9-25-2024

Environmental drivers of phytoplankton community succession and cyanobacterial harmful algal blooms along a fluvial-lacustrine continuum

Emily Marcella Varga
University of Windsor

Environmental drivers of phytoplankton community succession and cyanobacterial harmful algal blooms along a fluvial-lacustrine continuum

By

Emily Varga

A Dissertation

Submitted to the Faculty of Graduate Studies

through the Faculty of Science

And in support of the Great Lakes Institute for Environmental Research

in Partial Fulfillment of the Requirements for

the Degree of Doctor of Philosophy

at the University of Windsor

Windsor, Ontario, Canada

2024

Environmental drivers of phytoplankton community succession and cyanobacterial harmful algal blooms along a fluvial-lacustrine continuum

by

Emily Varga

APPROVED BY:

_____

C. Wigdahl-Perry, External Examiner
State University of New York

_____

G. Bullerjahn, Special Member
Bowling Green State University

_____

T. Noel
Department of Integrative Biology

_____

C. Febria
Great Lakes Institute for Environmental Research

_____

K. Stammler
Great Lakes Institute for Environmental Research

_____

R. McKay, Advisor
Great Lakes Institute for Environmental Research

September 9, 2024

DECLARATION OF CO-AUTHORSHIP / PREVIOUS PUBLICATION

I. Co-Authorship

I hereby declare that this thesis incorporates material that is result of joint research, as follows:

Chapter 2 of the thesis was co-authored with Z Song, P. Weidman, and R. M. McKay, under the supervision of R.M. McKay. In all cases, the key ideas, primary contributions, interpretation, and writing were performed by the author, and the contribution of co-authors was primarily through data analysis and the provision of grant funding alongside experimental design. All co-authors also provided feedback for the purpose of editing and refining the manuscript.

Chapter 3 of the thesis was co-authored with Z. Song, K. Brown, W. Cody, G. Bullerjahn and R. M. McKay under the supervision of R.M. McKay. In all cases, the key ideas, primary contributions, data analysis, interpretation, and writing were performed by the author, and the contribution of co-authors was primarily through the provision of grant funding alongside experimental design. All co-authors also provided feedback for the purpose of editing and refining the manuscript.

Chapter 4 of the thesis was co-authored with M. Swaleh, J. Stoll, and R. M. McKay under the supervision of R.M. McKay. In all cases, the key ideas, primary contributions, data analysis, interpretation, and writing were performed by the author, and the contribution of co-authors was primarily through the provision of grant

funding alongside experimental design. All co-authors also provided feedback for the purpose of editing and refining the manuscript.

I am aware of the University of Windsor Senate Policy on Authorship and I certify that I have properly acknowledged the contribution of other researchers to my thesis, and have obtained written permission from each of the co-author(s) to include the above material(s) in my thesis.

I certify that, with the above qualification, this thesis, and the research to which it refers, is the product of my own work.

II. Previous Publication This thesis includes 2 original papers that have been previously published/submitted for publication in peer reviewed journals, as follows:

Thesis Chapter	Publication title/ full citation	Publication status
Chapter [2]	Varga, E., Weidman, R. P., Song, Z. & McKay, R. M. (2024). Environmental drivers of phytoplankton community dynamics in an agriculturally – influenced tributary in the lower Great Lakes. *Science of the Total Environment, 939.* https://doi.org/10.1016/j.scitotenv.2024.173411	Published
Chapter [3]	Varga, E., Song, Z., Brown, K., Cody, B., Bullerjahn, G., McKay, R. M. (2024). Temporal and spatial differences in phytoplankton community structure along a fluvial-lacustrine continuum in the lower Great Lakes basin.	Submitted July 10, 2024

I certify that I have obtained a written permission from the copyright owner(s) to include the above published material(s) in my thesis. I certify that the above material describes work completed during my registration as a graduate student at the University of Windsor.

III. General

I declare that, to the best of my knowledge, my thesis does not infringe upon anyone's copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my thesis, published or otherwise, are fully acknowledged in accordance with the standard referencing practices. Furthermore, to the extent that I have included copyrighted material that surpasses the bounds of fair dealing within the meaning of the Canada Copyright Act, I certify that I have obtained a written permission from the copyright owner(s) to include such material(s) in my thesis.

I declare that this is a true copy of my thesis, including any final revisions, as approved by my thesis committee and the Graduate Studies office, and that this thesis has not been submitted for a higher degree to any other University or Institution.

ABSTRACT

The integrity of freshwater resources has been continually threatened by persistent human influence through increased urbanization, agriculture and the use of chemical fertilizers. One manifestation of anthropogenic influence in aquatic ecosystems in recent decades is an increased prevalence of cyanobacterial harmful algal blooms (cyanoHABs) due to eutrophication. Proliferation of cyanoHABs can alter aquatic food webs and create low oxygen zones, which can be detrimental to freshwater life. These blooms can potentially produce toxins with the most commonly found group of toxins in the lower Great Lakes being microcystins. Surveys within Lake Erie have shown that *Microcystis* can contribute over 40% of cyanobacterial abundance creating communities of both toxin-producing and non toxin-producing strains. However, toxicity can be difficult to predict, as community composition depends on multiple environmental factors. According to the Paradox of the Plankton, competitive exclusion should result in phytoplankton species out-competing each other for resources and reaching eventual equilibrium; however, it is known that well-mixed bodies of water can support a vast number of species. Phytoplankton communities in the Great Lakes follow seasonal and temporal succession, and limited surveys have found that phytoplankton community structure differs between rivers and their respective lakes. The research that comprises this dissertation was conducted to gain an understanding of the drivers of these temporal and spatial differences by examining nutrients, water quality parameters and phytoplankton community composition along the continuum. Results of microcosm experiments conducted in the Thames River found there was a reduction in overall abundance and diversity of phytoplankton, and changes in community composition with

lower light availability. Experimental units with higher light availability were dominated by colonial cyanobacteria, mainly *Aphanocapsa*. Results from research done along the fluvial-lacustrine continuum found that water quality parameters differ temporally, likely driving the differences in phytoplankton community composition. River sites were generally characterized by higher nutrient concentrations and N:P ratio whereas the lake sites had lower nutrient concentrations and higher temperatures, and the river mouth represented a zone of mixing. Phytoplankton communities follow a seasonal succession, as well, mainly dominated by diatoms in the winter and spring and shifting to cyanobacterial dominance in summer and fall. The composition of cyanoHABs is often attributed to *Microcystis,* however, we found colonial communities to be more diverse than expected, often co-dominated by several genera. Similar research was conducted in the Sondu River- Lake Victoria continuum as the lake is plagued by cyanoHABS as we see in Lake Erie. Though we obtained only limited results due to a lack of field supplies, they suggest that the river is an important source of nutrients contributing to the degradation of the lake. We found similar water quality characteristics along the continuum as in Ontario. This laid important groundwork for continued research in the Lake Victoria catchment and highlighted the need for continued, place-based studies and investment in African-led research. There is a scarcity of in-depth ecosystem studies conducted in this region, and gaining a better understanding of these systems could inform policy development on freshwater management in African countries. These cumulative findings provide insight into the drivers of phytoplankton community composition along fluvial-lacustrine continuums and supported the notion that cyanoHABS often are more diverse than usually understood.

DEDICATION

I dedicate this dissertation to my wonderful, supportive husband and number one fan, Thomas Varga; my parents, siblings and kids, and my extended family and friends for all their support and encouragement.

ACKNOWLEDGEMENTS

First and foremost, I want to thank my advisor and mentor, Dr. R Michael McKay for taking me under his wing at a time that I was seriously reconsidering grad school. Mike has taught me almost everything I know about algae, field sampling techniques and processing samples in the lab. I didn't have the benefit of being in a lab group throughout my PhD work, and thus didn't have lab mates to help me out. Mike was always patient with me and made himself available to show me how to sample and process. Mike also made it possible for me to experience African culture and although the travel portion of that experience was not great, he checked in regularly on my 63 hour journey across the world. I will be forever grateful to him. I want to acknowledge the rest of my committee as well; Dr. George Bullerjahn, Dr. Catherine Febria, Dr. Katie Stammler and Dr. Tanya Noel.

George agreed to sit on my committee without even having met me and in the middle of the COVID pandemic which made it impossible to meet in person. Post lockdown, we finally did meet in person – in Africa. George was incredibly supportive and complimented my outfit every morning despite the fact I was wearing bits and pieces of other people's wardrobes. Except for my shoes. George knows way more about shoes than I do. He was also very accommodating, allowing me space in his lab in Bowling Green and making sure I was put up for the night in a hotel. He has always been easy to talk to and I will never forget his Dirty Harry impression while discussing what I should study for my comprehensive exam.

Catherine was quick to agree to being on my committee as well. I look up to Cat as she is a strong, empowered woman of science. She was also very supportive and while I was faltering, assured me that I belonged at the University and in the Environmental Science PhD program. She taught me how to look at data in a different way, particularly when there didn't seem to be enough data to tell the story I was trying to tell.

When I approached Katie about being on my committee, it was kismet. She was just about to reach out to Cat to ask if anyone was looking for committee members. Katie has been a great support, as she has put me in touch with people in the field who turned out to be valuable resources. We also forged a strong friendship and Katie was always there to listen, whether I needed to vent about academics or life in general. She taught me an appreciation for the philosophy of science and would share random philosophical statements, always when I seemed to need it the most. Katie also helped me through imposter syndrome by telling me "None of us knows what we're doing."

Tanya didn't hesitate to join my committee, even though I knew she was already busy enough. I do tell her all the time she is too nice. We share a nerdy love for microbiology and a birthday! Tanya has always been there to listen to whatever was on my mind and has referred to me as her emotional support GA. I guess going forward, I'll have to be her emotional support nerd. She taught me how to be the best GA I could be, which partially funded my journey through grad school. Because of her guidance and the amazing teacher that she is, I won an award for teaching practice (for which she nominated me). I owe so much to this amazing group of people.

I wouldn't be writing this dissertation if it weren't for my husband, best friend and number one fan, Tom Varga. Tom encouraged me to go back to school to finish my

bachelor's degree – little did he know it would turn into more than a decade of schooling. Tom has lifted me up every time I was down and encouraged me not to give up every time I doubted myself. He reminded me frequently that "I didn't come this far only to come this far." He has supported our family financially and emotionally, helped me get through chemistry and calculus and stuck by my side, even when I was "Exam Emily." Despite the roller coaster of undergraduate schooling, he still encouraged me to continue when I was debating the merits of quitting my full time job to attend graduate school. I will never forget his pep talk. Here, I am paraphrasing:

Find your wings and soar. Be an albatross. What is the worst that could happen? Either you leave the cliff and soar high above the ocean, or you crash violently into the waves and get slowly eaten by mollusks. Be an albatross.

I also want to thank the rest of my family, first, all four of our children; William, Alexia, Liam and Olivia. As much of my field work was done during the pandemic, there were many safety hoops we had to jump through, including not being out in the field with people outside "your bubble". This meant that I had to enlist the help of people *within* my bubble. My kids stepped up to the plate and together with my husband, became my number one sampling team. They never hesitated (and have become proficient at filtering very turbid river water). I want to thank my son William for being so willing to climb down into the river to collect samples on his own and bring them back to me! My youngest, Olivia, has an old soul, and always seems to know when I need encouragement. One day while looking up at my undergraduate diploma, she said, "Mom, I am so proud of you." That was all I needed to hear to keep going. My parents, Richard and Patricia, have shown unrelentless support for my academic endeavours, as have my siblings, Ron,

Adam and Jennifer. I remember how my dad's face lit up when I told him I was going back to school. I promised him I would see it through, and although it has taken me a long time to get here, he's never judged me for that. My mom has shown me constant encouragement and is usually the first to congratulate me when something great happens. My brother Ron often forwards me nerdy scientific articles and tidbits that he knows I will appreciate. My sister Jenn bought me my first lab coat, and even though she insisted it had to be designer, I still wear it. My brother Adam has shown his love and support through a personalized tattoo that includes the words "Dr. Em" and I couldn't be more appreciative of the sentiment! It would be remiss of me to not mention my Aunt Pat here, more like a second mom, as she checked in on my mental, emotional, physical, and academic well-being regularly, and was always available when I needed a shoulder to cry on.

I am very lucky to have people in my life whom I consider extended family. Our best friends on the entire planet, Shawn and Geri, deserve a special shout-out. These two people celebrate all of my achievements, big or small. Shawn is like a brother to me. For as long as I have known him, he has been there for me. I love that I can talk to him about my research and he seems genuinely interested. I've only known Geri for a couple of years but I appreciate her for constantly checking in on me and how she always referred to me as "Best Friend Almost Doctor Emily" (until very recently). They both always know when we need them and I can't imagine life without these two!

The GLIER support staff have been amazing and deserve a shout-out for helping me get where I am and keeping me on track. Although our grad secretaries changed several times, I am thankful for Kendra and Nia for keeping me on track with all the

requirements to obtain my PhD. Judy came in near the end, but was always kind and made herself available if needed. Last but definitely not least is Mary-Lou. I don't know where to start or end with the support that Mary-Lou has given me. She could always tell when I needed a hug and would ask me every time I saw her how I was doing, with my lab work, field work, thesis writing, or life at home. I think Mary-Lou knows everything about me; she would even ask about my kids by name. I cannot ever thank her enough or express in words what she means to me. I owe a special thank you to Dr. Nargis Ismail for training me on the SmartChem and always helping me troubleshoot when nutrient analysis didn't go my way (which was often). I will be forever appreciative of her patience and kindness.

There are too many individuals to list, whom I got to know and trust throughout grad school; other students at GLIER, staff, faculty, and the friends and colleagues I obtained on main campus. However, I must mention specifically, Nick Falk. Nick was just starting his PhD journey as I started my master's degree. Nick trained me in the lab on various techniques and was my right-hand man in the field. Our sampling days were very long, often in extreme heat or rain, and Nick never complained or ducked out early. Although, I did help him with field work during extreme cold, knee-high snow, flooded ditches and an incident where a GLIER truck may or may not have gotten seriously stuck in the mud. We traveled together and stayed in close quarters. Nick drove the two of us to Brockport, New York for IAGLR because I was afraid of American roads and we spent a month in New Zealand for research (although he forgets about this).

I also want to mention the GLIER Goddesses. Lauren and Alicia were my closest friends during my master's work. Lauren and I temporally shared an office, and

consequently many hugs and tears. Our research did not align, but I appreciated her letting me run ideas by her and have her proof-read my work. Lauren has since left for a career in England, but she will never be forgotten. Alicia, what can I say here that I haven't said to you in person a million times? We started our master's degrees at the same time and we quickly became close friends. She has been my confidante and soul sister for five years. We trained on the equipment in the OANL together (the "Robit") and she always helped me run my nutrient samples. The Robit can be a bit of a jerk sometimes and analysis that should have only taken a couple of hours often turned into entire days of frustration and failed attempts. But she never left my side. The laws of physics also don't apply in the OANL and slowly boiling 40 mL of water could literally take hours. Again, she never left my side. We are friends outside of GLIER as well. We have spent birthdays, Christmas eve parties, Alicia's wedding and dozens of half-day Fridays at Rock Bottom together. Alicia Lambier, thank you for everything. I love you, Rabbit.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS/SYMBOLS

ACARE	African Center for Aquatic Research and Education
ALH	Algal Loading Hypothesis
ANOVA	Analysis of Variance
BGSU	Bowling Green State University
BRWTP	Belle River Water Treatment Plant
cyanoHABs	Cyanobacterial Harmful Algal Blooms
Chl *a*	Chlorophyll *a*
db-RDA	Distance-Based Redundancy Analysis
DIN	Dissolved Inorganic Nitrogen
DO	Dissolved Oxygen
DOM	Dissolved Organic Matter
HSD	Honestly Significant Difference
KMFRI	Kenya Marine and Fisheries Research Institute
L.C.	Lighthouse Cove
LTVCA	Lower Thames Valley Conservation Authority
MCs	Microcystins
N	Nitrogen
NH_3	Ammonia
NMDS	Non-Metric MultiDimensional Scaling
NO_3-NO_2	Nitrate-Nitrite
P	Phosphorus
PCA	Principal Components Analysis
PERMANOVA	Permutational Multivariate ANOVA
P.S.	Prairie Siding
SPWTP	Stoney Point Water Treatment Plant
SRP	Soluble Reactive Phosphorus
TDS	Total Dissolved Solids
TP	Total Phosphorus

CHAPTER 1: INTRODUCTION

1.1 Thesis Overview

The integrity of freshwater resources is threatened on multiple fronts by persistent human influence. One manifestation of anthropogenic influence in aquatic ecosystems in recent decades is an increased prevalence of cyanobacterial harmful algal blooms (cyanoHABs) (Bullerjahn et al., 2016; Cheung et al., 2013; Ho et al., 2019). These blooms can potentially produce toxins (collectively known as cyanotoxins) and other secondary metabolites that can be harmful to humans, pets and wildlife (Paerl et al., 2001). The most commonly found, and the most well-studied, group of toxins in freshwater systems are known as microcystins, included in the category of hepatotoxins (Campos & Vasconcelos, 2010; Chaffin et al., 2023). However, some genera of cyanobacteria are capable of producing other toxins, including neurotoxins and dermatotoxins. Eutrophication is a term describing an over-enrichment of nitrogen (N) and phosphorus (P) related to anthropogenic activities. A general symptom of this increased loading is often the proliferation of algal blooms, and enhancement of cyanobacterial dominance of blooms (Conley et al., 2009; O'Neil et al., 2012). It can also alter aquatic food webs and create low oxygen zones, which can be detrimental to freshwater life (Paerl et al., 2011).

Surveys within Lake Erie have shown that *Microcystis* can contribute over 40% of cyanobacterial abundance, creating communities of both toxin-producing and non-toxin-producing strains (Rinta-Kanto et al., 2009; Yancey et al., 2022). Most of the time, cyanobacterial metabolites are not required for normal cell function, and though this is not well studied, may provide the organism some benefit, such as defense against

herbivory (Ae et al., 2009; Miller et al., 2017). Toxicity can be difficult to predict, with the ratio between toxin-producing and non toxin-producing strains likely to fluctuate over the algal growing season, as toxin-producing strains tend to be weaker competitors for light. This can result in lower microcystin concentrations despite increased overall biomass at the height of the growing season (Kardinaal et al., 2007). Adding to this complexity is the fact that there are over 300 microcystin congeners, with varying levels of toxicity. The highly toxic microcystin-LR (MC-LR) is the most abundant congener found in Lake Erie, but recent studies suggest that congeners which are more hydrophobic may be more toxic than MC-LR (Chaffin et al., 2023). Further, the available forms of N in lakes directly affect which algal species dominate, thereby indirectly influencing which congeners of microcystin are produced in a cyanobacterial community (Monchamp et al., 2014). However, specific congener production has been shown to track seasonal N availability with a shift to production of less N-rich congeners following depletion of N in Lake Erie (Chaffin et al., 2023).

The neurotoxin, saxitoxin, is another secondary metabolite produced by some cyanobacteria. There are more than 50 congeners of saxitoxin which were traditionally known to be widely distributed, though rarely in temperate lakes. However, with the expansion of the range of genera capable of producing saxitoxins into the Laurentian Great Lakes, it may become more commonly detected (Miller et al., 2017). Indeed, a study of the central basin of Lake Erie found that the saxitoxin gene *sxtA* was detectable and co-occurred with the presence of *Dolichospermum*, a cyanobacterium capable of saxitoxin production (Chaffin et al., 2019). However, samples were not analyzed for the

toxin itself and further work is needed to identify the abiotic factors associated with its production.

1.2 Phytoplankton Succession

According to the Paradox of the Plankton, competitive exclusion should result in phytoplankton species out-competing each other for resources and reaching eventual equilibrium; however, it is known that large, well-mixed bodies of water can support a vast number of species (Hutchinson, 1961). Thus, the ability of species to co-exist has been explained by multiple theories. One of them is competition theory, where two species require the same limited resource, resulting in lower fitness of both. This theory takes into consideration non-equilibrium conditions in mathematical models (such as differences in niche or natural predators) (Alley, 1982). Another theory is intermediate disturbance hypothesis (IDH), which suggests that maximum diversity of species occurs at intermediate disturbances (Flöder & Sommer, 1999). However, Darwinian ecology describes a theory of species succession, which occurs as a series of replacements, usually as a response to various physical factors (specifically with seasonal changes) but sometimes as a result of biotic interactions (Loewen et al., 2021). Symbiotic interactions between species are considered rare, due to competition for resources and grazing of one species by another. In their *Paradox Reconsidered*, Ghilarov considers the possibility of phytoplankton species with the same resource limitations as having evolved in parallel (Ghilarov, 1984), while other theorists describe succession as independent responses to physical and chemical factors or by existence of a hierarchy (Sommer, 1989).

Although cyanobacteria often decrease the diversity of phytoplankton communities due to their competitive advantages, they are susceptible to a variety of parasites, such as

viruses and fungi, causing downstream modifications in the food web (Haraldsson et al., 2018). Viruses, in particular, play a part in modulating phytoplankton succession through "boom and bust" cycles. A healthy population size of the would-be dominant, highly competitive species is reduced markedly in abundance by viral pathogens, essentially controlling the "winner" in phytoplankton communities. This top-down control of a particular genus or species generates diversity, temporally and spatially, with subsequent trophic cascades of other species due to changes in competition for nutrient availability and grazing (Flynn et al., 2022). Fungal pathogens are common in freshwater systems; some species of which are able to infect a variety of hosts while others target specific hosts (Gerphagnon et al., 2015). For example, several species of *Planktothrix* are a preferred host of the chytrid, *Rhizophydium*, which target and break down otherwise inedible filaments (Mckindles et al., 2021). Where filamentous cyanobacteria dominate a phytoplankton community, these fungal infections can render them susceptible to grazing by zooplankton (trophic upgrading), causing a shift in dominance, and providing nutrients to competitors (Frenken et al., 2018).

Studies conducted over the last couple of decades in the Great Lakes region have explored the seasonal shift in phytoplankton community succession. In Lake Erie in particular, we see a dominance of diatoms during the spring, green algae and cryptophytes in early summer, then cyanobacterial blooms in late summer (O'Donnell et al., 2023). The cyanobacterial communities may then experience successions from non-diazotrophic (non-N-fixing) taxa to diazotrophic, as nutrients become depleted (Kane et al., 2014). Although this has been a typical succession for Lake Erie since the early 2000s, a 20-year study reveals that the onset of cyanobacterial blooms has been starting

later into the summer, and there has been an overall increase in most phytoplankton taxa over time (O'Donnell et al., 2023).

Although consideration of phytoplankton succession is typically associated with the ice-free season, a growing number of studies recognize that plankton possess adaptive mechanisms to adjust to low temperatures and reduced light, permitting population growth during winter (Bertilsson et al., 2013; Edgar et al., 2016; Hampton et al., 2015; Salonen et al., 2009; Wilhelm et al., 2020). Whereas taxonomic composition may vary between lakes, winter algal communities share key functional traits addressing motility, nutritional mode and resting stage formation that facilitate their success in a given environmental gradient (Ozkundakci et al., 2016). Winter severity shapes the functional trait composition such that taxonomic organization can vary even within a lake between years of fluctuation in winter severity (Babanazarova et al., 2013; Lenard et al., 2013).

As part of the Great Lakes system, Lake St. Clair connects Lake Huron to Lake Erie. However, limited work examining community succession has been conducted in Lake St. Clair, with the majority of studies representing conditions pre-dreissenid invasion. Wallen (1977) reported that during ice cover in January, diatoms (mainly *Fragilaria* spp.) were the dominant phytoplankton taxon (60-80%), accumulating moderate Chl-*a* biomass (1-2 $\mu g\ L^{-1}$) and with rates of primary production comparable to open-water winter production in Lakes Ontario and Erie (Glooschenko et al., 2011). At one station, the diatom component of the algal assemblage declined by February to <50% being replaced mainly by green algae and filamentous cyanobacteria (Wallen, 1977). This coincided with a decline in reactive silicate at this site to $<100\ \mu g\ L^{-1}$ (Wallen & Tuppling, 1977), a threshold level below which diatoms are reported to be Si-limiting (Schelske & Stoermer,

1971). Subsequent microcosm studies supported limitation of the phytoplankton at this site by silicic acid (D. G. Wallen, 1979). However, in the post-dreissenid era, this may no longer be an issue as post-invasion silica concentrations have been substantially elevated (Barbiero et al., 2006; Makarewicz et al., 2000).

Experiments conducted in freshwater lakes have shown that the taxonomic composition of phytoplankton communities can shift in response to changes in physico-chemical conditions and mixing depths (Stockenreiter et al., 2021). Previous studies have shown that the shifts in community composition occur primarily by co-limitation of light, then nitrogen and phosphorus (Liu et al., 2021). P availability is considered a strong factor in determining phytoplankton species community composition, as storage specialists can benefit from luxury uptake, whereas affinity adapted species gain competitive superiority through induction of a high-affinity P uptake phenotype (Sommer, 1984). For example, *Dolichospermum (*formerly *Anabaena*) has the ability to take up more luxury P than other genera of cyanobacteria, storing it within cells for use during P depleted conditions (Carey, 2012). Some genera capable of luxury uptake will also contribute to P translocation by releasing stores back into the water column during colony recruitment (Cottingham, 2015). In contrast, when P is in low concentration, *Nostoc* has the ability to up-regulate high-affinity P transporters, pumping inorganic P into the cell, facilitating more efficient P scavenging (Solovchenko, et al., 2020). Some phytoplankton can also take advantage of luxury N through gene expression. Research conducted in Sandusky Bay found that *Planktothrix* had the ability to up-regulate the gene responsible for the synthesis of cyanophycin, used for N storage, when NH_4 and NO_3 concentrations are high. Conversely, when N concentrations were depleted, there

was no expression of the gene and cyanophycin was degraded as an adaptation to N limitation (Hampel et al., 2019).

Although it is conventionally thought that HABs can be controlled by the reduction of P only, recent studies highlight the importance of dual-nutrient reduction strategies targeting both N and P to mitigate cyanoHABs and their toxins (Hellweger et al., 2022; Paerl et al., 2016). While toxin and chlorophyll biomass are directly correlated to both nutrients, the ratio of microcystin to chlorophyll production is more highly correlated to dissolved inorganic nitrogen (DIN) (Chaffin et al., 2021). As microcystins are rich in N, their production will likely cause an increase in N requirements (Davis et al., 2015). Thus, it is important to consider the contributions of both N and P in freshwater systems to control the prevalence and impact of algal blooms.

Classically, the onset of spring cyanoHABs is most notably triggered by changes in temperature and light, when temperature-induced stratification of the water column reduces the mixing depth thereby increasing light exposure to algal cells (Winder et al., 2012). In-lab mesocosm experiments have been employed to investigate the phytoplankton response to warming and light intensity, however few studies have coupled the effects of these factors with nutrient availability (Winder et al., 2012).

1.3 Taxonomic Composition Along the Fluvial-Lacustrine Continuum

Fluvial-lacustrine continuums within the Laurentian Great Lakes are robust ecosystems consisting of river, outlet and near-shore area of the lake. The river mouth provides ecological benefits as here, the river plume delivers nutrients and other materials, and hydrologic mixing occurs, functioning much the same as a marine estuary.

More recent work has also shown that the river channel itself is also important for substantial mixing in this zone (Carlson Mazur et al., 2019; Larson et al., 2013).

Flow alteration is an important driving factor in shaping the phytoplankton community composition, spatially and temporally (Qu et al., 2018). Consistent with the Algal Loading Hypothesis (ALH), surveys have shown there is a transfer of cyanobacterial species as well as their associated toxins along the continuum, with a strong correlation between biomass and toxin concentrations (Bormans et al., 2019). Application of the ALH along the fluvial-lacustrine continuum would suggest that rivers contain phytoplankton propagules delivered to their downstream lakes where they are exposed to a more favourable light climate, along with nutrients, to promote blooms (Kane et al., 2014). However, there have been conflicting findings of whether or not rivers play a key role of delivering propagules to prime the blooms of downstream waters. For example, surveys along the Maumee River – Lake Erie continuum confirmed that the taxonomic composition of blooms in rivers differ from those in their receiving waters, suggesting that rivers do not directly seed the lakes (Kutovaya et al., 2012). Contrary to these findings, earlier studies have described the presence of particular cyanobacterial taxa within a river as suggestive as an important seed source to their later appearance in downstream lake waters (Bridgeman et al., 2012; Conroy et al., 2008), although in some cases, this can be attributed to buoyant lake biomass dispersed upstream by wind activity into the lower reaches of the river (Matson et al., 2020). Similarly, evidence from a laboratory study done on water samples from Lake Superior and its upstream rivers suggests that the rivers do play an important role in seeding the blooms found on the southern shore of the lake (Reinl et al., 2020).

In western Lake Erie and Lake St. Clair, cyanobacterial blooms tend to be dominated by colonial species, some producing hepatotoxins such as *Microcystis*, yet, the tributaries of these lakes that are impacted by agricultural runoff of nutrients are often dominated by various filamentous species of cyanobacteria. For example, in the Thames River, these communities tend to be dominated by *Planktothrix agardhii* and *Aphanizomenon flos-aquae* (McKay et al., 2020), commonly occurring in low light conditions (Bonilla et al., 2012). *Planktothrix* can utilize various sources of N whereas *Microcystis*, which thrives in eutrophic waters, relies more heavily on readily available sources of N from runoff (Davis et al., 2015; Hampel et al., 2019). *Microcystis* is found to succeed in warmer epilimnetic waters coincident with periods of reduced turbulence (Chaffin et al., 2013). In western Lake Erie, warmer temperatures usually begin in late July, promoting the formation of buoyant mats of *Microcystis* at the surface where light conditions are favourable (Guellati et al., 2017). While blooms of *Microcystis* have recurred annually in western Lake Erie for decades, reports of bloom events in tributaries are more recent (Conroy et al., 2014; Matson et al., 2020). Recent work done along the continuum connecting Lake Huron to Lake Erie investigated the environmental factors contributing to the drivers of the microbial community. However, there were no clear patterns found between nutrient concentration and cyanobacterial abundance but showed seasonal succession of the microbial community (Crevecoeur et al., 2023).

Although much of the attention on cyanoHABs in the Great Lakes region has focused on dominant, microcystin-producing genera, such as *Microcystis* and *Planktothrix*, blooms are generally composed of a diverse community of cyanobacteria as well as other co-occurring microbes (Pound et al., 2021). As it is well documented that

the transcriptional activity of certain genera of phytoplankton capable of producing secondary metabolites varies with N and P availability and competition, it is important to expand studies to other cyanobacteria (Yancey et al., 2023). Despite the amount of research done in this area, there is still uncertainty on the overall effect of abiotic factors as well as the microbiome in shaping these communities (Pound et al., 2021). We need to recognize that climate change will likely be increasing intensity and frequency of weather and precipitation events, and thus it is even more important to better understand the effects of multiple stressors on phytoplankton growth.

1.4 Thesis Objectives

This research intends to fill some of the gaps, as described above, in terms of the limited studies done in this particular system, and focusing on cyanobacterial genera other than *Microcystis*. We aimed to provide a better understanding of environmental drivers that affect the taxonomic composition and tendency of cyanoHABs to produce algal toxins globally. We employed a systems biology approach to investigate the complex interactions within the fluvial-lacustrine continuum by combining environmental chemistry, algal ecology and molecular biology. More specifically, we compared phytoplankton community composition along the Thames River - Lake St. Clair continuum in the Laurentian Great Lakes, as few studies have focused on the ecology of blooms in this location (Crevecoeur et al., 2023; Davis et al., 2014). This was achieved through microscopy to determine phytoplankton relative abundance and which groups of phytoplankton dominate spatially and temporally. Using qPCR, we investigated the potential toxicity of the blooms, targeting the *mcyE* and *sxtA* genes, involved in

production of microcystin and saxitoxin, respectively, and quantified the presence of total cyanobacteria, targeting the 16s rRNA gene.

To investigate differences in abiotic factors along the fluvial-lacustrine continuum, globally, we compared multiple water quality parameters in the Thames River – Lake St. Clair continuum in the Laurentian Great Lakes basin, as well as in the Sondu River - Lake Victoria continuum in the African Great Lakes basin. These included temperature, specific conductivity, nutrient concentrations and stoichiometry, chlorophyll *a*, and total microcystins. To address environmental factors promoting blooms in the river, in-situ microcosm experiments were conducted within the Thames River during the algal growing season (typically late June to late September) and in the Sondu River in June 2022. These were designed to investigate the effects of light availability as a driver of bloom formation. Finally, we addressed seasonal succession along the continuum by accessing samples from water utility intakes on the shores of Lake St. Clair and shoreside at several locations along the Thames River. We expanded on the temporal resolution of previous work by sampling at minimum, monthly, for three continuous years.

The main objectives of this research included gaining a better understanding of the environmental factors that contribute to phytoplankton biomass and toxin production in both river and lake, and along the continuum. We aimed to assess the distribution of possible toxigenic and non-toxigenic cyanobacteria and phytoplankton succession over time (seasonal comparison) and space (fluvial-lacustrine continuum). Our project also included use of remote data collection using a water quality sonde to monitor in real time changes in physico-chemical parameters and algal community dynamics. The sonde was deployed in the intake well of the Stoney Point Water Treatment Plant (Lake St. Clair)

where it logged and reported data to a LoRaWan gateway at high temporal frequency.

Collectively, the research in the following chapters provided important groundwork that could contribute to predictive modeling tools to better understand when cyanoHABs may develop and whether or not they may be toxigenic. It provided insight into the role that rivers play in the development of downstream blooms and the mechanisms contributing to phytoplankton growth and toxicity.

References

Alley, T. R. (1982). Competition theory, evolution, and the concept of an ecological niche. *Acta Biotheoretica*, *31*, 165–179.

Babanazarova, O., Sidelev, S., & Schischeleva, S. (2013). The structure of winter phytoplankton in Lake Nero, Russia, a hypertrophic lake dominated by *Planktothrix*-like cyanobacteria. *Aquatic Biosystems*, *9*(18).

Barbiero, R. P., Rockwell, D. C., Warren, G. J., & Tuchman, M. L. (2006). Changes in spring phytoplankton communities and nutrient dynamics in the eastern basin of Lake Erie since the invasion of *Dreissena* spp. *Canadian Journal of Fisheries and Aquatic Sciences*, *63*(7), 1549–1563. https://doi.org/10.1139/F06-059

Bertilsson, S., Burgin, A., Carey, C. C., Fey, S. B., Grossart, H. P., Grubisic, L. M., Jones, I. D., Kirillin, G., Lennon, J. T., Shade, A., & Smyth, R. L. (2013). The under-ice microbiome of seasonally frozen lakes. *Limnology and Oceanography*, *58*(6), 1998–2012. https://doi.org/10.4319/LO.2013.58.6.1998

Bonilla, S., Aubriot, L., Soares, M. C. S., González-Piana, M., Fabre, A., Huszar, V. L. M., Lürling, M., Antoniades, D., Padisák, J., & Kruk, C. (2012). What drives the distribution of the bloom-forming cyanobacteria *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*? *FEMS Microbiology Ecology*, *79*(3), 594–607. https://doi.org/10.1111/J.1574-6941.2011.01242.X

Bormans, M., Amzil, Z., Mineaud, E., Brient, L., Savar, V., Robert, E., & Lance, E. (2019). Demonstrated transfer of cyanobacteria and cyanotoxins along a freshwater-marine continuum in France. *Harmful Algae*, *87*.

Bridgeman, T. B., Chaffin, J. D., Kane, D. D., Conroy, J. D., Panek, S. E., & Armenio, P. M. (2012). From River to Lake: Phosphorus partitioning and algal community compositional changes in Western Lake Erie. *Journal of Great Lakes Research*, *38*(1), 90–97. https://doi.org/10.1016/J.JGLR.2011.09.010

Bullerjahn, G. S., McKay, R. M., Davis, T. W., Baker, D. B., Boyer, G. L., D'Anglada, L. V., Doucette, G. J., Ho, J. C., Irwin, E. G., Kling, C. L., Kudela, R. M., Kurmayer, R., Michalak, A. M., Ortiz, J. D., Otten, T. G., Paerl, H. W., Qin, B., Sohngen, B. L., Stumpf, R. P., … Wilhelm, S. W. (2016). Global solutions to regional problems: Collecting global expertise to address the problem of harmful cyanobacterial blooms. A Lake Erie case study. *Harmful Algae 54*, 223–238. https://doi.org/10.1016/j.hal.2016.01.003

Campos, A., & Vasconcelos, V. (2010). Molecular mechanisms of microcystin toxicity in animal cells. *Open Access Int. J. Mol. Sci*, *11*, 11. https://doi.org/10.3390/ijms11010268

Carey, C. C., Ibelings B. W., Hoffmann, E. P., Hamilton, D. P., Brookes, J.D. (2012). Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. Water Research,46 (5), 1394-1407. https://doi.org/10.1016/j.watres.2011.12.016.

Carlson Mazur, M. L., Schaeffer, J., Granneman, J. E., Goldstrohm, N., Fitzpatrick, F. A., Larson, J. H., Reneau, P. C., Kowalski, K. P., & Seelbach, P. W. (2019). Seasonal patterns in hydrochemical mixing in three Great Lakes rivermouth ecosystems. *Journal of Great Lakes Research*, *45*(3), 651–663. https://doi.org/10.1016/j.jglr.2019.03.009

Chaffin, J. D., Bratton, J. F., Verhamme, E. M., Bair, H. B., Beecher, A. A., Binding, C. E., Birbeck, J. A., Bridgeman, T. B., Chang, X., Crossman, J., Currie, W. J. S., Davis, T. W., Dick, G. J., Drouillard, K. G., Errera, R. M., Frenken, T., MacIsaac, H. J., McClure, A., McKay, R. M., … Zhou, X. (2021). The Lake Erie HABs Grab: A binational collaboration to characterize the western basin cyanobacterial harmful algal blooms at an unprecedented high-resolution spatial scale. *Harmful Algae*, *108*, 102080. https://doi.org/10.1016/J.HAL.2021.102080

Chaffin, J. D., Bridgeman, T. B., & Bade, D. L. (2013). Nitrogen constrains the growth of late summer cyanobacterial blooms in Lake Erie. *Advances in Microbiology*, *2013*(06), 16– 26. https://doi.org/10.4236/AIM.2013.36A003

Chaffin, J. D., Mishra, S., Kane, D. D., Bade, D. L., Stanislawczyk, K., Slodysko, K. N., Jones, K. W., Parker, E. M., & Fox, E. L. (2019). Cyanobacterial blooms in the central basin of Lake Erie: Potentials for cyanotoxins and environmental drivers. *Freshwater Ecology*. https://doi.org/10.1016/j.jglr.2018.12.006

Chaffin, J. D., Westrick, J. A., Reitz, L. A., & Bridgeman, T. B. (2023). Microcystin congeners in Lake Erie follow the seasonal pattern of nitrogen availability. *Harmful Algae*, *127*. https://doi.org/10.1016/j.hal.2023.102466

Cheung, M. Y., Liang, S., & Lee, J. (2013). Toxin-producing cyanobacteria in freshwater: A review of the problems, impact on drinking water safety, and efforts for protecting public health. *Journal of Microbiology 2013 51:1*, *51*(1), 1–10. https://doi.org/10.1007/S12275-013-2549-3

Conley, D. J., Paerl, H. W., Howarth, R. W., Boesch, D. F., Seitzinger, S. P., Havens, K. E., Lancelot, C., & Likens, G. E. (2009). Ecology - Controlling eutrophication: Nitrogen and phosphorus. *Science, 323* (5917), 1014–1015. https://doi.org/10.1126/science.1167755

Conroy, J. D., Kane, D. D., Briland, R. D., & Culver, D. A. (2014). Systemic, early-season *Microcystis* blooms in western Lake Erie and two of its major agricultural

tributaries (Maumee and Sandusky rivers). *Journal of Great Lakes Research*, *40*(3), 518–523. https://doi.org/10.1016/J.JGLR.2014.04.015

Conroy, J. D., Kane, D. D., & Culver, D. A. (2008). *Declining Lake Erie ecosystem health? Evidence from a multi-year, lake-wide, plankton study* (M. Munawar & R. Heath, Eds.). Aquatic Ecosystem Health & Management Society.

