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Title of Thesis: Phytoplankton-Removal Ecosystem Services of a Native Mussel in an Urban Estuary

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2. **Kido, A**; Using Mussels and Barnacles to Reduce Algae Blooms in Baltimore's Inner Harbor; Presented at: Spring Quarterly Meeting: Wildlife/Organismal Biology & Baltimore Communities, April 2023; Baltimore, MD.
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Title of Document: PHYTOPLANKTON-REMOVAL ECOSYSTEM SERVICES OF A NATIVE MUSSEL IN AN URBAN ESTUARY

Allyson Kimi Kido, M.S., 2024

Directed By: Dr. Eric Schott; Associate Research Professor; Marine, Estuarine, and Environmental Science

Baltimore Harbor is stressed by nutrients in stormwater runoff. Efforts to reduce nutrients focus on point sources, but native bivalves provide an additional nutrient-removal opportunity. The dark false mussel, *Mytilopsis leucophaeata*, is a native bivalve that grows abundantly in Baltimore Harbor. Using lab algae cultures and wild algae blooms, I examined the ability of *M. leucophaeata* from Baltimore Harbor to reduce algae levels, and thus the nutrients within the algae. First, I tested if *M. leucophaeata* can reduce cultured algae species. I then evaluated the ability of *M. leucophaeata* to reduce algae levels under different temperature and salinity conditions. Finally, I established that *M. leucophaeata* can reduce wild algae blooms. Results show that *M. leucophaeata* can reduce both lab grown and wild algae blooms. Overall, these results show that *M. leucophaeata* can provide similar ecosystem services to that of oysters and show promise for nature-based nutrient reduction.

PHYTOPLANKTON-REMOVAL ECOSYSTEM SERVICES OF A NATIVE MUSSEL IN AN URBAN ESTUARY

By

Allyson K. Kido

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore County, in partial fulfillment
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2024

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Chapter 1: Eutrophication in Urban Estuaries and the Dark False Mussel, *Mytilopsis leucophaeata*

The Chesapeake Bay is the largest estuary in North America, and has a large cultural, economic, and ecological significance for the Atlantic coast. Its watershed is home to over 17 million people, and within the water, there are more than 250 fish species that use the estuary for habitat (NOAA, 2021). However, the Chesapeake Bay has been plagued by contaminants from agricultural and urban runoff. Excess nutrients in the water can lead to intense phytoplankton blooms that directly shade submerged aquatic vegetation (SAV) habitat and contribute to fish kills as a result of microbial decomposition of the dead phytoplankton that drives down oxygen (Bricker et al., 2008; Lefcheck et al., 2018). While most of the nutrient contamination comes from agricultural land uses, urban environments also contribute nutrients to the Chesapeake Bay. Urban areas also have the added challenge of mitigating runoff from impervious surfaces that increase the runoff from precipitation. This leads to nutrients and contaminants entering the Bay. To combat the issue of increased nutrients in the water, attention needs to be paid to limiting and reducing the inputs into the bay and removing nutrients already in the water. While the reduction of nutrients from the watershed is essential, there is a need to mitigate the effects of nutrient pollution in urban waters. One promising approach to reduce nutrients and phytoplankton is through the ecosystem services of suspension feeding bivalves.

Baltimore Harbor is an Urban Estuary:

Baltimore Harbor is located on the Patapsco River in Maryland, in the upper portion of the Chesapeake Bay. The harbor spans about 6000 acres, and is classified as a mesohaline tidal, with a salinity of 6-15 ppt (Baltimore City and Baltimore Department of Public Works, 2019). Impervious surface in the immediate region surrounding the harbor is 38% (Figure 1). Most of the freshwater input to the Inner Harbor comes from the Jones Falls, which has a watershed that is 32% impervious surface. Freshwater entering the Middle Branch originates in the Gwynns Falls watershed, which is 33% impervious (Parsons Brickerhoff, 2004).

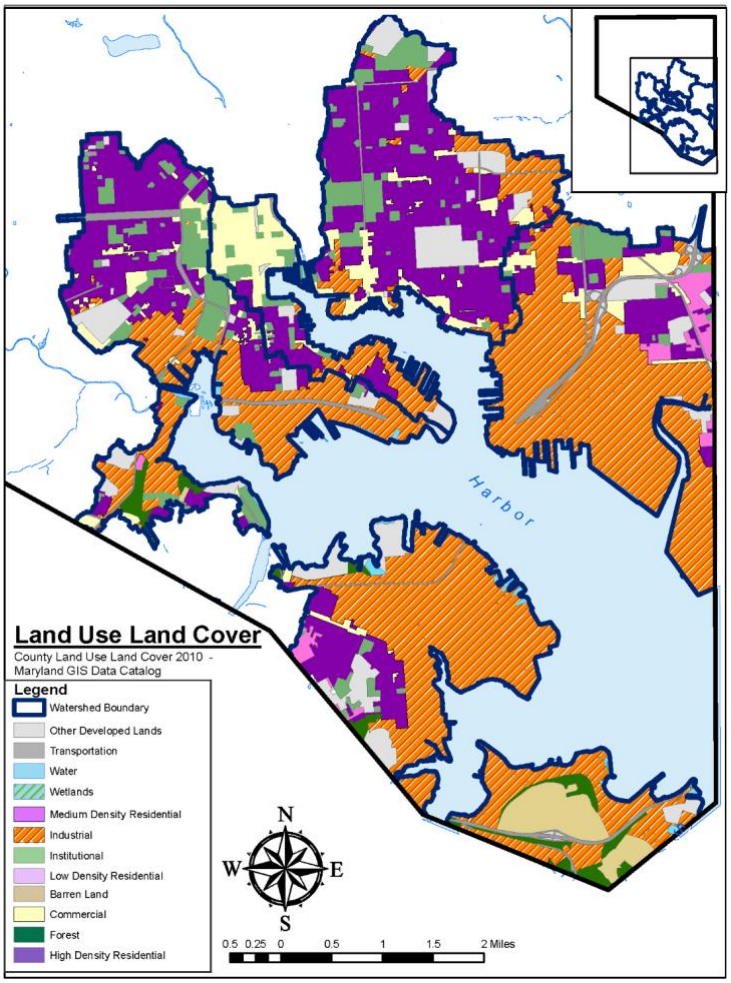


Figure 1. Land use and land cover surrounding Baltimore Harbor. (Baltimore City and Baltimore Department of Public Works, 2019)

Historically, Baltimore Harbor's economy was initially based on the canning factories and various industrial plants, such as chrome and coal, that took advantage of the harbor for shipping (Keith, 2005). Shipping and industrialization led to an increase in hardened shorelines and the need for dredging for even larger ships to pass through. While this aided with the shipping industry, natural habitats were lost. As industries grew and environmental regulations were few, dumping of waste, especially from the chromium

plant, into the water was a common occurrence (Travers, 2016). The legacy pollutants persist within the sediment of Baltimore Harbor and efforts to remove them are hampered by the lack of a proper area or method of disposal.

In addition to the historical contaminants, Baltimore Harbor is also plagued by contemporary contaminants conveyed by stormwater runoff. The combination of precipitation with a high percentage of impervious surfaces accelerates stormwater runoff and its contaminant delivery from roadways into the estuary. Additionally, failures in the sanitary system also bring contaminants from leaking pipes. Contaminants range from nutrients and bacteria to trash from the land resulting in marine debris. The Baltimore Harbor watershed lacks agricultural land, and thus the nutrients that result from farm manure and fertilizer use. However, fertilizer use on yards and parks adds to the nutrient accumulation in stormwater runoff.

Eutrophication and Phytoplankton Blooms:

Nitrogen and phosphorous are the major nutrients carried in stormwater; like fertilizer in a garden, these nutrients boost the growth of single celled photosynthetic organisms called phytoplankton, commonly referred to as algae. Phytoplankton blooms can consist of a variety or organisms from small cyanobacteria to larger dinoflagellates (Kemp et al., 2005). Regardless of the causal organism, when phytoplankton begin to die, they are decomposed by bacteria (Figure 2). The bacteria consume oxygen, and the water column can become hypoxic or even anoxic. Hypoxic and anoxic waters are not suitable for most aquatic organisms and can lead to fish kills (Tango et al., 2005).

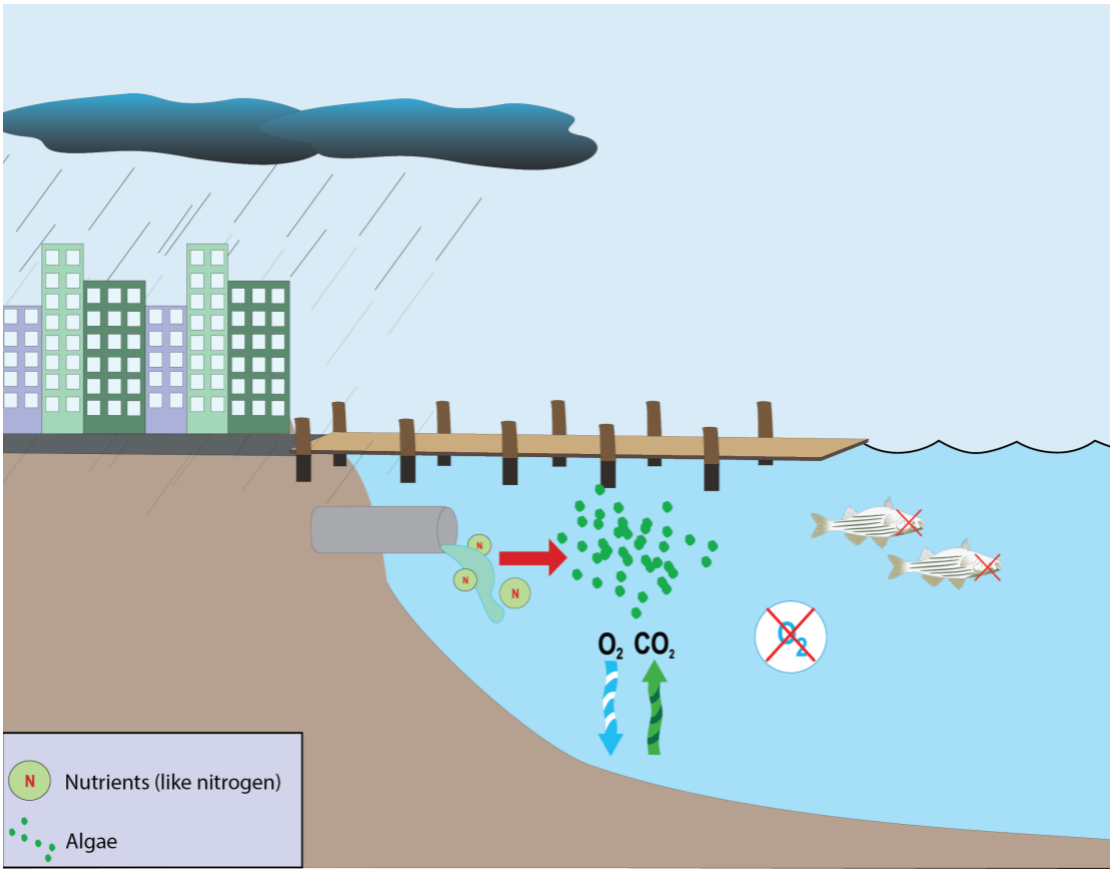
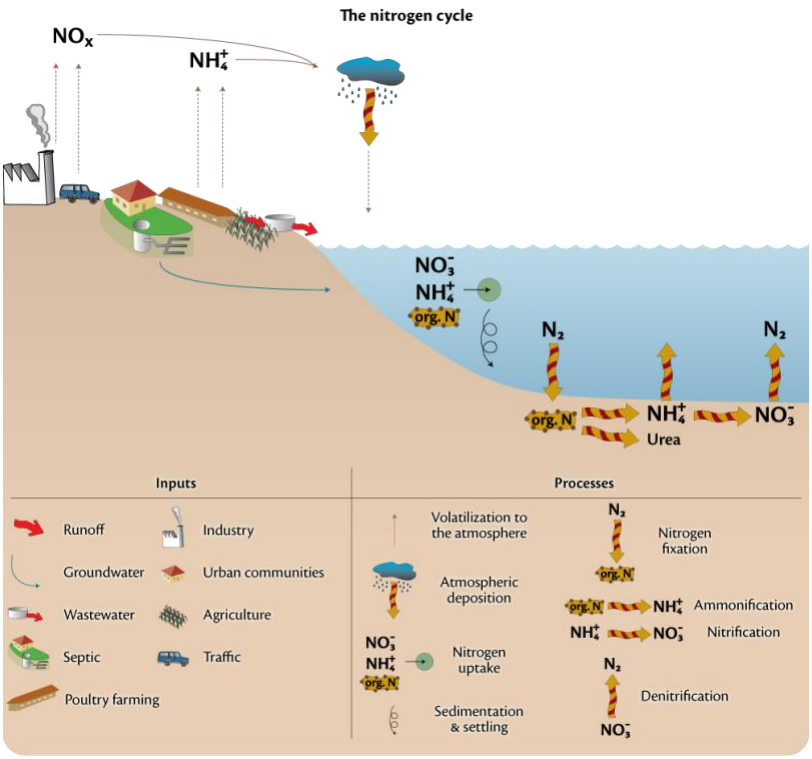


Figure 2. Algae Blooms in an Urban Estuary. (Images from Integration and Application Network (ian.umces.edu/media-library)).

Increases of nutrients into waterways can come from many sources. Even as the region has seen a decrease in nutrients from agricultural sources, there has been a rise in nutrient inputs from urban environments due to the new developments creating more impervious surfaces (Ator et al., 2019). Additionally, atmospheric deposition of nitrogen further increases total nitrogen in the water (Figure 3) (Carey et al., 2013). In Baltimore Harbor, the sediments also contain sediment-bound nutrients that can be re-suspended in the water column to trigger phytoplankton blooms.



Conceptual diagram illustrating the nitrogen cycle, and it's many transformations.
Diagram courtesy of the Integration and Application Network (ian.umces.edu), University of Maryland Center for Environmental Science. Source: *Dennison, W.C., J.E. Thomas, C.J. Cain, T.J.B. Carruthers, M.R. Hall, R.V. Jesian, C.E. Wazniak, and D.E. Wilson. 2009. Shifting Sands: Environmental and cultural change in Maryland's Coastal Bays. IAN Press, University of Maryland Center for Enviromental Science*

Figure 3. The Nitrogen Cycle. Nitrogen enters the water from a variety of sources like runoff, groundwater, and atmospheric deposition. Figure credit: Integration and Application Network, UMCES.

Algae blooms also pose an additional challenge as they are often comprised of toxic or harmful algae species, usually dinoflagellates. The toxins help the dinoflagellates capture their prey and deter potential filter-feeding predators. Common toxin producing dinoflagellates found in the Chesapeake Bay include *Karlodinium veneficum, Dinophysis spp.,* and *Alexandrium spp.* (Place et al., 2012; Tango et al., 2004). *K. veneficum* produces a karlotoxin and has been associated with fish kills and oyster larval mortality (Pease et al., 2021). Toxins produced by *Dinophysis* and *Alexandrium* species are responsible for paralytic shellfish poisoning and diarrhetic shellfish poisoning respectively.

Bivalve Filter Feeding and Associated Ecosystem Services:

Bivalves are filter feeders. They pull water in through their siphon and can sort through particles with their gills. Through this mechanism, bivalves can sort through suspended particles in the water (Shumway et al., 1985). This allows bivalves to retain food particles and reject non-food particles or sediments. Rejection of particles can be either through pseudo-feces if the rejection occurs prior to digestion or as feces if rejection occurs after digestion (Ward and Shumway, 2004). Through these processes, mechanism, bivalves can select for ideal food particles and reject others.

Bivalves vary in their response to toxic species. Responses to toxic phytoplankton range from reducing their clearance rate to completely closing to avoid interacting with the toxic species (Hégaret et al., 2007). When exposed to the toxic dinoflagellate *Alexandrium tamarense*, manila clams, *Ruditapes philippinarum,* showed a decline in their clearance rates (Li et al., 2002). However, mussels in this study did not show a decline in their clearance rates. Additionally, when exposed to toxin producing phytoplankton like *Karlodinium armiger*, blue mussels, *Mytilus edulis*, did not feed for the 6-hour experimental period (Binzer et al., 2018). However, when provided with a phytoplankton species that did not produce toxins, the mussels fed normally. Depending on the species of bivalve and the species of toxic algae, bivalves will express different abilities to reduce algae levels.

The Eastern Oyster is an ecosystem service provider in the Chesapeake Bay. Oysters create reef habitats for other species, filter the water, and aid with benthic-pelagic

coupling (Coen et al., 2007). Models show that restoration of oyster populations to historical levels can help increase SAV growth through water clarity improvements (Cerco and Noel, 2007; zu Ermgassen et al., 2013).

More specifically, oysters have been credited for their ability to reduce nutrients in the water. As mentioned previously, oysters, like other bivalves, are filter feeders and feed on phytoplankton in the water. Through this process they incorporate the nutrients from the algae into their tissue and shell, thus reducing the nutrients in the water (Kellogg et al., 2013). Any phytoplankton or particles that the oyster does not use are rejected as pseudofeces, which also removes the nutrients from the water column. When the pseudofeces reach the benthic layer, the nutrients in them can then be transformed into atmospheric nitrogen through anerobic processes or may be retained in the sediment for extended periods of time (Figure 3). Additionally, the natural community of microbes that grows on and around oysters provides another opportunity for denitrification to occur through an aerobic process (DePiper et al., 2017; Kellogg et al., 2013).

Oyster ecosystem services, specifically the nutrient removal, have made the oyster a prime candidate for nutrient credit trading for these services. In 2010, when the Environmental Protection Agency (EPA) created a total maximum daily limit (TMDL) for nitrogen and phosphorus in the Chesapeake Bay, the ability to reduce inputs of nitrogen and phosphorus became regulated (EPA, 2021). At first, nutrient reduction efforts focused on agriculture and promoted the use of cover crops to reduce the amount of nutrients entering the water. More recently, oysters have been included in the Maryland Department

of the Environment's Water Quality Trading Program where oyster farmers can sell credits generated from the oysters they grow (Cornwell et al., 2016). While these programs focus on the harvest of the oyster, and thus the nutrients within it, there is denitrification that occurs within oyster reefs that would supplement current nitrogen management strategies (Kellogg et al., 2013; Rose et al., 2021).

Programs like this create opportunities for growers to profit more from the nutrients their oysters remove, but oysters do not grow well in certain parts of the Chesapeake Bay. Specifically, these are areas of lower salinity and near urban centers. Without the natural populations of oysters in these areas, these areas are also not receiving the ecosystem services provided by oysters. Instead, the focus should shift toward other native bivalves, and this has already proven to be useful in certain areas of New York Harbor where oysters are unable to grow.

In New York Harbor, the Billion Oyster Project aims to bring back oysters to the harbor ("Billion Oyster Project"). However, some areas of New York Harbor are too contaminated by bacteria for shellfish harvest and researchers have started to look toward other naturally occurring bivalves for their ecosystem services. The Bronx River Estuary is one of the locations in New York Harbor that has too much bacterial contamination for oyster restoration to occur. In this area, researchers are studying the naturally occurring ribbed mussel, *Guekensia demissa,* for its ability to remove nutrients from the water (Galimany et al., 2017). Further, there is no commercial market for *G. demissa,* so there is no worry that these might be harvested for consumption in this estuary. Using field

experiments, Galimany et al. (2017) calculated the nutrient removal from a deployed aquaculture raft with adult mussels attached. In the field, they allowed the mussels to grow on the raft in the estuary and harvested them after 6 months to assess particulate carbon and nitrogen analysis. They also performed flow-through experiments using the mussels on the raft mussels to obtain a clearance rates. Using these two measurements, they could calculate the volume of water cleared by the raft as well as the amount of nutrient removal from the water. The results showed that *G. demissa* can provide valuable ecosystem services for this urban estuary.

The Dark False Mussel (Mytilopsis leucophaeata):

Mytilopsis leucophaeata (Conrad, 1831), also known as the dark false mussel, is a small mussel that occurs naturally in Baltimore Harbor. Their size ranges from 1 – 2 cm in length, and in Baltimore Harbor, they appear to have a spring and fall reproductive season (personal observation). The native range of *M. leucophaeata* is from the New England Coast in the United States of America to lagoons in Mexico (Kennedy, 2011). They are short-lived, rarely achieving 2 years of age. The mussels tend to occur in low salinity habitats (1 to 15 ppt) and in low abundance usually attached to oyster shells or other hard substrates such as pier pilings or ropes submerged in the water.

Abroad, *M. leucophaeata* is a common biofouling pest in the Netherlands and South America (Neves et al., 2020; Rajagopal et al., 2005a). Experiments conducted in the Netherlands examined the physiological response of dark false mussels to a range of temperatures (Rajagopal et al., 2005a). As a temperate species, *M. leucophaeata* is tolerant

to a wide temperature range and with increasing acclimation time, there was an increase in the survival time of the mussels. Additional research conducted by this group also found that byssal attachment was important for physiological response in the mussels (Rajagopal et al., 2005b). They found that detached mussels had higher filtration rates. Much of the research conducted in the Netherlands focused on physiological changes in the mussel to better understand strategies to manage their populations.

Experiments conducted in Brazil have shown that the dark false mussel, while invasive in that region, can improve water clarity and reduce suspended particulate matter (Neves et al., 2020; Rodrigues et al., 2023). Neves et al. (2020), used historical datasets of water quality at the Rodrigo de Freitas Lagoon in Brazil to examine the effect of the introduction of the dark false mussels. This data was also paired with a laboratory experiment looking at changes to lagoon water when exposed to dark false mussels. Their historical analysis shows that there was a significant reduction in chlorophyll a, phytoplankton, and total coliform levels. This also correlated with an increase in water clarity and dissolved oxygen. In laboratory experiments, they found there to be an increase in water clarity and decrease in coliform bacteria in the treatments that had mussels. Work later conducted by Rodrigues et al. (2023), examined the clearance rates of the dark false mussels at different concentrations of hypereutrophic lagoon water. They found that mussels could remove suspended particulate matter (SPM), but at higher SPM levels, the mussel's clearance rate was lower, which could be due to environmental cues. These results prove promising for the use of dark false mussels for this ecosystem service. However, as

these are invasive species in those areas, extreme care must be taken in the consideration of using these for ecosystem services.

