WILD AND FARM EASTERN OYSTER (CRASSOSTREA VIRGINICA)

CONTRIBUTIONS TO IMPROVED WATER QUALITY IN THE MID-ATLANTIC:

CONTEMPORARY AND FUTURE CLIMATE ESTIMATES

By

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And approved by

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ABSTRACT OF THESIS

Wild and farm Eastern oyster (*Crassostrea virginica*) contributions to improved water quality in the mid-Atlantic: contemporary and future climate estimates

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Globally it is estimated that 85% of oyster reef ecosystems have been lost over the past 130 years as a result of overharvesting, changes in freshwater inflows, alterations to shorelines, disease, and other factors. This decline has resulted in a loss of the ecosystem services oysters provide such as water quality improvements. Restoration of natural oyster reefs has been a common solution, but research suggests oyster aquaculture could be providing equivalent or greater water quality benefits than reefs. However, the scale at which oyster farms provide important ecosystem services is not adequately known. Furthermore, there is a lack of data concerning site-specific effects of changes in salinity on oyster filtration. Oysters are generally resilient to changes in salinity, but prolonged exposure to low salinity conditions have caused severe mortality events in oyster populations. Low salinity stress will likely increase with climate change as the frequency and intensity of extreme rainfall events increases across the mid-Atlantic.
This thesis aims to address the aforementioned concerns by (1) quantifying farm-specific year-round Eastern oyster (*Crassostrea virginica*) filtration at three oyster farms in the mid-Atlantic and (2) assessing the effects of decreased salinity on the filtration services provided by a wild oyster bed and an oyster farm in Delaware Bay, New Jersey. Field experiments were conducted seasonally using a flow-through filtration chamber with ambient water to calculate individual oyster filtration physiology. The experiments discussed in Chapter 1 provide a robust dataset of oyster feeding behavior observed under natural conditions across oyster farms. The results show oyster filtration physiology differed among locations and through the year. Collectively, an increase in salinity and temperature was associated with an increase in oyster physiological activity across all farms, but physiological activity at each farm was associated with a different suite of environmental variables (including total particulate matter and the organic content of seston). These data may be used in a broader framework to inform development of nutrient management strategies in the mid-Atlantic.

The experiments discussed in Chapter 2 estimate the impacts of seasonal climatic stress (*i.e.*, increased extreme precipitation events) on wild and farm oyster filtration. The results show wild and farm oyster populations provide reduced water quality benefits during a Hurricane Sandy scale salinity disturbance occurring between the spring and the late-fall. Individual wild and farm oyster responses to the low salinity conditions were varied highlighting the importance of gathering oyster- and site-specific feeding behavior data. These data may be used to inform proactive, long-term management of wild and farm oyster populations in the region to achieve water quality and oyster stock goals.

Together, these chapters highlight the utility of wild and farm oysters as a nature-based water quality management tool and the importance of making conservative estimates of
ecological filtration to account for days of low oyster physiological activity triggered by possible environmental stressors.
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DEDICATION

To Mom and Dad for their unwavering and loving support,
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CHAPTER 1 – SEASONAL FEEDING BEHAVIOR AND WATER QUALITY BENEFITS PROVIDED BY EASTERN OYSTER (CRASSOSTREA VIRGINICA) AQUACULTURE IN THE MID-ATLANTIC

1.1 ABSTRACT

The Eastern oyster (Crassostrea virginica) is a commercially important aquaculture species and food resource along the Atlantic and Gulf coasts of the United States. In addition to its economic value, oyster aquaculture provides ecological value such as water quality improvement. Oyster filtration is influenced by environmental conditions such as water temperature, hydrodynamics, salinity, food quality and quantity, as well as oyster size and energetic demands. Although filtration is highly variable, average rates generated in laboratory experiments are often used to estimate the ecological impact of oyster filtration; therefore, there is a need for field-based, farm-specific estimates of filtration that account for this variation. In this study, field experiments were conducted between September 2020 and September 2021 to estimate seasonal farm physiology (i.e., filtration, clearance, rejection, and absorption rates) at three oyster farms in the mid-Atlantic. The farms studied included a subtidal farm in Barnegat Bay, an intertidal farm in Delaware Bay, and a subtidal farm in Rehoboth Bay. The physiological activity of oysters at each farm was varied such that oysters at Barnegat Bay were the most active and oysters at Rehoboth Bay were the least active. Seasonal physiological trends were observed such that filtration behavior generally increased in warmer months. An increase in physiological activity across all farms was associated with an increase in salinity and temperature, but physiological activity at each farm was associated with a different suite of environmental variables including total particulate matter and the organic content of seston. This study provides a robust dataset which can be implemented in models estimating ecological filtration rates and adds to
the growing body of evidence supporting bivalve aquaculture as a nutrient pollution mitigation tool.
1.2 INTRODUCTION

The critical importance of oysters to coastal ecosystems and the impact of anthropogenic stressors on oyster reefs are well documented (Beck et al. 2011). Globally it is estimated that 85% of oyster reef ecosystems have been lost over the past 130 years as a result of overharvesting, changes in freshwater inflows, alterations to shorelines, disease, and other factors (Beck et al. 2011). This decline has resulted in a loss of the ecosystem services oysters provide such as water quality improvements (Grabowski et al. 2012). This ecosystem service is particularly important as nutrient pollution continues to be a global water quality concern for aquatic and marine environments (Howarth 2008; Wurtsbaugh et al. 2019). Excess nutrients in a water body, a condition called eutrophication, can be harmful to aquatic and marine life (Bricker et al. 1999; de Jonge et al. 2002; Ferreira et al. 2011). Restoration of natural oyster reefs is one approach to the nutrient pollution problem (Hernández et al. 2018; Duarte et al. 2020) and studies suggest oyster aquaculture could provide equivalent or greater water quality benefits than reefs (Zu Ermgassen et al. 2012; Froehlich et al. 2017; Campbell and Hall 2019).

A growing body of evidence demonstrates the value of bivalve aquaculture to mitigate eutrophication and nutrient pollution in the United States and abroad via bioextraction (Lindahl et al. 2005; Rose et al. 2014; Rose et al. 2015; Ferreira and Bricker 2016; Bricker et al. 2018; Clements and Comeau 2019; van der Schatte Olivier et al. 2020). Bioextraction refers to the permanent removal of nutrients and other particulate matter from the water column when certain aquaculture species are harvested, including bivalves. As suspension feeders, bivalves remove nutrients from the water column directly (i.e., feeding on phytoplankton sized nutrient rich particles) and indirectly (i.e., feeding on phytoplankton, which have already assimilated nutrients into their cellular structure). Bioextraction is an affordable and innovative option to mitigate nutrient pollution when implemented into water quality management strategies (Rose
et al. 2014) and bivalves are beginning to be introduced into such strategies (Cornwell et al. 2016; Reitsma et al. 2017).

While it is clear that bivalve aquaculture can mitigate eutrophication, the scale at which oyster farms provide water quality benefits is not well understood (Gentry et al. 2019). One reason being estimates of oyster clearance rates (CR) and filtration rates (FR), and other physiological measures of interest (Table 1.1), often do not account for seasonal variation in suspension feeding. Site-specific environmental variables are known to influence oyster feeding behavior (Cranford et al. 2011) such as hydrodynamics (Campbell and Hall 2019), water temperature (Comeau et al. 2008; Pernet et al. 2008), salinity (Casas et al. 2018a), and quantity and quality of suspended particles (Navarro and Iglesias 1993). Although suspension feeding is variable over both space and time, average physiological rates are often used in estimates of ecological filtration. Furthermore, traditional methods for measuring oyster CRs and FRs may overestimate oysters’ water quality benefits due to biases in sampling protocol where static water or laboratory diets, or both, were used (Kreeger et al. 2018). Comprehensive strategies for measuring oyster feeding behavior have been developed recently that overcome these biases (Hoellein et al. 2015; Galimany et al. 2018). Therefore, a detailed understanding of oysters’ CRs and FRs, under site-specific conditions, is possible and essential for a comprehensive estimation of oysters’ impact on water quality (Ehrich and Harris 2015).

Oyster aquaculture also serves as a mechanism for benthic-pelagic coupling such that oyster feces and pseudofeces (collectively known as “biodeposits”) settle to the sediment beneath and near oyster farms. These biodeposits introduce a new source of energy and nutrients to the benthic environment. Some studies have expressed concern for the potential negative effects of excessive biodeposits to local ecosystems and water quality such that biodeposits may inhibit denitrification processes at the benthic-pelagic boundary, cause benthic anoxia, or be
resuspended into the water column via local hydrology (Dame and Libes 1993; Kreeger et al. 2018). Additional studies have shown that these effects can be minimized if biodeposits are adequately dispersed and aquaculture gear appropriately stocked (Testa et al. 2015) and overall oyster aquaculture is considered ecologically sound (Hilborn et al. 2018). Moreover, in certain locations, biodeposits from restored or farmed oyster can enhance sediment denitrification thus providing another means to reduce nutrient pollution separate from bioextraction (Humphries et al. 2016; Donnelly 2021; Rose et al. 2021). The negative impacts that have been observed tend to be associated with areas of high oyster density, are found in poorly flushed ecosystems, and are limited to localized areas directly adjacent to the oyster farm (Lunstrum et al. 2018; Turner et al. 2019). To more holistically understand the role of oyster aquaculture in a coastal ecosystem, it is important to quantify what oysters are taking out of the water column via suspension feeding and putting back into the environment via biodeposition. This can be achieved by analyzing various physiological components of oyster feeding behavior.

The present study quantifies farm-specific year-round filtration services provided by three Eastern oyster (Crassostrea virginica) farms in the mid-Atlantic. To do this, filtration experiments were conducted seasonally in the field using ambient farm water to estimate FR, CR, rejection rate (RR), and absorption rate (AR) (hereafter collectively “filtration physiology”) of farm oysters (Table 1.1). Oyster filtration physiology was analyzed under a range of environmental conditions and associations with oyster behavior were explored. These experiments provide a robust dataset of oyster filtration physiology observed under natural conditions across farms and may be used in a broader framework to inform development of nutrient management strategies in the region.
Table 1.1 Definitions of the physiological components of absorptive balance for oysters (Iglesias et al. 1998). Modified from Galimany et al. 2017b.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ab.</th>
<th>Units</th>
<th>Definition</th>
<th>Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance rate</td>
<td>CR</td>
<td>L h⁻¹</td>
<td>The volume of water cleared of particles per unit of time.</td>
<td>(mg inorganic matter from both feces and pseudofeces per unit of time [mg h⁻¹]) / (mg particulate inorganic matter (PIM) in bay water [mg L⁻¹])</td>
</tr>
<tr>
<td>Filtration rate</td>
<td>FR</td>
<td>mg h⁻¹</td>
<td>The biomass of particles removed from water column per unit of time.</td>
<td>CR x total particulate matter (TPM) in bay water [mg L⁻¹]</td>
</tr>
<tr>
<td>Rejection rate</td>
<td>RR</td>
<td>mg h⁻¹</td>
<td>TPM that has been retained in the gills but rejected prior to ingestion</td>
<td>mg inorganic and organic matter from pseudofeces per unit of time</td>
</tr>
<tr>
<td>Absorption rate</td>
<td>AR</td>
<td>mg h⁻¹</td>
<td>Biomass of organic particles ingested and not egested as feces per unit of time.</td>
<td>(CR x particulate organic matter (POM) in bay water) – (mg organic matter from pseudofeces [mg h⁻¹]) – (mg organic matter from feces [mg h⁻¹])</td>
</tr>
</tbody>
</table>
1.3 MATERIALS AND METHODS

1.3.1 Study Site and Frequency

Seasonal, filtration experiments were conducted in the field at three different oyster farms in the mid-Atlantic between September 2020 and September 2021. These farms were located in Barnegat Bay (39° 36' 10.8'' N, 74° 18' 7.2'' W), Delaware Bay (39° 4' 16.4'' N, 74° 54' 47.5'' W), and Rehoboth Bay (38° 39' 0.3'' N, 75° 7' 41.8' W) (Figure 1.1). Each location had two sites: the oyster farm where oysters were collected and the experiment site where the filtration experiments were conducted. Each farm and experiment site pair were less than 1.4 km apart (1.27, 1.03, and 1.36 km respectively) such that no significant difference in water temperature was found between each farm/experiment pair (Barnegat Bay: t(2)=-1.07, p=0.40; Delaware Bay: t(3)=-0.61, p=0.58; Rehoboth Bay: t(1)=0.20, p=0.87) (Appendix A). The three farms represent a range of water quality conditions and used different farming methods: a coastal backbay habitat supporting a subtidal farm using floating and cage culture; a mudflat habitat supporting an intertidal farm using rack-and-bag culture methods; and an inland bay habitat supporting a subtidal farm using floating and longline culture, respectively.

Three filtration experiments were conducted at Barnegat Bay (July 2021, September and November 2020), four experiments at Delaware Bay (April, June, and August 2021; October 2020), and four experiments at Rehoboth Bay (April, July, and September 2021; November 2020). An additional experiment was attempted in late-November 2020 in Delaware Bay but the experiment failed due to an insufficient mass of biodeposit collection.
Figure 1.1. Locations of the three Eastern oyster farms where filtration experiments were conducted (created with Datawrapper). The northern site is a coastal backbay habitat in Barnegat Bay, New Jersey using floating and cage culture; the central site is a mudflat habitat in Delaware Bay, New Jersey using intertidal rack-and-bag culture methods; and the southern site is an inland bay habitat in Rehoboth Bay, Delaware using floating and longline culture. Color indicates farm location (blue represents Barnegat Bay, orange represents Delaware Bay, and grey represents Rehoboth Bay). Three experiments were conducted at Barnegat Bay, four experiments at Delaware Bay, and four experiments at Rehoboth Bay.
1.3.2 Physiology Experiments

A flow-through filtration chamber with ambient water was used during each experiment (n=11) to calculate filtration physiology values for three oyster farms (Galimany et al. 2018). The flow-through filtration chamber consisted of a central PVC reservoir tank and twenty smaller PVC feeding chambers (Figure 1.2). A submersible pump was used to pump ambient water from approximately 30 cm below the surface through a coarse filter (100 μm mesh) into the reservoir tank. The coarse filter prevented large pieces of detritus from clogging the connection tubing between reservoir tank and feeding chambers, and the sealed reservoir served to maintain constant water pressure and flow. Water flow through each of the twenty feeding chambers was set at 12 L hr⁻¹. This flow rate allowed a homogeneous distribution of particles among feeding chambers and precluded water recirculation within a feeding chamber (Galimany et al. 2011). The reservoir tank was aerated with two air stones to suspend particles throughout the tank allowing equal distribution of particles to each feeding chamber. The reservoir tank and filtration chambers were leveled prior to setting flow rates and introduction of oysters into the feeding chambers. Shade boxes were also placed over the feeding chambers (Figure 1.3).

Oysters were collected from the farm no more than four hours prior to the start of each experiment. Once collected, oysters were rinsed in ambient bay water, cleaned of all detritus, and all fouling organisms removed. Oysters used in each experiment represented the full range of sizes on the farm and were held out of water in a shaded area until the experiment began.

Prior to the start of an experiment, oyster gut transit time (GTT) was estimated following the methods detailed in Galimany et al. (2018) with the exception that water flow in the chamber was achieved with recirculation. In summary, five oysters from the farm were placed in a 30 L gut transit chamber (GTC) filled with 13 L of ambient bay water filtered to 1 μm (Figure 1.4). Each oyster was placed into one of five smaller containers (14 x 14 x 5 cm) located in the GTC. An
aquarium pump (Eheim’s CompactON 300, 79 GPH) continually circulated the filtered bay water through a baffle into each of the smaller containers, creating posterior to anterior flow over each oyster. This created a flow-through environment similar to that in the filtration chamber, while allowing cultured algae to be used.
Figure 1.2. Schematic of flow-through filtration chamber design. Panel (A) shows the entire apparatus including reservoir tank with 20 feeding chambers (modified from Galimany et al. 2018), (B) shows the side view of the head tank with arrows indicating flow of water, and (C) shows the front view with arrows indicating the flow of water from head tank to feeding chamber with baffles to provide appropriate water turbulence. Panel (A) feeding chambers outlined in red indicate location of oyster blanks during experiments.

