Investigating the Distribution of *Nitellopsis obtusa* in Ontario Lakes and its role as an Ecosystem Engineer

by

Tyler J. Harrow-Lyle

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Submitted by: **Tyler J. Harrow-Lyle**

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An oral defense of this thesis took place on August 16, 2021, in front of the following examining committee:

Examining Committee:

Chair of Examining Committee	Dr. Janice Strap
Research Supervisor	Dr. Andrea Kirkwood
Examining Committee Member	Dr. Robert Bailey
Examining Committee Member	Dr. Dario Bonetta
University Examiner	Dr. Ève Gilroy
External Examiner	Dr. Paul Frost, Trent University

The above committee determined that the thesis is acceptable in form and content and that a satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate during an oral examination. A signed copy of the Certificate of Approval is available from the School of Graduate and Postdoctoral Studies.

ABSTRACT

Nitellopsis obtusa is a non-native macrophyte introduced into the Laurentian Great Lakes in 1974. Over the last decade, increasing reports emerged for *N. obtusa* within lakes in the Great Lakes Basin. Despite *N. obtusa* being in North America since the 1970s, little is known about the ecosystem- and community-level impacts upon establishment. My thesis aimed to elucidate these potential effects by focusing on both within-lake and regional scale *N. obtusa* populations. I conducted a comprehensive investigation into ecosystem impacts of *N. obtusa* across 12 sites in Lake Scugog, Ontario, Canada, from spring to fall over four years (2016-2019). Upon *N. obtusa* establishment, diversity across lower aquatic food web (LAFW) communities significantly (p-value < 0.05, multiple linear regression) decreased. Despite community diversity decreased across the LAFW, *Microcystis* spp. blooms emerged where they were previously unreported. To assess drivers of bloom development, a structural equation model demonstrated that *N. obtusa* had a direct faciliatory (p-value < 0.05) role in *Microcystis* spp. blooms. In addition, *N. obtusa* biomass was a moderately strong negative predictor of near-bed oxygen concentration ($R^2 = 0.59$, p-value < 0.001), which likely promoted internal loading of phosphorus. Supporting this conclusion was a strong predictive model showing that *N. obtusa* biomass explained 90% of pore water soluble reactive phosphorus concentrations ($R^2 = 0.90$, p-value < 0.001). During a narrow sampling window (3 weeks) in 2019, I sampled 60 lakes across a geological transition zone in Ontario, Canada. Calcium is for *N. obtusa*, thus a strong response related to calcium availability was expected. *Nitellopsis obtusa* was documented at 37 sites in 19 of the study lakes. Interestingly, an ecological niche model demonstrated that depth and the cations potassium, magnesium, and sodium were better predictors of *N. obtusa* presence than calcium. Strong positive associations between *N. obtusa* and native Characeae members were revealed through general linear latent variable modelling, in addition to strong negative associations with other macrophyte taxa such as *Myriophyllum spicatum*. In combination, these findings indicate that *N. obtusa* may be an ecosystem engineer in invaded lakes by affecting key lake elements and altering community composition throughout the LAFW community.

Keywords: *Nitellopsis obtusa*; Invasive species; Ecological niche model; Internal loading; Ecosystem Engineer.

AUTHOR'S DECLARATION

I hereby declare that this thesis consists of original work of which I have authored. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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STATEMENT OF CONTRIBUTIONS

The work described in Chapter 2 has been submitted to the journal Freshwater Biology as a research article: **FWB-P-Jun-21-0384**. Work described in Chapter 2 was performed in partnership with the Scugog Lake Stewards. In addition, a portion of this chapter was used in a technical report prepared for the Scugog Lake Stewards: **Harrow-Lyle, Tyler J and Kirkwood, A. (2020). *An assessment of Lake Scugog offshore water quality and ecological condition (2017-2019).*** I was responsible for collecting field samples, conducting laboratory analyses, curating, and analyzing data.

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LIST OF ABBREVIATIONS AND SYMBOLS

%	Percent
°	Degrees
α	Alpha
µg	Micrograms
µm	Micrometers
µS	Micro Siemens
ANOVA	Analysis of variance
AUC	Area under the curve
BF	Bag Fraction
BRT	Boosted regression tree
C	Celsius
Ca	Calcium
CD	*Ceratophyllum demersum*
CFI	Comparative fit index
CHL a	Chlorophyll α
Cl	Chloride
cm	Centimeters
Con	Conductivity
CV	Cross validation
CV	*Chara vulgaris*
DFBL	Distance from boat launch

DO	Dissolved oxygen
EC	*Elodea canadensis*
Fe	Iron
g	Gravity
GIS	Geographic information systems
GLLVM	General linear latent variable model
glmm	General linear mixed model
K	Potassium
km	Kilometer
L	Litres
LAFW	Lower aquatic food web
LM	*Limna minor*
LR	Learning rate
m	Meter
MB	*Myriophyllum sibiricum*
Mg	Magnesium
mL	Millilitres
ML	Maximum likelihood
mm	Millimeters
Mn	Manganese
MS	*Myriophyllum spicatum*
MV	*Myriophyllum verticulatum*
N	Nitrogen
N:P	Nitrogen to phosphorus
Na	Sodium
NF	*Najas flexilis*
NH3	Ammonia
NH4	Ammonium
NL	*Nuphar lutea*
nm	Nanometer
NO	*Nitellopsis obtusa*
NO2	Nitrite
NO3	Nitrate
P	Phosphorus
PA	*Potamogeton amplifolius*
PCA	Principal component analysis
permanova	Permutational analysis of variance
PF	*Potamogeton foliosus*
PN	*Potamogeton natans*
PP	*Potamogeton pusilus*
PZ	*Potamogeton zosteriformis*
R^2	Coefficient of determination
RDA	Redundancy analysis

RMSEA	Root mean square error of approximation
rpm	Revolutions per minute
SEM	Structural equation model
Spp.	Species
SRMR	Standardized root mean square residual
SRP	Soluble reactive phosphorus
SSRP	Sediment soluble reactive phosphorus
TC	Tree complexity
Temp	temperature
TI	*Tolypella intricata*
TN	Total nitrogen
TOC	total organic carbon
TP	total phosphorus
TSP	total soluble phosphorus
TSW	Trent Severn waterway
Tur	turbidity
UV	*Utricularia vulgaris*
VA	*Vallisneria americana*
x	Times

Chapter 1. General Introduction

1.1 Introduction

Freshwater is essential for supporting life on Earth. However, only two and a half percent of Earth's water is considered fresh water. Canada holds 20% of the world's freshwater resources in a variety of ecosystems including lakes, streams, rivers, and wetlands. In Ontario alone, there are 250,000 lakes, which are under increasing threat every day. Threats range from climate change, habitat loss, pollution, to invasive species.

Organisms that are transported to habitats previously not occupied, often through anthropogenic vectors, and establish self-sustaining populations are described as invasive species. Invasive species are known to negatively impact ecosystems throughout the world (Schultz & Dibble, 2012), but are often difficult to manage to diminish effects on natural ecosystems and biodiversity. Potential effects can range from untraceable to dramatic, affecting multiple levels of organization given the invasive species of interest (Mack et al., 2000). Taxa specific interactions, both native and non-native, can be disrupted upon introduction of a new non-native species which may trigger trophic cascades (Simon & Townsend, 2003), resulting in dramatic shifts of food web dynamics. Additionally, introduction of non-native species can also result in ecosystem-level consequences, often affecting the movement of energy and nutrients within a given habitat (Simon & Townsend, 2003).

Understanding the biology of new invaders can provide a comprehensive approach for identifying management strategies and mitigating impacts on natural systems. *Nitellopsis obtusa* is a relatively new non-native species to Ontario lakes. This thesis aims to elucidate the invasion biology and associated effects of *N. obtusa* by focusing on both within-lake and regional scale populations.

1.2 *Nitellopsis obtusa*

1.2.1 Taxonomic Classification

Nitellopsis obtusa is the only extant member of the genus *Nitellopsis* and belongs to the Characeae family. Characeae are composed of several groups of green (Chlorophyceae) algae, which share a common ancestor with land plants (Graham, 1993). Characeae are referred to as stoneworts or brittleworts that include 400 species worldwide (Blaženčić, Stevanović, Blaženčić, & Stevanović, 2006). Unfortunately, over 80% of these taxa are now considered endangered due to climate change and anthropogenic stressors (Blaženčić et al., 2006).

Traditionally, *N. obtusa* had been classified under several different genera. For instance, Deslongchamps initially categorized *N. obtusa* as a member of the genus *Chara* in 1810. Thereafter, *N. obtusa* was classified as a member of four different genera including *Lychnothamnus*, *Tolypellosis*, *Nitella*, and *Nitellopsis* (Larkin et al., 2018). Due to inconsistent grouping, the subtribe *Nitellopsinae* was recommended, which joined the remaining genera from the subtribe *Charinae* (Wood, 1962). Historically, there were 16 designated species within the genus *Nitellopsis*, however, upon review many of these species were moved to other genera apart of Characeae (Wood, 1962). The genus *Nitellopsis* remained distinct, consisting of *Nitellopsis obtusa* and *Nitellopsis sarcularis*, as members are ecorticate, with slim bract cells separate from the branching node (Wood, 1962).

1.2.2 Native Distribution and Status

The native distribution of *N. obtusa* extends from Britain through most of Europe and Asia, including Japan. Across most of its native range, *N. obtusa* is classified as threatened or endangered (Table 1.1). Nevertheless, due to climate change, new populations of *N. obtusa* are being found within the native range, leading to the re-examination of conservation status within many countries (Larkin et al., 2018). Most notably, new populations of *N. obtusa* were reported in the Jura Mountains of Switzerland and France (Bailly, Ferrez, Guyonneau, & Schaefer, 2007). In addition, new populations were identified in eastern and southern France (Bailly & Schäfer, 2010; Mouronval et al., 2015). Similarly, *N. obtusa* populations have been found to reside in newly developed floodplains within Germany, and large eutrophic lakes in Switzerland (Rey-Boissezon & Auderset Joye, 2015; Larkin et al., 2018). New populations of *N. obtusa* have also been discovered in Japan, where it was previously believed extinct (Kato et al., 2014).

Table 1.1. Conservation status of *Nitellopsis obtusa* across its native range.

Location	Conservation Status	Reference
Switzerland	Near Threatened	Auderset Joye & Schwarzer, 2012
Germany	Vulnerable, Critically Endangered	Hamann & Garniel, 2002; Kabus & Mauersberger, 2011; Korsch, Doege, Raabe, & van de Weyer, 2013
Eastern Europe	Regionally Extinct	Blaženčić et al., 2006; Caisová & Gabka, 2009
Nordic Countries	Vulnerable	Johansson et al., 2010
Britain	Critically Endangered	United Kingdom Legislation, 1981
Japan	Critically Endangered	Kato et al., 2014

1.2.3 Morphology

Depending on environmental conditions, *N. obtusa* can appear bright green to yellowish-brown with slender thalli. This macroalga is a dioecious species, but is known for its conspicuous white star-shaped bulbils, which serve as asexual reproduction structures (Figure 1.1) (Bharathan, 1987). The bulbils arise from the rhizoid nodes and monofilaments, which are typically suspended above the lake bottom (Figure 1.1). Identification based only on the presence of these characteristic bulbils can prove difficult, as bulbils are only found within mature, established beds (Bonis & Grillas, 2002).

When bulbils are lacking, *N. obtusa* can be identified by its tangled mass appearance. Thalli can be encrusted in a thick marl, composed of calcium bicarbonate and phosphorus

(Blindow, 1992). Although representatives of the Characeae family produce a characteristic sulfurous or musky smell, *N. obtusa* does not exude a prominent odour. Branchlets can grow up to nine cm in length, and be arranged five to eight per whorl in two to three segments (Karol & Sleith, 2017). Each node can form solitary or paired gametangia. Despite oogonia not being observed within North America, they can vary in colour from light green-red, and appear almost spherical with the presence of a five-celled coronula (Larkin et al., 2018). Antheridia that have matured can be orange to bright red (Figure 1.1).

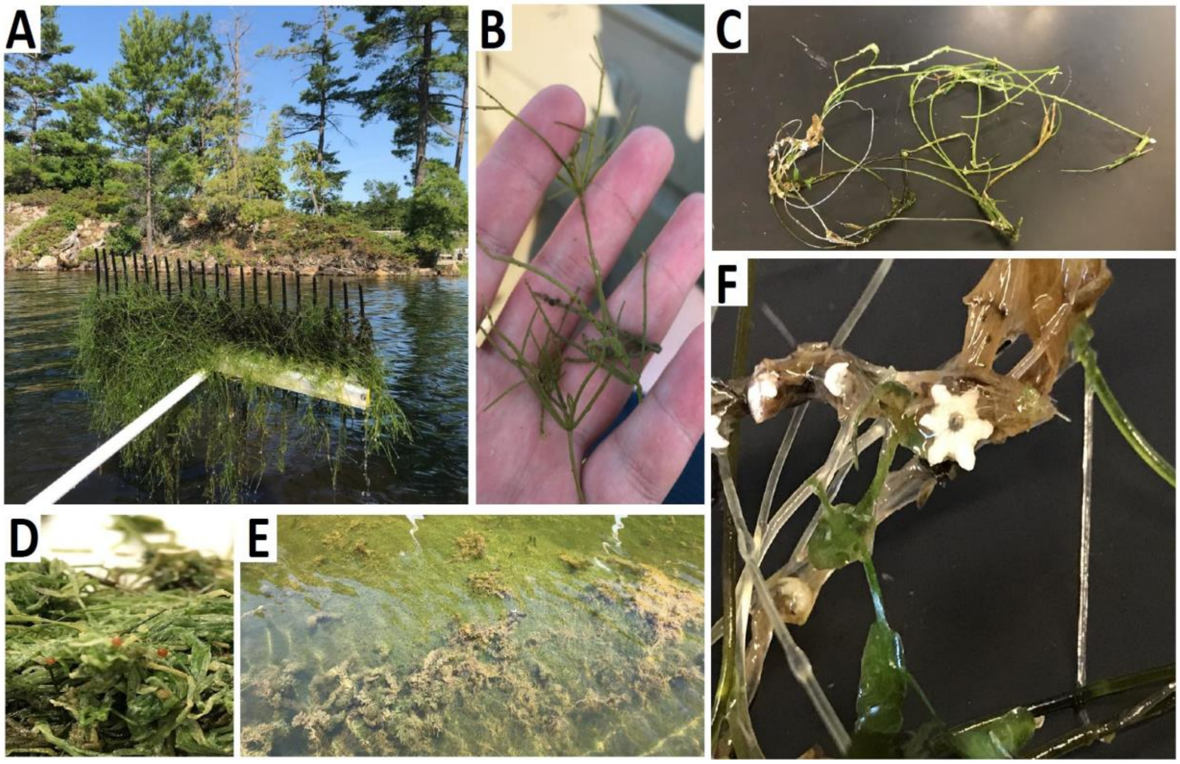


Figure 1.1. *General field and morphological observations for* N. obtusa throughout this research. ***A.*** *Depicts a lake-rake collection of a monoculture stand of* N. obtusa. ***B.*** *Classic rough calcareous branch morphology observed* ***C.*** *Typical form of* N. obtusa *after washing with reverse osmosis water. Note the monofilament and presence of bulbils.* ***D.*** *Image of the male antheridia present on the samples collected throughout the study.* ***E.*** *High growth characteristic of* N. obtusa¸ *where these dense benthic mats grew from the sediment water interface into surface waters.* ***F.*** *Close-up image of the white star-shaped bulbils present on a monofilament node. Image was taken prior to rinsing off detritus.*

1.2.4 Reproduction Biology and Dispersal

Members of the Characeae family undergo both asexual and sexual reproduction. Within *N. obtusa*’s native range, reproduction occurs primarily through vegetative fragments, consisting of calcified asexual and sexual components deposited within the sediments, referred to as propagules (Migula, 1897). Sediments can host transient propagule banks that reproduce within one year of deposition (Thompson & Grime, 1979), or persistent propagule banks that can last up to 89 years and possibly more (Bonis & Grillas, 2002). Despite the ability to persist within sediments for extended periods of time, propagules must remain within the surface sediments or the potential to germinate is usually lost (Bonis & Grillas, 2002). This is likely due to the requirement of light and anoxia at the sediments, which are essential for propagule germination (Bonis & Grillas, 2002).

In contrast, a shift in reproduction has been observed when *N. obtusa* colonizes shallow environments (Krause, 1985). The development of gametangia and fertility within such environments has been linked to warmer water temperatures, and sunny growing seasons (Willén, 1960; Boissezon, Auderset Joye, & Garcia, 2018). However, despite sexual reproduction being beneficial within shallow environments, low fertility has been reported as early as the 1800s (Migula, 1897).

Initial reports suggest both male and female specimens of *N. obtusa* were present within North America, however, examination of suspected oogonia revealed that they were male antheridium (Sleith, Havens, Stewart, & Karol, 2015). Currently, only specimens that do not produce sexual organs, or male individuals, have been documented in North America (Mann, Proctor, & Taylor, 1999; Sleith et al., 2015). Normally, male specimens have been recognized to emerge first, while females arise later in the growing season (Boissezon et al., 2018). Therefore, it is postulated that there are no female specimens of *N. obtusa* in North America due to unfavourable environmental conditions or protandry (Larkin et al., 2018). Populations of *N. obtusa* in North America appear to be a result of clonal reproduction and dispersal thus far.

Within the native range, propagules have been known to be dispersed through epizoochory and endozoochory (Bonis & Grillas, 2002). However, there is mounting evidence that the majority of *N. obtusa* dispersion within North America is due to the movement of watercraft. Midwood et al., (2016) demonstrated that *N. obtusa* was found in areas with higher dock densities. Clumps of *N. obtusa*, which are often found on watercraft and trailers, remain viable for clonal reproduction after several days (Glisson et al., 2019). Furthermore, research from invaded regions within the United States demonstrate that *N. obtusa* populations are not found in isolated lakes (Sleith et al., 2015).

1.3 *Nitellopsis obtusa* Invasion History

The means by which *N. obtusa* was introduced into North America is not known, however, it has been hypothesized that unintentional introduction occurred via the ornamental gardening trade and trans-oceanic shipping (Kay & Hoyle, 2001; Padilla & Williams, 2004). The first record of *N. obtusa* within North America comes from a specimen catalogued at the New York Botanical Garden, New York, USA. This specimen was collected from the St. Lawrence River near Montreal, Quebec, in 1974, but was first misidentified as a *Chara* spp. (Karol & Sleith, 2017). In 1978, *N. obtusa* was documented in New York State, along the St. Lawrence (Geis, Schumacher, Raynal, & Hyduke, 1981), and presence was confirmed within the St. Clair-Detroit River system in 1983 (Schloesser, Hudson, & Nichols, 1986; Griffiths, Thornley, & Edsall, 1991).

By 2005, *N. obtusa* had successfully moved into inland lakes of New York State, USA (Sleith et al., 2015). Meanwhile, from 2012 – 2015, *N. obtusa* was discovered in Pennsylvania, Indiana, Michigan, Wisconsin, Minnesota, and Vermont inland lakes (Larkin et al., 2018). In Ontario, *N. obtusa* was found in Lake Simcoe as early as 2008 (Ginn, Dias, & Fleischaker, 2021), and within Presqu'ile Bay of Lake Ontario since 2015 (Midwood, Darwin, Ho, Rokitnicki-Wojcik, & Grabas, 2016). Currently, there are confirmed reports of *N. obtusa* in seven states and two provinces (Larkin et al., 2018).

With increasing awareness of this new invasive macrophyte, anecdotal reports of *N. obtusa* began to surge over the last decade across the southern Great Lakes Basin in North America. Currently, reports from Ontario include Sturgeon, Pigeon, Upper Buckhorn, Lower Buckhorn, Ston(e)y, and Big Cedar Lake (Kawartha Lake Stewards Association per comms.). Several reports are now suggesting *N. obtusa* has become prolific throughout most of the Trent-Severn waterway and Kawartha Lakes. However, these occurrences require investigation, as *N. obtusa* is frequently misidentified (Larkin et al., 2018).

1.4 Ecology and Growth Characteristics of *Nitellopsis obtusa*

1.4.1 Native Range

Nitellopsis obtusa is found in a variety of habitats within its native range, including channels, rivers, gravel pits, estuaries, and lakes. Generally, Characeae have been known to colonize a variety of depths upwards of 65 m (Spence, 1982; Kufel & Kufel, 2002). The ability to colonize such depths has been attributed to Characeae's high degree of shade tolerance, and the ability to regulate buoyancy while growing towards light sources (Kufel & Kufel, 2002). Despite the ability to withstand shaded environments, light is a critical component for Characeae establishment (Kufel & Kufel, 2002). As such, Characeae, and *N. obtusa*, are frequently found within shallow environments ranging from 0.5 – 1.5 m (Janauer, Schmidt-Mumm, & Schmidt, 2010). With a high affinity for cold water, as well as the capability to survive over winter (Boissezon et al., 2018), *N. obtusa* has been classified as a boreal taxon (Corillion, 1957).

For *N. obtusa* to become dominant within a given habitat, hard water is required. Stroede (1937) stated the minimum concentration of calcium necessary for *N. obtusa* establishment in an environment was between 15 and 25 $mg \cdot L^{-1}$. Calcium cations of hardwater lakes are generally accompanied by bicarbonate anions, which are the main source of inorganic carbon for most macrophytes (Kufel & Kufel, 2002). Characeae have a better affinity for bicarbonate compared to vascular macrophytes, with bicarbonate being exhausted within dense charophyte meadows (Hutchinson, 1975).

Initially, elevated phosphorus was thought to be toxic to members of Characeae (Forsberg, 1964; Hough & Putt, 1988), however, Blindow (1992) documented that Characeae are able to withstand soluble reactive phosphorus (SRP) concentrations upwards of 370 $mg\ P\ m^{-3}$. Interestingly, *N. obtusa* has been used as an indicator species for oligotrophic to mesotrophic systems in the past (Kasaki, 1962). Nevertheless, *N. obtusa* has also been found in mesotrophic to eutrophic ecosystems, without a decline of biomass (Bailly et al., 2007; Hutorowicz & Dziedzic, 2008). As such, *N. obtusa* is now recognized as a nutrient tolerant species.

Macrophytes are an integral part of aquatic ecosystems, providing habitat to several other aquatic communities. Characeae have been regarded as important components to shallow lake dynamics (Kufel & Ozimek, 1994; Van Den Berg, Scheffer, Coops, & Simons, 1998; Christensen, Sand-Jensen, & Staehr, 2013). Generally, Characeae are known to prevent internal loading by stabilizing the sediments, and increasing water clarity (Søndergaard et al., 1990). Usually, internal loading results in phosphorus being released from the sediments under hypoxic-anoxic conditions.

Characeae modify nutrient cycles in several ways. Essential nutrients such as phosphorus and nitrogen are taken up and incorporated into Characeae biomass, which limits available nutrients for other organisms (Kufel & Kufel, 2002). Generally, Characeae decompose more slowly than vascular macrophytes, which increases retention time of sequestered nutrients (Kufel & Kufel, 2002). When Characeae precipitate the marl encrustation, phosphorus is co-precipitated into the mixture, further removing available phosphorus from other organisms (Otsuki & Wetzel, 1972; Murphy, Hall, & Yesaki, 1983).

1.4.2 Invaded Regions

Within invaded regions, *N. obtusa* has been found in several different habitats ranging from Great Lakes coastal wetlands (Midwood et al., 2016), to inland lakes (Sleith et al., 2015; Ginn et al., 2021). *Nitellopsis obtusa* populations have been reported at a variety of depths extending from shallow littoral environments (< 1 m) to depths of 7 m within occupied lakes (Geis et al., 1981; Pullman & Crawford, 2010; Sleith et al., 2015; Ginn et al., 2021). Pullman & Crawford (2010) have suggested that *N. obtusa* preferentially takes over shallow habitats within invaded ecosystems, and only when there is no further available space, will the taxa begin invading the deeper reaches of a lake.

Generally, *N. obtusa* also tends to avoid growing in boat lanes, until all other habitable area is crowded (Pullman & Crawford, 2010), as macrophyte stands in these areas are prone to disturbance and removal. Due to the high shade tolerance of Characeae, no preference for clearwater systems within invaded regions has been observed (Pullman & Crawford, 2010). *Nitellopsis obtusa* is found within mesotrophic to eutrophic hard water systems, with calcium concentrations going as high as $190 \text{ mg} \cdot \text{L}^{-1}$ (Pullman & Crawford, 2010; Larkin et al., 2018).

The overall ecological threat that *N. obtusa* presents in freshwater ecosystems of North America is similar to other non-native invasive macrophytes (Pullman & Crawford, 2010; Hackett, Caron, & Monfils, 2014; Brainard & Schulz, 2017). Pullman & Crawford (2010) have stated that *N. obtusa* is the most aggressive macrophyte ever observed within Michigan State, USA, including all invasive species such as *Myriophyllum spicatum*, *Potamogeton crispus*, and *Cambomba caroliniana*. The invasion biology of *N. obtusa* in Ontario, and other parts of North America, remains largely unknown.

Nitellopsis obtusa has been suggested to modify sediment dynamics after successful establishment (Pullman & Crawford, 2010). Dense mats of *N. obtusa* are thought to create a layer of anoxia at the sediments, which would facilitate internal loading and the release of bioavailable nutrients from the sediments (Pullman & Crawford, 2010). Consequently, anecdotal evidence also suggests cyanobacterial blooms increase in occurrence within invaded lakes post *N. obtusa* establishment (Pullman & Crawford, 2010). If *N. obtusa* were to facilitate benthic anoxia, negative impacts on macroinvertebrate and fish communities would be expected (Caraco & Cole, 2002; Murphy, Schmieder, Baastrup-Spohr, Pedersen, & Sand-Jensen, 2018).

The structural complexity of macrophyte communities are essential for ensuring habitat heterogeneity, that in turn supports diverse aquatic systems. Characeae are rapid colonizers as they are superior competitors over vascular macrophytes for space and nutrients (Blindow, 1992; Van Den Berg et al., 1998). Naturally, *N. obtusa* has been observed to grow taller than other Characeae species, growing into surface waters, while forming thick benthic mats (Figure 1.1) (Larkin et al., 2018). Characeae can reduce phytoplankton density by competing for available space, taking available nutrients into their biomass and marl precipitate, and produce allelopathic substances to inhibit growth (Blindow, 1992; Berger & Schagerl, 2004).

Competing macrophytes have been known to decline upon *N. obtusa* invasion (Pullman & Crawford, 2010; Brainard & Schulz, 2017; Ginn et al., 2021). However, some macrophytes such as *Utricularia vulgaris* and *Ceratophyllum demersum*, have been observed to reach nuisance levels within *N. obtusa* beds (Pullman & Crawford, 2010). Regardless, negative impacts on fish spawning habitat are probable due to the formation of a physical barrier, reducing nesting area, and spawning activities (Pullman & Crawford, 2010).

Despite extensive discussion emphasizing proposed impacts of *N. obtusa* invasion on aquatic communities and ecosystem processes, very little quantitative information is available. Most of the information available for community impacts remains anecdotal. For instance, even with *N. obtusa* being labeled as the most aggressive macrophyte introduced into North America, only 2 peer-reviewed studies have reported effects exerted on macrophyte communities across invaded regions (Brainard & Schulz, 2017; Ginn et al., 2021). No studies have investigated the suggested effects to ecosystem services, including hypoxia-anoxia at the sediment water interface and internal loading. Furthermore, the full extent of *N. obtusa* distribution across Ontario and Canada remains unknown. In Ontario, only 2 confirmed populations have been reported within Lake Simcoe and Presqu'ile Bay, yet there are increasing reports emerging for inland lakes in proximity and within the Trent Severn Waterway. With Characeae altering their appearance based on environmental conditions, and Ontario's heterogenous landscape, skilled investigation and confirmation of reports is essential.

1.5 Research Objectives

The overall goals of my thesis research were to (1) document the distribution of *N. obtusa* in south-central Ontario lakes to determine the scope of invasion, as well as (2) assess the potential role of *N. obtusa* as an ecosystem engineer in invaded lakes. An ecosystem engineer is defined as an organism that has direct or indirect effects on ecosystem processes, causing significant habitat modification or destruction. My thesis research aimed to achieve these goals by focusing on both within-lake and regional scale populations of *N. obtusa*. Lake Scugog, a headwater reservoir, was selected as a sentinel lake study-system because of confirmed reports of *N. obtusa* beginning in 2015. This provided an ideal opportunity to intensively study the intra-lake distribution and dynamics of *N. obtusa* over space (12 sites) and time (annual and seasonal). The regional scale study involved a synoptic survey of 60 lakes with notable shifts in calcium availability across a geological gradient. Cations such as calcium are important in supporting the growth of all macrophyte species, but certain Characeae species are especially dependent on adequate concentrations being available.

Several lines of investigation were considered necessary to provide evidence that *N. obtusa* is an ecosystem engineer, including data from the sentinel lake study and the regional 60 lake study. Below are my thesis research objectives, which are primarily aimed at collecting and analyzing field data to determine *N. obtusa*'s role as an ecosystem engineer:

1. Evaluate the impact of *N. obtusa* invasion on the lower aquatic food web and habitat condition in Lake Scugog;
2. Determine the role of *N. obtusa* in emerging *Microcystis* spp. blooms in Lake Scugog;
3. Map the distribution of *N. obtusa* in south-central Ontario lakes, and identify regional habitat constraints; and
4. Distinguish the role of spatial and environmental factors on *N. obtusa*'s influence on macrophyte communities.

These objectives provide a unique opportunity to answer critical questions about the invasion and impact of *N. obtusa* as a non-native species within Ontario such as:

1. How prevalent is *N. obtusa* across a representative sample of Ontario lakes?
2. What are the physical and chemical habitat characteristics that promote and constrain *N. obtusa* dominance in Ontario lakes?
3. How is *N. obtusa* disrupting ecosystem cycles and lower aquatic food web communities?
4. Is *N. obtusa* an ecosystem engineer in Ontario lakes?

To visualize the central role *N. obtusa* may have as an ecosystem engineer in lakes where it has successfully invaded in Ontario, I developed a conceptual model (Figure 1.2). To be considered an ecosystem engineer, *N. obtusa* would need to significantly alter ecosystem processes, such as sediment biogeochemistry, and modify habitat resulting in biotic assemblage changes. I propose that *N. obtusa* will impact two key lake-ecosystem compartments: sediments and the aquatic food web (Figure 1.2).

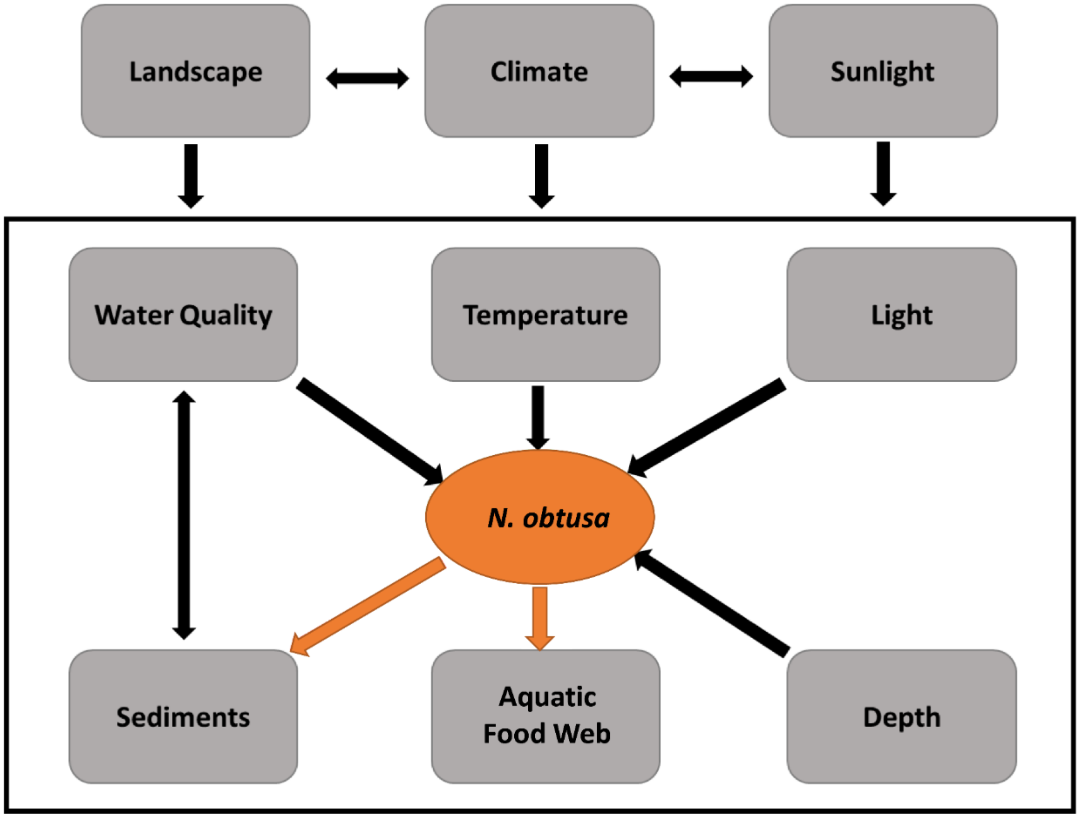


Figure 1.2. *Proposed conceptual model for the role of* N. obtusa *as an ecosystem engineer in Ontario lakes. Direction of arrows reflect the direct influence of a model compartment on another compartment, two-sided arrows indicate dual interactions between model compartments. The black box is representative of an ecosystem scale.*

By exploring the invasion biology of *N. obtusa* in Ontario lakes, the subsequent chapters aim to address the previously identified research gaps. Chapter 2 investigates the lower aquatic food web (LAFW) community dynamics of Lake Scugog, in response to *N. obtusa* establishment. Chapter 3 reveals how *N. obtusa* is altering the biogeochemical cycles of oxygen and phosphorus at the sediments, a key lake ecosystem compartment. Chapter 4 considers the emergence of *Microcystis* spp. blooms within Lake Scugog in response to *N. obtusa* invasion. Chapter 5 determines the distribution of *N. obtusa* within Ontario lakes, and the habitat preferences associated with *N. obtusa*'s invasion. Finally, Chapter 6 focuses on the effect of *N. obtusa* on macrophyte communities across Ontario.

Chapter 2. Evaluating changes to the lower aquatic food web following *Nitellopsis obtusa* establishment in Lake Scugog

2.1 Introduction

The spread and impact of invasive species is a global issue (Schultz & Dibble, 2012). Lake Scugog, a large, shallow lake in Southern Ontario, Canada has had its share of invasive species introductions due to its connection to the expansive Trent Severn Waterway, which links lakes Huron and Ontario in the Great Lakes Basin. Though Lake Scugog has established populations of well-known non-native invaders such as *Myriophyllum spicatum* (Eurasian watermilfoil) and *Dreissena polymorpha* (zebra mussel), the lake has generally been considered productive, supporting a popular sport-fishing industry for many decades (Kawartha Conservation, 2010). In recent years, however, *Nitellopsis obtusa* (starry stonewort), a non-native species, has become established in the lake, coinciding with observed changes in lake water quality and algal bloom eruptions (Chapter 4).

A member of the Characeae family, *N. obtusa* is classified as threatened through critically endangered in parts of its native range in Eurasia (Blaženčić et al., 2006; Auderset Joye & Schwarzer, 2012; Korsch et al., 2013; Kato et al., 2014). The first report of *N. obtusa* in North America was in Quebec, Canada, where it was found in the St. Lawrence River in 1974 (Karol and Sleith, 2017). Over the intervening 40 years, *N. obtusa* seemed to be relegated only to embayment’s throughout the lower Laurentian Great Lakes, but this is now thought to have been an artifact of under-reporting due to misidentification (Larkin et al., 2018). Over the last decade, there has been increased reports of *N. obtusa* establishment in dozens of inland lakes throughout the southern portion of the Great Lakes Basin (Midwood, Darwin, Ho, Rokitnicki-Wojcik, & Grabas, 2016; Larkin et al., 2018; Ginn, Dias, & Fleischaker, 2021).

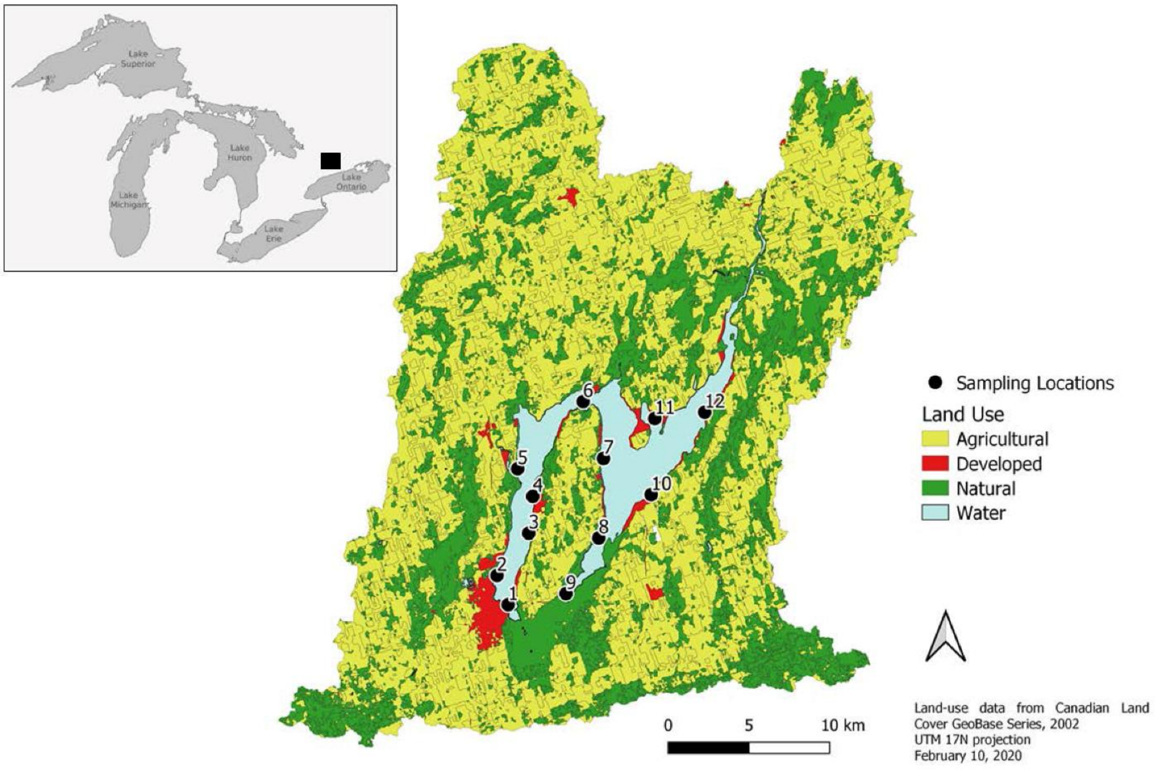
Despite emerging reports of expansion and establishment in North American lakes, there have been very few studies evaluating the impact of *N. obtusa* on lake communities, particularly from the point of early establishment to dominance in the macrophyte community. Some authors have suggested that *N. obtusa* could have negative effects on communities (phytoplankton, macroinvertebrates, and macrophytes) throughout the lower aquatic food web (Pullman & Crawford, 2010; Larkin et al., 2018). These suppositions are based on observations of lower macroinvertebrate and fish abundance in or near macrophyte beds dominated by *N. obtusa*. Of the few quantitative studies performed, Brainard & Schulz (2017) documented negative effects of *N. obtusa* on macrophyte richness in four lakes in New York. Similarly, Ginn et al., (2021) confirmed that macrophyte communities in Lake Simcoe, Ontario shifted in response to *N. obtusa* invasion. However, the broad-scale effects of *N. obtusa* after establishment on the LAFW community have not been evaluated.