In Baltimore Harbor, *M. leucophaeata* would be the ideal bivalve to perform phytoplankton reduction ecosystem services. They naturally live and recruit to surfaces in the water, thus there would be no need for transportation of mussels from a hatchery or aquaculture setting. Historically in years when there was higher than average precipitation many parts of the Chesapeake Bay saw an increase in dark false mussels population as well as an increase in water clarity (Bergstrom et al., 2010). Even in high salinity years *M. leucophaeata* can be found on substrates in the water. However, not much research has been conducted on this species in its native range, nor has there been any experiments to measure uptake of live phytoplankton. This data is crucial for resource managers to evaluate the potential of *M. leucophaeata* to provide ecosystem benefits. My research aims to generate the data resource managers need to evaluate the ecosystem services from *M. leucophaeata*.

Motivations:

Urban estuaries worldwide face problems associated with excess nutrient loads, and Baltimore Harbor is no different. In Baltimore Harbor, algae blooms are intense and frequent and can lead to hypoxic and anoxic conditions (Maryland Department of Natural Resources). These low oxygen conditions can lead to fish kills and make the water unsuitable for many animals that call Baltimore Harbor their home (Baltimore Sun, 2009). Baltimore City also has a goal to make the harbor swimmable and fishable for the public

use, and while great progress has been made toward cleaning up trash and mitigating sewage leaks, solutions to algae blooms and anoxia will require additional strategies to ensure safe swimmable and fishable waters.

The aim of this thesis is to test the potential of *M. leucophaeata* to reduce algae levels in an urban estuary. In Chapter 2, I detail the results of a lab clearance rate experiments that establish that *M. leucophaeata* can reduce the levels of lab-grown algae cultures. I then test the mussels' ability to reduce algae levels at different temperatures and salinities. In Chapter 3, I tested *M. leucophaeata* ability to reduce algae levels of wild algae blooms collected from Baltimore Harbor. For the experiments in Chapter 3, I also measured nutrient removal by assessing changes to the content of carbon and nitrogen in phytoplankton over the course of the uptake experiment. In Chapter 4, I briefly summarize my results from Chapter 2 and Chapter 3, suggest areas for future studies, and address how to use these mussels as a best management practice for nutrient uptake.

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Chapter 2: Measurement of Phytoplankton Uptake by *Mytilopsis leucophaeata* Using Cultured Algae

Introduction:

Estuaries are the interface between land and sea. About 40% of the US population lives in coastal counties with the population density in shoreline counties is four times higher than the national average (Crossett et al., 2013). Runoff from coastal development causes intense stress on urban estuaries, which are already heavily modified by historical and ongoing land uses. The understanding of estuary ecology was developed in un-impacted areas, and while the impacts of urbanization are also well-studied, their effects on the ecology of urban estuaries is not well studied (Graells et al., 2021; Pickett et al., 2017). All urban estuaries share the stress of intense nutrient inputs, leading to a cycle of algae blooms that can cause anoxic and hypoxic zones in the water and shade submerged vegetation (Bricker et al., 2008; Kemp et al., 2005; Lefcheck et al., 2017). Baltimore Harbor is one of many urban estuaries struggling with increased nutrients from stormwater runoff that causes algae blooms. While there are ongoing efforts to reduce nutrient inputs from point and non-point sources (Maryland Department of the Environment, 2022), resource managers should also consider in-water practices to reduce algae levels in Baltimore Harbor. One unexplored strategy is to enhance the inherent ability of the urban estuarine ecosystem to remove excess algae, such as through the use of native bivalve species.

From habitat formation to water quality improvements, bivalves provide numerous ecosystem services. Research has focused on commercially valuable bivalve species such as oysters and mussels (Cubillo et al., 2023; Van Den Burg et al., 2022). In the Chesapeake Bay, where oyster aquaculture is an economically important industry, researchers and managers have quantified ecosystem services of oysters to create a nutrient trading system (Cornwell et al., 2016; Kellogg et al., 2013). However, oysters may not grow well in polluted urban environments like Baltimore Harbor, and there is a public health risk should people harvest and consume oysters grown for ecosystem services and restoration purposes. Therefore, using an alternative bivalve species in these urban estuaries to reduce phytoplankton and nitrogen levels would reduce potential harm to humans.

One promising bivalve for this type of ecosystem service in Baltimore Harbor is the Dark False Mussel, *Mytilopsis leucophaeata*. *M. leucophaeata* is native to the eastern coast of North America and the lagoons in Mexico (Kennedy, 2011a). The mussels are small, about 1-2 cm long, and will settle on any hard substrate in the water. They can tolerate salinities from 5 to 20 and a wide temperature range (Kennedy, 2011b; Rajagopal et al., 2005). In the Chesapeake Bay, when salinity has been low, irruptions of *M. leucophaeata* are often seen in urbanized areas (Bergstrom et al., 2010). *M. leucophaeata* has expanded past its native range and become an invasive pest in South America and the Netherlands. Research in Brazil, where *M. leucophaeata* is invasive, has shown that the mussel holds promise for improving water quality (Neves et al., 2020; Rodrigues et al., 2023). Baltimore Harbor proves to be a prime candidate to study the ecosystem service

potential of these bivalves due to its history of industrialization and the natural population of Dark False Mussels.

Baltimore Harbor is a complex and dynamic body of water. Tidal forces and flash freshwater inputs cause shifts in salinity (6 – 15 ppt), temperature (summer peaks as high as 30˚C), and chlorophyll. In the past the inner harbor was a major port and dredging for shipping has changed the landscape away from a natural soft shoreline with submerged aquatic vegetation to a hardened shoreline devoid of abundant underwater grasses. Despite this, many species still inhabit Baltimore Harbor, including noticeable populations of iconic Chesapeake Bay life, such as striped bass, menhaden, and blue crabs, and the occasional cow-nosed rays (Maryland Department of Natural Resources, n.d.; Miller et al., 2004; Sharma, 2023). However, like many urban estuaries, Baltimore Harbor faces excessive stormwater runoff that leads to eutrophication. Eutrophication fuels algae blooms, which in Baltimore Harbor are intense and frequent (MD - DNR, Eyes on the Bay). These algae blooms can lead to anoxic and hypoxic zones that make the water unsuitable for the fish and crabs to live there. Baltimore Harbor has a recurring population of *M. leucophaeata* making these mussels an ideal species to study for ecosystem services, beginning with their ability to reduce algae levels in the water.

My study aims to determine if *M. leucophaeata* can reduce algae levels and if environmental factors impact the clearance rate of *M. leucophaeata*. To test for this, I conducted three experiments to better understand the filtering capabilities of *M. leucophaeata*. First, I conducted a baseline clearance rate experiment with two different

cultured algae species. Second, I tested clearance rates at three different temperatures that reflect conditions experienced in the Baltimore Harbor. Third, I tested clearance rate at three different salinities that fall in the range found in Baltimore Harbor. I hypothesized that warmer temperatures would increase the clearance rate of the mussels and cooler temperatures would reduce the clearance rates. These results provide quantitative information needed to evaluate how *M. leucophaeata* can contribute to phytoplankton and thus nutrient removal from Baltimore Harbor.

Methods:

I performed three different experiments to test the clearance rate of *M. leucophaeata*: 1) mussels and different cultured algae species, 2) mussels at three different temperatures, and 3) mussels at three different salinities. The first experiment tested two algae species and were conducted at 20˚C in 10 ppt artificial seawater (ASW). The second experiment examined the effect of temperatures (10˚C, 20˚C, and 30˚C) on the mussel clearance rate. The third experiment examined the effect of salinities (5 ppt, 10 ppt, and 15 ppt) at 20˚C on mussel clearance rate. Experimental temperatures and salinities were all chosen based on the range these parameters recorded by the Eyes on the Bay continuous water quality sondes in Baltimore Harbor from 2018 to 2022 (S. Figure 1).

Animal Care and Collection:

Naturally recruited *M. leucophaeata* were collected from rigid substrates in 0.25 to 1.0 m depth at three locations in Baltimore Harbor: 1) The Downtown Sailing Center, 2) National Aquarium East, and 3) the IMET (Table 1).

Table 1. Mussel Collection Locations and Experiments.

Location	Experiment	Coordinates
Downtown Sailing Center	Salinity Trial 1, Temperature Trial 1, and Trial 2	(39.274458, -76.600185)
Aquarium East	Algae Experiment	(39.285473, -76.607978)
IMET	Salinity Trial 2	(39.286472, -76.606360)

Mussels were brought back to the lab to acclimate to experimental conditions for a minimum of 10 days before starting any experiment. Mussels were detached from their original substrate and reattached to plastic surfaces, taking advantage of their ability to form new byssal threads. For the algae species experiment, mussels were attached to 10 cm diameter acrylic discs. For the salinity and temperature experiment, mussels were attached to ~6 cm square polypropylene panels. Approximately 15 +/- 3 mussels were used in the algae species experiment and 10 +/- 2 for the temperature and salinity experiment. During acclimatization, water was changed every other day with water collected from Baltimore Harbor.

Salinity and temperature experiments used water from Baltimore Harbor, which was adjusted to target temperature and salinity using water baths and ASW produced by the Aquaculture Research Center (ARC) at IMET. For the temperature and salinity experiments, mussels were acclimated to the appropriate temperatures and salinity for 10 days prior to the experiment. To adjust the temperature, water baths were gradually shifted to the target temperatures of 10˚C and 30˚C in 2 degree increments every other day. Approximately 22˚C conditions were maintained at room temperature without a water bath. For the salinity experiment, I acclimated mussels to 5 ppt, 10 ppt, and 15 ppt using ASW. Using a similar method as for the temperature experiment, I gradually added the ASW of the corresponding salinity to harbor water to acclimate the mussels. ASW was made by diluting 35 ppt ASW with deionized water from ARC.

Experimental Design of Phytoplankton Uptake Experiments:

Phytoplankton used in the experiments were obtained from the oyster hatchery at the UMCES Horn Point Laboratory in Cambridge, MD. Two algae species were compared, the flagellate *Isochrysis* sp. and the diatom *Chaetoceros* sp. For temperature and salinity experiments, only *Isochrysis* sp. was used. *Isochrysis* is about 4-8 microns long and 4-6 microns wide, and *Chaetoceros* is about 4-9 microns long and 4-10 microns wide. Algae cultures were maintained for up to two weeks at room temperature (~22˚C) in f/2 medium at 10 ppt salinity under constant illumination from a 60-watt equivalent white LED light at a distance of 30-50 cm. To calculate the algae needed for the experiment, a Lugol's fixed suspension was counted on a hemocytometer and a standard curve of cell counts versus in vitro chlorophyll (IVCH) was constructed, and then used to calculate the amount for algae culture needed for a target of 4000 relative fluorescence units (RFU). Due to the variable density and age of the algae cultures, the target of 4000 RFUs was not always met; I detail any deviances below.

Prior to the start of a clearance rate experiment, mussels were placed in fresh ASW for a minimum of 12 hours. All experiments had four replicates per treatment conditions of: 1) algae and mussels (Mussels), 2) algae and no mussel (No Mussel), and 3) mussel only control (Figure 4). All experiments were conducted in 2-liter (algae species experiment) or 1 liter (temperature and salinity experiment) polypropylene containers.

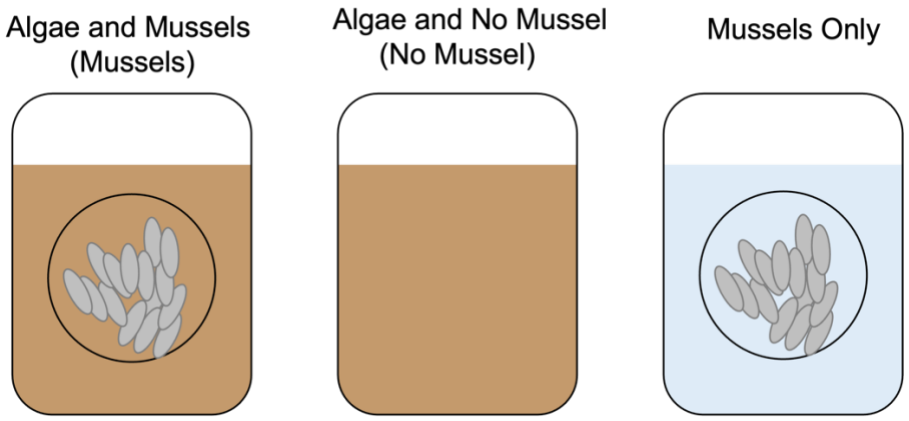

Figure 4. Example treatment conditions. Algae and mussels, algae and no mussels and mussels only.

Algae Species Experiment:

Both *Isochrysis* sp. and *Chaetoceros* sp. algae cultures were used in this experiment. The treatment groups consisted of the mussels and individual algae species. The control treatments were the algae alone or mussels on their own. Each treatment group had four replicates. At the beginning of the experiment the mussel discs were suspended in individual containers with 1000 ml ASW to acclimate for an hour. A T0 timepoint water collection was collected for immediate measurement of IVCH reading. Then algae culture was added to a target of 4000 RFUs based on the hemocytometer cell counts and standard curve measurements. Approximately ten minutes after adding the algae, I took the T1 water samples for IVCH measurements, chlorophyll extractions. I collected additional water samples at 2, 4, 6, and 22.5 hours. IVCH and Lugol's storage occurred at all the water collections. Water samples for later chlorophyll extractions were collected at the T1, T4, and T5 timepoints (Figure 5).

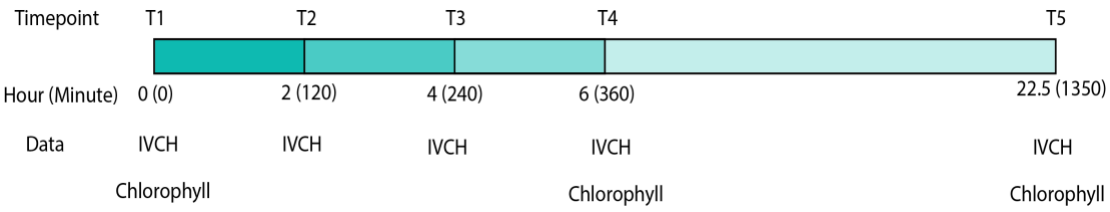

Figure 5. Timeline for algae species experiment

Chlorophyll measurements:

In vitro chlorophyll (IVCH) was measured using a Turner Design Aquaflour® and measurements were recorded in RFU. In addition to the IVCH measurements, I collected water at the T1, T4, and T5 time periods for measurements of extracted chlorophyll. The amount collected was either 4, 10, or 15 ml and amount filtered was increased using ASW so that the total volume filtered was 15 ml. For extracted chlorophyll measurements, samples (adjusted 15 ml) were filtered onto GF/C filters (Whatman) and stored at -20˚C until processing. I sent the frozen samples to the Chesapeake Biological Laboratory Nutrient Analytical Services Laboratory (Solomons, MD) for chlorophyll a quantification using the EPA 445.0, SM10200H.3 method.

Temperature Experiment:

Temperature records in Baltimore Harbor were examined from 2018 to 2022 using the high frequency Eyes on the Bay dataset from the Aquarium East location (Maryland Department of Natural Resources, Eyes on the Bay). I plotted a histogram of the continuous

temperature data rounded to the nearest whole number to visualize the temperature range that the mussels experience. The data show that there is a mean of 16˚C and a range from 0˚C to 31˚C (S. Figure 1). I selected temperatures of 10˚C, 20˚C, and 30˚C as they fell within the range that naturally occurs and were within the range the water baths I used could achieve. I conducted two trials to test the effect of temperature on clearance rates of *M. leucophaeata.* Both trials occurred in July 2023 using mussels also collected in July 2023. The first trial used 800 ml of water and the second trial used 500 ml, to accommodate for the differences in the density of the *Isochrysis* sp. cultures.

At the beginning of each experiment, mussels affixed to plastic panels were placed in the water to acclimate for 1 hour. After that the T0 time point was collected for IVCH measurements and cell counts. Then *Isochrysis* was added to the containers, and water was collected for IVCH measurements and cell counts. IVCH was recorded at all timepoints, while cell count collections only occurred at the first and last timepoint (Figure 6).

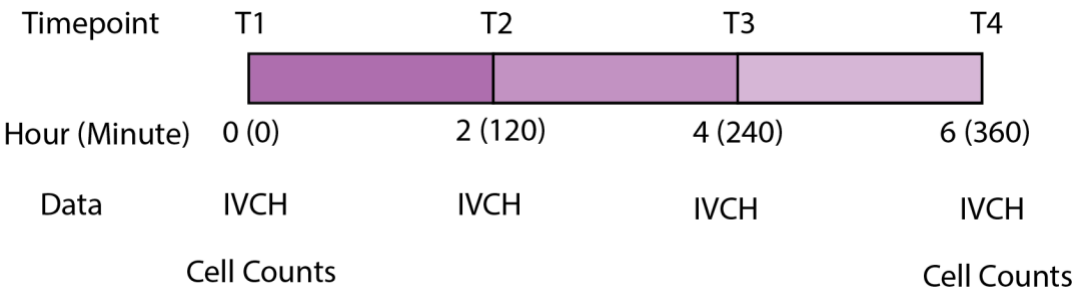

Figure 6. Timeline for Temperature and Salinity Experiments

Salinity Experiment:

Experimental salinities were chosen in the same way as in the temperature experiment described above. I used the Eyes on the Bay data to determine that the 5 years mean salinity was 7 ppt and the total salinity ranged from 1 to 17 ppt (S. Figure 2). Salinities of 5, 10, and 15 ppt were picked for the experiments. Like with the temperature experiment, two separate trials were performed. Trial 1 was conducted in July 2023 and used mussels collected in July 2023. Trial 2 occurred in October 2023 with mussels collected at the end of September 2023. The first trial used 500 ml of water and the second trial used 800 ml. This was to adjust for the density of algae stocks used in the experiment. Experimental conditions and procedures for collecting water samples and experiment set-up was the same as the temperature experiment.

Cell Counting – Flow Cytometer:

Water samples for cell counting were stored in 1% glutaraldehyde at 4˚C until processing and analysis. All cell counting was done by flow cytometry on an AccuriC6 (BD Biosciences). I ran the samples using the "Fast" fluidics setting and analyzed 300 µl of sample. Counts were then gated to remove any background noise of small particles or those that did not fluoresce. Gated particle counts were converted to counts per ml for statistical comparisons.

Clearance rate calculation:

Clearance rates (R_c) for the salinity and temperature experiments were calculated using the following equation (Jacobs et al., 2015):

$$R_c = \frac{V}{nt}\left(\ln\left(\frac{C_0}{C_t}\right) - \ln\left(\frac{C_{0'}}{C_{t'}}\right)\right)$$

Where V = volume in liters, n = number of mussels, t = time in hours, C_0 = concentration at the beginning for the treatment, C_t = concentration at the end for the treatment, $C_{0'}$ = concentration at the beginning for the control, and $C_{t'}$ = concentration at the end for the control. I calculated the clearance rates using the first timepoint (T1) and the final timepoint (T4).

Statistics:

All statistical tests were run in R version 4.2.1. Algae species experiment data were analyzed by algae species type for all collected data. IVCH data for the temperature experiment was analyzed as a group, but cell count data were analyzed separately due to differing algae culture concentrations between the trials. Since salinity experiments were conducted at separate times of the year, data were analyzed by trial.

To assess the effect of mussel or no mussels on IVCH levels I performed a Kruskal-Wallis ("Stats") since data was not parametric. I then performed a post-hoc pairwise Wilcoxon rank sum test ("Stats"). To test for the effect of time on IVCH levels, I performed a one-way repeated measures ANOVA ("Rstatix") and a post-hoc pairwise t-test ("Rstatix") to test for individual comparisons. Each treatment group was tested

independently. If assumptions were violated, I instead used the Friedman Rank Sum test ("Stats") followed by a Wilcoxon rank sum test ("Stats").

To test if treatment (mussels or no mussels) effected total extracted chlorophyll concentrations, I performed a Kruskal-Wallis ("Stats") test followed by a post-hoc Wilcoxon rank sum test ("Stats"). To test for the effect of time on total chlorophyll concentrations, I performed a repeated measures ANOVA ("Rstatix") and a post-hoc pairwise t-test ("Rstatix"). If data was non-parametric, a Friedman test ("Stats") and Wilcoxon rank sum test ("Rstatix") were performed instead. For the control *Isochrysis* group, I removed entries from the first replicate due to unequal data points for the time comparisons.

For the cell count data, a paired t-test ("Stats") was used to measure the effect of time between the first and last time point for the salinity and temperature treatments. Each trial was analyzed separately for this analysis.

To test for the effect of salinity or temperature on the IVCH-based and count-based clearance rate, I used a one-ANOVA ("Stats", "car") followed by a Tukey's HSD test ("Stats"). The ANOVA was changed to a Type III ANOVA for the salinity trial 1 data due to missing data points. All other ANOVAs were Type II.

Results:

Algae species experiment:

The presence of mussels had a significant effect on the average IVCH levels and the total chlorophyll levels. Treatment showed a significant effect on IVCH levels for both *Isochrysis* and *Chaetoceros* (Kruskal-Wallis, $p < 0.05$; Figure 7, Table 2). Time also was shown to be a significant factor for both treatment groups of *Isochrysis* ($p < 0.05$;Table 3). Pairwise comparisons show that in the mussel treatment there is a significant difference between the first timepoint (T1) and the other four timepoints (T2, T3, T4, and T5), but no other pairwise comparison was significant (S. Table 1). A similar pattern is observed with the no mussel group and the first timepoint (T1) is statistically different than the T2, T3, and T4 time point ($p < 0.05$; S. Table 2), but no difference was found between the first and final time point. Furthermore, the T2 and T4 time points were statistically different from each other for the no mussel treatment.

Time had a significant effect on IVCH levels for the mussel treatment using *Chaetoceros* algae, yet the no-mussel treatment was not significant (Table 3). Pairwise comparisons of the mussel treatment show significant differences between the T1 and the T3 and T5 timepoint ($p < 0.05$; S. Table 3).