Figure 1.3. Wooden shade boxes were placed over each feeding chamber assembly to maintain ambient water temperature throughout the flow-through filtration chamber system.
Figure 1.4. Schematic of the gut transit chamber (GTC) used prior to each experiment to estimate the time it takes oysters to generate biodeposits from time of ingestion. Panel (A) is a birds-eye-view of the GTC where the black box is an aquarium pump and dashed arrows indicate flow of water. Panel (B) is the side view with dashed arrows indicating flow of water through a small container. Panel (C) shows a picture of the GTC during an experiment.
Oysters were acclimated to the GTC for 10 minutes before algae (LPB Frozen Shellfish Diet, Reed Mariculture) was introduced to the system at a concentration of 300,000 cells mL\(^{-1}\). GTT was determined based on the time an oyster opened to the time the oyster started to produce green feces. When valve opening could not be accurately observed, the time elapsed between initial and green feces deposit was used as GTT. The average GTT of oysters in the GTC was used to offset the time of biodeposit collection in the filtration chamber. Oysters in the GTC that did not produce biodeposits, or produced biodeposits that did not turn green, were omitted from the final GTT calculation.

To begin each experiment, 18 oysters were placed in the filtration chamber between 120 and 10 minutes before high tide (t=0). Nine oysters were placed in feeding chambers on either side of the reservoir tank and one oyster blank (dead, clean shells glued together) was placed in the remaining feeding chambers indicated in red in Figure 1.2. Oyster blanks were used to account for particle deposition due to hydrodynamics around each oyster. Temperature, dissolved oxygen (DO), total dissolved solids (TDS), salinity, and pH of the incoming water was measured every 20 minutes starting at t=0 using a YSI handheld multiparameter meter (YSI ProDSS Water Quality Meter #626973) submerged adjacent to the submersible pump.

When the GTT had elapsed (t=GTT), all detritus and biodeposits that settled to the bottom of each feeding chamber were removed with minimal disturbance to the oysters. For the remainder of the experiment, the feces and pseudofeces produced by each oyster were carefully collected with a glass transfer pipette and stored separately in 50 mL Falcon tubes. Pipettes were rinsed in distilled water before collecting each biodeposit to protect samples from cross contamination. Care was taken to minimize the bay water deposited into each tube during biodeposit collection. When biodeposits could not be conclusively identified as feces or pseudofeces, the biodeposit was not collected. After two hours had elapsed (t = GTT + 2 hours),
oysters were removed from feeding chambers and the experiment terminated. Two hours was
generally sufficient time to collect enough biodeposits for processing; however, when needed,
biodeposits were collected for an additional 20 minutes.

Bay water samples (250 mL each) were collected every 20 minutes starting from t=0 from
three sources: the filtration chamber inflow (after the 100μm filter) and the outflow of the two
feeding chambers holding oyster blanks. If the experiment ran for 2 hours and 20 minutes, an
additional water sample was taken. Oysters, biodeposits, and water samples were stored on ice
and transported to the lab for further processing.

Each oysters’ FR, CR, RR, AR, absorption efficiency (AE), inorganic egestion rate (IER),
organic egestion rate (OER), inorganic rejection rate (IRR), and organic rejection rate (ORR) were
calculated using the biodeposition method detailed in Iglesias et al. (1998). This required the total
particulate matter (TPM), particulate organic matter (POM), and particulate inorganic matter
(PIM) content of the (1) 250mL water samples, (2) pseudofeces samples, and (3) feces samples
(hereafter “biological samples”) to be measured for each oyster in each experiment. To do this,
each biological sample was filtered through a pre-ashed (450°C), pre-weighed (10⁻⁵ g) 1.2 μm glass
microfiber filter (GF/C 25mm diameter) using a filtration manifold and vacuum pump (MultiVac
300-MS 3-Branch Stainless Steel Manifold, filtration flask, and Rocker300 oil-free vacuum pump)
consistent with Galimany et al. (2018). All feces and pseudofeces samples were diluted using
isotonic 1 μm filtered seawater to achieve a volume suitable to filter. When biological samples
contained a greater mass than the filter paper could support, a subsample was processed. Once
filtered, biological samples were rinsed with 5 mL of isotonic ammonium formate. All biological
samples were processed within 13 hours of experiment termination then stored frozen.
Frozen filters were dried at 60°C until a constant weight was achieved (~4.5 days). Each dry filter plus sample were weighed to determine TPM (mg L\(^{-1}\)) of each biological sample (TPM = dry filter plus sample weight – initial filter weight). Dried filters were then burned in a muffle furnace at 450°C for 4 hours and weighed a third time to determine PIM (mg L\(^{-1}\)) of each biological sample (PIM = burn filter plus sample weight – initial filter weight). POM was calculated by subtracting the burned sample weight from dry sample weight (Galimany et al. 2018).

Various measurements were also taken from the 23 oysters used in each experiment (18 oysters in filtration chamber, 5 oysters in the GTC). Shell length (umbo to growing edge) of the right valve was measured. Each oyster was shucked and dry tissue weight (DTW), as well as dry shell weight, were measured after drying for 72 hours at 60°C. Oyster condition (= DTW [g] / dry shell weight [g]) was also calculated. All physiological variables were standardized to 1 g DTW using the following equation:

\[ Y_s = Y_e \left(1/W_e\right)^b \]

where \(Y_s\) is the standardized physiological rate, \(Y_e\) is the experimentally determined rate, \(W_e\) is the measured dry tissue weight, and \(b = 0.73\) as determined by Riisgård (1988).

Physiology values were only determined for oysters that produced both feces and pseudofeces during an experiment. Likewise, oysters that produced both feces and pseudofeces at any point during an experiment were considered open (i.e., gaping) for the duration of the experiment as the feeding chambers with shade boxes made it difficult to observe gaping with the naked eye.

### 1.3.3 Farm Environmental Conditions

The water quality measurements made during each experiment (temperature, salinity, DO, pH, TDS, TPM, and the organic content of suspended solids in the water column [i.e.,
WC.Org], were also made monthly at each experiment location starting in May 2021. The goal of these water quality measurements was to quantify the environmental conditions oysters experienced between experiments. WC.Org [%] was calculated by dividing POM of a water sample by TPM. An additional monthly measurement was made at the Delaware Bay experiment site in July 2020, as well as an opportunistic set of measurements in early-May 2021 taken during an experiment not reported here. All discrete samples were taken in the 3 hours before high tide.

Loggers (Onset HOBO Conductivity Logger) were also deployed at each farm to measure temperature and salinity data at 30-minute intervals between July 2020 and September 2021 (Appendix B).

1.3.4 Statistical Analyses

All analyses were performed using RStudio version 4.1.1 (R Core Team 2021), and a p-value less than 0.10 was considered significant.

1.3.4.1 Water Quality

Water and seston characteristics (i.e., temperature, salinity, DO, pH, TDS, TPM, and WC.Org) were compared among experiment sites with a one-way analysis of variance (ANOVA) followed by post hoc Tukey’s test when significant differences were found. Water and seston measurements collected during an experiment were averaged prior to the ANOVA. Water and seston values collected during and between experiments were included in analyses.

1.3.4.2 Oyster Filtration Physiology

Median filtration physiology values (i.e., FR, CR, RR, and AR) and average oyster condition were compared among experiment sites with a one-way ANOVA followed by a post hoc Tukey’s test when significant differences were found.
Analyses were performed to relate water quality with oyster filtration physiology at two scales: local and global. “Local” indicates each farm was analyzed separately, while “global” indicates data from the three farms were analyzed together. Four water quality parameters (i.e., average temperature, salinity, TPM, and WC.Org) were compared against oyster filtration physiology to mitigate autocorrelation biases in analyses. TPM and WC.Org are inversely correlated; both seston variables remained in analyses. Median filtration physiology values were included in all analyses, as opposed to oyster-specific filtration physiology values, to avoid pseudoreplication and better represent the central tendency of oyster behavior in each experiment.

First, linear regression analyses were used to relate the environmental characteristics (i.e., average temperature, salinity, TPM, and WC.Org) with each filtration physiology at the local and global scale.

Next, relationships between the environmental variables and filtration physiology were compared with analysis of covariance (ANCOVA). Median FR, CR, RR, and AR as well as average temperature, salinity, TPM, and WC.Org were examined for normality (Shapiro-Wilk Test) and homogeneity of variance (Bartlett’s Test) and were square root transformed as required. In each ANCOVA, a median filtration physiology was compared over the experiment site (the factor) and one environmental variable (the continuous independent variable or “covariate”). Each ANCOVA produced two outputs concerning the global dataset. Using median FR and TPM as an example, an ANCOVA determined (1) whether TPM significantly influenced median FR across all farms and (2) whether there was a significant difference in FR among farms after controlling for TPM. A local post hoc Tukey’s test was performed when median FR, CR, RR, or AR differed significantly by location. ANCOVAs were not performed with more than one covariate due to small sample size.
Coefficient of variation (CV) values were also calculated for each experiment to quantify
the dispersion of FR, CR, RR, and AR values (hereafter FR.CV, CR.CV, RR.CV, and AR.CV).

1.3.4.3 Gaping

The proportion of oysters opened during each experiment were compared among
experiment sites with a one-way ANOVA followed by a post hoc Tukey’s test when significant
differences were found. Linear regression and ANCOVA (followed by a post hoc Tukey’s test as
necessary) were used to test the influence of average temperature, salinity, TPM, and WC.Org on
proportion of open oysters at the local and global scale.
1.4 RESULTS

1.4.1 Water Quality

No significant difference in water quality was found between Barnegat and Rehoboth Bays. Temperature, DO, and pH were not significantly different among the three farm locations; however, Delaware Bay had significantly lower salinity ($F(2,17)=23.84$, $p<0.0001$; Tukey HSD, $p=0.00014$ and $p<0.0001$), WC.Org ($F(2,17)=3.14$, $p=0.02$; Tukey HSD, $p=0.29$ and $p=0.07$), and TDS ($F(2,17)=4.39$, $p=0.03$; Tukey HSD, $p=0.10$ and $p=0.04$) and significantly higher TPM ($F(2,17)=4.90$, $p=0.02$; Tukey HSD, $p=0.06$ and $p=0.04$) than Barnegat and Rehoboth Bays, respectively. Seasonal trends in temperature and DO were evident at all farms with increased temperature and decreased DO in the summer (Figure 1.5).

The ordinal days displayed on Fig. 1.5, and throughout this thesis, are not from one continuous calendar year. Data collection began in September, July, and November 2020 at Barnegat, Delaware, and Rehoboth Bays, respectively, and ended in August 2021 at Barnegat and Delaware Bays and in September 2021 in Rehoboth Bay.
(A) Average Temperature (°C)
(B) Average Salinity (ppt)
(C) Average TPM (mg L⁻¹)
(D) Average WCOG (%)
(E) Average DO (mg L⁻¹)
(F) Average pH
(G) Average TDS (mg L⁻¹)
(H) Average Condition Index

**Location:**
- Barnegat Bay
- Delaware Bay
- Rehoboth Bay
Figure 1.5. Water quality measurements recorded at three oyster farms during, and between, filtration experiments including (A) temperature, (B) salinity, (C) TPM, (D) WC.Org, (E) DO, (F) pH, and (G) TDS. Measurements collected during experiments were averaged over ~3.5 hour period and have standard deviation (SD) bars displayed. Measurements collected between experiments were opportunistic and have no SD bars. All water quality measurements were taken within 3 hours of high tide. (H) Oyster condition (or condition index [CI]) was also measured for the oysters in each experiment. In all panels blue indicates data from Barnegat Bay, orange indicates data from Delaware Bay, and grey indicates data from Rehoboth Bay. In each panel, data to the left of the vertical dashed line were collected in 2020 and data to the right of the vertical dashed line were collected in 2021.
1.4.2 Oyster Filtration Physiology

Oyster filtration physiology was successfully calculated for all experiments (n=11, Figure 1.6). Results for other oyster filtration physiological parameters (IER, OER, IRR and ORR) can be found in Appendix C.

The Barnegat and Rehoboth Bay oysters showed a general seasonal trend in average filtration physiology where average FR, CR, RR, and AR were largest in the summer (June 21 – September 22) (Figure 1.6). This seasonal trend was not observed from Delaware Bay oysters as average filtration physiology was lower in August relative to the values observed in June and October. Average AE for oysters from Delaware Bay followed the same trend with 54.5, 87.3 and 85.6% AE respectively (Figure 1.7). Average condition increased seasonally at Delaware Bay (i.e., 6.44 to 7.32 from April to June) and dropped to 6.45 in August (Figure 1.6). Delaware Bay oysters had significantly higher condition than the other farms (F(2,227)=90.6, p=<0.0001; Tukey HSD, p<0.0001).

Oyster physiological activity at each farm varied across the calendar year. Oysters from Barnegat Bay were, on average, the most active, Delaware Bay oysters were moderately active, and Rehoboth Bay oysters were the least active (Figure 1.6). Median CR for oysters at Barnegat Bay was significantly higher than for oysters at Delaware and Rehoboth Bays (F(2,8)=4.01, p=0.06; Tukey HSD, p=0.08 and p=0.09, respectively).

Additional analyses comparing oyster median filtration physiology and farm location yielded conflicting results. No significant difference in oyster median FR (F(2,8)=1.36, p=0.31), RR (F(2,8)=1.12, p=0.37), or AR (F(2,8)=1.84, p=0.22) among farms was found. However, oyster median AR at Rehoboth Bay was significantly lower than at Barnegat and Delaware Bays after controlling for temperature and salinity, and oyster median RR at Delaware Bay was significantly
higher than Rehoboth Bay (Tukey HSD, p=0.08) after controlling for salinity (Table 1.2). Nevertheless, differences in average filtration physiology among farms and seasons were apparent.
Figure 1.6. Seasonal patterns in average filtration physiology parameters measured at three oyster farms including average (A) filtration rate (FR), (B) clearance rate (CR), (C) rejection rate (RR), and (D) absorption rate (AR). Filtration physiology was measured in the field during eleven seasonal experiments. Color indicates farm location (blue represents Barnegat Bay, orange represents Delaware Bay, and grey represents Rehoboth Bay) and error bars indicate standard deviation. In each panel, data to the left of the vertical dashed line were collected in 2020 and data to the right of the vertical dashed line were collected in 2021.
Figure 1.7. Absorption efficiency (AE) varied among location and time with no clear pattern. Each dot represents one oyster and color indicates oyster farm location. Extreme outlier values are included as colored triangles with the AE annotated adjacent to the corresponding point. Negative AE values indicate the oyster was not efficient in absorbing nutrients during the experiment. Data to the left of the vertical dashed line were collected in 2020 and data to the right of the vertical dashed line were collected in 2021.
Table 1.2. Results of ANCOVAs performed on five filtration behaviors. A yellow shaded cell under the “variable” column indicates a given environmental variable has a significant influence on the physiological variable regardless of location. A shaded cell under the “location” column indicates a significant difference in physiological variable among farms when the respective environmental variable is controlled for. A shaded cell under the “site” column contains the location pairs (blue for Barnegat Bay, orange for Delaware Bay, and grey for Rehoboth Bay) that differ via a post hoc Tukey’s test; “--” indicates no significant difference in the post hoc Tukey’s test.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Location</th>
<th>Site</th>
<th>Variable</th>
<th>Location</th>
<th>Site</th>
<th>Variable</th>
<th>Location</th>
<th>Site</th>
<th>Variable</th>
<th>Location</th>
<th>Site</th>
<th>Variable</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp.</td>
<td></td>
<td></td>
<td>F(1,7)=3.19, p=0.117</td>
<td>F(1,2)=1.37, p=0.316</td>
<td></td>
<td>--</td>
<td></td>
<td></td>
<td>F(1,7)=9.22, p=0.013</td>
<td>F(1,2)=5.26, p=0.040</td>
<td></td>
<td>F(1,7)=7.51, p=0.029</td>
<td>F(1,2)=2.39, p=0.162</td>
</tr>
<tr>
<td>Salinity</td>
<td></td>
<td></td>
<td>F(1,7)=4.31, p=0.078</td>
<td>F(1,2)=2.41, p=0.092</td>
<td></td>
<td>--</td>
<td></td>
<td></td>
<td>F(1,7)=8.33, p=0.023</td>
<td>F(1,2)=5.95, p=0.011</td>
<td></td>
<td>F(1,7)=14.2, p=0.007</td>
<td>F(1,2)=8.73, p=0.013</td>
</tr>
<tr>
<td>TPM</td>
<td></td>
<td></td>
<td>F(1,7)=5.01, p=0.060</td>
<td>F(1,2)=1.99, p=0.207</td>
<td></td>
<td>--</td>
<td></td>
<td></td>
<td>F(1,7)=2.81, p=0.127</td>
<td>F(1,2)=1.04, p=0.298</td>
<td></td>
<td>F(1,2)=2.72, p=0.133</td>
<td>F(1,2)=1.43, p=0.229</td>
</tr>
<tr>
<td>WC.Org</td>
<td></td>
<td></td>
<td>F(1,7)=3.12, p=0.121</td>
<td>F(1,2)=1.41, p=0.307</td>
<td></td>
<td>--</td>
<td></td>
<td></td>
<td>F(1,7)=0.07, p=0.500</td>
<td>F(1,2)=3.22, p=0.102</td>
<td></td>
<td>F(1,7)=1.69, p=0.252</td>
<td>F(1,2)=2.35, p=0.098</td>
</tr>
</tbody>
</table>
A range of physiological activity was observed during each experiment regardless of farm and time of year as indicated by the large standard deviation evident in Fig. 1.6. Qualitatively, this variation was driven by oysters that would either never, intermittently, or constantly produce biodeposits over the duration of each experiment. Differences in oyster FR.CV, CR.CV, RR.CV, and AR.CV were found among locations such that CVs at Rehoboth Bay were largest across all filtration physiologies and CVs at Barnegat Bay were consistently the smallest (Table 1.3).

