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Benthic Algal and Macroinvertebrate Response to the Removal of Dreissenid Mussels in the Nearshore Zone of Lake Michigan

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BENTHIC ALGAL AND MACROINVERTEBRATE RESPONSE TO THE REMOVAL OF DREISSENID MUSSELS IN THE NEARSHORE ZONE OF LAKE MICHIGAN

by

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ABSTRACT

BENTHIC ALGAL AND MACROINVERTEBRATE RESPONSE TO THE REMOVAL OF DREISSENID MUSSELS IN THE NEARSHORE ZONE OF LAKE MICHIGAN

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Tyler Kunze

The University of Wisconsin-Milwaukee, 2023
Under the Supervision of Professor Harvey Bootsma

Dreissenid mussels (*Dreissena polymorpha* and *Dreissena rostriformis bugensis)* have changed the fundamental community structure and biogeochemical processes of the Lake Michigan nearshore zone. There is evidence that dreissenids promote the nuisance growth of benthic algae, especially *Cladophora sp.* In addition, benthic macroinvertebrates have benefitted from the presence of dreissenid mussels due to the increased structural complexity along with greater nutrient availability in the benthos. These effects of the dreissenid mussel invasion are well documented; however, little is known about how these systems respond to the removal of dreissenid mussels from a once-populated area. Therefore, it is important to further our understanding of the role dreissenid mussels play in the benthic ecosystem as we contemplate future management strategies in Lake Michigan.

There have been few attempts to remove large areas of dreissenid mussels in the Great Lakes: however, mussel removal attempts have been successful in both initially removing the populations from an area and preventing the re-establishment of those populations. This may be due to heavy predation of juvenile dreissenid mussels by the round goby (*Neogobius melanostomus*) which may inhibit the repopulation of dreissenids once they are removed. In 2021, we removed a 140 m^2 area of dreissenid mussels by depriving them of oxygen and nutrients using a benthic barrier membrane. This was followed by monitoring the response of

the benthic community over a one-year period and comparing community composition to that on a control site where mussels were still present in large numbers (5471 ± 1709 individuals m^{-2}). The goal of this project was to further our understanding of the relationship between dreissenids, benthic algae, and benthic macroinvertebrates in the Lake Michigan nearshore zone in Good Harbor Bay near Sleeping Bear Dunes National Lakeshore.

Benthic invertebrate abundance and algae biomass were measured by gathering *in situ* samples. Additionally, *in situ* incubations and oxygen measurements along with laboratory experiments were used to determine the production of the algae. The internal phosphorus in the algal tissue was measured and compared to the algae production to determine how the concentration of internal phosphorus affects algae production. These experiments showed an increase in algae production when in the presence of mussels compared to algae grown in the absence of mussels. The results of *in situ* and lab experiments were comparable and revealed that Lake Michigan benthic algae has a minimum internal cell P quota (the minimum required to support net growth) that is lower than previously reported values of *Cladophora* and corresponds with the very low molar carbon: phosphorus ratios frequently measured in Lake Michigan *Cladophora*, while the measured maximum growth rate was similar to previously reported values. We used the results of *in situ* and laboratory experiments in a *Cladophora* growth model to highlight the sensitivity of benthic algal community response to small internal P changes in extremely low P environments and explore the response of the benthic algal community to light and nutrient conditions.

Throughout the summer, we observed depressed non-dreissenid invertebrate abundances, lower algae productivity, and major differences in algal community composition on the mussel-free site compared to the control site. We show that this may be attributed to a lack of nutrient

cycling and availability, in the absence of dreissenid mussels, accompanied by reduced habitat structural complexity. The algae on the mussel-free site had lower particulate phosphorus concentrations than that on the control site. Lower ^{13}C-enrichment of algae in the presence of mussels is interpreted as reflecting the photosynthetic fixation of isotopically light CO_2 respired by mussels, highlight the dependence of benthic algae on both carbon and nutrients released by dreissenids. These findings can be used to explore the effects of future large scale dreissenid removal efforts on nuisance benthic algae and nearshore food web structure.

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CHAPTER 1: INTRODUCTION AND GENERAL METHODS

Introduction

There are greater than 180 aquatic invasive species and counting in the Laurentian Great Lakes (Escobar et al. 2018). Some of these species, such as the Pacific salmonids, were intentionally introduced, and some gradually migrated into the Great Lakes through a series of constructed shipping canals. The majority of these non-native species entered the Great Lakes via ballast water of ships crossing the Atlantic Ocean (Grigorovich et al. 2003). This was the case for zebra (*Dreissena polymorpha*) and quagga (*Dreissena rostriformis bugensis*) mussels, which are arguably the most transformative species of all the invaders. The transformation began in 1988, when zebra mussels were first discovered in Lake St. Claire (Hebert et al. 1989). Zebra mussels prefer shallower and warmer water and are relegated to inland lakes and the littoral zone of Lake Michigan (Karatayev et al. 2015). Zebra mussels were soon followed their close taxonomic relative, the quagga mussels, who were first observed in Lake Michigan in 2000 (Nalepa et al. 2001). This intensified dreissenid mussel colonization as quagga mussels not only have outcompeted zebra mussels in the nearshore zone (Mills et al. 1999; Karatayev et al. 2015), but due to their ability to actively filter in very low water temperatures, they have expanded to cover the entire bottom of Lake Michigan (Vanderploeg et al. 2010).

Typically, there are roughly 6500 quagga mussels m^{-2}, and abundances can be near 20,000 individuals m^{-2} in some locations in Lake Michigan (Nalepa et al. 2009, 2020). These vast abundances have caused several issues with the economic and ecological functions of the lake. For example, the yearly cost of managing aquatic invasive species in the Great Lakes is greater than 200 million U.S. dollars, most of which is spent controlling the proliferation of dreissenid mussels (Rosaen et al. 2012). Some of these costs include research about dreissenid

mussel monitoring and control ($1.7 million year^{-1}), while much of the cost is to physically remove dreissenid mussels from infrastructure: $1.2 million year^{-1} per power plant, $500,000 year^{-1} per water treatment plant, and $1.97 million from just one Lake Michigan paper plant (Rosaen et al. 2012).

The physical presence of dreissenid mussels is not the only cause for concern when it comes to their invasion. Invasive mussels also affect the fundamental function and ecosystem processes of environments in which they are abundant. Dreissenid mussels are filter feeders, and due to their sheer abundance, they can filter immense amounts of water. When filtering the water, they filter nutrients and plankton from the water column, which deprives the offshore ecosystem of these nutrients and small organisms, which are the base of the offshore food web (Hecky et al. 2004; Higgins and Vander Zanden 2010; Bootsma and Liao 2013). Since the dreissenid invasion to the offshore and the subsequent decline in offshore nutrients and plankton, there have been lower abundances of many fish populations and fish recruitment has been decreasing (Bunnell et al. 2009; Cunningham and Dunlop 2023). These changes in the offshore production in lakes such as Lake Michigan have further diminished the regional economy in regard to recreational fishing and tourism (Rosaen et al. 2012).

Because of all this damage and the costs associated, groups such as the Invasive Mussel Collaborative (IMC) were assembled, which have the goal of improving methods of invasive mussel management and control. Groups like these, in addition to other researchers, have helped facilitate mussel removal pilot studies. Two of these pilot studies tested a dreissenid-specific molluscicide on Lakes Erie and Michigan, (Weber 2015; LimnoTech 2020) which were successful at removing the invasive mussels in small test areas. However, without further innovation, this method is likely not physically or financially feasible over larger areas of the

lake due to effort required for the administration of the molluscicide and the necessity of structures at the bottom of the lake to contain the chemicals. Another attempt at removing invasive mussels occurred in 2016, when divers physically scraped a comparatively small area of rocks clean of mussels in Lake Michigan (Bootsma, unpublished). This method was also successful in removing the invasive mussels but is too labor intensive to apply to a larger area of the lake. With these few attempts at removing dreissenid mussels from the Great Lakes, there remain large knowledge gaps in other mussel removal methods, and how the benthic ecosystem might respond to larger scale removal of this dominant benthic organism.

The research described in this thesis fills some of these knowledge gaps by implementing a new method for dreissenid removal and observing the response of the nearshore benthic community. The goal of this project was to further our understanding of the relationship between dreissenids, benthic algae, and macroinvertebrates in the Lake Michigan nearshore zone. The response of the benthic community to mussel removal was compared among a site that has been mussel-free for six years, a site that has been mussel-free for one year, and a control site. I hypothesize that each of the three sites will have different densities of dreissenid mussels, due to the repopulation time, therefore, I predict there will be different levels of nutrients and habitat available to the algae and non-dreissenid benthic invertebrates. Where there are more mussels, I predict there will be increased available nutrients, higher algal productivity, and higher benthic invertebrate populations. Where there are little to no dreissenid mussels after removal, I predict there will be lower nutrient availability, lower algal production, and less non-dreissenid benthic invertebrates due to the loss of mussel excretion and habitat. The second chapter focuses on dreissenid-altered nutrient dynamics and the resulting benthic algae production and community composition in the presence and absence of dreissenid mussels. We

also explored algal phosphorus requirements and growth rates dependent on varying irradiance and phosphorus concentrations for Lake Michigan benthic algae. The third chapter focuses on mussel recolonization, non-dreissenid macroinvertebrate community composition, and energy flow in response to dreissenid removal. Both of these two chapters are independent and include motivation, methods, results, and discussions, with a chapter 4 summary integrating all results.

General Methods

Mussel Removal

Dreissenid mussels were removed using a benthic barrier membrane on a rocky nearshore reef in Good Harbor Bay, Lake Michigan near Sleeping Bear Dunes National Lakeshore (SLBE) outside of Leland, Michigan (Figure 1). The benthic barrier membrane covered a 140 m^2 area and was deployed by University of Wisconsin – Milwaukee (UWM) divers in July 2021 in conjunction with the National Park Service (NPS)

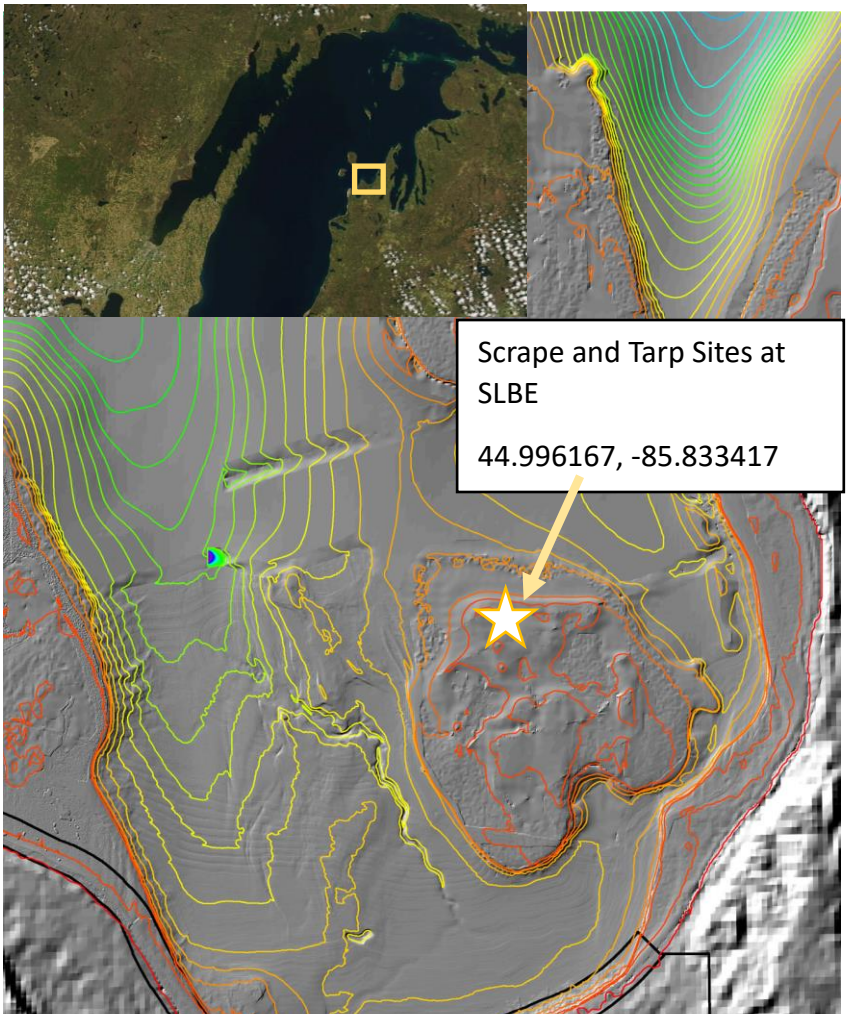

Figure 1. Experimentation site where mussels were removed via physical scraping (2016) and benthic barrier membrane (2021). The site is on a rocky reef in Good Harbor Bay near Sleeping Bear Dunes National Lakeshore at a depth of 10 meters. Bathymetric map credit to the National Park Service.

and Michigan Department of Natural Resources (MDNR). The membrane, or tarp (Figure 2), was removed in September 2021, after being deployed for slightly less than two months. The tarp site is located at a latitude of 44.996167, and a longitude of -85.833417 (Figure 1). This site (SLBE) is an ideal location as it is part of a long-term monitoring project and is home to a Great Lakes Observing System (GLOS) buoy which measures the water temperature at various depths, surface turbidity, pH, dissolved oxygen, wave height, and weather conditions. This site is also near the 2016 scraped mussel removal site and it is on a rocky reef at approximately 10 meters in depth.

Sample Collection

Samples at SLBE were collected four times throughout the 2022 sampling season: May, July, August, and October. The control and tarp sites were sampled during each the four sampling trips, while the scrape site was only sampled in July, August and October. Triplicate benthic algae and invertebrate samples were collected by divers from the tops of rocks on the mussel-free site. The divers placed a 0.04 m^2 quadrat on top of a representative rock of the site where there was a relatively flat, upward facing surface with an area at least twice as big as the quadrant, and carefully scraped the samples into a Whirl-Pak® bag. In the same way, algae samples were collected in July on the tarp site at the edge (0 meters from the perimeter), two meters from the perimeter, and towards the center of the site at 4 meters from the

Figure 2. Benthic Barrier membrane used to exterminate dreissenid mussels.

perimeter of the tarp site/control site border. This was done to determine whether proximity to the surrounding mussel bed might affect the abundance and P content of benthic algae. Triplicate samples of algae on the control site were plucked off the hard substrate (rocks and mussels) inside the $0.04m^2$ quadrat, collected with a small net, and carefully placed in a Whirl-Pak® bag, ensuring no algae floated away. The algae were collected seperately from the mussels and invertebrates on the control site to avoid any phosphorus uptake by algae that might occur if they were temporarily strored in the same sample bag as mussels. Triplicate samples of mussels and benthic invertebrates on the control site were then scraped from the same area from which the algae had been collected.

Water was collected from both the surface (1 m) and near-bottom (1 m above bottom) of the water column using a Niskin bottle from the boat. The water samples were placed in acid-washed and de-ionized water-rinsed 4-liter high density polyethylene bottles and stored in a cooler. After returning to land, the water samples were filtered onto three separate glass fiber filters (GF/F) for analysis of particluate phosphorus (PP), carbon and nitrogen stable isotopes, and chlorophll-*a*. Some filtered water from each depth was placed in 500 mL acid-washed, de-ionized water-rinsed bottles for analysis of total dissolved phosphorus (TDP) and soluble reactive phosphorus (SRP). After filtering, filters for stable isotope and PP analyses were placed in a container with desiccant and the filters for chlorophyll-*a* anlysis were wrapped in aluminum foil and placed in the freezer. Algae, benthic invertebrates, and mussel samples were frozen until the return to the lab in Milwaukee and chlorophyll-*a* analysis samples were stored frozen and kept in the dark to prevent the degredation of chlorophyll-*a*. Tests on subsamples indicated that freezing did not significantly affect the particulate P content of the algae. All samples were taken back to the UWM School of Freshwater Sciences (SFS) for futher analyses.

Sample Analysis

The benthic algae, mussel, and invertebrate samples collected at SLBE were analyzed at SFS. When the samples were thawed, the benthic invertebrates, algae, and mussels were separated. Benthic invertebrates were taxonomically sorted; isopods and amphipods were identified to the order, oligochaetes to the subclass, and chironomid to the family. Each taxon was counted and placed in separate vials. Algae samples were rinsed with filtered lake water and a sub-sample was used for algal community composition identification. Both the algae and benthic invertebrate samples were then freeze-dried in darkness. Dreissenid mussels were counted and the length was measured with a ruler to the nearest 1 mm.

SRP analysis was completed using the method of (Stainton et al. 1977). A standard curve with eight concentrations of orthophosphate (0, 1, 2.5, 5, 10, 20, 50, 100 μg L^{-1}) was created and 15 mL of of each filtered sample was added to acid-washed scintillation vials. A mixed reagent of freshly made ascorbic acid and pre-made antimony molybdate were combined to create a 4:1 ratio of anitimoney-molybdate : ascorbic acid. This mixed reagent was added to each standard and sample. After 30 minutes to allow for color development, the standards and samples were analyzed on a spectrophotometer at 885 nm. TDP samples were digested by adding 62.6 μL 4N H_2SO_4 and 62.6 μL 30% H_2O_2 to 15 mL of the sample in a quartz tube and then photo-oxididized for 2 hours, after which SRP was measured as described above (Stainton et al. 1977).

After freeze drying, algal samples were weighed to measure biomass to the nearest 10 mg. They were then homogenized into a powder using a blender and a mortar and pestle. Once powderized, sub-samples of homogenized algae from each sample were weighed for paticulate phosphorus, stable isotopes, and chlorophll-*a* analysis. Algae PP was digested by combusting 4-

7 mg in a muffle furnace at 550°C for 1 hour, followed by heating at 105°C for 2 hours with dilute hydrochloric acid, and then analyzed for SRP (Stainton et al. 1977). Standards were made by adding 2 mL of 1N HCL and 10 mL de-ionized water into 6 standard vials. They were spiked with phosphate at concentrations of 0, 5, 10, 20, 50, and 100 μg L^{-1}.

The taxonomically sorted benthic invertebrates and dreissenid mussel tissues were homogenized using a mortar and pestle. If there was enough biomass, stable C and N isotopes were analyzed for each of the sample types. Before analysis, algal tissue was weighed out, placed on a GF/F filter and wetted with 5% hydrochloric acid to remove any inorganic carbon. The acid was in contact with the algal tissue for 2 minutes before being triple rinsed with de-ionized water. Benthic invertebrate and mussel tissue was weighed into small tin capsules, compressed into a small cube, and kept in a 96-well plate stored in a desiccator until analysis. Post-acidified algae filters were freeze-dried and wrapped in a flat tin disc, folded into a compressed cylinder, and placed into the 96-well plate in the desiccator. In the same way, water column filters were wrapped in tin discs in preparation for analysis. After the samples were prepared, five acetanilide standards ranging from 0.1-1.1 mg were prepared by weighing the acetanilide and wrapping it in tin cups. The samples and standards were then run on a Finnigan MAT delta S SIR-MS isotope ratio mass spectrometer (IRMS) fitted with a Carlo Erba NA 1500 NCS elemental analyzer and Conflo IV interface. ^{13}C:^{12}C ratios are measured relative to PDB (Pee Dee Belemnite) carbonate standard, and reported as the per mil (‰) difference of sample ratio to standard ratio. ^{15}N:^{14}N ratios are relative to the ratio of air, reported as the per mil (‰) difference of sample ratio to standard ratio (Equation 1).

$$\delta^{13}C \text{ or } \delta^{15}N = \left(\frac{R_{sample}}{R_{standatd}} - 1\right) x1000 \quad (1)$$

Where R = $^{13}C/^{12}C$ for $\delta^{13}C$ and R = $^{15}N/^{14}N$ for $\delta^{15}N$.

Chlorophyll-*a* was analyzed within two weeks of sample collection. Under the illumination of red-light, filters from water column samples were ground up in 10 mL of extraction fluid (68% methanol, 27% acetone, 5% distilled de-ionized water). Benthic algae samples were weighed out (4-7 mg) and combined with 10 mL of extraction fluid. The samples remained in extraction fluid in the freezer overnight (24 hours) to allow for complete chlorophyll extraction. After centrifuging the samples at 3000 rpm for 5 minutes, 5 mL of supernatent was pipetted into a quartz fluorometer tube and the fluoresence was measured on a Turner Designs fluorometer, model 10-000.

CHAPTER 2: DREISSENID EFFECTS ON BENTHIC ALGAL COMMUNITY COMPSITION, PHOSPHORUS CONTENT, AND PRODUCTIVITY

Abstract

After years of nutrient loading reductions in Lake Michigan, benthic algae, especially *Cladophora sp.*, have been once again growing at nuisance levels similar to those observed in the 1960s and 1970s. Apparent causes include increased water clarity and increased nutrient retention in the nearshore, largely promoted by the invasive quagga mussel (*Dreissena rostriformis bugensis*). In this study, we examined whether dreissenid mussels are a source of phosphorus to benthic algae. From these findings, a potential solution for managing nuisance benthic algae production was investigated. After removing dreissenid mussels over a large 140 m^2 area in Good Harbor Reef, Lake Michigan, samples of benthic algae were collected at mussel-free sites and control sites over the course of a six-month period. Measurements included benthic algal biomass, productivity, algal community composition, phosphorus (P) content, and stable Carbon (C) and Nitrogen (N) isotope ratios. While algae biomass on the mussel-free site remained comparable to that on the control site, algal internal P content and productivity were significantly lower on the mussel-free site. There were also major differences between the algal community composition between sites, with diatoms usually being more dominant on the mussel-free site.

In addition to measuring benthic algal production *in situ*, laboratory experiments were conducted to measure benthic algal growth rates over dissolved phosphorus and light gradients. Results of these experiments were used to parameterize the algal growth response to irradiance and internal tissue P content. Productivity measurements from *in situ* and lab experiments were comparable and revealed that Lake Michigan benthic algae have a minimum internal cell P quota (the minimum required to support net growth) that is lower than previously reported values for

Cladophora cultures. We used *in situ* and lab measurements to parameterize a benthic algal growth model similar in structure to previously developed *Cladophora* growth models and explore the response of the benthic algal community to changing light and nutrient conditions caused by dreissenid mussels. These results demonstrate the importance of dreissenid enhanced nutrient cycling for supporting nuisance algal growth and can be used to explore the effects of any future dreissenid removal efforts on nuisance benthic algae.

Introduction

Dreissenid mussels have changed the benthic structure and the fundamental functioning of Lake Michigan and other Great Lakes (Hecky et al. 2004; Ward and Ricciardi 2007). Dreissenid mussels are efficient filter feeders and mature individuals are capable of filtering greater than one liter of water every day (Horgan and Mills 1997). Due to their filtering capabilities and abundance, dreissenid mussels are a major sink for energy and nutrients (Hecky et al. 2004; Bootsma and Liao 2013; Brothers et al. 2016). This high clearance rate transfers the particulate matter from the pelagic water column to the benthic and profundal zones of the lake (Karatayev et al. 1997; Chapra and Dolan 2012), resulting in higher water clarity and lower

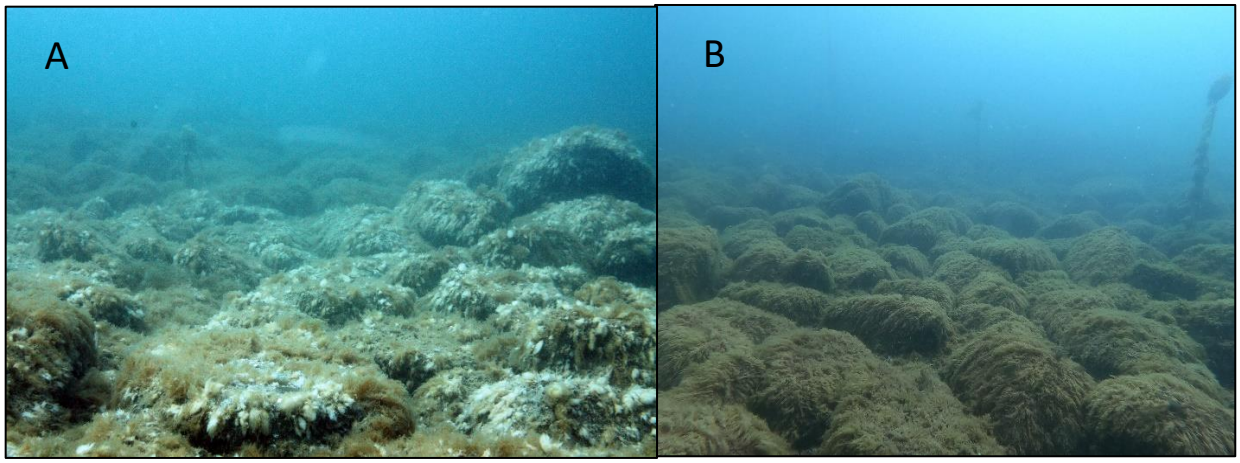

Figure 3. Time lapse camera images at Good Harbor Reef, Sleeping Bear Dunes National Lakeshore, Leland, Michigan, comparing *Cladophora* abundance in early summer (A) and late summer (B) at a depth of 10 meters.

concentrations of phytoplankton and total phosphorus (P) in the water column (Madenjian et al. 2002; Mida et al. 2010; Vanderploeg et al. 2010; Higgins and Vander Zanden 2010).

One of the most important nutrients affected by invasive mussels is P. Typically, phosphorus is the nutrient that limits algal growth in freshwater ecosystems (Schindler 1977; Blomqvist et al. 2004) and increased phosphorus causes an increase in algal growth (Schindler 1974). When there are excess nutrients such as phosphorus, the consequential excess algal growth is naturally followed by the death of large amounts of algae. The decay of algae creates higher oxygen consumption and potential hypoxic or anoxic conditions. The Great Lakes Water Quality Agreement (GLWQA) was established in part to combat this issue (Bruce and Higgins 1978; Dodds et al. 2009). Since then, there has been a gradual decline in allochthonous P loading into Lake Michigan, and total P concentration in the lake has been below target levels for decades (Dove and Chapra 2015).

Today, Lake Michigan is facing a different problem: oligotrophication, or the loss of nutrients. Offshore waters have become increasingly nutrient-limited and there are fewer available nutrients. Because of the decreased nutrients and increased phytoplankton grazing by dreissenid mussels, there is less phytoplankton in the pelagic zone compared to pre-dreissenid invasion. This can harm fish populations higher up in the food web (Shen et al. 2018); Lake Michigan is already seeing a decline in pelagic fish biomass (Bunnell et al. 2009). Oligotrophication in the pelagic zone of Lake Michigan is caused by the water column clearance by dreissenid mussels (Bunnell et al. 2009; Higgins and Vander Zanden 2010). Since the invasion, mean areal planktonic community abundance in the water column has declined [phytoplankton (-35% to -78%) and zooplankton (-40% to -77%)] (Higgins and Vander Zanden 2010), and the total production in the deep chlorophyll layer (DCL) has been reduced by half

(Fahnenstiel et al. 2010; Bockwoldt et al. 2022). Invasive mussels have increased the dissolved : particulate phosphorus ratio in the hypolimnion, and destroyed the profundal nepheloid layer in Lake Michigan (Mosley and Bootsma 2015). This demonstrates the incredible ability of quagga mussels change the fundamental processes of nutrient cycling as they remove particulate nutrients from the water column above them up to 11 times faster than the natural settling rate of phosphorus (Mosley and Bootsma 2015).

