THESIS APPROVAL SHEET

Title of Thesis: Oyster gardening in the Baltimore Harbor: Quantifying oyster growth & influencing factors

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Graduate Program: Marine Estuarine Environmental Science

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Jessica Diaz

EDUCATION

M.Sc. Marine, Estuarine, & Environmental Sciences (May 2023)
University of Maryland, Baltimore County (UMBC); Baltimore, MD
Thesis: Oyster Gardening in the Baltimore Harbor: Quantifying oyster growth & influencing factors

B.S. Fisheries & Wildlife (April 2021)
Michigan State University (MSU); East Lansing, MI
Minors: Science, Technology, Environment, & Public Policy; Music

RESEARCH EXPERIENCE

UMBC Interdisciplinary Consortium for Applied Research in the Environment (ICARE) Traineeship
(Aug. 2021 – Present); Baltimore, MD
Trainee

- Co-create research with the Environmental Justice Journalism Initiative & other South Baltimore community partners on restoration in the Baltimore Harbor
- Participate in cohort-based weekly discussions and semester courses on aspects of justice, equity, diversity, and inclusion in the environmental field

MSU Data-Intensive Landscape Limnology Lab (Oct. 2018 – Aug. 2021); East Lansing, MI
Research Assistant; *Research Experience for Undergraduates (REU) Student (*May – Aug. 2020)

- Developed a research project (under review for publication) assessing the equity of lake monitoring in different racial and ethnic communities in the US
- Contributed to LAGOS-NE & LAGOS-US databases of lake characteristics in the conterminous U.S.
- Conducted background literature review, data analysis (RStudio), spatial modeling (GIS), and documentation for various projects

Northern Arizona University Doris Duke Conservation Scholars Program (June 2018 – Jan. 2020); Flagstaff, AZ
Doris Duke Conservation Scholar

- Participated in a two-year environmental leadership program and eight-week summer immersion experience focused on equitable and just conservation science and policy in the Southwestern U.S.
- Discussed conservation challenges with agencies, Indigenous groups, and conservation organizations

WORK EXPERIENCE

MSU Office of Undergraduate Research (March 2018 – Aug. 2021); East Lansing, MI
Undergraduate Assistant

- Managed a database of research funding distribution and undergraduate student research participation
- Helped plan and execute the annual UURAF and Mid-SURE research symposiums for up to 1000 presenters

The Wilderness Society (June – Aug. 2019); Bozeman, MT
Cultural Areas Protection & Tribal Outreach Intern

- Drafted legislation creating a process to identify and rename offensively named landmarks and public lands, igniting a campaign which helped encourage 2021 Secretarial Orders 3404 & 3405 on the issue
- Created and maintained a database of Indigenous organizations and leaders to guide outreach efforts

Texas Wildlife Rehabilitation Center (May – Aug. 2017); Houston, TX
Wildlife Intern

ORGANIZATIONS

Doris Duke Conservation Scholars (DDCSP) Alumni Network – Alumni Council (Aug. 2022 – Present)
Council Member

- Elected by the Alumni Network to assist with budget management, event planning, and community building for the Alumni Network
- Co-lead the regional meet-ups team in planning meaningful experiences focused on the environment for alumni

American Fisheries Society (Jan. 2020 – Present)
MI Chapter Student Subunit Co-President (Jan. 2020 - May 2021)

Sunrise Movement (Jan. 2019 – Present)

Hub Coach (Aug. 2021 - Present)*; Michigan State University Hub Leader* (Aug. 2019 - July 2021)

- Guide hub leaders in movement building, relational organizing, and action strategy for a stronger movement
- Created and distributed press releases in advance of several actions and served as group's spokesperson

Minorities in Agriculture, Natural Resources, and Related Sciences (Aug. 2019 – 2021); East Lansing, MI
Vice President (April 2020 - April 2021)*; President* (April 2019 - April 2020)

- Collaborated with administrative staff to promote diversity, equity, and inclusion practices
- Facilitated community spaces, networking opportunities, skills building workshops between marginalized students, faculty, and alumni

PUBLICATIONS (* denotes first author)

Díaz Vázquez, J.[*], McCullough, I.M., Haite, M., Soranno, P.A. & Cheruvelil, K.S. US lakes are monitored disproportionately less in communities of color. *(Under Review in Frontiers in Ecology and the Environment*).

Soranno, P.A.[*], Webster, K.E., Smith, N.J., **Díaz Vázquez, J**. and Cheruvelil, K.S. (2020). What Is in a "Lake" Name? That Which We Call a Lake by Any Other Name. L&O Bulletin 29:1-7.
**Selected as one of 18 outstanding articles published in 2020–21 ASLO journal portfolio and invited to present at a special session for the 2023 ASLO Aquatic Sciences Meeting*

McCullough, I.M.[*], King, K., Stachelek, J., **Diaz, J.,** Soranno, P.A. & Cheruvelil, K.S. (2019). Applying the patch-matrix model to lakes: a connectivity-based conservation framework. Landscape Ecology 34:2703–2718.

PRESENTATIONS (* denotes poster presentations, all others are oral presentation)

Díaz Vázquez, J. (2022). "Investigating oyster growth and survival in the Baltimore Harbor." Chesapeake Oyster Science Symposium. Virtual.

Díaz Vázquez, J, Haite, M., McCullough, I.M., Soranno, P.A. & Cheruvelil, K.S. (2022). "Environmental justice: Communities of color & Hispanic communities lack data on lake health." Joint Aquatic Sciences Meeting. Grand Rapids, MI.

Díaz Vázquez, J., Haite, M. (2021). "Trends in monitoring of lake water quality in different racial communities." MSU University Undergraduate Research & Arts Forum. East Lansing, MI.

McCullough, I.M., Cheruvelil, K.S., **Díaz, J.**, et al. (2020). "Data synthesis: No picnic, but no need to panic." Ecological Society of America annual meeting. Virtual.

***Díaz, J.**, Aslan, C.E. (2019). "Impact of Fire Management on the Kaibab Plateau Vegetation Community." MSU University Undergraduate Research & Arts Forum. East Lansing, MI.

AWARDS & ACCOMPLISHMENTS

UMBC Interdisciplinary Consortium for Applied Research in the Environment NSF Fellowship — Aug. 2021 – Present
MSU College of Agriculture & Natural Resources Outstanding Graduating Senior Finalist — April 2021
MSU CANR STEM-FEE Scholars Program — Aug. 2020 – April 2021
Doris Duke Conservation Scholar, Northern Arizona University — June 2018 – Jan. 2020
MSU College of Agriculture & Natural Resources Undergraduate Research Grant Program — Aug. – Dec. 2019
Michigan State University Dean's List — All Semesters Aug. 2017 – April 2021
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ABSTRACT

Title of Document:	OYSTER GARDENING IN THE BALTIMORE HARBOR: QUANTIFYING OYSTER GROWTH AND INFLUENCING FACTORS
	Jessica Diaz, M.S., 2023
Directed By:	Mercedes Burns, Assistant Professor, Biological Sciences, and Marine, Estuarine, and Environmental Sciences

In Baltimore, industrialization has degraded the ecosystem and displaced communities from the waterfront. Organizations like the Great Baltimore Oyster Partnership and the Environmental Justice Journalism Initiative aim to address these problems via restoration efforts. While oyster gardening has occurred in the Inner Harbor since 2013, the health and growth of the oysters has never been quantified, nor are any programs established in the Middle Branch. The goal of this study was to understand location-specific factors contributing to growth differences of the Eastern oyster (*Crassostrea virginica*). Oysters were deployed in the Harbor for 7 months and measurements for oyster growth and influencing factors collected. Oyster shell growth varied by site, but was not explained solely by temperature, salinity, and dissolved oxygen. Through phytoplankton metabarcoding, the preferred food sources of oysters were detected at all sites. These findings provide a baseline for quantifying oyster restoration initiatives in the Baltimore Harbor.

OYSTER GARDENING IN THE BALTIMORE HARBOR: QUANTIFYING OYSTER GROWTH AND INFLUENCING FACTORS.

By

Jessica Diaz.

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, Baltimore County, in partial fulfillment
of the requirements for the degree of
Master of Sciences
2023

Dedication

Para mi mama y papá, Bertha y Miguel Díaz, y mis ancestros, que por sus logros y esfuerzos estoy aquí. For my sisters, Angela & Rebeca, my extended family, and chosen family whose unconditional love and support made this degree a reality.

To my younger self and the brown, first-generation, children of immigrants who never believed they were good enough to be a scientist. I am worthy and I am a scientist.

Acknowledgements

I am deeply grateful to my advisor, Dr. Mercedes Burns, who took me into her lab unexpectedly and has been a wonderful mentor and role-model. My success in this program is largely due to her support; she was the advisor I did not know I needed. My committee and ICARE team also played a role in ensuring that I was equipped with the tools and plan to succeed in this short timeline. Thank you to Dr. Allison Colden for training me as an oyster researcher, answering all of my oyster questions, and being a mentor in my marine conservation career. Thank you to Dr. Louis Plough for his support as I navigated the world of metabarcoding and genomics and for being so enthusiastic about my research. While not on my committee, I am grateful to Jennifer Wolny for sharing her expertise on phytoplankton and helping me learn all about phytoplankton in the Chesapeake Bay. Thank you to Dr. Tamra Mendelson for joining my team calls and providing support to ensure I had the network, tools, and anything else I needed amid unexpected circumstances.

The ICARE program is the reason that I was able to conduct this research and for that I am forever grateful. Thank you to my ICARE cohort for providing a safe space to be a person of color in science, in the environment, and in academia. A special shoutout to Darryl Acker-Carter, my fellow oyster lover, who I truly leaned on for everything. I am so lucky to have gained a life-long friend and colleague as amazing as you. My other source of support in academia was the Burns Lab and I am always

in awe of your never-ending enthusiasm for all things science. Thank you to the 2021 – 2023 Burns undergraduate researchers and fellow graduate students for expanding my worldview and inspiring me to like arachnids so much more.

This research would not have been possible without the many partners, including Chesapeake Bay Foundation, the Great Baltimore Oyster Partnership, the Environmental Justice Journalism Initiative, the Lakeland Community Association Partnership & STEAM Center, and Solar Oysters, LLC. Thank you all for providing guidance, resources, a location to conduct my research, and more.

Gracias a mi familia por su apoyo y amor. No tengo muchas palabras, pero los amo mucho y sin ustedes no estaría aquí. Thank you to my family for their unconditional love. I might not have many words, but I love you so much and I could not have done this without you. To my partner, Cameron, I am so appreciative and thankful of you for your support of me, from helping me with field work to letting me talk through my research problems, I love you.

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The Eastern oyster (*Crassostrea virginica*) is a keystone species of the Chesapeake Bay (Raj 2008). Oysters promote healthy ecosystems by filtering the water column, forming reefs that provide habitat for other species, and stabilizing shorelines to prevent erosion (Grabowski et al. 2012, Newell 2004). Although oysters thrived in the Chesapeake Bay through the 1800s, anthropogenic activities, such as overfishing, pollution, and the introduction of diseases, brought a large decline of the oyster population (Rick et al 2016, Rothschild et al 1994). Oyster restoration efforts in the Bay have shown success in restoring the services that oysters provide (Bruce et al. 2021). However, highly urbanized areas like the Baltimore Harbor are often overlooked when implementing any environmental restoration initiatives.

1. Oyster Life History

The Eastern oyster is a filter-feeding bivalve mollusk. Adult oysters in the Chesapeake Bay and Mid-Atlantic will typically spawn around the spring and summer (Galtsoff 1964). As broadcast spawners, their eggs are fertilized in the water column rather than inside the organism. The larvae are free-swimming, pelagic, and undergo three stages over 2 - 3 weeks: trochophore, veliger, and pediveliger (Galtsoff 1964). During the pediveliger stage, the larvae develop a foot that facilitates

movement for finding suitable substrate. Once attached and metamorphosed, the larvae become spat or juvenile oysters, transitioning from pelagic and free-swimming existence to a benthic and sessile one. It can take juvenile oysters from 1 – 3 years to grow into adults and then the cycle begins again. Various factors including temperature, oxygen, and phytoplankton abundance influence spawning and settlement (Nelson 1928, Loosanoff and Engle 1940). This repeated settlement of oysters on other oysters and their continued growth over decades creates the 3-D structure of reefs that is observed in near shore environments (Woods et al. 2005).