Table 1.3. Coefficient of variation for each farm location and filtration physiology.

<table>
<thead>
<tr>
<th>Location</th>
<th>Filtration Rate CV</th>
<th>Clearance Rate CV</th>
<th>Absorption Rate CV</th>
<th>Rejection Rate CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barnegat Bay</td>
<td>0.47</td>
<td>0.48</td>
<td>0.66</td>
<td>0.60</td>
</tr>
<tr>
<td>Delaware Bay</td>
<td>0.58</td>
<td>0.59</td>
<td>0.83</td>
<td>0.61</td>
</tr>
<tr>
<td>Rehoboth Bay</td>
<td>0.74</td>
<td>0.77</td>
<td>2.04</td>
<td>0.83</td>
</tr>
</tbody>
</table>
Locally, the oyster population at each farm had unique physiological responses to environmental conditions (Table 1.4). Values reported in Table 1.4 represent all significant correlations present. Median filtration physiology of oysters from Delaware Bay was positively correlated with salinity, while median filtration physiology of oysters from Rehoboth Bay was positively correlated with temperature (Table 1.4). However, oysters from Barnegat Bay showed a mixed response such that median FR was negatively correlated with WC.Org and median CR, RR and AR were positively correlated with salinity, TPM, and temperature, respectively (Table 1.4).

When all farms were analyzed collectively (i.e., globally) a significant influence of salinity and temperature on oyster filtration physiology was found (Table 1.2) with the exception that temperature does not significantly influence median FR (F(1,7)=3.19, p=0.12) (Table 1.2). Most median filtration physiology parameters were positively associated with temperature and salinity, and negatively associated with WC.Org (Figure 1.8, Table 1.5). Associations were sometimes found globally between average TPM and median filtration physiology (Table 1.2, in contrast to Table 1.5); however, as oysters were exposed to higher TPM loadings individual oyster CRs decreased and FRs increased (Appendix C, Figure C.2).
Table 1.4. Results of linear regression of median physiology and environmental variables at each farm location. Correlations listed are the best fit environmental parameter for the given farm and physiology (e.g., the WC.Org is the best fit for Barnegat Bay oyster filtration rate). Shaded cells indicate significant correlation. Where more than one significant correlation was found, it is listed below. Correlations are positive unless specified by a “(−)” sign at the start of the cell.

<table>
<thead>
<tr>
<th>Filtration Physiology Variable</th>
<th>Barnegat Bay</th>
<th>Delaware Bay</th>
<th>Rehoboth Bay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median FR</td>
<td>(-) WC.Org: ( F(1,1)=263.4, p=.04, R^2=.99 )</td>
<td>Salinity: ( F(1,2)=2.22, p=.27, R^2=.52 )</td>
<td>Temp.: ( F(1,2)=16.4, p=.06, R^2=.89 )</td>
</tr>
<tr>
<td>Median CR</td>
<td>Salinity: ( F(1,1)=4.35, p=.28, R^2=.81 )</td>
<td>Salinity: ( F(1,2)=7.08, p=.12, R^2=.78 )</td>
<td>Temp.: ( F(1,2)=4.10, p=.18, R^2=.67 )</td>
</tr>
<tr>
<td>Median RR</td>
<td>TPM: ( F(1,1)=2026, p=.01, R^2=.99 )</td>
<td>Salinity: ( F(1,2)=1.73, p=.32, R^2=.46 )</td>
<td>Temp.: ( F(1,2)=19.9, p=.05, R^2=.91 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TPM: ( F(1,2)=8.6, p=.10, R^2=.81 )</td>
</tr>
<tr>
<td>Median AR</td>
<td>Temp.: ( F(1,1)=1956, p=.01, R^2=.99 )</td>
<td>Salinity: ( F(1,2)=39.2, p=.02, R^2=.95 )</td>
<td>Temp.: ( F(1,2)=10.8, p=.08, R^2=.84 )</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TPM: ( F(1,2)=11.0, p=.08, R^2=.85 )</td>
</tr>
<tr>
<td>Proportion Opened</td>
<td>(-) TPM: ( F(1,2)=19.5, p=.14, R^2=.95 )</td>
<td>Salinity: ( F(1,2)=76.1, p=.01, R^2=.97 )</td>
<td>(-) WC.Org: ( F(1,2)=20.8, p=.04, R^2=.91 )</td>
</tr>
</tbody>
</table>
Figure 1.8. Linear regressions performed globally (across all farms) between median filtration physiology and environmental variables including temperature (light blue), salinity (black), TPM (brown), WC.Org (dark green). Each colored dot represents one experiment (n=11 for each environmental variable). Statistical results of each regression are listed in Table 1.5.
Table 1.5. Results of linear regressions performed globally (across all farms) between median filtration physiology and environmental variables (expressed visually in Figure 1.8).

<table>
<thead>
<tr>
<th>Physiological Variable</th>
<th>Average Temperature (°C)</th>
<th>Average Salinity (ppt)</th>
<th>Average TPM (mg/L)</th>
<th>Average WC.Org (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median FR</td>
<td>$F(1,9)=3.64, p=.09, R^2=.29$</td>
<td>$F(1,9)=.87, p=.38, R^2=.09$</td>
<td>$F(1,9)=1.48, p=.25, R^2=.14$</td>
<td>$F(1,9)=3.44, p=.10, R^2=.28$</td>
</tr>
<tr>
<td>Median CR</td>
<td>$F(1,9)=4.60, p=.06, R^2=.34$</td>
<td>$F(1,9)=3.60, p=.09, R^2=.29$</td>
<td>$F(1,9)=.42, p=.53, R^2=.04$</td>
<td>$F(1,9)=.005, p=.95, R^2&lt;.001$</td>
</tr>
<tr>
<td>Median RR</td>
<td>$F(1,9)=2.58, p=.14, R^2=.22$</td>
<td>$F(1,9)=.44, p=.52, R^2=.05$</td>
<td>$F(1,9)=2.05, p=.19, R^2=.19$</td>
<td>$F(1,9)=4.15, p=.07, R^2=.32$</td>
</tr>
<tr>
<td>Median AR</td>
<td>$F(1,9)=7.18, p=.03, R^2=.44$</td>
<td>$F(1,9)=2.36, p=.16, R^2=.21$</td>
<td>$F(1,9)=.17, p=.69, R^2=.02$</td>
<td>$F(1,9)=1.06, p=.33, R^2=.10$</td>
</tr>
<tr>
<td>Proportion Opened</td>
<td>$F(1,9)=.83 p=.38, R^2=.08$</td>
<td>$F(1,9)=.05, p=.82, R^2&lt;.01$</td>
<td>$F(1,9)=.28, p=.61, R^2=.03$</td>
<td>$F(1,9)=.95, p=.36, R^2=.10$</td>
</tr>
</tbody>
</table>
1.4.3 Gaping

At no point during a filtration experiment did all oysters (n=18) open and actively filter (Figure 1.9). The average proportion open during a filtration experiment was 0.70 ± 0.20 SD and a significant difference among farms (F(2,9)=4.53, p=0.04) was observed where a larger proportion of oysters from Barnegat Bay opened than Rehoboth Bay (0.90 and 0.54 respectively; Tukey HSD, p=0.04). When controlling for the influence of salinity, the proportion of oysters from Rehoboth Bay that opened was significantly less than oysters from the other two bays (F(1,7)=7.75, p=0.02) (Table 1.2).

A seasonal trend in proportion open was evident at Rehoboth Bay where more oysters opened in the warmer months (Figure 1.8a). No seasonal trend in proportion of open oysters was evident at Delaware or Barnegat Bay (Figure 1.8a).

Locally, the proportion of oysters open at Delaware Bay were significantly positively associated with salinity and at Rehoboth Bay the proportion open were significantly negatively associated with WC.Org (Table 1.4). Globally, the proportion of opened oysters did not correlate with temperature, salinity, TPM, or WC.Org (Tables 1.2 and 1.5).
Figure 1.9. Gaping behavior of oysters among farms. (A) Seasonal patterns in the proportion of oysters that opened during each filtration experiment. Color indicates farm location (blue indicates data from Barnegat Bay, orange indicates data from Delaware Bay, and grey indicates data from Rehoboth Bay). (B) Linear regressions performed globally (across all farms) between proportion of open oysters and environmental variables including temperature (light blue), salinity (black), TPM (brown), WC.Org (dark green). Each colored dot represents one experiment (n=11 for each environmental variable). Data to the left of the vertical dashed line were collected in 2020 and data to the right of the vertical dashed line were collected in 2021. Statistical results of each regression were all non-significant.
1.5 DISCUSSION

1.5.1 Oyster Filtration Physiology

The filtration physiology of oysters analyzed in this study differed among locations and across seasons. The range in physiological activity observed in this study is within the range of filtration physiology values reported across the Eastern oyster range via comparable field conditions (Table 1.6). A robust review of existing filtration physiology values is outside the scope of this study, but a brief comparison between the results listed in Table 1.6 and the oysters analyzed in this study verifies that these fall among those previously reported values.
Table 1.6. Site-specific filtration physiology and environmental values that have been reported in other studies from across the range of Eastern oyster habitat.

<table>
<thead>
<tr>
<th>Location</th>
<th>Date</th>
<th>Temp. (°C)</th>
<th>Oyster Source</th>
<th>CR (L h⁻¹ g⁻¹)</th>
<th>Additional Physiologies (mg h⁻¹ g⁻¹)</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Natural Diet - Laboratory Experiments</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prince Edward Island, Canada</td>
<td>Sept. 2012</td>
<td>16</td>
<td>Farm, Bottom-grown</td>
<td>6.3</td>
<td>Not reported.</td>
<td>Comeau 2013</td>
</tr>
<tr>
<td>Price Edward Island, Canada</td>
<td>Sept. 2012</td>
<td>16</td>
<td>Farm, Floating-cage</td>
<td>4.3</td>
<td>Not reported.</td>
<td>Comeau 2013</td>
</tr>
<tr>
<td><strong>Natural Diet – Field Experiments</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Hampshire (low nutrient site)</td>
<td>July 2012</td>
<td>25</td>
<td>Wild, Subtidal bed</td>
<td>3.51</td>
<td>FR: 38.01 AR: 5.27</td>
<td>Hoellein et al. 2015</td>
</tr>
<tr>
<td>South Carolina (Intertidal reefs)</td>
<td>May 2005</td>
<td>22-27</td>
<td>5 constructed and 3 natural reefs</td>
<td>1.0</td>
<td>Not reported.</td>
<td>Grizzle et al. 2008</td>
</tr>
<tr>
<td>Florida (Mosquito Lagoon)</td>
<td>March and August 2015</td>
<td>23.3 and 29.6</td>
<td>Farm, Subtidal</td>
<td>2.06</td>
<td>FR: 40.18 AR: 5.21</td>
<td>Galimany et al. 2017a</td>
</tr>
<tr>
<td>Florida (Mosquito Lagoon)</td>
<td>3 days in March 2015</td>
<td>27.8</td>
<td>Farm, Subtidal</td>
<td>2.75</td>
<td>FR: 47.12 RR: 28.76 AR: 5.60</td>
<td>Galimany et al. 2017b</td>
</tr>
<tr>
<td>Florida (Banana River)</td>
<td>March and August 2015</td>
<td>24.1 and 29.2</td>
<td>Farm, Subtidal</td>
<td>2.35</td>
<td>FR: 17.84 AR: 3.43</td>
<td>Galimany et al. 2017a</td>
</tr>
<tr>
<td>Florida (Indian River)</td>
<td>March and August 2015</td>
<td>25.3 and 30.5</td>
<td>Farm, Subtidal</td>
<td>2.85</td>
<td>FR: 36.92 AR: 4.69</td>
<td>Galimany et al. 2017a</td>
</tr>
</tbody>
</table>
The difference in filtration physiology evident among farms suggests the oyster populations analyzed in this study were acclimated to their own unique environment and could alter their feeding behavior to meet changing metabolic demands. Indeed, Barnegat and Delaware Bay oysters had high median AE, 72.6 and 82.9% respectively, which suggests these oyster populations optimized feeding when exposed to endogenous and exogenous stressors (Bayne et al. 1987; Bayne and Hawkins 1988; Ibarrola et al. 2000; Hall et al. 2020). Barnegat and Delaware Bay oysters had the greatest physiological activity and the lowest CVs, further supporting the acclimation of these populations to their habitat. Oysters from Rehoboth Bay had a median AE of 56.4% suggesting these oysters did not absorb nutrients optimally as reflected in the overall low physiological activity at that farm (Figure 1.5), which could be linked to oyster acclimation to the high WC.Org values seen in Rehoboth Bay (Cammen 1980, Galimany et al. 2017a; in contrast Taghon 1981, Bayne et al. 1984). The site-specific nature of oyster physiology is recognized broadly (see references in Table 1.6).

Across the farms analyzed in this study, temperature, salinity, TPM, and WC.Org were associated with changes in filtration physiology, but temperature and salinity had the most prominent association (Table 1.2). As temperature and salinity decreased so did median FR, CR, RR, and AR, regardless of farm location (Figure 1.8).

This positive relationship between temperature and oyster physiological activity is well known (Casas et al. 2018b; Kinsella 2019). In this study a linear relationship was observed; however, seasonal CR experiments using farmed Crassostrea angulata oysters in southern China showed a non-linear trend between CR and temperature with CR measurements made in the field between 16.7 and 30.2°C such that maximum CR was found at 24.9°C (Yu et al. 2017). This non-linear relationship between oyster physiological activity and temperature is reflected elsewhere in a Dynamic Energy Budget model for Crassostrea virginica (Filgueira et al. 2014). Studies suggest
the optimal temperature for all Eastern oyster physiology lies between 16-28°C (Loosanoff 1958; Casas et al. 2018a). The highest experimental temperature in this study was 29°C in Rehoboth Bay which may not have been high enough above the thermal optimum (16-28°C) to see a decrease in oyster physiological activity. The lowest experimental temperature in this study was recorded for Delaware Bay oysters examined in November 2020 (10.5°C, Figure 1.1) and biodeposit production was so low for this experiment, filtration physiology could not be calculated. Cooler temperatures slow the ectothermic oyster metabolic rate and reduces growth (Zuo et al. 2012) and the viscosity of the cooler water increases the energy necessary for cilia to function (Ward and Shumway 2004; Humphries 2013). Warmer temperatures with corresponding low oxygen levels may require oysters to transition into anaerobic respiration (less energetically favorable than aerobic respiration) (Pörtner 2010), which was likely not observed in this study as the filtration chamber was aerated.