Cottingham, K. L., H. A. Ewing, M. L. Greer, C. C. Carey, and K. C. Weathers. 2015. Cyanobacteria as biological drivers of lake nitrogen and phosphorus cycling. *Ecosphere 6*(1):1. http://dx.doi.org/10.1890/ES14-00174.1

Crevecoeur, S., Edge, T. A., Watson, L. C., Watson, S. B., Greer, C. W., Ciborowski, J. J. H., Diep, N., Dove, A., Drouillard, K. G., Frenken, T., McKay, R. M., Zastepa, A., & Comte, J. (2023). Spatio-temporal connectivity of the aquatic microbiome associated with cyanobacterial blooms along a Great Lake riverine-lacustrine continuum. *Frontiers in Microbiology*, *14*, 1073753. https://doi.org/10.3389/FMICB.2023.1073753/FULL

Davis, T. W., Bullerjahn, G. S., Tuttle, T., McKay, R. M., & Watson, S. B. (2015). Effects of increasing nitrogen and phosphorus concentrations on phytoplankton community growth and toxicity during *Planktothrix* blooms in Sandusky Bay, Lake Erie. *Environmental Science and Technology*, *49*(12), 7197–7207. https://doi.org/10.1021/acs.est.5b00799

Davis, T. W., Watson, S. B., Rozmarynowycz, M. J., Ciborowski, J. J. H., & McKay, R. M. (2014). Phylogenies of microcystin-producing cyanobacteria in the lower Laurentian Great Lakes suggest extensive genetic connectivity. *PLoS ONE*, *9*(9), 106093. https://doi.org/10.1371/journal.pone.0106093

Edgar, R. E., Morris, P. F., Rozmarynowycz, M. J., D'souza, N. A., Moniruzzaman, M., Bourbonniere, R. A., Bullerjahn, G. S., Phuntumart, V., Wilhelm, S. W., & McKay, R. M. L. (2016). Adaptations to photoautotrophy associated with seasonal ice cover in a large lake revealed by metatranscriptome analysis of a winter diatom bloom. *Journal of Great Lakes Research*, *42*(5), 1007–1015. https://doi.org/10.1016/j.jglr.2016.07.025

Flöder, S., & Sommer, U. (1999). Diversity in planktonic communities: An experimental test of the intermediate disturbance hypothesis. *Limnology and Oceanography*, *44*(4), 1114– 1119. https://doi.org/10.4319/lo.1999.44.4.1114

Flynn, K. J., Mitra, A., Wilson, W. H., Kimmance, S. A., Clark, D. R., Pelusi, A., & Polimene, L. (2022). 'Boom-and-busted' dynamics of phytoplankton–virus interactions explain the paradox of the plankton. *New Phytologist*, *234*(3), 990–1002. https://doi.org/10.1111/NPH.18042

Frenken, T., Wierenga, J., van Donk, E., Declerck, S. A. J., de Senerpont Domis, L. N., Rohrlack, T., & Van de Waal, D. B. (2018). Fungal parasites of a toxic inedible cyanobacterium provide food to zooplankton. *Limnology and Oceanography*, *63*(6), 2384–2393. https://doi.org/10.1002/LNO.10945

Gerphagnon, M., Macarthur, D. J., Latour, D., Gachon, C. M. M., Van Ogtrop, F., Gleason, F. H., & Sime-Ngando, T. (2015). Microbial players involved in the decline of filamentous and colonial cyanobacterial blooms with a focus on fungal parasitism. In *Environmental Microbiology* (Vol. 17, Issue 8, pp. 2573–2587). https://doi.org/10.1111/1462-2920.12860

Ghilarov, A. M. (1984). The paradox of the plankton reconsidered; or, why do species coexist? *Oikos*, *43*(1), 46. https://doi.org/10.2307/3544244

Glooschenko, W. A., Moore, J. E., Munawar, M., & Vollenweider, R. A. (2011). Primary production in Lakes Ontario and Erie: A comparative study. *Journal of the Fisheries Research Board of Canada, 31*(3), 253–263. https://doi.org/10.1139/F74-045

Guellati, F. Z., Touati, H., Tambosco, K., Quiblier, C., Humbert, J. F., & Bensouilah, M. (2017). Unusual cohabitation and competition between *Planktothrix rubescens* and *Microcystis* sp. (cyanobacteria) in a subtropical reservoir (Hammam Debagh) located in Algeria. *PLoS ONE*, *12*(8). https://doi.org/10.1371/journal.pone.0183540

Hampel, J. J., McCarthy, M. J., Neudeck, M., Bullerjahn, G. S., McKay, R. M. L., & Newell, S. E. (2019). Ammonium recycling supports toxic *Planktothrix* blooms in Sandusky Bay, Lake Erie: Evidence from stable isotope and metatranscriptome data. *Harmful Algae*, *81*, 42–52. https://doi.org/10.1016/j.hal.2018.11.011

Hampton, S. E., Moore, M. v, Ozersky, T., Stanley, E. H., Polashenski, C. M., & Galloway, A. W. E. (2015). Heating up a cold subject: prospects for under-ice plankton research in lakes. *J. Plankton Res*, *37*(2), 277–284. https://doi.org/10.1093/plankt/fbv002

Haraldsson, M., Gerphagnon, M, Bazin, P., Colombet, J., Tecchio, S., Sime-Ngando, T., & Niquil, N. (2018). Microbial parasites make cyanobacteria blooms less of a trophic dead end than commonly assumed. *The ISME Journal*, *12*, 1008–1020. https://doi.org/10.1038/s41396-018-0045-9

Hellweger, F. L., Martin, R. M., Eigemann, F., Smith, D. J., Dick, G. J., & Wilhelm, S. W. (2022). Models predict planned phosphorus load reduction will make Lake Erie more toxic. *Science*, *376*(6596), 1001–1005.

https://doi.org/10.1126/science.abm6791/suppl_file/science.abm6791_mdar_reproducibility_checklist.pdf

Ho, J. C., Michalak, A. M., & Pahlevan, N. (2019). Widespread global increase in intense lake phytoplankton blooms since the 1980s. *Nature*, *574*. https://doi.org/10.1038/s41586-019- 1648-7

Hutchinson, G. E. (1961). The Paradox of the Plankton. *The American Naturalist*, *95*(882), 137– 145.

Kane, D. D., Conroy, J. D., Peter Richards, R., Baker, D. B., & Culver, D. A. (2014). Re-eutrophication of Lake Erie: Correlations between tributary nutrient loads and phytoplankton biomass. *Journal of Great Lakes Research*, *40*(3), 496–501. https://doi.org/10.1016/J.JGLR.2014.04.004

Kardinaal, W. E. A., Tonk, L., Janse, I., Hol, S., Slot, P., Huisman, J., & Visser, P. M. (2007). Competition for light between toxic and nontoxic strains of the harmful cyanobacterium *Microcystis*. *Applied and Environmental Microbiology*, *73*(9), 2939–2946. https://doi.org/10.1128/AEM.02892-06

Kutovaya, O. A., McKay, R. M. L., Beall, B. F. N., Wilhelm, S. W., Kane, D. D., Chaffin, J. D., Bridgeman, T. B., & Bullerjahn, G. S. (2012). Evidence against fluvial seeding of recurrent toxic blooms of *Microcystis* spp. in Lake Erie's western basin. *Harmful Algae*, *15*, 71–77. https://doi.org/10.1016/J.HAL.2011.11.007

Larson, J. H., Trebitz, A. S., Steinman, A. D., Wiley, M. J., Carlson Mazur, M., Pebbles, V., Braun, H. A., & Seelbach, P. W. (2013). Great Lakes rivermouth ecosystems: Scientific synthesis and management implications. *Journal of Great Lakes Research*. https://doi.org/10.1016/j.jglr.2013.06.002

Lenard, T., Paul, J., & Wojciechowska, W. (2013). Phytoplankton diversity and biomass during winter with and without ice cover in the context of climate change. *Polish Journal of Ecology (Pol. J. Ecol.)*, *61*, 739–748. https://www.researchgate.net/publication/263430018

Liu, X., Chen, L., Zhang, G., Zhang, J., Wu, Y., & Ju, H. (2021). Spatiotemporal dynamics of succession and growth limitation of phytoplankton for nutrients and light in a large shallow lake. *Water Research*, *194*, 116910. https://doi.org/10.1016/j.watres.2021.116910

Loewen, C. J. G., Vinebrooke, R. D., & Zurawell, R. W. (2021). Quantifying seasonal succession of phytoplankton trait-environment associations in human-altered landscapes. *Limnology and Oceanography*, *66*(4), 1409–1423. https://doi.org/10.1002/lno.11694

Makarewicz, J. C., Bertram, P., & Lewis, T. W. (2000). Chemistry of the offshore surface waters of Lake Erie: Pre- and post-*Dreissena* introduction (1983-1993). *Journal of Great Lakes Research*, *26*(1), 82–93. https://doi.org/10.1016/S0380-1330(00)70675-7

Matson, P. G., Boyer, G. L., Bridgeman, T. B., Bullerjahn, G. S., Kane, D. D., McKay, R. M. L., McKindles, K. M., Raymond, H. A., Snyder, B. K., Stumpf, R. P., & Davis, T. W. (2020). Physical drivers facilitating a toxigenic cyanobacterial bloom in a major Great Lakes tributary. *Limnology and Oceanography*, *65*(12), 2866–2882. https://doi.org/10.1002/lno.11558

McKay, R. M., Frenken, T., Diep, N., Cody, W. R., Crevecoeur, S., Dove, A., Drouillard, K. G., Ortiz, X., Wintermute, J., & Zastepa, A. (2020). Bloom announcement: An early autumn cyanobacterial bloom co-dominated by *Aphanizomenon flos-aquae* and *Planktothrix agardhii* in an agriculturally-influenced Great Lakes tributary (Thames River, Ontario, Canada). *Data in Brief*, *30*, 105585. https://doi.org/10.1016/j.dib.2020.105585

Mckindles, K. M., Jorge, A. N., McKay, R. M., Davis, T. W., & Bullerjahn, G. S. (2021). Isolation and characterization of *Rhizophydiales* (Chytridiomycota), obligate parasites of *Planktothrix agardhii* in a Laurentian Great Lakes embayment. *Applied and Environmental Microbiology*. https://doi.org/10.1128/AEM.02308-20

Miller, T. R., Beversdorf, L. J., Weirich, C. A., Bartlett, S. L., Eikrem, W., & Glaser, K. B. (2017). Cyanobacterial toxins of the Laurentian Great Lakes, their toxicological effects, and numerical limits in drinking water. *Marine Drugs*. https://doi.org/10.3390/md15060160

Millie, D. F., Fahnenstiel, G. L., Bressie, J. D., Pigg, R. J., Rediske, R. R., Klarer, D. M., Tester, P. A., & Litaker, R. W. (2009). Late-summer phytoplankton in western Lake Erie (Laurentian Great Lakes): bloom distributions, toxicity, and environmental influences. *Aquatic Ecology*, *43*, 915–934. https://doi.org/10.1007/s10452-009-9238-7

Monchamp, M.-E., Pick, F. R., Beisner, B. E., & Maranger, R. (2014). Nitrogen forms influence microcystin concentration and composition via changes in cyanobacterial community structure. *PLOS One*. https://doi.org/10.1371/journal.pone.0085573

O'Donnell, D. R., Briland, R., Budnik, R. R., Ludsin, S. A., & Hood, J. M. (2023). Trends in Lake Erie phytoplankton biomass and community structure during a 20-year period of rapid environmental change. *Journal of Great Lakes Research*, *49*, 672–684. https://doi.org/10.1016/j.jglr.2022.12.011

O'Neil, J. M., Davis, T. W., Burford, M. A., & Gobler, C. J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae*, *14*, 313–334. https://doi.org/10.1016/j.hal.2011.10.027

Ozkundakci, D., Gsell, A. S., Shintze, T., Teauscher, H., & Adrian, R. (2016). Winter severity determines functional trait composition of phytoplankton in seasonally ice-covered lakes. *Glob Chang Biol*. https://doi.org/10.1111/gcb.13085

Paerl, H., Fulton Iii, R., Moisander, P., & Dyble, J. (2001). *Harmful freshwater algal blooms, with an emphasis on cyanobacteria.* https://doi.org/10.17615/ndpc-5j58

Paerl, H. W., Hall, N. S., & Calandrino, E. S. (2011). Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Science of the Total Environment, The*, *409*, 1739–1745. https://doi.org/10.1016/j.scitotenv.2011.02.001

Paerl, H. W., Thad Scott, J., McCarthy, M. J., Newell, S. E., Gardner, W. S., Havens, K. E., Hoffman, D. K., Wilhelm, S. W., & Wurtsbaugh, W. A. (2016). It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. *Environ. Sci. Technol*, *50*, 10805–10813. https://doi.org/10.1021/acs.est.6b02575

Pound, H. L., Martin, R. M., Sheik, C. S., Steffen, M. M., Newell, S. E., Dick, G. J., McKay, R. M. L., Bullerjahn, G. S., & Wilhelm, S. W. (2021). Environmental studies of cyanobacterial harmful algal blooms should include interactions with the dynamic microbiome. *Cite This: Environ. Sci. Technol*, *55*, 12776–12779. https://doi.org/10.1021/acs.est.1c04207

Qu, Y., Wu, N., Guse, B., & Fohrer, N. (2018). Riverine phytoplankton shifting along a lentic-lotic continuum under hydrological, physiochemical conditions and species dispersal. *Science of the Total Environment*, *619–620*, 1628–1636.

Reinl, K. L., Sterner, R. W., Lafrancois, B. M., & Brovold, S. (2020). Fluvial seeding of cyanobacterial blooms in oligotrophic Lake Superior. *Harmful Algae*, *100*, 101941. https://doi.org/10.1016/J.HAL.2020.101941

Rinta-Kanto, J. M., Konopko, E. A., DeBruyn, J. M., Bourbonniere, R. A., Boyer, G. L., & Wilhelm, S. W. (2009). Lake Erie *Microcystis*: Relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake. *Harmful Algae*, *8*(5), 665–673. https://doi.org/10.1016/J.HAL.2008.12.004

Salonen, K., Leppäranta, A. M., Viljanen, A. M., & Gulati, A. R. D. (2009). Perspectives in winter limnology: closing the annual cycle of freezing lakes. *Aquatic Ecology*. https://doi.org/10.1007/s10452-009-9278-z

Schelske, C. L., & Stoermer, E. F. (1971). Eutrophication, silica depletion, and predicted changes in algal quality in Lake Michigan. *Science*, *173*(3995), 423–424. https://doi.org/10.1126/SCIENCE.173.3995.423

Solovchenko A, Gorelova O, Karpova O, Selyakh I, Semenova L, Chivkunova O, Baulina O, Vinogradova E, Pugacheva T, Scherbakov P, Vasilieva S, Lukyanov A, Lobakova E. (2020). Phosphorus feast and famine in cyanobacteria: is luxury uptake of the nutrient just a consequence of acclimation to its shortage? *Cells*, *9*(9). https://doi: 10.3390/cells9091933.

Sommer, U. (1984). The paradox of the plankton: Fluctuations of phosphorus availability maintain diversity of phytoplankton in flow-through cultures. *Limnology and Oceanography*, *29*(3), 633–636. https://doi.org/10.4319/LO.1984.29.3.0633

Sommer, U. (1989). *Plankton Ecology : Succession in Plankton Communities* (U. Sommer, Ed.). Springer Berlin Heidelberg.

Stockenreiter, M., Isanta Navarro, J., Buchberger, F., & Stibor, H. (2021). Community shifts from eukaryote to cyanobacteria dominated phytoplankton: The role of mixing depth and light quality. *Freshwater Biology*, *66*(11), 2145–2157. https://doi.org/10.1111/FWB.13822

Wallen, D. G. (1977). Production processes under the ice in Lake St. Clair I. Irradiation and temperature. *Water Resources Bulletin*, *13*(2). https://doi.org/10.1111/j.1752-1688.1977.tb02025.x

Wallen, D. G. (1979). Nutrients limiting phytoplankton production in ice-covered Lake St. Clair. *Journal of Great Lakes Research*, *5*(2), 91–98. https://doi.org/10.1016/S0380-1330(79)72133-2

Wallen, D., & Tuppling, E. (1977). Production processes under the ice in Lake St. Clair II. Nutrients (silicate) as a limiting factor. *Journal of the American Water Resources Association*, *13*(3), 512–520.

Wilhelm, S. W., Bullerjahn, G. S., Michael, R., & McKay, L. (2020). The Complicated and Confusing Ecology of *Microcystis* Blooms. *mBio, 11*(3), 1-5. https://doi.org/10.1128/mBio

Winder, M., Berger, S. A., Lewandowska, A., Aberle, N., Lengfellner, K., Sommer, U., & Diehl, S. (2012). Spring phenological responses of marine and freshwater plankton to changing temperature and light conditions. *Marine Biology*, *159*(11), 2491–2501. https://doi.org/10.1007/S00227-012-1964-Z

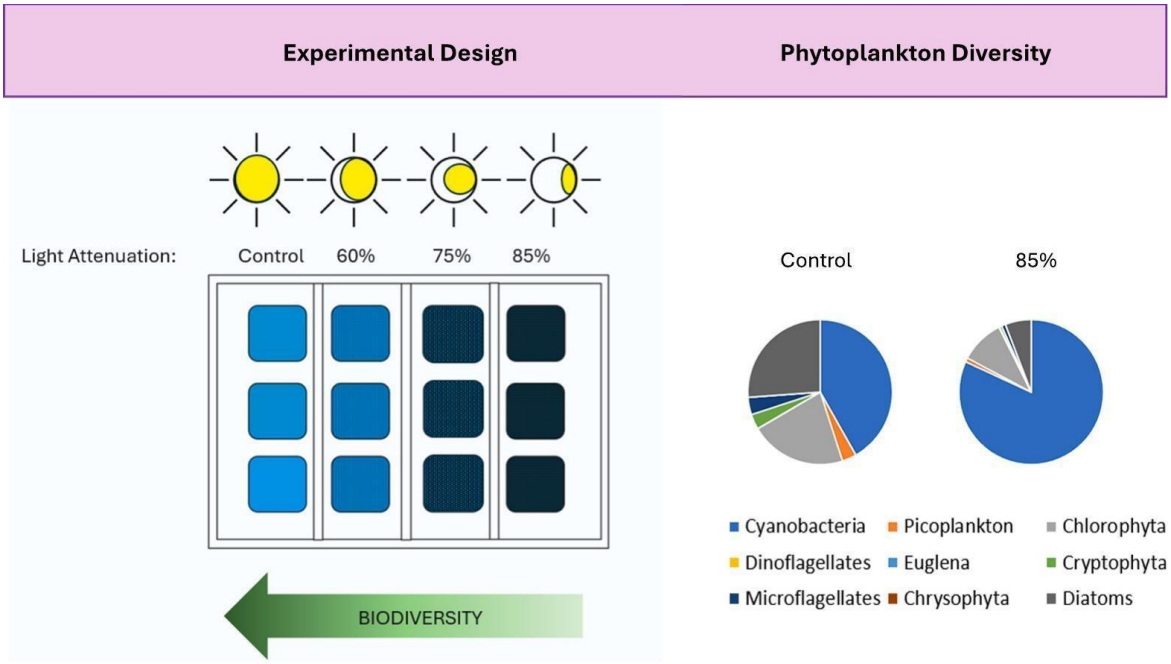
Yancey, C. E., Mathiesen, O., & Dick, G. J. (2023). Transcriptionally active nitrogen fixation and biosynthesis of diverse secondary metabolites by *Dolichospermum*

and *Aphanizomenon*- like Cyanobacteria in western Lake Erie *Microcystis* blooms. *Harmful Algae*, *124*, 102408. https://doi.org/10.1016/j.hal.2023.102408

Yancey, C. E., Smith, D. J., Den Uyl, P. A., Mohamed, O. G., Yu, F., Ruberg. Steven A., Chaffin, J. D., Goodwin, K. D., Tripathi, A., Sherman, D. H., & Dick, G. J. (2022). Metagenomic and metatranscriptomic insights into population diversity of *Microcystis* blooms: spatial and temporal dynamics of *mcy* genotypes, including a partial operon that can be abundant and expressed. *Applied and Environmental Microbiology*, *88*(9), e02464-21.

CHAPTER 2: Environmental drivers of phytoplankton community dynamics in an agriculturally-influenced tributary in the lower Great Lakes

GRAPHICAL ABSTRACT



2.1 Introduction

The integrity of freshwater resources is threatened globally on multiple fronts by persistent human influence. One widespread manifestation of anthropogenic influence in recent decades is an increased prevalence of cyanobacterial harmful algal blooms (cyanoHABs) in freshwater bodies (Bullerjahn et al., 2016; Cheung et al., 2013; Ho et al., 2019). Proliferation of algal blooms, as a general symptom of eutrophication, can alter aquatic food webs and create low oxygen zones, which can be detrimental to freshwater life (Carpenter et al. 1995; Paerl et al., 2011; Sterner et al. 2021). These blooms can also produce toxins (collectively known as cyanotoxins) and other secondary metabolites which are dangerous to many organisms. The most commonly found, and the most well-studied group of toxins in freshwater systems are hepatotoxins, collectively known as microcystins. Eutrophication occurs due to over-enrichment of phosphorus (P) and nitrogen (N) due to anthropogenic activities, which can enhance cyanobacterial dominance of algal blooms (Paerl et al., 2016). However, interactions among multiple factors including light availability, water mixing, and bioavailability of various chemical species of N and P are increasingly being considered to manage the proliferation of cyanoHABs (Graeber et al., 2024; Hellweger et al., 2022; Liu et al., 2021; Paerl et al., 2016).

Multiple environmental factors may drive changes in cyanoHABs and phytoplankton community composition along the tributary to lake continuum. Phytoplankton communities in tributaries differ from those in their receiving waters, likely due to sediment resuspension and resulting light attenuation in rivers as opposed to nutrient limitation under more optimal light conditions in lakes (Davis et al., 2014). In agriculturally-influenced rivers, much of the spatial and temporal variation in

phytoplankton community composition can be attributed to variation in light availability (Davis et al., 2014). These fluvial systems are often characterized by high turbidity and low light levels due to high sediment loads (McKay et al., 2018; Reynolds et al., 1994). Fluctuations in discharge rate can cause changes in nutrient supply and light conditions, and drive shifts in phytoplankton community composition and biomass (Domingues et al. 2005; Laiveling et al., 2022). Surveys along the fluvial-lacustrine continuum confirm this difference in taxonomic composition, reinforcing the idea that rivers do not primarily seed phytoplankton communities in lakes in the Great Lakes region (Crevecoeur et al., 2023; Kutovaya et al., 2012). However, rivers deliver nutrients required for the occurrence of cyanoHABs in lakes where phytoplankton are exposed to more favourable light conditions, thus promoting the formation of blooms (Kane et al., 2014).

In shallow eutrophic lakes, shifts in phytoplankton community composition can occur due to co-limitation of light, nitrogen and phosphorus (Liu et al., 2021). Mixing depth also plays a key role in community composition, with a dominance of motile taxa found under shallow mixing regimes, and diatoms predominating in deeper mixed layer environments (Jäger et al., 2008). In deeper lakes, the onset of spring cyanoHABs can be triggered by changes in temperature and light, when temperature-induced stratification of the water column reduces mixing depth, thereby increasing light exposure of algal cells (Winder et al., 2012). Alterations to light regimes caused by vertical stratification can cause changes in phytoplankton biomass, community composition, and physiology (Marzetz et al., 2020). For example, the peak depth of the distribution of phytoplankton chlorophyll within the water column, referred to as the deep chlorophyll maximum (DCM), is determined by stratification and is usually dominated by diatoms,

dinoflagellates and cryptophytes (Leach et al., 2017). Phytoplankton communities are also susceptible to daily (fast) and seasonal (slow) light fluctuations. Fast fluctuations can delay the timing of competitive exclusion, whereas slow fluctuations can allow for a stable co-existence between taxa (Litchman & Klausmeier, 2001). Slow fluctuations have the greatest impact on species diversity particularly when phytoplankton are competing for nutrients (Flöder & Burns, 2005). Mesocosm experiments conducted in lakes have also shown that the taxonomic composition of phytoplankton communities can shift in response to changes in physico-chemical conditions and mixing depths (Stockenreiter et al., 2021).

In western Lake Erie and Lake St. Clair, cyanobacterial blooms tend to be dominated by colonial species, such as *Microcystis*, whereas cyanobacterial assemblages in the tributaries of these lakes that are directly impacted by agricultural runoff of nutrients are often dominated by various filamentous taxa. For example, communities in the Thames River tend to be dominated by taxa such as *Aphanizomenon flos-aquae* and *Planktothrix agardhii* (Crevecoeur et al., 2023; McKay et al., 2020), commonly occurring in low light conditions (Bonilla et al., 2012; Scheffer et al., 1997). *Microcystis* is found to proliferate in warmer epilimnetic waters coincident with periods of reduced turbulence (Chaffin et al., 2013). In western Lake Erie, warmer temperatures usually begin in late July, promoting the formation of buoyant mats of *Microcystis* at the surface where light conditions are favourable (Guellati et al., 2017; Wilhelm et al., 2020). While blooms of *Microcystis* have recurred annually in western Lake Erie for decades, reports of bloom events in tributaries are more recent (Conroy et al., 2014; Matson et al., 2020).

The main objective of this study was to determine how multiple environmental factors, including light attenuation and nutrient concentrations, promote or constrain phytoplankton community dynamics in an agriculturally-influenced tributary of the lower Great Lakes. We conducted *in situ* microcosm experiments to determine the effects of light attenuation on the proliferation of the phytoplankton community of the Thames River in southwestern Ontario. We deployed 10 L containers in a floating frame in the Thames River in early, mid and late summer of 2021 and 2022, when peak algal biomass was expected (Crevecoeur et al., 2023; McKay et al., 2020), in order to test two main hypotheses. First, we hypothesized that the abundance of the dominant taxa and diversity of the phytoplankton community within the experimental containers would vary with respect to light attenuation and the availability and stoichiometric ratio of N and P. Second, we expected that a reduction in light availability would serve as a cue for the cyanobacterial community to shift from buoyant colonial cyanobacterial taxa to filamentous forms more tolerant of low light.

2.2 Materials and Methods

2.2.1 Study site

The Thames River is a Great Lakes Water Quality Agreement Annex 4 priority tributary located in southwestern Ontario (Figure 1). It is 273 kilometres in length with an average depth of 1.2 metres, and flows southwesterly from the headwater near the township of Tavistock and drains into southern Lake St. Clair. The drainage basin of the Thames is 5825 km^2, with the land use dominated by row crop agriculture (McKay et al., 2020). Our study site was located in Chatham, Ontario, adjacent to the Lower Thames Valley Conservation Authority (LTVCA) facility (42.4076° N, 82.1853° W), one of two conservation authorities with jurisdiction over the Thames River watershed. The

experimental containers were deployed at this site and monitored from the northern shore of the river.

2.2.2 Effects of light attenuation on phytoplankton abundance

Field trials were conducted using 10 L low-density polyethylene containers that were fixed within a three-row floating PVC frame tethered to a retaining wall on shore (Appendix A, Figure S1). The tether had approximately 4 m of slack, allowing movement of the frame on the river surface to prevent batch effects. Likewise, at several intervals over the incubation, the polyethylene containers were randomly distributed throughout the frame. Three of the containers were unamended as controls. The remaining nine containers were wrapped in neutral density fiberglass window screen to attenuate ambient light by 60%, 75% and 85% (i.e., each attenuation level had three containers) as determined using a QSL-101 light meter (Biospherical Instruments, Inc., San Diego, CA, USA). Total solar radiation was determined for each deployment in 2021, using data from Environment and Climate Change Canada, and despite the linear decrease from June to September, ANOVA found no statistically significant differences between deployments ($F = 3.3$, $p > 0.05$, Appendix A, Table S1). Data were not available for the July 2022 deployment and thus solar radiation comparisons between deployments were not made for year two.

Prior to the start of the experiment, the containers were washed with dilute acid (5% HCl) and then thoroughly rinsed in distilled water. Prior to filling the units at the study site, the containers were conditioned by rinsing them with water from the Thames River several times. River surface water was then pumped into a clean 100 L plastic barrel, which was continuously stirred with a plastic paddle to ensure homogenization

while filling the containers. Each container was filled with 7 L of river water prior to being deployed in the floating frame. Time-zero samples were taken, in triplicate, from the barrel at the start of the experiment to measure ambient nutrients and chlorophyll *a* (Chl *a*) concentrations and determine phytoplankton taxonomy. Accessory chlorophylls were not measured as Chl *a* is the most abundant pigment found in all algal species and regularly used as a proxy for algal biomass, whereas other photosynthetic pigments are not distributed uniformly across phyla (Kumar Patel, et al., 2022). A handheld model 600QS sonde (YSI, Yellow Springs, Ohio, USA) was used to determine river temperature and specific conductivity at the deployment location at the beginning and end of each experiment as well as interim sampling events (Appendix A, Table S2).

The first deployment began on June 23, 2021, and was incubated for nine days prior to removal, with sampling occurring at time zero, day three, day six, and day nine. Prior to sampling, the containers were shaken to maintain homogeneous distribution of nutrients and phytoplankton cells. There were no significant differences found in Chl *a* concentrations between day three and subsequent sampling; however growth rates declined after day three. Because of this, the incubation time of the following experiment was shortened. The second deployment began on July 27, 2021, and was incubated for five days, with sampling occurring at time zero, day three and day five. The third deployment began on September 20, 2021, but high river levels created a hazard for sampling, so sampling occurred at time zero, day three, and day nine as the frame was inaccessible for sampling on day 5. In order to maintain consistency in the second year, all deployments were incubated for nine days and began on July19, August 24 and September 28, 2022.

2.2.3 Sample processing and analyses

For each sample, water was filtered through 0.22 µm Sterivex cartridge filters (MilliporeSigma, Burlington, MA, USA) and frozen at -20°C for later analysis of soluble reactive P (SRP), nitrate plus nitrite (NO_3-NO_2,) and ammonia (NH_3). Raw water was frozen at -20°C for later analysis of total P (TP). For Chl *a* quantification, samples were filtered through a 0.2 µm PCTE filter, then frozen at -20°C until ready for analysis. For total microcystins, 10 mL of raw water was transferred to a glass scintillation vial and frozen at -20°C for later analysis. For phytoplankton taxonomic identification, 50 mL of raw water was pooled from each set of triplicates and preserved with Lugol's iodine solution for phytoplankton identification and enumeration.

Water samples were analysed for major nutrients using a SmartChem 170 Discrete Analyzer (KPM Analytics, Westborough, MA, USA), using colorimetric determination where samples were analyzed in discrete mode and each reaction took place in an individual cuvette. The methods were based on the following: Soluble reactive phosphorus (SRP) concentrations were analysed as orthophosphate with a method detection limit of 0.0019 mg P L^{-1} (EPA 365.1 rev. 02, 1993); NO_3-NO_2 by reducing nitrate to nitrite with a detection limit of 0.0028 mg N L^{-1} (EPA 353.2 rev. 02, 1993); total phosphorus (TP) after manual sulfuric acid digestion with a detection limit of 0.0015 mg P L^{-1} (EPA 365.1 rev. 02, 1993) and NH_3 with a detection limit of 0.014 mg NH-N L^{-1} (SM4500).

Samples for Chl *a* analysis were extracted overnight in dimethyl sulfoxide (DMSO) and analyzed fluorometrically using a TD-700 fluorometer (Turner Designs, San Jose, CA, USA). Cyanotoxins were analyzed following manufacturer's instructions using a Microcystins/Nodularins (ADDA) ELISA kit (Gold Standard Diagnostics,

Warminster, PA, USA) with a detection limit of 0.016 µg L^{-1}. Whole water preserved with Lugol's iodine was analyzed by microscopy for algal taxonomy (Aquatic Taxonomy Specialists, Malinta, OH, USA). Phytoplankton cells were identified and enumerated in a measured aliquot using the Utermöhl method with a magnification modification of the stratified counting technique of Munawar and Munawar (1976) as described elsewhere in (McKay et al., 2020).

2.2.4 Statistical Analyses

All statistical analyses were conducted in R programming environment (https://www.r-project.org/). Alpha biodiversity metrics (i.e., Shannon and Simpson indices, richness and evenness) were calculated using R function 'diversity' in the package 'vegan'. All water quality parameters that were not normally distributed (e.g., TP, NO_3-NO_2, SRP, and NH_3) were ln-transformed before univariate and multivariate analyses to improve normality and homogeneity of variance. For univariate analyses, analysis of variance (ANOVA) was used to detect the difference in water quality parameters among treatment levels within each month (June, July, and September). When ANOVA detected statistical significance for a water quality parameter, a Tukey's test was used to do follow-up multiple comparisons. For SRP and NH_3, we used ANOVA for censored data, followed by Tukey's multiple comparison test to detect treatment-level differences (Helsel, 2012).

Multivariate analyses were conducted on the entire experimental dataset (three monthly deployments × five treatment levels) on phytoplankton abundance data and abiotic data including light attenuation, water quality parameters, stoichiometric ratios, and number of sampling days. Multivariate analyses were not run on June, July, and

September data separately, due to small sample size (five samples per month). Nonmetric multidimensional scaling (NMDS) was performed using Bray-Curtis dissimilarity to identify variation in phytoplankton community composition across treatment levels and in the three monthly deployments. NMDS stress values were used to assess whether NMDS provided acceptable ordinations of phytoplankton composition. Similarly, principal component analysis (PCA) was carried out to show abiotic variation among treatment levels and months. Permutation multivariate ANOVA (PERMANOVA) was performed to detect statistical differences in phytoplankton composition and abiotic factors among treatment levels and months. Before PERMANOVA, permutational analysis of multivariate dispersion (PERMDISP) was conducted to assess the homogeneity of variance in phytoplankton composition and abiotic factors. There were no significant differences in heterogeneity of variance in phytoplankton composition among treatment levels and months.

To study the relationships between phytoplankton assemblages and abiotic factors, distance-based redundancy analysis (db-RDA) was performed using Bray-Curtis dissimilarity, a type of constrained ordination similar to RDA. The difference between RDA and db-RDA is that RDA relies on Euclidean distance, while db-RDA can incorporate other distance metrics such as Bray-Curtis dissimilarity. Because the length of the first axis of detrended correspondence analysis (DCA) < 3, unimodal methods (e.g., CCA) were not appropriate for the data and not used (Leps & Smilauer, 2003). For water quality parameters, PCA, PERMANOVA and db-RDA were conducted based on means of three replicates within each treatment level. Specifically, mean TP and NO_3-NO_2 were calculated using the arithmetic mean, while mean SRP and NH_3 were

estimated using regression on order statistics (ROS) due to the presence of non-detects in their datasets (SRP: ~37%; NH_3: ~14%) (Helsel, 2011).

2.3 Results and Discussion

Phytoplankton community composition in tributaries of the Great Lakes differs from the lakes themselves due to a number of factors including light limitation from suspended particles and variation in dissolved nutrients (Kane et al., 2014). This study was designed to determine the effects of reduced solar radiation on phytoplankton community composition motivated by specific interest on cHAB-forming taxa, while also tracking the concurrent effects of variation in dissolved nutrient levels, in an agricultural tributary of the lower Great Lakes. This study showed that light attenuation was important for limiting total phytoplankton abundance in the Thames River. Although total phytoplankton abundance increased over time under all light attenuation treatments (relative to time zero), total abundance in the highest light attenuation treatment (85%) was less than half of the controls. Increased light attenuation reduced phytoplankton diversity, as species richness and evenness was lowest in the 85% light attenuated treatments in all three experimental trials of 2021. Results were less consistent in 2022, suggesting that biodiversity cannot be attributed to light availability alone. Greater light attenuation increased the abundance of cyanobacteria in five of six trials , although relative abundance of cyanobacteria was low in early summer and early fall.

2.3.1 Nutrients and chlorophyll *a*

2021:

There were no significant differences in concentrations of SRP between time zero, control or treatment containers during any of the trials (SRP: $t = -1.6 – 1.3$, $p > 0.5$),

though SRP was below detectable levels for the majority of samples in July and September (Figure 2).

There were no statistical differences in TP concentrations in any of the samples in June ($t = -2.5 – 0.72$, $p > 0.05$) or July ($t = -2.9 – 1.3$, > 0.05), however, in September, they were much higher at time zero and 85% light attenuation compared to control samples (Table 1), suggesting a lower draw down of P with higher light attenuation (time zero vs control: $t = -6.2$, $p < 0.01$; 85% vs control: $t = 4.6$, $p < 0.05$).

In all three trials, NO_3-NO_2 showed a positive trend with increased light attenuation after an initial decrease from time zero to the control samples, although not significant in July (Figure 2). In September, control samples had significantly lower concentrations than time zero ($t = -5.1$, $p < 0.01$), 75% ($t = 4.2$, $p < 0.05$) and 85% treatments ($t = 5.3$, $p < 0.01$). In June, control samples had marginally lower concentrations than time-zero ($t = -3.9$, $p = 0.07$) and 85% ($t = 3.9$, $p = 0.07$). This could be interpreted as a reduced uptake of nitrate in lower light conditions. Conversely, in September, NH_3 concentrations were significantly lower in the 75% light attenuated treatment compared to time-zero ($t = -4.9$, $p < 0.01$), control ($t = -5.2$, $p <0.01$) and 60% attenuated treatment ($t = -5.2$, $p<0.01$).

The dissolved inorganic nitrogen (DIN):SRP ratio at time-zero was almost seven times higher in July than in June, and mean NO_3-NO_2 concentrations approximately double (Table 1; Figure 2), suggesting more intense P limiting conditions at the start of the July experiment. This is also reflected in the mean SRP concentration, with a range from below detectable levels to 6.3 µg L^{-1} compared to 10.8 to 21.5 µg L^{-1} in June.

Chlorophyll data were not available for the July deployment due to instrument failure and subsequent sample degradation, but concentrations were significantly different between some of the samples in September (Table 1, Appendix A, Figure S2). Concentrations were higher in control samples and 60% compared to time zero ($t = 6.2$, $p < 0.01$; $t = 7.1$, $p < 0.01$) and in 60% compared to 85 % ($t = 4.2$, $p < 0.05$), suggesting that phytoplankton abundance increased during the experiment, with higher light levels, and lower light availability had a negative impact on production. Earlier work examined the differences in phytoplankton productivity in relation to light penetration along a river continuum within the Lake Erie basin. Similar to the findings in this study, Conroy's study found that when light limitation was relieved to mimic the off-shore light climate, productivity increased, whereas decreasing light penetration in off-shore samples decreased productivity (Conroy, 2007; Conroy et al., 2008).

2022:

Similar to 2021 results, there were no significant differences in concentrations of SRP, or in NO_3- NO_2 between samples in any of the trials (SRP: $t = -2.4 – 2.7$, $p > 0.5$; NO_3-NO_2: $t = -1.8 – 2.4$, $p > 0.5$), and SRP was again below detectable levels for time zero samples in the later trials (Figure 3). This could indicate depletion of the bioavailable P compared to the beginning of the summer, as often occurs during the algal growing season (Xu et al., 2010). There were no statistical differences in TP concentrations in any of the samples in August, however, in July, they were approximately three times higher in the 60% light attenuation treatment compared to time zero ($t = 4.2$, $p = 0.007$; Table 2) and in the September – October deployment, approximately three times higher in the 60% treatment compared to the control samples (t

= 4.2, p = 0.007). This is in contrast to 2021 results where TP was highest in the highest attenuated containers rather than the lowest attenuated. As in 2021, NH_3 concentrations in September were significantly lower in the 75% light attenuated treatment but only when compared to 60% (t = 3.6, p = 0.03). The dissolved inorganic nitrogen (DIN):SRP ratio at time-zero was more than 30 times higher in August than in July. Mean SRP concentrations were more than 30 times lower in August at 0.95 µg L^{-1} than in July at 33.5 µg L^{-1}, suggesting more intense P limiting conditions later into the summer, in line with the 2021 results and supporting the idea that the bioavailable P pool is being depleted over the algal growing season. The DIN:SRP at time zero in September was six times lower, and SRP concentrations were eight times higher than in August, but considerably lower than in July.

Chlorophyll *a* concentrations in July were highest in the controls and decreased with increasing light attenuation (Table 2). They were significantly lower in the 85% attenuated treatment compared to time zero and control samples (t = -3.7, p = 0.021; t = -3.9, p = 0.01) suggesting that lower light availability had a negative impact on productivity (Appendix A, Figure S3). Similar results were found in the September-October deployment with a linear decrease in productivity with light attenuation, but all treatments had non-significantly higher concentrations than control samples. This further supports the results found in 2021 where less chlorophyll was being produced in the higher light attenuated treatments.

2.3.2 Phytoplankton community composition and biodiversity

2021:

The three most abundant major phytoplankton groups were cyanobacteria, diatoms, and chlorophyta, which accounted for ~90% of mean relative abundance (~43%, ~35%, and ~13%, respectively) across all months and treatments (Figure 4). Microflagellates, picoplankton, and cryptophyta accounted for an additional ~9% of mean abundance (~4%, ~3%, and ~2%, respectively). Dinoflagellates, euglenophyta, and chrysophyta accounted for <1% of mean abundance.

Eighteen of the most abundant individual phytoplankton taxa (comprising >1% of relative abundance) made up ~90% of total relative abundance across all months and treatments (Appendix A, Table S3). Seven cyanobacteria taxa made up ~38% of total mean abundance; these taxa included *Aphanocapsa* spp. (~14%), *Merismopedia tenuissima* and *Merismopedia* sp. (~14%), *Planktothrix agardhii* (~4%), *Aphanizomenon* sp. (~2%), *Chroococcus microscopicus* (~2%), and *Romeria* sp. (~1%). Four diatom taxa made up ~37% of mean abundance, including *Skeletonema* spp. (~21%), *Cyclotella* spp. (~11%), a centric diatom sp. (~3%), and *Nitzschia* spp. (~2%). Four chlorophyta taxa made up ~8% of mean relative abundance, including *Scenedesmus* spp. (~3%), a coccoid sp. (~2%), *Dictosphaerium* spp. (~2%), and *Sphaerocystis* spp. (~1%). Overall, the high proportion of cyanobacteria (except in June; discussed below) suggested eutrophic conditions, although the presence of diatoms, chlorophyta and a variety of other phytoplankton taxa reflects a diverse community.

Total phytoplankton abundance (measured as cells mL^{-1}) increased in all containers relative to time-zero conditions in all three deployments, although total

abundance was lowest in high light attenuation treatments (Table 3). This finding was similar to earlier research which showed that light attenuation of ≥ 50% significantly reduced total phytoplankton biomass in Lake Taihu, China (Zhou et al., 2014).

All four alpha biodiversity indices were generally highest in the controls and lowest in the 85% light attenuation treatment in all three trials (Appendix A, Table S4). As one of multiple factors controlling phytoplankton community composition, light limitation is proportional to species diversity (Flöder & Burns, 2005) and light availability can play a significant role in shaping community structure through competition (Litchman & Klausmeier, 2001). Diversity was highest in September, specifically in the control compared to treatment groups, with a linear decrease in total abundance with increased light attenuation.

In all three months, increasing light attenuation tended to favour the dominate phytoplankton group in two or more of the light treatments relative to the controls (Figure 4). In June, diatoms were the dominant group in all treatments, representing ~40% of the taxa in control treatments, at time-zero and more than 60% in all light attenuation treatments (Figure 4A). In contrast, in July and September, most light attenuation treatments were dominated by cyanobacteria (Figure 4B and 4C). This was expected as diatom taxa often dominate phytoplankton communities in spring and early summer, whereas cyanobacteria become more dominant later in summer under eutrophic, lower light conditions, and higher temperatures (Lowe, 1974; Mancuso et al., 2021).

In the July experiment, relative abundance shows all treatments, with the exception of the 75% attenuation were dominated by cyanobacteria (Figure 4B). Compared to control samples, there were lower abundances of colonial *Aphanocapsa* in

the treatment containers. Diatoms were present at the start of the experiment, increased slightly in the control and 60% and dominated in the 75% attenuation. However, the July time zero samples had the highest N:P ratio, suggesting intense P limiting conditions, under which chlorophyta tend to have a competitive advantage over cyanobacteria (Andersen et al., 2020).

In the September experiment, cyanobacteria again dominated with an increase in relative abundance with light attenuation, when compared to time-zero samples (Figure 4C). The most abundant was the filamentous *Planktothrix,* a genus known to be more shade tolerant and a better competitor at low light than other cyanobacterial genera (Torres et al., 2016; Visser et al., 2020). The increase in *Planktothrix* was coincident with a decrease in DIN:SRP, and this genus is known to be a more efficient scavenger of N than other cyanobacteria and is more tolerant of N limitation (Hampel et al., 2019). These findings support the hypothesis that dominance of phytoplankton taxa would shift with light availability.

