EFFECTS OF EELGRASS PRESENCE AND ESTUARINE ABIOTIC FACTORS ON OYSTER PHYSIOLOGY

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by

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DEDICATION

This thesis is dedicated to Umihiko Hoshijima, a mentor and friend who “shore”-ly made waves in my life, believed in my potential. and nurtured my dreams. As I navigate the currents of marine science, I carry your spirit, passion, and puns with me.

ABSTRACT OF THE THESIS

Effects of Eelgrass Presence and Estuarine Abiotic Factors on
Oyster Physiology
by
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Seagrass meadows and oyster beds are in decline because of anthropogenic climate change and human impacts on coastal habitats. In Newport Bay, California, restoration of both the native eelgrass (*Zostera marina*) and the native Olympia oyster (*Ostrea lurida*) was conducted starting in 2016. This paired restoration not only aimed to restore both historically abundant species to functional population sizes, but also intended to return valuable ecosystem services to Newport Bay. However, excess sedimentation by *Z. marina* can smother oysters and prevent filter feeding, counteracting efforts to restore *O. lurida*. I studied the performance of *O. lurida* planted in three habitat types: *Z. marina* beds, *O. lurida* beds, and mudflats, and monitored temperature and rainfall effects on *O. lurida* performance. Oyster performance was evaluated through variations in valve gaping and heart rate measurements. Under stressful conditions bivalves close their shell valves to wait for favorable conditions, but in doing so, can no longer filter feed or aerobically respire. Stressors can also affect heart rates as oysters can increase or lower their heart rate to cut their energetic losses, with greater variations in heart rate relating to higher stress. The loss of feeding time and need to divert energy to maintenance can potentially impact growth and reproduction.

Over the course of 11 months in three different habitats and four sites in Newport Bay, there was no effect of site or habitat on the physiology of the oysters. There were seasonal effects, linked to rainfall, on the proportion of valve gaping, as oysters were more closed in the winter season than summer, fall, and spring. Although rainfall significantly increased shell closure and prevented oysters from feeding or respiring, these changes were temporary. In addition, growth metrics were not affected by seasonal differences, so the rain events may only affect oyster physiology in the short term. My findings may direct future restoration of *O. lurida* in Newport Bay to continue paired restoration with *Z. marina*.

TABLE OF CONTENTS

PAGE

LIST OF TABLES

PAGE

LIST OF FIGURES

INTRODUCTION

LIVING SHORELINES

As the effects of climate change and sea level rise threaten ecologically important coastal vegetated habitats, scientists have turned to more novel approaches to protect against shoreline erosion (Crosby et al., 2016). Previously, buffers such as seawalls or riprap were implemented to protect the coast behind the wall (Kraus & McDougal, 1996), but these armoring strategies are associated with habitat loss and fragmentation due to the physical structures separating upland from wetland ecosystems (Patrick et al., 2014) and an increase in wave energy from reflecting waves that promote down shore erosion (Bozek & Burdick, 2005). Living shorelines were introduced as an alternative strategy to prevent erosion through the use of natural habitat as buffers (Bilkovic et al., 2016). Living shorelines are structures made of natural materials and living organisms that grow over time. Materials used for living shorelines can vary but include soft materials (marsh grasses, upland trees and shrubs), biodegradable materials while vegetation becomes established (coir fiber logs and matting), or hard structures (oyster reefs and rock sills; O'Donnell, 2017). Natural flora, fauna, and other organic materials can reduce erosion risk and increase wave attenuation and habitat heterogeneity, which is especially important in habitats prone to intense storms (Bilkovic et al., 2016; Narayan et al., 2016). In North Carolina, a rock sill living shoreline, established between 1991-2006, was more resilient to erosion during the 2016 Hurricane Matthew than hardened shorelines and natural marshes (Smith et al., 2018). The rock sill living shoreline recovered from hurricane damage naturally and provided habitat for important marsh plants (Smith et al., 2018). In Chesapeake Bay, the establishment of various living shorelines habitats were shown to expand the functional value of the shoreline (Davis, 2008). Restored vegetation significantly increased densities of various fish species within two months after restoration and restored oyster reefs provided the greatest refuge for the commercially valuable blue crabs (Davis, 2008). In combination with erosion prevention, living shoreline initiatives can also restore ecologically extinct or rare species back into ecosystems where they were once prevalent.

One successful living shorelines initiative took place in Newport Bay, California in 2016 with multiple ecological goals in mind. Scientists combined the practice of living shorelines with the restoration of declining populations of seagrasses and oysters (Zacherl et al., 2015). Oyster and seagrass beds were restored in pairs with the aim to synergistically provide ecosystem benefits. Research in Newport Bay has continued to evaluate the effects of these species on each other and their potential synergistic ecosystem services. Seagrass beds have grown widely and expanded into the restored oyster beds, potentially affecting the oysters. Information on the physiology of the oysters in this restoration, and subsequent response to stressful events, is lacking. It will be important to evaluate the effects of this paired restoration on the performance of the oyster species, especially given their important ecological role as filter feeders and builders of emergent reef structure that can help moderate wave action.

OYSTERS AND SEAGRASS ECOSYSTEM EFFECTS

Oysters and seagrasses are common foundation species in estuarine environments (Howard, 2019). Eelgrass (*Zostera marina*) provides key habitat for a diverse assortment of infaunal and epifaunal species worldwide, but due to overfishing, habitat loss, water quality degradation, and other anthropogenic factors, *Z. marina* populations have declined (Heide et al., 2007; Lefcheck et al., 2017; Sharma et al., 2016). Tall, dense blades of *Z. marina* beds create nursery habitat for juvenile fish and invertebrates, along with the potential to attenuate waves, decrease turbidity, and lower nutrient levels in the water column (Heck et al., 2003; Moore, 2004). *Z. marina* is an ecosystem engineer, with the ability to physically create microhabitats for a multitude of organisms within its beds (Koivisto & Westerbom, 2010). Along with providing oxygen and organic matter to nearby species, *Z. marina* increases the pH of the water in its meadows (Hendriks et al., 2014; Ricart et al., 2021). This increase in pH allows for more carbonate ions to be available in the water column, potentially benefiting nearby oysters that need carbonate to build their calcium carbonate shells (Ricart et al., 2021). These associated shell-building organisms also provide important functions and services for the system.

Oysters improve water quality through filter feeding, removing pathogens, providing habitat for other species by aggregating into reefs, stabilizing muddy substrate, and increasing habitat complexity in threatened estuaries (P. Baker, 1995; Blake & Bradbury, 2012; Howard, 2019). Adjacent oyster beds may benefit *Z. marina* beds due to their filter feeding activity. *Z. marina* are usually limited by light availability, whereas high densities of oysters have been shown to remove suspended sediments, plankton, and other organic debris from the water column and improve light availability for photosynthesis (van der Heide et al., 2012).

In addition to providing valuable ecosystem services, oysters are economically valuable through fisheries and aquaculture practices. The California oyster fishery began in the 1850s with the harvesting of the Olympia oyster, *Ostrea lurida,* the only native oyster to the west coast of North America (California Department of Fish & Wildlife, 2008). But due to the rapid expansion of this fishery, *O. lurida* populations crashed (California Department of Fish & Wildlife, 2008). The non-native Pacific oyster, *Crassostrea gigas* was imported from Japan to be used as a replacement for the fishery and subsequent aquaculture practices on the west coast. As a larger and more robust species, *C. gigas* quickly outcompeted dwindling populations of *O. lurida* (Buhle & Ruesink, 2009). Through a combination of habitat loss from coastal developments, overharvesting, and the introduction of *C. gigas, O. lurida* populations declined to less than 1% of their previously documented size in 1996 and were later defined as functionally extinct (Beck et al., 2011; Wallace, 1966). Due to their ecological and economic importance, multiple efforts to restore the species have taken place across the west coast of North America with over $1 million invested by NOAA to facilitate restoration (Bulseco, 2012).

OYSTER-SEAGRASS PAIRED RESTORATION IN NEWPORT BAY

Paired restorations of foundation species in particular have been shown to have synergistic benefits to their ecosystem, allowing for one species to fulfill an ecosystem service where another may be lacking (Gedan et al., 2011; van Wesenbeeck et al., 2013). Due to their declining populations, potential mutualistic associations, and ecosystem services, *O. lurida* and *Z. marina* have been restored in proximity to one another in multiple locations including Washington State (Hood Canal) and California (Griffith, 2018; San Francisco Bay and Newport Bay; Sharma et al., 2016; Valdez et al., 2017). In Newport Bay, California, restoration of *Z. marina* and *O. lurida* was conducted in 2016 and 2017, respectively. As the second largest estuarine environment in southern California, Newport Bay experiences heavy boat traffic and substantial coastal development. Restoration of native species that protected the coastline from erosion and brought back ecosystem services was the main goal of the restoration project (Howard, 2019; Sutula et al., 2006).

By utilizing multi-habitat restoration techniques, beds of *Z. marina* and *O. lurida* were successfully restored in multiple sites in Newport Bay (Howard, 2019). *Z. marina* restoration took place in May 2016 with individual shoots transplanted from healthy beds at two local donor sites to the restored bed locations. Recruitment of *O. lurida* populations requires hard substrate to attach to and Newport Bay consists of mostly mudflat substrate in the intertidal zone (Trimble et al., 2009). To promote *O. lurida* settlement on hard substrate, coco coir bagged empty shells of *C. gigas* were planted in Newport Bay (Nichols et al., 2019; Zacherl et al., 2015). The shells of the non-native species were both abundant and large, ideal for creating substrate for *O. lurida* recruits. Both oyster species are found to overlap between +0.3 m above mean lower low water level (MLLW) and +0.7 m above MLLW but *O. lurida* density is highest at or below +0.2 m MLLW while *C. gigas* density is highest at or below +0.4 m MLLW (Tronske et al., 2018). To prevent competition from *C. gigas* and promote *O. lurida* growth at their optimal tidal elevation, the coco coir shell bags were placed at -0.15 m MLLW (Griffith, 2018).

Some studies have found the overlapping distributions of *Z. marina* and *O. lurida* lead to competition for space and increased sediment levels in oyster beds as a result of *Z. marina* sediment deposition (Wagner et al., 2012). As *Z. marina* blades slow water movement, sediment falls out of the water column and onto the substrate. Increased sedimentation can prevent erosion in valuable estuary habitat but can also smother oyster gills with excess sediment (Pritchard et al., 2015; Wasson, 2010). The restoration project in Newport Bay attempted to prevent this by planting *Z. marina* beds lower in the intertidal, from ~ -0.3 m MLLW through -3.6 m MLLW, and restoring *O. lurida* reefs at -0.15 m MLLW, the highest tidal height possible while still promoting settlement (D. Zacherl, personal communication, 2022; Howard, 2019; Valdez et al., 2017). Since the original restoration, *O. lurida* beds have migrated ~3 m vertically towards the shoreline (reaching ~ +0.1 m MLLW). *Z. marina* beds have grown outside of the original plots, expanding upshore between and next to oyster beds in 3 out of 4 of the original sites. As *Z. marina* beds move closer to *O. lurida* individuals, the opportunity for smothering due to sedimentation may increase.

OYSTER PERFORMANCE IN DIFFERENT HABITATS

Within the restored sites in Newport Bay, three potential habitats exist for *O. lurida* to reside in: restored *O. lurida* beds, restored *Z. marina* beds, and pre-existing mudflats, which make up much of the remaining shoreline. Currently, the majority of *O. lurida* individuals have settled in the restored oyster beds where there is ample hard substrate to attach to, in the form of other oyster shells. As *O. lurida* expands upshore and laterally, *O. lurida* individuals may move into mudflat areas, a terrain that oysters are usually absent from due to a lack of hard substrate to settle on and the potential for burial under fine sediments (Wasson, 2010). Wasson et al. (2015) noted that in the absence of hard substrate, sediment burial is an extremely important factor and the most commonly encountered stressor for *O. lurida*. In Newport Bay, nearly the only hard substrate available to *O. lurida* is the loose shell placed down during the initial restoration process.

