Assessment of Ecosystem Management Strategies and Stakeholder Needs for Harmful Algal Blooms in the Great Lakes

by

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Abstract

This Master's Project focused on improving the understanding of harmful algal blooms (HABs) within Lake Erie and Saginaw Bay as well as characterizing needs of water utility managers for information products. This research addressed four different topics: 1) In Chapter 2: Quantifying the impact of nutrient availability and form on the growth of HABs in Lake Erie and Saginaw Bay, we found that phosphorus was the main limiting nutrient based on nutrient addition treatments, but with nutrient reduction treatments, nitrogen appeared to be co-limiting, suggesting reductions in phosphorus alone may not eliminate blooms. 2) In Chapter 3: Mechanistic Assessment of Dreissenid Mussel Grazing Impacts on Phytoplankton Growth Rates, we found that the synergistic effect of nutrient excretion and dilution through grazing had the largest impact on total phytoplankton growth, followed by nutrient excretion and dilution through grazing, respectively. The overall impact of grazing by Dreissenid mussels on cyanobacteria growth rates was limited, and mussel-mediated nutrient recycling was the strongest explanatory mechanism. 3) In Chapter 4: Comparative Analysis of *Microcystis* Colony Buoyant Velocities in Western Lake Erie and Saginaw Bay of Lake Huron: Effects of Colony Size and Light Intensity, we show that Western Lake Erie and Saginaw Bay differ in the way that *Microcystis* buoyancy responds to light and may therefore ultimately affect how the bloom is transported throughout each ecosystem. Understanding the effects of light on buoyancy will help explain observed differences in vertical distribution and movement of *Microcystis* in the two lakes. 4) In Chapter 5: Characterization of Public Water System Needs and Attitudes Related to HAB Toxicity in Saginaw Bay and Western Lake Erie, we describe a focus group and survey of water system managers in Saginaw Bay about how HABs impacted them and if a HAB forecast model would be useful. Our results show that managers believed the forecast would be useful, and that they would be willing to use it. It also suggested that managers feel unprepared in the event of a HAB beyond what has occurred recently in the Bay, so efforts should be taken to increase their knowledge and preparedness.

Acknowledgements

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Executive Summaries

Chapter 2: Responses of Algal Communities in Lake Erie and Saginaw Bay to Changes in Phosphorus Levels, Nitrogen Levels, and Ammonium to Nitrate Ratio

The typical approach for managing harmful algal blooms in most lakes has been reducing the input of phosphorus into the lake. However, there has been a growing body of evidence that suggests nitrogen may be secondarily limiting or co-limiting alongside phosphorus. Nitrogen has several bio-available forms in lakes, namely nitrate and ammonium, which could potentially play a role in the size and severity of blooms. This experiment sought to determine how additions and reductions in dissolved inorganic nitrogen and soluble reactive phosphorus impact the growth of cyanobacteria in Lake Erie and Saginaw Bay. In addition, the impact of the form of inorganic nitrogen on growth was studied by adjusting the ratio of ammonium to nitrate, without increasing the ambient lake levels of total nitrogen. The results showed that nitrogen form had little to no impact on growth rates of cyanobacteria in either lake. In Saginaw Bay, phosphorus appeared to be the main limiting nutrient based on nutrient additions, but when nutrients were reduced below ambient levels, nitrogen appeared to be co-limiting. This was consistent with prior studies , and suggests that dual nutrient reductions could potentially reduce the size of harmful algal blooms in Saginaw Bay.

Chapter 3: Mechanistic Assessment of Dreissenid Mussel Grazing Impacts on Phytoplankton Growth Rates

The establishment and proliferation of the invasive dreissenid mussels, quagga mussels (*Dreissena rostriformis bugensis*) and zebra mussels (*Dreissena polymorpha*), have resulted in many pervasive and irreversible ecological impacts in the Great Lakes. At least three mechanisms have been proposed which implicate dreissenid mussels in exacerbating harmful algal blooms. First, the release of key nutrients (ammonium and soluble reactive phosphorus) in mussel excretions. Second, the mussels' ability to selectively feed on their preferred phytoplankton, such as diatoms and cryptophytes, while rejecting less favorable *Microcystis*, has been credited for reducing the cyanobacteria's competition. Third, the mussels' grazing activity has increased water clarity and, thus light availability, supporting the growth of phytoplankton. The objective of the mussel bioassay experiments was to isolate and quantify the relative importance of these mechanisms and to determine the role of potential interactions and synergies between them in the promotion of cyanobacteria growth. The results from these experiments showed that the relationship between dreissenid mussels and phytoplankton growth is proportional to mussel grazing and excretion rates. When these rates were high, the nutrient excretion and increased water clarity explained the increased growth rate of total phytoplankton; however, nutrient enrichment alone was the explanatory mechanism in promoting cyanobacterial growth. These experiments showed that the relationship between mussels and phytoplankton growth is more complicated than expected and that further exploration into the relative importance of these mechanisms is warranted.

Chapter 4: Comparative Analysis of Microcystis Colony Buoyant Velocities in Western Lake Erie and Saginaw Bay of Lake Huron: Effects of Colony Size and Light Intensity

Microcystis aeruginosa, the predominant species of harmful cyanobacteria in both Lake Erie and Saginaw Bay, has the capacity to regulate the buoyancy of its cells and colonies – sinking under certain conditions while floating towards the surface in others. Understanding the factors that control buoyant velocity is critical for modelling and forecasting harmful algal blooms within these two systems. In order to understand whether *Microcystis* colony buoyant velocities in the two lakes respond similarly to diurnal light cycles, we executed a replicated experiment in which we manipulated light intensities in outdoor mesocosms, then tracked colony size and velocity using micro-cinematography. Overall, colonies in Lake Erie were more likely to be positively buoyant (floating) than in Saginaw Bay, where most colonies were negatively buoyant. In Lake Erie we found that within each light treatment (i.e. ambient, 30% ambient, and 10% ambient) colonies had a significantly higher buoyant velocity in the morning compared to the afternoon, where most colonies were sinking in all but the lowest light treatments. In addition, we found that although colony velocity was positively correlated to colony size in Lake Erie in accordance with Stoke's law, this relationship was very weak in Saginaw Bay. This finding indicates potentially different *Microcystis* movement dynamics between lakes and highlights the need to investigate variables other than colony size that could be impacting velocities in Saginaw Bay. When looking at colony density, our data exhibit a pattern of higher variability among small colonies compared to large colonies in both Lake Erie and Saginaw Bay, suggesting a common pattern of colony density becoming less plastic as colonies increase in size. The results of this study show that Western Lake Erie and Saginaw Bay differ in the way that *Microcystis* responds to light and may therefore ultimately affect how the bloom is transported throughout each system. Understanding the effects of light on buoyancy will help explain observed differences in vertical distribution and movement of *Microcystis* in the two lakes.

Chapter 5: Characterization of Public Water System Needs and Attitudes Related to HAB Toxicity in Saginaw Bay and Western Lake Erie

In order to provide stakeholders with up to date forecasts and reports about harmful algal blooms in Lake Erie, NOAA/GLERL developed the experimental Lake Erie HAB Tracker in 2014. As there is also concern about HABs in Saginaw Bay, there is interest in seeing if applying a similar forecasting product forSaginaw Bay would be useful and effective for various stakeholders, such as water treatment plant operators. To gauge this interest, we conducted a focus group and survey with 10 stakeholders from water plants and the Michigan Department of Environment, Great Lakes, and Energy. The goal was to answer two overarching research questions: 1. Would a HAB forecast similar to the experimental Lake Erie HAB Tracker be useful for Saginaw public water system employees and 2. How are public water systems in Saginaw Bay impacted by HABs? Participants believed that a HAB forecast product in Saginaw Bay would be useful for them, and expressed a willingness to use the product. Participants said that HABs were not currently impacting their plants, but expressed a desire to learn more about

HABs, as well as determining ways to prepare for and respond to potential future HAB events. Based on these responses, a HAB forecast would be beneficial to water managers in Saginaw Bay. Efforts should be made to provide some guidelines or procedures in the event of a HAB at one of these plants, so that managers feel better prepared to respond.

Chapter 1. Introduction

The Laurentian Great Lakes contain 18% of the world's surface freshwater. The lakes provide drinking water, fisheries, recreation, navigation, and ecosystem services to eight US states and two Canadian provinces. Lake Erie is the smallest Great Lake by volume, and is exposed to the largest amount of urbanization and agriculture (Waples et al. 2008), particularly the lake's western basin. Saginaw Bay, in western Lake Huron, is located between the "thumb" of Michigan and the rest of the Lower Peninsula, and the Bay provides drinking water to nearly 100,000 people.

Algal blooms began occurring in Lake Erie and Saginaw Bay during the late 1960s and early 1970s (Vollenweider et al 1974) due to eutrophication, or the influx of anthropogenic-sourced nutrients such as phosphorus which promote the excessive growth of algae. During the 1970s efforts were made in order to attempt to limit these phosphorus inputs through national (Clean Water Act of 1972) and international policy initiatives (Great Lakes Water Quality Act 1972). It was during this period that both Lake Erie and Saginaw Bay experienced the establishment and proliferation of invasive Dreissenid mussels (Benson, 2013). These mussels have been linked to increases in cyanobacteria blooms through various direct and indirect mechanisms (Bierman et al. 2005) (Vanderploeg et al. 2001).

By the 1990s, eutrophication and phosphorus levels had been reduced in both Lake Erie (Richards & Baker, 1993) and Saginaw Bay (Stow et al. 2014). However, despite the reduction in total phosphorus, algal blooms remain an issue in both systems. The phytoplankton species primarily responsible for these blooms is the cyanobacterium *Microcystis aeruginosa.* Prevalent in many aquatic systems, these cyanobacteria produce a harmful hepatotoxin known as microcystin (Figueiredo et al. 2004). Because of this, blooms caused by *Microcystis* are referred to as harmful algal blooms (HABs).

Exposure to microcystin can result in a multitude of symptoms, including irritation to the lungs, skin, and eyes, nausea, fever, and liver inflammation (McLellan & Manderville, 2017). There is evidence that chronic exposure can lead to liver and colorectal cancer (Figueiredo et al. 2004). Because the risk to human health is so high, it is particularly dangerous if microcystin infiltrates public drinking water systems. In 2014, the water treatment plant in Toledo, Ohio detected microcystin in the drinking water that surpassed health and safety thresholds, resulting in a "do not drink" order to be issued to nearly a half million people (McCarty et al. 2016).

Public health is not the only factor impacted by HABs. The economic costs of blooms are very large, with blooms in freshwater systems across the United States costing some $2.2 billion (Dodds et al. 2009). Lake Erie provides some $2 billion in revenue from hunters and anglers, and potential blooms that degrade water quality could cause a decline in this revenue (Great Lakes Commision 2014). The 2014 bloom alone was estimated to have cost some $65 million due to losses in property value, recreation, tourism, and the cost of updating treatment facilities (Bingham et al. 2015). As of 2019, no large harmful algal bloom has occurred within Saginaw Bay; however, as climate change and further development in the region continue, the potential for a large HAB to occur in Saginaw Bay increases (Moore et al. 2008).

In order to mitigate the risks imposed by HABs in western Lake Erie, forecasts such as the Lake Erie HAB Bulletin and the experimental Lake Erie HAB Tracker were developed and made available for public use (Gill et al. 2017). These models provide stakeholders within the affected region with information regarding bloom size and location, both geographically and vertically within the water column itself (Rowe et al. 2016). Due to the success of these models and the predicted increase in the occurrence of HABs, it is necessary to understand the perceived risks and impacts caused by HABs on public drinking water systems within Saginaw Bay to ensure that potential future products would be utilized by utility managers in the region.

Chapter 2. Responses of algal communities in Lake Erie and Saginaw Bay to changes in phosphorus levels, nitrogen levels, and ammonium to nitrate ratio

2.1 Introduction

The large harmful algal blooms (HABs) that have occurred in Lake Erie since the mid-nineties are a major public health, ecological, and economic concern (Michalak et al. 2013; Steffen et al. 2017). These blooms are caused by the growth of the cyanobacterium *Microcystis aeruginosa*, which can produce a hepatotoxin called microcystin. While Lake Erie has received much attention, HABs also occur in Saginaw Bay of Lake Huron and other locations in the Great Lakes. Efforts to understand what factors contribute to these HABs have focused on light (Kardinaal et al. 2007), temperature (Davis et al. 2009), trace elements such as iron (Xu et al. 2012), invasive mussels (Vanderploeg et al. 2001), nitrogen, and phosphorus (Elser et al. 1990; Hamilton et al. 2016). As in many other freshwater lakes, the management strategy for controlling these HABs in the Great Lakes has focused on limiting cultural eutrophication, caused by activities in the watershed which lead to excessive inputs of nutrients, particularly phosphorus (Stumpf et al. 2012).

Nitrogen and phosphorus are of particular interest for HABs because these nutrients are critical to the growth of algae and come from human activities (Lewis et al. 2011). Much of the management strategies in Western Lake Erie and Saginaw Bay have focused on limiting phosphorus inputs into the system (Stow et al. 2014). The Environmental Protection Agency created an action plan for the reduction of HABs in Lake Erie by focusing strictly on a reduction of phosphorus inputs. Recently, some scientists have begun to question whether managing P alone will be sufficient to control HABs (Cotner, 2017; Paerl et al. 2016; Newell et al. 2019). In many lake ecosystems, nitrogen and phosphorus are co-limiting, or nearly so, based on experiments where the nutrients are added individually or in combination (Elser et al. 2007). However, it has been shown that reductions of phosphorus below ambient levels might be less effective at reducing algal growth than reductions to both phosphorus and nitrogen (Baer, 2019; Paerl et al. 2016, 2019). Thus there is a need to compare the outcomes from nutrient addition bioassays with those from nutrient depletion bioassays.

The impact of nitrogen on algal growth is further complicated as it exists in multiple accessible forms in lakes, namely ammonium and nitrate. Ammonium is favored by *Microcystis* (Chaffin & Bridgeman 2014), and elevated levels could potentially exacerbate blooms. Most studies that look at how nitrogen impacts blooms focus solely on the total inorganic N concentration in the water, with no consideration for the form the nitrogen is in. There is increasing evidence that the availability of inorganic nitrogen, particularly in the form of ammonium, has a role in triggering the onset of bloom conditions in eutrophic lakes (Chaffin and Bridgeman, 2014). Prior studies that have analyzed the impact of nitrogen forms on *Microcystis* growth rate either have measured their impact individually (Chaffin and Bridgeman, 2014) or in fixed ratios (Kim et al. 2017). Prior experiments that have examined the impact of ammonium to nitrate ratio, have done so by increasing the total amount of ammonium or nitrate in the system (Chaffin and Bridgeman, 2014). This means that not only was the ratio changed, but total inorganic nitrogen concentrations increased, making it difficult to say what caused the response in growth (Figure 2.1). For example, a bioassay in which ammonium or nitrate are added to concentrations above ambient in lake water tests whether the ratio of NH_4:NO_3 affects growth if the total inorganic N concentration were higher than ambient. Given that many lakes are at or near co-limitation for N and P, this approach may give misleading conclusions. To determine if nitrogen form truly has an impact on the growth of cyanobacteria, the ratio must be altered without changing the amount of total inorganic nitrogen in the system (Figure 1).

In order to test the impact of phosphorus and nitrogen form on the development of HABs, two experiments were performed. The first experiment tested how differing concentrations of dissolved inorganic nitrogen (DIN) and soluble reactive phosphorus (SRP) influence the growth of algae in western Lake Erie and Saginaw Bay. To determine which nutrient was limiting, both additions and reductions were performed. If a nutrient was limiting, one would expect an increase of that nutrient to result in increased growth. Conversely, if a limiting nutrient is reduced, growth should be reduced. The algal growth rates in Saginaw Bay were expected to be more responsive to the total concentration of phosphorus than the algal community within Lake Erie, where secondary limitation via N might occur. This is due to the high phosphorus inputs from the Maumee and other rivers that drain into western Lake Erie (Baker et al. 2014; Michalak et al. 2013).

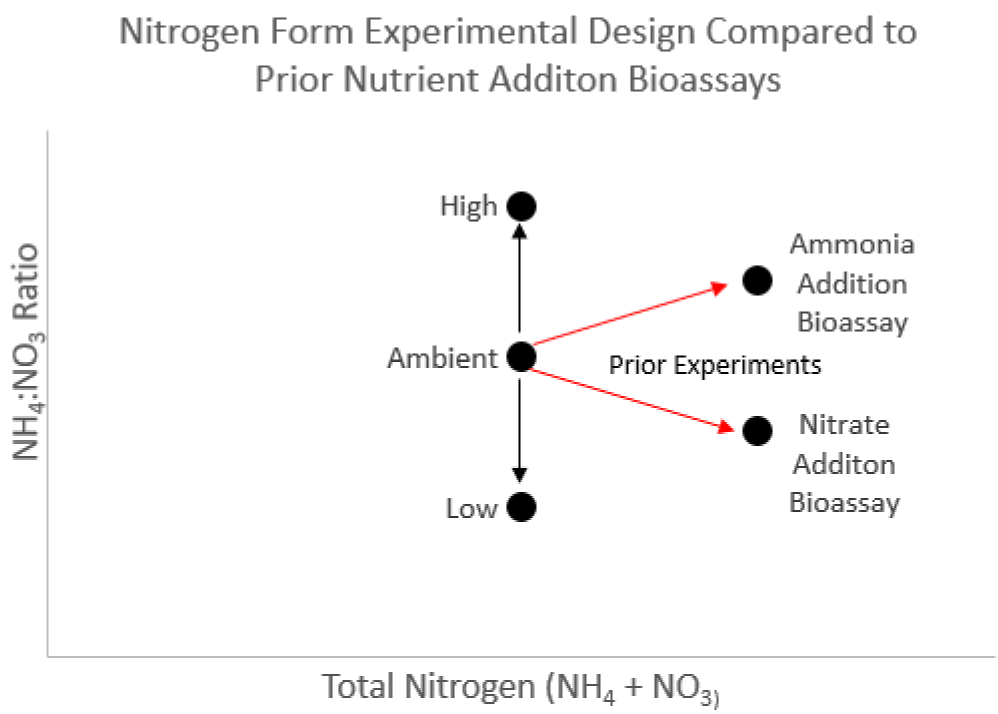


Figure 2.1: The experimental design allows for the manipulation of the NH_4:NO_3 ratio, while maintaining a consistent total nitrogen concentration (NH4+NO3,.

The second experiment explored how nitrogen form impacts algal growth. To avoid increasing total nitrogen while adjusting the NH_4:NO_3 ratio, lake samples were diluted, reducing total inorganic nitrogen levels by 40%. From there, the nitrogen levels are brought back up to match ambient conditions in the lake at the time of sampling. For example, to achieve a high NH_4:NO_3 ratio, the sample would be diluted by 40%, then ammonium would be added to the system to bring it back up to the levels in the lake at the time of sampling. This way, the form of nitrogen can be adjusted without adding a confounding change in nitrogen concentration. High, low, and ambient lake ratios were tested. Phosphorus levels were tested at both ambient lake conditions and a 40% addition. This addition was tested in case the algal community was limited by phosphorus, which could potentially mask any effect from the nitrogen form. Adding more phosphorus should alleviate P limitation and allows for observation of how N form impacts algal growth.

2.2 Methods

Surface water was collected from Saginaw Bay and Lake Erie near NOAA-GLERL station (https://www.glerl.noaa.gov/res/HABs_and_Hypoxia/habsMon.html) SB14 (43.738,-83.641) (Burtner et al. 2020) and WE2 (41.762,-83.330) (Burtner et al. 2019) respectively using a 5L Niskin bottle 1m below the surface during the late morning or early afternoon from June to August. 10L of water was collected in a carboy and stored in a cooler until arrival at the laboratory. Three experiments were performed in Saginaw Bay, and three were performed in Lake Erie.

In order to maintain nutrient levels at ambient conditions, the nutrient concentrations in the lake at the time of collection were determined. A 10ml sample of water was filtered to 0.2μm and the nutrient content was immediately measured using a SEAL AutoAnalyzer 3 HR (Seal Analytical, 2012). These values were used to determine how much N, P as well as the form of N to be added to reach the desired treatment. On the day of the experiment, prior to the arrival of the samples, a 10L solution was made that contained no nitrogen or phosphate but provides major ions found within the lakes, such as Mg^{2+} and HCO_3^- (Baer, 2019). It has final concentrations of 13.5mg/L $MgSO_4$, 31.5 mg/L of $NaHCO_3$ and 27.6mg/L of $CaCl_2$. Working stocks of ammonium chloride, sodium nitrate, and potassium phosphate were made and were used for the nutrient additions.

The SRP and DIN experiment was tested by performing a combined SRP-DIN reduction, an SRP reduction, ambient lake levels, a DIN increase and an SRP increase. These were done by diluting samples by 40% with the ion solution, and then adding nutrients back to the desired levels. This 40% reduction was chosen as it is the goal for phosphorus reduction in the most recent BMP and represents a realistic reduction within Lake Erie.