The oyster shell has a lower valve that is cup-shaped and deeper than the top shell, which is relatively flat or curved. The two valves come together at the hinge axis or umbo. The umbo is also the origin of the shell and from where it grows outward. The wider end opposite the umbo is the bill, which will open slightly for feeding. The dimensions of oysters, and bivalves generally, are measured using height, length, and width (Figure 1, Galtsoff 1964). Height is the distance from the umbo to the bill. Length is perpendicular to height and the greatest distance from the anterior to posterior shell edge. Lastly, width is the maximum thickness of the closed valves.

The native geographical range of the Eastern oyster is from Canada down the East coast of the United States to the Gulf of Mexico (Carriker and Gaffney 1996). This means that oysters can live in and tolerate a wide range of conditions. Salinity,

temperature, oxygen, and food supply are the factors predominantly impacting oysters. Oysters are typically found in salty waters ranging from 5 – 40 ppt, but the optimal salinity range is 14 – 28 ppt. However, oysters can survive for short periods of time in conditions less than 1 ppt (Shumway 1996). The optimal temperature range for growth is between 3 – 20 ℃, although oysters can tolerate between -2 – 30 ℃ (Galtsoff 1964, Marshall et al. 2021). At higher temperatures, the oyster's metabolic rate is increased so its oxygen and energy requirements also increase (Gosling 2003). Although oxygen consumption is dependent on the temperature and salinity conditions, oysters are able to use the available oxygen in all conditions to maintain an energy gain (Shumway 1996). The minimum dissolved oxygen requirement for oyster survival is 2 mg/l, however, oysters often experience waters less than 2 mg/l for short periods of time in the Chesapeake Bay (Kennedy & Breisch 2001).

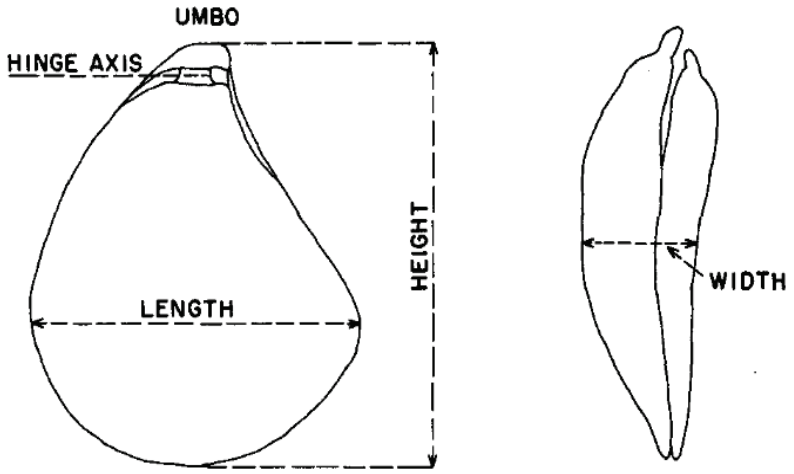

Figure 1. Measurement of height, length, and width for oyster shells (Galtsoff 1964)

2. Oyster Food Source: Phytoplankton

Temperature, salinity, and dissolved oxygen are also important factors in the food source of oysters, which is primarily phytoplankton. Since oysters intake all the particles in the water, including sediment and contaminants, they are able to efficiently select those particles that are nutritious from those that are not. The nutritious particles are digested while the rest are excreted as pseudofeces (Gosling 2004). Studies have shown that the preferred food source of oysters are eukaryotic phytoplankton, like diatoms and dinoflagellates, while prokaryotic phytoplankton, like cyanobacteria, are not utilized (Davis 1953, Langdon & Newell 1990, Martin 1923). High biomasses of phytoplankton occur during the spring and summer months

when temperature is high and stay very low during the winter months when temperatures are also low (Langdon & Newell 1990). This coincides with the seasonal energy and nutrient demands of oysters; however, the phytoplankton community biomass and composition can quickly change due to factors like heavy rainfall that lowers salinity or algal blooms that deplete the dissolved oxygen.

3. Historical Importance of Oysters in the Chesapeake Bay

Long before European colonists settled on present-day North America, the Eastern oyster was central to the culture of Indigenous Peoples of the Chesapeake Bay (Reeder-Myers et al. 2022, Rick et al. 2016). The Kecoughtan, Algonquians, and Powhatan all collected oysters for raw eating, smoking and drying, and for trading. In Maryland, archaeological sites along rivers, including the Rhode and Patuxent Rivers, have been documented with anywhere from 2 – 40 million oysters dating from 1250 – 950 years ago (Reeder-Myers et al., 2022). Contrary to most theories, Indigenous people of the Chesapeake Bay maintained a range of practices, including large-scale harvesting, that supported a sustainable oyster population (Rick et al. 2016). With the arrival of Europeans, the oyster population in the Chesapeake Bay quickly declined and is now estimated to be about 1% of the historic population (Wilberg et al., 2011).

A combination of overharvesting, habitat loss and degradation, and disease have contributed to the population decline. Harvest has been the main proxy for quantifying oyster populations, with harvests reaching a high of 15 million bushels in Maryland during the late 1800s (Kennedy and Breisch 1983, Kirby 2004). This largely coincided with the development of new technologies for harvesting oysters in the late 1800s to early 1900s, mainly oyster dredges and patent tongs (Rothschild et al. 1994). Compounding this was the habitat destruction caused by those practices through reducing natural shell replacement and increasing siltation (Jackson et al 2001, Wilberg et al. 2011). Shells were also taken for non-consumption purposes; for example, to make by-products, including as crop fertilizer and to build roads (Hargis and Haven 1999). A more recent stressor is the MSX and Dermo diseases discovered in the late 20th century. Although these came into play after the major population decline, they thrive in higher salinity and are highly infectious and deadly, further reducing the resilience of the oyster population (Ford & Trip 1996, Paynter 1991, Wilberg et al. 2011).

The social pressures on the industry were also mirrored in the decline of oyster harvest. In the early to mid 1800s, New England businessmen traveled down to the Chesapeake Bay in search of a new area to establish the oyster industry after depleting the oyster beds of New England (Botwick and McClane 2005). The

commercial oyster fishery quickly developed throughout the Chesapeake Bay with Baltimore becoming the hub for packing and canning. Thousands of Baltimoreans were employed at the facilities which canned oysters during the winter and fruit and vegetables during the summer (Mackenzie 2007). However, in the early 1900s demand for oysters heavily slowed due to pollution and disease scares as well as competition by other affordable foods and widespread economic depressions. The decline of the oyster population also had numerous ecological implications, many which were not quantified due to the focus on harvest rather than ecosystem services (Coen et al. 2007).

4. Oyster Restoration: Options & Opportunities

Restoring oyster reefs in Chesapeake Bay tributaries is a step towards restoring the complex ecosystem of the Bay due to their various ecosystem services (Newell 2004). Oysters and oyster reefs can reduce nitrogen and phosphorus pollution, which currently contributes to harmful algal blooms and hypoxic events that often result in die-offs of aquatic life (Kellog et al 2018). Reefs also promote biodiversity by supporting a wide variety of organisms including juvenile fish and crabs. Under climate change, reefs are especially important as they protect the coastline from erosion and storm surges (Kellog et al 2018).

Urban oyster restoration initiatives improve water quality for a healthier ecosystem while connecting people to their waters. This is evident in various initiatives already underway in the Chesapeake Bay and Atlantic Coast. Lafayette River, an urban waterway in Norfolk, Virginia now hosts Virginia's first large-scale oyster restoration tributary considered "restored", which was found to support higher macrofauna density and diversity, and more diverse fish species while improving water clarity and nitrogen loads (Bruce et al. 2021). In the New York Harbor (New York, NY), the Billion Oyster Project has demonstrated that oysters can grow and survive in permanently deployed restoration structures for over a decade (McCann 2019). In recent years, the Billion Oyster Project observed naturally occurring spat in locations along the Hudson River, Jamaica Bay, and Bronx River, an indication of success of those restoration efforts. Oysters in the New York Harbor are also known to bioaccumulate mercury (Kim et al 2017) which can benefit water quality long-term by removing heavy metal pollutants. The Billion Oyster Project also employs a unique multi-level engagement strategy where K-12 teachers and students, summer interns, volunteers, and a team of scientists all participate in the daily and long-term activities. These success stories in urban areas provide a framework for implementing oyster restoration initiatives in the Baltimore Harbor.

Common restoration methods in Maryland and the Chesapeake Bay include habitat enhancement, seeding, and off-bottom farming. Habitat enhancement involves building and maintaining reefs with natural or artificial substrates where larvae can settle (Kennedy et al 2011). Seed (spat) that is harvested from existing reefs or produced in hatcheries is transplanted or planted to areas where survival is expected to be high (Kennedy et al 2011). Off-bottom (floating or suspended) growing systems, adapted from small-scale aquaculture techniques, are utilized in oyster gardening to grow mature oysters for reef planting and to engage the public (Brumbaugh and Coen 2009). Most common in the Chesapeake Bay and Mid-Atlantic region, oyster gardening programs supply spat-on-shell (spat settled on recycled shells) to volunteer gardeners who grow oysters in Taylor floats or suspended cages for about 8 – 9 months (Brumbaugh and Coen 2009, Goldsborough and Meritt 2001). The short timeline reflects the primary goal of growing oysters in cages, which is to reduce mortality after reef planting by growing oysters to a larger size before planting occurs. Oyster gardening in Maryland is successful through the partnerships between the Maryland Department of Natural Resources, Oyster Recovery Partnership, Chesapeake Bay Foundation (CBF), and University of Maryland's Horn Point Hatchery that coordinate permits, seed production, transportation of oysters to gardeners and reefs, and establishment of gardening programs.

5. Motivations: Why Baltimore?

The Baltimore Harbor has historically benefited from a high oyster population in the Bay. In the 1800s it became a hub for the oyster industry, providing many jobs in packaging, shipping, local markets, and beyond. However, the Baltimore Harbor never hosted oyster reefs and the closest documented oyster bars were near Sparrows Point and further out near the mouth of the Patapsco River (Cumming 1916). Ultimately, the Baltimore industry shut down with lowered economic demand and oyster landings (harvest) in the early 20th century. The purpose of establishing oyster restoration initiatives in the Baltimore Harbor is for (re)establishing critical ecosystem services in the upper Chesapeake Bay and to (re)connect Baltimoreans to their waterways, rather than the reestablishment of a historic oyster population.

The Baltimore harbor is created by the Patapsco River and has two main branches, the Northwest Branch and the Middle Branch. Currently, oyster restoration efforts in Baltimore are in the Northwest Branch and led by the Great Baltimore Oyster Partnership. Launched in 2013 by the CBF and the Waterfront Partnership of Baltimore, the focus of this partnership is to connect Baltimoreans to the water while supporting oyster restoration. The timeline follows typical oyster gardening timelines where new spat-on-shell oysters are deployed in the Harbor every fall. In the spring, after about 8 months, they are transported to a reef, in this case, the Fort Carroll

sanctuary reef. During that time, limited data is collected on oyster growth and survival because it is primarily a volunteer program with an objective of cleaning the oyster cages to minimize biofouling. To date, there is no initiative focused on maintaining oysters year-round in the Baltimore Harbor for a dual restoration and community-engagement purpose.

The Middle Branch of the Patapsco River is of particular interest for restoration because of the Reimagine the Middle Branch initiative to transform this forgotten waterfront into a thriving place for outdoor recreation, environmental value, and economic development. Unlike the Northwest Branch, there is minimal to no investment in the Middle Branch for environmental restoration and engagement. The Environmental Justice Journalism Initiative (EJJI), in partnership with Reimagine the Middle Branch, aims to change the narrative around the Middle Branch by developing a central hub on the waterfront for environmental justice storytelling, youth leadership development, and environmental science. The goals of Reimagine the Middle Branch, EJJI, and CBF serve as the motivation for this thesis.

The purpose of this thesis is to quantify oyster growth and the factors influencing oyster growth in the Baltimore Harbor to fill the data gaps present in current oyster gardening initiatives in the Northwest Branch and to explore the possibility of oyster restoration initiatives in the Middle Branch. In chapter 2, I detail a 7-month study of

oysters at four locations within the Baltimore Harbor and the abiotic and biotic factors potentially affecting their growth, mortality, and condition. In chapter 3, I conclude by summarizing findings and providing considerations for current oyster restoration efforts in the Baltimore Harbor and for developing restoration efforts in the Middle Branch.