2022:

Nineteen of the most abundant individual phytoplankton taxa (comprising >1% of relative abundance) made up ~90% of total relative abundance across all months and treatments (Appendix A, Table S5). Seven cyanobacteria taxa made up ~25% of total mean abundance; these taxa included *Aphanocapsa* spp. (~2%), *Chroococcus microscopicus* (~1.5%), *Merismopedia* sp. (~7.8%), *Merismopedia tenuissima* (~5.6%), *Microcystis* sp. (~2.4%), *Planktolyngbya minor* (4.2%) and *Planktothrix* sp. (~1.5%; Figure 5). Three diatom taxa made up ~30% of mean abundance, including *Skeletonema* spp. (~10.6%), centric diatom sp. (~13.5%), and *Nitzschia* spp. (~5.8%). Five chlorophyta

taxa made up ~22% of mean relative abundance, including *Crucigenia* spp. (~1.2%), a coccoid sp. (~9.8%), *Dictosphaerium* spp. (~3.8%), *Micractinium* spp. (~1.2%), and *Scenedesmus* spp. (6.1)%. Two cryptophyta made up ~ 3%, including *Cryptomonas* sp. (~1.4%) and *Cryptomonas erosa* (~1.7%). Species of picoplantkon and microflagellates made up 6.7 and 2.7% of total abundance, respectively. Similar to 2021 findings, the variety of taxa present shows a diverse phytoplankton community growing under different nutrient and light availability.

The two most abundant major phytoplankton groups in the July experiment were diatoms, which accounted for a minimum of 50% of mean relative abundance across all treatments, and chlorophyta, which accounted for 13% (Figure 5A). Dinoflagellates accounted for ~5% of the time zero samples but were otherwise in very low abundance across all treatments and months (< 0.4%). Cyanobacteria made up only 0.2% of the time zero samples, but some growth occurred across all treatments, making up 14% and 16% of the 60% and 75% attenuated treatments, respectively. In the August - September and September - October deployments, cyanobacteria, chlorophyta and diatoms were the most abundant groups (Figure 5B), as was found in all three deployments in 2021. At time zero, cyanobacteria accounted for 23% and 60%, respectively. Compared to August time zero, cyanobacterial growth occurred in all treatments and control, but in September, cyanobacterial growth was considerably less in all containers than time zero with 60% dominance (Figure 5C). Control samples and all treatments were dominated by diatoms and diverse chlorophyta communities, with a linear increase of diatoms and decrease of chlorophyta with increased light attenuation. For both August and September deployments, overall cyanobacterial growth was lowest in the 85% attenuation. Relative

abundance of microflagellates, picoplankton, and cryptophyta varied across all treatments and months, and chrysophyta were found only in very low abundance (0% to 0.2%). These results were consistent with year one findings, where diatoms dominated in early summer and cyanobacteria later into the season, and earlier research highlighting the seasonal shifts between major phytoplankton groups (Wilhelm et al., 2020).

The dominance in diatoms for all three experiments was driven mainly by the presence of the same genera found in 2021; *Nitzschia, Skeletonema,* and unidentified centric species, though not *Cyclotella*. The cyanobacterial community in the July time zero samples was comprised of only *Pseudanabaena*, but with a very low cell count (~80). *Planktolyngbya* spp dominated the 60% and 85% light attenuated samples at 82% and 100% relative abundance, respectively. The cyanobacterial community in August – September was dominated by *Merismopedia* spp at time zero, in control and 60% attenuated containers and *Merismopedia tenuissima* and *Planktolyngbya minor* in the higher attenuated containers. In September, the dominant cyanobacteria varied amongst treatments. At time zero, only three types of taxa were present; *Planktothrix* at 20% and *Pseudanabaena* and unidentified coccoid species at 40%, each. As in 2021, DIN:SRP decreased compared to mid-summer, coinciding with a decrease in abundance of non-N fixing *Merismopedia* and an increase in *Planktothrix* (an efficient N-scavenger) and *Pseudanabaena*, capable of N fixation. The most abundant in the control and 60% attenuated samples were *Merismopedia* spp, *Microcystis* and *Planktothrix.* In the 75% treatment, they were *Microcystis* and *Planktothrix*. The most abundant in the 85% treatment were *Cuspidothrix, Planktothrix* and *Phormidium*. Although *Merismopedia* spp were found in high abundance in both 2021 and 2022, the cyanobacterial communities

varied considerably between the two years; most notably with the appearance of *Microcystis* in year two.

During the July experiment, total phytoplankton abundance (measured as cells mL^{-1}; Table 2) increased in control samples and decreased in treatments relative to time-zero, in contrast to the 2021 findings. Similar to the previous year, however, growth increased in all containers in the second and third deployments relative to time-zero. (Table 2). The lowest total abundance occurred in the highest light attenuated treatments (85%) in July and September, supporting the 2021 findings that lower light resulted in less overall growth. Results were inconsistent among deployments for highest total abundance, and therefore cannot be attributed to light attenuation alone.

In the July experiment, alpha biodiversity metrics showed the highest overall diversity and evenness occurred in the highest light attenuated treatment (85%), inconsistent with year one findings, but species richness was highest in the 60% attenuation. (Appendix A, Table S4). Conversely species richness in the August-September deployment was highest in 85% attenuated samples over other treatments and diversity was highest in the 60% attenuation relative to other treatments, but showed an overall decrease from time zero. Generally speaking, between trials, overall diversity was highest in the September-October deployment, specifically in the 60% light attenuation. As diversity was highest in September 2021 as well, but general findings were inconsistent between the two years, this suggests that diversity cannot be attributed to light availability alone with temperature/seasonality playing an important role in shaping these communities.

2.3.3 Relationships among phytoplankton, nutrients and water quality

2021:

In an NMDS analysis of major taxonomic groups, variation in phytoplankton composition associated with light attenuation occurred mainly along axis 1 (Figure 6A); time-zero samples were located towards the left of axis 1, control and intermediate light attenuation treatments were located towards the right, and the highest light attenuation treatment was located in the middle of axis 1. Variation in community composition associated with month occurred mainly along NMDS axis 2, where diatoms were most abundant in June towards the top of axis 2 and cyanobacteria were most abundant in July and September towards the bottom of axis 2. This analysis indicated that the high light attenuation treatment (85%) tended to promote the relative abundance of cyanobacteria, relative to the controls, particularly in July and September.

NMDS analysis of genus/species level community composition showed that dominance by diatoms in June (towards top of axis 2) was driven mainly by the relative abundance of *Skeletonema* (da11) and, to a lesser extent, *Cyclotella* (da4), which were highest in the control and low attenuation (60%) treatments (Figure 6B). Similarly, *Skeletonema costatum* has shown to prevail under low light conditions due to competitive advantage over other genera of phytoplankton (Shoman & Akimov, 2022). In July, the cyanobacterial dominance can be attributed to the presence of several of the most common freshwater cyanobacterial genera, including *Aphanocapsa* (cy2), *Merismopedia* sp. (cy10), *Merismopedia tenuissima* (cy11), and *Chroococcus microscopicus* (cy4). In September, the cyanobacterial community shifted towards greater abundance of genera which are common in eutrophic freshwater systems. These are, primarily, *Planktolyngbya* spp. (cy,13, cy14, cy15), *Planktothrix agardhii* (cy17), *Aphanizomenon* (cy1), and

Dolichospermum (cy7). This shift may have been in response to changing nutrient conditions, with a significantly lower DIN:SRP than in July and highest TP concentrations across the three deployments. *Planktothrix,* which often dominates cyanobacterial blooms when TP concentrations are high, and *Aphanizomenon*, a taxon capable of N fixation, were present at all levels of light attenuation; both are known to thrive in low light conditions (Bonilla et al., 2012; de Nobel et al., 1997).

RDA (i.e., dbRDA) analysis of covariance between major phytoplankton groups and environmental factors showed increased light attenuation was most strongly associated with increased abundance of cyanobacteria, increased DIN:SRP, and decreased SRP:TP (Figure 7A). Previous research has also found that cyanobacteria are favoured by lower light conditions compared to some other major groups of algae (Zhou et al., 2014). At the genus/species level, this change in cyanobacteria was explained by increased abundance of *Aphanocapsa* (cy2), *Merismopedia tenuissima* (cy10), *Merismopedia sp*. (cy11), and *Chroococcus microscopicus* (cy4; Figure 7B). The diatom *Cyclotella* (da4) also increased under higher attenuation conditions.

2022:

In an NMDS analysis of major taxonomic groups, variation in phytoplankton composition associated with light attenuation treatments occurred mainly along axis 2 (Figure 8A); with 60% attenuation towards the bottom, 85% towards the top and intermediate light attenuation treatments located in the middle of axis 2. Both time zero and control samples overlapped the light treatments. Variation in community composition associated with month occurred mainly along NMDS axis 1, where diatoms were most abundant in July towards the left of axis 1 and cyanobacteria were most abundant in the

August-September experiment, associated with intermediate light attenuation towards the right of axis 1. In the September-October experiments, chlorophyta were most abundant towards the right of axis 1 and associated with all levels of light attenuation. This analysis indicated that all treatments tended to promote the relative abundance of cyanobacteria, compared to time zero in August-September in the warmer months. The opposite occurred in September-October when cyanobacteria may have lost their competitive advantage with an average water temperature of 16° C (sub-optimal for cyanobacterial growth). (Lürling et al., 2013).

NMDS analysis of genus/species level community composition showed that dominance by diatoms in July (towards top of axis 2) was driven mainly by the relative abundance of centric diatoms (di3; Figure 8B). As in year one, the phytoplankton community shifted to greater abundances of genera which are common in eutrophic freshwater bodies later in the summer. In the August- September experiment, the cyanobacterial dominance at the lowest light attenuation can be attributed to the presence of colonial *Merismopedia* sp. (cy9) and filamentous *Planktolyngbya* (cy 14). At intermediate attenuation, it was driven by the abundance of *Merismopedia tenuissima* (cy10), as it was in the highest light attenuation, to a lesser extent. The cyanobacterial dominance in the highest light attenuation was driven mainly by the presence of filamentous *Aphanizomenon* (cy1). During the September-October deployment, the cyanobacterial community was primarily dominated by colonial *Microcystis* spp (cy11) and filamentous *Planktothrix* spp, specifically associated with control samples, 60% and 75% light attenuation.

These community shifts were likely in response to changing environmental conditions, consistent with the 2021 microcosm experiments. Temperature may have been an important driver in the reduction in cyanobacteria with reduced light intensity in the September deployment compared to August. Average river temperatures were optimal for cyanobacterial growth and competition in August (25° C) and significantly colder in September (15° C; Appendix A, Table S2) (Lürling et al., 2013). DIN:SRP was significantly lower in September than August, possibly promoting the cyanobacterial community shift from non-N fixing *Merismopedia* to N-fixing *Pseudanabaena*.

RDA (i.e., dbRDA) analysis of covariance between major phytoplankton groups and environmental factors showed increased light attenuation was most strongly associated with increased abundance of cryptophyte and diatoms, increased NH_3, NO_3-NO_2 and DIN:SRP. (Figure 9A). The presence of cyanobacteria was most strongly correlated to elevated levels of SRP and lower light attenuation. At the genus/species level, the diatom taxon most associated with increased light attenuation was *Skeletonema* (di10) and the cyanobacterial community shifted from a dominance of *Merismopedia* spp (cy9, cy 10) and *Plantktolyngbya minor* (cy15) in the August-September deployment to *Microcystis* (cy11) and *Planktothrix* (cy15) in the September-October deployment. (Figure 9B).

Differences in relative abundance of phytoplankton taxa between the all six trials may be explained by a combination of environmental factors, which supports our first hypothesis that phytoplankton communities would vary with respect to light and nutrient availability. Previous research has also found that certain species of cyanobacteria are better adapted to low light conditions compared to other groups of algae (Zhou et al.,

2014). Changes in seasonal temperatures may be a secondary effect, explaining the community shift to cyanobacterial dominance in mid-summer of both years, as studies have shown that cyanobacteria exhibit a higher tolerance to increased temperatures than diatoms, and higher nitrate availability under more intense P limitation (Lürling et al., 2013). Over the course of both studies, a higher abundance of non-N fixing genera was observed when N levels were highest relative to P.

Despite increased abundance of cyanobacteria in our experimental trials, concentrations of total microcystins remained low, within the range of 0.03 μg L^{-1} – 0.14 μg L^{-1} and 0.05 μg L^{-1} – 0.68 μg L^{-1} across trials in 2021 and 2022, respectively. These concentrations did not exceed the maximum acceptable level of 10 μg L^{-1} for recreational exposure in Canada (https://www.canada.ca). Future testing should be expanded to include additional toxins, such as saxitoxin or other secondary metabolites of concern (Chaffin et al., 2019).

2.4 Conclusions

Recognizing that it is difficult to design a microcosm experiment to mimic major ecosystem processes and community compositions, these experiments were performed to gain some insight into the basic mechanisms involved in algal growth with the manipulation of only one factor (e.g., solar irradiance). As these experiments capture only a snapshot of phytoplankton community at a given time and place, they do not necessarily represent the broader river system or address hydrologic factors and processes occurring at the sediment-water or land-river interface. As such, similar studies in larger experimental units reveal that scaling correction is necessary when extrapolating from smaller experiments (Schindler, 1998), however, many ecologists accept the use of

microcosm studies due to their replicability, statistical power and short time frame in which they can be carried out (Carpenter, 1996; Schindler, 1998).

The results of this study support the hypothesis that phytoplankton communities vary with respect to light and nutrient availability as well as nutrient stoichiometry. More specifically, there was a reduction in overall abundance and diversity of phytoplankton with increasing light attenuation as phytoplankton abundance, Chl *a*, and diversity were lowest in highest light attenuated treatments in most trials. The results also support the hypothesis that increased light attenuation will be accompanied by a shift in community composition to a dominance of taxa more tolerant of lower light levels. Diatoms dominated in the June trial and are often among the first groups of phytoplankton to appear in spring and early summer. The relative abundance of diatoms increased in the light attenuated treatments compared to time zero and control containers. In July 2021 and August 2022, nutrient conditions at time zero were suggestive of more intense P limitation and the community shifted to cyanobacterial dominance. In September of both years, the cyanobacterial dominance generally increased with light attenuation but was mostly attributed to filamentous forms. Although these findings did not consistently support the hypothesis that the community would shift from colonial cyanobacterial forms to filamentous taxa with increased light attenuation, in 2021 there was a lower abundance of a colonial genera (*Aphanocapsa*) in the reduced light treatments in July and a higher abundance of filamentous forms in September (*Planktothrix)* contributing to the overall cyanobacterial dominance. Similarly, in August 2022, there was a lower abundance of *Aphanocapsa* and an increase in *Planktolyngbya* in the reduced light treatments.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada, Discovery Grant Program. The authors thank the sampling team, Tom Varga, William Delaurier, Alexia Varga, William Varga, and Olivia Varga.

Author Contributions

Emily Varga: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. R. Paul Weidman: Writing – review & editing, Validation, Investigation, Formal analysis. Zhuoyan Song: Writing – review & editing, Validation, Investigation, Formal analysis, Data curation. R. Michael McKay: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

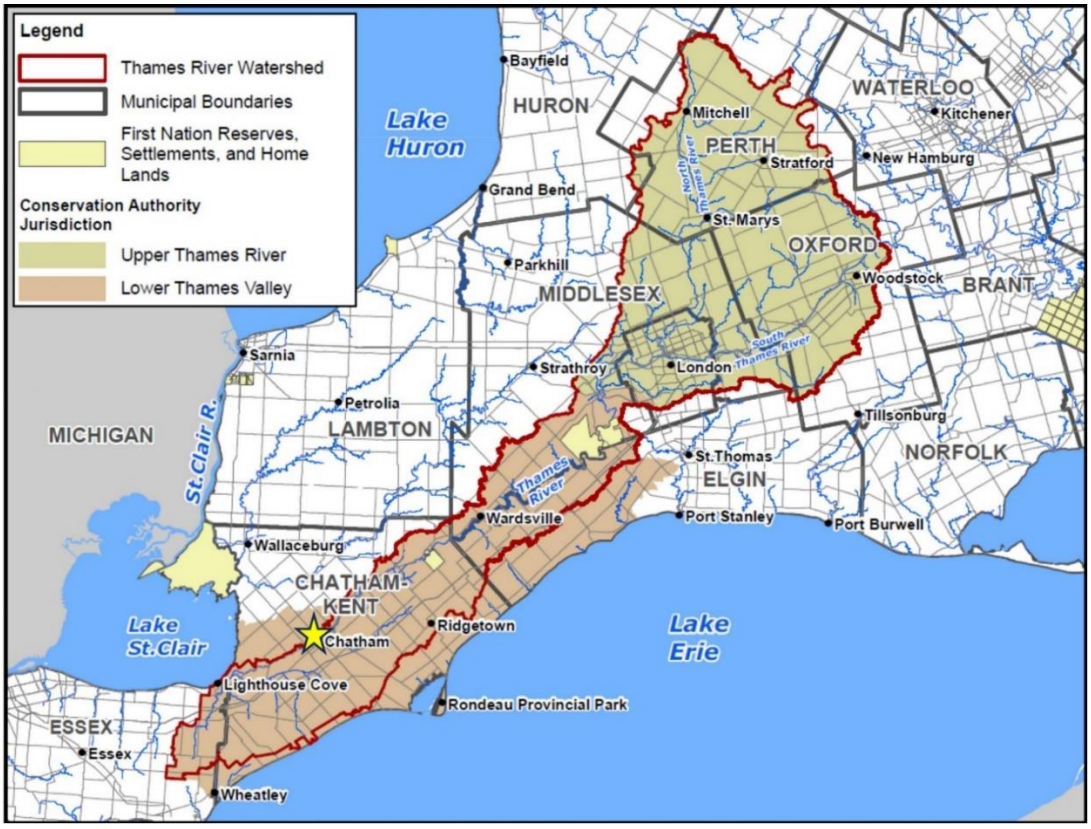
Conflicts of interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: R. Michael McKay reports financial support was provided by Natural Sciences and Engineering Research Council of Canada. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

FIGURES & TABLES

Figure 1:

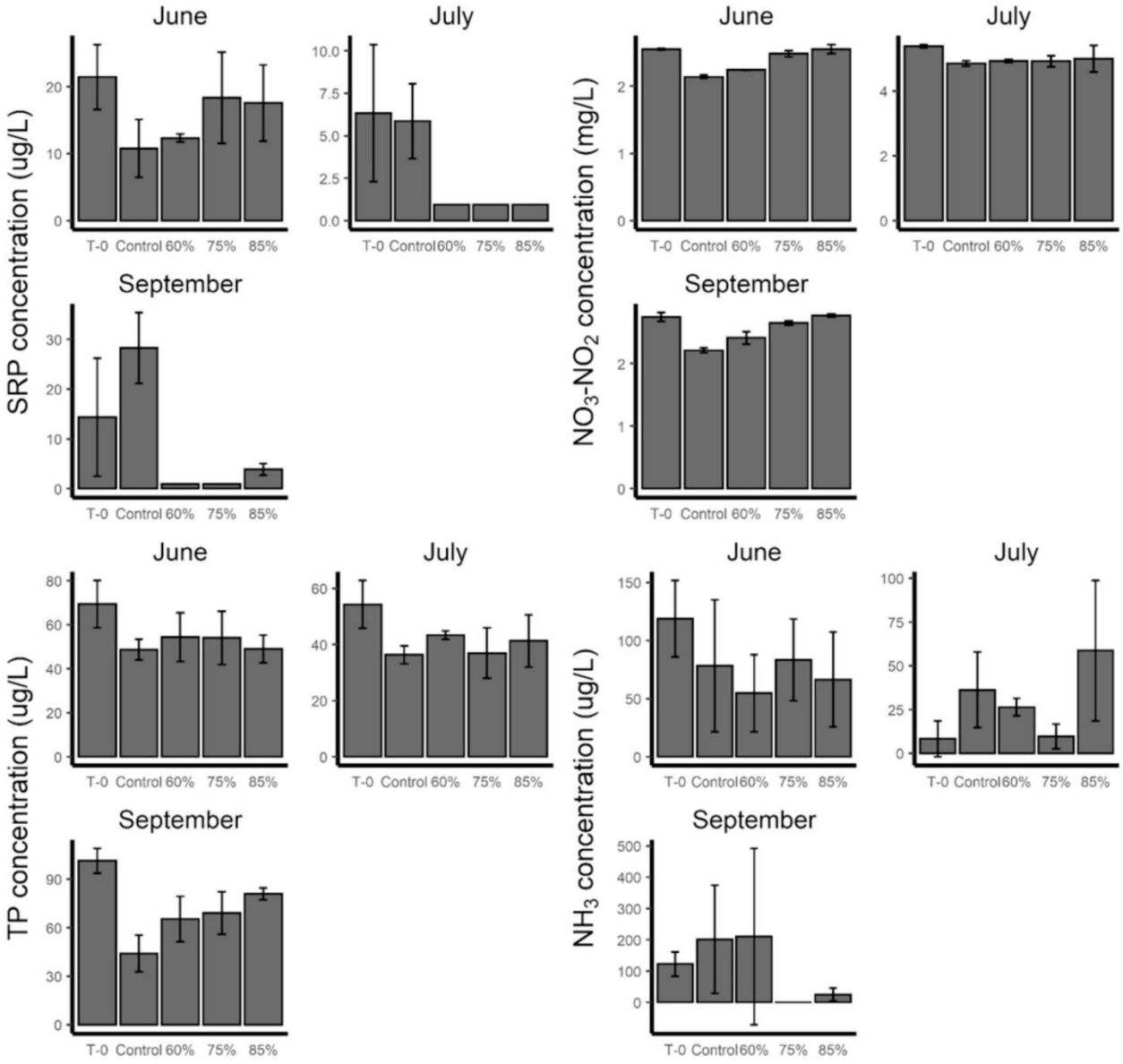
Map of the Study Area Within the Thames River Watershed



NOTE: Map of the study area within the Thames River watershed (outlined in red) and nearby towns in southwestern Ontario, Canada. The yellow star indicates the town of Chatham, where the Lower Thames Valley Conservation Authority is located.

Figure 2

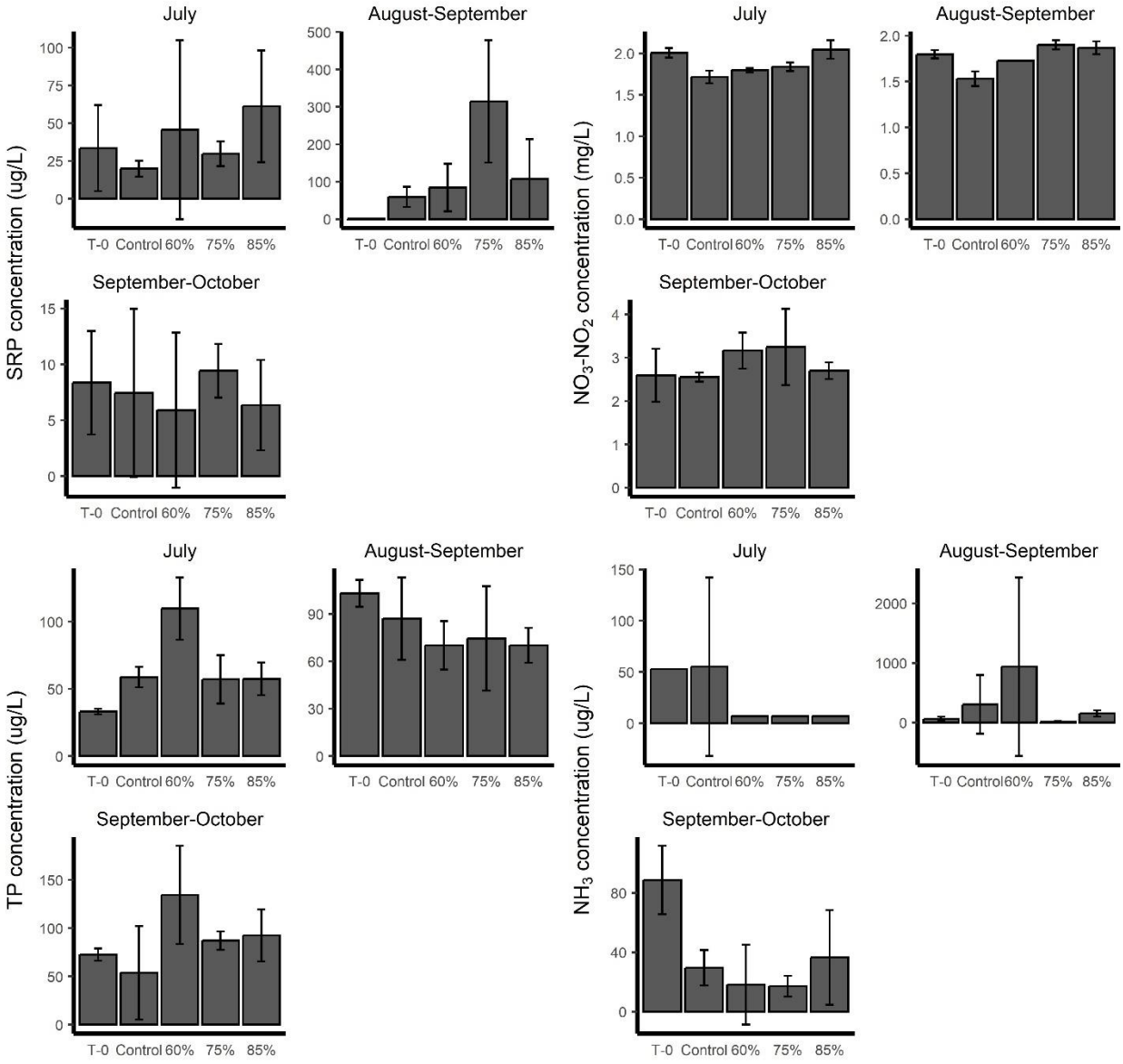
Nutrient Concentrations for 2021 Deployments



NOTE: Mean and standard deviation of SRP and NH_3 are estimated using regression on order statistics (ROS) due to the presence of non-detects in their datasets. When three replicates within a treatment level all fall below detection limit, half of the detection limit is used to represent mean with a standard deviation of zero.

Figure 3

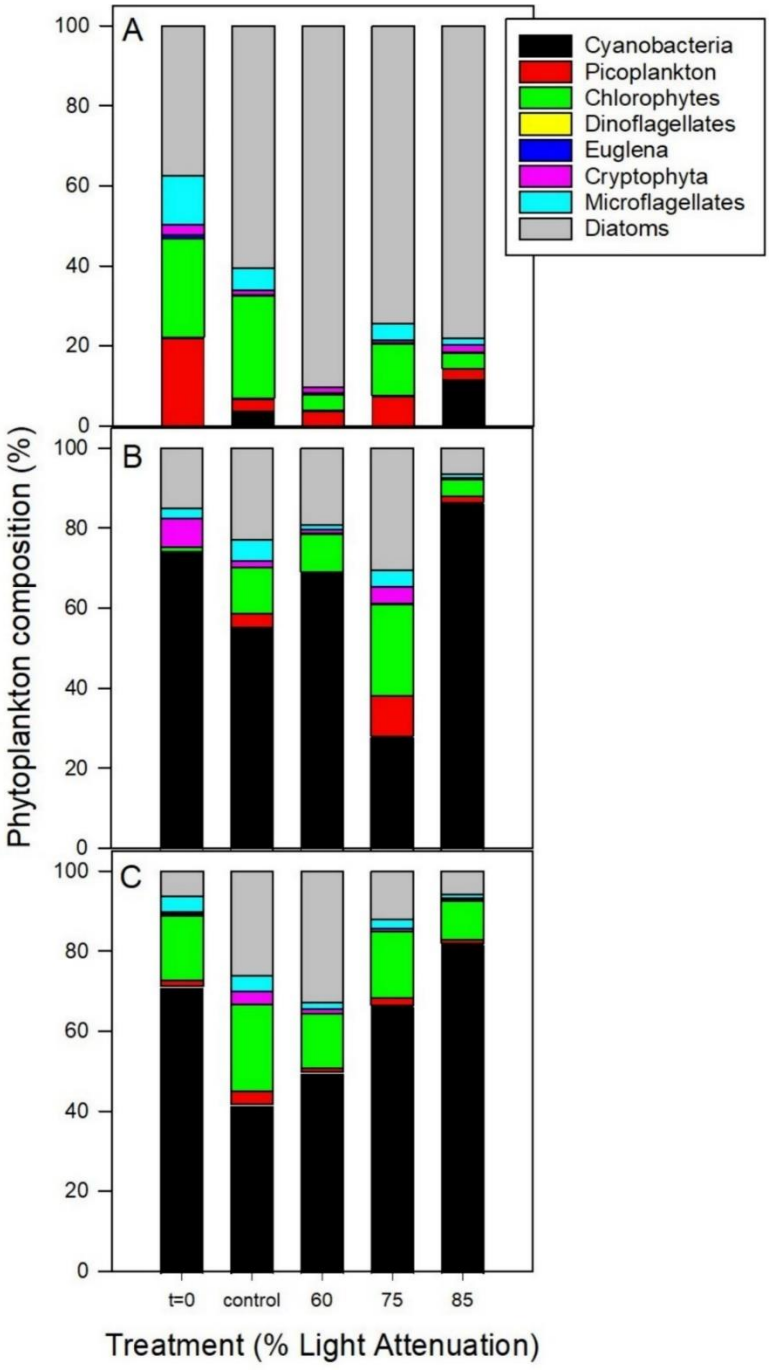
Nutrient Concentrations for 2022 Deployments



NOTE: Mean and standard deviation of SRP and NH_3 are estimated using regression on order statistics (ROS) due to the presence of non-detects in their datasets. When three replicates within a treatment level all fall below detection limit, half of the detection limit is used to represent mean with a standard deviation of zero.

Figure 4:

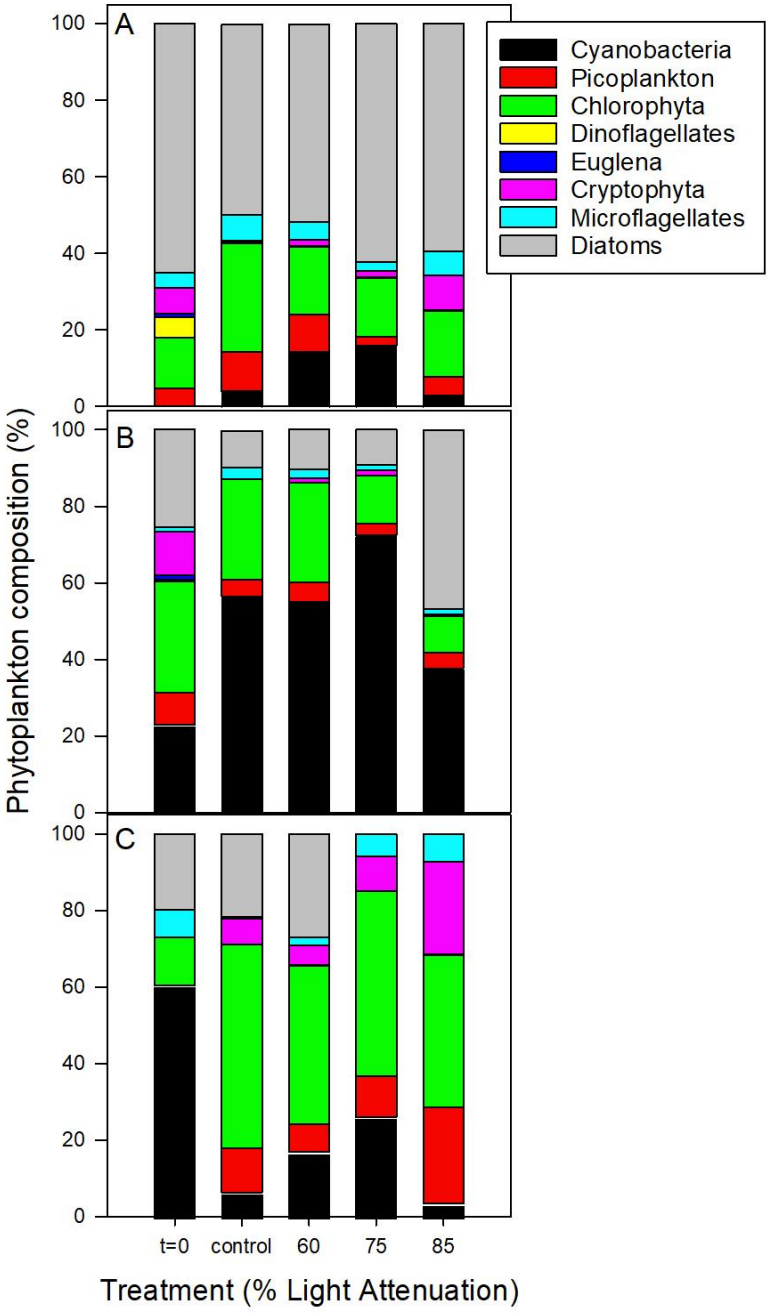
Phytoplankton Community Composition 2021



NOTE: Phytoplankton community composition, by taxonomic group, for June (A), July (B) and September (C) 2021.

Figure 5:

Phytoplankton Community Composition 2022

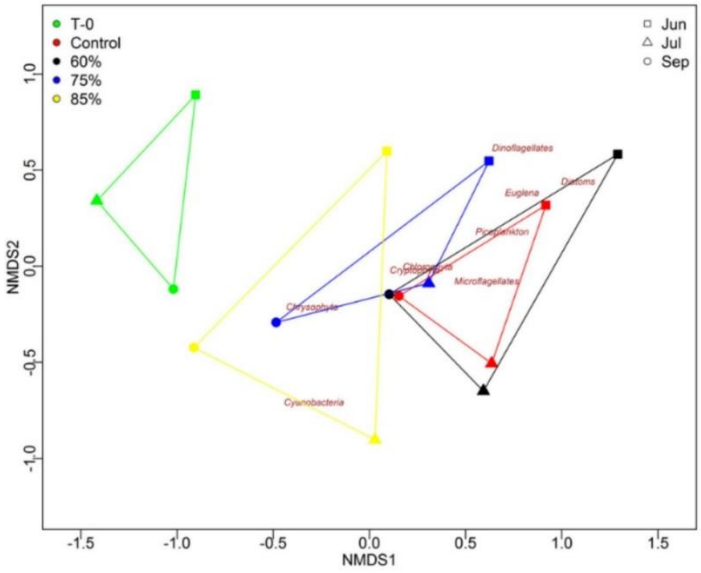


NOTE: Phytoplankton community composition, by taxonomic group, for July (A), August-September (B) and September-October (C) 2022.

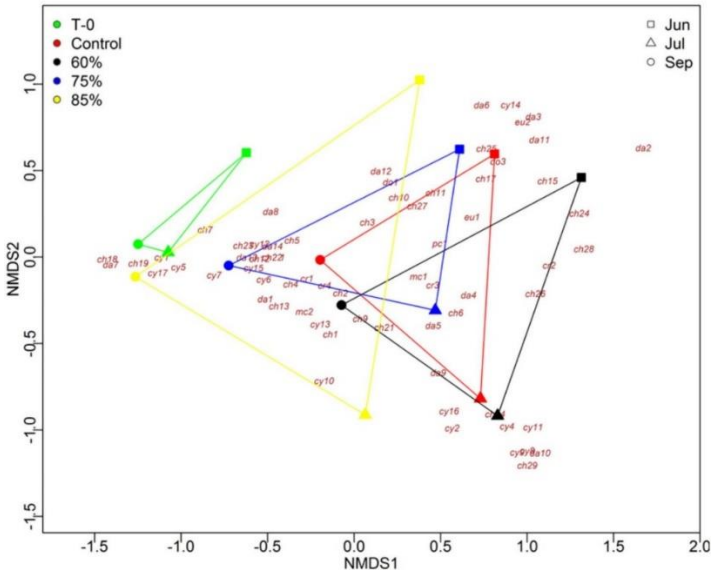
Figure 6:

Non-Metric Multidimensional Scaling (NMDS) 2021

A.



B.

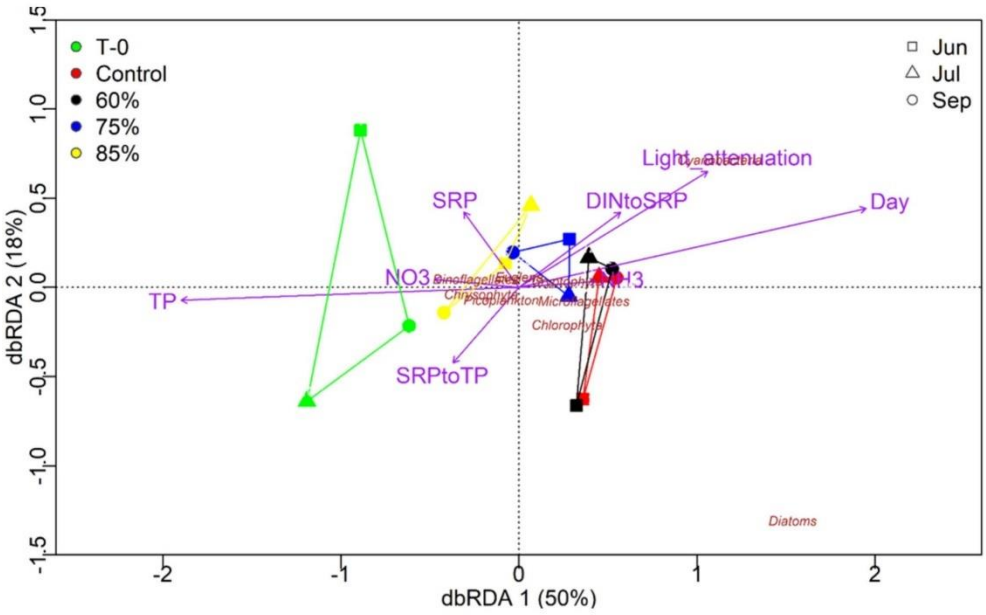


NOTE: Non-Metric Multidimensional Scaling (NMDS) analysis of community composition based on major phytoplankton taxonomic groups (A) and individual phytoplankton genera/species (B) in three months (June, July, Sept 2021) and five experimental treatments (time-zero, control, and 60 %, 75 %, and 85 % light attenuation; see Appendix A, Table S3 for genus/species codes in appendices).

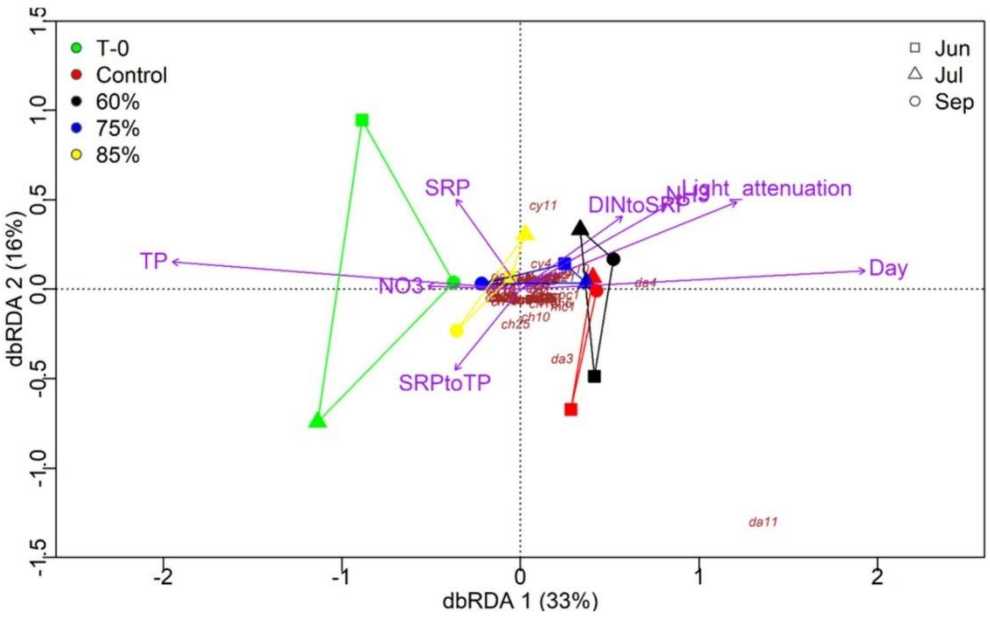
Figure 7:

Distance-Based Redundancy Analysis (db-RDA) of Environmental Drivers 2021

A.



B.



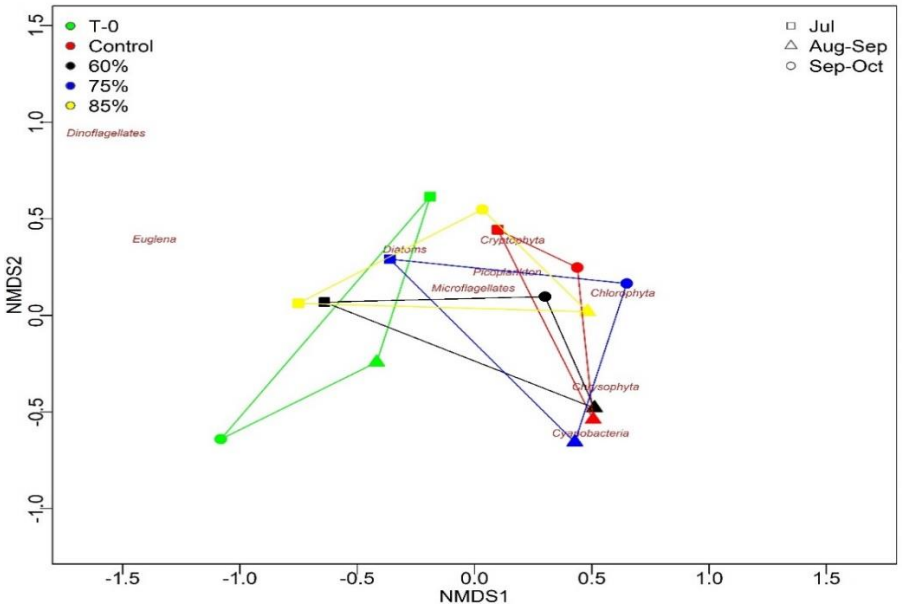
NOTE: Distance-based redundancy analysis (db-RDA) of environmental drivers of phytoplankton community composition, based on major taxonomic groups (A) and phytoplankton genera/species (B) over three deployments (June, July, Sept 2021) and five experimental treatments (T=0, control, 60 %, 75 %, and 85 % light attenuation). See

Appendix A, Table S3 for genus/species codes in appendices. See text for description of nutrient variables.