The effect of NH_4:NO_3 ratio and phosphate was tested by performing low ratio, ambient lake condition ratio, and high ratio treatments. These experiments were performed with ambient lake phosphate levels, and also with elevated SRP concentrations (40% above ambient). Similarly to the SRP and DIN experiment, lake water was diluted 40% using the ion solution. The nitrogen level was brought back up to ambient lake conditions using the necessary form of nitrogen, ammonium for high ratio, nitrate for low ratio, and a combination for ambient. Phosphate was added for the desired treatment as well.

Before use, all glass and plastic that would come in contact with the water was acid washed in 1N HCl to ensure any traces of phosphate were removed. First, 300mL of water was added to nine 500mL bottles in a random order. To these bottles, the appropriate amount of standard and ion solution was added to make the desired treatment effect based on the nutrient data collected from the auto analyzer. The bottles were then mixed and 100mL from each was transferred to a set of three 100mL borosilicate glass Erlenmeyer flasks. These flasks were assigned random numbers and capped with aluminum foil. These flasks were then transferred to an incubator set to the temperature of the water at time of collection, and with LED light panels set to 300μmol/m^2/s (5 hours of light and 9 hours of darkness).

Microcystis population density was quantified using in vivo fluorescence, converted to a chlorophyll concentration. Fluorescence was measured using a BBE Moldaenke FluoroProbe, a fluorometer capable of distinguishing between multiple classes of algae. The initial fluoroprobe measurement was taken after the samples were in the incubator for 30 minutes . The samples were measured using the FluoroProbe for 30 seconds while stirring, recording a measurement every second. Fluoroprobe readings were taken every morning (~8am) and evening (~5pm) over the course of 3 days for a maximum of 5 readings during the experiment. This time frame was chosen to avoid bottle artifacts and to focus only on the short-term growth response of the algal community (Baer 2019).

2.2.1 Statistical Analysis

The densities at the final time points were used in all analyses, as they would reflect the short-term growth across all treatments. Since the bottles were filled from the same source water, we assumed that the initial densities of algae were the same across all treatments. Final densities were analyzed using linear mixed effect models to account for random effects across replicates using the lmer package in R (Bates et al. 2015). The response variable for each flask was the 30 seconds of fluoroprobe acquisitions. The fixed effect for the nitrogen ratio test was the nitrogen ratio with the phosphate concentration. The random effects were the individual flask identities, which accounts for non-independence of measurements made on the same sample. The package lsmeans was used to determine the mean values of the mixed effect models (Russel, 2016).

2.3 Results and Discussion

2.3.1 Community Composition

	1 Sag Bay	2 Sag Bay	3 Sag Bay	4 West Erie	5 West Erie	6 West Erie
% Cyanobacteria	18.34	55.62	64.06	4.33	74.13	94.36
% Green Algae	22.72	n.d.	n.d	29.93	23.57	0.49
% Diatoms	52.14	29.11	13.20	65.74	1.01	1.41
% Cryptophytes	6.80	15.28	22.74	n.d.	1.30	3.74

Table 2.1: The percentage breakdown of community composition at the final time point across all experiments. Not detected (n.d) values were recorded as 0 by the fluoroprobe.

The algal community composition at the final time point of each experiment is summarized in Table 2.1. Previous studies on algal community composition found that cyanobacteria did not typically dominate the systems until July (Allinger & Reavie, 2013; Stoermer & Theirot, 1985). The community make-up of both lakes began with very low percentages of cyanobacteria. (18.34% for Sag Bay, 4.33% for West Erie) (Table 2.1) By July, cyanobacteria had come to dominate both communities (55.62% for Sag Bay, 74.13% for West Erie), and by August, Sag Bay saw a further increase in cyanobacterial concentration (64.06%),and n Lake Erie, cyanobacteria was nearly the only algae present within the community. (94.36%) by this time.

2.3.2 SRP and DIN Experiments

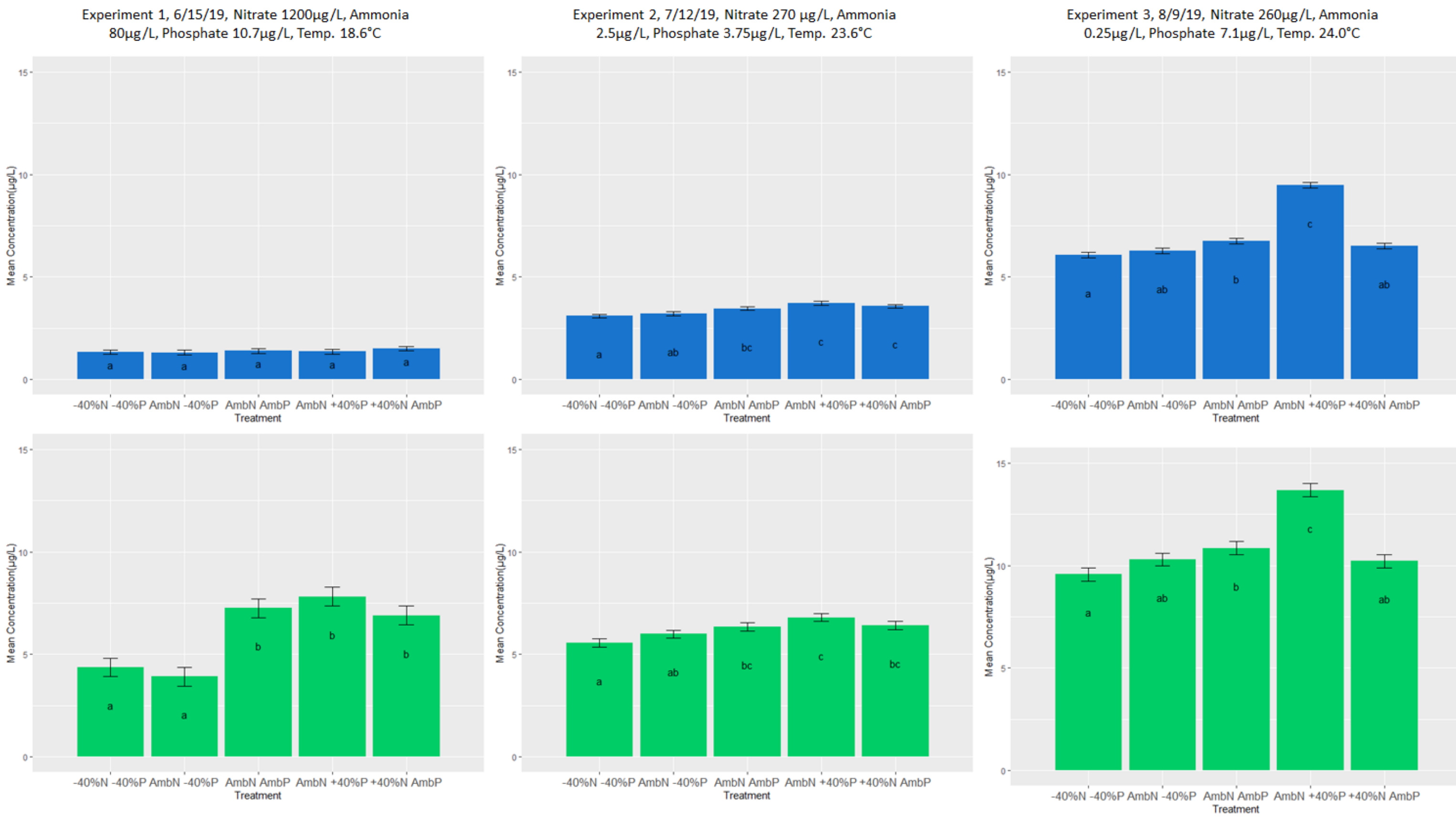


Figure 2.2: Saginaw Bay SRP and DIN experiments. Bars are the marginal means acquired from the lmer test. Blue bars represent cyanobacterial density, green bars are total algal density (µg/L). Error bars represent the standard error of the mixed effects model. Different letter labels signify a significant difference. Typically experiments lasted for approximately 62 hours, except for Experiment 4, which was stopped after about 48 hours. Experiment 7 (Western Erie) was not included as the algal growth was negative throughout that experiment.

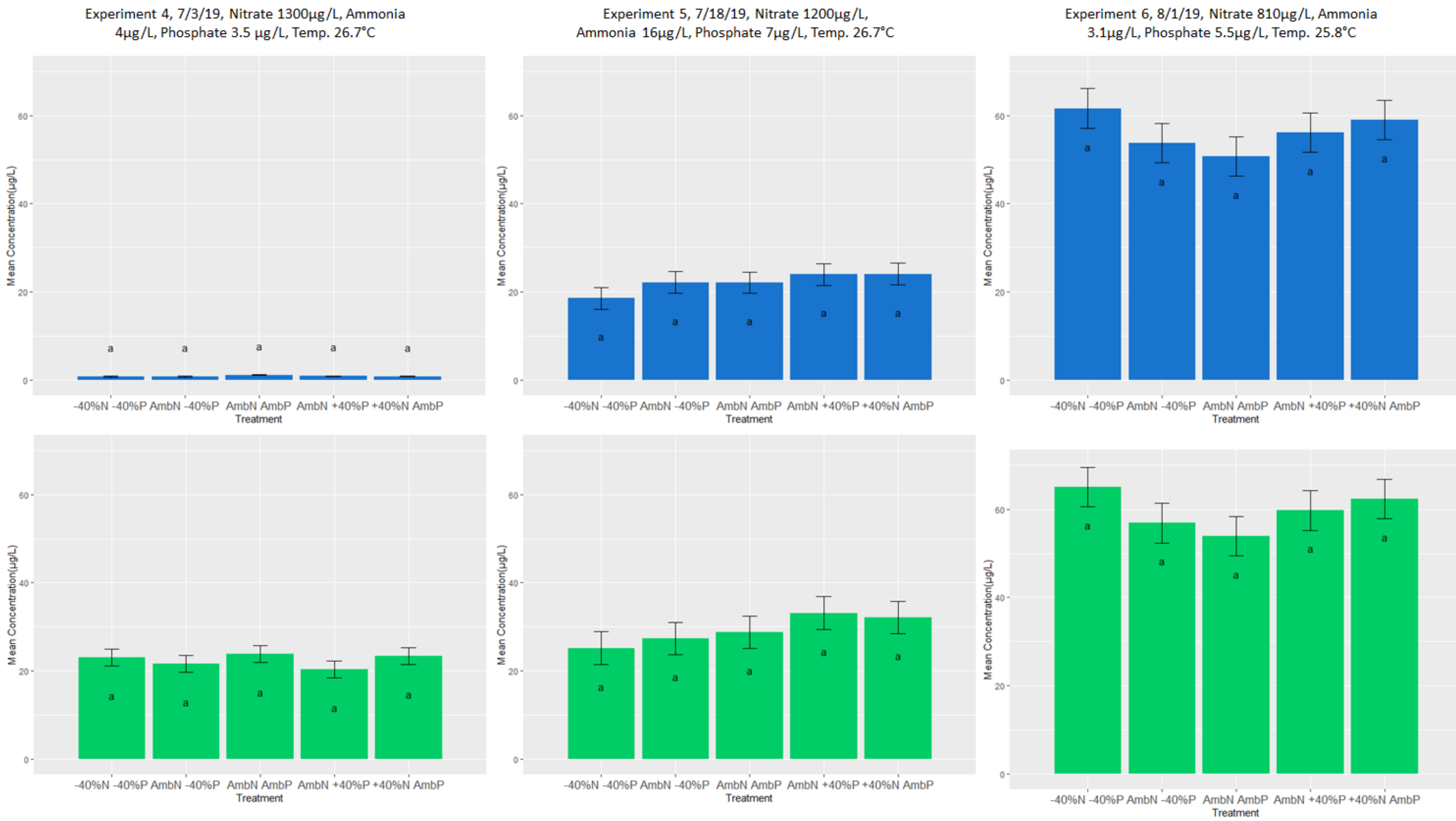


Figure 2.3: Lake Erie SRP and DIN experiments, refer to figure 2 for details.

The Saginaw Bay SRP and DIN experiment showed significant differences across all three experiments. For total algae in Experiment 1, ambient conditions, +40% P and +40% N had higher growth than nutrient reductions. Experiment 2 showed the -40%N -40%P treatment was significantly lower than ambient. In Experiment 3, the +40% P treatment had significantly higher growth than other treatments. Early in the season in Saginaw Bay, when ambient nutrients were high and total chlorophyll was low, increasing either nutrient had no significant impact on growth. Experiment 1 showed that the 40% reduction of N+P and the 40% reduction of P alone both produced a significant decrease in algal growth from ambient conditions. The P reduction and the N+P reduction were not significantly different from each other, implying that early in the season, the algal community in Saginaw Bay is likely P-limited only. In Experiment 2, a 40% reduction in N+P showed a significant decline in growth, suggesting the algal community could be near N+P co-limitation.

Based on P-addition, the algal community in Saginaw Bay appeared to be increasingly limited by phosphorus later in the season (Figure 2.2, Experiment 3), as the P addition had a significant increase in mean algal concentration when compared to ambient conditions. However, the community showed a significant decline in growth when N+P were reduced by

40%, but not when P alone was decreased by 40%. The increase of growth after the P addition implies primary P limitation of phytoplankton growth. However, if this were the case, one would expect the reduction of phosphorus in the system to have a significant decline on growth, which was not the case in the experiment. Experiments on nutrient limitation have typically analyzed changes in growth after the addition of nutrients (Elser et al. 2007), rather than the reduction (Xu et al, 2009). It is likely that the algal community in Saginaw Bay is limited by phosphorus, but further experiments need to be performed to see how the algae responds to reductions in phosphorus levels (Baer, 2019). The decline of growth in response to N +P reduction could mean ambient nitrogen concentrations were just low enough to cause secondary limitation, or a potential co-limitation when both phosphorus and nitrogen are reduced.

Lake Erie showed no significant difference between any of the treatments at any time during the DIN and SRP experiments (Figure 2.3). Prior studies in Lake Erie and other lakes have shown that phosphorus is limiting early in the season and nitrogen later in the season (Chaffin et al. 2013; Ding et al. 2018; Matthew et al. 2002). Those previous studies added a fixed amount that was greater than the 40% additions of P and N described here. It Is possible that the addition of 40% nitrogen was too small to see any significant effect on algal growth.

2.3.3 NH_4:NO_3 Ratio and Phosphate Experiment

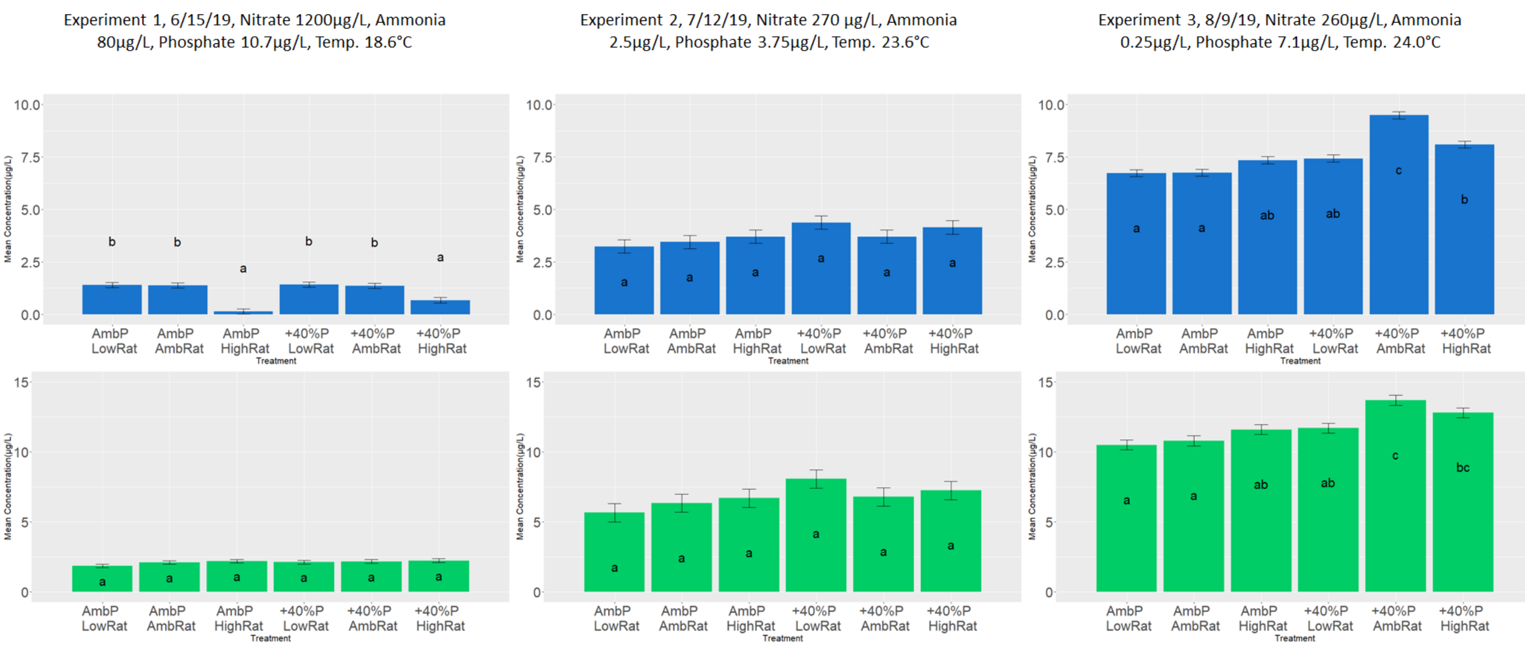


Figure 2.4: Saginaw Bay NH_4:NO_3 Ratio and Phosphate Experiment, refer to Figure 2 for details.

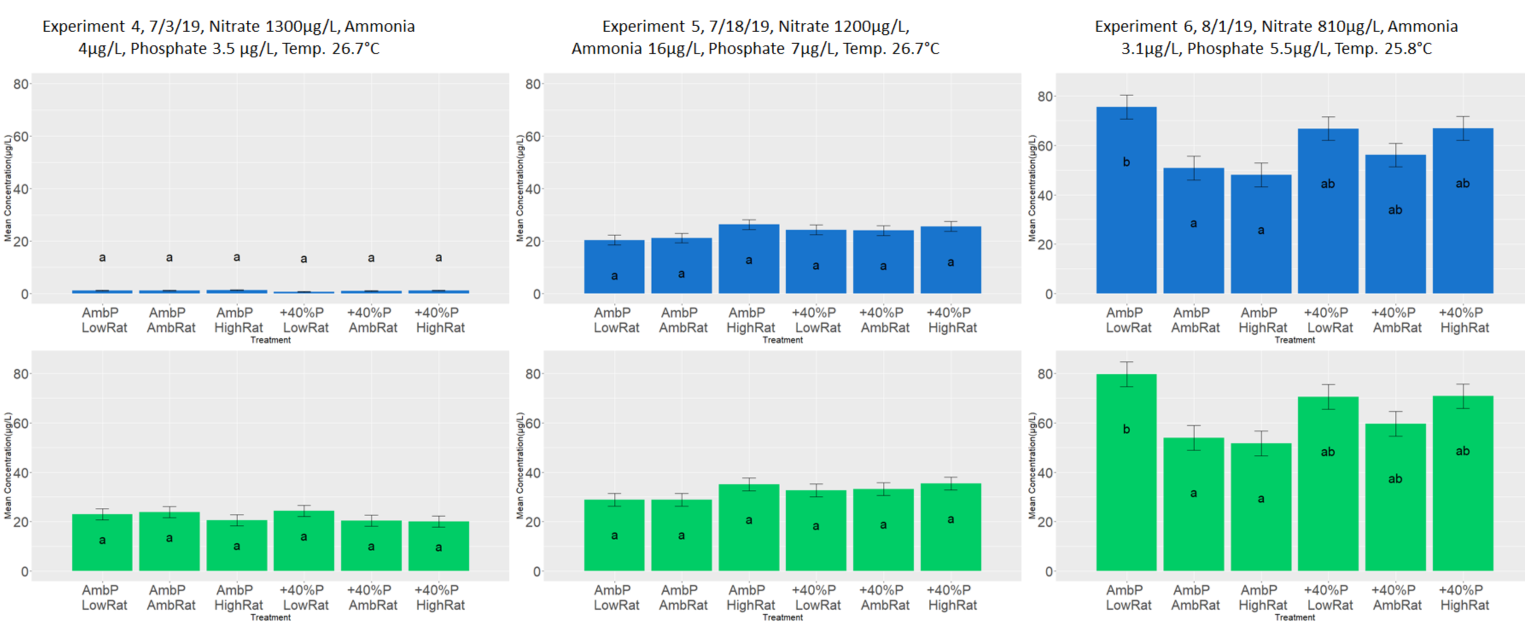


Figure 2.5: Lake Erie NH_4:NO_3 Ratio and Phosphate Experiment, refer to Figure 2 for details.