Table 2. Kruskal-Wallis analysis of IVCH data in algae species experiment

Algae	df	Chi-squared	p-value	Wilcoxon Rank Sum p-value
Isochrysis	1	16.684	**4.42E-05**	**1.30E-05**
Chaetoceros	1	17.132	**3.487E-05**	**3.7E-05**

Table 3. Repeated Measure ANOVA analysis of IVCH data in algae species experiment

Algae	Treatment	DFn	DFd	F	P
Isochrysis	Mussels	4	12	199.441	**7.32E-11**
Isochrysis	No Mussels	4	12	10.117	**0.000806**
Chaetoceros	Mussels	4	12	22.17	**1.8E-05**
Chaetoceros	No Mussels	4	12	2.617	0.088

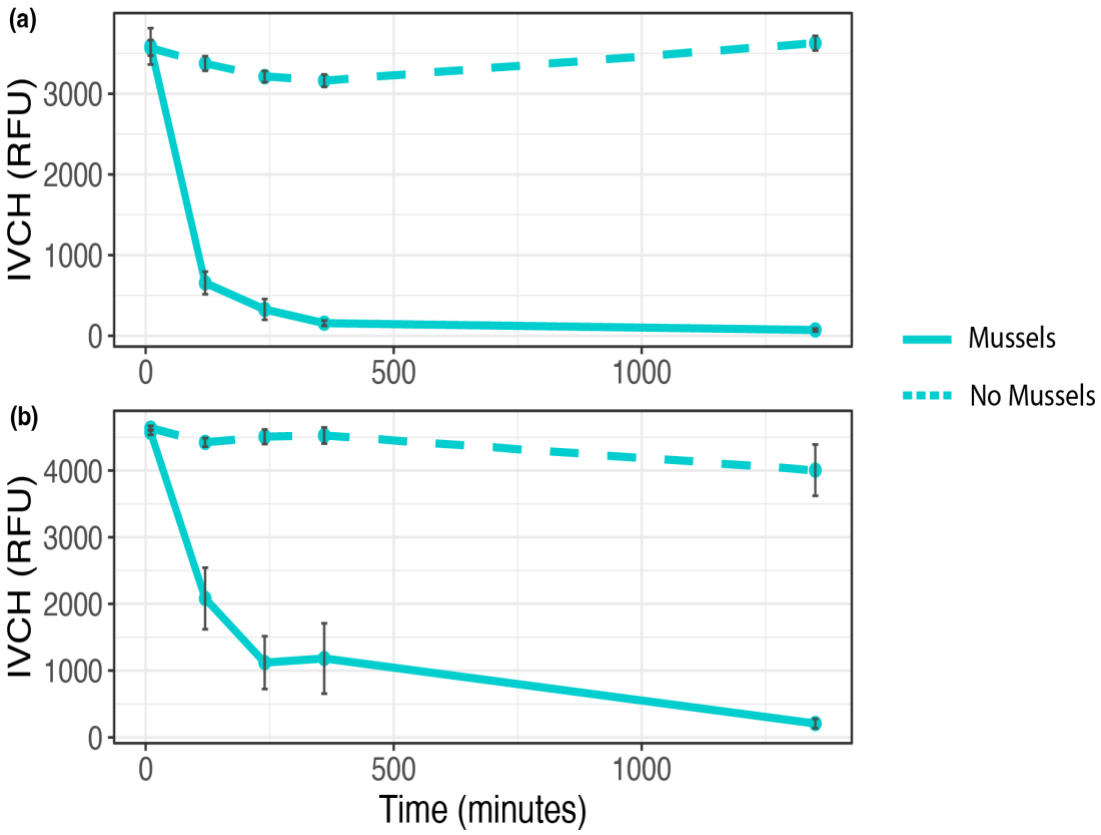

Figure 7. Average IVCH for a) *Isochrysis*, b) *Chaetoceros* experiments. Error bars are standard error mean (SEM).

Mussels also had a significant effect on the total chlorophyll levels ($p < 0.05$; Figure 8,Table 4). Time also had a significant effect on the total chlorophyll level for the treatments with mussels but not in the treatments without mussels (Table 5). Pairwise comparisons between time for the *Isochrysis* treatment with mussels did not show any significant difference between time despite the Friedman's rank sum test showing a significant effect of time (S. Table 5). Comparisons between time for the *Isochrysis* no-mussel treatment were also not significant. For *Chaetoceros*, the mussel treatment showed

significant differences between the T1 and T4 timepoints (S. Table 6), and the no-mussel treatment showed no significant differences (S. Table 7).

Table 4. Kruskal-Wallis Results for Total Chlorophyll

Algae	**df**	**Chi-squared**	**p-value**	**Wilcoxon Rank Sum p-value**
Isochrysis	1	6.3674	**0.01162**	**0.011**
Chaetoceros	1	9.2	**0.00242**	**0.0015**

Table 5. Friedman rank sum test results and †repeated measures ANOVA for total chlorophyll. *Chaetoceros* mussel treatment repeated measures ANOVA: DFn = 2, DFd = 6, F = 23.299.

Algae	**Treatment**	**Chi-squared**	**DF**	**p-value**	**Statistical Test**
Isochrysis	Mussels	6.5	2	**0.03877**	Friedman
Isochrysis	No Mussels	0.66667	2	0.7165	Friedman
Chaetoceros	Mussels	-	-	**0.001†**	Repeated measures ANOVA
Chaetoceros	No-mussels	4	2	0.1353	Friedman

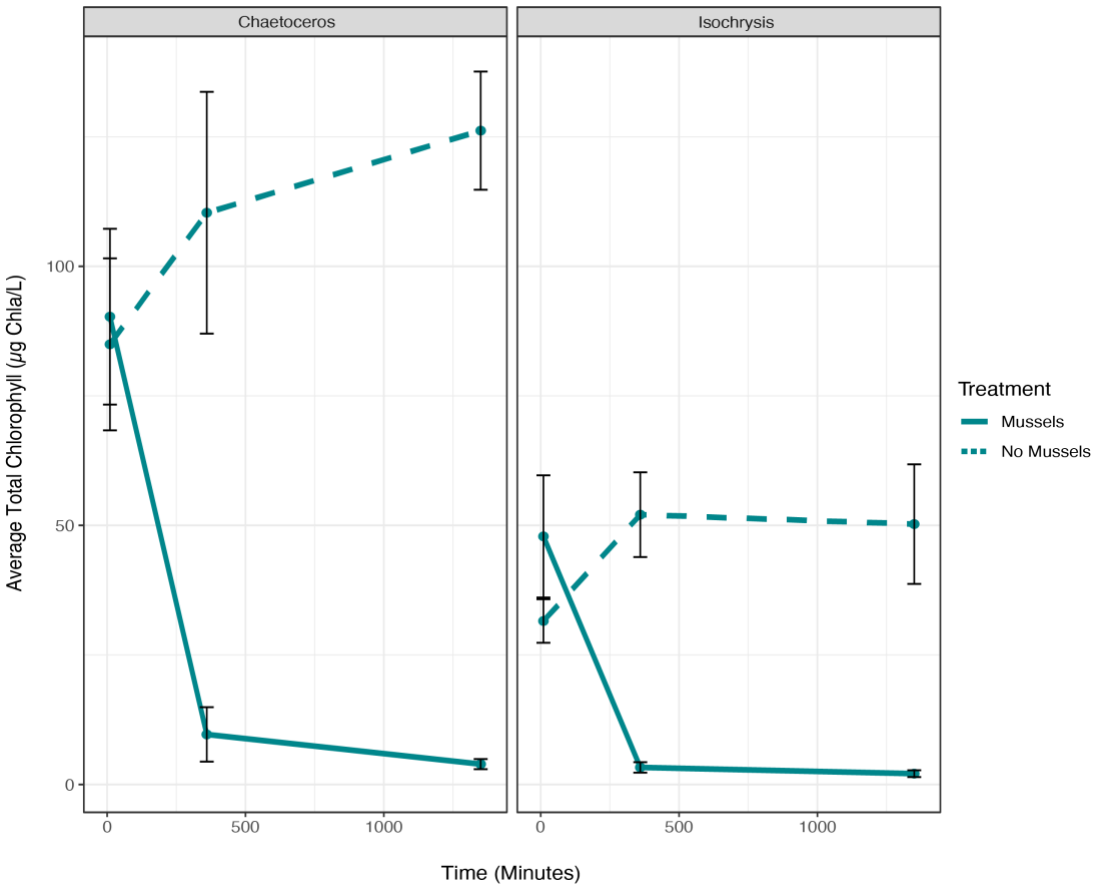

Figure 8. Total extracted chlorophyll change over time. Left panel) *Chaetoceros;* Right panel) *Isochrysis*. Dashes lines the no-mussel treatment, solid lines are the mussel treatment. Error bars are SEM.

Temperature experiments:

Mussels were able to reduce the IVCH at all temperatures tested ($p < 0.05$) (Figure 9, Table 6). For trial 2, the mussel treatment data was log transformed to meet normality assumptions. Time significantly affected IVCH levels for the mussel treatment in both trial 1 and 2 but was not significant for the no-mussel treatment (Table 7). Indeed, all pairwise comparisons of timepoints for the mussel treatments proved to be significant ($p < 0.05$; S. Table 8, S. Table 9, S. Table 10), and no significant pairwise comparison was found for

the no-mussel treatment. These results parallel the initial findings from the algae species experiment that mussels alter IVCH levels of phytoplankton cultures.

Table 6. Kruskal-Wallis Results for Treatment effect on IVCH for the temperature experiment

Trial	df	Chi-squared	p-value	Wilcoxon Rank Sum p-value
1	1	54.017	**1.99E-13**	**<2E-16**
2	1	55.645	**8.68E-14**	**8.90E-14**

Table 7. Repeated measure ANOVA results for time effect on IVCH and †Friedman's rank sum test (chi-squared = 3.3, df = 3) for the temperature experiment

Trial	Treatment	Dfn	DFd	F	P	Statistical Test
1	Mussel	1.45	16	33.253	**6.94E-16**	Repeated Measure ANOVA
	No-mussel	-	-	-	0.3476†	Friedmans
2	Mussel	1.17	12.51	42.587	**1.54E-05**	Repeated Measure ANOVA
	No-mussel	3	33	1.824	0.06	Repeated Measure ANOVA

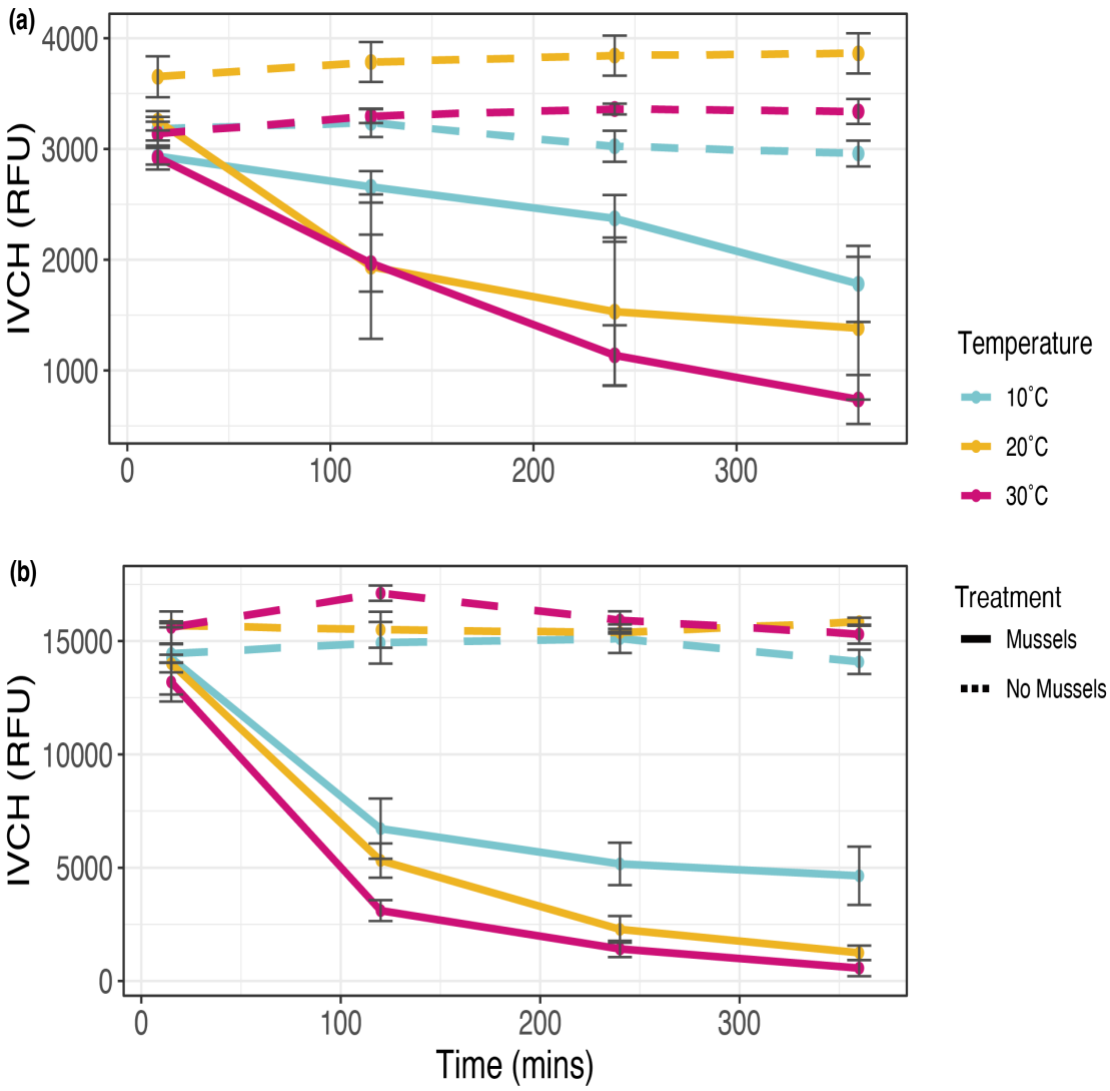

Figure 9. Average IVCH Change at different temperature a) trial 1, b) trial 2. Error bars are SEM.

Due a processing error, the first timepoint for the 30˚C trial 1 was unable to be counted on the flow cytometer. Paired t-tests show that there was a significant effect of time on the mussel treatment of both trials and the no-mussel treatment of the second trial ($p < 0.05$; Figure 10, Table 8). As shown by the IVCH data, the mussel treatment decreases

as expected, but the no-mussel treatment for trial 2 also shows there is a significant difference between the time points. Visually it appears that the last timepoint is increasing in cell number.

Table 8. Paired t-test results for temperature cell counts

Trial	Treatment	t	df	p-value
1	Mussel	4.1029	7	**0.004556**
	No mussel	0.8883	7	0.4039
2	Mussel	6.263	11	**6.15E-05**
	No mussel	-8.7991	11	**2.61E-06**

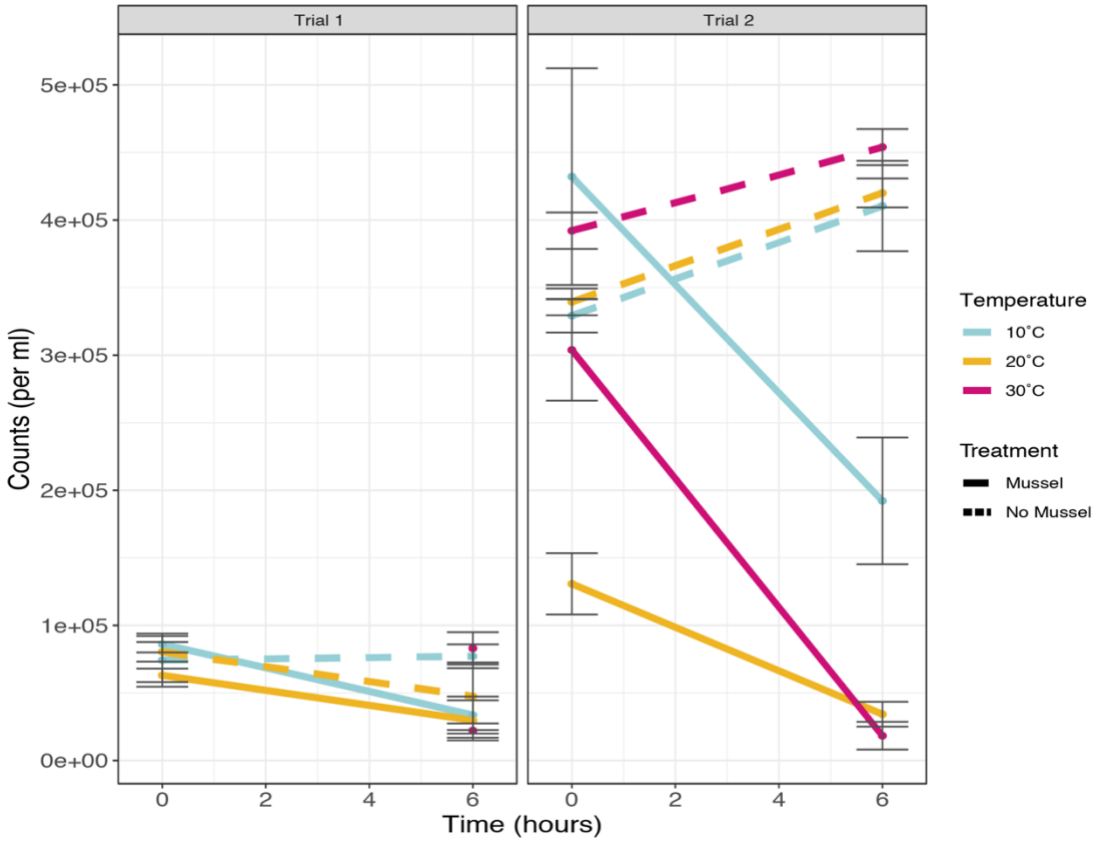

Figure 10. Average cell counts over time for temperature experiments. Error bars are SEM.

To analyze the effect of temperature on IVCH-based clearance rate, a one-way ANOVA was performed. Since both trials took place during the summer, the data was pooled together. Initially starting algae concentration was included in the model due to the differing starting concentrations between the trials, but this was found to be not significant and removed from the model ($p = 0.5482$). Temperature was found to have a significant effect on IVCH-based clearance rates ($p = 0.0143$), and conducting a Tukey HSD post-hoc test found the 10˚C and 30˚C comparison was significant but no other comparisons were found (Figure 11, Table 9). However, there was not a significant effect of temperature on

the cell count-based clearance rate (chi-squared = 3.6, df = 2, p – value = 0.1653, Figure 12). This is confirmed by a Wilcoxon rank sum test that showed no significant differences between temperatures (Table 9).

Table 9. Pairwise comparisons for clearance rates. IVCH p-value results from Tukey HSD test; Cell Count p – value from Wilcoxon rank sum test

Comparison	IVCH p - value	Cell Count p -value
20˚C-10˚C	**0.0719177**	1
30˚C-10˚C	**0.0142055**	0.33
30˚C-20˚C	0.727973	0.33

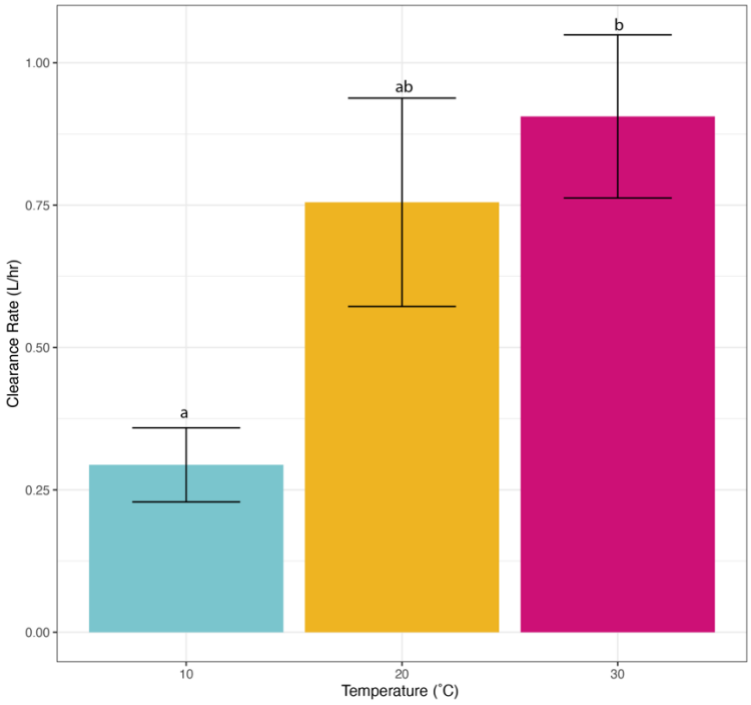

Figure 11. IVCH-based clearance rates at different temperatures Error bars are SEM.

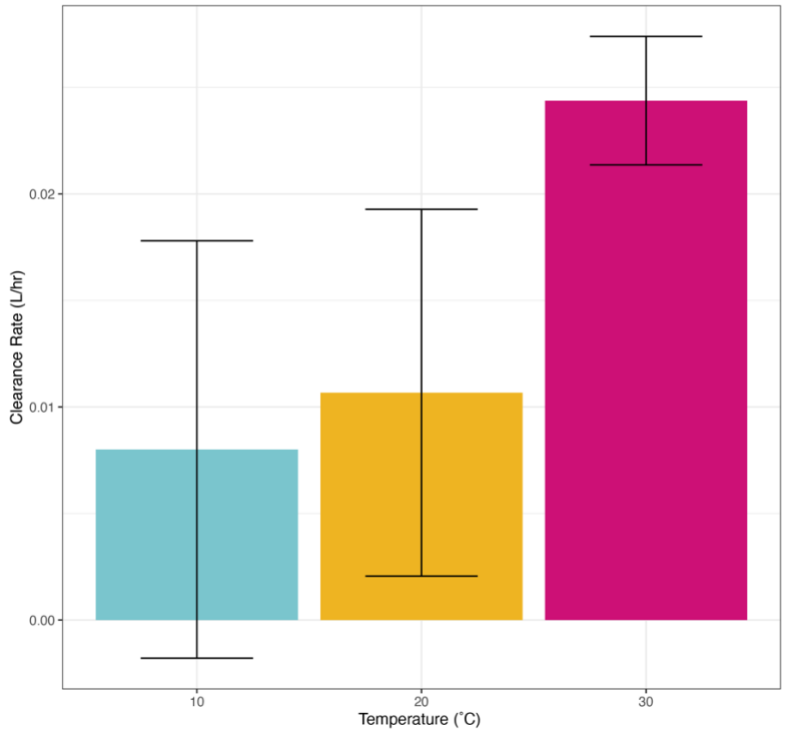

Figure 12. Cell count-based clearance rates at different temperature. Error bars are SEM.