It is possible the thermal tolerance of oysters examined in this study influenced how strongly oyster filtration physiology was associated with temperature. Oysters at Delaware Bay may have a higher thermal tolerance because the farm is intertidal and the oysters are exposed to extremely high temperatures during low tide exposure, whereas oysters from the other subtidal farms are not (Appendix B). This elevated thermal tolerance may explain why filtration physiology of oysters from the Delaware Bay farm was not associated with temperature. In contrast, filtration physiology of oysters from Rehoboth Bay increased in concert with temperature more so than the oysters at the other two farms. This may be related to the genetic diversity of Rehoboth and Barnegat Bay oyster stocks. Rehoboth Bay oyster seed largely came from one supplier while Barnegat Bay seed came from multiple suppliers: this may lead the oyster population at Rehoboth Bay to be less genetically diverse than at Barnegat Bay. Greater genetic diversity, presumably, would allow an oyster population to be physiologically resilient over
greater temperature extremes because each genetic strain may have a unique thermal tolerance. It is therefore possible the Rehoboth Bay oyster population was dominated by a genetic strain with lower thermal tolerance and were thus more sensitive to local changes in temperature.

Increased oyster physiological activity with increased salinities has been previously reported (Gray and Langdon 2019). Seasonal experiments conducted in Louisiana found oysters kept at about 22.8 ppt and subsequently exposed to three weeks of low salinity conditions (i.e., 3, 6, 9, 15, and 25 ppt) had larger CRs at 15 and 25ppt than in the lower salinity conditions (Casas et al. 2018a). These results of higher CR in higher salinity were more pronounced in summer than winter (Casas et al. 2018a), when the influence of temperature on oyster filtration physiology discussed above may play a role. In contrast, a Florida field study of oysters naturally exposed to salinity conditions between 21.9 and 33.1 ppt found that while salinity was positively correlated with absorption efficiency, no correlation was found with CR, FR, or AR (Galimany et al. 2017a). Although no significant correlation among salinity and physiology was observed, Galimany et al. (2017a) note that oyster physiological rates were highest in experiments with the highest salinity.

Oysters are known to tolerate 5 to 40 ppt (Shumway 1996) with an optimum salinity range estimated between 10 and 28 ppt (Loosanoff 1965). Low salinity conditions are harmful to oysters because the water chemistry hinders metabolism and intracellular ion and acid base regulation (Ballantyne and Berges 2011; Dickinson et al. 2012). High salinities are therefore generally favorable for oysters. As such, it is expected that filtration physiology of oysters examined in this study would be depressed by low salinity conditions, which is seen clearly in the global association between median CR and average salinity in this study. The more muted influence of salinity on median FR, RR, and AR globally in this study may be because salinities tested were all within the oysters’ optimal salinity range (Hoellein et al. 2015, Galimany et al. 2017a) and that oysters were not exposed to multiple weeks at dramatically lower salinity conditions as seen in Casas et al.
(2018a). However, the filtration physiology of oysters in this study was tested over an 8.6 ppt range in salinity (experiments conducted between 21.0 - 29.6 ppt) which was sufficient to detect the influence of salinity on clearance rate. Filtration physiology of oysters from Delaware Bay was the most positively influenced by salinity, which may be due to the fact that Delaware Bay had significantly lower salinity than the other two locations. Bivalves that experience conditions closer to the thresholds of their environmental optimum (like the 10-28 ppt optimum salinity for oysters) have been found to be more sensitive to changes in those environmental conditions (Galimany et al. 2017a).

The trends in filtration physiology were not uniform among locations with respect to TPM and WC.Org. While an increase in TPM corresponded to an increase in median FR, RR, and AR, this relationship was clearest in Barnegat and Rehoboth Bays for median RR. Furthermore, while an increase in WC.Org was linked with a decrease in median FR and RR at all farms, the relationship to WC.Org and median FR was strongest in Barnegat Bay. These trends are likely related to the selective feeding ability of oysters. Oysters have several mechanisms by which they can maximize the organic:inorganic ratio of particles ingested (Newell and Jordan 1983; Shumway et al. 1985; Ward et al. 1998; Espinosa and Allam 2021) including rejecting excess inorganic matter as pseudofeces through pre-ingestive selection (Newell and Jordan 1983; Hawkins et al. 1996; Ward and Shumway 2004). Pre-ingestive selection is particularly beneficial in turbid environments with elevated TPM (Hawkins et al. 1996) and evidence suggests oysters can be more effective at this selection mechanism compared to other bivalves (Galimany et al. 2017b). Oysters in Barnegat and Rehoboth Bays perform as expected: as TPM increased so did RR, suggesting efficient pre-ingestive selection. This trend was not seen in oysters from Delaware Bay, possibly because environmental conditions there preclude efficient pre-ingestive selection. Delaware Bay had significantly higher TPM and lower organic content than the other two farms. At a certain species-
specific particle concentration, it becomes energetically unfavorable for oysters to feed as their gills become clogged with high particle concentrations (Widdows et al. 1979; Bayne and Newell 1983; Gray and Langdon 2019). *Crassostrea gigas*, for example, can maintain constant feeding up to a natural seston TPM of 50 mg L⁻¹, but CR declines as TPM increases above that threshold (Barillé and Prou 1993). In this way the environmental conditions at the Delaware Bay site may be less favorable to oysters that may also be experiencing low salinity as discussed earlier.

The lack of a seasonal trend in oyster filtration physiology at Delaware Bay may be explained by rain events that occurred the day before the August 2021 experiment. Water quality measurements taken during the August rain event recorded the lowest salinity measurement (17.8ppt), higher than average TPM (413.7 mg L⁻¹), and low WC.Org (13.0%) at the Delaware Bay site. While conditions during the August experiment date were relatively normal, it is possible the storm the day prior disturbed the oysters in such a way that depressed feeding behavior the following day. This hypothesis agrees with CRs measured seasonally in Virginia (via a mesocosm experiment) which showed a similar seasonal trend in CR to Barnegat and Rehoboth Bay oysters, even in the summer when total suspended solids (TSS) were high (Kelly et al. 2011). Unlike Delaware Bay, CRs reported by Kelley et al. (2011) may not have been depressed in the summer because maximum TSS (49.3 mg L⁻¹) remained below the 50mg/L (Barillé and Prou 1993) and 90 mg L⁻¹ (Barillé et al. 1997) thresholds estimated for *Crassostrea gigas*. This underscores the value of making conservative estimates of ecological filtration to account for days of low physiological activity triggered by increased sedimentation (Poirier et al. 2021) or other environmental stressors.

A range of filtration physiology values were observed among individual oysters during each experiment. Previous studies have anticipated feeding rates in nature would vary more than in a laboratory physiology experiment (Grizzle et al. 2008). Other experiments have shown a range
of filtration physiology values similar to this study (Kelly et al. 2011), with some studies showing less variability than those reported here (Galimany et al. 2017a; 2017b). Moreover, bivalves are hypothesized to operate at two levels of filtration activity independent of season and food quality and quantity: a high and a low “gear” as a result of an unidentified physiological switch (Powell et al. 1992). Low gear CRs align with values observed in the field which suggests oysters are not feeding at their maximum rates in-situ (Powell et al. 1992). This bimodal behavior was observed when measuring CR of individual subtidal wild oysters in aquaria over a period of 24 to 33 hours (Palmer 1980). All oysters were fed algae and most oyster feeding was discontinuous such that CR fluctuated from high to low gears over three- to twelve- hour periods with no definitive evidence of exogenous factors controlling the switch (Palmer 1980). Each experiment in this study lasted approximately two hours so oysters observed may have been in a mix of high and low gear behavior. This bimodal behavior does not negate the relevance of the results discussed in this study as these two-hour experiments provide a random, but robust, snapshot of oyster filtration physiology.

1.5.2 Gaping

The opening behavior of oysters observed in this study was site-specific and varied with local environmental conditions. This lack of a global association between oyster opening behavior and an environmental parameter suggests there may be an exogenous or endogenous variable not examined in this study that controls the proportion of oysters that open at any given time. However, higher salinities (Casas et al. 2018a) and warmer temperatures (Comeau et al. 2012) are correlated with increased duration of time an oyster remains open. In this study, an increase in salinity was associated with an increase in the proportion of oysters open in Delaware Bay; but this may be related to hypothesized sensitivity of oysters to salinity in this population. Specifically, oyster valve opening behavior in Delaware Bay may have been more sensitive to increases in
salinity than oysters in Barnegat or Rehoboth Bays because oysters in Delaware Bay experienced significantly lower salinity than the other two farms. Casas et al. (2018a) and Comeau et al. (2012) continuously monitored oyster valve opening over a period of 10 and 147 days respectively, suggesting the opening behavior examined herein may have been collected over too short a period to identify opening in response to environmental conditions. Handling oysters within hours of these observations may also have altered their opening behavior.

The only significant difference in proportion of open oysters was evident between subtidal farms such that a greater proportion of open oysters occurred in Barnegat than Rehoboth Bay. This suggests subtidal and intertidal oysters can have similar gaping behavior during high tide, although differences in oyster opening behavior during low tide at subtidal farms were not examined in this study.

In general, the proportion of oysters open and actively feeding varied among locations in this study. The same has been observed in other studies, ranging from: 38.9 to 74.1% of oysters opened across three populations in Florida (Galimany et al. 2017a); 63% of oysters opened over a period of three days in Florida (Galimany et al. 2017b); and oysters examined for a long duration (113 days and 34 days) were open 68.6 and 79.7% of the time respectively in the Gulf of St. Lawrence (Comeau et al. 2012). These results, in combination with the results of this study, suggest oysters do not all feed at the same time.

**1.5.3 Implications to the Biodeposition Method**

Improvements can be made to the biodeposition method that may increase the accuracy of future filtration physiology studies. The largest inconsistency among biodeposition experiments concerns when to start collecting biodeposits. In this study, biodeposits were collected after an experiment-specific GTT had elapsed to ensure biodeposits collected were a
product of ambient water flowing through the filtration chamber. The GTT protocols used in this study provide a good estimate of GTT, but the methods herein assumed all oysters in the filtration chamber started to feed when submerged in the filtration chamber. Qualitative observations indicated some oysters did not begin to feed until the final 20-minutes of the experiment, but those biodeposits were still analyzed as time of initial biodeposit production were not recorded in this study. This likely contributed to some of the variation observed in filtration physiology during each experiment. This is because it is possible the initial biodeposits produced by an oyster in the flow-through chamber were generated from farm water in the hours or day before an experiment (oysters can remain closed for 8 hours at a time under ambient conditions [Poirier et al. 2021] and seston can remain in an oyster’s digestive system for more than 7 hours [Bayne et al. 1984]). Water quality measurements were generally not taken prior to each experiment, so it is not possible to confirm similarity with water samples taken during each experiment. As such, if an oyster’s initial biodeposits were used to calculate filtration physiology, these biodeposits may have been generated from water of drastically different quality than that observed during an experiment thus biasing results.

Other studies using varying approaches may likewise bias experimental results by omitting oysters from analyses based on specific behavioral traits, such as: removing oysters from analyses if biodeposits were not produced in the first hour (Hoellein et al. 2015); removing a set of oysters from a physiological model when measured CRs at 20°C were too low (Newell and Koch 2004); or not accounting for GTT at all. While no method may overcome all biases, possible solutions to minimize bias include taking biodeposits from multiple oysters at once and averaging those values (Zu Ermgassen et al. 2013; Gray and Langdon 2019) or measuring filtration over a period of days (Palmer et al. 1980; Yu et al. 2017). Observers should also take care in identifying
a feeding oyster based on valve gape via the naked eye as oysters may clear water with a gape as small as 0.2 μm (Gray and Langdon 2019).

1.5.4 Implications to Ecological Estimates of Filtration

The filtration physiology data collected in this study will help to improve ecological estimates of oyster water quality benefits in several ways. First, the seasonal CR values could be used in models to more accurately estimate water quality improvements provided by local oyster populations. When site-specific data are unavailable, models typically use a maximum CR from the literature derived under laboratory conditions (e.g., Gray et al. 2021) and modify the maximum CR value across the spatiotemporal limits established in the model based on environmental variables (e.g., water temperature, salinity, current speed, chlorophyll a, POM, TPM, TSS) (Ehrich and Harris 2015; Gray et al. 2021). The seasonal CR values calculated in this study not only provide the requisite site-specific CR data, these CR values could also be used broadly as a more robust “maximum CR” because the values are based on field experiments.

Furthermore, the observed relationships between environmental parameters and filtration physiology could also be used to improve the process of modifying the “maximum CR” value. It is well understood that models estimating ecological filtration need to incorporate the influence of environmental drivers on individual oyster CRs rather than using average physiological rates (Ferreira et al. 2007; Ehrich and Harris 2015; Cubillo et al. 2018; Gray et al. 2021), but some existing models do not include sensitivity thresholds reflective of those observed in this study. For example, CR at the farms examined in this study decreased in salinities ranging from between 21.0 - 29.6 ppt, yet previous models have assumed salinities above 7.5, 10.3, and 12.1 ppt have no impact on CR (Powell et al. 1992; Cerco and Noel 2005; Fulford et al. 2007; Ehrich and Harris 2015). Temperature and salinity had the largest influence on oyster filtration
physiology in this study and models may therefore benefit from physiological model parameters that respond to these two environmental variables.

Incorporating information regarding the proportion of oysters opened would also improve the accuracy of models estimating ecological filtration. In this study the proportion of open ranged from 47.1 – 94.4% indicating oysters in a population do not all feed at the same time. Although the proportion of oysters open has been used to model clearance in oyster populations (Comeau et al. 2013), it remains an important yet frequently overlooked component of modeling oyster ecological filtration.

Finally, the filtration physiology collected in this study captures seasonal information regarding what oysters remove from the water column and what they put back into the environment via biodeposition. Ecological estimates of oyster water quality benefits would be improved by this information as models estimating population- or ecosystem-scale oyster filtration tend to focus on CR and FR, without also estimating for the biomass of deposits to the benthic system. While the impacts of bivalve aquaculture are localized (Lunstrum et al. 2018; Turner et al. 2019) and biodeposits can effectively enhance net ecosystem losses of nitrogen and phosphorus (Newell et al. 2005; Humphries et al. 2016; Rose et al. 2021) the reintroduction of nutrients to the benthos should not be ignored. Indeed, a recent study found juvenile oysters (<14 months) contribute more nutrient rich biodeposits to the benthic environment than older oysters (Locher et al. 2021) which could be relevant to the young age classes typically found on oyster farms. Furthermore, reporting CR and FR estimates alone may give the false impression that bivalves absorb all of what they take in, whereas a comparison of FR and RR is more accurate for bioextraction purposes. For example, an oyster observed in July 2021 from Barnegat Bay deposited at least 218.1 mg h⁻¹ g⁻¹ (RR includes information regarding pseudofecal biodeposits only) but removed 253.6 mg h⁻¹ g⁻¹ when feeding. The farms examined in this study generally show
a seasonal trend in FR and RR such that a larger biomass of particles was removed from the water column and reintroduced into the benthic environment in warmer months. This increase in biodeposit production has been linked elsewhere with an increase in chlorophyll a in the water column (Hayakawa et al. 2001) which agrees with the correlation among median RR and WC.Org in this study (Table 1.2 and 1.5). While stand-alone models to predict biodeposition at bivalve farms (Hayakawa et al. 2001; Weise et al. 2009; Testa et al. 2015) and models to connect estimates of water quality improvements and settling biodeposits (Granada et al. 2018) exist, additional models are needed to provide a more holistic understanding of oyster farms’ ecological influence (Newell 2007).

