a later date. For sample analysis, zooplankton samples were lightly shaken to uniformly distribute organisms in the bottle, and then 1 mL of sample was placed in a Sedgewick Rafter counting chamber and topped with a glass cover slide. Then using an inverted phase contrast microscope, all zooplankton present on the slide were counted and identified to species or genus level, except for adult copepods, which were identified as either calanoids or cyclopoids. This was repeated until either 100 animals were counted, or the entire sample was depleted. Zooplankton were identified using a key by Haney (2013).

4.2.2 Data Analysis

Statistical analyses were performed using R version 4.0.4 (R Core Team, 2021) and SigmaPlot version 14.0. In order to visualize community composition across study lakes, both species and genus relative abundance plots were constructed in R using packages ggplot2 and cowplot (Wickham, 2016; Wilke, 2020). Simpson's diversity index (1-D) was calculated for each site in order to account for not only the number of species found at each site, but also their relative abundances (Simpson, 1949). Although rotifer and cladoceran taxa were identified to species, not all species were present in all lakes, thus regional scale multivariate analyses used zooplankton community data at the genus level.

Spatial scale is an important component in species distribution models as well as establishment. As the study area covered a large region of central Ontario, implicit spatial structure within my data was investigated. To determine whether there was spatial autocorrelation within an environmental parameter, Moran's positive and negative coefficients were calculated, and those that were identified to autocorrelate spatially were

removed from subsequent analyses. Moran's eigenvector mapping was conducted in the R packages *ade4*, *adespatial*, *spdep*, *adegraphics*, and *maptools* (Bivand et al., 2016; Bivand & Wong, 2018; Dray et al., 2019; Dray & Dufour, 2007; Siberchicot et al., 2017). The spatial model was built with irregular sampling excluding distance-based methods to account for unbalanced study design, and neighbourhood criteria was based on nearest neighbours due to the clustering of the study lakes. Moran's eigenvector mapping was conducted to investigate whether there was an implicit spatial structure present which influenced the species distributions of zooplankton communities across central Ontario lakes. Due to the underlying study design, which had a strong emphasis on exploiting the natural gradient of cations present within central Ontario lakes due to parent geology, no spatially implicit structures were identified to influence species distributions ($p > 0.05$) and therefore I was able to exclude spatial parameters from further analysis.

A detrended correspondence analysis was run to determine whether the relationship between zooplankton communities and the water quality and land-use parameters was linear or unimodal. This step evaluated if the data was more suited to a canonical correspondence analysis (CCA) or a redundancy analysis (RDA). Because the resulting longest axis-gradient length was smaller than 3, an RDA was used (Šmilauer & Lepš, 2014). A Hellinger transformation was applied to zooplankton count data in order to give low weights to rare taxa in the RDAs, and RDAs were constructed using R packages *ggord*, *stringr*, *dplyr*, *tidyr*, *vegan*, and *ggplot2* (Beck, 2016; Oksanen et al., 2019; Wickham, 2010, 2016; Wickham et al., 2019; Wickham & Henry, 2018). One RDA used zooplankton count data at the group level (cladoceran, copepod, rotifer) in

order to examine group-level responses to environmental parameters while another used zooplankton count data at the genus level to examine fine differences between taxa.

To explore relationships between zooplankton genera and environmental parameters including water quality and land-use, Kendall correlation analysis was conducted using R packages ggplot2, corrplot, and Hmisc (Harrell & Dupont, 2016; Wei & Simko, 2017; Wickham, 2016). Kendall correlation was used due to the non-normal distribution of the environmental data, and Kendall's coefficient produces narrower confidence intervals than Spearman's coefficient (Bishara & Hittner, 2017).

Multiple linear regression was used to predict zooplankton biomass. Multiple linear regressions were run between zooplankton biomass (μg) data and environmental parameters including phytoplankton biomass (mg) using R package vegan (Oksanen et al., 2019). All environmental and biomass data was $\log(x+1)$ transformed. Variance inflation factors (VIF) were calculated and used to assess whether any of the variables included in the models exhibited collinearity, but because none of the values had a VIF value above 4, none were omitted (James et al., 2013). A multiple linear regression predicting zooplankton diversity using Simpson's diversity index calculated in R package vegan (Oksanen et al., 2019) was attempted, but a significant model could not be found using the data currently available.

4.3 Results

4.3.1 Characterizing zooplankton communities across TLB

Relative abundance of zooplankton genus counts is presented in Figure 4.1. Community composition was highly variable across the 60 lakes, with the most common taxa across all lakes being the rotifer *Keratella sp.* and copepod nauplii. Low-calcium

lakes (< 20 mg/L of calcium) varied but were commonly dominated by small taxa such as rotifers and copepod nauplii. The most common cladocerans in low-calcium lakes were *Daphnia sp.*, *Bosmina sp.*, and *Holopedium sp.*, with *Holopedium sp.* being rare in medium-calcium lakes and absent in high-calcium lakes. Medium-calcium lakes (20 – 40 mg/L of calcium) were dominated by copepods, with nauplii again being the most abundant followed by adult cyclopoids. *Daphnia sp.* and *Chydorus sp.* were the most common cladocerans in medium-calcium lakes. High-calcium lakes (>40 mg/L of calcium) had a relatively small lake sample size (n=7), but they were also dominated by *Keratella sp.* and copepod nauplii, as well as *Polyarthra sp.* and *Bosmina sp.*, with *Bosmina sp.* being the most common cladoceran in high-calcium lakes. For finer resolution of community composition, species-level abundance plots are presented in Appendix A2.

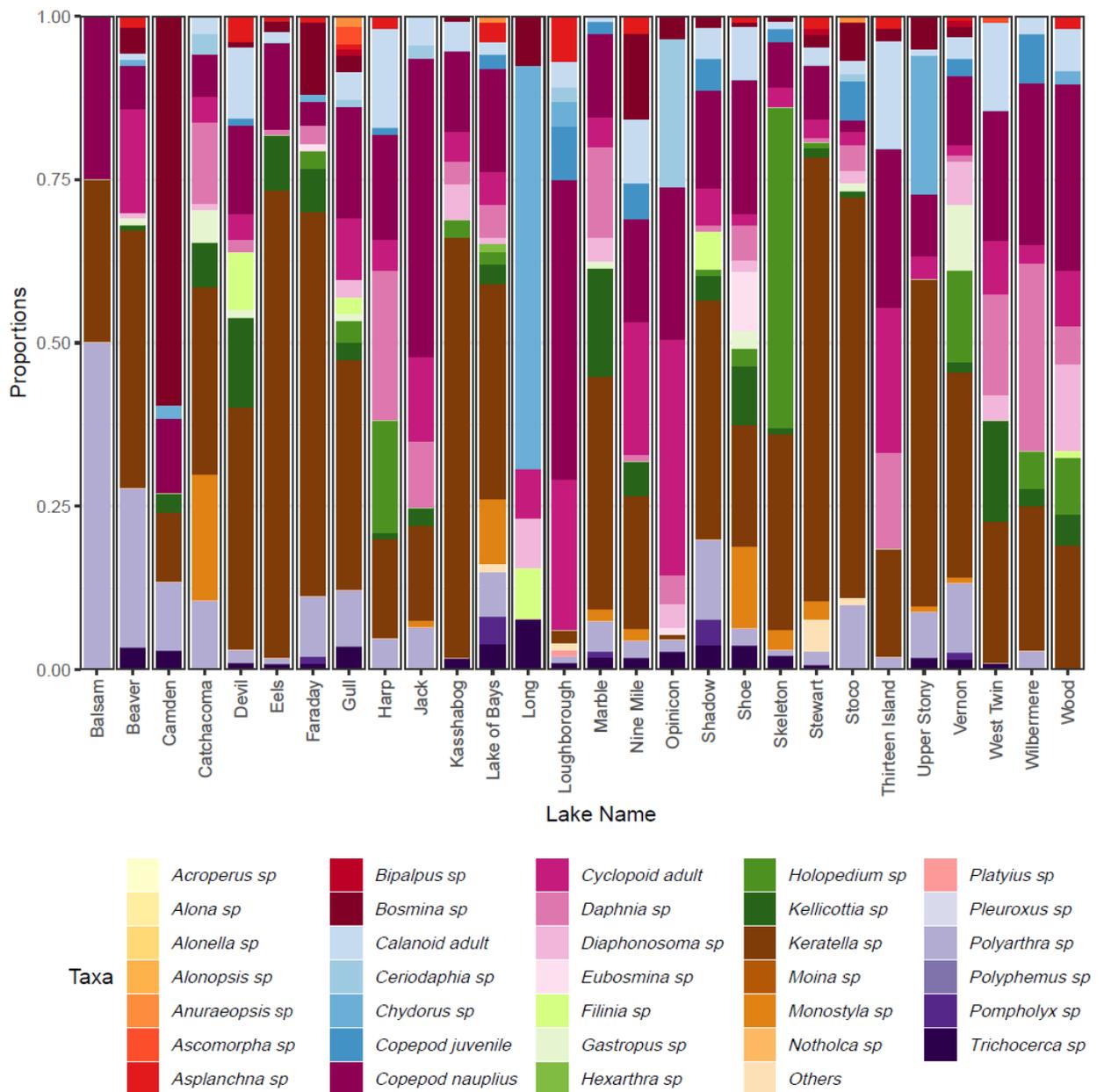


Figure 4.1a Relative abundance plot for zooplankton at the genus level in low-calcium lakes based on counts (<20mg/L).

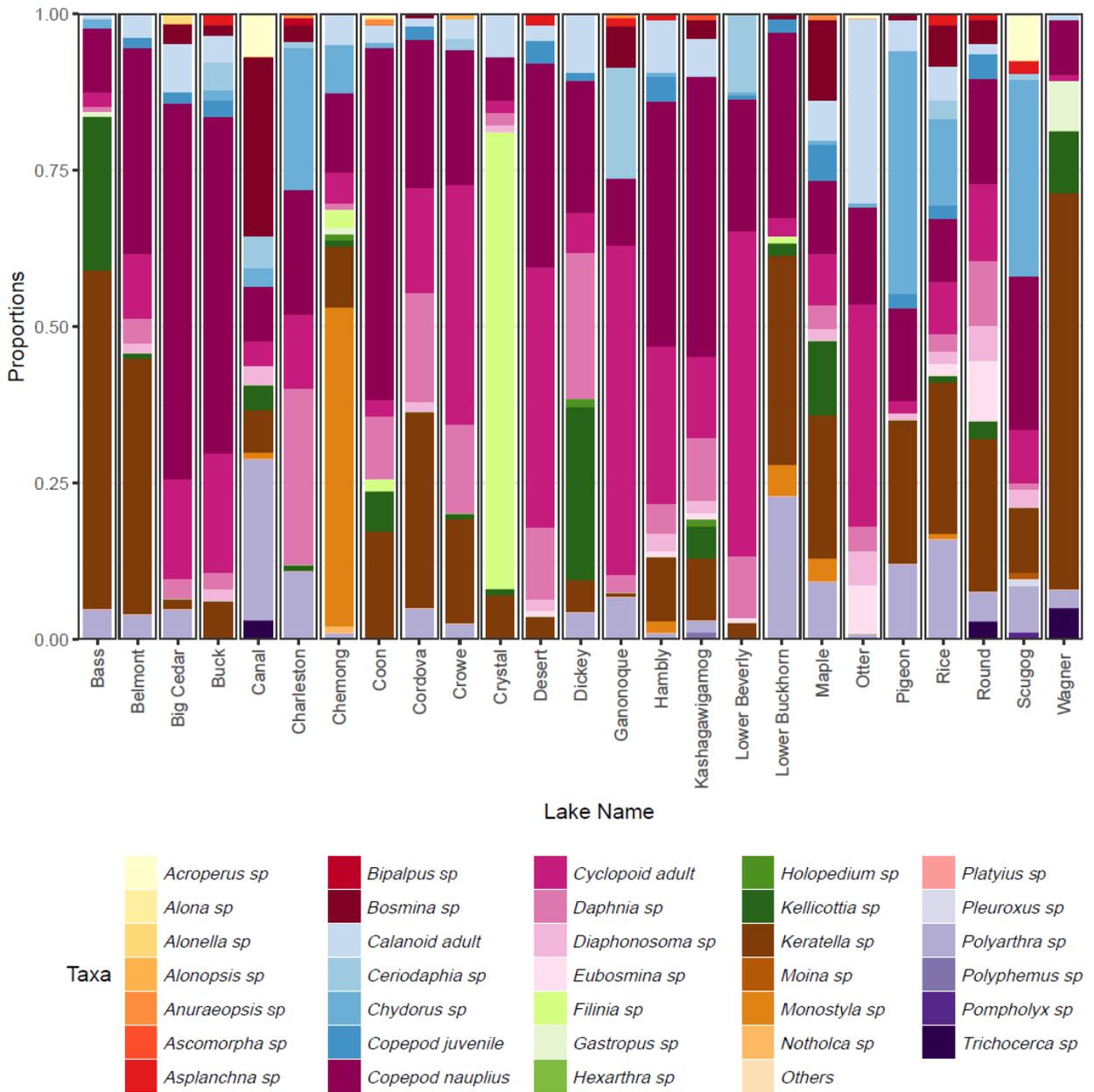


Figure 4.1b Relative abundance plot for zooplankton at the genus level in medium-calcium lakes based on counts (20 – 40 mg/L).

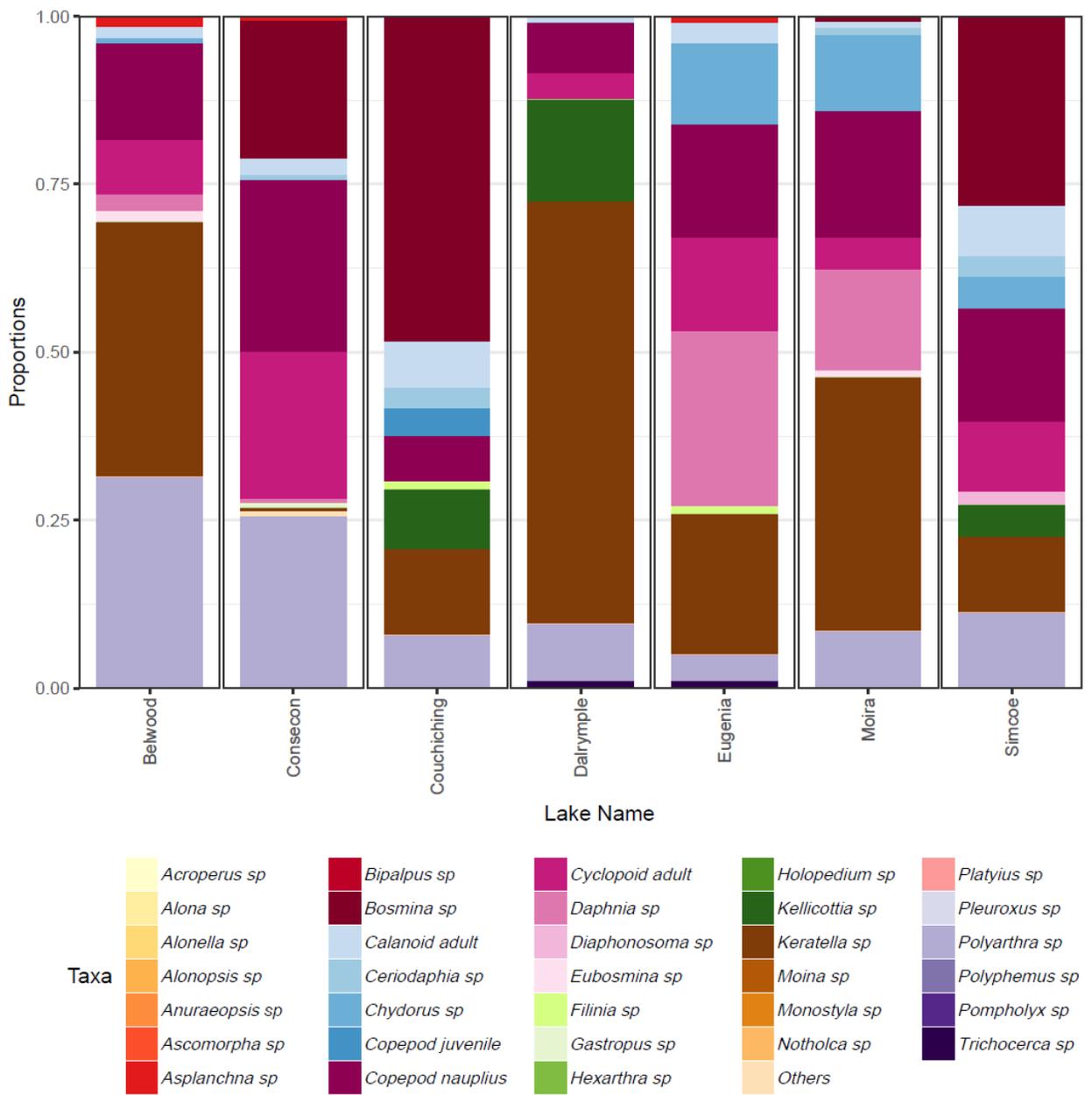


Figure 4.1c Relative abundance plot for zooplankton at the genus level in high-calcium lakes based on counts (> 40 mg/L).

4.3.2 Investigating drivers of zooplankton diversity and abundance across TLB

Zooplankton communities across the 60 study lakes were not found to be driven by spatial structures based on Moran's eigenvector mapping, therefore I was able to focus on the local water quality conditions as drivers of zooplankton community structure across TLB. Zooplankton biomass varied from site to site (see Figure 4.2), with Otter Lake having the highest overall zooplankton biomass.

