EVALUATING THE ROLE OF INVASIVE DREISSENID MUSSELS ON HARMFUL ALGAL BLOOM FORMATION AND TOXICITY USING CITIZEN SCIENCE DATA

by

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# Copyright Page

Acknowledgements

# Table of Contents

[Copyright Page ii](#_Toc43901847)

[Acknowledgements iii](#_Toc43901848)

[Table of Contents iv](#_Toc43901849)

[List of Tables vi](#_Toc43901850)

[List of Figures vii](#_Toc43901851)

[Abstract viii](#_Toc43901852)

[Chapter 1: Introduction 1](#_Toc43901853)

[1.1 Harmful Algal Blooms (HABs) 1](#_Toc43901854)

[1.1.1 Description of cyanobacterial HABs 1](#_Toc43901855)

[1.1.2 Temporal trends in HABs 1](#_Toc43901856)

[1.1.3 Factors associated with the formation of HABs 3](#_Toc43901857)

[1.2 Dreissenids 4](#_Toc43901858)

[1.2.1 Introduction to Great Lakes and inland lakes of New York 4](#_Toc43901859)

[1.2.2 Biological description 5](#_Toc43901860)

[1.3 Dreissenids as a biological driver of HABs in low nutrient lakes 5](#_Toc43901861)

[1.3.1. Selective filtration 5](#_Toc43901862)

[1.3.2. Nutrient alteration and redistribution 6](#_Toc43901863)

[1.4 Citizen Science 7](#_Toc43901864)

[1.4.1. History and citizen science in New York State 7](#_Toc43901865)

[1.4.2 Monitoring and sampling dreissenid mussels 8](#_Toc43901866)

[1.5 Research Motivation / Objectives 8](#_Toc43901867)

[1.6 References 11](#_Toc43901868)

[Chapter Two: Using citizen-science data to evaluate the role of dreissenid mussels in harmful algal bloom formation in low-nutrient lakes in New York State 20](#_Toc43901869)

[2.1 Abstract 21](#_Toc43901870)

[2.2 Introduction 22](#_Toc43901871)

[2.3 Methods 26](#_Toc43901872)

[2.3.1 Sample acquisition and analyses 26](#_Toc43901873)

[2.3.2 Lake selection 28](#_Toc43901874)

[2.3.3 Statistical analyses 28](#_Toc43901875)

[2.4 Results 30](#_Toc43901876)

[2.4.1 Changes in water quality after dreissenid invasion (historical analyses) 31](#_Toc43901877)

[2.4.2 Comparing water quality and HABs parameters between invaded and uninvaded lakes in New York (2012-2017) 31](#_Toc43901878)

[2.4.2 Effects of eutrophication on microcystin concentration 32](#_Toc43901879)

[2.5 Discussion 32](#_Toc43901880)

[2.6 References 38](#_Toc43901881)

[Chapter Three: Use of artificial substrate to monitor dreissenid populations to supplement an existing citizen-science water quality collection program 47](#_Toc43901882)

[3.1 Abstract 48](#_Toc43901883)

[3.2 Introduction 48](#_Toc43901884)

[3.3 Methods 54](#_Toc43901885)

[3.3.1 Site selection 54](#_Toc43901886)

[3.3.2 Artificial substrate 54](#_Toc43901887)

[3.3.3 Summer variations in abundance and shell length 55](#_Toc43901888)

[3.4 Results 56](#_Toc43901889)

[3.4.1 Variation in abundance and shell lengths 56](#_Toc43901890)

[3.4.2 Shell length distributions 57](#_Toc43901891)

[3.5 Discussion 57](#_Toc43901892)

[3.5.1 Variation in abundance and shell lengths 58](#_Toc43901893)

[3.5.2 Lake-wide abundance estimation 60](#_Toc43901894)

[3.5.3 Artificial substrates as a methodology for citizen science 61](#_Toc43901895)

[3.6 References 62](#_Toc43901896)

[Chapter Four: Synthesis and Conclusions 72](#_Toc43901897)

[4.1 Review of Research Objectives 72](#_Toc43901898)

[4.2 Chapter Two: Using citizen-science data to evaluate the role of dreissenid mussels in harmful algal bloom formation in low-nutrient lakes in New York State 72](#_Toc43901899)

[4.2.1 Main findings 72](#_Toc43901900)

[4.2.2 Future research 73](#_Toc43901901)

[4.3 Chapter Three:Use of artificial substrate to monitor population dynamics of dreissenids to supplement an existing citizen-science water quality collection program 73](#_Toc43901902)

[4.3.1 Main findings 73](#_Toc43901903)

[4.3.2 Future research 73](#_Toc43901904)

[Appendices 74](#_Toc43901905)

[Curriculum Vita 75](#_Toc43901906)

# List of Tables

**Table 2-1a.** Summary statistics of water quality and bloom variables for the global dataset

**Table 2-1b.** Summary statistics of water quality and bloom variables for the reduced dataset

**Table 2-2a.** Results of global mixed effects model with dreissenids, catchment area to surface area ratio, and mean depth as fixed effects

**Table 2-2b.** Results of reduced mixed effects model with dreissenids as fixed effect

**Table 2-S1.** Descriptions of each lake (list of lakes alphabetically)

**Table 2-S2.** Description, unit, source, and sampling frequency of variables

**Table 3-1.** Site descriptions (location, depth, distance from shore, sediment, and macrophytes)

**Table 3-2.** Summary statistics for shell length (mm).

**Table 3-3.** Sampling methods and results for Owasco, Song and Honeoye lakes.

**Table S3-1.** Lake descriptions (location, physical characteristics, invasion status)

**Table S3-2.** CSLAP trophic status guidelines

# List of Figures

**Fig 2-1.** TP and microcystin regression for the global dataset

**Fig 2-2.** Chlorophyll and microcystin regression for the global dataset

**Figure 3-1.** Map of lakes where artificial substrates were deployed.

**Figure 3-2.** Abundance grouped by length of deployment

**Figure 3-3.** Average abundance across all lakes per length of deployment time. Error bars are mean ± 1 s.d.

**Figure 3-4.** Average abundance across all sampling points per lake. Abundance ordered by year of invasion. Error bars are mean ± 1 s.d.

**Figure 3-5.** Average abundance across all lakes per length of sampling time. Error bars are mean ± 1 s.d.

**Figure S3-1.** Shell length distributions for each lake where mussels were found

# Abstract

V.G. Field. Evaluating the Role of Invasive Dreissenid Mussels on Harmful Algal Bloom Formation and Toxicity Using Citizen Science Data, XX pages, XX tables, XX figures, 2020. APA style guide used.

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# Chapter 1: Introduction

## 1.1 Harmful Algal Blooms (HABs)

### 1.1.1 Description of cyanobacterial HABs

Harmful algal blooms (HABs) are characterized as an excess growth of cyanobacteria or algae. Cyanobacteria (formerly referred to as blue-green algae) constitute a large and diverse group of photosynthetic prokaryotes (Stanier and Cohen-Bazire 1977). Some genera of cyanobacteria (including *Microcystis*, the genus that this thesis will focus on) can produce hepatotoxins and neurotoxins (O’Neil et al. 2012). The hepatotoxin microcystin has been linked to human illness, pet illness and death, and livestock death (Trevino-Garrison et al. 2015). Further, beach closures and drinking-water advisories have resulted from the risk of toxins produced by HABs. HABs are characterized by the physical presentation of pea-soup green color and surface scum. The spatial distribution of HABs within a lake is influenced by fetch and HABs are usually detected near the shoreline where the mass of the bloom has been pushed by wind and currents (Figueiras et al. 2006; Wu et al. 2015).

### 1.1.2 Temporal trends in HABs

HABs in freshwater systems are a growing concern for stakeholders. A review (Harke et al. 2016) of the global distribution of the cyanobacteria *Microcystis aeruginosa* found that *Microcystis* blooms had been reported in over 100 countries, an increase from earlier reports which cited less than 30 countries reporting *Microcystis* blooms (Zurawell et al. 2005). In the United States in the mid 20th century, HABs were prevalent in freshwater systems due to increased eutrophication. By the 1970s, lake management practices as well as legislation such as the Clean Water Act (1972) and the Great Lakes Water Quality Agreement (1978) to reduce nutrient input to freshwater systems initially led to reduction of HABs through the late 20th century (De Pinto et al. 1986). This reduction proved to be temporary as the U.S. has seen increases in HABs in not only eutrophic, but also oligotrophic systems in the past two decades (Brooks et al. 2017). The increase in HABs prevalence cannot be attributed to one or a few factors alone, but rather the interactive effects of factors like global climate change (Paerl and Huisman 2008; O’Neil et al. 2012) and food web modifications caused by anthropogenic interference (e.g., overfishing, invasive species) (Heisler et al. 2008; O’Neil et al. 2012). In the northeast U.S., HABs in the Laurentian Great Lakes have garnered extensive coverage and awareness. Lake Erie, the shallowest and most eutrophic Great Lake has experienced multiple HABs dominated by *Microcystis*. A large bloom of high toxin concentration in 2011 covered nearly the entire western basin (Michalak et al. 2013). After a bloom in 2014, microcystin was detected in the drinking water in Toledo, Ohio at levels that exceeded the World Health Organization’s guidelines (Steffen et al. 2015). This led to a drinking water advisory for 400,000 residents for more than 2 days. This event brought national attention to the threats posed by HABs and prompted research into the conditions that promote HABs formation (Steffen et al. 2015; Watson et al. 2016)

The negative effects of HABs are seen in a variety of sectors including threats to public health, recreation, and the freshwater ecosystem. Toxins from HABs present a variety of risks for the public health sector including contaminated drinking water, direct contact via swimming, fishing, and boating. If exposure occurs, cyanotoxins may cause adverse human health effects including nausea, gastrointestinal discomfort, and dermal irritation, especially in children (Weirich and Miller 2014). Cyanotoxins produced during HABs are linked to livestock deaths and illness in dogs (Trevino-Garrison et al. 2015). Lake recreation is also adversely affected by HABs due to beach closures (New York State Department of Health 2020). Currently there is some rapid analysis for HABs toxicity available, but efficacy is limited (Watson et al. 2017); therefore, state and local agencies will close public beaches during a bloom event to significantly reduce or eliminate risk (New York State Department of Environmental Conservation 2019). Furthermore, HABs can impact the aquatic ecosystem. The bacterial decomposition of cyanobacteria leads to reduced oxygen concentration and periods of local anoxia which result in fish kills (Anderson et al. 2002).