Z. marina has been shown to increase pH, aiding in calcium carbonate precipitation for oysters, but the degree to which *Z. marina* raises pH is likely to change depending on size and density of *Z. marina* beds, waterflow, and other factors (Ko et al., 2014; Ricart et al., 2021; Timmins-Schiffman et al., 2014; Wright et al., 2014). *O. lurida* individuals in these estuaries already experience a wide range of pH conditions, and the effect of future ocean acidification on *O. lurida* is predicted to be low (Wasson et al., 2015). With these factors in mind, pH is not likely to be a major stressor for *O. lurida* in Newport Bay. A. Lowe et al. (2018) planted *O. lurida* individuals in *Z. marina* beds and in unvegetated habitat in Washington State and found survival of *O. lurida* was lower in *Z. marina* habitat but found no overall patterns for shell and tissue growth, shell strength, and stable isotope and fatty acid biomarkers. This indicated the effect of *Z. marina* on *O. lurida* was mediated by environmental conditions and predation from other invertebrates, important factors to consider when observing *O. lurida* performance. In Newport Bay, environmental conditions are variable and likely to influence *O. lurida* performance. The predators present in Washington, such as oyster drills (*Urosalpinx cinerea*), sea stars (class *Asteroidea*), and moon snails (family *Naticidae*), are not prevalent in Newport Bay so the effect of predation is not likely to be as extreme (D. Zacherl, personal communication, 2022; Peter-Contesse & Peabody, 2005). Although A. Lowe et al. (2018) found lower survival in their transplant, they did not explicitly account for increased sedimentation effects from *Z. marina* that may smother *O. lurida* gills and lower performance, so the importance of this factor in their experiment is unclear (A. Lowe et al., 2018; Wasson, 2010).

Performance in each of these three habitats can also be affected by varying environmental conditions in the estuary such as temperature, salinity, food supply, oxygen levels, tidal elevation, and flow rates.

OYSTER PERFORMANCE IN DIFFERENT ABIOTIC CONDITIONS

Temperature can greatly impact the physiology of oysters. In the lab, mortality occurs when air temperature reaches around 40°C (Wasson et al., 2015). Newport Bay air temperatures rarely exceed 23°C during the year, but when coupled with other stressors and considering body temperatures of intertidal organisms can be much greater than the surrounding air temperatures at low tide, these temperatures could be stressful (Bible et al., 2017; Helmuth, 1998; National Oceanic and Atmospheric Administration, 2023). Similar patterns are found with water temperature, in which *O. lurida* grows faster in temperatures greater than average (24°C) but experiences stress at higher temperatures (38-39°C; Brown et al., 2004). Newport Bay water temperatures range from 12°C to 22°C during the year, rarely hitting *O. lurida*'s thermal maximum (National Oceanic and Atmospheric Administration, 2023).

The sensitivity of *O. lurida* to different abiotic conditions was determined by Wasson et al. (2015) for *O. lurida* in Northern California and Oregon estuaries. Salinity exposure below 25 psu had strong negative effects on *O. lurida*, but thresholds varied across regions due to local adaptation (Oates, 2013; Wasson et al., 2015). Salinity ranges in Newport Bay have been shown to vary from 22.58 ± 1.87 psu to 31.91 ± 1.83 psu in 2005 (Nezlin et al., 2009). The lower salinity ranges are likely to be observed during southern California's rainy season, which is projected to become shorter and sharper with changes in climate trends (Luković et al., 2021).

As a filter feeder, *O. lurida* depends on a constant supply of phytoplankton and organic matter (Lucas, 2012). Although *O. lurida* has significantly lower filtration rates than other species, a limited food supply may lead to reduced growth (Gray & Langdon, 2019; Hettinger et al., 2013; zu Ermgassen et al., 2013). In using chlorophyll *a* as a proxy for phytoplankton biomass, Riisgård et al. (2006) found other filter feeders reduce their gaping when chlorophyll *a* concentrations were low. Feeding time can also be limited by tidal elevation changes since low intertidal individuals have more time to feed than higher intertidal individuals (Deck, 2011). The phytoplankton concentrations necessary for optimum growth have not been determined for *O. lurida* (Wasson et al., 2015). Levels of chlorophyll *a* of <5 μg L^{-1} were found to be associated with reduced growth of *O. lurida* in Northern California and Oregon (Wasson et al., 2015). But in Upper Newport Bay, chlorophyll *a* levels averaged 2.8 ± 1.9 μg L^{-1} (mean ± SD) with a range of 1.7–3.66 μg L^{-1} in 2006 (Sutula et al., 2006). With levels above thresholds set by Wasson et al. 2015, the restored *O. lurida* may not experience stress due to reduced phytoplankton concentrations.

Estuaries can often experience hypoxic events in which dissolved oxygen (DO) levels decrease and create stressful conditions for organisms. At low oxygen levels, bivalves can close their valves and lower their heart rates to cut their energetic losses (Porter & Breitburg, 2016; Trueman, 1967). Oyster settlement, juvenile growth, and juvenile survival have all been shown to be negatively affected by hypoxia (S. M. Baker & Mann, 1992). *O. lurida* thresholds have been found to exist around 2-5mg/L but can vary depending on the amount of time an oyster spends at that oxygen level (Wasson et al., 2015). DO levels in Newport Bay were found to vary widely but exhibited ranges between 2.04 ± 1.01 mg/L and 7.56 ± mg/L in 2005 (Nezlin et al., 2009).

Optimal tidal elevation for *O. lurida* in Newport Bay was found to be around -0.15 m MLLW when restoration took place (Griffith, 2018). At this elevation, *O. lurida* are mostly underwater except during negative spring tides. This increases time for filter feeding and decreases potential stress due to low temperatures. In other restoration efforts, *O. lurida* recruitment was more successful when beds of empty shell were placed even lower in the intertidal zone (Fuentes et al., 2020). But since their initial restoration, *O. lurida* beds in

Newport Bay have expanded higher in the intertidal, likely due to the effects of wave action generated by passing boats dislodging material from the restored shell mounds (D. Zacherl, personal communication, 2022).

Water flow across *O. lurida* beds can be an important factor, given that flow may influence a sessile filter feeder's response to sedimentation rates, salinity levels, chlorophyll *a* levels, and DO levels (Grizzle et al., 1992; Riisgård et al., 2006). High flow habitats may enhance feeding and oxygenation of *O. lurida*'s gills (Wasson et al., 2015). Oysters thrive with flow rates of 15 cm s^{-1}, but are able to survive and grow between 1 and 22 cm s^{-1} (Campbell & Hall, 2019; Gray & Langdon, 2019). General flow rates for Newport Bay, which would primarily be driven by tidal exchange, are not known. Optimum flow rate is variable due to other potential factors such as chlorophyll *a* content, turbidity, oxygen, etc. that can vary depending on the environment (Campbell & Hall, 2019). These varying environmental conditions can influence each other and oyster performance in different ways so it will be important to understand how oysters respond to a suite of environmental conditions.

CARDIAC AND VALVE GAPE MEASUREMENTS OF OYSTER PERFORMANCE

Common metrics to study oyster performance include measuring length, weight, and recruitment rates (Livingston et al., 2000). These variables integrate oyster physiological responses to external pressures over longer periods of time. In contrast, heart rate and valve gape in intertidal invertebrates is widely accepted as a proxy for immediate physiological response (Bayne, 2000; Frank et al., 2007). Organisms have been found to mitigate stress through behavioral responses, such as curtailing feeding or increasing their heart rate under stressful conditions. Under non-stressful conditions, oysters can allocate more energy to growth and reproduction, instead of maintenance, which can have important implications for restoration success (Bayne, 2000).

In bivalves, higher heart rates may be indicative of higher performance under favorable conditions such as higher feeding activity from food availability (Widdows, 1973), while under some circumstances, an increase in heart rate range can demonstrate lower performance due to metabolic stress at high temperatures (Hui et al., 2020), or the need to replenish oxygen stores after closure from a low tide or rainfall events (Byrne et al., 1990). Lower heart rates may be indicative of non-stressful conditions (Andrews et al., 1959), but can also indicate stressful conditions such as when bivalves enter a state of dormancy in low salinity (Andrews et al., 1959) or during low tide (Bayne, 2000), in order to reduce energy usage as metabolism switches over to less efficient anaerobic glycolysis during prolonged closures. Since high or low heart rates can be indicative of stressful conditions, where an oyster is allocating more energy toward staying alive or recovering from stress, an oyster with a wide range of heart rates may be exhibiting lower overall performance. Valve gape can be an indicator of feeding and respiration activity and is one of the most important and practical measurements of behavioral response in bivalves (Frank et al., 2007). Under stressful conditions, such as low salinity levels, low dissolved oxygen levels, or emersion, oysters close their valves to protect their tissue (Dowd & Somero, 2013; Porter & Breitburg, 2016). Bivalves with closed valves exhibit lower performance since they spend less time feeding and respiring, and devote less energy toward growth and reproduction (Ballesta-Artero et al., 2018).

HYPOTHESES

As the *O. lurida* beds continue to move upshore and expand laterally, it is important to understand how these individuals are performing in mudflat habitat when compared to individuals in the middle of the *O. lurida* beds. Similarly, as the *Z. marina* beds move upshore and between *O. lurida* beds, it is important to note how the development of new *Z. marina* habitat will affect *O. lurida* individuals. Physiological performance of restored oysters has not been studied when nearby its neighboring species, *Z. marina*. If *O. lurida* individuals prove to have high individual performance near or away from *Z. marina* beds, then they will be more likely to have greater spawning numbers and extended spawning seasons when restored in that area (Seale & Zacherl, 2009). If there is no difference in individual performance, *Z. marina* restoration efforts may continue as *Z. marina* benefits from *O. lurida* populations (van der Heide et al., 2012), but if oyster performance is lower near *Z. marina,* then separate restoration of *O. lurida* might be necessary to increase oyster success. Improved understanding of organism responses to restoration efforts can inform future attempts to rehabilitate endangered and economically valuable species. This motivated my field experiment to measure the physiological performance of *O. lurida* within three habitats (mudflat, *Z. marina* beds, and *O. lurida* beds) at four restored sites in Newport Bay. The hypothesized measures of higher performance included more time with open valves, moderate heart rates, lower heart rate ranges, and higher shell growth rates. I hypothesized that oyster performance would be highest in *O. lurida* bed habitat, then *Z. marina* bed habitat, and lastly mudflat habitat. I also tested the hypothesis that *O. lurida* individuals that experienced fewer extreme fluctuations in environmental conditions such as temperature and salinity would experience higher performance than those in more extreme environmental conditions.

METHODS

SITE SELECTION AND OYSTER COLLECTION

I conducted my field experiment in upper Newport Bay (33.620272 N, -117.891842 W), an urbanized low-inflow estuary in Newport Beach, California. Upper Newport Bay is part of the Newport Bay watershed, which spans a 752-acre estuary, the largest protected estuary in southern California (California Department of Fish & Wildlife, 2023; Seale & Zacherl, 2009). Upper Newport Bay includes multiple environments such as tidal flats, coastal marshes, and upland bluffs that provide habitat for animals such as the endangered Ridgway's rail, the California least tern, and other small mammals and wetland fish (California Department of Fish & Wildlife, 2023). Efforts to create a living shoreline through the restoration of *O. lurida* and *Z. marina* took place at four sites within upper Newport Bay: Pacific Coast Highway (PCH), Westcliff (WC), DeAnza Peninsula (DA), and Shellmaker Island (SM; Howard, 2019; Fig. 1).

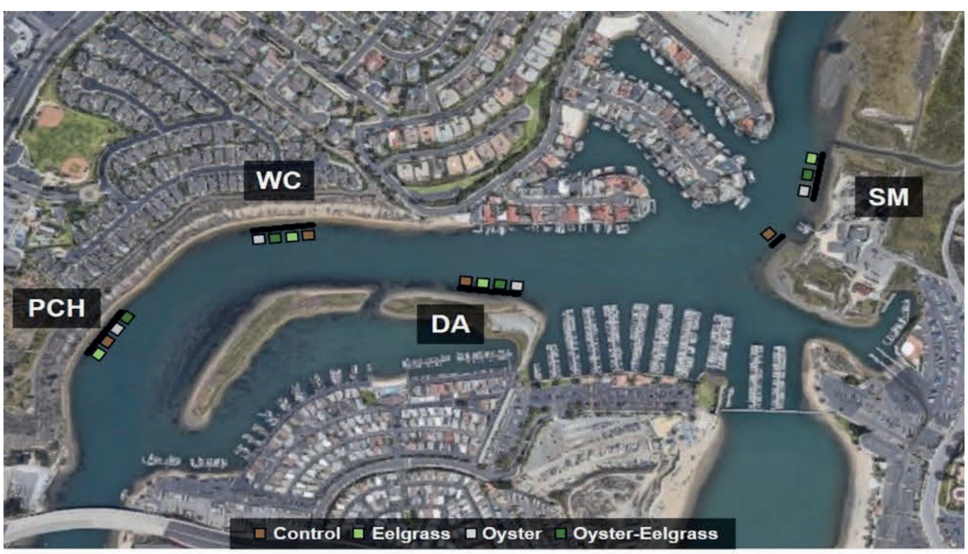

Figure 1. Map of restoration sites in Newport Bay, CA. Source: Howard 2019.