1.5.5 Implications to Aquaculture

The seasonal water quality benefits described in this study via FR, CR, and RR support the use of aquaculture as a tool for nutrient management in coastal waterbodies. The impact of oyster farms to local water quality can be negligible given site-specific conditions like high flushing rates that dilute improvements over a greater volume of water (Turner et al. 2019), but understanding how individual farms contribute to water quality improvement can help water quality managers incorporate oyster aquaculture into robust nutrient reduction strategies. A robust nutrient reduction strategy is one that compares the expected water quality benefits generated by all feasible nutrient reduction best management practices (BMPs) for an area on a per-acre basis. In reality, the nutrient reduction strategy for a water body will be a combination of complementary BMPs: oyster aquaculture is a tool, not the “silver bullet”, in the nutrient mitigation toolbox.

An estimate of year-round water quality benefits per-acre show the farms in this study collectively cleared $8.6 \times 10^9$ L acre$^{-1}$ year$^{-1}$ of water, removed 293.4 tons acre$^{-1}$ year$^{-1}$ of particulate matter, and deposited 229.3 tons acre$^{-1}$ year$^{-1}$ of pseudofeces into the water column (Appendix D). These values were based on the standing stock of oysters at each farm (each farm managed
between 100,000 and 1 million oysters) and farm acreage (oysters, in farming gear, covered no more than 1.5 acres at each farm). Thus, the farms examined in this study collectively removed an estimated 64.1 tons acre\(^{-1}\) year\(^{-1}\) of particulate matter from the ecosystem. Interestingly, Delaware Bay farm was estimated to provide similar water quality benefits to the Rehoboth Bay farm when comparing FR and RRs, which suggests intertidal farms can provide water quality benefits at a similar scale to subtidal farms.

When planning a new oyster farm, the location and size will influence water quality benefits (Ferreira et al. 2007). Location, as discussed in this study, impacts oyster filtration physiology by exposing oysters to a site-specific mixture of environmental conditions. Size of an oyster farm impacts the biomass of oysters on the farm and thus influences the number of oysters feeding at any given time. A model based on the values calculated in this study (Appendix D) indicated that water quality benefits scaled with biomass on each farm such that when more oysters were located on a given farm the CR, FR, and RR would increase. Comparisons like this may help water quality managers and farmers plan future farms.
1.6 CONCLUSION

Oyster farms in the mid-Atlantic exhibit similar seasonal trends in filtration physiology, but these trends are driven by site-specific environmental conditions. Increased feeding activity was associated with increased temperature and salinity. Additional environmental variables were found to influence filtration physiology (i.e., TPM and WC.Org), but not as ubiquitously as temperature and salinity. Beyond seasonal trends, each farm had a different level of filtration activity highlighting the importance of conducting filtration physiology studies in the field on a site-specific basis. Delaware Bay oysters did not demonstrate a clear seasonal trend as oysters analyzed in August 2021 exhibited depressed filtration physiology which may be attributed to a precipitation event that disrupted local water quality the day prior to the experiment. As the intensity and frequency of rainfall events along the east coast of the United States increases with climate change (Sanderson et al. 2019; Maxwell et al. 2021) conservative estimates of ecological filtration may be necessary.

Our results support the use of oyster aquaculture as a means to mitigate nutrient pollution in coastal waterbodies and provide data modelers can use to improve ecological estimates of aquaculture water quality benefits. While no field-based study is perfect, this study provides one of the first estimates of year-round filtration activity of Eastern oysters and will inform the sustainable growth of the United States oyster aquaculture industry.
1.7 REFERENCES


CHAPTER 2 – CHANGES IN WILD AND FARM EASTERN OYSTER (*CRASSOSTREA VIRGINICA*)

FEEDING BEHAVIOR IN RESPONSE TO SIMULATED PRECIPITATION EVENTS IN THE FIELD

2.1 ABSTRACT

In the Delaware Bay, like many major watersheds along the east coast of the United States, climate change is projected to increase the frequency and intensity of storms (*i.e.*, precipitation). These storms will result in more low salinity events across the system which may impact local ecology. The Delaware Bay has a large Eastern oyster (*Crassostrea virginica*) population which consists of a fishery, estimated to include over 3 billion adult oysters, and a growing aquaculture industry. Oyster filtration is known to vary in response to a number of local conditions including water temperature, food quality and quantity, and salinity. It is generally accepted that oysters thrive in higher salinity conditions, but the impact of short-term low salinity events on oyster feeding behavior is not well understood. In this study, seasonal field experiments were conducted between August 2020 and August 2021 to estimate the impacts of a Hurricane Sandy scale salinity disturbance on wild and farm oyster filtration physiology (*i.e.*, filtration, clearance, rejection, and absorption rates). These experiments show wild and farm oyster populations in the Delaware Bay provide reduced water quality benefits during a Hurricane Sandy scale salinity disturbance occurring between the spring and the late-fall. Individual oysters across the year of this study both increased and decreased their filtration physiology in response to three days under storm like conditions highlighting the importance of gathering oyster- and site-specific feeding behavior data. Gaping behavior of oysters was also quantified and the proportion of opened oysters did not always decrease when oysters went from normal to low salinity conditions. These experiments provide a robust dataset of oyster filtration observed under
contemporary and future climate conditions which may be used in a broader framework to inform long-term oyster contributions to nutrient management.
2.2 Introduction

Globally, nutrient pollution is a water quality concern in the marine environment (de Jonge et al. 2002; Howarth 2008; Wurtsbaugh et al. 2019). Land use changes as well as increases in impervious land cover, precipitation, and resulting runoff in some regions has increased nutrient loads to waterbodies. Excess nutrients in a coastal waterbody, a condition referred to as eutrophication, has been linked to environmental problems such as hypoxia, nuisance and/or toxic algal blooms, that are harmful to marine life (de Jonge et al. 2002; Bricker et al. 2008; Ferreira et al. 2011). Technology and stringent water quality regulations have reduced the amount of point source nutrient pollution across the United States and European waterways (Rose et al. 2014; Ferreira and Bricker 2016), but nutrient pollution remains a problem (Manuel 2014; Martinez-Dalmau et al. 2021) where water quality standards for nutrients are not met (Loop 2012). A diversity of nature-based nutrient management tools exists (Keesstra et al. 2018) and can complement technology-based nutrient management strategies to further improve local water quality. The use of suspension feeding oysters is among those nature-based nutrient management tools (Officer et al. 1982; Rose et al. 2014; Clements and Comeau 2019). Restoration of natural oyster reefs is one approach to mitigating nutrient pollution (Newell 1988; Hernández et al. 2018; Duarte et al. 2020), and a growing body of research suggests oyster aquaculture could provide equivalent or greater water quality benefits than reefs (Zu Ermgassen et al. 2012; Froehlich et al. 2017; Campbell and Hall 2019). However, the scale at which oyster farms provide ecosystem services is not well understood (Hernández et al. 2018; Gentry et al. 2019). Additionally, there is a lack of data concerning site-specific effects of salinity on wild and farm oyster filtration (Casas et al. 2018). The latter makes it difficult to forecast the utility of bivalves as a nutrient management strategy under climate change conditions.
Climate change is expected to increase the frequency and intensity of rainfall events along the east coast of the United States (Sanderson et al. 2019; Maxwell et al. 2021). This will result in an increase in short-term (days or weeks) low salinity events in coastal habitats occupied by Eastern oysters (*Crassostrea virginica*). Oysters are generally tolerant of variations in water properties, such as salinity, temperature, seston concentrations, and pH, since these occur regularly due to tidal fluctuations in oysters’ estuarine habitats (Shumway 1996). However, prolonged exposure to extreme low salinity over a period of weeks has caused severe mortality events across the Eastern oyster range (Butler 1952; May 1972; Andrews 1973; Burrell 1977; Pollack et al. 2011; Levinton et al. 2011; Munroe et al. 2013; U.S. Department of Commerce 2019; Du et al. 2021).

These mortality events occur because a common defense mechanism of bivalves in unfavorable environmental conditions is to close their shells to protect themselves and conserve energy by not osmoconforming to their extreme surroundings (Hand and Stickle 1977; Shumway 1996). Low salinity conditions are harmful to oysters by creating water chemistry that hinder metabolism and intracellular ion and acid base regulation (Ballantyne and Berges 2011; Dickinson et al. 2012). As such, high salinities are generally favorable for oysters (Shumway 1996; Kraeuter et al. 2007). If low salinity conditions are of sufficient duration that oysters consume all their energy reserves via anaerobic respiration to maintain muscular closure of their shells, the oyster will not survive the low salinity event (Munroe et al. 2013; Rybovich et al. 2016). This is more likely to occur at warmer temperatures because anaerobic metabolism will increase as temperature rises, as opposed to cooler temperatures where oysters can enter an energy saving hypometabolic state (Heilmayer et al. 2008). The impacts of short-term extreme low salinity events lasting a period of days on oyster filtration in the field remain poorly understood (McFarland et al. 2013; Pourmozaffar et al. 2020).
Oyster feeding behavior varies in response to changes in local environmental conditions, including: salinity (Casas et al. 2018), water temperature (Loosanoff 1958; Comeau et al. 2008; Pernet et al. 2008; Zuo et al. 2012), quantity and quality of suspended particles (Palmer and Williams 1980; Navarro and Iglesias 1993; Beninger and St-Jean 1997). Although filtration is variable over both space and time as these environmental conditions change, average rates are often used in estimates of ecological filtration. A detailed understanding of oyster filtration throughout the year, under site-specific conditions, is essential for a comprehensive baseline estimation of the impact of oysters on water quality (Ehrich and Harris 2015). Furthermore, filtration data should account for anticipated climatic trends (for example increases in extreme precipitation events) to ensure field data have longevity under climate change conditions.

Oysters in the Delaware Bay can be found on wild reefs or on aquaculture farms. The wild reefs harvested by the fishery are subtidal and are located in the lower salinity (upbay) portions of the bay. The wild stock that is part of the fishery is estimated to include over 3 billion adult oysters (Morson et al. 2021). Oyster farms are both subtidal and intertidal, and are located downbay of the wild fishery beds in higher salinity waters. Oyster farmers harvest oysters planted as seed and cultivated using a variety of aquaculture methods (e.g., rack-and-bag). The largest intertidal oyster farm on the New Jersey side of Delaware Bay can have upwards of 1 million oysters on site at any given time. Salinity in the Delaware Bay is expected to shift as a result of climate change, with some predicting salinity will decrease overall due to increased freshwater events (Sanderson et al. 2019), while others predict sea level rise may bring higher salinity water further upbay (Ross et al. 2015). This slow increase in Delaware Bay salinity from sea level rise would not preclude the short-term salinity stress on oysters induced by extreme precipitation events – both can occur together. Indeed, Hurricane Sandy (2012) reduced salinity by approximately 5ppt over a period of three-days in certain coastal areas of the Delaware Bay (Shinn
2013, unpublished). In contrast, the same storm showed a muted salinity signal in Barnegat Bay (Taghon et al. 2017) showing the site-specific influence precipitation events have on salinity. Prolonged exposure to low salinity can be detrimental to oyster reefs. In the fall of 2011, the Delaware Bay experienced a low-salinity event where oysters upbay experienced salinities that remained below 7ppt for more than 20 days and suffered very high mortality, with some beds exceeding 70% mortality by spring of 2012 as overwintering delayed the freshet mortality (Munroe et al. 2013).

This study aims to quantify the impact of a Hurricane Sandy scale salinity disturbance on oyster filtration in the Delaware Bay. Water quality managers in Delaware Bay have supported past nature-based solutions to mitigate the waterbody’s longstanding and widespread nutrient pollution issue (Denmark Jr. et al. 2019), but the scale of water quality benefits provided by wild and farmed oyster populations in the Delaware Bay remains limited. Furthermore, there is a lack of data concerning how said water quality benefits may be impacted by precipitation events. The experiments in this study provide a robust dataset of oyster filtration observed under natural and climate change conditions which may be used in a broader framework to inform long-term oyster contributions to nutrient management in the Delaware Bay.
2.3 MATERIALS AND METHODS

2.3.1 Study Location and Frequency

Seasonal filtration experiments were conducted in the field using wild and farmed oysters in the Delaware Bay between August 2020 and August 2021. Each paired salinity experiment had three sites: the site oysters were collected from, the site the normal salinity experiment was conducted, and the site the low salinity experiment was conducted (Figure 2.1). Wild oysters were collected from a subtidal reef (3.10 m ± 0.84 average depth) which is managed as part of the New Jersey Delaware Bay commercial oyster fishery (39° 16’ 42”N, 75° 14’ 54” W). Farm oysters were collected from the same intertidal rack-and-bag farm on a coastal mudflat in Delaware Bay as identified in Chapter 1 (39° 4’ 16.4” N, 74° 54’ 47.5” W).

Four paired filtration experiments were conducted using wild oysters (April and June 2021; August and October 2020). Four paired filtration experiments were conducted using farm oysters (April, June, and August 2021; October 2020). Additional paired experiments were attempted in early-December (wild oysters) and late-November (farm oysters) 2020, but the experiments at this time of year failed due to an insufficient mass of biodeposit collection.
Figure 2.1. Locations of seasonal filtration experiments conducted in the field using wild and farmed Eastern oysters in the Delaware Bay between August 2020 and August 2021 (n=4 wild, n=4 farm experiments). Wild oysters were collected from a subtidal reef and farm oysters were collected from an intertidal rack-and-bag farm. The shape of the point represents the site oysters were collected from (circle) as well as the site normal (triangle) and low (square) salinity experiments were conducted. Color indicates source of oyster (blue=wild, orange=farm).
2.3.2 Experimental Design for the Wild Oysters

For each set of paired wild oyster experiments, two filtration experiments were completed. The first experiment of a pair was conducted at the normal salinity experiment site following the protocols outlined in Chapter 1 (section 1.3.2) with one exception: oysters were not sacrificed for anatomical measurements when the first filtration experiment was terminated.

The 23 oysters that were used in the experiment (n=18 oysters in the filtration chamber, n=5 in the GTC) were labeled then held at the wild low salinity experiment site for approximately three days (69 ± 2.45 hours), then the second filtration experiment of the pair was conducted at the wild low salinity experiment site (following the protocols outlined in Chapter 1, section 1.3.2). Each oyster was placed in the same feeding chamber for both the normal and low salinity experiments. When the low salinity experiment was terminated, the biological samples and oysters were processed as outlined in Chapter 1 (section 1.3.2).

Wild oysters were collected from the subtidal reef via dredge 2 to 4 days before an experiment began, at which time 10 L of raw bay water was also collected from the reef. Wild oysters were transitioned into the normal salinity experiment site’s water via a controlled 12-hour acclimation period. Within 9 hours of collection from the reef, cleaned oysters were placed in a 5-gallon bucket filled with raw reef water and aerated with one air stone to begin the 12-hour acclimation period. Oysters remained in this bath for 4-hours before moving to baths containing 50% and 25% reef water diluted with raw water collected from the normal salinity experiment site. Wild oysters held at the normal and low salinity experiment site were continuously submerged, consistent with the subtidal nature of the oyster reef from which they were collected.

2.3.3 Experimental Design for the Farm Oysters

For each set of paired farm experiments, two filtration experiments were completed. Methods were consistent with the protocols above (section 2.3.2) with the following exceptions.
Farm oysters did not have an acclimation period prior to the start of the normal salinity experiment due to the proximity of the farm site and normal salinity experiment site (1.03 km). Farm oysters were collected during the low tide preceding the normal salinity experiment. Farm oysters held at the low salinity experiment site experienced a tidal cycle consistent with that at the intertidal farm (i.e., oysters were out of the water for approximately 5 hours during each low tide). Farm oysters were held at the low salinity site for 64.5 ± 2.63 hours.

2.3.4 Environmental Conditions

In addition to the water quality measurements made during each experiment as detailed in Chapter 1 (section 1.3.2), a logger (Onset HOBO Conductivity Logger) was deployed with oysters after collection from the reef and farm to record temperature and salinity data at 30-minute intervals until the conclusion of each low salinity experiment.