While profundal quagga mussels have decreased the pelagic production, nearshore quagga mussels have done the opposite in the littoral benthic zone of Lake Michigan (Brothers et al. 2016). Typically, nutrients are more abundant in the nearshore compared to the offshore (Pothoven and Vanderploeg 2020); therefore, the offshore mussels utilize a higher percentage of their filtrate for growth. After mussels consume phosphorus from the water column, the nutrients are either used by the mussels for reproduction and growth, egested as particulate P, or excreted as dissolved P (Bootsma and Liao 2013). When there is a relative abundance of nutrients like there is in the nearshore compared to the offshore, the mussels use a lower percentage of the total amount of filtered nutrients for survival and growth, and they egest more biodeposits into the benthic ecosystem which are available for other organisms to use (Hecky et al. 2004). Furthermore, most of the allochthonous nutrients enter the system via rivers and streams (Mooney et al. 2020). As particulate nutrients enter the Lake Michigan nearshore water, they are consumed by the dreissenid mussels, excreted as dissolved nutrients and egested as biodeposits, and recycled by other benthic organisms, becoming retained in the nearshore much longer before eventually making it into the offshore pelagic water; this process is known as the nearshore phosphorus shunt (Hecky et al. 2004). This is yet another reason why the energy in

Lake Michigan is being transferred from the pelagic water column into the nearshore benthic zone (Higgins and Vander Zanden 2010; Brothers et al. 2016; Vadeboncoeur et al. 2021).

The combination of increased nutrients in the littoral benthos and increased water clarity has contributed to another problem in Lake Michigan. Benthic algae, particularly *Cladophora sp.*, has presented significant management problems (Vadeboncoeur et al. 2021). *Cladophora* is a native filamentous alga (Figure 3) that had grown at nuisance levels in the 1960's and 1970's due to high levels of phosphorus loading from agricultural fields, industry, and wastewater treatment plants. Since 1978 when the Great Lakes Water Quality Agreement (GLWQA) set targets for phosphorus loading into the Great Lakes (Bruce and Higgins 1978), benthic algal production has greatly decreased (Dove and Chapra 2015). However, dreissenid mussels have altered the nutrient dynamics and water clarity so that *Cladophora* and other benthic algae are once again growing at nuisance levels (Auer et al. 2010; Tomlinson et al. 2010; Turschak and Bootsma 2015; Francoeur et al. 2017; Bravo et al. 2019; Vadeboncoeur et al. 2021).

Cladophora growth is either light limited or nutrient limited. Between the high water column clearance rates and high P excretion rates, dreissenids solve both of those issues, giving *Cladophora* the resources it needs to be highly productive (Hecky et al. 2004; Tomlinson et al. 2010; Kuczynski et al. 2016; Vadeboncoeur et al. 2021). Since the proliferation of dreissenid mussels, there have been higher P concentrations in the nearshore benthos (Hecky et al. 2004; Higgins and Vander Zanden 2010; Dayton et al. 2014) and dreissenids have caused greater water clarity; their substantial filtration levels allow light to penetrate to greater depths in the nearshore zone (Mida et al. 2010). When the benthic algae use these resources and biomass reaches a certain threshold, it can slough off the substrate (Kuczynski et al. 2022).

Nuisance algae becomes an issue when it sloughs off from the lake bottom or dies and decays; which can lead to problems such as beach fouling (Kuczynski et al. 2016), water intake clogs (Rosaen et al. 2012), and the formation of algal mats (Byappanahalli and Whitman 2009; Chun et al. 2013). The algal mats create suitable anoxic growing conditions for the bacterium *Clostridium botulinum* (Whitman et al. 2003; Yule et al. 2006). The bacterium can produce a neurotoxin that infects many benthic-feeding fishes and piscivorous birds through a proposed pathway of *Cladophora* to non-dreissenid benthic invertebrates to fish to birds, creating avian botulism die-offs in areas around the Lake Michigan coasts (Byappanahalli and Whitman 2009; Lafrancois et al. 2011; Chun et al. 2013; Essian et al. 2016; Shen et al. 2022). Dreissenids can cause higher *Cladophora* productivity and consequently larger and denser *Cladophora* mats.

These undesirable effects of increased benthic algae production prompted scientists and managers to search for a possible solution to the problem, as they did with the initial GLWQA (Bruce and Higgins 1978). The historical solution was to limit P loading into the lake as there was excess P throughout Lake Michigan. Since phosphorus is often the limiting nutrient in freshwater systems (Schindler 1974), these reductions in P loading resulted in reduced algae production. Today, some have suggested that further lowering the loading of phosphorus may once again be the optimal solution (Zhou et al. 2021). Others disagree about lowering P loading into the lake because it would likely intensify the ecological stress on the already nutrient limited pelagic water (Bootsma et al. 2015). This represents a “dual challenge”; nuisance levels of production in the nearshore zone of Lake Michigan concurrent with offshore waters increasingly becoming more oligotrophic, greatly disrupting the food web (Zhou et al. 2021).

Proponents of more stringent P loading restrictions recognize that the pelagic food web has gone into disarray, but argue that reducing local P loading is the only way to stop the

nuisance algal growth in the nearshore (Zhou et al. 2021). Others argue that the risk of further decimating the offshore food web is too great. When the initial P loading restrictions were put in place, they benefited the entire ecosystem. However, there may not be an ideal P loading target which is ideal for both the nearshore and pelagic zones (Bootsma et al. 2015).

Even as dreissenids are concentrating many of the nutrients in the nearshore lake-bottom (Hecky et al. 2004; Higgins and Vander Zanden 2010; Brothers et al. 2016; Zhou et al. 2021), *Cladophora* production is 44% lower than it was prior to the introduction of P loading restrictions through the GLWQA (Kuczynski et al. 2016). However, the *Cladophora*-colonizable depth has increased by a factor of 5, making the growing potential of *Cladophora* increase sixfold since the 1980s (Kuczynski et al. 2016). Because of the increased water clarity, there is still nuisance benthic algae growth, even though there is less phosphorus loading into Lake Michigan. This suggests that water clarity is indeed one of the driving factors in the proliferation of nuisance *Cladophora*. However, removing a small area of dreissenid mussels likely will not influence the water clarity of an area. In this study, I will focus on the nutrient dynamics at the near-bottom of the lake, not because water clarity is irrelevant in *Cladophora* growth, but because small scale dreissenid removal projects will have a greater impact on the nutrient availability to benthic algae if there is any impact at all.

The large-scale changes in the Great Lakes caused by the dreissenid mussel invasion are well-studied. However, the dreissenid mussel and benthic algae relationship is complex and many questions remain. The major question about this relationship is about the spatial scale in which mussels promulgate the growth of benthic algae because of biodeposit production. During quiescent conditions, dissolved P accumulates at the near-bottom of the water column (5-15 cm), forming a concentration boundary layer (CBL) (Dayton et al. 2014). However, this CBL created

by mussels was observed when the entire study site was covered with mussels. It is unknown whether a CBL would extend over an area where mussels are removed, even if mussels surround the entire mussel-free site. Turbulence also greatly effects this CBL, disrupting and diluting the dissolved P throughout the water column with very minimal wave activity (Dayton et al. 2014), making the formation of a CBL a relatively rare occurrence. Even so, these relatively few occurrences of the CBL can provide *Cladophora* with enough P to support their growth for long periods of time (Dayton et al. 2014). *Cladophora* is adept at storing P in times of abundance and using it for growth when there is less P availability; for example, *Cladophora* that is P-stressed which is exposed to external dissolved P concentrations of 50 μg L^{-1} for 14 hours can absorb enough P to increase their biomass by a factor of 10 (Auer and Canale 1982a). Therefore, it is important to explore whether the CBL produced by the dreissenid mussel population is localized directly over the mussel beds, or if it can be a regional effect, encompassing areas where mussels are removed. In this chapter, I present the results of a quagga mussel removal experiment designed to explore the relationship between quagga mussel nutrient recycling and benthic algal P content and productivity. I hypothesize that removing dreissenid mussels will create less P availability at the near-bottom of the nearshore and will decrease nuisance algae production in the nearshore benthos due to the loss of the relative P-rich excrement of the mussels.

Methods

In Situ Incubations

In situ incubations were conducted three times throughout the sampling season at Sleeping Bear Dunes National Lakeshore (SLBE) on the mussel-free tarp site and the control site where mussels were present. Each set of incubations consisted of one clear chamber and one dark chamber to measure both oxygen production and respiration (Figure 4). The oxygen

Figure 4. *In situ* incubations at Good Harbor Bay near Sleeping Bear Dunes National Lakeshore at a depth of 10 meters. We used pairs of light and dark benthic incubation chambers to measure the oxygen production and consumption by the benthic algae.

production and consumption were measured by fitting the incubation chambers with dissolved oxygen probes (PME mini DO_2T logger) which measures, displays, and records the dissolved oxygen concentration of the water in the incubation chamber at one-minute-intervals. Throughout the duration of the incubations, an Odyssey PAR (photosynthetically active radiation) logger was deployed to measure the light levels that the algae were exposed to during photosynthesis. Algae used for incubations were collected from the substrate. Divers used their best judgement to collect an amount on the substrate equivalent to the area of the incubator (0.0071 m^2). After the algae were extracted from the lake bottom, they were carefully placed inside the incubation chamber, which was then capped to avoid any water from the outside environment from entering. Each incubation lasted at least 14 minutes, and the water was gently stirred every 3-5 minutes to minimize algal settling, self-shading, and the formation of diffusive boundary layers. After the incubations were completed, the algae were transferred to a Whirl-pak® bag and frozen within 3 hours.

Laboratory Incubations

In addition to *in situ* measurements, a series of laboratory experiments were conducted to determine the effects that irradiance and phosphorus content have on algae photosynthesis.

Benthic algae used for these incubations were collected at two locations near Milwaukee (43.06112°N, -87.86413°W and 43.09183°N, -87.87068°W), and the SLBE site. Algae were collected on two different dates (September 14, 2022, and September 26, 2022) by divers who extracted algae from the lake-bottom. The algae were placed in a mesh bag underwater, stored in a cooler on the surface, and were immediately taken back to our laboratory at the School of Freshwater Science (SFS) (Milwaukee, WI, USA). Once in the lab, algae were separated into nine 1000 mL Erlenmeyer flasks filled with filtered Lake Michigan water, each with roughly 50 mg wet weight (WW) algae. Seven Erlenmeyer flasks were spiked with various levels of phosphate (0, 0.5, 1.0, 1.5, 2.0, 5.0, 10.0, 50.0 μg L^{-1}) and two were not spiked. One of these was used as a control and incubated immediately upon returning, and the other was incubated two days after collection in an attempt to produce as low a P content as possible. While bubbling to mix the water and provide oxygen to each flask, they were placed in a refrigerator to keep the water temperature between 8-15°C and the spiked flasks sat for 14 hours to give the algae time to take up the phosphorus. To determine the photosynthetic response to light, algal samples from each site were separated into five clear 500mL bottles and placed linearly in front of a 60-watt LED floodlight (Figure 5) for two hours. Irradiance was measured

Figure 5. Laboratory incubations using algae samples gathered from the field.

with a Biospherical™ optical sensor and the change in dissolved oxygen was measured using a Fisherbrand™ Traceable Portable Dissolved Oxygen Meter. The bottles were gently inverted every 15 minutes to minimize algal settling and the formation of diffusive boundary layers.

Benthic algae were collected from the mussel-free tarp site and a control site at SLBE in October 2022. The incubations of algae from each of the two sites were conducted in the same manner as the algae samples collected near Milwaukee.

Algae Growth Rate

Once the laboratory incubations were complete and the algae used for the *in situ* incubations were brought back to the SFS lab, the algae were freeze-dried, and the dry weight (DW) biomass was measured. After weighing, each sample was ground up and homogenized with a mortar and pestle. Algal internal P was measured using previously described methods in the general methods section (Chapter 1), and the carbon content was measured in 17 random samples from algae used for the lab experiments using a Carlo Erba NA 1500 NCS elemental analyzer. The carbon content in the algae was averaged from the random samples and the average carbon content was used to calculate the net specific growth rate (day^{-1}) from equation 2.

$$\mu_{net} = \frac{P^b}{C} * \theta \qquad (2)$$

Where μ_{net} is the net specific growth rate ($days^{-1}$), P^b is the net photosynthetic rate normalized to biomass ($mgO_2\ mgDW^{-1}\ day^{-1}$), C is the mean measured initial carbon content (20.6 ± 0.73% C), and θ is the photosynthetic quotient (12 mg C/ 32 mg O_2).

Once the net specific growth rates and irradiance were measured, production-irradiance (PI) curves were created for each experiment, and fit to a two-parameter model modified from Webb et al. (1974), Graham et al. (1982), and Bockwoldt et al. (2022):

$$\mu_{net} = \hat{\mu}_{net} * (1 - e^{-\alpha I}) \quad (3)$$

where $\hat{\mu}_{net}$ is the maximum net specific growth rate (day^{-1}), α is the initial slope of the PI curve and I is the irradiance. This model was fit to the data of each experiment by minimizing the sum of squares.

Results from the *in situ* incubations and optimal-light lab incubations (300-600 mmol photons m^{-2} s^{-1}) (Graham et al. 1982) were combined and fit to the Droop model (Droop 1968) (Equation 4), which compares the internal phosphorus content (Q, % mass) to the net specific growth rate (day^{-1}).

$$\mu_{net} = \hat{\mu}_{net} \left(1 - \frac{Q_0}{Q}\right) \quad (4)$$

The model was used to estimate the minimum cell quota (Q_0), which is the point below which there is no net growth, and the maximum growth rate ($\hat{\mu}_{net}$) which occurs as the algae becomes saturated with phosphorus.

Q_0 and $\hat{\mu}_{net}$ were calculated by using the linearized version of the Droop model (Droop 1973):

$$\mu_{net} Q = \hat{\mu}_{net}(Q - Q_0) \quad (5)$$

When $\mu_{net}Q$ is plotted against Q, they slope of the line is the maximum growth rate ($\hat{\mu}_{net}$) and the x-intercept is the minimum cell quota (Q_0). Maximum respiration ($\hat{R}$) can also be calculated

using a form of equation 5 by plotting RQ against Q instead of $\mu_{net}Q$ against Q and calculating the slope.

Great Lakes *Cladophora* Model

Results from the incubations were used to update the parameters of the Great Lakes *Cladophora* Model (GLCM). The GLCM was developed by Auer and Canale (1982b), which was created to study the impact of phosphorus on nuisance algal growth near a municipal wastewater treatment plant (Auer et al. 1982b). The GLCM has been updated to better reflect the changing lake conditions and as new information about *Cladophora* growth and physiology is discovered to more accurately determine the model parameters (Tomlinson et al. 2010; Kuczynski et al. 2016, 2020, 2022). Based on data collected in this study, the GLCM parameters (specifically, maximum growth rate and minimum tissue P quota) were further updated to better reflect the multi-species benthic algal community in the lake, which may differ physiologically from the monospecific *Cladophora* cultures used to parameterize previous versions of the GLCM.

Algal Community Composition

Benthic algae were sampled from three sites near SLBE (control, scrape, tarp) using the benthic scrape technique described in the general methods (Chapter 1). Upon returning to SFS, the algae were separated from the benthic invertebrates and 20-30 mg wet weight representative sub-samples using my best visual judgement, were used for community composition identification before freeze-drying. These sub-samples, taken from each triplicate sample from each site, for a total of nine sub-samples each sampling date, were homogenized by chopping the algae using a Kuchenprofi rolling 4-blade herb cutter. The algae were then placed in a small

beaker and suspended in 5 mL of tap water. The suspended algae were stirred vigorously to homogenize the chopped algae were placed in a glass plate. The objective was not to quantitatively determine the biomass of algae in each sub-sample, but to determine the relative abundance of the various algal taxa within each sub-sample. Three photos were taken from each sub-sample (nine photos from each site) using an EVOS® XL cell imaging microscope at 400x magnification.

Diatoms and filamentous green algae were identified using descriptions from Cox (1996) and identification keys from Prescott (1954). Percent volume represented by each taxa was determined by assigning taxon-specific volumes as designated by Spaulding et al. (2021). Each diatom was given a typical volume based upon their median size found in freshwater ecosystems. Filamentous green algae were measured using the program ImageJ based on a pixel:μm ratio of 7.635:1 at 400x magnification. Algae from three images at each triplicate were combined for the total percent composition. The three triplicates from each site were averaged together to find a mean percent composition. This was done for the months of May, July, August, and October, except for the scrape site in May as algal scrapes were not collected at that site for that month.

Results

Biomass and Nutrients

Benthic algal biomass on all three sampled sites was not significantly different ($p = 0.24$) based on a two-factor (site and date) ANOVA with replication. The biomass varied seasonally ($p < 0.001$) with the highest biomass in May and the lowest biomass in October (Figure 6). Inversely, the algal internal phosphorus concentration increased on the control and scrape sites as the sampling season progressed ($p < 0.001$), while algal P content on the tarp site remained

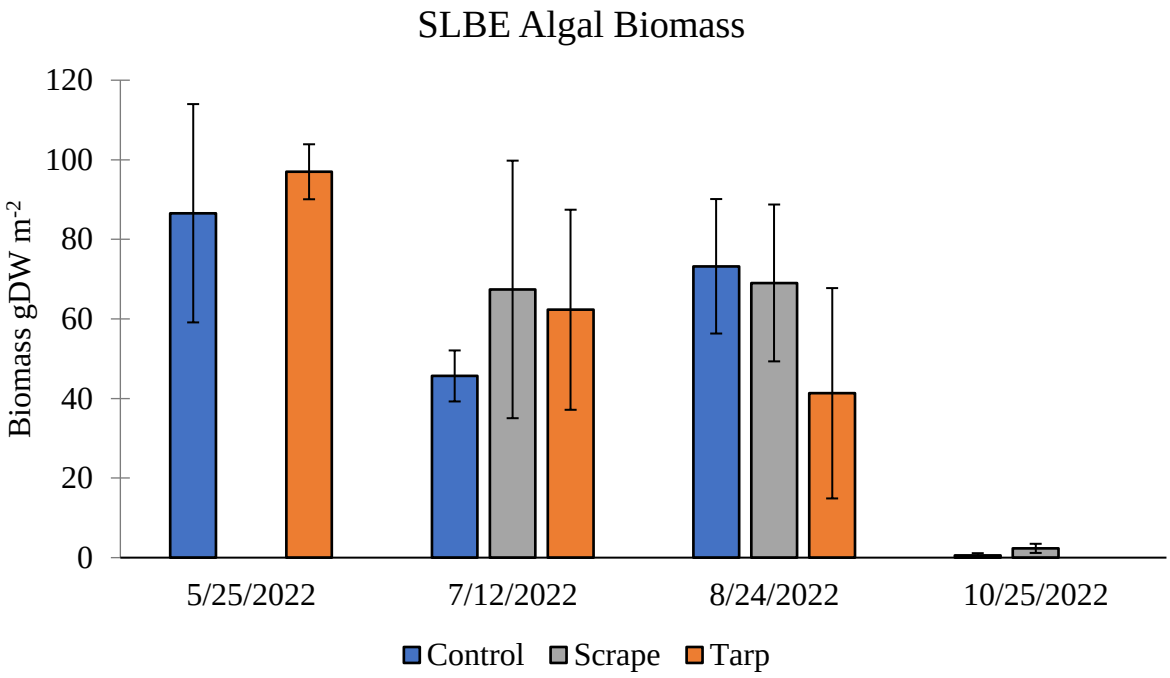

Figure 6. Algal biomass comparing a control site and two mussel-free sites at SLBE.

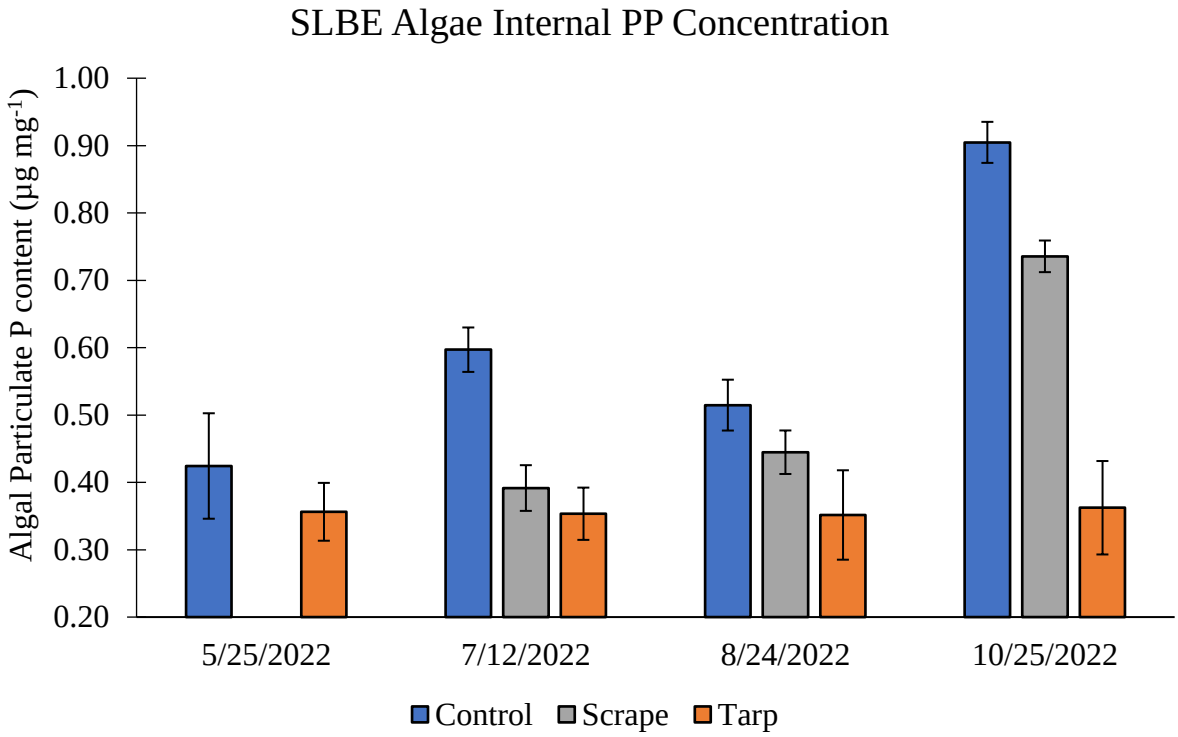

Figure 7. Algae internal phosphorus concentration comparing a control site and two sites where mussels were removed: one in 2016 via scraping by divers (Scrape), and one in 2021 via benthic barrier membrane (Tarp).

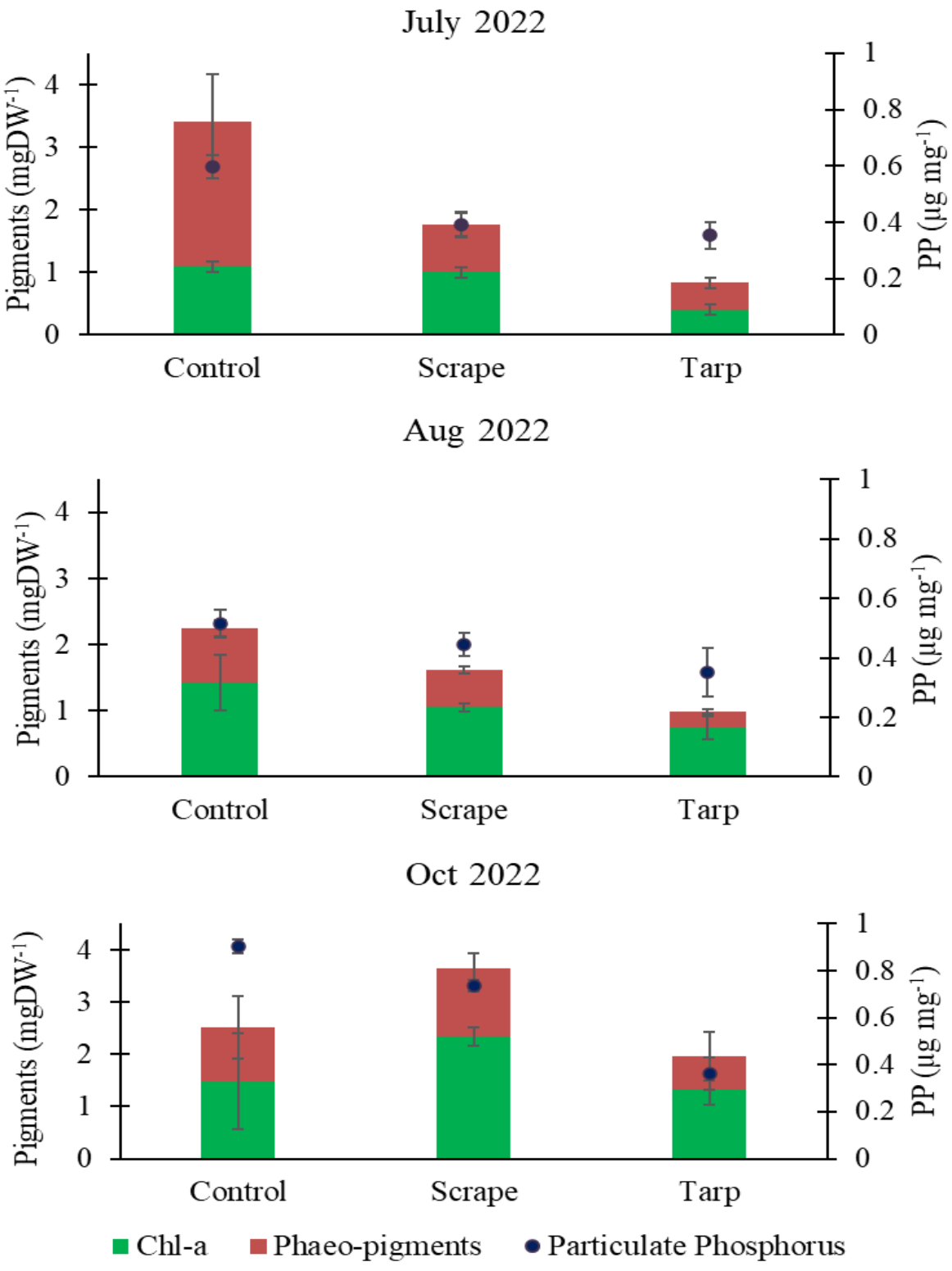

Figure 8. Chlorophyll-a, phaeo-pigments, and PP on three different sites at SLBE for each month in which chlorophyll-a was measured. Chlorophyll-a and phaeo-pigments are represented in a stacked bar graph to show both the photosynthetic and non-photosynthetic algal pigmentation.

constant throughout the season (Figure 7), and there was an interaction between the time of year and sampling site ($p < 0.001$). Algal P content was significantly different ($p < 0.01$) among sites and dates based on a two-factor ANOVA with replication. Post hoc paired t-tests indicate the control and tarp sites, and the control and scrape sites were significantly different in algal internal P concentration ($p = 0.002$, $p = 0.003$, respectively), while internal P concentrations between the tarp site and scrape site were not statistically different ($p = 0.06$).