These four restoration locations were chosen because they were thought to have similar environmental conditions such as water quality, depth, and sediment type across the estuary (Wood, 2018). The original restoration included four treatments at each site: oyster restoration alone, eelgrass restoration alone, paired oyster and eelgrass restoration, and unrestored mudflat that served as a control condition (Howard, 2019). Eelgrass restoration occurred in June-July 2016, and oyster restoration occurred in April 2017 (Wood, 2018). Coco coir bags filled with empty *C. gigas* shells, designed to promote *O. lurida* settlement, were planted intertidally at -0.15 m MLLW (Wood, 2018). In the paired treatment, one *Z. marina* bed was planted ~ -0.3 m MLLW through -3.6 m MLLW, a minimum horizontal distance of ~2.25 m away from the oyster bags. Currently, *Z. marina* beds have grown across both "Oyster/Eelgrass" and "Oyster only" treatments at three out of four of the sites, extending their perimeter upshore to the edges of *O. lurida* beds, and *O. lurida* individuals have recruited and grown outside the boundaries of the original beds, into unvegetated mudflat substrate (Tate-Pulliam, 2021). The *Z. marina* bed at SM died off, leaving no *Z. marina* near the *O. lurida* reefs.

I collected experimental oysters from a naturally occurring bed in Lower Newport Bay (33.617639 N, -117.905037 W) in June 2022 and transported them to San Diego State University's Coastal and Marine Science Institute Laboratory for sensor attachment. I collected individuals ranging from 35-60mm to allow for enough space to attach the sensors and maintain a minimum shell thickness for heart rate sensor measurements. I obtained permission to use this habitat from the California Department of Fish and Wildlife.

DEVELOPMENT OF PHYSIOLOGICAL SENSORS

Custom-made circuit boards were designed and soldered to power heart rate (VCNL4040, Vishay Intertechnology, Malvern, PA) and gape sensors (A1395, Allegro Microsystems, Manchester, NH). Techniques to measure physiological conditions have improved over time. Previous heart rate monitoring involved cutting into the bivalve's shell to expose the heart or implant electrodes into the pericardial cavity of the organism (Braby & Somero, 2006; G. Lowe, 1974). Modern techniques now include heart rate sensors that illuminate the heart and measure the amount of light reflected, measuring any movement on the heart's surface (Hellicar et al., 2015). This affordable technique allows for non-invasive, real-time monitoring and continuous measurements over longer periods of time. Heart rate sensors used an infrared LED emitter to shine light through the shell of the oyster and measure its reflection. The movement of oyster tissue from its heart beats changes the amount of light absorbed by the sensor, permitting the estimation of heart rate of the oyster through the rhythmic output of the sensor (Miller, 2022).

Electronic sensors, glued to the outside of a bivalve's shell, have been used to observe valve gaping (de Zwart et al., 1995). The most common system uses a Hall effect sensor and magnet placed on opposite valves of a bivalve's shell to measure the strength of the magnetic field (Wilson et al., 2005). When an oyster begins to gape, the magnet moves farther from the sensor and the magnetic field becomes weaker. When an oyster closes its shell, the magnet moves closer to the sensor and the magnetic field becomes stronger (Miller & Dowd, 2017). Due to the low power mode on the circuit boards, batteries (2200 mAh Lithium-Ion rechargeable, Adafruit Industries, New York, NY) lasted from 1-2 months. I collected data on a microSD card and retrieved the data approximately every month. Circuit boards were programmed using Arduino open-source software (Miller, 2022). Gape sensor circuit boards also included a temperature sensor, TMP107 (Texas Instruments, Dallas, TX). The temperature sensor was used to estimate water temperature in Newport Bay when sampling during tide heights greater than 0.5 m. Refer to Miller (2022) for more detail on the circuit board processors and components.

I outfitted over 100 *O. lurida* individuals with heart rate and gape sensors over the course of the 11-month experiment. Shells were cleaned and slightly thinned to provide a smooth surface for sensor attachment and penetration of the heart rate sensor's infrared emitter. All oysters were thinned in the same manner to account for handling stress. I placed heart rate sensors by identifying the location that provided the strongest heart rate signal using a pulse wave plot. Gape sensors were attached to the posterior end of the valves, at the thinnest part of the shell, with a magnet on the opposite valve (Miller & Dowd, 2017). Sensors and magnets were attached with cyanoacrylate glue and A-788 splash zone epoxy. Excess epoxy was placed on the outside of the heart rate sensor to reduce ambient light interfering with the sensor.

I attached waterproof sensors to the circuit board and battery and then placed within a waterproof PVC pipe. The compact instrumentation allowed for the PVC pipe to be buried in the sediment with the oyster resting at the surface of the substrate, reflecting their placement in natural conditions (Fig. 2).

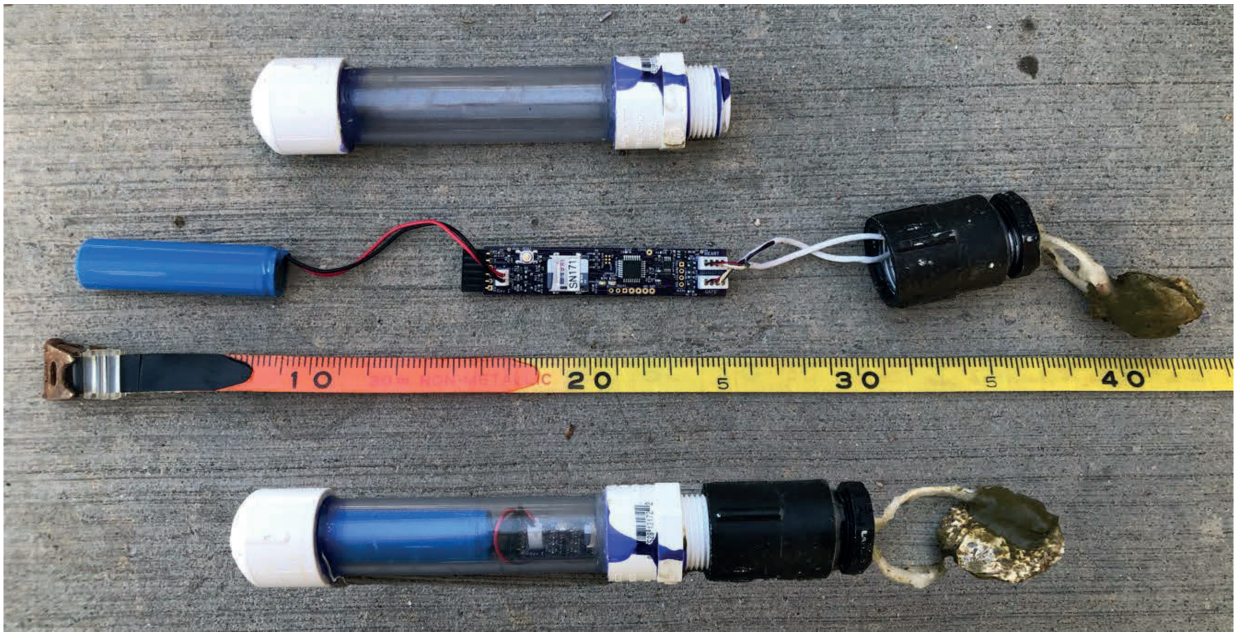

Figure 2. Picture of battery, circuit board, and housing unit connected to an oyster.

FIELD EXPERIMENT

At De Anza, PCH, and Westcliff, I chose three areas with distinct substrate types at the same tidal elevation- *Z. marina* bed, *O. lurida* bed, and mudflat area with neither *Z. marina* nor *O. lurida* present. At Shellmaker, only two treatment areas, *O. lurida* beds and mudflat areas, were selected because the restored *Z. marina* bed had completely died back since restoration. Each treatment area was a minimum of 30x4 m (length alongshore x width). In each treatment, oysters with attached sensors were randomly placed along a 10 m transect placed at -0.15 m MLLW shore height (Griffith, 2018). In the oyster bed and mudflat treatments, oysters were placed at least 1m away from subtidal seagrass beds to account for potential edge effects (Griffith, 2018). The number of oysters present in each treatment within a site varied for the duration of the experiment but averaged 3 oysters at a time, with a maximum of 6 oysters per treatment within a site. Sample size varied due to flooded sensors or dead oysters, but replicates were replaced as often as possible. Oysters with attached sensors were deployed starting July 15, 2022, and all removed by June 19, 2023, for a continuous 11 months of data collection.

The heart rate data was recorded at 8 Hz for 30 seconds every 5 minutes, while the valve gape and temperature data were recorded once every minute. I serviced oysters and their attached sensors approximately every month. During maintenance visits, I replaced dead batteries, downloaded data off each sensor, and replaced dead oysters and flooded or broken sensors. After 11 months of data collection, I removed oysters from Newport Bay and brought them back to SDSU's marine lab for dry weight analysis. Remaining live oysters were opened and soft tissue was removed. Soft tissue was dried in an oven at 65°C for 48 hours before being weighed (Langevin, 2019).

I measured oyster length and width using calipers (measured to the nearest mm) before deployment, during deployment, and post deployment. Length was measured as the distance from the umbo to the distal margin, width was measured as the perpendicular distance across the widest part of the shell (Andersen, 2018). Growth rates were calculated by using the difference in length or width of individual oysters between each measurement and its previous measurement, and dividing that by the number of days between measurements (Rimler, 2014).

The distance between the two valves at the distal end of the shell was estimated using the Hall effect sensor signal. Dead oysters with working sensors attached were used for calibrations to relate the sensor magnetic field strength reading to an estimate of valve gape distance in millimeters. Aluminum rods of known diameters were placed between the valves of the oyster shell adjacent to the Hall effect sensor and magnet location while the Hall sensor measurement was recorded. The slope of the line of the regression between the raw Hall sensor output and measured valve gape distance was used to determine the gape opening for the field data. Based on the minimum gape distance and maximum gape distance measured for each oyster, all gape distance estimates were converted into a percentage opening (0 to 100%) to provide a standardized metric that could be used to compare different oysters. For the analyses carried out below, oysters were classified as "Closed" or "Open" using an estimated gape value threshold of 5% of maximum gape width. Oysters that were <5% open at the time of a gape measurement were scored as "Closed", oysters that were >5% open at the time of measurement were scored as "Open". This binary variable was used instead of the continuous percentage open value because small differences in opening (i.e., the difference between 25% and 30% opening) don't have clear implications for oyster physiology. Some species of clams have been shown to significantly reduce feeding and respiring at around 20% gape opening (Ballesta-Artero et al., 2017; Jou et al., 2013). I used a threshold of 5% as a conservative value for feeding and respiring since *O. lurida* are the smallest species of oysters in North America and have a smaller distal opening than other bivalves, which could overemphasize the physical distances in percent opening (Gillespie, 2009). While the importance of small differences in the degree of valve opening aren't known for *O. lurida*, the open/closed dichotomy I have chosen for analysis does have necessary effects on the ability to feed, respire, eliminate waste, and successfully reproduce.

Heart rate data were filtered to define and count peaks in the oyster's heart rate. The daily average and daily range of heart rate was used as a proxy for stress levels. The greater the range, or change in heart rate, an oyster undergoes, the more stressed the oyster is likely to be. Bivalve heart rates increase with increasing temperature until a certain point when activity falls sharply (Braby & Somero, 2006). Extreme temperatures and salinity levels can cause heart rate to behave arrhythmically, as the heart has the potential to enter cardiac arrest under stressful conditions (Braby & Somero, 2006). Sensors glued to oysters recorded the amount of light reflection every 5 minutes for 30 seconds, at 8 measurements per second (240 measurements per 30 seconds). Raw sensor data were low-pass filtered to remove high frequency noise from the samples, and peak-detection algorithms were used to determine beats per minute (bpm) by counting the peaks for each 240 measurements and doubling the result. Three algorithms (*pracma* package; Borchers, 2022; *forecast* package; Hyndman et al., 2023; *signal* package; Signal Developers, 2013) with slightly different peak detection sensitivity were used simultaneously and were flagged if the results of the algorithms varied more than 2 bpm from each other. If flagged, the light reflection plot was then visually inspected to determine true beats or categorized as "NA" if the plot showed no pattern to the peaks, or if the amplitude of peaks was too low (range <20). The sampling duration of 30 seconds necessarily limited the ability to discern very slow heart rates, so all plots with two peaks or fewer in the sampling window (<4 bpm) were removed from the dataset.