Discrete measurements of temperature, salinity, dissolved oxygen (DO, [mg L\(^{-1}\)]), pH, total dissolved solids (TDS, [mg L\(^{-1}\)]), total particulate matter (TPM, [mg L\(^{-1}\)]), and organic content of suspended solids in the water column (WC.Org [%] = particulate organic matter [mg L\(^{-1}\)] / TPM [mg L\(^{-1}\)]) where possible were also made throughout the study period. These water quality measurements were used to compare environmental conditions (1) between the wild oyster reef and the wild normal salinity experiment site due to the distance between locations (19.0 km), (2) between the wild and farm oyster normal salinity experiment sites, (3) between the wild and farm oyster low salinity experiment sites, (4) between the wild normal and low salinity site, and (5) between the farm normal and low salinity site.

Long-term water quality monitoring was not possible at the wild oyster reef, but a logger (Onset HOBO Conductivity Logger) was deployed at the oyster farm to collect temperature and salinity data at 30-minute intervals between July 2020 and October 2021.
2.3.5 Statistical Analyses

Analyses were performed using RStudio version 4.1.1 (R Core Team 2021) or Excel (Version 2111), and a p-value less than 0.10 was considered significant.

2.3.5.1 Water Quality

Water and seston characteristics (temperature, salinity, DO, pH, TDS, TPM, and WC.Org) were compared between (1) the wild oyster reef and the wild normal salinity experiment site, (2) the wild and farm normal salinity experiment site, (3) the wild and farm low salinity experiment site, (4) the wild normal and low salinity site, and (5) the farm normal and low salinity site using a Student’s t-test. Water and seston measurements repeatedly collected during an experiment were averaged prior to statistical analysis.

2.3.5.2 Oyster Filtration Physiology

Oyster condition index (CI = dry tissue weight [g] / dry shell weight [g]) between wild and farm oysters was compared with a Student’s t-test. A one-way ANOVA, and a post hoc Tukey’s test as necessary, was used to test if oyster CI differed among paired experiments. Shell thickness was compared between wild and farm oysters using average shell weight:shell length ratios with a Student’s t-test.

Impacts of low salinity on oyster physiology were examined at the population and individual oyster level. At the population level, oysters that opened and filtered during any experiment were included in analyses. At the individual level, comparisons were only made using oysters that opened and filtered during both the normal and low salinity paired experiments. The following analyses were conducted at the population and individual level as specified in each header.
2.3.5.2.a Oyster Population Filtration Physiology at Unchanged Salinity

A comparison between wild and farm oyster median filtration physiology values (i.e., filtration rate (FR), clearance rate (CR), rejection rate (RR), and absorption rate (AR)) at normal salinity experiments were made with a Student’s t-test. Median filtration physiology values for each experiment were used in this analysis to avoid pseudoreplication and better represent the central tendency in each experiment. A comparison between wild and farm oyster median filtration physiology at low salinity experiments were made in the same way.

Coefficient of variation (CV) of FR, CR, RR, and AR values (hereafter FR.CV, CR.CV, RR.CV, and AR.CV) for wild and farm oysters were also calculated for normal as well as low salinity experiments.

2.3.5.2.b Differences in Oyster Population Filtration Physiology between Normal and Low Salinity Experiments

Across the year of experiments, median filtration physiology for both wild and farm oysters were compared between normal and low salinity experiments via separate Student’s t-tests. Student’s t-tests were also used to compare oyster filtration physiology between each paired normal/low salinity experiment.

2.3.5.2.c Differences in Individual Oyster Filtration Physiology Between Normal and Low Salinity Experiments

Median percent change in filtration physiology and percent change in four environmental parameters (i.e., temperature, salinity, TPM, and WC.Org) between normal and low salinity experiment pairs were used to test the influence of environment on filtration physiology. Median percent change was selected for each experiment among those oysters that opened in both experiment pairs (i.e., only individuals that opened in both could be used to calculate percent change). Environmental measures used were limited to temperature, salinity, TPM, and WC.Org
to minimize autocorrelation biases. TPM and WC.Org are inversely correlated; both seston variables remained in analyses.

Linear regression was used to compare percent change in each environmental parameter and the median percent change in filtration physiology across paired experiments, separately for farm and wild oysters. Linear regression analyses were also used to compare the percent change in each environmental parameter and percent change in filtration variability (FR.CV, CR.CV, RR.CV, and AR.CV) using the same methods.

Median percent changes in each of the four filtration physiologies were grouped by oyster source (i.e., wild and farm oysters) then averaged (e.g., median FR of wild oysters from April and June 2021 as well as August and October 2020 were averaged) and used to test whether median percent change in filtration physiology for wild or farm oysters differ using a Student’s t-test. The range in percent changes (i.e., the difference between the maximum and minimum for a given experiment pair) of FR, CR, RR, and AR were compared between wild and farm oysters via a Student’s t-test. Extreme outliers were not included in range calculations (i.e., percent changes greater than 590% were not included).

### 2.3.5.3 Gaping

The proportion of oysters opened during each experiment were compared between (1) wild and farm oysters during normal salinity, (2) wild and farm oysters during low salinity, (3) wild oysters between normal and low salinity, and (4) farm oysters between normal and low salinity using Student’s t-test. Linear regressions were used to test the influence of average temperature, salinity, TPM, and WC.Org on proportion of open oysters among wild and farm oysters separately.
2.4 RESULTS

2.4.1 Water Quality

2.4.1.1 Comparing Water Quality Between Sites

No significant difference in temperature, TPM, or WC.Org were found between the wild oyster reef and the wild normal salinity experiment site (temperature: \( t(10) = -0.32, p = 0.75 \); TPM: \( t(5) = -1.81, p = 0.13 \); WC.Org: \( t(4) = 0.42, p = 0.70 \)). Significantly higher salinity \( t(10) = 2.99, p = 0.01 \) was observed at the wild oyster reef \((12.8 – 19.8 \text{ ppt})\) than the wild normal salinity experiment site \((6.44 – 17.45 \text{ ppt})\).

All experiment locations experienced a seasonal trend such that temperatures increased and DO decreased in the summer months (Figure 2.2). Continuous water quality data at the oyster farm was limited to temperature (Appendix B) as attempts to collect continuous salinity data were unsuccessful due to biofouling of loggers.

The wild normal salinity experiment site experienced significantly lower salinity, DO, pH, and TDS than the farm normal salinity experiment site (salinity: \( t(13) = 2.39, p < 0.001 \); DO: \( t(12) = 2.65, p = 0.02 \); pH: \( t(13) = 2.39, p = 0.03 \); TDS: \( t(9) = 4.26, p = 0.002 \)). No significant difference in temperature or TPM was found between the normal salinity experiment sites.

The wild low salinity experiment site experienced significantly lower salinity and TDS than the farm low salinity experiment site (salinity: \( t(8) = 4.06, p = 0.004 \); TDS: \( t(8) = 3.58, p = 0.007 \)). No significant difference in temperature, TPM, WC.Org, DO, or pH was found between the low salinity experiment sites (temperature: \( t(11) = 0.26, p = 0.80 \); TPM: \( t(8) = 0.18, p = 0.86 \); WC.Org: \( t(9) = -0.23, p = 0.82 \); DO: \( t(11) = 2.01, p = 0.60 \); pH: \( t(8) = 0.34, p = 0.74 \)).
Figure 2.2. Water quality measurements recorded during, and between, paired normal/low salinity filtration experiments including (A) temperature, (B) salinity, (C) TPM, (D) WC.Org, (E) DO, (F) pH, and (G) TDS. Measurements collected during experiments were averaged over ~3.5 hour period and have standard deviation (SD) bars displayed. Measurements collected between experiments were opportunistic and have no SD bars. All water quality measurements were taken within 3 hours of high tide. (H) Oyster condition index (CI) was also measured for the oysters in each experiment. In all panels blue indicates data from Barnegat Bay, orange indicates data from Delaware Bay, and grey indicates data from Rehoboth Bay. In each panel, data to the left of the vertical dashed line were collected in 2020 and data to the right of the vertical dashed line were collected in 2021.
2.4.1.2 Quantifying Environmental Changes Between Paired Salinity Experiments

The wild normal salinity experiment site experienced significantly higher salinity and TDS than the wild low salinity experiment site (salinity: $t(5)=2.96$, $p=0.02$; TDS: $t(6)=2.25$, $p=0.07$). The farm normal salinity experiment site experienced a significantly higher salinity, DO, pH, and TDS than the farm low salinity experiment site (salinity: $t(6)=5.23$, $p=0.002$; DO: $t(9)=3.15$, $p=0.01$; pH: $t(8)=3.51$, $p=0.01$; TDS: $t(5)=3.54$, $p=0.02$). No significant difference was found in the remaining environmental variables between normal/low salinity experiment sites ($p>0.30$).

While both wild and farm oysters experienced a decrease in salinity from their normal to low salinity sites, farm oysters experienced a larger decrease than the wild oysters (Table 2.1, Figure 2.3). Temperature changed no more than $±2.5°C$ between normal and low salinity experiment sites (Table 2.1, Figure 2.3). The magnitude of WC.Org changes were close to zero except for three paired experiments that experienced a large decrease in WC.Org between normal and low salinity experiments ($6.6–14.9\%$ decrease) (Table 2.1). The magnitude of changes in TPM between paired normal/low salinity experiments varied (Table 2.1).
Table 2.1. Average change in water quality between the normal and low salinity conditions across all paired experiments (n=4 for farm, n=4 for wild). Negative values indicate a decrease from the normal to low salinity condition.

<table>
<thead>
<tr>
<th>Oyster Source</th>
<th>Temperature (°C)</th>
<th>Salinity (ppt)</th>
<th>TPM (mg L⁻¹)</th>
<th>WC.Org (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild</td>
<td>0.50 (2.12)</td>
<td>-3.69 (1.99)</td>
<td>-6.66 (32.7)</td>
<td>-1.23 (3.69)</td>
</tr>
<tr>
<td>Farm</td>
<td>1.13 (1.52)</td>
<td>-8.51 (2.50)</td>
<td>-6.94 (81.3)</td>
<td>-6.28 (6.97)</td>
</tr>
</tbody>
</table>
Figure 2.3. Continuous temperature and salinity observations for wild oysters (panels A and C) after being deployed at the normal salinity experiment site. Continuous temperature and salinity observations for farm oysters (panels B and D) after being deployed at the low salinity experiment site. Approximate times of deployment at normal salinity experiment site (long dashed lines), the normal salinity experiment (Exp. 1), deployment at the low salinity experiment site (dotted lines), and the low salinity experiment (Exp. 2) are noted in grey above each plot. Attempts to collect continuous data at the farm oyster normal salinity experiment site were unsuccessful due to biofouling of loggers. The influence of tides on salinity for both wild and farm oysters is evident. The paired normal/low salinity experiment in August was conducted in 2020 for wild oysters and 2021 for farm oysters. Long-term temperature data for the farm oysters is reported in Appendix B.
2.4.2 Oyster Filtration Physiology

Cl of wild oysters was significantly lower than that of farm oysters (t(160)=24.1, p<0.0001) (Figure 2.2), while oyster shell weight:oyster length ratios for wild oysters was significantly higher than the farm oysters (t(83)=-14.0, p<0.0001). Cl of wild oysters in August and October was significantly higher (F(3,68)=7.73, p<0.001) than April (Tukey HSD p=0.002 and p=0.01) and June (Tukey HSD p=0.004 and p=0.02). Cl of farm oysters was significantly higher in October than in April and August (F(3,67)=3.02, p=0.04; Tukey HSD p=0.09 and p=0.09) (Figure 2.2).

2.4.2.1 Oyster Population Filtration Physiology at Unchanged Salinity

Wild oyster filtration physiology varied seasonally with higher average FR, CR, RR, and ARs in the warmer months (Figure 2.4); however, this seasonal trend is not observed in farm oysters (Figure 2.4).

CV in filtration physiology fluctuated independent of season, and at normal salinity wild oysters had larger CV values for all filtration physiologies at normal salinity (mean CV = 0.92) than farm oysters (mean CV = 0.61). However, no significant differences were found in oyster median filtration physiology between wild and farm oysters at normal salinity (all p>0.56).

Wild and farm oyster filtration physiology varied seasonally at low salinity sites such that higher average FR, CR, RR and ARs occurred in warmer months; this trend was most evident among farm oysters (Figure 2.4). No significant differences were found in median filtration physiology between wild and farm oysters at low salinity (all p>0.28).
Figure 2.4. Relationship between month of paired normal/low salinity experiment and filtration physiology including average (A) filtration rate, (B) clearance rate, (C) rejection rate, and (D) absorption rate. Color indicates source of oyster and salinity condition. Error bars indicate standard deviation values. All experiments in April and June were conducted in 2021, the farm experiments in August were conducted in 2021, all other experiments were conducted in 2020. All values are standardized to one gram of dry tissue weight. (*) indicates a significant difference in filtration physiology between individual paired normal/low salinity experiments.
2.4.2.2 Differences in Oyster Population Filtration Physiology between Normal and Low Salinity Experiments

At the population level, wild oysters were physiologically more active (higher average FR, CR, RR, and AR) under normal salinity than at low salinity (with the exception of October 2020, Figure 2.2). As a population, farm oysters were physiologically more active (higher average CR and AR) under normal compared to low salinity conditions, but had larger average FR and RR values under low salinity conditions in June and August 2021 (Figure 2.2).

No significant differences in filtration behavior between normal and low salinity was observed among wild oysters, nor farm oysters, across all paired experiments (Table 2.2). However, with the exception of those measured in April, significant differences in filtration physiology were found between individual paired normal/low salinity experiments (Figure 2.4).
Table 2.2. Results of comparison (Student’s t-test) of oyster population filtration behavior between normal and low salinity experiments across one full year. No significant differences were found between normal and low salinity experiments’ median FR, median CR, median RR, or median AR. Separate analyses were conducted on physiological variables for wild and farm oysters.

<table>
<thead>
<tr>
<th>Physiological Variable Analyzed Between Normal and Low Salinity Experiments</th>
<th>Wild Oysters</th>
<th>Farm Oysters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median FR</td>
<td>t(5)=.39, p=.71</td>
<td>t(5)=.36, p=.73</td>
</tr>
<tr>
<td>Median CR</td>
<td>t(5)=.24, p=.82</td>
<td>t(4)=.87, p=.43</td>
</tr>
<tr>
<td>Median RR</td>
<td>t(5)=.35, p=.74</td>
<td>t(6)=.25, p=.81</td>
</tr>
<tr>
<td>Median AR</td>
<td>t(3)=.70, p=.53</td>
<td>t(3)=1.70, p=.19</td>
</tr>
</tbody>
</table>
2.4.2.3 Differences in Individual Oyster Filtration Physiology Between Normal and Low Salinity Experiments

At the individual level, oyster filtration physiology varied widely (Figure 2.5). Only in a few instances did all oysters in a paired experiment show a negative percent change in filtration physiology, and there were no experiment pairs in which all oysters showed an increase in percent change in physiology (Figure 2.5). The median percent difference in filtration physiology across individuals for wild oysters was significantly more negative than that of farm oysters ($t(5)=-2.41$, $p=0.06$). Additionally, the range of physiological responses within paired normal/low salinity experiments was significantly larger for wild compared to farm oysters ($t(5)=-4.39$, $p=0.007$).

Percent changes in wild oyster median FR and RR were significantly negatively associated with a percent change in temperature (FR: $F(1,2)=56.8$, $p=0.02$; RR: $F(1,2)=8.42$, $p=0.10$) and percent changes in median CR and AR were significantly negatively associated with a percent change in TPM (CR: $F(1,2)=26.2$, $p=0.04$; AR: $F(1,2)=156$, $p<0.01$) (Figure 2.6). Although the percent change in oyster median filtration physiology at the oyster farm is positively associated with a percent change in salinity, these were not significantly correlated (FR: $F(1,2)=3.68$, $p=0.20$; CR: $F(1,2)=3.03$, $p=0.22$; RR: $F(1,2)=3.79$, $p=0.19$; AR: $F(1,2)=1.25$, $p=0.38$) (Figure 2.6).