The Saginaw Bay NH_4:NO_3 ratio and phosphate experiment showed significant results during Experiment 1 for cyanobacteria only, where both high NH_4:NO_3 ratio treatments had significantly lower growth than the other treatments. Experiment 2 had no significant changes. In Experiment 3, the ambient ratio +40% phosphorus treatment was significantly higher than all other treatments in cyanobacteria. For total algae, growth in the ambient ratio +40% phosphorus treatment was only significantly increased compared to the low ratio ambient phosphorus, and ambient ratio ambient phosphorus treatments. In cyanobacteria, the high NH_4:NO_3 ratio +40% phosphorus treatment was significantly higher than the low ratio ambient phosphorus, and ambient ratio ambient phosphorus treatments, but lower than the ambient ratio +40% phosphorus treatment. For total algae, the high NH_4:NO_3 ratio +40% phosphorus treatment was only significantly higher than the low ratio ambient phosphorus, and ambient ratio ambient phosphorus treatments.

For the NH_4:NO_3 ratio and phosphate experiment, the early cyanobacteria community in Saginaw Bay had the strongest response (Figure 2.4). However, this response ran counter to what was expected, as growth was hindered by the higher ammonium to nitrate ratio (Chaffin and Bridgeman, 2014; Kim et al. 2017). There are several possibilities for this response. The addition of ammonium was particularly high to make the ratio, (~18 mmol/L) due to the high amount of nitrate present in the lake at the time. This high concentration could have been toxic to the cyanobacterial community which has shown a reduction of growth when levels reach 10 mmol/L (Dai et al. 2008, 2012).

The other significant change from ambient conditions was the +40% phosphorus ambient nitrogen ratio treatment in Experiment 3, though this was limited to cyanobacteria. The ambient NH_4:NO_3 ratio provided significantly higher growth than the other two +40% phosphate treatments. This runs counter to expectations, because if the ratio were to have an impact, it would likely be the higher NH_4:NO_3 ratio, as ammonium is the more bioavailable form. The total algal community did not have a significant difference between the ambient NH_4:NO_3 ratio and the high ratio in Experiment 3. This might suggest that the other algae species, likely diatoms and cryptophytes, were faster and more efficient at assimilating ammonium under high NH_4:NO_3 ratio, high phosphate conditions.

The Lake Erie NH_4:NO_3 ratio and phosphate experiment had only one significant difference among treatments (Figure 2.5). In Experiment 6, the low NH_4:NO_3 ratio ambient phosphorus treatment was significantly higher than the other two ambient phosphorus treatments. This runs counter to what was hypothesized to happen, and what prior nitrogen form studies had found.

2.3.4 Seston and Dissolved Ratios

Experiment	Seston N (PON) µg/L	Seston P (TP-TDP) µg/L	Seston Molar Ratio (Seston N:Seston P)	Dissolved Inorganic N µg/L	Soluble Reactive P µg/L	Ambient Inorganic Molar Ratio (N:P)
1 Sag Bay	180	23.6	16:1	1280	10.7	264:1
2 Sag Bay	370	8.2	99:1	273	3.75	161:1
3 Sag Bay	350	12.1	64:1	260	7.1	81:1
4 West Erie	290	52.9	12:1	1304	3.5	823:1
5 West Erie	430	43.3	22:1	1216	7.0	384:1
6 West Erie	1260	89.0	31:1	813	5.5	326:1
7 West Erie	600	36.0	37:1	2.25	4.1	1.2:1

Table 2.2: Molar Ratios in Seston and Ambient Molar Ratios. Seston ratios were determined using data from CIGLR's weekly HAB sampling. Experiment 7 in Lake Erie was included as it (Saginaw Bay-https://data.nodc.noaa.gov/cgi-bin/iso?id=gov.noaa.nodc:0209220, Lake Erie-https://data.nodc.noaa.gov/cgi-bin/iso?id=gov.noaa.nodc:0187718)

One explanation for the inconsistent responses to P manipulation is the capacity for phytoplankton, including cyanobacteria like *Microcystis* (Kromkamp et al. 1989), to assimilate intracellular phosphorus in excess of their immediate needs for growth (Keenan & Auer, 1974). This mechanism can allow phytoplankton to continue growth for a limited time despite low P concentrations in their environment. We analyzed the dissolved inorganic nutrients and the seston N and P concentrations in order to determine if there was a discrepancy between the two, since these can give different indications about limitation. Early in the season, the ratio of dissolved inorganic nitrogen to soluble reactive phosphorus measured in both lakes was above 100, which suggests P limitation (Liu et al., 2011) (Table 2.2). Only late in the season did this pattern change, with Saginaw Bay seeing a decline to 81:1, still consistent with P limitation, and Lake Erie declined to 1.2, indicative of N limitation. When looking at the seston N:P ratios, which is the ratio of N and P in living and nonliving organic matter, the ratios are far lower. This suggests that while the N:P ratios in the water may suggest P limitation; the phytoplankton are less limited and may not respond to shifts in nutrient concentration. This might explain why Lake Erie showed no clear response to nutrient additions, as the seston ratios were fairly low. Saginaw Bay, on the other hand, had seston ratios at 99:1 and 64:1 in the mid and late season respectively, which suggests that the community would be primarily phosphorus limited, consistent with what was observed.

2.4 Conclusion

This study highlights that the attempts at reducing phosphorus input into Saginaw Bay to reduce potential blooms is a good starting point but may be less impactful than expected if N also regulates growth rates (Paerl et al. 2019). While adding more phosphorus led to higher growth in Saginaw Bay, reducing phosphorus alone did not have a significant impact. The most significant decline in growth occurred when both nitrogen and phosphorus levels were reduced. This suggests that the algal community might be secondarily limited by nitrogen, or potentially co-limited by nitrogen and phosphorus. If these findings scale to ecosystem-level responses, it would suggest that future efforts to reduce blooms in Saginaw Bay should focus on the reduction of both nutrients to see the greatest effect on growth.

While this study did not show significant increases in growth with higher ratios of ammonium to nitrate, nitrogen form cannot yet be ruled out as a potential factor in exacerbating algal blooms. Attempts should be made in future nutrient studies to maintain ambient lake total nitrogen concentrations when adjusting NH_4:NO_3 ratios to prevent false positive results. If future studies show results similar to what we found, it is possible that nitrogen form is less impactful to algal growth than previously believed.

Chapter 3 : Mechanistic Assessment of Dreissenid Mussel Grazing Impacts on Phytoplankton Growth Rates

3.1 Introduction

The invasive dreissenid mussels, quagga mussels (*Dreissena rostriformis bugensis*) and zebra mussels (*Dreissena polymorpha*), rapidly increased in abundance and distribution across European and North American waters after their introduction (Higgins and Zanden, 2010). In the Laurentian Great Lakes, dreissenid mussels have caused many pervasive and irreversible ecological impacts that have cascaded through food webs (e.g. Vanderploeg et al. 2002, Higgins and Vander Zanden 2010). Due to these impacts, they have been labelled as one of "the world's 100 most troublesome biological invaders" according to the International Union for Conservation of Nature in 2005. Many of the mussels' direct impacts on the invaded ecosystems have been well studied. As prolific filter-feeders, the dreissenid mussels have greatly increased water clarity by reducing phytoplankton biomass in many offshore regions (Johengen et al. 2013, Vanderploeg et al. 2017). Because of their widespread distribution and abundance in the Great Lakes, the mussels' grazing behavior has greatly altered food webs and the flow of nutrients throughout the invaded lake systems (Fahnenstiel et al. 1995; Johengen et al. 2013; Vanderploeg et al. 2002, 2010). Additionally, the mussels have been shown to exhibit selectivity in their grazing behavior, which may also create conditions favorable to the nuisance cyanobacterium, *Microcystis aeruginosa* (Vanderploeg et al. 2001).

Microcystis aeruginosa populations can grow rapidly and produce harmful algal blooms (HABs), which are considered to be one of the greatest concerns in the Great Lakes region. In the Great Lakes and other ecosystems, HABs have significant socioeconomic and ecological impacts on inland waters by causing fish kills, beach closures, aquaculture losses, and threats to drinking water (Paerl, 2008; Cheung et al., 2013; Carmichael and Boyer. 2016). According to the modeling projections by Chapra et al, the effects of climate change will likely exacerbate the blooms, causing them to last longer with higher cyanobacteria concentrations (Chapra et al., 2017). In light of these threats, various forecasts and models have been produced, ranging from short-term bloom-tracking (Rowe et al 2016) to the NOAA Seasonal Forecast and Lake Erie bulletin (Wynne et al., 2013). Saginaw Bay of Lake Huron also experiences HABs but there is a need to understand whether the drivers and models of HABs based on Lake Erie can be extended to Saginaw Bay. Since dreissenid mussels are abundant (Benson, 2013) and likely exacerbate HABs in both lakes, it is important to understand the mechanisms by which mussels impact phytoplankton communities.

Previous studies have suggested at least three mechanisms by which dreissenid mussels may promote the growth of *Microcystis*. These mechanisms include the increase of sunlight availability and decrease in competition due to mussels feeding on the cyanobacteria's competitors, such as diatoms and cryptophytes, while selectively rejecting *Microcystis* (Vanderploeg et al. 2001, 2009; Johengen et al. 2013). Moreover, it was found that mussel excretions contain many nutrients that are essential to the growth of phytoplankton, such as soluble reactive phosphorus (SRP) and ammonium (Vanderploeg et al. 2001, 2009; Johengen et al. 2013). Based on the experiment by Johengen et al. 2013, the excretion of nutrients by mussels and its potential effects on HABs were influenced by multiple factors, including the mussel's food quality (algal community composition), feeding rates, temperature, and seston stoichiometry (Johengen et al. 2013). It is also possible that this influx of nutrients may work synergistically with the grazing effects on competition and light availability, further exacerbating the growth of cyanobacteria.

Given the different ways that mussels may affect HABs, it is essential to disentangle the proposed mechanisms by which invasive dreissenid mussels may promote the occurrence of HABs. In this study, ex situ mussel feeding and nutrient excretion experiments were conducted to quantify the nutrient influx caused by mussels and the impact of their grazing on phytoplankton communities. Phytoplankton bioassay experiments were then conducted using mixtures of grazed water and different treatments of ungrazed water in order to test three hypotheses examining the relationship between mussel grazing and phytoplankton growth rates:

- H_1- Nutrients released in mussel excretions increase the growth rate of *Microcystis* relative to other phytoplankton.
- H_2 - Feeding by mussels reduces total phytoplankton community density, decreasing competition and increasing light availability, which favors the growth of *Microcystis* relative to other phytoplankton.
- H_3 - Nutrient excretions and reduced phytoplankton density due to mussel grazing have a synergistic effect that increases the growth rate of *Microcystis* relative to other phytoplankton.

3.2. Methods

3.2.1 Experimental design

In order to quantify the impacts of mussel grazing on phytoplankton growth rates, six bioassay experiments were conducted in the laboratory using lake water and dreissenid mussels collected from western Lake Erie and Saginaw Bay. A total of three experiments were conducted for western Lake Erie and three experiments for Saginaw Bay. In each bioassay experiment, mussel-grazed lake water was mixed with one of four other water types (3 treatments and 1 control) in concentrations of 100%, 66%, 33%, and 0% (Figure 3.1). The purpose of mixing grazed water with treatment or control water is to mix the impact of grazing across a gradient from 100% ungrazed treatment or (control) to 100% grazed water. This gradient of grazing impacts can then be attributed to each of the proposed mechanisms via experimental manipulations which attempt to re-create those mechanisms. Mixtures of the control water with grazed water will reflect the impact of all three mechanisms and their interactions. The first treatment water type was ungrazed lake water that had been amended with ammonium chloride and potassium phosphate in order to match the ammonium and SRP concentrations of grazed water. These concentrations were derived from parallel excretion experiments in which we measured the release rates of nutrients from individual mussels (Johengen et al. 2013), which we then scaled to the number of animals and volume of water in the grazing experiment. This water type was called the '+ nutrients' treatment, and was used to test hypothesis 1. The second treatment water type was ungrazed lake water that was diluted with cell-free, ungrazed lake water in order to match the chlorophyll concentrations of grazed lake water at the end of mussel feeding. This water type was called the 'diluted' treatment and was used to test hypothesis 2. The third treatment water type was ungrazed lake water that had been both diluted with cell-free lake water and also amended with ammonium chloride and potassium phosphate so that it matched the chlorophyll concentration and the nutrient concentration of grazed lake water. This water type was called the 'diluted+nutrients' treatment and was used to test hypothesis 3. After the completion of each bioassay experiment, the net growth rates of total chlorophyll and cyanobacteria were calculated for each sample flask.

Linear models were generated to quantify changes in growth rates across the concentration gradients within each water type. Analyses of covariance (ANCOVA) were then performed to compare the effect of dilution on growth rates between each of the water types. By comparing the slopes of phytoplankton growth rates exhibited in the treatment regressions across the gradients of 100% treatment water to 100% grazed water, with the slope of phytoplankton growth rates exhibited in the control regression across the gradient of 100% raw ungrazed water to 100% grazed water, we were able to quantify the mechanisms of grazing impact on phytoplankton growth rates. If mussels exacerbate growth of phytoplankton, we expect there to be a positive slope of growth rates versus proportion grazed water. Then, if we 'remove' the effects of nutrients, dilution, or both, we would expect that this slope would decrease and the ungrazed phytoplankton growth rates would resemble those of the grazed treatment.

In order to replicate the nutrient and chlorophyll concentrations of grazed water, mussel excretion and feeding experiments were conducted prior to the bioassays to provide data for the rates of grazing and nutrient excretion.

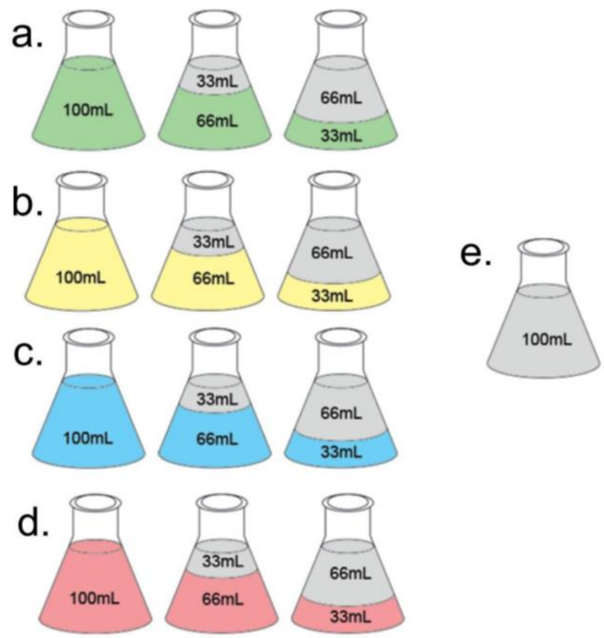


Figure 3.1 Experimental design. Each of the concentration treatment flasks shown here consisted of three replicates for experiments 1 - 4 and two replicates for experiments 5 & 6. **a.** Raw, ungrazed water (green) served as the control water type. **b.** The '+ nutrients' treatment, where yellow represents ungrazed lake water amended with ammonium chloride and potassium phosphate. **c.** The 'diluted' treatment, where blue represents ungrazed lake water that has been diluted with cell-free, ungrazed lake water. **d.** The 'diluted+nutrients' treatment, where red represents ungrazed lake water that has been both diluted with cell-free, ungrazed lake water and amended with nutrients. **e.** Mussel-grazed lake water in grey, is mixed with each of the treatment types in concentrations of 0%, 33%, 66%, and 100%.

3.2.2 Water and mussel collection

The source water and Dreissenid mussels used for these experiments were collected from western Lake Erie and Saginaw Bay between June and August of 2019 (Figure 3.2). The mussels were collected using a benthic dredge and lake water was pumped into 20 L polycarbonate carboys as outlined in Vanderploeg et al., 2009. The carboys of lake water were stored in plastic coolers while the mussels and their attached substrate were stored inside of a plastic cooler on damp paper towels over ice packs to maintain proper temperatures during transit. Following collection, the mussels and water were transported back to the Great Lakes Environmental Research Laboratory. Upon their arrival at the laboratory, the live mussels were sorted by size and species and were then scrubbed of any periphyton and other attached debris using a toothbrush while their byssal threads were removed with a razor blade. The mussels were then placed in a tank of lake water from the region where they were collected and incubated in a temperature-controlled room, set to match ambient temperature of their source lake, overnight for acclimation (Johengen et al., 2013).

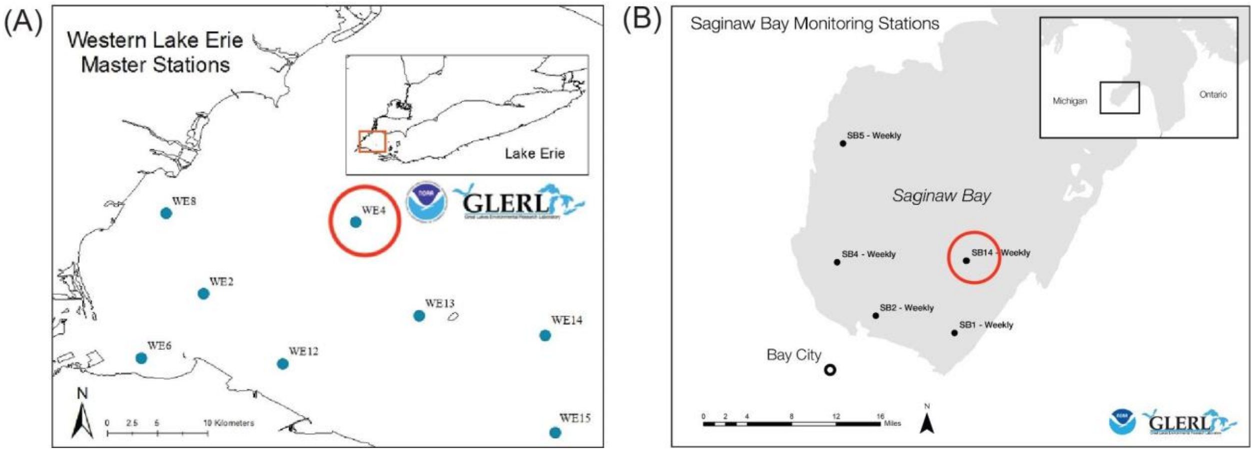


Figure 3.2 (A) Map of sampling stations in western Lake Erie. Mussels and water for the grazing bioassays were sourced from WE4. (B) Map of sampling stations in Saginaw Bay. Mussels and water for the grazing bioassays were sourced from near SB14.

3.2.3 Excretion experiments

Beginning the morning following collection, mussel excretion experiments were conducted in order to quantify the ammonium and SRP excreted by the mussels using a variation of the methods established by Johengen et al., 2013. In the cited study, filtered lake water was used to collect excretions, whereas in this study we used a synthetic hard water mussel medium (HWMM) (Baer 2019). Mussels (n=5) of similar body size were placed in each of four glass bottles containing 70 mL of HWMM, with two additional bottles containing only 70 mL of HWMM and treated identically as a control. Immediately after the mussels were added to the bottles, 20mL of HWMM were removed by syringe and analyzed using an AA3 Segmented Flow Analyzer (Seal Analytical) to quantify the initial concentrations of ammonium and SRP. The control bottles also had 20mL of HWMM removed and sampled identically. The bottles were left to incubate in a temperature-controlled room set at lake-ambient temperature for 2 hours. Following incubation, 20 mL of HWMM were collected from each bottle and the difference in nutrient concentrations was used to calculate excretion rates (i.e. µg N/animal/hour).

3.2.4 Grazing experiments

Beginning the morning following collection, mussel grazing experiments were conducted in order to quantify the rate that mussels reduce phytoplankton concentrations using a variation of the methods established by Vanderploeg et al., 2001. In this study, plastic baskets containing mussels (n=20) were placed in each of 4 experimental buckets containing 10L of lake water. Two control buckets, also containing 10 L of lake water but without mussels, were treated identically to those containing mussels. The buckets were placed in a dimly lit and temperature-controlled room, set to ambient lake temperature. Bubblers in each bucket kept the water gently mixed. The grazing experiments lasted 3 - 5 hours and concluded when either about 50% of the total chlorophyll content had been reduced or when it became clear that the mussels were not feeding. Phytoplankton concentration was measured using a FluoroProbe (BBE instruments) throughout the experiment as well as at the beginning and the end. Following the conclusion of the mussel grazing experiments, the grazed and ungrazed lake water remaining in the treatment and control buckets became the source water for the phytoplankton bioassays.

3.2.5 Phytoplankton bioassays

Experimental units were 125 mL Erlenmeyer flasks and contained a total of 100 mL of water. Each of the concentration treatments consisted of 3 replicates in experiments 1 - 4 and two replicates in experiments 5 and 6. Treatments were randomly assigned to the flasks. In order to prevent potential nutrient contamination, all glassware and laboratory equipment used for preparing treatments and replicates were acid-washed prior to use. After the sample flasks were filled with their designated treatments, their necks were covered with aluminum foil and they were placed in Percival Incubators (I-36LLVL) for 3 days under high-intensity light (300 µmoles m-2 s-1) delivered by light-emitting diode arrays (Waveform Lighting). The light panels were set on a 14-hour photoperiod using a light timer and the incubator temperature was set to match the ambient lake temperature where the water and mussels were initially collected. During the incubation period, 25mL of water from each flask was sampled with a Fluoroprobe instrument to track phytoplankton concentration and community composition, both in the morning and in the afternoon each day.