Due to the failure of a data logging water quality sonde, measurements of salinity, phytoplankton concentrations, pH levels, DO levels, and water flow were not available for Newport Bay during the dates of the field experiment. To serve as a proxy for salinity, precipitation data from the duration of the experiment was retrieved from the John Wayne

Airport rain gauge located approximately 6 km north of the field sites, within the Newport Bay watershed.

STATISTICAL ANALYSIS

Statistical analysis was conducted in R (version 4.3.1; R Development Core Team, 2023). Analysis of variance (ANOVA) tests were used to compare differences of the proportion of open valves, average daily heart rates, average daily range of heart rates, field deaths, and average growth of oyster width and length between seasons, sites, and treatments. Field deaths were analyzed as the number of deaths divided by the number of oysters deployed, since sample size differed by season, treatment, and site. ANOVA tests were also used to compare the proportion of open valves, daily average heart rate, and daily average heart rate range of oysters between the weeks of October 30-November 5, 2022, November 6-12, 2022, and November 13-19, 2022. Shapiro-Wilk's and Levene's tests were used to test assumptions of normality and heteroscedasticity, respectively. When data did not meet assumptions, was independent, and transformed data did not meet assumptions, the Kruskal-Wallis rank sum test was used to compare differences between seasons, sites, and treatments. Dunn's tests were used when Kruskal-Wallis tests were significant to measure differences between factors. An α-level of 0.05 was used for all statistical tests.

Correlation between average daily oyster temperatures and daily rainfall, average daily proportion of open valves, average daily heart rates, and daily heart rate ranges did not meet assumptions for normality so were conducted with the Kendall's Tau non-parametric test.

I analyzed repeated measures of gape and heart rate measurements in a generalized linear mixed-effects model (GLMM) from the *lme4* library (Bates et al., 2015). For the gape measurement model, I analyzed the relationship of open/closed values versus rain, site, and treatment using a binomial error distribution. I used binary response data from individual oysters (0=closed and gaping lower than the 5% threshold, 1=open and gaping greater than the 5% threshold) as the response variable. Fixed factors were a categorical rainfall variable ("Yes" if it rained that day or the day before, "No" if it did not rain on that day or day before), Treatment (three categories: oyster bed, seagrass meadow, mudflat), and Site (four locations: PCH, DA, SM, WC). Individual oyster ID was a random factor to account for repeated measures. To test the significance of the fixed factor, I used marginal mean sums of squares ("type=3" in the *Anova* function) with the *car* package (Fox & Weisberg, 2011), since the data was unbalanced. Dispersion was tested with the DHARMa nonparametric dispersion test and the DHARMa bootstrapped outlier test (*DHARMa* package; Hartig, 2022).

To understand the interactive effects between season and treatment, I ran a GLMM with the binary gape data as the response variable. Season (four categories: summer, fall, winter, and spring) and treatment were used as interactive fixed effects separately. Individual oyster ID was a random factor to account for repeated measures. To test the significance of the fixed factor, I used marginal mean sums of squares ("type=3" in the *Anova* function) with the *car* package (Fox & Weisberg, 2011), since the data was unbalanced. Pairwise analyses of the interaction values were conducted using a least-squares means test from the *lsmeans* package (Lenth, 2018) with "tukey" adjustment methods.

For the heart rate measurement model, I fit a negative binomial GLMM to understand the effect of rain, site, and treatment on average daily heart rates. The negative binomial model was used to deal with overdispersion. The response variable was either the average daily heart rate or the average daily range of heart rate of individual oysters. Fixed factors were a categorical rainfall variable ("Yes" if it rained that day or the day before, "No" if it did not rain on that day or day before), Treatment (three categories), and Site (four categories). Individual oyster ID was the random factor. I used marginal mean sums of squares to test significance of fixed factors with the *car* package (Fox & Weisberg, 2011) since the data was unbalanced.

RESULTS

PRECIPITATION AND TEMPERATURE

To account for seasonal variation in precipitation and water temperature, summer, fall, winter, and spring season data was separated into July 15, 2022- September 30, 2022, October 1, 2022- December 31, 2022, January 1, 2023-March 31, 2023, and April 1, 2023-June 19, 2023, respectively. When separated by season, Orange County received significantly higher cumulative rainfall during the winter months of January 2023-March 2023 compared to the rest of the year (Fig. 3A, Kruskal-Wallis (KW), $H_{(3)}$=23.80, p=2.753e-05). Cumulative rainfall was significantly different between Winter and Summer (Dunn's post-hoc, p=1.0e-5), Winter and Fall (Dunn's post-hoc, p=0.032), and Winter and Spring (Dunn's post-hoc, p=0.013). There was no significant difference between Fall and Summer (Dunn's post-hoc, p=0.201), Spring and Summer (Dunn's post-hoc, p=0.552), and Spring and Fall (Dunn's post-hoc, p=1.00).

Average daily water temperature in Newport Bay also differed significantly by season (Fig. 3B, KW, $H_{(3)}$=254.15, p<2.2e-16). Summer temperature was significantly different from Fall, Winter, and Spring, while Winter months were significantly different from Fall and Spring (all comparisons made using Dunn's test post-hoc, p<0.0001). Fall and Spring months did not significantly differ (Dunn's test post-hoc, p=1.00). Summer months had the highest daily average water temperature (22.9 °C), then Fall and Spring months (17.7 °C and 17.9 °C, respectively), and last Winter months (14.5 °C). Water temperature was retrieved from temperature sensors attached to oysters during high tides greater than 0.5 m.

For all boxplot graphs in this thesis, the box or interquartile range (IQR) represents the 50% of the data lying within the 25^{th}-75^{th} percentile of the dataset. The black bar within the IQR represents the median of the data. The whiskers on the lower end and upper end represent either the most extreme data points or points within 1.5x the IQR, while points outside the whiskers represent outliers of the data. The diamond symbols represent the mean of the data.

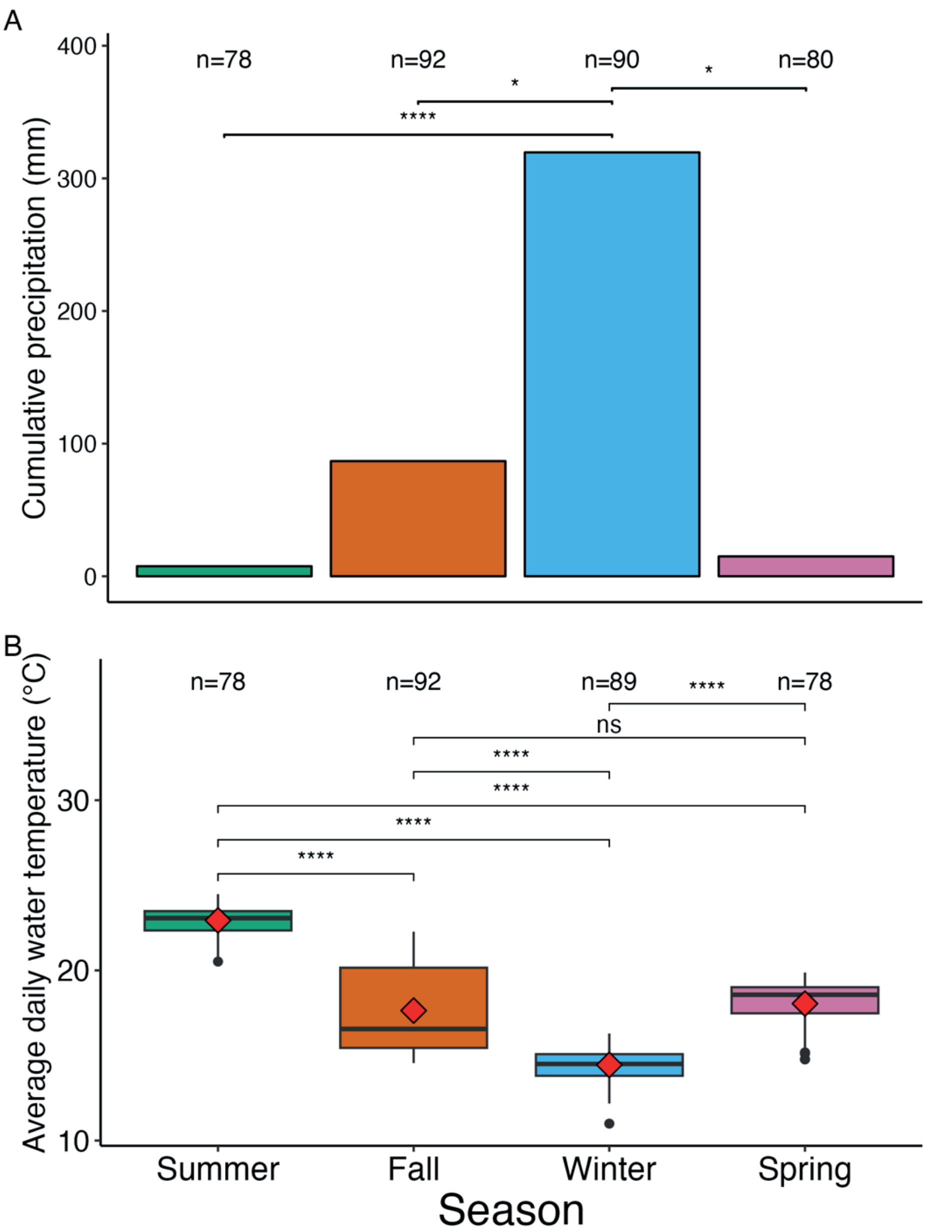

Figure 3. Plot of A) Cumulative precipitation in Orange County, CA. and B) average daily water temperature in Newport Bay, CA. separated by season from June 2022-June 2023. Precipitation data retrieved from John Wayne Airport rain gauge. Red diamonds represent averages and black bars represent medians. N = number of days. Significant differences as determined by Dunn's post-hoc comparisons, *=p<0.05, ****=p<0.0001.

Oyster temperature during high tide (greater than 0.5 m) did not significantly vary by site (Fig. A. 6A, KW, $H_{(3)}$=7.52, p=0.057) or treatment (Fig. A. 6B, KW, $H_{(2)}$=5.84, p=0.054). Oyster temperatures at low tide (less than -0.1 m) did not significantly vary by site (Fig. A. 6C, KW, $H_{(3)}$=7.84, p=0.853) or treatment (Fig. A. 6D, KW, $H_{(2)}$=1.73, p=0.424). Oyster temperatures across all tide levels did not significantly vary by site (Fig. A.6E, KW, $H_{(3)}$=6.42, p=0.093) or treatment (Fig. A.6F, KW, $H_{(2)}$=5.84, p=0.054).

There was a weak but significant correlation between average daily oyster temperature and daily rainfall (Kendall Tau Beta correlation coefficient, τ=-0.19, p<2.2e-16). Oyster temperatures decreased with increasing amounts of rainfall.

VALVE GAPE ANALYSIS

The proportion of open valves (>5 % open), averaged across all oysters, differed across seasons (Fig. 4A, KW, $H_{(3)}$=34.33, p=1.687e-07). The proportion of open valves was significantly different between Winter and Summer (Dunn's post-hoc, p=1.737e-07), Winter and Fall (Dunn's post-hoc, p=0.002, and Winter and Spring (Dunn's post-hoc, p=1.066e-03), and Fall and Summer (Dunn's post-hoc, p=5.365e-03). There was no significant difference between Spring and Summer (Dunn's post-hoc, p=1.000), and Spring and Fall (Dunn's post-hoc, p=0.434). The proportion of open valves did not differ by treatment (Fig. 4B, KW, $H_{(2)}$=0.81, p=0.669) and site (Fig. 4C, KW, $H_{(3)}$=8.04, p=0.061). I did not observe interactive effects between season, treatment, and site. The daily average proportion of open valves was weakly positively correlated with daily average oyster temperature (Kendall Tau Beta correlation coefficient, τ=0.25, p<2.2e-16).

When considering factors of rain, treatment, and site, the binary gape response was only significantly affected by rain ($\chi^2_{(1)}$=42782.50, p=<2.2e-16) and not affected by treatment ($\chi^2_{(2)}$=0.31, p=0.857) and site ($\chi^2_{(3)}$=2.82, p=0.420).

There was a significant effect of the interaction between season and treatment on the proportion of open valves ($\chi^2_{(6)}$=19356.93, p=<2.2e-16) and season ($\chi^2_{(3)}$=116124.81, p=<2.2e-16), but there was not an effect of treatment ($\chi^2_{(2)}$=1.211, p=0.546).