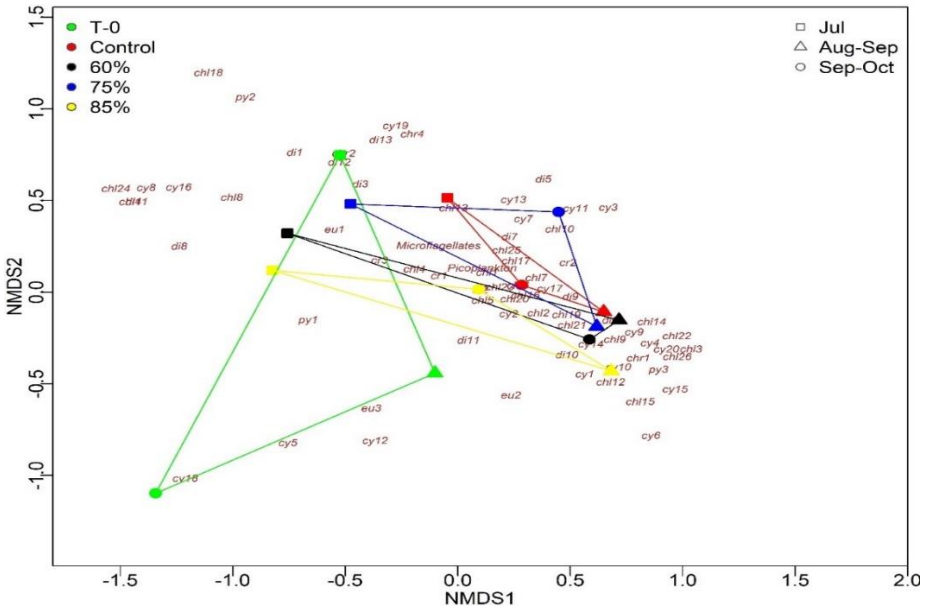
Figure 8

Non-Metric Multidimensional Scaling (NMDS) Analysis 2022

A



B.

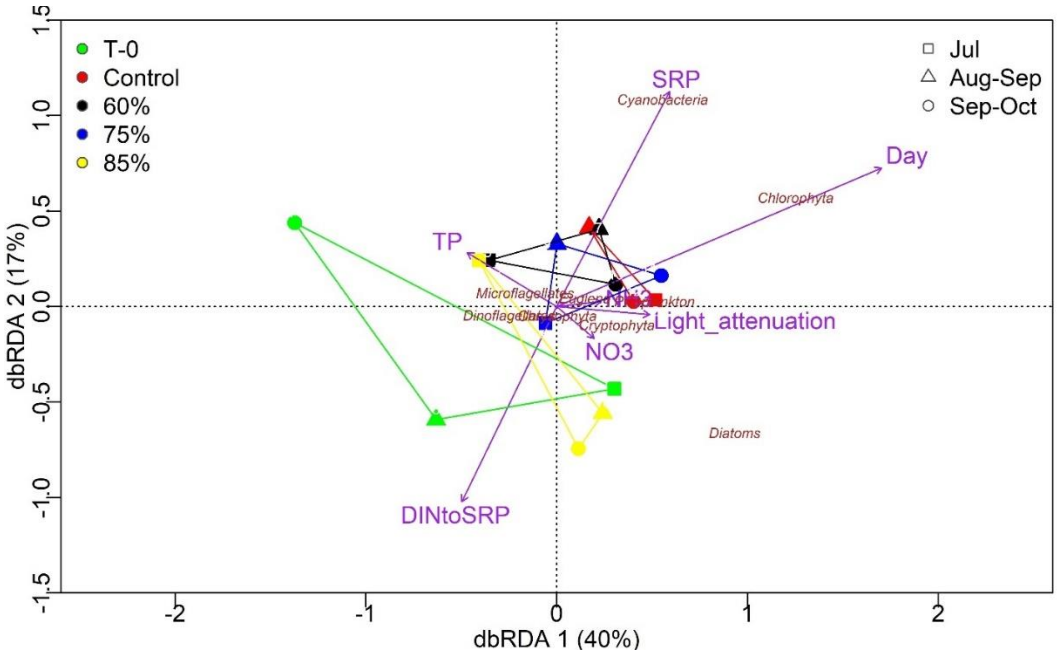


NOTE: Non-metric multidimensional scaling (NMDS) analysis of community composition based on major phytoplankton taxonomic groups (A) and individual phytoplankton genera/species (B) over three deployments (July, Aug - Sept, Sept - Oct, 2022) and five experimental treatments (time-zero, control, and 60%, 75%, and 85% light attenuation). (See Appendix A, Table S5 for genus/species codes).

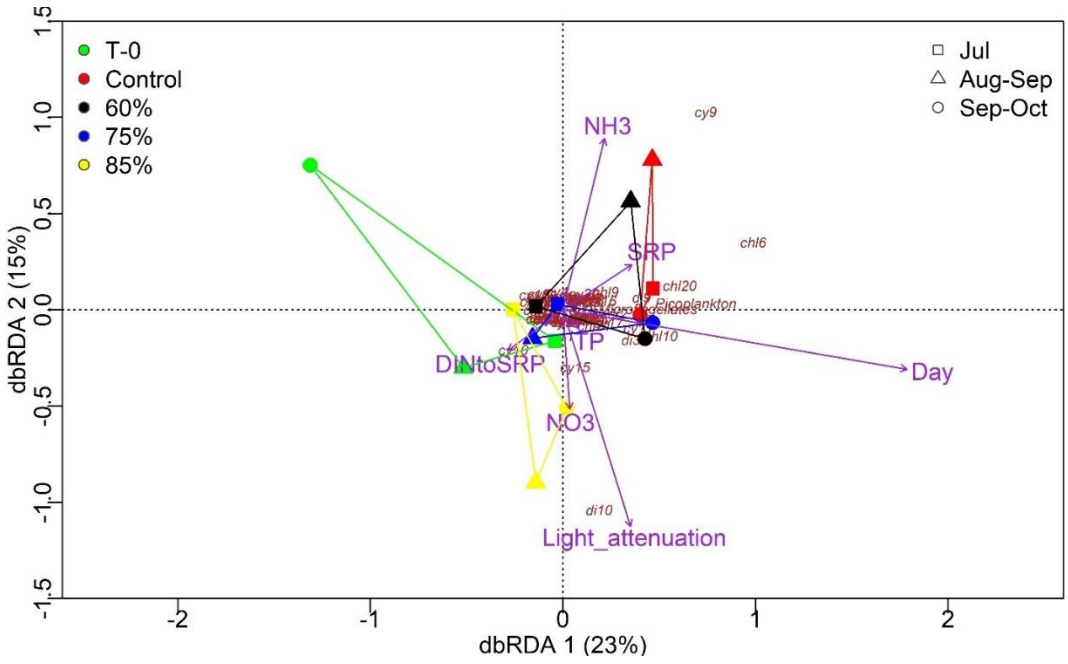
Figure 9:

Distance-Based Redundancy Analysis (db-RDA) of Environmental Drivers 2022

A.



B.



NOTE: Distance-based redundancy analysis (db-RDA) of environmental drivers of phytoplankton community composition, based on major taxonomic groups (A) and individual phytoplankton genera/species (B) in three months (July, Aug – Sept, Sept – Oct, 2022). See Appendix A, Table S5 for genus/species codes. See text for description of nutrient variables.

Table 1

Mean Chlorophyll and Nutrient Concentrations 2021

Month	Treatment	Dominant Taxa	Total Cells (cells/mL)	Chl *a* (µg/L)	TP (µg/L)	SRP (µg/L)	NO_3–NO_2 (mg/L)	NH_3 (µg/L)	DIN:SRP (molar)
June	Time-0	Diat, Chloro, Pico	10,869	2.2	69.3	21.5	2.6	118.9	125
	Control	Diat, Chloro	243,629	12.4	48.7	10.8	2.1	78.3	206
	60%	Diat	233,470	21.4	54.3	12.3	2.2	54.7	186
	75%	Diat	81,892	9.1	54	18.3	2.5	83.3	140
	85%	Diat	64,482	3.1	49	17.6	2.5	66.5	149
July	Time-0	Cyano	9,364	n/a	54.7	6.3	5.4	8.3	852
	Control	Cyano	252,162	n/a	167.3	5.9	4.8	36.3	833
	60%	Cyano	263,636	n/a	43.7	0.95	4.9	26.4	5219
	75%	Diat, Chloro, Cyano	139,841	n/a	37.3	0.95	4.9	9.7	5,190
	85%	Cyano	157,078	n/a	41.7	0.95	5	58.7	5,329
September	Time-0	Cyano	24,875	19.7	101.7	14.3	2.7	122.7	200
	Control	Diat, Cyano	127,682	96.3	44.3	28.3	2.2	201.3	85
	60%	Diat, Cyano	120,014	122	65.7	0.95	2.4	210.03	2,760
	75%	Cyano	57,839	45.6	69.3	0.95	2.7	0.007	2,790
	85%	Cyano	33,135	41.7	81.3	3.9	2.8	25.3	716
	Mean		**121,331**	**37.4**	**65.5**	**9.6**	**3.3**	**73.4**	**1,652**
	SD		**46,104**	**39.2**	**9.2**	**8.8**	**1.4**	**65.4**	**2,055**

Note: Mean chlorophyll and nutrient concentrations, calculated DIN:SRP molar ratio and phytoplankton abundance in experimental treatments in June, July, and September 2021. Mean SRP and NH_3 concentrations are estimated using regression on order statistics (ROS) due to the presence of non-detects in their datasets. When three replicates within a treatment level all fall below detection limit, half of the detection limit is used to represent mean.

n/a =not available. Diat = diatoms, Chloro = chlorophyta, Pico = picoplankton, Cyano = cyanobacteria. DIN = NO_3-NO_2 + NH_3

Table 2

Mean Chlorophyll and Nutrient Concentrations 2022

Month	Treatment	Dominant Taxa	Total Cells (cells/mL)	Chl *a* (µg/L)	TP (µg/L)	SRP (µg/L)	NO_3–NO_2 (mg/L)	NH_3 (µg/L)	DIN:SRP (molar)	Total Microcystins (µg/L)
Jul-22	Time-0	Diat	40059	21.9	33.1	33.5	2	52.8	61.4	0.36
	Control	Diat, Chloro	53982	22.4	58.8	19.9	1.7	55.1	89.2	0.68
	60%	Diat	23169	10.3	109.8	45.7	1.8	7	39.5	0.12
	75%	Diat	35720	9	57.1	29.9	1.8	7	61.7	0.12
	85%	Diat	16091	5.3	57.5	61.2	2	7	33.5	0.13
Aug-22	Time-0	Chloro, Diat, Cyano	30,843	40.3	103.1	1	1.8	64.6	1959.1	0.05
	Control	Cyano, Chloro	86,257	32.5	87.1	59.1	1.5	305.3	31.1	0.09
	60%	Cyano, Chloro	94,025	24.1	70.1	84.2	1.7	937.9	31.6	0.08
	75%	Cyano	92,561	33.8	74.5	314.5	1.9	12.2	6.1	0.08
	85%	Diat, Cyano	92,581	27.2	70.1	107.2	1.9	153.9	18.9	0.05
Sep-22	Time-0	Cyano	8,906	12.7	72.5	8.4	2.6	88.7	320.9	0.48
	Control	Chloro, Diat	117,444	30.2	53.8	7.4	2.6	29.5	347.1	0.61
	60%	Chloro, Diat	85,158	34	134.4	5.9	3.2	18.1	537.6	0.36
	75%	Chloro, Diat	92,070	33.3	87.1	9.4	3.2	17.1	345.8	0.32
	85%	Diat	23,375	33	92.4	6.4	2.7	36.5	430	0.42
	Mean		**59,483**	**24.7**	**77.4**	**52.9**	**2.2**	**73.4**	**287.6**	**0.26**
	SD		**35,893**	**10.8**	**25.6**	**79.1**	**0.5**	**65.4**	**495.3**	**0.21**

NOTE: Mean chlorophyll and nutrient concentrations, calculated DIN:SRP molar ratio, total microcystins and phytoplankton abundance in experimental treatments in July, August – September and September – October 2022. Mean SRP and NH_3 concentrations are estimated using regression on order statistics (ROS) due to the presence of non-detects in their datasets. When three replicates within a treatment level all fall below detection limit, half of the detection limit is used to represent mean.

Diat = diatoms, Chloro = chlorophyta, Cyano = cyanobacteria. DIN = NO_3-NO_2 + NH_3

References

Andersen, I. M., Williamson, T. J., González, M. J., & Vanni, M. J. (2020). Nitrate, ammonium, and phosphorus drive seasonal nutrient limitation of chlorophytes, cyanobacteria, and diatoms in a hyper-eutrophic reservoir. *Limnology and Oceanography*, *65*(5), 962–978. https://doi.org/10.1002/LNO.11363

Bonilla, S., Aubriot, L., Soares, M. C. S., González-Piana, M., Fabre, A., Huszar, V. L. M., Lürling, M., Antoniades, D., Padisák, J., & Kruk, C. (2012). What drives the distribution of the bloom-forming cyanobacteria *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*? *FEMS Microbiology Ecology*, *79*(3), 594–607. https://doi.org/10.1111/J.1574-6941.2011.01242.X

Bullerjahn, G. S., McKay, R. M., Davis, T. W., Baker, D. B., Boyer, G. L., D'Anglada, L. V., Doucette, G. J., Ho, J. C., Irwin, E. G., Kling, C. L., Kudela, R. M., Kurmayer, R., Michalak, A. M., Ortiz, J. D., Otten, T. G., Paerl, H. W., Qin, B., Sohngen, B. L., Stumpf, R. P., … Wilhelm, S. W. (2016). Global solutions to regional problems: Collecting global expertise to address the problem of harmful cyanobacterial blooms. A Lake Erie case study. In *Harmful Algae* (Vol. 54, pp. 223–238). Elsevier B.V. https://doi.org/10.1016/j.hal.2016.01.003

Carpenter, S. R. (1996). Microcosm Experiments have limited relevance for community and ecosystem ecology. *Ecology*, *77*(3), 677–680. https://doi.org/10.2307/2265490

Carpenter, S. R., Christensen, D. L., Cole, J. J., Cottingham, K. L., He, X. I., Hodgson, J. R., Kitchell, J. F., Knight, S. E., Pace, M. L., Post, D. M., Schindler, D. E., & Voichick, N. (1995). Biological control of eutrophication in lakes. *Environ. Sci. Techno!*, *29*, 784–786. https://pubs.acs.org/sharingguidelines

Chaffin, J. D., Bridgeman, T. B., & Bade, D. L. (2013). Nitrogen constrains the growth of late summer cyanobacterial blooms in Lake Erie. *Advances in Microbiology*, *2013*(06), 16– 26. https://doi.org/10.4236/AIM.2013.36A003

Cheung, M. Y., Liang, S., & Lee, J. (2013). Toxin-producing cyanobacteria in freshwater: A review of the problems, impact on drinking water safety, and efforts for protecting public health. *Journal of Microbiology 2013 51:1*, *51*(1), 1–10. https://doi.org/10.1007/S12275-013-2549-3

Conroy, J. D., Kane, D. D., Briland, R. D., & Culver, D. A. (2014). Systemic, early-season *Microcystis* blooms in western Lake Erie and two of its major agricultural tributaries (Maumee and Sandusky rivers). *Journal of Great Lakes Research*, *40*(3), 518–523. https://doi.org/10.1016/J.JGLR.2014.04.015

Conroy, J. D., Kane, D. D., & Culver, D. A. (2008). *Declining Lake Erie ecosystem health? Evidence from a multi-year, lake-wide, plankton study* (M. Munawar & R. Heath, Eds.). Aquatic Ecosystem Health & Mgmt Soc.

Conroy, J. D. (2007). *Testing the algal loading hypothesis: the importance of Sandusky River phytoplankton inputs to offshore Lake Erie processes* [Doctoral dissertation, Ohio State University]. OhioLINK Electronic Theses and Dissertations Center. http://rave.ohiolink.edu/etdc/view?acc_num=osu1186592400

Crevecoeur, S., Edge, T. A., Watson, L. C., Watson, S. B., Greer, C. W., Ciborowski, J. J. H., Diep, N., Dove, A., Drouillard, K. G., Frenken, T., McKay, R. M., Zastepa, A., & Comte, J. (2023). Spatio-temporal connectivity of the aquatic microbiome associated with cyanobacterial blooms along a Great Lake riverine-lacustrine continuum. *Frontiers in Microbiology*, *14*. https://doi.org/10.3389/FMICB.2023.1073753

Davis, T. W., Watson, S. B., Rozmarynowycz, M. J., Ciborowski, J. J. H., Mckay, R. M., & Bullerjahn, G. S. (2014). Phylogenies of microcystin-producing cyanobacteria in the lower Laurentian Great Lakes suggest extensive genetic connectivity. *PLoS ONE*, *9*(9). https://doi.org/10.1371/journal.pone.0106093

de Nobel, W. T. (Pim), Huisman, J., Snoep, J. L., & Mur, L. R. (1997). Competition for phosphorus between the nitrogen-fixing cyanobacteria *Anabaena* and *Aphanizomenon*. *FEMS Microbiology Ecology*, *24*(3), 259–267. https://doi.org/10.1111/J.1574-6941.1997.TB00443.X

Domingues, R. B., Barbosa, A., & Galvão, H. (2005). Nutrients, light and phytoplankton succession in a temperate estuary (the Guadiana, south-western Iberia). *Estuarine, Coastal and Shelf Science*, *64*(2–3), 249–260. https://doi.org/10.1016/J.ECSS.2005.02.017

Flöder, S. C., & Burns, C. (2005). The influence of fluctuating light on diversity and species number of nutrient-limited phytoplankton. *Journal of Phycology*, *41*(5), 950–956.

Graeber, D., Mccarthy, M. J., Shatwell, T., Borchardt, D., Jeppesen, E., Søndergaard, M., Lauridsen, T. L., & Davidson, T. A. (2024). Consistent stoichiometric long-term rela-tionships between nutrients and chlorophyll-a across shallow lakes. *Nature Communications*, *15*(809). https://doi.org/10.1038/s41467-024-45115-3

Hampel, J.J., McCarthy, M.J., Neudeck, M, Bullerjahn, G.S., McKay, R.M.L., & Newell, S.E. 2019. Ammonium recycling supports toxic Planktothrix blooms in Sandusky Bay, Lake Erie: Evidence from stable isotope and metatranscriptome data.

Harmful Algae. 81:42-52. https://doi.org/10.1016/j.hal.2018.11.011. http://www.sciencedirect.com/science/arti cle/pii/S1568988318301720

Hellweger, F. L., Martin, R. M., Eigemann, F., Smith, D. J., Dick, G. J., & Wilhelm, S. W. (2022). Models predict planned phosphorus load reduction will make Lake Erie more toxic. *Science*, *376*(6596), 1001–1005. https://doi.org/10.1126/science.abm6791/suppl_file/science.abm6791_mdar_reproducibility_checklist.pdf

Helsel, D. R. (2011). *Statistics for Censored Environmental Data Using Minitab and R January 2012* (2nd ed.). Wiley.

Helsel, D. R. (2012). *Statistics for Censored Environmental Data Using MINITAB® and R* (2nd ed.). John Wiley & Sons.

Ho, J. C., Michalak, A. M., & Pahlevan, N. (2019). Widespread global increase in intense lake phytoplankton blooms since the 1980s. *Nature*, *574*. https://doi.org/10.1038/s41586-019-1648-7

Jäger, C. G., Diehl, S., & Schmidt, G. M. (2008). Influence of water-column depth and mixing on phytoplankton biomass, community composition, and nutrients. *Limnology and Oceanography*, *53*(6), 2361–2373. https://doi.org/10.4319/lo.2008.53.6.2361

Kane, D. D., Conroy, J. D., Peter Richards, Baker, D. B., & Culver, D. A. (2014). Re-eutrophication of Lake Erie: Correlations between tributary nutrient loads and phytoplankton biomass. *Journal of Great Lakes Research*, *40*(3), 496–501.

Kutovaya, O. A., McKay, R. M. L., Beall, B. F. N., Wilhelm, S. W., Kane, D. D., Chaffin, J. D., Bridgeman, T. B., & Bullerjahn, G. S. (2012). Evidence against fluvial seeding of recurrent toxic blooms of *Microcystis* spp. in Lake Erie's western basin. *Harmful Algae*, *15*, 71–77. https://doi.org/10.1016/J.HAL.2011.11.007

Laiveling, A., Nauman, C., Stanislawczyk, K., Bair, H. B., Kane, D. D., & Chaffin, J. D. (2022). The relationship between cyanobacterial toxins and environmental factors. *Journal of Great Lakes Research, 48,* 1587-1598. https://doi.org/10.1016/j.jglr.2022.08.015

Leach, T. H., Beisner, B. E., Carey, C. C., Pernica, P., Rose, K. C., Huot, Y., Brentrup, J. A., Domaizon, I., Grossart, H. P., Ibelings, B. W., Jacquet, S., Kelly, P. T., Rusak, J. A., Stockwell, J. D., Straile, D., & Verburg, P. (2018). Patterns and drivers of deep chlorophyll maxima structure in 100 lakes: The relative importance of light and thermal stratification. *Limnology and Oceanography*, *63*(2), 628–646. https://doi.org/10.1002/LNO.10656

Leps, J., & Smilauer, P. (2003). *Multivariate Analysis of Ecological Data using CANOCO*.

Litchman, E., & Klausmeier, C. A. (2001). Competition of phytoplankton under fluctuating light. *The American Naturalist*, *157*(2), 170–187.

Liu, X., Chen, L., Zhang, G., Zhang, J., Wu, Y., & Ju, H. (2021). Spatiotemporal dynamics of succession and growth limitation of phytoplankton for nutrients and light in a large shallow lake. *Water Research*, *194*, 116910. https://doi.org/10.1016/j.watres.2021.116910

Lowe, R.L. (1974). *Environmental Requirements and Pollution Tolerance of Freshwater Diatoms*, US Environmental Protection Agency, Cincinnati.

Lürling, M., Eshetu, F., Faassen, E. J., Kosten, S., & Huszar, V. L. M. (2013). Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshwater Biology*, *58*(3), 552–559. https://doi.org/10.1111/J.1365-2427.2012.02866.X

Mancuso, J. L., Weinke, A. D., Stone, I. P., Hamsher, S. E., Villar-Argaiz, M., & Biddanda, B. A. (2021). Cold and wet: Diatoms dominate the phytoplankton community during a year of anomalous weather in a Great Lakes estuary. *Journal of Great Lakes Research*, *47*(5), 1305–1315. https://doi.org/10.1016/J.JGLR.2021.07.003

Marzetz, V., Spijkerman, E., Striebel, M., & Wacker, A. (2020). Phytoplankton community responses to interactions between light intensity, light variations, and phosphorus supply. *Frontiers in Environmental Science*, *8*. https://doi.org/10.3389/fenvs.2020.539733

Matson, P. G., Boyer, G. L., Bridgeman, T. B., Bullerjahn, G. S., Kane, D. D., McKay, R. M. L., McKindles, K. M., Raymond, H. A., Snyder, B. K., Stumpf, R. P., & Davis, T. W. (2020). Physical drivers facilitating a toxigenic cyanobacterial bloom in a major Great Lakes tributary. *Limnology and Oceanography*, *65*(12), 2866–2882. https://doi.org/10.1002/LNO.11558

McKay, R. M., Frenken, T., Diep, N., Cody, W. R., Crevecoeur, S., Dove, A., Drouillard, K. G., Ortiz, X., Wintermute, J., & Zastepa, A. (2020). Bloom announcement: An early autumn cyanobacterial bloom co-dominated by *Aphanizomenon flos-aquae* and *Planktothrix agardhii* in an agriculturally-influenced Great Lakes tributary (Thames River, Ontario, Canada). *Data in Brief*, *30*, 105585. https://doi.org/10.1016/j.dib.2020.105585

McKay, R. M. L., Tuttle, T., Reitz, L. A., Bullerjahn, G. S., Cody, W. R., McDowell, A. J., & Davis, T. W. (2018). Early onset of a microcystin-producing cyanobacterial

bloom in an agriculturally-influenced Great Lakes tributary. *Journal of Oceanology and Limnology 2018 36:4*, *36*(4), 1112–1125. https://doi.org/10.1007/S00343-018- 7164-Z

Paerl, H. W., Hall, N. S., & Calandrino, E. S. (2011). Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Science of The Total Environment*, *409*(10), 1739–1745. https://doi.org/10.1016/J.SCITOTENV.2011.02.001

Paerl, H. W., Thad Scott, J., McCarthy, M. J., Newell, S. E., Gardner, W. S., Havens, K. E., Hoffman, D. K., Wilhelm, S. W., & Wurtsbaugh, W. A. (2016). It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. *Environ. Sci. Technol*, *50*, 10805–10813. https://doi.org/10.1021/acs.est.6b02575

Patel, A. K., Paolo, F., Albarico, J.B., Perumal, P., K., Vadrale, A. P., Nian, C. T., Chau, H. T. B., Anwar, C., Wani, H. M. U. D., Pal, A., Reetu Saini, R., Ha, L. H., Senthilkumar, B., Tsang, Y.-S., Chen, C.-W., Cheng-Di Dong, C.-D., &Singhania, R. R. (2022). Algae as an emerging source of bioactive pigments. *Bioresource Technology, 351.* 10.1016/j.biortech.2022.126910

Reynolds, C. S., Descy, J. P., & Padisak, J. (1994). Are phytoplankton dynamics in rivers so different from those in shallow lakes? *Hydrobiologia, 289,* 1-7.

Scheffer, M., Rinaldi, S., Gragnani, A., Mur, L. R., Van Nes, E. H. (1997). On the dominance of filamentous cyanobacteria in shallow, turbid lakes. *Ecology*, *78*(1), 272–282. https://dare.uva.nl

Schindler, D. W. (1998). Whole-ecosystem experiments: Replication versus realism: The need for ecosystem-scale experiments. *Ecosystems 1998 1:4*, *1*(4), 323–334. https://doi.org/10.1007/S100219900026

Shoman, N. Yu., & Akimov, A. I. (2022). Competitive advantages of the diatom *Skeletonema costatum* cleve, 1873 in the Black Sea in the winter–spring period. *Marine Biological Journal*, *7*(3), 103–106. https://doi.org/10.21072/MBJ.2022.07.3.09

Sterner, R. W. (2021). *The Laurentian Great Lakes: A Biogeochemical Test Bed*. https://doi.org/10.1146/annurev-earth-071420

Stockenreiter, M., Isanta Navarro, J., Buchberger, F., & Stibor, H. (2021). Community shifts from eukaryote to cyanobacteria dominated phytoplankton: The role of mixing depth and light quality. *Freshwater Biology*, *66*(11), 2145–2157. https://doi.org/10.1111/FWB.13822

Torres, C. de A., Lürling, M., & Marinho, M. M. (2016). Assessment of the effects of light availability on growth and competition between strains of *Planktothrix agardhii* and *Microcystis aeruginosa*. *Microbial Ecology*, *71*(4), 802–813. https://doi.org/10.1007/S00248-015-0719-Z

Visser, P. M., Jacquet, S., Schwarzenberger, A., Kurmayer, R., & Martin-Creuzburg, D. (2020). Toward disentangling the multiple nutritional constraints imposed by *Planktothrix*: The significance of harmful secondary metabolites and sterol limitation. *Frontiers in Microbiology*, *11*, 586120. https://doi.org/10.3389/fmicb.2020.586120

Wilhelm, S. W., Bullerjahn, G. S., & Mckay, R. M. L. (2020). The complicated and confusing ecology of *Microcystis* blooms. *mBio*, *11*(3). https://doi.org/10.1128/mBio

Winder, M., Berger, S. A., Lewandowska, A., Aberle, N., Lengfellner, K., Sommer, U., & Diehl, S. (2012). Spring phenological responses of marine and freshwater plankton to changing temperature and light conditions. *Marine Biology*, *159*(11), 2491–2501. https://doi.org/10.1007/S00227-012-1964-Z

Xu, H., Paerl, H., Boqiang, Q., Zhu, G., & Gao, G. (2010). Nitrogen and phosphorus inputs control phytoplankton growth in eutrophic Lake Taihu, China. *Limnology & Oceanography, 55*(1), 420-432.

Zhou, Q., Chen, W., Shan, K., Zheng, L., & Song, L. (2014). Influence of sunlight on the proliferation of cyanobacterial blooms and its potential applications in Lake Taihu, China. *Journal of Environmental Sciences (China)*, *26*(3), 626–635. https://doi.org/10.1016/S1001-0742(13)60457-X

Zohra Guellati, F., Touati, H., Tambosco, K., Quiblier, C., Humbert, J.-F., & Bensouilah, M. (2013). Unusual cohabitation and competition between *Planktothrix rubescens* and *Microcystis* sp. (cyanobacteria) in a subtropical reservoir (Hammam Debagh) located in Algeria. *Plos One.* https://doi.org/10.1371/journal.pone.0183540

CHAPTER 3: Temporal and spatial differences in phytoplankton community structure along a fluvial-lacustrine continuum in the lower Great Lakes basin

3.1 Introduction

Globally, freshwater resources are under constant stress due to anthropogenic influence related to multiple factors, including increased urbanization and eutrophication related to agriculture (Paerl et al., 2016). Climate change has led to an increase in frequency and severity of rainfall events, enhancing the mobilization of nitrogen (N) and phosphorus (P) from agricultural land into the surrounding tributaries. This increased nutrient loading and the stoichiometric ratio between N and P are important drivers of the growth and structure of phytoplankton communities (Keatley et al., 2011; Paerl et al., 2016). Coupled with increasing surface water temperatures consistent with climate warming, these factors can promote a shift in community structure to dominance by potentially harmful cyanobacterial blooms (cyanoHABs) (O'Neil et al., 2012). The Lake St. Clair to western Lake Erie corridor of the Great Lakes is particularly vulnerable to cyanoHABs, coincident with shallow bathymetry and eutrophication related to agriculture (Watson et al., 2016).

Hutchinson's (1961) Paradox of the Plankton predicts that competitive exclusion will lead to phytoplankton species outcompeting one another for light and nutrient resources and reaching equilibrium, yet it's observed that large, well-mixed water bodies can accommodate a diverse array of species (Hutchinson, 1961). Species succession has been investigated by many ecologists and theorists. Darwinian ecology describes it as a series of replacements, resulting from biotic interactions whereas others describe succession as independent responses to physical and chemical drivers (Sommer, 1989). Previous studies have shown that this succession occurs primarily by co-limitation of light and nutrients (Liu et al., 2021). Phytoplankton community composition in the Great

Lakes region tends to follow typical seasonal oscillations for north temperate lakes with a dominance of eukaryotic algae and diatoms in the spring, transitioning to cyanobacteria in summer coincident with increases in surface water temperature and light (Wilhelm et al., 2020). Typically, the onset of cyanoHABs is most notably triggered by changes in temperature and light, when stratification of the water column causes an increase in light and warming of surface waters, promoting their competitive dominance (Paerl & Paul, 2012; Winder et al., 2012).

Rivers are acknowledged as important contributors of nutrients which promote phytoplankton growth in downstream lakes, yet seeding of living algal cells from tributaries as an instigator of blooms is often overlooked. This process is known as fluvial seeding, and its significance, known as the Algal Loading Hypothesis (ALH) emphasizes the importance of delivery of propagules to downstream lakes where they are exposed to a more optimal light climate (Conroy et al., 2014; Conroy & Stein, 2007; Reinl et al., 2020). However, studies using DNA analysis along the Maumee River – Lake Erie continuum found that the taxonomic composition of blooms in rivers is generally not similar to those in their receiving waters (Chaffin et al., 2014; Kutovaya et al., 2012). An exception to this trend was detailed in September 2017 when identical populations of *Microcystis* were reported from Lake Erie and extending through the Maumee River estuary (Matson et al., 2020). In this case, it is believed the *Microcystis* colonies had originated in the lake and were transported upstream to the estuary by sustained easterly winds.

Similar to western Lake Erie, cyanobacterial blooms occurring along the southern shore of Lake St. Clair are reported to be dominated by toxigenic colonial taxa, such as

Microcystis, (Chaffin et al., 2013; Davis et al., 2014). In contrast, the agriculturally influenced tributaries of these lakes receive high nutrient inputs from runoff contributing to turbidity and consequent lower light climates, and promoting dominance of various filamentous species of cyanobacteria, fostering diversity and species succession along the fluvial-lacustrine continuum. *In-situ* microcosm experiments in which light intensity was manipulated found that lower light availability increased the dominance of cyanobacteria over other major phytoplankton groups (Varga et al., 2024). In the Thames River flowing into Lake St. Clair, blooms have been reported coincident with periods of low flow and were dominated by the filamentous cyanobacteria *Planktothrix agardhii* and *Aphanizomenon flos-aquae* (McKay et al., 2020). A recent study conducted along the Huron –Erie corridor inclusive of the Thames River-Lake St. Clair continuum examined the environmental drivers of planktonic community structure and found no clear patterns between nutrient concentration and cyanobacterial abundance, but showed seasonal succession of the microbial community (Crevecoeur et al., 2023). Research examining the ALH has been mostly limited to Lake Erie and with mixed findings, emphasizing the need for further research extended to other locations in the Great Lakes basin (Reinl et al., 2020). Observations of seasonal changes in phytoplankton dominance made in 2019 (McKay, unpublished) prompted an investigation of these oscillations in the river – lake continuum. The objectives of this research were to fill these gaps by analysing the spatial and temporal variation in water quality parameters (e.g., nutrients), phytoplankton community composition, biomass and cyanotoxin accumulation along the Thames River – Lake St. Clair continuum.

Here, results are reported from surveys conducted over three years at multiple sites to compare the differences in phytoplankton communities between lacustrine and riverine sites over different seasons. We hypothesized that phytoplankton communities follow a seasonal succession and vary between upstream river sites and lake sites due to differences in the light climate. We expected that more optimal light conditions in the lake will promote the growth of colonial cyanobacteria and the lower light in the river will favour filamentous taxa. We also expected that higher nutrient levels in the river will support higher phytoplankton diversity due to less competition for resources, and warmer temperatures during the expected algal growing season will support a higher diversity in the lake sites.

3.2 Materials and Methods

3.2.1 Study sites

Over the course of the three-year study, five survey sites were chosen along the Thames River – Lake St. Clair continuum (Figure 10, Appendix B, Table S6). The primary lake study site was located on the southern shore of Lake St. Clair, using samples collected from the intake well at Stoney Point Water Works in Pointe-aux-Roches, Ontario (SPWTP). Additional lake samples were collected from the Lakeshore Water Treatment Plant (John George Facility) in Belle River, Ontario (BRWTP). In both cases, the intake pipes extend approximately 1 km from shore. To evaluate differences along the river-lake continuum, sampling was also conducted at several locations along the lower Thames River. Furthest upstream, samples were collected adjacent to the Lower Thames Valley Conservation Authority (LTVCA) in Chatham, Ontario and from the shoreline near Prairie Siding, Ontario. Samples were also collected from the Thames River mouth where it flows into Lake St. Clair at Lighthouse Cove, Ontario.

3.2.2 Sample collection

Sampling from Stoney Point Water Works began in late September 2020 and continued weekly, from late spring to early fall, and monthly, mid-fall to mid-spring, until October 2023. Sampling from Lakeshore began in late July 2021 and continued until late September 2023. Thames River samples were collected monthly, during the algal growing season, from the LTVCA location from June 2021 to September 2023. To increase spatial resolution along the continuum, Prairie Siding and Lighthouse Cove sites were sampled from August 2022 to September 2023.

A handheld model 600QS sonde (YSI, Yellow Springs, Ohio, USA) was used to measure water quality parameters (temperature and specific conductivity) at each river sampling location (Appendix B, Table S7). Water quality data for Lake St. Clair were supplied by the Municipality of Lakeshore, Ontario, for dates when sampling occurred at either water treatment plant. Samples at each site were collected in triplicate in 1 L high-density polyethylene (HDPE) bottles, that had been washed in dilute acid (10% HCl) and rinsed with reverse osmosis water. Prior to sample collection, each bottle was rinsed with raw source water from the respective site, three times. Samples from the water treatment plants were collected directly from the intake wells; river samples were collected from the surface.

3.2.3 Sample processing and analyses

For each sample, water was filtered through 0.22 µm Sterivex cartridge filters (MilliporeSigma, Burlington, MA, USA) and frozen at -20°C for later nutrient analysis, including soluble reactive phosphorus (SRP), nitrate plus nitrite (NO_3-NO_2,) and ammonia (NH_3). Filters were frozen at -20°C for qPCR analysis. Raw water was frozen at

-20°C for analysis of total phosphorus (TP). For Chl *a* quantification, samples were filtered through a 0.45 μm PCTE filter, then frozen at -20°C until ready for analysis. For phytoplankton taxonomic identification, 50 mL of raw water was pooled from each set of triplicates and preserved with Lugol's iodine solution for phytoplankton identification and enumeration from a subset of samples. Beginning in June 2021, 10 mL of raw water was transferred to a glass scintillation vial and frozen at -20°C for analysis of total microcystins.

Water samples were analysed for major nutrients using a SmartChem 170 Discrete Analyzer (KPM Analytics, Westborough, MA, USA). Colorimetric determination was used, where samples were analyzed in discrete mode and each reaction took place in an individual cuvette. The methods were based on the following: SRP was analysed as orthophosphate with a method detection limit of 0.0019 mg P L^{-1} (EPA 365.1 rev. 02, 1993); NO_3-NO_2 by reducing nitrate to nitrite with a detection limit of 0.0028 mg N L^{-1} (EPA 353.2 rev. 02, 1993); TP after manual sulfuric acid digestion with a detection limit of 0.0015 mg P L^{-1} (EPA 365.1 rev. 02, 1993) and NH_3 with a detection limit of 0.014 mg NH-N L^{-1} (SM4500).

DNA extractions were performed on the Sterivex filters and concentrated filtrate using Qiagen's AllPrep PowerViral DNA/RNA kit (Qiagen, Germantown, Maryland, USA) following the manufacturer's instructions. Subsequent analysis was performed on a 4-channel 95900-4C Quantabio Q qPCR instrument (Quantabio, Beverly, Massachusetts, USA) using Phytoxigene Mastermix and cyanoNAS standards (Phytoxigene, Akron, Ohio, USA) following manufacturers' instructions. Targeted genes were *mcyE*, *sxtA* and

cyrA for potential production of microcystins, saxitoxins and cylindrospermopsins, respectively.

Samples for Chl *a* analysis were extracted overnight in dimethyl sulfoxide (DMSO) and analyzed fluorometrically using a TD-700 fluorometer (Turner Designs, San Jose, CA, USA). Whole water preserved with Lugol's iodine was analyzed by microscopy for algal taxonomy (Aquatic Taxonomy Specialists, Malinta, OH, USA). Phytoplankton cells were identified and enumerated in a measured aliquot using the Utermöhl method with a magnification modification of the stratified counting technique of Munawar and Munawar (1976) as described elsewhere (McKay et al., 2020). Cyanotoxins were analyzed following manufacturer's instructions using a Microcystins/Nodularins (ADDA) ELISA kit (PN520011, Gold Standard Diagnostics, Warminster, PA, USA) with a detection limit of 0.016 μg L^{-1}.

3.2.4 Statistical analyses

Nutrient concentrations and chlorophyll biomass were compared across sites and seasons to investigate spatial and temporal differences along the continuum. Specifically, results from Lake St. Clair (collected from SPWTP) were compared across four seasons from fall 2020 to summer 2021 and three growing seasons (late June to early October of 2021, 2022 and 2023). Mean values from the growing season of 2021, 2022 and 2023 were compared between SPWTP and BRWTP to identify spatial differences within Lake St. Clair. To evaluate potential spatial differences along the continuum from the Thames River to open lake, mean values from the growing seasons of 2022 and 2023 were compared among SPWTP, BRWTP, LTVCA and Lighthouse Cove, with the addition of Prairie Siding in 2023.

All univariate and multivariate analyses were performed in R programming environment (https://www.r-project.org/). For nutrient analyses, arithmetic means were calculated for each sample when all three replicates were above detection limits. When non-detects were present, least-square means were estimated using Tobit models (Helsel, 2012). Nutrient, Chl *a* analysis and biodiversity metric datasets were tested for homogeneity of variance, using the Levene's Test in Social Science Statistics online calculator. For univariate analyses, when the assumptions of normality and homogeneity of variance were met, analysis of variance (ANOVA) and post-hoc Tukey's Honestly Significant Difference (HSD) test were run to detect the difference in nutrient values among sites and seasons using Social Science Statistics online calculator.

Alpha biodiversity metrics (i.e., Shannon and Simpson indices, richness and evenness) were calculated for total phytoplankton communities and cyanobacterial communities using R function 'diversity' in the package 'vegan'. Where the assumption of homogeneity of variance was violated in diversity metrics, differences between sites and seasons were investigated using Kruskal-Wallis test and post-hoc Dunn's test. When significant differences were found, box plots to visualize these differences were generated in R, using function 'ggplot' in the package 'ggplot2.' Linear regression was run using R function 'lm' in the package 'vegan' to examine correlations between total microcystins and environmental factors. Nonmetric multidimensional scaling (NMDS) was performed using R function 'metaMDS' in the package 'vegan', based on the phytoplankton abundance. Principal component analysis (PCA) was conducted on standardized environment data to show their differences among study sites, using R function 'PCA' in the package 'FactoMineR.'

3.3 Results and Discussion

3.3.1 Nutrients and Chl *a*

In comparing mean nutrient concentrations across seasons at SPWTP, the data did not fit the assumption of normality and Levene's test showed that homogeneity of variance was not met. The Kruskal-Wallis test identified significant differences in NO_3 concentrations and dissolved N:P molar ratio ($H = 9.04$, $p = 0.011$; $H = 6.89$, $p = 0.032$, respectively). Dunn's tests identified significantly higher NO_3 values in winter and summer 2021 compared to fall 2020, and dissolved inorganic nitrogen (DIN):SRP was higher in winter than fall (Table 3). This is similar to seasonal patterns of nutrient loading into Lake Erie from the Maumee River with steady nitrate concentrations from December into June but lowest in August and September (Stow et al., 2015).

Chlorophyll data were only available for fall 2020 and summer 2021, as COVID-19 mitigation policies created limitations in lab processing during winter 2020 and spring 2021, but values were similar between the two seasons. There were no significant differences in nutrient or chlorophyll concentrations between the two Lake St. Clair sites across the three growing seasons. Across the three growing seasons at SPWTP, differences were found in NO_3 concentrations ($H = 11.24$, $p = 0.004$) where values were significantly higher in 2021 and 2023 when compared to 2022 (Table 4).