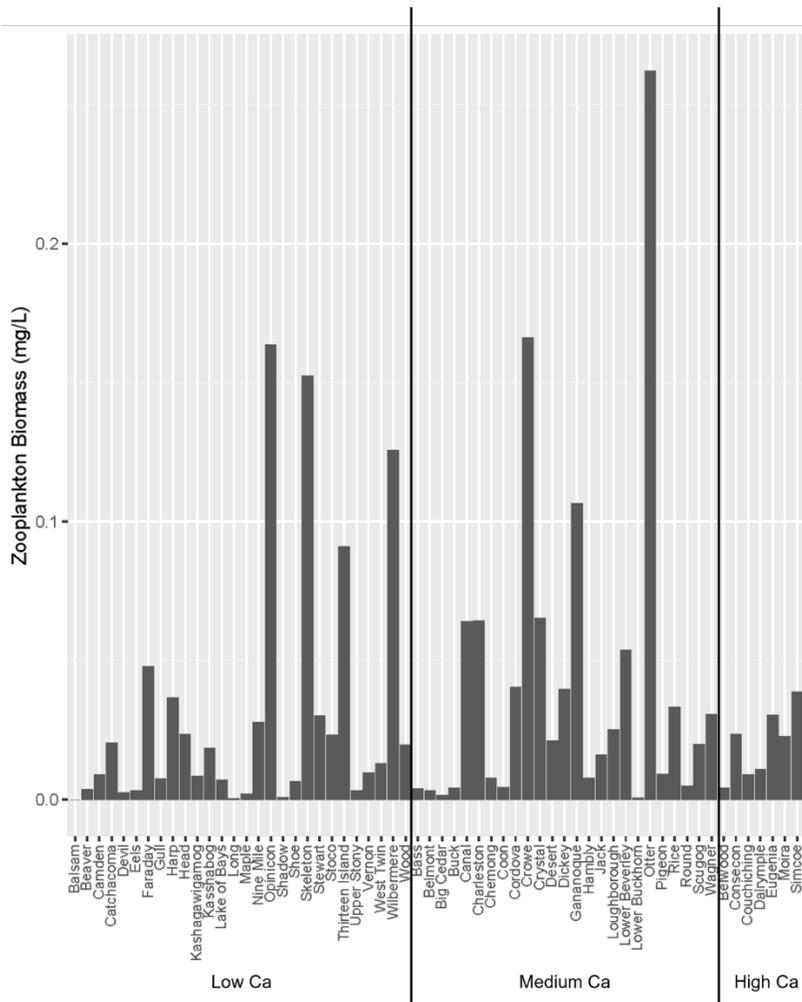


Figure 4.2 Bar plot depicting zooplankton biomass at each of the 60 study lakes.

The redundancy analysis using zooplankton families and water quality and land-use parameters is shown in Figure 4.3 and the first and second axes explain 97.08% of the variance in the data. Copepods, rotifers, and cladocerans are distinctly differentiated, with each in a different quadrant of the plot. Cladocerans appear to be positively associated with dissolved oxygen, total nitrogen, and total organic carbon, while being negatively associated with Secchi depth. Copepods are positively associated with natural land-cover, and negatively associated with total phosphorous, pH, and conductivity. Rotifers are positively associated with chlorophyll a, and negatively associated with agricultural land and calcium.

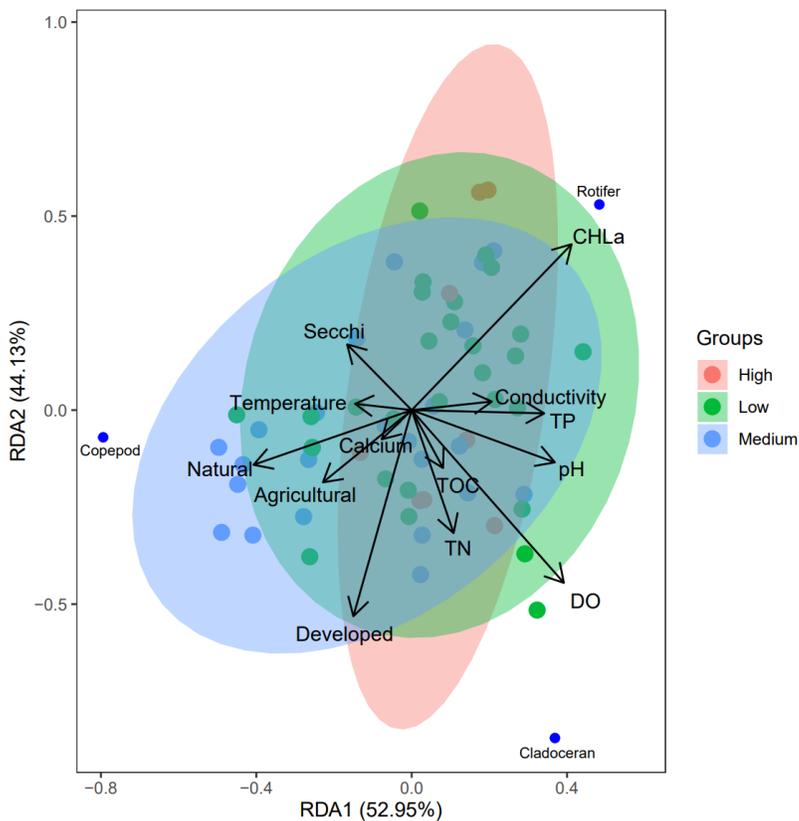
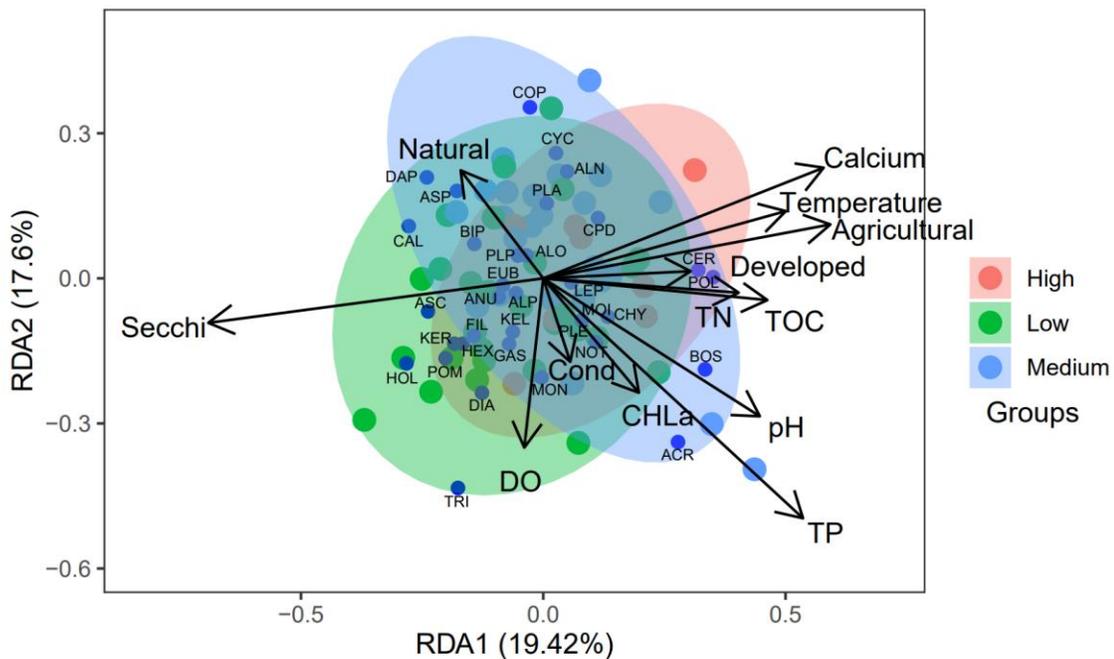


Figure 4.3 Redundancy analysis of zooplankton families with water quality and land-use overlaid with ellipses grouping low, medium, and high-calcium lakes. Zooplankton count data was transformed using Hellinger transformation to give low weights to rare species.

A redundancy analysis based on zooplankton genera, water quality, and land-use parameters is shown in Figure 4.4. This RDA explains 37.02% of the variance observed in the data. Lakes again do not distinctly cluster based on calcium groups. Strong drivers of zooplankton genera appear to be Secchi depth, total phosphorous, calcium, agricultural land-use, and pH. *Holopedium sp.* and several rotifer genera are negatively associated with calcium, and few taxa appear to have a strong positive association with calcium. A Kendall correlation matrix was produced which presents correlations between zooplankton genera and environmental parameters (Figure A3).



ACR = Acroperus, ALO = Alona sp., ALN = Alonella sp., ALP = Alonopsis sp., BOS = Bosmina sp., CER = Ceriodaphnia sp., CHY = Chydorus, DAP = Daphnia, DIA = Diaphanosoma, EUB = Eubosmina, HOL = Holopedium, MOI = Moina, PLE = Pleuroxus, PLP = Polyphemus, LEP = Leptodora, CAL = Calanoid adult, COP = Copepod young, CYC = Cyclopoid adult, CPD = Unknown Copepod, ANU = Anuraeopsis, ASC = Ascomorpha, ASP = Asplanchna, BIP = Bipalpus, FIL = Filina, GAS = Gastropus, HEX = Hexarthra, KEL = Kellicottia, KER = Keratella, MON = Monostyla, NOT = Notholca, PLA = Platyus, POL = Polyarthra, POM = Pompholyx, TRI = Trichocerca

Figure 4.4 Redundancy analysis of zooplankton genera with water quality and land-use overlaid with ellipses grouping low, medium, and high-calcium lakes. Zooplankton count data was transformed using Hellinger transformation to give low weights to rare species. Cond = conductivity, DO = dissolved oxygen, CHLa = chlorophyll a, TP = total phosphorous, TOC = total organic carbon, TN = total nitrogen

The MLR results displayed in Table 4.1 show predictor variables for zooplankton biomass. This model was found to explain 12.2% of the variance observed in zooplankton biomass across TLB ($p < 0.05$). Phytoplankton biomass was found to be a marginally significant negative predictor of zooplankton biomass ($p = 0.059$). Conductivity and calcium were found to be significant positive predictors of zooplankton biomass ($p < 0.05$).

Table 4.1 Summary Statistics for multiple linear regression of environmental parameters predicting zooplankton biomass in TLB.

	Estimate	Standard Error	t value	p-value (t-test)	Model Adjusted R ²	Model p-value
Phytoplankton Biomass	-0.274	0.142	-1.930	0.059	0.122	0.042
Temperature	-1.455	1.544	-0.943	0.350		
DO	1.031	0.690	1.493	0.141		
Conductivity	0.184	0.089	2.071	0.043		
Secchi	-0.531	0.538	-0.986	0.329		
Calcium	0.510	0.199	2.565	0.013		

4.4 Discussion

Environmental drivers of zooplankton community diversity and composition in TLB

Zooplankton communities across TLB were not spatially structured, indicating that habitat proximity (i.e., distance between lakes) did not play a role in zooplankton community composition, biomass, or diversity. Much like with phytoplankton as discussed in Chapter 3, zooplankton can be passively dispersed over a wide geographical area through several vectors (e.g., rivers/streams, wind, animals, humans) (Padisák et al., 2016; Shurin et al., 2009), and were therefore not expected to be limited by spatial structure in TLB. Instead, zooplankton communities appear to be structured by local environmental conditions as seen in Figures 4.3 and 4.4.

Zooplankton community composition appears to be influenced by a number of environmental variables, as can be seen in the RDAs. The calcium gradient across TLB appears to influence zooplankton communities in a number of ways. Calcium appears to be negatively associated with rotifers at the family-level RDA (Figure 4.3) and is negatively associated with most rotifer taxa at the genus-level RDA (Figure 4.4), indicating that rotifers are more common in low-calcium lakes and less common in high-calcium lakes. Rotifers have much lower calcium requirements than cladocerans and copepods, which likely provides them with a competitive advantage when calcium levels in the water column are low. Because rotifers are typically less competitive in food acquisition than filter-feeding cladocerans or copepods, which have efficient and/or diverse feeding patterns (Brooks & Dodson, 1965; Strickler & Bal, 1973b), they can be outcompeted by these groups when food is limited but can thrive when these groups are absent (Hurlbert et al., 1972). Shifts from the competitively superior large-bodied cladocerans to less competitive small-bodied rotifers have been observed in the past in response to declining calcium (Tessier & Horwitz, 1990), and this relationship between calcium and rotifers appears to emerge across TLB's calcium gradient.

The relationship between calcium and cladocerans (which typically have high calcium requirements) in TLB lakes is unclear, as few cladocerans at the genus-level were closely associated with calcium. The cladoceran genus *Daphnia sp.* is large-bodied and has high calcium requirements, thus it was expected to be positively correlated with calcium levels and found primarily in medium to high-calcium lakes. However, no significant correlations with calcium were detected in the study, and *Daphnia* were found in very low-calcium lakes, though at low abundance levels. Ashforth and Yan (2008)

found that *Daphnia pulex* required a calcium threshold of 1.5 mg/L to survive and reproduce, but Harp Lake, which had a calcium concentration of 0.43 mg/L, was found to have *Daphnia*. The *Daphnia* in Harp Lake were mostly *Daphnia mendotae* (see Figure A2), so it is possible that species differences played a role in their tolerance of low calcium conditions. Jeziorski et al. (2012) also found daphnids at calcium levels lower than the threshold posited by Ashforth & Yan (2008) and allotted this to species and clonal differences in calcium tolerance. This explanation is further supported by Azan et al. (2015) who found that some species of *Daphnia* were much less sensitive to decreasing calcium levels than others, including *Daphnia mendotae*. So, while these species may have been somewhat limited in low-calcium lakes leading to low abundances, they are apparently still able to survive and reproduce. Therefore, the lack of a relationship between large-bodied cladocerans and calcium concentration may simply be an indication that calcium is not at limiting concentrations for these zooplankton in TLB lakes.

While most cladocerans were scattered throughout the genus-level RDA, *Holopedium sp.* had a negative association with calcium (Figure 4.4). *Holopedium* require very little calcium compared to other common cladocerans due to their lack of a calcified exoskeleton, and often occur in low calcium conditions where highly competitive *Daphnia* are limited by their high calcium requirements (Jeziorski & Yan, 2006). It was expected that *Holopedium* would be a dominant taxon in low-calcium lakes, but this was not observed, possibly due to other abiotic or biotic factors. Since the study design was a “snap-shot” of communities across a large region, hence sacrificing seasonal dynamics for large-scale capture of community diversity, I may not have sampled when

Holopedium was at peak population level. Another potential explanation is that the neutral to slightly alkaline pH levels in the study lakes limited *Holopedium* populations, as pH levels above 8 have been found to be harmful to them (Hessen et al., 1995). The shift from *Daphnia* to *Holopedium* along a decreasing calcium gradient is often accompanied by decreases in pH, which provides the acid-tolerant *Holopedium* an advantage over the less-tolerant *Daphnia*. Instead, in TLB the two appeared to coexist at several sites, and neither cladoceran was dominant in any lakes except the low calcium Skeleton Lake, where *Holopedium* made up almost 50% of the zooplankton community.

Lake pH appeared to be a strong driver of variation in both the family-level RDA and genus-level RDA based on vector length. The ion exchange and respiration processes of zooplankton are dependent on lake pH, with different taxa having different optimal pH levels for these physiological processes. Generally, a majority of freshwater zooplankton species thrive in neutral to slightly alkaline pH levels due to the conditions being optimal for respiration and ion exchange, as well as the co-occurrence of alkaline conditions with productive phytoplankton populations (Ivanova & Kazantseva, 2006), though some are tolerant to either alkaline or acidic conditions. The study lakes varied in pH from neutral to alkaline with the exception of one slightly acidic lake (Beaver Lake, pH = 6.57), so the adverse effects of acidity were not observed. Copepods as a group were somewhat negatively associated with pH, indicating that they were less common in high pH lakes. While most studies examining the effects of pH on copepods focus on the effects of acidic conditions rather than alkaline ones, it has been recorded that marine copepods do not survive at $\text{pH} \geq 9$ in experimental conditions (Pedersen & Hansen, 2003). The

negative relationship detected between copepods and pH in TLB may be evidence that this relationship emerges in freshwaters as well.

In the genus-level RDA, pH appears to affect taxa differently, with some having a positive association and others having negative associations. Many cladoceran genera appear to be either positively or negatively associated with pH, with these relationships potentially being attributed to body size. The small-bodied *Bosmina* is strongly positively associated to pH, while the large-bodied *Daphnia* is negatively associated to pH. This is somewhat contradictory to other studies, for example, Labaj et al. (2021) found *Bosmina* to be associated with pH levels below 7 and *Daphnia sp.* to be associated with pH levels above 7. However, this may be attributed to the different pH range between their study (4.2 – 9.3, mean = 6.7 ± 0.9) and the present study (6.57 – 9.51, mean = 8.3 ± 0.66). All but one lake surveyed in TLB was above pH 7, which may be why such different results have been acquired between the two studies. The literature typically focuses on the effects of acidic pH on zooplankton communities, so these results demonstrate that future research should explore the effects of alkaline conditions on zooplankton communities and individuals. Rotifers also varied in their associations to pH, which further exacerbates the need for a better understanding of the effects of alkalinity on whole zooplankton communities, as rotifers are often neglected in experimental research in favor of the commonly studied cladocerans.