Both the total pigmentation (chlorophyll-*a* + phaeo-pigments) were measured to confirm the validity of the chlorophyll-*a* analysis. As chlorophyll-*a* degrades, it turns into the phaeo-pigments, therefore if there were any degradation during the time of transportation or analysis and the total pigment results and chlorophyll-*a* results disagree with each other, that could be explained if both were measured. The sum of chlorophyll-*a* and phaeo-pigments (the degradation product of algal chlorophyll pigments) was statistically different between the three sites ($p<0.001$) based on a two-factor ANOVA with replication and there was a significant interaction between time of year and site ($p < 0.001$). Algae on the tarp site had lower total chlorophyll-*a* and phaeo-pigments areal concentrations than algae found on both the control and scrape site ($p = 0.002$, $p < 0.001$, respectively) based on pos-hoc paired t-tests (Figure 8). When separating the chlorophyll-*a* from the phaeo-pigments, the chlorophyll-*a* remained statistically different ($p < 0.003$) based on a two-factor ANOVA with replication, however, there was no interaction between independent variables ($p = 0.09$). The tarp algae had lower chlorophyll-*a* than the other two sites ($p = 0.02$, $p < 0.001$, comparing tarp algae to control and scrape algae, respectively). Both chlorophyll-*a* and total pigments were not statistically different between the control site and scrape site ($p = 0.2$, $p = 0.3$, respectively). These results differ from algal PP results as the internal P concentrations were statistically different between the control and both

tarp and scrape sites, whereas internal P concentrations between tarp and scrape sites were not statistically different.

Algal C:N ratios were statistically different among the three sites ($p < 0.001$) and there was an interaction between site and month of collection ($p = 0.04$), according to a two-factor ANOVA with replication. The tarp site was lower than both the control and scrape site ($p = 0.002$, $p = 0.003$, respectively) based on paired t-tests (Figure 9A). There was no difference between the scrape and control site ($p = 0.06$). There was no statistical difference between SLBE algae chlorophyll-*a*: PP ratios ($p = 0.12$) according to a two-factor ANOVA with replication (Figure 9B). Algal C:P ratios were also not statistically different between sites ($p = 0.58$) according to a two-factor ANOVA with replication (Figure 9C). A two-factor ANOVA with replication indicated a statistical difference in the chlorophyll a : C ratio ($p = 0.03$) among sites, with the scrape site being higher than both the control ($p = 0.02$) and tarp ($p = 0.02$) sites based on post-hoc paired t-tests.

Chlorophyll-*a*: C ratios were different between sites ($p = 0.03$), but there was no interaction between site and date of collection ($p = 0.72$), according to a two-factor ANOVA with replication. The tarp and control site algae were not statistically different in Chlorophyll-*a*: C ratios ($p = 0.28$), according to a post-hoc paired t-test. N:P ratios in SLBE algae were statistically different ($p = 0.001$), and there was an interaction between the independent variables ($p < 0.001$) based on a two-factor ANOVA with replication. However, the tarp and scrape site were not significantly different after from each other ($p = 0.04$) after conducting a Bonferroni correction, while the control and tarp site ($p = 0.06$) and control and scrape site ($p = 0.07$) were not different before the correction, all based on post-hoc paired t-tests (Figure 9E). Lastly, the algal carbon concentrations were significantly different among sites ($p <0.001$) with no

interaction between site and time of collection ($p = 0.17$), according to a two-factor ANOVA with replication. All three sites differed from one another with results from post-hoc paired t-tests being statistically significant for comparisons of control and tarp, control and scrape, and tarp and scrape ($p < 0.001$, $p = 0.009$, $p = 0.009$, respectively). Algal carbon and nitrogen isotope ratios were measured at each site over the course of the summer. $\delta^{13}C$ between sites were statistically different (Figure 10A) based on a two-factor ANOVA with replication ($p = 0.004$). The control site had lower ^{13}C: ^{12}C than the scrape site ($p = 0.009$) based on a post hoc paired t-test. After conducting a Bonferroni correction, the control and tarp site $\delta^{13}C$ were not significantly different ($p = 0.03$) based on a post hoc paired t-test. The tarp and scrape sites were not different comparing carbon isotope ratios in algae ($p = 0.32$). Due to the high variability of the nitrogen ratios, there were no statistical differences among sites ($p = 0.13$), according to the two-factor ANOVA with replication (Figure 10B). The results of comparisons between the three sites are summarized below (Table 1).

Algae Transect

To determine whether proximity to dreissenids might affect algal nutrient content, algae collected in July from SLBE at each of the three sampling sites were additionally compared to a transect of samples at 0, 2, and 4 meters from the tarp/control site boundary. On this sampling date, the internal P content in algae between sites differed significantly ($p < 0.001$), according to a single factor ANOVA (Figure 11). The P content in the algae transect samples were not significantly different between transect locations ($p<0.001$). When combined, the algae transect P content (0.35 ± 0.01 μg mg^{-1}) was significantly different from the control algae P content (0.60 ± 0.04 μg mg^{-1}) ($p < 0.001$), according to student's t-test. Conversely, the transect algal P content was not different from either the tarp site or the scrape site ($p = 0.4$, $p = 0.07$,

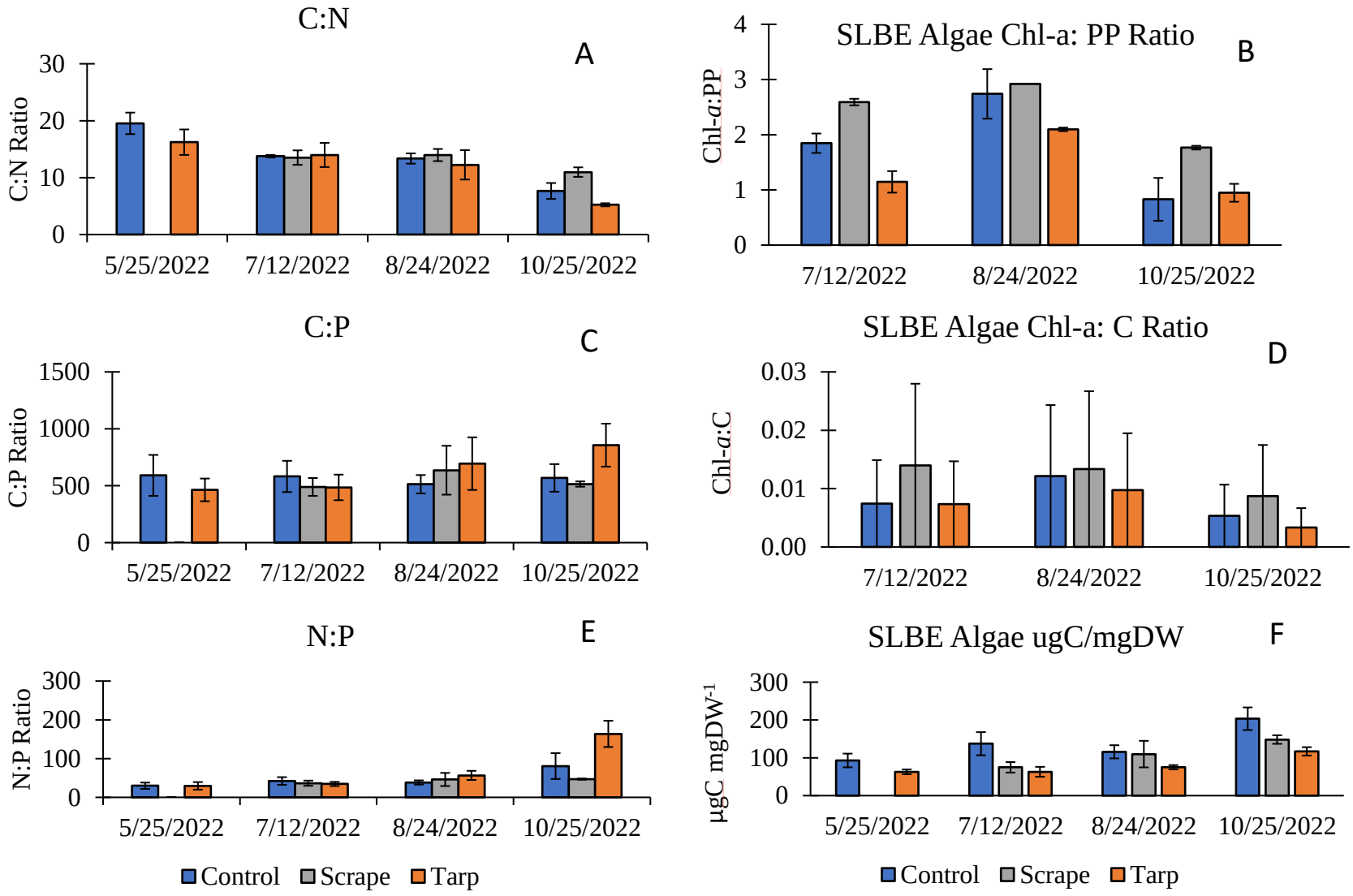

Figure 9. Nutrient ratios in benthic algae collected near SLBE throughout 2022. Includes nutrient levels of Carbon, Nitrogen, Phosphorus, and Chlorophyll-*a*.

respectively). Not only was the transect algae not statistically different from the tarp site, but each transect sample which were at 0 meters (0.34 µg mg^{-1}), 2 meters (0.36 µg mg^{-1}), and 4 meters (0.34 µg mg^{-1}) fell within the range of the range of the tarp samples on the same date (0.30 - 0.40 µg mg^{-1}), which were randomly sampled inside the tarp site area (Figure 10B).

Algae Composition

The algae which were gathered via benthic scrapes at SLBE were analyzed beyond the nutrient and isotope compositions. Benthic algae are often a mixture of dozens of diverse types of diatoms and filamentous green algae, each taxa preferring different environmental conditions and water chemistry. For this project, algae taxa were identified at each site, over the course of the sampling season. Once identified, the percent composition by volume was measured for each taxon according to sampling site and sampling date. Between the three study sites, the algal composition in terms of percent diatoms was significantly different ($p < 0.001$), according to a

Table 1. Summary of algae comparisons between sites at SLBE.

	Two-factor	Paired T-Test		
Analysis	ANOVA	Control/Tarp	Control/Scrape	Tarp/Scrape
Biomass	x	-	-	-
Internal P	✓	✓	✓	x
Chlorophyll-*a*	✓	✓	x	✓
Chlorophyll-*a* + phaeo-pigments	✓	✓	x	✓
C:N	✓	✓	x	✓
C:P	x	-	-	-
N:P	✓	x	x	x
Chlorophyll-*a* :PP	x	-	-	-
Chlorophyll-*a* :C	✓	x	x	x
µgC $mgDW^{-1}$	✓	✓	✓	✓
$\delta^{15}N$	x	-	-	-
$\delta^{13}C$	✓	x	✓	x
	α = 0.05	α = 0.017		
x = No statistical significance ($p > \alpha$)				
✓ = Statistical significance ($p \leq \alpha$)				

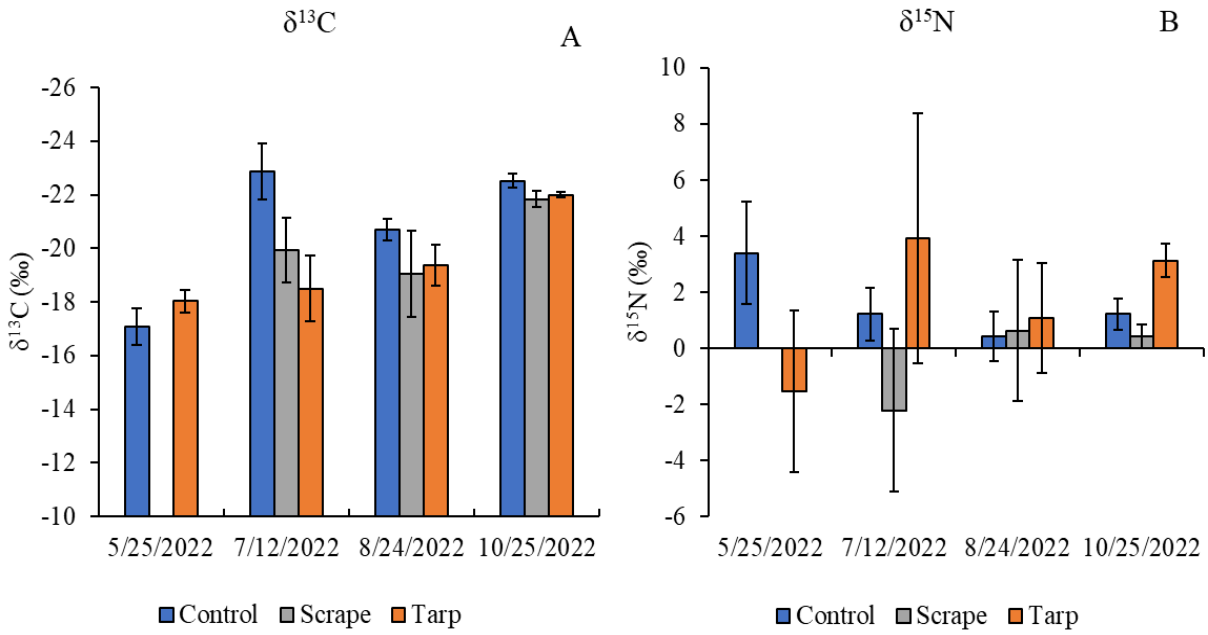

Figure 10. Carbon (A) and Nitrogen (B) stable isotope ratios from SLBE algae on three different study sites. Carbon isotopes were statistically different between sites, while nitrogen isotopes were not.

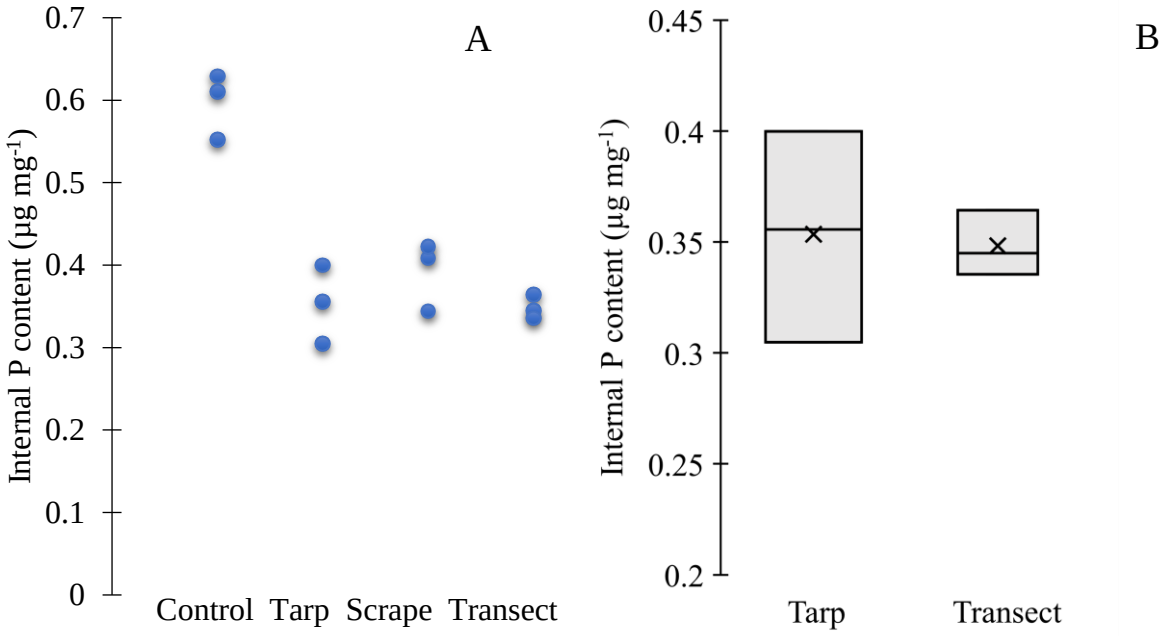

Figure 11. Internal P content from algae sampled at SLBE in July 2022. Each point represents a singular sample (A). Transect algae samples were gathered on the tarp site with increasing distance from the perimeter of the site: 0 meters, 2 meters, and 4 meters. Each transect algal sample fell within the samples that were randomly sampled on the tarp site (B).

two-factor ANOVA with replication. The mussel-free tarp site was different from both the control and scrape sites ($p = 0.002$, $p < 0.001$, respectively), based on paired t-tests. The scrape and control sites were not significantly different from each other ($p = 0.09$). Breaking down the composition by month shows a similar composition between the control and tarp sites in May. As the season progresses, the percentage of diatoms remains consistent at 75-90 percent on the

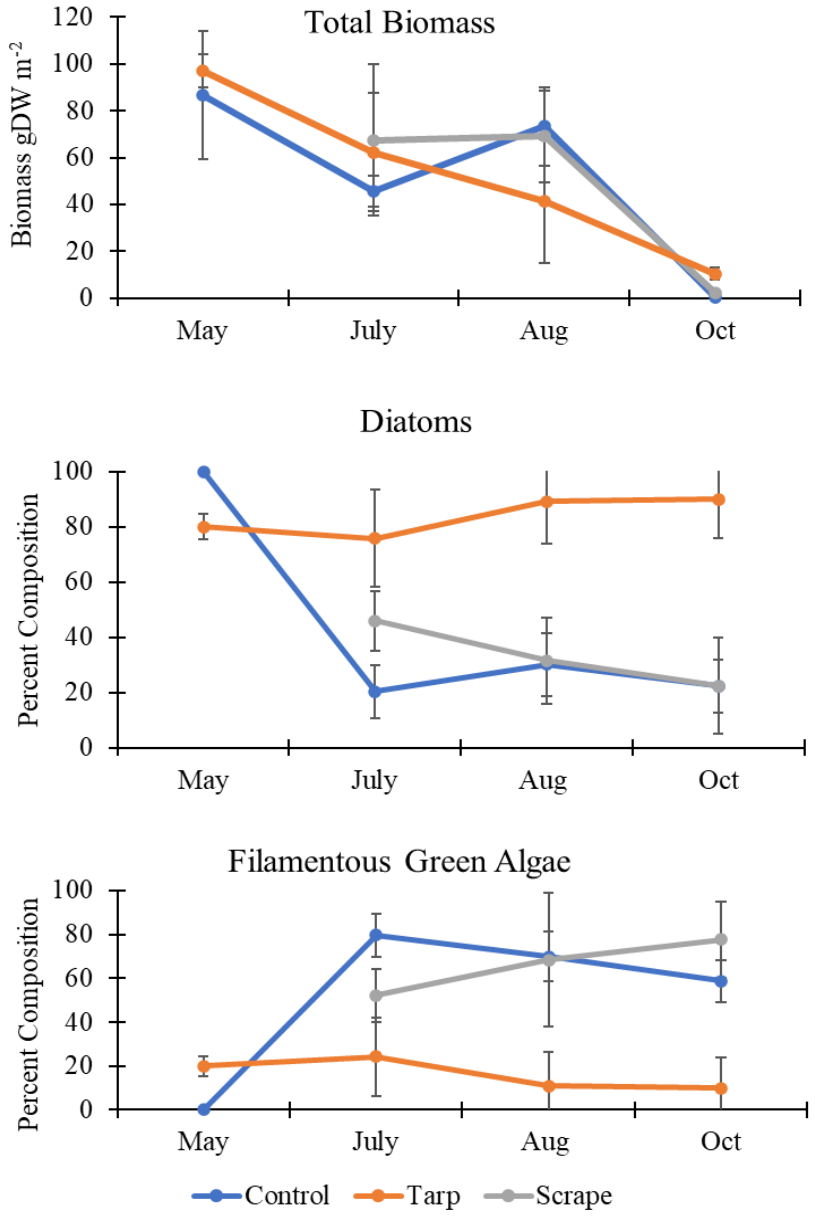

Figure 12. Algal community composition between three study sites at SLBE. Algal taxa were grouped into diatoms and filamentous green algae for the months of sample collection.

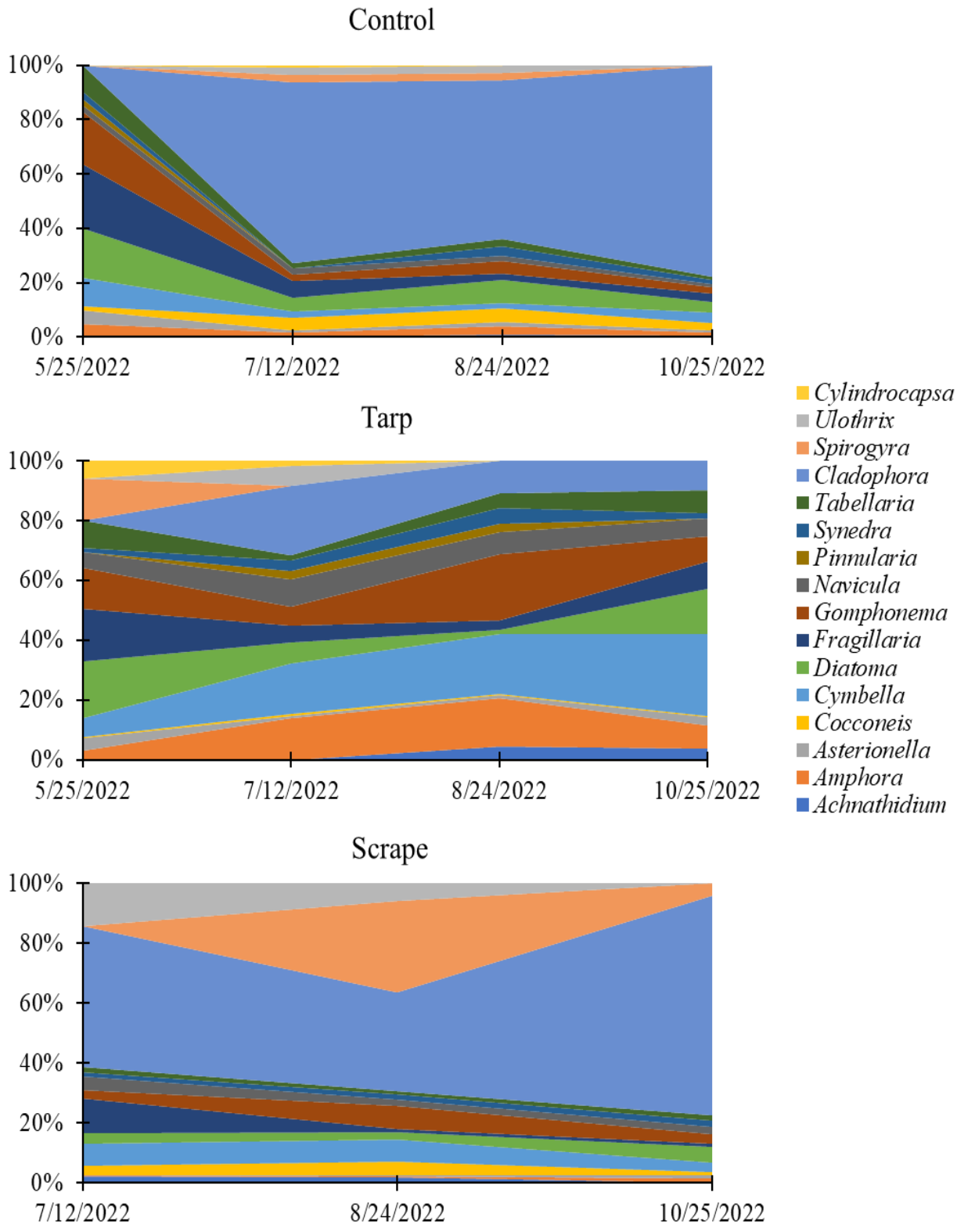

Figure 13. 100% stacked line graph showing algae composition separated by taxa during each month of the sampling season at SLBE. The taxa in the legend are ordered the same for each site from top to bottom.

tarp site, while the percentage of diatoms on the control site dramatically decreases from 100 percent to 20.4 ± 9.6 percent in July and exceeds 31 percent the remainder of the season (Figure 12). The most abundant diatoms in terms of percent volume were *Cymbella, sp., Diatoma sp., Fragilaria sp.,* and *Gomphonema sp.* Each of these taxa of diatoms nearly reached or exceeded 20 percent volume during at minimum one month during the sampling period (Figure 13, 14). Other notable diatoms which were observed in high individual numbers, but were not as abundant regarding percent volume due to the individual organisms being smaller on average were *Cocconeis sp., Amphora sp., Asterionella sp., Navicula sp., Synedra sp.,* and *Tabellaria sp. Cocconeis sp.* was always found in lower abundances on the tarp site than both the control and scrape sites (Figure 14D), *Navicula sp.* was always found in higher abundances on the tarp site compared to the control and scrape sites (Figure 14I), and all other diatoms which were identified at all three sites were not uniformly found in higher or lower abundances at one specific study site (Figure 14).

The most abundant filamentous green alga was *Cladophora sp.,* which generally increased on the control and scrape sites as the sampling season progressed. *Spirogyra sp.* was most abundant on the scrape site in July, having an abundance of 30.0 ± 21.9 percent by volume. The other notable filamentous green algae were *Ulothrix sp.* which had abundances of 12.0 ± 13.3 percent and 7.4 ± 10.5 percent on the scrape and tarp sites, respectively during the month of July, and *Cylindrocapsa sp.* which was found at 5.9 ± 8.4 percent by volume on the tarp site in May and decreased as the season progressed, not being observed at all after the month of July (Figure 14).

In Situ Incubations

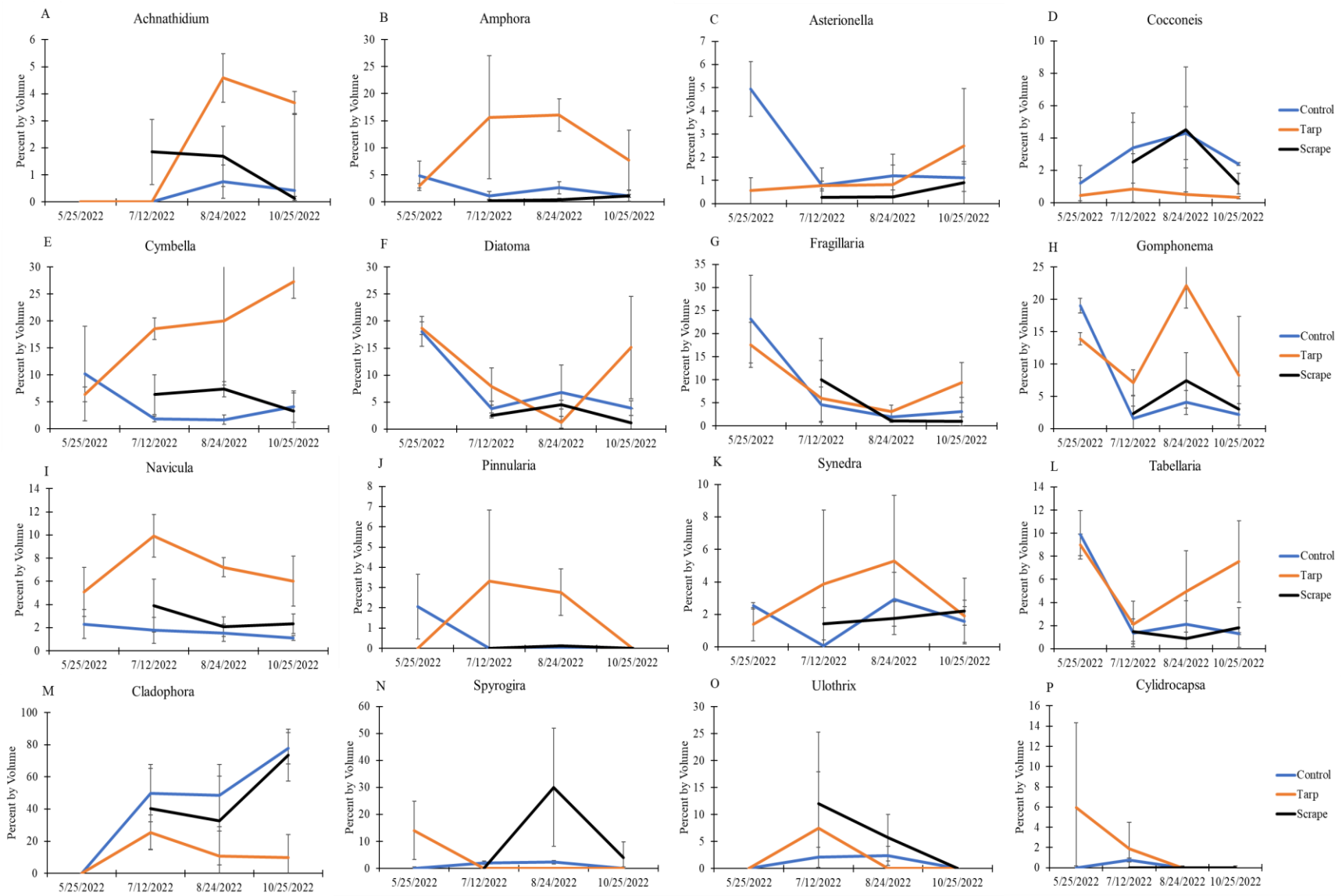

Figure 14. Percent volume of each taxa from algae collected from three sites at SLBE over the course of the sampling season.