In comparing means across all four sites in the growing season 2022, and all five in 2023, the assumptions of normality and homogeneity of variance were not met. For the 2022 data, Kruskal-Wallis tests revealed differences in NO_3, TP and Chl *a* concentrations and N:P ratio ($H = 211.23$, $p < 0.001$; $H = 10.9$, $p = 0.012$; $H = 13.67$, $p = 0.003$; $H = 16.06$, $p = 0.001$). NO_3, Chl *a* and N:P were all significantly higher at LTVCA and

Lighthouse Cove than at BRWTP (Table 5). NO_3 was also higher at LTVCA than SPWTP and TP was higher at Lighthouse Cove than SPWTP. This is consistent with other findings which show elevated nutrients in rivers and nearshore plumes, compared to the open lake (Makarewicz, et al., 2012). For the 2023 data, Kruskal-Wallis tests revealed differences in SRP, NO_3 and TP concentrations and DIN:SRP, ($H = 12.3$, $p = 0.015$; $H = 39.21$, $p < 0.001$; $H = 34.03$, $p < 0.001$; $H = 25.96$, $p < 0.001$). Dunn's test showed there was not enough evidence for a statistical difference in SRP between sites. Differences in nutrient values were found to be significantly lower in lacustrine sites when compared to riverine sites (Table 6). More specifically, NO_3 and TP values were lower at SPWTP and BRWTP than LTVCA, Lighthouse Cove and Prairie Siding. This is consistent with previous findings from the Thames River continuum, which showed a clear decrease in nutrient concentrations from river to lake (Crevecoeur et al., 2023) and higher TP concentrations at the river mouth than in Lake St. Clair (Davis et al., 2014). Chlorophyll concentrations were significantly higher at Prairie Siding than in lacustrine sites and further upstream at LTVCA. The mouth of the river at Lighthouse Cove also had higher concentrations than lacustrine sites. These findings are comparable to a correlation found between TP concentrations and Chl *a* in the Maumee River, suggesting decreasing P should result in lower primary production (Rowland et al., 2020).

3.3.2 Phytoplankton community composition and biodiversity along the continuum

Relative abundance of phytoplankton in Lake St. Clair, collected from SPWTP shows the expected seasonal oscillations of diatom dominance in the winter and spring months with a shift to cyanobacteria in the summer and fall over the three years (Wilhelm et al., 2020) (Figure 11A). Samples from BRWTP were only analyzed for taxonomy in

the summer of 2021 and show the same expected cyanobacterial dominance (Appendix B, Figure S4). The most abundant cyanobacterial taxa from both lacustrine locations were colonial forms, with filamentous taxa appearing in high abundance mainly in the cooler months when light availability is reduced (Yang et al., 2016; Figure 11B). The appearance of colonial cyanobacterial blooms in the Great Lakes region is often assumed to be attributed to *Microcystis*, as it is one of the most predominant genera and of high ecological concern (Yancey et al., 2023, Steffen et al., 2014). However, taxonomic analysis reported here from Lake St. Clair shows the most abundant and commonly occurring colonial taxa were not only *Microcystis*, but also *Aphanocapsa* and *Merismopedia*, often occurring at similar, if not higher abundances within samples. Previous work has examined the diversity within genera of colonial cyanobacterial communities, particularly that of *Microcystis*, (Kiledal et al., Matson et al., 2020; Yancey et al., 2023, Zhu et al., 2019) but this is one of the first studies to show that these communities can be co-dominated by different genera.

Although sequenced cyanobacterial genomes increased by more than 40% in recent years, there are still relatively few found within the NCBI database (Dvořák et al., 2023). Flow-imaging cytometers are commercially available, such as Flow Cam and Flow CytoBot, but at a high cost. Newer, inexpensive technologies are emerging using flow-through microscopy for taxonomic identification, but the software and training databases are still in development (Gincley et al., 2024). Thus, the taxonomic analysis by microscopy, which is labour- intensive, but less expensive, employed here allowed for assessment of the cyanobacterial diversity within communities that can be overlooked due to limited availability of reference genomes.

The diatom communities were comprised of mainly unidentified centric taxa, with a higher abundance of *Cyclotella* and *Fragilaria* occurring at both lacustrine sites in late summer 2021. Shifts in abundance of *Cyclotella* are mainly driven by changes in nutrients and light availability, with low to moderate nutrient requirements (Saros & Anderson, 2015). As such, their increased abundance late in the season may be due to a depletion of resources from summer phytoplankton growth. Similar seasonal oscillations were found in samples from the upstream riverine site at LTVCA from 2020 to 2023, and from the mouth of the river at Lighthouse Cove from 2022 to 2023 (Appendix B, Figures S5 and S6). However, diatoms were in high abundance in July 2022 at LTVCA (65%) and September 2023 at both sites (> 46%), when cyanobacteria dominated in the lake sites. Picoplankton was found to dominate late fall samples in 2022 at LTVCA, with a relative abundance of almost 50%.

Unlike all other sites, in summer 2023, the most dominant phytoplankton groups at the Prairie Siding site were diatoms and chlorophytes, with the exception of cryptophytes in late June and cyanobacteria in mid-July (Figure S7). This may be attributed to the afore mentioned higher nutrient levels at this site, as chlorophytes and diatoms are fast-growing phyla and can gain competitive advantage over other phytoplankton under high nutrients (Morris et al., 2006). The cyanobacterial dominance was attributed to only filamentous forms, specifically *Aphanizomenon* and *Planktolyngbya*. In late August, there was a higher abundance of cyanobacteria than diatom (~39% compared to ~29%), and was attributed to *Planktothrix agardhii*, another filamentous cyanobacterial taxa. The most commonly occurring genera of diatoms in the riverine sites were *Nitzschia*, *Skeletonema* and unidentified centric diatoms across all

three years. At LTVCA, *Cyclotella* appeared in high abundance in 2021 and *Aulacoseira* in 2023 at all three river sites. Unlike the lacustrine sites, the cyanobacterial communities at the river mouth and further upstream showed more diversity, with a variety of both colonial and filamentous forms dominating at different times.

Ellipses in the NMDS analysis of major taxonomic groups show that riverine sites cluster together and SPWTP shows an overlap with the river sites. Interpretation using the ellipses is difficult and could be due to the higher number of samples taken at SPWTP over all months of the year. (Figure 12). Lacustrine sites are more closely correlated to the occurrence of cyanobacteria, and diatoms were more closely correlated to riverine sites and found furthest from BRWTP. Various other major groups were more closely associated with riverine sites than lacustrine, suggesting that cyanobacteria may have a greater competitive advantage over other groups, particularly during the warmer months and higher light climate (Lürling et al., 2013, 2018; Paerl & Huisman, 2009). This may be attributed to their superior ability to regulate their buoyancy within a stable water column to access optimal sunlight and nutrients (Klemer et al., 1996; Reynolds et al., 1987).

NMDS analysis of genus/species level community composition showed similar clustering of the riverine sites, and again, SPWTP overlapped all sites. BRWTP clustered to the left of SPWTP, closely correlated to the samples taken in 2021 at both sites and with the mouth of the river (Figure 13). Cyanobacteria were more closely associated with lacustrine sites across years, with trends driven mainly by the relative abundance of colonial forms, such as *Aphanocapsa* spp (Cy3, Cy4), *Microcystis* spp (Cy20, Cy21, Cy22; Abbreviations are provided in Appendix B, Table S8) and unidentified coccoid

species (Cy11). Diatom dominance at all sites was closely associated with unidentified centric species (Di6), shown central to the plot. Other diatom genera more closely corelated to the riverine sites were *Nitzschia* and *Skeletonema* (Di15, Di17) as well as *Aulacoseira* (Di5) in 2023, and a *Cyclotella* sp (Di8) is more closely related to lacustrine sites.

Alpha biodiversity metrics were compared across sites and seasons to investigate spatial and temporal differences in overall diversity, taxon richness and evenness of major taxonomic groups along the continuum (Appendix B, Table S9). ANOVA detected significant differences in taxon richness across seasonal samples from SPWTP and along the continuum for the 2023 growing season ($F = 5.39$, $p < 0.05$; $F = 6.03$, $p < 0.05$). Tukey's HSD test showed that taxon richness from SPWTP was found to be significantly higher in fall 2020 and summer 2021 with averages of 19 and 16 individual taxa, respectively, when compared to spring 2021, with an average of eight taxa ($p = 0.007$ and 0.048, respectively; Table 7, Figure S8). Taxon richness during the 2023 growing season was found to be significantly higher in Prairie Siding and Lighthouse Cove, both with an average of 26 individual taxa, in comparison to SPWTP samples with an average of 17 taxa ($p = 0.007$ and 0.008, respectively; Figure S9). Shannon diversity did not meet the assumption of homogeneity of variance between datasets. Kruskal-Wallis results indicated there were significant differences in Shannon diversity between the different sites during the 2023 growing season ($H = 10.14$, $p < 0.05$). Overall diversity (H) was found to be higher at Prairie Siding and Lighthouse Cove when compared to SPWTP samples ($p = 0.005$ and 0.007, respectively; Appendix B, Figure S10).

To analyse the differences in the cyanobacterial communities, alpha biodiversity metrics were compared across sites and seasons. There were no statistical differences found between cyanobacterial diversity of the two lacustrine sites in 2021. Datasets met the assumptions of normality and homogeneity of variance and ANOVA results showed significant differences between sites and seasons (Table 8). Shannon diversity (H) was significantly higher in lake samples from SPWTP than at the river mouth at Lighthouse Cove during the 2022 growing season ($F = 5.61$, $p = 0.03$; Figure S11). Sample size from further upstream was too low in 2022 for statistical analyses (LTVCA). There was a significant difference detected in taxon richness across the different sites during the 2023 growing season ($F = 3.63$, $p = 0.031$), but Tukey's test found no significant differences between sites. There were differences found between seasons in some of the SPWTP samples for Shannon and Simpson diversity, evenness and richness ($F = 9.32$, $p < 0.05$; $F = 8.70$, $p < 0.05$; $F = 8.97$, $p < 0.05$, $F = 14.45$, $p < 0.05$). Dunn's post-hoc tests showed Shannon and Simpson diversity were significantly higher in fall 2020 and summer 2021 than spring 2021 (Appendix B, Figures S12 & S13). Shannon diversity was also higher in summer than winter. Due to there only being one cyanobacterial species present in most winter and spring samples, evenness could only be compared between summer and fall and was significantly higher in summer (Appendix B, Figure S14). Taxon richness was significantly higher in summer and fall compared to winter and spring (Appendix B, Figure S15). Across the continuum during the 2023 growing season, there were no significant differences in diversity of the cyanobacterial communities between sites.

3.3.4 Cyanobacterial toxins and gene expression

Total microcystins (MCs) were measured in samples from all sites. Samples exceeded the Government of Canada's guidelines for drinking water of 1.5 ppb during summer 2021 in lacustrine sites with an average of 2.75 ppb and 2.77 ppb at SPWTP and BRWTP, respectively. Using linear regression, there was a statistically significant positive correlation found between total microcystins and $NO_3 - NO_2$ concentrations ($F = 8.35$, $p = 0.044$; Figure 14), as shown in previous literature (Kieley et al., 2023). A key gene in the production of microcystins, *mcyE*, was detected through qPCR with concentrations of 6.4×10^5 and 1.3×10^5 gene copies/L, respectively at SPWTP and BRWTP. The following summer, total MC concentrations were mostly above the limit of detection (0.016 μg L^{-1}), but below drinking water guidelines, with the *mcyE* gene detected at lower average concentrations of 1.7×10^5 and 3.8×10^4 copies L^{-1} for SPWTP and BRWTP. However, transcriptionally active partial *mcy* operons have been identified through other studies and would not necessarily be detected through qPCR targeting only the *mcyE* gene (Yancey et al., 2022). Although microcystins have been the most reported cyanotoxin in the Great Lakes, the presence of genes involved in the synthesis of saxitoxins was recently reported in the central basin of Lake Erie and genetic potential in the western basin, creating a cause for concern (Boyer et al., 2007, Chaffin et al., 2019, Nauman et al., 2024). The occurrence of *Microseira* (formerly *Lyngbya*) *wollei*, a benthic cyanobacterium capable of saxitoxin production, has recently increased in western Lake Erie and US waters of Lake St. Clair (Bridgeman & Penamon, 2010, Butler et al., 2023) and was reported as washing up in large quantities on the northern shore (Vijayavel, et al., 2013). The gene responsible for the first step of saxitoxin synthesis, *sxtA*, was detected in SPWTP samples in summer 2021 and 2022, with averages of 3.8×10^4 and

3.9×10^4 copies L^{-1}. The presence of *sxtA* was detected in BRWTP samples in summer 2021 only with an average of less than 1000 copies L^{-1}. Although these values are considerably lower than those previously reported in Lake Erie between 2016 and 2019, these findings emphasize the need for further monitoring of *sxtA* as well as testing for the presence of the toxin, rather than focusing on microcystins only (Chaffin et al., 2019, Nauman et al., 2024). This is important as Lake St. Clair is one of the most heavily used lakes in the Great Lakes region for sport-fishing and other recreation, as well as a source of drinking water.

3.3.5 Spatial differences in phytoplankton, nutrients and water quality parameters along the Thames River – Lake St. Clair continuum

A principal component analysis (PCA) of water quality parameters across individual sites showed variability between sites occurred mainly along axis 1, accounting for 35.7% (Figure 15). Similar to the pattern shown in the NMDS, the ellipses show clustering of the lacustrine sites together and the upstream and mid-riverine sites together, with the river mouth overlapping. This would suggest that Lake St. Clair experiences water quality values distinct from those of the Thames River, and the river mouth represents a zone of mixing. The overlaid vectors show the river is more closely associated with elevated nutrients and DIN:SRP, and temperature does not appear to be a driver in differences between river and lake.

3.4 Conclusions

The findings of this study show that water quality parameters and nutrient availability differ greatly along the riverine-lacustrine continuum, likely driving the differences in phytoplankton community composition. Environmental conditions within the upstream Thames River sites varied considerably from those of its downstream Lake

St. Clair, with the river mouth sharing similarities between both. River sites were generally characterized by higher nutrient concentrations and N:P ratio than the lake sites.

Although production was expected to be higher in the lake sites than the river, in part related to nutrient loading and a more optimal light climate, chlorophyll biomass was found to be in higher concentrations in the Prairie Siding location and the river mouth, but not further upstream. However, flow was observed to be much faster upstream during most sampling events with dense tree canopy in the riparian zone, and this region likely receives much less light than the other riverine sites. While sampling depths also varied between sites, the shallow bathymetry of Lake St. Claire ensures the lake is holomictic during the ice-free season. Therefore, while the intake wells at SPWTP and BRWTP are located at depths of 1.94 m and 3.1 m respectively, samples collected from all sites reflect the mixed layer and thus are comparable to surface water samples collected elsewhere during the study.

The results of the relative abundance and biodiversity analyses support the hypothesis that phytoplankton communities along the continuum would follow a seasonal succession, and due to differences in the light climate would not be identical between river and lake sites. Results also support the hypothesis that conditions in the lake would promote the growth of colonial cyanobacteria over filamentous forms, and vice versa in the river. The Lake St. Clair communities were mainly dominated by diatoms in the winter and spring and shifted to cyanobacterial dominance in summer and fall. When cyanobacteria were present, they were found mainly in diverse colonial communities, such as *Microcystis*, *Aphanocapsa* and *Merismopedia.* In the colder months when there was less light intensity, lake sites showed an increase in filamentous forms, such as

Planktolyngbya. In the upstream and river mouth sites, there was a mixture of colonial and filamentous taxa in cyanobacterial communities, but at Prairie Siding, there were only filamentous.

Overall biodiversity of major phytoplankton groups and taxon richness were higher in river than lake sites, in general. This may suggest that a higher variety of taxa are supported in the river where they experience less competition with cyanobacteria due to the less favourable light climate for cyanobacteria. In Lake St. Clair, taxon richness was highest in summer and fall when compared to spring, and when examining the cyanobacterial community only, diversity, taxon richness and evenness were all highest in the warmer months, suggesting that warmer temperatures promote a wider range of phytoplankton taxa, supporting the third hypothesis.

In general, these results have implications for connectivity in the Laurentian Great Lakes as a whole. Previous research has found similar results with seasonal and spatial differences along the Maumee River-Lake Erie continuum. Ecosystem processes in Lake St. Clair likely are likely contributing to those in the Detroit River – Lake Erie continuum and beyond, to Lake Ontario (Crevecoeur et al., 2023; Davis et al., 2014). Similarly, upstream of this system, it is important to consider the processes occurring in Lake Huron, and likewise, the headwaters feeding the Thames River. Future work should consider the entire basin as one dynamic, connected system, where upstream perturbations will have cascading effects throughout, particularly in the face of climate change. Metagenomic and metabarcoding based on 16s and 18s rRNA analysis should be incorporated into the research to better understand metabolic processes of the microbial

communities in terms of nutrient cycling and to identify key producers of toxins, and to provide better taxonomic resolution of the phytoplankton communities.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada, Discovery Grant Program. The authors would like to thank the municipality of Lakeshore and staff at the water treatment plants for providing samples and water quality data for Lake St. Clair sites over the course of this study.

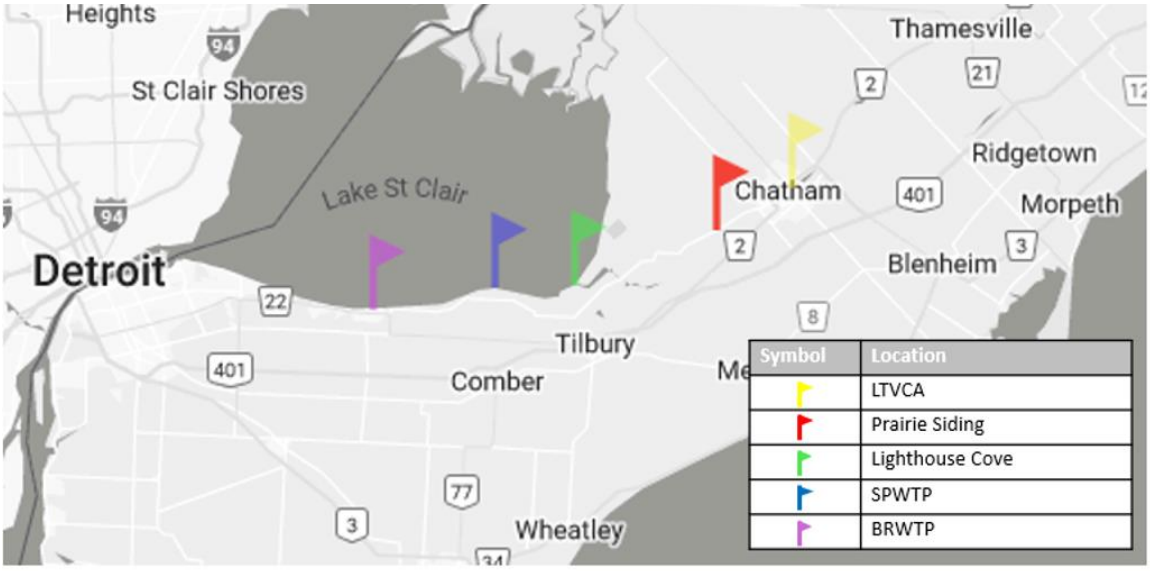
Author Contributions

Emily Varga: Writing – original draft, Writing – review & editing, Visualization, Conceptualization, Methodology, Investigation, Formal analysis, Data curation. Zhuoyan Song: Writing – review & editing, Validation, Investigation, Formal analysis. Katelyn Brown: Investigation, Formal analysis, Writing – review & editing. Bill Cody: Investigation, Formal analysis, Writing – review and editing. George Bullerjahn: Resources, Validation, Writing – review & editing. R. Michael McKay: Writing – original draft, Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

FIGURES & TABLES

Figure 10:

Map of the Thames River – Lake St. Clair Continuum

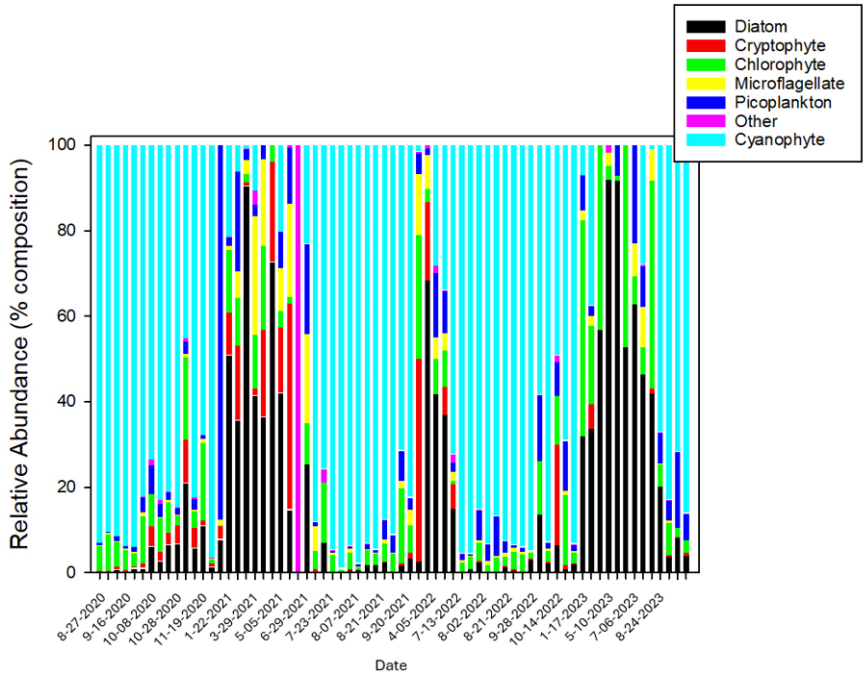


NOTE: Sampling sites are indicated by coloured flags. Map created with snazzymaps.com using a base map from Google Maps.

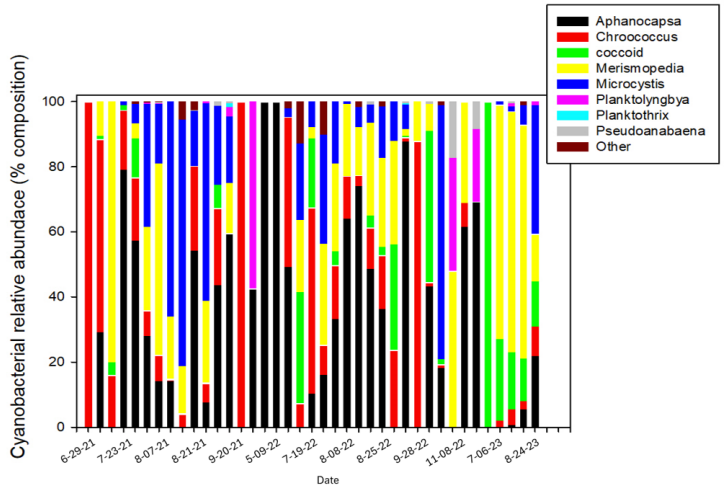
Figure 11:

Relative Abundance of Phytoplankton Communities in Lake St. Clair

A.



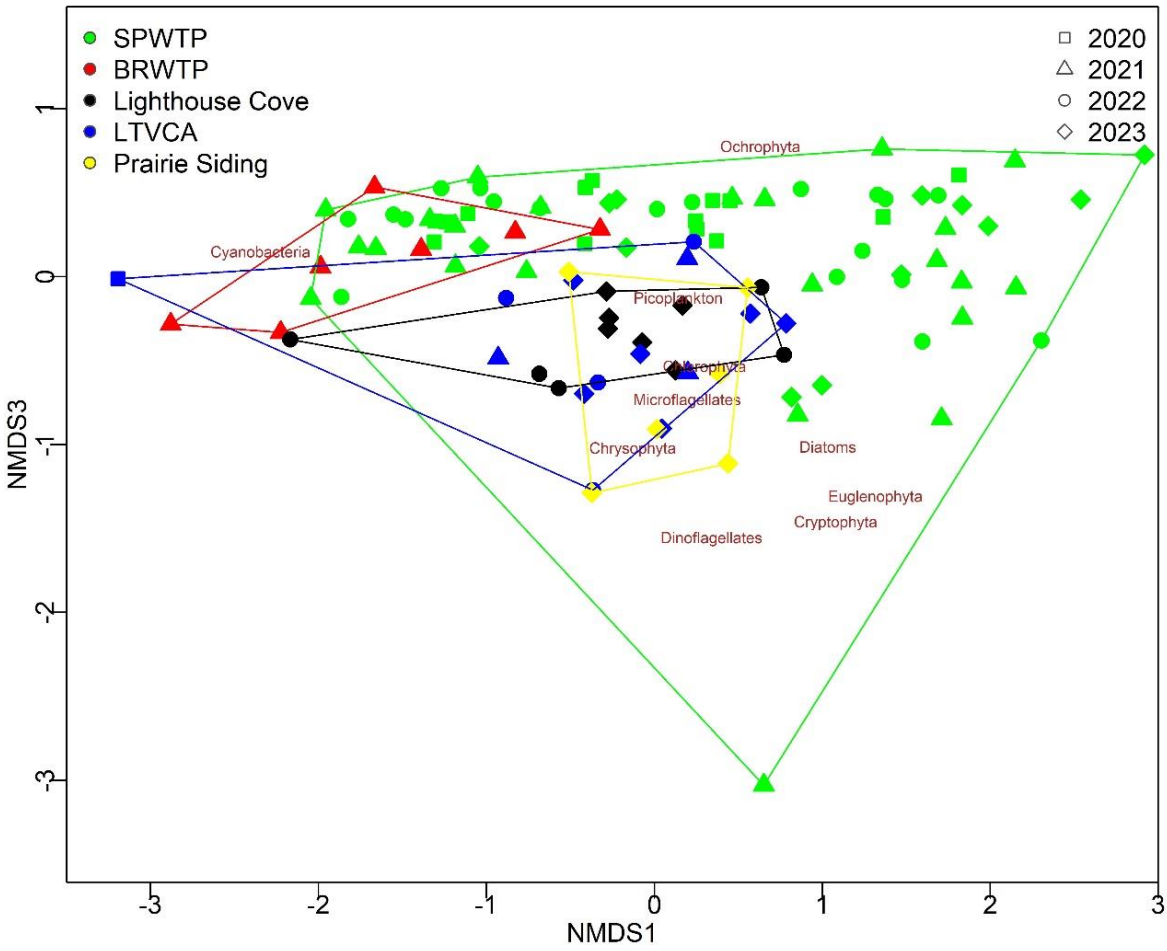
B



NOTE: Relative abundance of phytoplankton communities by major taxonomic group in Lake St. Clair at SPWTP showing seasonal oscillations from August 2020 to September 2023 (A) and by cyanobacterial genus from June 2021 to August 2023 (B).

Figure 12:

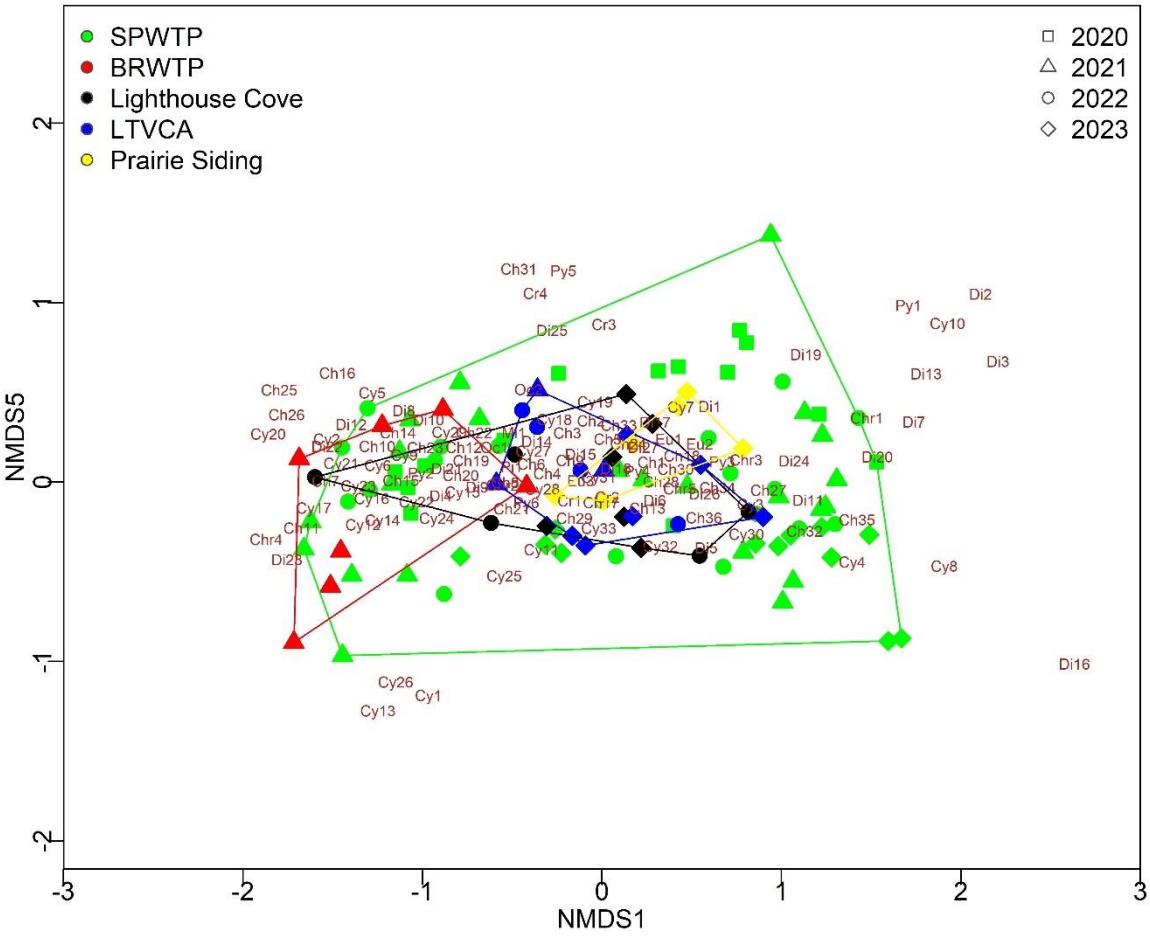
Non-metric Multidimensional Scaling Plot Comparing Major Taxonomic Groups Across Sites



NOTE: Non-metric multidimensional scaling (NMDS) plot comparing phytoplankton abundance by major taxonomic groups across sites along the Thames River-Lake St. Clair continuum over four years. Lacustrine sites are SPWTP and BRWTP. Riverine sites are Lighthouse Cove, LTVCA, and Prairie Siding.

Figure 13:

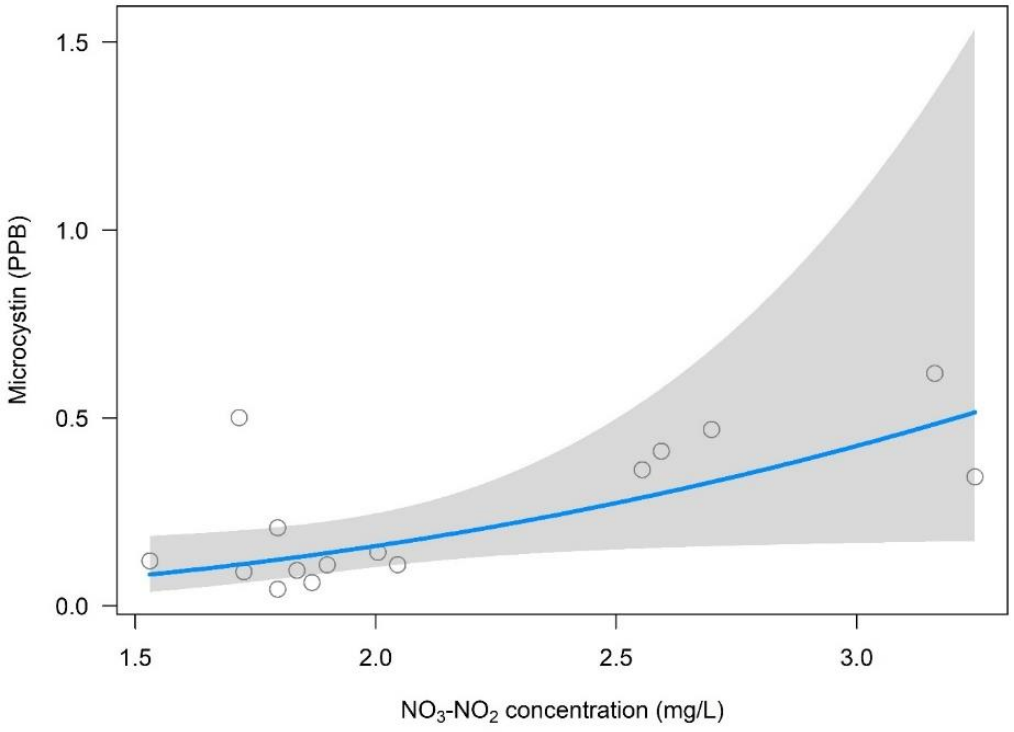
Non-metric Multidimensional Scaling Plot Comparing Genera/Species Across Sites



NOTE: Non-metric multidimensional scaling (NMDS) plot comparing phytoplankton abundance by genus/species across sites along the Thames River-Lake St. Clair continuum over four years. Lacustrine sites are SPWTP and BRWTP. Riverine sites are Lighthouse Cove, LTVCA, and Prairie Siding.

Figure 14:

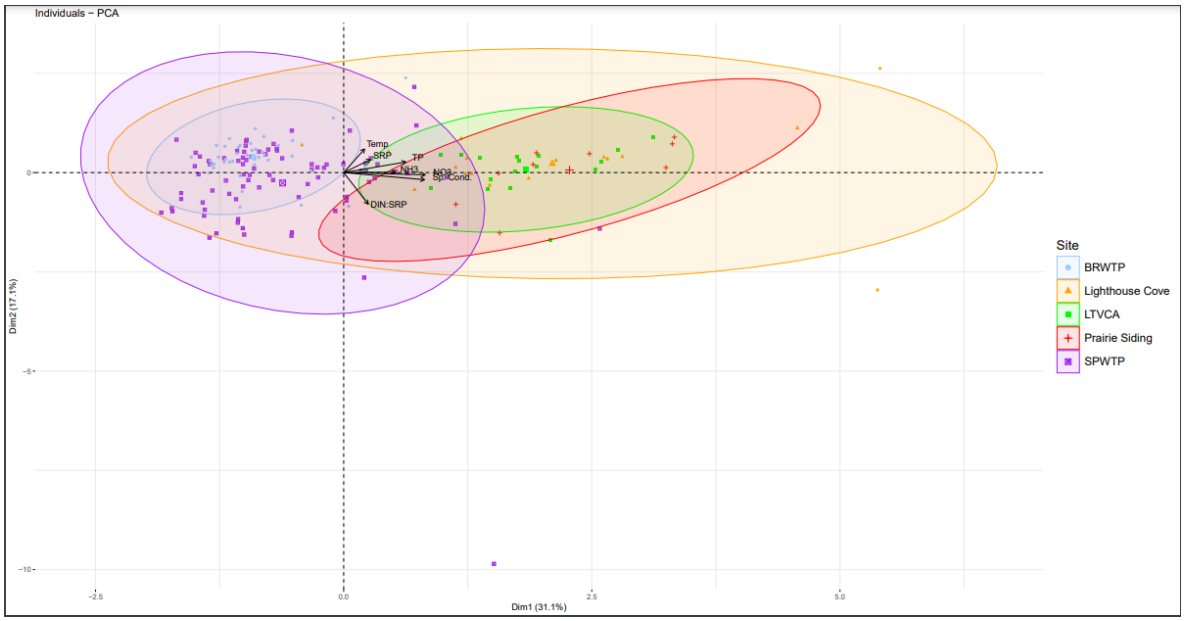
Scatterplot Representing the Correlation Between NO_3 and Microcystins



NOTE: Scatterplot with fitted trendline representing the positive correlation between NO_3 concentrations in mg/L and total microcystins in parts per billion (PPB).

Figure 15: Principal Component Analysis of Water Quality Parameters Across Sites

Principal Component Analysis of Water Quality Parameters Across Sites



NOTE: Principal component analysis of water quality parameters, nutrient concentrations and DIN:SRP across sites.

Table 3:

Kruskal-Wallis Test Scores for NO3 and DIN:SRP Across Seasons at SPWTP

NO3	p value	z value
Fall vs. Winter	0.006	2.77
Fall vs. Spring	n.s.	n.s.
Fall vs. Summer	0.023	2.28
Winter vs. Spring	n.a.	n.a.
Winter vs. Summer	n.s.	n.s.
DIN:SRP	p value	z value
Fall vs. Winter	0.011	2.53
Fall vs. Spring	n.s.	n.s.
Fall vs. Summer	n.s.	n.s.
Winter vs. Spring	n.s.	n.s.
Winter vs. Summer	n.s.	n.s.

*n.s. = non-significant

Table 4:

Kruskal-Wallis Test Scores for Nutrient Comparisons Across Algal Growing Seasons at SPWTP

NO3	p value	z value
2021 vs. 2022	0.002	3.07
2021 vs. 2023	n.s.	n.s.
2022 vs. 2023	0.008	2.62

*n.s. = non-significant

Table 5:

Kruskal-Wallis Test Scores for Nutrient/Chlorophyll a Across Sites 2022

NO3	p value	z value
SPWTP vs. BRWTP	n.s.	n.s.
SPWTP vs. LTVCA	< 0.001	3.45
SPWTP vs. L.C.	n.s.	n.s.
BRWTP vs. LTVCA	< 0.001	3.95
BRWTP vs. L.C.	0.004	2.92
LTVCA vs. L.C.	n.s.	n.s.
TP	p value	z value

SPWTP vs. BRWTP	n.s.	n.s.
SPWTP vs. LTVCA	n.s.	n.s.
SPWTP vs. L.C.	0.003	2.99
BRWTP vs. LTVCA	n.s.	n.s.
BRWTP vs. L.C.	n.s.	n.s.
LTVCA vs. L.C.	n.s.	n.s.
Chl *a*	p value	z value
SPWTP vs. BRWTP	n.s.	n.s.
SPWTP vs. LTVCA	n.s.	n.s.
SPWTP vs. L.C.	n.s.	n.s.
BRWTP vs. LTVCA	0.006	2.76
BRWTP vs. L.C.	0.006	2.75
LTVCA vs. L.C.	n.s.	n.s.
DIN:SRP	p value	z value
SPWTP vs. BRWTP	n.s.	n.s.
SPWTP vs. LTVCA	n.s.	n.s.
SPWTP vs. L.C.	n.s.	n.s.
BRWTP vs. LTVCA	0.003	2.96
BRWTP vs. L.C.	< 0.001	12.78
LTVCA vs. L.C.	n.s.	n.s.

*n.s. = non-significant; L.C. = Lighthouse Cove

NOTE: Kruskal-Wallis test scores for nutrient and chlorophyll a comparisons across four sites during the algal growing season 2022 (late June to early October).

Table 6:

Kruskal-Wallis Test Scores for Nutrients, Chlorophyll a Comparisons 2023

NO3	p value	z value
SPWTP vs. BRWTP	n.s.	n.s.
SPWTP vs. LTVCA	0.001	3.28
SPWTP vs. L.C.	< 0.001	3.41
SPWTP vs. P.S.	< 0.001	3.83
BRWTP vs. LTVCA	< 0.001	4.09

BRWTP vs. L.C.	< 0.001	4.23
BRWTP vs. P.S.	< 0.001	4.64
LTVCA vs. L.C.	n.s.	n.s.
LTVCA vs. P.S.	n.s.	n.s.
L.C. vs. P.S.	n.s.	n.s.
TP	p value	z value
SPWTP vs. BRWTP	n.s.	n.s.
SPWTP vs. LTVCA	0.004	2.85
SPWTP vs. L.C.	0.001	3.21
SPWTP vs. P.S.	< 0.001	3.75
BRWTP vs. LTVCA	< 0.001	3.56
BRWTP vs. L.C.	< 0.001	3.92
BRWTP vs. P.S.	< 0.001	4.46
LTVCA vs. L.C.	n.s.	n.s.
LTVCA vs. P.S.	n.s.	n.s.
L.C. vs. P.S.	n.s.	n.s.
Chl *a*	p value	z value
SPWTP vs. BRWTP	n.s.	n.s.
SPWTP vs. LTVCA	n.s.	n.s.
SPWTP vs. L.C.	0.002	3.04
SPWTP vs. P.S.	< 0.001	4.04
BRWTP vs. LTVCA	n.s.	n.s.
BRWTP vs. L.C.	0.035	4.2
BRWTP vs. P.S.	< 0.001	3.74
LTVCA vs. L.C.	n.s.	n.s.
LTVCA vs. P.S.	< 0.001	3.4
L.C. vs. P.S.	n.s.	n.s.

*n.s. = non-significant; L.C. = Lighthouse Cove, P.S. = Prairie Siding

NOTE: Kruskal-Wallis test scores for nutrient and chlorophyll *a* comparisons across five sites during the algal growing season of 2023 (late June to early October).

Table 7:

ANOVA Test Scores for Differences in Phytoplankton Diversity

Richness	p value	q value
SPWTP Fall vs. Winter	n.s.	n.s.
SPWTP Fall vs. Spring	0.007	5.42
SPWTP Fall vs. Summer	n.s.	n.s.
SPWTP Winter vs. Spring	n.s.	n.s.
SPWTP Winter vs. Summer	n.s.	n.s.
SPWTP Summer vs. Spring	0.048	4.07
SPTWP vs. L.C.	0.008	5.14
SPWTP vs. LTVCA	n.s.	n.s.
SPWTP vs. P.S.	0.007	5.24

Shannon Diversity (H)	p value	z value
SPTWP vs. L.C.	0.007	2.7
SPWTP vs. LTVCA	n.s.	n.s.
SPWTP vs. P.S.	0.005	2.82

*n.s. = non-significant; L.C. = Lighthouse Cove, P.S. = Prairie Siding

NOTE: ANOVA test scores for differences in richness and Simpson diversity (H) of major taxonomic groups between seasons at SPWTP in Lake St. Clair (fall 2020 to winter 2021) and sites along the Thames River – Lake St. Clair continuum during the algal growing season of 2023).