Another important factor in zooplankton community structure in TLB according to the RDAs was land-use. The relationships between zooplankton taxa and the land-use vectors are likely indirect, as land-use alters local environmental conditions and can cause bottom-up alterations in plankton communities (e.g., Dodson et al., 2005). As was

discussed in Chapter 2, lakes surrounded by natural land-cover had lower nutrient levels, while lakes surrounded by agricultural and developed land had higher nutrient levels and lower Secchi depth (or water clarity). These variables can impact zooplankton in a bottom-up manner by influencing phytoplankton and, in turn, zooplankton. Additionally, water clarity can affect zooplankton directly by influencing vertical migration patterns which depend on light (Leach et al., 2015).

Nutrient levels (TN and TP) are important limiting factors for phytoplankton, an important zooplankton food source. As can be seen in Figures 4.3 and 4.4, TP is closely associated with chlorophyll a, which is a proxy measure of phytoplankton biomass and lake productivity. A unimodal relationship has been noted between lake productivity and zooplankton species richness (Dodson et al., 2000), where richness rises with increasing lake productivity until eutrophic levels are reached, and then richness decreases. In the family-level RDA, TN and TP produced medium-sized vectors, with cladocerans being positively associated with TN and copepods being negatively associated with TP. In the genus-level RDA, TN produced a medium-sized vector while TP produced a long vector, indicating that TP was a stronger driver of the variation observed in the data than TN, though the latter still appears important. Several cladoceran taxa and one rotifer taxon were positively associated with TN and TP, while two other cladoceran taxa, two rotifer taxa, and calanoid copepods were negatively associated with them. These relationships to nutrients are likely the result of individual differences among these taxa, such as differences in size, that result in different competitive abilities in low or high nutrient conditions. For example, *Bosmina sp.*, which was positively associated with nutrients, is a small-bodied cladoceran that is often abundant in high nutrient conditions (Adamczuk,

2016), whereas *Holopedium sp.*, a large-bodied cladoceran that was negatively associated with nutrients, is commonly associated with low-nutrient conditions (Jeziorski et al., 2015). So, the zooplankton communities across TLB appear to be driven in part by lake trophic status.

Secchi depth was variable across TLB (0.85m to 8m) and appeared to affect zooplankton community structure in both the family-level and genus-level RDAs. Secchi depth was negatively associated with cladocerans in the family-level RDA, but at the genus-level, cladocerans varied in their associations to Secchi depth with some being positively associated and others being negatively associated. This differing relationship may again be owing to individual differences such as size among cladocerans, as the small-bodied cladocerans *Bosmina*, *Chydorus*, and *Ceriodaphnia* are somewhat negatively associated to Secchi depth. Small-bodied taxa tend to be favored in conditions with low Secchi depths (which are typically shallow and/or high nutrient conditions) (Forrest & Arnott, 2006; Jeppesen et al., 2001). Large-bodied cladocerans however, were variably associated with Secchi depth in the RDA, with some being slightly positively associated (e.g., *Holopedium*, which typically resides in deep, oligotrophic lakes (Jeziorski et al., 2015)), and some such as *Alona* having no strong relationship with Secchi depth. Several rotifer genera are somewhat positively associated with Secchi depth. Among copepods, only calanoid copepods appear to be positively affected by Secchi depth, while others lack any association. Calanoid copepods are more common in oligotrophic conditions (Sommer & Stibor, 2002), which may be why they exhibit a positive relationship with Secchi depth.

Dissolved oxygen appears as a sizeable vector in the family-level RDA and is positively associated with cladocerans. While zooplankton require oxygen for respiration, none of the water columns in the study lake had hypoxic conditions (dissolved oxygen below $2 \text{ mg}\cdot\text{L}^{-1}$) (see Table 2.1), so the size of the dissolved oxygen vector and its association with cladoceran zooplankton is surprising. Additionally, in spite of this association in the family-level RDA, only one cladoceran, *Diaphanosoma*, is strongly positively associated with dissolved oxygen in the genus-level RDA. This relationship may be the result of other undetected factors, such as macrophyte presence which could significantly affect dissolved oxygen (Caraco & Cole, 2002), but future research would be needed to better understand the reason for this relationship.

Water temperature did not emerge as a large vector in the family-level RDA, but it did in the genus-level RDA. Many taxa were negatively associated with temperature, including several rotifers and a few cladocerans. High water temperatures typically tend to favor small-bodied zooplankton (Mallin & Partin, 1989), so it is surprising that both large and small-bodied zooplankton appear negatively associated with water temperature. As was discussed in Chapter 3, temperature emerged as a marginally significant negative predictor of phytoplankton diversity and biomass, so its negative association with several zooplankton genera may be the result of bottom-up interactions such as loss of food or decrease in food quality in response to high water temperature.

A multiple linear regression predicting zooplankton diversity (Simpson's diversity index 1-D) was attempted using various environmental parameters, but the resulting models were weak, indicating that zooplankton diversity is driven by factors beyond what was captured in this survey. Variables which may influence zooplankton diversity that

could not be included due to study limitations include predation and habitat structure. Fish can exert significant grazing pressures on zooplankton, even exhibiting selective pressures on certain taxa (Brooks & Dodson, 1965) which can alter community dynamics. Fish will often selectively feed on larger plankton, such as cladocerans as opposed to rotifers, which alters community structure by favoring small species and introduces predator evasion as an important competitive factor determining species survival. Habitat structure, such as the presence or abundance of macrophyte species, can also have significant effects on zooplankton communities by providing shelter from predators. With the current results, the importance of these parameters as drivers of zooplankton diversity cannot be determined but based on the weak results of the attempted multiple linear regression using data captured in this survey, it is likely that they play an important role in determining zooplankton diversity in TLB.

Environmental drivers of zooplankton abundance in TLB

The MLR for zooplankton biomass included several statistically significant explanatory variables. Calcium had a significant positive effect on zooplankton biomass ($p < 0.05$), and this relationship is likely a size-spectra effect, where large bodied cladocerans disproportionately contribute more to biomass, and thus influence the relationship with calcium, unlike smaller bodied rotifers. As such, lakes where rotifers are more abundant than the other two groups, like in the low-calcium lakes, would be lower in overall biomass.

Conductivity emerged as a significant positive predictor of zooplankton biomass ($p < 0.05$), which is somewhat surprising due to the relationship between conductivity and salinity. Conductivity is often used as a metric for salinity, which typically negatively

affects cladoceran zooplankton (Hintz et al., 2016), with its effects on other zooplankton not yet fully studied. However, in TLB, the conductivity results obtained are more likely a result of a combined effect of ionic contribution from the underlying geology and salinity, especially since road salt application is likely low throughout TLB. As seen in Table 2.1, sodium concentrations in the study lakes range from 0.39 to 46.4 mg·L⁻¹. While chloride is the toxic component of road salts, sodium chloride is the most commonly applied road salt in Canada (Environment Canada, 2012), so sodium concentrations can potentially be used to estimate chloride concentrations in the lakes. The Canadian chronic threshold of chloride exposure for the protection of freshwater wildlife is 120 mg·L⁻¹ (Canadian Council of Ministers of the Environment, 2011), so using sodium concentrations as an estimate for chloride concentrations, none of the study lakes are at even half of the threshold for wildlife harm. As such, the positive relationship between conductivity and zooplankton biomass may instead be the result of increased nutrient content (minerals, inorganic compounds) associated with conductivity.

Phytoplankton biomass emerged as a marginally significant negative predictor of zooplankton biomass ($p = 0.059$). This was surprising, as the relationship between phytoplankton and zooplankton biomass is typically positive as zooplankton are commonly limited by food availability in oligotrophic and mesotrophic lakes. This relationship may have been the result of inedible or low-quality phytoplankton being common in certain lakes due to selective grazing. Colonial cyanobacteria were common in the study lakes, and they are found consistently to be nutritionally poor foods for zooplankton (Arnold, 1971) and can even be harmful in some cases by disrupting filtering mechanisms of large cladocerans (Lampert, 1987). Additionally, zooplankton

can only filter-feed on cells up to 50 μ m (Allan, 1976), so lakes with large phytoplankton taxa like the large dinoflagellate *Ceratium* (found in 5 out of 60 lakes), which can exceed greatly exceed that size, would have a high phytoplankton biomass without having a high food concentration for zooplankton. There could also be other factors not considered in this model due to the limitations of the study that could be influencing this relationship as well, such as fish predation or macrophyte presence. This relationship may also be an artifact of sample design, as zooplankton and phytoplankton communities are temporally dynamic and were only sampled once per lake. The reason for the negative relationship between phytoplankton biomass and zooplankton biomass in TLB warrants future research, and trophic interactions are often complex but valuable in lake management.

There are likely other factors not measured in this study that influence zooplankton biomass across TLB. It is widely known that predation by invertebrate and vertebrate predators can reduce zooplankton community biomass throughout the growing season. Fish predators often preferentially select large species over small species, potentially reducing overall biomass (Brooks & Dodson, 1965). While it is likely that some of the lakes in this study experienced these effects, further research would be required to determine the role of predation on zooplankton biomass in TLB.

Chapter 5: Investigating the Effects of Species-Specific and Trophic Interactions on Plankton Communities in The Land Between

5.1 Introduction

Plankton communities form the base of the aquatic food web, and they are the result of a complex interplay between abiotic drivers, which limit the species that can establish in a given ecosystem, and biotic drivers such as competition, which further limit species establishment and survival. Competition exists among plankton species with similar niches due to limited resource availability. Phytoplankton must compete for limited nutrients for growth, as well as for inorganic carbon and light for photosynthesis. Zooplankton, on the other hand, compete primarily for food, which is primarily edible (i.e., based on size) phytoplankton species. Plankton species vary in their competitive abilities for these resources, and when these resources are limited, better adapted taxa can exclude others in a process known as competitive exclusion (Hardin, 1960). For example, when food levels are low, cladoceran zooplankton tend to outcompete rotifers due to their more efficient feeding strategy (Hurlbert et al., 1972). Other times, plankton will have traits or adaptations that allow them to coexist with other species, such as phytoplankton species utilizing different wavelengths of light which minimizes competition between them for that resource (Stomp et al., 2004). These patterns of competition and coexistence can influence the structure of plankton communities, which in turn affects the whole lake system.

Trophic interactions between plankton are also important, as phytoplankton and zooplankton exert important influences on each other. Phytoplankton vary in edibility and nutritional value, so the types of phytoplankton available in an ecosystem can determine

which zooplankton are capable of establishing there (e.g., Francis et al., 2014). Zooplankton, in turn, exhibit selective grazing pressure on edible phytoplankton taxa, potentially altering phytoplankton community dynamics by exerting pressure on edible species and relieving inedible species from competition. For example, when grazing pressure from zooplankton is experimentally removed, phytoplankton communities can shift from large, inedible species to small edible species which were previously targeted for zooplankton consumption (McCauley & Briand, 1979). Some zooplankton are also capable of raptorial feeding and can prey on small rotifers, thus limiting their abundance (Williamson & Butler, 1986). As such, trophic interactions can have important roles in driving plankton structure and abundance, and these interactions influence the transfer of energy throughout the food web.

To add to the complexity of plankton interactions, they are sensitive to changing environmental conditions. Lakes are temporally dynamic systems, and the availability of resources throughout the water column varies daily and seasonally. The competitive outcomes and possibility for coexistence among species are therefore dynamic as well, which makes them difficult to study. For this reason, much research focuses exclusively on abiotic drivers of plankton communities while overlooking the effects of biotic interactions. However, doing so can limit our understanding of plankton communities. As plankton communities play an important role in lake health, attaining a better understanding of their drivers can provide insight for lake management.

Taxa-specific interactions may play a significant role in TLB's lakes, yet they are usually difficult to tease apart from abiotic factors. The goal of this chapter was to explore the role of taxa-specific interactions in plankton community structure in TLB,

and to compare the effects of abiotic and biotic drivers of plankton communities using a novel multivariate modelling technique called Generalized Linear Latent Variables Model (GLLVM). Thus, the research presented in this chapter aims to address two objectives:

1. Determine the relative roles of abiotic and biotic drivers of plankton community structure in TLB; and
2. Investigate co-occurrence patterns between zooplankton and phytoplankton taxa to better understand taxa-specific interactions TLB's plankton communities.

By integrating both abiotic and biotic data into a GLLVM framework, the roles of each in driving plankton communities may be elucidated. I expected that while environmental variables would continue to play an important role in plankton community structure, taxa-specific interactions would also emerge as having a significant role in driving community structure based on positive and negative co-occurrence patterns revealed in the analysis.

5.2 Methods

5.2.1 Data collection and processing

Water quality and land-use data were collected and processed according to the methods described in Chapter 2. Phytoplankton data was collected and processed according to the methods described in Chapter 3. Zooplankton data was collected and processed according to methods outlined in Chapter 4.

5.2.2 Data Analysis

Statistical analyses were performed using R version 4.0.4 (R Core Team, 2021). To examine variation between both phytoplankton and zooplankton communities in conjunction with environmental variables across TLB, I first used redundancy analysis (RDA) to visualize taxa associations in ordination space. The RDA biplots were composed using the 20 most abundant phytoplankton and zooplankton taxa at the genus level. This cut-off captured 42.4% of total phytoplankton biomass and 99.7% of total zooplankton biomass (see Figures 3.1 and 4.1 for phytoplankton and zooplankton relative abundance). An RDA was used rather than canonical correspondence analysis because the detrended correspondence analysis revealed the longest gradient was smaller than 3 (Šmilauer & Lepš, 2014). A Hellinger transformation was applied to plankton count data in order to give low weights to rare taxa in the RDAs, and RDAs were constructed using R packages `ggord` (Beck, 2016), `stringr` (Wickham, 2010), `dplyr` (Wickham et al., 2019), `tidyr` (Wickham & Henry, 2018), `vegan` (Oksanen et al., 2019), and `ggplot2` (Wickham, 2016).

To assess the interactions between plankton taxa in TLB, a relatively new multivariate approach, generalized linear latent variable modeling (GLLVM), was applied. A GLLVM is an extension of basic generalized linear models to multivariate data, which is done using a factor analytics approach that includes a small number of latent variables representative of environmental parameters at each lake accompanied by species-specific factor loadings to examine correlations between taxa. With this approach, it is possible to model taxa co-occurrence after factoring water quality

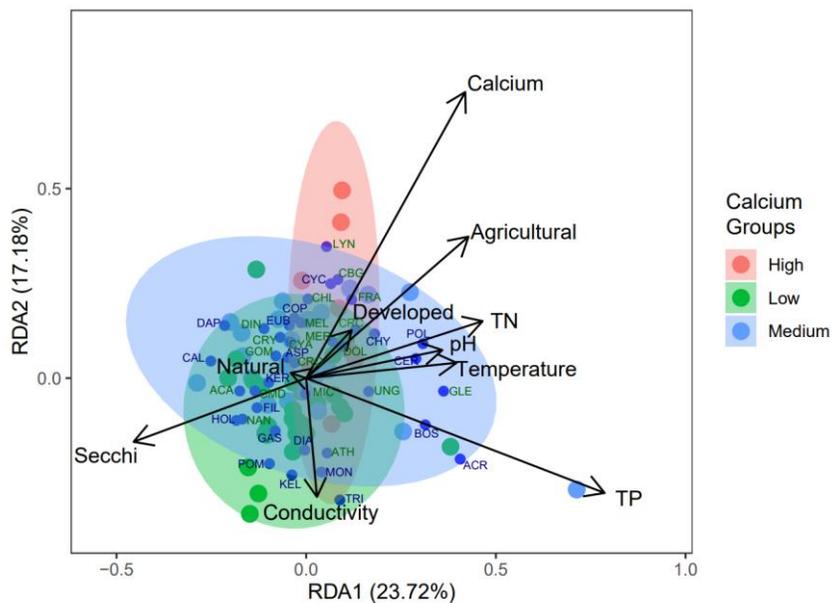
conditions in order to reveal the actual interactions between taxa, as opposed to other approaches which cannot separate latent effects and taxa interactions.

The GLLVM was modeled using the R package `gllvm` following methods described by Niku et al. (2019). Data from the top 20 zooplankton and phytoplankton taxa at the genus level across all study lakes was used in the GLLVM in order to investigate potential interactions and co-occurrence patterns between common taxa. Poisson, Tweedie, zero-inflated Poisson, and negative binomial distributions were fit to the data. Information criterion, Dunn-Smyth residual plots, and normal quantile-quantile plots with 95% intervals were used to assess the fit for TLB zooplankton and phytoplankton community distributions. It was determined that a negative binomial plot was the best fit for the given data and was thus used to construct the model. In order to account for abiotic influence on taxa-specific interactions, environmental parameters were added to the model. Based on residual analyses, seven water quality parameters (depth, Secchi depth, temperature, TP, TN, chlorophyll a, and chloride) were chosen for the final model as they indicated the most suitable mean-variance relationship for responses. The resulting correlation patterns between the selected taxa were plotted using the R packages `corrplot` and `gclus` (Hurley, 2012; Wei & Simko, 2017).

5.3 Results

The RDA of the 20 most abundant phytoplankton and zooplankton taxa across TLB is shown in Figure 5.1, and the first two axes explain 40.9% of the variance in the data. Based on the length of the vectors, calcium and TP appear to be strong drivers of the variation observed in the data. Zooplankton and phytoplankton taxa are spread throughout the plot with most taxa varying in their relationships with the environmental

parameters. Some taxa are strongly associated with one environmental variable, such as *Bosmina sp.* and *Acroperus sp.* being closely aligned with total phosphorous. Other taxa appear to be driven by several environmental variables, such as *Gleocapsa sp.* which is somewhat positively associated with temperature and total phosphorous, and negatively associated with natural land-cover. Genera from the same phytoplankton or zooplankton groups tended not to cluster together, but there were some notable exceptions. The only two diatom genera identified clustered together and were positively associated with calcium. All cryptophyte taxa were negatively associated with total phosphorous, and all rotifers, with the exception of one taxon, clustered together opposite the calcium vector.