In situ incubations were conducted during the months of July, August, and October on the mussel-free tarp site and the control site. These incubations revealed that for each month, as the internal phosphorus concentration (% mass) increased, so did the net specific growth rate (day^{-1}) (Figure 15). The slope in terms of net specific growth rate/internal P for the months of July, August, and October were 14.3, 11.3, and 15.9 respectively, with R^2 values in each month being 1, 0.56, and 0.86. Algae used for these incubations had significantly higher internal phosphorus concentrations ($p < 0.001$) on the control site when compared to algae from the tarp site, and net specific growth rate ($p = 0.015$) was also higher on the control site, according to paired t-tests.

Laboratory Incubations

To supplement the *in situ* incubations, incubations were done three separate times with four different algal samples to further examine the effects of light and internal phosphorus on

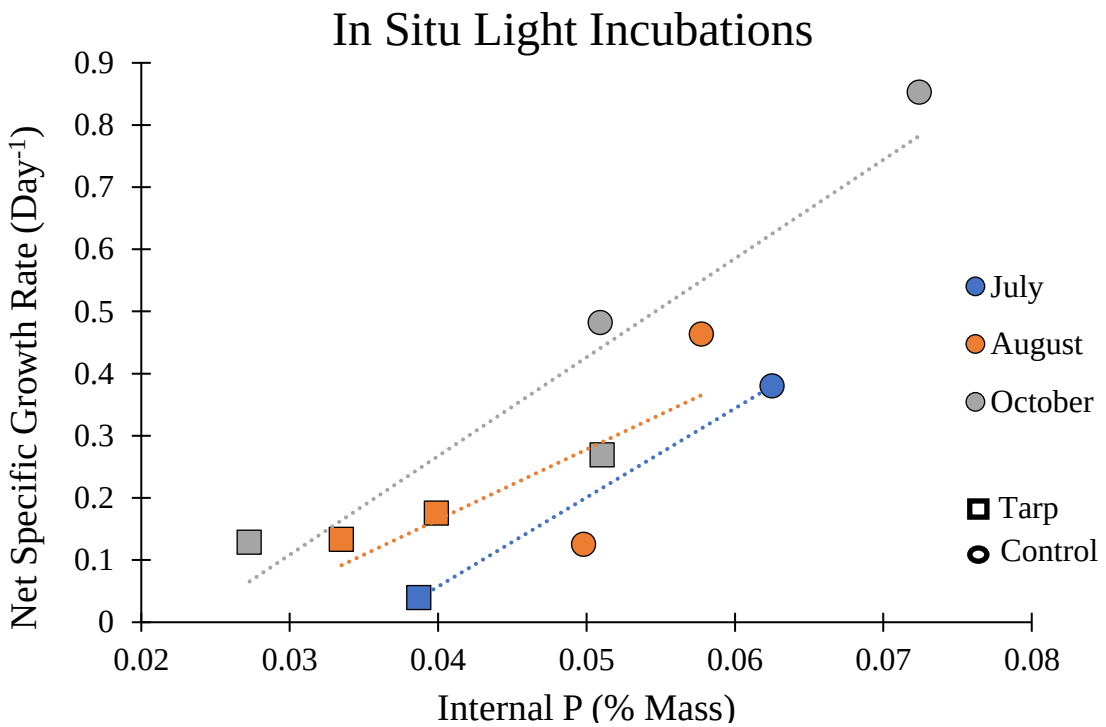

Figure 15. The net specific growth rate compared to internal P for algae collected at a mussel-free site and control site at SLBE. Growth was measured *in situ* using clear incubation chambers (Irradiance ranged from 48-200 mmol photons m^{-2} s^{-1}) and real-time dissolved oxygen loggers.

growth rate. For each incubation experiment, a production/irradiance (P-I) curve was created with a best fit line using the least sum of squares and equation 3 mentioned above. Each experimental algae sample had different P-I curves with different initial slopes (α) and maximum growth rates (μ_{mas}) (Figure 16). This is likely due to the differences in internal phosphorus contents between the algae (Table 2). According to a one-way ANOVA, there was a statistical difference between the algal P content between sites ($p < 0.01$), and after conducting post-hoc student's t-test for each pair, there were significant differences between each algal sample ($p < 0.01$ for every pair). This suggests large spatial and temporal heterogeneity in Lake Michigan benthic algae in terms of internal P and growth rate. Incubations done at SLBE comparing mussel-free tarp site algae and control site algae showed much lower max growth rates (0.19 day^{-1}) and P concentrations (0.038 ± 0.006 % P) on the tarp site compared to the control site (1.12 day^{-1}, 0.057 ± 0.007 % P).

After combining the results from both the *in situ* incubations and optimum light (300 - 600 mmol photons m^{-2} s^{-1}; Graham et al. 1982) lab incubations, the data were fit to the linearized Droop model where the minimum cell quota (Q_0) was calculated to be 0.03 %P and the maximum net growth rate ($\hat{\mu}_{net}$) was 1.14 day^{-1} ($R^2 = 0.95$; Figure 17). Using these calculations, the same data points from the incubations were fit to the Droop model (Equation 4; Figure 17).

Table 2. Summary of algae incubation PI curves for each lab incubation experiment.

Sample Collection Date	**Location**	**Depth (m)**	**Avg Internal P (% mass)**	**μ_{max}** (day^{-1})	**α** (**day^{-1}** mmol photons m^{-2} s^{-1})
9/14/2022	Milwaukee	5	0.075	0.87	0.0227
9/26/2022	Milwaukee	5	0.167	1.26	0.0047
10/25/2022	SLBE Tarp	10	0.038	0.19	0.0089
10/25/2022	SLBE Control	10	0.057	1.12	0.0105

Great Lakes Cladophora Model

The Great Lakes *Cladophora* Model was used to simulate *Cladophora* growth throughout the growing season based on *in s6565 2035itu* irradiance, temperature, and soluble reactive phosphorus concentration. Model results were compared with empirical measurements of benthic algal biomass. Water column SRP and light extinction coefficient were measured 4 times throughout the year on the same days that benthic algae were sampled. The average SRP concentration at the near-bottom of the water column was 0.28 ± 0.03 μg L^{-1}. The average light extinction coefficient was 0.18 ± 0.01. These values were used in the model for the duration of the growing season because of the minimal sampling dates and the lack of variability in the 2022 measurements. Surface light data from the buoy was used for the GLCM and using the combination of the surface irradiance with the light extinction coefficient, the bottom irradiance at a depth of 10 meters was calculated for use in the model.

Once the conditions of the lake were updated, the biomass throughout the growing season from May to October was modeled by the GLCM. *Cladophora* biomass was simulated for the period of May to October 2022, using the maximum growth rate (1.14 day^{-1}) and minimum cell quota (0.0325% P) determined in this study. The modeled biomass greatly exceeded the measured biomass for control site through the 2022 study period. Simulations were also conducted using past reported values of maximum growth rates and minimum cell quotas. Auer and Canale (1982b) report a minimum cell quota for *Cladophora* of 0.06% P and a maximum growth rate of 0.714 day^{-1}. Tomlinson et al. (2010) report a report a minimum cell quota for *Cladophora* of 0.035% P and a maximum growth rate of 1.3 day^{-1}. To evaluate the model sensitivity to these parameters, model simulations were run with various combinations of minimum cell quotas and maximum growth rates (Figure 18).

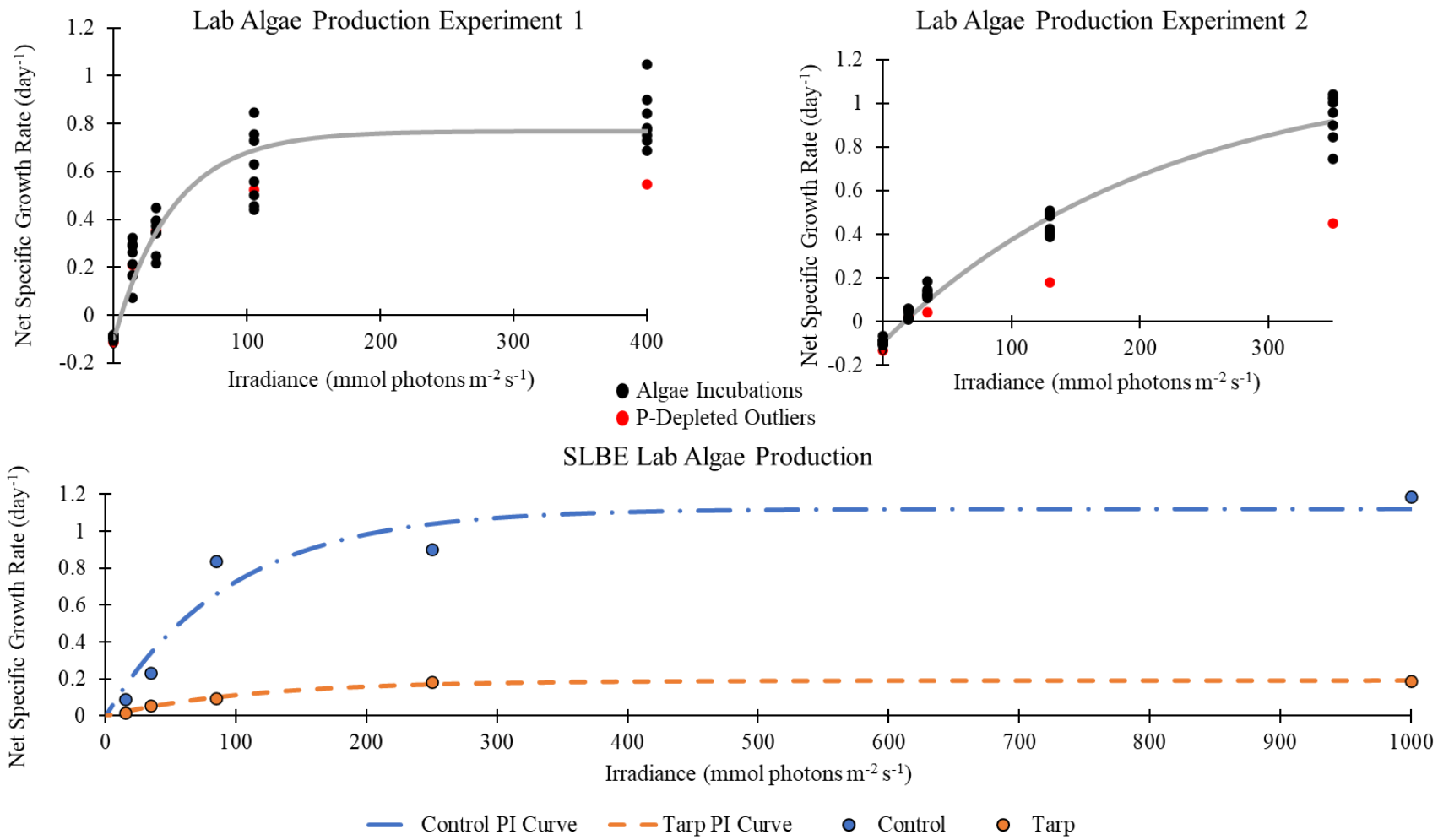

Figure 16. Production/Irradiance curves of three experiments with four different algae collections (Experiment 1 algae P content ranged from 0.62 – 0.92 µg mg^{-1}. Experiment 2 algae P content ranged from 1.49 – 1.96 µg mg^{-1}. SLBE control algae P content was between 0.46 – 0.69 µg mg^{-1}, while the SLBE tarp algae P content was between 0.30 – 0.45 µg mg^{-1}). PI curves are fit to least sum of square, the P-depleted sample was an outlier and not used in the sum of squares calculation (Red). Further results are summarized in table 1.

The modeled biomass from values of this study differed greatly from the measured biomass from SLBE in 2022; however, they did not differ significantly from the modeled biomass based on model parameters reported by Tomlinson et al. (2010) even as both the minimum cell quota and maximum growth rate are not equivalent between the two reports (Figure 18). The modeled biomass based off parameter values from Auer and Canale (1982b) were much lower than the simulated biomass based on parameter values from either Tomlinson et al. 2010 or this study. This is due to the lower maximum growth rate and lower minimum cell quota values applied by Auer and Canale (1982b). The model using all of the parameter values from Auer and Canale (1982b) predicted the values which most closely resembled the empirical biomass measured in SLBE throughout 2022. Changing the minimum cell quota in the model appears to affect the simulated biomass more than changing the maximum growth rate because of the further depressed predicted biomass values shown from changing only the minimum cell quota compared to changing the growth rates for both the reported values from Tomlinson et al. (2010) and Auer and Canale (1982b).

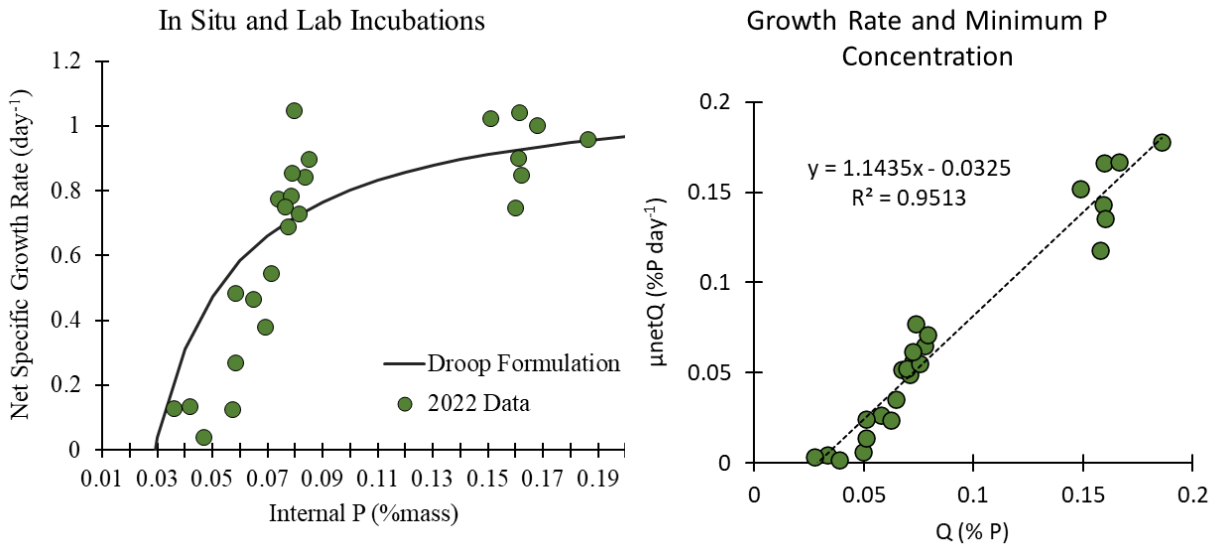

Figure 17. Droop Model (Original - Left, and Linearized - Right) fit to data collected in 2022 for *in situ* experiments and lab experiments with light levels between 300 and 600 mmol photons m^{-2} s^{-1}. The maximum growth rate is equal to the slope of the linearized Droop model and the minimum cell quota is the x-intercept.

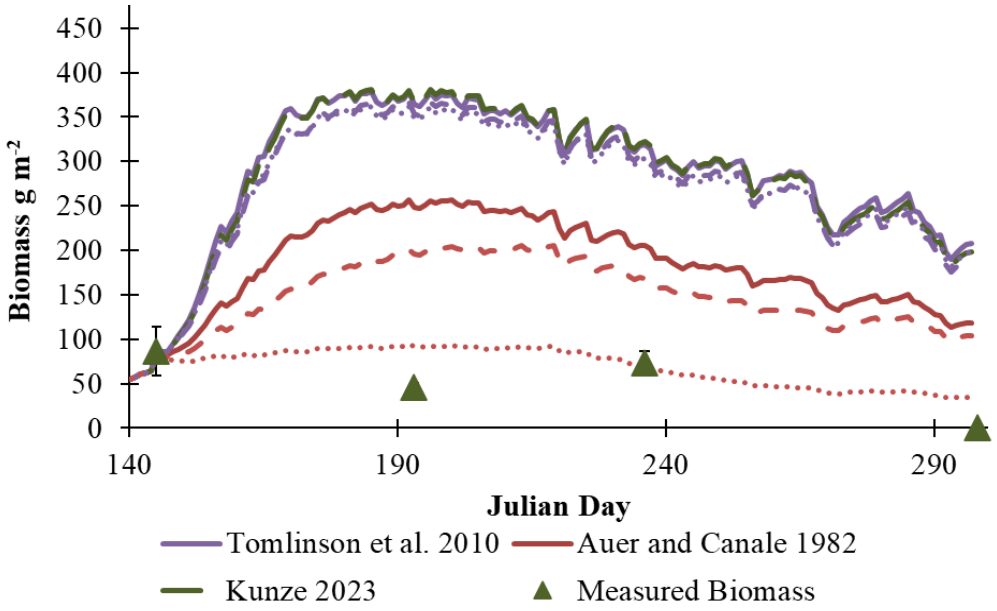

Figure 18. Simulations of *Cladophora* biomass using the Great Lakes *Cladophora* model with variable values for minimum cell P quota and maximum growth rate. Three different colors represent simulation results based on the three different reported maximum growth rates and P requirements for *Cladophora.* The solid lines are based on the maximum growth rate reported by the author of the corresponding color and the minimum cell quota reported in this study. The dashed lines are based on the minimum cell quota reported by the author of the corresponding color and the maximum growth rates reported in this study. The dotted lines represent simulation results based on the minimum cell quota and maximum growth rates reported by the author of the corresponding color.

Discussion

Nutrients and Community Composition

This study presents some of the most convincing evidence of dreissenid-induced benthic algal growth, specifically filamentous green algae which has grown at nuisance levels in the Great Lakes over the past two decades (Whitman et al. 2003; Higgins et al. 2005; Auer et al. 2010; Dayton et al. 2014; Bootsma et al. 2015; Kuczynski et al. 2016; Zhou et al. 2021). Chlorophyll-*a* is sometimes used as a proxy for biomass (Bothwell 1989; He et al. 2022) and it increases in concentration as P availability increases (Dillon and Rigler 1974; Kahlert and McKie 2014; Quinlan et al. 2021). This is exactly what happened in this study, asserting that the lack of mussels on the tarp site have altered the periphyton chlorophyll-*a*. Another proxy for biomass is carbon content (Riemann et al. 1989); the results of the carbon content in algae between sites resembles chlorophyll-*a* between sites with the mussel-free tarp site being depleted in carbon compared to the control site (Figure 9F). The carbon and chlorophyll-*a* results suggest a higher proportion of algae biomass on the control site actively photosynthesizing and taking up carbon. These results relate back to the available phosphorus at each site.

Phosphorus was the primary focus in this study because of the limited availability in Lake Michigan. The optimal C:N:P ratio for phytoplankton is 106:16:1, which is known as the Redfield ratio. The optimal C:N:P ratio for periphyton is roughly 119:17:1 (Hillebrand and Sommer 1999). In this study, while there was no difference in C:P ratios between sites; the average C:P ratio was 576.7 ± 166.6:1, which means that phosphorus is extremely depleted compared to carbon. Nitrogen was much more abundant. The average C:N ratios from each site were found in the range of 11.1:1 to 13.6:1. The apparently low concentrations of dissolved P in

the nearshore benthic system elevates the importance of the reduced P concentrations in algae tissue between sites because they are already so P-stressed.

Different types of benthic algae need different levels of nutrients to grow and be productive. Diatoms typically do better than filamentous green algae under low phosphorus conditions (van Donk and Kilham 1990; Litchman et al. 2003; Michel et al. 2006). Furthermore, when there is low phosphorus availability, initial population densities can strongly affect the outcome of algal competition (Hu and Zhang 1993). As diatoms historically dominate the algal community in spring (Kõiv and Kangro 2005), which is also the case in this study (Figure 12), they have an initial advantage in competition against filamentous green algae in extremely low phosphate environments. Previous studies report a minimum cell quota for *Cladophora* is 0.35 – 0.6 µg mg^{-1} (Auer and Canale 1982b; Tomlinson et al. 2010; Kuczynski et al. 2022). Varying

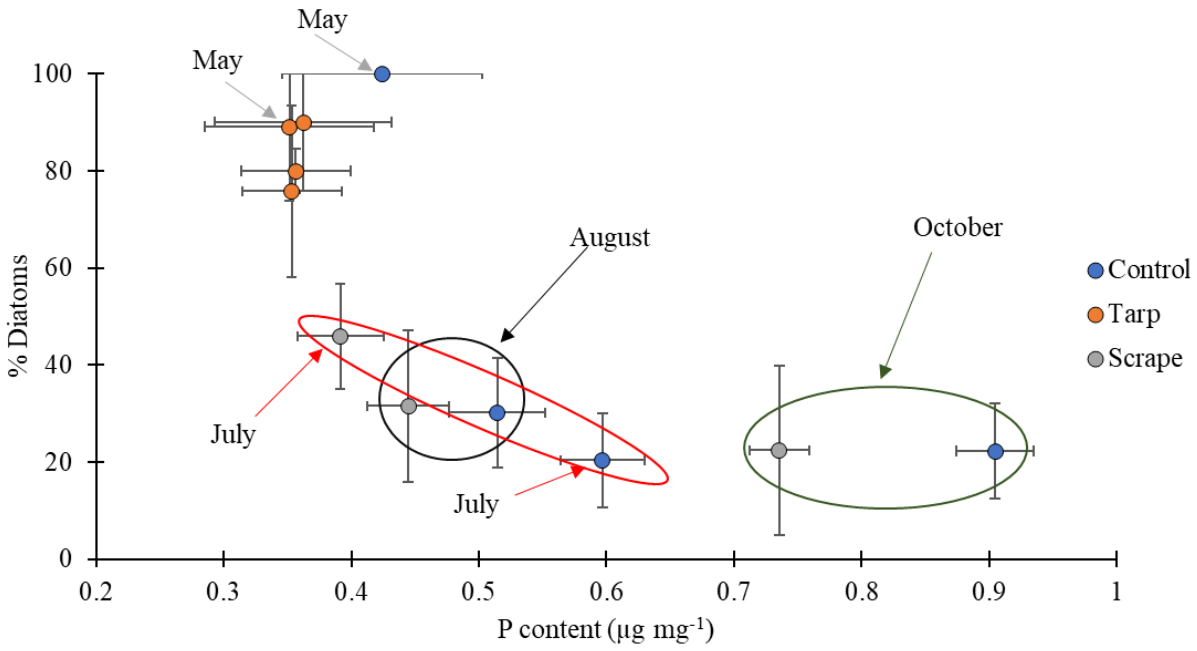

Figure 19. Algae internal P content compared to the ratio of diatoms to filamentous green algae. As P content decreases and reaches a threshold of about 0.4 µg mg^{-1}, diatoms appear to outcompete filamentous green algae, possibly due to green algae not being able to grow below certain P concentrations. Decreased P in diatom-dominated algae may also be due to increased Si in diatoms compared to filamentous green algae. When the percent diatoms are comparable in the same month between sites, the arrows and circles highlight samples from those months.

by month, the mean algal P content on the control site was 0.59 ± 0.04 μg mg^{-1}, while the mean algal P content on the mussel-free tarp site remained low throughout the season at 0.35 ± 0.05 μg mg^{-1} (Figure 7).

Mussels were removed from the scrape site six years before this sampling period. This gave dreissenid mussels some time to repopulate the sides of rocks and in crevices, therefore it provides an intermediate between the control and tarp site in many ways. While there were some mussels on the scrape site (700 ± 28 m^{-2}) in October 2022, their abundance remained well below that on the control site in the same month (6500 ± 260 m^{-2}). The average internal algal P content on the scrape site (0.52 ± 0.15 μg mg^{-1}) over the course of that sampling season was elevated compared to the tarp site but was lower than the control site. The intermediate P content on the scrape site and the intermediate mussel densities on that site support the hypothesis that algal P content is affected by dreissenid P recycling. As the season progressed, the scrape site algal P content increasingly resembled algal P content on the control site and distanced from the tarp site algal P content (Figure 7). In the same way, as the internal algal P content was low in July on the scrape site (0.39 ± 0.03 μg mg^{-1}), the diatom community made up nearly half of the population (45.9 ± 10.8 %) which is greater than double the abundance compared to the control site (Figure 12). As the summer continued and the internal P content in scrape site algae surpassed the minimum P quota for *Cladophora* (0.44 ± 0.04 μg mg^{-1} in July and 0.74 ± 0.03 μg mg^{-1} in October) (Figure 7), the algal composition became dominated by filamentous green algae, mostly *Cladophora*, closely resembling the algal community composition on the control site (Figure 12). One way to interpret these different algal P levels on each site is that there was enough available phosphate provided by the dreissenid mussels on the control site to support *Cladophora* growth, while the tarp site phosphate levels could not support dominant *Cladophora*

communities, which is why the tarp site algal composition continued to be composed of roughly 80% diatoms throughout the year, while the control site is dominated by *Cladophora* after the spring bloom.

A second possible explanation of lower internal P content in diatom-dominated populations is the difference in elemental makeup of different taxa. Diatom-dominated community compositions had lower P content, chlorophyll-*a*, and carbon content; however, the biomass was similar between sites. Diatoms are different from filamentous green algae in that they have a cell wall encasing the entire organism which is largely made up of silica (Lewin 1955). Because of the silicified cell wall, silica (Si) makes up a significant percentage of the diatom biomass. Si : C ratios in freshwater diatoms can be as high as 0.73 ± 0.43, reducing the percentage of other compositional elements of the organism (Conley et al. 1989). Therefore, if nutrient demands are indeed the same for diatoms and *Cladophora*, diatoms would appear to have depleted phosphorus, carbon, and chlorophyll-*a* when these variables are biomass-normalized. Support for this argument comes from the lack of C:P differences in the algae between the three sampled sites (Figure 9C, Table 1), while ratios of cellular components (C, P, chlorophyll-*a*) to dry biomass are different (Figure 9, Table 1).

An argument for the first interpretation: mussels providing the P needed for filamentous green algal growth, is that even in instances when diatom and green algae compositions are similar between sites during comparable months, algal P content is still higher on the control site than both the tarp and scrape site, suggesting dreissenid mussels providing some nutrients to the algae (Figure 19). Both of the above mechanisms may be important, with relative importance varying over time. Early during the algal growth period, mussels are providing increased dissolved P to benthic algae populations, due to increasing temperatures and phytoplankton

concentrations, facilitating higher internal algal P contents. As the internal P contents exceed the minimum quota for *Cladophora, Cladophora* outcompetes diatoms, but the *Cladophora* C:P ratio remains low because *Cladophora* growth dilutes internal P stores to a level near the minimum threshold for growth.