A decrease in FR.CV, CR.CV, AR.CV, and RR.CV between normal and low salinity conditions were associated with a decrease in WC.Org for wild oysters (Table 2.3).
Figure 2.5. Percent change in individual oyster filtration physiology including (A) filtration rate, (B) clearance rate, (C) rejection rate, and (D) absorption rate by month of experiment. Color indicates oyster source (blue = wild; orange = farm) and each black dot represents change in filtration physiology for an individual oyster. The zero-line is superimposed (yellow) for reference. Points below the zero-line represent oysters whose filtration physiology was lower during the low salinity experiment. Extreme values are included as colored triangles with the percent change annotated adjacent to the corresponding point.
Figure 2.6. Median percent difference in filtration physiology relative to percent changes in environmental variables for each paired experiment. Colors represent different environmental variables with temperature in light blue, salinity in black, TPM in brown, and WC.Org in green. Each colored dot represents one paired experiment (n=4 for farm and n=4 for wild, for each environmental variable). Wild oyster experiments are shown in the left column and farm oysters in the right column. Color coded statistics on the right of each panel are the results of linear regression analyses for each environmental variable. (*) indicates a significant correlation and (**) indicates the best fitting, but non-significant, correlation for a given panel.
Table 2.3. Results of linear regressions for wild and farm oyster CV values. Comparisons made between percent change (\%.Δ) in FR.CV, CR.CV, RR.CV, and AR.CV and percent change in four environmental variables. All wild correlations are positive unless otherwise noted. All farm correlations are negative unless otherwise noted. Shaded cells represent significant associations.

<table>
<thead>
<tr>
<th>Oyster Source</th>
<th>Physiological Variable</th>
<th>Percent Change in Average Temperature (%)</th>
<th>Percent Change in Average Salinity (%)</th>
<th>Percent Change in Average TPM (%)</th>
<th>Percent Change in Average WC.Org (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild</td>
<td>%Δ.FR.CV</td>
<td>F(1,2)=.30, p=.64, R²=.13</td>
<td>F(1,2)=5.28, p=.15, R²=.73</td>
<td>F(1,2)=.84, p=.84, R²=.30</td>
<td>(-) F(1,2)=21.8, p=.04, R²=.92</td>
</tr>
<tr>
<td></td>
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<td>(-) F(1,2)=23.57, p=.04, R²=.92</td>
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<tr>
<td></td>
<td>%Δ.RR.CV</td>
<td>F(1,2)=.43, p=.58, R²=.18</td>
<td>F(1,2)=4.78, p=.16, R²=.70</td>
<td>F(1,2)=1.04, p=.42, R²=.34</td>
<td>(-) F(1,2)=37.3, p=.03, R²=.95</td>
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<td>%Δ.AR.CV</td>
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<td>F(1,2)=8.84, p=.10, R²=.82</td>
<td>F(1,2)=4.07, p=.18, R²=.67</td>
<td>(-) F(1,2)=13.0, p=.07, R²=.87</td>
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<tr>
<td>Farm</td>
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<tr>
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<td>F(1,2)=1.40, p=.36, R²=.41</td>
<td>F(1,2)=2.58, p=.25, R²=.56</td>
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<td>F(1,2)=1.51, p=.34, R²=.15</td>
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<td>(+) F(1,2)=.54, p=.54, R²=.21</td>
<td>F(1,2)=.42, p=.58, R²=.17</td>
<td>F(1,2)=.24, p=.67, R²=.11</td>
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</tbody>
</table>
2.4.3 Gaping

At no point during any individual filtration experiment did all oysters \( n=18 \) open and actively filter (Figure 2.7). The proportion of oysters open during a normal salinity experiment averaged 0.74 ± 0.15 for wild and 0.75 ± 0.13 for farm oysters, and during a low salinity experiment averaged 0.67 ± 0.22 for wild and 0.64 ± 0.20 for farm oysters.

No significant difference in proportion of open oysters was found between wild and farm oysters under normal \( t(6)=0.12, p=0.91 \) nor low salinity conditions \( t(6)=-0.20, p=0.85 \). Likewise, no significant difference in proportion of wild oysters between normal and low salinity conditions \( t(3)=1.46, p=0.24 \), nor farm oysters between normal and low salinity conditions \( t(3)=1.14, p=0.34 \) was observed.

A seasonal trend in proportion of opened oysters was evident for wild oysters under normal and low salinity conditions wherein more oysters opened in the summer months (Figure 2.7). No significant correlations were found among the proportion of wild nor farm oysters open and changes in environmental variables for a given experiment.
Figure 2.7. Proportion of oysters that opened to feed during each filtration experiment by the month of the experiment. Color indicates source of oyster and salinity condition. Low salinity experiments were not successfully conducted in November and December due to low biomass collection.
2.5 DISCUSSION

2.5.1 Oyster Filtration Physiology

Oyster filtration physiology (i.e., FR, CR, RR, and AR) and CV were quantified seasonally for both wild and farm oysters in this study. There was a seasonal trend in filtration physiology (i.e., increased activity in warmer months), but among treatments (i.e., wild normal and low salinity, farm normal and low salinity) there was no significant difference in filtration physiology over the year-long study period. However, significant changes in oyster filtration physiology were found when comparing feeding behavior between individual paired normal/low salinity experiments. This suggests oysters alter their filtration physiology in response to acute environmental changes experienced over a three-day period.

During the three-day periods in this study, oysters experienced a range of environmental changes which did not universally align with the conditions expected during a precipitation event. Storms in the mid-Atlantic bring a decrease in water temperature along the continental shelf (Miles et al. 2015, Seroka et al. 2017) but these changes in temperature are muted in the Delaware Bay (Munroe et al. 2013). Storms also bring an increase in TPM (Thrush et al. 2004, Alberto et al. 2016) and a decrease in salinity (Munroe et al. 2013). Salinity in this study did decrease between all paired normal/low salinity conditions, but changes in temperature, TPM, and WC.Org were varied. As such the simulated storms in this study varied in their environmental signature, but the stressor of interest (i.e., low salinity) was represented consistently throughout.

This decrease in salinity, however, was not associated with consistent changes in oyster filtration physiology in contrast to other studies showing oysters are physiologically more active at higher salinities (Casas et al. 2018; Gray and Langdon 2019). Farm oyster filtration physiology in this study were positively associated with changes in salinity such that a larger percent decrease in salinity was associated with a larger percent decrease in filtration physiology, but this
association was weak. Moreover, wild oyster FRs and RRs decreased with an increase in temperature while wild oyster CRs and ARs decreased with an increase in TPM. Oysters typically increase their filtration physiology seasonally in response to rising temperatures (Yu et al. 2017) to meet metabolic demand which was supported by this study. Indeed, the lowest experimental temperatures in this study were recorded for wild oysters examined on December 2020 (7.98°C) and farm oysters examined on November 2020 (10.5°C) and biodeposit production was so low for these experiments that filtration physiology could not be calculated. The cooler April water conditions corresponded with the only month of experiments to show no significant difference between any paired normal/low salinity experiment, likely because cooler water conditions suppressed filtration activity (Zuo et al. 2012; Ward and Shumway 2004; Humphries 2013). However, this environmental analysis is focused on the impact of short-term environmental stress across the year.

Oysters upregulate aerobic metabolism as a short-term response to marine heatwaves that increase temperatures from 26 to 32°C in one day (Li et al. 2017; Leung et al. 2019). Under more extreme thermal conditions (i.e., 40°C thermal stress over four hours) oyster CRs decrease initially with CRs starting to recover within two hours of exposure (Giomi et al. 2016). These responses are expected given the estimated optimal temperature for oyster physiology lies between 16-28°C (Loosanoff 1958; Casas et al. 2018). It is therefore unclear why, after three days, percent change in FR and RR of wild oysters tended to become more negative as percent change in temperature became more positive in this study. These environmental associations should be interpreted with caution as small percent changes in temperature showed the greatest negative percent changes in FR and RR, which is an unlikely physiological response in terms of magnitude. This may suggest the changes observed in FR and RR cannot be solely attributed to temperature.
Instead, this may be indicative of a stress response of oysters to the collective, acute change in environmental conditions observed within a paired normal/low salinity experiment.

Percent changes in TPM were more strongly associated with percent changes in CR and AR than percent changes in temperature were with FR and RR in this study. An increase in TPM was associated with a decrease in CR and AR. High TPM concentrations can clog oyster gills and make it energetically challenging for oysters to feed (Widdows et al. 1979; Bayne and Newell 1983; Gray and Langdon 2019; La Peyre et al. 2020) which may be why a decrease in filtration physiology was evident for the wild oysters in this study. Moreover, the wild oyster reef ecosystem had lower TPM concentrations than the wild normal salinity experiment site possibly making those oysters less acclimated and more sensitive to increasing TPM between paired experiments (La Peyre et al. 2020). Percent changes in WC.Org were not associated with any change in filtration physiology which may suggest oysters were not food limited across the experiments.

Given the ubiquitous decrease in salinity between paired normal/low salinity experiments and the association of wild oyster CR and AR to TPM, it is possible a collective decrease in salinity and an increase in TPM may have stressed wild oysters in this study: indeed, wild oysters experienced these changes in salinity and TPM on June 2021 and August 2020 and a decrease in average filtration physiology was evident. Interestingly, a decrease in TPM was also observed within the wild oyster normal/low salinity in October 2020 when filtration physiology remained high during the low salinity condition. Short-term environmental perturbations including low salinity (Domíngues et al. 2020, clams and cockles) and increased TPM (Poirier et al. 2021, Eastern oyster) have been associated with reduced bivalve physiological activity during and in the days following the stress events. Additional studies are needed to fully understand the synergistic effects of these stressors, as most experiments of oyster physiology focus on the influence of
chronic temperature and salinity stress (Hutchinson and Hawkins 1982; Jones et al. 2019; Pack et al. 2021). This is understandable given the evidence that temperature and salinity are highly influential abiotic factors on oyster biology including respiration, utilization of food reserves, growth, and feeding (Shumway 1996), yet herein TPM also seems to be a prominent source of environmental stress.

It is possible the wild oysters experienced low salinity and high TPM stress prior to the start of each normal salinity experiment. During the 2- to 4-day acclimation period at the normal salinity experiment site, the wild oysters were held in water with lower salinity and higher TPM (averaged 67.4% higher) than the wild oyster reef. Differences in water quality between the oyster natural habitat and experiment site have been hypothesized to affect oyster feeding performance in other studies (Hall et al. 2020). Wild oysters had larger CV values than farm oysters at normal salinity experiment sites which could be an indication of oysters responding to a stressful environment. If these wild oysters were stressed due to being held in low salinity and elevated TPM, wild oyster filtration physiologies measured during normal salinity experiments may be underestimates.

An association between filtration physiology and TPM may also explain the observed increase in FR and RR during low salinity farm experiments observed in June and August 2021. The increase in physiologic activity may be due to uncommonly low TPM experienced by the farm oysters during the June and August 2021 normal salinity experiments. Typically, the farm normal salinity experiment site had higher TPM than the low salinity site, but the TPM conditions were opposite on June and August 2021 such that the farm low salinity site had higher TPM than the normal salinity site. Bivalves can easily adjust CRs in response to changes in TPM that are representative of the natural range in seston observed during tidal fluxes (Hawkins et al. 2001), but the TPMs observed during the June and August 2021 normal salinity experiments (averaged
39.6 mg L\(^{-1}\) ± 1.4) were outside of the normal TPM range at that site (all other TPM measurements at the normal salinity site, excluding one taken during a storm, averaged 117.11 mg L\(^{-1}\) ± 38.87). It is possible the farm oysters were stressed during the June and August 2021 normal salinity experiments from these low TPM conditions and the subsequent low salinity experiments, in which the TPM regime returned to conditions more similar to the normal farm environment, may have caused an increase in oyster feeding activity (La Peyre et al. 2020).

Collectively this analysis of environmental variables and filtration physiology may suggest wild and farm oysters respond differently to changes in their natural environment. However, these results are conservative because using median FR, CR, RR, and AR values reduces the range of individual responses in oyster behavior to that of one summary metric from each paired normal/low salinity experiment. These median differences in filtration physiology are more likely to represent oysters that are less sensitive to environmental pressures because they are median values, and do not reflect the extreme responses to environmental change.

A range of filtration physiology values were observed among individual oysters during each paired experiment. Previous studies have anticipated feeding rates in nature would vary more than in a laboratory (Grizzle et al. 2008). Other experiments have shown a range of filtration physiology values similar to this study (Kelly et al. 2011), or showing less variability than those reported here (Galimany et al. 2017a; 2017b). Interestingly, wild oysters showed a larger range in percent change in filtration physiology than farm oysters. Furthermore, when more organic rich seston was available at the low than the normal salinity experiment site, the variability among individual wild oyster physiological responses decreased. This may suggest extreme changes in filtration physiology could be tempered by increased food availability during low salinity conditions. Finally, the median percent difference in filtration physiology values for wild oysters, while generally negative, tended to be closer to zero than the corresponding values for farm
oysters. Disease likely did not influence oyster feeding behavior in this study as both the wild and farm populations were healthy (wild oysters had a lower condition, but this may be attributed to significantly heavier wild oyster shells). Together this suggests wild oysters in this study may have more diverse physiological responses to short-term environmental changes than farm oysters and that breadth of responses in the wild population may confer greater resilience or adaptive capacity to short-term environmental changes.

The diversity of physiological responses may also be related to the genetic diversity expected on a wild oyster reef exposed to locally specific conditions in comparison to farm oysters derived from selectively bred or limited seed sources. Wild oysters are known to be adapted to their own unique salinity and temperature regime (Burrell 1986; Stanley and Sellers 1986; White and Wilson 1996; Leonhardt et al. 2017). This adaptation likely also extends to turbidity (Loosanoff and Tomers 1948; La Peyre et al. 2020). For example, a *Crassostrea gigas* population from a turbid region of Japan has smaller gills and larger labial palps than oysters in low-turbidity areas nearby (Barillé et al. 2000). In contrast to the wild oysters, those from the farm are typically less than three years old and mortality on the farm is primarily by harvest and thus less related to local environmental conditions. In this way, wild oysters may be better able to handle the environmental stressors typical of the reef location and the wild population may have more genetic diversity to support resilience to a myriad of short-term environmental stressors. Genetic analyses of the wild and farm oysters used in this study were not performed, so these hypotheses cannot be confirmed.

### 2.5.2 Gaping

Wild and farm oysters had similar gaping behavior in this study: a clear seasonal trend was observed in wild oysters such that more oysters opened during warmer water conditions, but the seasonal trend at normal salinity in the farm oysters was interrupted by a low proportion
opened in August 2021. This may have been related to a storm event that occurred the day prior to the August 2021 farm normal salinity experiment discussed in Chapter 1 (section 1.5.1).

While the average proportion of wild and farm oysters open did decrease between normal/low salinity experiments across the year, the proportion of opened oysters did not always decrease between individual paired normal/low salinity experiments. This varied gaping behavior within paired experiments was not associated with any changes in environmental condition. This suggests that there may be an exogenous or endogenous variable not examined in this study that influences the proportion of oysters that open during stressful environmental conditions (Tran et al. 2011). However, higher salinities (Casas et al. 2018) and warmer temperatures (Comeau et al. 2012) are associated with increased duration of time an oyster remains open. Casas et al. (2018) and Comeau et al. (2012) performed valve opening experiments via continuous valve monitoring over a period of 10 and 147 days respectively, suggesting the opening behavior examined herein may have been collected over too short a period to identify opening in response to environmental conditions. It would be necessary to observe the oysters for a sufficient duration of time before and after exposure to the low salinity condition to accurately compare the influence the storm event. Monitoring oysters over a period of three days would likely lead to robust gaping results as oysters are known to osmoconform to high salinity conditions within three hours of valve opening (Strange and Crowe 1979).