### 1.1.3 Factors associated with the formation of HABs

There are many factors associated with the formation of HABs. Abiotic factors are among the most understood drivers of HABs formation. Increased water temperatures favor the growth of cyanobacteria over other phytoplankton like diatoms and green algae (Reynolds 2006; Paerl and Huisman 2008). Low winds might reduce vertical mixing in the water column leading to stratification. Some cyanobacteria, including *Microcystis* can migrate vertically by using intracellular gas vacuoles (reviewed in Harke et al. 2016). Increased buoyancy allows cyanobacteria to float to surface water and outcompete other phytoplankton for light (Reynolds and Walsby 1975; Huisman et al. 2006; Paerl and Huisman 2008). Nutrient enrichment is also associated with HABs formation. Total phosphorus (TP) (Schindler 1977, Correll 1998) and total nitrogen (TN) (Gobler et al. 2016, Newell et al. 2018) appear to play the largest role in HABs formation. Historically, HABs were seen predominantly in eutrophic systems. Those events were predictable given that increased nutrient loading can lead to increased phytoplankton abundance and productivity. However, HABs have been documented on occasion in oligotrophic and mesotrophic systems (Carey et al. 2012, Raikow et al. 2004). HABs in low nutrient (TP < 20 μg/L) lakes are especially perplexing because low-nutrient lakes do not have the expected nutrient conditions necessary for HABs.

Biotic factors also influence the formation of HABs in low nutrient contexts. Invasive dreissenid mussels (*Dreissena polymorpha* and *Dreissena bugensis rostriformis*; zebra and quagga, respectively) are implicated in the promotion of toxin producing HABs (Vanderploeg et al. 2001, 2013; Raikow et al. 2004; Knoll et al. 2008). In a study of 39 low-nutrient (<20 μg/L TP) lakes, Knoll et al. (2008) found that invasion by *D. polymorpha* resulted in 3.3 times higher microcystin concentrations than their uninvaded counterparts. Sarnelle et al. (2010) found that microcystin concentrations were significantly different between invaded and uninvaded lakes with TP <10 μg/L, with invaded lakes having greater microcystin concentration. Some proposed mechanisms of dreissenid promotion of HABs that will be discussed in this thesis include: selective filtration (i.e., rejecting toxin-producing cyanobacteria back into the water column) combined with overall phytoplankton and zooplankton reduction, redistribution of essential nutrients like P and N from the water column to the benthos, and increased water clarity.

## 1.2 Dreissenids

### 1.2.1 Introduction to Great Lakes and inland lakes of New York

Dreissenid mussels are among the most prolific invasive aquatic species in North America. Native to the Ponto-Caspian region, dreissenid mussels are thought to have first been introduced to North America via ballast water emptied into Lake St. Clair by shipping vessels in the late 1980s (Mackie 1991). Since their introduction into the Great Lakes, dreissenids are found in freshwater systems all across New York State (Figure 1-1; Benson et al. 2013) and new invasions are still occurring as anthropogenic activities like boating, recreation, and altered hydrology connect bodies of water (Karatayev et al. 2015). Dreissenids are considered both non-native and invasive because they readily outcompete native mussels for habitat, food, and other resources (Schloesser and Masteller 1999; Strayer and Smith 1996). Dreissenids are called “ecological engineers” (Coleman and Williams 2002) because they alter the physical, biological, and chemical environment of the systems they invaded. Shell litter from dead dreissenids provides further habitat for new mussels to attach and might edge out native benthic invertebrates (Ward and Ricciardi 2007). Filter feeding can reduce the abundance of phytoplankton in the water column and lead to increased light penetration (Higgins and Vander Zanden 2010). Filtering the water column can re-distribute nutrients to the benthos. These effects are just a few examples of the ways in which dreissenid mussels impact freshwater systems in North America.

### 1.2.2 Biological description

The biological characteristics of dreissenid mussels allow for quick and expansive invasion of freshwater systems. Its life cycle is represented by two distinct stages. The adult stage is a calciferous bivalve that attaches to surfaces via byssal threads, though further motility is possible should mussels seek better habitat. The larval stage is a free-floating planktonic veliger. The latter stage allows for a quick spread of mussels between and throughout systems. Dreissenid reproduction is primarily regulated by water temperature. Temperatures between 16 °C and 26 °C are optimal for the growth and reproduction of zebra mussels (Nalepa and Schloesser 2013). Dreissenids are obligate filter feeders. Markedly high filtration rates can cause drastic reductions in phytoplankton biomass in the water column (Nalepa et al. 1999; Noonburg et al. 2003). This is a characteristic that might be an important mechanism in the promotion of HABs.

## 1.3 Dreissenids as a biological driver of HABs in low nutrient lakes

### 1.3.1. Selective filtration

Observational and experimental literature attributes the promotion of cyanobacterial blooms to selective filtration by dreissenid mussels. Dreissenids directly affect phytoplankton assemblages via filter feeding as phytoplankton are a primary food source for zebra and quagga mussels (Karatayev et al. 2015). It is well-documented that the presence of dreissenids markedly reduces phytoplankton biomass (Strayer et al. 1999; Higgins and Vander Zanden 2010). Dreissenids are able to selectively feed and can excrete un-digested particles as pseudofeces (Vanderploeg 1999). Specifically, zebra mussels selectively reject colonies of toxin-producing *Microcystis aeruginosa* (Vanderploeg et al. 2001, 2013). Zebra mussels are especially inclined to reject those colonies/large masses because mussel feeding tends to be dependent on food particle size (Naddafi et al. 2007). These findings suggest one pathway by which dreissenids promote toxic cyanobacterial HABs.

### 1.3.2. Nutrient alteration and redistribution

Dreissenids may indirectly influence formation of HABs by altering nutrient availability and spatial distribution. Dreissenids can change the N:P ratio in the water column. Naddafi et al. (2008) found that zebra mussels reduced P availability from June to August by enhancing C:P and N:P ratios. Dreissenid mussel invasion is associated with changes in energy production from the pelagic region to the benthic littoral zone (Mayer et al. 2013). This phenomenon is called benthification. Depletion of P from the pelagic zone is well-described by the “nearshore phosphorus shunt” proposed by Hecky et al. (2004); nearshore druses of dreissenids capture allochthonous input via filter feeding, disallowing nutrients to be distributed to the open water. The outcome of these stoichiometric changes combined with nutrient benthification presents a competitive advantage to those cyanobacteria taxa in low-nutrient lakes who can move up and down in the water column to access nutrients (e.g., *Microcystis*).

## 1.4 Citizen Science

### 1.4.1. History and citizen science in New York State

Citizen science has become a growing source of data for many disciplines of science, and is increasingly used by scientists, lake managers, and stakeholders to gather valuable long-term information about freshwater systems (Miller-Rushing et al. 2012; Lottig et al. 2014; Vincent et al. 2017). Citizen science is the recruitment of non-scientists to collect high-quality, long-term ecological data (Bhattacharjee 2005). Examples of successful citizen science programs are reviewed in Bonney et al. (2009); in limnology, Sarnelle et al. (2010) found that water-quality data collected by citizen scientists was not more variable than data collected by trained professionals. Silvertown (2009) reviews three main drivers of the expansion of citizen science research. The first driver is the accessibility of technological advancements which allow volunteers to quickly and easily disseminate observations and findings; the second is the actual characteristics of public volunteers (e.g., little to no labor cost, a broad range of skill sets, and access to modern computational technologies); the third is that the common requirements of funded research projects to do some aspect of public outreach is inherent in citizen science. A successful citizen science program in New York has provided quality data from hundreds of inland lakes for nearly three decades. The Citizen’s Statewide Lake Assessment Program (CSLAP) provides long-term water quality and data on HABs to government, lake associations, stakeholders, and research scientists (Kishbaugh 1988). It is coordinated by the New York State Department of Environmental Conservation (NYSDEC) and the New York State Federation of Lake Associations Inc. (NYSFOLA). New York State has over 7500 water bodies, and collecting, analyzing, and understanding water quality data from these lakes is crucial for developing lake management plans. CSLAP enrolls lake residents who are interested in collecting data about their lakes. Through spring and summer, volunteers take measurements and collect data every two weeks. Residents are also asked to report their perceptions of the lake including suspected HABs. These measurements and reports, in conjunction with lab-analyzed samples, help the DEC and NYSFOLA meet the three main objectives of CSLAP: 1) collect lake data for lakes; 2) identify problems in lakes and any major changes in water quality; and 3) provide education and outreach to the public regarding lake science (New York State Department of Environmental Conservation 2019).

### 1.4.2 Monitoring and sampling dreissenid mussels

Citizen science has helped document and track geographic distributions of invasive species, but long-term data on invasive species population within a system is still needed. Advances in invasive species monitoring (e.g., New York’s *iMapInvasives* program) allow users (including volunteers, researchers, and governmental organizations) to report invasive species like terrestrial and aquatic plants and animals. In fact, information on dreissenid invasion used in this body of research was taken from reports documented in the *iMapInvasives* database. These reports rely on the will of volunteers to report observations. Currently in New York, there is no coordinated effort to monitor for dreissenids in uninvaded lakes. Furthermore, data on long-term dynamics of dreissenids in invaded systems is not readily available. The prior literature presented (Vanderploeg et al. 2001, 2013; Raikow et al. 2004; Knoll et al. 2008; Sarnelle et al. 2010) suggests a causative link between dreissenids and HABs and therefore warrants the need for more extensive and long term dreissenid data for lakes in New York.