Biomass and Production

For the past six years, observations between algal biomass and algal internal P content have not corresponded with each other when comparing algae from the mussel-free scrape site and the control site at SLBE. Biomass measurements were similar between sites, while P content was significantly lower on the mussel-free site (Bootsma, unpublished). This was puzzling because phosphorus is the limiting nutrient for primary production in Lake Michigan (Bootsma et al. 2012). Therefore, initial thinking would lead to the expectation of higher phosphorus leading to higher benthic algal biomass. However, that has not been the case, nor was it the case in this study (Figures 6, 7).

Standing biomass represents the balance between gain (growth) and loss (respiration; sloughing) processes. Sloughing is one potential cause of this apparent disconnect between biomass and internal P content. Sloughing is detachment of algal filaments caused by both physical processes such as wave-induced shear stress (Higgins et al. 2008; Tomlinson et al. 2010), and physiological processes because of high growth and self-shading resulting in filament senescence (Kuczynski et al. 2022). It is difficult to measure algal sloughing over time. However, a comparison of algal growth rates with biomass may provide some insight into the potential importance of sloughing as a regulator of standing biomass.

The *in situ* measurements done each month in this study show a linear increase in growth rate as P content increased (Figure 15). Algae on the control site consistently had higher internal P content and growth rates than algae growing on the tarp site. At low P levels, the increase in growth rate is very sensitive to small changes in P content. This linear relationship between growth rate and P content compares well with previous studies at very low P concentrations (< 0.1 %P) (Auer and Canale 1982b; Kuczynski et al. 2022). At higher internal P concentrations, the laboratory experiments showed that once P concentrations become elevated in algae (>0.1 %P), the growth rate levels off (Figure 17), which also compared well to previous studies (Auer and Canale 1982b; Kuczynski et al. 2022). This is typically the case for many marine and freshwater primary producers when it comes to growth rates and nutrient availability (Droop 1968). This suggests that the reduced supply of dissolved P that accompanies the removal of mussels from an area greatly reduces the benthic algae production. The fact that this reduction in production is not accompanied by a reduction in biomass suggests that algal sloughing on the mussel-free substrate is less than on mussel-covered rocks. This may be due to increased turbulence exposure and shear stress to the benthic algae growing on mussels as they are essentially on a platform provided by the mussels. On the other hand, benthic algae growing directly on rocks in the absence of mussels may be more protected from the physical shear stress. Another possibility to increased sloughing on the control site could be the stability of the substrate itself, whether it be the mussels or the rocks. Rocks are very solid compared to the mussels. If a mussel dies or there is some movement by the mussel, the algae go with it, creating increased algal loss with the increased variability of the mussel bed stability compared to the rocks without mussels.

Nutrient Sources and Stable Isotopes

Carbon isotope ratios are a very useful tool in exploring biogeochemical questions and analyzing ecosystem food web structure (Peterson and Fry 1987; Hecky and Hesslein 1995; Hobson et al. 1995; Bootsma et al. 1996; Post 2002; Turschak et al. 2014; Turschak and Bootsma 2015). The carbon-13 isotope (^{13}C) compromises a small percent of all the carbon in the environment and is typically conserved or slightly enriched (<1‰) from one source to another, making it a useful tracer to track the movement of energy in a system (Peterson and Fry 1987). $\delta^{13}C$ is also related to growth rate. As growth rate increases, periphyton and phytoplankton become enriched (heavier) in ^{13}C ratios compared to the same algae with lower growth rates (Takahashi et al. 1991; Burkhardt et al. 1999). In this study, ^{13}C values in benthic algae were enriched (isotopically heavier) on the tarp site compared to the control site. This suggests benthic algae on the two substrates are either obtaining inorganic carbon from different sources or growing at different rates. As algal growth rates showed in this study, algae on the tarp site are not growing faster, therefore algae in the absence of mussels are likely obtaining carbon from a different source.

Dreissenid mussels are filter feeders and phytoplankton are their main food source. Phytoplankton in Lake Michigan have a ^{13}C isotopic signature of roughly -24‰ (Camilleri and Ozersky 2019). This light ^{13}C isotopic signature is reflected in the dreissenid mussel carbon signature (-27 to -28‰) (Turschak et al. 2014; Turschak and Bootsma 2015). This may also explain the depleted ^{13}C signature in benthic algae on the control site compared to the tarp site. As dreissenid mussels excrete and egest nutrients and respire CO_2, benthic algal ^{13}C will reflect the carbon signature of the dreissenid mussels if they are utilizing those excreted nutrients and respired CO_2 to photosynthesize and grow. It is already known that a concentration boundary layer with increased phosphorus forms above mussel beds during quiescent conditions (Dayton

et al. 2014). It is also reasonable that in the same boundary layer at the near-bottom of the lake, there is isotopically light CO_2 which is respired by the mussels. Because there appears to be a shift in the ^{13}C:^{12}C ratios only in the algae growing in the mussel beds and not where mussels were removed, the high concentration P CBL along with isotopically light CO_2 appears not to extend over the mussel-free areas. The ^{13}C signatures, internal phosphorus concentrations, and community composition of the benthic algae suggest that dreissenid mussels are providing resources for nearby benthic algae and facilitating increased *Cladophora* growth which may be leading to the nuisance growth occurrences reported in the Great Lakes.

Spatial and Temporal Heterogeneity

Lake benthic ecosystems have large spatial and temporal variation in terms of nutrient availability and irradiance (Vadeboncoeur and Steinman 2002; Fink et al. 2006; Camilleri and Ozersky 2019; Quinlan et al. 2021). This was shown in this study in the laboratory and *in situ* production experiments (Figures 15 and 16), algal community composition between months (Figure 13), and algal nutrient compositions at SLBE (Figure 7). Because of the variation in environmental conditions, algae in the lake do not act alike in distinct locations over time. During the *in situ* experiments, the relationship between internal P content and growth rate was similar. However, according to a linear regression, the growth rate for algae was not affected by the light intensity on either the tarp site ($p = 0.3$) or the control site ($p = 0.9$). This seemingly contradicts previous studies (Graham et al. 1982), and the lab incubations which were done on a light gradient (Figure 16). However, each set of lab incubations which were used to produce a singular PI curve used algae which was collected from one location and at one moment in time. There were no temporal or spatial variation in those algae. However, when comparing different PI curves, there was great variation in terms of the maximum growth rates and initial slopes (α)

(Table 2). Each of the *in situ* incubations may have reacted differently to the light levels which they were exposed to because of the differences in collection location and date, potentially having different responses to the light levels like what is seen in the different lab incubation PI curves. Ultimately the results of this study demonstrate that algae in Lake Michigan do not act uniformly through space and time in terms of algal physiology, community composition, and response to the environment.

The spatial and temporal heterogeneity may not be purely due to the conditions during the incubation. Benthic macroalgae can have delayed responses to nutrient and light changes because of the continuously changing environment (Martínez et al. 2012). For example, if nutrient deficient algae encounter increased nutrients, the algae rapidly uptake those available nutrients. However, the growth rate remains as low or lower than before coming into contact with additional nutrients for several hours (Healey 1979). Furthermore, if the community composition is different, which appears to be the case at SLBE between sites and over time (Figure 12), the different taxa may have diverse growth rates, even if they are growing in comparable conditions (van Donk and Kilham 1990; Michel et al. 2006; Li et al. 2017). When dreissenid mussels are removed, this changes the conditions of the lake. This may be the case over much smaller areas than in this study because benthic algae sampled at the edge of the mussel-free tarp site had the same internal P content as algae sampled in the middle of the tarp site, while still having different P content than algae on the control site sampled just fractions of a meter away (Figure 11). This suggests that mussel excretion and egestion have a highly localized effect on benthic algae growth at SLBE.

Historical Comparisons

In the past 40 years, there have been numerous experiments and studies to measure the physiological properties and function of *Cladophora* in the environment. The Droop model, which describes the relationship between growth rate and internal concentration of a limiting nutrient, is the most widely utilized tool in describing the response of *Cladophora* to different nutrient levels (Droop 1968, 1973). The parameters of the Droop model are minimum cell quota (Q_0) which is the point where there are not sufficient nutrients to provide any net growth, and the maximum growth rate ($\hat{\mu}_{net}$) which occurs as the algae becomes saturated with phosphorus. 1982 was the first time that this model was used for *Cladophora* when Auer and Canale (1982b) examined the dependence of growth rates on the internal P pool size in *Cladophora*. They measured a maximum net specific growth rate of 0.77 day^{-1} and a minimum cell quota of 0.06% and applied these measurements to the "Great Lakes *Cladophora* Model." Tomlinson et al. (2010) revised this model using updated parameterization. They calculated a maximum growth rate of 1.53 day^{-1} and a minimum cell quota of 0.035%, which is a much higher maximum growth rate and a lower minimum P quota for *Cladophora* than previously reported. Lastly, Kuczynski et al. (2022) once again revised the GLCM using a combination of field and lab experiments. They applied a minimum P quota of 0.04% and reverted back to Auer and Canale's original maximum growth rate of 0.77 day^{-1}.

When comparing Droop model results using the parameters of these four studies, results from this study are most comparable to those from Tomlinson et al. (2010) (Figure 20). However, this study shows the highest growth rates for algae which have Internal P contents in the range found at SLBE (Figure 20). This is not to rebut previous reports, but to put these findings into perspective. All previous reports have utilized cultured *Cladophora* grown in a lab

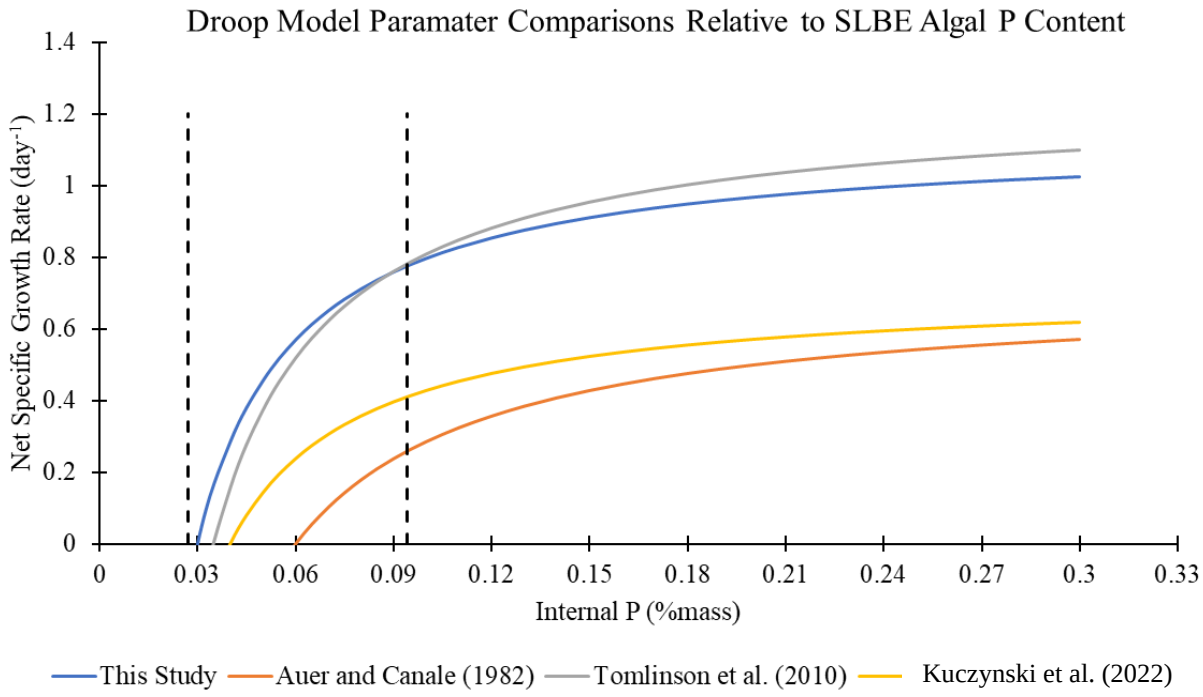

Figure 20. Droop model comparisons between this study and previous reports. Vertical dashed lines describe the range of benthic algae P content which was found at SLBE in 2022.

to measure the growth rates. This study used *in situ* benthic algae along with algae collected from the lake and incubated within 24 hours. This suggests the difference in growth rates and minimum cell quota between studies may be due to the conditions the algae were exposed to immediately prior to experimentation and the compositional makeup, not the physiology of *Cladophora*. Nevertheless, the Lake Michigan benthic community is not homogenous and uniform throughout, it is diverse and assorted with many different green algae and diatoms. Therefore, results from these *in situ* and lab experiments can be useful in attempting to predict the whole benthic algae community growth and abundance in Lake Michigan rather than only *Cladophora*.

The various reported values for benthic algae growth rates and minimum cell quotas highlight the importance of nuance in attempting to manage nuisance algal growth in Lake Michigan. Simply monitoring algal P content may not be an ideal way to determine the growth

potential of benthic algae because of the variety in reported responses to nutrient levels. Setting a specific P target will be a difficult management decision because there appears to be uncertainty about what the community growth response will be to a given internal P concentration. If algal tissue is measured at 0.05 %P, it is uncertain whether managers should assume that algae is growing at the predicted rates from this study, Tomlinson et al. (2010), or the much lower predicted growth rates reported by Kuczynski et al. (2022). Lastly, even if lake managers set a target P concentration for the water column, it is difficult to predict how that affects the nearshore benthic algal tissue P concentration due to the filtration and egestion of P by dreissenid mussels.

The offshore water column is already very P-limited and setting lower targets for P may further damage the offshore food web, without certainty of reducing nuisance benthic algal growth in the nearshore. This contradicts proposals by Higgins et al. (2012) and Zhou et al. (2021). These authors agree with each other that further decreasing P loading on a local level and abstaining from increasing P concentrations in the offshore water column will help reduce nuisance algal growth. They come to this conclusion because they correctly assert that dreissenid mussels filter out the particulate P being loaded into the lake via rivers and water treatment facilities and convert it into soluble reactive P (SRP) which is utilized by the algae (Higgins et al. 2012; Zhou et al. 2021). However, mussels may continue to transfer particulate P to SRP whether there are further restrictions or not and they will likely further deplete the offshore water column and continue providing benthic algae SRP. Evidence for this is shown in this study because there are no major rivers or other sources of P loading near SLBE, yet there are major differences in algal P content between algae growing on mussels and algae growing absent of mussels. P loading near SLBE cannot be further reduced and nuisance growth

continues. If dreissenid mussels remain a major factor in the nearshore, limiting nuisance algal growth by setting lower P targets in the water column will have unknown effects in both the nearshore and offshore.

Impact of Mussel Removal on Benthic Algae

The results of this study indicate that proximity to dreissenids affects the chemical composition, growth rate, and community composition of benthic algae. The evidence suggests that a main reason for these results is that mussels provide increased nutrients to the near-bottom of the nearshore water column, where benthic algae can locally uptake those nutrients, particularly phosphorus. When those mussels are removed, phosphorus supply to those algae decreases. On average, there was 0.25 μg mg^{-1} (37.1%) less phosphorus in algae on the tarp site compared to algae on the control site. Without the presence of mussels, benthic algae P levels are below the minimum cell quota for *Cladophora* according to Auer and Canale (1982b) and Kuczynski et al. (2022), while approaching *Cladophora* minimum cell quota estimates of Tomlinson et al. (2010). This is accompanied by a shift in the community composition. As the P content of the benthic algae communities approaches roughly 0.4 μg mg^{-1}, they increasingly are more diatom-dominated, while communities above this threshold are *Cladophora*-dominated (Figure 19).

Even if removing dreissenid mussels does not debilitate the *Cladophora* populations to the degree seen on the tarp site, small reductions in internal P content as seen between the control site and scrape site (0.15 μg mg^{-1}) theoretically should radically decrease growth rates in the benthic algal community. The Droop model results show that when algae have P contents in the range found at SLBE, they are very sensitive to minor changes in internal P content (Figure 20),

and so the removal of dreissenid mussels should have a significant negative effect on the growth rate of benthic algae. Further research should explore the effect that mussels have on benthic algae sloughing. In 2022, there was similar biomass on the control site and tarp site, but significantly higher growth rates on the control site. Algal sloughing may be the connection between these seemingly contradictory results, in addition to the connection between benthic algae production at the bottom of the lake, and algae washing up on the beaches near Sleeping Bear Dunes National Lakeshore.

CHAPTER 3: BENTHIC MACROINERTEBRATE DEPENDENCE ON INVASIVE DREISSENID MUSSELS IN NEARSHORE LAKE MICHIGAN

Abstract

There is strong evidence that some nearshore benthic invertebrates have benefited from the presence of dreissenid mussels. However, most studies documenting this relationship were conducted prior to the introduction of the round goby. Because round gobies prey on both dreissenids and other benthic macroinvertebrates, uncertainty remains about the relationship between the abundance of dreissenids and other benthic macroinvertebrates. Non-dreissenid benthic invertebrates are a large portion of round goby diets, and these invertebrates also rely upon dreissenids for food and habitat.. Therefore, any large-scale removal of dreissenids may have consequences for the entire nearshore food web. In this study, mussels were removed from a 140 m^2 area, followed by the monitoring of benthic invertebrates on the tops of rocks and at the rock-sediment interface. Benthic invertebrates from each site were identified and measured for biomass and stable isotopic composition. Benthic invertebrates were less abundant on the tops of rocks than beneath the rocks and were significantly less abundant at both the tops of rocks and underneath the rocks in the absence of dreissenid mussels. There was also a shift in ^{13}C: ^{12}C ratios in benthic invertebrates when mussels are absent, reversing the shift that was seen after the initial dreissenid mussel invasion, suggesting that these invertebrates cease to be supported by dreissenid populations, not only because of the loss of structural complexity, but also because of lack of food in the form of dreissenid biodeposits in the nearshore benthos.

Introduction

Dreissenid mussels first invaded the Great Lakes in the late 1980's. Since then, non-dreissenid benthic invertebrates (hereafter referred to as benthic invertebrates) have benefitted because of the increased benthic structural complexity and food availability in the form of biodeposits in the Lake Michigan nearshore (Thayer et al. 1997; Stewart and Haynes 1999; Bially and MacIsaac 2000; Ward and Ricciardi 2007). Changes in food web structure resulting from the dreissenid invasion are reflected in stable isotope ratios, with the ^{13}C: ^{12}C ratios in nearshore benthic invertebrates being slightly lighter after the invasion of dreissenid mussels (Turschak et al. 2014). These changes to the benthic ecosystem are why dreissenid mussels are considered effective ecosystem engineers and are the reason why benthic invertebrates have more taxonomic diversity and higher population abundances when dreissenids are present (Thayer et al. 1997).

While the effect of dreissenids on benthic invertebrates has been well studied, there are gaps in our knowledge of the effect of dreissenid removal on the benthic invertebrate

Figure 21. Round goby in Lake Michigan, resting in benthic algae.

populations. It is important to fill these knowledge gaps as future dreissenid removal management solutions are being inspected. Comparing sites with and without mussels may give the most insight into how dreissenid mussels impact the nearshore after the recent changes to the environment, including increased water clarity and further invasion of additional species. In this study, nearshore benthic invertebrate populations' response to a large area (140 m^2) of dreissenid mussel removal was studied to attempt to fill in these knowledge gaps in today's Lake Michigan nearshore zone.

Quagga mussels are the only bivalves who are able to attach to both hard and soft substrate, while having a planktonic larval stage, making them very effective at populating large areas over short periods of time (Karatayev et al. 1997). However, dreissenid mussel removal pilot projects have shown promising signs regarding the prevention of mussel repopulation (Bootsma, unpublished). Round gobies (*Neogobius melanostomus*) are another invasive species in the Great Lakes which, like the dreissenid mussels, arrived from the Black and Caspian Seas by way of ballast water in ships coming across the Atlantic Ocean (Grigorovich et al. 2003; Kornis et al. 2012). Round gobies are the only fish in the Great Lakes that feed on dreissenid mussels as a major part of their diet. This is likely due to the fact that both dreissenids and round gobies are native to the same region. Therefore, round gobies are already adapted to exploit dreissenids as an energy source (Kornis et al. 2012; Tarsa 2021). While round gobies feed on mussels, their ability to consume large dreissenids is limited, and they prefer small mussels and other benthic macroinvertebrates (Coulter et al. 2011; Kornis et al. 2012; Tarsa 2021). Even though gobies are capable of eating small dreissenid mussels, individual mussels may avoid goby predation by taking refuge under or in between rocks, or between large mussels on the tops of rocks. Therefore, round gobies by themselves likely cannot remove dreissenid mussels from a

habitat after the dreissenids have already been established, and the effect that round gobies have on dreissenid population densities varies depending on the habitat (Djuricich and Janssen 2001).

Despite the round goby's limited ability to regulate dreissenid abundances, recent research in Good Harbor Bay, Lake Michigan suggests that if the larger dreissenid mussels are removed, round gobies may prevent large-scale re-establishment by feeding on smaller mussels as they attempt to colonize the substrate. Near the tarp site, there is another site at a depth of 10 meters in Good Harbor Bay (Scrape) (Figure 1). There, a team of divers physically scraped off a 40 m^2 area of rocks and cleared the mussels from this area in 2016. Six years later, dreissenids have not re-populated the tops of these rocks (Bootsma, unpublished). Without the removal of the larger mussels, many small mussels would be protected from predation of the goby by those larger mussels (Djuricich and Janssen 2001). However, if those larger mussels are removed, it appears that the invasive round goby may serve as a useful management tool to control the invasive dreissenid mussel. On Good Harbor Reef, there are an estimated 2.3-2.9 round gobies per m^2 (Tarsa 2021). One third of the gobies' diet is dreissenid mussels and two-thirds are non-dreissenid benthic invertebrates (Tarsa 2021). If dreissenid mussels are removed in areas around Lake Michigan, round gobies may be a potential answer in the management of those mussels. However, little is known about how the trophic structure and nutrient dynamics of the nearshore benthos will respond to any large-scale loss of dreissenids.

While there is typically a strong negative influence of invasive species on other aquatic communities (Gallardo et al. 2016), many benthic invertebrates benefit from the presence of dreissenid mussels (Kuhns and Berg 1999; Ward and Ricciardi 2007). The presence of round gobies on the other hand, can have significant negative impacts on those same invertebrate populations. Specific invertebrate populations that are diminished in the presence of gobies are

amphipods, hydroptilid caddisflies, isopods, gastropods, trichopterans, and chironomids (Kuhns and Berg 1999; Lederer et al. 2006, 2008). When both gobies and dreissenids are present, there are conflicting reports on whether nearshore benthic invertebrate populations are affected positively or negatively. Kuhns and Berg (1999) found that the presence of both dreissenids and round gobies was associated with increased total benthic invertebrate biomass. Conversely, Lederer et al. (2008) found that all macroinvertebrates, including the zebra and quagga mussels, were negatively impacted by the presence of round gobies. These conflicting results may be due to the experimental setup of the studies. Lederer et al. (2008) completely separated the control and experimental mesocosms, while Kuhns and Berg (1999) used one mesocosm and separated the two sections with a course wire mesh which prohibited the movement of gobies, but allowed the movement of water and invertebrates between both sides of the pool.

Benthic invertebrates are an important part of the nearshore food web as they are a link from benthic algae and dreissenid biodeposits to higher trophic level fishes such as the yellow perch and round goby (O'Reilly et al. 2023). While pelagic primary production has been decreasing (Vanderploeg et al. 2002; Nalepa et al. 2009; Higgins and Vander Zanden 2010; Shen et al. 2018), increased littoral benthic production in the forms of benthic algae, invertebrates, and gobies have subsidized the lake-wide food web (Vadeboncoeur et al. 2002). Without the invertebrates, much of the energy from the benthic algae primary production would come to a dead end, potentially undoing some of the positive consequences of mitigating nuisance algal growth, which takes place when dreissenids are removed as shown in Chapter 2.

Dreissenids impact the Lake Michigan ecosystem in many different ways, but there have not been any major studies observing how benthic invertebrates respond to dreissenid removal in a once dreissenid-dominated area. The benthic ecosystem is part of the base of the food web in

which life in the nearshore zone of Lake Michigan is built upon. A collapse in the benthic invertebrate population may lead to a crumbing of higher trophic levels (Bunnell et al. 2014). Mussel removal operations are a potential option for management agencies around the Great Lakes to restore native habitats. However, more research is needed to determine how mussel removal efforts will affect the benthic community composition and productivity at the base of the food web in these newly restored habitats. I hypothesize that the removal of dreissenid mussels and subsequent loss of available nutrients and habitat in the benthic ecosystem will cause there to be decreased benthic invertebrate densities.

Methods

Field Sampling

In addition to the general benthic scrapes on the upper surfaces of rocks at each site as described above (Chapter 1), benthic invertebrates were sampled beneath the rocks on the mussel-free tarp site and the control site at SLBE using a benthic air lift device (Figure 22). This device utilizes a small air tank to create an air flow near the bottom of the device, which then rises toward the surface of the

Figure 22. Benthic air lift device used to collect benthic invertebrates beneath rocks.

water column and passes through a mesh bag, creating a suction from the nozzle of the device, giving the ability to sample invertebrates and mussels beneath rocks and in rock crevices. Using this air lift device, triplicate samples were collected from a 0.7 meter x 0.1 meter area along the rock – bottom interface at both the control site and tarp site in the months of July, August, and October 2022. Once these samples were in the mesh bags that were attached to top of the benthic air lift device, they were tied and placed in a Whirl-Pak® bag by divers while under water. The samples were then frozen upon returning to shore and brought back to SFS for further analysis. These samples were analyzed the same way in which the normal benthic scrape samples were analyzed: they were taxonomically sorted, counted, freeze dried, weighed to measure biomass, and analyzed for stable isotopic composition. These results were compared between sites and between location of collection (top of rocks and under rocks).

Mussel Killing Experiments

Four small tarps were deployed in Lake Michigan outside of Milwaukee to determine the time that tarps need to be deployed to eradicate the mussels in the natural environment. This was also supplemented with laboratory experiments to determine the length of time benthic tarps need to be deployed to achieve near-100% mussel mortality. Four tarps (2.67-meter diameter) were deployed alongside one another outside of Milwaukee (43.07833°N , -87.85523°W) on July 21, 2022. A dissolved oxygen logger (PME mini DO_2T logger) was placed beneath the tarp that would be recovered last. These tarps were weighed down with 3/8” chain around the perimeter of each tarp and rocks were placed on the middle of the tarps to minimize the amount of oxygenated water penetrating underneath. This procedure was similar to that used in Good Harbor Bay in 2021 (described in chapter 1). Once the tarps were down, divers gathered

triplicate control scrape samples of benthic invertebrates and mussels in the same manner as SLBE control scrape samples were gathered.

On July 23, 2022, two days after the initial deployment, the first tarp was recovered and triplicate scrape samples were collected from the area which was covered by the tarp, and from a control area near the tarps. This was done to continuously compare the tarp site to a control area on the same day, and to compare the temporal heterogeneity of benthic invertebrate populations in the same location on control sites. The following tarps were recovered on July 26, five days after the deployment; August 10, 20 days after deployment; and August 16, 26 days after deployment. Benthic scrapes were conducted in the same manner as the first-recovered tarp for each of the following tarps. The dissolved oxygen logger was recovered on the same date as the final tarp recovery.