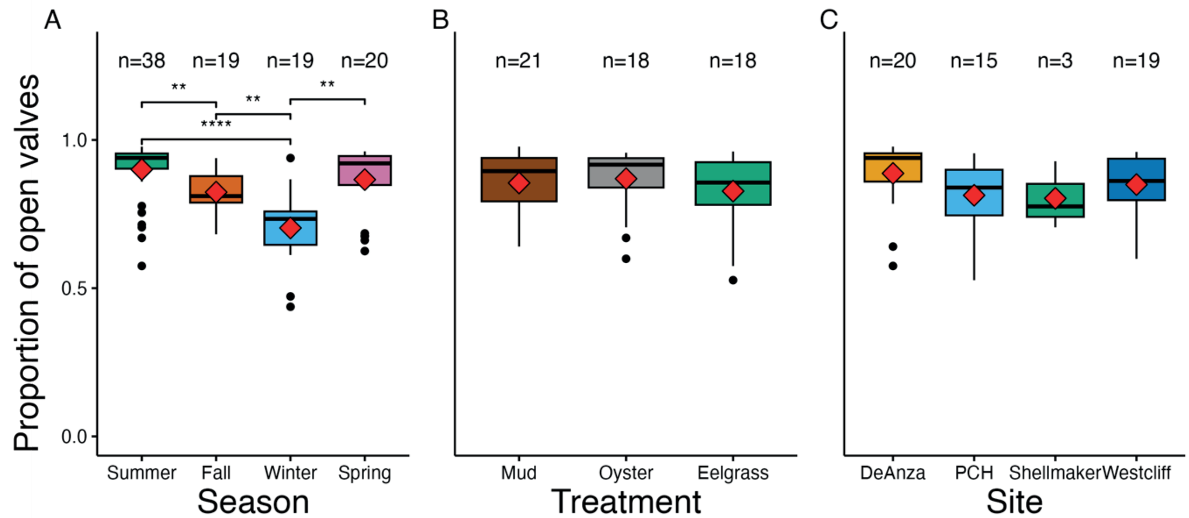

Figure 4. Proportion of open oyster valves across A) Season, B) Treatment, and C) Site. Summer, Fall, Winter, and Spring season represent data collected from July 15, 2022-September 30, 2022, October 1, 2022- December 31, 2022, January 1, 2023-March 31, 2023, and April 1, 2023-June 19, 2023, respectively. Red diamonds represent averages and black bars represent medians. N = number of oysters. Significant differences as determined by Dunn's post-hoc comparisons, **= p<0.01, ***=p<0.0001.

Heart rate analysis

I did not detect an effect of seasons (Fig. 5A, KW, $H_{(3)}$=0.58, p=0.901), treatments (Fig. 5B, KW, $H_{(2)}$=0.11, p=0.946) or sites (Fig. 5C, KW, $H_{(3)}$=1.78, p=0.620) on average daily heart rate. I did not observe interactive effects between season, treatment, and site. The average daily heart rate did not significantly correlate with daily average oyster temperature (Kendall Tau Beta correlation coefficient, τ=-0.02, p=0.124).

When considering factors of rain, treatment, and site, the average daily heart rate was not significantly affected by rain ($\chi^2_{(1)}$=0.40, p=0.527), treatment ($\chi^2_{(2)}$=1.93, p=0.381), or site ($\chi^2_{(3)}$=1.54, p=0.674).

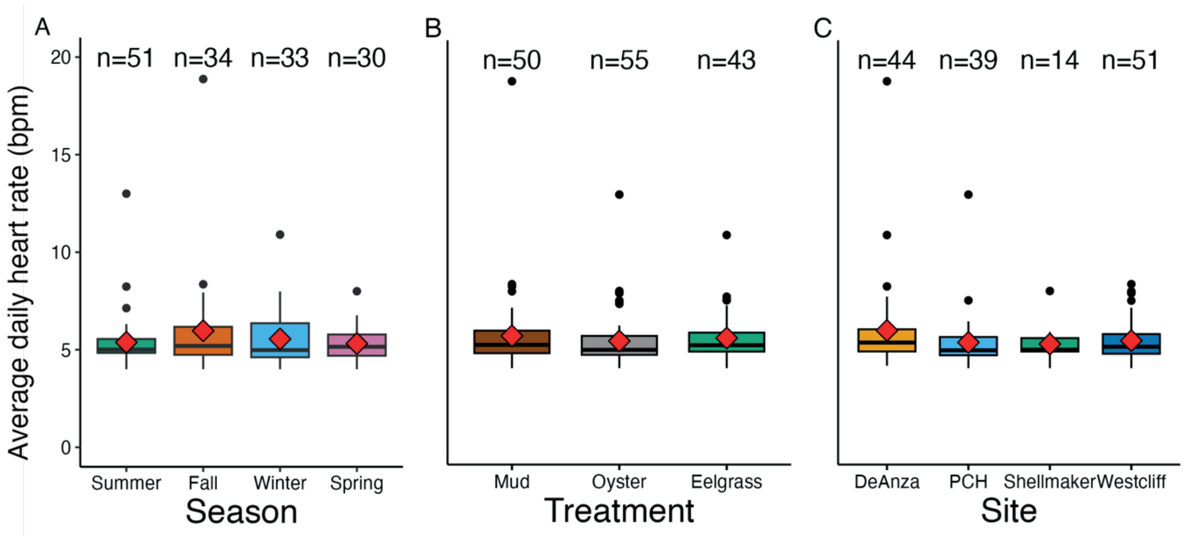

Figure 5. Average daily heart rate across A) Season, B) Treatment, and C) Site. Summer, Fall, Winter, and Spring season represent data collected from July 15, 2022-September 30, 2022, October 1, 2022- December 31, 2022, January 1, 2023-March 31, 2023, and April 1, 2023-June 19, 2023, respectively. Red diamonds represent averages and black bars represent medians. N = number of oysters.

Daily heart rate range, the difference between minimum and maximum heart rate observed on each day, did not significantly differ across seasons (Fig. 6A, KW, $H_{(3)}$=5.74, p=0.125), treatments (Fig. 6B, KW, $H_{(3)}$=2.69, p=0.260), or sites (Fig. 6C, KW, $H_{(3)}$=1.98, p=0.577). The daily heart rate range did not significantly correlate with daily average oyster temperature (Kendall Tau Beta correlation coefficient, τ=0.03, p=0.078).

When considering factors of rain, treatment, and site, the daily heart rate range was significantly affected by rain ($\chi^2_{(1)}$=6.94, p=0.008) but not treatment ($\chi^2_{(2)}$=0.17, p=0.917) or site ($\chi^2_{(3)}$=0.89, p=0.827). When considering treatment only, the daily heart rate range was not significantly affected by treatment ($\chi^2_{(2)}$=0.28, p=0.869).

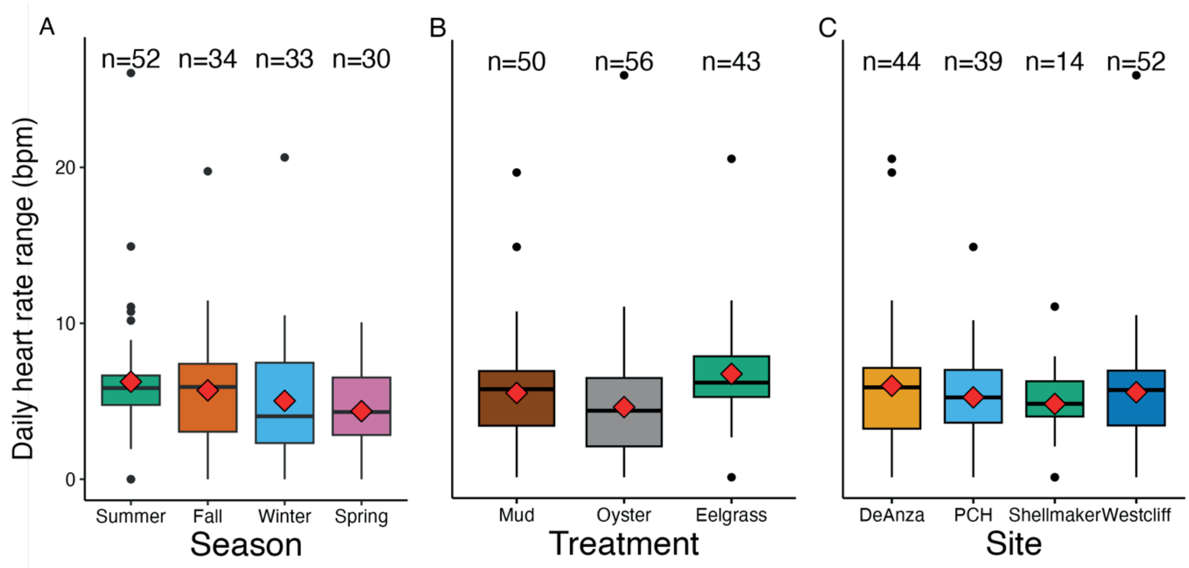

Figure 6. Daily heart rate range across A) Season, B) Treatment, and C) Site. Summer, Fall, Winter, and Spring season represent data collected from July 15, 2022- September 30, 2022, October 1, 2022- December 31, 2022, January 1, 2023-March 31, 2023, and April 1, 2023-June 19, 2023, respectively. Red diamonds represent averages and black bars represent medians. N = number of oysters.

GROWTH MEASUREMENTS AND FIELD DEATHS

Average length growth rates for individuals ranged from -0.258 mm/day to 0.158 mm/day. Average width growth rates for individuals ranged from -0.054 mm/day to 0.099 mm/day. Across all individuals, the average length growth rate was 0.000±0.008 mm/day (mean±1SE), and the average width growth rate was 0.011±0.005 mm/day (mean±1SE).

Growth rates (mm/day) of oyster shell length did not significantly differ across months (Fig. 7A, KW, $H_{(5)}$=2.77, p=0.735), treatments (Fig. 8B, KW, $H_{(2)}$=0.19, p=0.910) or sites (Fig. 8A, KW, $H_{(3)}$=2.15, p=0.542). Growth rates of oyster shell width also did not significantly differ across months (Fig. 7B, KW, $H_{(5)}$=1.55, p=0.908), treatments (Fig. 8D, KW, $H_{(2)}$=0.95, p=0.621) or sites (Fig. 8C, ANOVA, $F_{3,49}$=0.53, p=0.666).

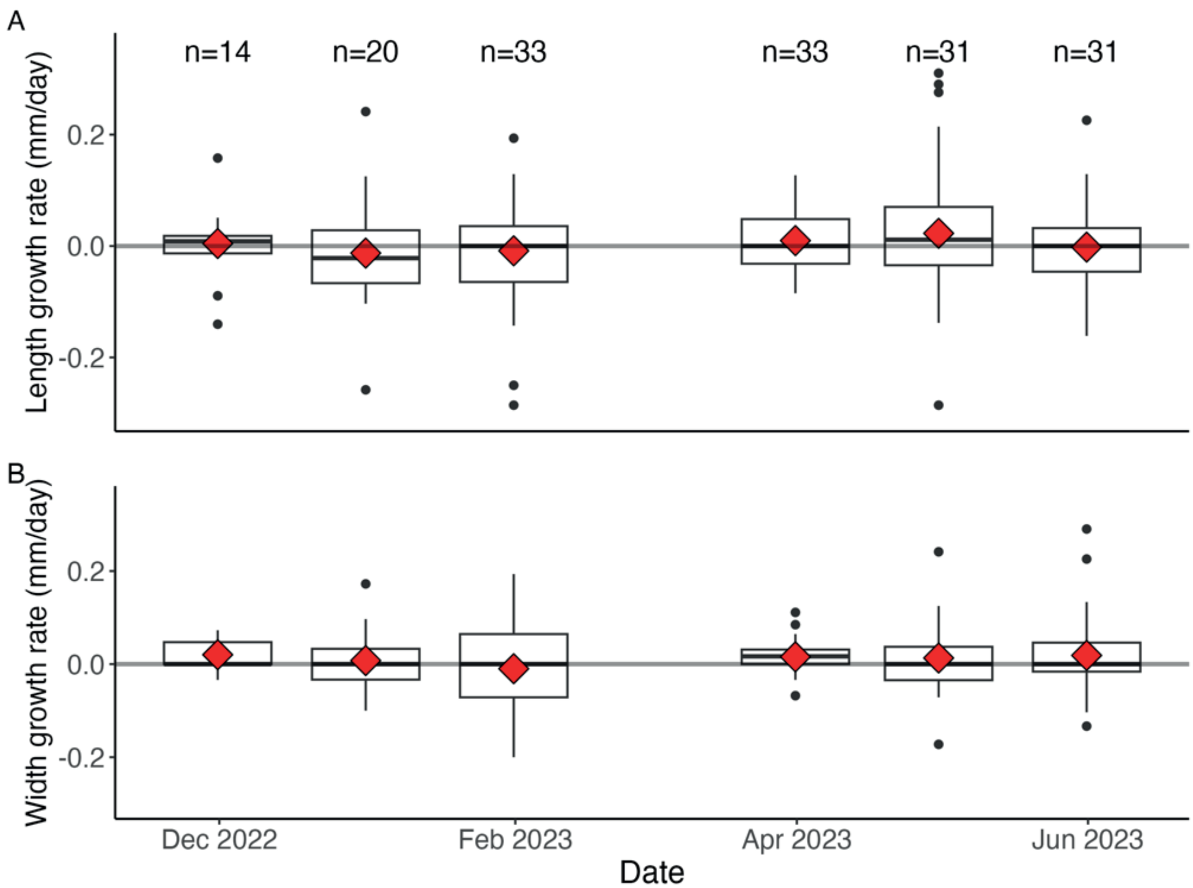

Figure 7. Plot of growth rates of A) oyster shell length and B) width across 6 months, December 2022-June 2023. No data was collected March 2023. Red diamonds represent averages and black bars represent medians. N = number of oysters. Light grey horizontal line is at 0 mm/week.