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Chapter 2: Investigating oyster growth geographical variation & influencing factors

Section 1. Introduction

The Baltimore Harbor is an urban estuary in Baltimore, Maryland fed by the Patapsco River. It is composed of two main tributaries: the Middle Branch and the Northwest Branch. As a result of its northern location in the Chesapeake Bay and the freshwater input from the Patapsco River, it is a mesohaline environment (salinity 5 – 18 ppt; STAC 2014). Despite being on the lower salinity range for the Eastern oyster, the Great Baltimore Oyster Partnership has successfully grown juvenile oysters in the Baltimore Harbor through oyster gardening. 'Oyster gardening' is a volunteer-led program in which spat-on-shell oysters are kept in cages and maintained clean until the oysters are about 10 – 12 months old and can be planted to a protected reef.

Growth metrics and mortality are unknown for oysters in the Baltimore Harbor. For this study oyster growth metrics include growth rate, shell height, and condition index. Growth rate and shell height are both measures of the nutrition status and energy allocated towards shell production and can reflect environmental stressors. Condition index is essential for assessing the whole health of the oyster by understanding the resource allocation into shell growth compared to tissue growth

(Abbe & Albright 2003). Generally, a higher condition index indicates a higher metabolism and energy allocation into shell and tissue growth, while a lower condition index indicates lower metabolism and higher energy allocation into shell growth.

Understanding the phytoplankton community available is important to understanding the influence of diet on oyster growth. In the Chesapeake Bay, five major taxonomic phyla of phytoplankton are found: Ochrophyta (mainly diatoms), Dinoflagellata, Chlorophyta, Cryptophyta, and Cyanobacteria (Buchanon et al. 2005). Various studies characterizing the phytoplankton community in the Chesapeake Bay show that diatoms dominate year-round with seasonal increases in dinoflagellates and cyanobacteria (Marshall et al. 2005, Marshall 2009). For mesohaline environments, a diatom bloom is often seen during the spring while summer blooms are often composed of dinoflagellates. Species within these groups are largely found in the oyster's diet. Specifically, oysters are known to preferentially select diatoms and dinoflagellates but reject cyanobacteria (Weissberger & Gilbert 2021). Within those dinoflagellates often consumed by oysters, several harmful algal bloom (HAB) - causing taxa are present (e.g. *Prorocentrum minimum*, *Karlodinium veneficum*) which may negatively affect oyster growth depending on the life stage (Basti et al. 2011, Wikfors and Smolowitz, 1995, Wikfors 2005).

The most common methods for studying phytoplankton as the diet of oysters are controlled feeding experiments and dissection or sequencing of oyster gut contents (Weissberger et al. 2021, Pierce & Ward 2019). A few studies (Clerissi 2020, Liu et al 2022, Li et al 2017) have utilized metabarcoding of phytoplankton for the purpose of understanding bivalve diets. Metabarcoding, anewer approach to studying algal communities, targets the 16S and 18S genetic regions that are universally present in the bacterioplankton and phytoplankton community (Harrison et al. 2021). It provides greater taxonomic resolution and can help assess seasonal trends in plankton diversity.

In the study, hatchery-reared, spat-on-shell oysters were deployed at four sites to investigate growth via shell height, mortality, and condition index in an urban estuary. To assess the factors directly influencing oysters, temperature, salinity, and dissolved oxygen were collected as well as water samples to characterize the phytoplankton community via DNA metabarcoding.

This chapter aims to address the following questions:

1) Does oyster growth, mortality, and condition change geographically within the Baltimore Harbor?

I hypothesize that spat-on-shell oysters will exhibit increased shell height and decreased mortality across time with oyster growth and condition index being different across locations. It is expected that at sites in the Northwest Branch, where oysters have been grown through the Great Baltimore Oyster Partnership, oyster growth, shell height, and condition index will be higher than at the other two sites where oysters have not been previously grown.

2) Water quality (temperature, salinity, dissolved oxygen) and food source (phytoplankton) will vary across sites and influence the oysters' resource allocation to somatic tissue and shell.

I hypothesize that temperature, salinity, and dissolved oxygen will vary geographically in the Baltimore Harbor with the Middle Branch experiencing higher freshwater inputs and the Northwest Branch lower dissolved oxygen creating different stressors in different locations. The differing water quality measures will in turn affect the phytoplankton community and the quality of food available to oysters.

Section 2. Methods

2.1. Site Description

The four locations chosen for this study were: 1. Downtown Sailing Center (DSC), 2. Lighthouse Point East Marina (LPE), 3. Maritime Applied Physics Corporation (MAPC), and 4. Middle Branch Marina (MBM) (Image a). The requirements considered when selecting sites were site and dock accessibility, water depth, and salinity. All sites had a dock or other structure that could support oyster cages for the duration of the project and permission was obtained to deploy oysters and gather data. Each site had a water depth of at least 3 ft which was necessary for the cages. The sites spanned a range of salinity with a minimum of 5 ppt.

Additionally, the sites were selected to be representative of the entire Baltimore Harbor environment and its varied use. DSC is located in the Northwest Branch, adjacent to the Domino Sugar Baltimore Refinery, and is primarily used for docking sailing boats. LPE is also located in the Northwest Branch as a part of the Canton waterfront area, with a large recreational boat club. MAPC is at the mouth of the Northwest Branch and Patapsco River in a predominantly industrial area with facilities and ports like the Masonville Dredged Material Containment Facility, Fairfield Marine Automobile Terminal, and Chesapeake Terminals adjacent to the

site. MBM is a small, privately owned marina that, despite being downstream of an incinerator, hosts the most natural landscape of the four sites with a living shoreline and wetlands. LPE and DSC, also referred to as the Northwest Branch sites, were two sites where the Chesapeake Bay Foundation (CBF) has established oyster gardening programs.

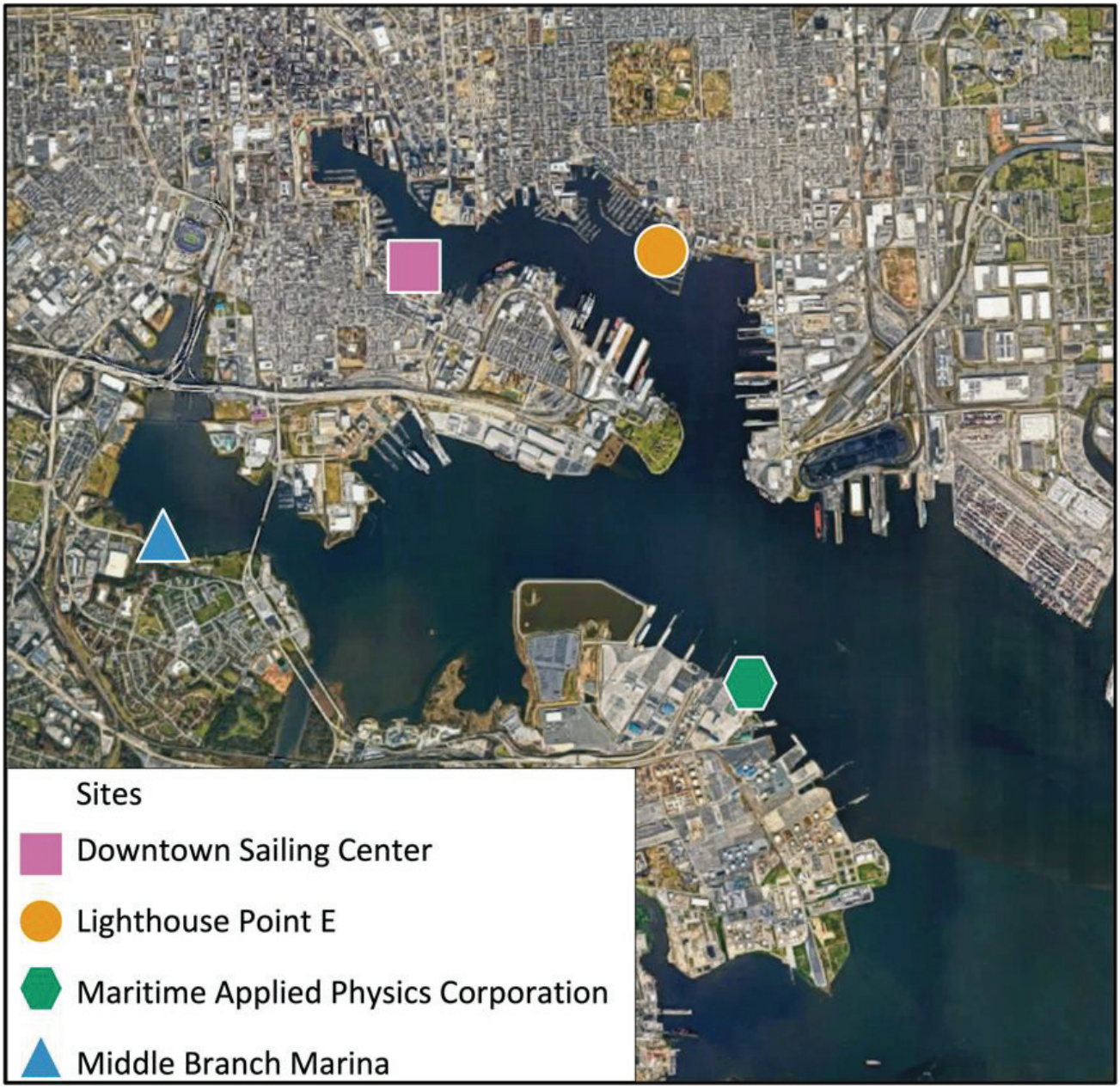

Image a. Map of the study locations in the Baltimore Harbor

2.2. Experimental Design

The study took place during the months of April – October 2022 to capture summer growth. Larvae for the spat-on-shell (SOS) oysters used in this study were spawned and reared by the Horn Point Oyster Hatchery (UMCES, Horn Point, MD) in summer 2021. Pediveliger larvae were transferred to CBF's Maryland Oyster Restoration Center in Shady Side, MD and set on recycled shell. Oysters were kept in hanging cages, measuring 0.5 m x 0.3 m x 0.3 m, provided by the CBF and intended for oyster gardening programs (Figure 2; Goldsborough & Meritt 2001). To maintain the cages and prevent excessive biofouling, the cages were manually scrubbed every 3 weeks. All cages were kept at LPE for an adjustment period of about three months (January – March 2022) until deployment at their respective sites. Each site hosted 5 cages with about 30 – 40 mother shells per cage. Oyster growth metrics (growth, shell height, condition), oyster mortality, water quality measures, and water samples were taken about every three weeks at each site (Table A.1) for a total of nine sampling periods as detailed below in sections 2.3, 2.4, and 2.5.

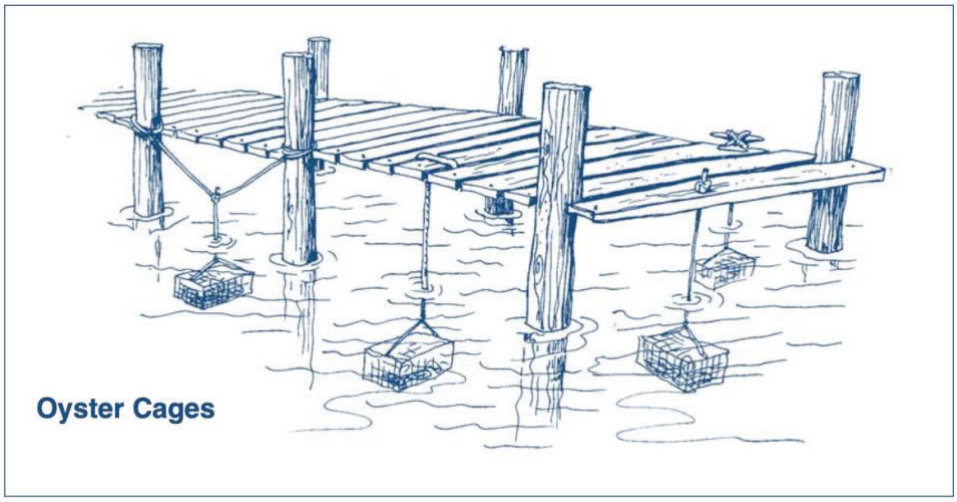

Figure 2. Hanging cages. Illustration by Cindy Fletcher-Holden (Goldsborough & Meritt 2001).