Salinity experiment:

Due to uncertainty created by a data collection error in trial 2, all IVCH data from the T2 time point were removed to uncertainty. The presence of mussels was found to have a significant effect on IVCH levels ($p < 0.05$;Table 10). Time had a significant effect on the IVCH levels for the mussel treatment for trial 1, the mussel treatment for trial 2, and the no-mussel treatment for trial 1 ($p < 0.05$; Table 11,Table 12). Pairwise comparisons show that there is a significant effect of all comparisons for the mussel treatment in both trials (S. Table 11, S. Table 12). For the trial 1 no-mussel treatment, most of the comparisons were significant except for the T1 and T2 comparison, and the T3 and T4

comparison (S. Table 11). Visually there is a slight increase in the IVCH levels of the no mussel trial 1 line.

Table 10. Kruskal-Wallis Results for treatment effect on IVCH levels for salinity experiment.

Trial	Chi-squared	Df	p-value
1	50.105	1	**1.46E-12**
2	30.703	1	**3.01E-08**

Table 11. Friedman rank sum test results for effect of time on IVCH salinity trial 1

Treatment	Chi-squared	Df	p-value
Mussel	36	3	**7.49E-08**
No mussel	6.3	3	**8.25E-06**

Table 12. Repeated measures ANOVA results for effect of time on IVCH salinity trial 2

Treatment	DFn	DFd	F	p-value
Mussel	1.18	13.02	94.781	**1.26E-07**
No mussel	2	22	0.877	0.43

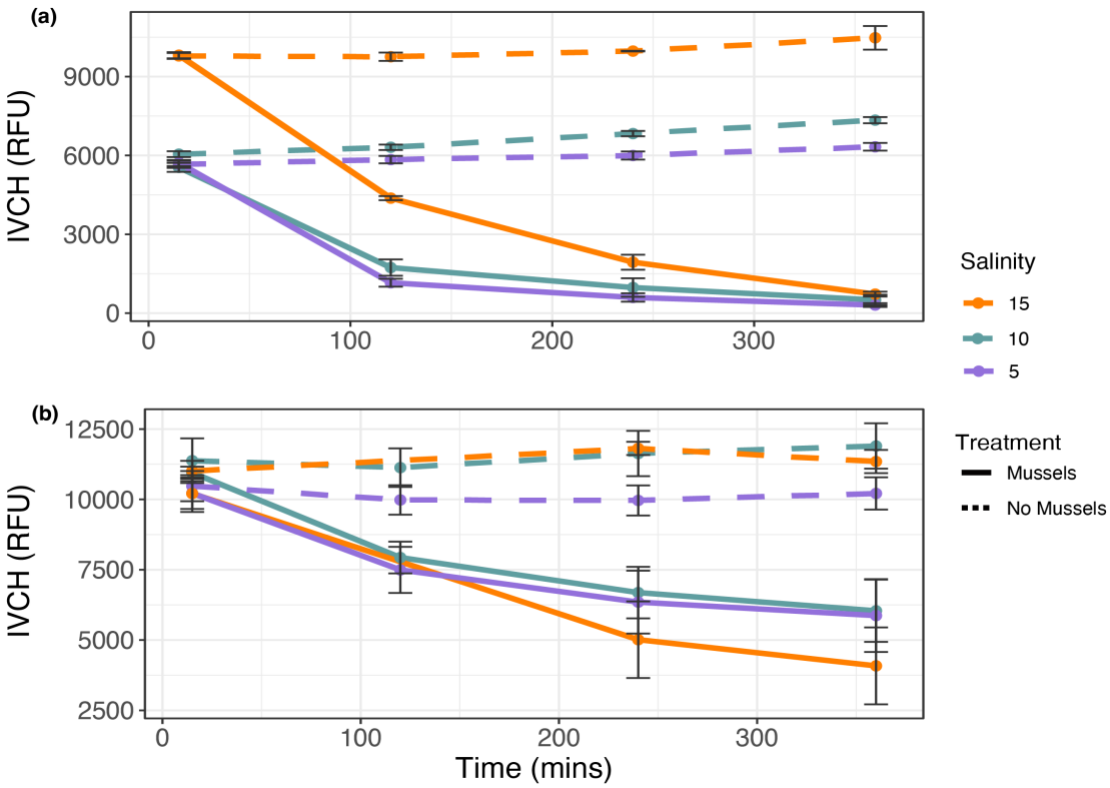

Figure 13. IVCH change at different salinities. Error bars are SEM.

Paired t-test for cell count at the first versus last time point show that there is a significant difference for both treatments in trial 1 and for the mussel treatment in trial 2 ($p < 0.05$; Figure 14, Table 13). Visually the no-mussel treatment of trial 1 does appear to increase in cell counts. Both mussel treatments decrease over time and supports the IVCH results findings.

Table 13. Paired t-test results for salinity cell counts

Trial	Treatment	t	df	p-value
1	Mussel	6.9932	11	**2.29E-05**
	No mussel	-5.0668	11	**0.0003624**
2	Mussel	11.061	11	**2.68E-07**
	No mussel	-0.56432	11	0.5839

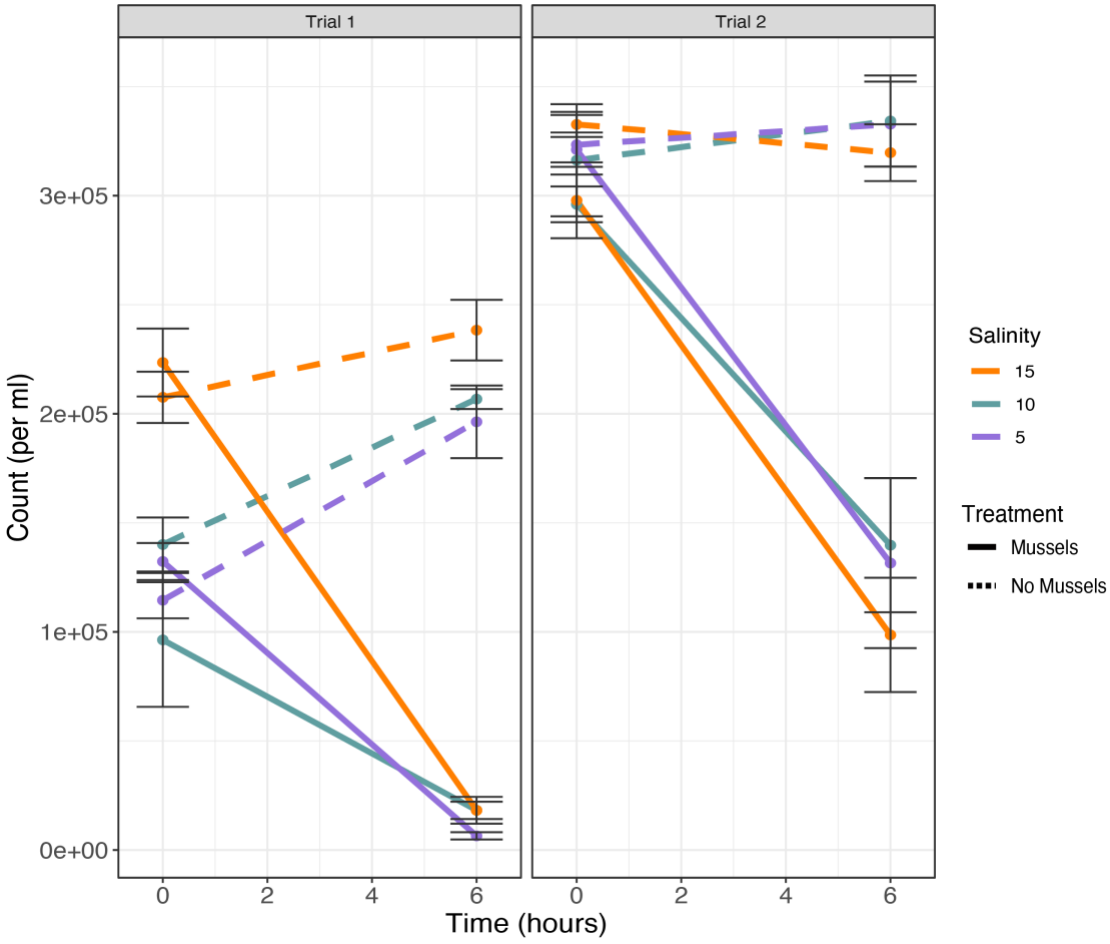

Figure 14. Change is cell count over time for salinity experiments. Error bars are SEM.

Salinity did not have a significant effect on the IVCH-based or the cell count-based clearance rates for either trial (One-way ANOVA, $p > 0.05$; Figure 15, Figure 16). Pairwise comparisons also did not find any significant difference between the clearance rates at different salinities (Table 14).

Table 14. Pairwise comparisons for clearance rates of salinity experiment

Comparison	Trial 1: IVCH p -value	Trial 2: IVCH p -value	Trial 1: Cell Count p -value	Trial 2: Cell Count p -value
5ppt – 10ppt	0.141	0.983	0.071	0.697
5ppt – 15ppt	0.455	0.140	0.502	0.975
10ppt – 15ppt	0.621	0.182	0.316	0.570

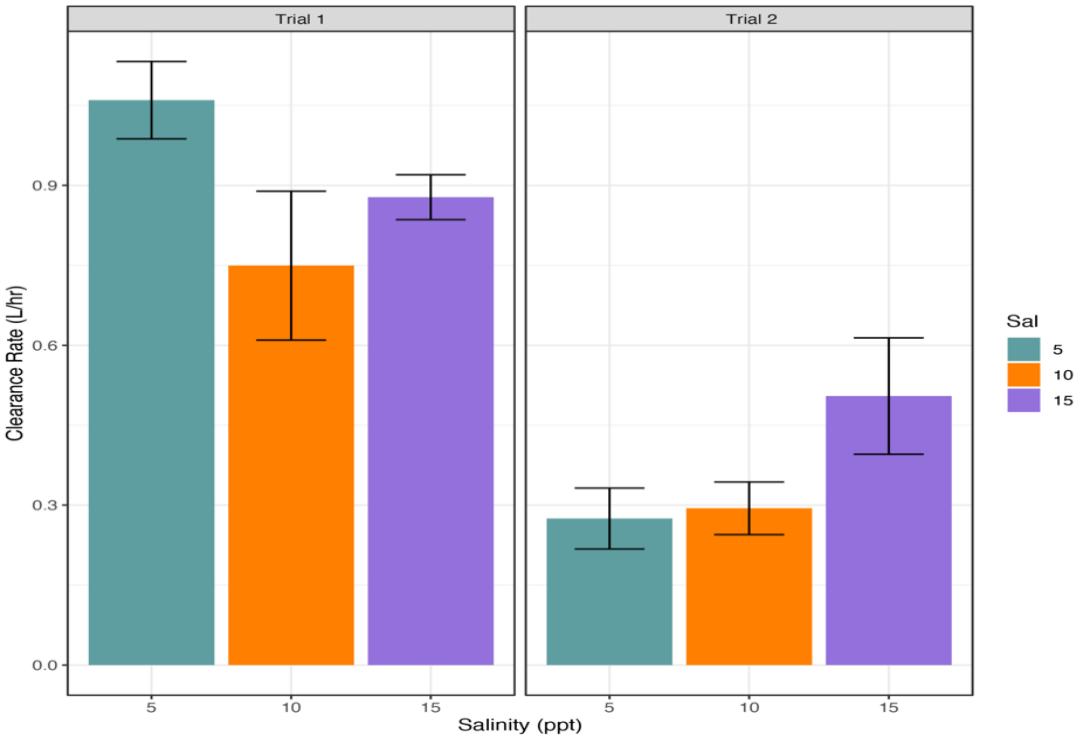

Figure 15. IVCH-based clearance rates at different salinities. Error bars are SEM.

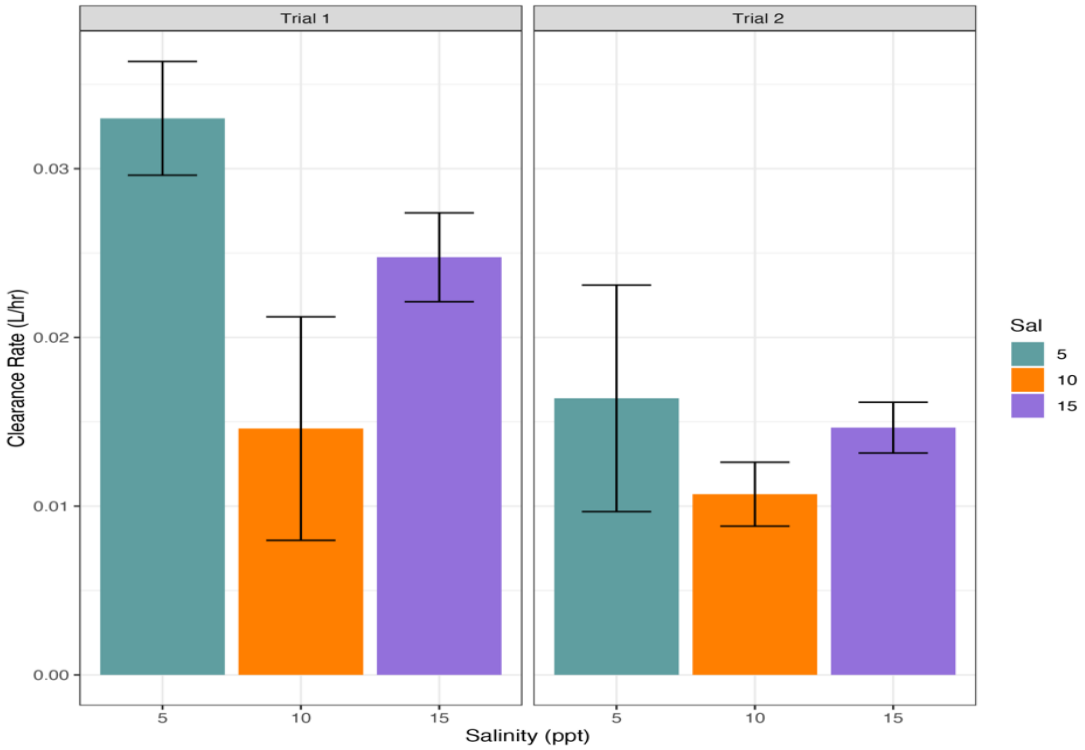

Figure 16. Count-based clearance rates at different salinities. Error bars are SEM.

Discussion:

Urban estuaries have high nutrient inputs and are greatly altered environments. This makes it hard to implement traditional nature-based solutions, like oyster reefs and floodplain wetlands, to remove nitrogen from the estuary. There is still a need to reduce the impacts of excess nutrients in highly modified urban waterways and my research shows that a native bivalve offers a possible solution. My study established a reproducible method for collecting and acclimating naturally recruited *M. leucophaeata* into a laboratory set-up. Similar to how they would be positioned in the environment, this set-up allows the animals to attach naturally to new substrates that can be placed vertically. The mussels appeared to be resilient to the manipulations in the laboratory, just as they appear to be hardy in the stressful environment of Baltimore Harbor.

My investigations show that *M. leucophaeata* consumed algae as indicated by decreasing IVCH and total chlorophyll readings. When I tested different environmental conditions, I found that salinity did not affect clearance rates. In looking at temperature, I found that there was a significant effect of temperature with the clearance rate at 10˚C being significantly lower than the clearance rates at 30˚C but not 20˚C. Additionally, the clearance rates at 20˚C and 30˚C were not significantly different from each other. My results indicate that salinity in the 5-15 ppt does not affect clearance rates. but mussels showed a significant effect on algae levels at all salinities.

The cell count data showed some discrepant results when compared to the IVCH data. That is, there were significant changes in IVCH but not cell counts as temperature

increased, although the apparent trend in cell counts resembled that of IVCH. The lack of statistical significance in cell counts could be due to the age and stage of the algae, preservation methods, and the nature of the assays. The IVCH method is direct and not as prone to noise or bias caused by preservation and later cell counting. Visually, many of the no-mussel controls that significantly changed between timepoints appeared to be trending upward. Samples were stored at 4˚C until they could be run on the flow cytometer, resulting in some samples being analyzed weeks following the experiment whereas others were run months later. Samples run months later might have had more degradation and might have been some loss of particle number to smaller sizes. Ideally, these samples would be run immediately after collecting, but due to a limited resources this could not be done in this study.

These results show that *M. leucophaeata* can significantly reduce cultured algae levels. Future research should investigate the ability of *M. leucophaeata* to reduce algae levels of natural blooms and in varying natural settings. Natural algae blooms are comprised of different species of varying sizes. In Baltimore Harbor, one of the most common small organisms (~6 microns) is the dinoflagellate *Prorocentrum minimum;* one of the larger (~50 microns) is *Akashiwo sanguinea* (personal communication). *M. leucophaeata* is a small mussel and while there has not been any literature on this species to date, studies conducted on other bivalve species suggest that there might be some physiological constraints to the size particles that they can ingest (Rosa et al., 2018; Shumway et al., 1985). Other species of bivalves are known to alter their filter feeding in

response to phytoplankton species (Binzer et al., 2018; Galimany et al., 2008). To date, there have been no studies looking at *M. leucophaeata* and its ability to filter larger or toxin-producing algae species.

Additional work should focus on the ability of *M. leucophaeata* to reduce algae blooms species in Baltimore Harbor and examine the effects of additional environmental factors. Dissolved oxygen in Baltimore Harbor can be depressed even near the surface due to microbial activity and stratification (Wicks et al., 2011). There is ample literature on the changes in feeding behavior of bivalves experiencing the stress of low oxygen (Kamermans and Saurel, 2022; Tang and Riisgård, 2018; Widdows et al., 1989). Future work should examine the effect of dissolved oxygen on mussel feeding behavior as it does fall below 2 mg/L in the summer months. Additionally, the experiments described in this chapter did not use algae blooms collected from the wild. The variety of phytoplankton found within natural algae blooms may affect feeding behavior of the mussels and thus their ability to clear the water. Overall, my results show *M. leucophaeata* can reduce algae levels of lab grown cultures across a variety of simulated environmental salinities and temperatures. Future work should focus on how *M. leucophaeata* feed with a natural algae community.

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Supplemental Information:

S. Table 1. Pairwise t-test for *Isochrysis* Mussel IVCH

group1	group2	n1	n2	statistic	df	p	p.adj
T1	T2	4	4	13.8	3	0.000816	**0.006**
T1	T3	4	4	19	3	0.000319	**0.003**
T1	T4	4	4	16.8	3	0.000456	**0.004**
T1	T5	4	4	16	3	0.000534	**0.004**
T2	T3	4	4	5.4	3	0.012	0.074
T2	T4	4	4	4.28	3	0.023	0.094
T2	T5	4	4	4.75	3	0.018	0.088
T3	T4	4	4	1.58	3	0.211	0.216
T3	T5	4	4	2.27	3	0.108	0.216
T4	T5	4	4	3.51	3	0.039	0.118

S. Table 2. Pairwise t-test for Isochrysis No Mussel IVCH

group1	group2	n1	n2	statistic	df	p	p.adj
T1	T2	4	4	9.55	3	0.002	**0.022**
T1	T3	4	4	8.22	3	0.004	**0.03**
T1	T4	4	4	11.6	3	0.001	**0.014**
T1	T5	4	4	-0.39	3	0.723	0.723
T2	T3	4	4	3.3	3	0.046	0.228
T2	T4	4	4	8.07	3	0.004	**0.03**
T2	T5	4	4	-1.9	3	0.153	0.459
T3	T4	4	4	1.47	3	0.239	0.478
T3	T5	4	4	-2.89	3	0.063	0.252

S. Table 3. Pairwise comparison of Chaetoceros Mussel Treatment IVCH

group1	group2	n1	n2	statistic	df	p	p.adj
T1	T2	4	4	5.22	3	0.014	0.096
T1	T3	4	4	9.01	3	0.003	**0.026**
T1	T4	4	4	6.73	3	0.007	0.054
T1	T5	4	4	50	3	1.760E-05	**1.76E-04**
T2	T3	4	4	2.7	3	0.074	0.369
T2	T4	4	4	1.05	3	0.373	0.746
T2	T5	4	4	4.77	3	0.018	0.105
T3	T4	4	4	-0.097	3	0.929	0.929
T3	T5	4	4	2.62	3	0.079	0.369
T4	T5	4	4	1.73	3	0.182	0.546

S. Table 4. Pairwise comparison of *Chaetoceros* No Mussel IVCH

group1	group2	n1	n2	statistic	df	p	p.adj
T1	T2	4	4	2.49	3	0.089	0.887
T1	T3	4	4	1.13	3	0.34	1
T1	T4	4	4	0.86	3	0.452	1
T1	T5	4	4	1.66	3	0.196	1
T2	T3	4	4	-1.39	3	0.259	1
T2	T4	4	4	-1.56	3	0.216	1
T2	T5	4	4	1.24	3	0.303	1
T3	T4	4	4	-1.21	3	0.313	1
T3	T5	4	4	1.81	3	0.169	1
T4	T5	4	4	1.93	3	0.15	1

S. Table 5. Pairwise comparisons for total chlorophyll - *Isochrysis*

Treatment	group1	group2	n1	n2	statistic	p	p.adj
Iso - Mussel	T1	T4	4	4	10	0.125	0.375
Iso - Mussel	T1	T5	4	4	10	0.125	0.375
Iso - Mussel	T4	T5	4	4	7	0.625	1
Iso – No Mussel	T1	T4	4	4	10	0.125	0.375
Iso – No Mussel	T1	T5	4	4	10	0.125	0.375
Iso – No Mussel	T4	T5	4	4	7	0.625	1