The number of oyster deaths proportional to the number of oysters deployed during the field experiment did not significantly differ across season (KW, $H_{(3)}$=5.65, p=0.130), site (KW, $H_{(3)}$=1.93, p=0.588), or treatment (KW, $H_{(2)}$=1.26, p=0.532).

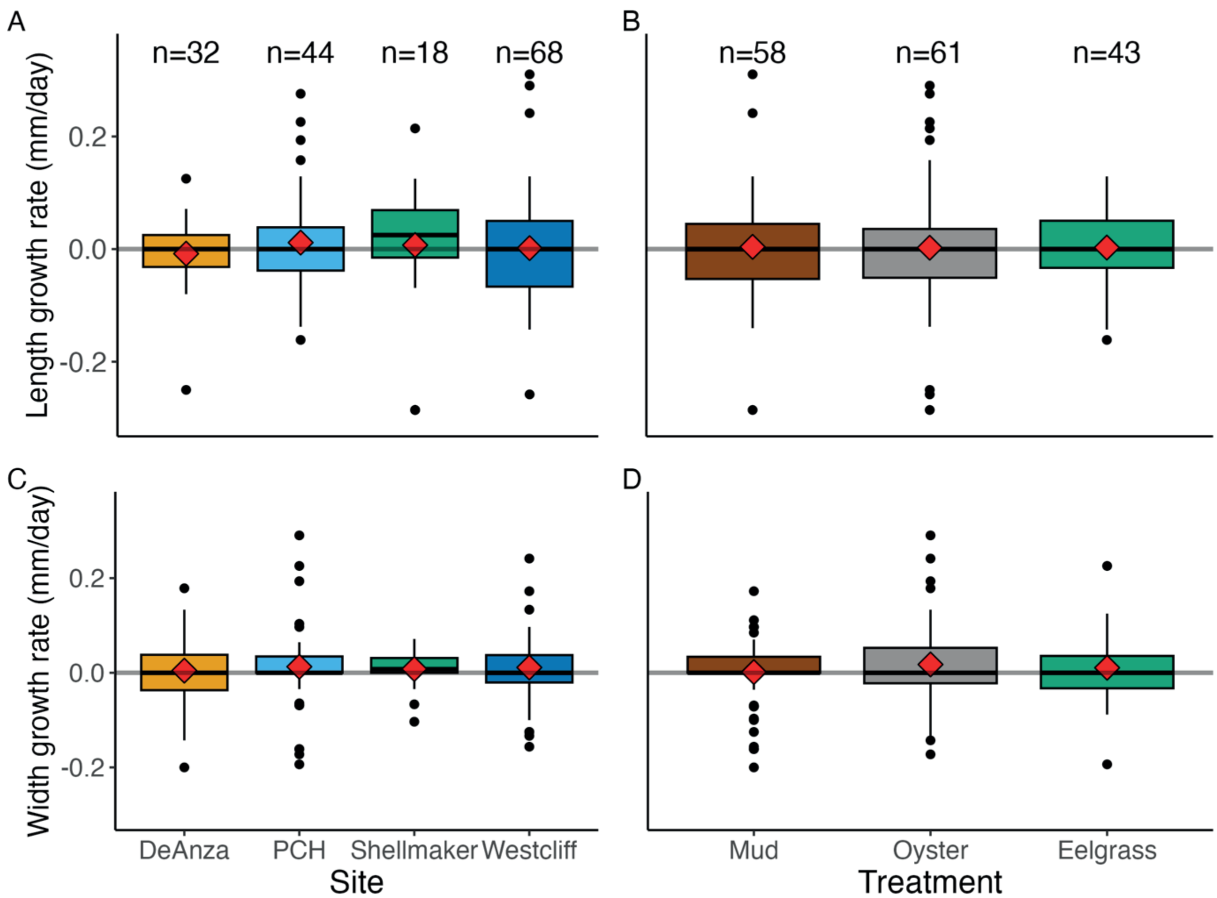

Figure 8. Plot of growth rates of oyster shell length between A) Sites and B) Treatment, and shell width between C) Sites and D) Treatment. Red diamonds represent averages and black bars represent medians. N = number of oysters. Light grey horizontal line is at 0 mm/week.

PHYSIOLOGICAL CHANGES AFTER RAIN EVENTS

In order to compare physiological differences before, during, and after a rain event, the proportion of open valves, average daily heart rate, and daily heart rate range were compared during the weeks of October 30, 2022 - November 5, 2022, November 6-12, 2022, and November 13-19, 2022. Orange County received 3.4 mm of rainfall from October 30, 2022 - November 5, 2022, 28.5 mm of rainfall from November 6-12, 2022, 0 mm of rainfall from November 13-19, 2022 (Fig. 9A). The proportion of open valves significantly differed between these three weeks (Fig. 9B, KW, $H_{(2)}$=15.26, p=4.9e-4), but average daily heart rate (Fig. 9C, KW, $H_{(2)}$=0.38, p=0.827) and daily heart rate range (Fig. 9D, KW, $H_{(2)}$=0.15, p=0.930) did not. The proportion of open valves did not significantly differ between October

30, 2022 - November 5, 2022 and November 13-19, 2022 (Dunn’s test post-hoc, $p=1.00$) but did differ between October 30, 2022 - November 5, 2022 and November 6-12, 2022 (Dunn’s test post-hoc, $p=0.014$), and between November 6-12, 2022 and November 13-19, 2022 (Dunn’s test post-hoc, $p=5.4e-4$).

There was a significant correlation ($n=54$ oysters) between daily average proportion of open valves and daily cumulative rainfall (Kendall Tau Beta correlation coefficient, $\tau=-0.13$, $p=<2.2e-16$).

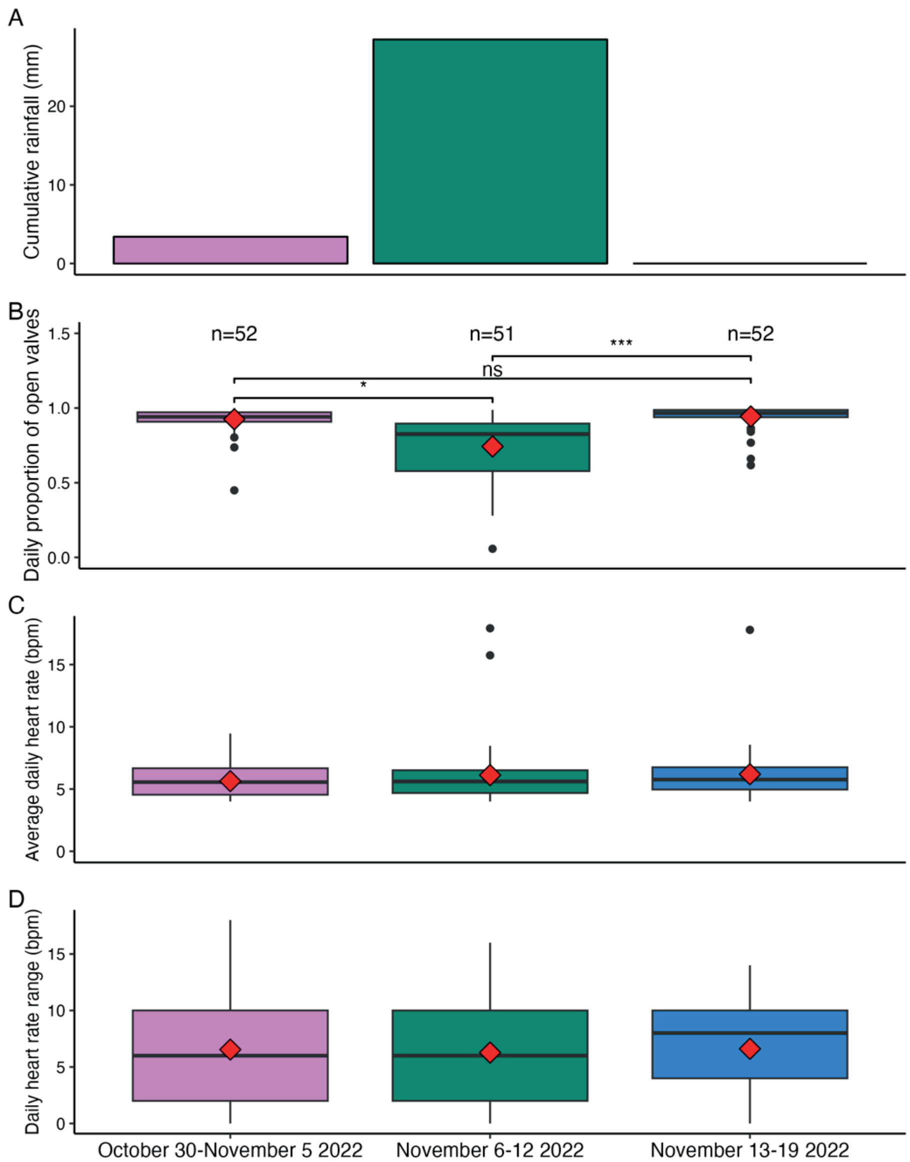

Figure 9. Plot of A) cumulative rainfall (mm), B) daily proportion of open valves, C) average daily heart rate (bpm), and D) daily heart rate range (bpm) of all oysters from October 30-November 5, 2022 (n=52 oysters), November 6-12, 2022 (n=51 oysters) and November 13-19, 2022 (n=52 oysters). Red diamonds represent averages and black bars represent medians. Significant differences as determined by Dunn's post-hoc comparisons, *= p<0.05, ***=p<0.001.

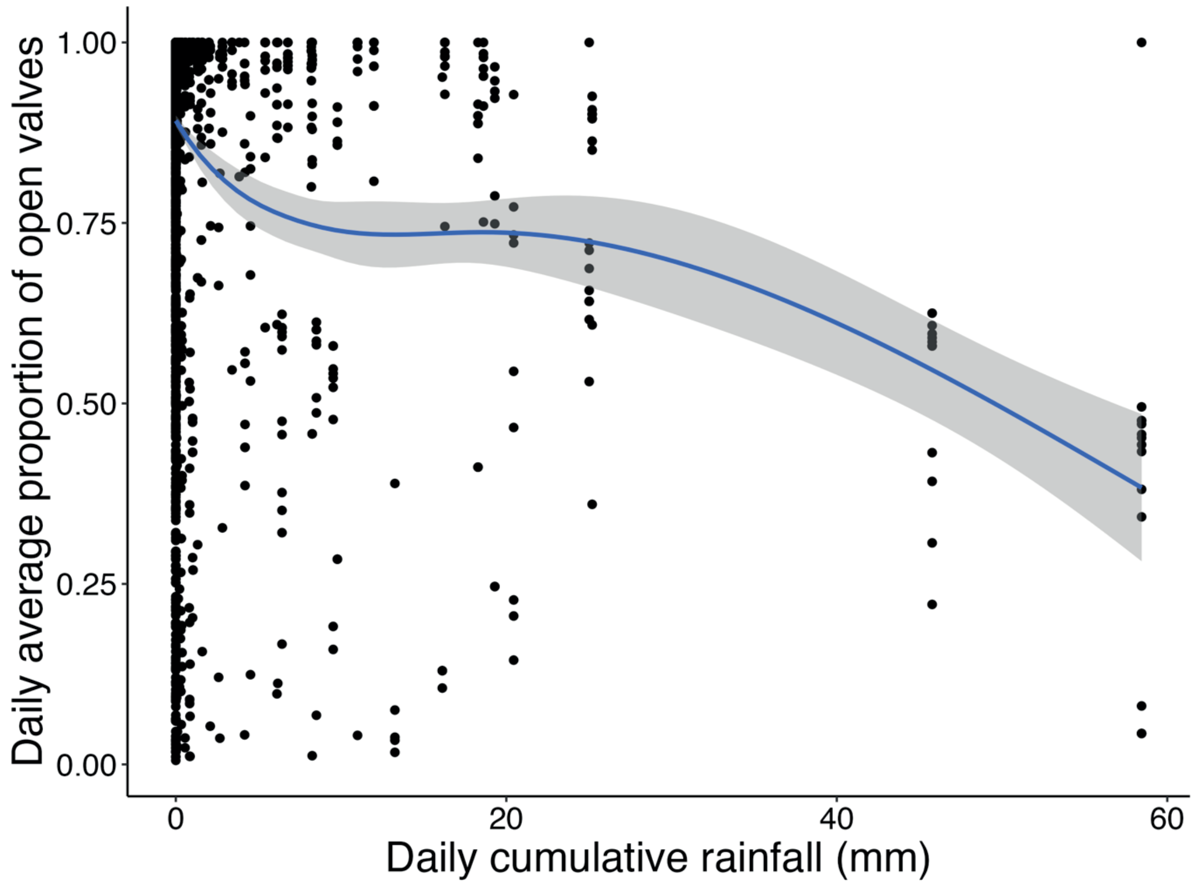

Figure 10. Proportion of open valves compared to the daily cumulative rainfall (mm). Gape data only used from oysters when submerged. Blue line represents a trend line. Shaded area represents standard error around the line.