2.3. Oyster Growth Metrics & Mortality

For each sampling period (Table A.1), a random sample of 10 mother shells with live or recently dead spat-on-shell oysters were selected from each of the five cages. Any blank shells, shells with no live or recently deceased spat, were returned to the cage and a new mother shell selected. A sample of 10 mother shells represented at least 30% of the population in a cage based on an initial survey of spat-on-shell oysters at MAPC and resulting assumptions that each mother shell initially had 2–4 spat settled on it and that about 30 mother shells with spat were placed in each cage. For each mother shell, measurements for the shell height of each oyster were taken to the nearest 0.1 mm and mortality was noted. It was assumed that oysters that were closed upon removal from the water were alive. Box (gaping) oysters were counted as dead

and oyster scars, single valves attached to the mother shell from which the second valve has been disarticulated, were not included. Box oysters are assumed to be deceased within the past week as the second valve is easily removed, therefore preventing resampling of dead oysters.

At the end of the study, oysters were taken from each site to determine the condition index (CI). At least 30 live oysters were collected at each site, sampling 10 oysters from each cage when possible. In some cases, a cage only had 10 or fewer live oysters. For LPE, oysters were collected one sampling period before the other sites (period 8 rather than 9) due to access issues at the site. After collection, oysters were frozen until further processing. During processing, the oysters were thawed for 24 hrs in a water bath. The whole oysters were weighed to the nearest 0.01 g then shucked and the wet shell weighed. The individual oyster meats were placed in a labeled aluminum foil boat with the corresponding shells to dry in a drying oven for at least 48 hours at 90 °C to reach a constant standardized weight. After reaching constant weight, the dry weight of the shells and the meat were taken. CI was assessed using the index of Abbe & Albright (2003) by the following equation:

$$\mathbf{CI} = \frac{\boldsymbol{Dry\ meat\ weight\ g}}{\boldsymbol{internal\ cavity\ capacity\ g}} * \mathbf{100}$$

2.4. Water Quality Measures (salinity, dissolved oxygen, & temperature)

Water quality measures of temperature (°C), salinity (ppt), and dissolved oxygen (DO; mg/l) were taken using a YSI ProDSS (YSI Inc., Ohio, US) handheld multiprobe data sonde with an ODO/CT probe. These measures were taken about every two weeks at each site when oyster metrics or water samples were taken (Table A.1). Generally, each sampling period has one measure for temperature, salinity, and DO, but some sampling periods have two in instances where oyster measures for a site were taken over two days or when water samples for metabarcoding were taken in the same period.

2.5. Phytoplankton Metabarcoding

Water samples for preservation and metabarcoding of phytoplankton were collected about every two weeks during one day for all sites. To ensure the phytoplankton being filtered by the oysters was captured, the samples were collected about 1 m below water near the oyster cages. Samples were transported on ice to the Environmental Systems Laboratory at the University of Maryland, Baltimore County for immediate filtration and preservation. Two 300 ml water sample were filtered separately for each site through a cellulose acetate filter (47mm diameter, 0.45 μm pore size) using a vacuum filtration manifold. Resulting filters were labeled and stored in a -80 °C freezer until extraction. To each 125 ml amber bottle (1 per site per

sampling event), 1.25 ml of 5% Lugol's iodine solution was added and stored at room temperature in a dark cabinet for microscopic validation of phytoplankton species (Anderson and Throndsen 2003).

At the end of the season, the filters underwent DNA extraction. Each filter was cut in half and the DNA extraction completed using Qiagen DNeasy plant pro kit following the manufacturer's instruction with some modifications: homogenization was performed using a vortexer, and purification was repeated a second time with half of the solution amount each time. Extracted samples were quantified and then stored in a -80 °C freezer.

The gene amplification and sequencing workflow followed a dual-end barcoding, two-step PCR workflow. The V9 region of the 18s rRNA genes were amplified in the first PCR step using the 1380F/1510R primers (Amaral-Zettler et al., 2009, Table 1). These gene-specific primers were modified by adding a 21nt universal tag (Utag) specific to the Burns Lab at UMBC. PCR 1 was set up as shown in Table 1. The second PCR step incorporated a barcoding primer to amplify the Utag on both forward and reverse ends. Barcodes were specific to each sample site and date, resulting in a total of 64 unique barcodes used. PCR 2 was set up using as shown in Table 1. For purification of the PCR amplified product, USB® ExoSAP-IT® (Affymetrix, Inc., Ohio, US) was added directly to the product. The DNA of each

product was then quantified and then pooled in equimolar amounts. This pooled DNA library was then sent to Genewiz (Azenta US, New Jersey, US) for index ligation and Illumina paired-end 150bp sequencing on the MiSeq platform.

The raw reads were processed using a custom script for Cutadapt v. 4.3 (Martin 2011) in two rounds: 1. remove the sample-specific barcode and demultiplex, and 2. remove the forward and reverse primers. The demultiplexed forward and reverse reads were then analyzed using the Quantitative Insights into Microbial Ecology (QIIME 2.0) software (Bolyen et al. 2019). The reads were denoised into amplicon sequence variants (ASVs) using the DADA2 plug-in (p-trunc-f 140, p-trunc-r 140 nts). The taxonomic classifier in QIIME2 was trained using the PR2 v 4.14.1 for 18S rRNA (Guillou et al. 2013) and the primers of the samples. Taxonomy was assigned to the denoised sequences and filtered for only eukaryotic phytoplankton classes (Chlorophyta, Cryptophyta, Dinoflagellata, and Ochrophyta). The resulting abundance data was downloaded as a *.csv file from the viewer for the abundance bar plots (view.qiime2.org).

Table 1. 18S rDNA primers used in this study.

Primer Name	Primer Sequence	Length (bp)	Cycling conditions
Burns Utag + 1380F	TGTGCACGATTTGCAGATATC + CCCTGCCHTTTGTACACAC	41	98°C for 25 cycles of: 98°C for 10 s 72°C for 20 s 72°C for 90 s
Burns Utag + 1510R	TGTGCACGATTTGCAGATATC + CCTTCYGCAGGTTCACCTAC	40	
Barcode + F/R Utag	XXXXXX + TGTGCACGATTTGCAGATATC	27	98°C for 25 cycles of: 98°C for 10 s 70°C for 20 s 72°C for 90 s

2.6. Analysis for oyster growth metrics and water quality

The observed increase in shell height (mm) between measurements describes growth over time (days) and was calculated for each sampling period (t) using the equation (Harding 2007):

Growth (mm/d) = (Average shell height$_{t1}$ – Average shell height$_{t0}$) / ($t_1 - t_0$)

An analysis of variance (ANOVA) on a linear mixed-effects model analyzed the effects of sample period (1 – 9) and site (DSC, LPE, MAPC, MBM) on oyster

growth, with five replicates at each site (5 x 4 = 20 cages total). Time period and site were predictor variables and cage was the grouping factor for oyster shell height (response variable) analysis.

A one-way ANOVA was used to compare mean shell height at death between sites and to compare mean shell height for oysters alive between sites. Any significance found for the "site" factor was investigated further through a Tukey's Honest Significant Difference (HSD) post-hoc pairwise comparison. A one-way ANOVA compared the spat per shell between sites for the first time period and last time period. A linear model analyzed the effects of the first and last sample period by site for dead spat on shell analysis and live spat on shell analysis. Further post-hoc Tukey's HSD comparisons were conducted for any significance.

Oyster condition index (CI) and water quality measures were both analyzed using a one-way ANOVA to compare CI, temperature, salinity, or DO between sites. A Tukey's HSD post-hoc test was conducted if significance was found.

Section 3. Results

3.1. Oyster Growth

Oyster growth rates ranged from 0.07 – 0.09 mm/d (Table 2, Figure 3). Oyster shell height over the entire study period varied significantly by site ($p < 0.01$) and period ($p < 0.01$; Table 3). Further post-hoc comparison between sites indicated that oyster shell height at LPE was significantly different from DSC ($p < 0.01$), MAPC ($p < 0.01$) and MBM ($p < 0.01$), and that shell height at MAPC was significantly different from MBM ($p < 0.01$; Fig. 3). For DSC the overall mean shell height was 33.2 mm with the lowest mean height at period 2 (27.5 mm) and highest mean height at period 9 (41.1 mm). For LPE the overall mean shell height was 34.7 mm with the lowest mean height at period 1 (28.6 mm) and highest mean height at period 7 (40.3 mm) and 8 (40.2 mm). For MAPC the overall mean shell height was 34 mm with the lowest mean height at period 2 (28.5 mm) and highest mean height at period 9 (44.2 mm). For MBM the overall mean shell height was 31.6 mm with the lowest mean height at period 3 (26.9 mm) and highest mean height at period 9 (39.2 mm).

Table 2. Summary of growth metrics for each site. Letters denote significance.

Site	Mean Shell Height (mm)	Growth Rate (mm/d)	Condition Index
DSC	33.21 *b*	0.08	9.79 *a*
LPE	34.73 *a*	0.07	11.59 *ab*
MAPC	34.03 *bc*	0.09	12.76 *b*
MBM	31.55 *bd*	0.07	12.94 *b*

Table 3. ANOVA and post-hoc Tukey's HSD for mean shell height between sites

Variable	Df	Chi Sq	Pr(>Chi Sq)
Period	8	202.52	**< 0.001**
Site	3	51.22	**< 0.001**
Contrast		Difference	p-value
DSC – LPE		-2.43	**<0.000**
DSC – MAPC		-0.71	0.540
DSC – MBM		1.27	0.061
LPE – MAPC		1.72	**0.009**
LPE – MBM		3.70	**<0.000**
MAPC – MBM		1.98	**0.001**

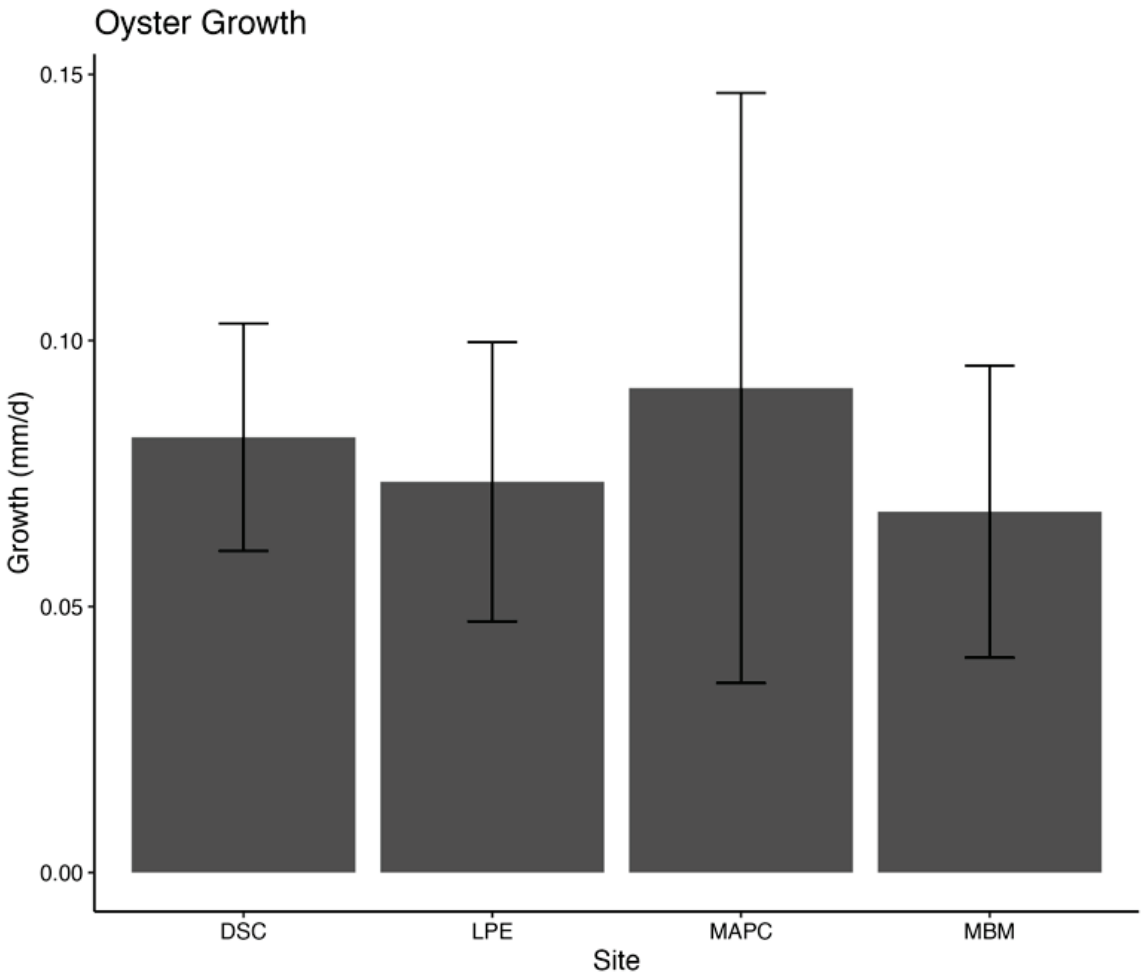

Figure 3. Oyster growth rate (mm/d) over the entire study period for each site. Error bars show standard error of the mean.