S. Table 6. Pairwise t-test for total chlorophyll *Chaetoceros* treatment with mussels

group1	group2	n1	n2	statistic	df	p	p.adj
T1	T4	4	4	5.13	3	0.014	**0.043**
T1	T5	4	4	4.86	3	0.017	0.05
T4	T5	4	4	0.945	3	0.414	1

S. Table 7. Pairwise comparisons from the Wilcoxon rank sum test for *Chaetoceros* treatment without mussels. Total chlorophyll

group1	group2	n1	n2	statistic	p	p.adj
T1	T4	2	2	0	0.5	1
T1	T5	2	2	0	0.5	1
T4	T5	2	2	0	0.5	1

S. Table 8. Pairwise t-test for temperature trial 1 mussel treatment IVCH

group1	group2	n1	n2	statistic	df	p	p.adj
T1	T2	12	12	3.7	11	0.004	**0.021**
T1	T3	12	12	5.31	11	0.00025	**0.002**
T1	T4	12	12	7.39	11	0.0000138	**0.0000828**
T2	T3	12	12	5.15	11	0.000319	**0.002**
T2	T4	12	12	7.34	11	0.0000147	**0.0000882**
T3	T4	12	12	4.96	11	0.000427	**0.003**

S. Table 9. Pairwise Wilcoxon rank sum test for temperature trial 1 no-mussel treatment IVCH

group1	group2	n1	n2	statistic	p	p.adj
T1	T2	12	12	16	0.077	0.463
T1	T3	12	12	24	0.266	1
T1	T4	12	12	33	0.677	1
T2	T3	12	12	43	0.791	1
T2	T4	12	12	51	0.38	1
T3	T4	12	12	44	0.733	1

S. Table 10. Pairwise t-test results for temperature trial 2. Treatment groups are tested individually. IVCH

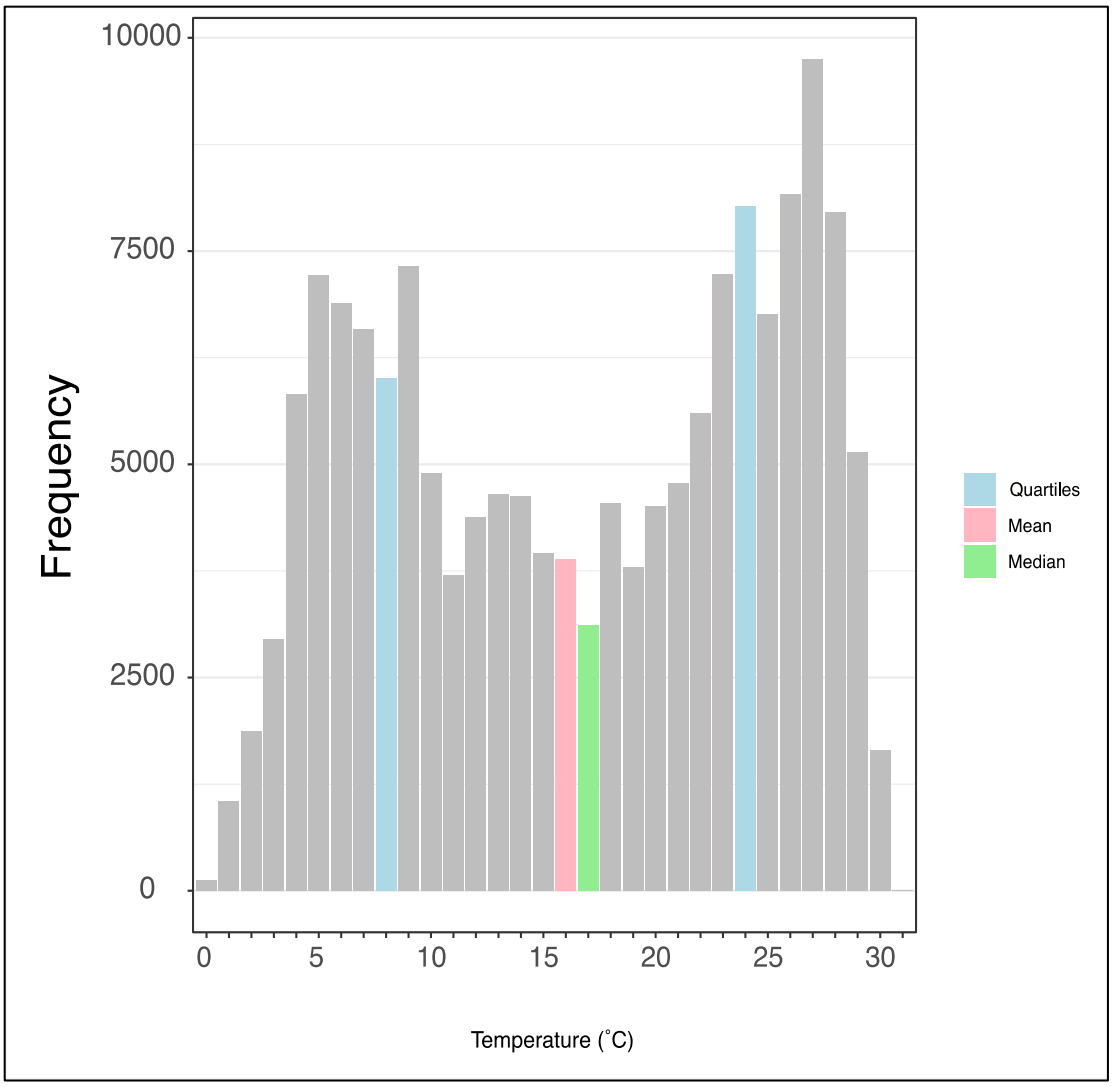
Treatment	group1	group2	n1	n2	statistic	p	p.adj
Mussel	T1	T2	12	12	78	0.000488	**0.003**
	T1	T3	12	12	78	0.000488	**0.003**
	T1	T4	12	12	78	0.000488	**0.003**
	T2	T3	12	12	78	0.000488	**0.003**
	T2	T4	12	12	78	0.000488	**0.003**
	T3	T4	12	12	76	0.001	**0.009**
No Mussel	T1	T2	12	12	-1.28	11	0.227
	T1	T3	12	12	-0.778	11	0.453
	T1	T4	12	12	0.713	11	0.491
	T2	T3	12	12	1.02	11	0.33
	T2	T4	12	12	1.78	11	0.102
	T3	T4	12	12	1.84	11	0.093

S. Table 11. Wilcoxon sum rank test results for salinity trial 1 – IVCH

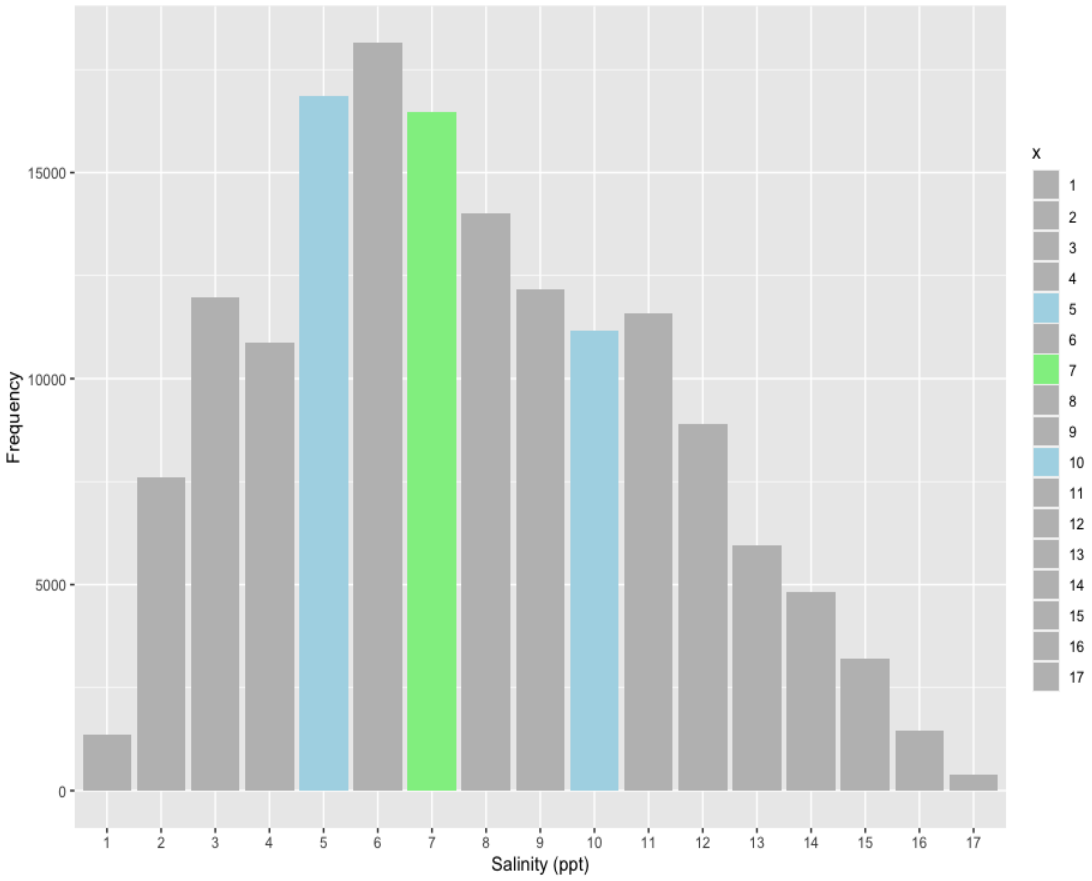
Treatment	group1	group2	n1	n2	statistic	p	p.adj
Mussel	T1	T2	12	12	78	0.000488	**0.003**
	T1	T3	12	12	78	0.000488	**0.003**
	T1	T4	12	12	78	0.000488	**0.003**
	T2	T3	12	12	78	0.000488	**0.003**
	T2	T4	12	12	78	0.000488	**0.003**
	T3	T4	12	12	78	0.000488	**0.003**
No mussel	T1	T2	12	12	10	0.021	0.126
	T1	T3	12	12	1	0.000977	**0.006**
	T1	T4	12	12	1	0.000977	**0.006**
	T2	T3	12	12	2	0.001	**0.009**
	T2	T4	12	12	0	0.000488	**0.003**
	T3	T4	12	12	9	0.016	0.097

S. Table 12. Pairwise t-test results for salinity trial 2 IVCH

Treatment	group1	group2	n1	n2	statistic	p	p.adj
Mussel							**4.52E-**
	T1	T3	12	12	10.5	11	**07**
							8.87E-
	T1	T4	12	12	9.82	11	**07**
	T3	T4	12	12	3.47	11	**0.005**
No Mussel	T1	T3	12	12	-0.945	11	0.365
	T1	T4	12	12	-1.25	11	0.237
	T3	T4	12	12	-0.117	11	0.909



S. Figure 1. Frequency of temperatures as captured by the eyes on the bay sonde. Temperatures are from 2018 – 2022. Temps are rounded up to the nearest whole number. Colors represent mean, median, and 1st and 3rd quartile.



S. Figure 2. Salinity from eyes on the bay data. Values rounded to the nearest whole number. From 2018 – 2022 eyes on the bay data for salinity at the aquarium east station. Green is mean and median. Blue is quartiles.

Chapter 3: Clearance of Natural Phytoplankton Blooms by the Dark False Mussel, *Mytilopsis leucophaeata*

Introduction:

Ecosystem services are the natural processes and functions that environments and the organisms living within provide. In aquatic environments, bivalves are one of the animals that perform numerous ecosystem services. Most notably, bivalves filter the water and improve water clarity and quality by removing phytoplankton and particulates, which also represents a removal of nitrogen (Newell 2004). In Maryland, this ecosystem service is monetized and recognized in a nutrient trading program. Oyster growers can sell nutrient credits from their oyster harvest to companies and municipalities that have a requirement to reduce nutrient inputs into the Chesapeake Bay (NOAA Office for Coastal Management, n.d.; Rose et al. 2021). However, oysters do not grow well in some parts of the Chesapeake Bay that have poor water quality or are heavily urbanized like Baltimore Harbor.

Baltimore Harbor is in the upper portion of the Chesapeake Bay on the Patapsco River in Maryland. Historically, Baltimore Harbor was a hub of shipping and industrial material factories such as chromium and steel (Travers 2016). For more than a century, these industries discharged contaminants into the water and sediments. Further, harbor dredging to accommodate larger ships created deeper channels and thus more opportunities for vertical stratification and accumulation of anoxic and hypoxic waters. Nutrient loading from urban runoff and wastewater discharges drive phytoplankton blooms, which senesce

and create biological oxygen demand in in these artificially deep waters. Currently, Baltimore City has a municipal separate storm sewer system (MS4) permit that requires the city to reduce nutrient inputs into the water (Maryland Department of the Environment 2021). The city achieves its required nutrient reduction through weekly street sweeping, litter and debris cleaning, and additional monitoring (Baltimore City Department of Public Works 2021). However, an untapped nutrient removal option lies below the surface of Baltimore Harbor with the native bivalve population.

The Dark False Mussel, *Mytilopsis leucophaeata*, is a small (1-3cm) mussel native to the east coast of North America and the Gulf of Mexico, that grows abundantly in upper Chesapeake Bay tributaries, including Baltimore Harbor. They have been reportedly associated with improvements in water clarity in Magothy Creek, MD (Kennedy 2011; Goldman 2007). In their invaded range in Brazil, studies show that *M. leucophaeata* can reduce chlorophyll levels of lagoon water (Neves et al. 2020; Rodrigues et al. 2023). This shows the promise of *M. leucophaeata* to reduce algae levels. Previous work shows that the mussels can reduce chlorophyll levels at different salinities and temperatures (Rajagopal, Van der Gaag, et al. 2005; Rajagopal, van der Velde, et al. 2005; Kido, et al, *in prep*). These studies examined clearance rates using either synthetic beads of defined sizes or cultured algae. However, no studies on the reduction of natural algae communities in the native range of *M. leucophaeata* have been conducted.

The objective of this study is to evaluate the ability of *M. leucophaeata* to reduce algae levels of natural algae blooms. To examine this, I conducted five clearance

experiments using algae blooms and mussels collected at different times from Baltimore Harbor. I monitored *in vitro* chlorophyll, and collected samples for chlorophyll extraction, algae cell counts and imaging, and analysis of carbon and nitrogen. These results show that mussels do reduce natural algae blooms, that nitrogen is removed, and point to additional studies needed to fully understand the nutrient removal ecosystem service.

Methods:

Animals care and condition

Mussels were obtained from underwater surfaces at the Downtown Sailing Center (1425 Key Hwy, Baltimore, MD, 21230) and from a floating dock in front of the Institute of Marine and Environmental Technology (IMET) (Pier 5, Baltimore, MD, 21202) (Table 15). Collections were made approximately every 30 days from June through September of 2023. Mussels were transported from their collection location back to IMET. In the lab, mussels were detached from their original substrate, placed on acrylic discs (approx. 10 cm diameter), and acclimated to lab conditions for a minimum of 10 days before an experiment could proceed. Mussels were kept at ambient lab temperature (~24˚C). Water changes occurred every other day using water collected from Baltimore's Inner Harbor. This constituted both water exchange and feeding from natural phytoplankton. On days when Harbor water chlorophyll levels were below 20 µg/L as indicated on the MD DNR Eyes on the Bay sonde for Aquarium East, supplemental cultured *Isochrysis* was provided to the mussels.

Table 15. Collection locations for mussels and wild algae blooms

COLLECTION TYPE	LOCATION	COORDINATES	EXPERIMENTS
Mussels	Downtown Sailing Center	(39.274458, -76.600185)	WB1, WB2, WB3
	IMET	(39.286472, -76.606360)	WB4, WB5
Algae	Aquarium East	(39.285473, -76.607978)	WB1
	Aquarium West	(39.285437, -76.608889)	WB2
	Middle Branch Marina	(39.258276, -76.625771)	WB3 and WB5
	IMET	(39.286472, -76.606360)	WB4

Collection of the algae:

Algae for the clearance rate experiment were collected from Baltimore's Inner Harbor or from the Middle Branch Marina (Table 15). To determine if there is a bloom occurring in the harbor, I used chlorophyll readings from Maryland Department of Natural Resources (MD DNR) Eyes on the Bay continuous monitoring sonde and visual identification (Maryland Department of Natural Resources, n.d.). If sonde readings were at or above 30 µg/L of chlorophyll then I would collect water from the respective location.

The IMET location was the exception as there is no monitoring sonde. Due to the proximity to IMET, water was visually monitored for an algae bloom. If surface water was a noticeably turbid and a brown-red color, water was collected and brought back to lab. Water quality parameters were measured using a YSI (Pro DSS) except for chlorophyll, which was instead estimated from in vitro chlorophyll (IVCH) recorded as relative fluorescence units (RFU) (Turner Instruments AquaFlor®). For collections made near an Eyes on the Bay sonde, DO, salinity, temperature, and chlorophyll were recorded from the Eyes on the Bay website (Table 16). In the lab, water was kept aerated with an air pump and a supplemental light (60-watt equivalent white LED light) was placed about 0.5 meters above the containers.

Table 16. Conditions of algae blooms used in clearance experiments

Exp.	Collection Date	DO (mg/L)	Salinity (ppt)	Temperature (˚C)	Chlorophyll (µg/L)	IVCH (RFU)
WB1	6/12/23	9.08	9.5	22.0	112.7	1825
WB2	6/23/23	11.72	8.1	25.2	45.6	1057
WB3	7/12/23	7.63	8.9	27.0	126.3	2905
WB4	7/31/23	6.4	10	27.7	N/A	976
WB5	9/20/23	13.12	15	25.1	32.63	856

Experimental Set Up:

Experiments took place between June through September of 2023 at IMET and are labeled as "WB" to signify these are wild algae blooms. Twenty-four hours before the start of the experiment, mussels were moved to a tank with artificial seawater (ASW) matching the salinity of the collected water. ASW (35 ppt) was made by the Aquatic Research Center at IMET and diluted with RO/DI water to the salinity that matched the algae bloom salinity. Water collected from the harbor was allowed to acclimate to lab temperature for a minimum of 12 hours before conducting the experiment. 1.5 l of the collected harbor water was added to a plastic jar to which the mussels would be added to. Air hoses were added to each container to ensure good mixing of the phytoplankton. On the day of the experiment, a T0 time point was collected before the mussels were added to the container. Water samples for IVCH measurements were collected at all timepoints and those measurements (RFU) were made immediately after collection. Samples for chlorophyll extractions, carbon and nitrogen analysis, and cell counts were collected at the T1, T4, and T5 timepoints (Figure 17). Cell counts were only collected for the WB3, WB4, and WB5 experiments. These samples were preserved in a 1% glutaraldehyde solution and stored at 4˚C.

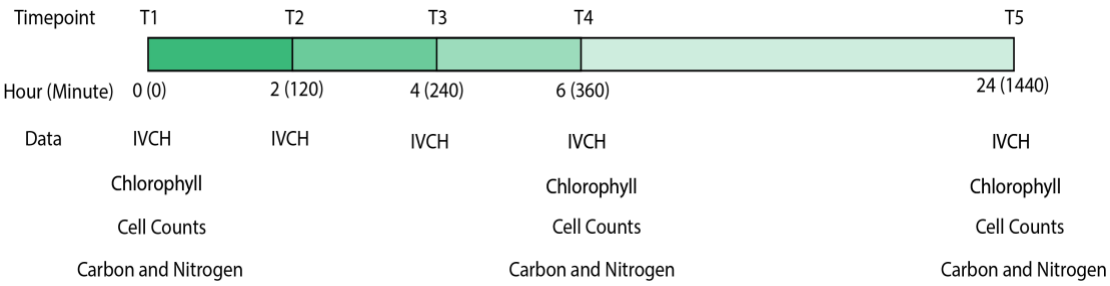


Figure 17. Timeline of data collection for wild bloom experiments

Cell Counts:

To measure cell counts, I ran the water samples stored in glutaraldehyde through a FlowCam (8000 Series, Yokogawa). I used the 10x magnification with the corresponding flowcell and syringe size. For fluidics, I used a flow rate of 0.15 ml/minute and used the auto-image mode to count and take images. The FlowCam ran for a total of 2 minutes for each sample (0.3 ml of sample). In between treatments, I ran 0.3 ml of deionized water to remove any lingering phytoplankton. In post analysis, I removed any images that were clearly not phytoplankton, such as debris or air bubbles, and used the new count as the cell count for that sample. Images were saved for future analysis and identification.

Carbon and Nitrogen Analysis:

For carbon and nitrogen analysis, I filtered water through a pre-combusted 25 mm diameter GF/C filter. To pre-combust the filters, I placed the filters (GF/C, Whatman) in a muffle furnace at 450˚C for 4 hours. The volume of water filtered varied for the first experiment (10-15 ml), but in the following experiments a standard of 20 ml was used.

Samples were placed into 2 ml tubes and stored in a -20˚C freezer until processing. Samples were sent to The Nutrient Analytical Services Laboratory (NASL) at the Chesapeake Biological Laboratory (Solomons, MD). Results were provided in mg C/L or mg N/L.

Chlorophyll Extractions and Analysis:

For the chlorophyll extractions, water was filtered through a regular GF/C filter and stored at -20 ˚C until processing. The volume filtered matches the volume used for carbon and nitrogen analysis. The samples were sent to the NASL at the Chesapeake Biological Laboratory (Solomons, MD). Results were provided as total chlorophyll in µg Chla/L.

Statistics:

All statistical tests were run on R (R version 4.3.1). Statistical tests were performed using the "Stats" package in R except when specified. For the pairwise t-tests and Wilcoxon rank sum test, p-values were adjusted using a Bonferroni correction.

The IVCH data collected to test for the effect of Treatment (mussels or no mussels) was non-parametric, so it was analyzed using the Kruskal-Wallis and a pairwise Wilcoxon rank sum test. To test for the effect of time on IVCH levels, a repeated measures ANOVA ("Rstatix") was performed with a post-hoc pairwise t-test ("Rstatix"). If data did not meet assumptions of normality or homogeneity of variance, a Friedman rank sum test was performed instead with a post-hoc pairwise Wilcoxon sum rank test.