ACA = Aphanocapsa, ATH = Aphanothece, CMD = Chlamydomonas, CHL = Chlorella, CRC = Chroococcus, CRO = Chroomonas, CRY = Cryptomonas, CYA = Cyanodictyon, DIN = Dinobryon, DOL = Dolichospermum, FRA = Fragillaria, GLE = Gleocapsa, GOM = Gomphosphaeria, LYN = Lyngbya, MEL = Melosira, MER = Merismopedia, MIC = Microcystis, NAN = Nanoflagellate, CBG = Colonial BG, UNG = Unicell Green, ACR = Acroperus, BOS = Bosmina, CER = Ceriodaphnia, CHY = Chydorus, DAP = Daphnia, DIA = Diaphanosoma, EUB = Eubosmina, HOL = Holopedium, CAL = Calanoid, COP = Copepod young, CYC = Cyclopid, ASP = Asplanchna, FIL = Filina, GAS = Gastropus, KEL = Kellcottia, KER = Keratella, MON = Monostyla, POL = Polyarthra, POM = Pompholyx, TRI = Trichocerca

Figure 5.1 Redundancy analysis of top 20 zooplankton and phytoplankton taxa at the genus level with water quality and land-use vectors. Overlaid ellipses group low, medium, and high-calcium lakes. Count data was transformed using Hellinger transformation to give low weights to rare species.

The correlation matrix resulting from the GLLVM revealed a variety of significant positive and negative interactions between plankton taxa once the influence of environmental parameters had been considered (Figure 5.2a). There is a cluster of negative interactions towards the center of the GLLVM triangle matrix involving several cladoceran and copepod taxa and several rotifer and colonial phytoplankton taxa. Clusters of positive interactions are present along the right side of the figure and occur between all kinds of taxa. When Figure 5.2a is compared to the correlation triangle matrix where environmental parameters as latent variables have not been considered (Figure 5.2b), many similar interactions are observed, indicating that community structure is influenced by both biotic interactions between taxa and environmental conditions in each lake.

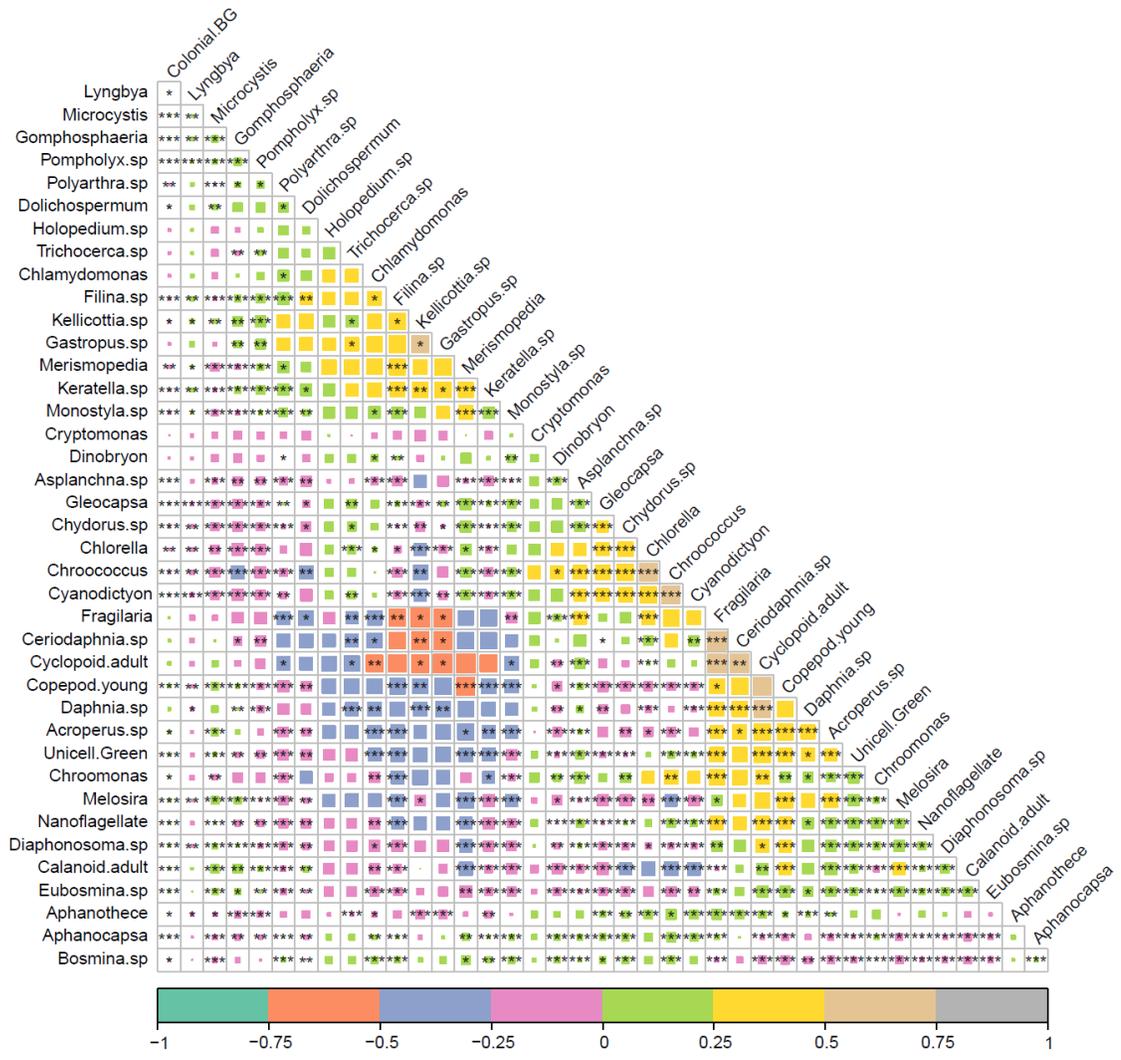


Figure 5.2a General linear latent variable model output to visualize taxa interactions across the top 20 phytoplankton and top 20 zooplankton across TLB study lakes (n=60) when environmental parameters are accounted for. The GLLVM model was constructed using a negative binomial distribution using seven of the original environmental covariates included in analysis: depth, Secchi depth, temperature, TP, TN, chlorophyll a, and chloride. A heatmap of the strength of correlations between the plankton taxa is presented, with correlations between species depicted by colors and asterisks (*) denoting statistical significance ($\alpha=0.05$): * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.001 .

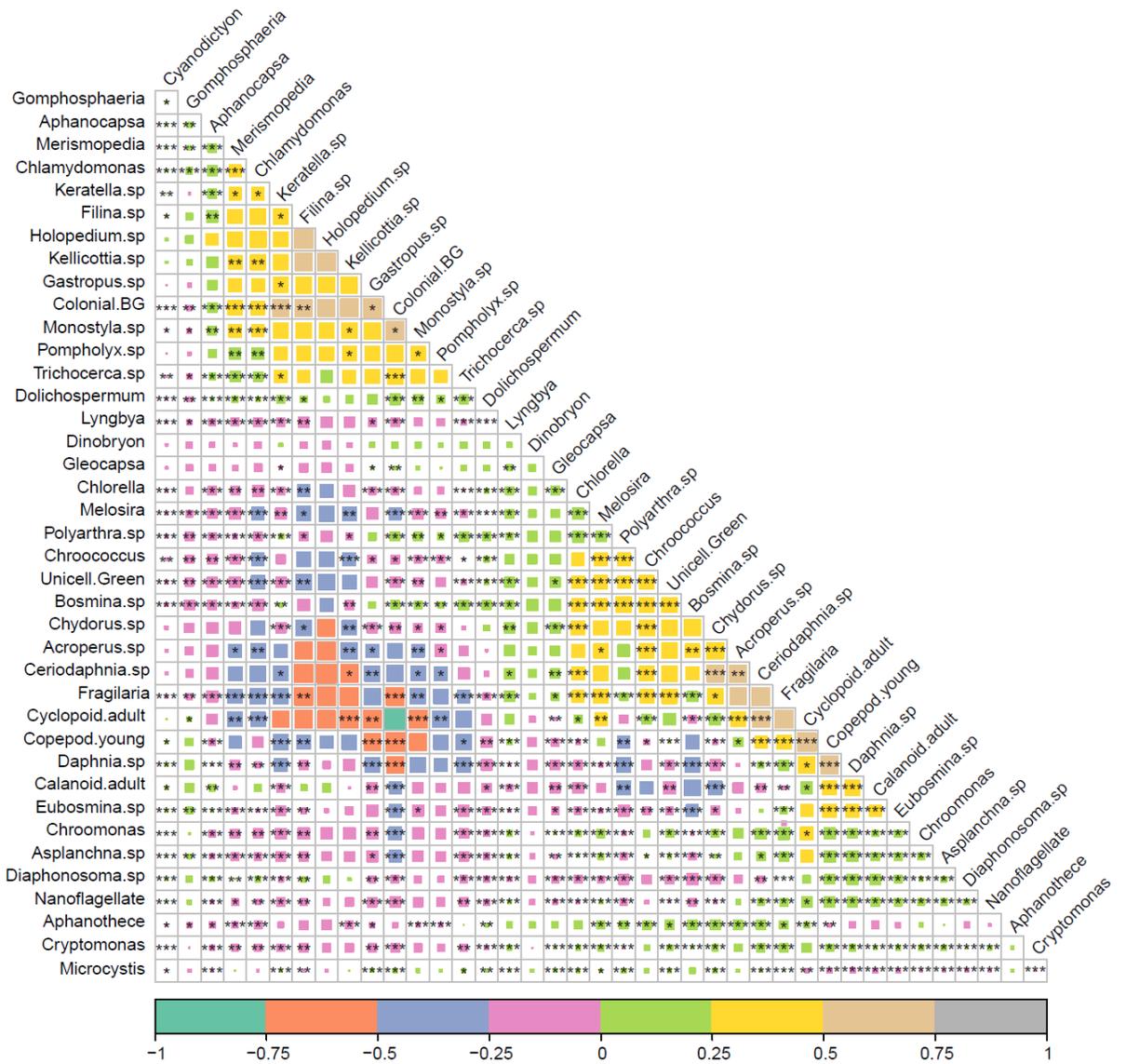


Figure 5.2b General linear latent variable model output to visualize taxa interactions across the top 20 phytoplankton and top 20 zooplankton across TLB study lakes (n=60) when environmental parameters are not accounted for. A heatmap of the strength of correlations between the plankton taxa is presented, with correlations between species depicted by colors and asterisks (*) denoting statistical significance ($\alpha=0.05$): * ≤ 0.05 , ** ≤ 0.01 , *** ≤ 0.001 .

5.4 Discussion

Examining the roles of abiotic and biotic drivers on plankton community structure

As can be seen in the RDA (Figure 5.1), abiotic drivers appear to have a varied influence on plankton community structure in TLB because many taxa are clustered towards the center of the biplot. Some taxa do pull out of the general cluster, but no clear community profiles can be associated with any one variable including calcium levels. Among the environmental parameters included in the analysis, calcium and total phosphorous appear to be the strongest drivers of variation in the data, though other variables contribute as well. Similar patterns emerged from what has been observed in previous chapters, such as several rotifers being negatively associated with calcium, and other individual zooplankton and phytoplankton taxa having a variety of positive and negative relationships with the environmental parameters in the biplot. Some habitat filtering may be occurring within TLB lakes, evidenced by the affiliation of certain taxa with individual water quality parameters (e.g., rotifers have a negative association with calcium, so appear more frequently in low-calcium lakes). However, aside from a few individual taxa, overall communities do not appear particularly affiliated with calcium categories or water quality profiles within the lakes. As most taxa appear to cluster together towards the center of the biplot, it is instead possible that other drivers such as variables not captured or not examined are structuring plankton communities.

The RDA cannot be used to determine factors beyond the effects of the selected abiotic drivers, such as the effects of taxa-specific interactions. For example, both cladoceran zooplankton *Bosmina sp.* and *Acroperus sp.* appear to be positively associated with TP, but their relationship with each other is unclear. Such taxa-specific interactions

may play an important role in determining community structure and should therefore be considered when investigating plankton communities in TLB.

Applying the same water quality and plankton data to a generalized linear latent variable model (GLLVM) allowed taxa-specific interactions to be investigated. Figure 5.2a represents taxa-specific correlations when environmental parameters have been considered, and therefore represents the actual correlations between plankton taxa. The role of environmental parameters in driving plankton community co-occurrence patterns can be examined by comparing Figure 5.2a to its counterpart 5.2b, which shows co-occurrence patterns when environmental parameters are not accounted for in the heatmap matrix (Niku et al., 2019). These two figures are very similar, which means that plankton community structure is not driven exclusively by environmental conditions, but rather biotic interactions that affect taxa co-occurrence are important as well. As such, while previous regression models and RDAs (see Chapters 3 and 4) indicated that environmental parameters are important drivers of plankton community abundance and composition, the plankton communities in TLB also appear to be influenced by taxa-specific interactions, and these interactions should be considered when characterizing these communities across TLB.

Investigating co-occurrence patterns between plankton taxa

The heatmap triangle matrix displayed in Figure 5.2a shows a number of positive and negative correlations between plankton taxa co-occurrence patterns. Among phytoplankton taxa, a variety of positive, negative, and neutral correlations emerged. The majority of perceived interactions between phytoplankton taxa were neutral, indicating that many of these species are capable of coexisting without any obvious effects of

competition or mutualistic relationships. These phytoplankton taxa may simply occupy different niches, where they have different requirements for survival and therefore do not overlap in ecosystems. This coexistence could also be the result of habitat heterogeneity, where variation in habitat conditions across space and time allows them to coexist. Since phytoplankton communities were based on composite water samples representing different depths in the water column, it is possible that taxa with high light requirements and low light requirements were both captured. These taxa would likely not co-exist together because they would be located in different parts of the water column according to light preference (Reynolds, 1997). Finally, there may be other factors that relieve these taxa from competition, such as predation which could keep competing populations from reaching abundances high enough to displace each other. Among the phytoplankton taxa included in the GLLVM, cryptophytes, chrysophytes, and dinoflagellates appeared to have almost exclusively neutral interactions with other phytoplankton. Chrysophytes and dinoflagellates are both mixotrophic (Wehr et al., 2015), potentially resulting in reduced competition with obligate autotrophs. Cryptophytes are motile and exhibit vertical migration, which could reduce competition with passively sessile taxa.

Some phytoplankton taxa did exhibit either positive or negative interactions with others, and these were mostly cyanobacteria, chlorophytes, and diatoms. Aside from cyanobacteria and diatoms, which had either neutral or negative interactions between taxa, taxa from these groups exhibited both positive and negative interactions with each other. Negative interactions among phytoplankton may indicate competition, where the two taxa are occupying similar niches and competing for the same resources. In these cases, one taxon could be able to outcompete the other, resulting in competitive

exclusion, where the less competitive taxa's population is either reduced or eliminated from the site. For example, the significant negative correlation detected between *Fragilaria sp.* and *Dolichospermum sp.* may be the result of both occupying similar mesotrophic sites (Li et al., 2016; Wehr et al., 2015), and therefore competing for the same resources. The positive interactions detected among phytoplankton taxa indicate potential mutualistic or commensal interactions. Although it is assumed most phytoplankton do not directly interact with one another, it may be possible that mutualism between different species does occur. Alternatively, these positive relationships may be the result of two coexisting taxa displacing a mutual competitor. For example, *Fragilaria sp.* and unicellular greens have a positive relationship, and both have a negative relationship with *Chlamydomonas sp.*, so they may be positively influencing each other by reducing *Chlamydomonas sp.* abundance. Alternatively, these positive relationships may be the result of undetected environmental effects that were not accounted for in the study, such as effects of bacteria, macrophytes, or grazing which could alter phytoplankton communities and species co-occurrence patterns.

Zooplankton taxa also showed a variety of correlations with other zooplankton. Rotifers appear to correlate with other rotifers mostly positively, but have primarily negative interactions with large cladocerans and copepods. This could be the result of competition, as zooplankton primarily compete for food, and cladocerans and copepods have more efficient feeding strategies than rotifers (Hurlbert et al., 1972). These negative correlations may also be the result of predation from copepods (Williamson & Butler, 1986) or physical damage to rotifer bodies caused by the feeding apparatus of large cladocerans (Gilbert & Stemberger, 1985). Rotifers appear to mostly coexist with small

cladocerans such as *Bosmina sp.*, which is consistent with a study by MacIsaac & Gilbert (1989) which found that small cladocerans can coexist somewhat with rotifers due to specialized feeding strategies. Copepods vary in their relationships with cladocerans; adult cyclopoids have strong positive correlations with cladocerans, while adult calanoids display varied relationships with cladocerans. This may be the result of the overlap between the feeding strategies between cladocerans and calanoids, which are both capable of filter-feeding, and this could create competition between the two. Cyclopoids are obligate raptorial feeders (Allan, 1976), so there would be less niche overlap between them and cladocerans, which are filter-feeders. Cladocerans have mostly positive correlations with each other, with the exception of *Bosmina sp.* and *Chydorus sp.* which are negatively correlated with most other cladocerans. It has been posited that coexistence among cladocerans is possible due to habitat partitioning and alterations in vertical migration patterns to minimize overlap in food sources (Lane et al., 1978), or the development of specialized feeding strategies (Demott & Kerfoot, 1982), which may be why these taxa appear to be capable of coexisting. Alternatively, other factors which could not be observed in the scope of the study such as the effects of habitat structure or fish predation may be influencing cladoceran taxa dynamics.