Table 8:

ANOVA Test Scores for Differences in Cyanobacterial Diversity Between Seasons

Shannon Diversity (H)	p value	q value
SPWTP Fall vs. Winter	n.s.	n.s.
SPWTP Fall vs. Spring	0.012	5.05
SPWTP Fall vs. Summer	n.s.	n.s.
SPWTP Winter vs. Spring	n.s.	n.s.
SPWTP Winter vs. Summer	0.032	4.37
SPWTP Summer vs. Spring	0.001	7.09
Simpson Diversity		
SPWTP Fall vs. Winter	n.s.	n.s.
SPWTP Fall vs. Spring	0.027	4.49
SPWTP Fall vs. Summer	n.s.	n.s.
SPWTP Winter vs. Spring	n.s.	n.s.
SPWTP Winter vs. Summer	n.s.	n.s.

SPWTP Summer vs. Spring	0.001	7.08
Evenness		
SPWTP Fall vs. Winter	n.a.	n.a.
SPWTP Fall vs. Spring	n.a.	n.a.
SPWTP Fall vs. Summer	0.017	8.97
SPWTP Winter vs. Spring	n.a.	n.a.
SPWTP Winter vs. Summer	n.a.	n.a.
SPWTP Summer vs. Spring	n.a.	n.a.
Richness		
SPWTP Fall vs. Winter	0.007	5.43
SPWTP Fall vs. Spring	0.008	6.93
SPWTP Fall vs. Summer	n.s.	n.s.
SPWTP Winter vs. Spring	n.s.	n.s.
SPWTP Winter vs. Summer	0.003	6.03
SPWTP Summer vs. Spring	0.0004	7.54

NOTE: ANOVA test scores for differences in richness and Simpson diversity (H) of the cyanobacterial communities between seasons at SPWTP in Lake St. Clair (fall 2020 to winter 2021).

References

Boyer, G. (2007). The occurrence of cyanobacterial toxins in New York lakes: Lessons learned from the MERHAB-Lower Great Lakes program. *Lake and Reservoir Management, 23,* 153-160.

Bridgeman, T. B. & Penamon, W. A. (2010). *Lyngbya wollei* in western Lake Erie. (2010). *Journal of Great Lakes Research, 36*(1), 167-171.

Butler, A., Thomas, C., Calomeni, A., McQueen, A., & Slack, T. (2023). *Microseira wollei (M. wollei*) Blooms in Freshwater Ecosystems in Lake St. Clair (Michigan, USA)– Impacts and Possible Management Approaches. https://hdl.handle.net/11681/47648.

Chaffin, J. D., Bridgeman, T. B., & Bade, D. L. (2013). Nitrogen constrains the growth of late summer cyanobacterial blooms in Lake Erie. *Advances in Microbiology*, *2013*(06), 16– 26. https://doi.org/10.4236/AIM.2013.36A003

Chaffin, J. D., Mishra, S., Kane, D. D., Bade, D. L., Stanislawczyk, K., Slodysko, K. N., Jones, K. W., Parker, E. M., & Fox, E. L. (2019). Cyanobacterial blooms in the central basin of Lake Erie: Potentials for cyanotoxins and environmental drivers. *Journal of Great Lakes Research*, *45*(2), 277–289. https://doi.org/10.1016/j.jglr.2018.12.006

Chaffin, J. D., Sigler, V., & Bridgeman, T. B. (2014). Connecting the blooms: tracking and establishing the origin of the record-breaking Lake Erie *Microcystis* bloom of 2011 using DGGE. *Aquatic Microbial Ecology*, *73*, 29–39.

Conroy, J. D., Kane, D. D., Briland, R. D., & Culver, D. A. (2014). Systemic, early-season *Microcystis* blooms in western Lake Erie and two of its major agricultural tributaries (Maumee and Sandusky rivers). *Journal of Great Lakes Research*, *40*(3), 518–523. https://doi.org/10.1016/J.JGLR.2014.04.015

Conroy, J. D., & Stein, R. A. (2007). *Testing the algal loading hypothesis: the importance of Sandusky River phytoplankton inputs to offshore Lake Erie processes [Doctoral Dissertation, Ohio State University].* OhioLINK Electronic Theses and Dissertations Center. http://rave.ohiolink.edu/etdc/view?acc_num=osu1186592400

Crevecoeur, S., Edge, T. A., Watson, L. C., Watson, S. B., Greer, C. W., Ciborowski, J. J. H., Diep, N., Dove, A., Drouillard, K. G., Frenken, T., McKay, R. M., Zastepa, A., & Comte, J. (2023). Spatio-temporal connectivity of the aquatic microbiome associated with cyanobacterial blooms along a Great Lake riverine-lacustrine continuum. *Frontiers in Microbiology*, *14*, 1073753. https://doi.org/10.3389/FMICB.2023.1073753/FULL

Davis, T. W., Watson, S. B., Rozmarynowycz, M. J., Ciborowski, J. J. H., Mckay, R. M., & Bullerjahn, G. S. (2014). Phylogenies of microcystin-producing cyanobacteria in the lower Laurentian Great Lakes suggest extensive genetic connectivity. *Plos One*, *9*(9), e106093. https://doi.org/10.1371/JOURNAL.PONE.0106093

Dvořák, P., Jahodářová, E., Stanojković, A., Skoupý, S. & Casamatta, D.A., 2023. Population genomics meets the taxonomy of cyanobacteria. *Algal Research*, 103128.

Gincley, B., Khan, F., Hartnett, E., Fisher, A., & Pinto, A. J. (2024). *Environmental Science & Technology*, *58*(30), 13540-13551. https://10.1021/acs.est.4c01928

Helsel, D. R. (2012). *Statistics for Censored Environmental Data Using MINITAB® and R* (2nd ed.). John Wiley & Sons.

Hutchinson, G. E. (1961). The Paradox of the Plankton. *The American Naturalist*, *95*(882), 137– 145.

Keatley, B. E., Bennett, E. M., Macdonald, G. K., Taranu, Z. E., & Gregory-Eaves, I. (2011). Land-use legacies are important determinants of lake eutrophication in the Anthropocene. *Plos One*, *6*(1). https://doi.org/10.1371/journal.pone.0015913

Kieley, C. M., Roelke, D. L., Park, R., Campbell, K. L., Klobusnik, N. H., Walker, J. R., Cagle, S. E., Kneer, M. L., Stroski, K. M., Brooks, B. W., & Labonté, J. M. (2023). Concentration of total microcystins associates with nitrate and nitrite, and may disrupt the nitrogen cycle, in warm-monomictic lakes of the southcentral United States. *Harmful Algae*, *130*, 102542. https://doi.org/10.1016/j.hal.2023.102542

Kiledal, E.A., Reitz, L.A., Kuiper, E.Q., Evans, J., Siddiqui, R., Denef, V.J. & Dick, G.J., 2024. Comparative genomic analysis of *Microcystis* strain diversity using conserved marker genes. *Harmful Algae*, *132*, 102580.

Klemer, A. R., Cullen, J. J., Mageau, M. T., Hanson, K. M., & Sundell, R. A. (1996). Cyanobacterial buoyancy regulation: the paradoxical roles of carbon. *Journal of Phycology, 32*(1), 47-53.

Kutovaya, O. A., McKay, R. M. L., Beall, B. F. N., Wilhelm, S. W., Kane, D. D., Chaffin, J. D., Bridgeman, T. B., & Bullerjahn, G. S. (2012). Evidence against fluvial seeding of recurrent toxic blooms of *Microcystis* spp. in Lake Erie's western basin. *Harmful Algae*, *15*, 71–77. https://doi.org/10.1016/J.HAL.2011.11.007

Liu, X., Chen, L., Zhang, G., Zhang, J., Wu, Y., & Ju, H. (2021). Spatiotemporal dynamics of succession and growth limitation of phytoplankton for nutrients and

light in a large shallow lake. *Water Research*, *194*, 116910. https://doi.org/10.1016/j.watres.2021.116910

Lürling, M., Eshetu, F., Faassen, E. J., Kosten, S., & Huszar, V. L. M. (2013). Comparison of cyanobacterial and green algal growth rates at different temperatures. *Freshwater Biology*, *58*(3), 552–559. https://doi.org/10.1111/J.1365-2427.2012.02866.X

Lürling, M., Mello, M. M., van Oosterhout, F., Domis, L. de S., & Marinho, M. M. (2018). Response of natural cyanobacteria and algae assemblages to a nutrient pulse and elevated temperature. *Frontiers in Microbiology*, *9*(AUG). https://doi.org/10.3389/fmicb.2018.01851

Makarewicz, J. C., Lewis, T. W., Boyer, G. L. & Edwards, W. J. (2012). The influence of streams on nearshore water chemistry, Lake Ontario. *Journal of Great Lakes Research, 38*(4), 62- 71.

Matson, P. G., Boyer, G. L., Bridgeman, T. B., Bullerjahn, G. S., Kane, D. D., McKay, R. M. L., McKindles, K. M., Raymond, H. A., Snyder, B. K., Stumpf, R. P., & Davis, T. W. (2020). Physical drivers facilitating a toxigenic cyanobacterial bloom in a major Great Lakes tributary. *Limnology and Oceanography*, *65*(12), 2866–2882. https://doi.org/10.1002/LNO.11558

McKay, R. M., Frenken, T., Diep, N., Cody, W. R., Crevecoeur, S., Dove, A., Drouillard, K. G., Ortiz, X., Wintermute, J., & Zastepa, A. (2020). Bloom announcement: An early autumn cyanobacterial bloom co-dominated by *Aphanizomenon flos-aquae* and *Planktothrix agardhii* in an agriculturally-influenced Great Lakes tributary (Thames River, Ontario, Canada). *Data in Brief*, *30*, 105585. https://doi.org/10.1016/j.dib.2020.105585

Morris, K., Bailey, P. C. E., Boon, P. I., & Hughes, L. (2006). Effects of plant harvesting and nutrient enrichment on phytoplankton community structure in a shallow urban lake. *Hyrdorbiologia, 571,* 77-91. https:// 10.1007/s10750-006-0230-0

Nauman, C., Stanislawczyk, K., Reitz, L. A., & Chaffin, J. D. (2024). The spatiotemporal distribution of potential saxitoxin-producing cyanobacteria in western Lake Erie. *Journal of Great Lakes Research*, *50*(3). https://doi.org/10.1016/j.jglr.2024.102342

O'Neil, J. M., Davis, T. W., Burford, M. A., & Gobler, C. J. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae*, *14*, 314–334. https://doi.org/10.1016/j.hal.2011.10.027

Paerl, H. W., & Huisman, J. (2009). Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environmental Microbiology Reports*, *1*(1), 27–37. https://doi.org/10.1111/j.1758-2229.2008.00004.x

Paerl, H. W., Paul, Valerie (2012). Climate change: links to global expansion of harmful cyanobacteria. *Water Research, 46*(5), 1349-1363.

Paerl, H. W., Thad Scott, J., McCarthy, M. J., Newell, S. E., Gardner, W. S., Havens, K. E., Hoffman, D. K., Wilhelm, S. W., & Wurtsbaugh, W. A. (2016). It takes two to tango: When and where dual nutrient (N & P) reductions are needed to protect lakes and downstream ecosystems. *Environ. Sci. Technol*, *50*, 10805–10813. https://doi.org/10.1021/acs.est.6b02575

Reinl, K. L., Sterner, R. W., Lafrancois, B. M., & Brovold, S. (2020). Fluvial seeding of cyanobacterial blooms in oligotrophic Lake Superior. *Harmful Algae*, *100*, 101941. https://doi.org/10.1016/J.HAL.2020.101941

Reynolds, C. S., Oliver, R. L., & Walsby, A. E. (1987). *New Zealand Journal of Marine and Freshwater Research, 21*(3), 379-390.

Rowland, F. E., Stow, C. A., Johengen, T. H., Burtner, A. M., Palladino, D., Gossiaux, D. C., Davis, T. W., Johnson, L. T. & Ruberg, S. (2020). Recent patterns in Lake Erie phosphorus and chlorophyll *a* concentrations in response to changing loads. *Environmental Science & Technology 54*(2), 835-841. https://doi.org/10.1021/acs.est.9b05326

Saros, J. E., & Anderson, N. J. (2015). The ecology of the planktonic diatom *Cyclotella* and its implications for global environmental change studies. *Biological Reviews*, *90*, 522–541. https://doi.org/10.1111/brv.12120

Sommer, U. (1989). *Plankton Ecology : Succession in Plankton Communities* (U. Sommer, Ed.). Springer Berlin Heidelberg.

Steffen, M. M., Belisle, B. S., Watson, S. B., Boyer, G. L., & Wilhelm, S. W. (2014). Status, causes and controls of cyanobacterial blooms in Lake Erie. *Journal of Great Lakes Research*, *40*(2), 215–225. https://doi.org/10.1016/J.JGLR.2013.12.012

Stow, C. A., Cha, Y, Johnson, L. T., Confesor, R. & Richards, R. P. (2015). *Environmental Science & Technology*, *49*(6), 3392-3400 DOI: 10.1021/es5062648

Watson, S. B., Miller, C., Arhonditsis, G., Boyer, G. L., Carmichael, W., Charlton, M. N., Confesor, R., Depew, D. C., Hö, T. O., Ludsin, S. A., Matisoff, G., McElmurry, S. P., Murray, M. W., Richards, R. P., Rao, Y. R., Steffen, M. M., & Wilhelm, S. W.

(2016). The re-eutrophication of Lake Erie: Harmful algal blooms and hypoxia. *Harmful Algae*, *56*, 44–66. https://doi.org/10.1016/j.hal.2016.04.010

Wilhelm, S. W., Bullerjahn, G. S., Michael, R., & Mckay, L. (2020). The complicated and confusing ecology of *Microcystis* blooms. *mBio*, *11*(3). https://doi.org/10.1128/mBio

Varga, E., Weidman, R. P., Song, Z. & McKay, R. M. (2024). Environmental drivers of phytoplankton community dynamics in an agriculturally – influenced tributary in the lower Great Lakes. *Science of the Total Environment, 939.* https://doi.org/10.1016/j.scitotenv.2024.173411

Vijayavel, K., Sadowsky, M. J., Ferguson & J. A., Kashian, D. R., (2013). The establishment of the nuisance *Lyngyba wollei* in Lake St. Clair and its potential to harbor fecal indicator bacteria. *Journal of Great Lakes Research, 39,* 560-568.

Winder, M., Berger, S. A., Lewandowska, A., Aberle, N., Lengfellner, K., Sommer, U., & Diehl, S. (2012). Spring phenological responses of marine and freshwater plankton to changing temperature and light conditions. *Marine Biology*, *159*(11), 2491–2501. https://doi.org/10.1007/S00227-012-1964-Z

Yancey, C.E., Smith, D.J., Den Uyl, P.A., Mohamed, O.G., Yu, F., Ruberg, S.A., Chaffin, J.D., Goodwin, K.D., Tripathi, A., Sherman, D.H., & Dick, G.J. (2022). Metagenomic and metatranscriptomic insights into population diversity of *Microcystis* blooms: spatial and temporal dynamics of *mcy* genotypes, including a partial operon that can be abundant and expressed. *Applied and Environmental Microbiology, 88* (9).

Yancey, C.E., Kiledal, E.A., Chaganti, S.R., Denef, V.J., Errera, R.M., Evans, J.T., Hart, L.N., Isailovic, D., James, W.S., Kharbush, J.J. & Kimbrel, J.A., 2023. The Western Lake Erie Culture Collection: a promising resource for evaluating the physiological and genetic diversity of Microcystis and its associated microbiome. *Harmful Algae*, *126*, 102440

Yang, Y., Stenger-Kovács, C., Padisák, J., & Pettersson, K. (2016). Effects of winter severity on spring phytoplankton development in a temperate lake (Lake Erken, Sweden). *Hydrobiologia, 780*, 47-57.

Zhu, B., Cao, H., Li, G., Du, W., Xu, G., Domingo, J. S., Gu, H., Xu, N., Duan, S., & Lu, J. (2019). Biodiversity and dynamics of cyanobacterial communities during blooms in temperate lake (Harsha Lake, Ohio, USA). *Harmful Algae*, *82*, 9–18. https://doi.org/10.1016/J.HAL.2018.12.006

CHAPTER 4: The importance of continued, place-based, and collaborative research in the African Great Lakes: A case study from the Lake Victoria catchment

4.1 Introduction

In many of our largest lakes, we are seeing a global increase in harmful cyanobacterial algal blooms (cyanoHABs). These blooms are threatening the quality of freshwater ecosystems and the sustainability of some of the world's Great Lakes, including Lake Erie in North America, and Lake Victoria, in Africa (Paerl et al., 2011). In Lake Victoria, as well as the Laurentian Great Lakes, this is due mainly to increased anthropogenic nutrient enrichment from rapid population growth and expansion of agricultural activities (Mahdiyan et al., 2021; Verschuren et al., 2002). The basin of Lake Victoria supports one of Africa's fastest growing populations and agricultural nutrient inputs are intensifying as a result (Simiyu et al., 2022). This urbanization, coupled with climate change, act as drivers of cyanoHABs with enhanced surface heating and intensification of rain events (Paerl & Scott, 2010). Frequent, heavy storms in the area cause nutrient loss through soil runoff into the surrounding tributaries which feed the lake (Majaliwa et al., 2005).

Though well-studied in the Lake Erie watershed, the issue of cyanoHABs in Africa has not been as deeply investigated (Svirčev et al., 2019). Since the 1960's, Lake Victoria has experienced increased eutrophication and of all the Great Lakes, is now considered to be the most eutrophic (Guildford & Hecky, 2000). This increased eutrophication has led to shifts in the composition of phytoplankton from a dominance of chlorophyta (green algae) to cyanobacteria. Lake Victoria has also experienced the greatest extent of water quality degradation due to these and other stressors, including species loss, species invasion, water level changes and intensive fishing, particularly in gulfs and bays (Hecky et al., 2010; Olokotum et al., 2020). Winam Gulf (formerly known

as Nyanza Gulf) on the southern border of Kisumu, is considered eutrophic, receiving more than 86% of its nutrient loading from industry and agriculture in surrounding towns (Olokotum et al., 2022; Simiyu et al., 2022). The gulf, similar to the western basin of Lake Erie, is found to be susceptible to recurring algal blooms, dominated by *Microcystis* spp. (Lung'ayia et al., 2000; Sitoki et al., 2012). A study of the Sondu-Miriu River indicates high nitrogen (N) loading from the river, likely due to high population density, agriculture, livestock and industry (Vuai & Mungai, 2012). Studies have also shown that cyanobacteria capable of forming blooms can persist in fluvial systems and that these systems likely seed their downstream lakes (Reinl et al., 2020). In temperate lakes and rivers, cyanobacteria tend to dominate with high temperature, light intensity, nutrient concentration and low turbulence (Wang et al., 2021). However, factors determining which cyanobacterial species will dominate in tropical freshwater systems has not been well-studied and is not yet understood (Aline et al., 2019).

There has been limited research conducted in the Lake Victoria region on ecosystem processes and water quality, with management practices mainly focused on the fisheries and quality of catch (Kundu, et al., 2017). Furthermore, while there has been a lot of nutrient data collected from the open lake, there is little data on the inputs from the surrounding catchment (Njagi et al., 2022). A lack of longer-term, place-based research on African Great Lakes systems limits insight into critical mechanisms shaping cyanobacterial dynamics, and thus prohibits effective management. The threats to the integrity of not only Lake Victoria, but all of the African Great Lakes, have been exacerbated due to the reasons mentioned above, and highlight the need for immediate

collective stewardship of these important resources to protect the livelihood of current and future generations (Lawrence et al., 2023; Obiero et al., 2020; Plisnier et al., 2023).

The role of research partnerships, particularly from northern countries, has been mainly to provide necessary funds and training to local scientists; however, local academic institutions need to have a say in research design and ownership in these partnerships (Ochola & Gitau, 2009). Place-based research should be conducted respectfully, and through co-production with the local communities. (Koster et al., 2012). It is imperative that any data collected is shared openly and honestly with the local community, and there is an acknowledgement of co-ownership of this data. Funding partners do not always commit to long-term studies, resulting in a lack of thorough understanding of ecosystem processes and consequentially, increases in ecosystem degradation (Achieng, 2023).

Higher education institutions play an important role in knowledge co-production. They can provide the much-needed training for specialists, scientists, and researchers to support development in countries in the Global South by generating new knowledge (Altbach & Salmi, 2011). Partnerships and collaboration across the globe can increase opportunities for securing funding to co-create consistent, comparable long-term and reliable datasets (Lawrence et al., 2023; Obiero et al., 2020). As a result of this research, we offer critical suggestions/ recommendations for priority place-based, locally-driven science efforts needed to overcome the science-management hurdles for this regionally- and globally-significant ecosystem.

4.2 Materials and Methods

Here, we discuss a case study of cooperative research and the pairing of traditional knowledge with western science. In June 2022, Bowling Green State University (BGSU), located in Ohio, USA, was awarded a National Science Foundation Advanced Studies Institute project on water quality and harmful algal blooms in Lake Victoria, Kenya (Bullerjahn, 2024). Together with Kenya's Kisii University, Technical University of Kenya, Kenya Marine and Fisheries Research Institute (KMFRI), and African Center for Aquatic Research and Education (ACARE), BGSU led a cross-cultural project to allow US-based students to expand their research into an important, yet under-studied system. To support regional collaboration, US students were paired with a Kenyan partner for reciprocal learning and cooperative research. We designed a study to collect preliminary nutrient and water quality data to compare results along the Sondu-Miriu River with those in Winam Gulf and deployed an exploratory microcosm experiment as an example of potential future research design.

4.2.1 Location and field methods

This study was conducted in the Winam Gulf, where Kenya borders Lake Victoria. The Winam Gulf is a shallow basin, with an average depth of 6 m and is found on the eastern part of Lake Victoria which is connected to several streams and major inflowing rivers (Mwamburi et al., 2020). The Sondu-Miriu River is one of the main rivers flowing directly into the Gulf, originating from the slopes of the Mau Escarpment. As the fourth largest basin in Kenya, it covers 3470 km^2 with various surrounding land use types (Masese et al., 2012). To gain a better understanding of the nutrient loading from the Sondu River into Winam Gulf of Lake Victoria, we collected water samples from various locations along the Sondu River – Lake Victoria continuum. Two of the

locations were within the Sondu River, along the Homo Bay County/Kisumu County border. The first was approximately 400 m downstream from the Kenya Marine Fisheries Research Institute (KMFRI), the second approximately 400 m downstream of the first (Figure 16). We collected samples by boat (supplied by KMFRI) from the mouth of both the Sondu River and Nyando River, Kendu Bay and mid-gulf for comparison. At each location, samples were collected for nutrient analysis, specifically, nitrates (NO_3), ammonia (NH_3) and soluble reactive phosphorus (SRP). Water quality parameters were recorded using handheld sondes, models YSI 650-MDS multi-parameter display system and YSI 6000 (Yellow Springs, Ohio, USA) including temperature, specific conductivity, total dissolved solids and dissolved oxygen. (Appendix C, Table S6).

To investigate the effects of light availability on in-river processes, we deployed four -day microcosm incubations at the two study sites located along the Homa Bay County/Kisumu County border from the northern shore of the river. These field trials were conducted using 1L low-density polyethylene containers contained within a floating PVC frame. Light treatments were applied to each experimental unit by wrapping them in mosquito netting. Three of the containers in each deployment were unamended as controls. Three of the remaining containers were wrapped in one layer of netting and three in two layers, to attenuate ambient light by approximately 50% and 70%, respectively, as determined using a QSL 101 light meter (Biophysical Instruments, Inc.). Time-zero samples were taken, in triplicate at the start of the experiment to measure ambient nutrients and chlorophyll, and identify phytoplankton assemblage.

4.2.2 Sample analysis

Nutrients analysis was done using methods described by Parsons and APHA was used to determine NO_3, NH_3 and SRP (APHA Method 2320, 1992). Orthophosphates concentrations were determined using the ascorbic acid method. Ammonium-N was determined using indophenol method. Dissolved (nitrate and nitrite)-N was determined using cadmium reduction method. A flow injection analyzer model QUAATRO39 autoanalyzer with a dual beam UV spectrophotometer detector was used to determine the levels of nutrients in the samples.

Whole and filtered water samples were collected at all study sites to measure background water chemistry (Appendix C, Table S7). Briefly, water was filtered through Sterivex filters (0.22 μm polyethersulfone membrane) and frozen, then shipped to Kent State University in Ohio. Shipping delays (8 d) caused samples to arrive thawed, but they were refrozen upon arrival (-20 °C) and stored until analysis. Filtered water samples were thawed and analyzed with a continuous flow analyzer (Skalar San++) for soluble reactive P (ISO 4-157), NO_3^- (ISO 4-129), and NH_4^+ (ISO 4-118).

Chlorophyll *a* was determined by filtering 100 ml of sample through a glass fiber filter, then digesting filters in 90% acetone overnight. Before reading, samples were sonicated at high frequency to lyse cells and break up filters. The mixture was then centrifuged and the supernatant was used to measure chlorophyl using a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) at the KMFRI laboratory. The sample absorbance was measured at 750 nm and 664 nm.

Principal component analysis (PCA) was conducted on standardized environment data to show their differences among study sites, using R function 'PCA' in the package 'FactoMineR.'

4.3 Preliminary Results

4.3.1 Nutrients and chlorophyll *a*

Although there were not enough data points collected during our survey to determine if there is a statistically significant difference between nutrient concentrations of the individual sampling sites, the values for NO_3, NH_3 and SRP were higher by more than an order of magnitude in the Sondu River at site 2 than any other site (Appendix C, Table S11). Chlorophyll *a* concentrations were below detectable levels within the river sites compared to those within the bay, with the highest at the Sondu River mouth.

4.3.2 Water quality parameters

In the PCA, Sites 1 and 2 from the Sondu-Miriu River lie to the right of the y-axis, more closely associated with higher total dissolved solids, specific conductivity, salinity and nutrient concentrations when compared to the river mouths and within the bay and gulf (Figure 17). The sites within the Gulf all lie to the left of the y-axis, more closely associated with higher temperatures, dissolved oxygen and Chl *a*.

4.3.3 Effects of light availability on in-river processes

Analysis of the nutrient concentrations, using a two-way ANOVA, show no statistically significant differences between any samples deployed at site 1 in the Sondu-Miriu River. (Appendix C, Table S12). However, differences were found in the concentrations for the microcosms deployed at site 2. To determine where these differences lie, we conducted paired, two-tailed T-Tests using mean values (Appendix C, Table S13). Concentrations of NO_3 and SRP were significantly higher at time-zero than in

control and treatment samples, and were significantly higher in the 70% light attenuated than control (p-value <0.05). NO_3 concentrations were also statistically higher in the 50% light attenuated than the control. Chlorophyll *a* biomass was below detectable levels in all samples.

4.4 Discussion

These preliminary findings showed higher levels of nutrients at Sondu River Site 2, which is located in an area that is densely populated and in close proximity to farming activities, which act as potential non-point sources of nutrients. A study investigating nitrate sources from rivers draining into Lake Victoria reported high contributions in rivers Nyando and Sondu were due to farming activities and domestic sewage (Nyilitya et al., 2020). This suggests that both rivers are important contributors to downstream nutrient inputs, playing a similar role of nutrient conduits in the development of lake cyanoHABs as the rivers that feed the Laurentian Great Lakes.

Chl *a* biomass was below detectable level at river sites but detectable in samples from the bay and the mouth. This supports observations made during sampling, where a phytoplankton bloom, which appeared to be cyanobacteria, (later identified as mainly *Dolichospermum;* Brown, 2024) was evident within the majority of Winam Gulf, but there was no visible evidence of a bloom in the river. This was likely due to high levels of turbidity and rapid flow that were observed during our surveys, creating an unfavourable light climate for phytoplankton growth. It is worth noting that other research identified that concentrations of Chl *a* were significantly higher in Homa Bay of Lake Victoria than in the Gulf (Brown, 2024). Whereas higher temperatures and dissolved oxygen were associated with the Gulf, higher TDS, specific conductivity, salinity and nutrients were

associated with river sites. This is consistent with previous research which found there were higher values of these water quality parameters in rivers where human population and agricultural activities are high (Raburu et al., 2006).

Samples from the exploratory microcosm experiment showed highest nutrient concentrations at time zero, indicating biological processes were occurring during the experiment. Nutrients were also higher in the darkest container compared to control samples, potentially suggesting lower depletion with less light availability. Phytoplankton identification and quantification through microscopy were an intended result of this study, but were unachievable due to the high amounts of silt in the samples. These results emphasize the need for a better understanding of nutrient cycling processes occurring in the Lake Victoria catchment. Chl *a* biomass being below detectable levels indicates a lack of primary production occurring in the river. This is likely due to the high turbidity of the river creating an unfavourable light climate for phytoplankton growth (Nunes et al., 2022).

4.5 Conclusions

Although we were unable to quantify and characterize phytoplankton community composition from river samples, these findings support the notion that conditions for growth are more favourable in the gulf than in the river, particularly for cyanobacteria. Nutrient data suggests that both the Nyando and Sondu River are important sources of nutrients to Lake Victoria, however there were complications involved in obtaining this data. Subsequent research at this location has found that the Nyando River mouth supported elevated levels of potential cylindrospermopsin-producing *Raphidiopsis* at the time of sampling (Brown et al., 2024). Due to shipping delays, samples were thawed

when they arrived at Kent University in Ohio, USA. Although the samples were immediately re-frozen upon arrival, they may have been compromised through continued nutrient cycling, particularly in the potential oxidation of reduced N species. Due to a baggage delay, there was a lack of field supplies, particularly for the microcosm study, resulting in small sample sizes and a low spatial resolution. The experiment had to be re-designed to incorporate the limited materials available to the researchers. The locations for the deployment were decided on months prior to the commencement of field work to ensure travel was arranged and the locations were accessible. However, when we arrived at the first site, we were informed by local citizens that a mother hippopotamus had recently given birth nearby, and it would be dangerous to approach. This further emphasizes the need to collaborate and incorporate local knowledge into research.

Through this research we obtained limited results, however they are suggestive that the rivers are important sources of nutrients contributing to the degradation of the open lake. We feel we have laid important groundwork and shown the need for continued research in the Lake Victoria catchment. Because of the challenges encountered during this study, we learned there is a need to be flexible in experimental design and consider having a backup plan. We also identified the importance of being aware of potential local issues that could present themselves during research in a foreign location. Place-based studies are important, and investment in African-led research is necessary. Northern collaborators can successfully fund these projects long-term, and provide much needed training to African scientists (Otieno, et al., 2022). We identified the benefits to focusing funding efforts on local infrastructure, as having the capacity and instrumentation to analyse samples on-site would reduce the risk of sample degradation during shipping to

North America. There is a scarcity of in-depth ecosystem studies conducted in this region, particularly on the effects of nutrient loading (Achieng et al., 2023). Gaining a better understanding of these effects could inform policy development on freshwater management, which would be beneficial not only to African countries, but others in the Global South which face similar challenges in research and data collection.

Acknowledgements

This work was supported by the National Science Foundation Advanced Studies Institute. The authors would like to thank the local people for assisting in field work and the KMFRI staff for organizing the Lake Victoria cruise and providing laboratory space for analysis.

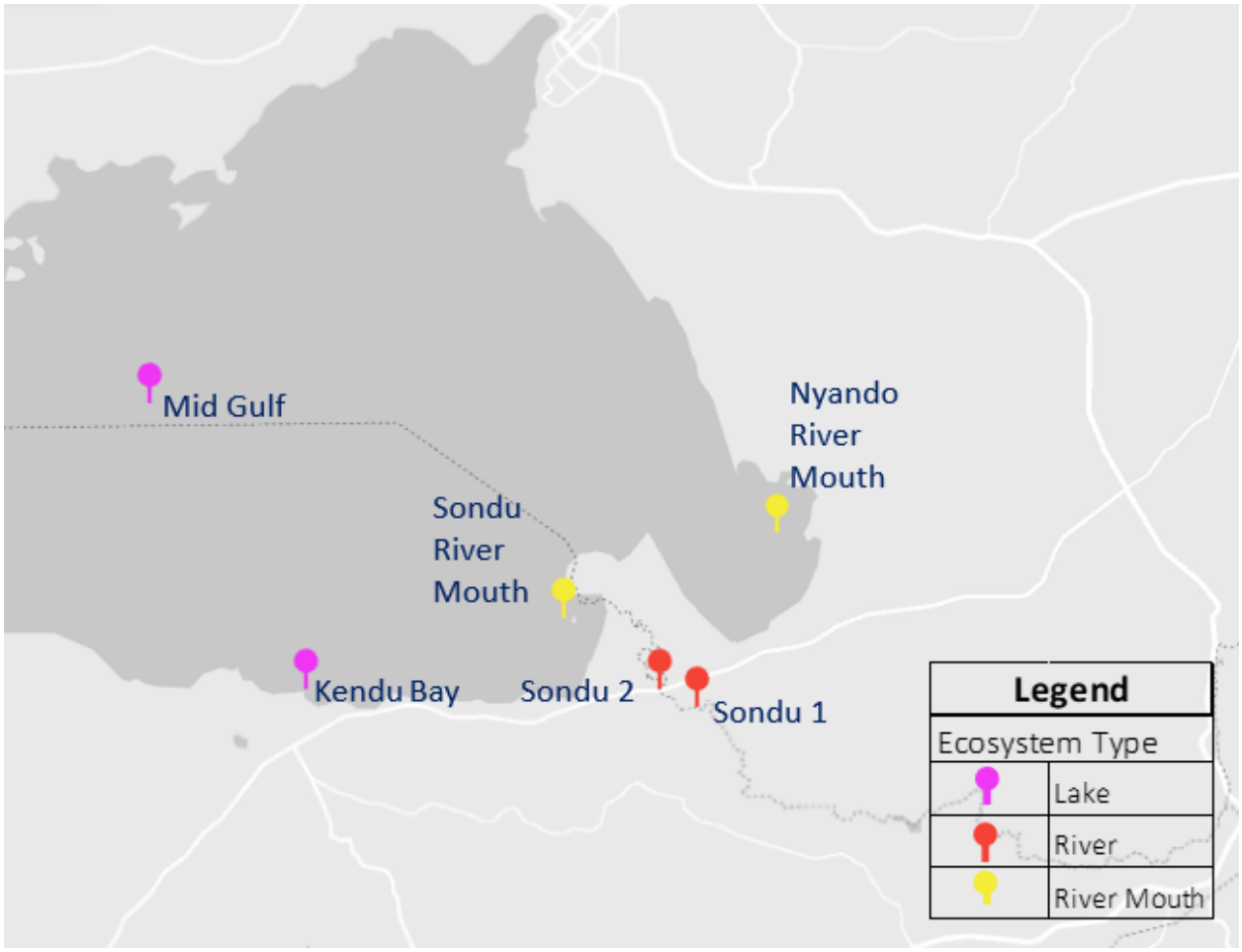
Author Contributions

Emily Varga: Writing – original draft, Writing – review & editing, Visualization, Conceptualization, Methodology, Investigation, Formal analysis, Data curation. Mariam Swaleh: Writing – review & editing, Conceptualization, Investigation, Formal analysis. Jordan Stoll: Formal analysis, Writing – review & editing. R. Michael McKay: Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

FIGURES

Figure 16:

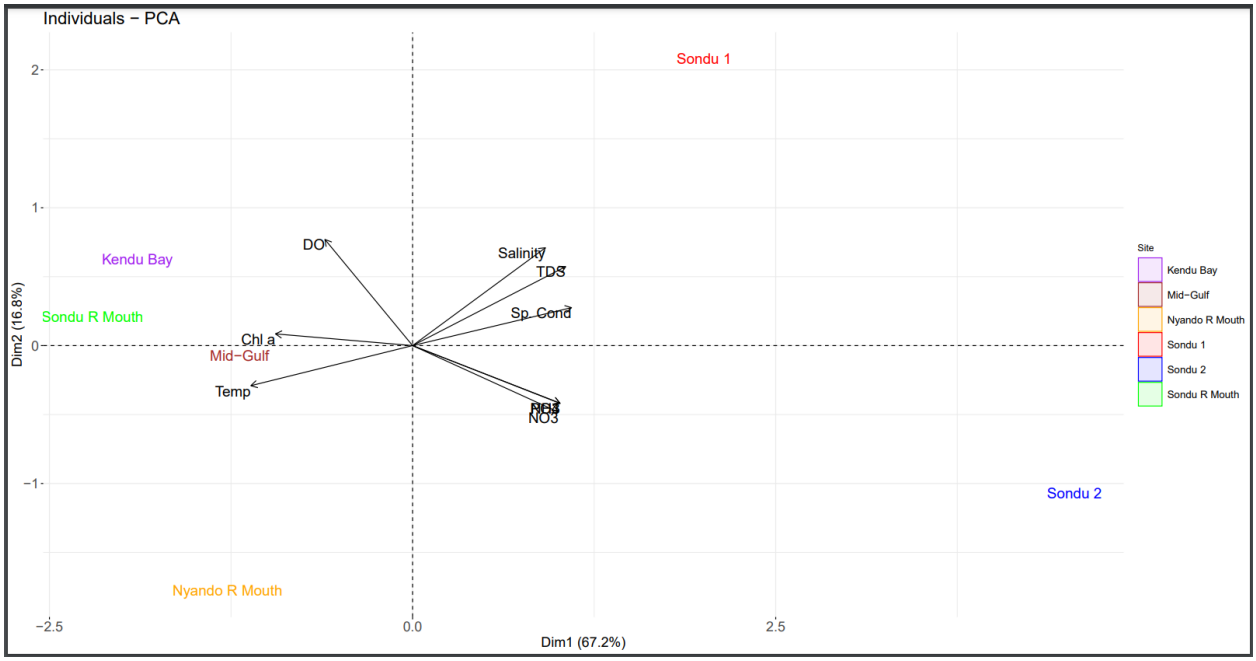
Sampling Sites Along the Sondu-Miriu River and in Winam Gulf



NOTE: Map created with snazzymaps.com using a base map from Google Maps.

Figure 17:

Principal Component Analysis Correlating Sites and Abiotic Factors



References

Achieng, A. O., Arhonditsis, G. B., Mandrak, N., Febria, C., Opaa, B., Coffey, T. J., Masese, F. O., Irvine, K., Ajode, Z. M., Obiero, K., Barasa, J. E., & Kaunda-Arara, B. (2023). Monitoring biodiversity loss in rapidly changing Afrotropical ecosystems: an emerging imperative for governance and research. *Philosophical Transactions of the Royal Society B*, *378*(1881). https://doi.org/10.1098/RSTB.2022.0271

Aline, A. A., da Luz, L. D., & de Aguiar Junior, T. R. (2019). Environmental factors driving the dominance of the harmful bloom-forming cyanobacteria *Microcystis* and *Aphanocapsa* in a tropical water supply reservoir. *Water Environment Research*, *91*(11), 1466–1478. https://doi.org/10.1002/WER.1141

Altbach, P. G. (2011). The road to academic excellence: The making of world-class research universities. *The World Bank*.

APHA Method 2320. (1992). Zigurat Roche.Pdf. *American Public Health Association*, *552*, 2–25 to 2–28. https://law.resource.org/pub/us/cfr/ibr/002/apha.method.2320.1992.pdf

Brown, K.M., K.B. Barker, R.S. Wagner, C.S. Ward, L. Sitoki, J. Njiru, R. Omondi, J. Achiya, A. Getabu, R.M. McKay & G.S. Bullerjahn. 2024. Bacterial community and cyanotoxin gene distribution of the Winam Gulf, Lake Victoria, Kenya. *Environmental Microbiology Reports.* 16: e13297. doi:10.1111/1758-2229.13297.

Bullerjahn (2024). Cyanobacterial blooms in the Winam Gulf of Lake Victoria Lessons for Western Lake Erie? Lakes Letter. https://iaglr.org/ll/2024-1_Winter_LL20.pdf

Guildford, S. J., & Hecky, R. E. (2000). Total nitrogen, total phosphorus, and nutrient limitation in lakes and oceans: Is there a common relationship? *Limnology and Oceanography*, *45*(6), 1213–1223. https://doi.org/10.4319/LO.2000.45.6.1213

Hecky, R. E., Mugidde, R., Ramlal, P. S., Talbot, M. R., & Kling, G. W. (2010). Multiple stressors cause rapid ecosystem change in Lake Victoria. *Freshwater Biology*, *55*(SUPPL. 1), 19–42. https://doi.org/10.1111/J.1365-2427.2009.02374.X

Koster, R., Baccar, K., Lemelin, R. H. (2012). Moving from research ON, to research WITH and FOR Indigenous communities: A critical reflection on community-based participatory research. *The Canadian Geographer, 56*(2), 195-210.

Kundu, R., Aura, C. M., Nyamweya, C., Agembe, S., Sitoki, L., Lung'ayia, H. B. O., Ongore, C., Ogari, Z., Werimo, K. (2017). Changes in pollution indicators in Lake Victoria, Kenya and their implications for lake and catchment management. *Lakes and Reservoirs, 22*(3), 199-214.

Lawrence, T. J., Achieng, A. O., Chavula, G., Haninga Haambiya, L., Iteba, J., Kayanda, R., Kaunda, E., Ajode, M. Z., Muvundja, F. A., Nakiyende, H., Nyamweya, C., Obiero, K., Plisnier, P. D., Phiri, H., Sibomana, C., Smith, S. (2023). Future success and ways forward for scientific approaches on the African Great Lakes. *Journal of Great Lakes Research,49*(6). https://doi.org/10.1016/j.jglr.2023.102242.

Lung'ayia, H. B. O., M'harzi, A., Tackx, M., Gichuki, J., & Symoensz, J. J. (2000). Phytoplankton community structure and environment in the Kenyan waters of Lake Victoria. *Freshwater Biology.* https://doi.org/10.1046/j.1365-2427.2000.t01-1-00525.x

Mahdiyan, O., Filazzola, A., Molot, L. A., Gray, D., & Sharma, S. (2021). Drivers of water quality changes within the Laurentian Great Lakes region over the past 40 years. *Limnology and Oceanography*, *66*(1), 237–254. https://doi.org/10.1002/LNO.11600

Majaliwa, J., Magunda, M., Tenywa, M., & Musitwa, F. (2005). *Soil and nutrient losses from major agricultural land-use practices in the Lake Victoria basin*. http://repository.eac.int/handle/11671/801

Masese, F. O., Raburu, P. O., Mwasi, B. N., & Etiegni, L. (2012). Effects of deforestation on water resources: Integrating science and community perspectives in the Sondu-Miriu River Basin, Kenya. *New Advances and Contributions to Forestry Research*. https://doi.org/10.5772/34373

Mwamburi, J., Basweti, G., Owili, M., Babu, J., & Wawiye, P. (2020). Spatio-temporal trends of nutrients and physico-chemical parameters on lake ecosystem and fisheries prior to onset of cage farming and re-opening of the Mbita passage in the Nyanza Gulf of Lake Victoria. *Lakes & Reserv*, *25*, 292–313. https://doi.org/10.1111/lre.12329

Njagi, D. M., Routh, J., Odhiambo, M., Luo, C., Basapuram, L. G., Olago, D., Klump, V., Stager, C. (2022). A century of human-induced environmental changes and the combined roles of nutrients and land use in Lake Victoria catchment on eutrophication. *Science of the Total Environment, 835,* 155425.