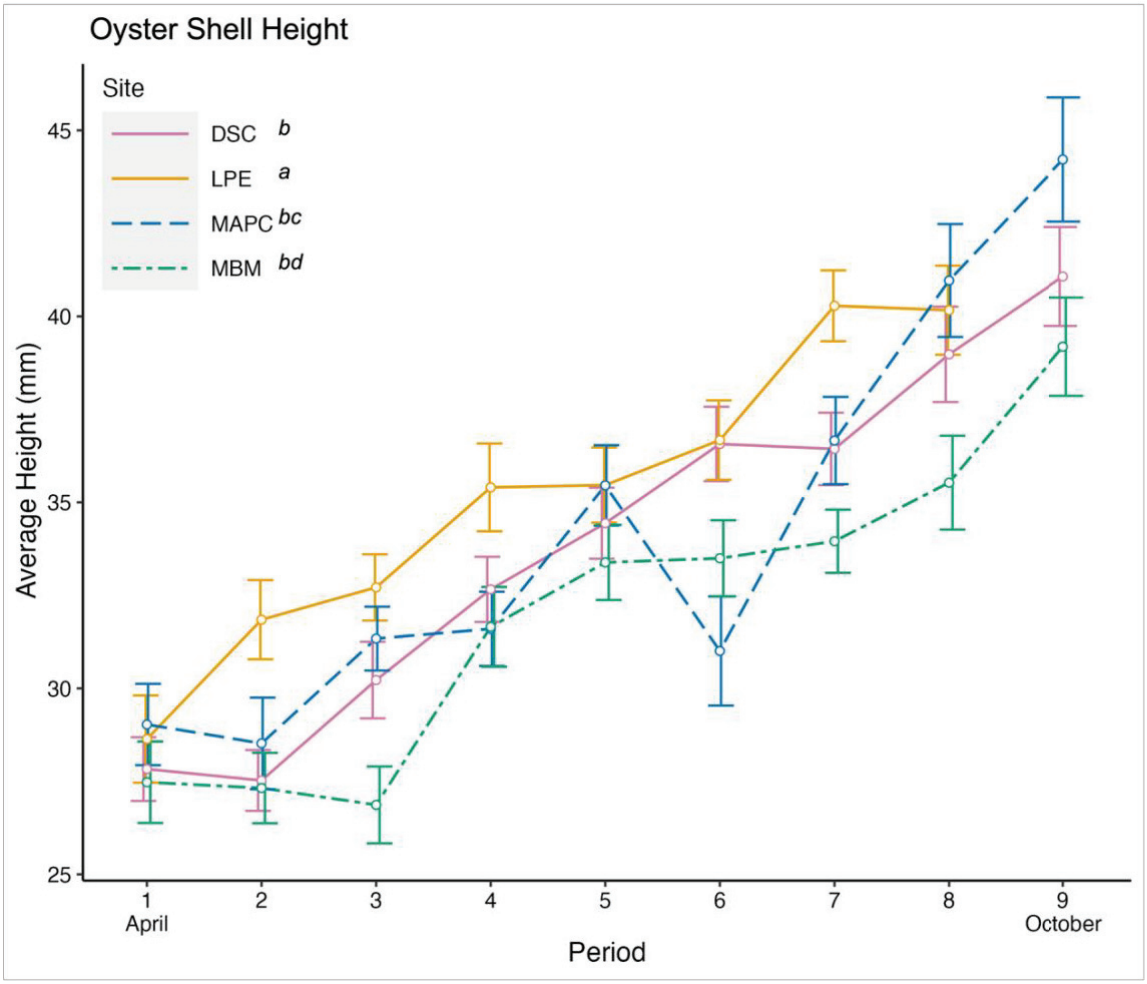

Figure 4. Oyster growth by site over sampling period. Error bars show standard error of the mean.

3.2. Oyster Mortality

Mortality over all time periods and sites ranged from 8% to 68%. DSC had the lowest average mortality of 31% while MBM had the highest average mortality of 50%. The

average mortality was similar for LPE (34%) and MAPC (33%). The number of spat per shell was looked at three ways for the first and last time periods: 1) spat per shell, 2) live spat per shell, and 3) dead spat per shell. The highest average spat per shell for the first period was about 2.2 and for the last about 1.4 at both DSC and MBM (Figure 5.a). These differences were not significantly different between sites for the first period nor the last period ($p > 0.05$). For live spat per shell, the highest average for the first period was at DSC (1.3) and MBM (1.4) and for the last was about 0.9 at DSC and LPE (Figure 5.b). In comparing between the first and last periods, the number of live spat per shell significantly decreased for DSC ($p = 0.02$), MAPC ($p = 0.02$) and MBM ($p < 0.001$) (Table 4). Third, the average dead spat per shell for the first period was about the same at DSC, LPE, and MBM (0.86 – 0.92) and for the last period was highest at MBM (0.85)(Figure 5.c). In comparing between the first and last periods, the number of dead spat per shell significantly decreased for DSC ($p = 0.01$) and LPE ($p = 0.001$) and remained unchanged for MAPC and MBM (Table 5).

The mean oyster shell height at death for the entire study period was 29.14 mm for DSC, 31.02 mm for LPE, 31.06 mm for MAPC, and 30.94 mm for MBM. This height at death was significantly different between sites ($p = 0.049$). Further post-hoc analysis showed that the shell height at death was significantly lower for DSC than MAPC ($p = 0.049$, Table 6, Figure 6). The mean shell height for surviving oyster over

the entire study period was 35.14 mm for DSC, 36.73 mm for LPE, 35.24 mm for MAPC, and 32.11 mm for MBM. The mean shell height of oysters surviving was significantly different between sites ($p < 0.001$). Further post-hoc analysis revealed that shell height for live oysters at MBM was significantly lower than DSC ($p < 0.001$), LPE ($p < 0.001$), and MAPC ($p < 0.001$, Table 7,Figure 6).

Table 4. ANOVA & Tukey's HSD for mean live spat per shell

Variable	Df	Sum Sq	Pr(>F)
Site	3	1.44	0.553
Period	1	17.0	**<0.001**
Site * Period	3	9.07	**0.005**
Contrast		Difference	p-value
DSC: First – Last		0.39	**0.02**
LPE: First – Last		0.02	0.9
MAPC: First – Last		0.38	**0.02**
MBM: First – Last		0.87	**<0.001**

Table 5. ANOVA & Tukey's HSD for mean dead spat per shell

Variable	Df	Sum Sq	Pr(>Chi Sq)
Site	3	16.75	**0.001**
Period	1	7.98	**0.005**
Site * Period	3		**0.033**
Contrast		Difference	p-value
DSC: First – Last		0.45	**0.011**
LPE: First – Last		0.56	**0.001**
MAPC: First – Last		-0.02	0.908
MBM: First – Last		0.00	0.987

Table 6. ANOVA & Tukey's HSD for mean shell height of dead oysters

Variable	Df	Sum Sq	Pr(>F)
Site	3	755	**0.05**
Contrast		Difference	p-value
DSC – LPE		-1.87	0.187
DSC – MAPC		-2.46	**0.05**
DSC – MBM		-1.80	0.133
LPE – MAPC		-0.58	0.931
LPE – MBM		0.08	0.999
MAPC – MBM		0.66	0.873

Table 7. ANOVA & Tukey's HSD for mean shell height of live oysters

Variable	Df	Sum Sq	Pr(>F)
Site	3	4330	**<0.001**
Contrast		Difference	p-value
DSC – LPE		-1.58	0.116
DSC – MAPC		-0.09	0.999
DSC – MBM		3.03	**<0.001**
LPE – MAPC		1.49	0.187
LPE – MBM		4.62	**<0.001**
MAPC – MBM		3.13	**<0.001**

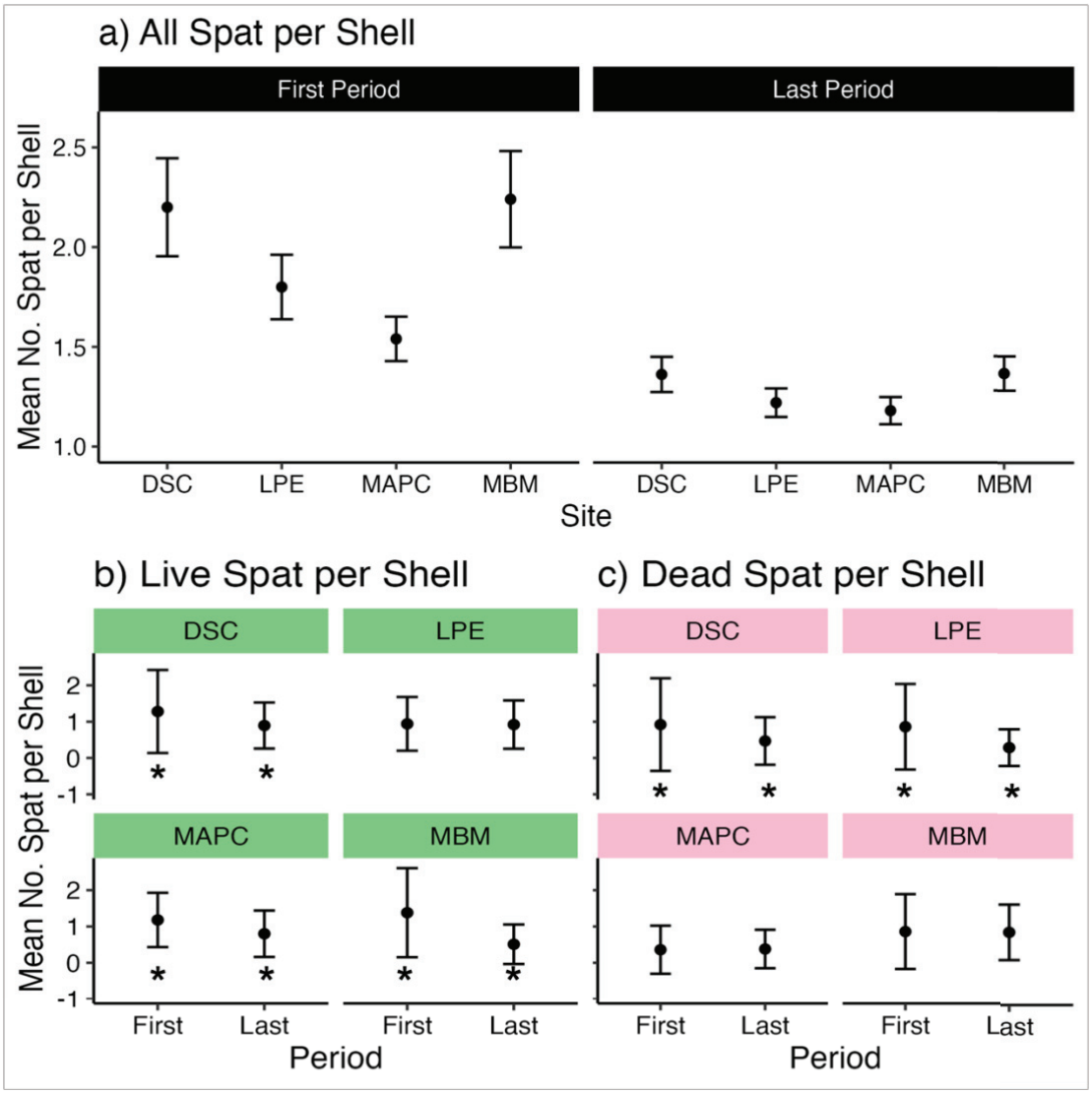

Figure 5. The average number of spat per mother shell for the first and last periods. a) all live and dead oysters between sites, b) live oysters within a site, and c) dead oysters within a site. Stars in b) and c) represent statistically significant differences between periods for a site ($p < 0.05$). The error bars in a) shows standard error and b) and c) show standard deviation.