To understand the effects of *N. obtusa* establishment and emerging dominance on LAFW community structure, I conducted a four-year study (2016-2019) in Lake Scugog that concurrently surveyed phytoplankton, macroinvertebrates, and the macrophyte communities at 12 sites distributed throughout the lake. The goal of this study was to evaluate spatial and temporal variation in LAFW community structure as a function of *N. obtusa* abundance. Additionally, I wanted to distinguish the influence of abiotic environmental parameters from species co-occurrence as drivers of LAFW structure. By assessing a suite of latent variables, I was able to elucidate the key role that *N. obtusa* had in changing LAFW community composition and structure over the course of the study period.

2.2 Methods

2.2.1 Study Location

Lake Scugog is a large (68 km^2), but shallow (mean depth = 1.4 m) hardwater lake located near Port Perry in southern Ontario, Canada. The land-use in Lake Scugog's catchment is largely agricultural, with some natural cover and urban development (Figure 2.1). Twelve sampling sites were selected within the pelagic zone of Lake Scugog, representing critical fish spawning locations. Additionally, sites were selected to cover the two lake basins equally, but also reflect continuous accessibility. Only sites 5 and 9 were inaccessible by boat once each year (September) due to lower water levels and dense macrophyte stands. Field sampling occurred monthly from May-September over four years (n = 232, 2016-2019).



Land-use data from Canadian Land Cover GeoBase Series, 2002
UTM 17N projection
February 10, 2020

***Figure 2.1**. Map of Lake Scugog with delineated watershed and land-use features. Sampling site locations are numbered 1-12. Map and land-use delineation was performed in QGIS version 3.12.0.*

2.2.2 Field Measurements and Sample Collection

Depth was measured using a Hawkeye handheld depth finder (DT1H, Hawkeye Electronics, Stuart, Florida, USA), while Secchi depth was determined with a standard Secchi disc at each site. Dissolved oxygen ($mg\cdot L^{-1}$), temperature (°C), conductivity ($\mu S\cdot cm^{-1}$), and pH were measured by a YSI 6 series multiparameter sonde at 0.5 m (YSI Inc., Yellow Springs, Ohio, USA). Water was collected at 0.5 m using a 4 L Van Dorn sampler. Lake water was transferred into 3 1 L Nalgene bottles and a 120 mL amber borosilicate bottle. The Nalgene bottles were detergent-washed using phosphate-free detergent and acid-washed, rinsing with Milli-Q filtered water to remove any residual acid prior to collection. Following collection, the Nalgene bottles were placed in a cooler with ice, and ~10-20 drops of Lugol's solution were added to the amber borosilicate bottle to preserve the phytoplankton community. Aquatic macrophyte samples were collected using the lake-rake method of Ginn (2011), consisting of three rake tosses extending to the lakebed. This method was determined to have easier application and comparable results with ponar-based methods (Ginn, 2011). Snorkeling or pulling macrophytes from quadrats in a large lake such as Scugog was not a practical approach for this study. All material captured on the rake (macroinvertebrates and macrophytes) were collected in Ziplock bags and placed in a cooler.

2.2.3 Sample Processing

Water and biological samples were transported back to the laboratory on the day of collection and stored in a walk-in refrigerator overnight. All samples were processed within 24 hours of collection. Each of the 1-L Nalgene bottles served as a technical replicate in determining chloride and chlorophyll α measurements. Chloride concentrations ($mg \cdot L^{-1}$) were determined using an Orion ion-selective electrode (ThermoFisher Scientific, Waltham, Massachusetts, USA). Total phosphorus concentrations ($\mu g \cdot L^{-1}$) were determined following a modified ascorbic acid method of Murphy & Riley (1962) developed by the Ontario Ministry of Environment (1983). Total nitrogen samples were sent to an accredited analytical laboratory (SGS Canada, Lakefield, Ontario) for processing and measurement. Lastly, 300 mL of each technical replicate was filtered through 47 mm GF/A glass fibre filters to collect seston and then extract chlorophyll a using 90 % acetone (Kirkwood, Chow-Fraser, & Mierle, 1999).

Phytoplankton genera were quantified in a 0.98 mL nanoplankton settling chamber using an EVOS XL core phase-contrast microscope (ThermoFisher) at 400 x magnification. Identification to genus and species (when possible) was performed following Prescott (1962), and Wehr et al., (2015). Macroinvertebrate taxa were carefully removed from macrophyte masses and rinsed in reverse osmosis water, then preserved in 70 % ethanol. Identification to species, where possible, was completed following Merrit et al., (1996) and Paulson (2011). Macrophytes were rinsed in reverse osmosis water, sorted, dried in a convection oven at 80 °C and weighed to determine relative abundance from dried biomass (Carr, Bod, Duthie, & Taylor, 2003). Macrophyte taxa were identified using a QZE stereo microscope (Walter Products, Windsor, Ontario) following Charophytes of North America (Wood, 1962), the common aquatic plants of Michigan guide (https://www.michigan.gov/documents/deq/wrd-anc-CommonPlant_408518_7.pdf), and status and strategy for Starry Stonewort (*Nitellopsis obtusa* (Desv. In Loisel.) J. Groves) management (Hackett et al., 2014).

2.2.4 Statistical Analyses

Statistical analyses were conducted in the open-sourced software platform R version 4.0.3 (R Core Team, 2019). To ensure figures were accessible for colour blindness, colour palettes were applied from the R package RColorBrewer (Neuwirth, 2014). Phytoplankton and macroinvertebrate count data, and macrophyte relative abundances were used for Simpson diversity calculations (1-D). *Nitellopsis obtusa* abundance was excluded from diversity calculations, to avoid confounding effects as *N. obtusa* was used as a predictor variable within diversity analyses. To determine whether it was appropriate to pool data, or if the hierarchal study design resulted in dependence between time points, a Chi-square test of independence was conducted ($X^2 = 9951.4$, p-value = 0.45). This confirmed that running multiple linear regression was appropriate for my dataset. A multiple linear regression between Simpson diversity and log (x + 1) transformed water quality parameters, including *N. obtusa* biomass (dry weight g), was performed using the vegan package (Oksanen et al., 2013). To ensure variables included in the multiple linear regression did not exhibit collinearity, variance inflation factors were determined as previously described (James, Witten, Hastie, & Tibshirani, 2013).

Redundancy analyses (RDA) for each community apart of the LAFW were used to visualize and assess variation across study years. RDA was chosen because a detrended correspondence analysis found the longest gradient to be < 3. Hellinger transformations were used on phytoplankton and macroinvertebrate community data as this gave low weights to rare species. Macrophyte relative abundances were log transformed. Biplot site scores reflect weighted sums of species, as this measure is more robust to noise within the environmental variables. The RDA distance matrix was based on Bray-Curtis dissimilarity. The resulting RDAs were prepared using the packages stringr (Wickham, 2019), dplyr (Wickham & Francois, 2016), tidyr (Wickham & Henry, 2019), and vegan (Oksanen et al., 2013), and plotted in ggord (Beck, 2016). A permutational analysis of variance (permanova) was used to distinguish whether a significant shift in community composition was present between years (Oksanen et al., 2013).

Assessing interactions between taxa across communities is often difficult and complex. A relatively new multivariate approach that was developed to address this challenging issue is generalized linear latent variable modeling (GLLVM). A GLLVM is an extension of a general linear model applied to multivariate data using a factor analytic approach, which includes latent variables representative of water quality for each site complemented by species specific factor loadings to ascertain correlations between species (Niku, Hui, Taskinen, & Warton, 2019). In general, the latent variables can be interpreted as ordination axes, all while using standard model selection tools (Hui, Taskinen, Pledger, Foster, & Warton, 2015). An important advantage of this modelling technique is the ability to handle many species at once, compared to alternative methods, as the covariance model scales linearly (Warton et al., 2015). Data for the top ten abundant taxa from each LAFW community were used in the GLLVM to reveal taxa-specific interactions, while still accounting for influences exerted by water quality parameters characterized as latent variables.

The resulting GLLVM model was constructed following Niku et al., (2019). To determine whether mixed effects were required in the GLLVM model, a Chi-squared test of independence was conducted (X^2 = 6873.7, p-value = 0.07). Poisson, Tweedie, zero inflated Poisson, and negative binomial distributions were fit to the data. Information criterion, Dunn-Smyth residual plots, and normal quantile-quantile plots with 95% confidence intervals were used to assess fit for LAFW community distributions (Niku et al., 2019). A negative binomial distribution was identified to be the best fit and thus used in the production of the model. Environmental parameters as latent constructs were added into the model, to determine which latent variables had influences on species interactions. A model that used eight of the latent water quality parameters (depth, Secchi, temperature, TP, TN, chloride, chlorophyll α, and pH) were chosen based on residual analyses, which indicated the most suitable mean-variance relationship for responses. The resulting estimations for correlation patterns across frequently occurring species were plotted using the corrplot and gclus packages (Hurley, 2005; Wei et al., 2017).

2.3 Results

Summary statistics for the environmental parameters at each of the 12 sampling sites are presented in the supplementary materials (Table A1). *Nitellopsis obtusa* increased in relative abundance from 20% in 2016 to just over 80% in 2019 (Figure 2.2). However, it is evident over the 4-year study period that *M. spicatum* maintained about a 20% share of macrophyte community biomass (Figure 2.2).

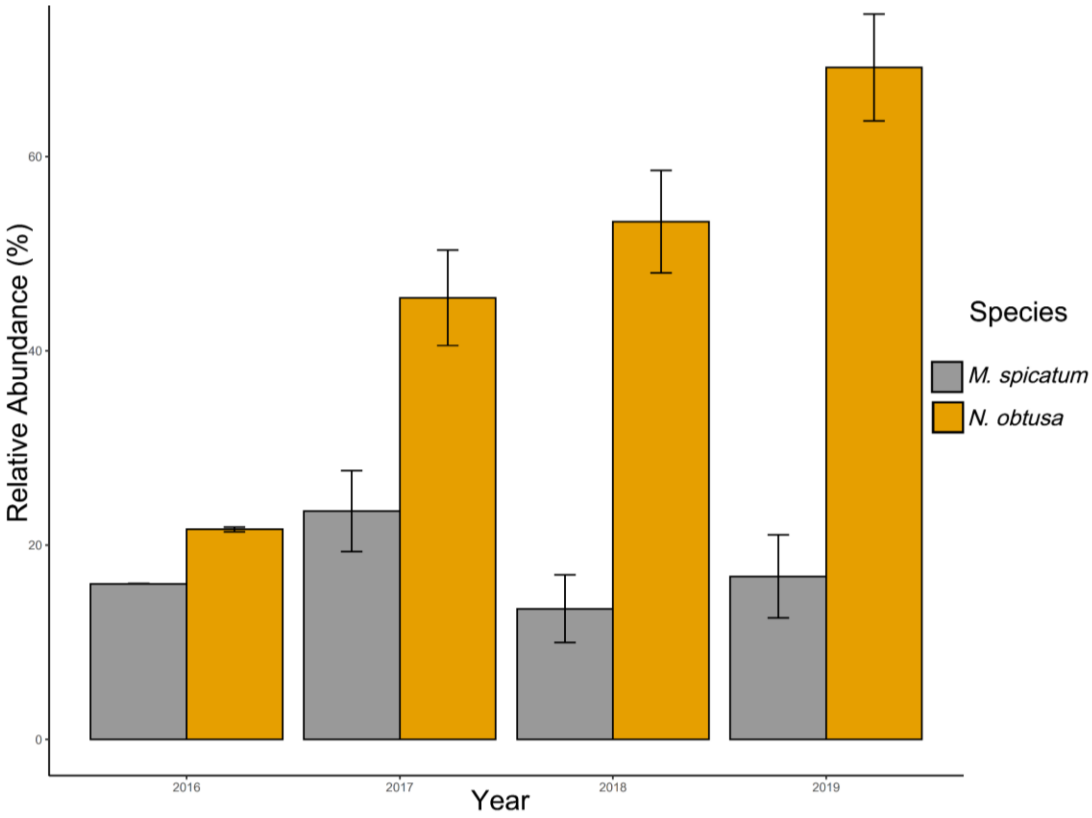


Figure 2.2. *A comparison of mean relative abundance with standard error bars for* Nitellopsis obtusa *(orange) and* Myriophyllum spicatum *(grey) across study years (2016-2019).*

Redundancy analysis allowed the visualization and evaluation of community profile shifts for each LAFW community over time. An RDA of the phytoplankton community shows axis one explaining 63.8% of the variance (Figure 2.3A). In comparison, it was revealed that axis one explained 92.27% of the variance in the macroinvertebrate community, (Figure 2.3B), and 39.3 % of the variance within the macrophyte community (Figure 2.3C). Axis two explained 18.73 % of the variance for phytoplankton (Figure 2.3A), 3.35 % of the variance for macroinvertebrates (Figure 2.3B), and 25.01 % of the variance for macrophytes (Figure 2.3C). Interestingly, over the course of the study, I observed shifts in community composition across the LAFW as *N. obtusa* prevalence increased (Figure 2.3). A permanova confirmed that the LAFW had statistically significant community shifts between years for the phytoplankton community (Figure 2.3A) ($F_{1,230}$ = 28.37, p-value < 0.001), macroinvertebrate community (Figure 2.3B) ($F_{1,230}$ = 20.56, p-value < 0.001), and macrophyte community (Figure 2.3C) ($F_{1,230}$ = 12.57, p-value < 0.001).

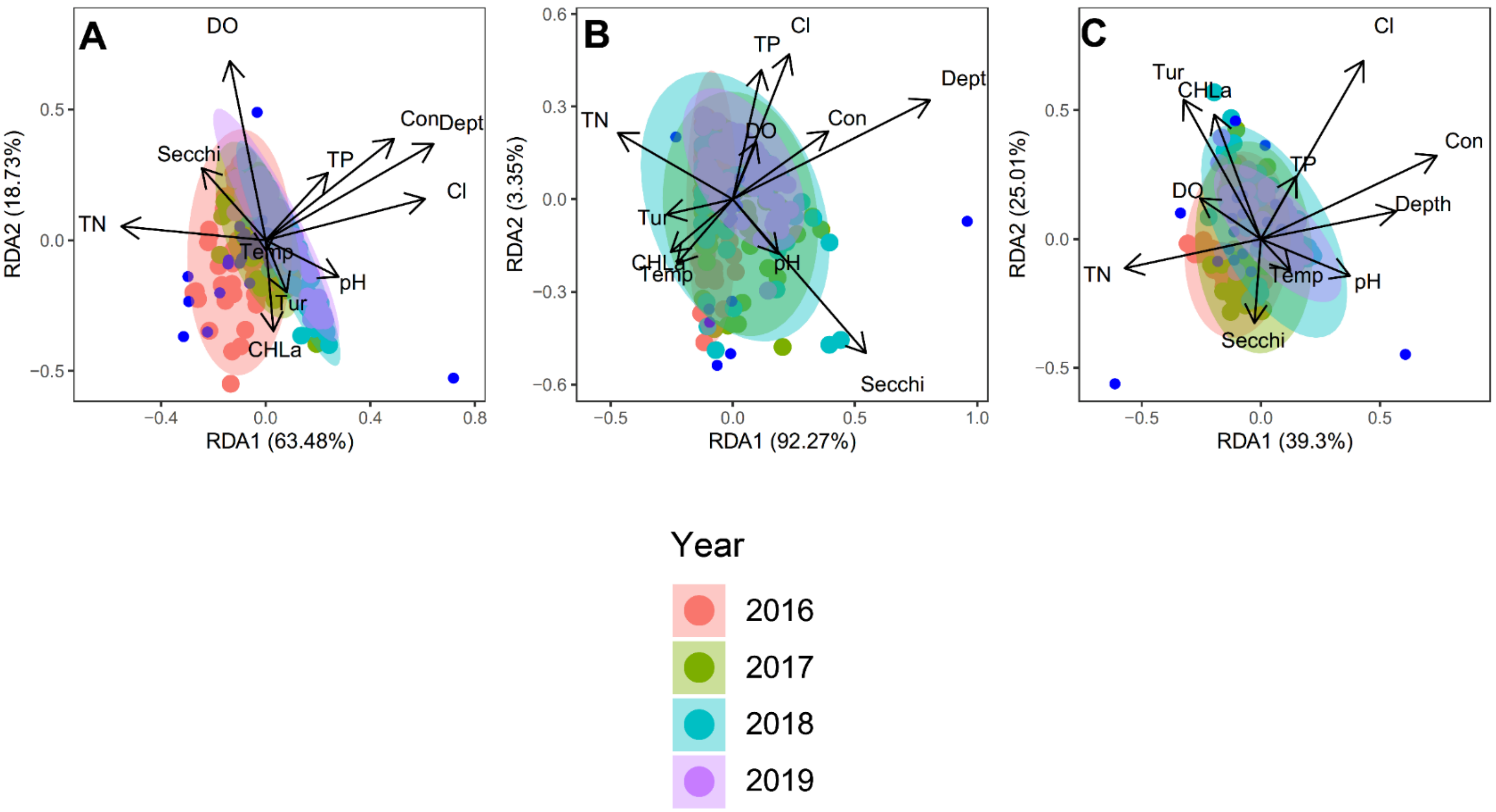


Figure 2.3. *Redundancy analysis (RDA) biplots,* ***A.*** *phytoplankton,* ***B.*** *macroinvertebrate, and* ***C.*** *macrophyte community profiles (n = 232) across 4 years (2016-2019). Blue dots in each biplot represent the mean for individual species in the community. Environmental parameter arrows pointing in the same direction reflect positive correlations, and arrows pointing in opposite directions indicate negative correlations. The length of the arrow is a direct representation of the variance explained by the environmental variable. TN = total nitrogen, DO = dissolved oxygen, TP = total phosphorus, Con = conductivity, Cl = chloride, Temp = temperature, Tur = turbidity, CHLa = chlorophyll α, and Secchi = Secchi depth.*

Table 2.1. Multiple linear regression models of Simpson's diversity for each LAFW community with environmental parameters and *Nitellopsis obtusa* biomass (dry weight) as independent variables. All variables were log (x + 1) transformed prior to analysis (n = 232).

	Phytoplankton (R^2 = 0.37)			**Macroinvertebrates (R^2 =0.33)**			**Macrophytes (R^2 = 0.21)**		
	Estimate	***t-value***	***p-value***	**Estimate**	***t-value***	***p-value***	**Estimate**	***t-value***	***p-value***
Depth	-0.67	-6.01	$\mathbf{7.37^{-09}}$	-0.32	-2.89	**0.0043**	-0.40	-3.59	**0.00054**
Secchi	0.88	4.95	$\mathbf{1.44^{-06}}$	-0.29	-1.61	0.11	-0.39	-2.18	**0.030**
Total Phosphorus	0.06	1.57	0.12	-0.11	-2.70	**0.0075**	-0.031	-0.79	0.43
Total Nitrogen	0.30	2.55	**0.011**	0.042	0.35	0.72	0.084	0.70	0.49
Chloride	-0.03	-0.79	0.43	-0.072	-1.87	0.063	-0.035	-0.89	0.37
Temperature	-0.52	-1.54	0.12	0.24	0.70	0.48	-0.51	-1.49	0.14
pH	0.05	0.41	0.68	0.11	0.94	0.35	-0.013	-0.12	0.91
Conductivity	-0.19	-1.46	0.15	-0.23	-1.74	0.083	-1.00	-0.75	0.45
N. obtusa	-0.05	-2.25	**0.026**	-0.11	-5.21	$\mathbf{4.27^{-07}}$	-0.035	-1.69	0.083

When assessing the environmental drivers of LAFW diversity, regression models revealed significant predictor variables (Table 2.1). Secchi depth and total nitrogen were positive explanatory variables, and depth and temperature were negative explanatory variables of phytoplankton Simpson’s diversity ($R^2 = 0.37$). Macroinvertebrate diversity was negatively influenced by total phosphorus and depth ($R^2 = 0.33$). Only Secchi depth and depth were found to have a negative influence on macrophyte diversity ($R^2 = 0.21$). Interestingly, *N. obtusa* biomass was a negative explanatory variable of diversity in all the multiple regression models, and statistically significant for 2 of the 3 communities (Table 2.1).

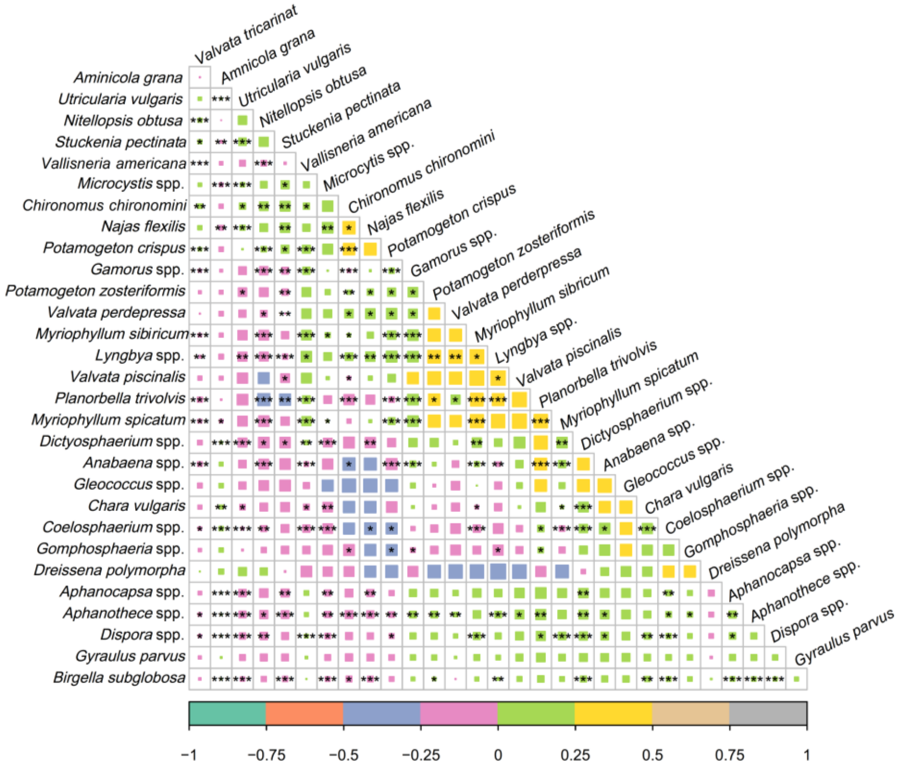


Figure 2.4. *General linear latent variable model output matrix to visualize species- specific interactions across the top 10 abundant taxa in lower aquatic food web communities of Lake Scugog when water quality (i.e., environmental co-variates) have been accounted for (n = 232). The GLLVM model represents a negative binomial distribution, with eight of the original environmental covariates included in the analysis: depth, Secchi, temperature, TP, TN, chlorophyll α, and pH. A heatmap depicting the strength of the correlation between the resulting species covariates is presented.*

Generalized linear latent variable modelling revealed that most taxa had weak to moderately strong positive co-occurrence relationships, except for *Dreissena polymorpha* (Figure 2.4). In contrast, *N. obtusa* mainly had significant negative interactions with several phytoplankton, macroinvertebrate, and macrophyte taxa. Most interestingly, the long established invasive macrophyte *M. spicatum* had largely positive interactions with other taxa in the LAFW yet had a significant (p-value < 0.001) negative association with *N. obtusa* (Figure 2.4). *Myriophyllum spicatum* also had negative interactions with native macrophytes such as *Stuckenia pectinata,* and *Utricularia vulgaris,* whereas these taxa were positively associated with *N. obtusa* (Figure 2.4). Furthermore, *M. spicatum* had negative interactions with *Microcystis spp.*, *Chironomus chironomini*, and *D. polymorpha*, while *N. obtusa* had positive associations with these taxa (Figure 2.4).

2.4 Discussion

Over the course of the 4-year study, *N. obtusa* not only increased in mean abundance from 20 – 80 % of the macrophyte community (Figure 2.2), but LAFW communities, particularly phytoplankton and macrophytes, shifted in community composition (Figure 2.3). To assess the role of *N. obtusa* in these community level changes, I determined that *N. obtusa* is a significant negative driver of Simpson's diversity across LAFW communities, although this effect on macrophyte diversity was marginally significant (p-value = 0.083). The GLLVM was a powerful joint modelling approach that allowed me to apply many response variables while concurrently demonstrating the correlations between them. The results showed important species relationships when accounting for water quality, including the generally negative relationship between *N. obtusa* and other LAFW taxa.

The overall impact to LAFW community structure reported here is in line with the few studies that have looked at community-level effects, albeit in macrophyte communities only. Brainard & Schulz (2017) found that native and non-native macrophyte richness declined in central New York state lakes where *N. obtusa* was present, and Ginn et al., (2021) documented reduced biomass of macrophytes coinciding with the colonization and establishment of *N. obtusa* in Lake Simcoe. There are likely several mechanisms by which *N. obtusa* displaces other macrophyte species. The first is the ability of *N. obtusa* to form dense benthic mats which can suppress the growth of emerging vascular macrophytes (Larkin et al., 2018). The second is the ability of *N. obtusa* to grow throughout the entire water column across a range of depths, causing a light-limited environment for other macrophytes (Larkin et al., 2018).

In my study, *N. obtusa* had a significant negative co-occurrence with the non-native invasive macrophyte *M. spicatum*, as well as the native taxon *Myriophyllum sibiricum*. Previously, *M. spicatum* was believed to be the most invasive aquatic macrophyte introduced to North America (Smith, Smith, Barko, & Barko, 1990), but Pullman & Crawford (2010) noted that *N. obtusa* was the most aggressive macrophyte they had ever documented in Michigan lakes. In my study, *N. obtusa* appeared to be a superior competitor to *M. spicatum* by increasing in relative abundance year over year (Figure 2.2). A recent study in Lake Simcoe, a large inland lake in Ontario, also had a similar pattern, where *M. spicatum* declined in abundance as *N. obtusa* became established (Ginn et al., 2021).

It is important to note that there were several native macrophyte species that positively co-occurred with *N. obtusa*, including *Stuckenia pectinata*, *Potamogeton crispus, Najas flexilis,* and *Utricularia vulgaris*. Interestingly, in the technical report of Pullman & Crawford (2010), they noted a unique interaction between *U. vulgaris* and *N. obtusa* in Michigan lakes, where both species increased to nuisance levels when they co-occurred. Interestingly, I observed a weak negative co-occurrence between the native taxon *Chara vulgaris* and *N. obtusa*. Ginn et al., (2021) also recently reported a reduction of native *Chara* spp. in Lake Simcoe as *N. obtusa* increased in abundance. In contrast, Brainard & Schulz (2017), found that *N. obtusa* was often discovered within *C. vulgaris* beds in their study lakes. Within its native range, it has been reported that *N. obtusa* and *Chara globularis* can co-occur in stands dominated by *Ceratophyllum demersum* (Pełechaty, Pronin, & Pukacz, 2014). Although *C. demersum* was observed in this study, it was a rare member of the community. I think these similarities and differences found across studies reflect different times of establishment and dominance. In my study, I was able to confirm that as *N. obtusa* increased in dominance, several early co-occurrences shifted to negative relationships with *N. obtusa*.

The negative effect of *N. obtusa* on phytoplankton and macroinvertebrate community diversity (Table 2.1) as well as certain taxa (Figure 2.4) is a novel finding. In a study from *N. obtusa*'s native range, Berger & Schagerl (2004) demonstrated that *N. obtusa* can secrete allelopathic compounds, which selectively inhibited growth of several phytoplankton strains within a laboratory setting. *Nitellopsis obtusa* has been observed to grow into the surface waters, allowing direct competition with phytoplankton for available light, habitat, and nutrients (Larkin et al., 2018). The GLLVM results identify a possible synergistic relationship between *N. obtusa* and *Microcystis* spp., coinciding with the observations of emergence in recent years (Figure 2.4). This relationship is investigated further in Chapter 4, but Berger & Schagerl (2004) did show *Microcystis aeroginosa* to be tolerant of *N. obtusa* allelopathic compounds, unlike the other phytoplankton strains tested.

Despite the lack of studies investigating the impact of *N. obtusa* invasion on macroinvertebrate communities from macrophyte beds, the formation of dense *N. obtusa* mats and anticipated decrease in oxygen concentrations are thought to be a detrimental environment for sensitive macroinvertebrate taxa (Larkin et al., 2018). The GLLVM results showed *N. obtusa* having a positive association with *Chironomus chironomini*. Generally, *Chironomid* spp. are bioindicators of lake oxygen concentrations, where several species are tolerant of low oxygen conditions (Davis, Golladay, Vellidis, & Pringle, 2003). The GLLVM also showed a positive association between *N. obtusa* and the non-native bivalve *D. polymorpha*. Anecdotal reports (e.g., Pullman & Crawford, 2010) have mentioned the proliferation of *D. polymorpha* on *N. obtusa*, likely because the calcified architecture of *N. obtusa* is ideal for *D. polymorpha* attachment. Indeed, throughout this study, *D. polymorpha* was consistently found attached on *N. obtusa* specimens.

In summary, my findings show for the first-time significant effects of *N. obtusa* at the species- to community-level across the LAFW in an invaded lake in North America. Despite reducing the overall diversity of the LAFW, *N. obtusa* also had positive associations with certain taxa, including native and non-native species. In combination, these results suggest that *N. obtusa* may act as an ecosystem engineer from the time of establishment to dominance in macrophyte beds. Shifts in lake productivity and energy flow across the LAFW are expected due to the alteration of communities. This has important implications for the fish community in Lake Scugog, and by extension, other lakes invaded by *N. obtusa*. The LAFW communities are essential to all fish species in the lake since parts or all their life cycles depend on the LAFW for food and/or habitat. Further research will be necessary to determine if *N. obtusa* invasion poses a threat to the fish community, especially in lakes like Lake Scugog that support an important sport fishery.

Chapter 3. Low Benthic Oxygen and High Internal Phosphorus-Loading are Strongly Associated with the Invasive Macrophyte *Nitellopsis obtusa* in Lake Scugog.

3.1 Introduction

Nitellopsis obtusa (Desv.) J. Groves, 1919, is a non-native invasive macrophyte in lakes and coastal wetlands of eastern North America. Native to Eurasia, *N. obtusa* is a macroalga belonging to the Characeae family. Initially identified in the St. Lawrence River in 1974 (Karol & Sleith, 2017), rapid expansion to lakes of the United States and Canada began just over a decade ago (Larkin et al., 2018). In Ontario, *N. obtusa* populations have been confirmed in Lake Simcoe as early as 2008 (Ginn et al., 2021), and Presqu'ile Bay in 2015 (Midwood et al., 2016). Despite an increasing range of distribution in the Great Lakes Basin, information pertaining to the negative effects on ecosystem processes, habitat structure, and biota associated with *N. obtusa* invasion are in short supply and remain largely anecdotal.

When dense beds of *N. obtusa* form in invaded lakes, other macrophyte taxa, non-native and native alike, are displaced and native fish habitat is severely altered, as seen in Chapter 2 and reported by others (Pullman and Crawford, 2010; Brainard and Schulz, 2017; Ginn et al., 2021). The *N. obtusa* invasion of Lake Scugog, a large, shallow lake in southern Ontario, Canada, likely occurred in the early-2000s, but was first documented in 2015. From 2016 – 2019, I monitored *N. obtusa* and have seen a clear increase in the dominance of this invasive species in the macrophyte community (Chapter 2). I also documented the first known occurrences of *Microcystis* spp. blooms in Lake Scugog, and determined that *N. obtusa* was a significant biological driver of *Microcystis* spp. biomass (Chapter 4).

To improve our understanding of the ecosystem-level impacts of *N. obtusa* and its role in *Microcystis* spp. blooms in Lake Scugog, I analyzed three-years (2017 – 2019) of benthic dissolved oxygen (DO) and sediment soluble reactive phosphorus (SRP) data from sites spanning the entire lake. Given the emergence of thick *N. obtusa* beds in Lake Scugog over the study period, I wanted to test the hypotheses that (1) low benthic oxygen concentrations across Lake Scugog were associated with *N. obtusa* presence, and (2) benthic DO decreased in response to increasing *N. obtusa* biomass. Although benthic anoxia has previously been reported under charophyte beds (Kufel & Kufel, 2002), there have been no studies to date showing benthic anoxia or low DO occurring under *N. obtusa* beds in its native or introduced ranges.

Additionally, I examined sediment pore water SRP in response to *N. obtusa* biomass, based on the premise that depleted benthic DO concentrations would enhance internal phosphorus loading (Lake, Coolidge, Norton, & Amirbahman, 2007). The hypolimnion of productive lakes can typically become anoxic by mid-summer due to high biological oxygen demand. When oxygen is depleted near the lakebed, redox conditions shift and trigger sediment-release of dissolved phosphorus, including SRP. Lake Scugog is a highly productive lake, but due to a long fetch and shallow mean-depth, it has been designated as polymictic with no persistent thermal stratification throughout the summer months (Kawartha Conservation, 2010). However, with dense beds of *N. obtusa* now occurring in parts of the lake, I conjectured that water column mixing with the atmosphere may be more subdued at those sites.

Here I report for the first-time clear differences in benthic DO at sites with and without *N. obtusa*. I also detected strong statistical relationships between DO and SRP with *N. obtusa* biomass, respectively. These results infer a mechanistic role for this invasive macrophyte that impacts near-bed habitat condition. A stabilized water column and internal loading of phosphorus are also conditions known to promote *Microcystis* spp. blooms (Chung, Imberger, Hipsey, & Lee, 2014). Based on the physical effect that dense beds of *N. obtusa* could have on water column mixing and near-bed biogeochemistry, I propose that *N. obtusa* may be an ecosystem engineer in invaded lakes and wetlands.

3.2 Methods

Lake Scugog is a large (68 km^2), shallow (mean depth = 1.4 m) headwater reservoir located in southern Ontario, Canada (Figure 2.1). Twelve sampling sites were monitored as described in Chapter 2, with site depths spanning 1 – 3 m. DO measurements were taken at 0.5 m depth intervals at each of the 12 sites using a YSI 6 series multiparameter probe (YSI Inc., Yellowsprings, Ohio, USA). Benthic DO concentrations were measured 0.5 m above the sediment-water interface to minimize sediment disturbance during the reading. The sonde was suspended at each depth until the reading stabilized, before recording the value.

Sediment cores were taken at each site using an NLA Gravity Corer (Hoskin Scientific, Burlington, Canada). The top 10 cm of each sediment core was sectioned into acid washed tubes and stored on ice until returning to the laboratory on the same day. Tubes were acid-washed to remove residual nutrients and were well rinsed with Milli-Q water to remove residual acid. Sediment cores were collected to measure pore water phosphorus concentrations at each site using a NLA gravity Corer (Hoskin Scientific, Burlington, Canada). Sediment samples were centrifuged at 12,000 rpm for 10 minutes to separate the pore water and particulate sediment. Pore water was stored at – 20 °C until phosphorus was analyzed as described in Chapter 2.

Statistical analyses were conducted in R version 4.0.0 (R Core Team, 2019) using the packages ggpubr (Kassambara, 2020), and ggplot2 (Wickham, 2016). To ensure visual accessibility of the oxygen profiles, an accessible palette was applied from the RColorBrewer package (Neuwirth, 2014). DO profiles were averaged for each sampling date and separated by year for sites with and without *N. obtusa* presence. SRP concentrations were separated into two categorical classifications (*N. obtusa* present/absent) and a Kruskal-Wallis H test was conducted. A non-parametric test was used as SRP concentrations for sites without *N. obtusa* presence often had values lower than the methods detection limit (1 µg · L^{-1}). To assess differences in benthic dissolved oxygen between sites with and without *N. obtusa*, a Welch's t-test was used due to unequal sample sizes. Linear regressions were performed with *N. obtusa* biomass (dry-weight in grams) as the independent predictor variable of benthic oxygen concentrations (mg · L^{-1}) and sediment pore water SRP (µg · L^{-1}).

3.3 Results and Discussion

Studies documenting connections between aquatic macrophytes and sediment anoxia are limited (Atapaththu, Parveen, Asaeda, & Rashid, 2018). However, dense stands of macrophytes, including those of Characeae and *Myriophyllum spicatum*, are often described to regulate dissolved oxygen profiles (Frodge, Thomas, & Pauley, 1990;

Cardinale, Burton, & Brady, 1997; Unmuth, Lillie, Dreikosen, & Marshall, 2000; Kufel & Kufel, 2002). Consistently over the three-year study, sites with *N. obtusa* had lower DO concentrations throughout the water column (Figure 3.1 A, C, E). Generally, sites without *N. obtusa*, frequently dominated by *M. spicatum*, had DO concentrations in the supersaturated range, and maintained higher concentrations throughout the water column than sites with *N. obtusa* (Figure 3.1 B, D, F).