A parallel laboratory mussel experiment was done to compare mussel mortality in the natural environment to mussel mortality in a controlled environment. This was done by gathering dreissenid mussels from Lake Michigan and placing 10 mussels in each of 15 500-mL plastic bottles filled with filtered lake water. Five sets of three bottles were filled with the mussels and filtered lake water, capped, and placed in a dark cooler held between 15-17°C. The dissolved oxygen concentration and temperature in three random bottles were measured daily during weekdays for the next six days using a Fisherbrand™ Traceable™ Portable Dissolved Oxygen Meter. As the water temperature and dissolved oxygen were measured, the mussels were poked and observed to see if they were alive. If the mussels responded to the disturbance by closing their shells, they were deemed to be alive. The percent mortality was calculated for each of the three bottles for that day. The average between the three bottles for all these measurements was calculated and the mussel mortality, dissolved oxygen concentration, and

water temperature were reported for that day. Measurements continued until there was 100% mussel mortality.

Mussel Recolonization

Hester Dendy traps were placed at increasing heights, starting at zero meters, going to one meter, and ending at two meters from the bottom of the lake at SLBE. These traps have various sized slots where dreissenid mussels can populate, escaping the predation of the round goby. The Hester Dendy traps were cleaned in the summer of 2021 and mussels were allowed to populate for one year. After a year, the mussels were collected from each of the Hester Dendy traps at the three heights above the bottom. Mussels were placed in a Whirl-pak® bag and were brought back to SFS where they were measured and counted. The Hester Dendy traps provide an effective method of goby exclusion in two ways. Gobies cannot consume mussels growing in the slots of the trap, and gobies have trouble swimming to the higher heights above the lake bottom, providing multiple comparisons of how dreissenid mussel recolonization can be suppressed because of round goby predation.

Results

SLBE Mussel Recolonization

In 2022, one year after the original removal of dreissenids using benthic tarps, there was a 99.8% reduction in dreissenid abundance on the tarp site compared to the control site. On the scrape site (from which mussels were removed in 2016), the mussel abundance in October 2022 was 700 ± 267 individuals m^{-2}, which represented an 89.2% reduction in density in compared to the control site in that month (6500 ± 1420 m^{-2}). According to paired t-tests where the pairing was by date to allow for the removal of temporal variation when comparing among sites, there

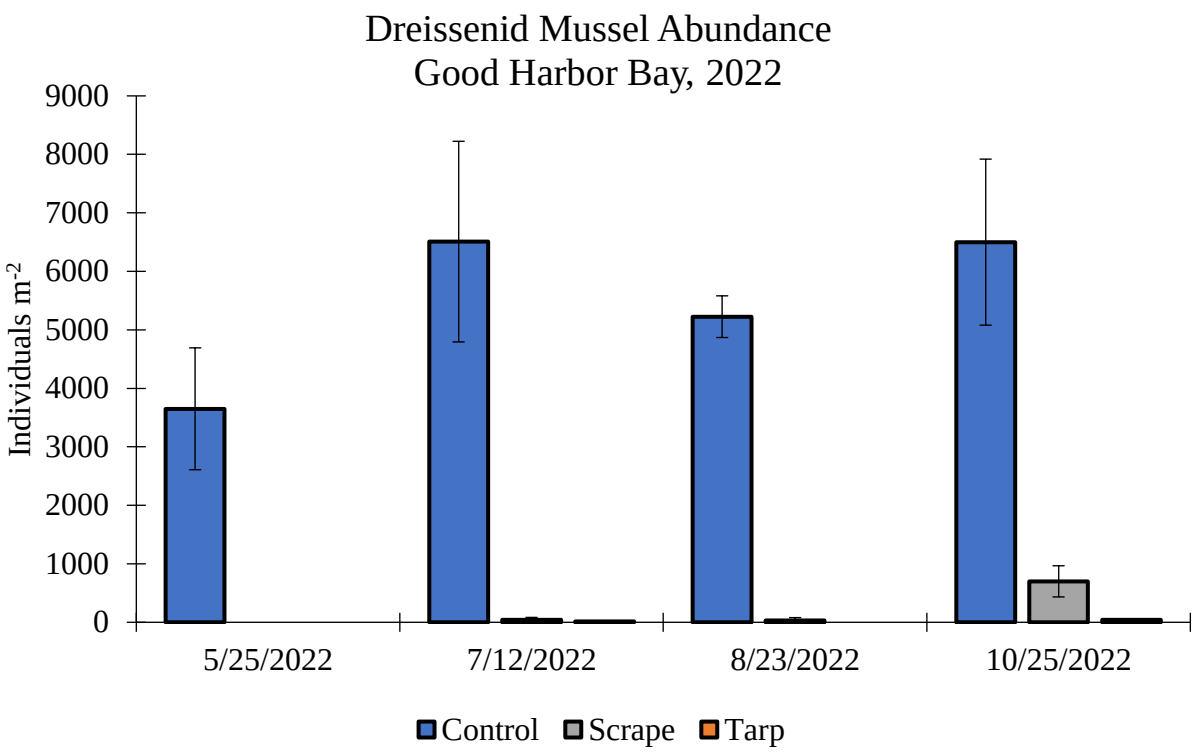

Figure 23. Dreissenid mussel abundance at each SLBE site.

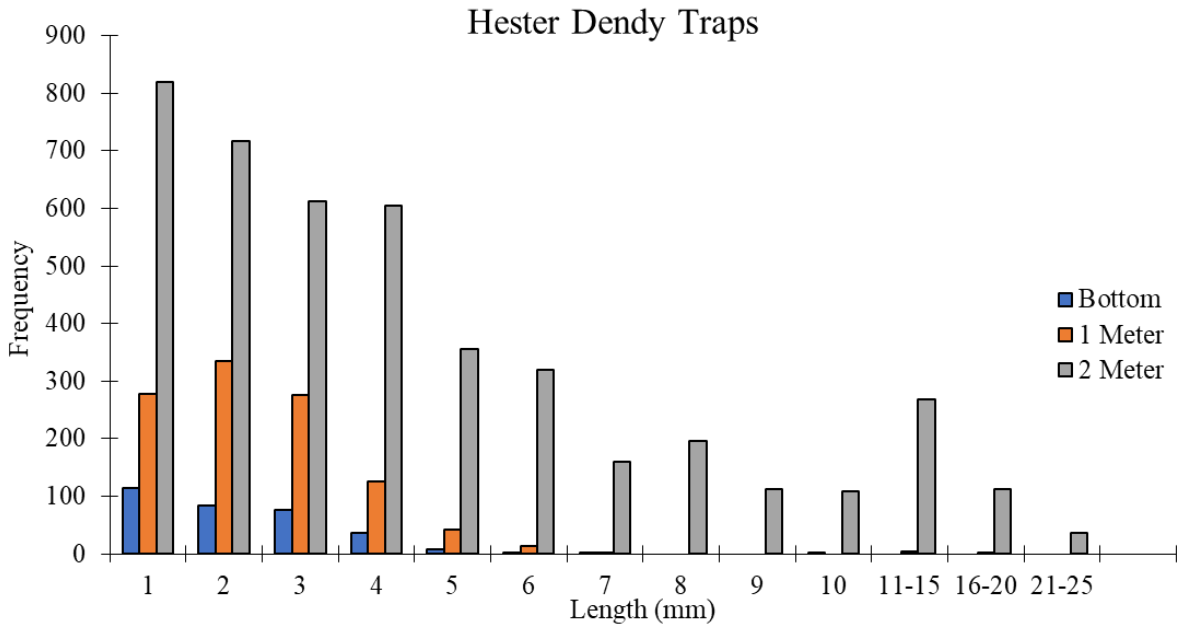

Figure 24. Histogram of mussel frequency at different lengths. Each set of bars are from Hester Dendy traps set at different heights above the bottom of the Lake.

were statistically many more mussels on the control site (5470 ± 1709 m^{-2}) than both the tarp site (14.6 ± 21.6 m^{-2}) ($p < 0.01$) and scrape site (258 ± 350 m^{-2}) ($p < 0.01$).

The mussels that populated the bottom Hester Dendy trap were on average the smallest between the three heights above the bottom (2.27 mm) and the bottom Hester Dendy trap had the fewest number of mussels (322). This was compared to the 1-meter Hester Dendy trap which had three times as many mussels (1077), but with similar length distribution (2.47 mm). The mussels were most abundant on the 2-meter Hester Dendy trap (4420), where the largest average mussels were also found (4.8 mm). The 2-meter site had the greatest amount of mussels for all size classes (Figure 24), and each Hester Dendy trap had many more small mussels compared to large mussels.

Mussel Mortality Experiments

Underneath the tarps in the Lake Michigan nearshore, it took less than two days for the water to become anoxic. It is unknown for certain how long it took because the oxygen logger was not properly deployed at first, but the issue was resolved when the first tarp was removed, two days after initial deployment (Figure 25). Similarly, it took about three days for the water in the laboratory experiment to become hypoxic. The two experiments indicated differing lengths of time needed to produce 100% mortality. In the natural environment, some mussels were still alive after 20 days beneath the tarp (89.4 ± 5.1% mortality), and 100% mortality was not observed until day 26 (Figure 25B). This is likely from short bursts of oxygen breaching the constraints of the tarp during high energy events. Oxygen levels beneath the tarp trials outside Milwaukee were very similar to the oxygen levels beneath the SLBE tarp in that there was an initial rapid decline in oxygen levels which then remained low for long periods of time, with intermittent pulses of oxygen, likely due to incursions of fresh water (Figure 25). The laboratory

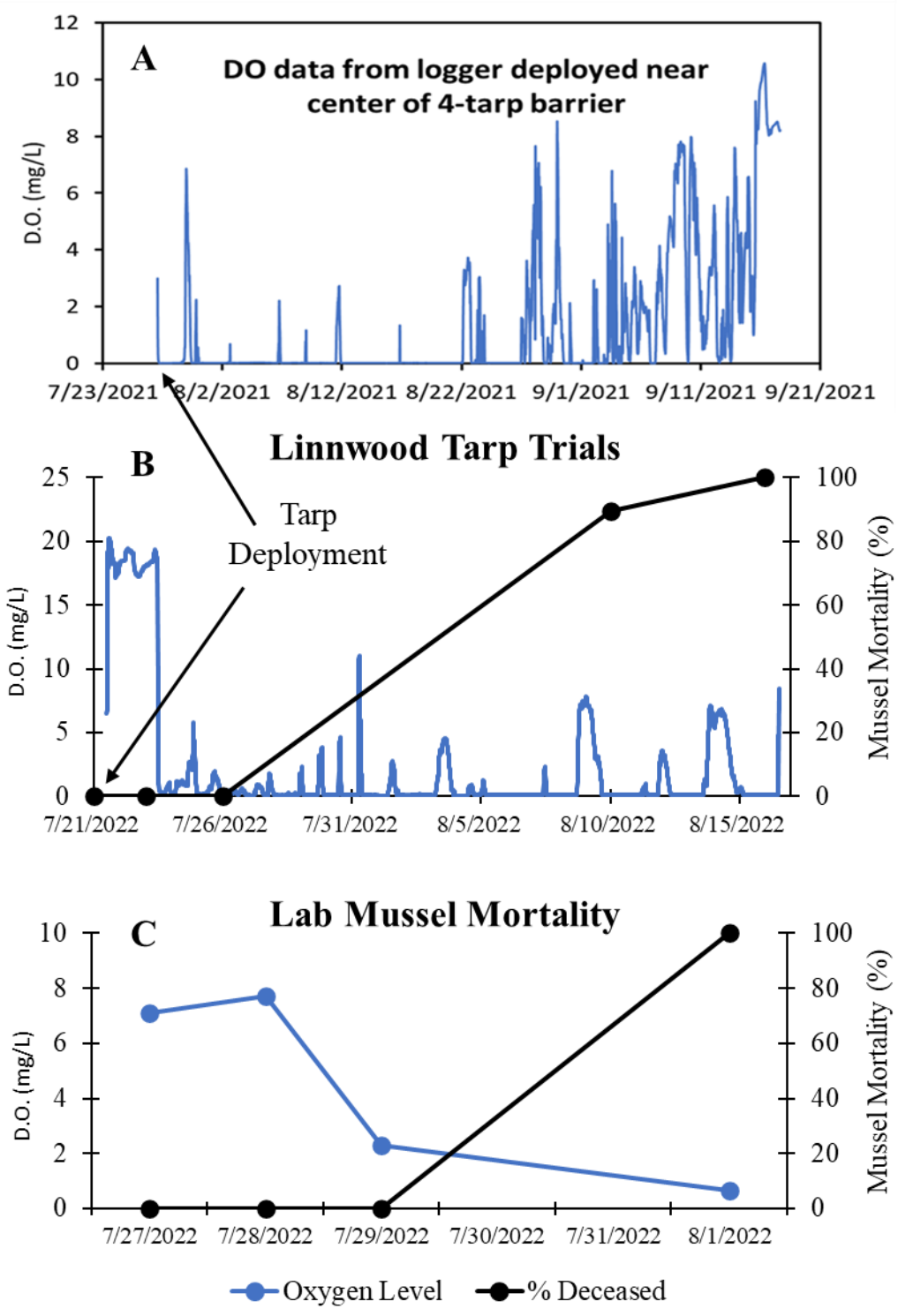

Figure 25. Oxygen concentration beneath the tarps at SLBE (A) and outside Milwaukee along with mussel mortality (Black) over time plotted with the dissolved oxygen levels (Blue) for both *in situ* tarp trials (B) and laboratory-controlled environments (C). Note the differences in dates between figures. It took 4-6 days for the mussels to die in a controlled environment, whereas it took roughly three weeks for the mussels to die in the lake.

experiments, on the other hand, showed that 100% of the mussels died somewhere between 4-6 days.

Benthic invertebrates were also measured over time beneath the experimental tarps and were compared with control samples. The taxa observed were similar to those typically found at SLBE: *Amphipoda, Isopoda, Chironomidae,* and *Oligochaete.* Each of these taxa, besides the isopods ($p = 0.07$) were found in lower abundances on the tarp site compared to control site (Amphipod $p < 0.01$, Chironomid $p = 0.007$, Oligochaete $p = 0.01$) according to paired t-tests. When separating the abundances by date, the total benthic invertebrate densities were not

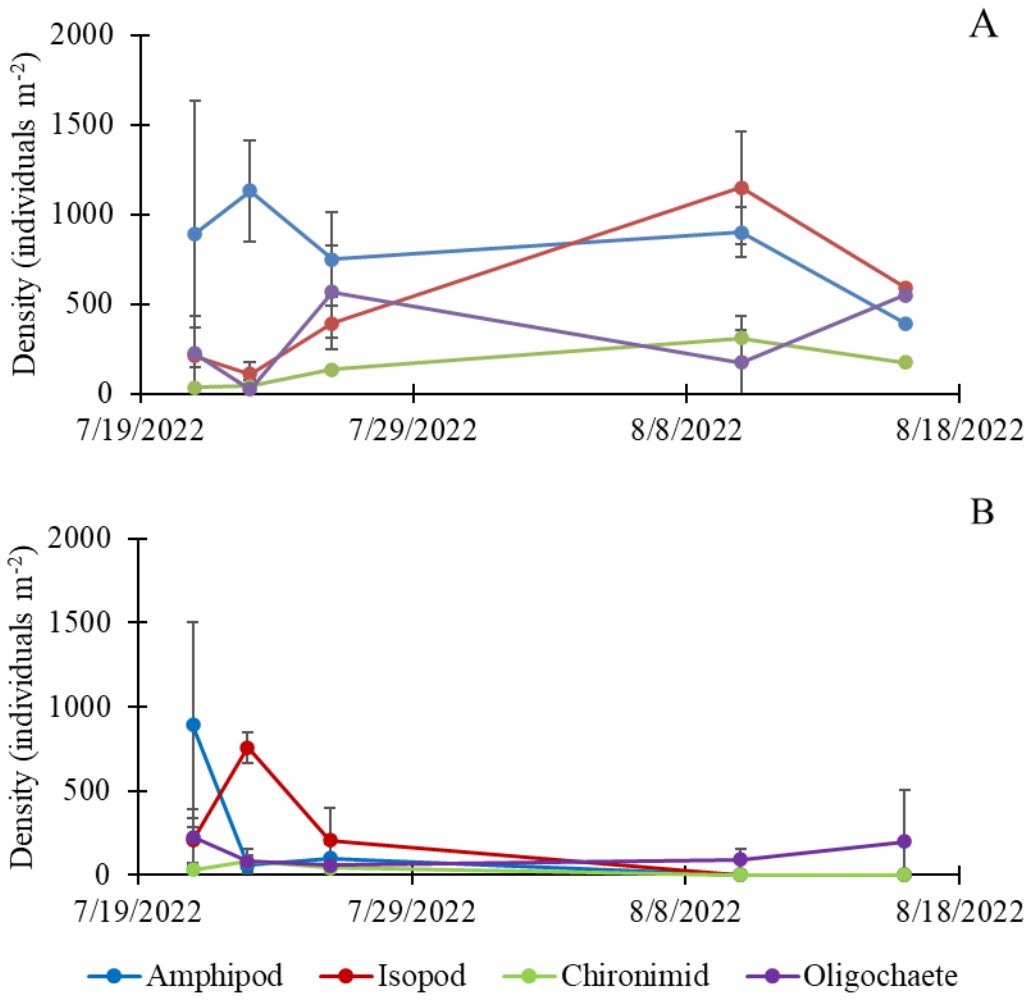

Figure 26. Benthic invertebrate abundances separated by taxa comparing the control site (A) and tarp trial experiments (B) over time.

statistically different between the tarp and control sites until 7/26/2022, five days after the initial tarp deployment. Thereafter, there were always more invertebrates on the control site than the tarp sites (Figure 26). By August 10th, 20 days after the initial deployment, only Oligochaetes were present on the tarp site.

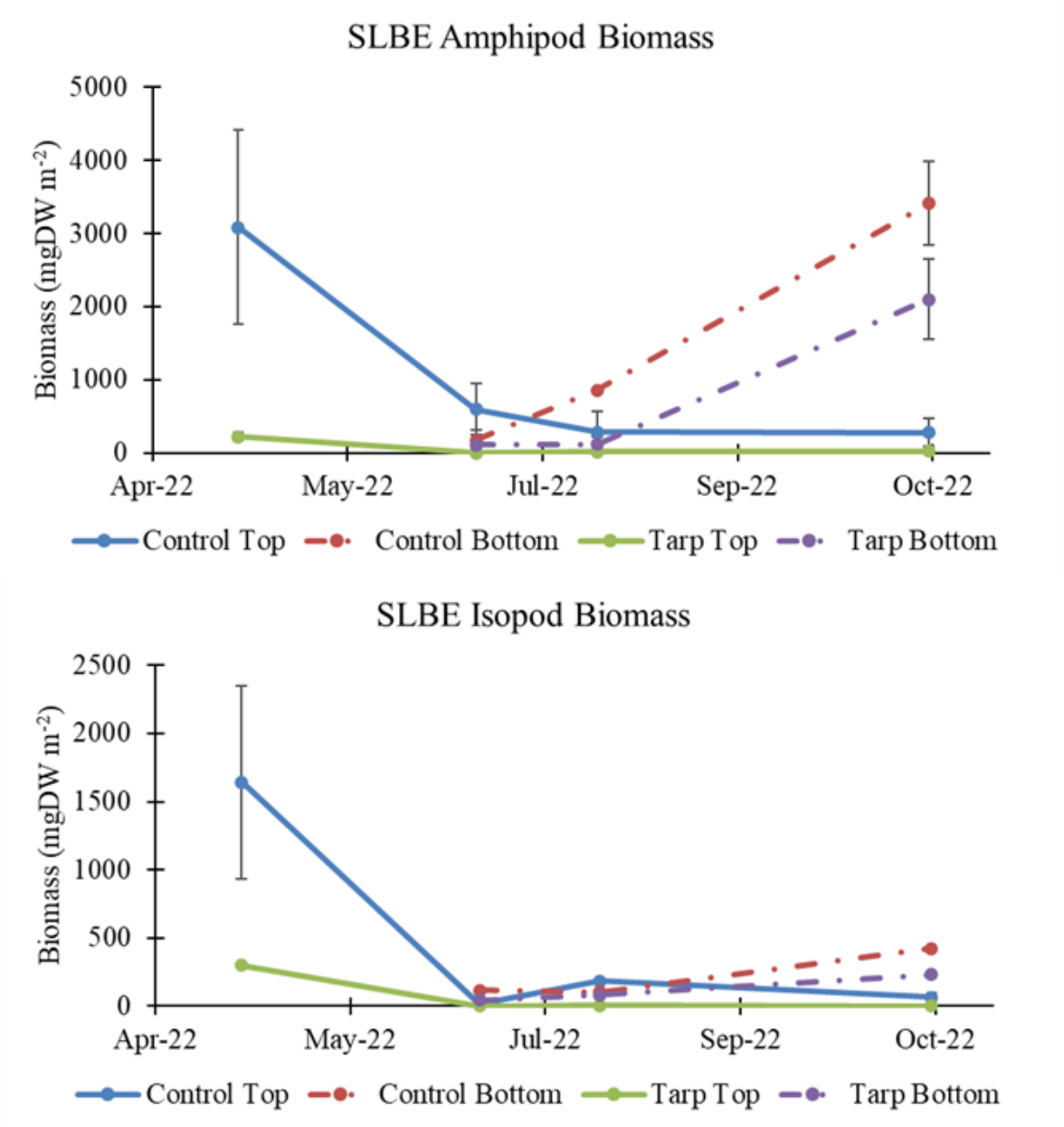

Figure 27. Benthic invertebrate biomass for amphipods (top) and isopods (bottom) normalized to area sampled. For both amphipods and isopods, the biomass is compared between four sampling locations: the top and bottom of rocks on the control site and the top and the top and bottom of rocks on the tarp site over the course of the 2022 sampling season at SLBE.

SLBE Benthic Invertebrate Abundances

At SLBE, amphipods and isopods were the dominant taxa throughout the sampling season, both on the tops of rocks and underneath rocks. For both amphipods ($p = 0.003$) and isopods ($p = 0.004$) on both sites, there were more invertebrates in terms of biomass living underneath the rocks compared to on top of the rocks, according to paired t tests. On the tops of rocks, there were more amphipods ($p = 0.02$) and isopods ($p = 0.04$) on the control site when compared to the tarp site, according to paired t-tests between sites. Similarly, underneath the rocks, greater biomass was observed for both amphipods ($p = 0.02$) and isopods ($p =0.02$) on the control site compared to the tarp site. Overall, the greatest biomass of invertebrates were amphipods found on the control site, underneath the rocks (1487 ± 1537 mgDW m^{-2}), and the lowest invertebrate biomass was also amphipods, but on the tarp site on top of rocks (43.5 ± 88.3 mgDW m^{-2}).

There were large temporal variations observed on most sites in both amphipods and isopods. On top of control rocks, there were much more abundant invertebrates in May 2022 than there were the rest of the year (Figure 27). The opposite was the case for amphipods underneath the rocks, where there were much fewer invertebrates in July (control: 172 ± 139 mgDW m^{-2}, tarp: 108 ± 141 mgDW m^{-2}) and August (control: 879 ± 407 mgDW m^{-2}, tarp: 115 ± 96 mgDW m^{-2}) compared to October (control: 3409 ± 575 mgDW m^{-2}, tarp: 2100 ± 545 mgDW m^{-2}) (Figure 27).

Nutrient Concentrations and Stable Isotopes

Many of the benthic invertebrate samples did not have enough biomass to measure carbon and nitrogen content or stable isotope ratios. Therefore, statistical analysis could not be

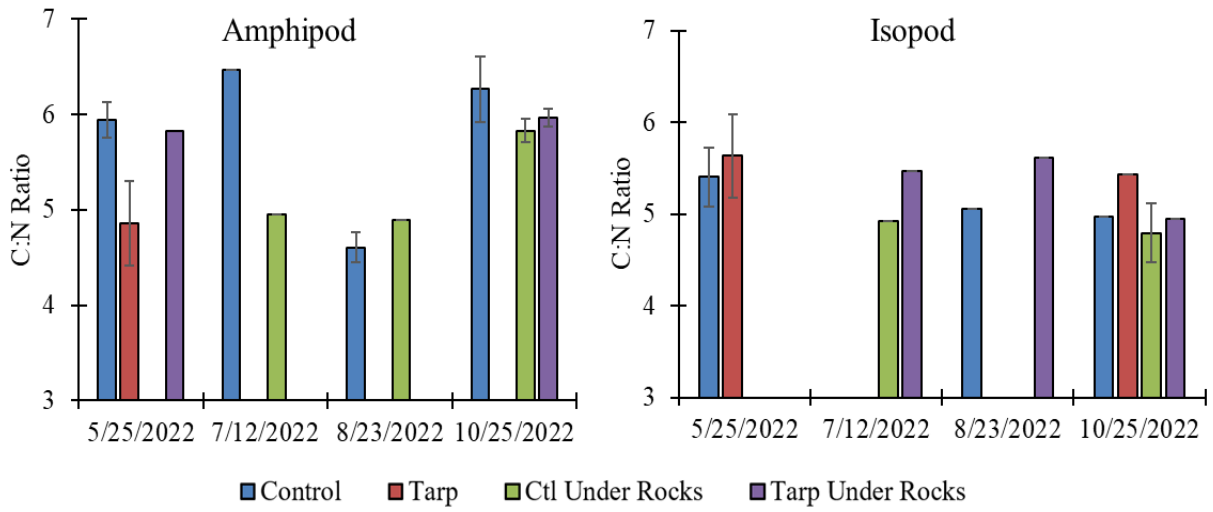

Figure 28. Benthic invertebrate Carbon: Nitrogen ratios in amphipods and isopods at four different sampling locations at SLBE. Note: many of the samples did not have enough biomass to complete C:N analysis.

completed in those instances. In amphipods, C:N ratios were often highest on the control site on top of rocks, and often the lowest on the control site underneath the rocks. Isopod populations on the tarp site appeared to have higher C:N ratios compared to isopod populations on the control site (Figure 28). Some statistical analyses of stable isotope data were also not possible due to the lack of abundance during certain times of the year at each site. There were no statistical differences in ^{15}N ratios between amphipods ($p = 0.16$, $p = 0.18$) or isopods ($p = 0.30$, $p = 0.20$) when comparing populations collected on differing sites or locations (top or bottom of rocks, respectively) according to Student's t test. However, $\delta^{13}C$ appeared to be influenced by the presence/absence of quagga mussels. In May, the $\delta^{13}C$ was significantly lower in amphipods collected on the control site (-21.47 ± 0.51‰) than on the tarp site (-20.13 ± 0.17‰) on the tops of rocks ($p = 0.04$), according to Student's t-test. The overall $\delta^{13}C$ for amphipods on the tops of rocks between the tarp and control site were not significantly different ($p = 0.38$), according to student's t test. Underneath the rocks, amphipods had depleted $\delta^{13}C$ on the tarp site compared to the control site throughout the sampling season ($p = 0.015$), according to a Student's t-test.

Isopod $\delta^{13}C$ values did not differ significantly between the tarp and control sites ($p = 0.38$), according to Student's t test. However, the ^{13}C ratios between the top and bottom of rocks were different for both amphipods and isopods. On the tarp site, both amphipods ($p = 0.01$) and isopods ($p = 0.002$) had enriched $\delta^{13}C$ values underneath the rocks compared to scraped samples collected from the tops of rocks according to Student's t test. Conversely, there were no differences in $\delta^{13}C$ ratios in amphipods ($p = 0.23$) or isopods ($p = 0.28$) on the control site top samples compared to invertebrates beneath those rocks, according to Student's t test. Overall, benthic invertebrates, along with benthic algae were more ^{13}C-depleated in the absence of dreissenid mussels, specifically in the months of July, August, and October (Figure 29). Dreissenid mussels had $\delta^{13}C$ values much lower than algae and invertebrates ($-26.2 \pm 0.69‰$) and similar to the $\delta^{13}C$ of suspended phytoplankton $\delta^{13}C$ ($-26.4 \pm 1.5‰$) over the course of the sampling season (Figure 29).