## 1.5 Research Motivation and Objectives

The presence of invasive dreissenid mussels is suggested as a causal factor of HABs, especially in low-nutrient lakes where typical factors (i.e., high nutrients) that lead to HABs are not present. I wanted to examine the differences in water quality and HAB parameters between low-nutrient lakes with dreissenids and low-nutrient lake without dreissenids. Furthermore, I wanted to see if there was an easy method for citizen volunteers to use that would help lake associations and managers collect data on dreissenid mussels, including monitoring for dreissenids in uninvaded but lakes at risk of invasion due to proximity to invaded systems. I used a long-term water quality dataset (CSLAP) for oligotrophic and mesotrophic lakes in New York State to evaluate difference in water quality and HAB parameters as a result of dreissenid invasion. An additional group of 10 CSLAP lakes was used to test a methodology for detecting and tracking dreissenids. I used this data to attempt to:

**1.** Describe how lakes change after dreissenid invasion (Chapter Two)

**2.** Describe differences among water quality parameters, including HAB frequency and microcystin concentration, between low-nutrient lakes with and without dreissenid mussels. (Chapter Two)

**3.** Evaluate if and how the effects of eutrophication (measured by TP and chlorophyll-a) on microcystin concentrations varies between invaded and uninvaded lakes. (Chapter Two)

**4.** Design and evaluate citizen-accessible method for detecting and monitoring dreissenid mussels. (Chapter Three)

**5.** Describe variations in mussel abundance and mussel length distributions through the summer. (Chapter Three)

**6.** Determine the best length of deployment time for artificial substrates to collect dreissenid data. (Chapter Three)

The data and insights collected from this thesis will help inform the impacts of dreissenids on low-nutrient lakes. Because the effects of dreissenids are generally well understood, I hope to specifically enlighten the causative role they might play in HABs formation and toxicity in low-nutrient lakes. The results of these analyses will ideally bring additional attention of lake managers to the effects of invasive species. Further, this body of work could help to inform management practices regarding monitoring for dreissenids pre-invasion and long-term monitoring of dreissenids post-invasion.

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# Chapter Two: Using citizen-science data to evaluate the role of dreissenid mussels in harmful algal bloom formation in low-nutrient lakes in New York State

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## 2.1 Abstract

Harmful algal blooms (HABs) are often attributed to nutrient enrichment by eutrophication, but HABs in low-nutrient lakes are becoming more prevalent. We sought to see if a biological driver, invasive dreissenid mussels, promoted toxic HABs in lakes of New York State, USA. We evaluated long-term water quality and HAB data from 68 low-nutrient lakes in New York sampled from 2012 to 2017. We evaluated how the presence of dreissenids affects water quality and found that true color differs significantly between invaded and uninvaded lakes. An analysis was conducted on the global dataset (n=68) as well as a reduced dataset (n=16) using a matching approach to identify the uninvaded lakes most similar to the invaded lakes in catchment to surface area ratio, mean depth, and percent agricultural use in the watershed. We found that neither chlorophyll-a nor microcystin concentrations from both shoreline blooms or open water were significantly different between invaded and uninvaded lakes in both the global and reduced datasets. Further, we did not find differences in frequency of HABs between invaded and uninvaded lakes. We found that TP significantly influenced open water microcystin concentrations in invaded lakes but not uninvaded lakes, which deviates from the expectation of eutrophication having less pronounced effects on microcystin concentrations in invaded lakes than uninvaded lakes.

## 2.2 Introduction

Harmful algal blooms (HABs) of cyanobacteria are occurring more frequently in North America and pose risks to human and ecosystem health (Winter et al. 2011; Brooks et al. 2016). Common genera of cyanobacteria, including *Microcystis, Anatoxin,* and *Aphanizomenon*,can produce hepatotoxins and neurotoxins such as microcystin (O’Neil et al. 2012). Cyanotoxins have been linked livestock deaths and dog illnesses (Trevino-Garrison et al. 2015) in addition to ecological, economic, and human health implications in regions affected by HABs (Pimentel 2005; Connelly et al. 2007).).

While HABs have historically occurred absent of human causes (summarized in Hallegraeff 1993), the interaction of eutrophication, anthropogenic land-use changes, invasive species, and climate change appear to promote the formation of HABs at an increased rate in inland surface waters (Anderson et al. 2004; Huisman et al. 2018; O’Neil et al. 2012). Nutrient enrichment, specifically from phosphorus (P) (Schindler 1977; Correll 1998), and nitrogen (N) (Gobler et al. 2016; Newell et al. 2018), are known to control the development of HABs. Additional factors implicated in HABs formation include increased water temperatures from anthropogenic climate change, and calm conditions (Paerl and Huisman 2008). Lastly, physical characteristics, including mean depth, lake catchment area to surface area ratio (CA:SA), and watershed agricultural use can lead to increased nutrient inputs and therefore susceptibility to HABs. Lakes of shallower depths tend to have higher concentrations of chlorophyll-a, total phosphorus (TP), and total nitrogen (TN) (Phillips et al. 2008) and lakes of larger CA:SA tend to have greater nutrient input (Jacquemin et al. 2019). Agricultural use in a watershed is associated with lake nutrient input; P export from an agricultural watershed is expected to be greater than another land-use types (e.g., forested watershed) (Vaithiyanathan and Correll 1992).

HABs are most likely to occur in high nutrient conditions. Recent examples of HABs in the Laurentian Great Lakes basin include events on Lake Erie, the shallowest and most productive Great Lake (Michalak et al. 2013; Ho and Michalak 2017). Lake Erie has frequently experienced HABs dominated by *Microcystis aeruginosa*. Blooms of large spatial extent in 2011 covered nearly the entire western basin, and water column integrated samples for microcystin concentrations ranged from 0.1 μg/L to 8.7 μg/L (Michalak et al. 2013). In 2014, microcystin was detected in the drinking water in Toledo, Ohio which brought increased societal and scientific attention to HABs (Steffen et al. 2015). Michalak et al. (2013) concluded that increased spring precipitation along with long-term agricultural land-use contributed to a marked pulse of allochthonous P into the lake which may have led to the 2011 event. However, high nutrient lakes are not the only lakes experiencing HABs. Increasingly, oligotrophic and mesotrophic systems appear susceptible to HABs (Carey et al. 2012; Raikow et al. 2004). HABs in low nutrient (TP < 20 μg/L) lakes are especially perplexing because low-nutrient lakes do not have the expected nutrient conditions necessary for HABs.

Previous studies provide a biological explanation for HABs in low-nutrient systems. Specifically, invasive dreissenid mussels (zebra; *Dreissena polymorpha* and quagga mussels; *Dreissena bugensis rostriformis*) are implicated in HABs formation in low-nutrient lakes (Vanderploeg et al. 2001, 2013; Raikow et al. 2004; Knoll et al. 2008). Invasive dreissenid mussels are considered ecological engineers because they alter the physical, chemical, and biological environment (Coleman and Williams 2002). Dreissenids are obligate filter feeders who reduce overall phytoplankton abundance which can lead to increased Secchi depth (Karatayev et al. 1997). Dreissenid feeding can affect the redistribution of nutrients from offshore pelagic zones to the nearshore benthic zone, increasing primary production in the benthos while reducing productivity (TP, TN, and chlorophyll-a) in the open water (Hecky et al. 2004; Vaughn and Hoellein 2018). There is experimental evidence to show that dreissenids alter the stoichiometric availability of P and N. Nutrient regeneration via excretion by dreissenids decreased dissolved inorganic N to P ratios (Benelli et al. 2019); further, Ruginis et al. (2014) showed that the presence of dreissenids significantly affected N and P benthic fluxes either directly (e.g., ammonium excretion) or indirectly (e.g., sediment P release due to anoxic conditions of the benthos).

Proposed mechanisms of HABs promotion by dreissenid mussels include selective filtration and selective rejection of toxin-producing taxa (Vanderploeg et al. 2001, 2013).In a study of 39 low-nutrient (< 20 μg/L TP) lakes, Knoll et al. (2008) found that lakes invaded with *D. polymorpha* had 3.3 times higher microcystin concentrations than their uninvaded counterparts. Sarnelle et al. (2010) confirmed this trend by finding microcystin concentrations were significantly different between invaded and uninvaded lakes with TP <10 μg/L, with the former having greater microcystin concentration. Furthermore, Sarnelle et al (2010) found that the effects of eutrophication on microcystin concentrations were limited in invaded lakes; i.e., TP as a limiting nutrient was not significantly correlated with microcystin concentrations in invaded lakes. We sought to see if HABs in invaded low-nutrient lakes had higher microcystin concentration than uninvaded low-nutrient lakes in New York State.

In New York State, water quality monitoring dates back to the 1920s. Sampling efforts have continued through the decades and important data on parameters like chlorophyll-a, TP, TN, Secchi depth and true color are routinely collected. By the mid 2010s, HABs in low-nutrient lakes in New York were increasingly documented and sampled (REF). In 2012, 58 waterbodies had blooms and in 2016, 174 waterbodies had blooms (New York State Department of Environmental Conservation 2019). Notably, Skaneateles Lake, an oligotrophic Finger Lake with an established dreissenid population, had a HAB event in 2017. This was especially concerning because unfiltered water from Skaneateles Lake is the primary source of drinking water for the city of Syracuse and surrounding areas.