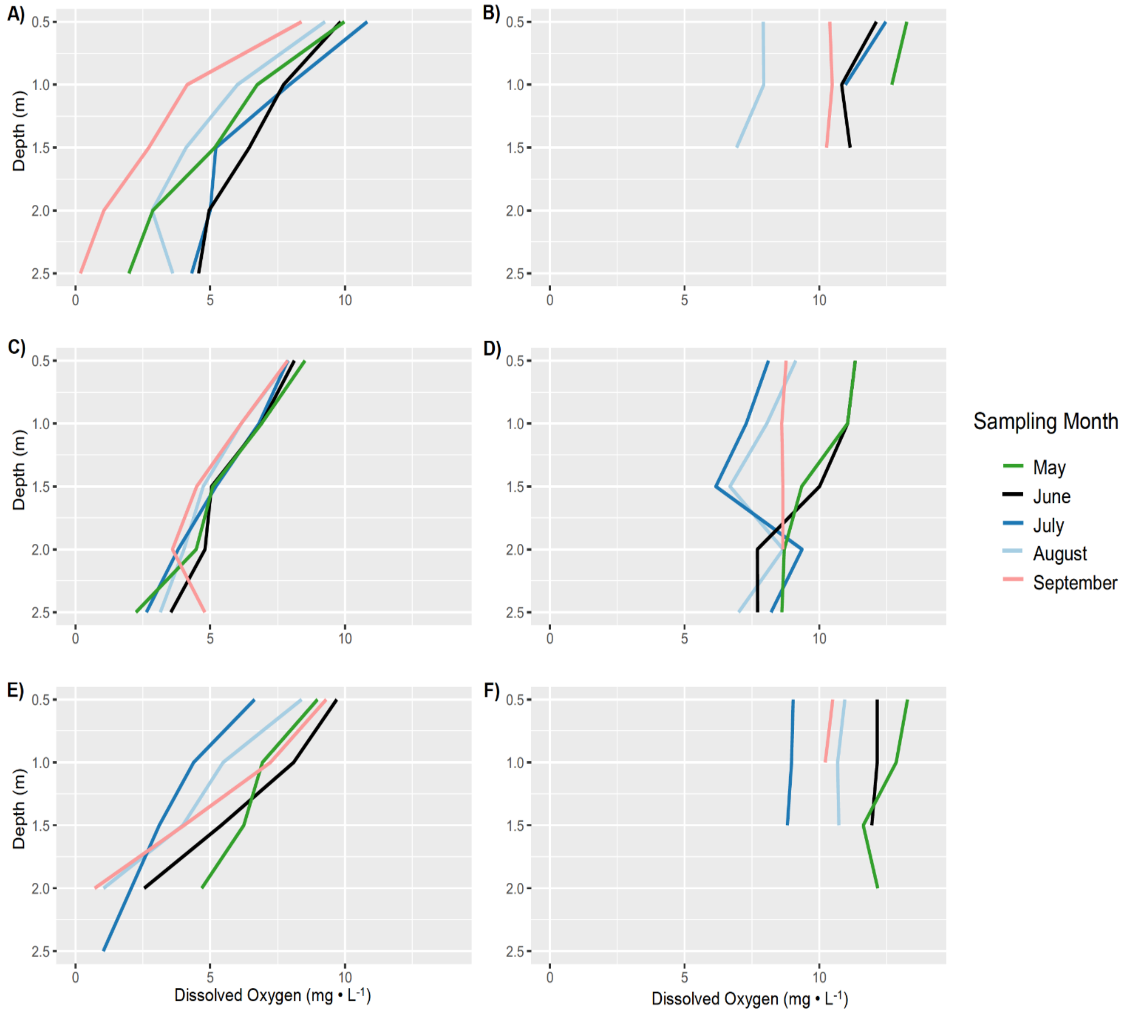


Figure 3.1. *Averaged dissolved oxygen profiles (mg · L^{-1}) for sites with (n = 138) and without (n = 36)* N. obtusa *presence per month and year.* ***A.*** *2017 dissolved oxygen profiles for sites with* N. obtusa*,* ***B.*** *2017 dissolved oxygen profiles for sites without* N. obtusa*,* ***C.*** *2018 dissolved oxygen profiles for sites with* N. obtusa*,* ***D.*** *2018 dissolved oxygen profiles for sites without* N. obtusa*,* ***E.*** *2019 dissolved oxygen profiles for sites with* N. obtusa*, and* ***F.*** *2019 dissolved oxygen profiles for sites without* N. obtusa*.*

Benthic DO concentrations were significantly different (Welch's t-test p-value < 0.001) between sites with and without *N. obtusa* (Figure 3.2). Similar to my results, Unmuth et al., (2000) demonstrated dense stands of *M. spicatum* within a Wisconsin lake often resulted in super saturated dissolved oxygen concentrations. Furthermore, Atapaththu et al., (2018) concluded that hypoxic-anoxic conditions negatively affect *M. spicatum* growth.

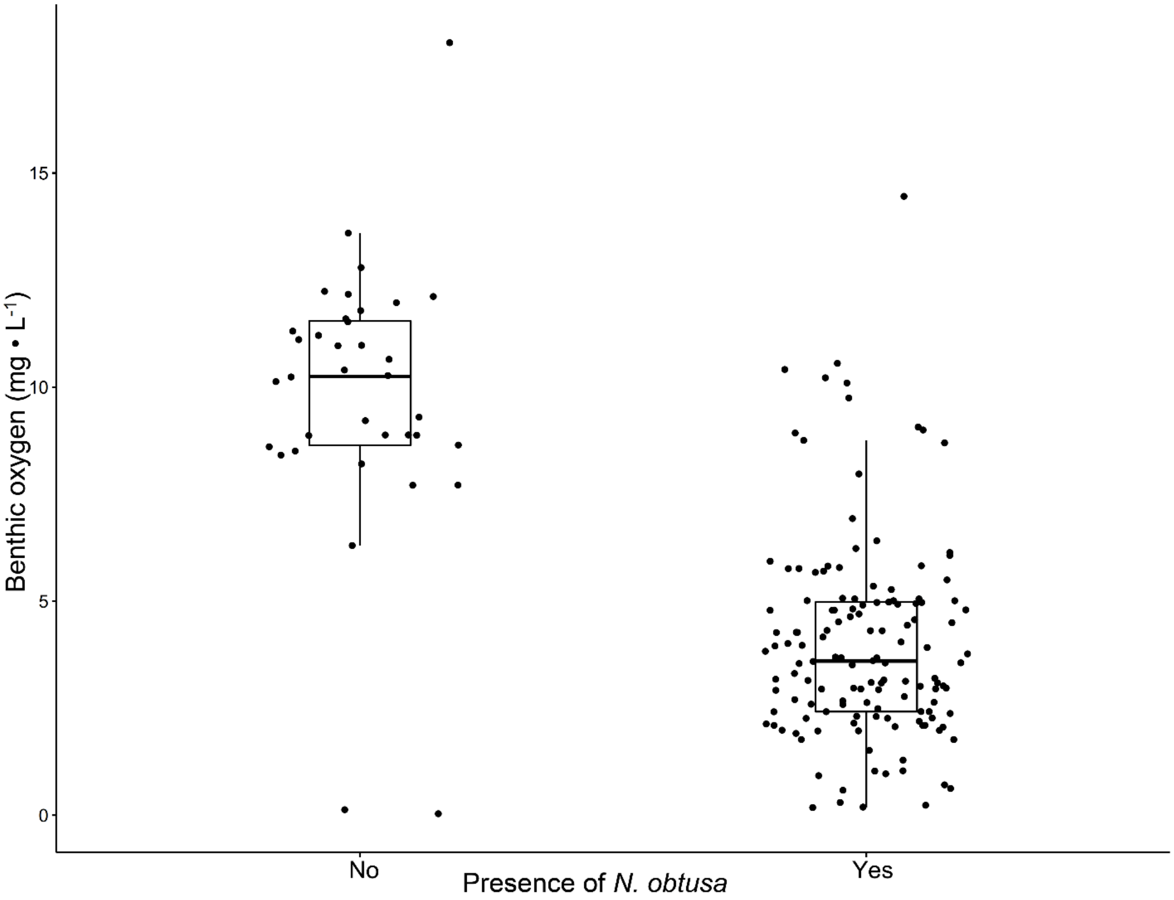


Figure 3.2. *Boxplots comparing benthic oxygen concentrations for sites with (n = 138) and without (n = 36)* N. obtusa *presence. A Welch's t-test was used to compare sample means (α = 0.05).*

Unlike other macrophytes, the life cycle and reproductive strategies of some Characeae are dependent on hypoxic-anoxic events at the sediment-water interface.

Generally, Characeae that reproduce through vegetative propagules rely on viable propagule banks within the sediments (Migula, 1897). Propagules can persist in sediments for extended periods of time, however, when buried deeper than 2 cm, the potential to germinate is lost (Bonis & Grillas, 2002). This is attributed to the necessity of hypoxic-anoxic conditions at the sediment-water interface to initiate germination (Bonis & Grillas, 2002). The strong association of reduced benthic DO with increasing *N. obtusa* abundance in this study suggests that *N. obtusa* may alter local habitat conditions to promote propagule bank germination. This positive-feedback system may explain why *N. obtusa* is initially delayed in becoming dominant in the macrophyte community because it takes time for there to be sufficient biomass to induce hypoxic-anoxic conditions for propagules to germinate.

Not only was low benthic DO associated with *N. obtusa* presence, but *N. obtusa* was also associated with significantly elevated concentrations of SRP within the sediment pore water (p-value < 0.05, Kruskal-Wallis H test). Furthermore, *N. obtusa* biomass was a negative explanatory variable for benthic DO ($R^2 = 0.59$) (Figure 3.3A) and positive explanatory variable for sediment pore water SRP ($R^2 = 0.90$) (Figure 3.3B). These results clearly show a distinct water quality profile associated with *N. obtusa* in Lake Scugog, where *N. obtusa* appears to be a driver of benthic hypoxia-anoxia, a necessary condition for internal loading of bioavailable phosphorus from sediments.

Brainard & Schulz (2017) suggested that when dense benthic mats of *N. obtusa* undergo senescence, nutrients are released from sediments. Despite *N. obtusa* not undergoing senescence in this study, *N. obtusa* biomass was a very strong predictor of sediment pore water SRP concentration. Based on the negative relationship with DO, it is likely that *N. obtusa* is indirectly facilitating sediment phosphorus release into the water column. Internal loading of bioavailable phosphorus is regarded as a primary driver of cyanobacterial blooms (Bormans, Maršálek, & Jančula, 2016). Thus, this prominent association between *N. obtusa* and internal phosphorus loading in Lake Scugog infers an indirect faciliatory role in *Microcystis* spp. bloom development (Chapter 4).

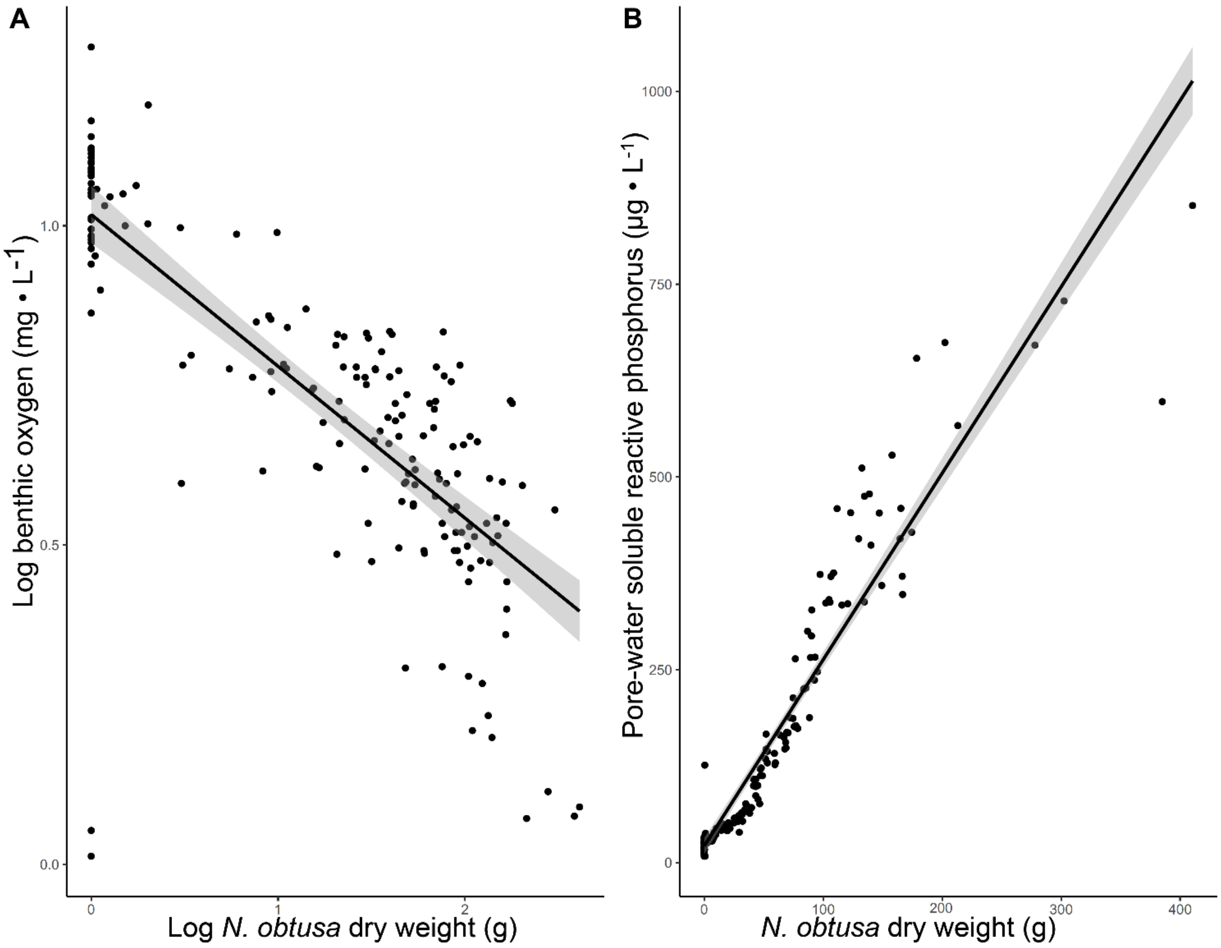


Figure 3.3. A. *Scatter plot with regression line visualizing the relationship between Log transformed benthic DO and* N. obtusa *dry-weight (R^2 = 0.59, p-value < 0.001),* ***B.*** *Scatter plot with regression line visualizing the relationship between pore water SRP and* N. obtusa *dry-weight (R^2 = 0.9, p-value < 0.001) (n = 138).*

Nevertheless, given the strong inference from my results that *N. obtusa* is altering the biogeochemical fate of oxygen and phosphorus in Lake Scugog, I propose that *N. obtusa* is acting like an ecosystem engineer of internal biogeochemical processes. With increasing biomass and dominance in aquatic weed beds, *N. obtusa* may be reducing water-column mixing and exchange with atmospheric oxygen. Although depleted near-bed DO is known to drive internal phosphorus loading in lakes, there is also the negative impact that water column hypoxia-anoxia can have on biota. Studies are now emerging that show the negative effects of *N. obtusa* on aquatic communities (e.g., Brainard and Schulz, 2017; Ginn et al., 2021) as discussed in Chapter 2, but much remains unknown about the effects of *N. obtusa* on the aquatic food web, especially fish. Considering the extent of hypoxia to anoxia reported here in a lake designated as polymictic, this poses serious concerns regarding the quality of sportfish habitat.

These findings not only raise questions about habitat condition in Lake Scugog, but other lakes in the region where *N. obtusa* has become established. With the distribution of *N. obtusa* expanding across Ontario lakes (Chapter 5), my study provides clear observational data that raise serious concerns for biogeochemical cycles, benthic habitat structure, and other biota in invaded lakes. These results also support previous work, which implicated *N. obtusa* as a biotic driver of *Microcystis* spp. blooms in Lake Scugog (Chapter 4). Here I provide evidence of a possible mechanism for promoting bloom development, whereby *N. obtusa* drives down benthic DO to facilitate internal phosphorus loading from sediments as well as possible propagule germination. Further studies should be conducted to verify if these inferred effects by *N. obtusa* in Lake

Scugog are occurring in other invaded lake ecosystems in North America. If such notable impacts to lake biogeochemistry are documented region-wide, there will be more certainty that *N. obtusa* is acting as an ecosystem engineer in invaded lakes.

Chapter 4. Elucidating the Role of *Nitellopsis obtusa* in the Recent Emergence of *Microcystis* Blooms in Lake Scugog.

4.1 Introduction

Aquatic invasive species in Canadian inland waters are an increasing problem that pose significant socio-ecological impacts. The invasive mussel *Dreissena polymorpha* is no exception, and was first discovered in the Laurentian Great Lakes in 1988 (Hebert, 1989). *Dreissena polymorpha* has been shown to have negative effects on ecosystem biodiversity and function, including: biofouling (Clarke, 1952; Greenshields & Ridley, 1957; Morton, 1969; Afanas'yev & Protasov, 1987), displacement of native species (Sebestyen, 1938; Arter, 1989), and alteration of nutrient dynamics (Stanczykowska, 1977; Stanczykowska & Planter, 1985). Another non-native invasive species from Eurasia is *Nitellopsis obtusa*, a freshwater macroalga first introduced to the Laurentian Great Lakes Basin in the 1970s, when it was discovered in the St. Lawrence River (Karol & Sleith, 2017). By the 1980s, *N. obtusa* was documented in Lake Ontario and Lake St. Clair (Schloesser et al., 1986; Midwood et al., 2016).

Over the intervening decades, *D. polymorpha* has successfully invaded inland lakes in Ontario such as Lake Simcoe and the Trent Severn Waterway (TSW). It was confirmed in 1991 that *D. polymorpha* had invaded Lake Scugog, a large headwater reservoir to the TSW (Hincks & Mackie, 1997). In fact, Lake Scugog's water chemistry was determined to be ideal for dreissenid mussel invasion, but the prevalence of soft substrate throughout the lake has likely kept numbers in check. This assumption is based on anecdotal reports by the Scugog Lake Stewards, which describe *D. polymorpha* as known to be present, but not at nuisance levels.

Few published reports have documented the spread of *N. obtusa* to inland lakes in the Great Lakes Basin. However, by the mid-2000s, conspicuous stands became apparent in Michigan and New York lakes (Larkin et al., 2018). On the Canadian side, there has been mounting anecdotal reports of *N. obtusa* in Ontario inland lakes connected to the TSW, including Lake Simcoe (Ginn et al., 2021). The scarcity of data on *N. obtusa* spread and establishment in North America is likely due to both a lack of awareness of this non-native taxon, as well as frequent misidentification (Larkin et al., 2018). There is growing consensus, however, that it has become the dominant aquatic macrophyte in many inland lakes across the east-central United States (Larkin et al., 2018).

As a member of the Characeae family, *N. obtusa* can undergo both sexual and asexual reproduction, depending on environmental conditions (Schloesser et al., 1986). Although a dioecious organism, *N. obtusa* is more commonly associated with its white star-shaped bulbils, which function as asexual reproductive and hibernation organs. The overall ecological threat that *N. obtusa* poses in North America is similar to other non-native invasive macrophyte taxa (Pullman & Crawford, 2010; Hackett et al., 2014; Brainard & Schulz, 2017). In shallow ecosystems, *N. obtusa* has been observed to grow taller than other Characeae species and vascular macrophytes, causing native species to become light limited (Larkin et al., 2018). When dense benthic mats of *N. obtusa* form in lakes, they typically displace native macrophytes (Pullman & Crawford, 2010; Larkin et al., 2018) causing a decline in species richness (Brainard & Schulz, 2017).

Nitellopsis obtusa was first reported and confirmed in Lake Scugog, Ontario in 2015 by the Scugog Lake Stewards. A large headwater reservoir of the TSW system, Lake Scugog has been a macrophyte-dominated ecosystem since its impoundment in the early 1800s (Irwin, 1984). Similar to other macrophyte-dominated ecosystems (Sand-Jensen & Borum, 1991), algal blooms have generally not been an issue in Lake Scugog, as far as reports go. In support of this assumption is the fact that there have been no historical reports of phytoplankton blooms in the lake. As a weedy lake, Lake Scugog has also supported an economically important sport fishery (Kawartha Conservation, 2010).

To address concerns about the establishment of a new non-native macrophyte in Lake Scugog, particularly a species with the potential to impact fish habitat (Pullman & Crawford, 2010; Larkin et al., 2018), I initiated a 3-year study in 2016. The goal of this study was to document the spatial distribution and temporal dynamics of *N. obtusa* in relation to the lower aquatic food web community (i.e., phytoplankton, macroinvertebrates, and macrophytes). Not only would this offer some insight into the establishment success of *N. obtusa* in Lake Scugog, but it would serve as the first systematic survey targeting *N. obtusa* in a large inland lake in Canada.

Over the 3-year study period, there were periodic eruptions of *Microcystis* spp. blooms in Lake Scugog that were captured during the sampling regime. Since algal blooms had not been historically reported in the lake, and the occurrence of *Microcystis* spp. blooms was regarded as a new phenomenon by the Scugog Lake Stewards and the lake monitoring agency Kawartha Conservation, a primary focus of my research turned to assessing the possible drivers of *Microcystis* spp. blooms in Lake Scugog. Here I present for the first-time supporting evidence of an invasive species facilitation scenario, where the invasive species *N. obtusa* positively influences *D. polymorpha* and *Microcystis* spp. blooms in a large, shallow lake ecosystem.

4.2 Methods

4.2.1 Sampling Location and Processing

The 12 study sites presented in this chapter are the same as Chapter 2, which are depicted in Figure 2.1. Environmental parameters were collected and analyzed as described in Chapter 2. In addition to environmental parameters discussed in Chapter 2, monthly precipitation data spanning the field season (May – September) over three years (2016 – 2018) was obtained from the Government of Canada historical climate data website (http://climate.weather.gc.ca/historical_data/search_historic_data_e.html), using the Oshawa airport station. This weather station was the closest to the Lake Scugog region that had consistent data spanning the course of this study. Monthly precipitation averages were used in all subsequent statistical analyses. In 2017 and 2018, sediment cores were collected as described in Chapter 3. Total phosphorus and soluble reactive phosphorus were determined from pore water isolated from sediment cores as described in Chapter 2.

4.2.2 Statistical Analyses

All statistical analyses were conducted using the open-sourced software platform R version 3.5.1 (R Development Core Team 3.0.1., 2013). Analysis of Variance (ANOVA) and Pearson correlation analyses were performed using the 12 environmental variables presented in Table 4.1. Biological data were log-transformed to comply with parametric assumptions of normality. Resulting correlation coefficients were plotted as a heatmap using ggplot2 (Wickham, 2016).

4.2.3 Structural Equation Modelling (SEM)

Structural Equation Modelling (SEM) is a useful approach in assessing direct and indirect biotic and abiotic interactions in ecological communities (Mitchell, 1992). In this study, SEM was used to analyze the role of the lower food web community in facilitating *Microcystis* spp. blooms. Even in complex models, SEM allows for a complete explanation using each variable, while also using confirmatory factor analysis as a multivariate approach to test interactions between factors (Wan, 2002). SEM was conducted using the R packages: lavaan, semPlot, OpenMX, tidyverse, knitr, kableExtra, and GGally (Boker et al., 2011; Rosseel, 2012; Xie, 2012; Epskamp, 2015; Wickham, 2017; Zhu, 2018; Schloerke et al., 2020). Multivariate normality was evaluated in R with the MVN package using the Mardia approach (Korkmaz, Goksuluk, & Zararsiz, 2014). However, because construction of this modelling technique involved using biological data with a negative binomial distribution, the SEM was constructed using a maximum likelihood (ML) model estimator.

A ML estimator was chosen as it is considered to be relatively robust for models with deviations away from multivariate normality (Diamantopoulos & Siguaw, 2000; Bollen, 2014). Furthermore, Monte-Carlo experiments have shown no significant differences in constructed SEM that use the ML estimator with varying skewness and kurtosis levels defining multivariate normality (Reinartz, Haenlein, & Henseler, 2009). Multi-collinearity of variables was assessed as described above and bootstrapping was performed to determine if it could help with multivariate normality (Preacher & Hayes, 2004). Notably, there were no differences between the bootstrapped data and data used for the construction of the SEM. The final SEM presented is based on assumed theoretical relationships between factors, as recommended by Fan et al., (2016).

To ensure the resulting SEM was not over or under specified, a combination of two fit indices were used as suggested by Hu & Bentler (1999). Standardized root-mean square residual (SRMR) and the comparative fit index (CFI) were used to evaluate the final SEM. In brief, SRMR is a fit index to represent a badness of fit, where 0 is a perfect fit and 1 shows the model does not fit the data at all (Browne & Cudeck, 1992; Hu & Bentler, 1999). This fit index was chosen because it is useful in identifying mis-specified models and is less sensitive to sample size (unlike the Chi-squared test), while also being comparable between models. A value less than 0.09 was chosen to evaluate the fit of the SEM as previously described (Hu & Bentler, 1999). The CFI was also chosen as it is less sensitive to sample size than the Chi-squared test (Fan, Thompson, & Wang, 1999; Tabachnick & Fidell, 2007). The CFI represents the amount of variance that has been accounted for within the covariance matrix, using a minimum of 0.95 to evaluate the fit of the SEM (Hu & Bentler, 1999).

4.3 Results

Average physical and water quality variables for each sampling site over the 3-year study period are presented in Table 4.1. An ANOVA determined that only chloride and pH were significantly different between sites (p-value < 0.05). Sites closest to urban development in the west basin (sites 1 – 5) tended to have higher chloride concentrations than the east basin sites. All sites shared average values within the same order of magnitude, apart from sediment phosphorus (TSP and SSRP). Sediment phosphorus was highly variable across sites, but some sites had notably higher concentrations (Sites 5, 9, and 11). No appreciable trends in nutrients were noted over the 3-year study period.

Population trends for *N. obtusa*, *D. polymorpha*, and *Microcystis* spp. over three-years are presented in Figure 4.1. Generally, all taxa increased from 2016 to 2018 (ANOVA, p-value < 0.05). *N. obtusa* and *Microcystis* spp. median biomass was highest in 2018, whereas *D. polymorpha* median density was highest in 2017. However, all taxa experienced maximum levels in 2018 (Figure 4.1). When assessing potential relationships between environmental variables and each study taxon on an inter-annual basis, Pearson correlation analysis deemed several parameters statistically significant, but only in a single year (2017) (Figure 4.2). In 2017, precipitation weakly correlated with *N. obtusa*, and conductivity with *Microcystis* spp. Several environmental variables strongly correlated with *D. polymorpha* in 2017, including chlorophyll a (-), depth (+), Secchi depth (+) and turbidity (-) (Figure 4.2).

Table 4.1. Seasonal means (May – September) reflecting 3-years of pooled data (2016-2018) for twelve physical and water quality parameters measured concurrently in Lake Scugog, Ontario. Standard deviation is in brackets. Temp = temperature, Con = conductivity, DO = dissolved oxygen, TN = total nitrogen, TP = total phosphorus, TSP = total sediment phosphorus, SSRP = sediment soluble reactive phosphorus, and Chl a = chlorophyll α. Note that TSP and SSRP reflect 2 years of data (2017 & 2018) and that sample size was slightly smaller for sites 5 and 9 because they were consistently inaccessible in September throughout the 3-year study.

Site No.	**n**	**Depth (m)**	**Secchi (m)**	**Temp (°C)**	**pH***	**Con (μS·cm^{-1})**	**Chloride* (mg·L^{-1})**	**DO (mg·L^{-1})**	**TN (mg·L^{-1})**	**TP (μg·L^{-1})**	**TSP (μg·L^{-1})**	**SSRP (μg·L^{-1})**	**Chl a (μg·L^{-1})**
1	15	1.16(0.67)	1.3(0.38)	21.54(2.97)	7.7(2.18)	492(165)	101.1(105.4)	10.1(3.1)	0.77(0.91)	41(34.4)	61.4(44.9)	25.5(18.6)	91.9(25)
2	15	1.06(0.65)	1.04(0.35)	21.90(3.3)	7.78(2.21)	530(199)	171.4(144.4)	11.7(5.2)	0.78(1.08)	65.1(53.7)	106.4(77.9)	13.7(10)	41(56.5)
3	15	1.68(0.9)	1.48(0.60)	22.37(2.72)	7.73(2.17)	531(182)	139.8(133)	10(2.4)	0.61(0.69)	38.4(39.2)	55(40.3)	38.7(28.3)	22.7(49.2)
4	15	1.71(0.94)	1.62(0.49)	22.15(3.05)	7.63(2.16)	540(181)	114.5(93.4)	9.1(1.4)	0.76(0.98)	42(46.9)	58.2(42.6)	9.1(6.7)	99.7(340)
5	12	0.72(0.69)	0.75(0.41)	22.11(2.55)	7.43(2.15)	534(224)	96.4(70.9)	8.9(5.6)	0.82(0.85)	72.4(65.3)	127.4(93.3)	74(54.1)	14.4(41.7)
6	15	1.59(0.85)	1.5(0.51)	21.98(3.4)	7.57(2.14)	475(156)	97.3(90.6)	9.2(1.6)	0.96(1.77)	45.9(54.1)	83.8(53.4)	56.9(38.5)	11.9(78.1)
7	15	1.19(0.62)	1.23(0.41)	22.18(3.14)	8.1(0.42)	477(163)	70.7(56.3)	9.6(2.5)	1.19(2.2)	31.6(41.8)	53.7(35.8)	52.6(38.9)	23.1(28.9)
8	15	1.45(0.88)	1.31(0.48)	22.41(3.11)	8.32(0.33)	442(164)	68.3(71.5)	10.5(2.5)	1.12(0.79)	39.7(41.3)	58.3(44.4)	26.5(14.6)	14.2(74.4)
9	12	0.95(0.57)	1.08(0.37)	22.67(2.92)	8.20(0.39)	365(142)	28.5(56.3)	9.4(2.3)	0.90(1.27)	37.7(31.3)	148(183.6)	64.7(47.4)	6.4(40.8)
10	15	1.31(0.82)	1.11(0.56)	21.77(2.35)	8.24(0.32)	482(170)	74.6(94.6)	9.7(2)	0.63(0.8)	46.2(56)	89.1(89)	16.4(3.9)	27.8(71.4)
11	15	1.38(0.71)	1.32(0.45)	22.22(2.39)	8.19(0.34)	461(173)	83.6(91.3)	9.6(2.8)	0.71(1.07)	37.3(35.7)	146.9(109)	35.7(19.7)	16.2(25.3)
12	15	1.9(1.1)	1.46(0.61)	22.48(2.56)	8.25(0.37)	492(183)	69.4(95.9)	9.5(4.6)	0.82(1.29)	38.2(43)	80(54.7)	2.4(6.9)	23.3(33.9)

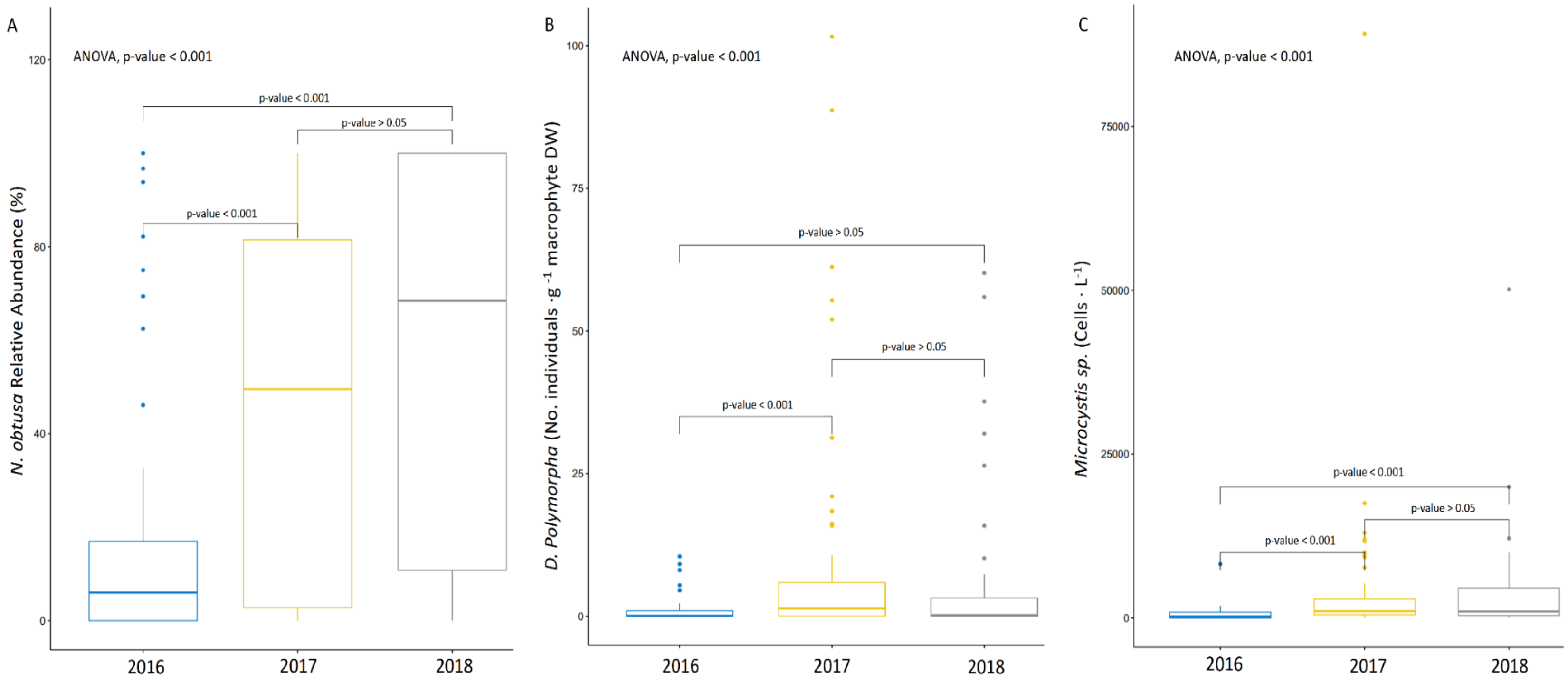


Figure 4.1. *Boxplots comparing seasonal abundance (May-September) of* ***A.*** N. obtusa, ***B.*** D. polymorpha, *and* ***C.*** Microcystis *spp. over the 3-year study period (n = 174).*

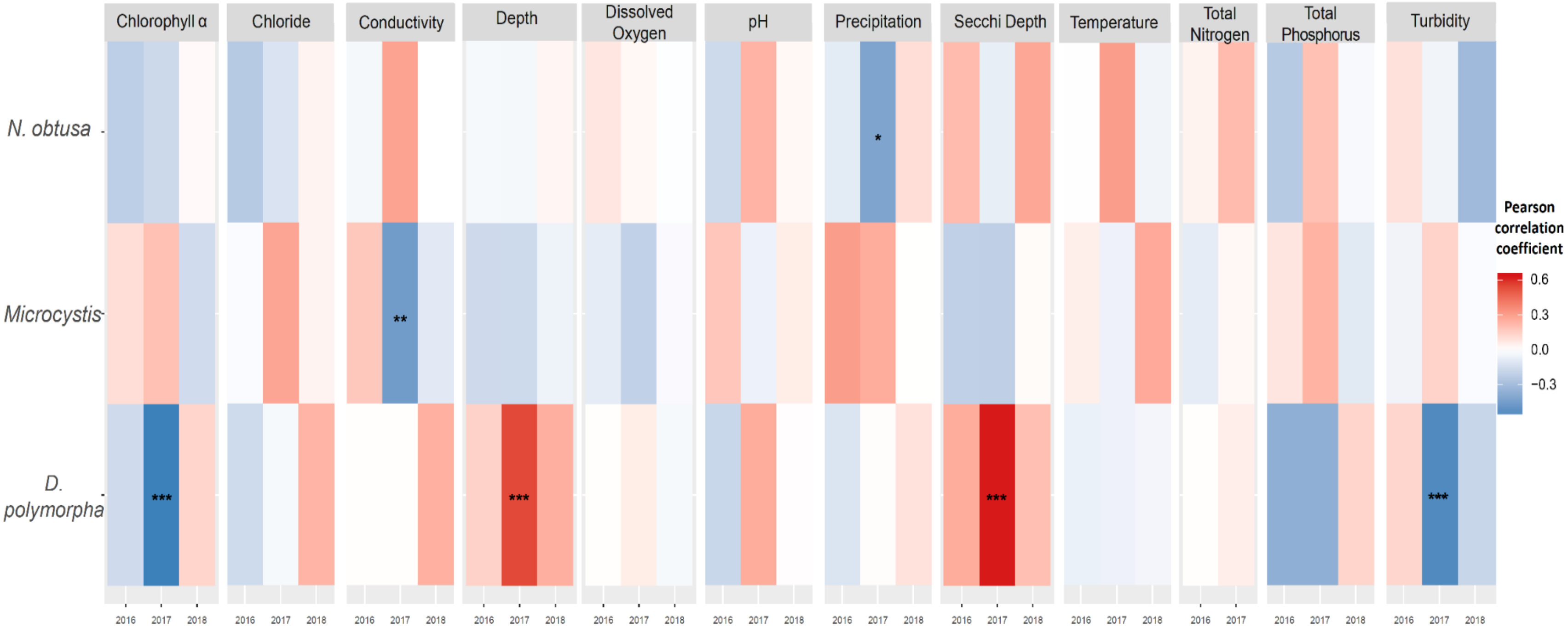


Figure 4.2. Pearson correlation heatmaps visualizing relationships between environmental variables and N. obtusa, D. polymorpha, *and* Microcystis *spp. (n=58). Asterisks (*) denote statistical significance (α = 0.05): * ≤ 0.05, ** ≤ 0.01, *** ≤ 0.001.*

The SEM results presented in Figure 4.3 show the direct positive and negative relationships between the abiotic and biotic variables included in the model. Depth was a statistically significant positive explanatory variable for all biotic components, including *N. obtusa*, *D. polymorpha*, and *Microcystis* spp. In addition to depth, two other abiotic factors (precipitation and chloride) were positive explanatory variables of *Microcystis* biomass. Total nitrogen was a negative explanatory variable for *N. obtusa* and *Microcystis* spp. Most interestingly, *N. obtusa* was the only biotic factor to serve as a positive explanatory variable for both *D. polymorpha* and *Microcystis* spp. Overall, the SEM explained 27 % (p-value < 0.05) of the variation in *Microcystis* spp. biomass in Lake Scugog.