Discussion

Mussel Mortality

In situ mussel survival under tarps differed greatly from mussel survival in closed containers in the lab. It took nearly three weeks for most of the mussels to die under the benthic barrier membranes in Lake Michigan, whereas in the lab, it was less than one week before all the mussels were dead (Figure 25). While quagga mussels are less tolerant of hypoxic conditions than zebra mussels, quagga mussels still can survive in water with as low as 6% dissolved oxygen for 60 days (De Ventura et al. 2016). On the other hand, when the water is anoxic, dreissenid mussels cannot survive for more than a few days (Karatayev et al. 2018), which corresponds well with the results from this lab experiment (Figure 25). The reason for the

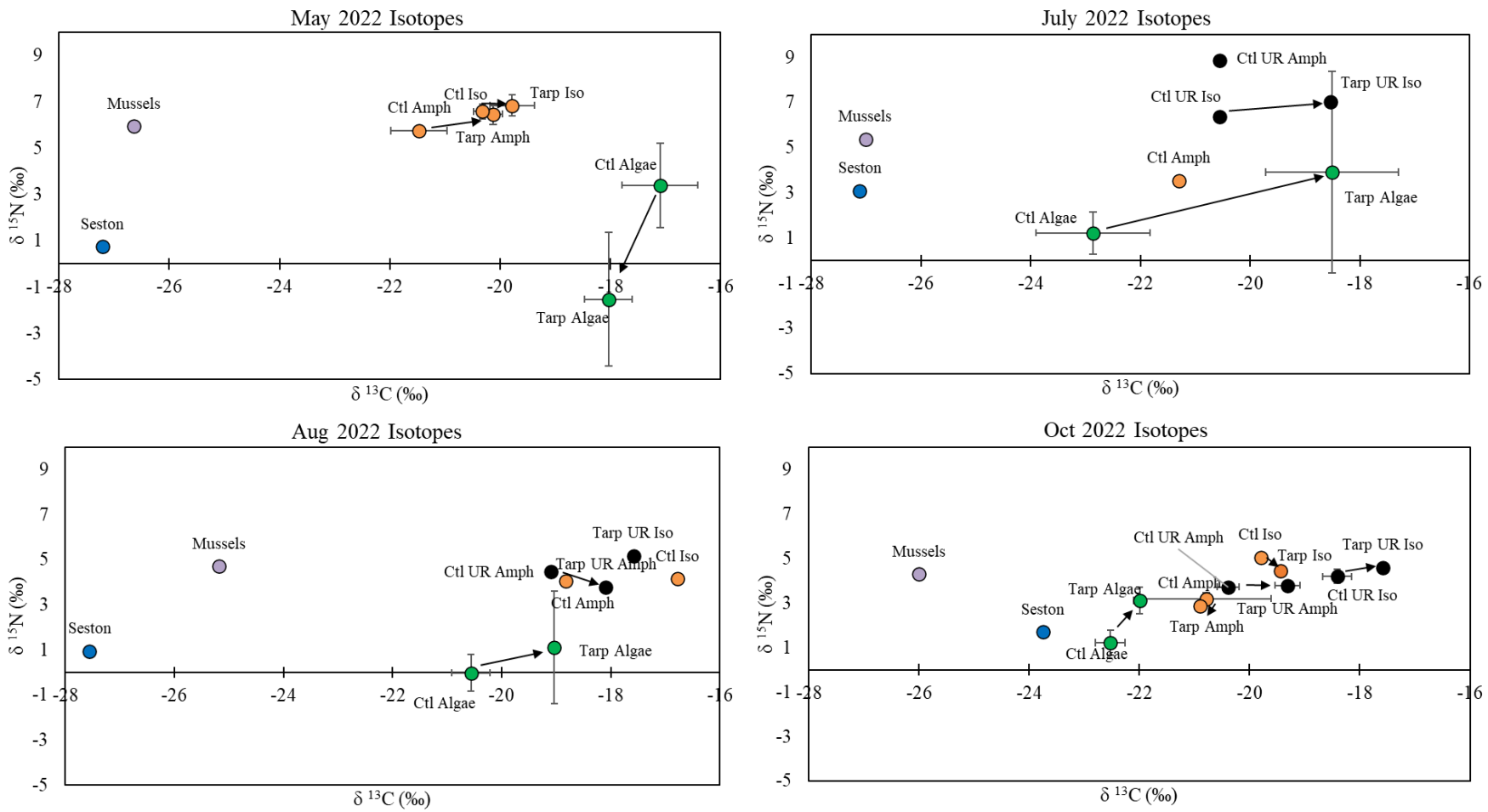

Figure 29. Stable isotope biplot illustrating mean (±SD) $\delta^{13}C$ and $\delta^{15}N$ of Lake Michigan benthic invertebrates from the tops of rocks (orange) and under rocks (black), dreissenid mussels (purple), benthic algae (green), and seston (blue). Invertebrates and benthic algae were collected at the tarp site and control site,. Black arrows highlight the difference in isotopic ratios between the control and tarp sites in each month, whenever there were comparable samples from each site.

discrepancy in mussel survival in the lake compared to controlled conditions is likely the oxygen fluctuations under the tarp, due to incursions of oxygenated lake water. Even a small amount of oxygen supplied to the mussels can extend their survival as they only require 1.0-1.7 mg l^{-1} of oxygen at optimal water temperatures (Yu and Culver 1999). This suggests that when using a benthic barrier membrane technique to eradicate mussels from an area, there must be continuously anoxic conditions for at least several days to cause mussel mortality, which was the case for the initial tarp deployment at SLBE, and throughout the mussel removal trials in Lake Michigan near Milwaukee (Figure 25). Because the deeper water is typically less turbulent and flatter than the nearshore (Zhu et al. 2021), removing mussels with benthic barrier membranes may work better in deeper environments.

Gobies preventing dreissenid recolonization

Results from this study help explain why a site in which mussels were removed six years prior does not have mussel population abundances near to the degree of the control area (Figure 23). The spaced-out Hester Dendy traps showed that higher abundances and larger individual mussels grew as the height above the lake-bottom increased (Figure 24). Round gobies, who incorporate dreissenid mussels as a major part of their diet (Tarsa 2021), do not have a swimbladder and typically only rise as far as a few inches above the lake-bottom (Phillips et al. 2003; Kornis and van der Zanden 2010). However, they are very aggressive and proficient swimmers (Tierney et al. 2011), allowing them to reach higher in the water column for short periods of time. The lack of swimbladder in the round goby may explain why the bottom Hester Dendy trap had the fewest number of mussels. Consuming mussels on the bottom does not take much energy for the gobies. Gobies were still able to reach the 1-meter Hester Dendy, even though it may be more energy intensive; the lack of dreissenid mussels growing at the 1-meter

Hester Dendy trap suggest that the energy gained from the gobies swimming up one meter is likely equal to or less than the energy the mussels provide to the round goby. Conversely, the 2-meter Hester Dendy trap was covered in mussels, which had grown inside the slots of the trap and on the outside, covering the entire trap and line it was attached to (Figure 30). The extreme colonization of mussels at two meters compared to one meter and at the bottom suggest that between one meter and two meters, there is a threshold where the energy gained is surpassed by energy used to get to the mussels, disincentivizing the gobies to swim that high.

These Hester Dendy observations point to gobies preventing the recolonization of mussels, both on the traps, and at the mussel-removed sites from 2016 and 2021. Zebra mussels are typically very fast growing (20 mm yr^{-1}), and grow to a maximum length of roughly 35 mm (Mackie 1993). Little is known specifically about quagga mussel age to sexual maturity (Karatayev and Burlakova 2022), however, zebra mussels can be sexually mature within the first year of life, sometimes reproducing multiple times every year (McMahon 2002). Dreissenid mussels are estimated to live between 4 and 7 years (McMahon 2002). All this suggests that if

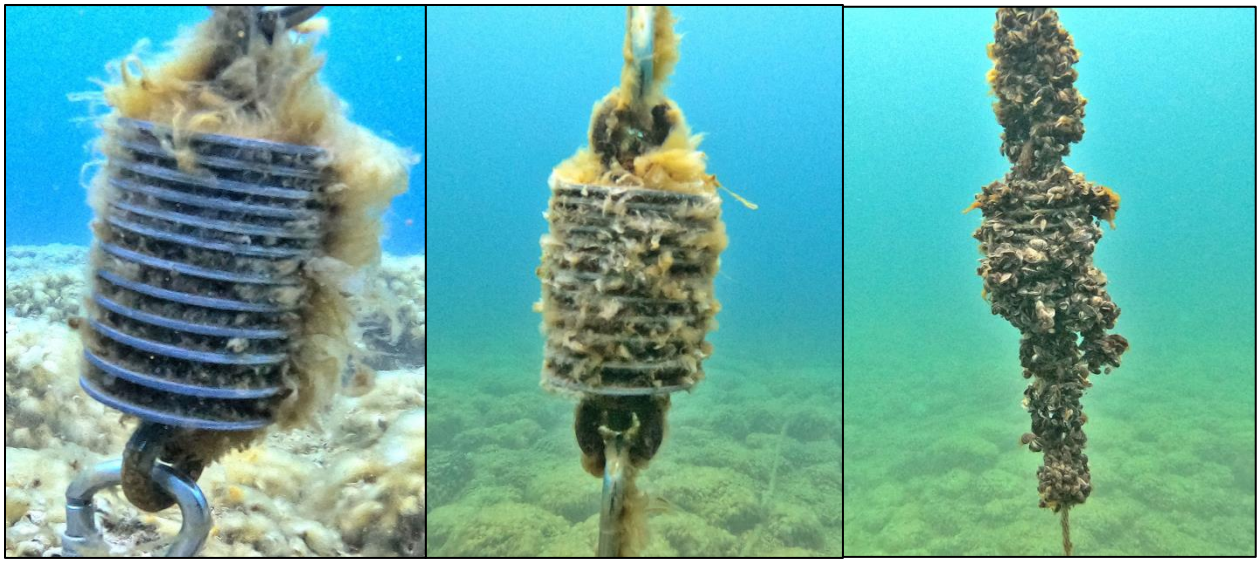

Figure 30. Hester Dendy traps populated with dreissenid mussels at SLBE. Bottom Hester Dendy trap (left) had mussels only growing between slots, 1-meter (middle) Hester Dendy had few mussels growing outside slots, and 2-meter (right) Hester Dendy trap had an abundance of dreissenid mussels growing inside and out of trap.

mussels were to repopulate without predation pressure, they certainly would have covered the scrape site, and we would have likely seen many sexually mature mussels growing on the tarp site too, even one year after removal.

While it is plausible that round goby predation is preventing mussel re-colonization, it appears that, six years after initial removal, some mussels are starting to grow on the bottom and sides of the rocks on the scrape site. This may be due to the removal method, as divers may not have eradicated all the mussels towards the bottom of rocks on the scrape site. Another possibility for the slow recolonization on the bottom and sides of the scrape site, which may also eventually happen on the tarp site, is mussels growing in crevices of rocks where they are protected from goby predation. From there, they could slowly spread over time. This was observed in Green Bay during a field study that measured dreissenid population on a site where round gobies were already present (Houghton & Janssen, 2013). These authors found that dreissenid mussels did not populate the lake to the same degree in the presence of gobies compared to when gobies were not present, however, there was a correlation between number of mussels and vugs (pits and crevices in rocks caused by differential erosion of component minerals). This suggests that mussels can partially repopulate an area if there are micro-refugia in which they can avoid predation by gobies, but gobies may prevent a complete recolonization of dreissenid mussels in the nearshore zone.

Benthic Invertebrate Populations

Deployment of a barrier membrane over a mussel bed results in rapid anoxia. This eradicates not only the mussels, but also most of the benthic invertebrates after a relatively short period of time (Figure 26). The typical life span of nearshore Lake Michigan amphipods and isopods is about 1 year (Hatchett 1947; Winnell and White 1984) and they reach adulthood as

early as 12 weeks of age (Hatchett 1947). After the mussel removal on the tarp site at SLBE, benthic invertebrates were observed to have repopulated the tarp site by the following spring. However, further study is needed to determine the time scales over which benthic invertebrate repopulation occurs.

Round gobies utilize dreissenid mussels as roughly 1/3 of their overall diet, the other 2/3 are mainly made up of non-dreissenid macroinvertebrates (Tarsa 2021). Therefore, when mussels are removed from a site, the gobies either must move to an area that has both dreissenids and invertebrates, or they must make up for the loss of energy from dreissenid mussels by consuming greater amounts of benthic invertebrates. Additionally, benthic invertebrates are aided by dreissenid mussels in terms of habitat structural complexity and refuge, protecting them from predation (Thayer et al. 1997; Bially and MacIsaac 2000; Ward and Ricciardi 2007). When dreissenids are removed, a double-edged sword falls upon the invertebrate community: they lose their structural protection, and gobies are forced to look harder for invertebrates to fill the void in energy consumption.

Comparisons in the benthic invertebrate abundances among sites show the importance of dreissenid mussels for those populations. There were consistently more amphipods and isopods on the control site compared to the tarp site throughout the sampling season. Invertebrates were most abundant on the tops of rocks in the Spring (Figure 27). This is likely because round gobies migrate to the deeper offshore waters during the late fall and winter seasons (Carlson et al. 2021), allowing invertebrate populations to take advantage of the Spring diatom bloom on the rocks when predation by gobies is minimal. As the season progresses and the water warms, gobies migrate back to the warmer nearshore water (Carlson et al. 2021) and feed on the accessible benthic invertebrates. This may explain the decreased invertebrate abundances found

at SLBE in July and through the rest of the sampling season (Figure 27). However, under the rocks, invertebrate abundances increased as the season progressed (Figure 27). This is likely because the invertebrates have increased refuge under the rocks and in the sediment where they can more easily avoid predation.

Ecosystem energy transfer

Unlike $\delta^{15}N$, $\delta^{13}C$ is conserved or slightly enriched (<1‰) between trophic levels, and therefore the $\delta^{13}C$ of a predator may reflect the $\delta^{13}C$ of its prey (Peterson and Fry 1987; Wada et al. 1993). Because of the conservation of carbon, carbon isotope ratios can be a useful tool to track some of the movement of energy through a food web. The base of the food web consists of primary producers; in the water column they are phytoplankton or seston, and at the bottom of the lake, they are periphyton or benthic algae. Depending on the degree of fractionation, primary producers' $\delta^{13}C$ may reflect some of the $\delta^{13}C$ in the CO_2 that is used for photosynthesis. However, these primary producers may obtain their CO_2 from different sources. $\delta^{13}C$ in phytoplankton can spatially and temporally vary depending on the system they are in (Wada et al. 1993) because phytoplankton obtain CO_2 from the water in which it is suspended. As Lake Michigan completely mixes every year, there is little spatial or temporal variation in the phytoplankton carbon isotopic composition (Turschak and Bootsma 2015). Moreover, phytoplankton discriminate strongly against ^{13}C, giving them a very light $\delta^{13}C$ ratio between -24‰ and -38‰ (France 1995).

Periphyton, or benthic algae, show much greater $\delta^{13}C$ isotopic variation than phytoplankton. This is because of the characteristics of the benthic ecosystem and enrichment or depletion of $\delta^{13}C$ depending on algal growth rates. In the benthic ecosystem, a diffusive boundary layer forms directly around the algae while it is photosynthesizing. Because of this

boundary layer, the algae cannot be nearly as discriminative as phytoplankton, typically giving the benthic algae a heavier $\delta^{13}C$ (France 1995; Hecky and Hesslein 1995; Rau et al. 1996). Algal growth rate also impacts the $\delta^{13}C$ in benthic algae. As algae are more productive and fixing carbon faster, they must become even less selective and discriminatory in taking up ^{13}C because of the deplete CO_2 in the cellular boundary layer (Takahashi et al. 1991; Burkhardt et al. 1999).

Dreissenid mussels are great filter feeders and they filter phytoplankton, which is isotopically light relative to benthic algae (Hecky et al. 2004; Bootsma and Liao 2013; Brothers et al. 2016). Mussels reflect the isotope ratios of the phytoplankton and are also isotopically light (Figure 29). Furthermore, dreissenid mussels respire CO_2 into the benthic ecosystem. This CO_2, along with the other nutrients (Atkinson et al. 2021), is likely utilized by nearby benthic algae for photosynthesis. This suggests that the respired CO_2 by the mussels is giving the benthic algae depleted $\delta^{13}C$ compared to algae that are not growing directly near dreissenid mussels. This is likely what is causing the shift in $\delta^{13}C$ for benthic algae when comparing the control algae to algae on the tarp site. Continuing up a trophic level, the primary consumers in the benthic ecosystem at SLBE are benthic amphipods and isopods. These invertebrates mainly feed on filamentous green algae and benthic diatoms (Fenoglio et al. 2020). Benthic invertebrates do not have a very large habitat range and may live in small microhabitats for the majority or all of their lives (Covich et al. 1999). This makes them useful in measuring the effect of dreissenid mussels or lack thereof, because the invertebrates likely do not migrate back and forth from the control site to the tarp site. Therefore, the shifts seen in the $\delta^{13}C$ of invertebrates suggest a transfer of carbon (initially in the form of CO_2) and nutrients from dreissenid mussels, through benthic algae, to benthic invertebrates in locations where mussels are present.

Invasive species affect their new environments in many ways, especially when multiple new invasive species dominate an ecosystem as has occurred in Lake Michigan. For example, dreissenid mussels reduce offshore phytoplankton production (Fahnenstiel et al. 2010; Vanderploeg et al. 2010), increase water clarity (Fahnenstiel et al. 2010; Mida et al. 2010), and facilitate benthic production in invertebrates and algae (Bially and MacIsaac 2000; Ward and Ricciardi 2007; Brothers et al. 2016). Round gobies negatively impact other nearshore fish species because of competition and egg predation, and they negatively impact benthic invertebrate diversity and abundance because of persistent predation (Lauer et al. 2004; Burkett and Jude 2015; Astorg et al. 2022). However, the data presented here suggests that gobies drastically slow or prevent the repopulation of dreissenid mussels (Figures 23, 24) and mussels protect benthic invertebrates from over-predation by gobies (Figure 27). To put the matter another way, further changing an ecosystem by removing an invasive species like the dreissenid mussel does not mean the ecosystem will return to its pre-dreissenid state. Most aquatic ecosystem managers agree that the prevention of dreissenid mussel invasions is critical to prevent major disruptions to ecosystem structure and function. This can lead to the assumption that, once dreissenids are in a system, their removal is desirable and will have positive effects. This may be the case for many lakes, but in Lake Michigan and the other Great Lakes, invasion by dreissenids is not the only change that has occurred in the past several decades. In particular, the invasive round goby has become an important part of the food web that is taking advantage of the high nearshore productivity for which dreissenids are responsible (Tarsa 2021) and serving as a significant source of energy for some higher trophic level species, including lake trout and burbot (Jacobs et al. 2010; Kornis et al. 2012). The results presented here suggest that round goby production might be negatively affected following any large-scale dreissenid removal.

Further studies, perhaps including dreissenid removal at larger spatial scales, are needed to better understand what the effects on the nearshore food web might be, and how other aspects of nearshore fish ecology, including spawning and recruitment, might be affected by the mussel removal.

CHAPTER 4: SUMMARY

Dreissenid mussels have altered the fundamental structure and biogeochemical functions of Lake Michigan. Today, they are often found in abundances of 6500 individuals m^{-2} or more in the nearshore zone. Although, the impacts of zebra and quagga mussels have been studied extensively since they entered the Great Lakes, critical questions remain regarding their impact on the nearshore nutrient and benthic algal dynamics. The research presented here is based on a novel approach – the removal of dreissenids – to better understand their role in the nearshore ecosystem.

Based on a comparison of the in-lake vs lab experiments, there remains some uncertainty about the length of time required for 100% quagga mussel mortality under anoxic conditions. Laboratory experiments for quagga mussel mortality in this study (4-6 days to 100% mortality) compared well with past zebra mussel mortality studies [4-5 days to 100% mussel mortality (McMahon et al. 1995), 1-7 days to 100% mussel mortality, depending on water temperature (Matthews and McMahon 1995)]. Mussels beneath a benthic barrier membrane in Lake Michigan may live 3-5 times longer than mussels in the lab when no oxygen is being intentionally supplied. These differences are likely due to the physical movement of water and the rugosity of the lake-bottom. Oxygen spiked beneath the benthic barrier membrane episodically throughout the deployment, which likely allowed the mussels to extend the time of their survival. When the mussels are eventually eradicated from an area, it appears that the invasive round goby may help prevent their immediate and rapid re-establishment on the nearshore rocky substrate from which they were removed. When rocks are cleared of mussels, the new juvenile mussels do not have protection from goby predation, and the gobies are able to consume the easily attainable young mussels and stave off the recolonization of that area.

Because the mussels do not recolonize when gobies are present, mussel removal may have long-term effects.

Cladophora is not invasive, but due to the sequestering of nutrients in the nearshore benthos by dreissenids (Hecky et al. 2004; Bootsma et al. 2012, 2015) and increased water clarity (Auer et al. 2010; Fahnenstiel et al. 2010; Mida et al. 2010; Tomlinson et al. 2010; Kuczynski et al. 2016), it has been growing at nuisance levels in the Lake Michigan nearshore near SLBE, as well as other areas around the lake (Shuchman et al. 2013). However, nuisance *Cladophora* growth rate is severely diminished where dreissenid mussels are removed. Algae growing at sites where mussels are removed have significantly less internal P, and therefore, have much lower growth rates (Figures 15, 17, and 20) compared to algae growing where mussels are present. Benthic algae in the SLBE area of Lake Michigan are already P-stressed, and this is further exacerbated when quagga mussels are removed. Growth rates for benthic algae with internal P that is in the range found at SLBE are extremely sensitive to changes in P availability (Figure 20). Compared to algae growing on the control site, algae growing on the scrape site, which had mussels removed six years prior to sampling, had an average of 0.15 μg mg^{-1} less internal P (22% difference); and algae on the tarp site had an average of 0.25 μg mg^{-1} less internal P (37% difference). When fitted to the Droop model, the growth vs P content relationship observed in this study indicates that a change in algal internal P from 0.60 μg mg^{-1} to 0.45 μg mg^{-1} can reduce the maximum growth rate of that algae by 33%. A drop in algal P from 0.60 μg mg^{-1} to 0.35 μg mg^{-1} can reduce the maximum growth rate by 71%. This sensitivity of algal growth rates to internal P concentration changes can be a potentially significant benefit of dreissenid mussel removal, specifically when attempting to manage nuisance *Cladophora* growth.

Internal phosphorus concentration also affects the community composition of benthic algae found in Lake Michigan. There have been various reported values of the minimum phosphorus cell quota for *Cladophora*: 0.6 μg mg^{-1} (Auer and Canale 1982b), 0.4 μg mg^{-1} (Kuczynski et al. 2022), and 0.35 μg mg^{-1} (Tomlinson et al. 2010). When the benthic algae have internal P concentrations nearing the most recent reported *Cladophora* minimum cell quotas of 0.35 – 0.4 μg mg^{-1}, benthic algae are dominated by diatoms. This occurs most often in algal communities where mussels are absent (Figure 19), furthering the likelihood of managers curbing the nuisance *Cladophora* growth by removing mussels from an area.

It is important to note that the ecosystem would likely not return to its pre-dreissenid state if those mussels are removed. Since the initial invasion of dreissenids, other changes have occurred, including the establishment of other invasive species, such as the round goby, continued declines in external phosphorus loading (Madenjian et al. 2002; Chapra and Dolan 2012; Bunnell et al. 2014; Rowe et al. 2017), and changes in relative abundance of various food web components ranging from zooplankton to piscivores (Bunnell et al. 2014). Although round gobies may be the reason mussel removal projects may have long-term effects, they also may be reducing non-dreissenid benthic invertebrates after mussels are removed. This study shows benthic invertebrate populations crash when the mussels are removed, likely because they lose the structural protection and increased nutrient cycling that the mussels provide. Non-dreissenid benthic invertebrates are a large part of the nearshore benthic food web, and reductions in those populations may have widespread effects on upper trophic level fishes. It is important to further our understanding of the effects that mussel removal has on round goby populations and other higher level trophic level fish populations in the Lake Michigan nearshore food web, especially if larger-scale mussel removal projects of are to be attempted in the future.

Impacts of mussel removal were shown to percolate throughout the food web. $\delta^{13}C$ isotope values in benthic algae and non-dreissenid benthic invertebrates, specifically during months of high productivity (July and August), show a shift in $\delta^{13}C$ between the control site and mussel-free tarp site (Figure 29). Mussels filter the water, consuming and metabolizing isotopically light phytoplankton. This light $\delta^{13}C$ signature is reflected in dreissenid mussels because $\delta^{13}C$ is largely conserved throughout trophic levels (Peterson and Fry 1987). Furthermore, The low $\delta^{13}C$ values of algae growing in the presence of dreissenids may reflect the uptake of isotopically light CO_2 respired by dreissenids, just as the benthic algae appear to derive some of their P from dreissenid excretion. Benthic algae growing in the absence of mussels do not receive CO_2 directly respired by mussels, giving them a higher $\delta^{13}C$ signature compared to algae on the control site. This light isotopic signature was also observed in non-dreissenid benthic invertebrates living in mussel beds, suggesting they are strongly dependent on benthic algae as an organic carbon source. Invertebrates living in the absence of dreissenid mussels have a heavier $\delta^{13}C$ signature than invertebrates growing in the presence of dreissenid mussels, providing further evidence of dreissenid mussel induced nutrient cycling in the benthos.

This study furthers our understanding of how dreissenids affect nearshore dynamics of nutrients, benthic algae, and non-dreissenid benthic invertebrates in Lake Michigan. By removing dreissenid mussels, this study elucidates many questions regarding invasive mussels and their impact at SLBE. Some of the questions answered or partially answered include: How long does it take for mussels to repopulate after removal? To what degree do nearshore benthic algae rely on dreissenids as a phosphorus source? How do dreissenids affect the abundance and community composition of non-dreissenid benthic macroinvertebrates? Even as answers to all these questions are now recognized, many more questions remain. Future research should

examine the impact of mussel removal on the upper trophic levels in the food web. If the invertebrate populations decline because of mussel removal, do round goby or other nearshore fish populations suffer? Finally, all of this research was conducted at a depth of 10 meters Good Harbor Bay. Changes in depth greatly affect one of the main factors of *Cladophora* growth: light availability. In deeper water, where there is less light, removing mussels may not affect the benthic algae growth as much because there is already decreased growth. If there are similar negative impacts on non-dreissenid benthic invertebrates, more negative effects may arise from removal at those depths. However, in shallow waters, *Cladophora* is much more productive because it receives more light, therefore, removing mussels in shallow water may have disproportionate effects on the nuisance algal growth. Future work should focus on the effects of mussel removal at different depths, both in shallower and deeper waters.