The interactions between phytoplankton and zooplankton are quite variable. Many of the most abundant phytoplankton taxa selected for this analysis were colonial cyanobacteria, which are rarely the preferred food for zooplankton due to their poor nutritional value as well as their large colonies which can inhibit the filter-feeding apparatus of certain cladocerans (Burns, 1968). The relationships between cyanobacteria and zooplankton have thus been noted to be complex, as cyanobacteria are a poor food

source for zooplankton and can actively harm them in some cases (Burns, 1968; Nogueira et al., 2004), but zooplankton can indirectly promote their growth by selectively feeding on other phytoplankton and therefore reducing competition for cyanobacteria (Wang et al., 2010). In TLB, most correlations between zooplankton and cyanobacteria were neutral or weakly positive or negative, indicating that there may not be strong interactions between them. *Microcystis* and *Dolichospermum* are two cyanobacterial taxa that can produce toxins, but they were not particularly abundant in the study lakes, which may be why they lack strong interactions with zooplankton.

The unicellular green phytoplankter *Chlamydomonas* had moderate negative relationships with multiple copepod and cladoceran taxa, indicating that those zooplankton may be preferentially feeding on it. *Chlamydomonas* has been found to be a high-quality food source for zooplankton (Mitchell et al., 1992). It had a somewhat positive correlation with rotifers, indicating that they may not be feeding on it like cladocerans and copepods. Other potential high-quality foods such as *Chlorella* or *Chroomonas* appear to be negatively correlated with rotifers and positively correlated with cladocerans and copepods. This distinction is surprising, as it would be expected that all zooplankton would be negatively correlated with these phytoplankton as they are acceptable food sources. It is possible that some selective feeding is occurring where cladocerans and copepods select different types of foods than rotifers, or there may be other underlying effects that are not captured in the scope of this study. Rotifers also had strong negative relationships with *Fragilaria*, a large diatom, which was unexpected because large diatoms are typically only consumed by copepods (Knisely & Geller,

1986). Due to *Fragilaria* typically exceeding the maximal size that rotifers can consume (up to 20um) (Allan, 1976), it is likely that this relationship is indirect.

The nature of the relationships between phytoplankton and zooplankton are complex and may be influenced by factors beyond the scope of this study. Although it cannot be ascertained whether the correlations observed are direct or indirect, the finding that these communities are driven in part by taxa-specific co-occurrence is very notable. These results not only provide baseline information about taxon co-occurrence, but also the relative role of environmental variables such as calcium and nutrients. In combination, this information provides a more comprehensive picture of plankton communities across TLB and suggests that management strategies may want to consider both abiotic and biotic drivers to understand plankton dynamics in these lakes.

Chapter 6: General Conclusion

The Land Between ecotone is a valuable region in Ontario not only as a biodiversity hotspot, but the diverse landscape attracts visitors who support local economies through tourism revenue and seasonal property taxes. Since the lakes across TLB are an important feature attracting visitors to the area, as well as a growing permanent resident population, an improved understanding of lake ecological condition is warranted. The goal of my research was to characterize water quality and plankton communities across TLB ecotone for the first time, in order to not only characterize their community structure, but to determine what environmental parameters may be important for structuring these biological communities. This research provides valuable baseline information about these lakes, and also provides important information to lake managers that can be used to protect or maintain lake health.

When examining water quality across the study lakes, lakes in the low calcium category generally had more natural land-cover, higher water clarity, and fewer nutrients, while high-calcium lakes had more nutrients and more urban and agricultural land-use. Lakes with medium calcium levels were highly variable with respect to water quality profiles. I also found that when considering the effects of land-use on water quality, natural land-cover appears to play an important role in mitigating nutrient levels in lakes, which means that as land-use expands throughout TLB, lakes may experience a shift in water quality towards undesirable conditions.

When examining the influence of bottom-up drivers on phytoplankton, total phosphorous emerged as the limiting nutrient for phytoplankton biomass, while total nitrogen had a positive effect on phytoplankton diversity. Phytoplankton biomass appears

to be controlled somewhat by zooplankton grazing, indicating that top-down drivers of plankton communities are important as well. Additionally, temperature negatively impacted both phytoplankton diversity and biomass, which means that rising water temperatures which are anticipated with climate change, may significantly affect food webs in TLB and potentially harm higher trophic levels.

Much like phytoplankton, zooplankton communities were not driven by regional spatial structure, but instead were driven by local environmental conditions. While several water quality parameters influenced zooplankton community composition, calcium emerged as an important variable for rotifers, where rotifer biomass was negatively associated with calcium and community structure was highly variable along the calcium gradient. Overall, calcium and conductivity emerged as significant positive predictors of zooplankton biomass, while phytoplankton biomass was a significant negative predictor. The role of top-down effects on zooplankton could not be explored due to limitations in the study design.

When investigating the role of species-specific interactions on plankton community structure, I found that plankton co-occurrence patterns are not driven exclusively by environmental parameters, meaning that species-specific interactions are important to consider when studying lakes in TLB. Both species-specific (i.e., phytoplankton-phytoplankton, zooplankton-zooplankton) and trophic interactions (zooplankton-phytoplankton) emerged in this study. The role of taxa-specific interactions on biological communities are often overlooked in the literature in favor of studying bottom-up drivers. Therefore, the results of my research demonstrate that biological co-occurrence patterns should not be overlooked in the future as they do appear to play a

significant role in plankton community structure in TLB, even if the interactions are not fully understood at this time.

I recognize that there were certain limitations in this study related to trade-offs in study design and duration. Water quality and plankton communities change seasonally, and while capturing all the data in a three-week period provides comparable data across the 60 study lakes, singular sampling events in each lake likely resulted in certain temporal changes or population peaks being missed. Additionally, the macrophyte communities at each sampling location were not included in this study, but aquatic vegetation can play a role in phytoplankton and zooplankton community structure. For example, macrophytes compete with phytoplankton for nutrients and light, and also provide shelter to zooplankton from predation. Another factor which was not measured in this study was fish predation. Fish predation can play a significant role in the top-down control of plankton communities, such as driving plankton communities towards smaller species or relieving phytoplankton from grazing pressure. While bottom-up drivers of plankton communities were shown in this study to be important, top-down effects were limited to zooplankton-phytoplankton trophic interactions.

Future considerations which address the limitations of this study could provide valuable additions to the conclusions of my research. Determining how the water quality and plankton communities of these lakes vary temporally by studying a subset of lakes throughout a whole season or a whole year could provide additional knowledge regarding their water quality and plankton community drivers. Additionally, gathering data on more biological organisms such as macrophytes and fish could also fill in the gaps encountered

by this research, especially regarding the top-down effects of predation on zooplankton communities.

A conclusion which should be explored further is the relationship between land-use and nutrients. While it was expected that agricultural and developed land-use would be positively associated with nutrients, the stronger relationship was the negative association between natural land-cover and nutrients. While the nature of this relationship can be speculated, it cannot be known for sure why it emerged based on the current study design. In the current study design, all land-use and land-cover were pooled into either natural land-cover, agricultural land-use, or developed land-use to study high-level effects of land-use in whole watersheds. However, it is possible that different types of land-use impact lakes to varying degrees, and so pooling them into larger categories may have concealed major sources of nutrient pollution into the lakes. Breaking down the components of land-use and determining which are the biggest sources of nutrients would provide information to lake managers to manage and mitigate nutrient pollution into TLB lakes. Additionally, proximity of land-use to the lakes may influence this relationship as well, so studying land-use and land-cover of the direct shoreline of these lakes instead of the whole watershed may provide further insight about mitigating nutrient pollution into TLB lakes.

To conclude, my research has provided significant insight into the water quality and plankton communities of TLB. Increasing urbanization and the effects of climate change threaten TLB as a whole, and so it is important to preserve and maintain natural lake ecosystems as much as possible. My research has provided important baseline information regarding the current status of these lakes, as well as insight into the

important drivers of lake water quality and plankton communities, which can be used by lake managers to maintain the lakes of TLB in the future.

References

- Adamczuk, M. (2016). Past, present, and future roles of small cladoceran *Bosmina longirostris* (O. F. Müller, 1785) in aquatic ecosystems. In *Hydrobiologia* (Vol. 767, Issue 1). <https://doi.org/10.1007/s10750-015-2495-7>
- Agrawal, S. C. (2012). Factors controlling induction of reproduction in algae--review: the text. *Folia Microbiologica*, 57(5), 387–407. <https://doi.org/10.1007/s12223-012-0147-0>
- Alibone, M. R., & Fair, P. (1981). The effects of low pH on the respiration of *Daphnia magna* Straus. *Hydrobiologia*, 85(2), 185–188. <https://doi.org/10.1007/BF00006629>
- Allan, J. D. (1976). Life History Patterns in Zooplankton. *The American Naturalist*, 110(971), 165–180. <http://www.jstor.org/stable/2459885>
- Arnér, M., & Koivisto, S. (1993). Effects of salinity on metabolism and life history characteristics of *Daphnia magna*. *Hydrobiologia*, 259(2), 69–77. <https://doi.org/10.1007/BF00008373>
- Arnold, D. E. (1971). Ingestion, Assimilation, Survival, and Reproduction by *Daphnia pulex* Fed Seven Species of Blue-Green Algae. *Limnology and Oceanography*, 16(6), 906–920. <http://www.jstor.org.uproxy.library.dc-uoit.ca/stable/2834296>
- Ashforth, D., & Yan, N. D. (2008). The interactive effects of calcium concentration and temperature on the survival and reproduction of *Daphnia pulex* at high and low food concentrations. *Limnology and Oceanography*, 53(2), 420–432. <https://doi.org/10.4319/lo.2008.53.2.0420>
- Atkinson, D. (1994). Temperature and Organism Size—A Biological Law for Ectotherms? In M. Begon & A. H. Fitter (Eds.), *Advances in Ecological Research* (Vol. 25, pp. 1–58). Academic Press. [https://doi.org/https://doi.org/10.1016/S0065-2504\(08\)60212-3](https://doi.org/https://doi.org/10.1016/S0065-2504(08)60212-3)
- Azan, S. S. E., Arnott, S. E., & Yan, N. D. (2015). A review of the effects of *Bythotrephes longimanus* and calcium decline on zooplankton communities — can interactive effects be predicted? *Environmental Reviews*, 23(4), 395–413. <https://doi.org/10.1139/er-2015-0027>
- Baker, A. L. (2012). *Phycokey -- an image based key to Algae (PS Protista), Cyanobacteria, and other aquatic objects*. University of New Hampshire Center for Freshwater Biology.
- Balian, E. V, Segers, H., Lévèque, C., & Martens, K. (2008). The Freshwater Animal Diversity Assessment: an overview of the results. *Hydrobiologia*, 595(1), 627–637. <https://doi.org/10.1007/s10750-007-9246-3>

- Barica, J. (1975). Collapses of algal blooms in prairie pothole lakes: their mechanism and ecological impact. *SIL Proceedings, 1922-2010*, 19(1), 606–615. <https://doi.org/10.1080/03680770.1974.11896103>
- Baumann, P. C., & Kitchell, J. F. (1974). Diel Patterns of Distribution and Feeding of Bluegill (*Lepomis macrochirus*) in Lake Wingra, Wisconsin. *Transactions of the American Fisheries Society*, 103(2), 255–260. [https://doi.org/10.1577/1548-8659\(1974\)103<255:DPODAF>2.0.CO;2](https://doi.org/10.1577/1548-8659(1974)103<255:DPODAF>2.0.CO;2)
- Baustian, J. J., Kowalski, K. P., & Czayka, A. (2018). Using Turbidity Measurements to Estimate Total Phosphorus and Sediment Flux in a Great Lakes Coastal Wetland. *Wetlands*, 38(5), 1059–1065. <https://doi.org/10.1007/s13157-018-1044-3>
- Beck, M. W. (2016). ggord: Ordination Plots with ggplot2. *R Package Version 0.11*.
- Bishara, A. J., & Hittner, J. B. (2017). Confidence intervals for correlations when data are not normal. *Behavior Research Methods*, 49(1). <https://doi.org/10.3758/s13428-016-0702-8>
- Bivand, R. S., Lewin-Koh, N., Pebesma, E., Archer, E., Baddeley, A., Bearman, N., Bibiko, H.-J., Brey, S., Callahan, J., Carrillo, G., Dray, S., Forrest, D., Friendly, M., Giraudoux, P., Golicher, D., Rubio, V. G., Hausmann, P., Hufthammer, K. O., Jagger, T., ... Turner, R. (2016). Tools for Reading and Handling Spatial Objects: Package “maptools.” *R Documentation*.
- Bivand, R. S., & Wong, D. W. S. (2018). Comparing implementations of global and local indicators of spatial association. *Test*, 27(3). <https://doi.org/10.1007/s11749-018-0599-x>
- Brauns, M., Gücker, B., Wagner, C., Garcia, X.-F., Walz, N., & Pusch, M. T. (2011). Human lakeshore development alters the structure and trophic basis of littoral food webs. *Journal of Applied Ecology*, 48(4), 916–925. <https://doi.org/10.1111/j.1365-2664.2011.02007.x>
- Brett, M., & Müller-Navarra, D. (1997). The role of highly unsaturated fatty acids in aquatic foodweb processes. *Freshwater Biology*, 38(3), 483–499. <https://doi.org/https://doi.org/10.1046/j.1365-2427.1997.00220.x>
- Brooks, J. L., & Dodson, S. I. (1965). Predation, Body Size, and Composition of Plankton. *Science*, 150(3692), 28–35. <http://www.jstor.org.uproxy.library.dcuoit.ca/stable/1717947>
- Burns, C. W. (1968). Direct Observations of Mechanisms Regulating Feeding Behavior of *Daphnia*, in Lakewater. *Internationale Revue Der Gesamten Hydrobiologie Und Hydrographie*, 53(1). <https://doi.org/10.1002/iroh.19680530104>
- Butterwick, C., Heaney, S. I., & Talling, J. F. (2005). Diversity in the influence of temperature on the growth rates of freshwater algae, and its ecological relevance. *Freshwater Biology*, 50(2). <https://doi.org/10.1111/j.1365-2427.2004.01317.x>

- Campbell, P. G. C., & Stokes, P. M. (1985). Acidification and toxicity of metals to aquatic biota. *Canadian Journal of Fisheries and Aquatic Sciences*, 42(12). <https://doi.org/10.1139/f85-251>
- Canadian Council of Ministers of the Environment. (2011). Canadian Water Quality Guidelines for the Protection of Aquatic Life: Chloride. *Canadian Environmental Quality Guidelines*.
- Caraco, N. F., & Cole, J. J. (2002). Contrasting impacts of a native and alien macrophyte on dissolved oxygen in a large river. *Ecological Applications*, 12(5). [https://doi.org/10.1890/1051-0761\(2002\)012\[1496:CIOANA\]2.0.CO;2](https://doi.org/10.1890/1051-0761(2002)012[1496:CIOANA]2.0.CO;2)
- Carlson, R. E. (1977). A Trophic State Index for Lakes. *Limnology and Oceanography*, 22(2), 361–369. <http://www.jstor.org.uproxy.library.dc-uoit.ca/stable/2834910>
- Carrillo, P., Reche, I., Sanchez-castillo, P., & Cruz-pizarro, L. (1995). Direct and indirect effects of grazing on the phytoplankton seasonal succession in an oligotrophic lake. *Journal of Plankton Research*, 17(6). <https://doi.org/10.1093/plankt/17.6.1363>
- Chen, C. Y., & Durbin, E. (1994). Effects of pH on the growth and carbon uptake of marine phytoplankton. *Marine Ecology-Progress Series - MAR ECOL-PROGR SER*, 109, 83–94. <https://doi.org/10.3354/meps109083>
- C.O'Keefe, T., C.Brewer, M., & Dodson, S. I. (1998). Swimming behavior of Daphnia: its role in determining predation risk. *Journal of Plankton Research*, 20(5), 973–984. <https://doi.org/10.1093/plankt/20.5.973>
- Cottingham, K. L., Carpenter, S. R., & Amand, A. L. St. (1998). Responses of epilimnetic phytoplankton to experimental nutrient enrichment in three small seepage lakes. *Journal of Plankton Research*, 20(10), 1889–1914. <https://doi.org/10.1093/plankt/20.10.1889>
- Cowgill, U. M. (1976). The chemical composition of two species of Daphnia, their algal food and their environment. *Science of The Total Environment*, 6(1), 79–102. [https://doi.org/https://doi.org/10.1016/0048-9697\(76\)90008-5](https://doi.org/https://doi.org/10.1016/0048-9697(76)90008-5)
- Demott, W. R., & Kerfoot, W. C. (1982). Competition among cladocerans: nature of the interaction between Bosmina and Daphnia. *Ecology*, 63(6). <https://doi.org/10.2307/1940132>
- Deng, D., Xie, P., Zhou, Q., Yang, H., Guo, L., & Geng, H. (2008). Field and experimental studies on the combined impacts of cyanobacterial blooms and small algae on crustacean zooplankton in a large, eutrophic, subtropical, Chinese lake. *Limnology*, 9(1), 1–11. <https://doi.org/10.1007/s10201-007-0229-x>
- Dodson, S. I., Arnott, S. E., & Cottingham, K. L. (2000). The Relationship in Lake Communities between Primary Productivity and Species Richness. *Ecology*, 81(10), 2662–2679. <https://doi.org/10.2307/177332>