Nunes, P., Roland, F., Amado, A. M., da Silva Resende, N., Cardoso, S. J. (2022). Responses of phytoplanktonic chlorophyll-*a* composition to inorganic turbidity caused by mine tailings. *Frontiers in Environmental Science, 9.* https://doi.org/10.3389/fenvs.2021.605838

Nyilitya, B., Mureithi, S., & Boeckx, P. (2020). Land use controls Kenyan riverine nitrate discharge into Lake Victoria–evidence from Nyando, Nzoia and Sondu Miriu

river catchments. *Isotopes in Environmental and Health Studies*, *56*(2), 170–192. https://doi.org/10.1080/10256016.2020.1724999

Obiero, K., Lawrence, T., Ives, J., Smith, S., Njaya, F., Kayanda, R., Waidbacher, H., Olago, D., Miriti, E., Hecky, R. E. (2020).Advancing Africa's Great Lakes research and academic potential: Answering the call for harmonized, long-term, collaborative networks and partnerships. *Journal of Great Lakes Research*, *46*(5), 1240-1250. https://doi.org/10.1016/j.jglr.2020.02.002.

Ochola, L. I., & Gitau, E. (2009). Challenges in retaining research scientists beyond the doctoral level in Kenya. *PLOS Neglected Tropical Diseases.* https://doi.org/10.1371/journal.pntd.0000345

Oksanen, J., Blanchet, F., 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. (2018). *Vegan Community Ecology Package. R Package* (2.5-4).

Olokotum, M., Humbert, J. F., Quiblier, C., Okello, W., Semyalo, R., Troussellier, M., Marie, B., Baumann, K., Kurmayer, R., & Bernard, C. (2022). Characterization of potential threats from cyanobacterial toxins in Lake Victoria embayments and during water treatment. *Toxins*, *14*(10). https://doi.org/10.3390/toxins14100664

Olokotum, M., Mitroi, V., Troussellier, M., Semyalo, R., Bernard, C., Montuelle, B., Okello, W., Quiblier, C., & Humbert, J. F. (2020). A review of the socioecological causes and consequences of cyanobacterial blooms in Lake Victoria. *Harmful Algae*, *96*. https://doi.org/10.1016/J.HAL.2020.101829

Otieno, A., Lawrence T., Flavin, B., Ajode, Z., M., Coffey, T., Manyala, J., Stein, H., Irvine, K., Opaa, B. (2022). *Journal of Great Lakes Research, 49*(1). http://dx.doi.org/10.1016/j.jglr.2022.08.012

Paerl, H. W., Hall, N. S., & Calandrino, E. S. (2011). Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Science of The Total Environment*, *409*(10), 1739–1745. https://doi.org/10.1016/J.SCITOTENV.2011.02.001

Paerl, H. W., & Scott, J. T. (2010). Throwing fuel on the fire: synergistic effects of excessive nitrogen inputs and global warming on harmful algal blooms *Environ. Sci. Technol*, *44*(20), 40. https://doi.org/10.1021/es102665e

Plisnier, P.-D., Kayanda, R., MacIntyre, S., Obiero, K., Okello, W., Vodacek, A., Cocquyt, C., Abegaz, H., Achieng, A., Akonkwa, B., Albrecht, C., Balagizi, C., Barasa, J., Bashonga, R. A., Bashonga Bishobibiri, A., Bootsma, H., Borges, A. V., Chavula, G., Dadi, T., De Keyzer, E. L. R., Doran, P. J., Gabagambi, N., Gatare, R., Gemmell, A., Getahun, A., Haambiya, L. H., Higgins, S. N., Hyangya, B. L.,

Irvine, K., Isumbisho, M., Jonasse, C., Katongo, C., Katsev, S., Keyombe, J., Kimirei, I., Kisekelwa, T., Kishe, M., Koding, S. O. A., Kolding, J., Kraemer, B. M., Limbu, P., Lomodei, E., Mahongo, S. B., Malala, J., Mbabazi, S., Masilya, P. M., McCandless, M., Medard, M., Ajode, Z. M., Mrosso, H. D., Mudakikwa, E. R., Mulimbwa, N., Mushagalusa, D., Muvundja, F. A., Nankabirwa, A., Nahimana, D., Ngatunga, B. P., Ngochera, M., Nicholson, S., Nshombo, M., Ntakimazi, G., Nyamweya, C., Nyeko, J. I., Olago, D., Olbamo, T., O'Reilly, C. M., Pasche, N., Phiri, H., Raasakka, N., Salyani, A., Sibomana, C., Silsbe, G. M., Smith, S., Sterner, R. W., Thiery, W., Tuyisenge, J., Van der Knaap, M., Van Steenberge, M., van Zwieten, P. A. M., Verheyen, E., Wakjira, M., Walakira, J., Wembo, O. N., Lawrence, T. (2023). Need for harmonized long-term multi-lake monitoring of African Great Lakes. *Journal of Great Lakes Research, 49*(6). https://doi.org/10.1016/j.jglr.2022.01.016.

Raburu, A., Basin, V., & Raburu O, K. P. (2006). Impact of agro-industrial activities on the water quality of River Nyando, Lake Victoria Basin, Kenya. *Proceedings of the 11th World Lakes Conference,* 2. http://hdl.handle.net/1834/3501

Reinl, K. L., Sterner, R. W., Lafrancois, B. M., & Brovold, S. (2020). Fluvial seeding of cyanobacterial blooms in oligotrophic Lake Superior. *Harmful Algae*, *100*, 101941. https://doi.org/10.1016/J.HAL.2020.101941

Simiyu, B. M., Amukhuma, H. S., Sitoki, L., Okello, W., & Kurmayer, R. (2022). Interannual variability of water quality conditions in the Nyanza Gulf of Lake Victoria, Kenya. *Journal of Great Lakes Research*, *48*(1), 97–109. https://doi.org/10.1016/J.JGLR.2021.10.017

Sitoki, L., Kurmayer, R, & Rott, E. (2012). Spatial variation of phytoplankton composition, biovolume, and resulting microcystin concentrations in the Nyanza Gulf (Lake Victoria, Kenya). *Hyrdrobiologia*, *691*, 109–122. https://doi.org/10.1007/s10750-012-1062-8

Svirčev, Z., Lalić, D., Bojadžija Savić, G., Tokodi, N., Drobac Backović, D., Chen, L., Meriluoto, J., & Codd, G. A. (2019). Global geographical and historical overview of cyanotoxin distribution and cyanobacterial poisonings. *Archives of Toxicology*, *93*(9), 2429–2481. https://doi.org/10.1007/S00204-019-02524-4

Verschuren, D., Johnson, T. C., Kling, H. J., Edgington, D. N., Leavitt, P. R., Brown, E. T., Talbot, M. R., & Hecky, R. E. (2002). History and timing of human impact on Lake Victoria, East Africa. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1488), 289–294. https://doi.org/10.1098/RSPB.2001.1850

Vuai, S., & Mungai, N. W. (2012). Spatial variation of nutrients in Sondu-Miriu and Simiyu-Duma rivers: implication on sources and factors influencing the transportation into Lake Victoria. *Journal of Earth Sciences and Climatic Change 03*(02).https://doi.org/DOI: 10.4172/2157-7617.1000119

Wang, Z., Akbar, S., Sun, Y., Gu, L., Zhang, L., Lyu, K., Huang, Y., & Yang, Z. (2021). Cyanobacterial dominance and succession: Factors, mechanisms, predictions, and managements. *Journal of Environmental Management*, *297*, 113281. https://doi.org/10.1016/J.JENVMAN.2021.113281

CHAPTER 5: **Conclusions**

The topic of cyanobacterial harmful algal blooms (cyanoHABs) is a very important one, and is often the subject of news articles and current research within the Great Lakes region. It has become even more important in the last couple of decades, particularly in the face of global climate change, which has caused an increase in prevalence and severity of cyanoHABs. However, most of this research is conducted within the western basin of Lake Erie. Although Lake St. Clair is not considered to be one of the Great Lakes, it is known as "the Heart of the Great Lakes", connecting Lake Huron to Lake Erie. It is one of the most extensively used lakes in the region, providing important recreational, ecological and commercial benefits, as well as drinking water to millions of people on both the Canadian and US sides. Despite its importance, it is often overlooked in cyanoHAB research, though its southern shore is susceptible to the same types of blooms as Western Lake Erie. The relationship between the formation of cyanoHABs and this location is largely due to the nutrient plume received from the mouth of the Thames River, another overlooked system in the Great Lakes region. The research presented in this dissertation was conducted to address the environmental drivers of cyanoHAB formation and differences in community dynamics along the fluvial-lacustrine continuum, and to better understand how rivers contribute to blooms in lakes.

The first data chapter in this dissertation (Chapter 2) investigated the effects of nutrient concentrations, as well as light availability, on phytoplankton community dynamics within an agriculturally - influenced tributary. We deployed *in-situ* microcosms during the growing seasons of 2021 and 2022 to determine how light availability, in

conjunction with nutrient stoichiometry would promote or constrain phytoplankton growth and diversity.

Findings from both years of this study showed a depletion of bioavailable P mid-growing season, which correlated with a phytoplankton community shift from diatom to cyanobacterial dominance. In higher light attenuated treatments, this dominance was mainly attributed to filamentous taxa. Chl *a*, measured as a proxy for biomass, was shown to be produced proportional to light availability, and microcosms with the highest light attenuation had the least productivity. In five of the six trials, total phytoplankton abundance, measured as cells/mL, was lowest in the highest light attenuated containers, showing overall growth was positively correlated to light availability. Overall species diversity metrics across trials were inconsistent between the different treatments, suggesting that diversity cannot be attributed to light availability alone. In September of each year, there was a shift in the dominant cyanobacterial genera at time zero, coincident with a decrease in DIN:SRP compared to mid-growing season. The dominance of non N-fixing *Merismopedia* was replaced in 2021 by *Planktothrix*, a taxon that is more tolerant of N limitation and more efficient as an N scavenger, and by N-fixing *Pseudanabaena* in 2022.

Chapter 3 of this dissertation addressed the environmental drivers of spatial and temporal differences in phytoplankton community composition along the Thames River – Lake St. Clair continuum over the course of a three-year study. The key findings of this chapter showed that nutrient conditions vary significantly along the continuum as well as seasonally and that the river is an important source for nutrients leading to the formation of cyanoHABs downstream. Results showed that nutrient loading into Lake St. Clair

follows a seasonal pattern similar to the that of Lake Erie from the Maumee River, with higher concentrations found in winter and summer than in the fall (at the end of the growing season). In general, nutrient concentrations and N:P ratios were higher in river sites and at the river mouth where the plume enters the lake, showing a decreasing gradient towards the lake. Relative abundance of phytoplankton also showed a seasonal pattern of diatoms dominating in winter and spring, then shifting to cyanobacteria in summer and fall. The most dominant cyanobacteria in the lake were colonial forms, and communities were comprised of a variety of taxa, not only *Microcystis.* River sites supported a higher diversity of major taxonomic groups when compared to the lake.

The *mcyE* gene responsible for microcystin production was detected in lake sites, and during the 2021 growing season, total microcystins concentrations exceeded the Government of Canada's guidelines for drinking water. The *sxtA* gene responsible for the production of saxitoxin was detected in some of the lake sites, but in much fewer copies mL^{-1} than previously reported in Lake Erie. However, the presence of the gene is an indication that testing for the toxin itself should be considered in Lake St. Clair as several towns rely on it as a source for drinking water.

The final data chapter in this collection (Chapter 4) is a commentary from a case study conducted in Lake Victoria, Kenya investigating the importance of the Sondu-Miriu River and the Nyando River as sources of nutrients leading to cHAB formation and the degradation of the lake. Due to difficulties in carrying out the experiment, there were too few data points to test for significant differences in nutrient concentrations between river and lake sites. However, concentrations were an order of magnitude higher in Sondu River compared to any other site, suggesting that further research should be done. A

comparison of water quality parameters showed river sites were characterized by higher TDS, salinity and conductivity whereas the bay and gulf sites were characterized by higher temperatures, Chl *a* and dissolved oxygen.

The limited data obtained during this study came from the help of local citizens, proving the need for local knowledge and collaboration in research projects. Whereas northern countries can provide necessary funding for ongoing place-based research, local academic institutions should have a say in how and where research is conducted, and have equal ownership over the data collected from the research, particularly when involving Indigenous communities. This preliminary study showed that further research in the Lake Victoria catchment needs to be done to have a better understanding of in-river processes and their contributions to downstream nutrient loading leading the potential formation of cyanoHABs.

Collectively, these data chapters provide insight into the complicated and intricate chemical and biological processes occurring along fluvial-lacustrine continuums. The data chapters included in this dissertation provide a broad view of the importance of in-river processes and the role rivers play in the formation of downstream cyanoHABs. Due to the spatial and temporal resolution of chapters 2 and 3, we were able to collect a comprehensive dataset clearly outlining seasonal oscillations and temporal gradients in nutrient loading and phytoplankton community dynamics. Chapter 4 provided preliminary results demonstrating the importance of rivers as sources of nutrients to Lake Victoria and provided a compelling argument that continued, place-based African research needs to continue, with the inclusion of local scientists and knowledge.

Although this collection of work has provided important findings from lesser-studied locations, there are still unanswered questions. Most trials of the microcosm study yielded similar results, but there were some inconsistencies. Further research of this kind should continue and include RNA analysis to obtain higher resolution of taxonomic composition and insight into metabolic pathways. Having an understanding of the species present, not just genera, could tease out what competitive advantages particular phytoplankton taxa may have over others, and link this to specific water quality parameters and seasonality. The work done along the continuum is of high importance as fewer studies have focused on Lake St. Clair and the Thames River, compared to Lake Erie and several of its tributaries.

Future work should include metatranscriptomic analysis to examine gene up- and down-regulation, particularly in relation to nutrient cycling, and link this to seasonal oscillations of nutrient loading. As reports of the presence of saxitoxin and saxitoxin-producing genes are becoming more prevalent in Lake Erie, and the US side of Lake St. Clair, future work should not only focus on the presence of microcystins. With the geographical range of some phytoplankton species expanding, there is an increasing potential for production of multiple families of toxins in the Great Lakes region.

Chapter 4 outlined some very important lessons learned, including the need for local knowledge in research and learning to be flexible in experimental design. Although the African Great Lakes experience a much different climate than the Laurentian Great Lakes, specifically a lack of seasonality. Lake Victoria is susceptible to cyanoHABs as much as the western basin of Lake Erie. Much of the data available from research that has been conducted in Lake Victoria addresses the open lake and not the catchment. It is

important to continue African research, and extend it to include the tributaries flowing into Lake Victoria to better understand their contributions to nutrient loads. While this dissertation provides insight into how environmental factors drive phytoplankton succession along riverine-lacustrine continuums, there remain missing pieces to the puzzle.

APPENDICES

Appendix A (Chapter 2)

Figure S1: Photograph of Microcosms and Enclosure at LTVCA

Figure S2: Mean Chlorophyll September 2021

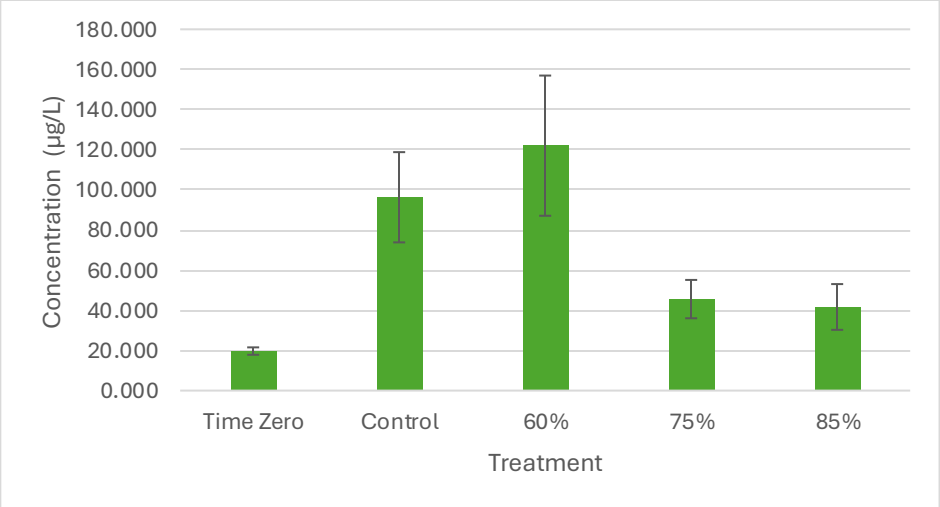


Figure S3: Mean Chlorophyll July 2022

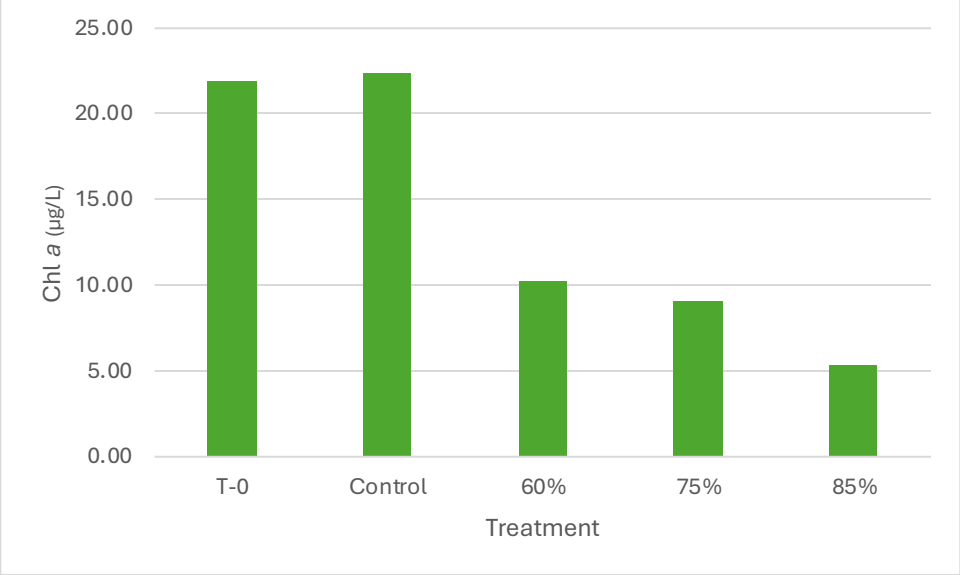


Table S1: Total Solar Radiation During the 2021 Deployments

Solar Radiation (KJ/m3)

Day	June	July	September
1	23,891	15,774	10,243
2	1,711	22,959	5,567
3	3,112	16,915	1,396
4	16,841	23,313	3,333
5	23,313	20,193	18,287
6	20,195		8,127
7	21,629		11,964
8	10,218		16,258
9	23,782		17,522
Sum	144,692	99,154	92,697

*Data obtained from Environment and Climate Change Canada

Table S2: Temperature and Specific Conductivity of Thames River

Deployment	Date	Temp (°C)	Sp. Cond (µS/cm)
Jun-21	06-23-2021	23.7	773
	06-25-2021	23.2	775
Jul-21	07-27-2021	25.3	753
	07-29-2021	25.4	765
Sep-21	09-22-2021	21.2	649
	09-28-2021	15.6	501
Jul-22	07-19-2022	26.3	849
	07-21-2022	25.8	835
	07-24-2022	27.3	843
	07-27-2022	26.3	797
Aug-22	08-24-2022	26.3	740
	08-26-2022	25.3	822
	08-29-2022	25	800
	09-01-2022	23.8	854
Sep-22	09-28-2022	17.14	766
	09-30-2022	15.61	788
	10-03-2022	15.81	883
	10-06-2022	15.23	746

Table S3: List of Phytoplankton Taxa, Codes and Mean Cell Density 2021

Group	Code	RDA	Genus species name	Mean (cells/L)	SD	% Mean
Cyano	cy1		*Aphanizomenon*	2,942	4,608	2.4%
Cyano	cy2	*	*Aphanocapsa*	17,261	31,684	14.3%
Cyano	cy3		*Chroococcus*	32	124	0.0%
Cyano	cy4	*	*Chroococcus microscopicus*	2,299	5,454	1.9%
Cyano	cy5		Coccoid	92	169	0.1%
Cyano	cy6		*Cuspidothrix*	298	789	0.2%
Cyano	cy7		*Dolicospermum*	872	1,836	0.7%
Cyano	cy8		*Jaaginema*	112	432	0.1%
Cyano	cy9		*Lyngbya contorta*	39	150	0.0%
Cyano	cy10	*	*Merismopedia* sp.	8,538	20,325	7.1%
Cyano	cy11	*	*Merismopedia tenuissima*	7,472	26,887	6.2%
Cyano	cy12		*Microcystis*	279	1,081	0.2%
Cyano	cy13		*Planktolyngbya* spp.	480	998	0.4%
Cyano	cy14		*Planktolyngbya contorta*	39	150	0.0%
Cyano	cy15		*Planktolyngbya minor*	83	261	0.1%
Cyano	cy16		*Romeria*	1,315	3,044	1.1%
Cyano	cy17		*Planktothrix agardhii*	5,351	8,334	4.4%
Cyano	cy18		*Pseudanabaena*	0	0	0.0%
Diato	da1		*Aulacoseira*	228	636	0.2%
Diato	da2		*Achnanthidium*	61	235	0.1%
Diato	da3	*	Centric diatom spp.	3,373	13,065	2.8%
Diato	da4	*	*Cyclotella*	13,928	16,352	11.5%
Diato	da5		*Cymatopleura*	2	9	0.0%
Diato	da6		*Encyonopsis*	8	31	0.0%
Diato	da7		*Gyrosigma*	1	5	0.0%
Diato	da8		*Navicula*	10	18	0.0%
Diato	da9		*Nitzschia*	2,338	3,615	1.9%
Diato	da10		*Reimeria*	524	2,030	0.4%
Diato	da11		*Skeletonema*	27,322	45,267	22.6%
Diato	da12		*Surirella*	7	19	0.0%
Diato	da13		*Synedra*	1	5	0.0%
Diato	da14		*Uroselena*	27	103	0.0%
Chloro	ch1		*Actinastrum*	157	333	0.1%
Chloro	ch2		*Ankistrodesmus*	665	774	0.6%
Chloro	ch3		*Chlamydomonas*	278	314	0.2%
Chloro	ch4		Chlorobiflagellates	43	107	0.0%
Chloro	ch5		*Closterium*	32	46	0.0%
Chloro	ch6		Coccoid spp.	2,988	4,157	2.5%
Chloro	ch7		*Coelastrum*	116	235	0.1%
Chloro	ch8		*Cosmarium*	0	0	0.0%

Group	Code	RDA	Genus species name	Mean (cells/L)	SD	% Mean
Chloro	ch9		*Crucigenia*	837	1,398	0.7%
Chloro	ch10	*	*Dictosphaerium*	1,960	4,133	1.6%
Chloro	ch11		*Dictosphaerium subsolitarium*	149	397	0.1%
Chloro	ch12		*Elakatothrix*	40	155	0.0%
Chloro	ch13		*Francia*	16	62	0.0%
Chloro	ch14		*Golenkenia*	559	1,399	0.5%
Chloro	ch15		*Micractinium*	600	1,138	0.5%
Chloro	ch16		*Mougeotia*	0	0	0.0%
Chloro	ch17		*Oocystis*	340	718	0.3%
Chloro	ch18		*Pandorina*	10	38	0.0%
Chloro	ch19		*Pediastrum*	78	164	0.1%
Chloro	ch20		*Quadricoccus*	0	0	0.0%
Chloro	ch21		*Scenedesmus*	3,109	2,893	2.6%
Chloro	ch22		*Schroderia*	13	52	0.0%
Chloro	ch23		*Selenastrum*	471	1,024	0.4%
Chloro	ch24		*Selenastrum minitum*	112	261	0.1%
Chloro	ch25	*	*Sphaerocystis*	1,718	5,076	1.4%
Chloro	ch26		*Spermatozopsis*	396	694	0.3%
Chloro	ch27		*Tetrastrum*	1,153	1,819	1.0%
Chloro	ch28		*Treubaria*	94	258	0.1%
Chloro	ch29		*Westella*	39	150	0.0%
Pico	pc1		Picoplankton spp.	3,885	4,254	3.2%
Microflag	mc1		Microflagellate spp.	3,527	4,425	2.9%
Microflag	mc2		*Mallomonas*	1	5	0.0%
Crypto	cr1		*Cryptomonas*	232	309	0.2%
Crypto	cr2		*Cryptomonas erosa*	159	285	0.1%
Crypto	cr3		*Rhodomonas komma*	1,419	1,609	1.2%
Crypto	cr4		*Rhodomonas minuta*	68	150	0.1%
Eugleno	eu1		*Euglena*	61	116	0.1%
Eugleno	eu2		*Trachelomonas*	15	36	0.0%
Dinoflag	do1		*Glenodinium*	15	32	0.0%
Dinoflag	do2		*Gymnodinium*	0	0	0.0%
Dinoflag	do3		*Peridinium*	75	154	0.1%
Dinoflag	do4		*Phaces*	1	5	0.0%
Chryso	ch1		Coccoid spp.	0	0	0.0%
Chryso	ch2		*Ophiocytium*	2	9	0.0%

NOTE: Density and % abundance were calculated across all months and treatment levels. The top 18 most abundant taxa (> 1% relative abundance, with names highlighted in yellow), accounted for ~90% of total abundance. * RDA = eight taxa that responded the most strongly to environmental drivers of community composition in the redundancy analysis (RDA).

Table S4: Alpha Biodiversity Metrics Across Treatments for 2021 and 2022 Trials

Month	Treatment	Shannon Index (H)	Evenness	Richness	Simpson Index (1-D)
Jun-21	Time-Zero	2.14	0.79	15	0.84
	Control	2.00	0.65	22	0.78
	60%	1.04	0.37	16	0.48
	75%	1.46	0.49	19	0.62
	85%	1.05	0.36	18	0.43
Jul-21	Time-Zero	1.49	0.60	12	0.68
	Control	2.01	0.67	20	0.77
	60%	1.97	0.59	28	0.77
	75%	2.71	0.82	27	0.91
	85%	1.42	0.45	24	0.64
Sep-21	Time-Zero	2.08	0.66	23	0.80
	Control	2.88	0.79	38	0.92
	60%	2.62	0.76	31	0.91
	75%	2.38	0.71	28	0.85
	85%	1.65	0.53	22	0.64
Jul-22	Time-Zero	1.70	0.60	17	0.66
	Control	2.08	0.59	33	0.76
	60%	1.89	0.58	26	0.73
	75%	1.67	0.52	25	0.63
	85%	2.24	0.73	22	0.82
Aug - Sept 2022	Time-Zero	2.76	0.79	33	0.91
	Control	2.10	0.62	30	0.75
	60%	2.55	0.74	31	0.86
	75%	2.30	0.67	31	0.80
	85%	1.84	0.53	33	0.73
Sept - Oct 2022	Time-Zero	2.05	0.76	15	0.84
	Control	2.31	0.69	29	0.84
	60%	2.78	0.77	37	0.92
	75%	2.67	0.78	31	0.91
	85%	2.26	0.67	30	0.85

Table S5: List of Phytoplankton Taxa, Codes and Mean Cell Density

Group	Code	RDA	Genus species name	Mean (cells/L)	SD	% Mean
Cyano	cy1		*Aphanizomenon*	455.2	759.2	0.7
Cyano	cy2		*Aphanocapsa*	1435.1	2023.8	2.2
Cyano	cy3		*Chroococcus*	33.6	104.8	0.1
Cyano	cy4		*Chroococcus microscopicus*	952.8	2807.8	1.5
Cyano	cy5		Coccoid spp	305.4	561.8	0.5
Cyano	cy6		*Cylindrospermopsis*	11.2	43.4	0
Cyano	cy7		*Cuspidothrix*	23.5	63.2	0
Cyano	cy8		*Lyngbya martensiana*	30.9	119.8	0
Cyano	cy9	*	*Merismopedia* spp	4965.3	12493.9	7.8
Cyano	cy10		*Merismopedia tenuissima*	3589.3	9676.4	5.6
Cyano	cy11	*	*Microcystis*	1508.3	3924.8	2.4
Cyano	cy12		*Oscillatoria*	8.1	31.5	0
Cyano	cy13		*Phormidium*	93.1	255.5	0.1
Cyano	cy14		*Planktolyngbya*	287.2	446.6	0.4
Cyano	cy15	*	*Planktolyngbya minor*	2667	6543.6	4.2
Cyano	cy16		*Planktolyngbya limnetica*	201.4	696.9	0.3
Cyano	cy17	*	*Planktothrix*	949.7	1945.5	1.5
Cyano	cy18		*Pseudanabaena*	264.8	577.9	0.4
Cyano	cy19		*Romeria*	20.6	79.8	0
Cyano	cy20		*Woronichia*	406.7	919.5	0.6
Diato	da1		*Attheya*	34.4	82.3	0.1
Diato	da2		*Aulacoseira*	127.3	135.8	0.2
Diato	da3		Centric Diatoms	8635.9	8694.7	13.5
Diato	da4		*Cymbella*	0.6		0
Diato	da5		*Gomphonema*	47.7	155.6	0.1
Diato	da6		*Gyrosigma*	2.8	6.4	0
Diato	da7		*Melosira*	69.8	146.9	0.1
Diato	da8		*Navicula*	59.5	161.9	0.1
Diato	da9		*Nitzschia*	3697.4	3421.7	5.8
Diato	da10	*	*Skeletonema*	6755.7	10551.6	10.6
Diato	da11		*Surirella*	16.1	21.5	0
Diato	da12		*Synedra*	53	132.7	0.1
Diato	da13		*Uroselena*	10	38.7	0
Chloro	ch1		*Actinastrum*	104.7	213.5	0.2
Chloro	ch2		*Ankistrodesmus*	668	490.1	1
Chloro	ch3		*Carteria*	1.2	4.6	0
Chloro	ch4		*Chlorobiflagellates*	270.7	256.6	0.4
Chloro	ch5		*Closterium*	3.2	6.7	0
Chloro	ch6		Coccoid spp	6271.2	9098.2	9.8

Chloro	ch7	*Coelastrum*	621.1	531.2	1
Chloro	ch8	*Cosmarium*	1.4	3.7	0
Chloro	ch9	*Crucigenia*	769.3	1514.4	1.2
Chloro	ch10	*Dictosphaerium*	2406.3	5664.8	3.8
Chloro	ch11	*Dictosphaerium subsolitarium*	6.1	23.5	0
Chloro	ch12	*Francia*	10.7	41.3	0
Chloro	ch13	*Golenkenia*	14.5	41.3	0
Chloro	ch14	*Gonium*	19.5	75.7	0
Chloro	ch15	*Lagerhemia*	16	44.8	0
Chloro	ch16	*Micractinium*	750.5	816.6	1.2
Chloro	ch17	*Oocystis*	209.5	349.4	0.3
Chloro	ch18	*Pandorina*	190	659.9	0.3
Chloro	ch19	*Pediastrum*	181.7	281.5	0.3
Chloro	ch20	*Scenedesmus*	3936.5	4314	6.1
Chloro	ch21	*Selenastrum*	536.9	663	0.8
Chloro	ch22	*Selenastrum minitum*	80	309.8	0.1
Chloro	ch23	*Sphaerocystis*	193.9	213.2	0.3
Chloro	ch24	*Spermatozopsis*	8	31	0
Chloro	ch25	*Tetrastrum*	208	496.7	0.3
Chloro	ch26	*Treubaria*	11.1	25.7	0
Pico	pc1	Picoplankton spp.	4301.9	3583.1	6.7
Micro	mc1	Microflagellate spp.	1733.2	1336.5	2.7
Pyrro	py1	*Gymnodinium*	2.8	6.7	0
Pyrro	py2	*Peridinium*	160.7	553.9	0.3
Pyrro	py3	*Woloszynakia*	4.1	8.4	0
Euglena	eu1	*Euglena*	65.2	102.1	0.1
Euglena	eu2	*Phacus*	2.3	4.3	0
Euglena	eu3	*Trachelomonas*	2.9	9.6	0
Crypto	cr1	*Cryptomonas*	914.1	1321.1	1.4
Crypto	cr2	*Cryptomonas erosa*	1118.7	2156.3	1.7
Crypto	cr3	*Komma - Rhodomonas*	485.8	707.6	0.8
Chryso	chr1	Coccoid spp	18.7	54.2	0
Chryso	chr2	*Dinobryon*	6.1	19.1	0
Chryso	chr4	*Mallomonas*	0.6	2.3	0

NOTE: Density and % abundance were calculated across all months and treatment levels. The top 19 most abundant taxa (> 1% relative abundance, with names highlighted in yellow), accounted for ~90% of total abundance. * RDA = five taxa that responded the most strongly to environmental drivers of community composition in the redundancy analysis (RDA).

Appendix B (Chapter 3)