DISCUSSION

The goal of this study was to provide insight into the physiological responses of oysters to seasons, habitat, and site 5-6 years post-paired restoration. I did not observe any differences in physiological effects of *O. lurida* due to habitat or site. Valve gape was lower during periods of rainfall, but heart rate was unaffected. My results can inform future restoration projects on the ideal conditions for *O. lurida* individuals in Newport Bay.

LITTLE PHYSIOLOGICAL DIFFERENCE BETWEEN HABITAT TREATMENTS

O. lurida individuals did not differ in valve gape (Fig. 4B), average daily heart rate (Fig. 5B), daily heart rate range (Fig. 6B), growth rates (Fig. 8B, 8D), nor mortality rates when living in *O. lurida* beds, or *Z. marina* beds, or mudflat habitats. A lack of physiological response of *O. lurida* to *Z. marina* beds is not unexpected since there are many conflicting studies on the effects of seagrass on oyster survival, growth, and shell strength (A. Lowe et al., 2018; Wagner et al., 2012). The differences in oyster response in these studies are mainly due to estuary-specific factors, such as predator behavior, water flow, and seagrass bed size and density. Some studies have found higher predation in mudflat areas in comparison to seagrass beds due to easier access to prey (McDevitt-Irwin et al., 2016), while others have found the opposite due to seagrass providing habitat for predators (A. Lowe et al., 2018). These prior studies were conducted in Washington state, where the prominent oyster predator is the Atlantic oyster drill. In Newport Bay, oyster drills are not present, and I did not directly observe predation. During my study I found drill holes in a small number of dead experimental oysters' shells across sites, which likely indicated death due to predation. I also observed *Octopus bimaculoides*, the California two-spot octopus, a known oyster predator that can drill holes through the shell using its abrasive radula, at the *Z. marina* treatment at

PCH. There is potential for predation events in Newport Bay, but even with these observations, I did not find that the number of deaths in the field differed between habitats.

Daily heart rate range can be used as a proxy for stress in oysters, since individuals will adjust their metabolic behavior, or heart rate, to mitigate stress. Oysters with greater daily heart rate ranges may be increasing or lowering their heart rate more during the course of a day, potentially due to stressful events they encounter. Heart rate range did not differ between *O. lurida* beds, *Z. marina* beds or mudflat habitat. *Z. marina* beds may have higher sedimentation rates than the mudflat areas, but since all other metrics of performance (valve gape, average daily heart rate, growth rates, and mortality rates) did not differ between treatments, it is possible sedimentation is not the main factor. I observed few differences in heart rate variation across individuals except for two outlier individuals that had much higher heart rate ranges than the others (Fig. A.1).

Studies within in Newport Bay also have conflicting results on the relationship between *O. lurida* and *Z. marina*. One study found positive associations with *O. lurida* density and *Z. marina* below-ground dry weight and rhizome growth rates, potentially due to bio-deposits of nitrogen onto *Z. marina* beds from oyster filter feeding (Emery, 2022). Preliminary results from another study found *O. lurida* biomass was higher when further from *Z. marina* beds (B. Quintana, personal communication, 2022). Other research has found varying positive and negative impacts of *O. lurida* and *Z. marina* on fish and infaunal communities (Howard, 2019; Tate-Pulliam, 2021). Howard (2019) found infaunal diversity was higher in *Z. marina* beds than subtidal mudflats, and fish abundance and diversity was greater in some *Z. marina* beds than in mudflats, but the patterns were not consistent over time and by site. Howard (2019) also found infaunal diversity decreased under *O. lurida* beds. One year later, Tate-Pulliam (2021) found invertebrate abundance and species richness below *O. lurida* beds had recovered and were greater than Howard's (2019) measurements. These inconsistent results suggest Newport Bay is a complicated system, with great variation across time and space.

Oyster physiology was also not substantially different in mudflat areas when compared to *O. lurida* beds and *Z. marina* beds. The lack of pattern here was unexpected since I initially hypothesized oyster physiology would be negatively impacted by mudflat habitat because of excess sedimentation. Sedimentation in Newport Bay has not yet been evaluated for 2022 but research with sedimentation pins was conducted. There may have not been enough difference in sedimentation between treatments to make a difference in oyster behavior or physiology.

NO PHYSIOLOGICAL DIFFERENCES BETWEEN SITES

Site did not significantly affect any measured physiological response (Fig. 4C, 5C, 6C) or short-term growth rates (Fig. 8A, 8C) of the oysters, but previous research has found differences in epifaunal community structure, fish community composition, and oyster densities across the four restoration sites (Griffith, 2018; Howard, 2019; Wood, 2018). These previous studies were conducted 1-year post-restoration while my experiment took place 5-6 years post-restoration, so differences between sites may have settled after the initial restoration.

SEASON AFFECTED VALVE GAPE

This study spanned four seasons during 2022-2023, and I did see differences in oyster performance across those seasons. However, I first acknowledge that because the study was not replicated across multiple years, my ability to attribute differences to true 'seasonality' is limited, and that the observed differences through time may be phenomenological events unique to this one sampling year. Season significantly affected valve gape measurements but not heart rate measurements in individuals. I found a significant interaction between season and treatment on valve gape measurements. The treatments with the highest median proportion of open valves differed depending on season (Fig. A.2). But there were no significant pairwise comparisons between treatments within the same season. Season alone also significantly affected valve gape, so even though an interaction existed between season and treatment, likely driven by specific pairwise comparisons, there was still an overall difference between seasons. Growth rates also did not differ over time (Fig. 7). I was surprised to find average daily heart rate and daily heart rate range did not differ by season since I hypothesized oyster individuals would increase or lower their heart rate due to stressors (Fig. 5A, 6A). Seasonal stressors may have not been enough to influence heart rate metrics.

Oysters were closed the most during the winter season (63% daily time spent open on average), then spring and fall (85% and 83% daily time spent open on average, respectively), and lastly the summer season (89% daily time spent open on average; Fig. 4A). Precipitation and temperature were seasonal factors that could have directly affected the seasonal changes in valve gape measurements (Fig. 3). I measured no effect of oyster temperature on heart rate or daily heart rate range, but there was a significant correlation of temperature with the proportion of open valves. As temperature decreased, oyster valves were more likely to be closed. This relationship was not strong, and since temperature and rainfall were also weakly correlated, this effect could be related to rainfall. Studies of other oyster species found valve opening durations differed significantly when temperatures increased by up to 10°C (Casas et al., 2018), but these trials were in the lab with consistent temperatures, while my oysters in the field experienced temperature fluctuations daily. The greatest temperature range observed by an individual in the field within one day was 10.9°C-25.8°C. The greatest temperature range observed by an individual oyster across my entire experiment was 6.5°C-26.6°C, for an oyster deployed from August 2022-June 2023. I measured large daily temperature changes, and a significant, but weak, relationship between temperature and proportion of open valves. Due to the weak relationship with temperature, other factors in the field may be equally or more important for driving oyster gaping. Visualizing oyster gaping in comparison to temperature, rainfall, and tide made it clear rainfall was an important factor in oyster physiology (Fig. 11).

RAINFALL HAD SIGNIFICANT BUT TEMPORARY IMPACTS ON PHYSIOLOGY

When observing overall physiological trends on a year-long timeline, experimental oysters closed their valves after major rain events (Fig. 11). I also found the daily average proportion of open valves was negatively correlated with daily cumulative rainfall (Fig. 10). These drops in gape are likely related to the declining salinity through the estuary. From November 6-12, 2022, Orange County experienced multiple rain events with cumulative rainfall reaching 28.5 mm. In the week previous, October 30-November 5, 2022, cumulative rainfall was significantly lower at 3 mm. During the week of November 13-19, 2022, cumulative rainfall dropped again to 0 mm (Fig. 9A). The proportion of open valves from

November 6-12, 2022 was significantly lower than October 30-November 5, 2022, and November 13-19, 2022 (Fig. 9B) but average daily heart rate and daily heart rate range did not differ between weeks (Fig. 9C, 9D). I observed oysters closing their valves for an extended period during the rain events, likely to avoid osmotic stress due to low salinity levels or other stressors (Fig. 11; Shumway, 1977). With a lower proportion of open valves, oysters are not feeding and respiring as often, which has the potential to shift the allocation of metabolic energy towards maintenance and recovery processes, and allocate less energy to growth and reproduction. These closures are important to note, but rainfall may affect oyster physiology only for short periods of time, since there is no difference in valve gape between the two drier weeks. Additionally, in Fig. 12, after each major rain event, oyster gape increased back to normal within two days.

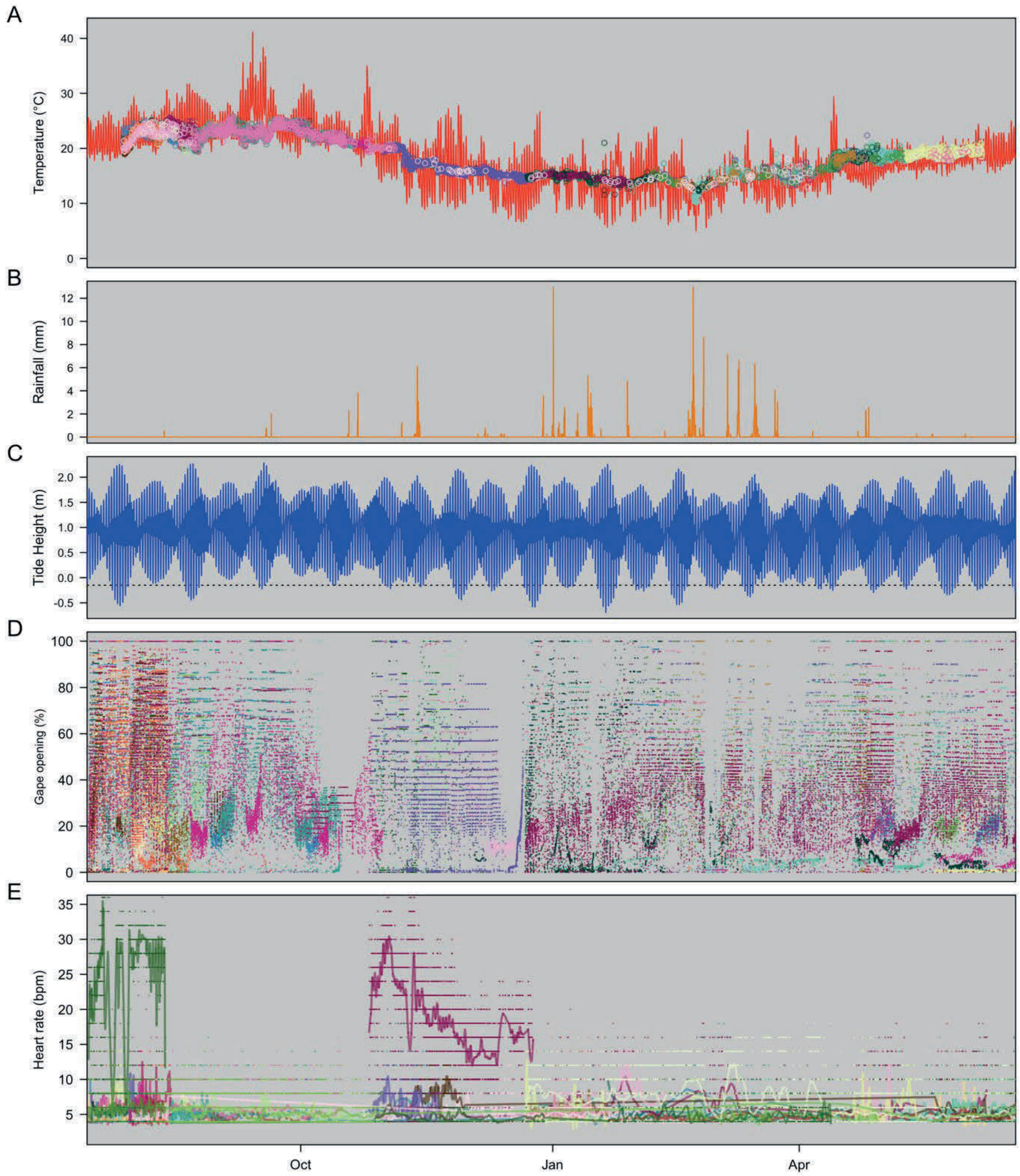

Figure 11. Plot of A) temperature, B) hourly rainfall, C) tide height, D) gape opening, and E) heart rate between July 15, 2022-June 19, 2023. Gape opening and heart rate points and lines (panels D and E) are colored by individual oysters across all sites and treatments. In the temperature graph (A), the red line is air temperature and colored dots are temperature of oysters, colored by individual oyster. Air temperature and rainfall data were retrieved from the John Wayne Airport weather station. Tide height data was retrieved from NOAA Los Angeles tide Station ID: 9410660. Dotted line in tide height panel is at -0.15m, the tide level of the experimental oysters.