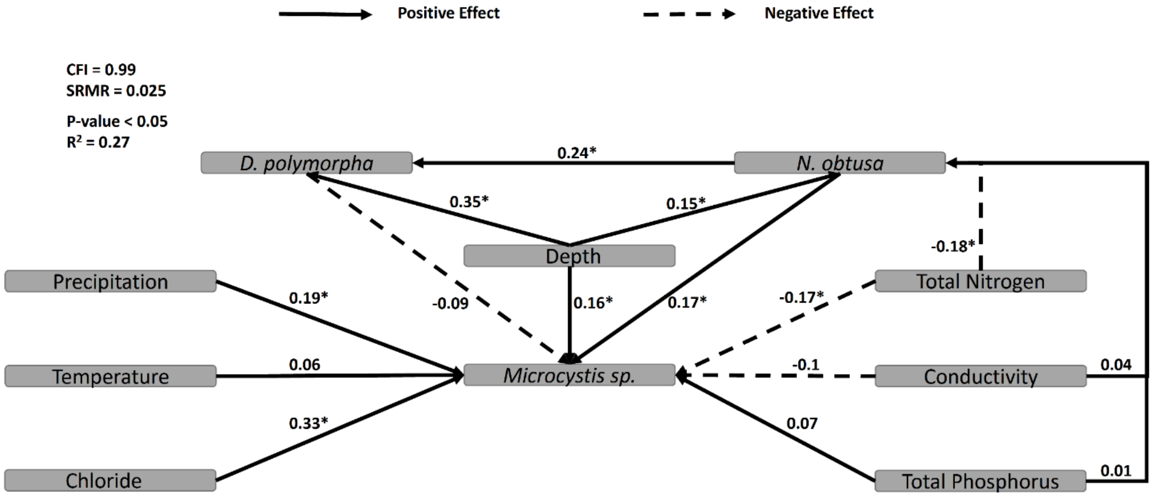


Figure 4.3. *Structural equation model (SEM) path diagram showing explanatory variables and their negative and positive effects on each other and* Microcystis *spp. (n = 174). Solid lines represent positive effects, and dashed lines represent negative effects. Path coefficients are provided for each factor in the model, and asterisk (*) signify statistically significant relationships (α = 0.05). CFI, comparative fit index, SRMR, standardized root-mean square residual.*

4.4 Discussion

Most inland lakes in Ontario are not monitored on a regular basis for water quality and biota, and as such, it is not known if the increases in *N. obtusa*, *D. polymorpha*, and *Microcystis* reported here reflect a region-wide trend. With respect to *Microcystis* spp. blooms, I could not find any reports on region-wide trends in Ontario since 2009 (Winter et al., 2011). Therefore, it is difficult to ascertain if the population increases documented in my study reflect a larger, regional pattern across the TSW. Increases in invasive species in Lake Scugog, however, does not bode well for downstream lakes in the TSW, especially when considering that boat traffic is a known vector for invasive species in the TSW (Kelly, Wantola, Weisz, & Yan, 2013). Thus, the popular navigation route between Lake Scugog and the TSW likely serves as an important conveyance pathway for *N. obtusa* and *D. polymorpha*.

As a first step in elucidating potential associations between water quality variables and each study taxon, Pearson correlation analyses detected some weak to moderate relationships, but for only one study year (2017) (Figure 4.2). This shows a large degree of inter-annual variation, which can influence the strength of potential drivers of taxa abundance. Even with differences in environmental conditions across years, the SEM was able to pull out direct drivers of each study taxon (Figure 4.3). Depth was a positive explanatory variable for all study taxa, which is quite interesting considering depth across sites only varied between 1-3 m. Depth largely infers available habitat space, so this finding suggests that increasing depth, even at a small scale, promoted the growth of all taxa. Precipitation and chloride were positive explanatory variables for *Microcystis* spp.

biomass, which is consistent with the literature. Several studies have shown a positive relationship between precipitation and *Microcystis* spp. blooms (Reichwaldt & Ghadouani, 2012; Pick, 2016), where the underlying mechanism is presumed to be increased nutrient loading with heavy rain events. In systems such as Lake Erie, it has been shown that the frequency and intensity of rainfall events can be an important model predictor of *Microcystis* spp. blooms (Michalak et al., 2013). Chloride at high enough concentration is toxic to algae, but *Microcystis* spp. are more tolerant of chloride than other freshwater species (Tonk, Bosch, Visser, & Huisman, 2007).

Interestingly, Total Nitrogen was a negative explanatory variable for both *N. obtusa* and *Microcystis* spp. in the SEM. Although nitrogen is an important nutrient for algae, elevated concentrations of specific forms of nitrogen (e.g., ammonia) can inhibit algal growth (Turpin, 1991). Alternatively, algal communities can shift in composition in response to shifting N:P due to physiological differences between species (Klausmeler, Litchman, Daufreshna, & Levin, 2004). Not knowing the form of nitrogen makes it difficult to ascertain the underlying mechanism of this negative relationship between Total Nitrogen and the study algae. Even so, further investigation is warranted to assess whether particular forms of nitrogen are playing a role in controlling nuisance algal growth in Lake Scugog.

Despite other studies showing clear positive relationships between water or sediment phosphorus and *Microcystis* spp. biomass (Jacoby, Collier, Welch, Hardy, & Crayton, 2000; Winter et al., 2011; Pick, 2016), my study did not show a strong link. This is not particularly surprising given the high water-column and sediment phosphorus concentrations, which reflect eutrophic to hypertrophic conditions (Carlson, 1977) in Lake Scugog. The relationship between algal biomass and phosphorus tends to asymptote at high phosphorus concentrations due to other limiting factors (Chow-Fraser, Trew, Findlay, & Stainton, 1994), which likely explains the lack of a significant relationship between *Microcystis* spp. and phosphorus in this study.

The only biotic factor found to serve as an explanatory variable for both *D. polymorpha* and *Microcystis* spp. biomass was *N. obtusa*. This finding is particularly noteworthy, because it is the first-time that a possible facilitative effect of *N. obtusa* on other species has been shown in a North American ecosystem. Anecdotal reports (e.g., Pullman & Crawford, 2010) have mentioned the proliferation of *D. polymorpha* on *N. obtusa*, likely because its calcified architecture is ideal for *D. polymorpha* attachment. Indeed, in this study, *D. polymorpha* was consistently found attached to *N. obtusa* specimens. Although other studies have shown *D. polymorpha* to have a positive effect on *Microcystis* spp. (Vanderploeg et al., 2001; Raikow, Sarnelle, Wilson, & Hamilton, 2004; Sarnelle, Wilson, Hamilton, Knoll, & Raikow, 2005), *D. polymorpha* did not play an explanatory role in this study.

The SEM delineated *N. obtusa* as being a direct explanatory variable of *Microcystis* spp. biomass in Lake Scugog. I hypothesize that *N. obtusa* could facilitate *Microcystis* spp. growth in several ways. One mechanism relates to the way *N. obtusa* can alter sediment water chemistry. It has been suggested that when forming dense stands, *N. obtusa* can create hypoxic to anoxic conditions at the sediment-water interface, as demonstrated in chapter 3 (Pullman & Crawford, 2010). When charophytes like *N. obtusa* create anoxic conditions, this stimulates internal phosphorus loading from the sediments (Kufel & Kufel, 2002). A significant relationship between sediment phosphorus and *Microcystis* spp. was not captured in this study, but *Microcystis* spp. resting cells are known to benefit from their proximity to sediment phosphorus in other ecosystems (Brunberg & Boström, 1992; Nalewajko & Murphy, 2001).

Another potential facilitation mechanism involves allelopathy. Charophytes like *N. obtusa* are known to exude bioactive substances that inhibit select phytoplankton species, in order to minimize competition for nutrients and light (Berger & Schagerl, 2003; Berger & Schagerl, 2004). Studies have shown that *N. obtusa* can produce and exude allelopathic substances that inhibit some aquatic plants and algae, while facilitating others (Blindow, Hargeby, & Hilt, 2014). In one particular study, bioactive exudates from *N. obtusa* inhibited several phytoplankton taxa, but not *Microcystis aeruginosa* (Berger & Schagerl, 2004). Therefore, it is possible that *Microcystis* spp., being tolerant of both chloride and allelopathic compounds produced by *N. obtusa*, can erupt in blooms in Lake Scugog because competition from other phytoplankton has been reduced.

In summary, I have documented increases in *D. polymorpha* and *Microcystis* spp. in a lake where historically, these species were not reported at nuisance levels. Applying an SEM approach allowed me to evaluate a network of relationships between abiotic and biotic variables in a relatively complex dataset. The resulting model explained 27% of the variation in *Microcystis* spp. biomass, highlighting key abiotic (depth, chloride, precipitation, Total Nitrogen) and biotic (*N. obtusa*) drivers. SEM also revealed a possible facilitative role for *N. obtusa* in supporting both *D. polymorpha* population growth and

Microcystis spp. blooms in Lake Scugog. These findings raise concerns for other lakes in the region, including Lake Simcoe and the Kawartha Lakes, which have all been invaded by *N. obtusa*. Even though nutrients and climate change are established drivers of *Microcystis* spp. blooms in other lakes, my results indicate that *N. obtusa* may be an additional factor to consider. Further studies across lake ecosystems that have both *N. obtusa* and *D. polymorpha* should be completed to confirm the relative role of each invasive species on *Microcystis* spp. bloom formation.

Chapter 5. Establishing the Distribution and Habitat Characteristics of *Nitellopsis obtusa* in Ontario Lakes.

5.1. Introduction

Nitellopsis obtusa (Desv.) J. Groves, 1919, is an ecorticated charophyte species native to Eurasia. First documented in North America along the St. Lawrence River in 1974 (Karol & Sleith, 2017). How *N. obtusa* was introduced to North America is not known, but unintentional introduction via the ornamental gardening trade and trans-oceanic shipping is suspected (Kay & Hoyle, 2001; Padilla & Williams, 2004). Although known to be in the lower Great Lakes since the 1970s, it was only about a decade ago that first reports of *N. obtusa* in inland lakes of the United States began to emerge (Larkin et al., 2018). In Canada, *N. obtusa* was reported as early as 2008 in Lake Simcoe (Ginn et al., 2021), with the first documentation of establishment in Lake Scugog in 2015 (Chapter 2). The forty-year latency between initial introduction and the surge of reports in inland lakes is not well understood, but may be due to its frequent misidentification as *Chara* spp. during early colonization and establishment (Larkin et al., 2018).

The common name of starry stonewort is due to conspicuous white star-shaped bulbils (Figure 1.1), which serve as asexual reproductive and hibernation structures. Members of the Characeae can undergo both sexual and asexual reproduction, depending on environmental conditions (Larkin et al., 2018). Within its native range, *N. obtusa* tends to reproduce primarily through vegetative propagules (Migula, 1897). When *N. obtusa* colonizes shallower waters, a shift to sexual reproduction has been observed (Krause, 1985). Although *N. obtusa* bulbil viability is not known, they can be relatively short-lived (a few months) (Bonis & Grillas, 2002; Boedeltje, Bakker, Bekker, Van Groenendael, & Soesbergen, 2003), or persist for several years (Bonis & Grillas, 2002). Bulbils can only be found within established beds of *N. obtusa*, which can disrupt identification during early establishment (Bonis & Grillas, 2002). When bulbils are lacking, *N. obtusa* can be identified through its disheveled look, with long slender thalli heavily encrusted in marl, composed of calcium bicarbonate (Blindow, 1992).

In its native range, *N. obtusa* is valued as habitat and food for native fish, but it is also classified as threatened or endangered (Wildlife and Countryside Act, 1981; Blaženčić et al., 2006; Caisová & Gabka, 2009; Kabus & Mauersberger, 2011; Auderset Joye & Schwarzer, 2012; Kato et al., 2014). In contrast, *N. obtusa* poses an ecological threat to North American freshwater ecosystems, including nuisance growth and the displacement of native species (Pullman & Crawford, 2010; Hackett et al., 2014; Brainard & Schulz, 2017). In shallow lakes and coastal wetlands, *N. obtusa* can grow taller than other Characeae species (Larkin et al., 2018). The formation of dense benthic mats of *N. obtusa* displaces other macroalgae and vascular macrophytes (Pullman & Crawford, 2010; Brainard & Schulz, 2017; Ginn et al., 2021). Dense beds of *N. obtusa* effectively displace other macrophytes, but they also may serve as an effective nutrient sink, which limits nutrient availability to other taxa (Blindow, 1992). This has important implications for benthic habitat structure, since submerged macrophytes provide substrate, food, and refuge for most members of the aquatic community (Noordhuis, Van der Molen, & Van den Berg, 2002; Van Nes, Scheffer, Van den Berg, & Coops, 2003).

To improve our understanding of the habitat preferences of *N. obtusa* in eastern North American lakes, I selected 60 candidate lakes along a natural geological transition zone in south-central Ontario, Canada. The study area includes a region known colloquially as The Land Between, because it reflects a south to north shift in limestone-dominated (St. Lawrence Lowlands) to granite-dominated (Canadian Shield) surficial geology. The Land Between geological transition zone is a mosaic of both bedrock types, which strongly influence the edaphic and physiographic characteristics of the region. As such, lakes situated in the transition zone reflect gradients in water hardness and nutrients. Since *N. obtusa* requires ecosystems with a minimum concentration of calcium between 15 – 25 mg · L^{-1}, documenting *N. obtusa* distribution along a natural calcium gradient in the same geographic and climatic region offered an ideal study location (Stroede, 1937). Additionally, the Land Between has varying land-use type and intensity (e.g., agriculture, urban development) that contribute to anthropogenic water quality gradients across the region. Based on this study design, I was able to not only document the distribution of *N. obtusa* in Ontario's inland lakes for the first-time, but I was also able to determine the key environmental factors influencing *N. obtusa*'s distribution using an ecological niche model approach.

5.2 Methods

5.2.1 Study Design

A synoptic survey of 60 lakes spanning a large geographic region (~65,000-km^2) in Ontario, Canada, was conducted over a constrained three-week period from August 1- 22, 2019. Due to the mix of underlying bedrock and unique physiographic features in the study region, there is a distinct water hardness gradient that varies from soft water to very hard water. Since calcium carbonate is required for *N. obtusa* growth, and hard water lakes are known to be a preferred habitat for certain charophytes, selecting lakes along a broad calcium gradient within the same geographic and climatic region was expected to reveal the relative importance of calcium and water hardness to *N. obtusa'*s invasion success.

Candidate lake selection was aided by Geographic Information System (GIS) analyses (QGIS Development Team, 2016) to minimize candidates with surface connectivity between lakes. Out of the 60 lakes in the study, only 3 were indirectly connected via surface water flow in the same catchment. Historical lake-calcium data garnered from a variety of sources (The Land Between Charity, Kawartha Conservation, and the Ontario Ministry of Environment, Conservation, and Parks) were used to ensure a broad calcium gradient was captured in the final set of study lakes.

Based on a summary of previously published calcium concentrations where *N. obtusa* was found in its invaded range (Larkin et al., 2018), candidate lakes were divided into three groups: calcium concentrations below threshold (0 - 20 $mg \cdot L^{-1}$), optimal conditions (20 - 40 $mg \cdot L^{-1}$), and above threshold (40 - 65 $mg \cdot L^{-1}$). Within these categories, 20 candidate lakes were selected to ensure a balanced study design representing the entire calcium gradient. The historical calcium gradient present throughout the candidate lakes can be observed in Figure 5.1.

The lowest lake calcium concentration was 0.43 mg · L^{-1}, and the highest was 83.7 mg · L^{-1}. The final study lake locations are presented in Figure 5.2.

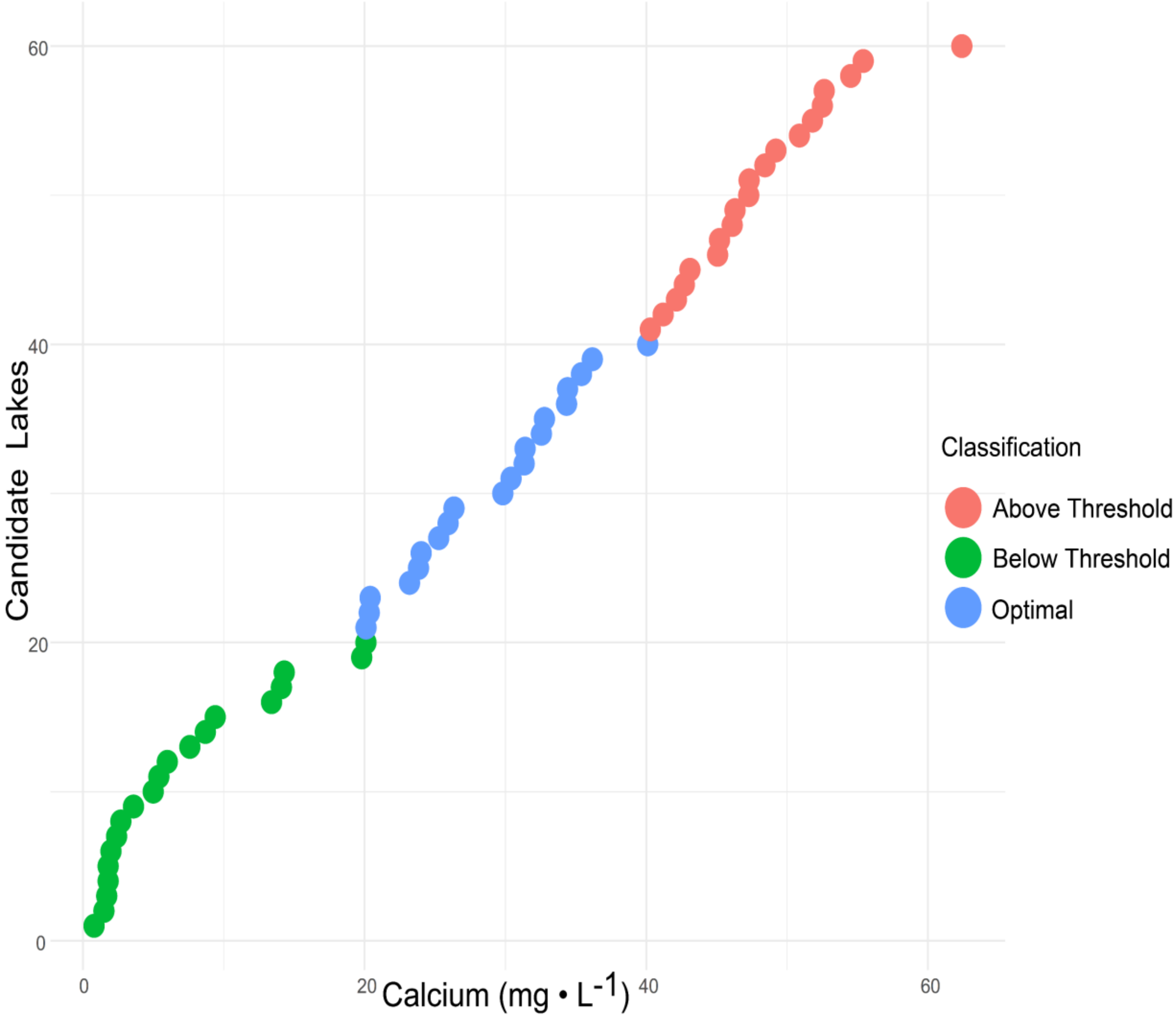


Figure 5.1*. Dotplot of historical calcium concnetrations for the 60 candidate lakes selected within this study. Colour corresponds to one of the three categorical classificaitons each lake was placed into. Green represents below threshold (< 20 mg · L^{-1}), blue corresponds to the optimal concentration range (20-40 mg · L^{-1}) and salmon corressponds to above threshold (> 40 mg · L^{-1}).*

5.2.2. Sampling Collection and Field Measurements

A consistent sampling effort occurred at four sites in each lake along a transect starting adjacent to the boat launch area. Sampling near the boat launch was intentional based on previous work, which showed an association between *N. obtusa* and proximity to boat launch sites (Midwood et al., 2016). However, sampling did not occur directly in a boat lane, as frequent traffic and harvesting would introduce bias into the samples, instead I sampled in the area adjacent to the boat launch. Sampling sites were along a transect which was based on known depth-profile bathymetry between 0.5 to 15 m, and ran perpendicular to the shoreline. Depth bathymetry was gathered from open-source bathymetric maps and the Anglers Atlas (https://www.anglersatlas.com). Depth was verified using a depth finder as described in Chapter 2. The distance between sampling sites in each lake was determined using GPS coordinates in QGIS 7.4.2 (QGIS Development Team, 2016). The distance from boat launch variable corresponds to the measured distance between sampling sites, from the first site. Environmental parameters that were measured in the field were assessed as described in Chapter 2. In instances where conductivity readings were high ($> 900\ \mu s \cdot cm^{-1}$), additional readings were taken back at the lab with a separate YSI 6-series multiparameter probe to confirm measurement accuracy.

Water samples were collected with a Van Dorn sampler at 0.5 m across all sampling locations. Samples were decanted into four 50 mL conical tubes. All tubes were previously acid washed and handled as described in Chapter 2. Macrophytes were collected using the lake rake method described in Chapter 2.

5.2.3. Sample Processing

Samples were transferred to the laboratory and processed as described in Chapter 2. Total organic carbon (TOC) and total nitrogen (TN) were determined following a combustion catalytic oxidation method using a TOC5000 series TOC analyzer (Shimadzu, Kyoto, Japan) at York University, Toronto, Canada. Water samples earmarked for cation analyses were shipped to an accredited analytical lab (SGS Canada, Lakefield, Ontario). Cations that were investigated in this analysis include: calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), iron (Fe), and manganese (Mn). Analyses for TP, TN, and cation suites were done on unfiltered water samples.

4.2.4 Statistical Analyses

All statistical analyses were performed using the open-sourced software R version 3.5.1 (R Development Core Team 3.0.1., 2013). Environmental and biological parameters were log-transformed to comply with parametric assumptions of normality. In order to assess statistical differences for each of the 16 environmental variables between sites with confirmed *N. obtusa* presence and those that did not have *N. obtusa*, a generalized linear mixed model (glmm) using binomial error was constructed with the glmmTMB package (Magnusson et al., 2020). Furthermore, since environmental conditions overlapped between sites with and without *N. obtusa*, a principal component analysis was done to assess co-variation of variables between site categories (i.e. presence versus absence) using the packages FactoMineR, factoextra, and ggplot2 (Lê, Josse, & Husson, 2008; Wickham, 2016; Kassambara & Mundt, 2017). Environmental parameters were standardized and corresponding ellipses depicting multivariate normality were displayed. A permutational analysis of variance was used to distinguish if a significant difference existed between site categories present within the principal component analysis using the vegan package (Oksanen, 2017).

To determine the ecological niche of *N. obtusa* in Ontario, boosted regression tree (BRT) modelling was performed. This method differs from traditional parsimonious models by using regression and decision trees, while boosting combines several simple models together to improve predictive performance (Elith et al., 2006; Leathwick, Elith, Francis, Hastie, & Taylor, 2006; Leathwick, Elith, Chadderton, Rowe, & Hastie, 2008). Boosting within a BRT model originates from machine learning (Schapire, 2003), but has been accepted within the statistical community as an advanced form of regression (Friedman, Hastie, & Tibshirani, 2000). By fitting multiple trees, BRT modelling overcomes limitations of single tree models (Elith, Leathwick, & Hastie, 2008). Furthermore, the process of building a BRT is stochastic, and this stochasticity improves predictive performance while reducing the variation observed in the final model (Friedman, 2002). During model fit, new trees are built upon previously fitted trees, which increases the models focus on the hardest observations to predict, which in turn provide a BRT that supports ecological insight (Elith et al., 2008). This statistical approach was chosen because it can fit complex non-linear relationships, and the data used does not need to be transformed. BRT also has a high predictive capability when compared to other statistical approaches (Elith et al., 2008). BRT modelling has been used to demonstrate which environmental factors were associated, and useful in predicting invasive species (Coutts, van Klinken, Yokomizo, & Buckley, 2011; Altartouri, Nurminen, & Jolma, 2014), including the presence of *N. obtusa* in a coastal wetland in Lake Ontario (Midwood et al., 2016), and predicting the distribution of *N. obtusa* in the Midwest of the United States (Muthukrishnan, Sleith, Karol, & Larkin, 2018).

A random subset of 180 sampling points for the 16 environmental parameters were used to fit a series of BRT models to determine the best value for each of the required parameters (bag fraction (BF), learning rate (LR), and tree complexity (TC)). BRT models were constructed using TC (3, 5, 7, 10, 20), LR (0.01, 0.005, 0.001), and BF (0.5, 0.6, 0.75). The resulting models were tested with the remaining 60 sampling points, and the model with the highest area under the curve (AUC) and highest cross-validation coefficient (CV) was investigated further.

Optimal parameters for BRT model construction were a TC of 10, a LR of 0.001, and a BF of 0.75. This model resulted in an AUC of 1 and a CV of 0.9. To create a less complex model for *N. obtusa*, the BRT model was simplified using *gbm.simplify* function as described by Elith et al., (2008). Upon simplification, five environmental parameters were removed from the model which included Fe, TOC, conductivity, TN, and DFBL. The analysis and construction of the BRT model for *N. obtusa* was done following Elith et al., (2008) using the dismo package (Hijmans & Elith, 2013).

5.3 Results

Summary statistics for each lake can be found in appendix B, Table B1. Overall, the presence of *N. obtusa* was documented at 37 sites in 19 of the 60 study lakes (Figure 5.2).

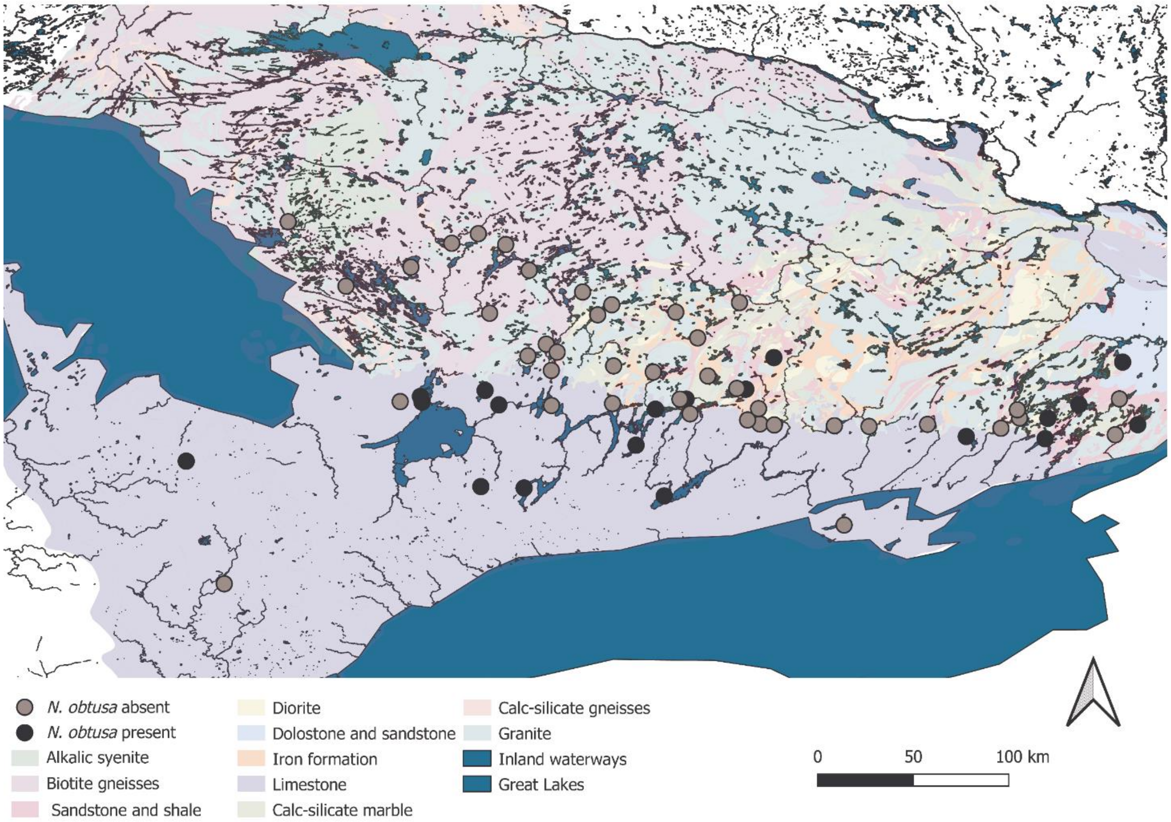


Figure 5.2. *Map of candidate lakes with underlying geology. Candidate lakes are shown as circles, where Black circles denote lakes with* N. obtusa, *and grey circles are lakes where* N. obtusa *was not documented. Underlying geology shape files were obtained from the Ontario Geological Survey (2011), which represents a 1: 250 000 scale in bedrock geology.*

Lakes that had *N. obtusa* present include Big Cedar, Buck, Camden, Canal, Charleston, Chemong, Couchiching, Dalrymple, Dickey, Eugenia, Loughborough, Lower Buckhorn, Opinicon, Otter, Rice, Scugog, Simcoe, Wagner, and West-Twin. Eleven of the study lakes had no macrophytes present at the time of sampling. *Nitellopsis obtusa* presence occurred over a relatively broad Ca concentration (15.9 - 57.9 mg · L^{-1}). Six out of the 16 environmental variables measured were significantly different between sites with and without *N. obtusa* including: depth, Secchi, Ca, K, Na, and Mg (glmm, p-value < 0.05) (Figure 5.3).

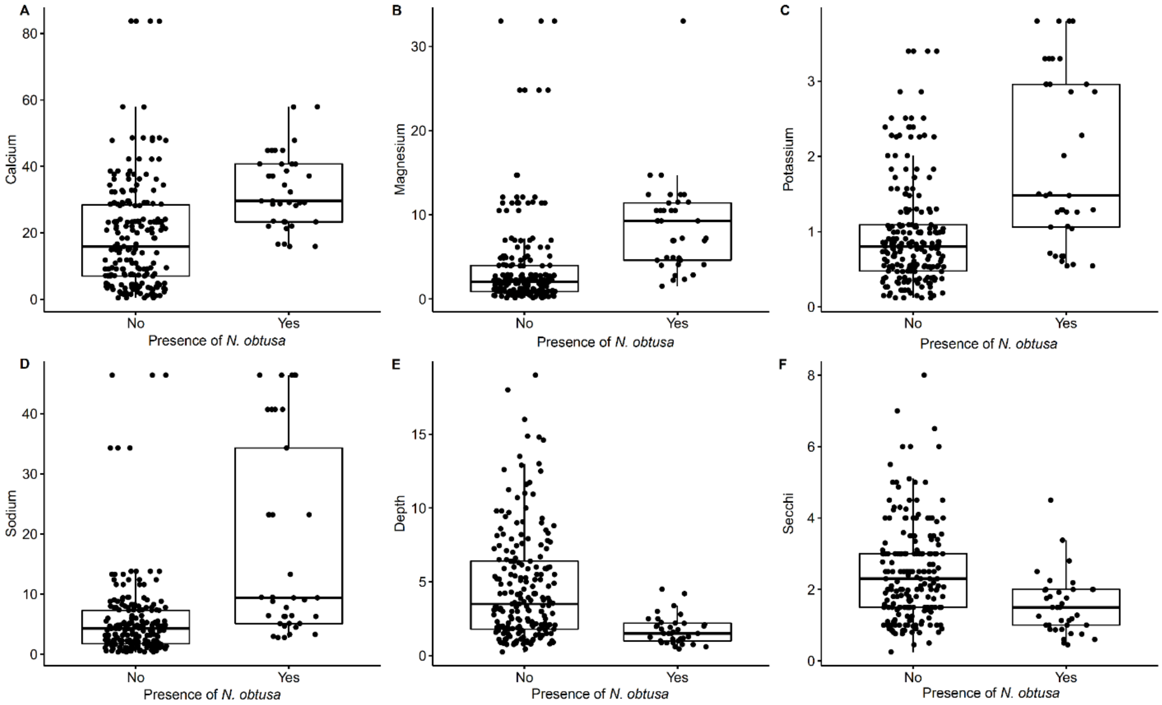


Figure 5.3. *Boxplots comparing environmental parameters that were determined to be significantly different with respect to* N. obtusa *presence (yes, n = 37) and absence (no, n = 203) using a general linear mixed model with binomial error (p-value < 0.05). Parameters include* ***A****. Calcium (mg · L^{-1})* ***B.*** *Magnesium (mg · L^{-1})* ***C.*** *Potassium (mg · L^{-1})* ***D.*** *Sodium (mg · L^{-1})* ***E.*** *Depth (m)* ***F.*** *Secchi (m). One sample per lake was sent for cation analysis (n = 60), while environmental parameters had a measurement at each of the four sites (n = 240).*

Environmental concentrations of Ca, K, Na, and Mg were significantly higher at sites with *N. obtusa*. In contrast, depth, and Secchi depth were significantly lower at sites with *N. obtusa* (Figure 5.3). A principal component analysis demonstrated that pH, nutrients (TP and TN), DFBL, TOC, Na, Mg, K, and Ca were positively associated with *N. obtusa* presence (Figure 5.4). In contrast, variables such as depth, Secchi, Fe, and conductivity were negatively associated with *N. obtusa* presence (Figure 5.4). The chemical and morphometric properties between site classification differed significantly, for sites with *N. obtusa* presence (permanova, p-value < 0.05) (Figure 5.4).

Figure 5.4. *Principal component analysis using standardized environmental parameters showing differences between sites with (n = 37)* N. obtusa *present to those without (n = 203). Relative contribution of each of the environmental parameters are visualized along a gradient, as well as vector transparency. Ellipses for each of the categorical groups represent multivariate normality. TP = total phosphorus, TN = total nitrogen, TOC = total organic carbon, DFBL = distance from boat launch.*

The final ecological niche model (BRT) included 9 of the 16 environmental variables and had an AUC score of 0.99 (Figure 5.5). This model consisted of 2700 trees and accounted for 91% of the variation of *N. obtusa* in the study lakes. Interestingly, depth and potassium had the highest relative influence on *N. obtusa* in the model, contributing 43.5% (Figure 5.5). In contrast, calcium had a relatively small influence (5.5 %) and was the lowest contributing parameter (Figure 5.5). Depth, magnesium, potassium, and total phosphorus contributed 64.6 % of the relative influence out of the nine factors defining the ecological niche for *N. obtusa* in the study lakes (Figure 5.5). The ecological niche model infers that *N. obtusa* prefers habitats with shallower depths and higher concentrations of magnesium, potassium, and sodium in the study region (Figure 5.5). *Nitellopsis obtusa* also seems to prefer habitats with lower manganese concentrations, and lower Secchi depths (Figure 5.5).

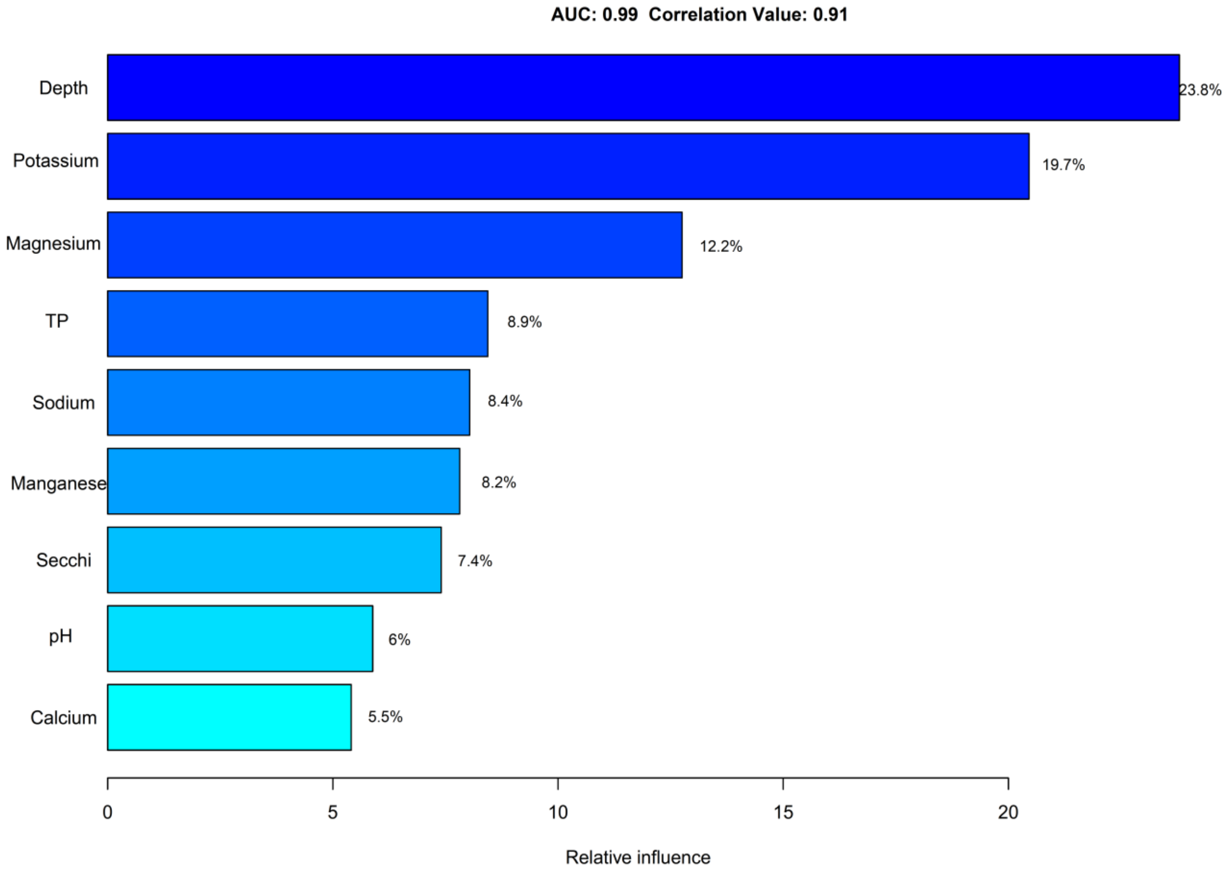


Figure 5.5. *Relative influence depicting the contribution each environmental variable has on* N. obtusa *within the simplified ecological niche model. AUC corresponds to the area under the curve obtained during model testing.*

5.4 Discussion

Commonly, most inland lakes in Ontario are not systematically monitored for invasive species, thus non-native invaders like *N. obtusa* can go unnoticed until it dominates the community it has invaded. The fact that I documented *N. obtusa* in one third of the study lakes is in stark contrast to what has been reported in Ontario. Most reports refer to *N. obtusa* invasion in lakes directly connected to the Trent Severn Waterway (TSW), as in Chapter 2 (Larkin et al., 2018). For the first-time, I report here that *N. obtusa*'s distribution across central-Ontario extends beyond lakes directly connected to the TSW (Figure 5.2). With other lakes in Ontario having favorable habitat conditions to support *N. obtusa* establishment and growth, management is essential. Therefore, it is my intention that the ecological niche model, be made available for use. Applying the forecasting component of the BRT, using long term water quality monitoring data, can help various stake holders to identify vulnerable areas for *N. obtusa* invasion, thus directing management and future research endeavours.

Dispersal and establishment of *N. obtusa* within the Great Lakes Basin is poorly understood. Generally, invasive macrophytes can be transported to new ecosystems by viable propagules or vegetative fragments (Reynolds, Miranda, & Cumming, 2015; Green, 2016). Within the native range, propagules of charophytes have been known to be dispersed through epizoochory (Bonis & Grillas, 2002). However, there is mounting evidence that the spread of *N. obtusa* into inland lakes is driven by watercraft movement and deployment across the Great Lakes Basin (Midwood et al., 2016). Sites where *N. obtusa* was detected were closer to boat launches along sampled transects (Figure 5.3). Generally, removing plant material can be an effective strategy for mitigating transport of invasive macrophytes, although recreational boaters often fail to follow clean and drain instructions (Rothlisberger, Chadderton, McNulty, & Lodge, 2010; Cimino & Strecker, 2018). Furthermore, Glisson et al., (2020) found that clumps of *N. obtusa*, similar to what is found on watercraft trailers, can remain sufficiently wet for days. Given the popularity of boating culture in Ontario, and the proximity of the study lakes to the Trent Severn Waterway, it is likely that recreational boaters are serving as a vector of *N. obtusa* spread throughout Ontario's inland lakes.