To test for the effect of Treatment (mussels or no mussels) on cell count levels, a one-way ANOVA was conducted with a Tukey's HSD test. If assumptions were not met,

non-parametric tests were performed instead. If the assumption for normality was not met, then a Kruskal-Wallis test and pairwise Wilcoxon rank sum test was performed. If the assumption for homogeneity of variance was not met, a Welch test was performed. To test for the effect of time on cell counts, a repeated measures ANOVA was performed ("Rstatix"). This was followed by a post-hoc pairwise t-test ("Rstatix"). If assumptions were not met for the repeated measures ANOVA, a Friedman Rank sum test and pairwise Wilcoxon sum rank test were conducted instead.

To assess the effect of Treatment and time on carbon and nitrogen levels, a mixed measures ANOVA was performed ("Rstatix"). This was followed by a pairwise t-test and one-way ANOVA ("Rstatix") to test for the effect of treatment on time and time on treatment respectively. If assumptions were not met for this test but did meet the assumptions for a one-way ANOVA, a one-way ANOVA was run to test for the effect of treatment on carbon and nitrogen levels. If assumptions were not met for the one-way ANOVA, then a Kruskal-Wallis rank sum test and Wilcoxon rank sum test were conducted instead. To test for the effect of time on carbon and nitrogen a repeated measures ANOVA and pairwise t-test was conducted ("Rstatix"), and if assumptions for this test were not met, a Friedman rank sum and Wilcoxon test were performed instead.

To test for the effect of time and treatment on total chlorophyll levels, a mixed measures ANOVA ("Rstatix") was performed and followed by a pairwise t-test ("Rstatix") and one-way ANOVA ("Rstatix") to more closely examine any significance found in the mixed measures ANOVA. If the assumptions for the mixed measures ANOVA were not

met, a Welch test was performed instead to test for the effect of treatment on total chlorophyll levels, and a repeated measures ANOVA was performed to test for the effect of time on total chlorophyll levels.

Results:

In vitro chlorophyll (IVCH):

Treatment (mussels or no mussels) had a significant effect on IVCH for all experiments except for WB1 (Figure 17, Table 17). Time had a significant effect on IVCH for all the mussel treatments and the no-mussel treatments for WB2 (Table 18). While the Kruskal-Wallis test did not show a significant effect of treatment on IVCH for WB1, the statistical tests for the effect of time on IVCH did show that the mussel treatment has a significant effect whereas the no-mussel treatment did not (Table 18). The pairwise t-test for mussel treatment of WB1 show that there is a significant effect of time for the T1 to T5, T2 to T5, T3 to T5, and T4 to T5 comparisons (S. Table 13). No significant pairwise comparisons were found with the no-mussel treatment of WB1 (S. Table 14). The tests for time cannot be directly compared for the two treatments, thus it cannot be said for certain that the treatment for WB1 has a significant effect.

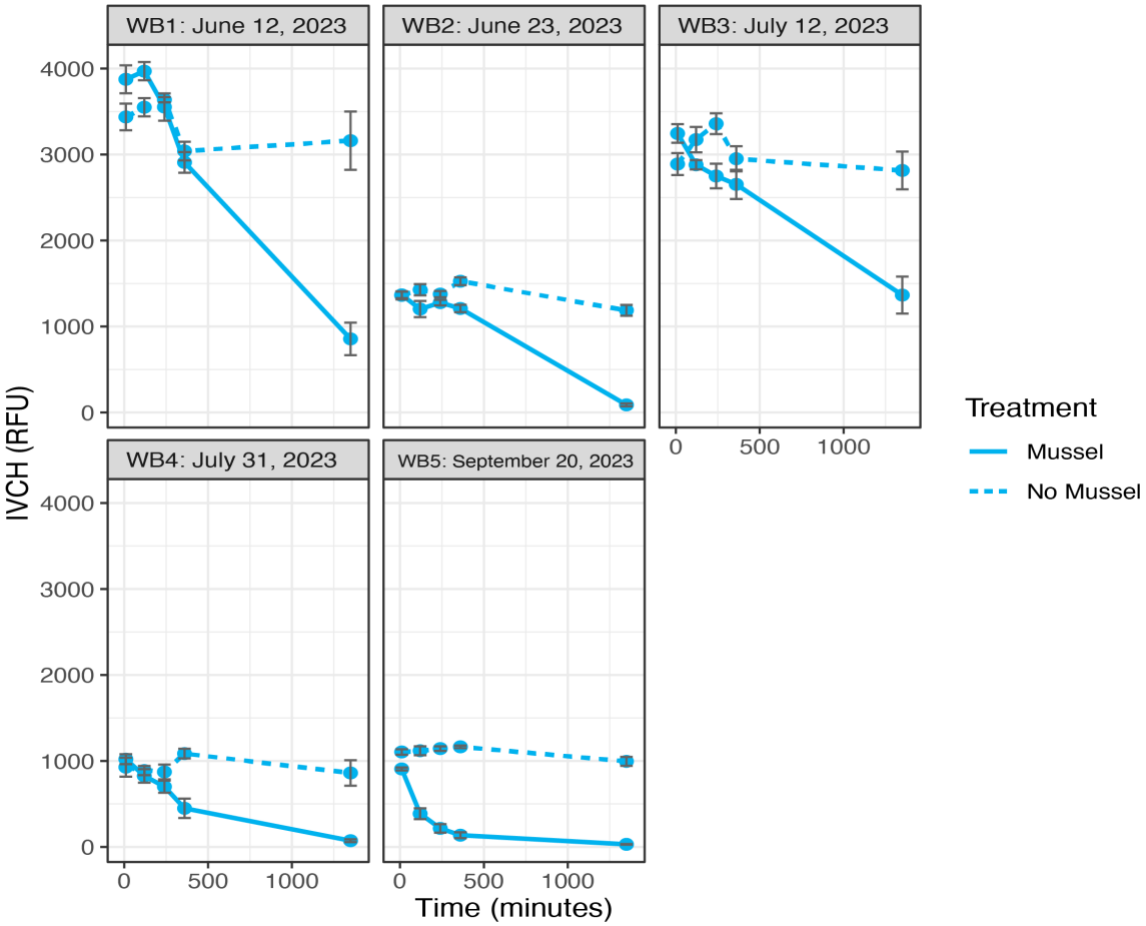


Figure 18. IVCH change over 24 hours. Error bars are standard error mean (SEM).

Table 17. Kruskal-Wallis and pairwise Wilcoxon sum rank test results for IVCH

Experiment	Chi-Squared	Df	p-value	Wilcoxon p-value
WB1	0.263	1	0.877	0.88
WB2	10.276	1	**0.001**	**0.001**
WB3	6.193	1	**0.013**	**0.012**
WB4	7.464	1	**0.006**	**0.007**
WB5	28.397	1	**9.88E-08**	**1.00E-10**

Table 18. Effect of Time on IVCH measurements. Tests are specified in the table. Repeated measures ANOVA (RMA) or Friedman rank sum

Exp.	Treatment	Test	Dfn	DFd	F	P-value	Chi-Squared	Df	P
WB1	Mussel	RMA	4	12	61.078	**7.08E-08**	-	-	-
	No Mussel	Friedman	-	-	-	-	4.911	4	0.297
WB2	Mussel	Friedman	-	-	-	-	13	4	**0.011**
	No Mussel	RMA	4	12	11.69	**4.19E-04**	-	-	-
WB3	Mussel	RMA	4	12	31.494	**2.80E-06**	-	-	-
	No Mussel	RMA	4	12	2.072	0.148	-	-	-
WB4	Mussel	RMA	4	12	49.876	**2.22E-07**	-	-	-
	No Mussel	RMA	4	12	1.5	0.25	-	-	-
WB5	Mussel	RMA	4	12	117.671	**1.62E-09**	-	-	-
	No Mussel	RMA	4	12	2.86	0.071	-	-	-

The WB2 experiment displayed a significant effect of time on IVCH for both the mussel and no-mussel treatments (Table 18). Examining the pairwise comparisons for each timepoint, no significant comparisons were found for the mussel treatment, and the no-mussel treatment show that T1 and T4 are significantly different (S. Table 15, S. Table 16).

For the WB3, WB4 and WB5 experiments time had a significant effect on IVCH for the treatment with mussels and the treatment without mussels did not show any significant effect of time on IVCH levels (Table 18). For WB3, the T1 to T5 comparison was statistically significant for the mussel treatment. None of the time comparisons for the no-mussel treatment were significant (S. Table 17). The WB4 experiment had significant comparisons between the T1 and T5 timepoints, T2 and T5 timepoints, and T3 and T5 timepoints (S. Table 17). The no-mussel treatment for WB4 did not show significant effects of time, treatment, or the interaction of time and treatment from the repeated measures ANOVA. However, there were significant pairwise comparison for T2 and T4 timepoints (S. Table 17). For the WB5 experiment, there was a significant difference between the T1 and T2, T3, T4, and T5 timepoints as well as between T2 and T3 (S. Table 17). There were no significant comparisons between timepoints for the WB3 and WB4 no-mussel treatments.

Cell counts:

Visually inspecting the data, all experiments show a decrease in cell counts for the mussel treatment. (Figure 18). However, for WB3, there was not a significant effect of treatment (mussels or no mussels) on cell counts, but there was a significant effect of time on cell counts in the mussel treatment (Table 19, Table 21). When making pairwise comparisons of timepoints for the mussel treatment of WB3, there were no significant differences between timepoints, but there was a significant difference between the T1 and T4 timepoints of the no-mussel treatment (S. Table 18).

Due to a missing data point for WB5, the replicate with missing data was removed from the analysis and the statistical tests were conducted with four replicates in the mussel treatment and three replicates in the no mussel treatment. WB 4 and WB5 experiments showed a significant effect of treatment (mussels or no mussels) on cell counts (Table 20).

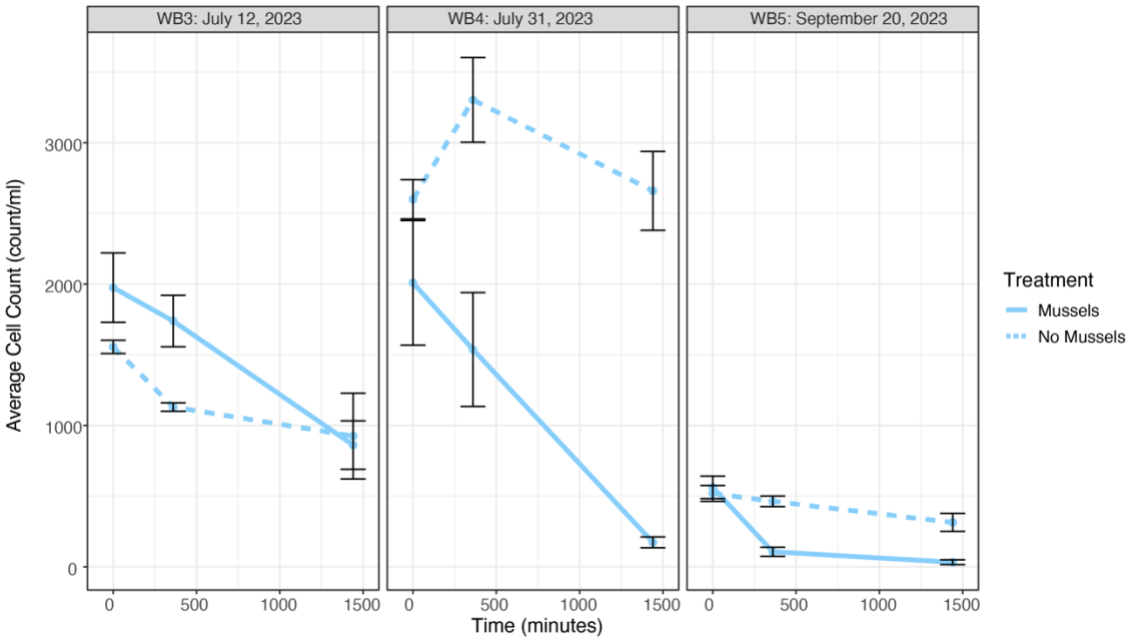


Figure 19. Change in cell count over time. Error bars are SEM.

Table 19. One-way ANOVA results for WB3 treatment (mussels or no mussels) effect on cell counts

	SumSq	Df	F value	p-value
Treatment	620312	1	2.2024	0.152
Residuals	6196228	22	-	-

Table 20. Non-parametric (Welch and Kruskal test) for treatment (mussels or no mussels) for effect on cell counts.

Experiment	Test	p-value
WB4	Welch	**1.71E-4**
WB5	Kruskal	**0.0422**

Table 21. WB3 repeated measure ANOVA for time effect on cell counts

Experiment	Treatment	Dfn	DFd	F	P-value
WB3	Mussel	2	6	6.059	**0.036**
	No Mussel	2	6	3.246	0.111

For WB4, both mussel and no-mussel treatments displayed a significant effect of time on cell counts (Table 22). However, the pairwise comparisons of time showed no significant differences between timepoints (S. Table 19). Time had a significant effect on the cell counts for the WB5 mussel treatment, but not the no-mussel treatment. There were no significant differences between timepoints in pairwise comparisons for WB5 no-mussel treatment and the mussel treatment (S. Table 19).

Table 22. Friedman rank sum and Wilcoxon rank sum test results for time effect on cell counts

Experiment	Treatment	Chi-Squared	Df	p-value
WB4	Mussels	6.5	2	**0.03877**
	No Mussels	6.5	2	**0.03877**
WB5	Mussels	6.5	2	**0.03877**
	No Mussels	2	2	0.3679

Carbon:

Quantitatively, the mussel treatments for all experiments have a lower carbon concentration at the final timepoint (1440 minutes) than the starting timepoint (Figure 19). In WB1, carbon showed no significant differences between the mussel and no-mussel

treatments, and no significant effect of timepoint on cell carbon concentration (Table 23, Table 24, S. Table 20).

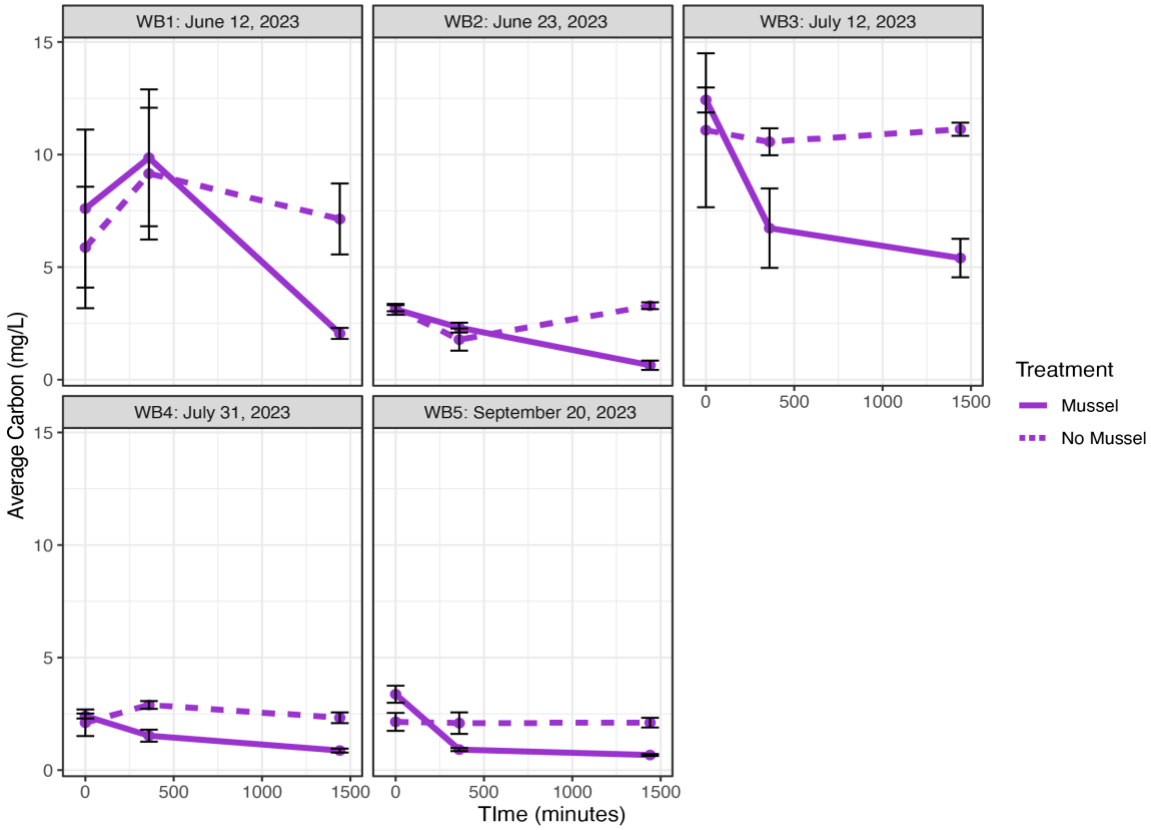


Figure 20. Change in carbon concentration over time. Error bars are SEM.

Table 23. Kruskal-Wallis for WB1 treatment effects on carbon concentration

Experiment	Chi-Squared	Df	p-value
WB1	0	1	1

Table 24. Repeated measures ANOVA results WB1 carbon concentration

Experiment	Treatment	Dfn	DFd	F	P-value
WB1	Mussel	2	6	2.699	0.146
	No mussel	2	6	0.815	0.091

For WB2, there was a significant effect of treatment, time, and the interaction between treatment and time on carbon concentration (Table 25). In comparing the treatments at the different timepoints, I found that there was a significant difference only at the T5 timepoint (Table 26). Additionally, both the mussel and no-mussel treatment had a significant effect of time (Table 27).

For WB3, only the interaction of treatment and time was found to have a significant effect on carbon concentrations (Table 25). The pairwise comparisons showed that there was a significant effect between treatments at the T5 timepoint (Table 26). The mussel treatment for the WB3 experiment showed a significant effect of time on carbon levels, but there was no significant effect of time on carbon levels for the no-mussel treatment (Table 27).

Table 25. Mixed measure ANOVA results for WB2, WB3, WB4, and WB5 for carbon concentration

Experiment	Effect	Dfn	DFd	F	P-value
WB2	Treatment	1	6	17.302	**0.006**
	Time	2	12	10.18	**0.003**
	Treat*Time	2	12	16.577	**0.000352**
WB3	Treatment	1	6	4.049	0.091
	Time	1.07	6.43	2.729	0.146
	Treat*Time	1.07	6.43	2.497	0.162
WB4	Treatment	1	4	8.467	**0.044**
	Time	2	8	3.186	0.096
	Treat*Time	2	8	6.629	**0.02**
WB5	Treatment	1	5	5.125	0.073
	Time	2	10	7.689	**0.009**
	Treat*Time	2	10	7.144	**0.012**

Table 26. Pairwise comparison of Treatment at timepoints (carbon)

Experiment	Time	group1	group2	n1	n2	p
WB2	T1	Mussel	No mussel	4	4	0.864
	T4	Mussel	No mussel	4	4	0.363
	T5	Mussel	No mussel	4	4	**0.0000476**
WB3	T1	Mussel	No mussel	4	4	0.711
	T4	Mussel	No mussel	4	4	0.0852
	T5	Mussel	No mussel	4	4	**0.000732**
WB4	T1	Mussel	No mussel	4	3	0.586
	T4	Mussel	No mussel	4	3	**0.0132**
	T5	Mussel	No mussel	3	3	**0.00883**
WB5	T1	Mussel	No mussel	4	4	0.0668
	T4	Mussel	No mussel	4	4	0.0505
	T5	Mussel	No mussel	4	4	**0.000611**

Table 27. Effect of time on each treatment for carbon levels

Experiment	Treatment	Effect	DFn	DFd	F	p
WB2	Mussel	Time	2	9	32	**0.0000809**
	No Mussel	Time	2	9	7.4	**0.013**
WB3	Mussel	Time	2	9	10	**0.005**
	No Mussel	Time	2	9	0.024	0.977
WB4	Mussel	Time	2	9	19.4	**0.000551**
	No Mussel	Time	2	9	1.03	0.412
WB5	Mussel	Time	2	9	44.9	**0.0000209**
	No Mussel	Time	2	9	0.005	0.995

The WB4 experiment had a significant effect of treatment and the interaction between treatment and time on carbon concentrations (Table 25). Pairwise comparisons reveal a significant effect of treatment at the T4 and T5 timepoint (Table 26). Only the mussel treatment had a significant effect of time (Table 27).

For the last experiment, WB5, time and the interaction of time and treatment had a significant effect on carbon concentrations, but there was no significant effect of treatment alone on carbon concentrations (Table 25). The pairwise comparisons show that there is a significant difference between the treatments at the T5 timepoint (Table 26). The mussel treatment showed a significant effect of time on carbon concentrations but not for the no-mussel treatment (Table 27).

Nitrogen:

Nitrogen concentrations are lower at the T5 (1440 minute) timepoint than at the T1 timepoint (Figure 20). For nitrogen levels, WB1 and WB3 did not show a significant effect of treatment, like the carbon results (Table 28, Table 29). For WB1, time also did not have an effect on nitrogen levels for either treatment and no pairwise significance was found (Table 30, Table 31, S. Table 21). WB3 did have a significant effect of time for the mussel treatment but not the no-mussel treatment (Table 31). Pairwise comparisons of the WB3 mussel treatment show that there is a significant difference between the T1 and T5 timepoints (S. Table 22).