2.5.3 Implications to Oyster Fishery and Farm Resilience

The paired normal/low salinity experiments conducted in this study on wild and farm oysters indicate individual oysters both increase and decrease their filtration physiology in response to three days under storm like conditions. These responses were associated with unique environmental drivers for wild (temperature and TPM) and farm (possibly salinity) oysters across the seasons of this study. In no instance did all oysters increase their filtration physiology during
a paired normal/low salinity experiment, which suggests the storm conditions in this study are unfavorable for wild and farm oysters. However, both wild and farm oyster populations maintained some amount of feeding behavior at low salinity with the proportion of opened oysters both increasing and decreasing between normal/low salinity experiments. Collectively, this suggests wild and farm oysters, at the population scale, are somewhat resilient to three days exposure to storm like conditions.

As the intensity and frequency of rainfall events along the east coast of the United States increases with climate change (Sanderson et al. 2019; Maxwell et al. 2021), these short-term low salinity stress events will become more common in the Delaware Bay. It is therefore important for managers of the wild oyster fishery to continue traditional ‘intermediate transplant’ activities where oysters found upbay in the Delaware Bay are transplanted downbay to enhance abundance and maintain a sustainable fishery (Morson et al. 2021). This will help maintain biodiversity of the stock (Eggleston 1999) and possibly integrate low salinity resilience to future oyster cohorts as upbay oysters inhabit a naturally lower salinity than the oysters harvested downbay. When planning a new oyster farm, care should be taken to select a location with naturally higher salinity. This may provide buffering capacity for oysters when a precipitation event occurs such that the minimum salinity the oysters experience during a storm will remain relatively high in the oyster’s optimum salinity range estimated to be between 10 and 28 ppt (Loosanoff 1965).

2.5.4 Implications to Nature Based Nutrient Management

The filtration physiology described in this study support the use of wild and farm oysters as a tool for nutrient management in coastal waterbodies and demonstrate wild and farm oysters provide similar water quality benefits under normal salinity conditions, which vary with the seasons. At the population scale, wild and farm oysters are negatively impacted by a Hurricane Sandy scale salinity disturbance evident in the reduced average physiological activity during low
salinity experiments. This pattern was not always observed as, in some instances, wild and farm oyster populations exhibited increased average filtration physiology during low salinity experiments which may be attributed to stressful environmental conditions during normal salinity experiments (see 2.5.1). At the individual scale, wild and farm oysters both increase and decrease their filtration physiology in response to a short-term low salinity event. Thus, during a salinity stress event comparable in scale to those examined in this study, managers can expect a range of oyster behavior: some oysters will be tolerant to and sensitive to low salinity conditions.

Overall, these results suggest while oysters have individual and varied responses to sublethal salinity stress, both wild and farm oyster populations provide reduced water quality benefits during periods of sublethal salinity stress. This underscores the value of making conservative estimates of ecological filtration to account for days of low physiological activity triggered by environmental stressors. Conservative estimates of oyster water quality benefits are common (Cornwell et al. 2016) but estimates may not directly address periodic and stochastic environmental perturbations common in ecological systems (Sabo and Post 2008), although interest in incorporating the impacts of storms on water quality does exist among oyster production modelers (Liu and Huang 2009; Livingston et al. 2010).

The site-specific filtration information generated from this study, including proportion of open oysters, may be integrated into ecological estimates of oyster water quality benefits and improve the estimates therein for reasons outlined in Chapter 1 (see 1.5.4). Changes in seasonal RRs in response to sublethal salinity stress events may also be useful for estimating the scale of biodeposition at bivalve farms (Hayakawa et al. 2001; Weise et al. 2009; Testa et al. 2015).
2.6 Conclusion

Wild and farm oysters in the Delaware Bay provide reduced water quality benefits during a Hurricane Sandy scale salinity disturbance occurring between the spring and the late-fall. Oysters in this study both increased and decreased their individual filtration physiology in response to low salinity conditions (i.e., decreased salinity and changes in temperature, TPM, WC.Org) occurring over a span of three days. At the population level, average physiologic activity was reduced in response to low salinity conditions when oysters were unstressed immediately preceding exposure to low salinity conditions. The sensitivity of oysters to abnormal environmental conditions highlights the need for conservative estimates of ecological filtration to account for periods of low physiological activity. The intensity and frequency of rainfall events along the east coast of the United States are expected to increase with climate change (Sanderson et al. 2019; Maxwell et al. 2021) which makes these conservative estimates all the more relevant.

Our results support the use of oyster fisheries and aquaculture as a means to mitigate nutrient pollution in coastal waterbodies and provide data modelers can use to improve ecological estimates of oyster water quality benefits under climate change conditions. This study is limited in that oyster physiology was examined over one rather than multiple years, the latter would be necessary to gain a wholistic understanding of oyster behavior. However, the oysters in this study did experience wetter than average conditions (Robinson 2020 (a-e), 2021(a-h)) and thus provide a reasonable estimate of feeding behavior under climate change conditions. This study provides one of the first estimates of year-round filtration activity of wild and farm oysters and will inform the sustainable growth of the United States oyster industry.
2.7 REFERENCES


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Robinson, D.A. 2020b. Up and down but in the end quite average: September 2020 recap. New Jersey State Climatologist, Center for Environmental Prediction, School of Environmental and


Paired t-tests were performed on available data to ensure water quality between each farm/experiment pair did not significantly differ. Due to failures of water quality measuring probes and human error in sample collection, comparisons between farm/experiment pairs were limited. Below is a summary of the differences in key environmental variables:

1. Temperature: No significant difference in temperature was found among the three farm/experiment pairs (Barnegat Bay: t(2)=-1.07, p=0.40; Delaware Bay: t(3)=-0.61, p=0.58; Rehoboth Bay: t(1)=0.20, p=0.87).
2. Salinity: No significant difference was found between the Rehoboth Bay farm/experiment pair (t(2)=-0.95, p=0.44). Failure of HOBO temperature/salinity loggers precluded a comparison of Barnegat Bay and Delaware Bay farm/experiment pairs.
3. TPM: No significant difference was found between the Rehoboth Bay farm/experiment pair (t(2)=1.04, p=0.41). Only one set of samples were collected at Barnegat Bay (TPM=76.82mg/L at farm and 99.2mg/L at experiment). However, samples were taken ~40 minutes apart and may have contributed to the difference in TPM. No samples were collected in Delaware Bay.
4. WC.Org: No significant difference was found between the Rehoboth Bay farm/experiment pair (t(2)=0.34, p=0.69). Only one set of samples were collected at Barnegat Bay (WC.Org=20.12% at farm and 18.15% at experiment). However, samples were taken ~40 minutes apart and may have contributed to the difference in WC.Org. No samples were collected in Delaware Bay.
Figure B.1. Results of long-term temperature/salinity readings at the (A) Barnegat Bay, (B) Delaware Bay, and (C) Rehoboth Bay farms site taken across the study period. Red vertical lines indicate when data collected in 2021 transitions into 2020 data. Higher temperatures at Delaware Bay farm site are attributed to the intertidal nature of this farm. Barnegat and Rehoboth Bay farms are subtidal. Failure of loggers via biofouling precluded the collection of accurate salinity data.
**APPENDIX C - Additional Oyster Physiological Parameters**

![Graphs showing physiological parameters](image)

Figure C.1. Relationship between day of experiment and additional physiology values including (A) average inorganic egestion rate, (B) organic egestion rate, (C) inorganic rejection rate, and (D) organic rejection rate. Color indicates farm location and error bars indicate standard deviation values. Data to the left of the vertical dashed line were collected in 2020 and data to the right of the vertical dashed line were collected in 2021.
Figure C.2. Relationship between clearance rate and filtration rate of all oysters examined in Chapter 1 of this study where the size of each dot represents the biomass of TPM suspended in the water column. As oysters were exposed to higher TPM loadings individual oyster CRs decreased and FRs increased.
APPENDIX D - Estimating Year-Round Water Quality Benefits Analyzed in Chapter 1

D.1. Purpose

A supplemental analysis was conducted to estimate the year-round water quality benefits provided by each of the three oyster farms using data collected in Chapter 1. To accomplish this goal, seasonal CR, FR, and RR values were scaled up to estimate the annual (acre⁻¹) volume of water cleared, biomass of particles removed from the water column, and the biomass of particles deposited into the environment at each farm, respectively. Estimates were used to compare water quality benefits among farm locations and oyster populations.

For the purposes of this Appendix, the methods for scaling up CR values at the Barnegat Bay farm to generate an estimate of the annual volume of water cleared (acre⁻¹) will be outlined. The following methods were repeated for FR and RR in Barnegat Bay, as well as for CR, FR, and RR values in Delaware and Rehoboth Bays. Where methods differ, refer to section D.4 Methods Modifications included in this Appendix.

D.2. Methods

To scale up the water quality benefits provided by Barnegat Bay, an estimate of oyster biomass at the farm was needed. No official cutoff size exists to differentiate between a sub-market and market sized oyster, but for the purposes of this analyses a common cutoff was used (i.e., 63.5 mm). All sub-market sized oysters on the farm were assumed to be 45 mm and all market sized oysters were assumed to be 80mm. The oyster sizes assumed for each group (45 and 80 mm) reflected the size distribution of oysters on the farm.

Biomass estimates of a 45 and 85 mm oyster was made by first using a relationship based on morphology observed across all Barnegat Bay oysters across all experiments (n=49). A power curve was fit to those observations to predict DTW from length, thereby generating an allometric
relationship of oyster biomass to length \( B = aL^b \) where \( B \) is oyster DTW or biomass, and \( L \) is oyster length) (Powell et al. 2016) specific to the oysters used in this study from the Barnegat Bay farm. The allometric equation for Barnegat Bay was \( y = 0.000036x^{2.3752} \) (Figure D.1). Using this equation, a 45 mm oyster found at the Barnegat Bay farm was estimated to have a 0.30 g DTW and a 80 mm oyster to have a 1.19 g DTW.
Figure D.1. The distribution of farm oyster DTW and length measured at (A) Barnegat Bay (n=49), (B) Delaware Bay (n=121), and (C) Rehoboth Bay (n=72). The best-fit power function for each location is superimposed on each panel and is the allometric equation used to estimate the biomass of a sub-market and market sized oyster at each farm. $R^2$ and p-values are also included on each panel.
To estimate the biomass on the entire farm, the recently calculated biomasses (0.30 and 1.19 g DTW) were scaled up using standing stock estimates provided by the farmer (sub-market = 570,000 and market = 30,000 oysters for Barnegat Bay). The biomass of sub-market oysters on the Barnegat Bay farm was estimated at 173,337 g and market oysters at 35,780 g. The sum of the sub-market and market biomasses provided an estimate of the total oyster biomass on the farm (209,117 g).

To estimate the volume of water cleared by the total biomass on the farm in one day an experiment specific formula was generated:

\[ V_{July.7.2021} = \frac{CR_{July.7.2021} \left( W_{July.7.2021} \right) \left( T_{July.7.2021} \right) (0.6958)}{A} \]

where \( V \) is the total volume of water cleared by the Barnegat Bay farm in one day [L day\(^{-1}\) acre\(^{-1}\)], \( CR \) is the median CR determined during the experiment [L h\(^{-1}\) g\(^{-1}\)], \( W \) is the estimated total biomass on the farm on that experiment day [g], \( T \) is the number of hours the oysters are underwater in one day, \( A \) is the total acreage of the farm, and 0.70 is the calculated average proportion of opened oysters determined in Chapter 1. In this example, the CR, W, and T values used are for the Barnegat Bay experiment conducted in July 2021. This process was repeated for all three experiments conducted at Barnegat Bay and an estimated 2.5x10\(^7\), 4.17x10\(^7\), and 9.41x10\(^6\) L of water were cleared on July 2021, September 2020, and November 2020, respectively.

The coldest recorded water temperature in Barnegat Bay was observed on January 30, 2021. It was assumed that this temperature prevented oyster feeding and therefore no water was cleared. These zero filtration days, along with the three experimentally derived estimates of total farm clearance volume were used to fit a second order polynomial curve that predicts volume filtered by the farm by ordinal day (Figure D.2). The area under this curve (-1160000 + 399021x + 956.8x\(^2\)) was then calculated to determine the annual volume of water cleared by the Barnegat
Bay farm. The two $y=0$ points were added to ensure the curve of best fit was a negative quadratic function.

Annual estimates of water quality benefits provided by the three oyster farms studied in Chapter 1 can be found in Table D.1.
Figure D.2. Estimated volume of water cleared by oysters at the (A) Barnegat Bay, (B) Delaware Bay, and (C) Rehoboth Bay farm in one day by ordinal day of experiment. In panel A, points at (30, 0) and (395,0) represent the coldest day of recorded water temperature at the Barnegat Bay farm (January 30, 2021). The area under each curve (defined by the superimposed equations, $R^2$ and p-values in each panel), represents the estimated total volume of water cleared by each farm in one calendar year. Note the scale of magnitude difference in panel A’s volume of water cleared values.
Table D.1 Estimated water quality benefits provided by the three oyster farms studied in Chapter 1 over a one-year period standardized per acre. Shaded boxes correspond to the biomass of the farm identified in the given row. White boxes correspond to estimated water quality benefits based on the biomass found at Barnegat Bay (small biomass), Rehoboth Bay (medium biomass), and Delaware Bay (large biomass). PF = pseudofeces.

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<tr>
<td>Rehoboth Bay</td>
<td>1.54</td>
<td>40.15</td>
<td>24.95</td>
<td>0.83</td>
<td>21.64</td>
<td>13.44</td>
<td>4.13</td>
<td>115.62</td>
<td>73.49</td>
</tr>
<tr>
<td>Delaware Bay</td>
<td>0.28</td>
<td>16.52</td>
<td>13.83</td>
<td>0.15</td>
<td>8.90</td>
<td>7.46</td>
<td>0.80</td>
<td>43.77</td>
<td>36.36</td>
</tr>
</tbody>
</table>


D.3. Water Quality Benefits with a Different Biomass

An additional way to compare water quality benefits among farms was to compare each farm with the same biomass. The calculations outlined in D.2 Methods above were repeated such that each farm was assigned the biomass of the other two farms. This is particularly helpful because each farm had a different biomass: Barnegat Bay farm biomass = 4.24x10^4 g (the small biomass farm), Delaware Bay farm biomass = 5.21x10^5 g (the large biomass farm), and Rehoboth Bay farm biomass = 1.05x10^5 g (the medium biomass farm).

For example, to calculate the volume of water cleared by oysters at the Barnegat Bay farm in one year based on the Delaware and Rehoboth Bay oyster biomasses, the corresponding Delaware and Rehoboth Bay “W” values were used in the equation instead of the originally discussed Barnegat Bay W value. Annual estimates for water quality benefits for each farm at different biomasses can be found in Table D.1.

D.4. Methods Modifications

The calculations for Delaware and Rehoboth Bays varied from the above methods in the following ways:

- Delaware Bay:
  - The oyster lengths used to estimate the average biomass of a sub-market and market sized oyster were 30 and 80mm respectively. This change was intended to incorporate the oyster seed on the Delaware Bay farm as no other farms held small seed.
  - Robust standing stock records were kept so estimates of total biomass on the Delaware Bay farm were calculated for each experiment. Total biomass at Barnegat and Rehoboth Bays was assumed constant for each experiment.
The Delaware Bay farm was intertidal. As such $T=14$ hours as opposed to the 24 hours used for the subtidal farms.

Four experiments were conducted at Delaware Bay, so four estimates of daily volume of water cleared, biomass of particles removed from the water column, and the biomass of particles deposited into the environment at each farm were generated.

The coldest water temperature was observed on January 24, 2021.

- **Rehoboth Bay**

  Four experiments were conducted at Rehoboth Bay, so four estimates of daily volume of water cleared, biomass of particles removed from the water column, and the biomass of particles deposited into the environment at each farm were generated.

  The coldest water temperature was observed on January 31, 2021.

**D.5. Results**

Similar water quality benefits were evident between Delaware and Rehoboth Bay farms. Barnegat Bay provided more water quality benefits and generated more biodeposits than the other two farms. As the biomass on a farm increased so did the estimated water quality benefits and biomass deposition.

**References:**