Pullman & Crawford (2010) found that *N. obtusa* usually colonizes shallower water (0.5 – 1.5 m) before moving to deeper sites as part of its invasion pattern. The highest biomass of *N. obtusa* detected in this study was from sites less than three meters deep (Figure 5.3). Within its native range, *N. obtusa* is found in both deep and shallow ecosystems (Janauer et al., 2010; Korsch et al., 2013), colonizing depths upwards of 65 m (Spence, 1982; Kufel & Kufel, 2002). Depths reported for the invaded range, where *N. obtusa* has become established, range from 0.5 to 7 meters (Geis et al., 1981; Sleith et al., 2015). Considering the prevalence of *N. obtusa* at shallower sites and sites closer to boat launches in this study, it is possible that *N. obtusa* is in the early stages of invasion in Ontario inland lakes. This coincides well with emerging reports, starting in 2015, and recent conclusions of other authors (e.g., Romero-Alvarez, Escobar, Varela, Larkin, & Phelps, 2017).

In its native range, *N. obtusa* has been classified as threatened to critically endangered due mainly to eutrophication (Watanabe, 2005). Further, *N. obtusa* has been classified as an indicator species for oligotrophic to mesotrophic ecosystems (Kasaki, 1962). Yet in this study, *N. obtusa* was found at sites with higher TP concentrations in the meso- to eutrophic range. Further studies are necessary to determine the key role of nutrients in *N. obtusa* invasion and spread, but it is clear from my study that *N. obtusa* is tolerant of a broad range of nutrient concentrations, and TP is a moderate, but statistically significant predictor variable in the BRT ecological niche model.

Until this study, calcium concentration has been denoted as a primary driving factor in *N. obtusa* establishment (Olsen, 1944; Pullman & Crawford, 2010; Auderset Joye & Rey-Boissezon, 2015; Rey-Boissezon & Auderset Joye, 2015). *Nitellopsis obtusa* was indeed found to be associated with higher calcium concentrations in the study lakes, but most interestingly, *N. obtusa* was also found to be associated with higher concentrations of other cations, including potassium, sodium, and magnesium. As far as I know, no other studies have investigated the role of other cations in *N. obtusa* distribution and establishment. In the BRT analysis, calcium was determined to be a minor predictor variable compared to potassium, sodium, and magnesium. Given the broad range of cation concentrations captured in this study, I serendipitously was able to tease out the relative importance of cations in predicting *N. obtusa* presence/absence across the study region.

It is not clear why cations such as potassium and magnesium would play a stronger role than calcium in *N. obtusa* distribution. Katsuhara & Tazawa (1988) did find that *N. obtusa* concentrated potassium in the cytoplasm as a mechanism of salt tolerance. However, *N. obtusa* samples collected from an oligohaline lake revealed the inability to accumulate potassium within the cytoplasm to regulate turgor (Winter et al., 1999). More recent work has explored why *N. obtusa* differs in turgor regulation compared to other charophytes, and perhaps this relates to differential cation requirements (Kisnieriene, Lapeikaite, Pupkis, & Beilby, 2019). Interestingly, the ecological niche model also indicated that *N. obtusa* presence was associated with lower levels of manganese. Although no studies have yet investigated the biological role that manganese plays in *N. obtusa* biology, manganese has been shown to have an inhibitory effect at higher concentrations for other charophytes (Lambert & Davy, 2011). Further studies are required to determine what ecophysiological role these cations play in *N. obtusa* growth and establishment.

Although earlier studies have modelled the distribution of *N. obtusa* in the Great Lakes Basin, they are limited to either a specific coastal area (e.g., Midwood et al., 2016) or defined geographic region (e.g., Muthukrishnan et al., 2018). In fact, Muthukrishnan et al., (2018) noted that ecological niche models based on one area should not be used for predicting occurrence in external regions. Since I was able to exploit a geological transition zone in one large geographic region across south-central Ontario, this served as a natural experiment to assess the relative influence of wide-ranging habitat characteristics within the same climatic zone. As such, I was able to confirm that habitat characteristics such as calcium concentration do indeed play a role in *N. obtusa* presence, but it was found not to be a particularly important predictor of *N. obtusa* presence. Ultimately, I have been able to show for the first-time on the Canadian side of the Great Lakes Basin what the possible physical and chemical constraints of *N. obtusa* invasion are, and these findings will inform the development of both a policy and guidance framework for *N. obtusa* management that currently does not exist in Ontario.

Chapter 6. Evaluating the impact of *Nitellopsis obtusa* on macrophyte diversity and community composition in lakes across a heterogeneous geological landscape.

6.1 Introduction

Nitellopsis obtusa is a non-native species in North America belonging to the Characeae family. Generally, Characeae are referred to as stoneworts or brittleworts, due to the common marl encrustation of thalli, which includes 400 species worldwide (Blaženčić et al., 2006). Unfortunately, over 80% of these taxa, including *N. obtusa*, are now considered endangered in their native ranges due to climate change and persistent anthropogenic stressors (Blaženčić et al., 2006). The native distribution of *N. obtusa* extends from Britain through most of Europe and Asia to Japan. Throughout its native range, *N. obtusa* is classified as threatened or endangered (Hamann & Garniel, 2002; Blaženčić et al., 2006; Caisová & Gabka, 2009; Johansson et al., 2010; Kabus & Mauersberger, 2011; Auderset Joye & Schwarzer, 2012; Korsch et al., 2013; Kato et al., 2014).

How *N. obtusa* came to North America is not known, however, it has been conjectured that accidental introduction occurred via the ornamental gardening trade and trans-oceanic shipping (Kay & Hoyle, 2001; Padilla & Williams, 2004). The first record of *N. obtusa* within North America is from a specimen catalogued at the New York Botanical Garden, New York, USA. This specimen was collected along the St. Lawrence River near Montreal, Quebec, in 1974, but was first misidentified as *Chara* spp., a common occurrence for *N. obtusa* (Karol & Sleith, 2017). In Ontario, *N. obtusa* has been documented in Lake Simcoe as early as 2008 (Ginn et al., 2021), within Presqu’ile Bay of Lake Ontario since

2015 (Midwood et al., 2016), and in Lake Scugog as early as 2015 (Chapter 2). Recently, *N. obtusa* was confirmed in 19 lakes across south-central Ontario (Chapter 5).

The common name of Starry Stonewort originates from the conspicuous white star-shaped bulbils. These bulbils arise from the rhizoid nodes and monofilament, and serve as hibernation cells and asexual reproductive structures (Bharathan, 1987). Despite the presence of bulbils being an important diacritical feature for distinguishing between Characeae, identification solely on the presence of bulbils may prove difficult, as bulbils are only found within established beds of *N. obtusa* (Bonis & Grillas, 2002). When bulbils are absent, *N. obtusa* can be identified through its characteristic tangled-mass appearance. Thalli are long and slender encrusted in a thick marl, composed of calcium bicarbonate and phosphorus (Blindow, 1992). Branchlets are arranged in 5-8 per whorl, and grow upwards of 9 cm (Karol & Sleith, 2017). Furthermore, in spite of other Characeae giving off a characteristic sulfurous aroma, *N. obtusa* does not exude a prominent smell (Pullman & Crawford, 2010; Hackett et al., 2014). Generally, *N. obtusa* resembles *Nitella* spp. and *Chara* spp. due to the whorled branches that arise from stem nodes. The high degrees of similarity between *N. obtusa* and native Characeae make identification difficult, leading to repeated misidentification and underreporting of this invasive taxon.

The threat that *N. obtusa* poses towards North American freshwater ecosystems is thought to be similar to other non-native macrophytes (Pullman & Crawford, 2010; Hackett et al., 2014; Brainard & Schulz, 2017). Pullman and Crawford (2010) concluded after several years of lake monitoring that *N. obtusa* was the most aggressive macrophyte ever observed within Michigan, United States. The invasion biology of *N. obtusa* in North

America is largely unknown. Few studies have documented community- to ecosystem-level impacts of *N. obtusa,* from early colonization to full establishment. However, some studies have observed displacement of other macroalgae and vascular macrophytes upon *N. obtusa* establishment (Pullman & Crawford, 2010; Brainard & Schulz, 2017; Ginn et al., 2021).

To improve our understanding of *N. obtusa* invasion biology and associated impacts on macrophyte communities, I surveyed 60 lakes across Ontario, Canada to (1) document the occurrence and abundance of *N. obtusa* and (2) evaluate macrophyte community composition and diversity as a function of environmental conditions and *N. obtusa* biomass. The study area stretches across a notable geological gradient, where the limestone-dominated bedrock of the St. Lawrence lowlands shifts to the granite-dominated bedrock of the Precambrian shield. By sampling over such a geologically diverse landscape, I was able to capture a wide range of habitats that helped to elucidate not only environmental drivers of macrophyte community structure, but also the biotic interactions between macrophytes within the study lakes.

6.2 Methods

6.2.1 Sample Collection and Processing

A survey of 60 lakes spanning a large geographic region (~ 65,000-km^2) in Ontario, Canada was conducted within a narrow time frame (August 1- 22, 2019) to minimize seasonal effects. Candidate lakes that were investigated are outlined in Chapter 5 (Figure 5.2).

Briefly, lake selection was based on capturing a broad water hardness gradient using historical calcium data collected from a variety of sources (The Land Between Charity, Kawartha Conservation, and the Ontario Ministry of Environment, Conservation, and Parks), while also minimizing surface connectivity through Geographic Information System (GIS) analysis (QGIS Development Team, 2016). Water and macrophyte communities were sampled along a depth transect as described in Chapter 5. All environmental and biological samples were collected as described in Chapters 2 and 5.

6.2.2 Statistical Analysis

Statistical analyses were done using the open-sourced platform R version 4.0.3 (R Core Team, 2019). To ensure colour-palette accessibility, figure colours were selected and applied using the R package RColorBrewer (Neuwirth, 2014). Macrophyte taxa abundance was averaged for each lake (n = 4). Simpson's diversity (1-D) was calculated for each lake using the vegan package (Oksanen et al., 2013). *Nitellopsis obtusa* biomass was removed from the macrophyte community matrix prior to Simpson diversity calculations to avoid confounding effects, as *N. obtusa* presence was used as a possible explanatory variable. Diversity for each candidate lake was split into three categorical classifications: No *N. obtusa* presence, low *N. obtusa* presence (< 50 % relative abundance), and high *N. obtusa* presence (> 50 % relative abundance). A Kruskal-Wallis H test was used to determine whether Simpson's diversity significantly differed between each categorical classification. Redundancy analysis (RDA) was used to visualize the variation in macrophyte community structure as described in Chapter 2. An RDA was chosen based on the results of a detrended correspondence analysis, which found the longest gradient to be < 3. Biplot scores are representative of the weighted sums of species, as this measure is robust to noise within environmental variables. RDA analysis was conducted using a Bray-Curtis dissimilarity matrix. The resulting RDA was prepared using the packages stringr (Wickham, 2019), dplyr (Wickham & Francois, 2016), tidyr (Wickham & Henry, 2019), vegan (Oksanen et al., 2013), and plotted in ggord (Beck, 2016). A permanova was used to distinguish whether a significant difference was evident within macrophyte community composition based on *N. obtusa* presence (Oksanen et al., 2013).

A GLLVM model was constructed as described in Chapter 2, following Niku et al., (2019). Briefly, Poisson, Tweedie, zero-inflated Poisson, and negative binomial distributions were fit to the data. Information criterion, Dunn-Smyth residual plots, and normal quantile-quantile plots with 95% confidence intervals were used to assess the fit for macrophyte community distributions (Niku et al., 2019). A negative binomial distribution was identified to be the best fit for the data and was used in the following analysis. Environmental parameters were added into the model, to account for the effects on species interactions. A model that used five of the environmental parameters (calcium, potassium, pH, manganese, and depth) were chosen based on residual analysis indicating the most suitable mean-variance relationship for the responses. Estimations for correlation patterns across species were plotted using the corrplot and gclus packages (Hurley, 2012; Wei et al. 2017).

6.3 Results

Nitellopsis obtusa was found in 19 of the 60 study lakes (Figure 5.2). The community proportion that *N. obtusa* comprised in each of the invaded lakes varied from

1% to 90% (Figure 6.1). Lakes Scugog, Simcoe, and Couchiching had the highest proportions (> 80%) of *N. obtusa* biomass (Figure 6.1). In contrast, lakes Canal, Eugenia, and Opinicon had the lowest proportions (< 5%) of *N. obtusa* biomass in this study (Figure 6.1). The remaining 13 study lakes had *N. obtusa* at variable proportions < 50% of the total macrophyte community (Figure 6.1).

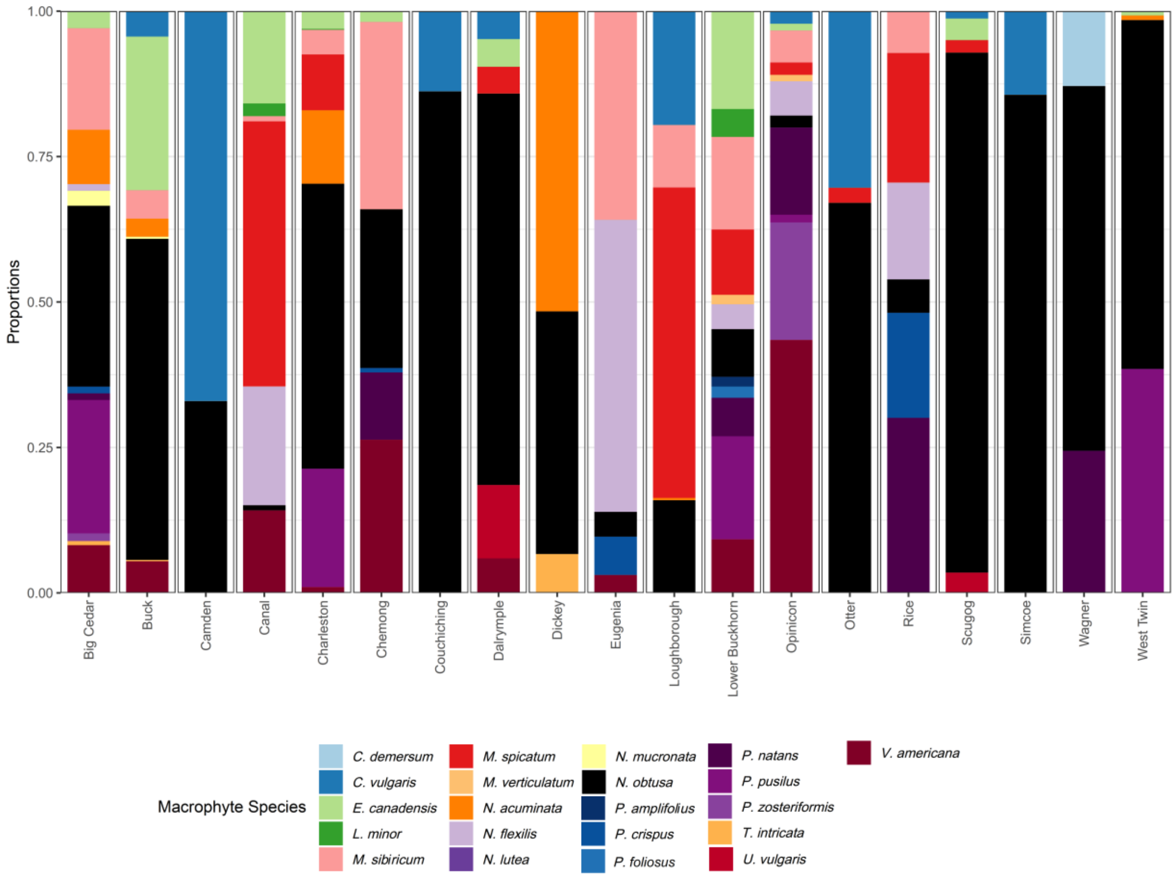


Figure 6.1. *Macrophyte community taxa plots for the 19 lakes where* N. obtusa *was identified in candidate lakes across south-central Ontario.*

Redundancy analysis allowed me to evaluate whether there were distinct community profiles associated with *N. obtusa* presence across Ontario. The RDA biplot shows that axis one explained 34.67 % of the variation observed and axis two explained 22.82 % of the variation observed (Figure 6.2). Macrophyte communities tended to have distinct community profiles when *N. obtusa* was present/absent (Figure 6.2). Increased developed and agricultural land-use in the watershed positively co-varied with macrophyte communities that had *N. obtusa* present (Figure 6.2). Additionally, concentrations of sodium, potassium, magnesium, and calcium were positively associated with *N. obtusa* presence within communities (Figure 6.2). In contrast, depth, Secchi depth, and iron concentrations were positively associated with macrophyte communities that did not have *N. obtusa* present (Figure 6.2). A permanova confirmed that the macrophyte communities had distinct profiles when *N. obtusa* was present or absent in those communities (Figure 6.2) ($F_{1,47}$ = 4.18, p-value = 0.002).

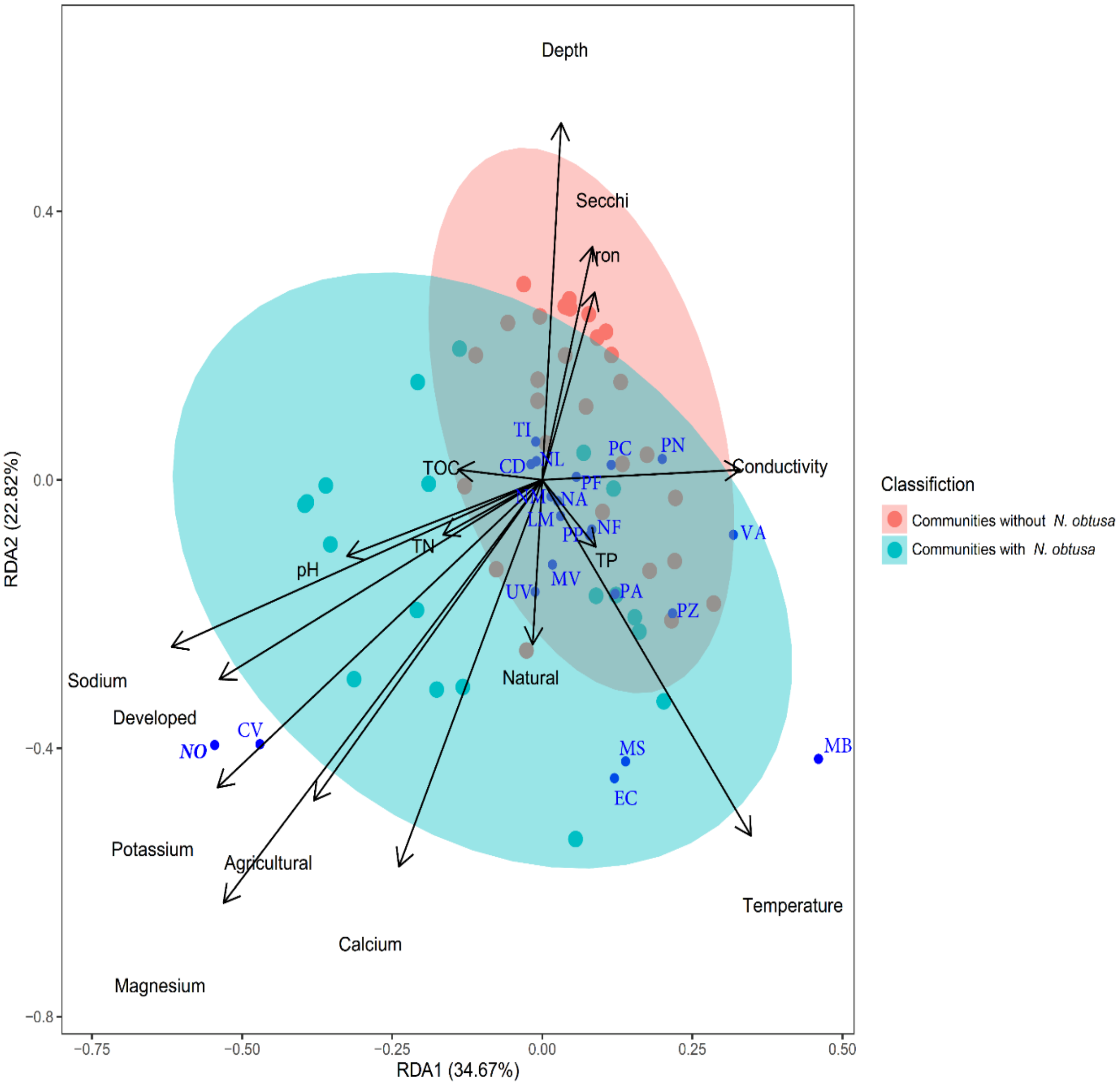


Figure 6.2. *Redundancy analysis (RDA) biplot presenting macrophyte community compositions where* N. obtusa *is present or absent. Blue dots in the biplot represent the mean of each individual species where NO =* N. obtusa, *CV =* Chara vulgaris, *MB =* Myriophyllum sibiricum, *MS =* Myriophyllum spicatum, *EC =* Elodea canadensis, *UV =* Utricularia vulgaris, *VA =* Vallisneria americana, *MV =* Myriophyllum verticulatum, *TI =* Tolypella intricata, *NL =* Nuphar lutea, *CD =* Ceratophyllum demersum, *PN =* Potamogeton natans, *PC =* Potamogeton crispus, *PF =* Potamogeton foliosus, *PA =* Potamogeton amplifolius, *PZ =* Potamogeton zosteriformis, *LM =* Lemna minor, *NF =* Najas flexilis, *and PP =* Potamogeton pusilus. *Arrows pointing in the same direction reflect positive correlations, and arrows pointing in opposite directions indicate negative correlations. The length of the arrow is a direct representation of the variance explained by the environmental variable. The first axis explained 34.67 % of the total canonical eigenvalues and the second axis explained 22.82 % of the total canonical eigenvalues.*

Macrophyte diversity was highly variable across the study region, particularly at sites without *N. obtusa* in the community (Figure 6.3). In the low *N. obtusa* presence classification, macrophyte diversity was demonstrated to be significantly (p-value = 0.023, Kruskal-Wallis) higher than other classifications (Figure 6.3). In contrast, communities with high abundance of *N. obtusa* tended to have lower macrophyte community diversity (Figure 6.3). Overall, there were significant differences (p-value = 0.023, Kruskal-Wallis) across the macrophyte community categories (Figure 6.3).

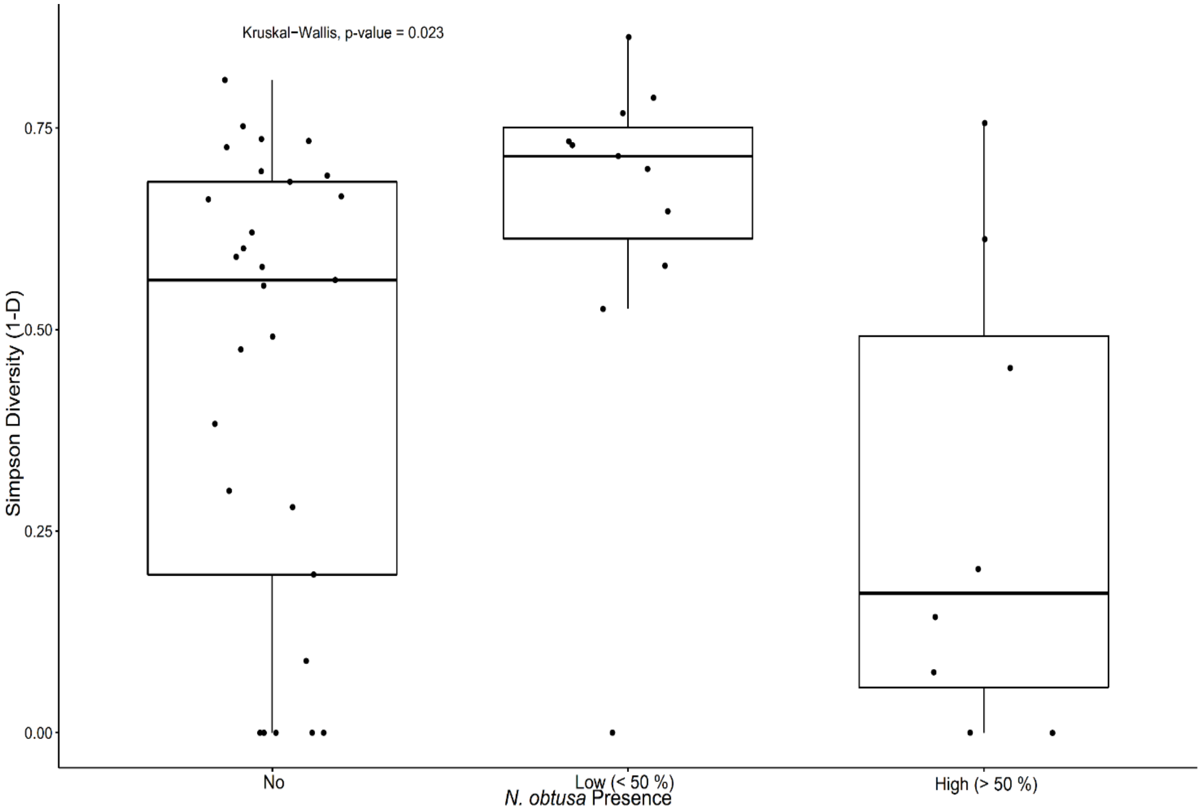


Figure 6.3. *Simpson's diversity boxplots for each categorical classification. Low (< 50 %) corresponds to macrophyte communities with* N. obtusa *presence comprising less than 50 % of the relative abundance (n = 11), and High (> 50 %) corresponds to communities with* N. obtusa *presence comprising greater than 50 % of the relative abundance for that candidate lake (n = 8).*

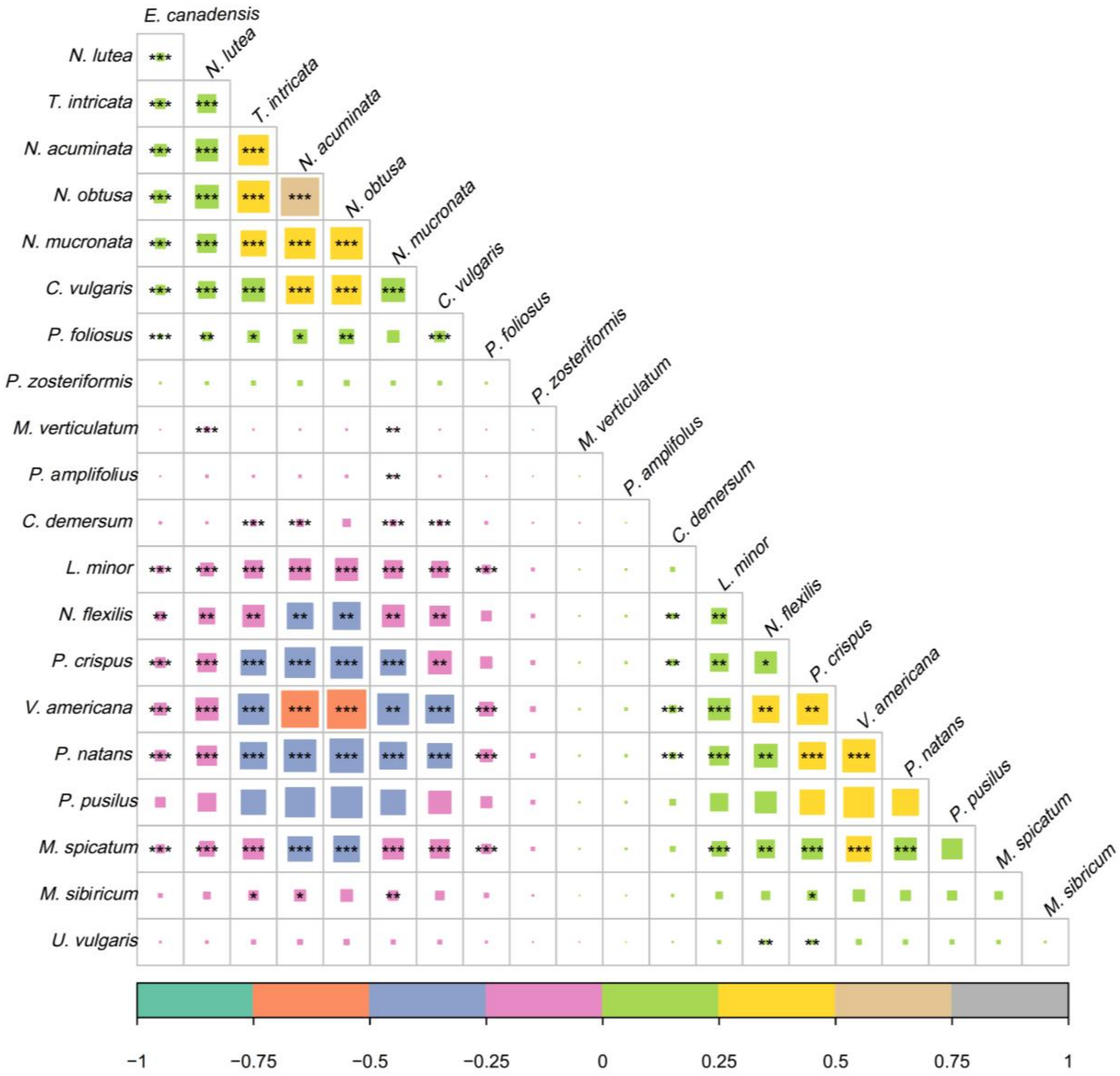


Figure 6.4. *General linear latent variable model (GLLVM) output matrix to visualize the species-specific interactions between macrophyte assemblages present within the candidate lakes (n = 49) when influencing environmental co-variates have been accounted for. The GLLVM model represents a negative binomial distribution, incorporating influences from five of the water quality parameters, calcium, potassium, pH, manganese, and depth. A heatmap depicting the strength of the correlation between the species covariates is presented.*

The GLLVM model demonstrated significant (p-value < 0.05) positive co-occurrences between *N. obtusa* and native Characeae such as *Nitella acuminata*, *Nitella mucronata*, *Chara vulgaris*, and *Tolypella intricata* (Figure 6.4). Significant (p-value <

0.05) positive associations between *N. obtusa* and other macrophytes were also found including *Elodea canadensis*, *Potamogeton foliosus*, and *Nuphar lutea* (Figure 6.4). In contrast, *N. obtusa* had significant (p-value < 0.05) negative co-occurrence patterns with the majority of other macrophyte taxa in the study lake communities, and most notably with *Myriophyllum spicatum* (Figure 6.4).

6.4 Discussion

In three of the nineteen study lakes where *N. obtusa* was found, it represented over 80% of macrophyte biomass. This is the first-time that near-monoculture dominance of *N. obtusa* in the macrophyte community of several Canadian lakes has been reported. In Ontario, aquatic ecosystems are not systematically monitored for non-native species, until dramatic community-level shifts are observed. Since most of the study lakes, including those with ideal growth conditions (Chapter 5), had no *N. obtusa* or less than 50% relative abundance, it is possible that the *N. obtusa* invasion front in Ontario lakes is still in its early stages.

A sleeper population is defined as a non-native species persisting at low abundances until environmental change triggers subsequent population increases (Spear, Walsh, Ricciardi, & Zanden, 2021). It is also plausible that *N. obtusa* has been inhabiting Ontario lakes for decades as sleeper populations. Generally, when a population explosion of a new non-native species occurs, it is assumed that the introduction occurred recently (Spear et al., 2021). The premise of non-native species persisting within habitats for lengthy periods of time prior to population eruption is often overlooked (Spear et al., 2021). Within my study, I observed 11 instances where *N. obtusa* populations were at low abundance, often mixed within beds of native Characeae. Given a history of misidentification, it is likely that *N. obtusa* has been present in inland lakes within the Great Lakes Basin well beyond the last decade and were missed due to inadequate community monitoring and skilled identification. Populations of *N. obtusa* discovered across the study period all had the presence of the star-shaped bulbils. Typically, bulbils can only be found within established beds, thus I conclude that *N. obtusa* has established populations within the study lakes (Bonis and Grillas, 2002). Future paleolimnological studies may be able to ascertain the period of initial colonization in invaded lakes via fossilized tissues, including bulbils.

The earliest record of *N. obtusa* within North America was first misidentified as a *Chara* spp. collected along the St. Lawrence River of Montreal, Quebec, and was later corrected through herbarium records (Karol & Sleith, 2017). In this study, *N. obtusa* had strong positive co-occurrences with *Chara vulgaris*, *Nitella accuminata*, and *Nitella mucronata*. These species are among the genera that are often mistaken as *N. obtusa*, which are commonly found in tangled masses of *N. obtusa*. This intermingling can thwart identification in the early stages of invasion by lake users and practitioners alike (Pullman & Crawford, 2010). A distinctive feature of *N. obtusa* is the presence of white star-shaped bulbils connected to the monofilament rhizoid. However, relying on bulbils alone for classification can prove difficult, as bulbils are only found within established beds of *N. obtusa* (Bonis & Grillas, 2002).

Although dispersal of *N. obtusa* within invaded regions is not well understood, it is thought to occur through distribution of propagules and vegetative fragments (Reynolds et al., 2015; Green, 2016). Propagules are known to spread through epizoochory (Bonis &

Grillas, 2002), however, there is growing evidence that the bulk of *N. obtusa* transfer is occurring by the movement of watercraft. Supporting this idea are several lines of evidence including one study that surveyed isolated ecosystems within invaded regions of the United States, but failed to detect *N. obtusa* populations (Sleith et al., 2015). Furthermore, Midwood et al., (2016) noted that *N. obtusa* was found in areas with higher dock densities, and docks are strongly tied to recreational watercraft activity. Masses of *N. obtusa* found on watercraft and trailers remain viable after several days, suggesting that proper clean and drain protocols are not being followed (Glisson et al., 2019). In addition, I found that populations of *N. obtusa* tended to have the highest biomass at sites nearest boat launches (Chapter 5). Given the proximity of invaded lakes documented in this study to the Trent Severn Waterway, it is probable that boats serve as an important vector of *N. obtusa* spread. The Trent Severn Waterway is a lake-canal navigation system that allows free movement of watercraft between Lake Huron and Lake Ontario, and has been implicated as an important conveyance system for invasive species spread (Kelly et al., 2013; Masson, Brownscombe, & Fox, 2016)

As more studies are published, it is becoming apparent that *N. obtusa* can displace other macroalgae and vascular macrophytes (Pullman & Crawford, 2010; Brainard & Schulz, 2017; Ginn et al., 2021). Total and native macrophyte biomass decreased in response to *N. obtusa* in invaded regions within the United States (Brainard & Schulz, 2017). In Ontario, Ginn et al., (2021) also described the displacement of native and invasive macrophytes upon *N. obtusa* establishment within Lake Simcoe. Similarly, the initial identification and subsequent increase of *N. obtusa* within Lake Scugog, Ontario, has resulted in the displacement of native and invasive macrophytes (Chapter 2).

In my region-wide study across south-central Ontario, I observed a reduction in macrophyte diversity with high *N. obtusa* abundance, however, there was still considerable variation. When examining the species-specific interactions revealed by GLLVM, *N. obtusa* had negative co-occurrences with most members of the macrophyte community, but also had significant positive associations with native Characeae across Ontario. Based on these findings, and the possibility that *N. obtusa* is still in its early stages of an invasion front, it is too early to conclude that *N. obtusa* has caused diversity loss and species displacement across Ontario lakes. However, more comprehensive in-depth lake studies such as those done in other invaded systems (Brainard & Schulz, 2017; Ginn et al., 2021) do offer supporting evidence of *N. obtusa*'s negative impacts to macrophyte community diversity.

In other invaded regions, *Ceratophyllum demersum* and *Utricularia vulgaris* were observed to reach nuisance levels in conjunction with *N. obtusa* growth (Pullman & Crawford, 2010). Furthermore, *N. obtusa* has also been documented in stands of *C. demersum* within its native range (Pełechaty et al., 2014). In Ontario, my results demonstrate *N. obtusa* negatively co-occurs with these taxa, along with most other members of the macrophyte community. Notably, this study highlights a negative relationship between *Myriophyllum spicatum*, a normally aggressive non-native macrophyte long established in many Ontario lakes over the last 60 years. Within two invaded systems in Ontario, Lake Scugog (Chapter 2) and Lake Simcoe, similar negative relationships have been observed between these two non-native macrophytes (Ginn et al., 2021).

Overall, *Nitellopsis obtusa* may be the most enigmatic, non-native macrophyte species to be introduced into North America. Early identification and mitigation is often foiled when *N. obtusa* is present within stands of native Characeae (Brainard and Schulz 2017). The reduction in macrophyte diversity shown to occur in this study and others, coupled with the frequent misidentification of *N. obtusa*, warrant increased monitoring efforts. Early detection, as with most invasive species, will be critical for lake management efforts aimed at mitigating the negative ecological effects of *N. obtusa* establishment and increasing dominance in inland North American lakes.

Chapter 7. General Discussion and Conclusions

There were two main goals for my thesis research, the first of which was to determine the distribution and prevalence of *N. obtusa* in south-central Ontario lakes. The second goal was to collect several lines of evidence to evaluate and determine if *N. obtusa* fulfills the criteria for being an ecosystem engineer. To achieve these goals, I set out to address four main research objectives that included: (1) evaluating the impact of *N. obtusa* invasion on the lower aquatic food web and habitat condition in Lake Scugog (Chapters 2 and 3); (2) determining the role of *N. obtusa* in emerging *Microcystis* blooms in Lake Scugog (Chapter 4); (3) mapping the distribution of *N. obtusa* in south-central Ontario lakes, and identifying regional habitat constraints (Chapter 5); and (4) distinguishing the role of spatial and environmental factors on *N. obtusa*'s influence on macrophyte communities (Chapter 6). Chapters 2 – 6 address all my research objectives, and in combination provide supporting evidence that *N. obtusa* is acting as an ecosystem engineer in Ontario lakes, particularly with respect to changing habitat conditions (near-bed hypoxia-anoxia and internal phosphorus loading) and affecting aquatic community diversity and community structure (Figure 7.1).