The raw, ungrazed water that was used as the control in the feeding experiments was used in preparing the raw concentration treatments. The water was gently mixed to resuspend any particles that may have settled during the feeding experiment. Raw ungrazed lake water was combined with grazed lake water in 125 mL Erlenmeyer flasks in ratios of 1:2, 2:1, and 1:0 (Figure 3.1).

Using the mean values of the initial and final concentrations of ammonium and SRP measured during the excretion experiments, we calculated the amount of ammonium chloride and potassium phosphate that would need to be added to the raw ungrazed water in order to match the nutrient content of the grazed water. To do this, we used the following equation:

$$\frac{mL\ stock}{L\ ungrazed\ water} = \frac{\left(\frac{\left(\frac{[final]-[initial]}{n_{exc}}\right)\times V_{exc}}{t_{exc}}\right)\times\frac{n_{grz}}{V_{grz}}\times t_{grz}}{[stock]}$$

Where [final]-[initial] refers to the difference in nutrient concentration from before and after the excretion experiments (µg/L), n_{exc} refers to the number of mussels in each excretion flask, V_{exc} refers to the volume of each excretion flask (L), t_{exc} refers to the duration of the excretion experiment (hrs), n_{grz} refers to the number of mussels in each grazing bucket, V_{grz} refers to the volume of each grazing bucket (L), t_{grz} refers to the duration of the grazing experiment (hrs), and [stock] refers to the concentration of the chemical standards used to amend the ungrazed water (µg/mL). The concentrations of both of the nutrient stocks that were used were 1µg/mL. The appropriate amounts of ammonium chloride and potassium phosphate were then added to the raw ungrazed water. The nutrient-amended, ungrazed lake water was combined with grazed lake water in ratios of 1:2, 2:1, and 1:0 (Figure 3.1).

Following the completion of the mussel grazing experiments, 30 seconds of fluoroprobe readings were taken from both the grazed and ungrazed lake water. We prepared cell-free lake water by filtering the ungrazed water to < 0.2 µm using a polycarbonate membrane filter. Using the mean concentrations of total chlorophyll, the following equation was used to determine the amount of cell-free lake water that we needed to add to ungrazed lake water in order to match the phytoplankton concentrations of the grazed lake water:

$$dilution\ factor = \frac{L\ cell\ free\ water}{L\ total} = \frac{[INITIAL] - [FINAL]}{[INITIAL]}$$

Where [initial] is the mean concentration of total chlorophyll in the ungrazed water and [final] is the mean concentration of total chlorophyll in grazed water. Diluted ungrazed lake water was combined with grazed lake water in ratios of 1:2, 2:1, and 1:0 (Figure 3.1).

The 'diluted+nutrients' treatment required ungrazed lake water with equal concentrations of ammonium, SRP, and chlorophyll as grazed lake water. Cell-free ungrazed water, ammonium chloride, and potassium phosphate were added to raw ungrazed water using the steps outlined above for the 'diluted' treatment and the '+ nutrients' treatment. 'Diluted+nutrients' treatment water was combined with grazed lake water in ratios of 1:2, 2:1, and 1:0 (Figure 3.1).

After filling each treatment flask and prior to the onset of incubation, 25 mL of water from one replicate flask of each treatment was sampled using a Fluoroprobe. Throughout the incubation period, each flask was sampled using a Fluoroprobe in the morning and afternoon each day. Every fluoroprobe sampling consisted of thirty consecutive seconds of readings. As the first and last three fluoroprobe readings of each of the 30-second sampling periods exhibited a large degree of noise, they were removed from the dataset. The mean total chlorophyll and cyanobacteria-specific chlorophyll values were then calculated for each flask from the remaining fluoroprobe readings.

The response variable that we summarized was an exponential growth rate over the duration of the experiment. Following each experiment, the natural log of the mean total chlorophyll and mean cyanobacteria was calculated for each flask at each sampling period. The net growth rates of total chlorophyll and cyanobacteria were then calculated for each flask using the slope of the natural log of total chlorophyll or cyanobacteria versus the duration of incubation, in hours. Growth rates (1/h) of total chlorophyll and cyanobacteria were calculated for each flask and these were then used as the response variables in linear models with the treatment types and the proportion of grazed water as interacting independent variables. These linear models were performed in R for each experiment separately and were then run in an ANOVA framework for analysis of covariance. If the ANCOVA results did not indicate a significant interaction term between treatment type and proportion grazed (alpha = 0.05), then the independent variables were treated separately in the linear regressions.

3.3 Results

3.3.1 Excretion experiments

Over the course of the six excretion experiments that were conducted during the grazing bioassay, the amount of ammonium and SRP that the mussels excreted were higher in Lake Erie relative to Saginaw Bay (Table 3.1). The average excretion rates were 1.27µg-N mussel^{-1} hour^{-1} and 0.39µg-P mussel^{-1} hour^{-1} in western Lake Erie and 0.538µg-N mussel^{-1} hour^{-1} and 0.0053µg-P mussel^{-1} hour^{-1} in Saginaw Bay. During the third excretion experiment, conducted on July 18 using mussels collected from western Lake Erie, the amount of ammonium excreted was about five times greater than any of the other excretion experiments conducted throughout the summer, while the excreted SRP was at least 12 times higher than in any of the other experiments. These results support previous studies that found that higher nutrient excretion was correlated with higher grazing activity, and that the high availability of SRP in Lake Erie seston relative to Saginaw Bay was responsible for the greater amounts of SRP in Lake Erie mussel excretions (Johengen et al. 2013).

Table 3.1 Results from the mussel excretion and feeding experiments. $[NH_4]_i$ and $[NH_4]_f$ represent mean initial and final concentrations of ammonium respectively and $[SRP]_i$ and $[SRP]_f$ represent mean initial and final concentrations of soluble reactive phosphorus respectively. Means were calculated from the four incubation bottles. $[Chlorophyll]_{grz}$ and $[Chlorophyll]_{ung}$ represent the mean concentration of total chlorophyll in the grazed and the ungrazed lake water respectively. The means were calculated from 30 seconds of Fluoroprobe readings.

Experiment #	Date	Collection Site	$[NH_4]_i$ (μg/L)	$[NH_4]_f$ (μg/L)	$[SRP]_i$ (μg/L)	$[SRP]_f$ (μg/L)	$[Chlorophyll]_{grz}$ (μg/L)	$[Chlorophyll]_{ung}$ (μg/L)
1	6/12/19	SB14	18	130	0.8	2	5.1	5.6
2	7/10/19	SB14	15.75	133.14	1.02	1.839	8.677	9.465
3	7/18/19	WLE4	4.024	529.195	0.6895	210.223	4.19	8.786
4	7/31/19	WLE4	2.142	111.218	0.5325	17.452	32.72	37.34
5	8/8/19	SB14	43.344	137.017	0.585	1.75	8.992	9.119
6	8/13/19	WLE4	21.349	149.71	1.295	9.417	3.3	4.449

3.3.2 Grazing experiments

Reductions in chlorophyll concentration were highest in both lakes from mid-late July and were quite low at the beginning and end of the summer, averaging 0.14μg chlorophyll grazed per mussel per hour (Table 3.1). For the majority of the experiments, there was not much change in relative community composition between grazed and ungrazed water (Figure 3.3). The only exception was that in Experiment 3 from Lake Erie the green algae biomass was reduced by 75% in grazed water.

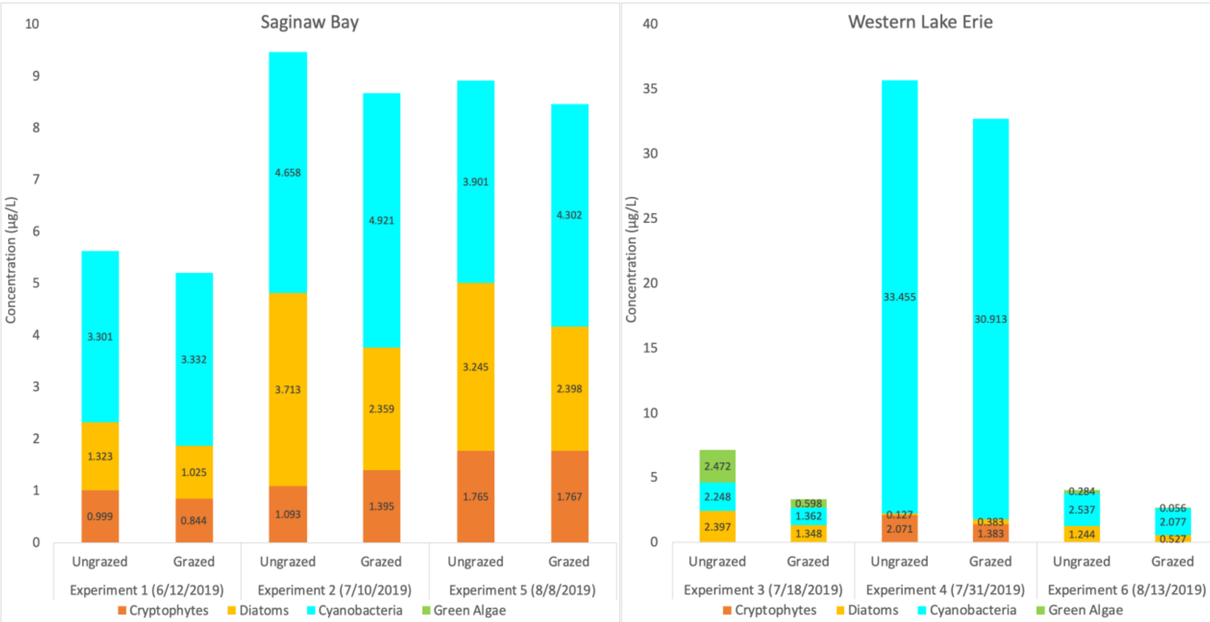


Figure 3.3 Phytoplankton community composition before and after the grazing experiments conducted with mussels and water from Saginaw Bay (left) and western Lake Erie (right).

3.3.3 Bioassay experiments: Total chlorophyll growth rates

The proportion grazed water was positively correlated with total chlorophyll growth rates in Experiments 1 and 5 in Saginaw Bay (slope = 0.045, p = 0.0001 & slope = 0.094, p = 3.47e-5 respectively) and in Experiment 6 from Lake Erie (slope = 0.22, p = 1.81e-12)(Figures 3.4 & 3.5). In every experiment except for Experiment 3, there was no significant interaction between the mix type and the proportion of grazed water. In Experiments 1, 5, and 6, the slopes of the linear regressions across treatments were not significantly different from one another, and in Experiments 2 and 4 the slopes of the linear regressions were not significantly different from zero (table 3.2). Only in Experiment 3, using water from Lake Erie, did the linear model yield significantly different slopes between the treatments (Figure 3.5a). The slope of growth rate across proportion grazed was the highest in the 'raw' control (slope = 0.57, p = 5.33e-13), followed by the 'diluted' treatment (slope = 0.54, p = 3.11e-12), and then by the '+ nutrients' treatment (slope = 0.12, p = 0.027). In the 'diluted+nutrients' treatment, the slope of growth rate across proportion grazed was not found to be significantly different from zero (p = 0.92), meaning there was no change in the growth rate of total chlorophyll across the gradient of grazed water and 'diluted+nutrients' water, or that the growth rates in both types of water were not different. In every experiment other than Experiments 3 and 6, many of the flasks exhibited negative growth rates of total chlorophyll, or declining populations of phytoplankton over the course of the bioassay. In experiments 4 and 5, all or nearly all of the flasks exhibited negative growth rates, yet total chlorophyll growth rate was still positively correlated with mussel grazing in Experiment 5 (slope = 0.094, p = 1.92e-6).

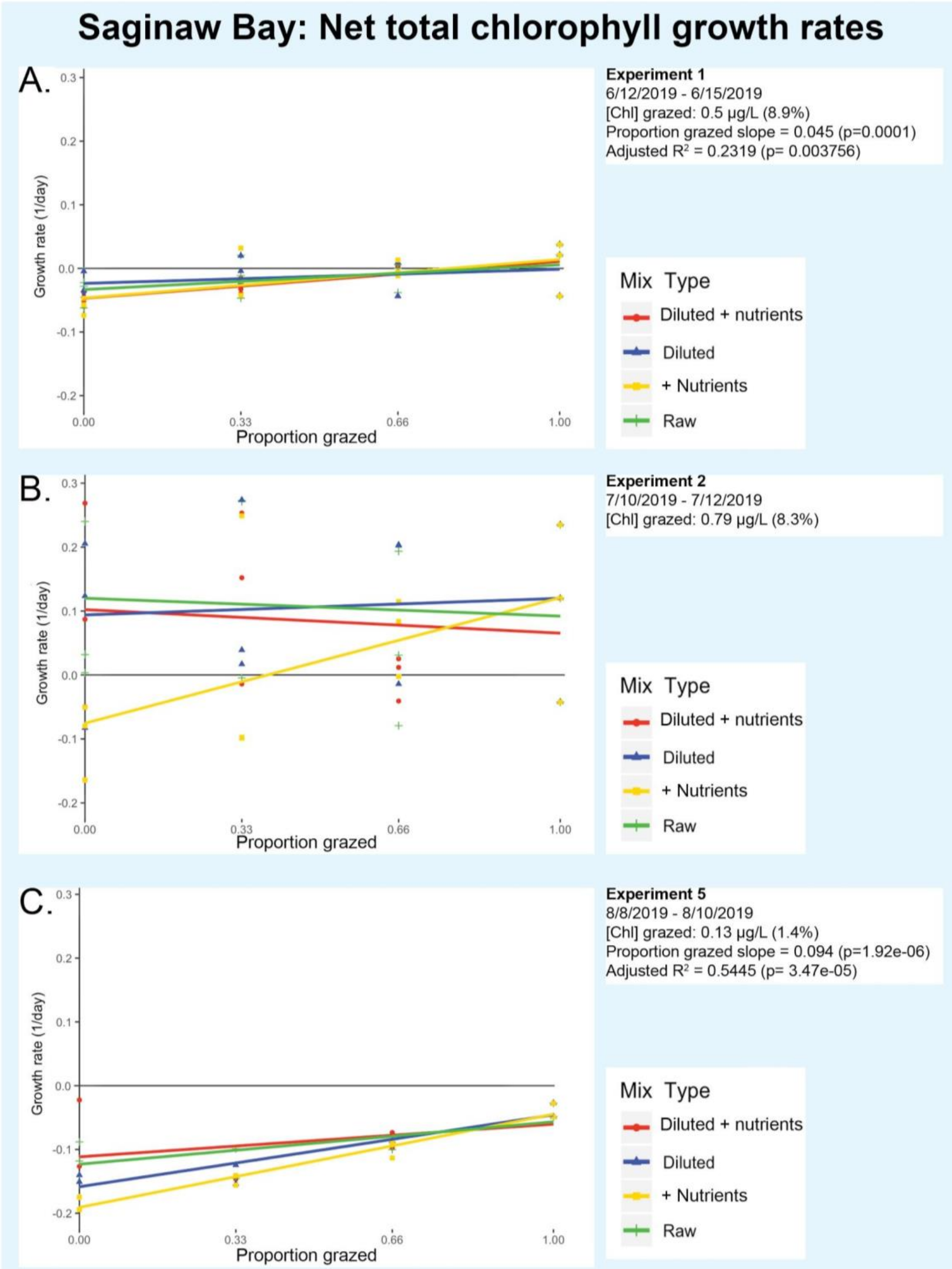


Figure 3.4 Linear models of total chlorophyll growth rates from the Saginaw Bay experiments. Significant terms from the linear regression are shown to the right of each chart. [Chl] grazed shows the amount of chlorophyll that was reduced by mussels during the grazing experiments. The interaction between treatment type and the proportion of grazed water had no significant impact on total chlorophyll growth rates in the Saginaw Bay experiments.

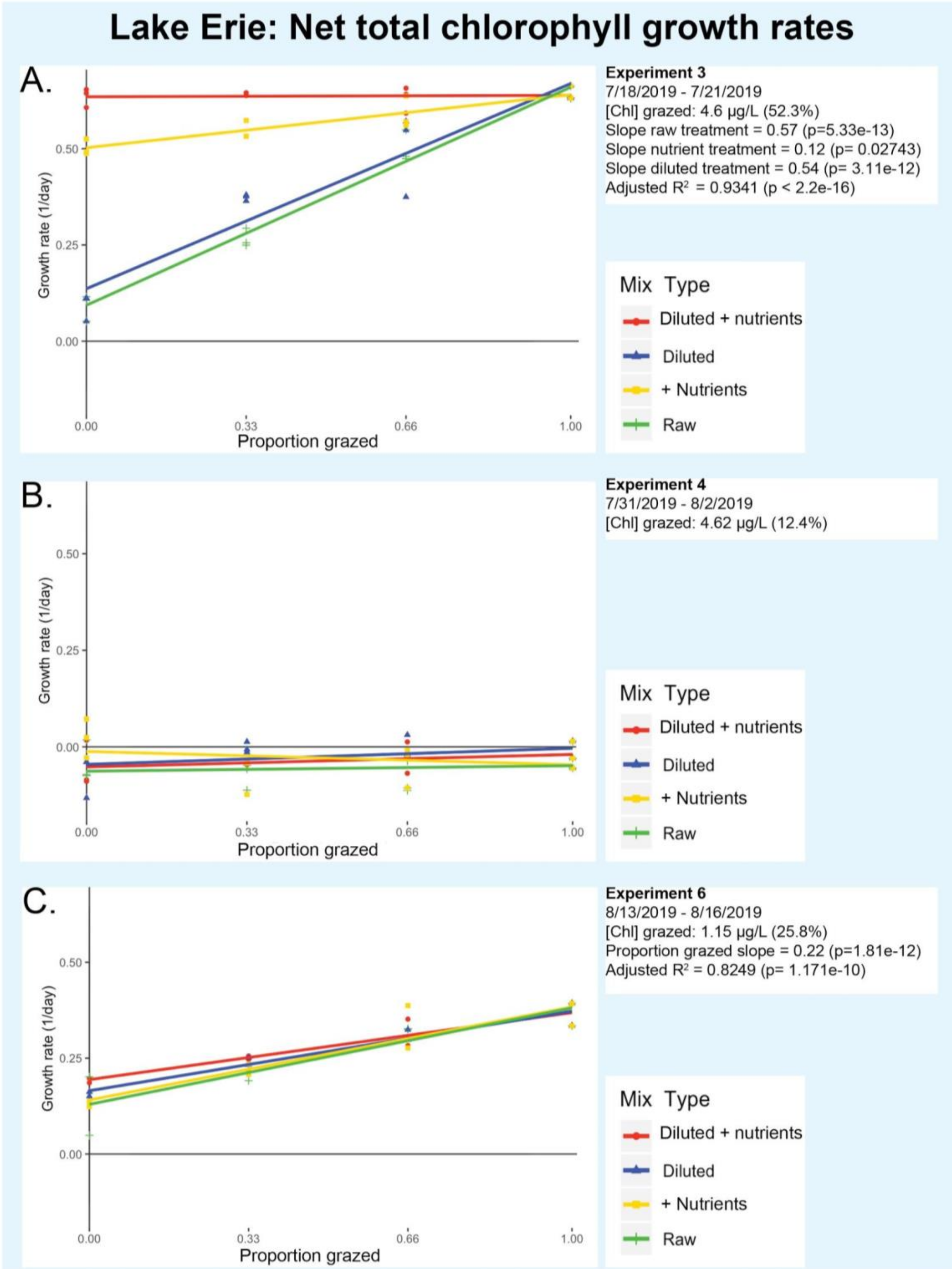


Figure 3.5 Linear models of total chlorophyll growth rates from the Lake Erie experiments. Significant terms from the linear regression are shown to the right of each chart. [Chl] grazed shows the amount of chlorophyll that was reduced by mussels during the grazing experiments. Experiment 3 (panel A) shows the only instance where the interaction between treatment type and the proportion of grazed water had a significant impact on total chlorophyll growth rates.

Table 3.2 Regression statistics from the linear models created for net total chlorophyll growth rates (left) and net cyanobacteria growth rates (right) in each experiment. Significant terms are in bold. Only in experiment 3 was the interaction between mix type and proportion grazed found to be significant, and the slopes for each treatment regression are listed below.