- Dodson, S. I., Lillie, R. A., & Will-Wolf, S. (2005). Land use, water chemistry, aquatic vegetation, and zooplankton community structure of shallow lakes. *Ecological Applications*, 15(4). <https://doi.org/10.1890/04-1494>
- Dray, S., Bauman, D., Blanchet, G., Borcard, D., Clappe, S., Guenard, G., Jombart, T., Larocque, G., Legendre, P., Wagner, N., & Wagner, H. H. (2019). Adespatial: Multivariate Multiscale Spatial Analysis. R package version 0.3-7. *Ecological Monographs*, 82(3).
- Dray, S., & Dufour, A. B. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22(4). <https://doi.org/10.18637/jss.v022.i04>
- Duarte, C., Agustí, S., & Agawin, N. S. (2000). Response of a Mediterranean phytoplankton community to increased nutrient inputs: A mesocosm experiment. *Marine Ecology-Progress Series - MAR ECOL-PROGR SER*, 195, 61–70. <https://doi.org/10.3354/meps195061>
- Elliott, J. A., Jones, I. D., & Thackeray, S. J. (2006). Testing the Sensitivity of Phytoplankton Communities to Changes in Water Temperature and Nutrient Load, in a Temperate Lake. *Hydrobiologia*, 559(1), 401–411. <https://doi.org/10.1007/s10750-005-1233-y>
- Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., & Smith, J. E. (2007). Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecology Letters*, 10(12), 1135–1142. <https://doi.org/https://doi.org/10.1111/j.1461-0248.2007.01113.x>
- Environment Canada. (2012). *Five-year review of progress: code of practice for the environmental management of road salts*.
- Evans, M., & Frick, C. (2001). The Effects of Road Salts on Aquatic Ecosystems. *WSTD Contribution No. 02-308, 02*.
- Fay, L., & Shi, X. (2012). Environmental impacts of chemicals for snow and ice control: State of the knowledge. *Water, Air, and Soil Pollution*, 223(5). <https://doi.org/10.1007/s11270-011-1064-6>
- Fischer, J. M., Klug, J. L., Ives, A. R., & Frost, T. M. (2001). Ecological history affects zooplankton community responses to acidification. *Ecology*, 82(11), 2984–3000. [https://doi.org/https://doi.org/10.1890/0012-9658\(2001\)082\[2984:EHAZCR\]2.0.CO;2](https://doi.org/https://doi.org/10.1890/0012-9658(2001)082[2984:EHAZCR]2.0.CO;2)
- Flöder, S., Urabe, J., & Kawabata, Z. (2002). The Influence of Fluctuating Light Intensities on Species Composition and Diversity of Natural Phytoplankton Communities. *Oecologia*, 133(3), 395–401. <http://www.jstor.org.uproxy.library.dcuoit.ca/stable/4223433>

- Forrest, J., & Arnott, S. E. (2006). Immigration and zooplankton community responses to nutrient enrichment: a mesocosm experiment. *Oecologia*, *150*(1), 119–131. <https://doi.org/10.1007/s00442-006-0490-4>
- Forster, J., Hirst, A. G., & Atkinson, D. (2012). Warming-induced reductions in body size are greater in aquatic than terrestrial species. *Proceedings of the National Academy of Sciences*, *109*(47), 19310. <https://doi.org/10.1073/pnas.1210460109>
- Francis, T. B., Wolkovich, E. M., Scheuerell, M. D., Katz, S. L., Holmes, E. E., & Hampton, S. E. (2014). Shifting regimes and changing interactions in the Lake Washington, U.S.A., plankton community from 1962-1994. *PLoS ONE*, *9*(10). <https://doi.org/10.1371/journal.pone.0110363>
- Gayosso-Morales, M. A., Nandini, S., Martínez-Jeronimo, F. F., & Sarma, S. S. S. (2017). Effect of organic and inorganic turbidity on the zooplankton community structure of a shallow waterbody in Central Mexico (Lake Xochimilco, Mexico). *Journal of Environmental Biology*, *38*(6 (Special Issue)), 1183–1196. [https://doi.org/10.22438/jeb/38/6\(si\)/03](https://doi.org/10.22438/jeb/38/6(si)/03)
- Ger, K. A., Hansson, L.-A., & Lüring, M. (2014). Understanding cyanobacteria-zooplankton interactions in a more eutrophic world. *Freshwater Biology*, *59*(9), 1783–1798. <https://doi.org/https://doi.org/10.1111/fwb.12393>
- Gerloff, G. C., & Fishbeck, K. A. (1969). Quantitative cation requirements of several green and blue-green algae. *Journal of Phycology*, *5*(2), 109–114. <https://doi.org/https://doi.org/10.1111/j.1529-8817.1969.tb02587.x>
- Gilbert, J. J., & Stemberger, R. S. (1985). Control of *Keratella* Populations by Interference Competition from *Daphnia*. *Limnology and Oceanography*, *30*(1), 180–188. <http://www.jstor.org.proxy.library.dc-uoit.ca/stable/2836225>
- Gildow, M., Aloysius, N., Gebremariam, S., & Martin, J. (2016). Fertilizer placement and application timing as strategies to reduce phosphorus loading to Lake Erie. *Journal of Great Lakes Research*, *42*(6), 1281–1288. <https://doi.org/https://doi.org/10.1016/j.jglr.2016.07.002>
- Gophen, M. (2018). Temperature dependence of food intake, ammonia excretion and respiration in *Ceriodaphnia reticulata* (Jurine) (Lake Kinneret, Israel)*. *Freshwater Biology*, *6*(5), 451–455. <https://doi.org/10.1111/j.1365-2427.1976.tb01634.x>
- Greenaway, P. (1985). Calcium Balance and Moulting in the Crustacea. *Biological Reviews*, *60*(3), 425–454. <https://doi.org/10.1111/j.1469-185X.1985.tb00424.x>
- Haney, J. F. et al. (2013). *An-Image-based Key to the Zooplankton of North America*.
- Hansson, L. A. (1992). The role of food chain composition and nutrient availability in shaping algal biomass development. *Ecology*, *73*(1). <https://doi.org/10.2307/1938735>

- Hansson, L.-A., Gustafsson, S., Rengefors, K., & Bomark, L. (2007). Cyanobacterial chemical warfare affects zooplankton community composition. *Freshwater Biology*, 52(7), 1290–1301. <https://doi.org/https://doi.org/10.1111/j.1365-2427.2007.01765.x>
- Hardin, G. (1960). The competitive exclusion principle. *Science*, 131(3409). <https://doi.org/10.1126/science.131.3409.1292>
- Harrell, F. E., & Dupont, C. (2016). Package ‘Hmisc’: Harrell Miscellaneous. *R Topics Documented*.
- Hays, G. C. (2003). A review of the adaptive significance and ecosystem consequences of zooplankton diel vertical migrations. *Hydrobiologia*, 503. <https://doi.org/10.1023/B:HYDR.00000008476.23617.b0>
- Henderson, R. J., & Mackinlay, E. E. (1989). Effect of temperature on lipid composition of the marine cryptomonad *Chroomonas salina*. *Phytochemistry*, 28(11), 2943–2948. [https://doi.org/https://doi.org/10.1016/0031-9422\(89\)80258-4](https://doi.org/https://doi.org/10.1016/0031-9422(89)80258-4)
- Hessen, D. O., Alstad, N. E. W., & Skardal, L. (2000). Calcium limitation in *Daphnia magna*. *Journal of Plankton Research*, 22(3), 553–568. <https://doi.org/10.1093/plankt/22.3.553>
- Hessen, D. O., Faafeng, B. A., & Andersen, T. (1995). Competition or niche segregation between *Holopedium* and *Daphnia*; empirical light on abiotic key parameters. *Hydrobiologia*, 307(1), 253–261. <https://doi.org/10.1007/BF00032019>
- Hintz, W. D., Mattes, B. M., Schuler, M. S., Jones, D. K., Stoler, A. B., Lind, L., & Relyea, R. A. (2016). Salinization triggers a trophic cascade in experimental freshwater communities with varying food-chain length. *Ecological Applications*, 27(3), 833–844. <https://doi.org/10.1002/eap.1487>
- 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. <http://www.jstor.org/stable/26155920>
- Hintz, W. D., & Relyea, R. A. (2019). A review of the species, community, and ecosystem impacts of road salt salinisation in fresh waters. *Freshwater Biology*, 64(6), 1081–1097. <https://doi.org/https://doi.org/10.1111/fwb.13286>
- Hörnström, E. (2002). Phytoplankton in 63 limed lakes in comparison with the distribution in 500 untreated lakes with varying pH. *Hydrobiologia*, 470. <https://doi.org/10.1023/A:1015619921119>
- Howarth, R. W., Sharpley, A., & Walker, D. (2002). Sources of Nutrient Pollution to Coastal Waters in the United States: Implications for Achieving Coastal Water Quality Goals. *Estuaries*, 25(4), 656–676. <http://www.jstor.org.uproxy.library.dc-uoit.ca/stable/1353025>

- Huisman, J., Jonker, R. R., Zonneveld, C., & Weissing, F. J. (1999). Competition for Light Between Phytoplankton Species: Experimental Tests of Mechanistic Theory. *Ecology*, 80(1), 211–222. [https://doi.org/https://doi.org/10.1890/0012-9658\(1999\)080\[0211:CFLBPS\]2.0.CO;2](https://doi.org/https://doi.org/10.1890/0012-9658(1999)080[0211:CFLBPS]2.0.CO;2)
- Hurlbert, S. H., Mulla, M. S., & Willson, H. R. (1972). Effects of an Organophosphorus Insecticide on the Phytoplankton, Zooplankton, and Insect Populations of Fresh-Water Ponds. *Ecological Monographs*, 42(3), 269–299. <https://doi.org/https://doi.org/10.2307/1942211>
- Hurley, C. (2012). gclus: Clustering Graphics. R package version 1.3. 1. *URL* [Http://CRAN.R-Project.Org/Package= Gclus](Http://CRAN.R-Project.Org/Package=Gclus).
- Ivanova, M. B., & Kazantseva, T. I. (2006). Effect of water pH and total dissolved solids on the species diversity of pelagic zooplankton in lakes: A statistical analysis. *Russian Journal of Ecology*, 37(4), 264–270. <https://doi.org/10.1134/S1067413606040084>
- James, G., Witten, D., Hastie, T., & Tibshirani, R. (2013). *An introduction to statistical learning* (Vol. 112). Springer.
- Jamieson, C., & Burns, C. (1988). The effects of temperature and food on copepodite development, growth and reproduction in three species of Boeckella (Copepoda; Calanoida). *Hydrobiologia*, 164(3), 235–257. <https://doi.org/10.1007/BF00005943>
- Jeppesen, E., Peder Jensen, J., SØndergaard, M., Lauridsen, T., & Landkildehus, F. (2001). Trophic structure, species richness and biodiversity in Danish lakes: changes along a phosphorus gradient. *Freshwater Biology*, 45(2), 201–218. <https://doi.org/10.1046/j.1365-2427.2000.00675.x>
- Jeziorski, A., Paterson, A. M., & Smol, J. P. (2012). Crustacean zooplankton sedimentary remains from calcium-poor lakes: complex responses to threshold concentrations. *Aquatic Sciences*, 74(1), 121–131. <https://doi.org/10.1007/s00027-011-0202-y>
- Jeziorski, A., Tanentzap, A. J., Yan, N. D., Paterson, A. M., Palmer, M. E., Korosi, J. B., Rusak, J. A., Arts, M. T., Keller, W. (Bill), Ingram, R., Cairns, A., & Smol, J. P. (2015). The jellification of north temperate lakes. *Proceedings: Biological Sciences*, 282(1798), 1–9. <http://www.jstor.org.uproxy.library.dc-uoit.ca/stable/43601865>
- Jeziorski, A., & Yan, N. D. (2006). Species identity and aqueous calcium concentrations as determinants of calcium concentrations of freshwater crustacean zooplankton. *Canadian Journal of Fisheries and Aquatic Sciences*, 63, 1007+. https://link.gale.com/apps/doc/A148482423/CPI?u=ko_acd_uoo&sid=bookmark-CPI&xid=097a9248
- Judd, K. E., Adams, H. E., Bosch, N. S., Kostrzewski, J. M., Scott, C. E., Schultz, B. M., Wang, D. H., & Kling, G. W. (2005). A Case History: Effects of Mixing Regime on Nutrient Dynamics and Community Structure in Third Sister Lake, Michigan During

- Late Winter and Early Spring 2003. *Lake and Reservoir Management*, 21(3), 316–329. <https://doi.org/10.1080/07438140509354437>
- Kassambara, A., Mundt, F., & Kassambara, A.; Mundt, F. (2017). Factoextra: extract and visualize the results of multivariate data analyses. *URL*
Http://Www.Sthda.Com/English/Rpkgs/Factoextra BugReports.
- Keller, W., & Yan, N. D. (1991). Recovery of Crustacean Zooplankton Species Richness in Sudbury Area Lakes following Water Quality Improvements. *Canadian Journal of Fisheries and Aquatic Sciences*, 48(9), 1635–1644. <https://doi.org/10.1139/f91-194>
- Knisely, K., & Geller, W. (1986). Selective feeding of four zooplankton species on natural lake phytoplankton. *Oecologia*, 69(1). <https://doi.org/10.1007/BF00399042>
- Knowlton, M. F., & Jones, J. R. (1996). Experimental evidence of light and nutrient limitation of algal growth in a turbid midwest reservoir mssub:1995-03-3. *Archiv Für Hydrobiologie*, 135(3), 321–335. <https://doi.org/10.1127/archiv-hydrobiol/135/1996/321>
- Korneva, L. G. (1996). Impact of acidification on structural organization of phytoplankton community in the forest lakes of the North-Western Russia. *Water Science and Technology*, 33(4), 291–296.
[https://doi.org/https://doi.org/10.1016/0273-1223\(96\)00243-0](https://doi.org/https://doi.org/10.1016/0273-1223(96)00243-0)
- Korosi, J. B., & Smol, J. P. (2012). A comparison of present-day and pre-industrial cladoceran assemblages from softwater Nova Scotia (Canada) lakes with different regional acidification histories. *Journal of Paleolimnology*, 47(1), 43–54.
<https://doi.org/10.1007/s10933-011-9547-4>
- Labaj, A. L., Jeziorski, A., Kurek, J., Bennett, J. R., Cumming, B. F., DeSellas, A. M., Korosi, J. B., Paterson, A. M., Sweetman, J. N., Thienpont, J. R., & Smol, J. P. (2021). Environmental drivers of cladoceran assemblages at a continental scale: A synthesis of Alaskan and Canadian datasets. *Freshwater Biology*, 66(5).
<https://doi.org/10.1111/fwb.13689>
- Lafrancois, B. M., Nydick, K. R., & Caruso, B. (2003). Influence of Nitrogen on Phytoplankton Biomass and Community Composition in Fifteen Snowy Range Lakes (Wyoming, U.S.A.). *Arctic, Antarctic, and Alpine Research*, 35(4).
[https://doi.org/10.1657/1523-0430\(2003\)035\[0499:ionopb\]2.0.co;2](https://doi.org/10.1657/1523-0430(2003)035[0499:ionopb]2.0.co;2)
- Lampert, W. (1987). Laboratory studies on zooplankton-cyanobacteria interactions. *New Zealand Journal of Marine and Freshwater Research*, 21(3).
<https://doi.org/10.1080/00288330.1987.9516244>
- Lampert, W. (1993). Ultimate causes of diel vertical migration of zooplankton: New evidence for the predator-avoidance hypothesis. *Arch. Hydrobiolo. Beih.*, 39.