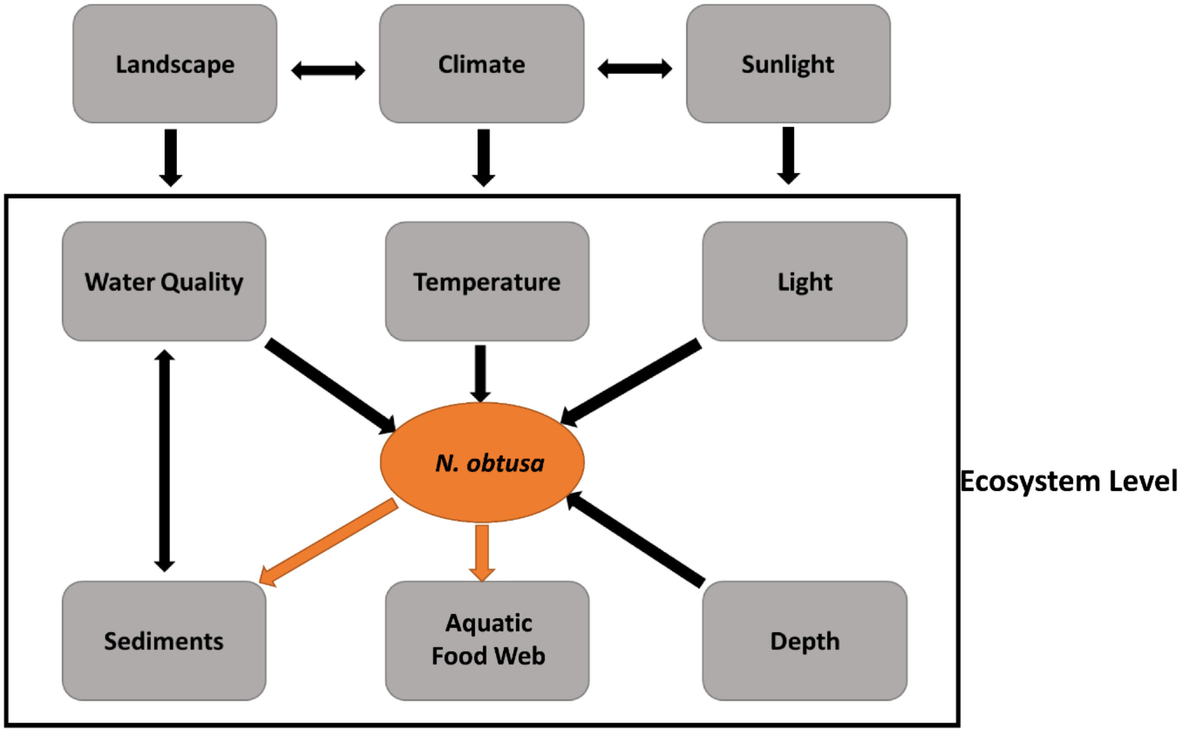


Figure 7.1. *Conceptual model for the role* N. obtusa *has as an ecosystem engineer in Ontario lakes. Direction of arrows reflect the direct influence of a model compartment on another compartment, two-sided arrows indicate dual interactions between model compartments.*

7.1 Mapping the Prevalence of *Nitellopsis obtusa* in Ontario Lakes

Previously, the distribution of *N. obtusa* in Ontario lakes was largely unknown. Prior to my thesis research, *N. obtusa* was most recently confirmed in two Ontario locations: Presqu'ile bay (Midwood et al., 2016), and Lake Simcoe (Ginn et al., 2021). Although confirmed presence of *N. obtusa* in Ontario lakes has remained low, unconfirmed reports have emerged with claims of widespread distribution (ex. Drew Monkman, the Peterborough Examiner). Given the difficulty in differentiating between Characeae taxa, especially in early stages of invasion, additional investigation was required to confirm its presence. My investigation found *N. obtusa* at 37 sites in 19 study lakes (Figure 5.2). This is in stark contrast to what has been confirmed within Ontario. This was not surprising as lakes in Ontario are rarely monitored for invasive macrophytes until drastic ecosystem changes occur. Unconfirmed reports of *N. obtusa* infestation continue to emerge, often referring to lakes directly connected to the Trent Severn Waterway (TSW). Nonetheless, this study confirmed for the first-time that in Ontario, *N. obtusa's* distribution extends beyond lakes connected to the TSW (Figure 5.2). Given the history of misidentification of *N. obtusa* within invaded regions, *N. obtusa* likely has invaded several other lakes across Ontario than what I could capture in my study design.

Generally, when rapid increases of invasive species occur, it is thought that the introduction of the non-native species occurred recently (Spear et al., 2021). Within communities where *N. obtusa* was present in this study, *N. obtusa*'s relative abundance was generally low. This infers that *N. obtusa* either recently colonized these lakes and is in the early stages of its invasion front, or that *N. obtusa* was introduced decades ago, persisting as a sleeper species until ecosystem disturbance provided a competitive advantage for subsequent population increases (Spear et al., 2021). Bulbils of Characeae are only present within established beds (Bonis & Grillas, 2002), however, all populations of *N. obtusa* throughout this study had apparent bulbils. This provides evidence that *N. obtusa* is established in lakes where it was detected, even at low biomass, and has likely been overlooked due to the lack of monitoring and/or ability to correctly identify *N. obtusa* in Ontario.

Historical misidentification and reporting inaccuracies for *N. obtusa* within Ontario have likely occurred, as strong positive co-occurrences with native Characeae (*Nitella* spp., *Chara* spp., and *Tolypella* spp.) were observed in this study (Figure 6.4).

Identification is known to be confounded by native Characeae (Pullman & Crawford, 2010; Brainard & Schulz, 2017; Ginn et al., 2021), especially when *N. obtusa* grows within stands of native Characeae during the early stages of invasion (Pullman & Crawford, 2010; Brainard & Schulz, 2017; Ginn et al., 2021). With Ontario having an abundant and diverse native Characeae assemblage, a concerted effort should be made to disseminate information about *N. obtusa* characteristics, including key aspects for differentiating *N. obtusa* from similar Characeae species.

7.2 Habitat Characteristics that Promote and Constrain *N. obtusa* Prevalence in Ontario Lakes

Until this study, calcium concentration had been considered the primary driving factor for *N. obtusa* establishment success in lakes (Olsen, 1944; Pullman & Crawford, 2010; Auderset Joye & Rey-Boissezon, 2015; Rey-Boissezon & Auderset Joye, 2015). A minimum calcium concentration between 15 – 25 mg · L^{-1} is required for *N. obtusa* to occupy a given habitat (Stroede, 1937). Due to capturing a very broad calcium gradient in my synoptic lake survey, I confirmed that *N. obtusa* was limited to lakes with calcium concentrations above 15 mg · L^{-1} (Figure 5.3, 5.5). However, I was also able to elucidate the role of other cations in *N. obtusa* establishment and distribution within the study region. In contrast to previous reports, it was determined that other cations such as potassium and magnesium had a stronger influence on *N. obtusa* occurrence (Figure 5.5). Regardless, calcium was still an essential contributing factor in explaining *N. obtusa* distribution across Ontario Lakes (Figure 5.5), but the influence of other cations must also be considered in the future.

It is not clear why cations such as potassium and magnesium would play a larger role than calcium for determining *N. obtusa* presence in Ontario lakes, but Katsuhara & Tazawa (1988) found that *N. obtusa* concentrated potassium in the cytoplasm as a mechanism of salt tolerance. With populations of *N. obtusa* being associated with increased urban development, the relationship observed may be a physiological response for higher chloride concentrations present within these systems. Interestingly, the ecological niche model also indicated that *N. obtusa* presence was associated with lower levels of manganese (Figure 5.5). Although no studies have investigated the biological role that manganese plays in *N. obtusa* biology, manganese has been shown to have an inhibitory effect at higher concentrations for other charophytes (Lambert & Davy, 2011).

Within invaded regions of the United States, it has been determined that *N. obtusa* generally colonizes shallow water prior to moving to deeper sites (Geis et al., 1981; Pullman & Crawford, 2010; Sleith et al., 2015). Within the native range, *N. obtusa* is found within both shallow and deep habitats, reaching depths upwards of 65 m (Spence, 1982; Kufel & Kufel, 2002; Janauer et al., 2010; Korsch et al., 2013). In Ontario, I found the highest densities of *N. obtusa* mainly within shallow environments (0.5 - 3 m). The pattern of *N. obtusa* colonizing shallow water prior to moving into deeper areas has been documented in the United States, thus it is possible that *N. obtusa* is still within the early stages of an invasion front in Ontario.

The preferential colonization of shallow habitats observed throughout this study hindered the ability to quantify the influential role that temperature and light have on *N. obtusa* establishment and persistence in Ontario lakes. However, *N. obtusa* is classified as a boreal taxon, due to the high thermal tolerance, and the ability to persist during under ice conditions (Corillion, 1957; Boissezon et al., 2018). *Nitellopsis obtusa* was found to occupy sites where temperatures approached 30 °C, and as such, it is likely temperature is not a critical constraint for *N. obtusa* invasion of Ontario lakes, at least currently. Furthermore, with an abundance of light at the sediments, no connection between light and *N. obtusa* establishment was identified. This is likely because light levels were not growth limiting at sampling locations without established beds of *N. obtusa*. Regardless, light is required for propagule germination in some Characeae (Bonis & Grillas, 2002), and as *N. obtusa's* invasion extends to deeper sites of Ontario, the crucial role of light maintaining populations may become evident.

7.3 Ecosystem Services and Habitat Disruption Following *N. obtusa* Establishment

One of the main outcomes of assessing *N. obtusa* as an ecosystem engineer within Ontario lakes was determining the associated changes to the lake benthic zone. It was confirmed that low benthic oxygen concentrations were associated with *N. obtusa* presence, and benthic DO decreased in response to increasing *N. obtusa* biomass. Sediment pore water SRP also increased with *N. obtusa* biomass, indicating that internal loading was occurring in response to rising *N. obtusa* biomass. Reduced benthic oxygen, and light triggers propagule germination within Characeae that are actively using propagules within the sediments (Bonis & Grillas, 2002). The strong association of reduced benthic DO with increasing *N. obtusa* abundance in this study suggests that *N. obtusa* may alter local habitat conditions to promote propagule bank germination. This positive-feedback system may explain why *N. obtusa* is initially delayed in becoming the dominant species in the macrophyte community because it takes time for there to be sufficient biomass to induce hypoxic-anoxic conditions for propagules to germinate. Nevertheless, this research demonstrates that *N. obtusa* has a direct link to the internal loading of nutrients leading to eutrophication of Ontario's lakes, which has not been documented previously.

Interestingly, *N. obtusa* is used as an indicator species for oligotrophic to mesotrophic ecosystems within the native range (Kasaki, 1962), where it is classified as threatened to critically endangered due to eutrophication (Watanabe, 2005). In this study, *N. obtusa* was found at sites with higher TP concentrations in the meso-eutrophic range, and TP was a significant positive explanatory variable identified within the ecological niche model. Despite *N. obtusa* being associated with facilitating the increase of phosphorus concentrations via internal loading, it is not known whether exposure to high phosphorus concentrations will have a detrimental impact on *N. obtusa* persistence within Ontario lakes either directly or through competition with weedy species. Over the course of this study, it did not appear to have a detrimental effect, suggesting *N. obtusa* is tolerant to high phosphorus concentrations in Ontario lakes (e.g., Lake Scugog).

Generally, Characeae have been regarded as important components to the biogeochemical cycling of phosphorus, often resulting in decreased phosphorus concentrations due to marl production (Blindow, 1992; Kufel & Ozimek, 1994; Kufel & Kufel, 2002). Nevertheless, *N. obtusa* not only maintained hypoxic-anoxic conditions at the sediment water interface in Lake Scugog, but also caused a noticeable increase in *Dreissena polymorpha* populations, which have also been recognized to alter phosphorus cycles within invaded systems (Chapters 2 and 4). Although the SEM results did not reveal a direct positive relationship between dreissenid mussels and phosphorus (Chapter 4), it is possible that *D. polymorpha* is contributing to SRP release due to its increased abundance associated with *N. obtusa* (Chapters 2 and 4). The excretion of phosphorus-rich feces and psuedofeces is known to affect phosphorus cycles (Caraco, Cole, & Likens, 1990; Mosley & Bootsma, 2015). A fraction of the soluble reactive phosphorus that was documented in this study could be due to egested materials rich in bioavailable phosphorus, similar to what has been found in other studies (Li et al., 2021). Further research is required to elucidate the direct, and indirect, mechanism(s) by which *N. obtusa* is affecting the biogeochemical cycling of phosphorus within invaded lakes.

7.4 LAFW Community Response to *N. obtusa* Presence and Abundance

The second mechanism by which *N. obtusa* is acting as an ecosystem engineer is its associated alteration of LAFW communities, including a decrease in overall community diversity (Chapters 2 and 6). Despite *N. obtusa* being introduced to North America over 40 years ago, very few quantitative studies have looked at LAFW effects (Pullman & Crawford, 2010; Brainard & Schulz, 2017; Ginn et al., 2021). My thesis research clearly demonstrates that *N. obtusa* is associated with notable changes to resident aquatic communities, including phytoplankton, macroinvertebrates, and macrophytes. The surprising outcome from this research was the positive links revealed between *N. obtusa* and other problematic species such as *D. polymorpha* and *Microcystis* species (Chapter 4). Not only was a direct positive relationship detected between *N. obtusa* and *Microcystis* spp., but the notable association between *N. obtusa* and internal phosphorus loading also infers an indirect faciliatory role in *Microcystis* spp. bloom development that has not been previously reported.

7.5 Implications for Future Range Expansion in North America

Establishment of non-native invasive species can have considerable spatial variation in a given habitat (Hunt & Behrens Yamada, 2003; DeRivera, Ruiz, Hines, & Jivoff, 2005; Jones & Ricciardi, 2005). This location-dependent variation is driven by the environmental parameters of a given site, often reflecting the physiological tolerance of the invasive species (Dunson & Travis, 1991; Moyle & Light, 1996; Von Holle, 2005). This is not to say invasive species cannot be found beyond their ideal habitat, but their ability to establish and persist outside transient populations in such environments is typically rare.

Several of the study lakes had ideal habitat characteristics for *N. obtusa* invasion, as identified by the ecological niche model, but *N. obtusa* populations were not detected. It is possible established populations were missed, since sampling intensity in each lake was restricted to only 4 sites, but lack of detection still may reflect unoccupied niche space. Several lakes within Ontario have conducive habitat conditions to support *N. obtusa* establishment and growth. Therefore, it is my intention that an important deliverable from my thesis research, the ecological niche model, be made available for use by various stake holders to identify vulnerable areas for *N. obtusa* invasion. Unfortunately, more ecosystems will likely become susceptible to invasion as increasing stressors from climate change, and a changing landscape effect ecosystem resiliency (Ricciardi & Macisaac, 2010).

Despite most of the candidate lakes being relatively pristine, with large portions of their watersheds remaining undeveloped, the stress that climate change and land-use exerts on a given ecosystem are expected to alter the ecological impacts of invasive species in the coming years (Hellmann, Byers, Bierwagen, & Dukes, 2008; Rahel & Olden, 2008; Walther et al., 2009; Ricciardi & Macisaac, 2010). In Ontario, as the landscape continues to change (i.e., more conversion of lands for urban and peri-urban development), biodiversity loss will be unavoidable, which has been suggested to make ecosystems more susceptible to invasion (Brauman, Daily, Duarte, & Mooney, 2007; Rands et al., 2010; Foley et al., 2011). Increased development and agricultural land-use within a lake's watershed had a positive association with the presence of *N. obtusa* in this study, indicating that Ontario lakes are already impacted by such stressors, making invasion and establishment more likely (Figure 6.3). When non-native taxa are introduced into impacted ecosystems, diversity is further degraded similar to what was observed across this study (Table 2.1). In lakes where *N. obtusa* became established, community diversity decreased (Table 2.1).

Habitat heterogeneity is important to support healthy functional ecosystems, with a decrease in diversity, the stability and health of an ecosystem is called into question. However, recent debate has questioned whether heterogenous habitats are more susceptible to invasion compared to homogeneous habitats (Davies et al., 2005; Kestrup & Ricciardi, 2009). Heterogeneous ecosystems facilitate species co-existence, but also offer neighbouring habitat that can be suitable for native or non-native species (Chesson & Huntly, 1997; Chesson, 2000; Davies et al., 2005; Kestrup & Ricciardi, 2009). Thus, a heterogenous environment may offer refuge for inferior competitors within a given community; a component that is largely overlooked for understanding the invasional success of non-native species upon introduction (Hunt & Behrens Yamada, 2003; Davies et al., 2005; DeRivera et al., 2005; Mitchell et al., 2006; Melbourne et al., 2007; Kestrup & Ricciardi, 2009).

This premise can be observed throughout my study of Lake Scugog. Within the first two years, 2016 and 2017, the composition of the Lake Scugog's macrophyte community was significantly more diverse (Figure 2.2C) (Appendix A, Figure A9-A12). However, as *N. obtusa* gained a foothold, and increased in prevalence from 2016-2019, diversity and habitat heterogeneity drastically decreased (Table 2.1, Figure A11-12). Unfortunately, invasional mechanisms for aquatic macrophytes have received limited attention, largely focusing on management and eradication strategies, instead of understanding the invasional biology of a particular species. Dispersal of non-native invasive macrophytes is one of the least studied and understood mechanisms in invasion biology.

Yet, insight on dispersal may be the most important factor for management of *N. obtusa* in Ontario. Even though dispersal and establishment of *N. obtusa* within invaded regions is poorly understood, macrophyte dispersal usually occurs through viable propagules or vegetative fragments (Reynolds et al., 2015; Green, 2016). Characeae have also been known to be dispersed through epizoochory (Bonis & Grillas, 2002). Thus far, there is increasing evidence that the majority of spread for *N. obtusa* within invaded regions is occurring through anthropogenic vectors, as inferred by this study (i.e., recreational watercraft). Supporting this notion is the fact that within invaded regions of the United States, *N. obtusa* populations were not found within isolated lakes (Sleith et al., 2015). Furthermore, Midwood et al., (2016) documented an association between dock density and

N. obtusa presence within Presqu’ile Bay of Lake Ontario. Populations of *N. obtusa* detected in this study were found most frequently at sites closest to boat launches. Given the popularity of boating culture in Ontario, it is likely that recreational boaters are serving as a vector of *N. obtusa* spread throughout Ontario’s lakes. Thus, implementing management programs that target boat launches may be effective in preventing *N. obtusa* spread, while also providing early detection for new populations within Ontario lakes.

7.6 Study Limitations and Future Directions

As in all field-based studies, certain trade-offs are inherent in the final study design. For example, the synoptic survey was designed to optimize finding *N. obtusa* populations while also identifying key habitat characteristics across a large spatial extent during a constrained period to minimize seasonal effects. Admittedly this was done at the cost of more frequent sampling within each study lake and additional lakes to the sampling pool. Although an increased sampling effort would have invariably detected more *N. obtusa* populations, I still think the study design, which exploited a geological gradient, allowed me to capture the environmental factors that influence *N. obtusa* establishment. Not only was this evident in the ecological niche model, but I was also able to capture additional factors (e.g., cations).

To further characterize the habitat preferences of *N. obtusa*, additional environmental parameters could be investigated. Pullman & Crawford (2010) have suggested *N. obtusa* populations within Michigan State, United States colonize sediments composed of sand, gravel, and unconsolidated organic matter. However, there is a lack of consensus for a preference of sediment types within invaded regions, hence inquiry into sediment preference could provide further information. Considering that this study showed *N. obtusa* being strongly associated with sediment pore water SRP in Lake Scugog, further experimental studies could confirm whether internal loading will occur in all invaded systems, or only within systems with a certain sediment type. With additional characterization of the sediments, the importance of propagule banks and their role in maintaining consistent *N. obtusa* populations in Ontario lakes can also be ascertained. Although beyond the scope of my thesis research, the next knowledge gap that should be addressed is the direct and indirect effect of *N. obtusa* invasion on fish communities. Considering the notable effects to the LAFW and dissolved oxygen in Lake Scugog, it is not unreasonable to conjecture that fish communities would be affected by *N. obtusa* as well.

7.7 Final Thoughts

Overall, my thesis research demonstrated that *N. obtusa* is more broadly established in Ontario inland lakes than previously thought and may be acting as an ecosystem engineer by altering the benthic biogeochemistry, as well as reducing diversity across the LAFW. This study also provides further evidence that dispersal is likely due to anthropogenic vectors such as recreational watercraft. Currently, no management strategies are in place for mitigating *N. obtusa* within Ontario, however, by increasing education and awareness of this non-native taxon, and enforcing clean, drain and dry protocols, dispersal, and successful establishment of *N. obtusa* could be diminished.

In closing, I think my thesis research provided important documentation on the distribution of *N. obtusa* in Ontario lakes and provided several lines of evidence supporting the designation of *N. obtusa* as an ecosystem engineer. Overall, these findings improve our collective understanding of the invasion biology of *N. obtusa* that is not only specific to Ontario lakes, but throughout the Great Lakes Basin.

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Appendix A.

Table A1. Seasonal means (May-September) reflecting four years of pooled data (2016-2019) for 11 physical and water quality parameters measured concurrently in Lake Scugog, Ontario (n = 20). Sites five and nine were inaccessible each year of sampling in the month of September due to water levels (n = 16). Standard deviation is presented in parenthesis. Temp = temperature, DO = dissolved oxygen, Con = conductivity, Tur = turbidity, TP = total phosphorus, TN = total nitrogen, CHLα = chlorophyll α, Cl = chloride.

Site	**Depth (m)**	**Secchi (m)**	**Temp (°C)**	**DO (mg · L^{-1})**	**pH**	**Con (µS · cm^{-1})**	**Tur**	**TP (µg · L^{-1})**	**TN (µg · L^{-1})**	**CHLα (µg · L^{-1})**	**Cl (mg · L^{-1})**
1	1.18 (0.60)	1.18 (0.38)	21.87 (2.66)	10.79 (2.90)	8.06 (1.96)	521.07 (161.60)	0.0027 (0.0002)	47.44 (31.82)	0.77 (0.73)	87.64 (83.44)	124.06 (117.75)
2	1.11 (0.61)	1.03 (0.34)	22.06 (2.93)	12.32 (4.69)	8.23 (2.04)	577.62 (199.62)	0.0061 (0.0014)	61.92 (46.93)	0.79 (0.87)	305.67 (470.37)	219.57 (148.75)
3	1.80 (0.81)	1.53 (0.57)	22.14 (2.82)	10.17 (2.14)	7.95 (1.94)	566.05 (182.74)	0.0024 (0.0022)	37.34 (35.02)	0.65 (0.55)	85.61 (96.03)	181.04 (143.78)
4	1.89 (0.89)	1.61 (0.49)	21.96 (3.03)	9.427 (1.38)	7.88 (1.93)	571.00 (178.66)	0.0028 (0.0025)	42.12 (40.96)	0.75 (0.81)	246.85 (493.40)	144.57 (99.67)
5	0.92 (0.62)	0.81 (0.41)	22.36 (2.41)	10.28 (6.16)	7.74 (2.16)	583.75 (236.79)	0.0029 (0.0036)	90.06 (89.88)	0.85 (0.72)	304.15 (658.14)	120.95 (78.05)
6	0.79 (0.79)	1.51 (0.39)	21.91 (2.85)	9.80 (2.02)	7.86 (1.92)	500.94 (159.32)	0.0019 (0.0022)	45.58 (49.44)	0.94 (1.45)	70.89 (71.08)	124.07 (98.36)
7	1.25 (0.57)	1.23 (0.38)	22.30 (3.33)	9.87 (2.13)	8.21 (0.53)	500.66 (162.53)	0.0027 (0.0021)	33.42 (30.51)	1.08 (1.33)	62.09 (54.69)	86.50 (55.30)
8	1.63 (0.83)	1.36 (0.45)	22.31 (2.87)	10.61 (2.27)	8.43 (0.51)	469.57 (158.95)	0.0025 (0.0019)	38.77 (36.79)	1.04 (1.59)	92.81 (85.74)	86.86 (82.22)
9	1.03 (0.48)	1.08 (0.31)	22.49 (2.68)	10.26 (2.66)	8.43 (0.67)	383.91 (115.39)	0.0024 (0.0023)	38.15 (29.63)	0.84 (1.18)	123.02 (145.29)	32.28 (21.76)
10	1.40 (0.68)	1.10 (0.51)	21.65 (2.85)	9.95 (2.00)	8.31 (0.43)	506.38 (172.27)	0.0035 (0.0025)	60.03 (88.86)	0.64 (0.66)	73.01 (58.16)	94.00 (79.48)
11	1.48 (0.74)	1.25 (0.49)	22.10 (2.68)	9.81 (1.67)	8.27 (0.44)	494.45 (166.69)	0.0025 (0.0022)	36.12 (30.90)	0.70 (0.86)	70.78 (57.19)	106.79 (88.84)
12	1.89 (0.99)	1.42 (0.58)	22.29 (2.47)	9.77 (1.66)	8.31 (0.41)	522.13 (180.11)	0.0030 (0.0025)	38.26 (38.32)	0.80 (0.96)	68.54 (52.58)	85.95 (94.56)

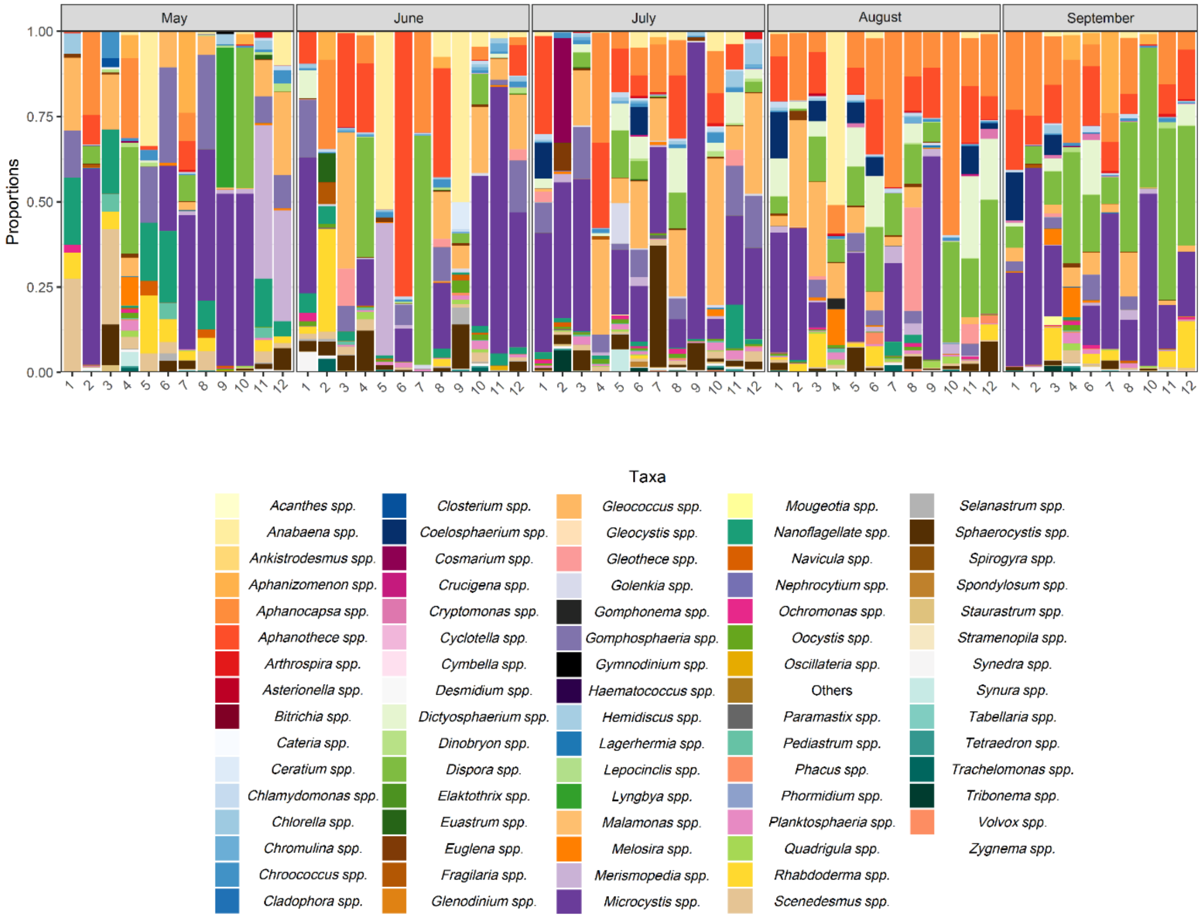

Figure A1. *Taxa plot for the phytoplankton community across the five months of sampling (May-September) in 2016 for Lake Scugog.*

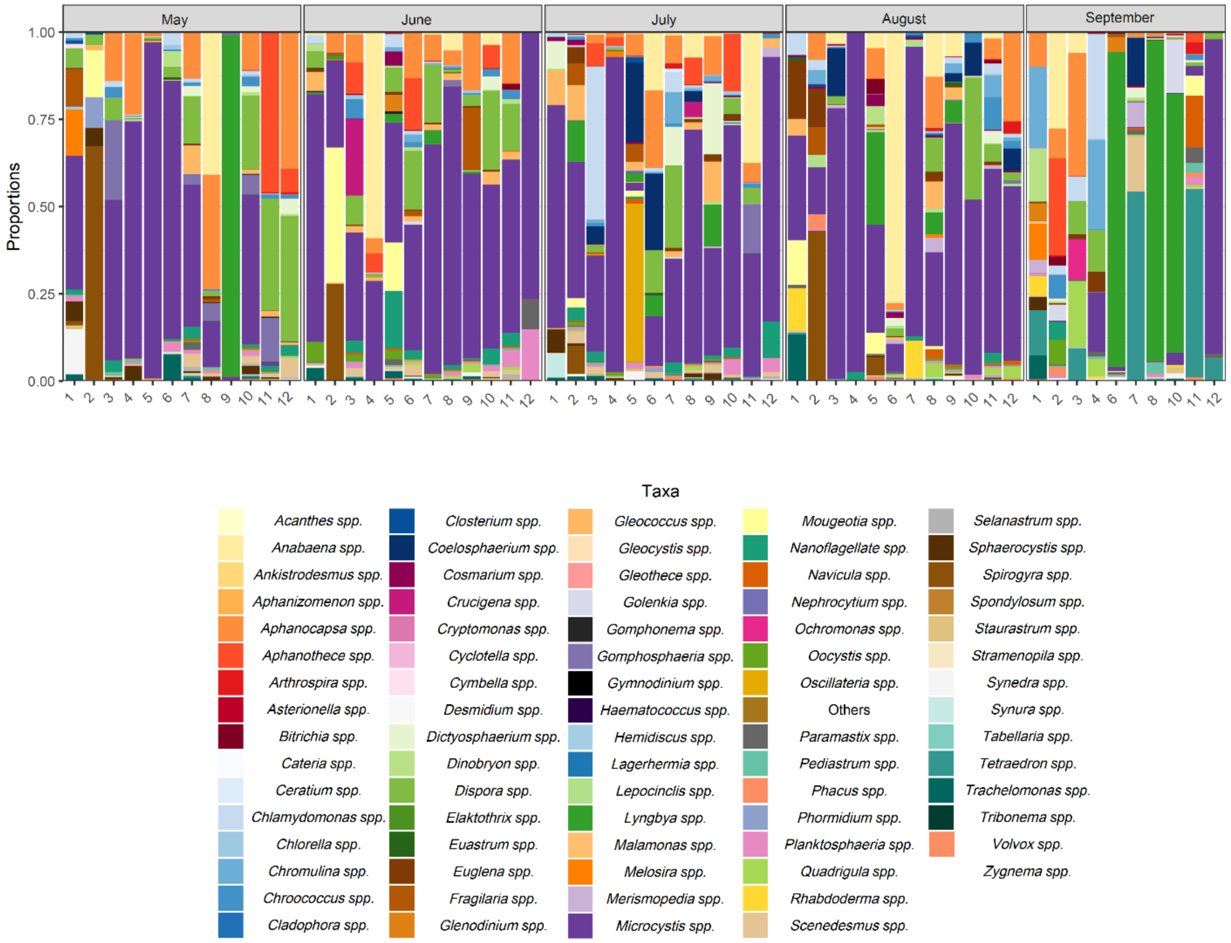

Figure A2. *Taxa plot for the phytoplankton community across the five months of sampling (May-September) in 2017 for Lake Scugog.*

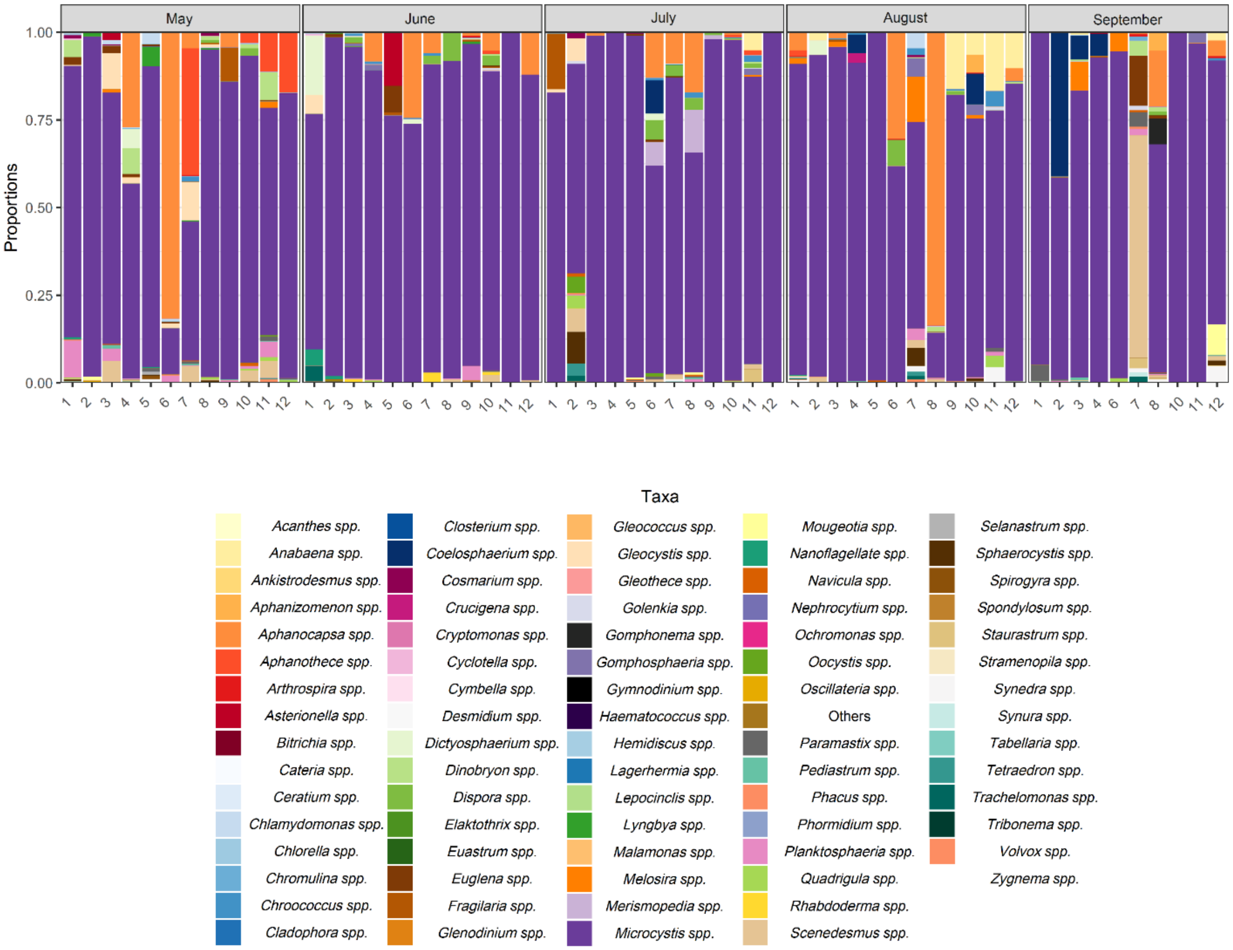

Figure A3. *Taxa plot for the phytoplankton community across the five months of sampling (May-September) in 2018 for Lake Scugog.*

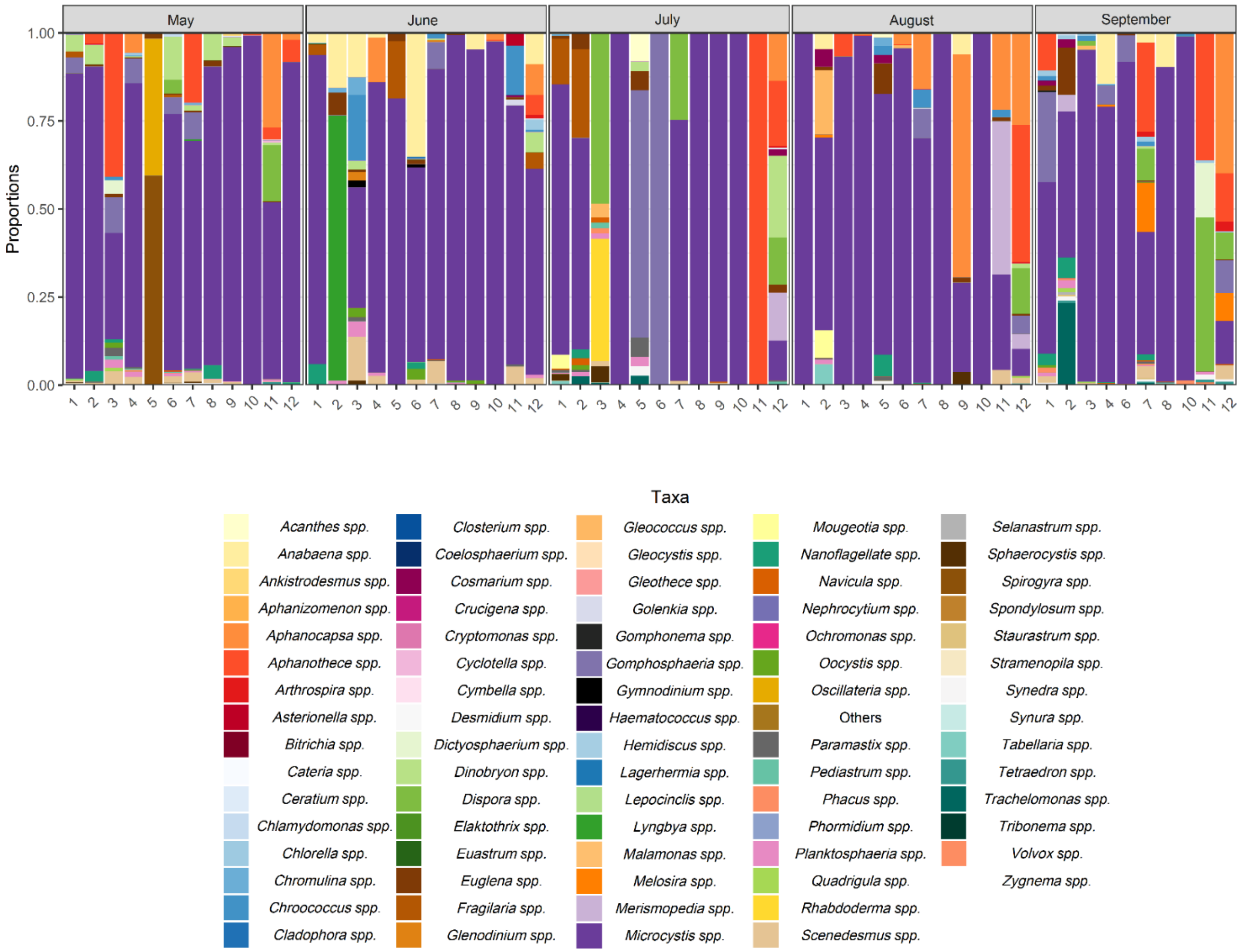

Figure A4. *Taxa plot for the phytoplankton community across the five months of sampling (May-September) in 2019 for Lake Scugog.*