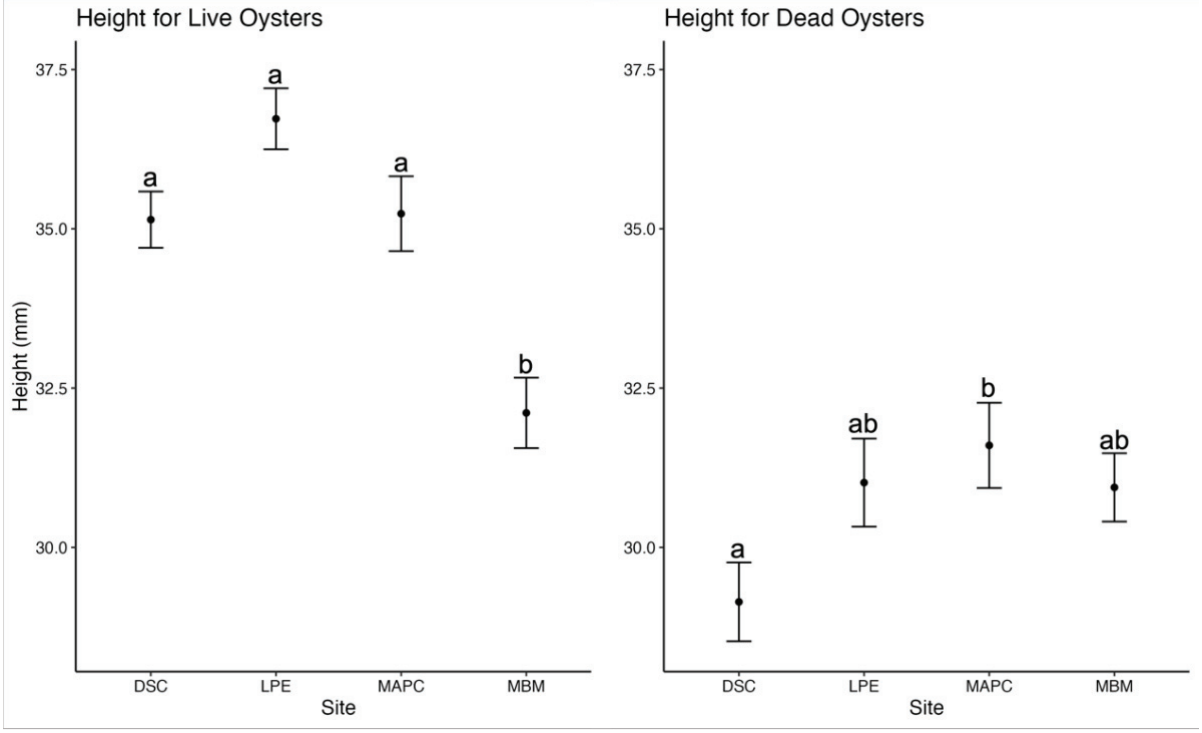

Figure 6. Oyster shell height for oysters live & dead by site. Letters above boxes represent statistically significant differences for shell height within group (live or dead, $p < 0.05$). Points represent mean with error bars showing standard error of the mean.

3.3. Oyster Condition Index

The lowest mean CI of 9.79 was at DSC. The two highest CIs were seen at MAPC (12.76) and MBM (12.94). The mean CI at LPE was 11.59. A one-way ANOVA found that the mean CI was significantly different between sites (Table 8, Figure 7). Further post-hoc analysis showed that CI was significantly lower for DSC than MAPC ($p = 0.001$) and MBM ($p = 0.003$; Table 6).

Table 8. Tukey's HSD for condition index (CI)

Contrast	Difference	p-value
DSC – LPE	-1.80	0.084
DSC – MAPC	**-1.97**	**0.001**
DSC – MBM	**-3.15**	**0.003**
LPE – MAPC	-1.17	0.520
LPE – MBM	-1.35	0.482
MAPC – MBM	-0.18	0.998

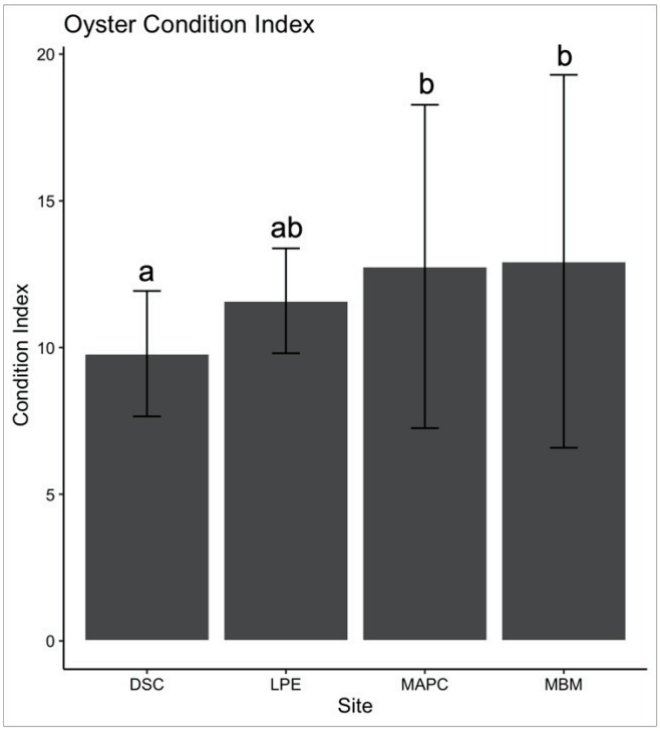

Figure 7. Condition index for each site. Letters above bars represent statistically significant differences between condition index at all sites and dates ($p < 0.05$).

3.4. Water Quality

The mean temperature for all sites was about 22 °C, with the highest temperature of 32 °C recorded at DSC (Table 9). The mean salinity at DSC and LPE was about the same while MBM was lower and MAPC was higher. The lowest recorded salinity was at MBM (0.05 ppt) and the highest at DSC (14.8 ppt). Dissolved oxygen seemed to vary the most between sites (Figure 8). DSC and LPE had the lowest mean DO (5.7 & 5.4 mg/l) while MAPC had the highest of 7.2 mg/l. The lowest recorded DO was at DSC (1.6 mg/l) and the highest at DSC and MBM of 12 mg/l. There was no significant difference between sites for mean temperature, salinity, and DO ($p = 0.06$).

Table 9. Summary statistics for temperature, salinity, & dissolved oxygen at each site

Site	Temperature (°C) Min, Mean, Max	Salinity (ppt) Min, Mean, Max	Dissolved Oxygen (mg/l) Min, Mean, Max
DSC	10.1, 23.3, 31.7	2.0, 7.8, 14.8	1.6, 5.7, 12.2
LPE	9.9, 23.7, 30.1	4.9, 7.9, 13.4	2.4, 5.4, 10.0
MAPC	10.8, 22.7, 29.0	2.7, 8.4, 14.1	5.0, 7.2, 11.1
MBM	11.1, 22.5, 29.7	0.5, 6.9, 13.1	2.4, 6.9, 12.3

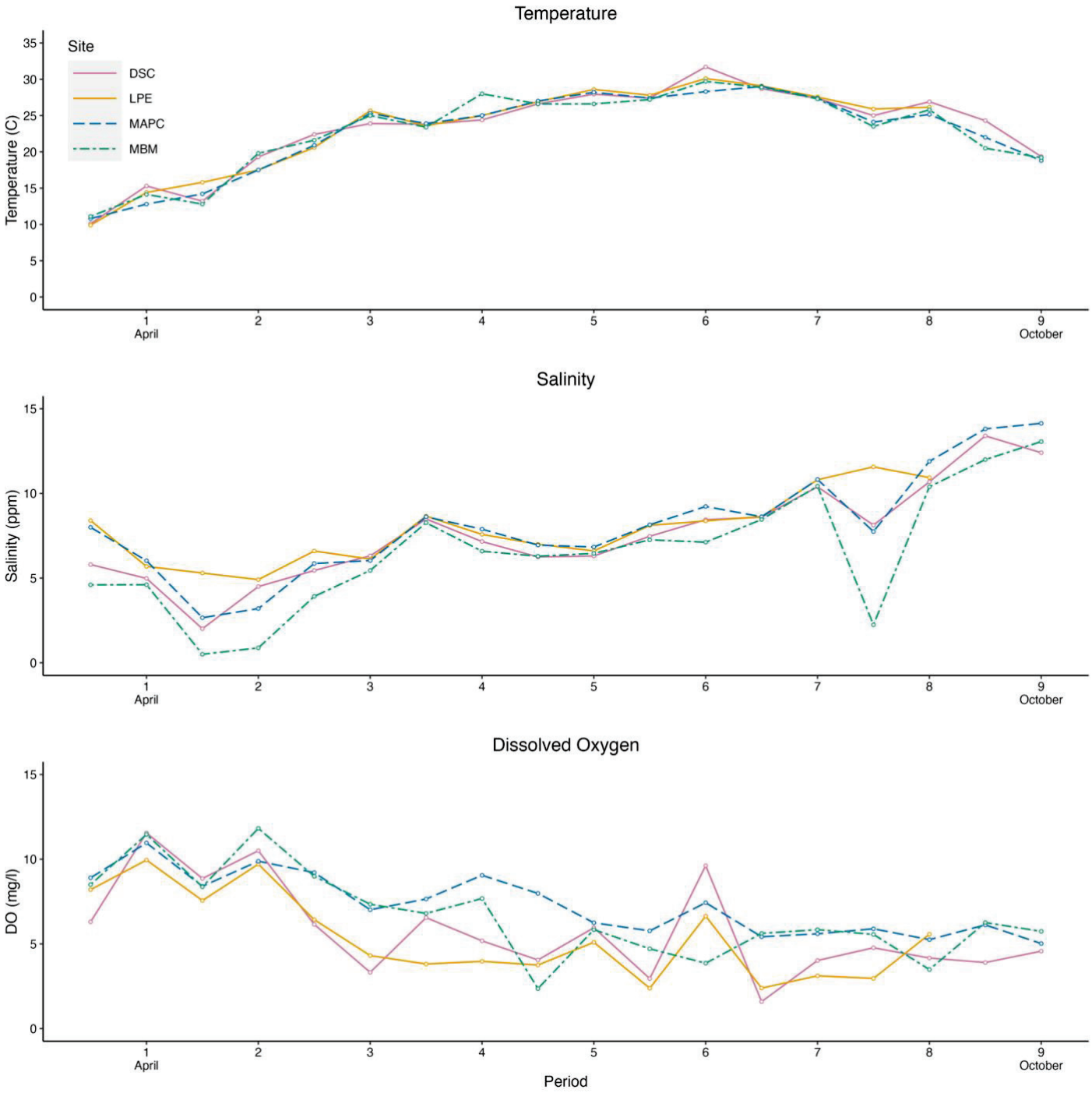

Figure 8. Water quality of temperature, salinity, & dissolved oxygen at each site over sampling period.

3.5. Food Availability: Phytoplankton Metabarcoding

Four major divisions of phytoplankton were detected via metabarcoding of the 18s locus: Chlorophyta, Cryptophyta, Dinoflagellata, Ochrophyta. The abundances of those groups varied slightly among sites (Figure 9). The abundances of division Chlorophyta was similar among the four sites. The division Ochrophyta was higher in DSC and MBM than in LPE and MAPC. The division Dinoflagellata was higher in LPE than the other three sites. Divisions Chlorophyta and Cryptophyta were assessed at the class level and divisions Dinoflagellata and Ochrophyta were assessed at the order level for a more diverse characterization (Figure 10). Each division was dominated by one or two classes or orders at each site. In Chlorophyta, the most abundant class was Pyramimondadophyceae. In Ochrophyta, the two most abundant classes were Bacillariophyta (diatoms) and Chrysophycea. In Cryptophyta, Cryptophyceae_X and Goniomonodales were most abundant orders. In Dinoflagellata, Gymnodiniales were most abundant at all sites, followed by Peridiniales at MAPC and Prorocentrales at DSC, LPE, and MBM.