Figure S4: Phytoplankton Relative Abundance at BRWTP

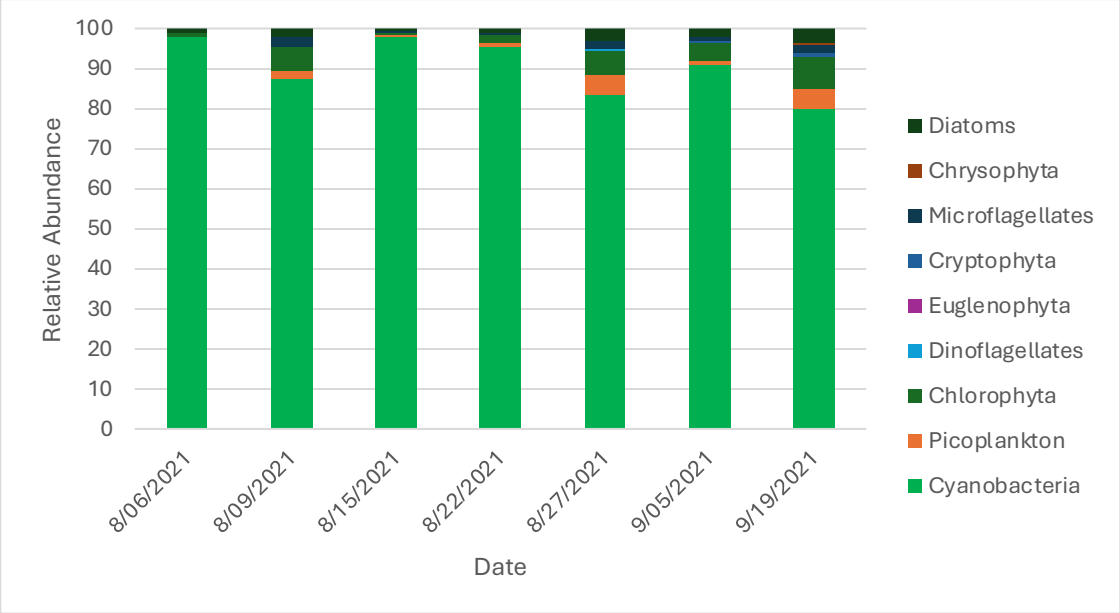


Figure S5: Phytoplankton Relative Abundance at LTVCA

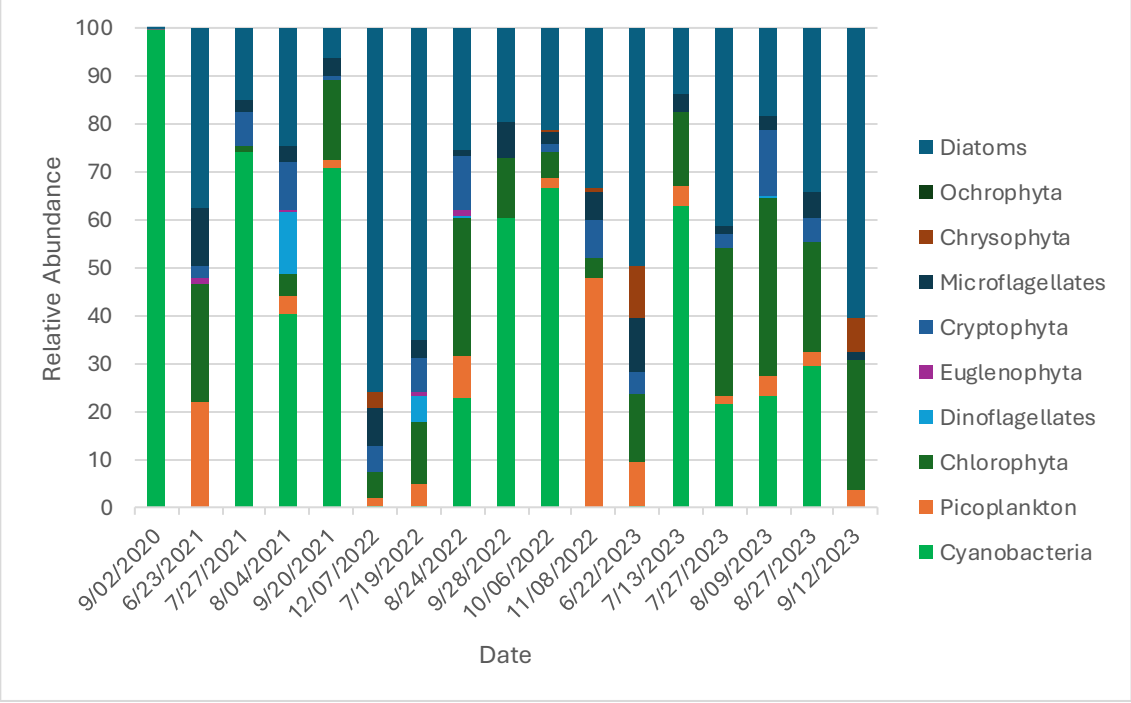


Figure S6: Phytoplankton Relative Abundance at Lighthouse Cove

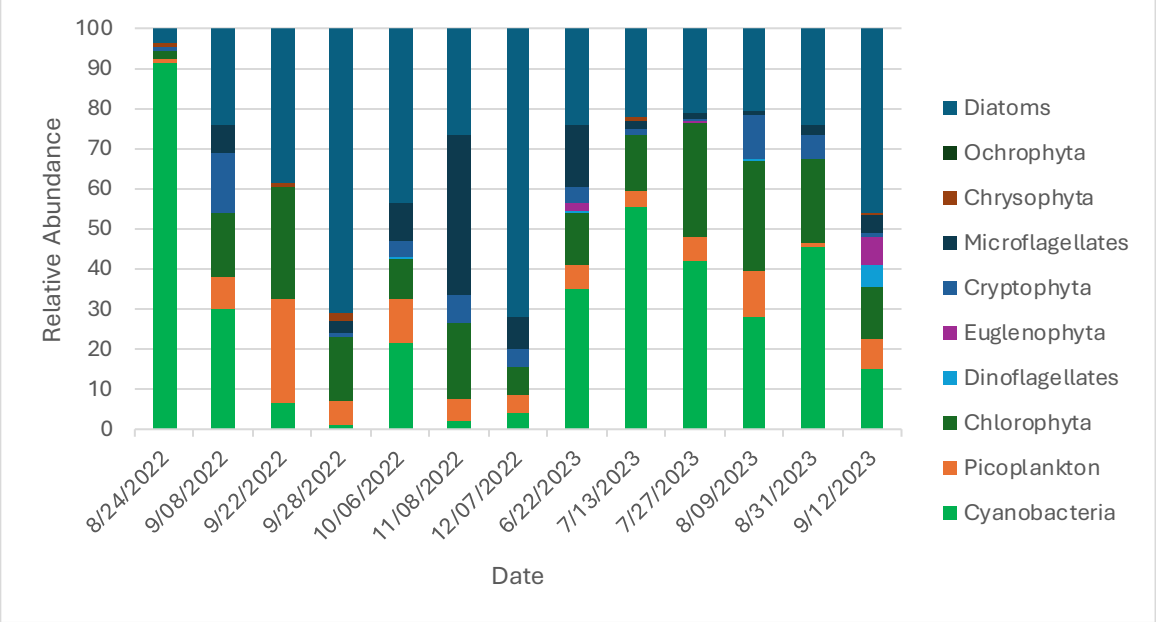


Figure S7: Phytoplankton Relative Abundance at Prairie Siding

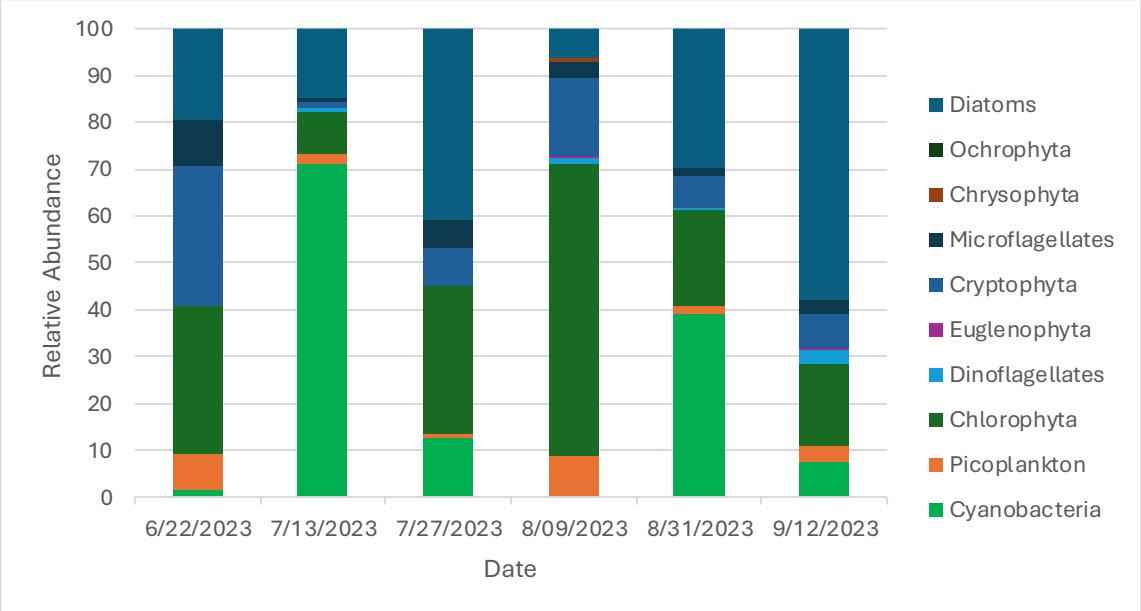


Figure S8: Boxplot Comparing Phytoplankton Richness Across Seasons

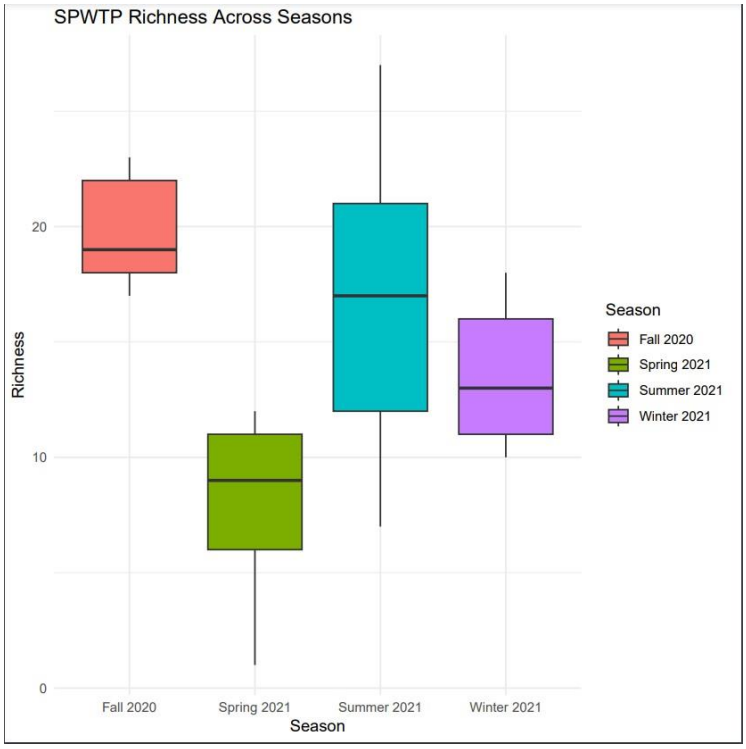


Figure S9: Boxplot Comparing Phytoplankton Richness Across Sites

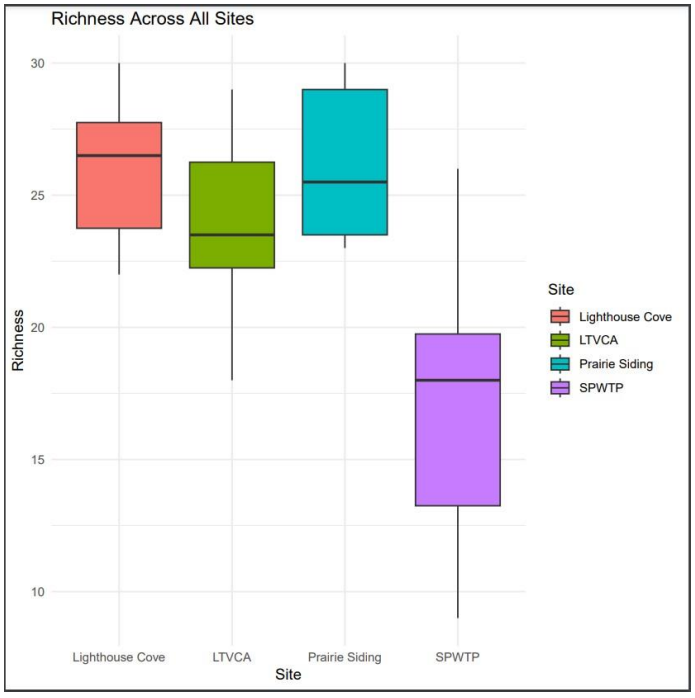


Figure S10: Boxplot Comparing Shannon Diversity Across Sites

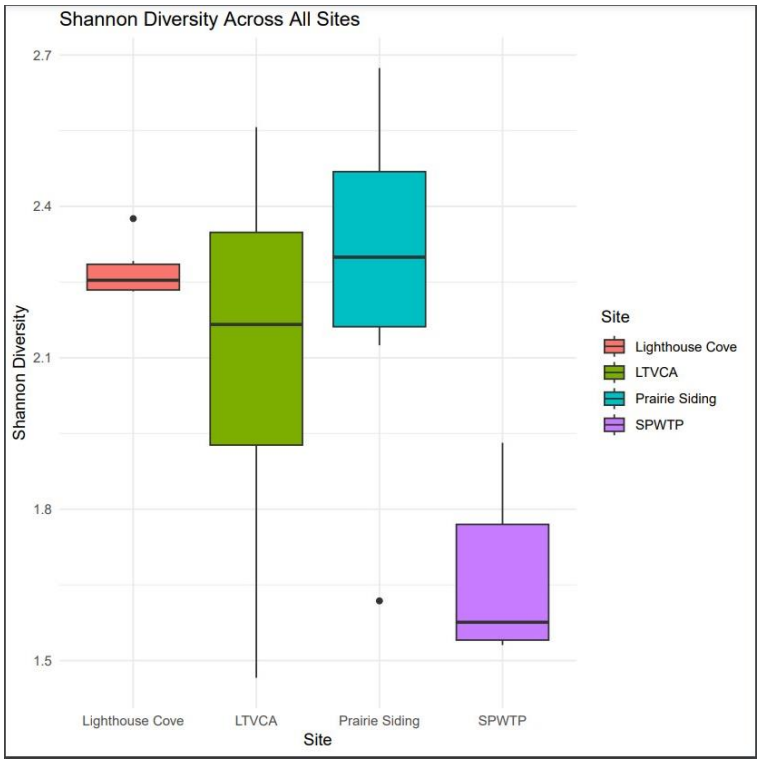


Figure S11: Boxplot Comparing Shannon Diversity Between Sites

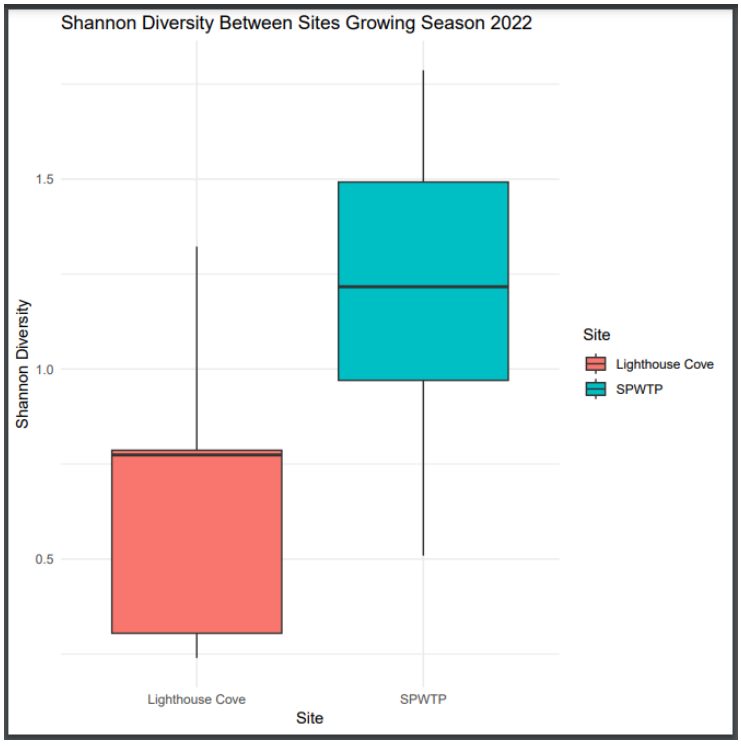


Figure S12: Boxplot Comparing Shannon Diversity Across Seasons

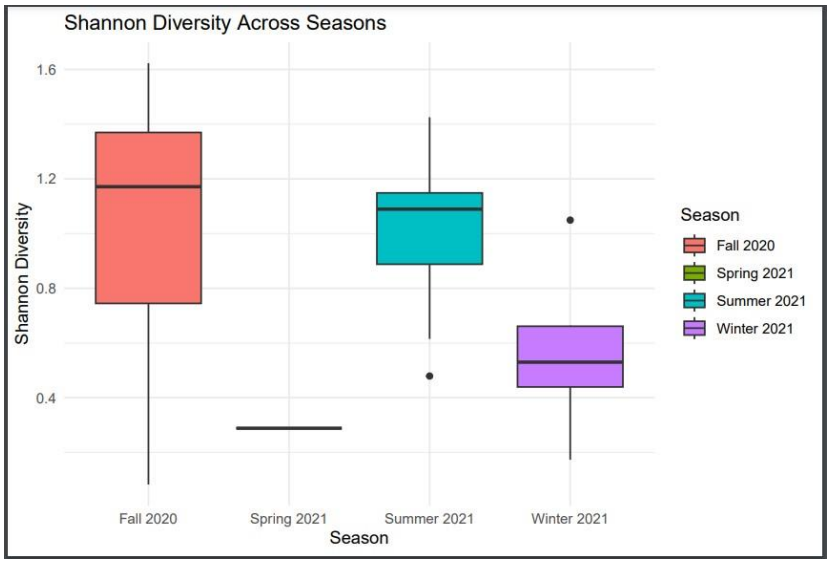


Figure S13: Boxplot Comparing Simpson Diversity Across Seasons

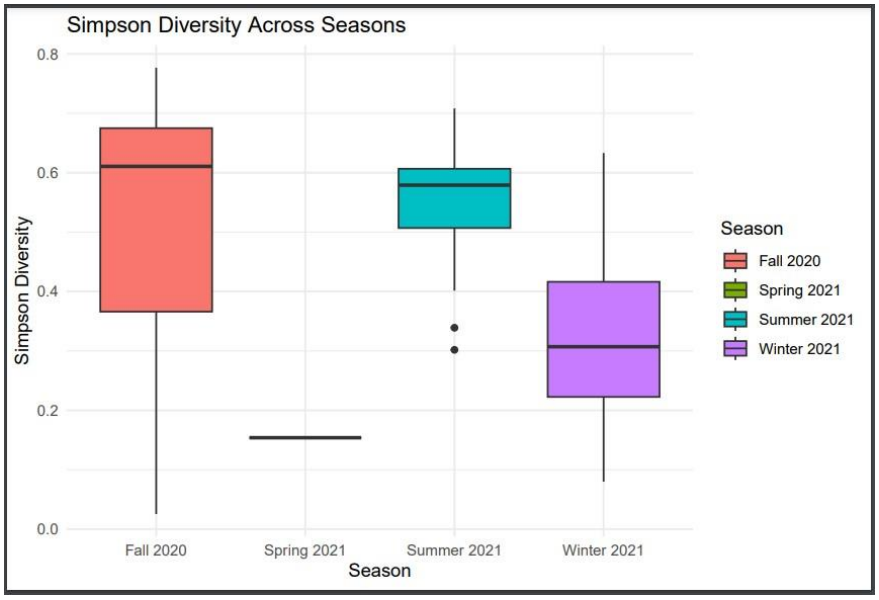


Figure S14: Boxplot Comparing Evenness Across Seasons

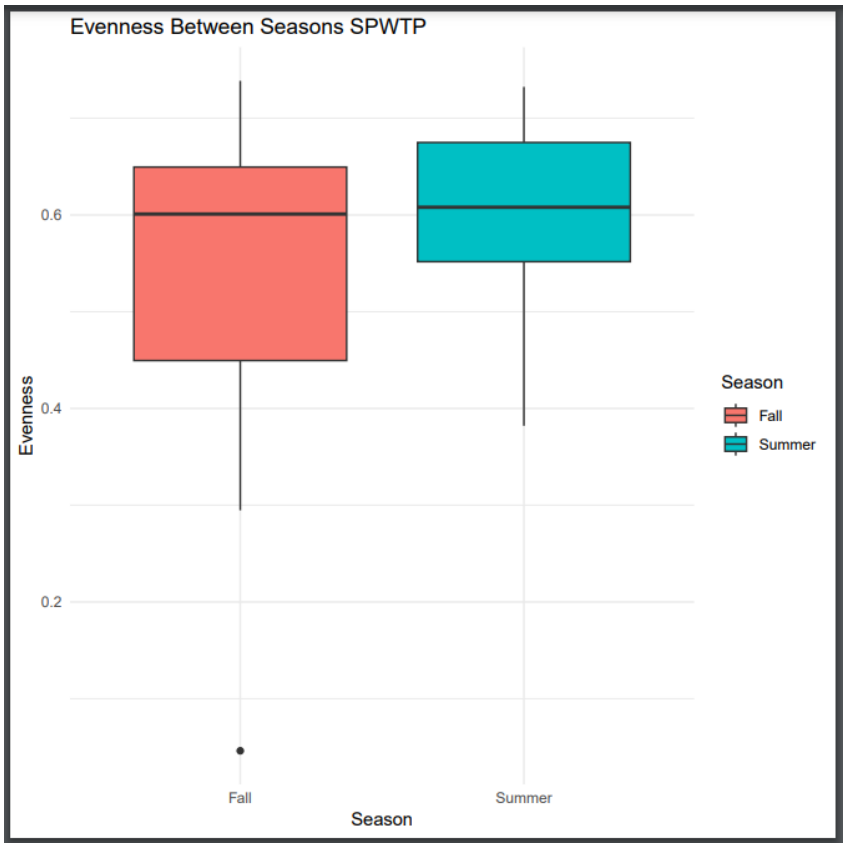


Figure S15: Boxplot Comparing Richness Across Seasons

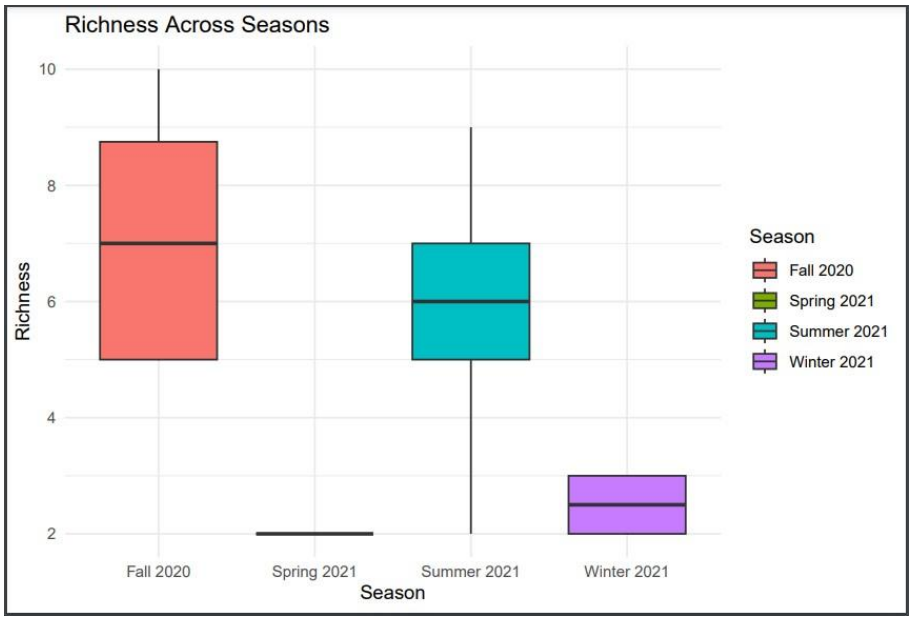


Table S6: Site Names and Geographical Coordinates Along the Continuum

Sample Site	Coordinates
SPWTP	42.3152° N, 82.5551° W
BRWTP	42.2973° N, 82.7060° W
LTVCA	42.4078° N, 82.1850° W
Prairie Siding	42.3691° N, 82.2794° W
Lighthouse Cove	42.3180° N, 82.4536° W

Table S7: Mean Temperature and Specific Conductivity Along the Continuum

Location	Date	Temp (°C)	Sp. Conductivity (µS/cm)
SPWTP	08-27-2020	25.3	196
SPWTP	09-02-2020	24.1	197
SPWTP	09-10-2020	21.2	416
SPWTP	09-16-2020	20.2	506
SPWTP	09-23-2020	17.6	448
SPWTP	09-30-2020	18.3	424
SPWTP	10-08-2020	15.6	425
SPWTP	10-14-2020	16.2	429
SPWTP	10-20-2020	12.0	237
SPWTP	10-28-2020	10.7	326
SPWTP	11-05-2020	8.5	288
SPWTP	11-12-2020	9.8	232
SPWTP	11-19-2020	6.6	210
SPWTP	11-25-2020	6.6	496
SPWTP	01-11-2021	0.9	414
SPWTP	01-22-2021	0.9	346
SPWTP	01-29-2021	0.8	279
SPWTP	02-23-2021	3.2	758
SPWTP	03-19-2021	5.6	887
SPWTP	04-13-2021	12.6	342
SPWTP	04-27-2021	10.2	218
SPWTP	05-05-2021	13.9	257
SPWTP	05-25-2021	18.8	22
SPWTP	06-25-2021	21.2	214
SPWTP	06-29-2021	23.6	200
SPWTP	07-08-2021	23.6	204
SPWTP	07-15-2021	23.6	388
SPWTP	07-23-2021	23.2	421
SPWTP	07-27-2021	24.5	374
SPWTP	07-29-2021	25.4	348

SPWTP	07-31-2021	23.6	336
SPWTP	08-07-2021	24.4	360
SPWTP	08-14-2021	25.7	288
SPWTP	08-16-2021	24.0	333
SPWTP	08-21-2021	24.5	354
SPWTP	08-23-2021	26.2	243
SPWTP	08-28-2021	26.6	242
SPWTP	08-30-2021	26.9	236
SPWTP	09-06-2021	22.7	660
SPWTP	09-13-2021	22.0	501
SPWTP	09-20-2021	21.9	564
SPWTP	09-27-2021	17.4	517
SPWTP	10-11-2021	20.2	537
SPWTP	10-25-2021	13.9	325
SPWTP	11-08-2021	10.3	353
SPWTP	12-08-2021	3.5	346
SPWTP	01-19-2022	2.0	568
SPWTP	02-15-2022	4.5	584
SPWTP	03-15-2022	1.9	523
SPWTP	04-05-2022	5.5	221
SPWTP	05-09-2022	12.3	266
SPWTP	06-08-2022	19.8	199
SPWTP	07-13-2022	24.0	443
SPWTP	07-19-2022	24.0	462
SPWTP	07-27-2022	25.1	369
SPWTP	08-02-2022	24.4	368
SPWTP	08-08-2022	26.2	329
SPWTP	08-16-2022	23.4	282
SPWTP	08-25-2022	24.4	232
SPWTP	08-31-2022	23.3	238
SPWTP	09-08-2022	22.6	280
SPWTP	09-13-2022	22.4	321
SPWTP	09-22-2022	21.2	259
SPWTP	09-28-2022	16.4	260
SPWTP	10-06-2022	15.7	271
SPWTP	10-14-2022	13.0	311
SPWTP	11-08-2022	12.3	363
SPWTP	12-07-2022	3.3	356
SPWTP	01-17-2023	1.6	326
SPWTP	02-23-2023	1.8	327
SPWTP	03-23-2023	3.7	256
SPWTP	04-20-2023	9.6	409
SPWTP	05-10-2023	13.2	623
SPWTP	06-06-2023	22.6	285
SPWTP	06-22-2023	21.5	277

SPWTP	07-06-2023	24.5	266
SPWTP	07-13-2023	22.7	260
SPWTP	07-22-2023	23.4	384
SPWTP	07-26-2023	24.2	434
SPWTP	08-03-2023	24.7	431
SPWTP	08-10-2023	24.5	435
SPWTP	08-15-2023	22.8	385
SPWTP	08-24-2023	21.1	346
SPWTP	08-31-2023	21.6	312
SPWTP	09-07-2023	22.9	327
SPWTP	09-13-2023	21.5	350
SPWTP	09-20-2023	21.1	342
SPWTP	09-26-2023	20.1	285
BRWTP	07-30-2021	25.1	251
BRWTP	08-06-2021	23.9	260
BRWTP	08-08-2021	24.4	278
BRWTP	08-09-2021	24.9	294
BRWTP	08-13-2021	25.2	198
BRWTP	08-15-2021	24.5	256
BRWTP	08-20-2021	24.3	259
BRWTP	08-22-2021	23.4	224
BRWTP	08-27-2021	26.4	212
BRWTP	08-29-2021	26.7	190
BRWTP	09-05-2021	23.0	282
BRWTP	09-12-2021	22.0	205
BRWTP	09-19-2021	21.6	218
BRWTP	09-24-2021	17.6	302
BRWTP	10-09-2021	19.0	336
BRWTP	10-26-2021	11.0	277
BRWTP	11-06-2021	8.9	314
BRWTP	07-19-2022	24.3	276
BRWTP	07-27-2022	25.0	218
BRWTP	08-03-2022	24.6	111
BRWTP	08-08-2022	26.3	229
BRWTP	08-16-2022	23.3	311
BRWTP	08-24-2022	24.0	226
BRWTP	08-31-2022	23.5	219
BRWTP	09-08-2022	21.9	236
BRWTP	09-13-2022	22.3	233
BRWTP	09-21-2022	22.3	247
BRWTP	09-27-2022	16.8	231
BRWTP	06-27-2023	21.3	225
BRWTP	07-04-2023	21.9	227
BRWTP	07-11-2023	22.9	206
BRWTP	07-18-2023	23.5	209

BRWTP	07-25-2023	24.9	278
BRWTP	08-09-2023	23.8	283
BRWTP	08-15-2023	23.9	262
BRWTP	08-24-2023	22.6	283
BRWTP	08-31-2023	21.3	238
BRWTP	09-07-2023	22.9	210
BRWTP	09-13-2023	20.8	263
BRWTP	09-20-2023	20.2	310
BRWTP	09-27-2023	19.0	393
Lighthouse Cove	08-17-2022	24.3	700
Lighthouse Cove	08-24-2022	27.3	430
Lighthouse Cove	08-29-2022	24.8	563
Lighthouse Cove	09-08-2022	23.4	696
Lighthouse Cove	09-14-2022	23.4	795
Lighthouse Cove	09-22-2022	23.1	796
Lighthouse Cove	09-28-2022	17.3	727
Lighthouse Cove	10-06-2022	16.8	807
Lighthouse Cove	06-06-2023	21.6	710
Lighthouse Cove	06-22-2023	22.5	759
Lighthouse Cove	07-06-2023	24.1	604
Lighthouse Cove	07-13-2023	22.7	614
Lighthouse Cove	07-22-2023	23.2	603
Lighthouse Cove	07-26-2023	24.8	590
Lighthouse Cove	08-09-2023	25.0	638
Lighthouse Cove	08-30-2023	21.0	515
Lighthouse Cove	09-12-2023	22.3	685
LTVCA	06-23-2021	23.3	774
LTVCA	07-27-2021	25.3	755
LTVCA	07-19-2022	26.3	849
LTVCA	08-24-2022	26.3	740
LTVCA	09-01-2022	23.8	854
LTVCA	09-28-2022	17.1	766
LTVCA	10-06-2022	15.2	746
LTVCA	06-06-2023	20.3	835
LTVCA	06-22-2023	22.5	715
LTVCA	07-06-2023	23.7	515
LTVCA	07-13-2023	22.8	672
LTVCA	07-22-2023	22.5	637
LTVCA	07-26-2023	25.1	656
LTVCA	08-09-2023	24.5	729
LTVCA	08-30-2023	20.2	600
LTVCA	09-12-2023	22.3	773
Prairie Siding	06-06-2023	15.1	830
Prairie Siding	06-22-2023	21.9	690
Prairie Siding	07-06-2023	23.3	567

Prairie Siding	07-13-2023	21.7	660
Prairie Siding	07-22-2023	22.4	613
Prairie Siding	07-26-2023	24.2	618
Prairie Siding	08-09-2023	25.8	700
Prairie Siding	08-30-2023	20.1	598
Prairie Siding	09-12-2023	22.4	742

Table S8: Abbreviations and Corresponding Taxa

Order	Abbreviation	Taxa
Cyanobacteria	Cy1	*Aphanizomenon*
Cyanobacteria	Cy2	*Aphanocapsa*
Cyanobacteria	Cy3	*Aphanocapsa planktonica*
Cyanobacteria	Cy4	*Aphanocapsa holsatica*
Cyanobacteria	Cy5	*Aphanothece*
Cyanobacteria	Cy6	*Chroococcus*
Cyanobacteria	Cy7	*Chroococcus aphanocapsoides*
Cyanobacteria	Cy8	*Chroococcus limneticus*
Cyanobacteria	Cy9	*Chroococcus microscopicus*
Cyanobacteria	Cy10	*Chroococcus minimus*
Cyanobacteria	Cy11	Coccoid Cyanobacteria
Cyanobacteria	Cy12	*Coelosphaerium*
Cyanobacteria	Cy13	*Cylindrospermopsis*
Cyanobacteria	Cy14	*Dolicospermum*
Cyanobacteria	Cy15	*Gloeocapsa*
Cyanobacteria	Cy16	*Jaaginema*
Cyanobacteria	Cy17	*Merismopedia*
Cyanobacteria	Cy18	*Merismopedia punctata*
Cyanobacteria	Cy19	*Merismopedia tenuissima*
Cyanobacteria	Cy20	*Microcystis*
Cyanobacteria	Cy21	*Microcystis aeruginosa*
Cyanobacteria	Cy22	*Microcystis smithii*
Cyanobacteria	Cy23	*Planktolyngbya limnetica*
Cyanobacteria	Cy24	*Planktolyngbya minor*
Cyanobacteria	Cy25	*Planktolyngbya*
Cyanobacteria	Cy26	*Planktothrix*
Cyanobacteria	Cy27	*Planktothrix agardhii*
Cyanobacteria	Cy28	*Pseudaanabaena*
Cyanobacteria	Cy29	*Pseudanabaena mucicola*
Cyanobacteria	Cy30	*Cuspidothrix*
Cyanobacteria	Cy31	*Oscillatoria*
Cyanobacteria	Cy32	*Phormidium*
Cyanobacteria	Cy33	*Romeria*
Picoplankton	Pi 1	Picoplankton

Chlorophyta	Ch1	*Ankistrodesmus*
Chlorophyta	Ch2	*Carteria*
Chlorophyta	Ch3	*Chladydomonas*
Chlorophyta	Ch4	*Chlorobiflagellates*
Chlorophyta	Ch5	Coccoid Chlorophyta
Chlorophyta	Ch6	*Coelastrum*
Chlorophyta	Ch7	*Cosmarium*
Chlorophyta	Ch8	*Crucigenia*
Chlorophyta	Ch9	*Dictosphaerium*
Chlorophyta	Ch10	*Dictosphaerium subsolitarium*
Chlorophyta	Ch11	*Francia*
Chlorophyta	Ch12	*Golenkenia*
Chlorophyta	Ch13	*Lagerhemia*
Chlorophyta	Ch14	*Micratinium*
Chlorophyta	Ch15	*Mougeotia*
Chlorophyta	Ch16	*Odeogonium*
Chlorophyta	Ch17	*Oocystis*
Chlorophyta	Ch18	*Pandorina*
Chlorophyta	Ch19	*Pediastrum*
Chlorophyta	Ch20	*Scenedesmus*
Chlorophyta	Ch21	*Selenastrum*
Chlorophyta	Ch22	*Sphaerocystis*
Chlorophyta	Ch23	*Staurastrum*
Chlorophyta	Ch24	*Tetrastrum*
Chlorophyta	Ch25	*Stigeoclonium*
Chlorophyta	Ch26	*Selenastrum minitum*
Chlorophyta	Ch27	*Schroderia*
Chlorophyta	Ch28	*Tetraedron*
Chlorophyta	Ch29	*Spermatozoopsis*
Chlorophyta	Ch30	*Trubaria*
Chlorophyta	Ch31	*Gonium*
Chlorophyta	Ch32	*Gymnodinium*
Chlorophyta	Ch33	*Actinastrum*
Chlorophyta	Ch34	*Closterium*
Chlorophyta	Ch35	*Elakatothrix*
Chlorophyta	Ch36	*Phacotus*
Pyrrophyta (Dinoflagellates)	Py1	*Glenodinium*
Pyrrophyta (Dinoflagellates)	Py2	*Gymnodinium*
Pyrrophyta (Dinoflagellates)	Py3	*Peridiniopsis*
Pyrrophyta (Dinoflagellates)	Py4	*Peridinium*
Pyrrophyta (Dinoflagellates)	Py5	*Glenodinium*
Pyrrophyta (Dinoflagellates)	Py6	*Woloszynakia*
Euglenophyta	Eu1	Euglena
Euglenophyta	Eu2	*Trachelomonas*
Euglenophyta	Eu3	*Phaces*

Cryptophyta	Cr1	*Cryptomonas*
Cryptophyta	Cr2	*Komma - Rhodomonas*
Cryptophyta	Cr3	*Rhodomonas minuta*
Cryptophyta	Cr4	*Cryptomonas erosa*
Microflagellates	Mi1	*Microflagellates*
Chrysophyta	Chr1	*Chrysococcus*
Chrysophyta	Chr2	Coccoid Chrysophyta
Chrysophyta	Chr3	*Mallonomonas*
Chrysophyta	Chr4	*Senura*
Ochrophyta	Oc1	*Mallomonas*
Ochrophyta	Oc2	*Dinobryon*
Bacillariophyta (Diatoms)	Di1	*Achnanthedium*
Bacillariophyta (Diatoms)	Di2	*Achnanthes*
Bacillariophyta (Diatoms)	Di3	*Asterionella formosa*
Bacillariophyta (Diatoms)	Di4	*Attheya*
Bacillariophyta (Diatoms)	Di5	*Aulacoseira*
Bacillariophyta (Diatoms)	Di6	Centric Diatoms
Bacillariophyta (Diatoms)	Di7	*Cocconeis*
Bacillariophyta (Diatoms)	Di8	*Cyclotella stephanodiscus*
Bacillariophyta (Diatoms)	Di9	*Cymatopleura*
Bacillariophyta (Diatoms)	Di10	*Diatoma*
Bacillariophyta (Diatoms)	Di11	*Gomphonema*
Bacillariophyta (Diatoms)	Di12	*Fragilaria*
Bacillariophyta (Diatoms)	Di13	*Hippodonta*
Bacillariophyta (Diatoms)	Di14	*Navicula*
Bacillariophyta (Diatoms)	Di15	*Nitzschia*
Bacillariophyta (Diatoms)	Di16	*Psammothidium*
Bacillariophyta (Diatoms)	Di17	*Skeletonema*
Bacillariophyta (Diatoms)	Di18	*Staurosira*
Bacillariophyta (Diatoms)	Di19	*Stephanodiscus*
Bacillariophyta (Diatoms)	Di20	*Surirella*
Bacillariophyta (Diatoms)	Di21	*Synedra*
Bacillariophyta (Diatoms)	Di22	*Uroselenia*
Bacillariophyta (Diatoms)	Di23	*Amphipleura*
Bacillariophyta (Diatoms)	Di24	*Asterionella*
Bacillariophyta (Diatoms)	Di25	*Cyclotella*
Bacillariophyta (Diatoms)	Di26	*Gyrosigma*
Bacillariophyta (Diatoms)	Di27	*Melosira*
Bacillariophyta (Diatoms)	Di28	*Selephora*

Table S9: Alpha Biodiversity Metrics Along the Continuum

Site	Date	Shannon	Simpson	Richness	Evenness
SPWTP	08-27-2020	1.547904	0.662365	26	0.475095
SPWTP	09-02-2020	1.75965	0.748059	29	0.522571
SPWTP	09-10-2020	1.822434	0.766123	34	0.516803
SPWTP	09-16-2020	1.892442	0.802865	26	0.580843
SPWTP	09-23-2020	1.619811	0.693286	24	0.509686
SPWTP	09-30-2020	1.41677	0.62594	17	0.500058
SPWTP	10-08-2020	1.932613	0.778659	17	0.682128
SPWTP	10-14-2020	1.939815	0.776016	24	0.610378
SPWTP	10-20-2020	1.581781	0.615148	24	0.49772
SPWTP	10-28-2020	1.887963	0.716798	24	0.594063
SPWTP	11-05-2020	1.949258	0.774601	18	0.674397
SPWTP	11-12-2020	1.448913	0.552526	20	0.483659
SPWTP	11-19-2020	1.584228	0.627715	19	0.538041
SPWTP	11-27-2020	0.299244	0.093845	19	0.10163
SPWTP	01-10-2021	0.546623	0.230149	12	0.219977
SPWTP	01-22-2021	1.736433	0.738663	15	0.641211
SPWTP	01-29-2021	1.891222	0.806407	12	0.761084
SPWTP	02-23-2021	0.528863	0.190799	19	0.179614
SPWTP	03-19-2021	1.93485	0.78892	18	0.669412
SPWTP	04-13-2021	1.920575	0.82338	10	0.834095
SPWTP	04-27-2021	1.17829	0.569254	7	0.605521
SPWTP	05-05-2021	1.938599	0.80832	13	0.755804
SPWTP	05-25-2021	1.527044	0.70151	12	0.614528
SPWTP	06-25-2021	0.636514	0.444444	2	0.918296
SPWTP	06-29-2021	1.859012	0.825283	8	0.893996
SPWTP	07-08-2021	1.42556	0.655263	12	0.573688
SPWTP	07-15-2021	1.817877	0.754758	11	0.758114
SPWTP	07-23-2021	0.858958	0.407949	12	0.34567
SPWTP	07-27-2021	1.400435	0.6326	13	0.545989
SPWTP	07-31-2021	1.595012	0.742486	18	0.551836
SPWTP	08-07-2021	1.283368	0.614406	22	0.415189
SPWTP	08-14-2021	1.224717	0.573188	18	0.423723
SPWTP	08-16-2021	1.064584	0.465645	19	0.361558
SPWTP	08-21-2021	1.563518	0.696507	18	0.54094
SPWTP	08-30-2021	1.400325	0.634428	16	0.505061
SPWTP	09-06-2021	2.252901	0.837571	28	0.676099
SPWTP	09-20-2021	1.844825	0.711553	30	0.542404
SPWTP	01-19-2022	1.850786	0.769902	13	0.721568
SPWTP	02-15-2022	1.2396	0.541944	13	0.483284
SPWTP	04-05-2022	2.34966	0.882161	14	0.890341
SPWTP	05-09-2022	1.916474	0.782094	20	0.639735
SPWTP	06-08-2022	1.271614	0.493543	14	0.481844

SPWTP	07-13-2022	1.414084	0.680511	14	0.535829
SPWTP	07-19-2022	1.99195	0.810448	22	0.644427
SPWTP	07-27-2022	1.721484	0.716876	17	0.607608
SPWTP	08-02-2022	1.850211	0.784117	23	0.590086
SPWTP	08-08-2022	1.873455	0.809575	20	0.625375
SPWTP	08-17-2022	1.343894	0.597429	19	0.456418
SPWTP	08-21-2022	1.204426	0.494994	25	0.374176
SPWTP	08-25-2022	1.762528	0.73676	16	0.635698
SPWTP	09-08-2022	1.716775	0.765916	30	0.504756
SPWTP	09-28-2022	0.888481	0.328094	23	0.283362
SPWTP	09-21-2022	2.102678	0.860639	16	0.758381
SPWTP	10-06-2022	1.927227	0.763159	22	0.623488
SPWTP	10-14-2022	1.882607	0.789764	18	0.651337
SPWTP	11-08-2022	1.052664	0.451433	18	0.364197
SPWTP	12-07-2022	1.97504	0.766935	20	0.659284
SPWTP	01-17-2023	2.205875	0.84514	17	0.778577
SPWTP	03-23-2023	1.298367	0.702111	5	0.806721
SPWTP	04-20-2023	0.651739	0.259696	8	0.31342
SPWTP	05-10-2023	0.575847	0.243772	7	0.295927
SPWTP	06-06-2023	1.624881	0.770153	7	0.835024
SPWTP	06-22-2023	1.281346	0.621267	7	0.658482
SPWTP	07-06-2023	1.918972	0.781775	13	0.748152
SPWTP	07-22-2023	1.726148	0.784743	10	0.749657
SPWTP	08-10-2023	1.569011	0.699218	21	0.515355
SPWTP	08-24-2023	1.54462	0.602855	27	0.468658
SPWTP	09-13-2023	1.621662	0.691159	18	0.561056
SPWTP	09-26-2023	1.951662	0.8093	20	0.651481
BRWTP	08-06-2021	1.3516	0.654975	22	0.437264
BRWTP	08-09-2021	1.016827	0.462527	20	0.339425
BRWTP	08-15-2021	1.773983	0.744648	23	0.565775
BRWTP	08-22-2021	0.562761	0.235815	20	0.187854
BRWTP	08-27-2021	1.829847	0.79331	17	0.645856
BRWTP	09-05-2021	1.865896	0.782778	25	0.579673
BRWTP	09-19-2021	2.119271	0.809367	29	0.629369
LTVCA	09-02-2020	0.740775	0.417294	17	0.261461
LTVCA	06-23-2021	2.239644	0.85299	18	0.774864
LTVCA	07-27-2021	1.63549	0.712435	14	0.619725
LTVCA	08-04-2021	2.413246	0.888675	21	0.792652
LTVCA	09-20-2021	2.116687	0.806104	27	0.64223
LTVCA	07-19-2022	1.864331	0.698982	20	0.622329
LTVCA	08-24-2022	2.831344	0.9204	36	0.790102
LTVCA	09-28-2022	2.054689	0.837581	16	0.741072
LTVCA	10-06-2022	2.156776	0.836163	22	0.69775
LTVCA	11-08-2022	1.769727	0.747443	19	0.601041
LTVCA	12-07-2022	1.528037	0.562146	27	0.463626

LTVCA	06-22-2023	1.967047	0.752919	24	0.618947
LTVCA	07-13-2023	1.470969	0.582401	25	0.456982
LTVCA	07-27-2023	2.620164	0.900041	26	0.804201
LTVCA	08-09-2023	2.32009	0.86803	29	0.689007
LTVCA	08-27-2023	2.475284	0.87477	32	0.714216
LTVCA	09-12-2023	2.114523	0.787725	18	0.731575
Lighthouse Cove	08-24-2022	1.180911	0.493252	37	0.327039
Lighthouse Cove	09-08-2022	2.251298	0.845553	26	0.690986
Lighthouse Cove	09-22-2022	2.015688	0.806971	19	0.684575
Lighthouse Cove	09-28-2022	1.604032	0.602725	16	0.578532
Lighthouse Cove	10-06-2022	2.277889	0.860432	27	0.691141
Lighthouse Cove	11-08-2022	1.976383	0.77981	21	0.64916
Lighthouse Cove	12-07-2022	1.286346	0.495981	19	0.436873
Lighthouse Cove	06-22-2023	2.236804	0.84867	23	0.713382
Lighthouse Cove	07-13-2023	2.268693	0.818163	28	0.680838
Lighthouse Cove	07-27-2023	2.379272	0.854152	27	0.721902
Lighthouse Cove	08-09-2023	2.235978	0.852862	24	0.703568
Lighthouse Cove	08-31-2023	2.245781	0.82306	29	0.666939
Lighthouse Cove	09-12-2023	2.297718	0.819478	31	0.669111
Prairie Siding	06-22-2023	2.33132	0.864162	32	0.672677
Prairie Siding	07-13-2023	1.664548	0.589258	32	0.480287
Prairie Siding	07-27-2023	2.595613	0.89441	28	0.778948
Prairie Siding	08-09-2023	2.704593	0.914024	27	0.820609
Prairie Siding	08-31-2023	2.368716	0.854406	25	0.735883
Prairie Siding	09-12-2023	2.230777	0.833826	25	0.69303

Appendix C (Chapter 4)

Table S10: Water Quality Parameters from Lake Victoria Catchment

Date	Site	Temp (°C)	Sp. Cond (µS/cm)	DO (%)	DO (mg/L)	TDS (mg/L)	Salinity (psu)
22-Jun-22	Sondu 1	20.53	284	83.5	6.16	150	0.14
22-Jun-22	Sondu 2	20.85	268	68.5	5.35	138	0.13
27-Jun-22	Sondu R Mouth	25.06	133	96	6.95	66	0.06
27-Jun-22	Nyando R Mouth	24.77	179	68.9	4.9	50	0
27-Jun-22	Kendu Bay	25.95	155	103.3	7.33	77	0.07
30-Jun-22	Mid-Gulf	24.94	146	88.9	6.34	73	0.07

Table S11: Nutrients, Microcystins and Chlorophyll *a* from Lake Victoria Sites

Date	Site	NO_3 (mg/L)	NH_3 (mg/L)	SRP (mg/L)	Microcystins (ppb)	Chlorophyll *a* (mg/m^3)
22-Jun-22	Sondu 1	0.040	0.030	0.035	0.033	BDL
22-Jun-22	Sondu 2	0.441	0.438	0.384	0.056	BDL
27-Jun-22	Sondu R Mouth	0.043	0.018	0.011	0.040	50.193
27-Jun-22	Nyando R Mouth	0.043	0.008	0.013	0.034	12.311
27-Jun-22	Kendu Bay	0.012	0.016	0.034	0.038	35.075
30-Jun-22	Mid-Gulf	0.032	0.009	0.044	0.109	16.308

*BDL = Below detectable level

Table S12: Nutrient Values from Microcosm Experiments in Sondu-Miriu River

Date	Treatment	NO3 mg/L	NH3 mg/L	SRP mg/L
22-Jun-22	Sondu 1 Time-Zero	0.040	0.030	0.035
25-Jun-22	Sondu-1-Control	0.042	0.031	0.021
25-Jun-22	Sondu-1-50%	0.026	0.034	0.045
25-Jun-22	Sondu-1-70%	0.025	0.030	0.027
22-Jun-22	Sondu 2 Time-Zero	0.441	0.438	0.384
25-Jun-22	Sondu 2 Control	0.037	0.035	0.036
25-Jun-22	Sondu 2 50%	0.211	0.222	0.070
25-Jun-22	Sondu 2 70%	0.206	0.206	0.125

Table S13: T-test Results for Nutrient Comparisons at Sondu Site 2

Comparison	NO_3	SRP
T-0 vs. Control	0.0001	0.0035
T-0 vs. 50%	0.0009	0.0085
T-0 vs. 70%	0.0015	0.0065
Control vs. 50%	0.0013	n.s.
Control vs. 70%	0.0012	0.0028
50% vs. 70%	n.s.	n.s.

*n.s. = not significant

VITA AUCTORIS

NAME:	Emily Varga
PLACE OF BIRTH:	Leamington, ON
YEAR OF BIRTH:	1982
EDUCATION:	Tilbury District High School, ON, OSSD, 2000
	Tilbury District High School, ON, OAC, 2001
	University of Windsor, Honours B.Sc., Windsor, ON, 2018