The Citizen’s Statewide Lake Assessment Program (CSLAP) provides long-term water quality data and HABs-related data and is coordinated by the New York State Department of Environmental Conservation (NYS DEC)) and the New York State Federation of Lake Associations Incorporated (NYSFOLA). Using data from low-nutrient lakes sampled by the CSLAP, our study objectives were to 1) use available historical data to quantify lake water quality changes post dreissenid invasion, 2) assess differences in water quality parameters, including microcystin concentrations, between invaded and uninvaded lakes while accounting for lake characteristics (i.e., mean depth, CA:SA, agricultural use in the watershed), and 3) investigate whether TP, TN:TP, and chlorophyll-a concentrations are predictors of microcystin concentration in uninvaded vs. invaded lakes. Water quality parameters were expected to significantly change after dreissenid invasion. Specifically, Secchi depth was expected to increase due to reductions in phytoplankton (chlorophyll-a was used as a proxy for phytoplankton abundance). Comparing currently invaded lakes with currently uninvaded lakes, we expected that invasion status would be a significant predictor for variations in bottom sample TP, TN:TP, Secchi depth, and chlorophyll-a. Specifically, bottom sample TP, TN:TP, and chlorophyll-a were expected to be lower in invaded lakes, while Secchi depth was expected to be greater in invaded lakes. Further, we hypothesized that invaded low-nutrient lakes would have more HABs with greater microcystin concentrations. For invaded lakes, it was expected that TP and chlorophyll-a would not be significant predictors for variations in microcystin concentrations, while they would be uninvaded lakes.

## 2.3 Methods

We sought to evaluate the effect of dreissenid mussels on water quality, HABs frequency, and microcystin concentrations in low-nutrient lakes. We used a 5-year (2012-2017) dataset from CSLAP. Our data set included 68 low-nutrient lakes in New York, 8 of which have confirmed dreissenid populations. Lakes were considered “uninvaded” for the years before invasion was reported, and “invaded” for the year of invasion and subsequent years. For the historical analyses, data for the 8 invaded lakes dated back to 1988.

### 2.3.1 Sample acquisition and analyses

Water quality data were obtained from the CSLAP. Each year, scientists and professionals host training sessions before the sampling season to ensure volunteers collect accurate, reproducible water quality parameter data. Water quality samples are taken bi-weekly from late May to late September by lake residents. Water samples are collected with a Kemmerer bottle over the deepest point of the lake. These “open water samples” are taken at a depth of 1.5 meters, except in lakes that are shallower than 1.5 meters. “Bottom” samples are taken from 1.5 meters above the bottom of the lake in stratified lakes only (n = 54 in our dataset). Water samples are then pre-processed on-shore by volunteers.

The data included in this study were from sampling years 2012-2017 because collection and analytical methods were most consistent during that time period (Table 2-1). Secchi depth, air and water temperature, and various metrics of lake perception are taken in the field and recorded. In the field, aliquots for TP, TN, and calcium are collected and frozen for shipment. Pre-processed samples, chain-of-custody forms, and related paperwork are then shipped in an insulted Styrofoam cooler to a pre-determined lab listed below. For on-shore processing of true color, 200 mL of water is vacuum-filtered using a 0.45 μm cellulose nitrate filter and frozen for shipment; the filter is retained and used for cyanobacterial abundance estimation by the Boyer Lab at the State University of New York College of Environmental Science and Forestry (SUNY-ESF) in Syracuse, New York using a FluoroProbe® (bbe Moldaenke 2014). For chlorophyll-a analysis, 6–10 drops of magnesium carbonate (MgCO3) are added to 100 mL of sample water and filtered through a 0.45 μm cellulose nitrate filter. The filter is folded, wrapped in foil, placed in a tube and frozen for shipment. Chlorophyll-a is analyzed using standard methods (USEPA 445.0, Rev 1.2). True color is estimated by visually comparing water sample to a scaled set of standards created from a platinum-cobalt solution (USEPA 110.2). For routine sampling, water quality analyses were performed at Upstate Freshwater Institute (UFI) in Syracuse, New York.

Shoreline bloom (SB) samples were taken only when a bloom was visually suspected by lake residents or volunteers. Samples are analyzed for chlorophyll (bbe Moldaenke 2014) and microcystin at SUNY-ESF. In 2012 and 2013, microcystin was analyzed using the protein phosphatase inhibition assay (PPIA) (An and Carmichael 1994). From 2014-2017, microcystin concentration was determined using liquid chromatography mass spectrometry (LC-MS) (Boyer 2007). For SB samples, no other water quality variables (e.g., TP, TN, etc.) were taken. Variability in sampling technique among volunteers likely creates variability in reported chlorophyll and microcystin concentrations. Volunteers are asked to sample shoreline blooms when they are visually suspected by scooping surface water from a dense area of the bloom, therefore chlorophyll and microcystin concentrations might vary from volunteer to volunteer.

Information about the presence of dreissenid mussels, including year of invasion, was retrieved from *iMapInvasives* (https://www.nyimapinvasives.org/), an online invasive species database managed by the New York Natural Heritage Program.

### 2.3.2 Lake selection

As of 2019, there are 158 lakes enrolled in CSLAP. For this study, lakes were categorized and subset by trophic status to include only oligotrophic (based on meeting all or some of the criteria: annual average TP < 10 μg/L; annual average chlorophyll-a < 2 μg/L; annual average Secchi depth > 5 m) and mesotrophic (10 < TP < 20 μg/L; 8< chlorophyll-a < 10 μg/L; 10 < Secchi depth < 20 m ) lakes (n=140). Then we further subset to include only lakes enrolled from 2012 to 2017 because of consistency in methods as described above (n=68; Supplemental Table 2-S1). Of these 68 lakes, 8 had established dreissenid populations between 2012-2017. We called this dataset of 68 lakes the “global” dataset. We used propensity score matching (Sekhon 2011) to create a smaller dataset where the number of invaded and uninvaded lakes was balanced (n=16; 8 invaded, 8 uninvaded). We used mean depth, catchment area to surface area ratio (CA:SA), and percent agricultural use to find matching pairs. We called this dataset the “reduced” dataset. The matchit function was used to find matching pairs using the package matchit in R (R Foundation for Statistical Computing 2018).

### 2.3.3 Statistical analyses

*Changes in water quality after dreissenid invasion (historical analyses)*

To address objective one, we compared water quality data from years before established dreissenid populations (pre-dreissenid) with data from years after populations were established (post-dreissenid). For the eight invaded lakes in the dataset, historical TP, TN, TN:TP, chlorophyll-a, and Secchi depth data (1988-2017) was used to evaluate compare the effect of dreissenids on water quality. True color was not evaluated for the historical analyses due to significant differences in methods from 1988 to 2017, which were expected to skew results towards greater true color for post-dreissenid years. We ran a mixed effects model with TP, TN, TN:TP, chlorophyll-a, and Secchi depth as the response variables, invasion status as the fixed effect, and lake as the random effect. All variables except Secchi depth were log-transformed to improve distribution of residuals. We created a linear mixed effects model (function lmer in packaged lme4 in R; R Foundation for Statistical Computing). Residuals of the model were evaluated for normality and heteroscedasticity.

*Comparing water quality and HABs parameters between invaded and uninvaded lakes in New York State (2012-2017)*

To address objective two, we used additional linear mixed effect models to evaluate the following response variables: bottom sample TP, TN:TP, Secchi depth, true color, open water chlorophyll-a, shoreline bloom chlorophyll-a (SB chlorophyll),and shoreline bloom microcystin concentrations (SB microcystin). Fixed effects were invasion status (discrete), mean depth (continuous, log-transformed for scale), CA:SA (continuous; log-transformed for scale), and percent agriculture (continuous). Random effects were lake, sample year, sample month, and the interactive effects of lake and sample year. Residuals of the model were evaluated for normality and heteroscedasticity. Response variables were log-transformed to improved fit. We created a total of 16 models (8 for global dataset, 8 for reduced dataset). For the reduced dataset, we eliminated the fixed effects of mean depth, CA:SA and agricultural use because these factors were used to create the matched pairs from the global dataset and therefore would be equal between invaded and uninvaded lakes.

*Comparing HABs and HABs with high toxin concentration frequency between invaded and uninvaded lakes in New York State (2012-2017)*

The DEC threshold concentrations for a bloom status of “confirmed bloom with high toxins” are 25 µg/L blue-green algae chlorophyll and 20 µg/L microcystin (New York State Department of Environmental Conservation 2019). Further, to evaluate whether the number of blooms and number of high toxin (HT) blooms differed between invaded and uninvaded lakes, a generalized linear mixed effects model from the Poisson family with a log link was used (function glmer; R). Events where blue-green algae chlorophyll concentrations were greater than 25 µg/L were marked as a bloom. Then, the frequency of blooms for each lake was calculated to generate a continuous variable. Within these bloom events, when microcystin concentration was greater than or equal to 20 µg/L, the observation was marked as an HT bloom. Then the frequency of HT blooms for each lake was calculated to generate a continuous variable. Two separate models were created and either bloom frequency or HT bloom frequency was used as the response variable, while invasion status was used as the fixed effect. Lake was included as a random effect.

*Effects of eutrophication on microcystin concentration*

The relationship between microcystin concentrations and TP, microcystin concentration and TN:TP, and microcystin cocnentration and chlorophyll-aconcentrations were analyzed using linear regression (all variables log-transformed). For each relationship, a model for open water samples was created and a model for shoreline bloom samples was created. For open-water microcystin concentrations, associated TP, TN:TP, and chlorophyll-avalues were used (i.e., those collected at the same time). For shoreline bloom microcystin concentrations (i.e., samples where other water quality data is not taken), the yearly average TP, and TN:TP for the lake was used in the regression analysis.

## 2.4 Results

### 2.4.1 Changes in water quality after dreissenid invasion (historical analyses)

Dreissenid invasion was found to be a significant predictor for variations in Secchi depth, chlorophyll-a, and bottom sample TP. Secchi depth was found to increase by ~0.7 m after invasion (*t* = -9.37, p < 0.0001). This corresponds with model predictions for chlorophyll-a, which found that chlorophyll-a levels were ~82% greater in pre-invasion years (*t* = 13.31, p < 0.0001). Bottom sample TP decreased by ~40% (*t* = 0.34, p = 0.0002). No significant differences in TN:TP were found.