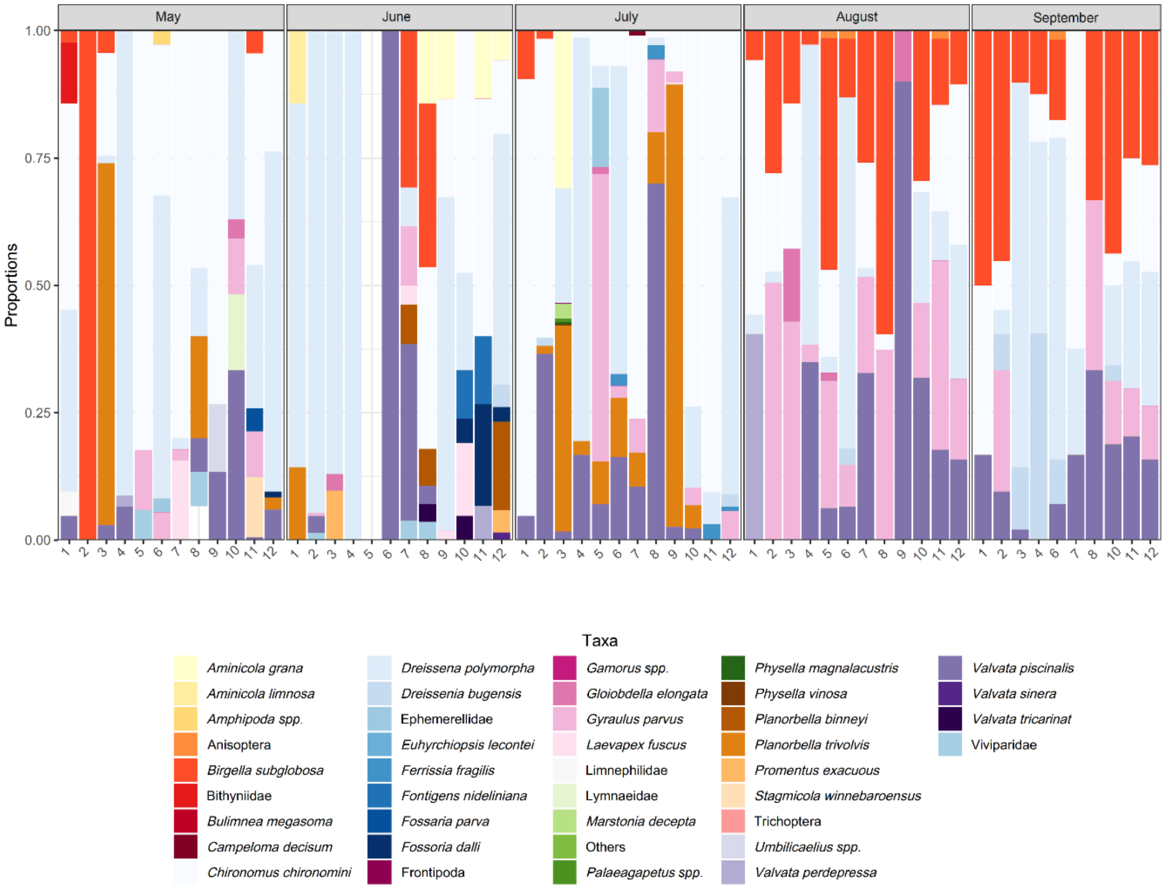

Figure A5. *Taxa plot for the macroinvertebrate community across the five months of sampling (May-September) in 2016 for Lake Scugog.*

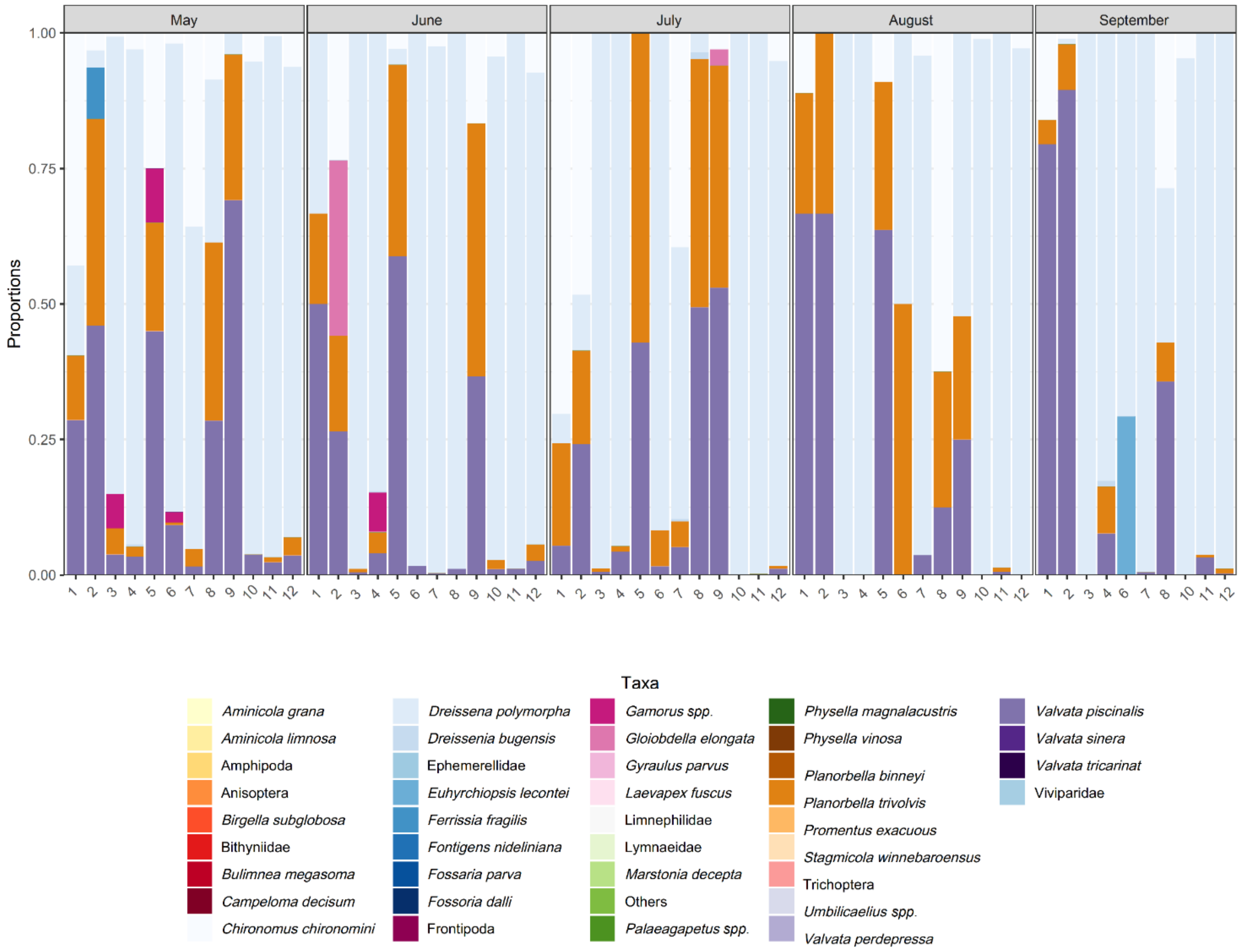

Figure A6. *Taxa plot for the macroinvertebrate community across the five months of sampling (May-September) in 2017 for Lake Scugog.*

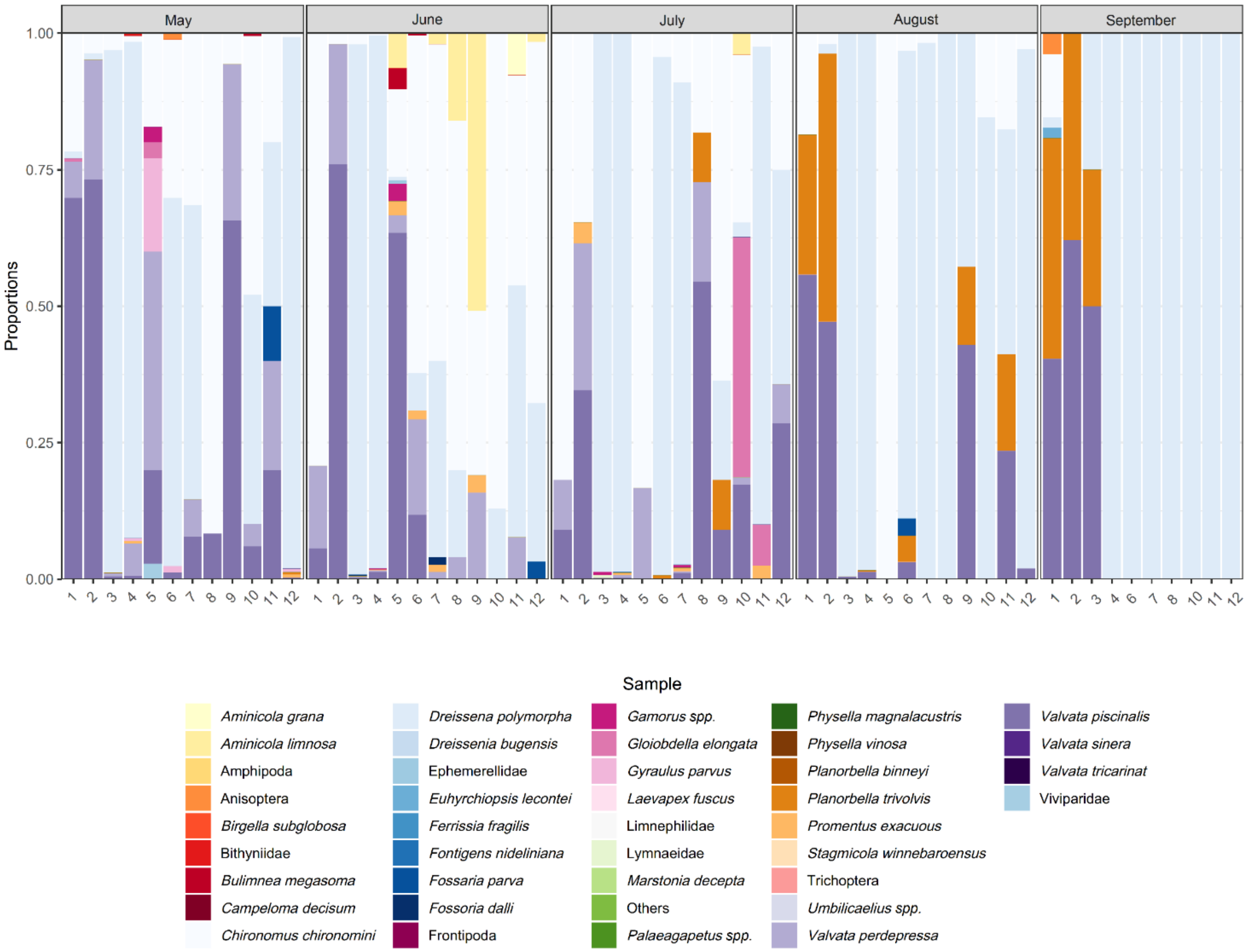

Figure A7. *Taxa plot for the macroinvertebrate community across the five months of sampling (May-September) in 2018 for Lake Scugog.*

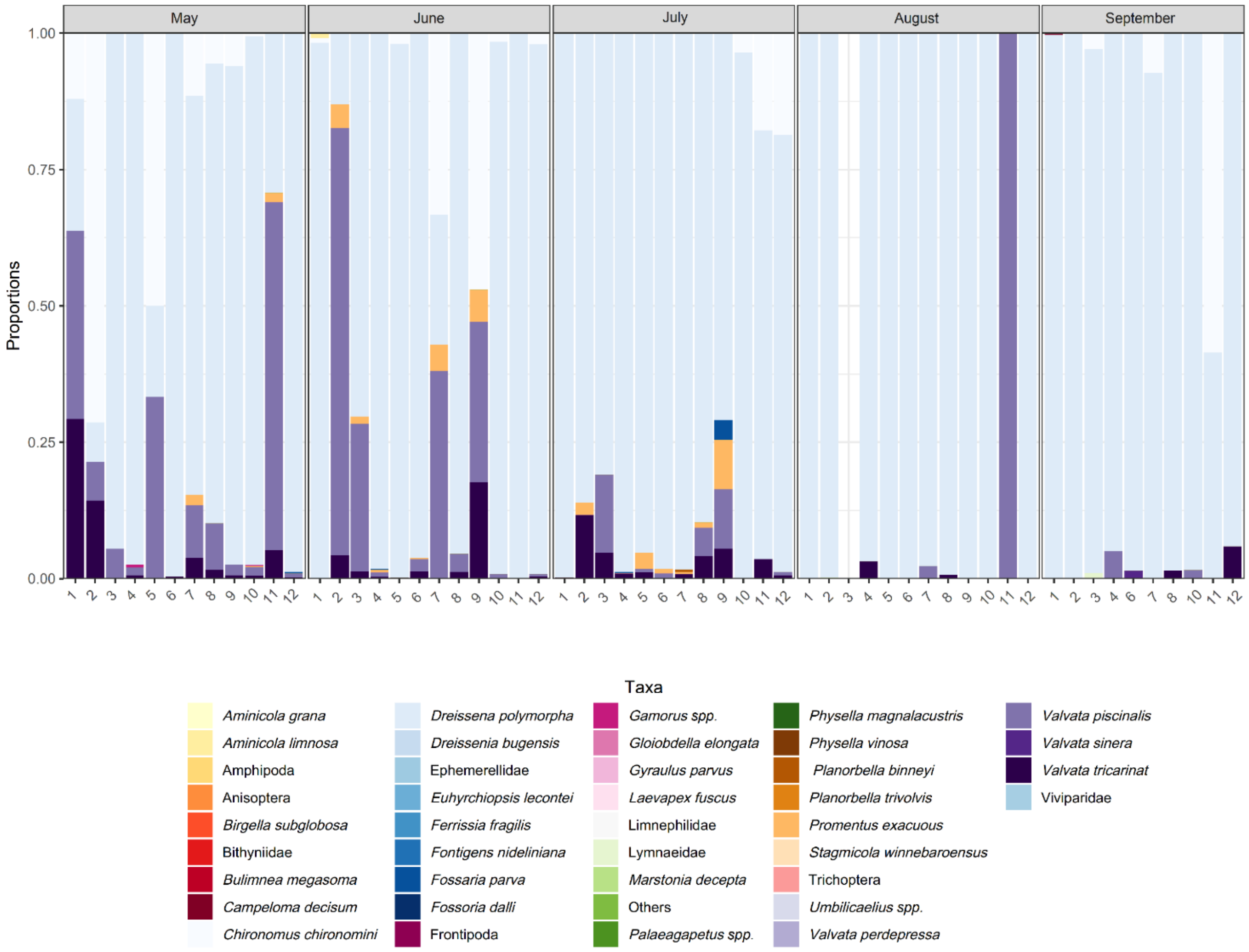

Figure A8. *Taxa plot for the macroinvertebrate community across the five months of sampling (May-September) in 2019 for Lake Scugog.*

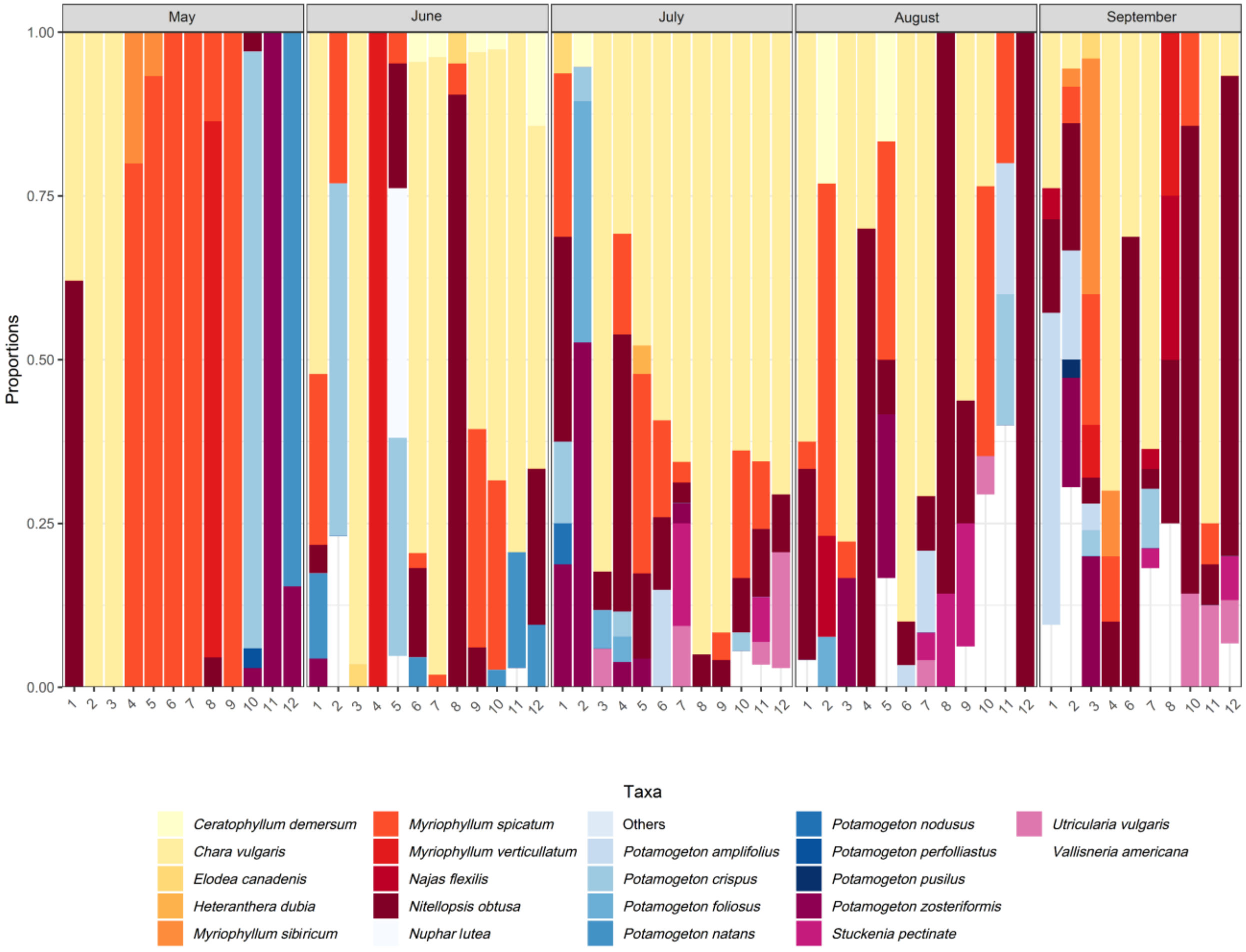

Figure A9. *Taxa plot for the macrophyte community across the five months of sampling (May-September) in 2016 for Lake Scugog.*

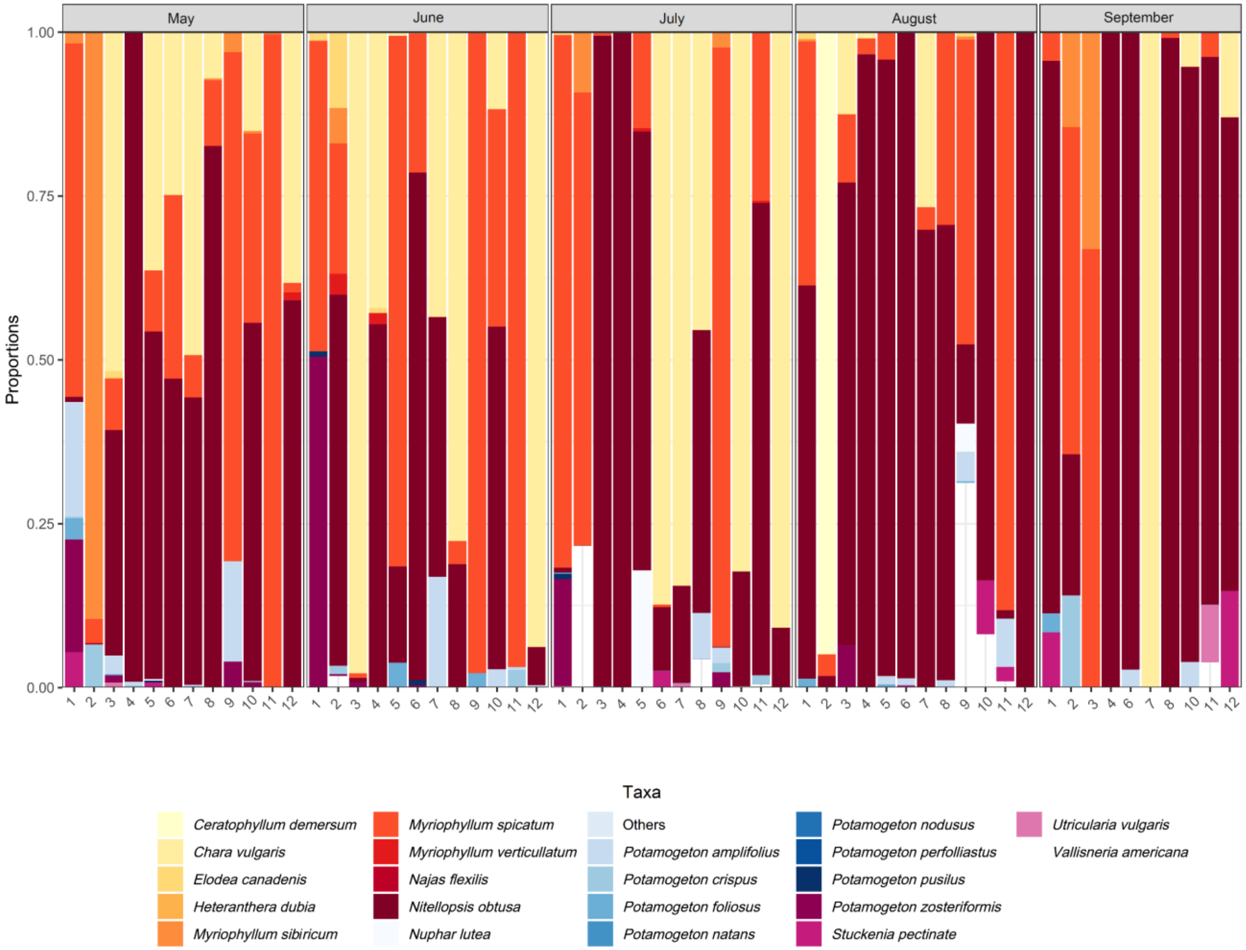

Figure A10. *Taxa plot for the macrophyte community across the five months of sampling (May-September) in 2017 for Lake Scugog.*

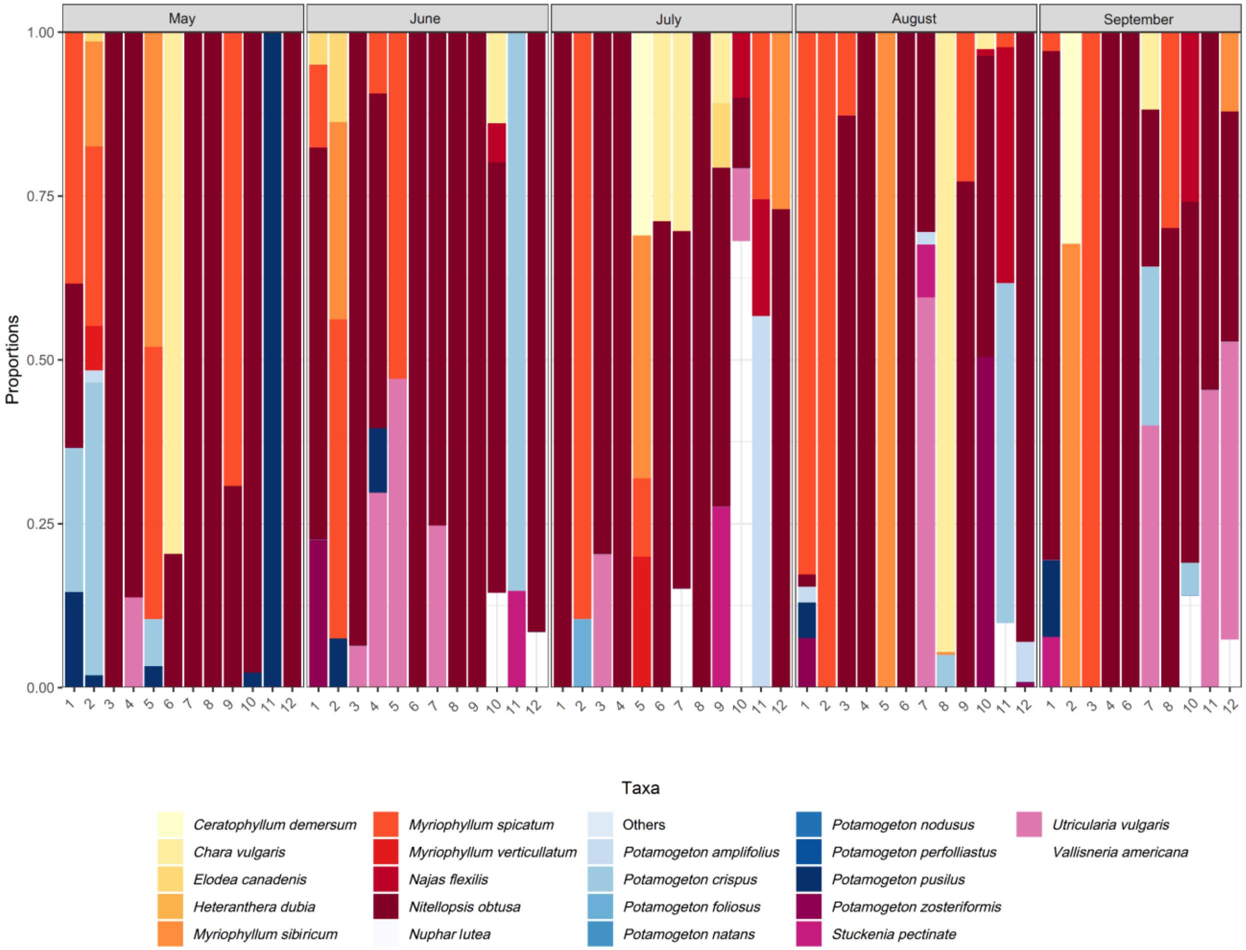

Figure A11. *Taxa plot for the macrophyte community across the five months of sampling (May-September) in 2018 for Lake Scugog.*

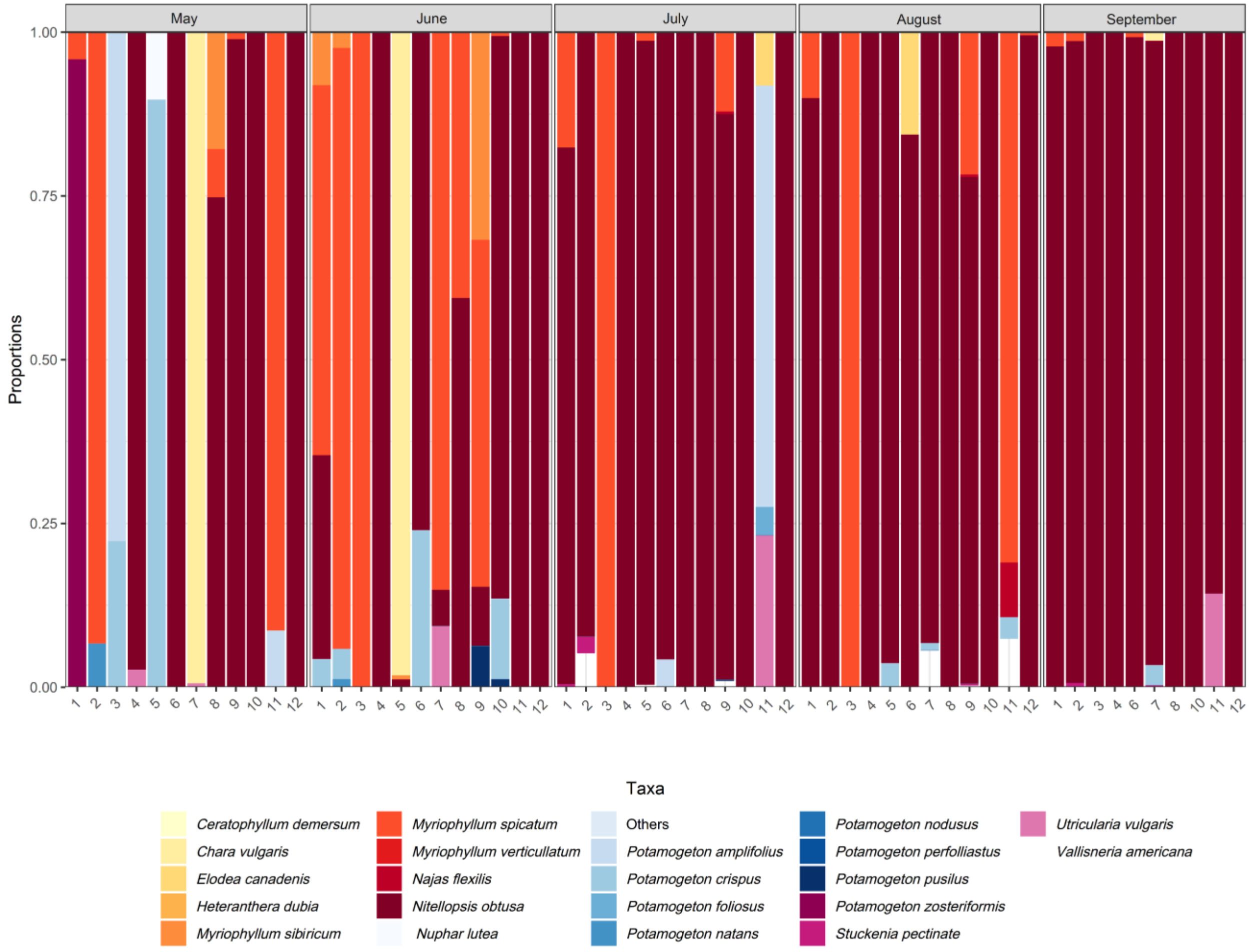

Figure A12. *Taxa plot for the macrophyte community across the five months of sampling (May-September) in 2019 for Lake Scugog.*

Appendix B

Table B1. Average water quality data reflecting the pooled four sites per lake (n = 4). Standard deviation is presented in brackets. Temp = Temperature, DO = dissolved oxygen, Con = Conductivity, Secchi = Secchi depth, TOC = total organic carbon, TN = total nitrogen, TP = total phosphorus, DFBL = distance from boat launch, Ca = calcium, Fe = iron, K = potassium, Mg= magnesium, Mn = manganese, Na = sodium. Ca, Fe, K, Mg, Mn, Na do not have corresponding standard deviations as one sample per lake, from the deepest spot, was sent away for analyses. Temp, DO, pH, Con, Depth, Secchi, TOC, TN, TP, and DFBL had a sample size of four per candidate lake. Ca, Fe, K, Mg, Mn, Na had a corresponding sample size of one per lake.

Lake Name	Temp (°C)	DO (mg · L^{-1})	pH	Con (µS cm^{-1})	Depth (m)	Secchi (m)	TOC (mg · L^{-1})	TN (mg · L^{-1})	TP (µg · L^{-1})	DFBL (m)	Ca (mg · L^{-1})	Fe (mg · L^{-1})	K (mg · L^{-1})	Mg (mg · L^{-1})	Mn (mg · L^{-1})	Na (mg · L^{-1})
Balsam	24.7 (0.2)	9.3 (0.7)	7.6 (0.3)	275.6 (0.9)	2.5 (2.3)	1.6 (0.6)	20.4 (10.3)	0.4 (0.2)	14.2 (4.0)	362.9 (233.8)	10.9	0.02	0.5	1.2	0.006	3.3
Bass	23.2 (0.06)	10.8 (0.7)	8.9 (0.07)	465.9 (3.5)	1.7 (0.3)	1.7 (0.3)	4.8 (1.4)	0.2 (0.06)	27.9 (9.3)	340.4 (198.06)	29.1	0.008	1.3	11.5	0.009	5.12
Beaver	23 (0.08)	8.3 (0.2)	8.6 (0.2)	246.5 (0.9)	2.3 (1.9)	1.4 (0.7)	5.6 (1.0)	0.9 (0.1)	38.8 (6.8)	197.6 (129.6)	7.0	0.02	0.3	1.2	0.02	1.8
Belmont	24.9 (0.1)	10.0 (0.2)	8.7 (0.1)	900.5 (123.7)	3.8 (3.9)	2.1 (0.6)	7.2 (0.9)	0.2 (0.06)	9.9 (1.1)	163.5 (118.7)	24.1	0.008	1.0	2.8	0.005	4.9
Belwood	23.3 (0.05)	11.6 (0.3)	9.2 (0.06)	741.8 (0.5)	11.5 (2.4)	1.4 (0.3)	7.5 (2.0)	0.9 (0.2)	40.2 (16.6)	267.0 (158.4)	83.7	0.03	3.4	24.8	0.005	13.8
Big Cedar	23.0 (0.1)	6.6 (0.1)	8.6 (0.1)	8329.8 (290.6)	2.1 (1.9)	1.6 (1.0)	5.4 (1.4)	0.3 (0.1)	22.7 (15.0)	215.0 (151.8)	21.3	0.07	0.7	2.8	0.01	7.8
Buck	25.9 (0.1)	9.5 (0.6)	8.3 (0.04)	368.0 (9.6)	3.0 (1.8)	2.8 (1.5)	5.3 (1.2)	0.2 (0.05)	18.4 (5.0)	244.8 (136.0)	23.3	0.01	1.3	6.9	0.003	6.3
Camden	23.1 (0.06)	9.4 (0.6)	9.5 (0.05)	403.6 (0.3)	1.0 (0.1)	0.9 (0.1)	24.6 (13.4)	1.1 (0.6)	36.1 (22.0)	149.9 (79.5)	16.6	0.02	0.7	4.9	0.01	2.8
Canal	23.2 (0.2)	8.9 (0.3)	9.5 (0.04)	7236.8 (456.1)	1.3 (0.9)	1.3 (0.9)	5.8 (1.5)	0.3 (0.09)	20.0 (6.2)	83.0 (71.4)	32.3	0.06	1.1	4.9	0.04	13.3
Catchacoma	24.1 (0.05)	8.9 (0.3)	8.2 (0.4)	86.0 (0.1)	3.0 (1.3)	1.4 (0.4)	7.5 (2.2)	0.3 (0.006)	14.3 (4.0)	514.2 (271.8)	7.4	0.04	0.5	0.9	0.002	1.8
Charleston	25.8 (0.4)	14.4 (1.4)	8.2 (0.1)	3710.5 (104.0)	3.9 (3.5)	2.1 (0.9)	8.2 (1.9)	0.3 (0.06)	25.75 (2.5)	486.9 (433.1)	28.8	0.008	1.3	11.4	0.009	5.1
Chemong	24.1 (0.1)	10.8 (1.2)	9.0 (0.4)	2514 (64.8)	2.4 (2.1)	1.2 (0.3)	8.4 (4.1)	0.2 (0.1)	36.0 (13.9)	190.4 (193.5)	29.1	0.05	2.3	2.9	0.03	9.0
Consecon	27.2 (0.2)	10.7 (1.9)	7.8 (0.1)	743.6 (15.8)	3.1 (2.1)	1.9 (0.6)	7.5 (0.7)	0.3 (0.06)	29.5 (11.3)	387.4 (340.3)	48.6	0.02	1.0	2.4	0.005	3.9
Coon	24.3 (0.08)	9.5 (0.2)	8.5 (0.1)	197.4 (0.3)	4.9 (2.7)	1.7 (0.3)	6.2 (0.9)	0.2 (0.05)	21.2 (15.0)	163.3 (133.8)	20.8	0.01	0.5	2.7	0.006	1.7
Cordova	23.9 (0.1)	9.3 (0.8)	8.9 (0.1)	5177.5 (25.0)	5.5 (3.7)	3.5 (2.1)	7.9 (1.3)	0.5 (0.5)	20.6 (4.6)	114.0 (126.0)	23.4	0.02	0.9	2.4	0.007	4.7
Couchiching	23.6 (0.2)	6.2 (0.4)	9.1 (0.1)	1539 (23.1)	2.4 (0.4)	2.4 (0.4)	5.1 (0.5)	0.3 (0.03)	24.2 (8.3)	245.9 (172.6)	40.7	0.009	3.8	12.4	0.003	46.4
Crowe	24.2 (1.6)	8.7 (0.5)	9.0 (0.2)	6230.8 (257.4)	4.1 (3.5)	2.2 (1.2)	7.6 (1.6)	0.3 (0.1)	40.7 (18.3)	144.9 (119.9)	23.2	0.03	0.4	1.8	0.009	3.2
Crystal	23.4 (0.9)	7.6 (0.4)	8.8 (0.04)	7579.8 (545.9)	6.8 (4.4)	3.7 (0.9)	5.3 (0.2)	0.2 (0.001)	21.7 (6.2)	383.5 (241.3)	23.3	0.007	0.8	2.1	0.001	1.7
Dalrymple	22.8 (0.1)	9.8 (0.7)	8.8 (0.05)	465.8 (5.4)	2.6 (1.6)	0.9 (0.4)	7.4 (0.5)	0.4 (0.09)	21.7 (9.9)	615.3 (208.4)	57.9	0.04	2.9	7.2	0.05	9.4
Desert	26.3 (0.1)	9.9 (0.3)	7.9 (0.2)	373.6 (1.7)	4.5 (4.0)	2.6 (1.5)	5.2 (1.1)	0.2 (0.04)	18.2 (5.2)	400.0 (297.6)	22	0.009	1.0	6.9	0.002	4.7
Devil	22.0 (0.5)	7.0 (3.9)	7.4 (0.7)	2174.3 (608.3)	3.2 (2.3)	2.3 (1.0)	5.5 (0.8)	0.1 (0.01)	24.1 (11.1)	163.6 (93.9)	1.5	0.1	0.2	0.5	0.007	0.5
Dickey	22.8 (0.05)	8.3 (0.5)	8.7 (0.08)	4790.3 (165.2)	9.6 (4.6)	4.1 (0.8)	6.8 (0.7)	0.1 (0.03)	15.7 (4.8)	369.5 (86.4)	23.5	0.05	1.1	1.5	0.004	3.0
Eels	22.7 (1.3)	9.4 (0.8)	7.8 (0.5)	1474 (257.9)	4.0 (3.2)	2.9 (1.2)	5.1 (0.4)	0.2 (0.02)	17.1 (4.7)	369.5 (234.6)	9.5	0.02	0.5	0.9	0.005	1.4
Eugenia	23.0 (0.2)	10.0 (0.2)	8.7 (0.4)	664.3 (0.5)	4.3 (2.1)	3.3 (1.4)	6.2 (3.1)	0.5 (0.3)	12.5 (3.3)	333.0 (143.1)	47.8	0.03	1.0	33.0	0.003	46.4
Faraday	22.5 (0.2)	9.2 (0.1)	8.3 (0.2)	1709.8 (326.4)	3.6 (2.8)	2.2 (1.0)	4.2 (0.5)	0.1 (0.04)	190.1 (32.7)	217.1 (194.7)	7.1	0.07	0.9	1.2	0.003	2.4
Gananoque	27.5 (0.4)	12.0 (2.4)	8.4 (0.2)	4642.3 (30.2)	4.6 (3.4)	2.0 (0.7)	20.5 (23.6)	0.9 (0.7)	32.1 (9.4)	475.8 (424.1)	37.6	0.02	2.5	12.1	0.02	7.8
Gull	22.9 (0.1)	8.6 (0.2)	8.1 (0.05)	2644.5 (205.7)	5.0 (3.1)	4.4 (2.4)	3.5 (0.4)	0.1 (0.01)	8.5 (3.8)	107.4 (97.0)	3.6	0.01	0.3	0.8	0.001	2.1
Hambly	27.0 (0.1)	9.3 (0.3)	7.9 (0.06)	766.5 (2.1)	4.1 (2.7)	3.2 (1.3)	8.0 (0.7)	0.4 (0.04)	35.3 (16.5)	94.3 (90.4)	36.2	0.007	1.8	11.4	0.004	12.3
Harp	22.8 (0.1)	10.0 (0.6)	7.7 (0.3)	5289.8 (759.4)	6.6 (4.5)	2.3 (1.1)	5.1 (0.3)	0.1 (0.03)	62.6 (9.3)	133.6 (80.1)	0.4	0.03	0.1	0.1	0.003	0.4
Head	23.8 (0.4)	9.6 (0.7)	8.5 (0.4)	2517.3 (93.0)	2.9 (1.0)	2.9 (1.0)	4.6 (0.2)	0.2 (0.04)	46.9 (20.0)	125.6 (92.6)	9.1	0.04	0.7	2.2	0.005	3.2
Jack	23.2 (0.2)	10.1 (0.6)	8.5 (0.1)	210.4 (0.8)	8.7 (3.2)	3.1 (0.2)	8.7 (3.7)	0.3 (0.04)	10.0 (1.4)	541.5 (391.4)	20.0	0.03	1.7	1.6	0.006	4.8
Kashabog	24.8 (0.2)	9.6 (0.1)	8.2 (0.1)	147.5 (0.5)	10.6 (7.7)	4.2 (2.7)	3.9 (0.8)	0.2 (0.06)	18.4 (10.1)	164.3 (103.6)	7.68	0.04	0.8	0.9	0.007	5.2
Kashagawigamog	23.7 (0.2)	9.2 (0.06)	8.5 (0.2)	4151.0 (1645.1)	1.7 (0.7)	1.7 (0.7)	5.6 (0.8)	0.2 (0.07)	21.4 (6.2)	280.9 (199.6)	14.0	0.07	2.3	2.9	0.01	7.4
Lake of Bays	22.6 (1.3)	9.9 (0.7)	7.9 (0.3)	7490 (51.9)	10.1 (6.1)	3.25 (1.2)	7.4 (6.8)	0.2 (0.07)	19.0 (13.4)	278.8 (117.9)	4.4	0.05	2.4	1.2	0.005	5.9
Long	23.2 (0.1)	7.9 (0.1)	7.1 (0.005)	460.4 (1.9)	4.2 (2.0)	3.1 (1.2)	6.2 (2.7)	0.3 (0.1)	5.5 (0.4)	101.1 (84.3)	1.72	0.1	0.3	0.5	0.007	0.7
Loughborough	25.9 (0.2)	10.3 (0.8)	7.86 (0.3)	573.8 (19.1)	3.4 (2.3)	2.0 (0.6)	12.2 (9.5)	0.3 (0.05)	30.1 (4.8)	338.0 (280.4)	22	0.03	1.5	4.6	0.003	6.43
Lower Beverly	26.5 (0.4)	14.0 (3.0)	8.5 (0.06)	4811.8 (26.7)	4.4 (3.3)	2.2 (1.1)	17.1 (12.0)	0.6 (0.3)	33.8 (20.9)	1046.3 (600.6)	23.3	0.05	0.8	5.1	0.02	4.4
Lower Buckhorn	23.3 (0.1)	10.4 (0.2)	9.1 (0.1)	326.5 (8.8)	1.2 (0.5)	1.2 (0.5)	8.0 (3.4)	0.4 (0.4)	25.7 (11.5)	304.2 (237.1)	28.6	0.01	0.7	4.0	0.008	8.8
Maple	23.5 (0.3)	9.8 (0.2)	8.1 (0.2)	1233.8 (23.5)	4.0 (2.6)	2.8 (1.1)	4.5 (0.4)	0.1 (0.03)	21.8 (5.1)	457.6 (330.0)	4.4	0.06	0.8	0.9	0.007	1.8