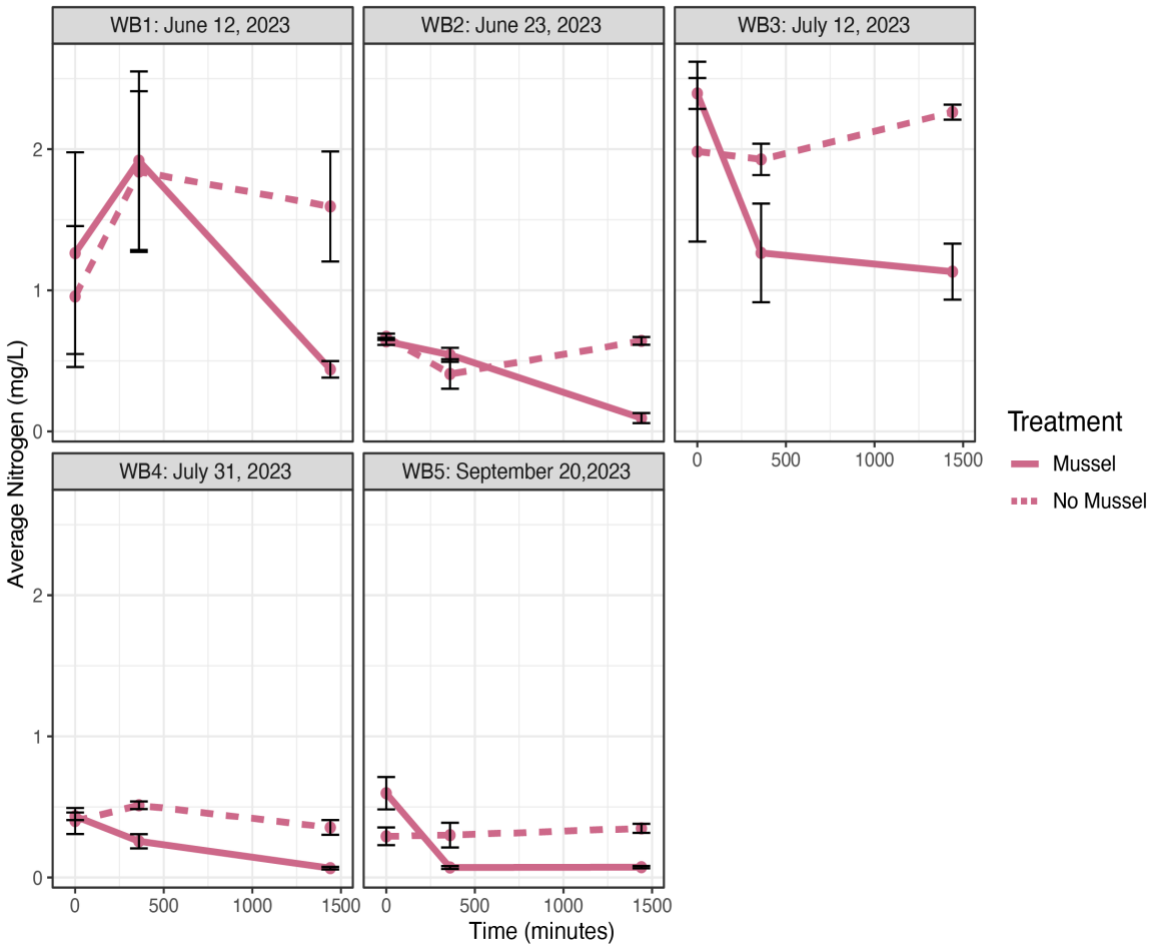


Figure 21. Change in nitrogen over time. Error bars are SEM.

Table 28. Kruskal-Wallis test results for the effect of treatment on nitrogen levels for WB1

Experiment	Chi-Squared	Df	p-value
WB1	0.1634	1	0.686

Table 29. One-way ANOVA results for the effect of treatment on nitrogen levels for WB3 and WB4

Experiment	Effect	SumSq	Df	F value	p-value
WB3	Treatment	1.2705	1	2.485	0.1292
	Residuals	11.2481	22	-	-
WB4	Treatment	0.1734	1	7.534	**0.01183**
	Residuals	0.5064	22	-	-

Table 30. Friedman rank sum test results for effect of time on nitrogen levels for WB1 mussel treatment and WB4 no-mussel treatment

Experiment	Treatment	Chi-Squared	Df	p-value
WB1	Mussels	1.5	2	0.4724
WB4	No Mussels	4.5	2	0.1054

Table 31. Repeated measures ANOVA results for time effects on nitrogen levels for WB1 (no-mussel treatment), WB3, and WB4 (mussel treatment)

Experiment	Treatment	Dfn	DFd	F	P-value
WB1	No mussel	2	6	0.815	0.091
WB3	Mussel	2	6	13.213	**0.006**
	No Mussel	1.01	3.04	0.206	0.683
WB4	Mussel	1.02	3.07	26.282	**0.014**

The WB4 experiment also had a significant effect of treatment on nitrogen levels (Table 29). Time had a significant effect on the mussel treatment but not the no-mussel treatment (Table 30, Table 31). Pairwise comparison of the timepoints show that there is a significant difference between the T1 and T5 timepoint for the mussel treatment (S. Table 21).

Results for WB2 and WB5 were analyzed using a mixed measures ANOVA. WB2 showed significant effects for treatment, time, and the interaction of treatment and time on nitrogen (Table 32). The T5 timepoint showed a significant difference between the two treatment groups, and overall, both treatment groups were significantly affected by time (Table 33, Table 34). WB5 did not show a significant effect of treatment (mussels or no-mussels) on nitrogen levels, but there was a significant effect of the interaction of treatment

and time (Table 32). WB2 also had a significant effect of time and the interaction of treatment and time.

The final experiment, WB5, showed no significant effect of treatment, but a significant effect of time and the interaction of time and treatment on nitrogen levels (Table 32). Between the two treatment groups, nitrogen was found to significantly differ at the T4 and T5 time point, and time had a significant effect on the mussel treatment nitrogen levels (Table 33, Table 34).

Table 32. Mixed measures ANOVA results for WB2 and WB5 Nitrogen

Experiment	Effect	Dfn	DFd	F	P-value
WB2	Treatment	1	6	18.839	**0.005**
	Time	2	12	12.802	**0.001**
	Treat*Time	2	12	19.342	**0.000176**
WB5	Treatment	1	6	1.593	0.254
	Time	2	12	9.21	**0.004**
	Treat*Time	2	12	11.751	**0.001**

Table 33. Pairwise comparisons of treatment at time WB2 and WB5 nitrogen

Experiment	Time	group1	group2	n1	n2	p
WB2	T1	Mussel	No mussel	4	4	0.377
	T4	Mussel	No mussel	4	4	0.285
	T5	Mussel	No mussel	4	4	**0.000019**
WB5	T1	Mussel	No mussel	4	4	0.058
	T4	Mussel	No mussel	4	4	**0.041**
	T5	Mussel	No mussel	4	4	**0.000165**

Table 34. Effect of time on treatment for WB2 and WB3 on nitrogen levels

Experiment	Treatment	Effect	DFn	DFd	F	p
WB2	Mussel	Time	2	9	57.7	**0.00000735**
	No Mussel	Time	2	9	5.13	**0.033**
WB5	Mussel	Time	2	9	20.7	**0.000429**
	No Mussel	Time	2	9	0.218	0.808

Chlorophyll:

Total extracted chlorophyll levels had a lower concentration at the T5 (1440 timepoint) than the T1 timepoint (Figure 21). For total chlorophyll, there was not a significant effect of treatment for WB1 and WB3 (Table 35,Table 37). For WB3, no significant effects were found for time nor the interaction for time and treatment (Table 37). However, WB1 did show a significant effect of time on total chlorophyll levels for both the mussel and no-mussel treatment (Table 36). Pairwise comparisons show that the T1 and T5 timepoints significantly differed from each other (S. Table 23).

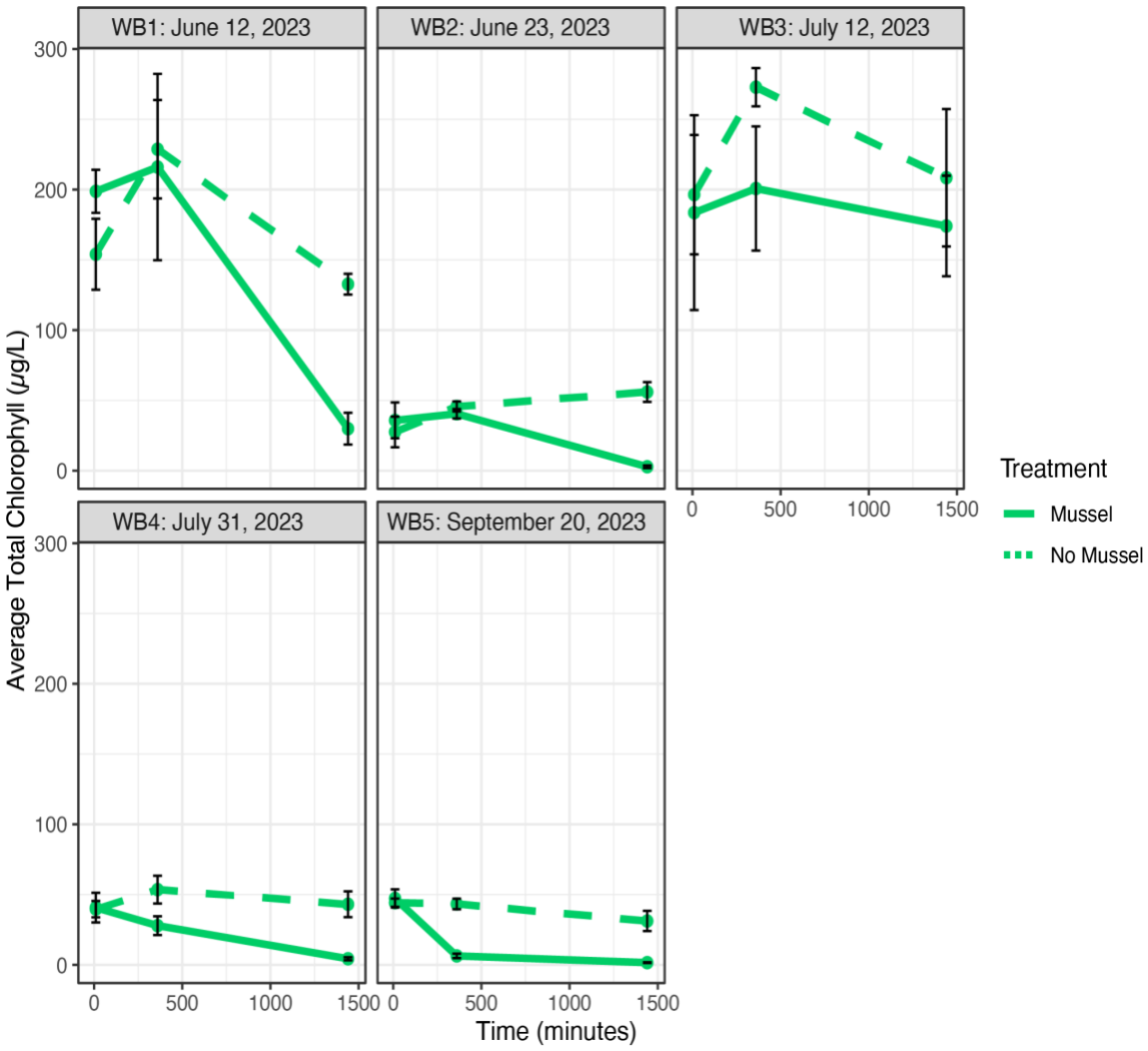


Figure 22. Total chlorophyll change over time. Error bars are SEM.

Table 35. Welch results for WB1 Chlorophyll

F	numDF	denomDF	p-value
0.39674	1.0	17.148	0.5371

Table 36. Repeated measures ANOVA results for WB1

Experiment	Treatment	Dfn	DFd	F	P-value
WB1	Mussel	2	6	6.46	**0.032**
	No Mussel	2	6	5.448	**0.045**

The interaction between treatment and time was the only variable that significantly influenced total chlorophyll levels for WB2 (Table 37). Pairwise comparisons show a significant difference between the treatments at the T5 timepoint, as well as a significant effect of time for the mussel treatment only (Table 38, Table 39).

Treatment was the only variable that significantly affected chlorophyll levels for WB4 (Table 37). At the T5 timepoint, the test shows a significant difference between treatments (Table 38). Time was also found to be significant for the mussel treatment but not for the no-mussel treatment (Table 39).

For the final experiment, WB5, all three variable terms (treatment, time, and treatment and time interaction) had a significant effect on chlorophyll levels (Table 37). Treatment differed significantly at the T4 and T5 timepoint and time had a significant effect for only the mussel treatment (Table 38, Table 39).

Table 37. Mixed measures ANOVA results for chlorophyll

Experiment	Effect	Dfn	DFd	F	P-value
WB2	Treatment	1	6	4.767	0.072
	Time	2	12	2.395	0.133
	Treat*Time	2	12	11.557	**0.002**
WB3	Treatment	1	6	0.897	0.38
	Time	2	12	0.799	0.472
	Treat*Time	2	12	0.253	0.781
WB4	Treatment	1	6	13.24	**0.011**
	Time	2	12	2.738	0.105
	Treat*Time	2	12	3.023	0.086
WB5	Treatment	1	6	42.073	**0.000638**
	Time	2	12	21.225	**0.000115**
	Treat*Time	2	12	10.544	**0.002**

Table 38. Effect of treatment at time point chlorophyll

Experiment	Time	group1	group2	n1	n2	p
WB2	T1	Mussel	No Mussel	4	4	0.642
	T4	Mussel	No Mussel	4	4	0.329
	T5	Mussel	No Mussel	4	4	**0.000293**
WB3	T1	Mussel	No Mussel	4	4	0.88
	T4	Mussel	No Mussel	4	4	0.17
	T5	Mussel	No Mussel	4	4	0.591
WB4	T1	Mussel	No Mussel	4	3	0.933
	T4	Mussel	No Mussel	4	3	0.0753
	T5	Mussel	No Mussel	3	3	**0.00576**
WB5	T1	Mussel	No Mussel	4	4	0.684
	T4	Mussel	No Mussel	4	4	**0.0000998**
	T5	Mussel	No Mussel	4	4	**0.00617**

Table 39. Effect of time on treatment - Chlorophyll

Experiment	Treatment	Effect	DFn	DFd	F	p
WB2	Mussel	Time	2	9	7.24	**0.013**
	No Mussel	Time	2	9	3.42	0.078
WB3	Mussel	Time	2	9	0.068	0.935
	No Mussel	Time	2	9	1.16	0.356
WB4	Mussel	Time	2	9	6.51	**0.018**
	No Mussel	Time	2	9	0.723	0.511
WB5	Mussel	Time	2	9	43.1	**0.0000246**
	No Mussel	Time	2	9	2.14	0.174

Discussion:

My results show that *M. leucophaeata* from Baltimore Harbor can reduce the phytoplankton levels of natural algae blooms in a mesocosm. Five different algae blooms from Baltimore Harbor varied in their intensities and likely their species composition, and in all instances, the treatment with mussels showed a reduction of the algae over time as measured by IVCH. In most cases, there were also declines in carbon, nitrogen, and extracted chlorophyll over the 24 hours of these experiments. This work shows that mussels in Baltimore Harbor have the potential to reduce algae levels in Baltimore Harbor, and in doing so, also the amount of nitrogen and carbon.

The IVCH measurements showed that there was a significant effect of treatment (mussels or no mussels) in four of the five experiments and that timepoint had a significant effect. For most of the experiments, as time increased there was a decrease in IVCH levels in the mussel treatment group. Most of the time the IVCH levels for the no-mussel treatment remained consistent. However, there were a few instances where there was a significant effect of time in the no-mussel treatment. Changes in the IVCH levels of the no-mussel group could be due to the dynamic of the algae community. It is known that estuarine waters contain variable numbers of micro grazers that can decrease phytoplankton levels (Calbet et al. 2003). Because of these interactions and any potential algae growth, IVCH levels may have been affected by these processes.

Measurements of the filterable carbon and nitrogen closely parallel each other across the different treatments and times. This is to be expected from the methodology of

analyzing carbon and nitrogen from particulates collected on the same GF/C filter. There was not a significant effect of treatment on nitrogen level in WB1 and WB3, and this is possibly related to the high variance of data for both carbon and nitrogen in these experiments. However, for WB3, there was a significant effect of time on carbon and nitrogen for the mussel treatment. The other three experiments show a significant effect of treatment and thus the reduction of their respective elements. Interestingly, these three experiments had low variance and started with blooms that had lower IVCH values (below 1500 RFU), whereas WB1 and WB3 had high variance and higher starting IVCH values (above 3000 RFU).

Total extracted chlorophyll levels were differentially affected by time and treatment across the experiments. Again, WB1 did not show a significant effect of treatment, but time for the mussel and no-mussel treatments was significant. WB3 also had a similar pattern of no significant effect of treatment or time for the no-mussel treatment, and time was significant only for the mussel treatment. Given that extracted chlorophyll was measured from GF/C filters similar to the carbon and nitrogen concentrations, the qualitative differences in slopes of experiment WB3 are noticeable. For the no-mussel treatment, carbon and nitrogen values increased from T4 (360 minutes) to T5 (1440 minutes) whereas the slope decreases in this interval for extracted total chlorophyll. For the mussel treatment, the chlorophyll values stay almost constant from T1 to T4 to T5, while the carbon and nitrogen values decrease sharply. Thus, there is an uncoupling of extracted chlorophyll

from the carbon and nitrogen for this experiment, which may be due to interactions within the phytoplankton community or a potential resuspension of deposited particles.

The FlowCam particle count data was highly variable. Only one of the three experiments (WB5) showed a significant effect of treatment on cell count and the effect of time was variable within and across the experiments. Part of this variation could be attributed to the FlowCam's limited ability to image algae of a certain size. The 10x objective is useful for a size range of 2 µm to 70 µm. Particles smaller than 2µm (such as cyanobacteria) could have been missed by the machine. Similar to the IVCH levels, there could have been natural interactions of the algae leading to the varied results. The identity of the particles imaged by the FlowCam are uncertain at this time, but one of the qualitative observations of size showed a proportional decrease in smaller particles over time for the WB3 treatment. This also corresponded with a proportional increase of larger particles in the T4 (360 minutes) and T5 (1440 minutes) timepoints. While it is uncertain why this proportional change occurred, I hypothesize that *M. leucophaeata* could be selecting for certain particles or are unable to ingest the larger particles due to size. Previous studies show that bivalves can select for particles based on size, if the algae are alive, and species (Shumway et al. 1985; Beninger et al. 2008; Qiao et al. 2022). The current study is unable to determine what the deciding factor may be for *M. leucophaeata* and future research is needed.

M. leucophaeata resides on hard underwater surfaces along with a wide range of species in Baltimore Harbor. When considering the use of these mussels for providing

algae reduction ecosystem services, resource managers should consider the contribution of other biofouling organisms like barnacles and bryozoans. The National Aquarium has seven years of data analyzing what species grow on acrylic "biodiscs" adjacent to the floating wetland in Baltimore Harbor. In some years, mussels are the dominant species, and other years barnacles are more prominent. Preliminary lab results show that barnacles can reduce IVCH of cultured algae species, and studies looking at their ability to reduce natural algae blooms are needed. However, due to their permanent attachment method to substrates in the water, it is hard to work with barnacles in a lab setting unless one can remove all other organisms from a permanently deployed surface (personal observation). Inter-annual species changes are important to consider when contemplating how the rates calculated in this project might change in a barnacle dominated year.

Overall, *M. leucophaeata* can reduce algae levels of a natural algae bloom. This research takes an important first step in using *M. leucophaeata* for their algae-reduction ecosystem services. There was a wide variation of how effectively mussels were able to reduce algae levels; this could be due to the species composition of the blooms. As demonstrated, the algae blooms used in these experiments had varying levels of IVCH, cell counts, carbon, nitrogen, and chlorophyll. The FlowCam data, showed that the species composition from experiment to experiment varied and the dominant species was not the same each time. From initial examination of the images, it appears that larger phytoplankton species are rejected or not consumed. This is reflected in the higher proportion of larger phytoplankton images at the final timepoint than compared to the first

timepoint. Adding to the complexity, *M. leucophaeata* does not grow in a monoculture in Baltimore Harbor and exists with other biofouling organisms. Future research should examine the ability of the entire biofouling community to consume phytoplankton blooms and sequester nitrogen.