### 2.4.2 Comparing water quality and HABs parameters between invaded and uninvaded lakes in New York (2012-2017)

Invasion status was a significant predictor for open water true color only. For the global dataset, true color was ~50% lower in invaded lakes (Table 2-3a). Invasion status was not a significant predictor for any of the models in the reduced dataset (Table 2-3b).

Lake characteristics used as fixed effects in the global dataset were significant predictors for open water TN:TP, bottom sample TP, open water true color, Secchi depth, and open water chlorophyll-a concentrations. CA:SA was a significant predictor for open water true color and Secchi depth. For every 20% increase in CA:SA, true color increased by about 8% and Secchi depth decreases by ~8%. Mean depth was a significant predictor for TN:TP, open water chlorophyll-a, true color, and Secchi depth. For every 1m increase in mean depth, TN:TP increased by ~.13%, open water chlorophyll-adecreased by ~3%, open water true color decreases by 4%, and Secchi depth increases by about 0.2 m. Percent agricultural use in the watershed was a significant predictor for bottom sample TP. For every 1% increase in agricultural use, bottom sample TP increases by ~2%.

### 2.4.2 Effects of eutrophication on microcystin concentration

We examined TP, TN:TP, and chlorophyll-a as predictors of microcystin concentrations. Overall, TP positively influenced microcystin concentrations; however, the relationship between TP and microcystin was statistically significant only in open water samples from invaded lakes (log microcystin = 8.6+ 4.2 log TP, R2 = 0.59, n = 8, *p* = 0.001) (Figure 2-1.). No significant relationships between TN:TP and microcystin concentrations were found for either invaded or uninvaded lakes (Figure 2-3). Similarly, no significant relationships between chlorophyll-a and microcystin were found for either invaded or uninvaded lakes.

## 2.5 Discussion

We examined low-nutrient lakes in New York State’s CSLAP monitoring program that were sampled from 2012 to 2017 to determine how dreissenid invasion influenced water quality and HABs. Historical data from invaded lakes showed that dreissenid invasion significantly changed water quality. However, when comparing currently invaded lakes to currently uninvaded lakes, we found that HABs in invaded lakes did not occur at greater frequencies nor did they have greater microcystin concentrations than uninvaded lakes. This finding is inconsistent with our hypotheses and previous research that shows dreissenid mussels promote toxic HABs in low nutrient lakes (Sarnelle et al. 2010; Vanderploeg et al. 2001). Further, we found that blooms occurring in invaded lakes did not have greater chlorophyll-a concentrations compared to uninvaded lakes. The regressions between microcystin and TP and microcystin and chlorophyll-a revealed that both relationships were positively correlated in invaded and uninvaded lakes. The relationship between TP and microcystin was not, however, significant for invaded lakes.

For the eight invaded lakes, it was found that Secchi depth was significantly different between pre-dreissenid and post-dreissenid years. Secchi depth increased, on average, by 0.7 m after invasion. Increased Secchi depth is likely a result of decreased primary productivity by phytoplankton and zooplankton, leading to increased water clarity (Karatayev et al. 1997), which is supported by our finding that chlorophyll-a concentrations were ~82% greater for pre-dreissenid years. The magnitude of the difference in Secchi depth found here is consistent with known impacts of dreissenids on water clarity. Higgins et al. (2008) found that for two morphometrically and physiochemically similar basins in a small Irish lake, Secchi depth was 0.93 greater for the invaded basin. Though not examined here, increased water clarity after dreissenid invasion should also consider turbidity. For lakes with high turbidity prior to invasion, the expected effect of increased water clarity via dreissenid filtration may be lessened (Higgins and Vander Zanden 2010). In the western and central basins of Lake Erie, water clarity has not increased since dreissenid invasion, in fact water clarity has decreased in these basins as the probable result of sediment loading (Barbiero and Tuchman 2004). Dreissenids have also been implicated in changes or re-distribution of nutrients. Bottom sample TP was significantly different between pre-dreissenid and post-dreissenid years. We found that bottom sample TP decreased by ~ 40%. This finding is in contrast to most published research on the effect of dreissenids on TP (Higgins and Vander Zanden 2010).

The presence of dreissenids was a significant predictor for variations in true color. True color is used to determine water quality resulting from dissolved substances only (i.e., particulate matter is filtered out before analysis for true color). This is different from perceived color, which can be influenced by suspended particles and algal matter. Model results showed that true color was ~50% lower in invaded lakes. This is unsurprising given the ecology of dreissenids but to our knowledge has not been reported elsewhere. True color is generally impacted by nutrient and run-off input from the watershed (Solomon et al. 2015). Dreissenids in the nearshore benthos are thought to capture allochthonous inputs, limiting offshore productivity (Hecky et al. 2004). Further, overall reductions in phytoplankton biomass by dreissenids can indirectly influence true color by decreasing the amount of dissolved organic matter from phytoplankton biological processes.

Dreissenid presences was not a significant predictor for the other water quality parameters tested (bottom sample TP, TN:TP, chlorophyll-a and Secchi depth). Prior research showed that dreissenids significantly decrease bottom sample TP, TN:TP and chlorophyll-a, while increasing Secchi depth (Fahnenstiel et al. 1995; Makarewicz et al. 2000; Higgins et al. 2007; Higgins and Vander Zanden 2010; Ruginis 2014). However, these studies considered spatial variations within lakes, i.e., taking multiple samples in littoral and pelagic zones. CSLAP open water and bottom samples are taken only in the pelagic zone, and therefore may limit the potential to detect variations in TP, TN:TP, and chlorophyll-a between invaded and uninvaded lakes. Further, because our study was limited to mesotrophic and oligotrophic lakes (median open water TP = 0.01 mg/L, range: 0.004–0.07), the effect of dreissenids on TP concentrations may be outweighed by environmental factors like allochthonous nutrient loading. Year of invasion may also impact our ability to detect differences between invaded and uninvaded lakes. Three of the eight invaded lakes in this dataset had been invaded for more than 10 years before our study period (Table 2-S1). While there are competing theories regarding the long-term population trends of dreissenids (reviewed in Burlakova et al. 2006), the most common feature is a rapid increase in populations 1–2 years after invasion (Strayer et al. 2019). Given the amount of time some of the study lakes had been invaded, major impacts of dreissenids could be minimized.

We found that microcystin concentrations were not significantly different between invaded and uninvaded lakes in either the global or reduced dataset. This is in contrast to previous findings that showed invaded low-nutrient lakes tend to have higher microcystin concentrations (Knoll et al. 2007; Raikow et al. 2004; Sarnelle et al. 2010). Dreissenid mussels exhibit a high degree of influence on the systems they inhabit by altering the physical, biogeochemical, and biological environment; the impacts of which are summarized in Higgins and Vander Zanden (2010). Our results suggest a need for more rigorous controlled studies to elucidate if and how dreissenids might influence HABs formation in low-nutrient lakes in New York. Vanderploeg et al. (2017) conducted a laboratory study in which mussels were exposed to different strains of *Microcystis*, as well as strains from different sites in the Great Lakes; from this study the authors determined that rejection of *Microcystis* by mussels can be both size- and toxicity- dependent. Extrapolating from lab experiments remains difficult because bloom formation as a result of selective rejection is also dependent on mussel abundance, available nutrients, and intra- and inter- taxa competition.

We looked at the relationships between TP and microcystin concentrations, TN:TP and microcystin concentrations, and chlorophyll-a and microcystin concentrations. We found that neither chlorophyll-anor TN:TP was a significant predictor for microcystin concentrations in either shoreline bloom or open water samples. This relationship held true in both invaded and uninvaded lakes. We found that TP was positively correlated with microcystin in open water samples, but the relationship was significant for invaded lakes only, which refutes the theory that dreissenid mussels limit the influence of TP on microcystin as was found in Raikow et al. (2004) and Sarnelle et al. (2010).

Our findings suggest dreissenid mussels alone might not promote HABs in low-nutrient lakes in New York, as suggested by prior research (Knoll et al. 2007; Raikow et al. 2004; Sarnelle et al. 2010).Dreissenid populations can be highly variable within a lake (Strayer et al. 2019), so future research should include mussel abundance as a continuous variable in HAB forecast models. This would also allow total filtration rates to be estimated, providing further insight into management. We acknowledge that determining lake-wide abundance requires time, funding, and expertise. In the case that no data on lake-wide abundance is available, we consider methods for detecting presence/absence to be sufficient for making inferences about HABs promotion by dreissenids. Citizen-monitoring should continue to be a source of long-term water quality data. Citizen-science has become an integral part of mass data collection across numerous ecological spheres (Silvertown 2009 and Bonney et al. 2009). Sarnelle et al. (2010) found that water-quality data collected by citizen scientists in Michigan was not more variable than data collected by trained professionals. New York State’s CSLAP program has provided standardized quality data for decades to lake managers, scientists, and citizens. In recent years, CSLAP has ramped up efforts to collect HABs related data and create platforms to which HABs information can be rapidly disseminated. Current CSLAP limitations specific to HAB research include lack of water-quality data during a shoreline bloom sample (i.e., TP and microcystin not collected concurrently), as well as non-standardized methods for sampling HABs. However, analyses presented above show that data from citizen-science can provide insightful information about water quality to lake managers and scientists.