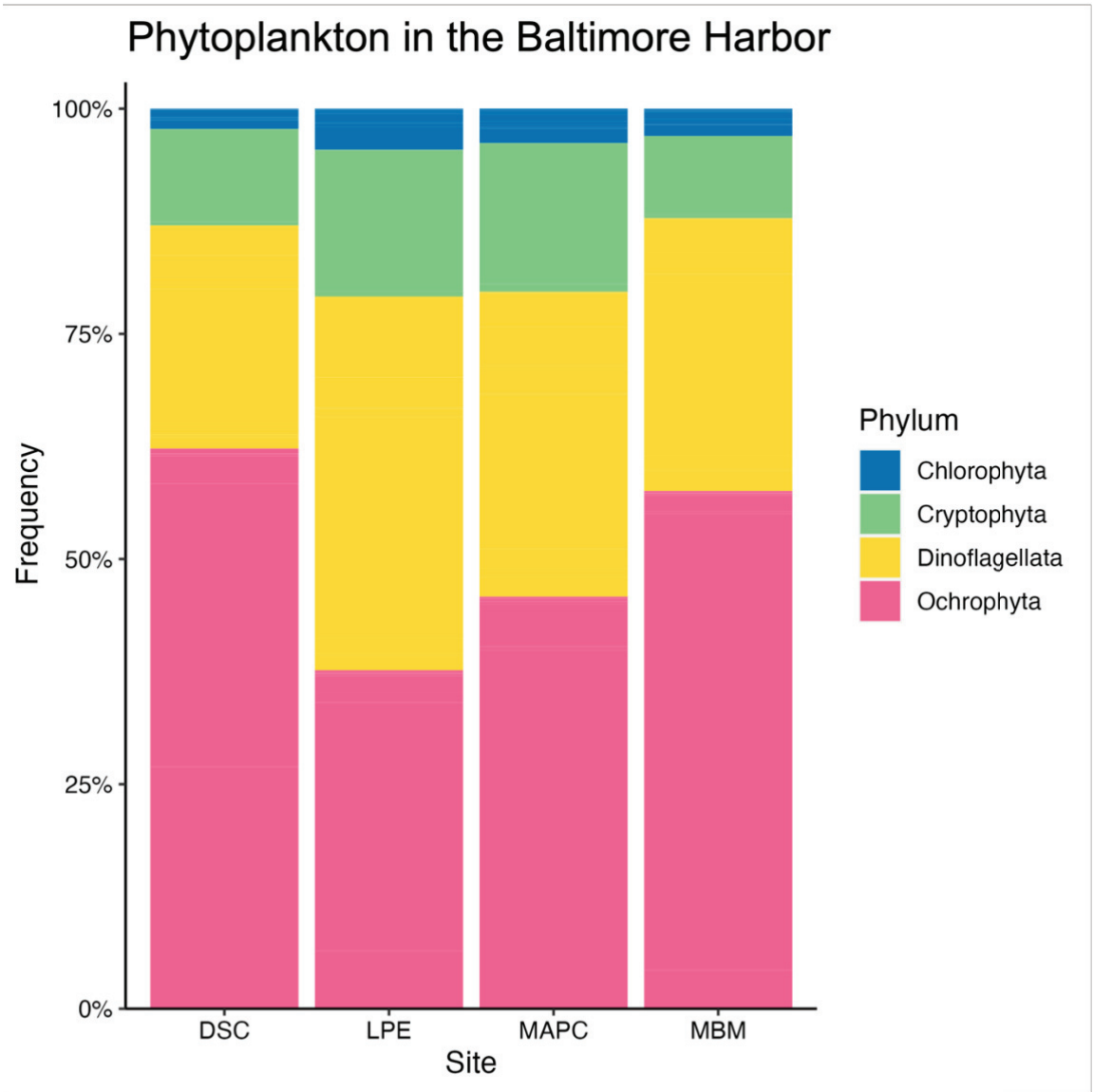

Figure 9. Frequency of phytoplankton phyla at each site.

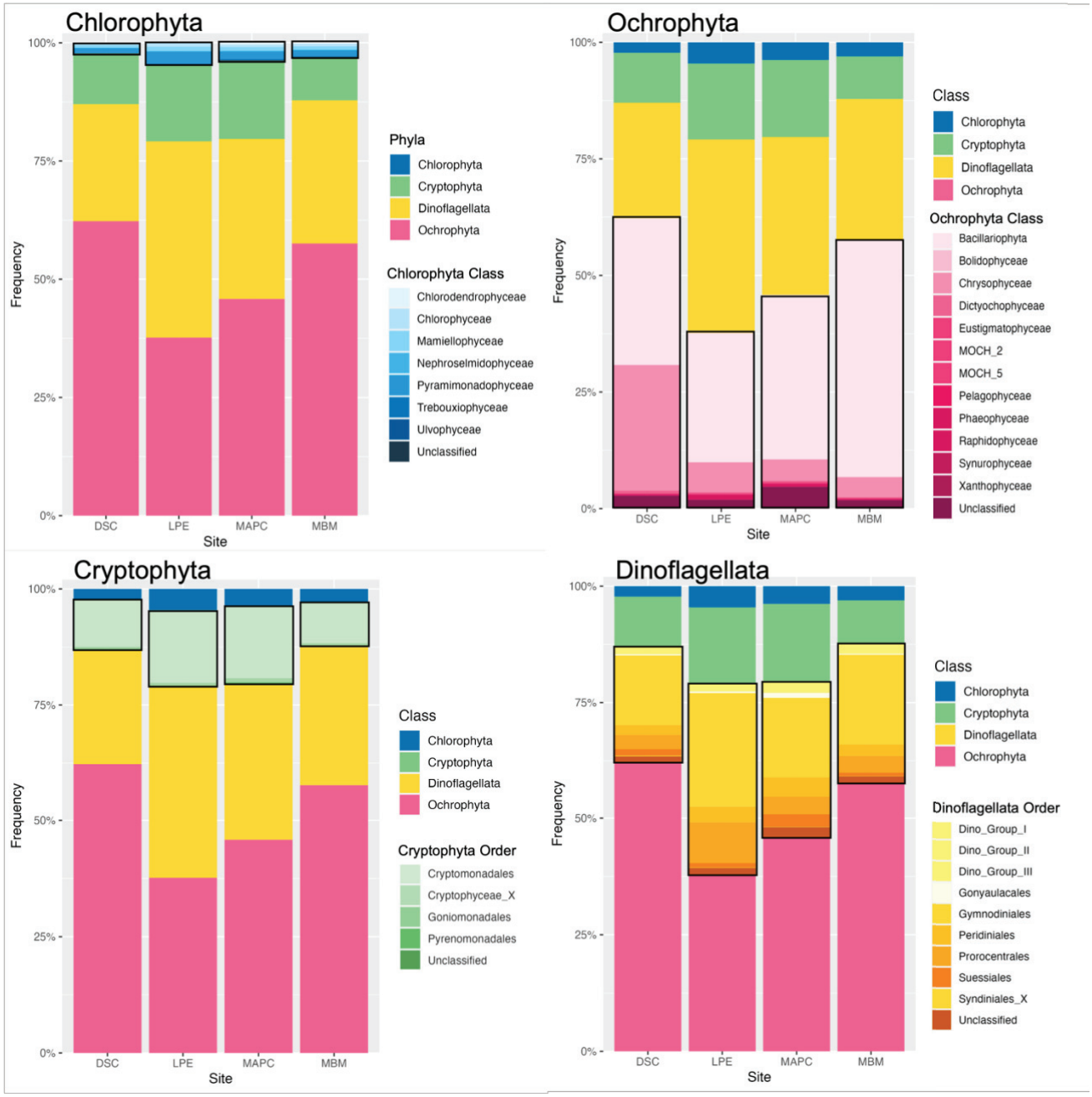

Figure 10. Frequency of Cryptophyta orders, Chlorophyta classes, Dinoflagellata orders, & Ochrophyta classes at each site.

Section 4. Discussion

The goal of this study was to establish a baseline for oysters growing via the oyster gardening method for restoration in a highly urbanized environment, the Baltimore Harbor. This was assessed using differences of oyster growth metrics and mortality between sites in the Baltimore Harbor and the abiotic (water quality) and biotic (food source) factors known to directly influence oysters. As previously stated, the four sites represent the various habitats and uses of the Baltimore Harbor (Image a). All oysters studied originated from the same brood stock that were then uniformly transported to different sites. Therefore, any differences in oyster growth and condition between sites can be attributed to the varying local environment rather than local adaptation or genetic factors as demonstrated in previous studies (McFarland & Hare 2018).

4.1. Does oyster growth, mortality, and condition change geographically within the Baltimore Harbor?

4.1.1. Oyster Growth Metrics

The oyster growth metrics findings partially support the hypothesis that the Northwest Branch sites (DSC & LPE) have higher growth rate, shell height, and condition index than sites in the Middle Branch (MBM) or mouth of the Harbor

(MAPC). LPE oysters exhibited the greatest shell height and DSC oysters had the second highest, but neither site was in the top two sites for condition index (CI). At MAPC, the mean shell height, the growth rate, and CI was always the highest or second highest compared to the other sites. MBM had the lowest shell height and growth rate, but highest condition index. While some differences were not significantly different, those that were can indicate potential differences between sites and the effect on oysters.

The lower CI at Northwest Branch sites potentially indicates a higher presence of predators than at other sites. Oysters are known to create larger, thicker shells and less tissue (i.e., lower condition index) when predation risk is high (Kimbro et al. 2020). Throughout this study, the presence of blue crabs was noted at LPE, DSC, and MAPC, but never at MBM. Further research on oysters in the Baltimore Harbor should quantify predator presence to better understand their effect on shell height and CI. Other stressors that could contribute to a lower CI in the Northwest Branch include low dissolved oxygen and poor diet. Oysters grown at MAPC grow better in both shell and tissue, potentially due to the location of MAPC at the mouth of the Harbor. This location possibly experiences more inputs from the main stem of the Bay estuary than from the Patapsco River leading to slightly higher water quality condition. The exposed nature of the site itself, might be conducive to increased water

flow which is known to increase the uptake of phytoplankton. Lastly, the high CI of oysters grown at MBM was surprising because the significantly lower shell height should indicate that the environmental conditions were less ideal for oyster growth than at other sites. This suggests that the limiting factor for oyster growth is not nutritional, but potentially related to water quality or other environmental factors.

It is important to discuss the difference between shell height and growth rate, particularly in cases where the shell height and growth rate ranking between sites did not match. The average shell height over the entire study is indicative of the average shell height for the population and the energy allocated towards shell production, however it is affected by outliers (i.e., heights extremely large or small compared to most of the sample). The growth rate (mm/d), also an indicator of energy allocation, reflects the speed and amount of shell production over time. For LPE, oyster shell height was significantly higher than all the other sites, yet its growth rate was in the bottom two. This suggests that LPE oysters grew more uniformly and without significant of variation between individual oyster shell height. MAPC oysters had the highest growth rate but second highest shell height. This could be attributed to the growth between periods 6 and 9. Between period 5 and 6, MAPC oyster growth rate was -0.2 mm/d, but the next two period intervals both had a growth of about 0.2. It is possible that the negative growth and extremely low shell height for period 6 is a

result of sampling a subset of the population that grew very little between period 4 and 6 and was not sampled in period 5. It also indicates that unlike oysters at LPE, oysters at MAPC grow less uniformly with more variation. The discrepancy between shell height and growth rates reflects the importance of collecting and calculating multiple growth measures and should be anticipated in the design of future studies.

4.1.2. Mortality & Spat-on-shell

The sampling method for this study created limitations for understanding mortality and are further detailed in section 4.1.3. The mean mortality percentage for each sample over the entire study for each site showed a stark difference between MBM (50% mortality) and the other sites (31 - 34% mortality).

To further investigate the causes of mortality, the mean shell height of oysters dead and live was compared between sites and the number of spat per shell between sites and across sites. It was expected that on average, dead oysters would be smaller than live oysters due to the larger filtration capacity and protection from predation of larger oysters. For all sites, the mean height at death was 31 mm or less, which indicates oysters with minimal growth are more susceptible to causes of death, considering that most oysters reached a size above 35 mm by the end of the study. The only significant difference found was in the lower shell height at death for DSC

than MAPC. This might indicate that there are some environmental conditions causing larger oysters to die at MAPC that are not present at DSC.

The goal in investigating the number of spat per shell is to understand the influence of initial density on survival and mortality under the conditions at each site. DSC and MBM showed a slight, but non-significant higher starting density than LPE and MAPC, which could be one reason why MBM experienced a higher mortality percentage. Overall, no significant difference in starting density was found between sites, which was expected because the shells were from the same brood stock and set. No significant difference was found between sites in ending density which suggests that the environmental conditions causing differences in shell height and condition index are not influencing the spat density on a shell. The differences within a site between starting and ending density can point to causes of mortality that are site-specific. For both live and dead spat per shell, a significant decrease over time is expected. Live spat should decrease with natural mortality of younger oysters and dead spat should decrease as oysters grow and are less susceptible to causes of juvenile mortality. At all sites, except LPE, a significant decrease of live spat per shell between the first and last periods was found, suggesting that the conditions at LPE support a constant population. This could also be reflected in the less varied shell height seen at LPE. For dead spat, a significant decrease was only seen at DSC

and LPE, which might indicate that a higher starting density (0.9 at DSC and 0.8 at LPE) leads to more initial competition and mortality. However, MBM also had a high starting density (0.86) but the number of dead spat did not change over the study. This, alongside the higher percent mortality than other sites, suggests that the conditions at MBM cause not just slower growth, but also more deaths. While the number of dead spat on shell at MAPC started lower than other sites (0.36), it also did not change over the study, suggesting that factors causing mortality at other sites might not be present at MAPC.