- Lane, P. A., Makarewicz, J. C., & Likens, G. E. (1978). Zooplankton niches and the community structure controversy. *Science*, *200*(4340). <https://doi.org/10.1126/science.200.4340.458>
- Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software*, *25*(1). <https://doi.org/10.18637/jss.v025.i01>
- Leach, T. H., Williamson, C. E., Theodore, N., Fischer, J. M., & Olson, M. H. (2015). The role of ultraviolet radiation in the diel vertical migration of zooplankton: an experimental test of the transparency-regulator hypothesis. *Journal of Plankton Research*, *37*(5), 886–896. <https://doi.org/10.1093/plankt/fbv061>
- Levine, S. N., Borchardt, M. A., Braner, M., & Shambaugh, A. D. (1999). The impact of zooplankton grazing on phytoplankton species composition and biomass in Lake Champlain (USA-Canada). *Journal of Great Lakes Research*, *25*(1). [https://doi.org/10.1016/S0380-1330\(99\)70717-3](https://doi.org/10.1016/S0380-1330(99)70717-3)
- Li, X., Dreher, T. W., & Li, R. (2016). An overview of diversity, occurrence, genetics and toxin production of bloom-forming *Dolichospermum* (*Anabaena*) species. In *Harmful Algae* (Vol. 54). <https://doi.org/10.1016/j.hal.2015.10.015>
- Litchman, E. (1998). Population and Community Responses of Phytoplankton to Fluctuating Light. *Oecologia*, *117*(1/2), 247–257. <http://www.jstor.org.proxy.library.dc-uoit.ca/stable/4222157>
- Litchman, E., Klausmeier, C. A., Schofield, O. M., & Falkowski, P. G. (2007). The role of functional traits and trade-offs in structuring phytoplankton communities: scaling from cellular to ecosystem level. *Ecology Letters*, *10*(12), 1170–1181. <https://doi.org/https://doi.org/10.1111/j.1461-0248.2007.01117.x>
- Lurling, M., Eshetu, F., Faassen, E., Kosten, S., & Huszar, V. (2013). Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshwater Biology*, *58*. <https://doi.org/10.1111/j.1365-2427.2012.02866.x>
- Lyons, D. A., Mant, R. C., Bulleri, F., Kotta, J., Rilov, G., & Crowe, T. P. (2012). What are the effects of macroalgal blooms on the structure and functioning of marine ecosystems? A systematic review protocol. *Environmental Evidence*, *1*(1), 7. <https://doi.org/10.1186/2047-2382-1-7>
- MacDougall, M. J., Paterson, A. M., Winter, J. G., Jones, F. C., Knopf, L. A., & Hall, R. I. (2016). Response of periphytic diatom communities to multiple stressors influencing lakes in the Muskoka River Watershed, Ontario, Canada. *Freshwater Science*, *36*(1), 77–89. <https://doi.org/10.1086/690144>
- MacIsaac, H. J., & Gilbert, J. J. (1989). Competition between rotifers and cladocerans of different body sizes. *Oecologia*, *81*(3). <https://doi.org/10.1007/BF00377074>

- Mallin, M. A., & Partin, W. E. (1989). Thermal Tolerances of Common Cladocera. *Journal of Freshwater Ecology*, 5(1), 45–51. <https://doi.org/10.1080/02705060.1989.9665212>
- Martínez-Jerónimo, F., & Martínez-Jerónimo, L. (2007). Chronic effect of NaCl salinity on a freshwater strain of *Daphnia magna* Straus (Crustacea: Cladocera): A demographic study. *Ecotoxicology and Environmental Safety*, 67(3), 411–416. <https://doi.org/10.1016/j.ecoenv.2006.08.009>
- McCauley, E., & Briand, F. (1979). Zooplankton grazing and phytoplankton species richness: Field tests of the predation hypothesis1. *Limnology and Oceanography*, 24(2), 243–252. <https://doi.org/https://doi.org/10.4319/lo.1979.24.2.0243>
- Mitchell, S. F., Trainor, F. R., Rich, P. H., & Goulden, C. E. (1992). Growth of *Daphnia magna* in the laboratory in relation to the nutritional state of its food species, *Chlamydomonas reinhardtii*. *Journal of Plankton Research*, 14(3). <https://doi.org/10.1093/plankt/14.3.379>
- Morgan, K., & Kalff, J. (2008). Effect on light and temperature interactions on growth of *Cryptomonas erosa* (Cryptophyceae). *Journal of Phycology*, 15, 127–134. <https://doi.org/10.1111/j.1529-8817.1979.tb02975.x>
- Moss, B. (1973). The Influence of Environmental Factors on the Distribution of Freshwater Algae: An Experimental Study: II. The Role of pH and the Carbon Dioxide-Bicarbonate System. *The Journal of Ecology*, 61(1). <https://doi.org/10.2307/2258925>
- Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 27, 31–36. [https://doi.org/https://doi.org/10.1016/S0003-2670\(00\)88444-5](https://doi.org/https://doi.org/10.1016/S0003-2670(00)88444-5)
- Natural Resources Canada. (2015). *Canadian Land Cover, Circa 2000 (Vector) - GeoBase Series, 1996-2005*. <https://Open.Canada.ca/Data/En/Dataset/97126362-5a85-4fe0-9dc2-915464cfd7bb7>.
- Niku, J., Hui, F. K. C., Taskinen, S., & Warton, D. I. (2019). gllvm: Fast analysis of multivariate abundance data with generalized linear latent variable models in R. *Methods in Ecology and Evolution*, 10(12). <https://doi.org/10.1111/2041-210X.13303>
- Nogueira, I. C. G., Saker, M. L., Pflugmacher, S., Wiegand, C., & Vasconcelos, V. M. (2004). Toxicity of the cyanobacterium *Cylindrospermopsis raciborskii* to *Daphnia magna*. *Environmental Toxicology*, 19(5). <https://doi.org/10.1002/tox.20050>
- Norris, V. (1993). The use of buffer zones to protect water quality: A review. *Water Resources Management*, 7(4), 257–272. <https://doi.org/10.1007/BF00872284>
- Novotny, E. v., & Stefan, H. G. (2012). Road Salt Impact on Lake Stratification and Water Quality . In *Journal of Hydraulic Engineering* (Vol. 138, Issue 12, pp. 1069–

- 1080). American Society of Civil Engineers .
[https://doi.org/10.1061/\(ASCE\)HY.1943-7900.0000590](https://doi.org/10.1061/(ASCE)HY.1943-7900.0000590)
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P. R., Hara, R. B. O., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Szoecs, E. (2019). *Package vegan: Community Ecology Package*.
- Ontario Ministry of Environment. (1983). Handbook of Analytical Methods for Environmental Samples. In *Laboratory Services and Applied Research Branch, Ontario Ministry of Environment*.
- Ontario Ministry of Natural Resources and Forestry. (2018). *Ontario Hydro Network (OHN) - Waterbody*. Ontario GeoHub.
<https://Geohub.Lio.Gov.on.ca/Datasets/Mnrf::Ontario-Hydro-Network-Ohn-Waterbody/About>.
- Ontario Ministry of Natural Resources and Forestry. (2020). *Ontario Watershed Boundaries (OWB)*. Ontario GeoHub.
<https://Geohub.Lio.Gov.on.ca/Maps/Mnrf::Ontario-Watershed-Boundaries-Owb/About>.
- Padisák, J., Vasas, G., & Borics, G. (2016). Phycogeography of freshwater phytoplankton: Traditional knowledge and new molecular tools. In *Hydrobiologia* (Vol. 764, Issue 1). <https://doi.org/10.1007/s10750-015-2259-4>
- Paerl, H. W. (1988). Nuisance Phytoplankton Blooms in Coastal, Estuarine, and Inland Waters. *Limnology and Oceanography*, 33(4), 823–847.
<http://www.jstor.org/proxy.library.dc-uoit.ca/stable/2837225>
- Pedersen, M. F., & Hansen, P. J. (2003). Effects of high pH on a natural marine planktonic community. *Marine Ecology Progress Series*, 260.
<https://doi.org/10.3354/meps260019>
- Poesch, M. S., Chavarie, L., Chu, C., Pandit, S. N., & Tonn, W. (2016). Climate Change Impacts on Freshwater Fishes: A Canadian Perspective. *Fisheries*, 41(7), 385–391.
<https://doi.org/https://doi.org/10.1080/03632415.2016.1180285>
- Porcella, D. B., Rixford, C. E., & Slater, J. v. (1969). Molting and Calcification in *Daphnia magna*. *Physiological Zoology*, 42(2), 148–159.
<http://www.jstor.org/stable/30158469>
- Proulx, M., Pick, F. R., Mazumder, A., Hamilton, P. B., & Lean, D. R. S. (1996). Experimental Evidence for Interactive Impacts of Human Activities on Lake Algal Species Richness. *Oikos*, 76(1), 191–195. <https://doi.org/10.2307/3545761>
- Pulina, S., Brutemark, A., Suikkanen, S., Padedda, B. M., Grubisic, L. M., Satta, C. T., Caddeo, T., Farina, P., & Sechi, N. (2016). Effects of warming on a Mediterranean phytoplankton community. *Web Ecology*, 16, 89.

https://link.gale.com/apps/doc/A482062472/AONE?u=ko_acd_uoo&sid=AONE&xid=b25cfbe0

- Puth, M. T., Neuhäuser, M., & Ruxton, G. D. (2015). Effective use of Spearman's and Kendall's correlation coefficients for association between two measured traits. In *Animal Behaviour* (Vol. 102, pp. 77–84). Academic Press.
<https://doi.org/10.1016/j.anbehav.2015.01.010>
- QGIS Development Team. (2020). *QGIS Geographic Information System*. Open Source Geospatial Foundation. <http://qgis.osgeo.org>
- R Core Team. (2021). *R: A Language and Environment for Statistical Computing* (4.0.4). R Foundation for Statistical Computing.
- Rajakaruna, H., & Lewis, M. (2018). Do yearly temperature cycles reduce species richness? Insights from calanoid copepods. *Theoretical Ecology*, *11*(1), 39–53.
<https://doi.org/10.1007/s12080-017-0347-y>
- Redfield, A. C. (1958). The biological control of chemical factors in the environment. *American Scientist*, *46*(3), 230A – 221. <http://www.jstor.org/stable/27827150>
- Reynolds, C. S. (1997). Vegetation processes in the pelagic: A model for ecosystem theory. In *Excellence in Ecology* (Vol. 9). <https://doi.org/10.2216/i0031-8884-37-1-70.1>
- Reynolds, C. S. (2006). The Ecology of Phytoplankton. In *The Ecology of Phytoplankton*. <https://doi.org/10.1017/CBO9780511542145>
- Risser, P. G. (1995). The status of the science examining ecotones. *BioScience*, *45*(5), 318–325.
- Ritchie, R. J. (2008). Universal chlorophyll equations for estimating chlorophylls a, b, c, and d and total chlorophylls in natural assemblages of photosynthetic organisms using acetone, methanol, or ethanol solvents. *Photosynthetica*, *46*(1), 115–126.
<https://doi.org/10.1007/s11099-008-0019-7>
- Robarts, R. D., & Zohary, T. (1987). Temperature effects on photosynthetic capacity, respiration, and growth rates of bloom-forming cyanobacteria. *New Zealand Journal of Marine and Freshwater Research*, *21*(3), 391–399.
<https://doi.org/10.1080/00288330.1987.9516235>
- Sarma, S. S. S., Nandini, S., Morales-Ventura, J., Delgado-Martínez, I., & González-Valverde, L. (2006). Effects of NaCl salinity on the population dynamics of freshwater zooplankton (rotifers and cladocerans). *Aquatic Ecology*, *40*(3), 349–360.
<https://doi.org/10.1007/s10452-006-9039-1>
- Schindler, D. W. (1977). Evolution of Phosphorus Limitation in Lakes. *Science*, *195*(4275), 260. <https://doi.org/10.1126/science.195.4275.260>

- Schindler, D. W., Wagemann, R., Cook, R. B., Rusczyński, T., & Prokopowich, J. (1980). Experimental Acidification of Lake 223, Experimental Lakes Area: Background Data and the First Three Years of Acidification. *Canadian Journal of Fisheries and Aquatic Sciences*, *37*(3), 342–354. <https://doi.org/10.1139/f80-048>
- Sharpley, A. N., & Smith, S. J. (1994). Wheat tillage and water quality in the Southern plains. *Soil and Tillage Research*, *30*(1), 33–48. [https://doi.org/10.1016/0167-1987\(94\)90149-X](https://doi.org/10.1016/0167-1987(94)90149-X)
- Shurin, J. B., Cottenie, K., & Hillebrand, H. (2009). Spatial autocorrelation and dispersal limitation in freshwater organisms. *Oecologia*, *159*(1). <https://doi.org/10.1007/s00442-008-1174-z>
- Siberchicot, A., Julien-Laferrière, A., Dufour, A. B., Thioulouse, J., & Dray, S. (2017). adegraphics: An S4 lattice-based package for the representation of multivariate data. *R Journal*, *9*(2). <https://doi.org/10.32614/rj-2017-042>
- Simpson, E. H. (1949). Measurement of Diversity. *Nature*, *163*(4148), 688. <https://doi.org/10.1038/163688a0>
- Šmilauer, P., & Lepš, J. (2014). *Multivariate Analysis of Ecological Data using CANOCO 5* (2nd ed.). Cambridge University Press. <https://doi.org/10.1017/CBO9781139627061>
- Smith, V. H. (1983). Low Nitrogen to Phosphorus Ratios Favor Dominance by Blue-Green Algae in Lake Phytoplankton. *Science*, *221*(4611), 669–671. <http://www.jstor.org.proxy.library.dc-uoit.ca/stable/1691193>
- Smith, V. H. (2003). Eutrophication of freshwater and coastal marine ecosystems a global problem. *Environmental Science and Pollution Research*, *10*(2), 126–139. <https://doi.org/10.1065/espr2002.12.142>
- Smith, V. H., Sieber-Denlinger, J., deNoyelles, F., Campbell, S., Pan, S., Randtke, S. J., Blain, G. T., & Strasser, V. A. (2002). Managing Taste and Odor Problems in a Eutrophic Drinking Water Reservoir. *Lake and Reservoir Management*, *18*(4), 319–323. <https://doi.org/10.1080/07438140209353938>
- Sommer, U., & Stibor, H. (2002). Copepoda - Cladocera - Tunicata: The role of three major mesozooplankton groups in pelagic food webs. *Ecological Research*, *17*(2). <https://doi.org/10.1046/j.1440-1703.2002.00476.x>
- Søndergaard, M., & Jeppesen, E. (2007). Anthropogenic Impacts on Lake and Stream Ecosystems, and Approaches to Restoration. *Journal of Applied Ecology*, *44*(6), 1089–1094. <http://www.jstor.org.proxy.library.dc-uoit.ca/stable/4539332>
- Søndergaard, M., Jeppesen, E., Mortensen, E., Dall, E., Kristensen, P., & Sortkjær, O. (1990). Phytoplankton biomass reduction after planktivorous fish reduction in a shallow, eutrophic lake: a combined effect of reduced internal P-loading and

- increased zooplankton grazing. *Hydrobiologia*, 200(1), 229–240.
<https://doi.org/10.1007/BF02530342>
- Soranno, P. A., Hubler, S. L., Carpenter, S. R., & Lathrop, R. C. (1996). Phosphorus Loads to Surface Waters: A Simple Model to Account for Spatial Pattern of Land Use. *Ecological Applications*, 6(3), 865–878. <https://doi.org/10.2307/2269490>
- Sprules, W. G. (1975). Midsummer Crustacean Zooplankton Communities in Acid-Stressed Lakes. *Journal of the Fisheries Research Board of Canada*, 32(3), 389–395. <https://doi.org/10.1139/f75-045>
- Stokes, P. M. (1986). Ecological effects of acidification on primary producers in aquatic systems. *Water, Air, and Soil Pollution*, 30(1), 421–438.
<https://doi.org/10.1007/BF00305212>
- Stomp, M., Huisman, J., de Jongh, F., Veraart, A. J., Gerla, D., Rijkeboer, M., Ibelings, B. W., Wollenzien, U. I. A., & Stal, L. J. (2004). Adaptive divergence in pigment composition promotes phytoplankton biodiversity. *Nature*, 432(7013).
<https://doi.org/10.1038/nature03044>
- Strickler, J. R., & Bal, A. K. (1973a). Setae of the First Antennae of the Copepod *Cyclops scutifer* (Sars): Their Structure and Importance. *Proceedings of the National Academy of Sciences of the United States of America*, 70(9), 2656–2659.
<http://www.jstor.org.proxy.library.dc-uoit.ca/stable/63073>
- Strickler, J. R., & Bal, A. K. (1973b). Setae of the First Antennae of the Copepod *Cyclops scutifer* (Sars): Their Structure and Importance. *Proceedings of the National Academy of Sciences of the United States of America*, 70(9), 2656–2659.
<http://www.jstor.org.proxy.library.dc-uoit.ca/stable/63073>
- Taranu, Z. E., & Gregory-Eaves, I. (2008). Quantifying Relationships among Phosphorus, Agriculture, and Lake Depth at an Inter-Regional Scale. *Ecosystems*, 11(5), 715–725. <http://www.jstor.org.proxy.library.dc-uoit.ca/stable/40296321>
- Tessier, A. J., & Horwitz, R. (1990). Influence of Water Chemistry on Size Structure of Zooplankton Assemblages. *Canadian Journal of Fisheries and Aquatic Sciences*, 47, 1937–1943.
- Wærvågen, S. B., A. Rukke, N., & Hessen, D. O. (2002). Calcium content of crustacean zooplankton and its potential role in species distribution. *Freshwater Biology*, 47(10), 1866–1878. <https://doi.org/https://doi.org/10.1046/j.1365-2427.2002.00934.x>
- Wang, X., Qin, B., Gao, G., & Paerl, H. W. (2010). Nutrient enrichment and selective predation by zooplankton promote *Microcystis* (Cyanobacteria) bloom formation. *Journal of Plankton Research*, 32(4). <https://doi.org/10.1093/plankt/fbp143>

- Webster, K. E., & Peters, R. H. (1978). Some Size-Dependent Inhibitions of Larger Cladoceran Filterers in Filamentous Suspensions. *Limnology and Oceanography*, 23(6), 1238–1245. <http://www.jstor.org.uproxy.library.dc-uoit.ca/stable/2835679>
- Wehr, J. D., Sheath, R. G., & Kociolek, J. P. (2015). Freshwater Algae of North America: Ecology and Classification. In *Freshwater Algae of North America: Ecology and Classification*. <https://doi.org/10.1016/C2010-0-66664-8>
- Wei, T., & Simko, V. (2017). *R package "corrplot": Visualization of a correlation matrix. R package version 0.84.*
- Whittier, T. R., Ringold, P. L., Herlihy, A. T., & Pierson, S. M. (2008). A Calcium-Based Invasion Risk Assessment for Zebra and Quagga Mussels (*Dreissena* spp). *Frontiers in Ecology and the Environment*, 6(4), 180–184. <http://www.jstor.org.uproxy.library.dc-uoit.ca/stable/20440869>
- Wickham, H. (2010). Stringr: Modern, consistent string processing. *R Journal*, 2(2). <https://doi.org/10.32614/rj-2010-012>
- Wickham, H. (2016). *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York. <https://ggplot2.tidyverse.org>
- Wickham, H., François, R., Henry, L., & Müller, K. (2019). dplyr: A Grammar of Data Manipulation. R package version. In *Media*.
- Wickham, H., & Henry, L. (2018). tidyr: Easily Tidy Data with “spread()” and “gather()” Functions. *R Package Version 0.8.0*. <https://CRAN.R-Project.Org/Package=tidyr>.
- Wiedner, C., & Nixdorf, B. (1998). Success of chrysophytes, cryptophytes and dinoflagellates over blue-greens (cyanobacteria) during an extreme winter (1995/96) in eutrophic shallow lakes. *Hydrobiologia*, 369(0), 229–235. <https://doi.org/10.1023/A:1017054011389>
- Wilke, C. O. (2020). cowplot: Streamlined Plot Theme and Plot Annotations for “ggplot2”. R package version 1.1.0. <https://CRAN.R-Project.Org/Package=cowplot>.
- Willén, E. (1991). Planktonic diatoms - an ecological review. *Algological Studies/Archiv Für Hydrobiologie, Supplement Volumes*, 62, 69–106. http://www.schweizerbart.de/papers/archiv_algolstud/detail/62/66691/Planktonic_diatoms_an_ecological_review
- Willén, E., Ahlgren, G., & Söderhielm, A.-C. (2000). Toxic cyanophytes in three Swedish lakes. *SIL Proceedings, 1922-2010*, 27(1), 560–564. <https://doi.org/10.1080/03680770.1998.11901298>
- Williamson, C. E. (1983). Invertebrate predation on planktonic rotifers. *Hydrobiologia*, 104(1), 385–396. <https://doi.org/10.1007/BF00045996>