## 2.6 References

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# Chapter Three: Use of artificial substrate to monitor dreissenid populations to supplement an existing citizen-science water quality collection program

**For submission to: Lake and Reservoir Management**

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## 3.1 Abstract

Dreissenid mussels are a nuisance invasive species capable of altering the physical, chemical, and biological environment they invade. Monitoring for presence/absence and sampling for long-term population dynamics is imperative for informing lake management decisions regarding prevention and mitigation. New York State has an expansive and informative network of citizen volunteers who collect water quality and harmful algal blooms (HABs) data, but do not regularly monitor or sample for dreissenid mussels. We deployed artificial substrates in 10 lakes in New York to evaluate how mussel abundance and size vary with deployment time. We found that the average abundance among lakes was greatest with a deployment time of 16 weeks, while shell length was greatest at a deployment time of 12 weeks. Abundance and shell lengths were highly variable within individual lakes, suggesting a need for in-lake replication across multiple sites. Overall, we concluded that artificial substrates deployed in this way do not provide sufficient information to lake associations.

## 3.2 Introduction

The zebra mussel (*Dreissena polymorpha*) was first reported in the Great Lakes region in Lake St. Clair in 1988 (Hebert et al. 1989), followed by the quagga mussel (*Dreissena rostriformis bugensis*) in 1989. These species are native to the Ponto-Caspian region, and were likely brought to North America via ballast water from shipping vessels (Hebert et al. 1989; Carlto 2008). Dreissenids are considered one of the most aggressive invasive species in freshwater systems and continue to spread globally via anthropogenic activities including shipping channels, reservoir construction, human migration, and changes in environmental regulations (Karatayev et al. 2007). Since their introduction into the Great Lakes, dreissenids have been found in inland freshwater systems across New York State (Benson et al. 2013)

The ability of dreissenids to rapidly colonize new habitat and readily outcompete native unionid mussels is facilitated by dreissenid life histories. Understanding dreissenid ecology is imperative for lake managers who who wish to prevent or mitigate the impacts of dreissenid invasion. Zebra mussels require hard, rocky substrate for attachment and are almost exclusively found in littoral waters at depths between 1 and 6 m, though deeper settlement is possible (Burlakova et al. 2006). Quagga mussels are able to colonize soft, silty substrate at greater depths and cooler temperatures (Karatayev et al. 2015). Generally, zebra mussels are the first to invade a system, followed quagga mussels (D’Hont et al. 2018). Following quagga invasion, there tends to be a drastic reduction in zebra mussel biomass due to quagga mussels’ increased biomass and spatial distribution (Karatayev et al. 2011). Both species are obligate filter feeders and exhibit high filtration rates, leading to drastic reductions in phytoplankton biomass (Nalepa et al. 1999; Noonburg et al. 2003). Dreissenid reproduction is primarily regulated by water temperature (Nalepa and Schloesser 2013) and characterized by two distinct stages: the first is a calciferous bivalve attached to substrate (though adult dreissenids are motile and can re-locate to optimize feeding), and the second is a free-floating planktonic larvae (veliger; Nalepa and Schloesser 2013).

Invasive dreissenid mussels are called “ecological engineers” because their life history affects the physical, biological, and chemical environment (Coleman and Williams 2002). Mussels aggregate to form druses which, at a minimum, are a nuisance and at a maximum can disrupt processes like water treatment by physically clogging water intake valves (Elliot et al. 2005). Mitigation of mussels in industrial water intake facilities is necessary to reduce biofouling (Elliot et al. 2005). Reductions in phytoplankton by mussels also leads to increased water clarity and therefore increases the potential for nuisance macrophyte or algal growth (Higgins and Vander Zanden 2010).

Dreissenids have been implicated as a factor in the formation of cyanobacterial HABs. HABs have become increasingly common in North America (Winter et al. 2011; Brooks et al. 2016; Wurtsbaugh et al. 2019). Various mechanisms of dreissenid promotion of HABs have been proposed. Dreissenids may alter the nitrogen to phosphorus ratio (N:P) in the water column (Conroy et al. 2005; Gergs et al. 2009; Hamilton et al. 2020). Dreissenids can re-distribute limiting nutrients including carbon (C) to the benthos and promote growth of benthic algae (Hecky et al. 2004). Armenio et al. (2016) found that the benthic algae *Lyngbya wollei* (cyanobacteria) grown in tanks with live dreissenids had significantly higher concentrations of C, N, and P relative to control treatments without dreissenids. In systems where invasive dreissenids co-exist with native unionid mussels, soluble reactive phosphorus can be significantly higher in dreissenid-inhabited sediments versus unionid-inhabited sediments (Benelli et al. 2018). Naddafi et al. (2008) found that zebra mussels reduced P availability from June to August by enhancing C:P and N:P ratios. Another proposed mechanism of HABs promotion by dreissenids is selective rejection of toxin-producing species by dreissenids (Vanderploeg et al. 2001).

The three main stages of invasive species management are prevention, detection, and mitigation or eradication. The foremost priority of lake managers should be prevention. While preventing introduction of dreissenids should remain the priority of lake managers, early detection is imperative for mitigating the impacts of dreissenid invasion. The cost of monitoring for invasive species should be balanced against cost of mitigation should a species become established (Counihan and Bollens 2017). Lake-wide eradication of dreissenids is not currently thought to be possible. Mitigation of druses at water intake facilities and other points-of-interest through the use of biocides and molluscicides is effective (Costa et al. 2011) but is not practical or safe for use in open waters. Early detection of invasive dreissenids is important even if eradication is not practical or possible. Detection of mussels can help lake managers predict changes that might occur as a result of mussel invasion, including increased water clarity (Binding et al. 2015; Geisler et al. 2016), trophic level changes (Barbiero et al. 2018; Gudimov et al. 2015), and macrophyte growth (Vaughn and Hoellein 2018).

The impacts of dreissenid invasion on lakes warrant appropriate methods for detecting and sampling dreissenids. Quadrat sampling via SCUBA is one of the most effective and accurate methods for estimating lake-wide dreissenid abundance (Wisniewski 1974; Mellina and Rasmussen 1993). While experimental designs vary, the basics of this method involve random placement of a quadrat, and a SCUBA diver collects all mussels within that quadrat. Ferguson et al. (2019) suggests that distance sampling along a transect line via SCUBA is also an efficient method for estimating mussel abundance. Though these methods are regarded as the most accurate, they are labor, time, and resource intensive. Ponar grabs along depth-integrated transects can provide estimation of mussel abundance and spatial dispersion (Marsden 1992). Given the non-homogenous spatial distribution of mussels, this method is particularly susceptible to either over-estimation or under-estimation of mussel abundance. Furthermore, grabs can be both time and labor intensive to collect and process. Underwater videography is a novel method for assessing spatial distribution and abundance of dreissenid mussels (Mehler et al. 2018). Underwater videography can provide valuable data alone, or in conjunction with classic methods including sonar and Ponar grabs (Mehler et al. 2018). A 2015 study from the Laurentian Great Lakes found that when compared with triplicate Ponar grabs, 500-m videography transects captured more area and gave increased precision to dreissenid biomass and abundance estimations (Karatayev et al. 2018). Availability of low-cost cameras and ease of use makes this method ideal for citizen science; however, analysis of video footage for abundance estimations remains time-consuming and requires expert examination (Raoult et al. 2016).

Artificial substrates serve as a method for estimating mussel biomass and abundance (Marsden 1992). This method requires the placement of material for a given amount of time in order to recruit settling veligers and adults. The substrate can then be removed and analyzed for target data. A wide variety of options exist for artificial substrate; mussels preferentially settle on upper versus lower horizontal surfaces and show no preference among materials including wood, fiberglass, concrete, aluminum, raw steel, but strongly avoided galvanized steel (Marsden and Lansky 2000). Artificial substrates are an easy, low-cost, and accessible method for dreissenid data collection. Developing a standard sampling protocol for dreissenids is important for comparing data among sites and between sampling periods (Marsden 1992; Ferguson et al. 2019). Lake associations and lake managers should consider artificial substrates as a sampling protocol for monitoring and tracking existing dreissenid populations.

After evidence of invasion, lake managers need to understand long-term population dynamics of dreissenid mussels because these dynamics can help determine ecological and economic impacts of invasion. Researchers have long sought to understand and characterize population dynamics of dreissenids in a given system (Burlakova et al. 2006; Karatayev et al. 2014; Karatayev et al. 2015), but consistent trends in population dynamics among all systems are less understood. Strayer et al. (2019) conducted a cross-system analysis of dreissenid population dynamics trends and reported the following findings: 1) populations increase drastically 1-2 years after initial introduction; 2) quagga mussels invade after zebra mussels, and; 3) quagga mussels tend to outcompete zebra mussels when both species are in a system. Quaggas likely outcompete zebra mussels in the long-term because quaggas invest more heavily in biomass and glycogen storage over byssal attachment strength (Balogh et al. 2019). Long-term population dynamics are inherently unique to a given system and along with the general trends in population dynamics discussed above, can be impacted by local ecological drivers like predation (e.g., Barton et al. 2005; Lederer et al. 2008), nutrient inputs, and water temperature.

We tested the use of artificial substrates as a sampling protocol for dreissenid mussels. Substrates were deployed in lakes enrolled in the Citizen’s Statewide Lake Assessment Program (CSLAP). CSLAP participants provide long-term water quality data and HABs-related data and is coordinated by the New York State Department of Environmental Conservation (NYS DEC) and the New York State Federation of Lake Associations Inc. (NYSFOLA). CSLAP volunteers are typically lake association members who live on or near their lake. From May-October, volunteers collect bi-weekly water samples, as well as document any notable changes in, or perceptions of, the lake. Volunteers help sample and track HABs. Currently, there is no mandated sampling of dreissenids in CSLAP, but volunteers are encouraged to report presence or absence of dreissenids.