4.1.3. Notes on Mortality - Limitations

As described in the methods section 2.3. Oyster Growth Metrics & Mortality, a sample of the population rather than the total population was taken for measurements at each sampling period. This created limitations in understanding mortality because individuals and mother shells were not marked and tracked across time. While resampling of dead oysters was not possible due to only sampling box oysters (i.e., both valves attached, with the upper valve dehiscing naturally between sampling periods) that are recently deceased, it is impossible to know if the oysters marked dead or their mother shell were previously sampled. Additionally, the sample size was based on the mother shells rather than individuals, meaning that the actual oyster sample size is different over time. For example, a 50 % mortality at period 1 and

period 9 would not be comparable because at time period 1 there could be 100 individuals (50 dead) and at period 9, 20 individuals (10 dead). Therefore, the estimated percent mortality should not be taken as a direct measurement of mortality. This method of sampling based on mother shell does facilitate understanding if the initial spat density influences final spat density and mortality over time.

4.2. How does water quality (temperature, salinity, dissolved oxygen) and food source influence the oysters' resource allocation to somatic tissue and shell?

4.2.1. Water Quality

While no significance among site differences were detected for temperature, salinity, and dissolved oxygen over the entire study period and temporal variations are similar across sites, there were some differences that could have influenced the oyster growth metrics and mortality between sites. MBM experienced a short period of extremely low salinity (< 5 ppt) between the start of the study and period 3 while the other sites only had below 5 ppt salinity at period 1 and 1.5. Previous studies of oysters in urban environments reveal low salinity to be a primary factor in mortality events (Levington et al. 2013, McFarland & Hare 2018). However, low salinity is particularly lethal under high temperature (> 25 °C; La Peyre et al. 2013) which was not the case until

after the salinity rose above 5 ppt (period 3). Additionally, higher salinity (> 10 ppt) typically correlates to increasing growth (Kreauter et al. 2007, Rybovich et al. 2016).

Temperature did not vary over time between sites, so it is not considered a factor in the site variation. Dissolved oxygen (DO) fluctuated substantially at each site across time and although not significant between sites, should be considered a factor in oyster growth in the Harbor. DSC, LPE, & MBM all had instances of hypoxia (< 2 – 3 ppm) which can be tolerated by oysters for short durations, but at longer durations is considered a primary factor in slow growth and mortality (Johnson et al. 2009). This is possibly reflected in the lower condition index at DSC & LPE. Under conditions of hypoxia, oysters do not filter-feed because they close their shells to survive, leading to lowered intake of phytoplankton.

4.2.2. Food Availability: Phytoplankton Community Detection

The phytoplankton metabarcoding results did not fully support the hypothesis that food availability explains the different stressors and energy allocations of oysters to somatic tissue and shell growth. All four major classes of eukaryotic phytoplankton known in the Chesapeake Bay were present at each site and the almost the same orders and families were present at each site. The abundances of classes Dinoflagellata and Ochrophyta were about the same within LPE. The species within

Dinoflagellates complicate the diets at all sites. These included the species *Levanderina fissa* known to negatively affect oysters, especially when in high abundances and for younger oysters, as well as species known to support oyster growth, like *Gyrodinium*. More research is needed to fully understand at what frequency or abundance detrimental phytoplankton will actually negatively impact oysters, especially when phytoplankton supporting oyster growth are overwhelmingly present. MBM which was expected to provide a less nutritious diet, had a phytoplankton community composed primarily of the class Ochrophyta, which includes the preferred food source diatoms. Ochrophyta was also most frequent in the samples of DSC and MAPC. The most common Ochrophyta group was diatoms commonly fed in hatchery operations, *Cyclotella* and *Skeletonema*, at all sites, this does provide one possible reason for the high CI of oysters at MBM and MAPC. However, if higher diatom frequency suggests higher CI, then DSC with the highest frequency of diatoms would also showcase the highest CI, but this is not the case. On its own, the phytoplankton community as the diet of oysters is not the sole driving force of differences in oyster growth and condition between sites. It could play a role when considered with other water quality or environmental factors.

Section 4. Conclusions

This study represents the first research conducted on oysters in the Baltimore Harbor. The main takeaway is that oysters do grow in the Harbor throughout the typical oyster growing season, despite the highly industrialized landscape of Baltimore and the many environmental conditions potentially influencing oysters (Image a). In the Northwest Branch (DSC & LPE), oysters have higher shell height, similar growth to other sites, similar mortality and spat-on-shell density, yet lower condition index. At MAPC, conditions seem to be most ideal for growing oysters as reflected in the high shell height, growth, and condition index and minimal difference in spat per shell density over time. Oysters at MBM, exhibited slower growth, a decrease in live spat per shell and no difference in dead spat per shell between first and last time periods, but high condition index. The causes of varying oyster growth, mortality, and condition between sites cannot be attributed to solely water quality measures nor food source as there were minimal differences in those between sites. The oysters do have a variety of phytoplankton available to filter, including diatoms known to support oyster growth.

Other environmental factors not quantified in this study that should be considered are predators and competitors, cyanobacteria, biofouling, and industrial contaminants. Blue crabs were often present at LPE, DSC, and MAPC, but never at MBM.

Quantifying predator presence and abundance will allow for further correlation between condition index and predators as previous studies have shown (Kimbro et al. 2020). Mud worms, *Polydora websteri*, which drill into the oyster and live symbiotically were also present at all sites. While mud worms are not expected to be a leading cause of mortality, they can act as competitors with the oyster tissue inside the oyster shell and have negative effects when in high abundance and on young oysters or when oysters are already vulnerable. In terms of other phytoplankton not quantified in this study, cyanobacteria are of particular concern because they are not easily digestible or nutritious for oysters. It could be possible that when cyanobacteria are in high abundance, the oysters have lowered feeding efficiency and cyanobacteria accumulate in their digestive organs. Biofouling is a serious concern for oyster gardening cages and other growing systems because excessive biofouling prevents oxygen and water flow to oysters. *Victorella*, a biofouling Bryozoan organism, appeared around halfway through the study at all sites for a few weeks, but persisted throughout the rest of the study in high amounts at MBM. It is unclear the impact of *Victorella* on the oyster, but as a biofouling organism on the growing cages it could have prevented adequate waterflow to oysters at MBM. Lastly, Baltimore and most of the locations in this study were industrial with proximity to cargo boats and polluting facilities. It is possible that some of the differences between oysters at locations were

due to the different pollutants, contaminants, or sediments present. Further research should investigate the role of these factors on oysters in the Baltimore Harbor.

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Chapter 3: Recommendations for oyster gardening in the Baltimore Harbor based on a 7-month study of spat-on-shell

1. Findings

This study aimed to establish a baseline for oyster gardening at various locations in the Baltimore harbor. The investigated measures of oyster growth, shell height, condition index, and mortality are all indicative of oyster health and reflect potential stressors present at each location. Additional factors of water quality (temperature, salinity, & dissolved oxygen) and phytoplankton community composition at each site gave insight into the similarities and differences between known stressors of oyster health.

Spat-on-shell oysters (<1 – ~1 year old) can grow in the Baltimore Harbor during a critical growth period (April – September). Increases in shell height were observed at all sites, with MAPC and DSC exhibiting the highest growth. Condition index, a measure of tissue-to-shell ratio, was highest at MAPC (12.76) & MBM (12.94). Observed mortality was highest at MBM (50 %) than at the other three sites (~ 30%), but due to sampling method, this measure is not indicative of true mortality. Average dead spat per shell density did not change between the beginning and end of study at MBM, although live spat per shell did decrease indicating that mortality should be a

concern at MBM. These results suggest that multiple and different stressors are present at each site and show the importance of collecting all measures of oyster health.

Temperature and salinity did not vary between sites; therefore, they do not explain differences in oyster health. Dissolved oxygen (DO) did vary, although not significantly, but should be considered to partially influence oyster health in the Harbor. Under condition of extremely low DO (< 2 – 3 ppm), which was seen at DSC, LPE, & MBM, oysters are unable to maintain their metabolism long-term and respond by decreasing or completely stopping respiration, leading to low growth in tissue compared to shell and ultimately, mortality. This is possibly reflected in the lower condition index at DSC & LPE than at MBM & MAPC where DO was on average higher. It could also explain the higher mortality (50%) at MBM.

The composition of the phytoplankton community dictates the quality of the oyster's diet. At all sites, the phytoplankton community was primarily composed of diatoms or brown algae (Ochrophyta) & dinoflagellates (Dinoflagellata), which include much of the preferred food source of oysters. Two other groups, the cryptomonads (Cryptophyta) and green algae (Chlorophyta) were also present at all sites and are also a known food source. This finding demonstrates that oysters are not diet limited in these locations.

2. Implications & Recommendations

For all current and future programs growing oysters in the Baltimore Harbor, oyster growth metrics should be regularly collected to assess the success of the program. At minimum, oyster shell height should be measured for at the beginning and end of the oyster's residence in the Harbor if it is temporary, like with current oyster gardening initiatives. For longer term initiatives, shell height should be measured at least once during the spring and fall when oysters are expected to grow the most. The frequency and type of growth metrics collected must be tailored towards program goals.

The Great Baltimore Oyster Partnership is a member of the Chesapeake Oyster Alliance, a coalition with the goal of adding 10 billion oysters to the Bay by 2025. The current Baltimore oyster gardening locations through the partnership are at DSC and LPE, both which had minimal mortality (~30%) in this study. This program should continue at both locations with extension into summer months rather than removing oysters in May. The oysters at the beginning of the study (April/May) were still small (< 35 mm) and are most likely still susceptible to predation on reefs. Extending the program to grow oysters into the early fall will ensure that oysters grow to a size that meets the goal of reducing predation on the restoration reef. The Great Baltimore Oyster Partnership should also consider extending to locations in the Middle Branch for oysters with higher somatic (tissue) quality and different

community engagement opportunities. With the increased interest in Middle Branch restoration activities, including from the Environmental Justice Journalism Initiative, the Partnership can play a role in (re)connecting environmental justice communities to a previously neglected waterway. Additionally, continued partnership between CBF and Solar Oysters to grow oyster at MAPC will result in oysters that are larger than other places and potentially less stressed due to MAPC's location at the mouth of the Harbor.

In the Middle Branch, EJJI, other organizations, and researchers should account for the potentially high mortality in this waterway when deploying spat-on-shell oyster. However, the high condition index for oysters at MBM shows that oysters are efficiently utilizing the available food source under lower dissolved oxygen conditions. This suggests that in the long-term, a large quantity of oysters at MBM could improve water quality.

Lastly, it is important to keep in mind that this 7-month study could not account for all environmental stressors and site differences that potentially impact oysters, nor should these findings be heavily weighted for an organism that can live up to 20 years. The findings from this study are a snapshot of juvenile spat-on-shell and are intended to be built on through further research and monitoring. Future studies should consider structural & industrial differences in the Harbor (e.g., boat traffic, floating

vs. static dock), contaminant presence, predators, and cyanobacteria presence and abundance.

Appendix

Table A. 1. Data collection timeline

Period	Oyster Height Samples	Water Samples - Phytoplankton	Water Quality
0.5		April 6	April 6
1	April 19, 20, 21, 23	April 21	April 19, 20, 21, 23
1.5		May 9	May 9
2	May 12, 13		May 12,13
2.5		May 24	May 24
3	June 2, 3, 4	June 2	June 2,3,4
3.5		June 22	June 22
4	June 24, 28, July 1		June 4,28, July1
4.5		July 6	July 6
5	July 13, 14, 15	July 14	July 13,14,15
5.5		July 27	July 27
6	August 5, 6	August 10	August 5,6,10
7	August 26, 27	August 25	August 25,26,27
7.5		September 8	September 8
8	September 13,15	September 16	September 13,15,16
8.5		September 23	September 23
9	October 4, 6	October 6	October 4,6