		Total Chlorophyll Growth Rate Models						Cyanobacteria Growth Rate Models					
Exp	Treatment	Slope	T	P	Adj R^2	F	P	Slope	T	P	Adj R^2	F	P
1	Pooled	0.045	4.23	**0.001**	0.23	4.55	**0.004**	1E-03	3.09	**0.003**	0.11	2.50	0.056
2	Pooled	0.039	0.80	0.429	0.003	1.04	0.400	2E-03	1.07	0.291	0.04	1.43	0.244
3	Raw	0.570	10.45	**5.3E-13**	0.93	96.13	**< 2.2e-16**	2E-02	5.41	**3.6E-06**	0.72	17.42	**4.3E-10**
	+ Nutrients	0.125	2.29	**0.027**				4E-03	1.09	0.281			
	Diluted	0.537	9.84	**3.1E-12**				2E-02	4.92	**1.7E-05**			
	Diluted+ nutrients	-0.004	-0.10	0.921				-1E-02	-4.67	**3.7E-05**			
4	Pooled	0.014	0.78	0.438	0.002	1.02	0.407	6E-04	0.87	0.390	0.003	1.04	0.399
5	Pooled	0.094	6.04	**1.9E-06**	0.54	10.27	**3.5E-05**	6E-04	0.59	0.561	-0.04	0.73	0.58
6	Pooled	0.219	12.17	**1.8E-12**	0.82	37.52	**1.2E-10**	7E-03	9.15	**9.3E-10**	0.73	22.25	**3.2E-08**

3.3.4 Bioassay experiments: Cyanobacteria growth rates

Only in Experiment 1 from Saginaw Bay and in Experiment 6 from Lake Erie was the proportion of grazed water found to be significantly correlated with cyanobacteria growth rates (Table 3.2). The slopes of the regressions as the proportion of grazed water increased however, were very small (slope = 0.001, p = 0.003; slope = 0.007, p = 3.2e-8 respectively) (Table 3.2). This shows that the difference in the net growth rate of cyanobacteria between grazed water and ungrazed water, when present, was very small. Similar to the total chlorophyll models, it was only in Experiment 3 that the interaction between mix types and the proportion of grazed water was significantly correlated with the net cyanobacteria growth rate. The linear model for this experiment yielded significantly different slopes between the treatment regressions (Table 3.2). As mentioned above, the regression of total chlorophyll growth rate in the 'diluted+nutrients' treatment had a slope not different from zero. Here however, the regression of cyanobacteria growth rate in the '+ nutrients' treatment had a slope not significantly different from zero (p = 0.28), meaning that there was no difference in cyanobacteria growth rate between grazed water and '+ nutrients' water (Table 3.2). In contrast to Experiment 3, the regression for the 'diluted+nutrients' treatment was found to be negatively correlated with cyanobacteria growth rate (slope = -0.011, p = 3.7e-5) as the proportion of grazed water increased (Table 3.2). This shows that the 'diluted+nutrients' water yielded higher net growth rates of cyanobacteria than did grazed water.

3.4 Discussion

3.4.1 Grazing impact on total chlorophyll growth rate

These experiments predicted that Dreissenid mussels would affect phytoplankton growth rates through one or more mechanisms that we could identify experimentally. It was only possible to isolate the proposed mechanisms in Experiment 3 (Figure 3.5a). The regression of total chlorophyll growth rate in raw ungrazed water had the greatest slope as the proportion of grazed water increased (slope = 0.57, p = 5.33e-13). By diluting the ungrazed water with cell free ungrazed water so that the concentration of total chlorophyll equaled that of the grazed water, we were able to decrease community density and thus competition, and the slope of the regression decreased by only 5%. By adding ammonium and SRP to raw ungrazed water to match the concentrations excreted by mussels in grazed water, we were able to decrease the slope of the regression by 79%. And by manipulating both dilution and nutrients, we were able to reduce the slope of the regression to zero. Considering Experiment 3 alone, it appears that the nutrients excreted in mussel waste have the greatest impact on phytoplankton growth rates followed by the reduction of competition by grazing, and that the synergistic effect between these two mechanisms can explain 100% of the increased phytoplankton growth rate observed in grazed water, supporting our third hypothesis. It is important to note that the mussel excretion experiment that informed the nutrient amendments for Experiment 3 also found the highest concentrations of ammonium and SRP and highest reduction of total chlorophyll. In contrast, when the mussels exhibited very little excretion or grazing, the positive relationship between grazing and phytoplankton growth rate was reduced but not absent in all experiments, and the differences between the treatment types disappeared. This means that the effect of mussels on total phytoplankton growth rates is proportional to grazing activity.

3.4.2 Grazing impact on cyanobacteria growth rate

Grazing had very little impact, if any, on the growth rate of cyanobacteria in our bioassay experiments. Similar to the impact on total chlorophyll growth rates, it was only in Experiment 3 that we were able to isolate the mechanisms by which mussel grazing affects cyanobacteria growth rate. However, unlike the pattern exhibited in total chlorophyll growth rates, the relative importance of the explanatory mechanisms was not the same. The linear model for cyanobacteria growth rates in Experiment 3 showed that diluting ungrazed lake water reduced the slope of the regression by 10%. Amending ungrazed water with SRP and ammonium to match the concentrations of grazed water reduced the slope to zero. And by both, diluting and amending the nutrients in ungrazed water, the growth rate of cyanobacteria was actually higher in the ‘diluted+nutrients’ treatment than the grazed water, leading to a slope of less than zero. In contrast to the pattern observed in total chlorophyll growth rate, by looking at Experiment 3 alone, it could be surmised that mussel-induced nutrient recycling can explain 100% of the impact on cyanobacteria growth rates, supporting our first hypothesis. The reduction of competition by grazing, to a much lesser degree, can also positively impact cyanobacteria growth rates, but unlike the relationship observed in total chlorophyll growth rates, the combination of these two mechanisms yields a growth rate of cyanobacteria greater than that observed in grazed water.

Cyanobacteria growth rates were positively correlated with mussel grazing in Experiment 1 from Saginaw Bay and Experiment 6 from western Lake Erie (Table 3.2). This observed relationship is notable because it differs from enclosure experiments conducted by Sarnelle et al. (2005). In the cited study, mussel grazing was found to be positively correlated with *Microcystis* growth in enclosures with high concentrations of SRP and negatively correlated with *Microcystis* growth in enclosures with low concentrations of SRP (Sarnelle et al. 2005). In our study, the results from Experiment 6 reflect this positive relationship in western Lake Erie, a system known to have high concentrations of SRP. Conversely, the positive relationship we observed between cyanobacteria growth rates and mussel grazing in Experiment 1 from Saginaw Bay contradict the results of Sarnelle et al. (2005) because Saginaw Bay typically has low concentrations of SRP compared to Lake Erie. However, considering that we were limited in our sampling approach and observing trends in phytoplankton growth at a more coarse taxonomic scale, it is possible that *Microcystis* was negatively impacted by mussel grazing in Saginaw Bay and that this effect was obscured by a larger, positive effect exhibited by other species of cyanobacteria.

3.4.3 The residual impact of grazing on phytoplankton growth rates

Experiments 1, 5, and 6 all found a significant positive relationship between mussel grazing and phytoplankton growth rates. This relationship did not differ significantly between treatment types within each experiment, meaning that we were not able to isolate the mechanisms by which mussels impacted the growth rates of phytoplankton in these three experiments. Considering that we were only looking at the relationship between mussel-grazing and phytoplankton, it is possible that we were missing an important impact of grazing on microzooplankton populations. Microzooplankton has been shown to be an important food source for dreissenid mussels in Saginaw Bay, representing up to 77% of their diet at times, and microzooplankton themselves have been found to consume up to 30% of phytoplankton standing stock per day (Lavrentyev, 2013). If the mussels were selectively grazing on significant proportions of microzooplankton during the feeding experiments, that may have led to a cascade effect whereby the mussels indirectly benefit the phytoplankton by removing their predators and converting them into nutrients.

Alternatively, the positive relationship between the growth rate of phytoplankton and mussel grazing could be due to excreted nutrients other than ammonium and SRP. Recent research has shown that *D. polymorpha* can mobilize and accumulate micronutrients such as iron and copper in their soft tissues and shells when exposed to sediments containing these metals (Schaller & Planer-Friedrich, 2017). Perhaps before the mussels were brought back to the laboratory, such assimilations of micronutrients already took place between the mussels and the lake sediments in the field. It is likely that the grazed water in our experiments was enriched through the active excretion or passive leaching of these extra nutrient sources from the soft tissues and shells of the mussels. Expanding the scope of the mussel excretion experiments beyond quantifying only the concentrations of macronutrients to looking also at potential excretions of micronutrients could provide a more holistic view of mussel-induced nutrient enrichment. Freshwater cyanobacteria have indeed been found to be especially sensitive to limiting micronutrients such as iron and copper, so if this were the case, we would expect to see a higher contribution to the increased growth rates of total chlorophyll by cyanobacteria relative to other phytoplankton (Facey et al., 2019). This was not the case in our bioassay experiments, but further investigation could be warranted.

3.5 Conclusion

The results from the mussel bioassay experiments show that the relationship between mussel grazing and phytoplankton growth rates is more complicated than previously expected. The overall impact of grazing by Dreissenid mussels on *Microcystis* growth rates was limited compared to the impact on total chlorophyll growth rates. Furthermore, the growth rate patterns observed in most of our experiments suggest that there are potentially more mechanisms that influence phytoplankton growth rates than the three tested in this study. In the one experiment in which we were able to isolate the three mechanisms that were proposed, the relative importance of these mechanisms was not consistent between total phytoplankton growth rate and cyanobacteria growth rate.

Future studies should aim to reduce as much variation in the experimental design as possible. In our experiments, the sampling processes did not take place on a regular basis either temporally or spatially throughout the whole summer. And the sampling was not conducted in the other three seasons. For each experiment we only sampled water from one lake, and the intervals between experiments were not consistent. Bioassay experiments could be repeated using mussels and water from a single lake throughout the whole year. Having a more regular sampling schedule from both lake systems may help identify seasonal variations in mussel grazing and excretion rates, which in turn impact *Microcystis* growth. The enclosure experiments by Sarnelle et al mentioned that the vulnerability of *Microcystis* to mussel grazing may vary between experiments (Sarnelle et al., 2005), depending on microcystis colony size and other chemical deterrents (Vanderploeg et al. 2001). As Sarnelle et al noticed in their experiment, the total phosphorus concentrations fluctuated despite their experimental design to keep them constant (Sarnelle et al., 2005). It is unlikely that the controlled laboratory conditions in our experiments recreated the complicated fluctuations of the environmental conditions in the lakes. Additional environmental fluctuations, such as disturbance events like storms, may also affect the lake ecosystem before collection and therefore should be recorded in order to improve accuracy of the experiments and catch any potential impacts they may have on phytoplankton communities or mussel behavior. Future studies may consider conducting enclosure experiments in the Great Lakes during the phytoplankton growth or cyano blooming period, wherein mussels can continuously graze on seston and excrete nutrients throughout the duration of a longer bioassay, rather than a one-time addition at the beginning of the bioassay.

Chapter 4: Comparative Analysis of *Microcystis* Colony Buoyant Velocities in Western Lake Erie and Saginaw Bay of Lake Huron: Effects of Colony Size and Light Intensity

4.1 **Introduction**

Harmful algal blooms, or HABs, are a problem in marine and freshwater ecosystems across the planet (Paerl and Otten 2013; Harke et al. 2016). In particular, blooms of the freshwater cyanobacterium *Microcystis* can produce a toxic peptide which poses a danger to humans and wildlife. Within warmer eutrophic lakes *Microcystis* frequently dominates other species of freshwater cyanobacteria in HABs and is responsible for the toxic compounds like microcystin (Jöhnk et al. 2008; Litchman et al. 2010; Deng et al. 2014). HABs can cause major disruptions in ecological communities and pose health risks to both animals and humans (Paerl et al. 2001; Hudnell 2008). While much of the research on freshwater HABs and *Microcystis* in particular has focused on its toxicity and growth in response to nutrients (Stumpf et al. 2012; Watson et al. 2016), these microbes also exhibit important behaviors like vertical migration. Colonies of *Microcystis*, along with other phytoplankton, actively regulate their position within the water column of lakes and the ocean by adjusting their buoyancy (Ganf and Oliver 1982; Konopka et al. 1988; Nakamura et al. 1993b; Aparicio Medrano et al. 2013). In *Microcystis* this regulation is partly due to inclusions of gas within the cells (Wallace et al. 2000) and allows for colonies to migrate vertically within the water column – moving towards the surface to receive light for photosynthesis while moving downwards to take up nutrients and avoid damaging effects of very high light intensity and short wavelengths (Reynolds and Walsby 1975).

While vertical migration is not unique to cyanobacteria or even HABs, *Microcystis* shows particularly flexible buoyancy regulation and can move at rates greater than one meter per hour under certain conditions (Reynolds et al. 1987; Aparicio Medrano et al. 2013; Xiao et al. 2018). Previous studies have shown that in a stable water column, *Microcystis* colonies exhibit a daily pattern of vertical migration due to increased accumulation of carbohydrates resulting in higher surface cell densities during daytime photosynthesis followed by the utilization of carbohydrates and decreasing cell densities in the dark (Kromkamp and Mur 1984; Visser et al. 1997). This continuous fluctuation of cell densities, together with colony size, influences colony vertical migration (Wu and Kong 2009; Aparicio Medrano et al. 2013). In addition to light, *Microcystis* buoyancy has been shown to be significantly affected by temperature (Paerl and Huisman 2008; You et al. 2018), wind (Ibelings et al. 1991; Wynne et al. 2010), as well as chemical factors such as nutrient availability (Harke et al. 2016). These factors affect buoyancy of *Microcystis* colonies by influencing their size and density (Nakamura et al. 1993b; Li et al. 2016). The buoyancy of *Microcystis* colonies affects the location of the bloom vertically within the water, which determines how wind and water currents will move the bloom horizontally within the lake (Reynolds et al. 1987; Wu and Kong 2009; Aparicio Medrano et al. 2013, 2016; Rowe et al. 2016).

HABs are a well-known symptom of eutrophication in Lake Erie (Watson et al. 2016; Steffen et al. 2017a) in part due to media exposure surrounding the Toledo water crisis of 2014 (Steffen et al. 2017b); however, several other areas of the Great Lakes experience *Microcystis* blooms. In particular, Saginaw Bay has experienced HABs and other eutrophication issues (Blerman et al. 1984; Stoermer and Theriot 1985). Although controls on point source inputs of phosphorus were successful at decreasing eutrophication starting in the mid-1970s (Blerman et al. 1984; Watson et al. 2016), blooms remain a concern in both ecosystems. To date, the vertical migration of buoyant *Microcystis* colonies has been studied under a limited set of environmental conditions for Lake Erie and there are no measurements from Saginaw Bay.

Here we present the results of a study to understand *Microcystis* buoyancy behavior in both Lake Erie and Saginaw Bay as a function of colony size, time of day, and light intensity. We used microscopic cinematography to measure both size and velocity of intact lake water samples during short-term incubations at different light intensities. Much of the previous studies on movement within *Microcystis* colonies have detailed the positive relationship between *Microcystis* colony size and density and that these factors influence colony diel migration in response to light (Nakamura et al. 1993b; Wallace et al. 2000; Rowe et al. 2016). Our study shows that while different colony size classes are responsive to light, the density of colonies also differs according to their size. In addition, we found that on average, *Microcystis* colonies in Lake Erie are positively buoyant whereas colonies in Saginaw bay are negatively buoyant.

4.2 **Methods**

4.2.1 Field Sampling

We collected samples of whole lake water at one station in Western Lake Erie (WE2, 41˚45′ 44.4″N 83˚19′54.2″W) and another in Saginaw Bay (SB14, 43˚44’19.6”N 83˚38’025.6”W) during the summer bloom season. We performed two experiments in each lake: Saginaw Bay was sampled on July 11th and August 7th, 2019 and Lake Erie was sampled on July 30th and August 13th, 2019. Water was collected via a peristaltic pump from three discrete depths: surface, 1m below surface, and 1 m above the lake bottom, mixed, and stored in coolers at ambient temperature for transport to the Great Lakes Environmental Research Laboratory. Incubations began 3-5 hours after collection. We collected the initial measurements on the following morning following collection (T_0+1), which was approximately 16 hours after the start of the incubation.

4.2.2 Outdoor Incubation Set Up

We subjected the lake water samples to 3 treatments: Ambient light intensity, medium intensity, and low light intensity in order to simulate light exposure at the surface, middle, and bottom depths. Experimental units were 2-liter borosilicate acid-cleaned bottles, which were covered in LEE neutral light density films to achieve light levels of 30% (medium), and 10% (low) compared to ambient.

The six bottles were then submerged upside down in an outdoor incubation tank (4,392 m^3) filled with water. Water temperature within the tank was maintained at in-situ ambient lake temperature according to the temperature at the time of collection via a water chiller (Cole Parmer, Polystat) and continuously circulated by two submersible pumps. Temperature within the incubation tank was monitored with an RBR solo logger (RBR Ltd.) at 10-minute intervals and light levels were quantified as Photosynthetically Active Radiation (PAR), measured using an underwater spherical sensor (LI-COR, LI-192 Underwater Quantum Sensor) which recorded light within the tank.

Date	Time of day	Lake	Average Field Temperature	Average Incubation Temperature	Average Incubation PAR (µmol m-2 s-1)
7/10/19	Afternoon	Saginaw	23.17	22.82	428 .97
7/11/19	Morning	Saginaw	23.71	22.33	572.37
7/11/19	Afternoon	Saginaw	22.98	22.98	1227.99
7/29/19	Afternoon	Erie	25.77	25.35	441.49
7/30/19	Morning	Erie	25.61	25.42	474
7/30/19	Afternoon	Erie	26.27	25.93	1102.01
8/6/19	Afternoon	Saginaw	24.28	23.82	598.70
8/7/19	Morning	Saginaw	24.07	22.33	516.70
8/7/19	Afternoon	Saginaw	24.49	22.90	1138.09
8/12/19	Afternoon	Erie	25.11	24.96	560.00
8/13/19	Morning	Erie	24.81	24.81	270.59
8/13/19	Afternoon	Erie	24.96	24.79	970.72

Table 1. Average field and incubation temperature for Lake Erie and Saginaw Bay on the afternoon of initial collection and the following morning and afternoon sampled time points. Field data was sourced from GLERL Buoy ReCON data (www.glerl.noaa.gov). Photosynthetically Active Radiation [PAR] was measured in the incubation tank.

4.2.3 Microcystis Velocity Measurements

Sub-samples were analyzed by micro-cinematography the day after field collection (T_0+1) in the morning (0900) and afternoon (1500). At each time point, bottles were gently inverted several times to mix the contents before 25mL of each sample was then poured into a 25mL cuvette (Rowe et al. 2016).Buoyant velocity of *Microcystis* colonies were measured using a microscopic cinematography consisting of two main components – a camera system and motion analysis software (Nakamura et al. 1993a; Rowe et al. 2016). The camera system was housed inside a temperature-controlled room set to ambient lake temperature at collection. The experimental setup utilized the Shadowgraph optics system (Rasenat et al. 1989; Trainoff and Cannell 2002), a light source in the form of a laser (400-710nm, Stocker Yale Canada Inc., LASIRIS^{TM}), a digital video camera (Basler acA1300 – 60g mNIR) for digital recording. The camera system was mounted on a 3D positioning frame which was controlled from the outside of the temperature controlled room via joystick which allowed for the user to focus an move the camera system along the sample contained in a 25mL quartz cuvette (10mm X 10mm inner dimension X 305mm tall, Friedrich & Dimmock, Inc.). The cuvette was housed inside a thermal water jacket (140mm X 140mm inner dimension X 400mm tall) that was placed inside the room the previous night as to acclimate to incubation temperature (Table.1).

The camera system was connected to a computer outside the chamber where the user coordinated digital recordings of *Microcystis* movement with image capturing software (Contemplas, GmbH, Germany). For each sample, a clear ruler with mm graduations was placed inside the thermal water jacket to allow for calibration before each set of recordings. Ten video segments were taken along the length of the cuvette. Following video recording, buoyant velocities were measured using the motion analysis software Vicon Motus (Contemplas, GmbH, Germany). Colony size was quantified by their equivalent spherical diameter (ESD). During the buoyant velocity measurements, colonies were assigned identities in calibrated image stills from Vicon Motus and were then imported into ImagePro Insight (Media Cybernetics) to obtain colony ESD. The sizes were then paired with the velocity data.

The influence of size on velocity is described by Stoke's law where the force of gravity of a particle balances with the fluid drag force, the particle reaches its terminal velocity V:

$$V = \frac{g\Delta\rho D^2}{18\phi\mu} \qquad \textit{Equation 4.1}$$

V is the velocity of the colony (m/s), g is the gravitational acceleration constant (9.8 m s^{-2}), $\Delta\rho$ is the effective density of the particle ($\rho water - \rho colony$), $\rho water$ is the density of freshwater (1000 kg/m^3), ρcolony is colony density(kg/m^3), μ is the viscosity of water(Pa*S), D is the diameter of the particle(m), and φ is the shape coefficient set to 1 (Reynolds et al. 1987). Reynolds et al. 1987, further developed an equation to describe colony density from the floatation velocity base of Stokes law that allows us to solve for ρcolony:

$$\rho colony = \rho water - \frac{18\varphi\mu v}{gD^2} \qquad \textit{Equation 4.2}$$

4.2.4 Statistical Analysis

In order to describe the effects of light and time of day within each experiment we analyzed the velocity data using general linear models for each of the four experiments to determine significant differences between colony buoyant velocity and density as functions of light condition and time of day. Light condition and time of day were combined into a single categorical explanatory variable. We fitted the models assuming a normal error distribution using the *lm* function in R (R Core Team, 2019). Post-hoc analysis was done by conducting multiple pairwise comparisons of the estimated marginal means with Tukey HSD adjustment with the R package emmeans (Russel Lenth, 2020).