We sought to evaluate if artificial substrates could be a beneficial addition to this existing citizen science water quality program. Specifically, we wanted to determine how the amount of time artificial substrate is deployed for (deployment time) affects mussel settlement and size. Furthermore, we quantified the effect of invasion time (years) on overall abundance and size of mussels. Lastly, we deployed artificial substrates in uninvaded lakes to see if mussel presence could be detected. Artificial substrates were initially deployed in May and it was expected that the greatest abundance and size would be found between deployment times of 12 and 16 weeks, corresponding with the warmest part of the year. It was expected that lakes invaded for longer periods of time would have lower recruitment than those invaded more recently because dreissenid populations tend to level out and decrease over time due to resource depletion. Overall, we expected that artificial substrates would be an accessible method for citizen volunteers to use to collect data on dreissenid mussels because it is low-cost, relatively easy, and can be done by citizens who are already involved in a citizen science program.

## 3.3 Methods

### 3.3.1 Site selection

For this study, we selected 10 lakes in Central New York that were sampled as part of CSLAP in 2019 (**Table 3-1)**. Docks adjacent to properties owned by CSLAP volunteers were used as the sampling site. Lakes were selected based volunteer willingness and dreissenid invasion status. and lake volume. Trophic status varied among lakes from mesotrophic to highly eutrophic. We applied classifications to the study lakes based on data from CSLAP that are determined using New York State trophic guidelines (**Table 3-3**). Eight lakes had established dreissenid populations, and two lakes had no documented presence of dreissenids but are highly susceptible to invasion based on their proximity to invaded lakes.

### 3.3.2 Artificial substrate

Five clay bricks were deployed in each lake between late-May and early-June 2019. Five bricks were placed on the lake bottom and secured with a marine-grade rope to the dock. The depth at which the bricks were situated varied among lakes (mean 1.15m ± 0.35m; **Table 3-2**). After approximately four weeks, one brick was removed from each lake; we removed one brick at a time, every four weeks, until all bricks were removed by mid-October giving five sampling points (**Table 3-4**). This methodological design was chosen to examine how mussel abundance and population dynamics change through the season, as well as determining an optimal deployment time. This method was meant to be feasible for citizens to utilize because the sampling coincided with an existing lake-monitoring program.

Bricks were removed from the water and transported back to the lab where they were kept wrapped in foil and frozen until analysis. All visible mussels were removed and shell length was measured using digital calipers (Thomas 6-inch digital calipers; accuracy: ±0.025 mm). Shell length constitutes the greatest length along the ventral side of the mussel. Shell length was taken as an estimate for age. Mussels were identified to the species level. To obtain an abundance estimation we took the total number of mussels per brick divided by the available surface area (m2) of the brick (mussels/m2).

### 3.3.3 Summer variations in abundance and shell length

To evaluate variations in settling through the season, we used a generalized linear mixed effects model in the negative binomial family with a log link using the function glmmTMB in the package glmmTMB in R (R Foundation for Statistical Computing 2018). The number of mussels settled was the response variable. For the fixed effect we used length of sampling time (number of weeks in water since deployment; continuous variable) and the number of years a lake had been invaded (continuous). Since lakes were sampled repeatedly, lake was used as a random effect. The mixed effects approach then treats individual lakes as a part of a collection of lakes drawn from a population. We expected our data to have an excess of zeros from uninvaded lakes. To check for zero-inflation, we used the testZeroInflation function from the package DHARMa, which compares the distribution of expected zeros against the observed zeros and found that our data was not significantly inflated with zero values. Model assumptions were tested by visually examining residuals QQ plots and residual vs. predicted plots.

To evaluate variations in shell length among lengths of deployment time, we used a generalized mixed effects model using the function lmer in the package lme4 in R. Shell length (mm) was used as the response variable. Deployment time was used as the fixed effect (weeks; continuous). Lake was used a random effect. Model assumptions were tested by visually examining residuals QQ plots and residual vs. predicted plots. Significance for all statistical analyses was set at α=0.05

## 3.4 Results

### 3.4.1 Variation in abundance and shell lengths

Abundance varied greatly both among lakes and sampling time (**Figure 3-2)**. Abundance was generally positively correlated with deployment (**Figure 3-3**); though Honeoye Lake abundance was greatest after four weeks and showed a general decline as deployment time increased and Silver Lake had an increase in abundance until a decrease from deployment time 3 to 4 (z=3.087, *p*=0.002; **Figure 3-4**). No mussels were recruited at any deployment time in Craine Lake, though there is an established dreissenid population. No mussels were recruited in either Crooked or Tully lakes where invasions have not been reported. Abundance ranged from 0/m2 to 2684/m2 with a median value of 818/m2.Average abundance across all lengths of sampling times appears to be negatively correlated with the number of years a lake has been invaded (z=2.095, *p*=0.036; **Figure 3-5**); however, Song Lake, the most recently invaded lake in this study, had lower mussel abundance than lakes invaded earlier. The results of the generalized linear mixed effects model fornumber of mussels settled showed that the number of mussels settled varied significantly depending on the length of deployment time (z=3.087, *p*=0.002) and the number of years a lake has been invaded (z=2.095, *p*=0.036). The model estimates that for every additional week of deployment time, the mean number of mussels recruited increased by 8%; for every additional year of invasion, the mean number of mussels recruited increases by 29%.

### 3.4.2 Shell length distributions

Dreissenid shell lengths ranged from 0.86­ mm (Honeoye) to 20.38 mm (Eaton Brook). Average shell length for each deployment time ranged from 8.56 ± 3.11 mm (4 weeks) to 9.39 ± 3.62 mm (12 weeks), while average shell length for each lake ranged from 4.43 ± 2.26 mm (Cazenovia Site 1) to 10.57 ± 3.56 mm (Silver) (**Table 3-4**). Variations in shell lengths among lengths of deployment time were not statistically significant (t= 1.16, *p*=0.246). For about half of invaded lakes (Cazenovia, Eaton Brook, Honeoye), shell length decreased as length of deployment time increased, while for the other half (Owasco, Silver, and Song, and Upper Little York), shell length increased as length of deployment time increased (**Figure 3-6**).

## 3.5 Discussion

Clay bricks were deployed in 10 lakes in New York State to evaluate the efficacy of artificial substrates to detect and recruit adult and settling juvenile dreissenids and its ease-of-use for citizens. Further, we investigated the effect of substrate deployment time on abundance and shell-length distribution. We found that these substrates actively recruited settling juvenile and adult dreissenids. While abundance was highly variable among deployment times and lakes, overall abundance correlated positively with deployment time. Shell length distributions were similarly variable among deployment times and lakes. Three lakes showed increased shell lengths as deployment time increased, and four lakes showed decreased shell lengths as deployment time increased.

For an uninvaded system, lake managers should be aware of the lake’s susceptibility to dreissenid mussels as well as the pathways for invasion. Because dreissenids are already established in many lakes in New York State, management efforts are focused on reducing secondary spread to uninvaded systems. Dreissenid mussels can be dispersed in two ways: 1) natural dispersion of veligers via downstream movement and 2) anthropogenic overland dispersal via vector-attachment. Evidence for the former has been estimated using 3-D models and shows that planktonic larvae can travel between 1 km to tens of kilometers in merely 2-3 weeks (Beletsky et al. 2017). The latter accounts for a large portion of secondary spread in invaded regions (Kraft et al. 2002; De Ventura et al. 2016). Transportation of boats and other recreational vehicles from one lake to another provides a pathway for veligers to disperse into uninvaded systems, especially zebra mussels because they exhibit a greater attachment rate than quagga mussels (Collas et al. 2018).

### 3.5.1 Variation in abundance and shell lengths

We found that while it was highly variable (**Figure 3-2**), the number of mussels settled was positively correlated with length of deployment time. For every additional week of deployment, the mean number of mussels recruited increased by 8%. The median abundance across all deployment times and invaded lakes was 818 mussels/m2, with the greatest abundance of 2,684 mussels/m2. Unsurprisingly, abundance estimations from other artificial substrate studies vary greatly. For example, Enders et al. (2019) found abundances reaching a maximum of 160,000 individuals per m2 in the south basin of Lake Winnipeg, Manitoba, Canada. For an amenity lake in Cardiff Bay, Wales, United Kingdom, two studies report differing maximum settlement by mussels: Alix et al. (2016) found maximum abundance of settling juvenile mussels to be 54700 ± 700 per m2 between 2008 and 2009, while Rolla et al. (2019) found abundances reaching 17,960 individuals per m2 in Cardiff Bay, United Kingdom between 2017 and 2018. Our reported abundances appear to be much lower than other artificial substrate studies. The substrates deployed in this study were exposed to the environment, so predation and excess sunlight may have contributed to reduced recruitment of mussels.

While abundance was generally positively correlated with deployment time, some lakes did not follow this trend. For Honeoye lake, the greatest abundance of 2454 mussels/m2 was found at a deployment time of 4 weeks. The following retrieval period of 8 weeks deployment time showed a drastic reduction in abundance (829 mussels/m2). The final deployment times (12, 16, and 20 weeks) then showed a decreasing trend in abundance. A similar trend was found by Alix et al. (2019) for settling veligers; the greatest amount of settlement occurred in June and was followed by decreasing settlement through the season. Silver Lake abundances showed an initial increase with deployment time, reaching a maximum abundance of 2293 mussels/m2 at 12 weeks deployment, and then a decrease in abundance through 16 weeks and 20 weeks deployment time. The shell length distribution for Silver Lake shifted towards larger mussels as total abundance decreased, suggesting that mussels invested more heavily in mass than reproductive efforts. These variations may be representative of the idea that spawning activity of dreissenids is heavily regulated by climatic and ecological factors including water temperature (Karatayev et al. 1998) and planktonic food supplies (Galbraith and Vaughn 2009). The lowest number of mussels were recruited in Song Lake (380–732 mussels/m2). This follows expected population dynamics for a recently invaded lake. Reports of zebra mussels in Song Lake were first made in 2017. Given that mussels tend to reach peak populations after a few years (Strayer et al. 2017), it is unsurprising that this recently invaded lake did not exhibit abundances as great as the other lakes. Though Craine Lake has had zebra mussel populations since 2007, no mussels were recruited at any deployment time.