Moira	22.6 (1.5)	8.7 (0.4)	9.7 (0.1)	482.2 (0.4)	4.0 (1.8)	2.1 (1.2)	9.3 (2.4)	0.3 (0.08)	29.7 (11.7)	253.9 (178.3)	42.2	0.02	1.3	6.2	0.03	7.3
Nine mile	23.5 (0.05)	9.0 (0.1)	8.4 (0.5)	2776.0 (28.6)	4.8 (2.0)	2.5 (0.2)	8.4 (2.6)	0.3 (0.1)	20.9 (5.4)	190.8 (127.8)	1.1	0.06	0.5	0.3	0.008	1.1
Opinicon	27.8 (0.3)	12.7 (0.6)	8.6 (0.2)	3144.5 (128.6)	1.4 (0.6)	1.4 (0.6)	11.7 (6.8)	0.5 (0.1)	40.8 (17.6)	1029.6 (285.4)	15.9	0.01	0.5	4.1	0.003	3.3
Otter	25.4 (0.3)	9.6 (0.6)	8.0 (0.4)	430.5 (20.3)	5.4 (4.4)	2.9 (1.4)	8.0 (1.2)	0.4 (0.1)	20.3 (6.1)	361.8 (285.4)	29.6	0.007	1.5	14.7	0.003	9.5
Pigeon	24.4 (0.8)	9.0 (1.4)	7.4 (0.3)	470.3 (7.5)	2.7 (2.5)	1.9 (0.9)	6.9 (1.4)	0.2 (0.002)	15.3 (4.8)	1524.8 (837.5)	32.8	0.02	1.1	3.9	0.008	8.3
Rice	24.7 (0.3)	10.2 (0.1)	9.1 (0.02)	448.7 (2.3)	1.2 (0.6)	1.2 (0.6)	6.3 (1.4)	0.3 (0.05)	39.0 (26.9)	263.7 (176.8)	34.4	0.05	0.6	2.2	0.03	4.7
Round	24.1 (0.3)	10.0 (0.2)	8.7 (0.07)	350.8 (10.4)	3.6 (1.2)	2.1 (0.3)	6.8 (2.1)	0.3 (0.1)	23.2 (12.5)	280.9 (199.2)	28.2	0.04	0.6	2.3	0.01	4.5
Scugog	21.7 (0.1)	10.6 (0.8)	9.5 (0.07)	574.2 (65.7)	2.2 (0.7)	1.4 (0.4)	9.7 (3.3)	0.5 (0.2)	53.3 (23.2)	1407.8 (320.8)	37.1	0.01	3.0	9.3	0.03	23.2
Shadow	22.6 (0.1)	9.1 (0.2)	8.1 (0.2)	1961.9 (1198.6)	6.9 (3.0)	4.8 (1.3)	5.4 (1.6)	0.2 (0.05)	17.1 (5.4)	808.8 (240.1)	2.98	0.02	0.2	0.3	0.003	0.9
Shoe	23.6 (0.4)	9.6 (0.8)	7.3 (0.5)	5116.7 (384.2)	4.0 (2.0)	4.0 (1.0)	5.8 (1.0)	0.2 (0.07)	32.0 (25.4)	57.8 (53.9)	2.23	0.01	0.5	0.7	0.004	1.2
Simcoe	23.3 (0.5)	7.7 (0.6)	9.1 (0.2)	1541.0 (27.1)	1.5 (0.4)	1.5 (0.4)	5.6 (1.8)	0.2 (0.03)	30.7 (12.5)	340.2 (232.8)	44.8	0.007	3.3	10.5	0.002	40.7
Skeleton	22.8 (0.05)	9.3 (0.8)	8.5 (0.1)	1251.2 (85.4)	5.0 (2.9)	2.1 (0.9)	2.6 (0.1)	0.3 (0.03)	37.7 (32.0)	129.5 (101.5)	7.26	0.03	1.1	1.1	0.003	8.0
Stewart	23.2 (0.06)	8.2 (0.5)	8.4 (0.1)	3547.0 (26.3)	4.0 (2.2)	2.5 (1.0)	8.6 (5.2)	0.2 (0.8)	23.6 (22.8)	191.5 (176.7)	4.84	0.03	0.4	0.4	0.004	5.5
Stoco	23.0 (0.1)	8.3 (0.9)	8.7 (0.1)	366.4 (1.5)	3.5 (0.6)	1.4 (0.2)	5.5 (3.4)	0.3 (0.2)	32.7 (15.9)	289.3 (193.9)	18.4	0.04	0.4	2.3	0.05	2.3
Thirteen Island	26.2 (0.1)	9.6 (0.2)	7.9 (0.2)	478.9 (1.1)	4.4 (3.7)	3.3 (2.8)	4.9 (0.5)	0.2 (0.05)	27.7 (9.0)	552.3 (424.9)	15.1	0.02	1.6	1.9	0.004	2.4
Upper Stoney	25.8 (0.3)	10.4 (0.6)	7.5 (0.2)	442.3 (15.3)	3.5 (2.1)	2.45 (0.5)	6.8 (0.8)	0.2 (0.05)	18.9 (8.3)	572.1 (474.5)	11.8	0.01	0.3	0.4	0.01	1.1
Vernon	22.7 (0.05)	9.2 (0.2)	7.8 (0.3)	8748.7 (75.4)	5.0 (6.6)	2.0 (1.4)	6.1 (0.8)	0.2 (0.01)	18.6 (2.4)	307.3 (243.3)	3.36	0.2	0.6	1.0	0.006	4.0
Wagner	23.1 (0.05)	13.7 (0.3)	9.0 (0.05)	947.2 (1.0)	5.4 (2.2)	0.9 (0.3)	9.0 (1.6)	0.3 (0.05)	50.5 (26.7)	141.3 (119.2)	38.6	0.008	2.0	10.5	0.003	34.3
West Twin	24.9 (0.3)	9.1 (0.1)	8.0 (0.1)	149.1 (1.5)	3.8 (0.8)	3.3 (0.6)	4.8 (1.0)	0.2 (0.08)	14.5 (4.0)	210.0 (173.6)	19.11	0.06	0.8	0.9	0.004	11.6
Wilbermere	22.2 (0.7)	8.7 (0.3)	7.9 (0.2)	3496.2 (186.5)	8.3 (7.2)	2.8 (1.6)	4.6 (0.3)	0.1 (0.02)	24.2 (16.2)	219.4 (154.3)	14.9	0.04	0.9	2.1	0.002	4.3
Wood	23.4 (0.4)	10.4 (0.5)	7.8 (0.2)	73.1 (186.5)	2.6 (7.2)	1.5 (1.6)	23.8 (0.3)	0.5 (0.02)	18.9 (5.6)	204.6 (124.4)	3.88	0.007	0.1	1.7	0.001	0.6

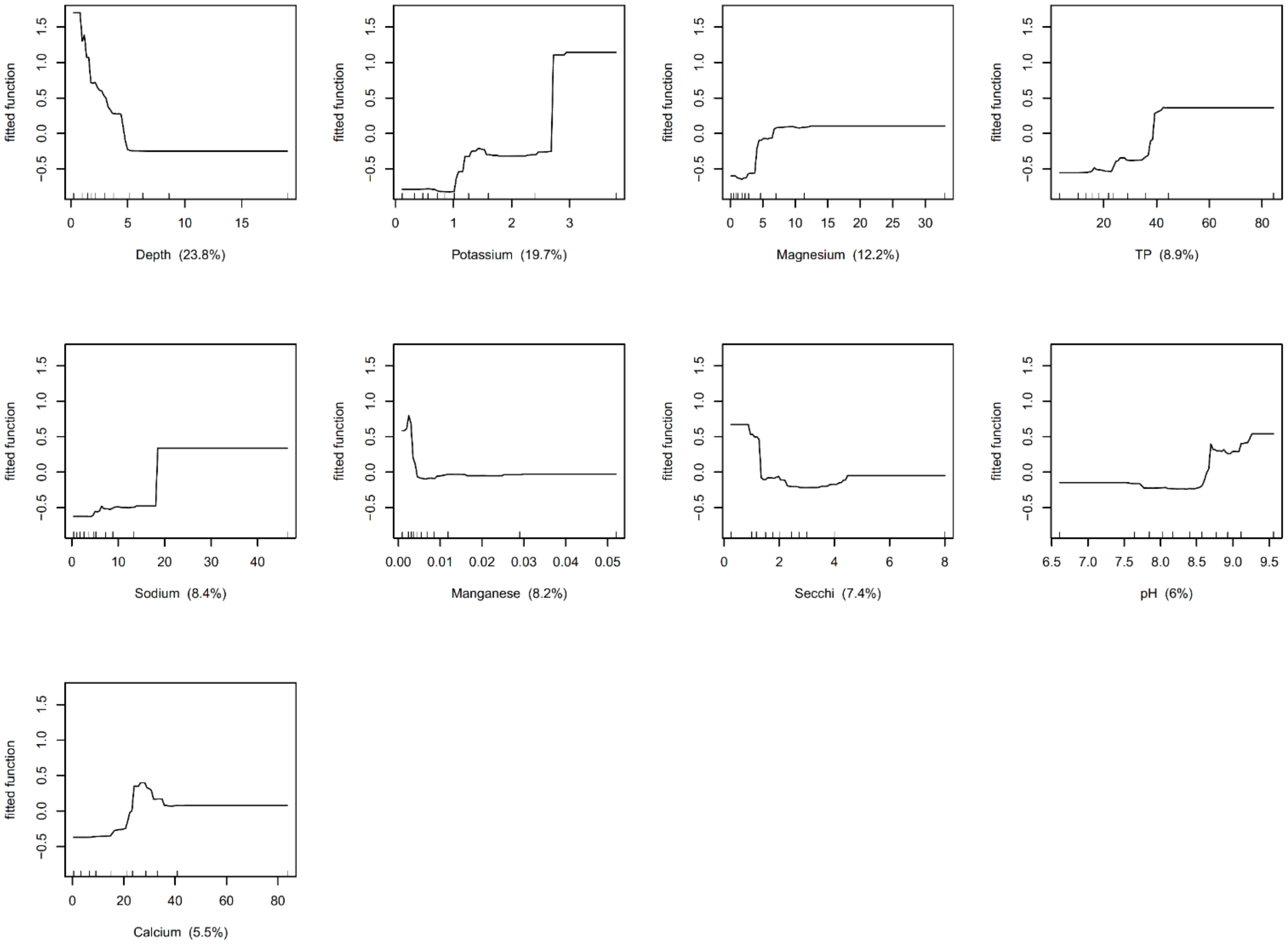

Figure B1. *Partial dependence plots for the nine environmental variables used in the BRT model for* N. obtusa*. They y-axis is standardized across the corresponding variable on a logit scale and is centered to have a mean of zero for the data distribution of the fitted function.*

Appendix C

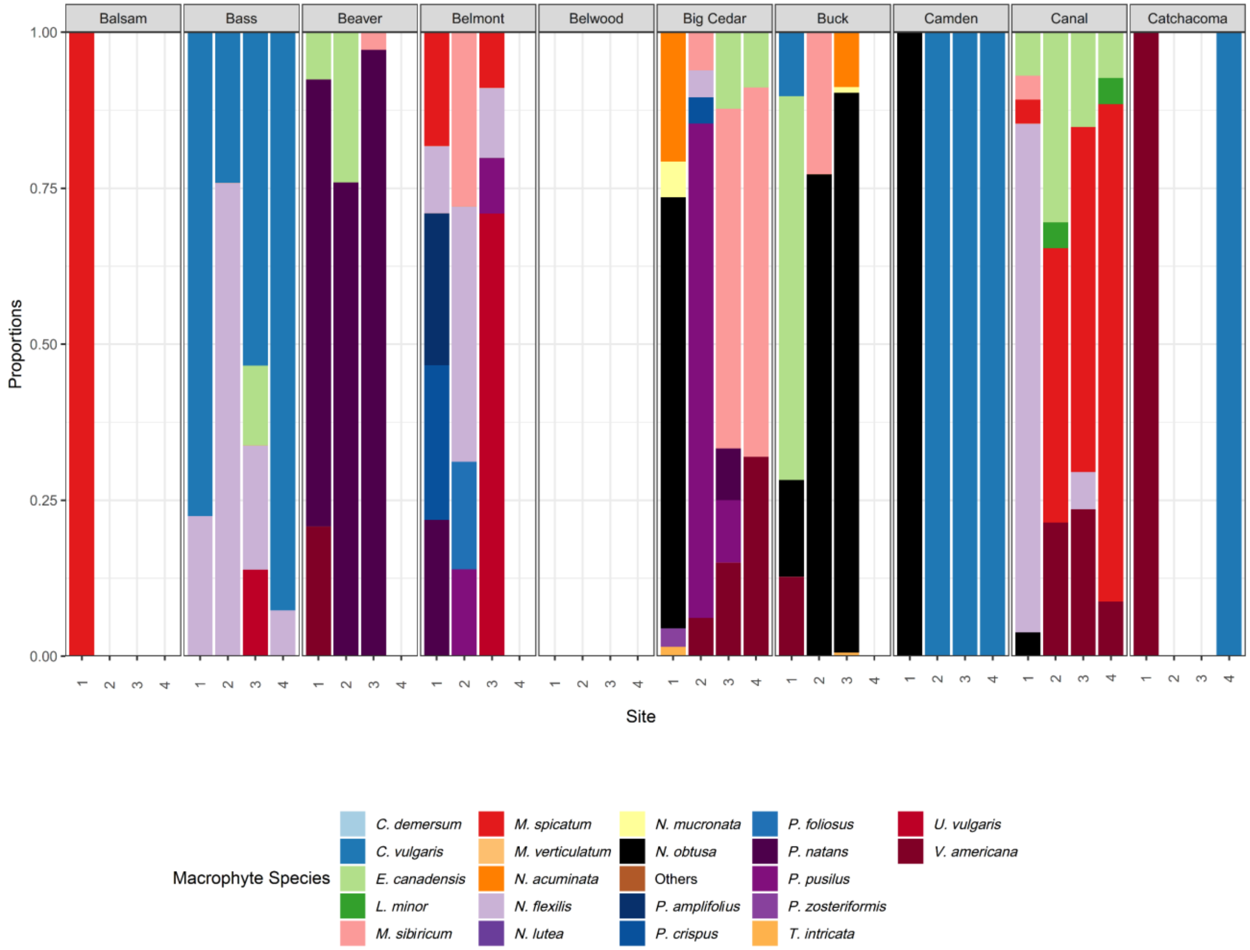

Figure C1. *Taxa plot depicting the macrophyte community composition across the first 10 study lakes investigated for the presence of* N. obtusa.

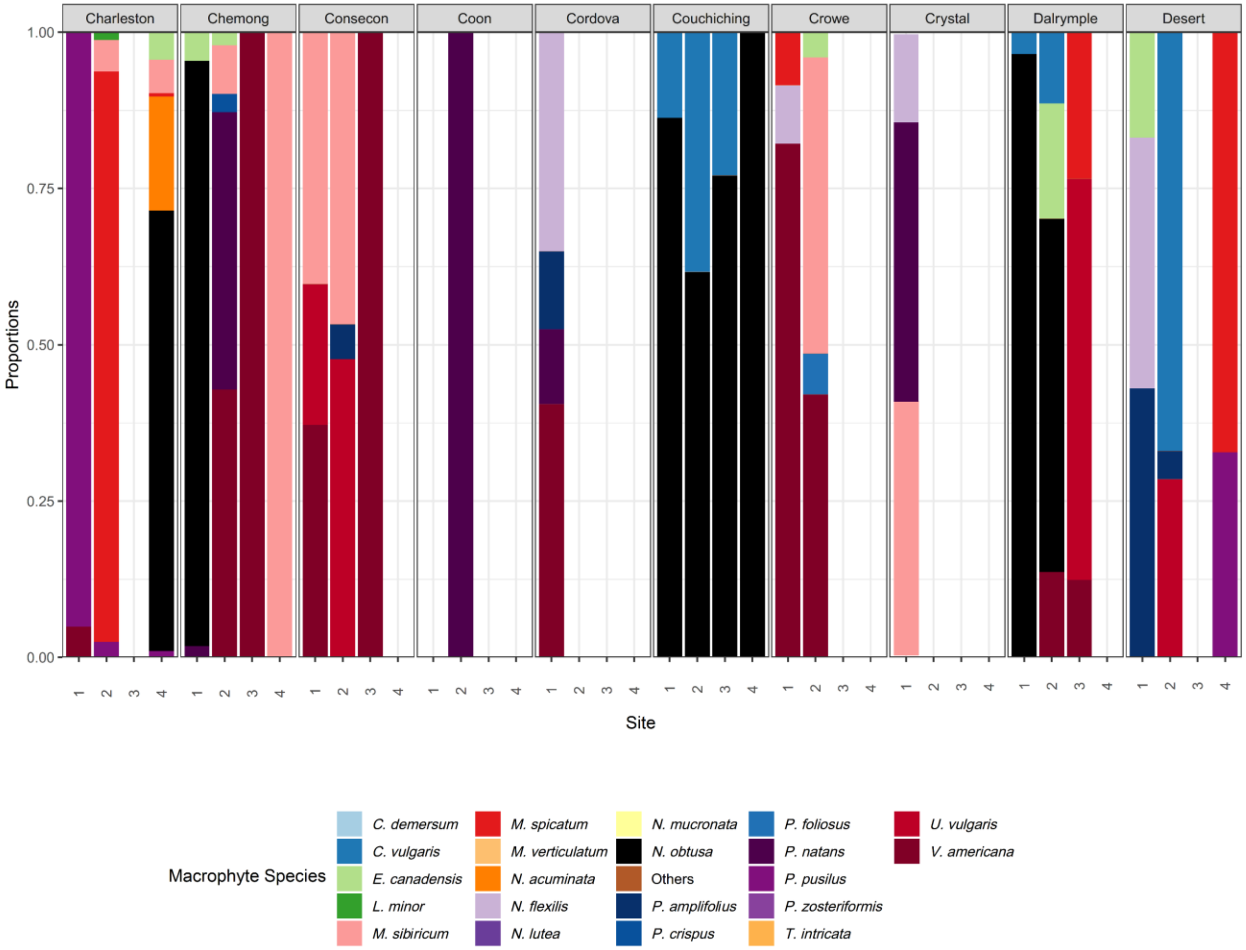

Figure C2. *Taxa plot depicting the macrophyte community composition across study lakes 11-20 investigated for the presence of* N. obtusa.

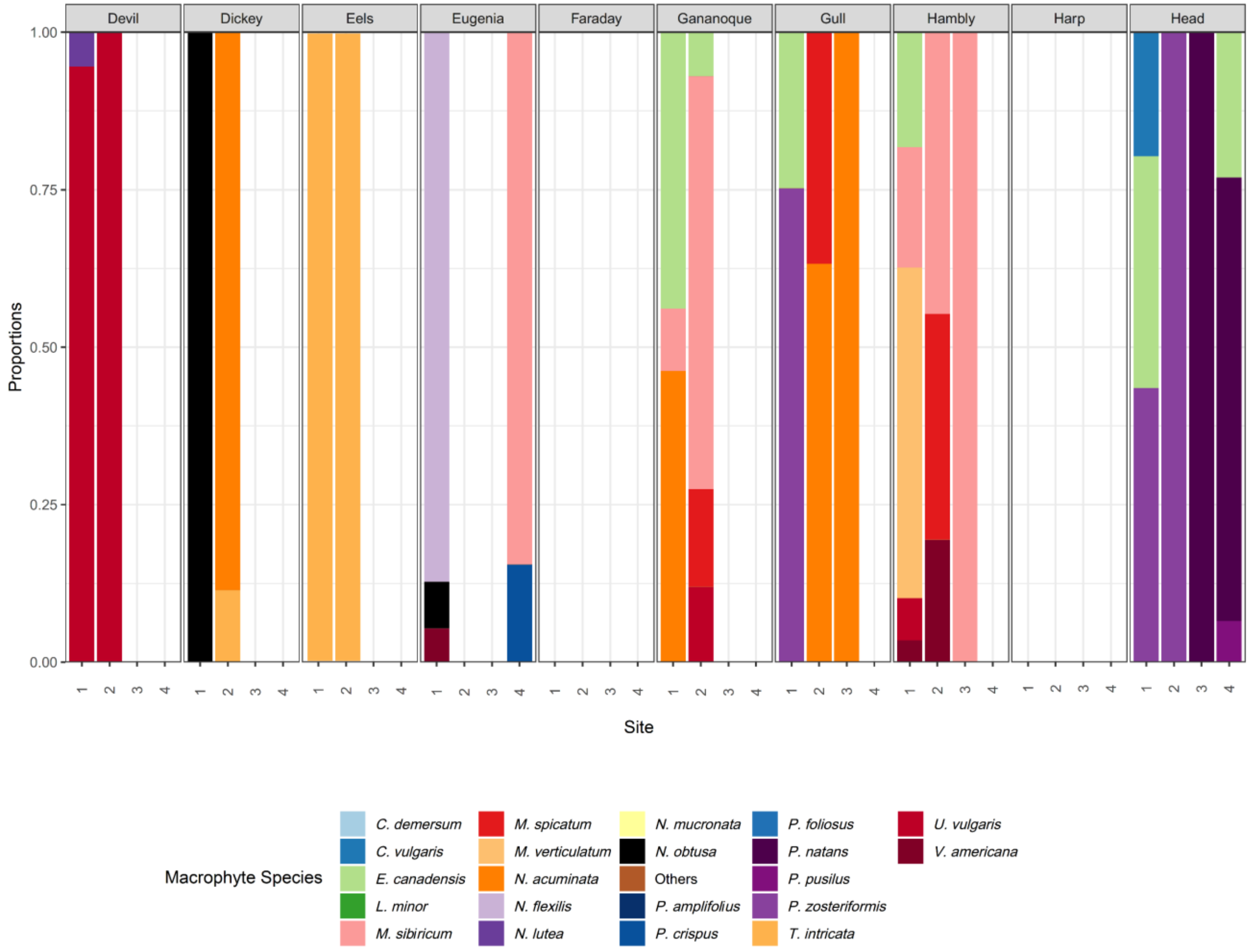

Figure C3. *Taxa plot depicting the macrophyte community composition across study lakes 21-30 investigated for the presence of* N. obtusa.

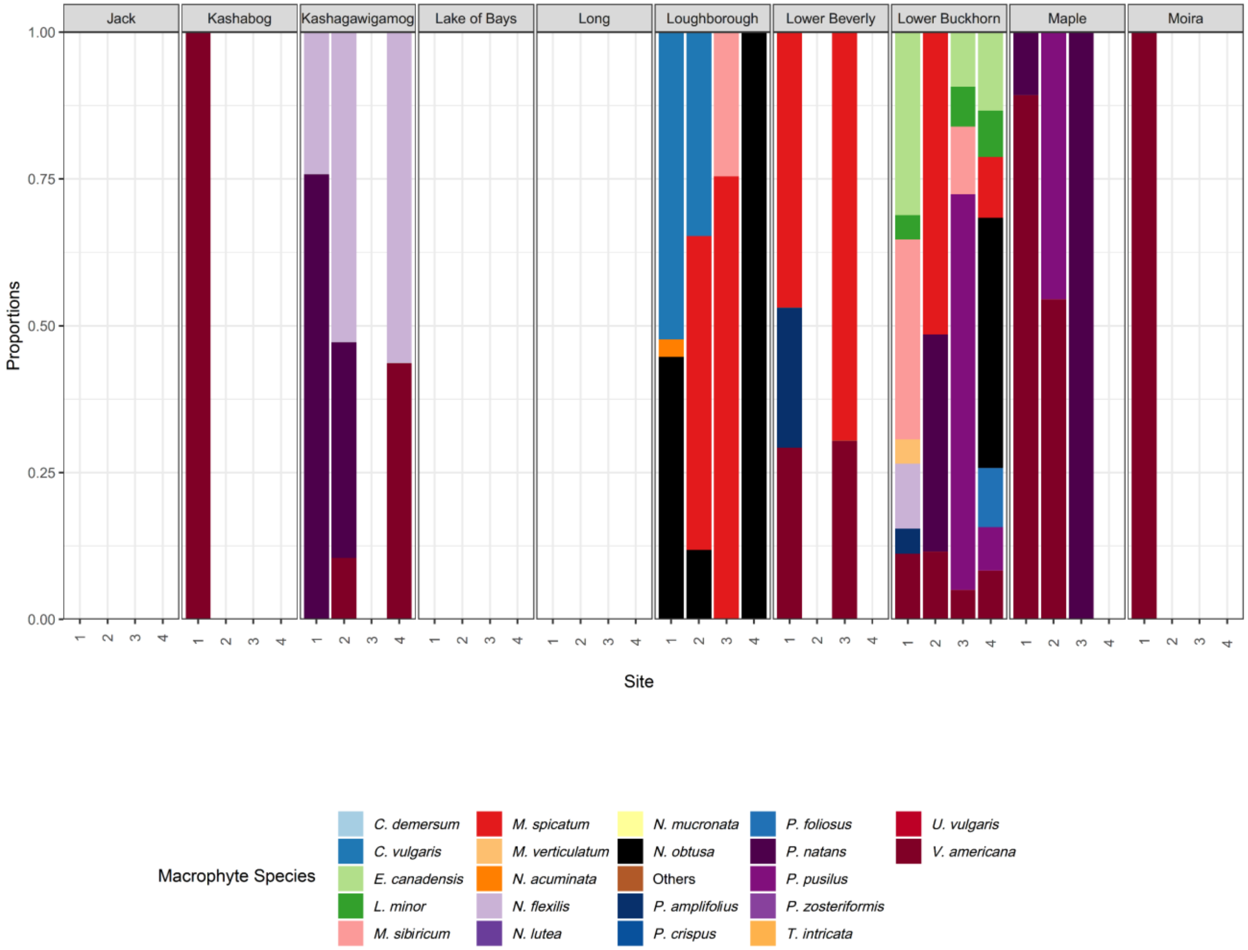

Figure C4. *Taxa plot depicting the macrophyte community composition across study lakes 31-40 investigated for the presence of* N. obtusa.

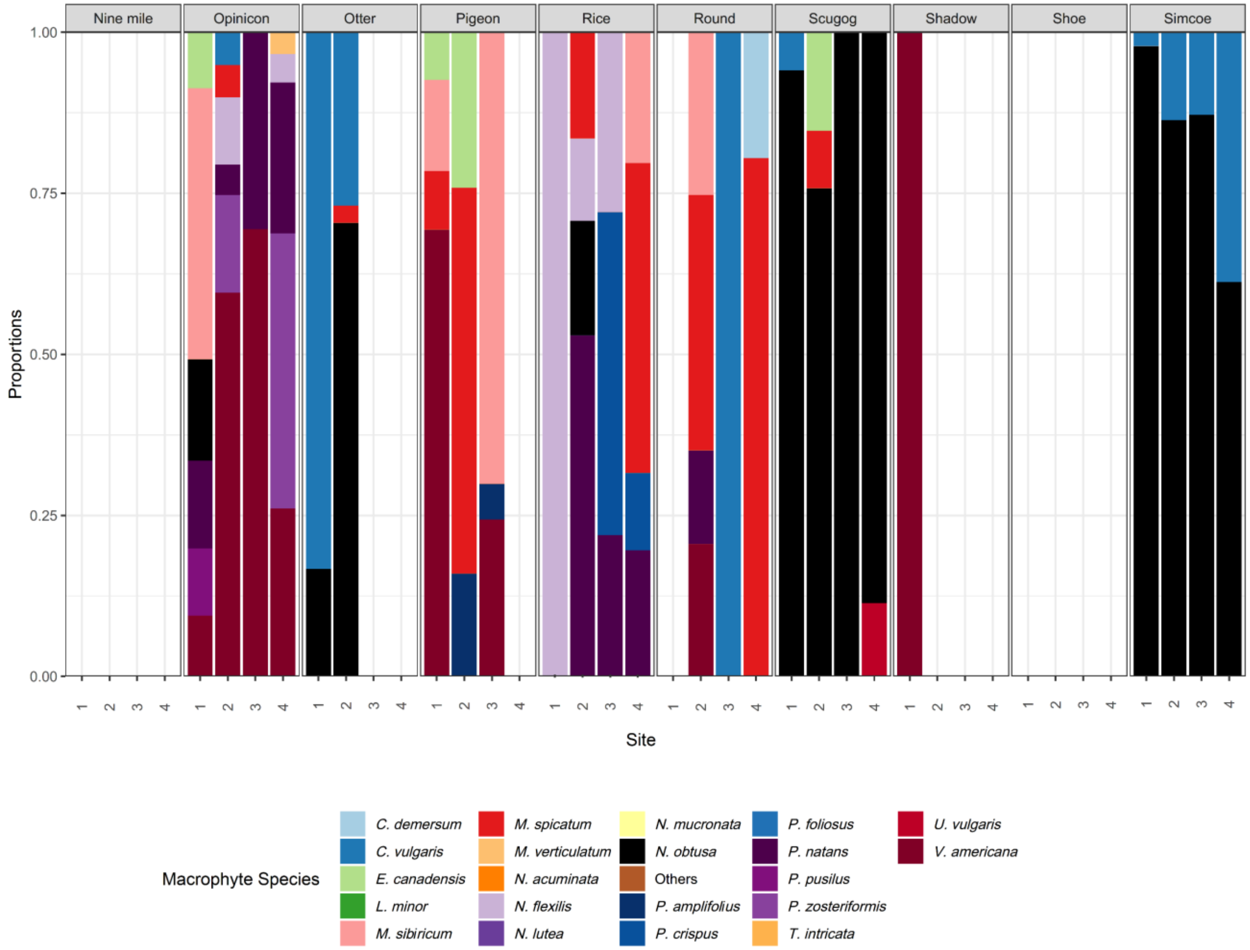

Figure C5. *Taxa plot depicting the macrophyte community composition across study lakes 41-50 investigated for the presence of* N. obtusa.

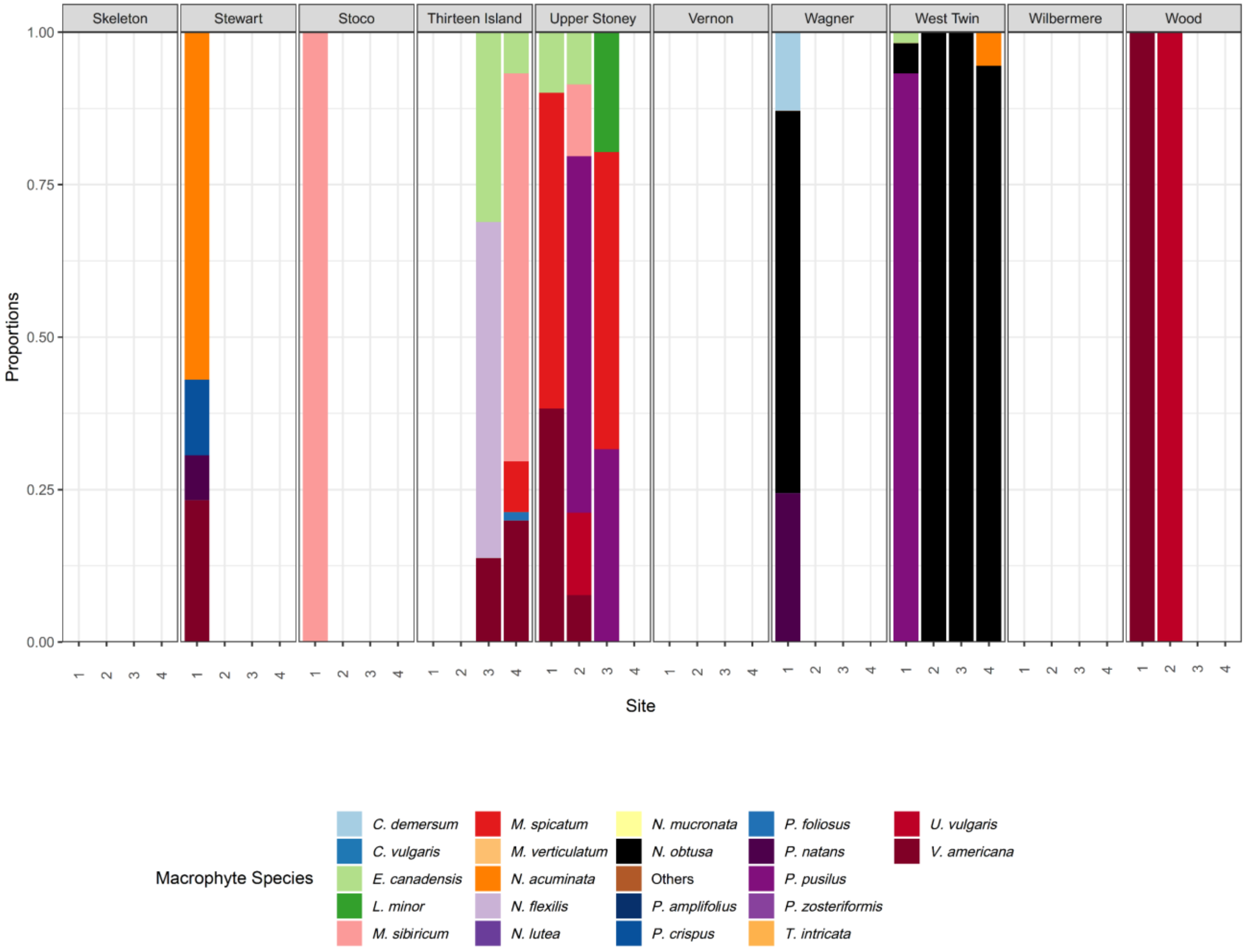

Figure C6. *Taxa plot depicting the macrophyte community composition across study lakes 51-60 investigated for the presence of* N. obtusa.