4.3 Results and Discussion

4.3.1 Colony Velocity, Density, and Size

Lake Erie

Consistent with previous studies on diel buoyancy movement by *Microcystis* colonies, we found significant differences in colony buoyant velocities in Lake Erie in response to light intensity and time of day. These effects were present on both July 30th 2019 ($F_{5,498} = 47.98$, $p < 0.001$) and August 13th 2019 ($F_{5,355} = 19.5$, $p < 0.001$) (Figure 1). Within each light treatment, we found that colonies had a significantly higher buoyant velocity in the morning compared to the afternoon (full ambient: p<0.001 on July 30th and August 13th; 30% ambient: p<0.001 on July 30th and August 13th; 10% ambient p<0.001 on July 30th and p=0.216 on August 13th, Table 2,

Appendix 1.1). This suggests that as sunlight increases from morning to afternoon, buoyant velocity decreases.

During the morning, all treatments were positively buoyant on average. During the afternoon, however, only the 10% ambient treatment remained positively buoyant and the treatments with increased light intensity were sinking on average. For each morning and afternoon time point, we found a negative effect of light intensity on buoyancy, whereby the 10% ambient light treatment had significantly higher colony velocity than the other two treatments (Table 2, Appendix 1.1). However this pattern was not significant on the morning of August 13th (Figure 1). This discrepancy is most likely due to the accumulation of carbohydrates under higher light intensities which could result in lower buoyant velocity.

Saginaw Bay

In contrast to Lake Erie, we found no significant difference in colony buoyant velocity in response to light intensity and time of day in Saginaw Bay on July 11th 2019 ($F_{5,197}$ = 0.99, p = 0.42) and August 7th experiment ($F_{5\text{-}448}$ = 2.19, p = 0.062) (Figure 1). In both the morning and afternoon all treatments were largely negatively buoyant with only a few colonies in each treatment exhibiting positive buoyancy.

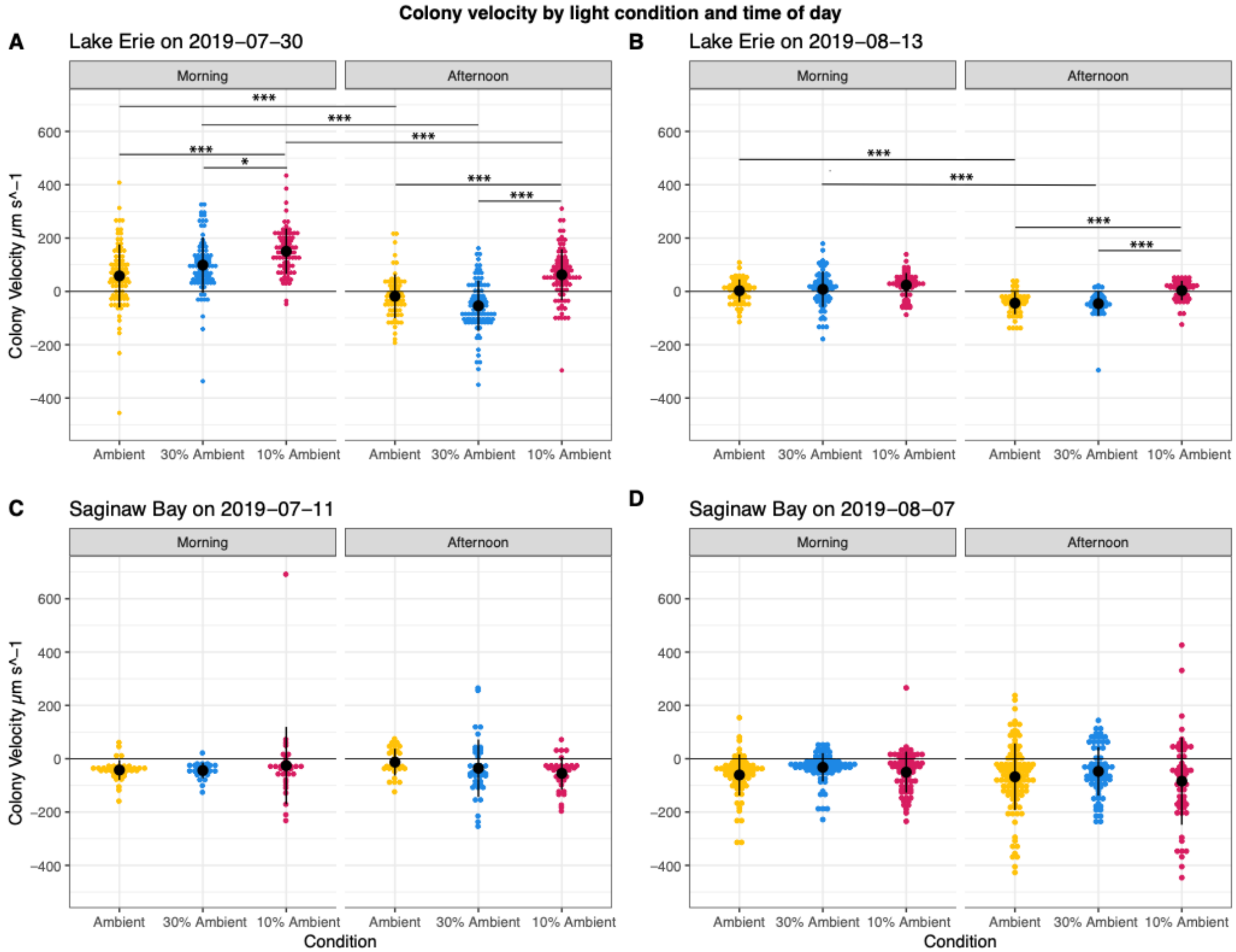


*Figure 1. Colony buoyant velocity (µm s^{-1}) as a response to ambient, medium, and low light conditions during the morning and afternoon for experiments performed in Lake Erie (A, B) and Saginaw Bay (C,D). Each point represents a measurement for a single colony, the black points and whiskers represent the mean and standard deviation. Values above zero on the x-axis indicate buoyant colonies and values below zero indicate sinking colonies. * Indicates significant differences found only in pairwise post hoc tests comparing groups either within the same time of day or within the same light treatment.*

We investigated the relationship between *Mycrocystis* colony velocity and size by constructing log-log plots for both lakes (Figure 6). Negative velocities of sinking colonies were converted to their absolute values to display the relationship on a log-log plot (lower panels in Figure 6). According to Stoke's law the velocity of floating and sinking spherical particles in a fluid, there should be a positive relationship between colony velocity and size (Equation 1.), which was found to be the case in previous *Mycrocystis* studies (Nakamura et al. 1993b; Rowe et al. 2016). In our data, we also found evidence of a significant positive relationship between colony velocity and size in Lake Erie, with a slope of 0.74 (95% CI=[0.58, 0.91]) for floating colonies and a slope of 0.48 (95% CI=[0.27, 0.68]) for sinking colonies. However, we found significantly negative relationships between colony velocity and size in Saginaw Bay, with a slope of -0.54 (95%CI = [-1.00, -0.07] and a slope of -0.45 (95% CI=[-0.69, -0.20]).

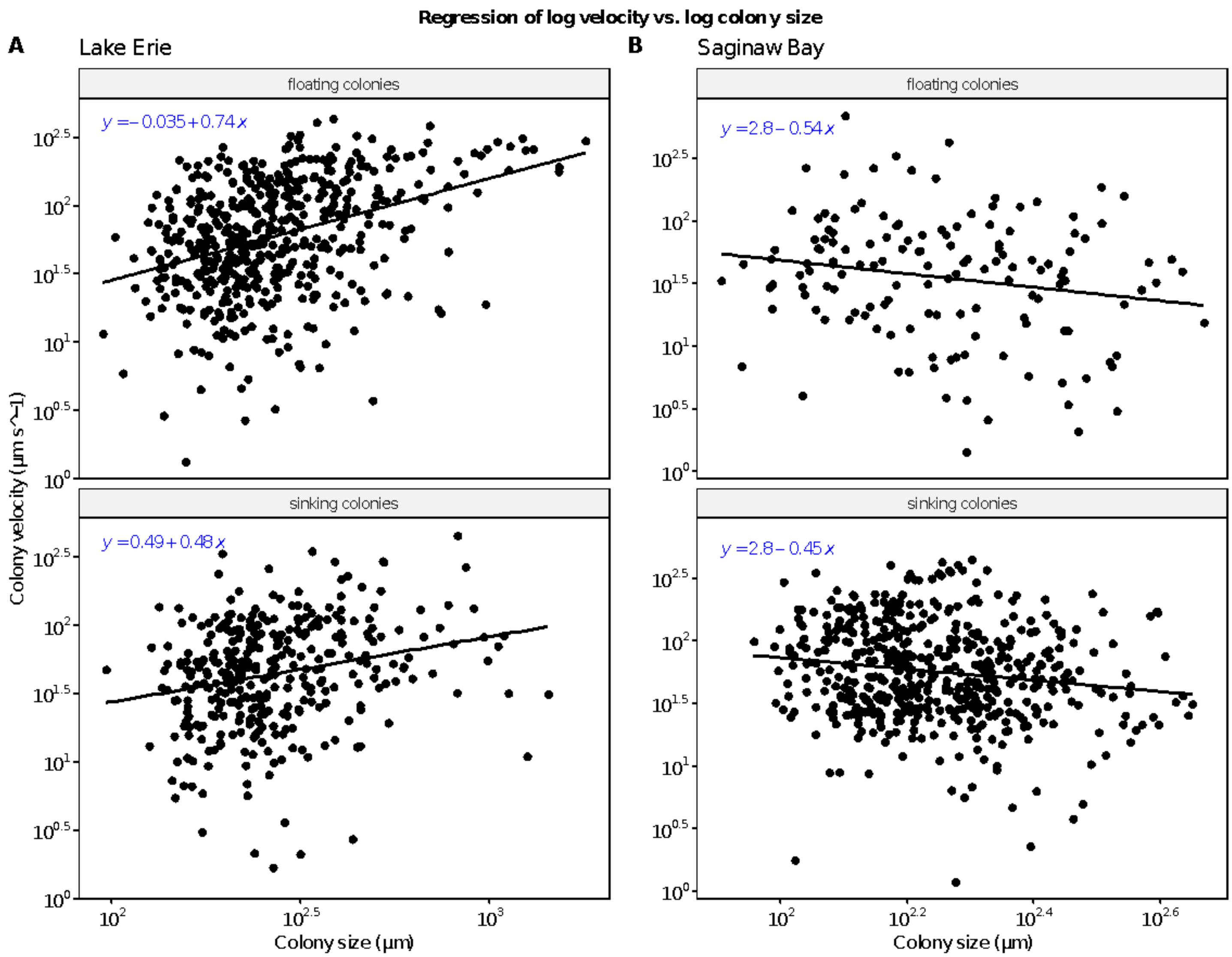


Figure 2. Log-Log plot of colony floating and sinking buoyant velocity as a function of colony size in Lake Erie (A) and Saginaw Bay (B). Regression line equations are shown in blue.

The presence of both positively and negatively buoyant colonies, combined with the poor correlation between velocity and equivalent spherical diameter suggests that colony density may differ by size. A plot of colony density versus equivalent spherical diameter shows similar decrease in variance with size (Figure 3). The inconsistency in density and size among small *Microcystis* colonies is most likely due to the fact that larger colonies tend to have voids among their mucilage and cells, which means that colony density will be less reflective of cellular density and more similar to water. This result indicates that smaller colonies may be more representative of variation in cell density. This is reflected in the vertical migration model from Visser et al. 1997 which predicts that small colonies have a higher net increase in cell density than large colonies, but are inhibited at higher light intensities. A study by Li et al. 2016 found that while the densities of *Microcystis* species *wesenbergi* and *ichthyoblabe* increased linearly with colony size, no such correlation existed for *M. aerginosa.* This pattern of increased variability in density of small colonies has important implications for the ecology of Microcystis blooms. To date, diel vertical migration has been assumed to be a function primarily of colony size and light, but our study indicates that the density of colonies is independent of their size. This finding could aid efforts in modelling the intensity and distribution of the bloom.

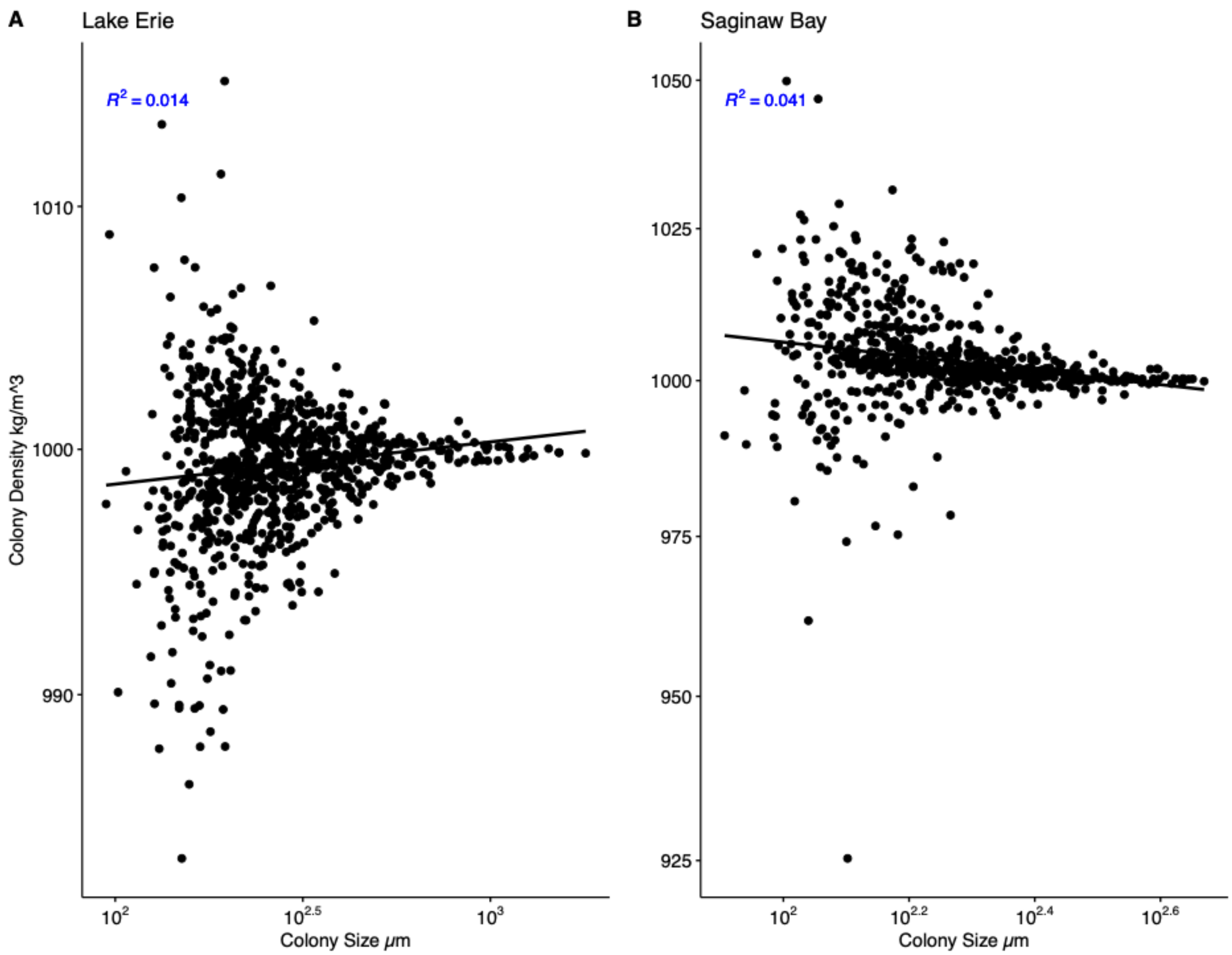


Figure 3. Plot of colony density as a function of logged colony size in Lake Erie (A), and Saginaw Baw (B) with respective coefficient of determination (R^2)

4.3.2 Colony Density, Size, and Light Intensity

Lake Erie

In addition to the effects of light intensity and time of day on buoyant velocity, these factors also significantly affected colony density in Lake Erie on July 30th 2019 ($F_{5,498} = 34.28$, $p < 0.001$) and August 13th 2019 ($F_{5,355} = 15.33$, $p < 0.001$, Figure 4). Within each light treatment colonies in the afternoon were significantly more dense than in the morning (Figure 4, Table 2, Appendix 1.2). This may be due to accumulation of carbohydrates as sunlight increases from morning to afternoon. During both the morning and afternoon, there was a positive effect of light intensity on colony density whereby the 10% ambient light treatment was significantly less dense than all other treatments (Figure 4). However this pattern was not significant on the morning of August 13th between the 10% and 30% treatments (Figure 4).

Saginaw Bay

In Saginaw Bay, we found no significant difference in colony density in response to light intensity and time of day for July 11th 2019 ($F_{5,197} = 1.37$, $p = 0.23$). However, a slight significant difference was found on August 7th 2019 ($F_{5,448} = 2.28$, $p < 0.05$, Figure 4). After post-hoc analyses, we found that the only significant difference in density was between the 30% ambient in the morning and 10% in the afternoon ($p = 0.0141$). Thus,colonies from Saginaw Bay were overall less responsive to light treatments and time of day than those from Lake Erie. Further hydrodynamic modeling would be required to determine whether the reduced densities observed in some conditions are sufficient to overcome turbulence and lead to substantial vertical migration in Saginaw Bay.

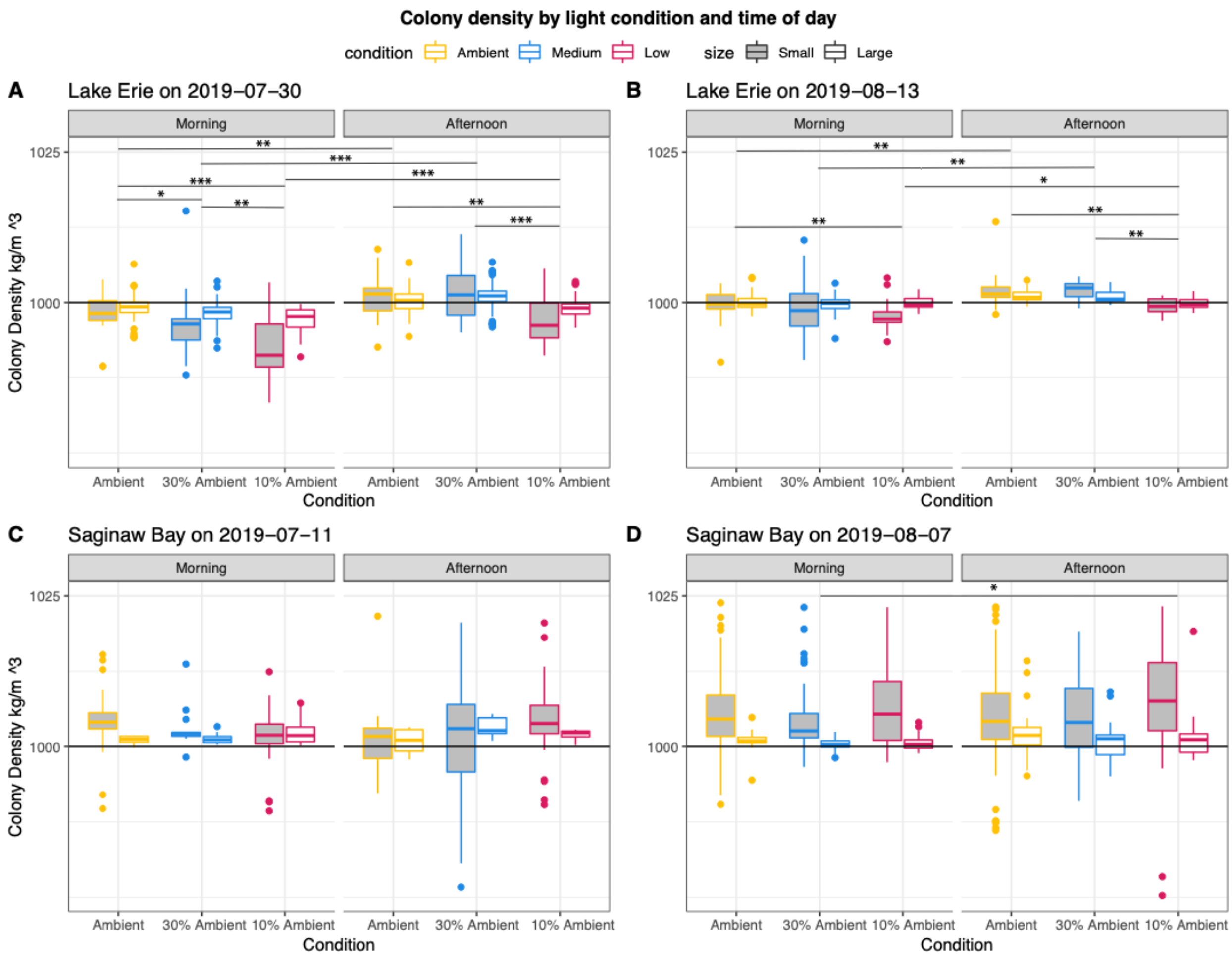


*Figure 4. Box and whisker plots showing colony densities (kg/m^3) as a response to ambient, medium, and low light conditions during the morning and afternoon for experiments performed in Lake Erie (A, B) and Saginaw Bay (C, D). Boxes represent the median, and the first and third quantiles. Grey boxes represent colonies smaller than 200µm while white boxes are colonies larger than 200µm. * Indicates significant differences found only in pairwise post hoc tests comparing groups either within the same time of day or within the same light treatment.*

Condition - Time	W. Erie 1 2019-07-30		W. Erie 2 2019-08-13		Saginaw Bay 2019-07-11		Saginaw Bay 2 2019-08-07	
Response Variable	Velocity	Density	Velocity	Density	Velocity	Density	Velocity	Density
Ambient-Morning	58.0 (37.8, 78.11)	999.0 (998.1, 999.3)	2.5 (-9.19, +14.2)	999.93 (999.4, 1000.4)	-42.4 (-69.0, -15.71)	1003.5 (1000.9, 1006)	-60.9 (-85.0, -36.8)	1003.9 (1002.1 , 1006.8)
Ambient-Afternoon	-18.1 (-41.7, +5.47)	1000.6 (999.9, 1001.3)	-43.86 (-56.6, -31.1)	1001.43 (1000.83, 1001.97)	-12.8 (-43.2, +17.6)	1000.9 (998.0, 1004)	-67.8 (-87.1, -48.4)	1003.80 (1002.3, 1005)
Medium-Morning	98.7 (+77.8, +119.5)	997.7 (997.1, 998.3)	7.94 (-3.10, +19.0)	999.53 (999.06, 1000.00)	-44.1 (-76.7, -11.41)	1002.2 (999.0, 1005)	-32.2 (-55.2, -9.19)	1002.5 (1000.7, 1004.3)
Medium-Afternoon	-54.1 (-73.4, -33.9)	1001.0 (1000.4, 1001.6)	-45.97 (-60.23, -31.7)	1001.27 (1000.67, 1001.88)	-35.6 (-61.9, -9.31)	1002.0 (999.4, 1005)	-47.4 (-71.7, -23.13)	1004.0 (1002.1, 1005.8)
Low-morning	150.4 (128.5, 172.24)	995.9 (995.4, 996.6)	23.42 (+11.36, +35.5)	998.64 (998.13, 999.15)	- 25.7 (-54.7, +3.30)	999.5 (996.6, 1002)	-50.4 (-74.8, -25.92)	1003.6 (1001.7, 1005.5)
Low - Afternoon	62.4 (42.0, 82.89)	998.7 (998.1, 999.3)	3.18 (-9.72, +16.1)	999.75 (999.20, 1000.30)	-54.9 (-83.0, -26.75)	1004.0 (1001.2, 1007)	-84.3 (-111.4, -57.13)	1007.1 (1005, 1009.2)

Table 2. Estimated marginal means for each experiment and their respective treatments with upper and lower confidence intervals of 95 percentiles.