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APPENDIX A: SLBE Algae Data

Replicate	Date	Site	Area Sampled (m2)	Dry mass (g)	Biomass (gDW m-2)	P content (µg mg-1)	δ13C	δ15N
A	5/25/2022	Control	0.04	1.91	47.80	0.39	-16.20	4.14
B	5/25/2022	Control	0.04	4.29	107.35	0.35	-17.25	0.88
C	5/25/2022	Control	0.04	4.18	104.54	0.53	-17.86	5.17
A	5/25/2022	Tarp	0.04	3.52	87.99	0.35	-17.49	-2.09
B	5/25/2022	Tarp	0.04	3.93	98.19	0.41	-18.54	2.25
C	5/25/2022	Tarp	0.04	4.19	104.79	0.31	-18.07	-4.73
A	7/12/2022	Control	0.04	1.89	47.16	0.55	-23.53	0.63
B	7/12/2022	Control	0.04	2.05	51.20	0.61	-22.04	0.36
C	7/12/2022	Control	0.04	1.55	38.64	0.63	-24.56	3.01
A	7/12/2022	Tarp	0.04	2.30	57.56	0.36	-18.65	8.21
B	7/12/2022	Tarp	0.04	1.59	39.87	0.30	-20.00	10.81
C	7/12/2022	Tarp	0.04	3.58	89.48	0.40	-18.95	-4.58
A	7/12/2022	Scrape	0.04	9.03	225.73	0.34	-18.24	-5.31
B	7/12/2022	Scrape	0.04	3.61	90.30	0.41	-20.61	-3.00
C	7/12/2022	Scrape	0.04	1.78	44.53	0.42	-20.93	1.68
A	8/24/2022	Control	0.04	2.27	56.80	0.51	-20.90	0.97
B	8/24/2022	Control	0.04	3.62	90.56	0.56	-20.73	-0.03
C	8/24/2022	Control	0.04	2.89	72.33	0.47	-20.07	-1.01
A	8/24/2022	Tarp	0.04	2.83	70.71	0.43	-19.07	-0.98
B	8/24/2022	Tarp	0.04	0.78	19.49	0.27	-18.99	-0.30
C	8/24/2022	Tarp	0.04	1.35	33.76	0.36	-19.05	4.63
A	8/24/2022	Scrape	0.04	2.90	72.38	0.43	-20.57	-2.47
B	8/24/2022	Scrape	0.04	1.92	47.88	0.49	-16.82	0.70
C	8/24/2022	Scrape	0.04	3.48	86.88	0.41	-19.80	3.69
A	10/25/2022	Control	0.04	0.01	0.30	N/A	-22.44	1.07
B	10/25/2022	Control	0.04	0.00	0.11	0.87	-22.89	1.97

C	10/25/2022	Control	0.04	0.05	1.20	0.94	-22.25	0.63
A	10/25/2022	Tarp	0.04	0.46	11.46	0.45	-22.15	2.69
B	10/25/2022	Tarp	0.04	0.30	7.57	0.29	-21.92	2.72
C	10/25/2022	Tarp	0.04	0.50	12.47	0.34	-21.92	3.96
A	10/25/2022	Scrape	0.04	0.11	2.83	0.70	-22.24	0.87
B	10/25/2022	Scrape	0.04	0.13	3.15	0.74	-21.71	0.52
C	10/25/2022	Scrape	0.04	0.04	1.02	0.76	-21.53	-0.14

APPENDIX B: *In Situ* Incubations

These incubations were done at Good Harbor Bay near Sleeping Bear Dunes National Lakeshore at a depth of 10 meters. The tarp site indicates the site where mussels were removed in the Summer of 2021. The control site is where the mussels were not removed.

Month	Incubation	Irradiance (mmols photons m^{-2} s^{-1})	Δ D.O. ($mg\ L^{-1}$)	Dry mass (mg)	Time (min)	Time (hr)	P-content (μg P mg^{-1})	θ (12 mgC 32 mg O_2^{-1})	Net Photosynthetic growth rate ($mgO_2\ mgDW^{-1}\ hr^{-1}$)	Net Specific Growth Rate (day^{-1})
Aug	1 Light Tarp	130	8.25	1215.0	61	1.02	0.40	0.38	0.004	0.175
Aug	1 Dark Tarp	130	-1.01	669.3	61	1.02	0.40	0.38	-0.001	-0.051
Aug	2 Light Tarp	180	2.46	1326.5	22	0.37	0.33	0.38	0.003	0.133
Aug	2 Dark Tarp	180	-0.34	911.2	22	0.37	0.35	0.38	-0.001	-0.034
Aug	1 Light Ctl	200	5.65	737.4	26	0.43	0.58	0.38	0.010	0.464
Aug	1 Dark Ctl	200	-0.07	618.8	26	0.43	0.41	0.38	0.000	-0.009
Aug	2 Light Ctl	70	1.06	948.5	14	0.23	0.50	0.38	0.003	0.125
Aug	2 Dark Ctl	70	-0.37	1291.0	14	0.23	0.48	0.38	-0.001	-0.042
July	Light Tarp	200	0.52	676.7	54	0.90	0.39	0.38	0.000	0.039
July	Dark Tarp	200	-0.69	1366.7	54	0.90	0.30	0.38	0.000	-0.026
July	Light Ctl	200	2.27	1023.1	16	0.27	0.63	0.38	0.005	0.380
July	Dark Ctl	200	-0.28	112.6	16	0.27	0.62	0.38	-0.007	-0.423
Oct	1 Light Tarp	48	1.41	154.4	112	1.87	0.27	0.38	0.003	0.128
Oct	1 Dark Tarp	48	-0.11	140.5	112	1.87	0.27	0.38	0.000	-0.015
Oct	2 Light Tarp	85	4.27	287.1	87	1.45	0.51	0.38	0.006	0.269
Oct	2 Dark Tarp	85	-0.43	233.0	87	1.45	0.36	0.38	-0.001	-0.043
Oct	1 Light Ctl	48	2.40	69.8	112	1.87	0.51	0.38	0.011	0.482
Oct	1 Dark Ctl	48	-0.73	39.5	112	1.87	0.55	0.38	-0.007	-0.340
Oct	2 Light Ctl	85	5.13	108.6	87	1.45	0.72	0.38	0.019	0.853
Oct	2 Dark Ctl	85	-0.32	171.7	87	1.45	0.70	0.38	-0.001	-0.044

APPENDIX C: SLBE Lab Incubation Experiment

These algae samples were collected in Good Harbor Bay near Sleeping Bear Dunes National Lakeshore at a depth of 10 meters in October 2022. The tarp site indicates the site where mussels were removed in the Summer of 2021. The control site is where the mussels were not removed.

Site	Irradiance	Time	Water Volume	Δ D.O.	Δ D.O.	Dry mass	P-content	θ	Net Photosynthetic growth rate	Net Specific Growth Rate
	(mmols photons m^{-2} s^{-1})	(hr)	(L)	(mg)	(mg L^{-1})	(mg)	(µg P mg^{-1})	(12 mgC 32 mg O_2^{-1})	(mgO_2 $mgDW^{-1}$ hr^{-1})	(day^{-1})
Control	1000	2	0.55	1.6	2.91	30.29	0.617	0.375	0.0264	1.19
Control	250	2	0.55	1.8	3.27	44.85	0.581	0.375	0.0201	0.903
Control	85	2	0.55	1.1	2.0	29.61	0.461	0.375	0.0188	0.836
Control	35	2	0.55	0.4	0.727	38.22	0.521	0.375	0.00523	0.235
Control	15	2	0.55	0.2	0.364	48.59	0.668	0.375	0.00206	0.0926
Tarp	1000	2	0.55	1.7	3.10	202	0.319	0.375	0.00421	0.189
Tarp	250	2	0.55	1.5	2.72	185	0.454	0.375	0.00405	0.182
Tarp	85	2	0.55	0.8	1.45	190	0.301	0.375	0.00211	0.0947
Tarp	35	2	0.55	0.4	0.727	158	0.444	0.375	0.00127	0.0570
Tarp	15	2	0.55	0.1	0.182	148	0.364	0.375	0.00034	0.0152

APPENDIX D: Milwaukee Lab Incubations: Experiment 1

These algae were collected in Lake Michigan near Milwaukee at a depth of 5 meters on September 14, 2022.

Irradiance (mmols photons m^{-2} s^{-1})	Water Volume (L)	Time (hr)	Δ D.O. (mg)	Δ D.O. (mg L^{-1})	Dry mass (mg)	P-content (µg P mg^{-1})	θ (12 mgC 32 mg $O2^{-1}$)	Net Photosynthetic growth rate (mgO2 $mgDW^{-1}$ hr^{-1})	Net Specific Growth Rate (day^{-1})
0	0.55	2	-0.7	-1.27	185.2	0.620	0.375	-0.0019	-0.0850
0	0.55	2	-0.7	-1.27	182.9	0.708	0.375	-0.0019	-0.0861
0	0.55	2	-0.6	-1.09	127.1	0.634	0.375	-0.0024	-0.1062
0	0.55	2	-0.5	-0.91	136.1	0.644	0.375	-0.0018	-0.0827
0	0.55	2	-0.7	-1.27	167.0	0.709	0.375	-0.0021	-0.0943
0	0.55	2	-1.1	-2.00	262.3	0.841	0.375	-0.0021	-0.0944
0	0.55	2	-0.7	-1.27	167.3	0.792	0.375	-0.0021	-0.0941
14	0.55	2	1.4	2.55	194.5	0.818	0.375	0.0036	0.1620
14	0.55	2	1.4	2.55	148.8	0.742	0.375	0.0047	0.2117
14	0.55	2	2	3.64	140.2	0.840	0.375	0.0071	0.3210
14	0.55	2	1.8	3.27	141.0	0.750	0.375	0.0064	0.2872
14	0.55	2	1.8	3.27	155.1	0.758	0.375	0.0058	0.2611
14	0.55	2	0.5	0.91	159.0	0.711	0.375	0.0016	0.0708
14	0.55	2	1	1.82	135.4	0.923	0.375	0.0037	0.1662
14	0.55	2	2.2	4.00	168.2	0.859	0.375	0.0065	0.2943
32	0.55	2	2.8	5.09	160.4	0.707	0.375	0.0087	0.3928
32	0.55	2	2.8	5.09	185.0	0.811	0.375	0.0076	0.3405
32	0.55	2	2.6	4.73	156.6	0.737	0.375	0.0083	0.3736
32	0.55	2	2.6	4.73	130.5	0.728	0.375	0.0100	0.4483
32	0.55	2	2.6	4.73	150.2	0.765	0.375	0.0087	0.3895
32	0.55	2	1.6	2.91	146.9	0.727	0.375	0.0054	0.2451
32	0.55	2	1.4	2.55	146.6	0.904	0.375	0.0048	0.2149

32	0.55	2	2.7	4.91	176.4	0.826	0.375	0.0077	0.3444
105	0.55	2	3.9	7.09	157.4	0.731	0.375	0.0124	0.5575
105	0.55	2	4.1	7.45	184.6	0.780	0.375	0.0111	0.4997
105	0.55	2	4.6	8.36	164.3	0.831	0.375	0.0140	0.6299
105	0.55	2	4.9	8.91	145.8	0.745	0.375	0.0168	0.7562
105	0.55	2	4.3	7.82	132.7	0.707	0.375	0.0162	0.7291
105	0.55	2	2.9	5.27	148.6	0.693	0.375	0.0098	0.4391
105	0.55	2	3.3	6.00	162.7	0.801	0.375	0.0101	0.4564
105	0.55	2	6.6	12.00	175.7	0.777	0.375	0.0188	0.8452
400	0.55	2	3.7	6.73	107.3	0.672	0.375	0.0172	0.7759
400	0.55	2	4.5	8.18	147.3	0.711	0.375	0.0153	0.6874
400	0.55	2	6.4	11.64	183.8	0.721	0.375	0.0174	0.7835
400	0.55	2	7.1	12.91	152.6	0.735	0.375	0.0233	1.0469
400	0.55	2	6.2	11.27	165.6	0.775	0.375	0.0187	0.8424
400	0.55	2	5.6	10.18	167.7	0.698	0.375	0.0167	0.7513
400	0.55	2	5.8	10.55	179.0	0.754	0.375	0.0162	0.7291
400	0.55	2	6.8	12.36	170.4	0.789	0.375	0.0200	0.8979

APPENDIX D: Milwaukee Lab Incubations: Experiment 2

These algae were collected in Lake Michigan near Milwaukee at a depth of 5 meters on September 26, 2022.

Irradiance	Water Volume	Time (hr)	Δ D.O.	Δ D.O.	Dry mass	P-content	θ	Net Photosynthetic growth rate	Net Specific Growth Rate
(mmols photons m^{-2} s^{-1})	(L)		(mg)	(mg L^{-1})	(mg)	(µg P mg^{-1})	(12 mgC 32 mg $O2^{-1}$)	(mgO2 $mgDW^{-1}$ hr^{-1})	(day^{-1})
0	0.55	2	-0.8	-1.45	208.8	1.683	0.375	-0.0019	-0.0862
0	0.55	2	-0.9	-1.64	193.1	1.669	0.375	-0.0023	-0.1049
0	0.55	2	-0.8	-1.45	271.3	1.646	0.375	-0.0015	-0.0663
0	0.55	2	-0.8	-1.45	212.4	1.586	0.375	-0.0019	-0.0847
0	0.55	2	-0.8	-1.45	172.5	1.718	0.375	-0.0023	-0.1043
0	0.55	2	-0.8	-1.45	196.9	1.695	0.375	-0.0020	-0.0914
0	0.55	2	-0.9	-1.64	197.1	1.929	0.375	-0.0023	-0.1027
20	0.55	2	0.5	0.91	207.5	1.690	0.375	0.0012	0.0542
20	0.55	2	0.2	0.36	232.3	1.537	0.375	0.0004	0.0194
20	0.55	2	0.1	0.18	228.8	1.645	0.375	0.0002	0.0098
20	0.55	2	0.4	0.73	216.3	1.685	0.375	0.0009	0.0416
20	0.55	2	0.6	1.09	228.8	1.718	0.375	0.0013	0.0590
20	0.55	2	0.5	0.91	193.0	1.697	0.375	0.0013	0.0583
20	0.55	2	0.2	0.36	232.8	1.957	0.375	0.0004	0.0193
35	0.55	2	1.6	2.91	197.4	1.693	0.375	0.0041	0.1824
35	0.55	2	1.1	2.00	204.6	1.539	0.375	0.0027	0.1210
35	0.55	2	1.2	2.18	213.2	1.572	0.375	0.0028	0.1266
35	0.55	2	1.1	2.00	213.4	1.694	0.375	0.0026	0.1160
35	0.55	2	1.3	2.36	199.1	1.722	0.375	0.0033	0.1469
35	0.55	2	1.4	2.55	226.6	1.788	0.375	0.0031	0.1390
35	0.55	2	1.1	2.00	225.6	1.854	0.375	0.0024	0.1097

130	0.55	2	4.1	7.45	224.0	1.565	0.375	0.0092	0.4118
130	0.55	2	4.7	8.55	208.8	1.656	0.375	0.0113	0.5065
130	0.55	2	3.6	6.55	208.4	1.734	0.375	0.0086	0.3887
130	0.55	2	4.7	8.55	263.2	1.619	0.375	0.0089	0.4018
130	0.55	2	4	7.27	212.5	1.676	0.375	0.0094	0.4235
130	0.55	2	4.7	8.55	213.5	1.653	0.375	0.0110	0.4953
130	0.55	2	3.7	6.73	172.0	1.641	0.375	0.0108	0.4840
350	0.55	2	7.9	14.36	238.0	1.579	0.375	0.0166	0.7468
350	0.55	2	8.3	15.09	179.3	1.596	0.375	0.0231	1.0416
350	0.55	2	8.8	16.00	220.1	1.592	0.375	0.0200	0.8996
350	0.55	2	8.4	15.27	184.7	1.486	0.375	0.0227	1.0233
350	0.55	2	8.6	15.64	228.6	1.601	0.375	0.0188	0.8465
350	0.55	2	8.9	16.18	199.9	1.664	0.375	0.0223	1.0018
350	0.55	2	8.4	15.27	197.1	1.857	0.375	0.0213	0.9589

APPENDIX E: SLBE Benthic Invertebrates Abundance

These non-dreissenid benthic invertebrates were collected at Good Harbor Bay near Sleeping Bear Dunes National Lakeshore at a depth of 10 meters. The tarp site indicates the site where mussels were removed in the Summer of 2021. The control site is where the mussels were not removed. Top indicates invertebrate samples collected on top of rocks via benthic scrapes and bottom indicates invertebrates collected underneath the rocks at the rock-sediment interface using a benthic air lift device.

Repl. No.	DATE collected	Station	Group	Count	Area Sampled (m^2)	Density (ind m^{-2})	Dry Weight (mg)	Biomass(mgDW m^{-2})
A	5/25/2022	Control Top	Amphipod	58	0.04	1450.0	141.4	3535.0
A	5/25/2022	Control Top	Isopod	14	0.04	350.0	42.2	1055.0
A	5/25/2022	Control Top	Planaria	1	0.04	25.0	0.0	0.0
B	5/25/2022	Control Top	Amphipod	33	0.04	825.0	51.0	1275.0
B	5/25/2022	Control Top	Isopod	19	0.04	475.0	49.5	1237.5
C	5/25/2022	Control Top	Amphipod	68	0.04	1700.0	177.9	4447.5
C	5/25/2022	Control Top	Isopod	41	0.04	1025.0	105.4	2635.0
C	5/25/2022	Control Top	Leech	1	0.04	25.0	1.0	25.0
A	5/25/2022	Tarp Top	Amphipod	3	0.04	75.0	11.4	285.0
A	5/25/2022	Tarp Top	Isopod	3	0.04	75.0	10.8	270.0
A	5/25/2022	Tarp Top	Oligochaete	2	0.04	50.0	0.0	0.0
B	5/25/2022	Tarp Top	Amphipod	1	0.04	25.0	6.4	160.0
B	5/25/2022	Tarp Top	Isopod	3	0.04	75.0	16.6	415.0
B	5/25/2022	Tarp Top	Chironomid	1	0.04	25.0	0.0	0.0
C	5/25/2022	Tarp Top	Isopod	2	0.04	50.0	8.7	217.5
A	7/12/2022	Control Bottom	Amphipod	18	0.03	600.0	4.0	133.1
A	7/12/2022	Control Bottom	Isopod	63	0.03	2100.0	5.3	175.9
B	7/12/2022	Control Bottom	Amphipod	12	0.03	400.0	9.8	326.5
B	7/12/2022	Control Bottom	Isopod	18	0.03	600.0	2.1	69.3
C	7/12/2022	Control Bottom	Amphipod	21	0.03	700.0	1.7	56.9
C	7/12/2022	Control Bottom	Isopod	44	0.03	1466.7	3.3	109.2

A	7/12/2022	Tarp Bottom	Amphipod	12	0.03	400.0	0.7	22.0
A	7/12/2022	Tarp Bottom	Isopod	12	0.03	400.0	2.5	82.5
B	7/12/2022	Tarp Bottom	Amphipod	3	0.03	100.0	0.9	31.4
B	7/12/2022	Tarp Bottom	Isopod	2	0.03	66.7	0.1	3.9
C	7/12/2022	Tarp Bottom	Amphipod	13	0.03	433.3	8.1	271.2
C	7/12/2022	Tarp Bottom	Isopod	8	0.03	266.7	1.6	53.4
B	7/12/2022	Control Top	Amphipod	43	0.04	1075.0	39.1	977.9
B	7/12/2022	Control Top	Isopod	1	0.04	25.0	0.2	3.8
C	7/12/2022	Control Top	Amphipod	21	0.04	525.0	11.3	283.7
C	7/12/2022	Control Top	Isopod	2	0.04	50.0	1.5	38.6
A	7/12/2022	Scrape Top	Amphipod	0	0.04	0.0	0.0	0.0
A	7/12/2022	Scrape Top	Isopod	1	0.04	25.0	1.1	26.3
A	7/12/2022	Scrape Top	Chironomid	1	0.04	25.0	0.2	5.2
A	7/12/2022	Tarp Top	Amphipod	0	0.04	0.0	0.0	0.0
A	7/12/2022	Tarp Top	Isopod	1	0.04	25.0	0.1	1.5
B	7/12/2022	Tarp Top	Amphipod	0	0.04	0.0	0.0	0.0
B	7/12/2022	Tarp Top	Isopod	0	0.04	0.0	0.0	0.0
B	7/12/2022	Tarp Top	Chironomid	1	0.04	25.0	ND	N/A
C	7/12/2022	Scrape Top	Amphipod	0	0.04	0.0	0.0	0.0
C	7/12/2022	Scrape Top	Isopod	0	0.04	0.0	0.0	0.0
B	7/12/2022	Scrape Top	Amphipod	0	0.04	0.0	0.0	0.0
B	7/12/2022	Scrape Top	Isopod	1	0.04	25.0	0.9	21.3
C	7/12/2022	Tarp Top	Amphipod	1	0.04	25.0	0.1	1.3
C	7/12/2022	Tarp Top	Isopod	0	0.04	0.0	0.0	0.0
A	7/12/2022	Control Top	Amphipod	22	0.04	550.0	21.2	528.9
A	7/12/2022	Control Top	Isopod	3	0.04	75.0	0.4	10.7
A	8/23/2022	Control Top	Isopod	12	0.04	300.0	8.2	203.8
A	8/23/2022	Control Top	Amphipod	22	0.04	550.0	7.3	182.7
A	8/23/2022	Control Top	Oligochaete	4	0.04	100.0	ND	N/A

A	8/23/2022	Control Top	Gastropod	3	0.04	75.0	28.8	719.6
B	8/23/2022	Control Top	Isopod	5	0.04	125.0	1.5	37.5
B	8/23/2022	Control Top	Amphipod	8	0.04	200.0	2.6	64.5
B	8/23/2022	Control Top	Oligochaete	2	0.04	50.0	ND	N/A
B	8/23/2022	Control Top	Chironomid	1	0.04	25.0	ND	N/A
B	8/23/2022	Control Top	Gastropod	4	0.04	100.0	34.7	866.6
C	8/23/2022	Control Top	Isopod	17	0.04	425.0	12.7	318.4
C	8/23/2022	Control Top	Amphipod	52	0.04	1300.0	23.9	596.6
C	8/23/2022	Control Top	Chironomid	2	0.04	50.0	0.4	9.6
C	8/23/2022	Control Top	Gastropod	17	0.04	425.0	103.2	2580.7
A	8/23/2022	Scrape Top	Isopod	1	0.04	25.0	0.4	9.2
A	8/23/2022	Scrape Top	Amphipod	1	0.04	25.0	0.2	6.2
A	8/23/2022	Scrape Top	Chironomid	1	0.04	25.0	0.2	5.0
B	8/23/2022	Scrape Top	Amphipod	1	0.04	25.0	0.5	11.7
B	8/23/2022	Scrape Top	Oligochaete	11	0.04	275.0	0.1	2.1
C	8/23/2022	Scrape Top	Isopod	1	0.04	25.0	0.3	6.7
A	8/23/2022	Tarp Top	Amphipod	1	0.04	25.0	0.5	12.5
A	8/23/2022	Tarp Top	Chironomid	2	0.04	50.0	0.2	5.7
B	8/23/2022	Tarp Top	Oligochaete	1	0.04	25.0	ND	N/A
C	8/23/2022	Tarp Top	Isopod	1	0.04	25.0	0.3	7.5
C	8/23/2022	Tarp Top	Oligochaete	1	0.04	25.0	0.1	1.3
A	8/23/2022	Tarp Bottom	Isopod	9	0.03	300.0	2.1	53.2
A	8/23/2022	Tarp Bottom	Amphipod	8	0.03	266.7	1.8	59.7
B	8/23/2022	Tarp Bottom	Isopod	8	0.03	266.7	2.1	70.6
B	8/23/2022	Tarp Bottom	Amphipod	30	0.03	1000.0	6.8	226.3
C	8/23/2022	Tarp Bottom	Isopod	5	0.03	166.7	3.1	101.7
C	8/23/2022	Tarp Bottom	Amphipod	9	0.03	300.0	1.8	59.0
A	8/23/2022	Control Bottom	Isopod	5	0.03	166.7	ND	N/A
A	8/23/2022	Control Bottom	Amphipod	60	0.03	2000.0	ND	N/A

A	8/23/2022	Control Bottom	Oligochaete	1	0.03	33.3	ND	N/A
B	8/23/2022	Control Bottom	Amphipod	17	0.03	566.7	ND	N/A
B	8/23/2022	Control Bottom	Isopod	2	0.03	66.7	ND	N/A
C	8/23/2022	Control Bottom	Amphipod	37	0.03	1233.3	3.1	104.0
C	8/23/2022	Control Bottom	Isopod	5	0.03	166.7	25.7	856.1
A	10/25/2022	Control Top	Amphipod	14	0.04	350.0	8.6	215.2
A	10/25/2022	Control Top	Isopod	5	0.04	125.0	4.2	106.2
B	10/25/2022	Control Top	Amphipod	5	0.04	125.0	3.2	79.8
B	10/25/2022	Control Top	Isopod	3	0.04	75.0	1.4	35.3
C	10/25/2022	Control Top	Amphipod	28	0.04	700.0	21.4	535.4
C	10/25/2022	Control Top	Isopod	4	0.04	100.0	2.0	50.8
A	10/25/2022	Scrape Top	Amphipod	1	0.04	25.0	0.2	3.8
A	10/25/2022	Scrape Top	Isopod	0	0.04	0.0	0.0	0.0
B	10/25/2022	Scrape Top	Amphipod	3	0.04	75.0	0.3	7.0
B	10/25/2022	Scrape Top	Isopod	0	0.04	0.0	0.0	0.0
C	10/25/2022	Scrape Top	Amphipod	2	0.04	50.0	2.6	65.3
C	10/25/2022	Scrape Top	Isopod	0	0.04	0.0	0.0	0.0
A	10/25/2022	Tarp Top	Amphipod	2	0.04	50.0	1.3	31.4
A	10/25/2022	Tarp Top	Isopod	0	0.04	0.0	0.0	0.0
B	10/25/2022	Tarp Top	Amphipod	2	0.04	50.0	0.9	21.9
B	10/25/2022	Tarp Top	Isopod	1	0.04	25.0	0.4	10.1
C	10/25/2022	Tarp Top	Amphipod	3	0.04	75.0	0.4	10.4
C	10/25/2022	Tarp Top	Isopod	0	0.04	0.0	0.0	0.0
A	10/25/2022	Control Bottom	Amphipod	134	0.03	4466.7	126.7	4221.8
A	10/25/2022	Control Bottom	Isopod	19	0.03	633.3	14.0	465.3
B	10/25/2022	Control Bottom	Amphipod	86	0.03	2866.7	90.1	3003.0
B	10/25/2022	Control Bottom	Isopod	10	0.03	333.3	6.3	210.4
C	10/25/2022	Control Bottom	Amphipod	97	0.03	3233.3	90.0	3000.9
C	10/25/2022	Control Bottom	Isopod	24	0.03	800.0	18.0	599.8

A	10/25/2022	Tarp Bottom	Amphipod	66	0.03	2200.0	51.3	1711.3
A	10/25/2022	Tarp Bottom	Isopod	9	0.03	300.0	8.0	267.5
B	10/25/2022	Tarp Bottom	Amphipod	70	0.03	2333.3	51.5	1717.9
B	10/25/2022	Tarp Bottom	Isopod	4	0.03	133.3	5.2	173.3
C	10/25/2022	Tarp Bottom	Amphipod	101	0.03	3366.7	86.1	2870.4
C	10/25/2022	Tarp Bottom	Isopod	11	0.03	366.7	7.5	251.6