### 3.5.2 Lake-wide abundance estimation

We compared abundance from artificial substrates to abundance estimates from previous research that calculated dreissenid abundance estimates for three of the study lakes, namely Owasco, Song, and Honeoye lakes (**Table 3-3.**). These studies estimated dreissenid abundance using rigorous sampling methods (e.g., ponar grabs at multiple sites, SCUBA collection, and replicate artificial substrate deployment within a lake). The results of these studies were compared with our abundance estimates to determine if artificial substrate abundance estimations from one site in a lake can be used as a proxy for lake-wide abundance. For Song Lake, Caves (2019) found zebra mussel abundances ranging from 308–3694/m2 in shallow (< 2 m) sites across the entire lake. Caves (2019) evaluated the difference in recruitment between the top and the bottom of floating substrate plates and found abundances (304–572/m2)comparable to the findings in this study (772/m2). Their estimates from mid-September are particularly interesting considering their substrate deployment methods were not floating and therefore did not provide a bottom surface onto which mussels could settle. For Honeoye Lake, Gilman (2018) found mussel abundances ranging from 0–7492/m2 in the month of July. Abundance estimations from this study ranged from 829–2454/m2 with the lowest abundance detected in July (i.e., 8 weeks deployment time) and the greatest abundance detected in mid-June (i.e., sample time one). Average abundance across all sample times in Honeoye Lake in 2018 was 2034/m2, compared to our 2019 average of 1472/m2. We suspect that while abundance estimation from artificial substrates at one site in a given lake might be at least the same order of magnitude as lake-wide abundance, replication at multiple sites within a lake would provide evidence to more accurately estimate lake-wide abundance.

### 3.5.3 Artificial substrates as a methodology for citizen science

Artificial substrates can be an easy and effective way for citizens to collect dreissenid data in their lakes. Though the design of the artificial substrates can vary greatly among size, shape, position in the water, and material (e.g., Marsden 1992, Martel et al. 1994, Marsden and Lansky 2000, Borcherding and Strum 2002), we propose that a simple substrate composed of a cored clay brick and marine-grade rope secured to any available fixture is sufficient to recruit dreissenid mussels and inform citizens and lake managers about the state of dreissenid populations in a system.

The advantages of the methodology presented here are low-cost, minimal time required, utilization of volunteers who are already involved in water quality sampling, and the collection of data informing population dynamics of dreissenid mussels. Fabricating a set of five artificial substrates can be done for as little as $10.00 using materials found at any hardware or home improvement store. Further, removing and measuring mussels requires no additional materials or tools. We considered counts to include only those mussels visible to the naked eye. This method could be used in conjunction with an existing water quality sampling program. New York’s CSLAP uses volunteers to sample water bodies bi-weekly through the summer. We believe that recruiting these volunteers who are already invested in their lake’s health to deploy this methodology would be an easy and ideal way to increase replication and collect quality dreissenid data. Combining information about dreissenid populations with long-term water quality and HABs data can provide lake managers and associations with important information about lake health. This method could be especially informative in systems where dreissenids are not currently present but are susceptible to invasion. Lastly, the benefits of collecting large quantities of mussels could be extended to interested parties (e.g., researchers, environmental education programs, etc.)

Some consideration of the limitations of this methodology should be taken by managers or associations who wish to implement this method for their lake. While zebra mussels prefer hard substrate, zebra mussels and quagga mussels are able to colonize soft silty substrate and macrophytes (Karatayev et al. 2015). Further, dreissenids can be spatially distributed within a system in non-homogenous fashion (Burlakova et al. 2012; Karatayev et al. 2015). Deploying artificial substrates such as those described here may therefore underestimate or overestimate lake-wide abundance depending on the amount of available substrate for colonization in the lake. It is therefore imperative that in-lake replications at various locations be deployed to best capture lake-wide abundance. While minimal training would be required, accurate identification of dreissenid species is important. Volunteers should be trained to recognize differences between zebra and quagga mussels, especially in lakes where zebra mussels are present, but no confirmed populations of quagga mussels have established. We acknowledge that removing, counting, and measuring every recruited mussel may be time consuming, so volunteers may also randomly subset a given surface area and extrapolate from those counts and measurements.

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# Chapter Four: Synthesis and Conclusions

## 4.1 Review of Research Objectives

There were six main goals of this research project. I sought to evaluate how dreissenid mussels affects low-nutrient freshwater lakes in New York State. Using historical water quality data, I analyzed how water quality parameters changed in 8 lakes after dreissenid invasion (Objective 1, Chapter 2). Then I compared the current (2012-2017) water quality and HABs parameters of those 8 invaded lakes to 60 uninvaded lakes (Objective 2, Chapter 2). I then inspected how eutrophication (measured by TP, TN:TP, and chlorophyll-a) affects microcystin concentrations, and whether the effect is the same in both invaded and uninvaded lakes (Objective 3, Chapter 2). I also tested a citizen-science based methodology for detecting and monitoring dreissenid mussels (Objective 4, Chapter 3). Using data collected from this methodology, I sought to describe how mussel settling (measured as abundance) and mussel length distribution changes through the summer months (Objective 5, Chapter 3). Lastly, we used those analyses describing settling and length to determine the ideal artificial substrate deployment time (Objective 6, Chapter 3).

## 4.2 Chapter Two: Using citizen-science data to evaluate the role of dreissenid mussels in harmful algal bloom formation in low-nutrient lakes in New York State

### 4.2.1 Main findings

I used historical data to evaluate how a lake changes after dreissenid invasion in 8 lakes in New York State. Physical, biological, and biogeochemical changes are expected when a lake is invaded with dreissenid mussels.Increased Secchi depth coupled with reduced chlorophyll-a concentration is one of the most well-documented examples of the impacts of dreissenid invasion

We found that dreissenid invasion was a significant predictor for variations in Secchi depth and chlorophyll-a concentration. Secchi depth significantly increased and chlorophyll-a concentrations significantly decreased after dreissenid invasion. Secchi depth was found to increase by ~0.7 m after invasion (*t* = -9.37, p < 0.0001). This corresponds with model predictions for chlorophyll-a, which found that chlorophyll-a levels were ~82% greater in pre-invasion years (*t* = 13.31, p < 0.0001). It was found that bottom sample TP significantly decreased after invasion.

Using a mixed effects framework, we evaluated how lake characteristics (invasion status, mean depth, CA:SA, and agricultural land use) predict variations in open water TN:TP concentration, bottom sample TP concentration, Secchi depth, chlorophyll-a concentration, true color, SB chlorophyll-a concentrations, SB microcystin concentrations, and open water microcystin concentrations. The random effects of lake, sample month, sample year, and the interactive effect of lake and sample year were all initially included. Random effects that explained very little (<.0001) variance within a model were dropped. It was found that dreissenid invasion was a significant predictor for variations in true color only. Lake and watershed characteristics appear to explain more variations in these water quality parameters. Mean depth was a significant predictor for TN:TP, chlorophyll-a concentrations, true color, and Secchi depth. CA:SA was a significant predictor for true color and Secchi depth. Percent agricultural use was a significant predictor for bottom sample TP.

I compared HABs frequency and HABs with high toxin concentration frequency between invaded and uninvaded lakes in New York State (2012-2017). It was found that neither HABs nor HABs with high toxin concentrations occur at differing rates between invaded and uninvaded lakes. This finding was consistent for both the global and the reduced datasets.

The effects of eutrophication on HABs microcystin concentration was evaluated. It was found that TP was a significant predictor for microcystin concentrations from open water samples in invaded lakes, but not uninvaded lakes. Yearly average TP was not a significant predictor for microcystin concentrations from shoreline bloom samples in either invaded or uninvaded lakes. TN:TP was not a significant predictor for either open water or shoreline bloom microcystin concentrations in both invaded and uninvaded lakes. Likewise, chlorophyll-a was not a significant predictor for microcystin concentrations from either open water or shoreline bloom samples in both invaded and uninvaded lakes.

### 4.2.2 Future research

Interestingly, dreissenid invasion status was only a significant predictor for variations in true color. The effects of dreissenid invasion on a lake are well known and well-documented; however, further research into changes in true color might inform a more nuanced effect of dreissenids. Literature regarding true color changes as an effect of dreissenids is limited. It was found that lake and watershed characteristics explained more variations in water quality than dreissenid invasion status. This finding might inform best management practices for lake associations or agencies wishing to prevent or mitigate HABs. I propose that further research is undertaken to identify thresholds for mean depth, CA:SA, and percent agricultural use to identify lakes that may be susceptible to HABs.

## 4.3 Chapter Three:Use of artificial substrate to monitor population dynamics of dreissenids to supplement an existing citizen-science water quality collection program

### 4.3.1 Main findings

A citizen-friendly methodology for detecting and monitoring dreissenid mussels was tested in summer of 2019. Using a mixed effects framework, we identified factors that accounted for variation in mussel abundance through the summer season. It was found that for every additional week of deployment time, the mean number of mussels recruited increased by 8%; for every additional year of invasion, the mean number of mussels recruited increases by 29%. Variations in shell-length distribution through the season were not statistically different between deployment times. Half of sample lakes showed tendencies towards increased shell length as deployment time increased, while the other half tended towards decreased shell length as deployment time increased.

### 4.3.2 Future research

This study is currently being extended to the 2020 season. Efforts to improve study efficacy are being undertaken. These efforts include in-site replication of substrates and lake-wide replication of sites. Replication will increase statistical power and accuracy in evaluating differences in mussel settlement through the summer season. Further, lake-wide replication will help inform managers of spatial variations of dreissenid populations. Quality assurance of citizen-collected data should also be considered in future works because of the potential of citizens to miscount or mis-identify dreissenid mussels. Despite the drawbacks of this methodology presented in Chapter Three, I expect that the use of this method could be informative when used in conjunction with long-term water quality data that is already being collected in CSLAP.

# Appendices

# Curriculum Vita