4.4 Conclusion

The results of our study show that overall, *Microcystis* colonies from Lake Erie were more positively buoyant than those from Saginaw Bay. Consistent with diel vertical migration exhibited in many species of phytoplankton, *Microcystis* colonies in Lake Erie became more positively buoyant in the morning as ambient light increased while sinking in the late afternoon. In contrast, we found no significant difference in colony buoyant velocity in response to light intensity and time of day for *Microcystis* colonies in Saginaw Bay where colonies were largely negatively buoyant in both morning and afternoon with only a few colonies in each treatment displaying positive buoyancy.

While previous studies have shown that buoyant velocity of *Microcystis* colonies are positively correlated to colony size, consistent with Stoke’s law, this relationship was in fact slightly negative for floating colonies from Saginaw Bay. An analysis of colony density revealed higher variability among smaller colonies compared to large colonies in both Lake Erie and Saginaw Bay. This finding suggests a common pattern of colony density becoming less plastic as colonies increase in size.

Our findings show that *Microcystis* colonies in the Western Lake Erie and Saginaw Bay differ in their response to light and may therefore ultimately affect how HABs are transported throughout each system or experienced on the surface of the lake. Understanding the effects of light on the diel vertical migration of *Microcystis* may explain the observed differences in colony distribution and movement of *Microcystis* within the two lakes. As understanding the mechanisms that induce these patterns is critical to improving forecasting efforts in freshwater lakes globally, these findings may improve how the movement of HABs are influenced by varying environmental conditions.

Chapter 5. Characterization of public water system needs and attitudes related to HAB toxicity in Saginaw Bay and Western Lake Erie

5.1 Introduction

5.1.1 Background

Community engagement in research is essential to effectively address water management issues within the Great Lakes (Krantzberg et al 2015). Due to the socio-economic and health ramifications of harmful algal blooms, a holistic multidisciplinary approach that not only includes natural sciences but aspects of social science and humanities is required to ensure adaptive resource management through addressing human causes, consequences, and responses to HABs (Bauer et al. 2010). In regions of the Great Lakes, including Lake Huron's Saginaw Bay, high concentrations of harmful algal blooms result from a suite of ecological factors including nutrients, light, and mussel grazing (Johengen et al. 2013). Complex interactions among these factors can lead to uncertainty in the extent and duration of blooms underscoring the need for forecasting products for water systems management. Such forecasting products combine current remote sensed observations of HABs, hydrodynamic models, and meteorological forecasts to provide advanced forecasts of the potential location and intensity of blooms five days in advance. Understanding the perspectives of public water utility managers and their abilities to mitigate impacts of HABs is necessary to develop a robust forecasting system. Our study explored the impacts of HABs in public water systems in Saginaw Bay and whether these systems would benefit from a Saginaw Bay HAB forecast.

In Saginaw Bay harmful algal blooms are predominantly composed of the cyanobacteria, *Microcystis,* which can produce a group of toxins called microcystins. Microcystins have substantial negative impacts on the communities in which they occur. These negative impacts range from aesthetic issues resulting from the reduction in water clarity that affect recreation, tourism, property values and fishing (Dodds et al. 2008; Hamilton et al. 2013; Gill et al. 2018) to severe health risks for humans and mammals (Rastogi and Incharoensakdi, 2014). Coming in contact with water contaminated with microcystin can lead to irritation of the skin, eyes, and throat (W.H.O., 1999) and ingestion can lead to headache, fever, stomach cramps, vomiting, and weakness (Carmichael, 2001; Carmichael and Boyer, 2016). These issues lead to substantial economic costs due to the loss of property values and the decrease in fishing license revenue in areas impacted by HABs (Wolf, 2018). Additionally, intensive water treatment is required to prevent disruption within drinking-water supply systems.

In August 2014, the City of Toledo, located on the coast of Lake Erie, issued a "do not drink" order due to microcystin contamination in the public water supply. This resulted in a three day ban on municipal water use for the city's 500,000 residents and commercial customers due to large scale disruptions within water treatment operations (Steffan et al. 2014). In response, Congress passed new legislation addressing algal toxins in drinking water (Drinking Water Protection Act of 2015. Pub. L. 114-45 129) which amended the Safe Drinking Act to include

“Algal Toxin Risk Assessment and Management”. Additional steps allocated increased funding to promote public health and protection of the environment (Water Infrastructure Improvements for the Nation Act of 2016 Pub. L. 114-322 130). In 2014, Researchers from NOAA and the Cooperative Institute for Great Lakes Research launched an experimental harmful algal bloom forecast, the experimental Lake Erie HAB Tracker, to provide a nowcast and a five-day forecast of the concentration and physical transport of cyanobacteria within Lake Erie. While HABs are recognized nationally as a problem in Lake Erie, harmful cyanobacterial blooms occur not only throughout the Great Lakes but worldwide. Their distribution range is continuing to extend in response to increasing ambient lake temperatures, CO_2, and eutrophication associated with climate change on a global scale (Paerl and Huisman, 2009; Visser et al., 2016). Lessons from the development of the experimental Lake Erie HAB Tracker can be applied to the design of HAB forecast models elsewhere, including potentially Saginaw Bay.

During the initial development of the experimental Lake Erie HAB Tracker, water utility managers represented a key demographic among intended users. Providing information on the current extent and potential trajectory of the bloom allows water utility managers to determine whether preventative measures are necessary to ensure the continued distribution of safe drinking water. While the experimental Lake Erie HAB Tracker has proven to be a widely used and important tool for water utility managers in Lake Erie (Wynne et al., 2013), the necessity of a Saginaw Bay HAB forecast has yet to be realized. In order to characterize public water system needs related to HAB toxicity in Saginaw Bay, a focus group took place on October 23rd, 2019 consisting of eight water utility managers, representing four regional water treatment plants, and two members from the State of Michigan’s Department of Environment, Great Lakes, and Energy (EGLE). The research questions below informed the development of focus group methodology.

5.1.2 Research Questions

1. How are public water systems in Saginaw Bay impacted by HABs?
2. Would a HAB forecast similar to the experimental Lake Erie HAB Tracker be useful for Saginaw public water system employees?

5.2 Methods

Mixed research methods were used in this study. A focus group was held to understand and collect empirical data on stakeholder information needs, knowledge, and perceptions of HABs, as well as evaluating the potential utility of a Saginaw Bay HAB forecast tool similar to the experimental Lake Erie HAB Tracker. The discussion was designed to create a permissive, nonthreatening environment that encouraged the stakeholders to share perceptions and points of view without pressuring them to reach consensus (Kruegar & Casey, 2015). Additionally, pre- and post-focus group surveys were administered in order to assess any changes in stakeholder needs, perceptions, knowledge, and willingness to engage with researchers as a result of their participation in the focus group. The analysis was done using an inductive research approach to find emerging themes and avoid subjective bias (Patton 2002).

5.2.1 Focus group

The focus group was held at the Saginaw Water Treatment Plant in Saginaw, Michigan on October 23rd, 2019. Ten stakeholders participated, including eight water utility managers and two representatives of Michigan's Department of Environment, Great Lakes, and Energy (EGLE). Water utility managers and the regulators at EGLE play critical roles in managing the quality of water distributed to the public. In order to develop a forecast product that effectively supports drinking water treatment in the event of a bloom, understanding the perceptions and decision-making processes of these target stakeholders is key. Focus groups provide an interactive environment and allow people to ponder, reflect, listen, and to compare experiences and opinions of others (Krueger and Casey, 2015). The discussion between these key stakeholders provided many details regarding their daily work routine, including decision making, communication with the public or other managers, and how they use management protocol to respond to HABs or other unforeseen events.

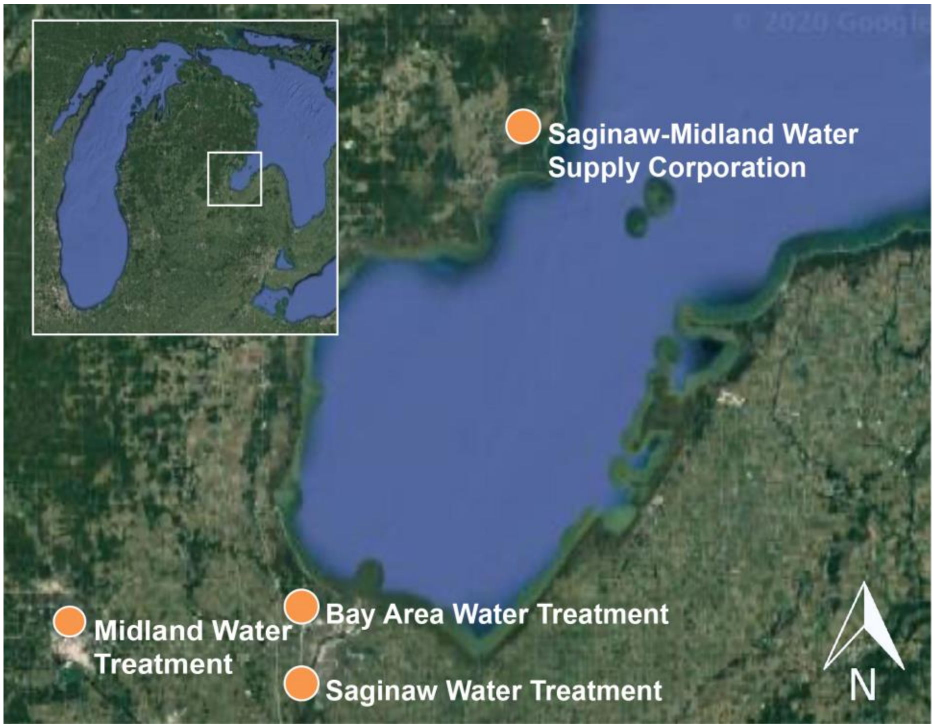


Figure 5.1. Map of water treatment plants represented in the focus group that was hosted by the Saginaw Water Treatment Plant. Map created using Google Earth satellite images.

The duration of this focus group was approximately three and a half hours. Two presentations were delivered by researchers from the NOAA Great Lakes Environmental Research Laboratory and the Cooperative Institute for Great Lakes Research. One presentation outlined the ecology of harmful algal blooms in Saginaw Bay and the other presentation detailed the HAB forecast models used by public water systems serving western Lake Erie. Each presentation lasted approximately twenty minutes. Following the presentation, facilitated discussions were directed using a semi-structured interview guide (Appendix II).

5.2.2 Survey

Surveys were conducted before and after the focus group to assess changes in knowledge or perceptions as a result of their participation. The surveys provide additional information by allowing us to determine what individual participants know or believe without being influenced by the others in the focus group (Lune and Berg, 2017). For example, the pre-focus group survey may reveal the true willingness of the water system managers to use a HAB forecast before everyone in the later discussion mentioned the benefits of using a HAB forecast. The eight survey questions were designed to assess stakeholders' knowledge of HABs, their perceptions of HAB impacts on their water plants, their preparedness to deal with a HAB intrusion, and their perceptions of the usefulness of a hypothetical HAB forecast for Saginaw Bay. Due to the sensitivity of discussing public drinking water in the Great Lakes region in light of recent public health crises (Ames, 2018), each participant was assigned a code for anonymous response to ensure confidentiality. Additionally, this anonymous coding eliminates the risk of subjective bias in subsequent survey analysis and reporting. Survey responses were converted to numeric values, allowing for quantitative analysis, which can facilitate comparison and statistical aggregation of the data (Patton, 2002). The surveys also helped fill information gaps regarding topics not explicitly covered in the focus group discussions. The pre-survey revealed differences in participant backgrounds, and the post-survey provided feedback on the effectiveness of the focus group in identifying stakeholder concerns.

5.2.3 Data analysis

The focus group discussion and all researcher notes were recorded and subsequently transcribed using NVivo 12 (QSR International Pty Ltd Version 12, 2018), a qualitative data analysis software. Themes relevant to our research questions were identified from the transcribed, anonymized text using Conventional Content Analysis (Kondracki & Wellman, 2002). Materials from the focus group such as the discussion content and researcher notes were compiled and coded using an inductive research approach (Patton 2002). During the coding process, data or quotes discussing similar topics were organized and labeled into nodes. (Dongen et al. 2016; Logan et al. 2005). This classification was dependent on shared qualities between quotes and topics, and how frequently these quotes and topics were brought up. For example, the theme of treatment response was identified due to the high frequency of nodes that related to plant treatment response, namely activated carbon and reservoir storage.The relevant themes uncovered during the focus group were ultimately synthesized in a concept map framework in order to answer our two research questions.

The survey results were organized manually and entered into Microsoft Excel. Participants' responses were coded and listed on spreadsheets anonymously. Every response under each survey question was converted to a numeric value for analysis. This analysis highlights any change in participants' self-reported knowledge of HABs, or their attitude towards a potential HAB forecast for Saginaw Bay.

5.3 Results and Discussion

Seven key themes relevant to our research questions were identified during the coding of the focus group (Figure 4.2). By drawing connections between these themes in the framework of the concept map, we answer our research questions (1) what is the impact of HABs on Saginaw Bay public water systems? and (2) would a HAB forecast be useful to Saginaw Bay public water systems?

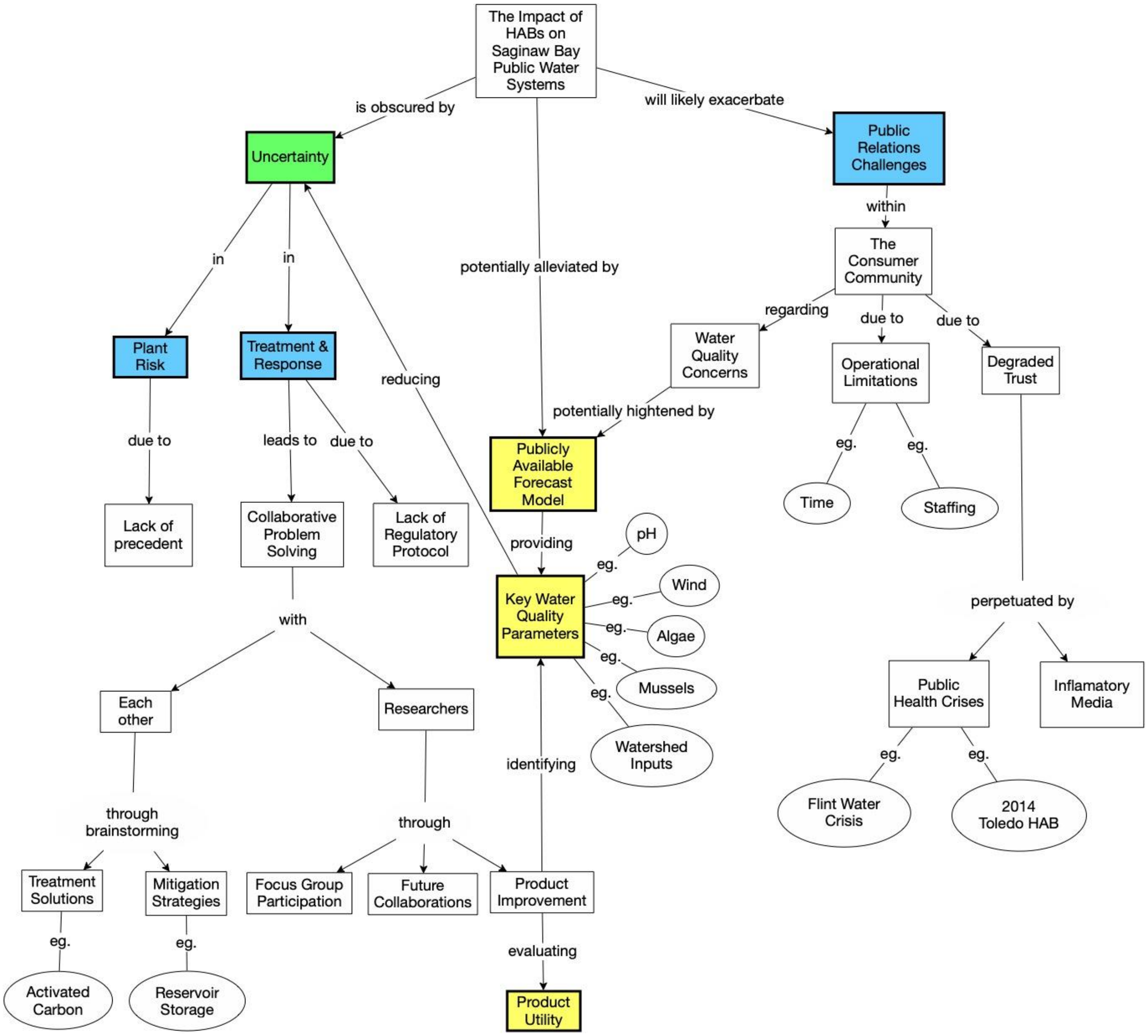


Figure 5.2. Concept map of themes identified through inductive coding of our focus group. Blue rectangles represent the major themes associated with the first research question, while the yellow rectangles represent the major themes associated with the second research question. The green rectangle represents the only major theme associated with both research questions.

5.3.1 What is the impact of HABs on Saginaw Bay public water systems?

To explore the impact of HABs on Saginaw Bay public water systems, participants were asked what their greatest concerns were regarding harmful algal blooms on plant operations. Concerns mentioned most frequently were identified as the following nodes: 1) uncertainty regarding plant risk, 2) uncertainty regarding operational treatment response to HABs, and 3) exacerbating existing public relations challenges regarding water quality if a HAB were to occur near plant intakes.

Uncertainty regarding plant risk

Of the 29 times plant operations were mentioned with regards to harmful algal blooms, 14 instances referred to an uncertainty or a fear in how HABs would impact the plant. As one participant explained, "...We need to know if it's a real threat for our water systems, because we're expected to be able to respond." The plants have yet to detect a bloom in their intakes, and many participants expressed uncertainty about their current risk. The participants also expressed uncertainty at how changing lake conditions due to climate change may lead to an increased risk of HABs impacting their plants.

> *...We feel pretty confident because we haven't had any problems, but is this stuff marching north? Are we having global climate change which is gonna warm up Lake Huron...Well, then it's fine until it's not fine. So it's not just public perception, it's an actual problem in our plant if it gets warmer and warmer...*

Compounding this uncertainty is the lack of mandated response protocols and a feeling of unpreparedness if a bloom were to impact the plant. As one participant explained, "*If we ever had harmful algae or cyano toxin coming our way, I don't think our plant would be prepared to fight that...If we have this in the future, it's definitely something that we would have to plan for and look ahead."* In Ohio, the state mandates that every public water system that has ever detected microcystin must draft a HAB response plan and follow it when needed. No such mandate currently exists for the state of Michigan.