- Williamson, C. E., & Butler, N. M. (1986). Predation on rotifers by the suspension-feeding calanoid copepod *Diaptomus pallidus*. *Limnology and Oceanography*, 31(2). <https://doi.org/10.4319/lo.1986.31.2.0393>
- Wilson, D. S. (1992). Complex Interactions in Metacommunities, with Implications for Biodiversity and Higher Levels of Selection. *Ecology*, 73(6), 1984–2000. <https://doi.org/10.2307/1941449>
- Winder, M., Reuter, J. E., & Schladow, S. G. (2009). Lake Warming Favours Small-Sized Planktonic Diatom Species. *Proceedings: Biological Sciences*, 276(1656), 427–435. <http://www.jstor.org.uproxy.library.dc-uoit.ca/stable/30244877>
- Winkenbach, F., & Wolk, C. P. (1973). Activities of Enzymes of the Oxidative and the Reductive Pentose Phosphate Pathways in Heterocysts of a Blue-Green Alga 1. *Plant Physiology*, 52(5), 480–483. <https://doi.org/10.1104/pp.52.5.480>
- Xu, H., Paerl, H. W., Qin, B., Zhu, G., & Gao, G. (2010). Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. *Limnology and Oceanography*, 55(1), 420–432. <https://doi.org/https://doi.org/10.4319/lo.2010.55.1.0420>
- Yamamoto, J., Kishimoto, N., Ichise, S., & Furuta, S. (2016). Effects of environmental factors on microalgal biomass production in wastewater using cyanobacteria *Aphanothece clathrata* and *Microcystis wesenbergii*. *Environmental Technology (United Kingdom)*, 37(4). <https://doi.org/10.1080/09593330.2015.1099605>
- Yang, Y., & Gao, K. (2003). Effects of CO₂ concentrations on the freshwater microalgae, *Chlamydomonas reinhardtii*, *Chlorella pyrenoidosa* and *Scenedesmus obliquus* (Chlorophyta). *Journal of Applied Phycology*, 15(5), 379–389. <https://doi.org/10.1023/A:1026021021774>
- Zohary, T., & Robarts, R. D. (1990). Hyperscums and the population dynamics of *Microcystis aeruginosa*. *Journal of Plankton Research*, 12(2). <https://doi.org/10.1093/plankt/12.2.423>

Appendix

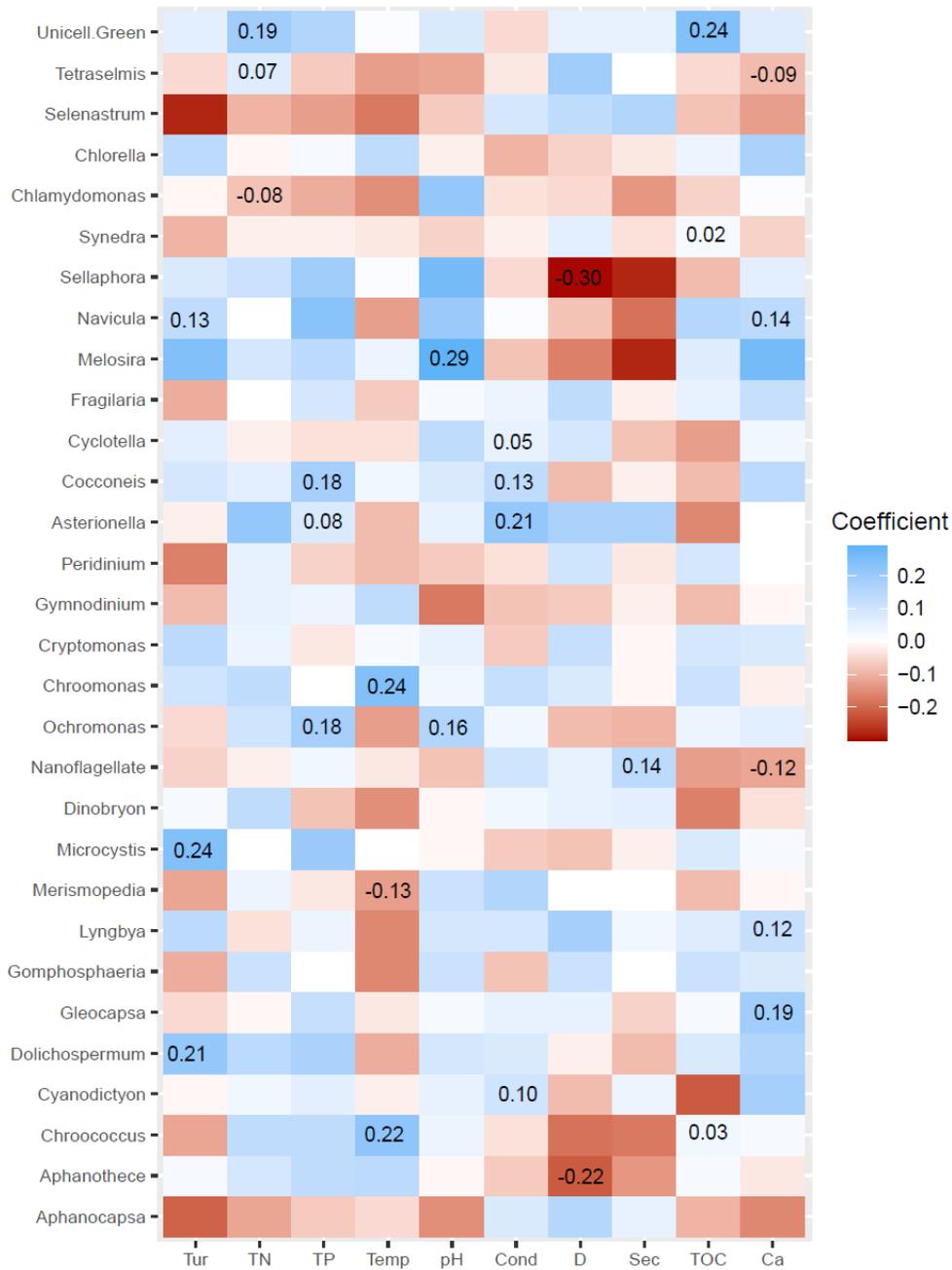
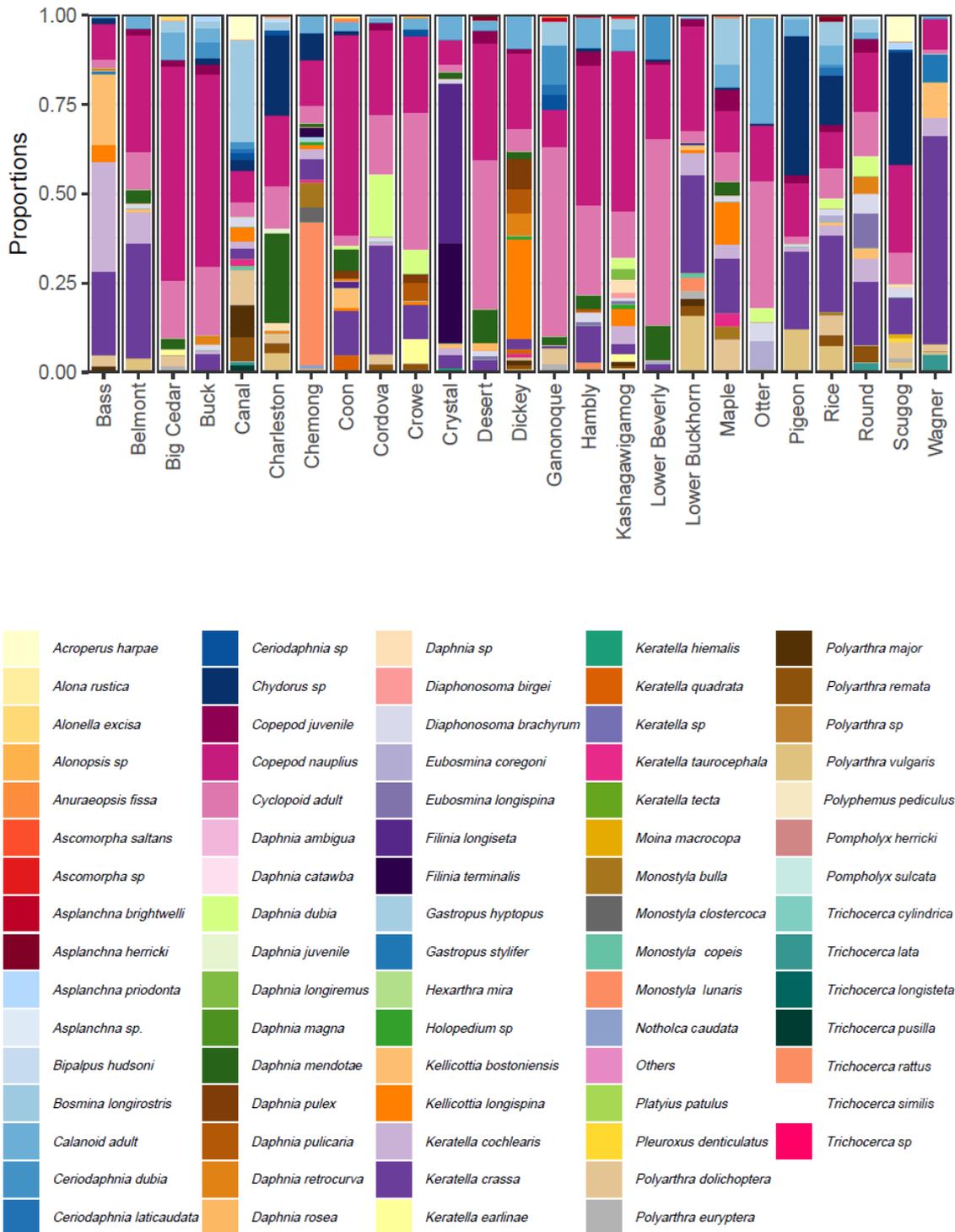


Figure A1. Kendall correlation matrix showing relationships between zooplankton taxa and water quality parameters and land-use. Tur = Turbidity, TN = Total Nitrogen, TP = Total Phosphorous, Temp = Temperature, Con = Conductivity, D = Depth, Sec = Secchi, TOC = Total Organic Carbon, Ca = Calcium



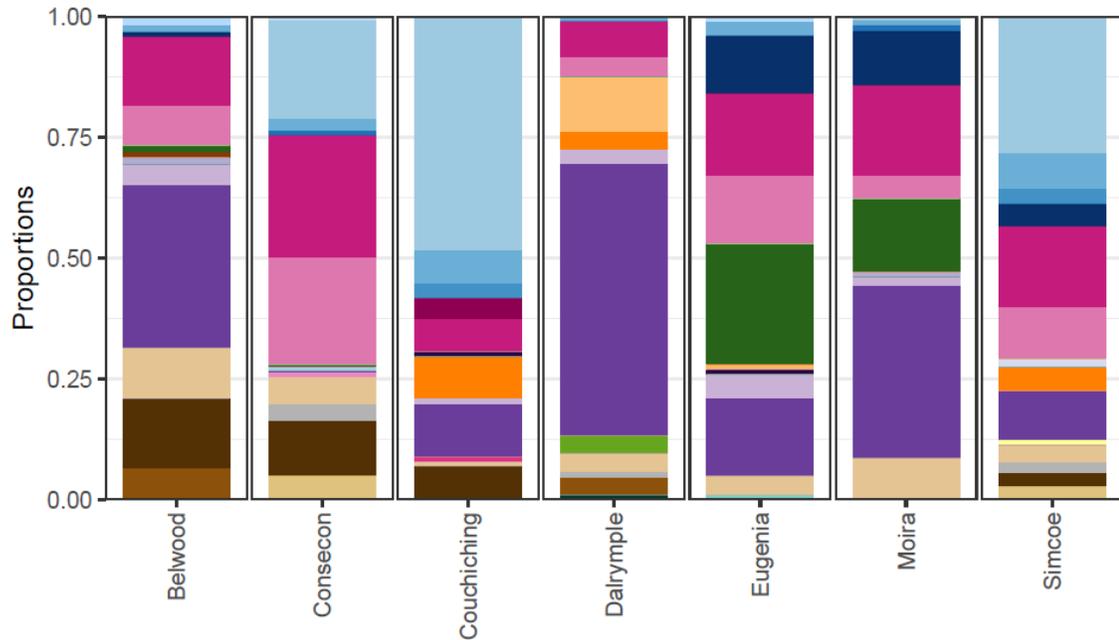


Figure A2c. Relative abundance plot for zooplankton at the species level in high-calcium lakes >40 mg/L).

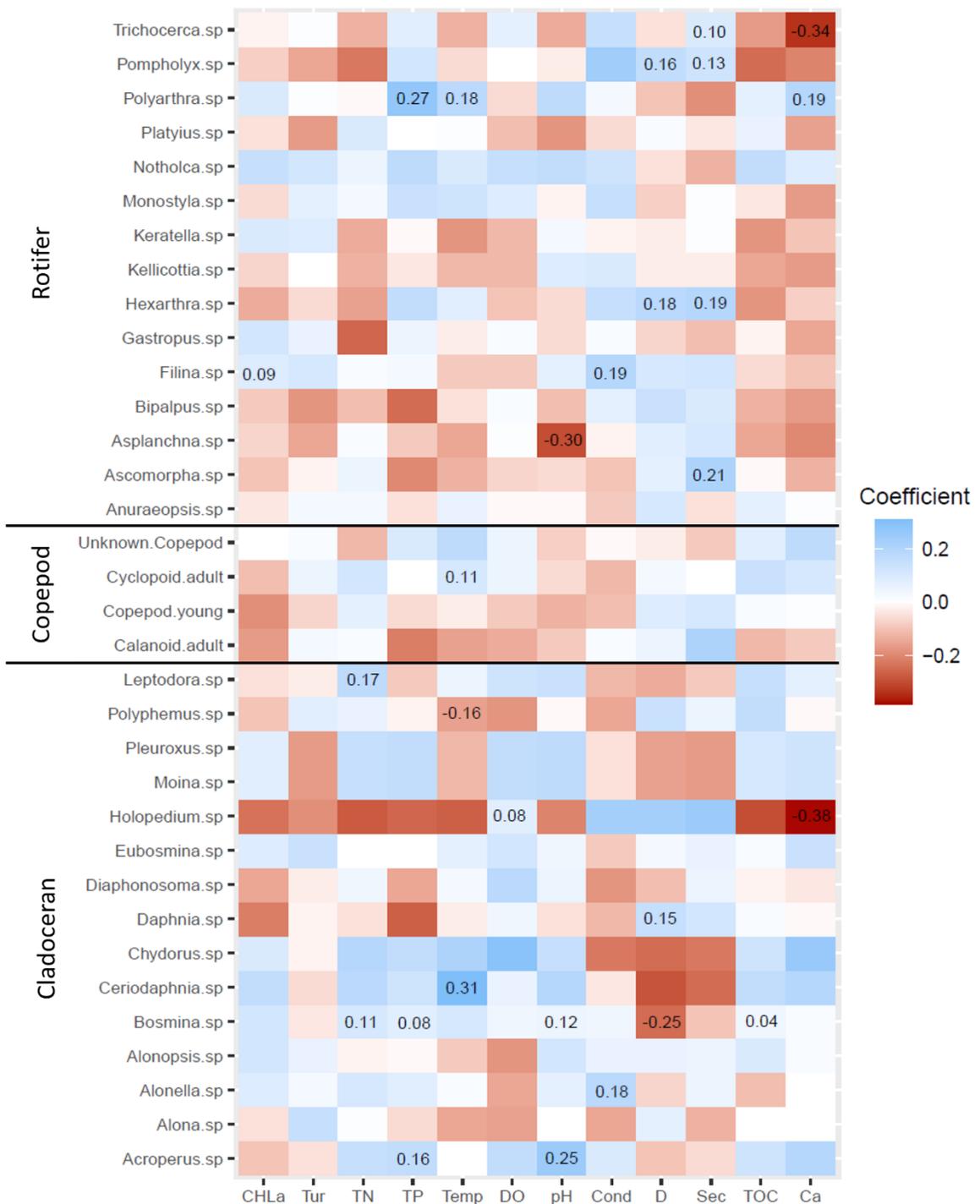


Figure A3. Kendall correlation matrix showing relationships between zooplankton taxa and water quality parameters and land-use. CHLa = Chlorophyll a, Tur = Turbidity, TN = Total Nitrogen, TP = Total Phosphorous, Temp = Temperature, DO = Dissolved Oxygen, Con = Conductivity, D = Depth, Sec = Secchi, TOC = Total Organic Carbon, Ca = Calcium