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Supplemental Information:

S. Table 13. Pairwise t-test WB1 mussel treatment for IVCH

group1	group2	n1	n2	statistic	df	p	p.adj
T1	T2	4	4	-0.374	3	0.733	1
T1	T3	4	4	1.13	3	0.341	1
T1	T4	4	4	6.93	3	0.006	0.062
T1	T5	4	4	11.6	3	0.001	**0.014**
T2	T3	4	4	2.17	3	0.119	1
T2	T4	4	4	5.36	3	0.013	0.127
T2	T5	4	4	22.3	3	0.000196	**0.002**
T3	T4	4	4	2.7	3	0.074	0.741
T3	T5	4	4	8.32	3	0.004	**0.036**
T4	T5	4	4	9.08	3	0.003	**0.028**

S. Table 14. Wilcoxon rank sum test WB1 no-mussel treatment for IVCH

group1	group2	p
T1	T2	1
T1	T3	1
T1	T4	1
T1	T5	1
T2	T3	1
T2	T4	0.29
T2	T5	1
T3	T4	0.29
T3	T5	1
T4	T5	1

S. Table 15. Wilcoxon sum rank test WB2 mussel treatment for IVCH

group1	group2	p
T1	T2	1
T1	T3	1
T1	T4	0.29
T1	T5	0.29
T2	T3	1
T2	T4	1
T2	T5	0.29
T3	T4	1
T3	T5	0.29
T4	T5	0.29

S. Table 16. Pairwise t-test WB2 no-mussel treatment for IVCH

group1	group2	n1	n2	statistic	df	p	p.adj
T1	T2	4	4	-1.81	3	0.169	1
T1	T3	4	4	-0.412	3	0.708	1
T1	T4	4	4	-9.15	3	0.003	**0.028**
T1	T5	4	4	2.43	3	0.094	0.936
T2	T3	4	4	1.48	3	0.236	1
T2	T4	4	4	-1.8	3	0.17	1
T2	T5	4	4	4.24	3	0.024	0.24
T3	T4	4	4	-3.13	3	0.052	0.519
T3	T5	4	4	5.17	3	0.014	0.14
T4	T5	4	4	4.07	3	0.027	0.267

S. Table 17. Pairwise t-test for WB3, WB4, and WB5. Both treatments for IVCH

Exp	Treatment	group1	group2	n1	n2	statistic	df	p	p.adj
	Mussels	T1	T2	4	4	2.69	3	0.075	0.747
		T1	T3	4	4	3.64	3	0.036	0.358
		T1	T4	4	4	3.87	3	0.03	0.305
		T1	T5	4	4	13.3	3	0.000923	**0.009**
		T2	T3	4	4	0.834	3	0.465	1
		T2	T4	4	4	1.54	3	0.22	1
		T2	T5	4	4	6.68	3	0.007	0.068

Exp	Treatment	group1	group2	n1	n2	statistic	df	p	p.adj
WB3		T3	T4	4	4	0.798	3	0.483	1
		T3	T5	4	4	5.1	3	0.015	0.146
		T4	T5	4	4	5.37	3	0.013	0.126
	No mussels	T1	T2	4	4	-1.06	3	0.367	1
		T1	T3	4	4	-2.1	3	0.127	1
		T1	T4	4	4	-0.296	3	0.787	1
		T1	T5	4	4	0.375	3	0.732	1
		T2	T3	4	4	-1.23	3	0.305	1
		T2	T4	4	4	1.62	3	0.204	1
		T2	T5	4	4	1.27	3	0.292	1
		T3	T4	4	4	1.82	3	0.166	1
		T3	T5	4	4	2.16	3	0.12	1
		T4	T5	4	4	0.635	3	0.571	1
WB4	Mussels	T1	T2	4	4	3.2	3	0.049	0.492
		T1	T3	4	4	4.24	3	0.024	0.24
		T1	T4	4	4	5.4	3	0.013	0.125
		T1	T5	4	4	14.5	3	0.000713	**0.007**
		T2	T3	4	4	2.22	3	0.113	1
		T2	T4	4	4	7.03	3	0.006	0.059
		T2	T5	4	4	10.5	3	0.002	**0.019**
		T3	T4	4	4	4.58	3	0.02	0.196
		T3	T5	4	4	9.68	3	0.002	**0.023**
		T4	T5	4	4	3.65	3	0.035	0.355
	No mussels	T1	T2	4	4	0.486	3	0.66	1
		T1	T3	4	4	0.824	3	0.471	1
		T1	T4	4	4	-2.06	3	0.132	1
		T1	T5	4	4	0.346	3	0.752	1
		T2	T3	4	4	0.437	3	0.692	1
		T2	T4	4	4	-15	3	0.000639	**0.006**
		T2	T5	4	4	0.218	3	0.841	1
		T3	T4	4	4	-5.06	3	0.015	0.149
		T3	T5	4	4	0.0944	3	0.931	1
		T4	T5	4	4	1.62	3	0.203	1
WB5	Mussels	T1	T2	4	4	8.23	3	0.004	**0.038**
		T1	T3	4	4	14.1	3	0.000781	**0.008**

Exp	Treatment	group1	group2	n1	n2	statistic	df	p	p.adj
		T1	T4	4	4	18.4	3	0.000351	**0.004**
		T1	T5	4	4	50.3	3	0.0000173	**0.000173**
		T2	T3	4	4	12.1	3	0.001	**0.012**
		T2	T4	4	4	4.9	3	0.016	0.163
		T2	T5	4	4	5.71	3	0.011	0.107
		T3	T4	4	4	1.92	3	0.151	1
		T3	T5	4	4	3.81	3	0.032	0.317
		T4	T5	4	4	3.37	3	0.044	0.435
	No mussels	T1	T2	4	4	-0.177	3	0.871	1
		T1	T3	4	4	-0.865	3	0.45	1
		T1	T4	4	4	-2.06	3	0.131	1
		T1	T5	4	4	1.68	3	0.192	1
		T2	T3	4	4	-0.404	3	0.713	1
		T2	T4	4	4	-0.705	3	0.532	1
		T2	T5	4	4	2.81	3	0.067	0.674
		T3	T4	4	4	-1.09	3	0.354	1
		T3	T5	4	4	2.71	3	0.073	0.731
		T4	T5	4	4	2.87	3	0.064	0.64

S. Table 18. WB3 cell count repeated measures ANOVA results for cell counts

Experiment	Treatment	group 1	group 2	n 1	n 2	statistic	df	p	p.adj
WB3	Mussels	T1	T4	4	4	0.565	3	0.612	0
		T1	T5	4	4	3.19	3	0.05	0.149
		T4	T5	4	4	4.19	3	0.025	0.074
	No Mussels	T1	T4	4	4	7.92	3	0.004	**0.013**
		T1	T5	4	4	2.09	3	0.128	0.384
		T4	T5	4	4	0.66	3	0.557	1

S. Table 19. Wilcoxon rank sum results WEB4 and WB5 cell counts

Experiment	Treatment	group1	group2	p-value
WB4	Mussels	T1	T4	0.886
		T1	T5	0.086
		T4	T5	0.086
	No Mussels	T1	T4	0.17
		T1	T5	0.93
		T4	T5	0.57
WB5	Mussels	T1	T4	0.086
		T1	T5	0.088
		T4	T5	0.177
	No Mussels	T1	T4	1
		T1	T5	0.8
		T4	T5	0.3

S. Table 20. Pairwise comparison (repeated measures ANOVA) for carbon WB1

Treatment	group1	group2	n1	n2	statistic	df	p	p.adj
Mussel	T1	T4	4	4	-0.628	3	0.575	1
	T1	T5	4	4	1.58	3	0.212	0.636
	T4	T5	4	4	2.39	3	0.096	0.289
No mussel	T1	T4	4	4	-1.47	3	0.239	0.717
	T1	T5	4	4	-0.407	3	0.712	1
	T4	T5	4	4	0.861	3	0.452	1

S. Table 21. Wilcoxon rank sum test for WB1 and WB4 for nitrogen

Experiment	Treatment	group1	group2	p-value
WB1	Mussel	T1	T4	0.93
		T1	T5	1
		T4	T5	1
WB4	No Mussel	T1	T4	1
		T1	T5	1
		T4	T5	0.34

S. Table 22. Pairwise t-test for Nitrogen levels

Experiment	Treatment	group 1	group 2	n1	n2	statistic	df	p	p.adj
WB1	No Mussel	T1	T4	4	4	-1.99	3	0.14	0.42
		T1	T5	4	4	-0.99	3	0.395	1
		T4	T5	4	4	0.535	3	0.63	1
WB3	Mussel	T1	T4	4	4	3.01	3	0.057	0.171
		T1	T5	4	4	7.22	3	0.005	**0.016**
		T4	T5	4	4	0.61	3	0.585	1
	No mussel	T1	T4	4	4	0.0734	3	0.946	1
		T1	T5	4	4	-0.468	3	0.672	1
		T4	T5	4	4	-2.1	3	0.126	0.378
WB4	Mussel	T1	T4	4	4	2.51	3	0.087	0.261
		T1	T5	4	4	13.4	3	0.000891	**0.003**
		T4	T5	4	4	4.28	3	0.024	0.071

S. Table 23. Pairwise t-test for WB1 for chlorophyll

Experiment	Treatment	group 1	group 2	n1	n2	statistic	df	p	p.adj
WB1	Mussel	T1	T4	4	4	-0.248	3	0.82	1
		T1	T5	4	4	6.57	3	0.007	**0.022**
		T4	T5	4	4	2.82	3	0.066	**0.2**
	No mussel	T1	T4	4	4	0.0734	-2.35	3	0.101
		T1	T5	4	4	-0.468	0.956	3	0.409
		T4	T5	4	4	-2.1	2.67	3	0.075

Chapter 4: Findings and Future Directions

Urban estuaries are plagued by excess nutrients that contribute to algae blooms and hypoxic and anoxic waters. Especially in places like Baltimore Harbor, algae blooms are intense and frequent. While efforts to reduce nutrients from point sources are ongoing, nature-based solutions may provide an opportunity for in-water removal.

Nature-based solutions, in this case filter-feeding bivalves, provide a natural mechanism to remove nutrients from the waterway. In most of the Chesapeake Bay, the Eastern oyster, *Crassostrea virginica,* has served as the nature-based solution for nutrient and algae mitigation as well as providing a fishery product and habitat. For highly modified and contaminated urban estuaries without hard bottom for oyster culture, and where any bivalve product would be not edible, the dark false mussel, *Mytilopsis leucophaeata,* is an attractive alternative to mitigate eutrophication and algae blooms. These mussels are very low cost because they naturally recruit to virtually any solid substrates in the water, meaning that there is no need for aquaculture to grow bivalve seed. Similar to systems for growing other alternate bivalves – ribbed mussels or edible mussels – engineered surfaces for growing dark false mussels can be as simple as nylon straps or PVC pipes (Galimany et al. 2017).

Although dark false mussels have the fundamental characteristics to be an economical and practical bivalve solution to nutrient and algae removal, there are many details to be understood and measured to move this potential best management practice towards implementation. In this chapter, I review the findings from my project, suggest

future research areas, and discuss the implications the data has for use of the dark false mussel and other natural suspension feeders for nitrogen management in the Chesapeake Bay.

Findings:

The results described in Chapters 2 and 3 of this thesis show that *M. leucophaeata* can reduce algae concentrations. In studies with lab-cultured algae, IVCH levels and chlorophyll levels decreased in the presence of mussels, and time had a significant effect on IVCH for all mussel treatments (Table 40). I found that the clearance of phytoplankton was resilient to changes in salinity but responded to temperature differences. The clearance rate at 10˚C was significantly lower than the clearance rate at 30˚C. Spring and fall temperatures in Baltimore Harbor are typically around 10˚C – 15˚C and summer temperatures range from about 17˚C – 30˚C. Harbor temperatures only drop below 10˚C from December to February. Summer is typically the time when algae blooms occur consistently in Baltimore Harbor, although there are occasional winter bloom events. Overall if the clearance rates of *M. leucophaeata* in Baltimore Harbor are similar to what I measured in the lab then we can expect that the uptake of phytoplankton by these mussels to be the highest when the blooms are most frequent in the summer.

Table 40. Summary of results from Chapter 2: Was there a significant effect of treatment or time?

Experiment	Data	Treatment	Time (Mussel/ No-mussel treatment)
Experiment 1 - *Isochrysis*	IVCH	Yes	Yes/Yes
	Chlorophyll	Yes	Yes/No
Experiment 1 - *Chaetoceros*	IVCH	Yes	Yes/No
	Chlorophyll	Yes	Yes/No
Temperature - Trial 1	IVCH	Yes	Yes/No
	Counts	-	Yes/No
Temperature - Trial 2	IVCH	Yes	Yes/No
	Counts	-	Yes/Yes
Salinity Trial - 1	IVCH	Yes	Yes/Yes
	Counts	-	Yes/Yes
Salinity Trial - 2	IVCH	Yes	Yes/No
	Counts	-	Yes/No

In addition to clearing lab cultured algae, *M. leucophaeata* can reduce wild algae concentrations. Compared to the experiments with cultured algae, there was much more variation in the IVCH, cell count, carbon, nitrogen, and extracted chlorophyll results (Table 41). In general, most of the experiments showed mussels reducing one or more of these metrics. The WB1 experiment did not demonstrate a significant effect of mussels by any

metric, and the WB3 experiment only demonstrated a significant effect of mussels on IVCH levels. These two experiments also had the highest starting IVCH levels and had high variance among replicates. These results suggest that there may be an effect of the algae bloom composition on the mussel's ability to clear the water. This is consistent with research on a better-known small mussel, the zebra mussel. In freshwater systems, the zebra mussel can reduce plankton and shows a preference for algae species between 7 and 50 microns (Fahnenstiel et al. 1995; Naddafi, Pettersson, and Eklöv 2007).

Table 41. Summary table for Chapter 3. Was there a significant effect of treatment or time on measured parameter?

Experiment	Data Parameters	Treatment	Time (Mussel/No-mussel treatment)
WB1	IVCH	No	Yes/No
	Cell Counts	-	-
	Carbon	No	No/No
	Nitrogen	No	No/No
	Total Extracted Chlorophyll	No	Yes/Yes
WB2	IVCH	Yes	Yes/Yes
	Cell Counts	-	-
	Carbon	Yes	Yes/Yes
	Nitrogen	Yes	Yes/Yes
	Total Extracted Chlorophyll	No	Yes/No
WB3	IVCH	Yes	Yes/No
	Cell Counts	No	Yes/No
	Carbon	No	Yes/No
	Nitrogen	No	Yes/No
	Total Extracted Chlorophyll	No	No/No
WB4	IVCH	Yes	Yes/No
	Cell Counts	Yes	Yes/Yes
	Carbon	Yes	Yes/No
	Nitrogen	Yes	Yes/No
	Total Extracted Chlorophyll	Yes	Yes/No
WB5	IVCH	Yes	Yes/No
	Cell Counts	Yes	Yes/No
	Carbon	No	Yes/No
	Nitrogen	No	Yes/No
	Total Extracted Chlorophyll	Yes	Yes/No

In summary, *M. leucophaeata* can provide a viable solution for mitigating algae blooms in Baltimore Harbor. However, more research must be conducted before implementing these mussels as a best management strategy. Below, I expand on possible reasons why the wild bloom experiments had varying results, suggest future studies, and discuss the implications of this work for a best management practice (BMP) for nutrient management.

Environmental Considerations:

Preliminary results from the summer natural algae bloom experiments show evidence of selection by the mussels as to the types of phytoplankton they consume. A FlowCam retrieved images of the algae to obtain cell counts from the wild bloom experiment. Based visual examination of images at the first time point versus the last time point, it seemed that there was an increase in the larger particles and a decrease in smaller diatoms (personal observations). These larger particles were identified as potentially *Akashiwo,* which matches with previous research and is a known algae bloom species in Baltimore Harbor. Future work should examine the mussels' ability to reduce these species from a monoculture in addition to other algae species commonly found in Baltimore Harbor. Earlier preliminary work at IMET used a qPCR assay to quantify two algae species in the harbor water and in the dark false mussel stomachs at the same time points. It was seen that *Prorocentrum minimum* (10-20 microns) was present in a much higher abundance in the stomachs of the mussel than its proportion in the water. *Akashiwo sanguinea* (50-60 microns) was the second species assayed, and there was a lower signal for *A. sanguinea* in

the mussel stomach than was seen in the water. Both of these bloom species have potentially harmful effects for other life. *P. minimum* blooms dive anoxia through the decomposition of senescent blooms, while *A. sanguinea* is reported to produce surfactant-like compounds harmful to birds (Jones et al. 2017).

Future Studies:

For *M. leucophaeata* to be used for algae and nutrient removal, the drivers of recruitment of the mussels to substrates needs to be further understood. The National Aquarium's 7-year video archive of sessile community data shows juvenile mussels recruiting to acrylic "biodiscs" in the spring and fall. Lab spawning experiments show that juvenile mussels reach about 2.6 mm after 30 days post fertilization (Kennedy 2011). *M. leucophaeata* appears to prefer low salinities and populations tend to increase during periods with high rainfall (Bergstrom et al. 2010). In years with lower rainfall, recruitment may be reduced and thus the algae and nutrient removal would be lower. A solution for this would be to obtain an algae and nutrient removal clearance rate for the entire biofouling community.

The National Aquarium's 7-year data set shows a diverse set of biofouling organisms in Baltimore Harbor. These organisms inhabit the same environment where the mussels occur and compete for food and habitat space. However, the clearance rates of these communities are yet to be studied. Preliminary lab experiments have shown that barnacles can reduce the IVCH of cultured algae species in a similar manner to the mussels. Challenges related to experimentation with barnacles or bryozoans make obtaining

clearance rates for the individual species difficult. Unlike Dark False Mussels, which could be removed from their original structure and attach to a new structure with new byssal threads, removing barnacles from their original surface risks damaging the animal and positioning barnacles to stay upright proved challenging (personal observation). The preliminary experiment I conducted with barnacles was performed with biodiscs that had experienced anoxia in the harbor before being transferred to a recirculating aquaculture tank for two months. During this time much of the other biofouling died off, leaving only the barnacles. Mixed-species community level clearance rates can still be obtained by using biodics with the biofouling community and measuring phytoplankton removal. The in-situ representation of each specific species can be approximated through similar methods used by the National Aquarium to understand which species are most likely driving the change. I believe that a community level clearance rate would provide the most realistic estimation of algae and nutrient removal from the water for the purpose of modeling the potential of this approach to provide ecosystem services.

There are additional ecosystem services to explore in this system such as habitat formation, bacterial reduction, and nutrient removal. To increase the abundance of mussels, and other biofouling organisms, structures must be created for recruiting the organisms. Prototype structures consist of wooden frame with bamboo poles suspended across and can be altered to match the environment they are placed (Figure 22A). The bamboo poles have nylon straps hanging that provide the surface for organisms to recruit to (Figure 22B). The structures would be placed out in late March or early April to grow. In November, all the

material that has grown can be removed for composting. These structures can create habitat areas, which is lacking in Baltimore Harbor. Underwater videos show fish and crabs interacting with the growing platforms suggesting that the structure can provide shallow habitat (personal communication – J. Diaz). Future studies should examine the habitat potential for fish and other larger aquatic organisms in Baltimore Harbor.

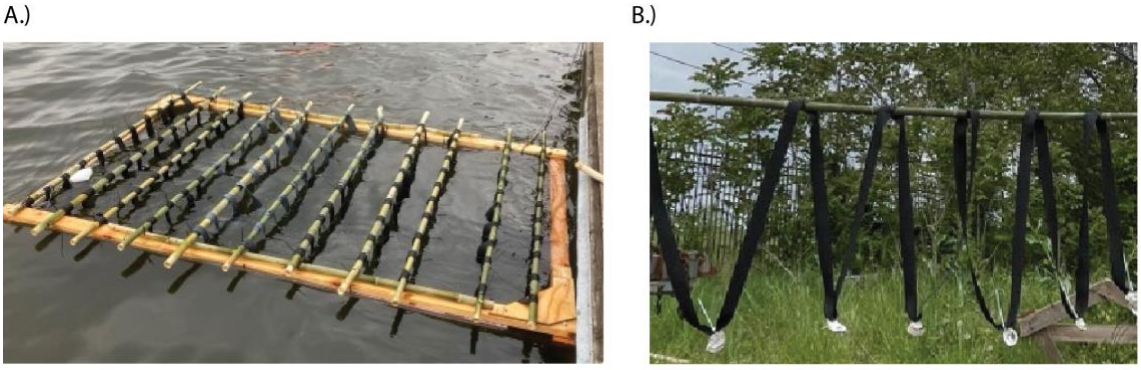

Figure 23. Figure of A) raft structure, and B) nylon straps for biofouling recruitment

Additional concerns in Baltimore Harbor revolve around bacteria contamination. Studies show that oysters can accumulate harmful bacteria like *Vibrio vulnificus* and *V. parahaemolyticus* (Froelich and Noble 2016). While this is not a desired trait in aquaculture settings, non-commercial and non-edible species, such as *M. leucophaeata*, may prove to be another option for bacteria removal. Neves et al., (2020) found a significant decrease in total coliform bacteria after *M. leucophaeata* introduction. Bacteria removal or amplification by these communities should also be considered in a BMP for this service.

Nutrient Removal Best Management Practices:

Nutrients in the Chesapeake Bay have been monitored and regulated after the creation of the Clean Water Act. Excess nutrients in the water lead to a decrease in submerged aquatic vegetation and abundance of hypoxic and anoxic zones (Lefcheck et al. 2018; Cornwell et al. 2016). These events can then lead to the decrease in other key species in the Chesapeake Bay. Efforts to reduce nutrients focus on limiting inputs from point sources like wastewater treatment plants (WWTP) and nonpoint sources like urban stormwater and agriculture.

In Baltimore, stormwater runoff is regulated by a National Discharge Elimination System Municipal Separate Storm Sewer System (MS4) permit. Through this permit, Baltimore City must take steps to reduce the amount of nutrients entering the bay from their drainage systems. Some items in the most recent iteration of this permit include street sweeping and inlet cleaning, stormwater best management practices (BMPs), litter and debris removal, and additional monitoring and outreach (Maryland Department of the Environment 2021).

For municipalities with a regulatory obligation to reduce their nutrient inputs into the bay, nutrient credits could be an appealing option in addition to their permit action items. My research shows that *M. leucophaeata* can reduce the algae levels that represents as nitrogen and carbon levels. On average 20 mussels can clear about 0.70 mg/L of nitrogen within 24 hours. Theoretically, within a day if there are 20,000 mussels growing on a substrate, then about 700 mg of nitrogen will be removed from the water column by the

mussels. These are in-water removal levels, and the nutrients are most likely being included in the mussel tissues and shell as it grows as well as being sent to the sediment layer as biodeposits (feces). Upon removal of the mussels, the nutrients are also removed. The total nitrogen in the biofouling material still needs to be assessed and future work should start by calculating the nutrient removal by the biofouling community. Additionally, within the biofouling material there is a denitrification at the microbial level that is further reducing nitrogen from the water (Schott, unpublished data). Nutrient removal through biofouling harvest and denitrification while in the water may provide cities with MS4 permits an additional option to meet their nutrient reduction quota.

In Maryland there is a system for oyster nutrient credit trading where oyster growers can get paid for the nutrients removed from the water by their oyster harvest. Businesses and municipalities with permits for nutrient reduction buy the nutrient credits from oyster harvest to help meet their permit requirements (NOAA Office for Coastal Management, n.d.). For some of these groups, this can be a more cost-effective way to meet their permit goals as other infrastructure improvements for nutrient reduction can be costly. Additionally in water denitrification occurring on oyster reefs is also being considered for credits (Rose et al. 2021; DePiper, Lipton, and Lipcius 2017). With a framework in place for nutrient credits, *M. leucophaeata* may provide an additional nutrient credit for trading and help with the eutrophication in Baltimore Harbor.

My research shows that *M. leucophaeata* can provide valuable algae and nitrogen reduction ecosystem services. Since dark false mussels do not grow in isolation from other

species, future research should examine the clearance rates of the entire biofouling community and the nitrogen reduction through feeding. Additionally, more studies should be conducted using algae species commonly found in Baltimore Harbor to better understand phytoplankton reduction by the biofouling community. Overall, my research provides an initial step to create a nutrient removal best management practice for this ecosystem service in Baltimore Harbor and similar estuaries.

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