The uncertainty and challenges to operations in the event of a HAB are explainable as at the time of this study no plants in Saginaw Bay have detected microcystin within their intakes. Michigan EGLE has tested Saginaw Bay for harmful algal blooms since 2016, and in that time has only detected low concentrations (Parker, 2016). One of the participants did mention the testing EGLE had done at the Whitestone input.

> *The state has been doing some testing monthly... we have some (microcystin), it was very minimal. We didn't detect anything at our individual systems and at the intake they found more levels, about two hits.*

These plants have never experienced a bloom, and have been told repeatedly by the state that there is no actionable amount of microcystin in the Bay. Due to the lack of detected microcystin, these plants had dedicated time and resources to more visible and immediate threats over HABs. This explains why participants expressed that planning and protocols were lacking, but efforts should be made to increase monitoring and testing.

These challenges posed to plant operations by HABs is further expressed in participant responses to pre-survey question 5 (Figure 4.3), in which half of the participants reported they have only occasionally talked about HAB response strategies, and the other half report never having discussed a response plan at their plant at all.

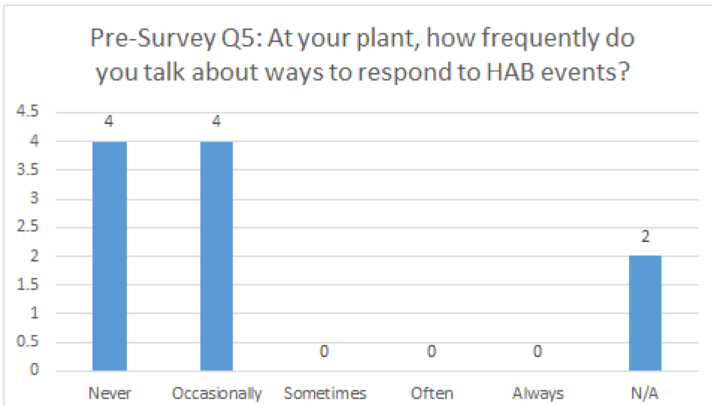
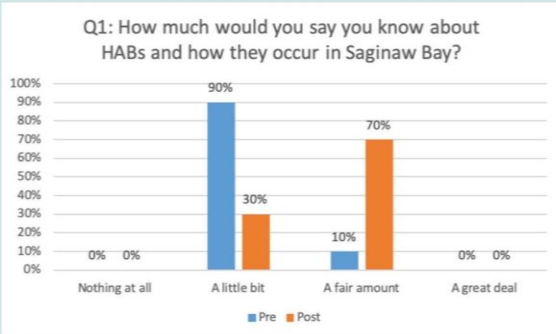
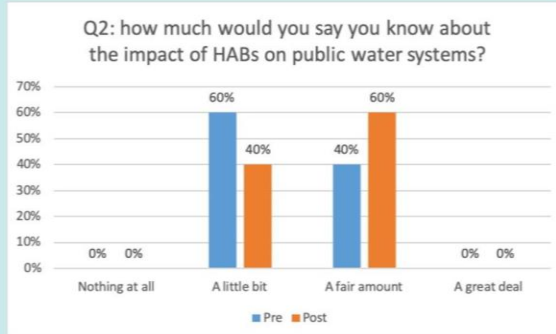


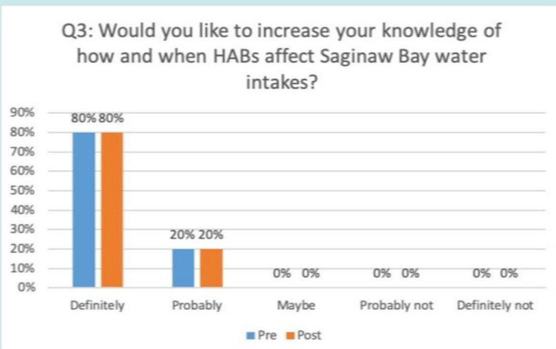
Figure 5.3. Plant operator responses prior to the focus group illustrate the lack of preparedness to deal with a HAB intrusion.

As was seen in survey question 4, a majority of participants believed that their plants had not been impacted by HABs at all, while fewer believed it was only a slight or neutral impact. It should be noted that this uncertainty does not mean there is no desire to create plans and further knowledge of potential blooms in the future. Throughout the focus group there was a common sentiment: there is a need to create a plan going forward to prevent any major consequences from a HAB event. As one participant expressed, "...we can't just assume that because we haven't had a problem, we won't have a problem... we need to develop a plan as well."

The surveys that were conducted further expressed participants' desire to address potential operational challenges in the event of a microcystin detect. Participants said their knowledge of HABs in Saginaw Bay increased after participating in the focus group, with 70% of participants believing they knew a fair amount (Box 1). 40% of participants reported that their knowledge of HABs in Saginaw Bay increased after participating (Box 1). A smaller percentage of participants (20%, n=2) reported that they learned something about the impact of HABs on water systems as a result of participating in the focus group (Box 1). At the conclusion of the focus group, 80% expressed a willingness to continue to learn more about HABs (Box 1). These survey results support the sentiments expressed by participants during the focus group that they felt there was more to learn about if and how their plants should prepare for future HAB intrusion events. This desire for more information supports the recommendation that Saginaw Bay HAB forecast product be developed to help public water systems reduce uncertainty regarding when an operational response may be needed, and to help them prepare for a potential future HAB event.







Box 1. Question 1 post-focus group survey responses show an increase in HAB knowledge compared to pre-focus group survey responses. Question 2 post-focus group survey responses show a slight increase in knowledge regarding potential impacts HABs have on public water systems. Question 3 post-focus group survey responses show no change from pre-focus group survey responses showing the continued desire to learn more about HABs and how to deal with HABs intrusion.

Uncertainty regarding HABs treatment and response

Much of the uncertainty about HABs related to how and when to go about treating HABs if they impact an intake. Potential solutions and strategies to combat a HAB if microcystin were to intrude into the water system was a common topic brought up during the focus group. The two most commonly discussed strategies were the use of activated carbon to remove the microcystin and the use of a reservoir to dilute the amount of toxin in the water to make treatment easier.

> *Participant A: Saginaw and Midland do have activated carbon as you mentioned...If we could find a way to react to it (microcystin) quickly before we pull in significant amounts at the intake, I feel like that's the direction.*

> *Participant B: ...Midland also has a fairly large reservoir and they could...use their raw reservoir to help dilute the water coming in to make it more manageable.*

Of these proposed solutions, the use of activated carbon would likely be the most feasible. Many water treatment plants, including those in Saginaw Bay, have activated carbon systems already in place.This form of treatment tends to be cost effective and has been shown to adequately remove microcystin from water. This is the method most commonly used by water treatment plants in Lake Erie to deal with the HABs (Ohio Sea Grant, 2018). The reservoir option might work for some plants, but other participants expressed their belief that this would likely be impossible due to infrastructure limitations:

“We’ve got 10 million gallons on hand, and in the middle of the summer we are using all of that and then some. The water coming to us from Saginaw is being used within a day, so we don’t have this option.”

At present, the state of Michigan has no protocol for how to treat water contaminated with microcystin, and the EGLE website simply refers to Ohio’s EPA guidelines. There is no universal plan in place if a bloom occurs, which has led to a lack of clarity in how plants should respond should a HAB intrusion occur. This puts the communities served by these public water systems at risk of a public health emergency or disruption in service, if microcystin was to be found in the raw water used by these plants.

Public relations

Throughout the focus group, general interactions with the public and/or the media was discussed a total of 21 times. The majority of these cases were specifically regarding issues that plant operators have had with negative public perception of water quality and a perceived lack of trust from the communities they serve: “I feel like what I run into with public perception is that when we tell people that the water is fine, it’s self-serving and people don’t tend to believe us.”

The lack of trust in public drinking water systems is a common theme among Great Lakes public water consumers, and has been exacerbated by public health crises such as the City of Toledo’s HAB-induced “do not drink” advisory of 2014 (Ames et al., 2019) and the Flint water crisis (Pieper et al., 2017). This perception was further evidenced in our focus group when participants expressed that public trust had degraded following the Flint water crisis: “When Flint was going on, people were calling right away [in Saginaw] wondering what to do with their water.”

Due to perceived distrust among the local community, focus group participants expressed their desire to improve public relations while acknowledging institutional shortcomings. There is a general lack of designated public relations or communications staff at the represented water plants, and the technicians themselves most often had to field calls from concerned members of the public. This can lead to feelings of frustration when plant operators engage suspicious or concerned consumers. This frustration was expressed when one participant said: “That’s because we’re all technical people, we aren’t communication majors.” The feeling amongst participants seemed to be that due to things beyond their control, they were inundated with questions and concerns that not only eroded good will with the public, but potentially pulled their attention away from plant operations.

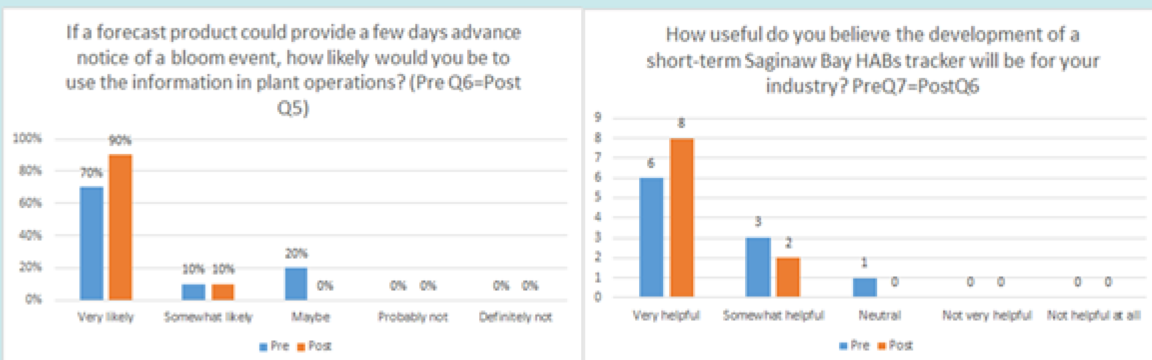
5.3.2 Would a HAB forecast be useful to Saginaw Bay public water systems?

A HAB forecast may have the potential to support information needs expressed by public water systems, particularly related to early detection of HABs and reducing uncertainties. When asked directly about the potential usefulness of a Saginaw Bay HAB forecast, the focus group participants explained the role it may play in reducing operational uncertainty and discussed potential public perceptions of a HAB forecast. Lastly, they discussed what specific data would be useful in a HAB forecast product for managers.

Forecast Utility and Public Access

When asked if a HAB forecast similar to the models serving Lake Erie would be useful for managers in Saginaw Bay, participants said it would be a useful resource even if the perception amongst the managers was that the risk of a HAB event seemed low.

> *...From what we've seen in your historical data, it doesn't look like it [*HABs*] would be an issue. But like we said before, something could always change, or a warming event could greatly affect this coupled with wind direction. It [*a HAB forecast] *would be nice to see.*



Box 2. Pre-Q6/Post-Q5 suggests that most participants felt they would likely use a HAB forecast in Saginaw Bay. Pre-Q7/Post-Q6 suggests that most participants feel a Saginaw Bay HAB forecast would be useful.

This sentiment was repeated in the survey questions relating to a HAB forecast (Box 2). The survey responses suggested that not only were participants very likely to use a Saginaw Bay HAB forecast (90%) (Box 2) , there was a belief that such a forecast would be very helpful for the managers in Saginaw Bay (80%) (Box 2).

Despite this strong support, there was an instance of apprehension regarding the HAB forecast. The concern being that if a HAB forecast was made publicly available, public drinking water plants would be flooded with calls from concerned consumers and staff would be stretched beyond operational capacity: "...If it's [a HAB forecast] available to the public...I'm thinking about the conversation before about perceptions or misconceptions, and not understanding why it's [the HAB forecast] being presented...operators are going to have a multitude of phone calls." With trust between plants and the public already perceived as being low, it is possible that a tool available to the public could lead to more calls and concerns, especially if a HAB is detected by the forecasting model. The benefit of the forecast is that the plants can have access to the same information, and can respond to the issue and the public in a manner that potentially re-establishes trust.

Forecast and information product needs

When it was established that a HAB forecast could be useful for the participants, questions were raised about what kind of data they would like to see provided by the forecast. Three fourths of the HAB forecast discussion with the participants were related to data that participants would like to have more information on. These data parameters included measures of *Microcystis* and microcystin, measures of data uncertainty, as well as other water quality parameters beyond the scope of a HAB forecast. These additional water quality parameters including pH, winds, and watershed data were noted by researchers, but are outside the scope of this results discussion.

The need for algal parameters specifically related to the HAB forecast was only mentioned once during the focus group. This is likely because it is implied that something called a HAB forecast would be tracking HABs. Beside this one mention however, there was no mention of microcystin, other algae, or any other measurement associated with HABs. Again, this could be due to the name of the forecast, or it could be further indication that the participants want and need more knowledge regarding HABs and how they could impact their plants.

Reducing uncertainty

One thing that all of these desired features have in common is that these are issues that regularly impact plant operations and are only detectable for the plants after the water has entered the intakes: “we just measure what’s coming into our intake. We’re blind on a lot of it. Once it gets into our plant we’re doing a lot of tests on it, before then no, that’s why you guys are out there collecting this information I guess it does have value for us”.

5.4 Conclusion

In summary, water managers around Saginaw Bay expressed a strong interest to learn and understand the risks of harmful algal blooms to their plants and the communities they serve. However, due to a lack of HAB events in the past and no guidelines from the state regarding treatment options, many water system managers are uncertain about what to do in the event of a bloom, and expressed their desire to be more prepared if one were to occur. States like Ohio only set guidelines after the disaster in 2014 in Toledo. These managers expressed a need for a plan, either one they make themselves or with the assistance of the state.

Not only do these managers feel that a future HAB forecast for Saginaw Bay would be useful, they claim that they would use it. They expressed many concerns regarding various water quality problems that occur in the Bay, and a forecasting HABs model could alleviate some of the burdens and lead to preventative actions. This preparedness in turn could help the plants manage one of their largest concerns, the difficulty engaging with the public. The other portions of this project contribute to potentially strengthening the predictive capabilities of the Saginaw Bay HAB forecast product. By offering more accurate and earlier predictions, the HAB forecast can allow managers a faster and more effective response to potential HAB events in the future. Until then, the monitoring of the Bay should continue, and in the event a threatening bloom is detected, the plants and the state should hopefully be able to respond quickly and effectively.

Chapter 6: Appendix

Appendix I.

	Ambient-Morning	Medium-Morning	Low-Morning	Ambient-Afternoon	Medium-Afternoon	Low-Afternoon
Ambient-Morning		-40.69	92.40	-76.09	112.10	4.45
Medium-Morning	0.06		51.71	-116.79	-152.79	-36.24
Low-Morning	<.0001*	0.0106*		168.55	204.50	-87.95
Ambient-Afternoon	<.0001*	<.0001*	<.0001*		36.01	80.54
Medium-Afternoon	<.0001*	<.0001*	<.0001*	0.20		116.55
Low-Afternoon	0.99	0.14	<.0001*	<.0001*	<.0001*	

Appendix 1 Lake Erie July 30th 2019. Estimated Marginal Means for colony velocity. The p-values are below the diagonal and above are their respective difference in estimated marginal means.

	Ambient-Morning	Medium-Morning	Low-Morning	Ambient-Afternoon	Medium-Afternoon	Low-Afternoon
Ambient-Morning		-5.44	20.91	-46.35	48.47	0.67
Medium-Morning	0.42		15.47	-51.80	-53.91	-4.76
Low-Morning	0.14			67.27	69.38	-20.23
Ambient-Afternoon	<.0001*	<.0001*	<.0001*		2.11	47.03
Medium-Afternoon	0.98	<.0001*	<.0001*	<.0001*		49.14
Low-Afternoon	1.00	0.99	0.21	<.0001*	<.0001*	

Appendix 1.2 Lake Erie August 13th 2019. Estimated Marginal Means for colony velocity. The p-values are below the diagonal and above are their respective difference in estimated marginal means.

	Ambient-Morning	Medium-Morning	Low-Morning	Ambient-Afternoon	Medium-Afternoon	Low-Afternoon
Ambient-Morning		1.288	-3.07	1.59	-2.01	-0.32
Medium-Morning	0.03		-1.78	2.88	3.30	0.96
Low-Morning	<.0001*	0.0012*		-4.66	-5.08	2.74
Ambient-Afternoon	0.0079*	<.0001*	<.0001*		-.041	-1.92
Medium-Afternoon	<.0001*	<.0001*	<.0001*	0.94		-2.33
Low-Afternoon	0.97	0.23	<.0001*	0.0006*	<.0001*	

Appendix 1.3 Lake Erie July 30th 2019. Estimated Marginal Means for colony density (kg/m^3). The p-values are below the diagonal and above are their respective difference in estimated marginal means.

	Ambient-Morning	Medium-Morning	Low-Morning	Ambient-Afternoon	Medium-Afternoon	Low-Afternoon
Ambient-Morning		0.403	-1.28	1.50	-1.34	-0.18
Medium-Morning	0.85		-0.88	1.90	1.74	0.221
Low-Morning	.005*	0.12		-2.79	-2.63	1.10
Ambient-Afternoon	.0010*	<.0001*	<.0001*		0.160	-1.68
Medium-Afternoon	.0103*	<.0001*	<.0001	0.99		-1.52
Low-Afternoon	0.99	0.99	0.04*	0.0003*	0.0036*	

Appendix 1.4 Table of Estimated Marginal Means of colony density top right (blue) and their respective p-values bottom left (red) for Lake Erie August 13th 2019.

	Ambient-Morning	Medium-Morning	Low-Morning	Ambient-Afternoon	Medium-Afternoon	Low-Afternoon
Ambient-Morning		1.46	3.16	-0.17	-0.02	3.16
Medium-Morning	0.87		1.08	1.28	1.48	4.62
Low-Morning	0.23	0.96		-0.20	-0.39	3.53
Ambient-Afternoon	1.00	0.88	1.00		-0.19	3.33
Medium-Afternoon	1.00	0.87	0.99	1.00		3.13
Low-Afternoon	0.23	0.0141*	0.14	0.11	0.25	

Appendix 1.5 Table of Estimated Marginal Means of colony density top right (blue) and their respective p-values bottom left (red) for Saginaw Bay August 7th 2019.

Appendix II.

a) Saginaw Bay HAB Forecast Public Water Systems Workshop Interview Guide 10/23/2019

<u>*Research Questions:*</u>
1) How are public water systems in Saginaw Bay impacted by Harmful algal blooms (HABs)?
2) Would a harmful algal bloom forecast be useful for public water system employees?

Knowledge

1. How did you first hear about Harmful Algal Blooms occurring in the Great Lakes?

    Potential follow up:

    a. How long ago?

    b. From which sources? (news/ through your line of work, fishing buddies)

- **Presentation: HAB Research in Saginaw Bay -**

Questions from the audience:

Perceptions

2. What problems do you think HABs pose for Saginaw Bay?

3. What concerns you about the impact that HABs may have on your water treatment plant?

Protocol

4. Has this plant ever detected harmful algae at the water intake?

    a. Yes:

    i. How did you first become aware of the detect?

    ii. How did the plant respond to the detect?

    iii. Can you describe the different roles that you and your staff undertake when responding to a HAB event?

    Do you keep a record of detected microcystin levels?

    - Do you remember what the microcystin level was?

    iv. Who do you notify when you detect a HAB at your intake?

v. How do you communicate these events to the community that you serve?

- What are the community's interactions with the plant?

b. No:

i. Do you monitor for harmful algae?

ii. How prepared do you feel if a HAB were to occur?

iii. Can you walk me through a scenario of what would occur if the plant were to experience a HAB threat at the water intake?

iv. Who will you report the problem to if this plant does experience a HAB?

v. How would you communicate a HAB event to the community that you serve?

- What are the community's interactions with the plant?

Information Resources

5. If you were in danger of experiencing HABs near your water intake, where would you go for resources and information?

a. Websites? Agencies? Professional contacts?
b.
c. Do you communicate with other regional water managers?

6.How often do you communicate with state or federal agencies regarding HABs?

a. What types of information do you share with one another?

b. Don't communicate:
   i. How would you feel if state agencies became more involved in this plant's operations regarding HABs? (Ohio example).

- **Presentation of experimental Lake Erie HAB Tracker -**

Questions from audience:

HABs Forecast

7. Prior to this meeting, had you heard of the experimental Lake Erie HAB Tracker?

8. If this product was available for Saginaw Bay, would you use it?

a. Yes:

i. How far in advance would you need to know if a HAB is coming in order for you to adjust your treatment operations?

Ii. What kind of water quality parameters are most important for your work?

Iii. At what scale would the HAB forecast be most helpful to you? - Entire bay level or region specific?

iv. How would you like to access the information? (home/work computer, smartphone)

b. No:

i. Why not?

9. As we work to develop the Saginaw Bay HAB forecast, would you be willing to use it and share feedback in terms of intake water quality monitoring data?

Chapter 7: References

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