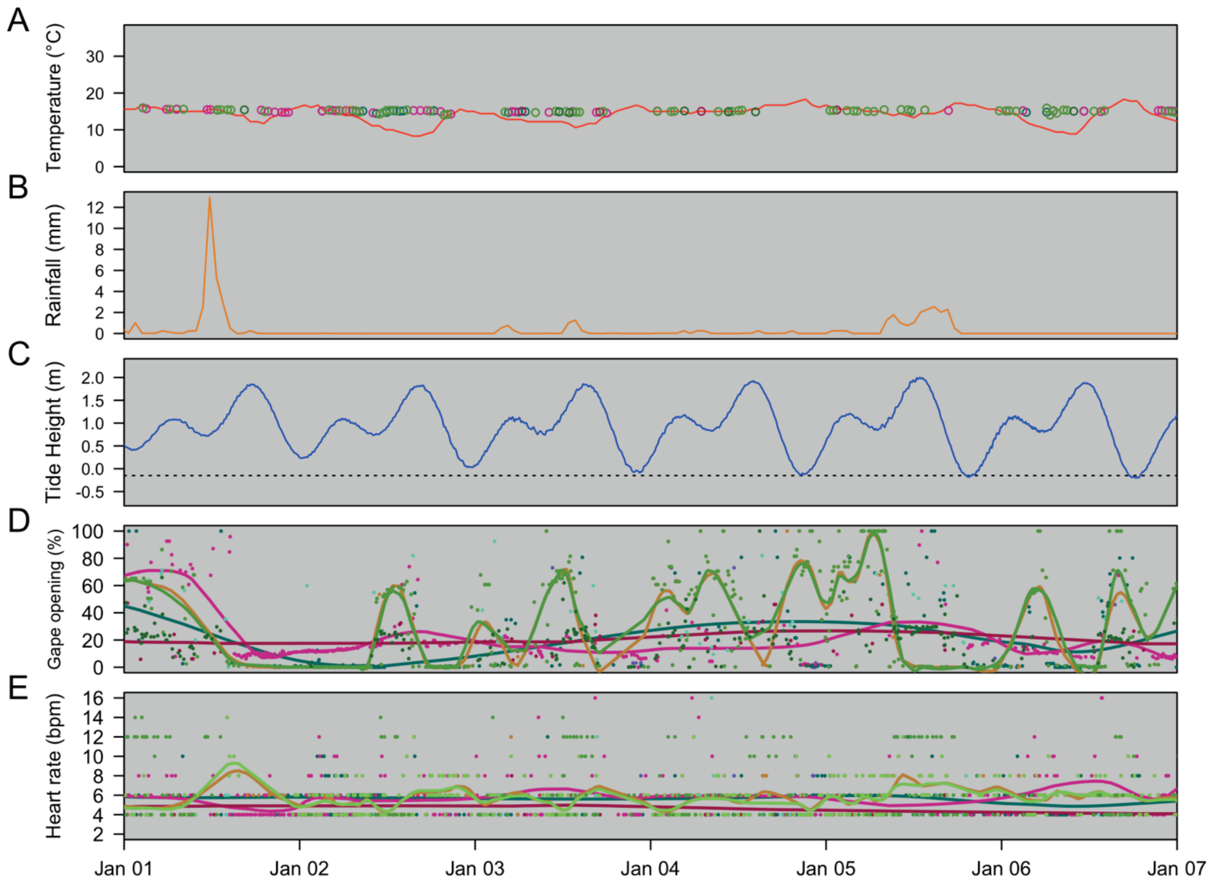

Figure 12. Plots of A) temperature, B) hourly rainfall, C) tide height, D) gape opening, and E) heart rate between January 1 and January 7, 2023. Gape opening and heart rate points and lines (panels D and E) are colored by individual oysters across all sites and treatments. In the temperature graph (A), the red line is air temperature and colored dots are temperature of oysters, colored by individual oyster. Air temperature and rainfall data were retrieved from the John Wayne Airport weather station. Tide height data was retrieved from NOAA Los Angeles tide Station ID: 9410660. Dotted line in tide height panel is at -0.15m, the tide level of the experimental oysters.

Average daily heart rate and daily heart rate range did not vary between seasons (Fig 5A, Fig. 6A), but when considering factors of rain, treatment, and site, the daily heart rate range was significantly affected by rain. It's possible heart rates only changed for short periods of time when it rained, and quickly returned to a less-stressful state. These quick changes in heart rate range were not common enough to influence seasonal averages but were significantly related to salinity stress associated with rainfall.

Rain events significantly affected valve gape and daily heart rate range, but the impact of rain may not be important for future restoration efforts. In comparison to other restoration locations for *O. lurida*, such as Washington and Oregon, rain events in Newport Bay are scarce, with a historical average yearly precipitation total of just 329 mm, while sites in Washington and Oregon frequently exceed 1000 mm yr^{-1} (National Oceanic and Atmospheric Administration, 2023). With less frequent rain and no measurable effect on growth rates or mortality, I don't recommend Newport Bay restoration efforts prioritize rainfall in planning. It's also important to note that during October 2022 to late March 2023, rainfall in southern California was 150% above average, so seasonal valve gape differences may not be as extreme in other years (National Oceanic and Atmospheric Administration, 2023). To properly account for seasonality, future research should sample across multiple years. As an estuarine species, *O. lurida* is tolerant to short-term changes in salinity levels (Wasson et al., 2015). Long-term exposure to low salinity can cause die offs (Grosholz et al., 2008), but there were no major die offs during or after heavy rain events in this experiment, and field deaths were not related to season. But as climate change increases the frequency and intensity of rain events in southern California, restored *O. lurida* individuals may be more negatively affected in the future.

NO SIGNIFICANT CHANGES IN GROWTH

Large changes in growth rates were not observed in this study (Fig. 7). Individual *O. lurida* growth rates had not previously been measured in Newport Bay, but other studies in Washington and Oregon measured growth rates of the length of *O. lurida* in the field ranging from 0.01-0.53 mm day^{-1} (Dinnel et al., 2009; Rimler, 2014; Trimble et al., 2009). My study found lower rates, with the oyster length growth rates ranging from -0.258 mm day^{-1} to 0.158 mm day^{-1}. These other studies measured growth rates of oysters during the summer only, when oysters have been shown to grow faster (Sellers & Stanley, 1984). My study measured growth rates from December 2022-June 2023 and did not include summer months. Further, *O. lurida* shells are relatively small and fragile compared to other oyster species, so handling during maintenance visits, could easily cause damage to the shell edge. This damage could have caused the negative growth rates I observed in the study.

OBSERVED HEART RATES

There are few published studies on the heart rates of *O. lurida* species. I found the average heart rate across all oysters at all sites and treatments was ~7 bpm, with maximum heart rates reaching up to ~30 bpm. My estimated minimum heart rates for all individuals were functionally limited to 4 bpm since all measurements spanned 30 seconds and the peak detection software could not reliably detect fewer than 2 cycles within that 30 second sampling period. Most heart rates for individuals ranged between 4-20 bpm, and two outlier individuals ranged between 4-40 bpm and 4-36 bpm (Fig. A.1). Hopkins (1936) measured the pulsation of blood vessels in *O. lurida* and found a complete contraction of the large medial vein varied between 100 and 150 seconds, but they did not measure heart rate. Heart rate measurements on other oyster species have found basal rates to be between 5-26 bpm (*Crassostrea virginica*) and an averaged 44 bpm (*Crassostrea gigas*; Feng, 1965; Ha Park et al., 2004).

POTENTIAL IMPORTANCE OF SHORE HEIGHT

Due to sample size constraints, my experimental oysters were placed along the same tidal elevation at all sites. Different tidal elevations may impact oyster performance differently at each site such as variations in community composition, grain size, and flow Tidal elevations are also well known to affect oyster growth rates (Gillmor, 1982; Roegner & Mann, 1995; Sumner, 1981; Walne, 1958), predation rates (Lin, 2022), sedimentation (Baillie & Grabowski, 2019), mortality rates (Bartol et al., 1999), and fouling (Chestnut & Fahy, 1953). I recommend that future studies measure the physiology of restored *O. lurida* oysters at different tidal heights across the four sites to accommodate for these potential differences.

CONCLUSION

I observed *O. lurida*'s immediate physiological response to multiple stressors and found small but variable effects of habitat type and abiotic factors on their physiology. By using high-frequency measurements that strongly correlate with energy used for growth and reproduction, these results may inform future restoration in Newport Bay. Future restoration of *O. lurida* may continue paired restoration with *Z. marina* beds and allow *O. lurida* beds to shift naturally into mudflat habitat since there was little effect of habitat on oyster valve gape and heart rate metrics. Rainfall appears to affect *O. lurida* behavior for brief periods, but it does not appear to have lasting effects on physiological performance. If *O. lurida* beds positively impact other valuable species, then living shorelines projects are encouraged to continue paired restorations to protect and improve estuarine habitat.

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DATA AVAILABILITY

Data and code for the project are available at https://github.com/lstrope/newport and permanently archived at https://doi.org/10.5281/zenodo.8411222. More information on the data logger and specific components are available at https://github.com/millerlp/BivalveBit and https://github.com/millerlp/BivalveBit_lib.

REFERENCES

Andersen, H. (2018). Quality assurance project plan Olympia oyster restoration in Sequim Bay. *The Northwest Straits Commission*.

Andrews, J., Haven, D., & Quayle, D. (1959). Fresh-water kill of oysters (*Crassostrea virginica*) in James River, Virginia, 1958. *Proceedings of the National Shellfisheries Association*, *49*, 29–49.

Baillie, C. J., & Grabowski, J. H. (2019). Factors affecting recruitment, growth and survival of the eastern oyster *Crassostrea virginica* across an intertidal elevation gradient in southern New England. *Marine Ecology Progress Series*, *609*, 119–132. https://doi.org/10.3354/meps12830

Baker, P. (1995). Review of ecology and fishery of the Olympia oyster, *Ostrea lurida* with annotated bibliography. *Journal Of Shellfish Research*, *14*(2), 501–518.

Baker, S. M., & Mann, R. (1992). Effects of hypoxia and anoxia on larval settlement, juvenile growth, and juvenile survival of the oyster *Crassostrea virginica*. *The Biological Bulletin*, *182*(2), 265–269. https://doi.org/10.2307/1542120

Ballesta-Artero, I., Janssen, R., van der Meer, J., & Witbaard, R. (2018). Interactive effects of temperature and food availability on the growth of *Arctica islandica* (Bivalvia) juveniles. *Marine Environmental Research*, *133*, 67–77. https://doi.org/10.1016/j.marenvres.2017.12.004

Ballesta-Artero, I., Witbaard, R., Carroll, M. L., & van der Meer, J. (2017). Environmental factors regulating gaping activity of the bivalve *Arctica islandica* in northern Norway. *Marine Biology*, *164*(5), 116. https://doi.org/10.1007/s00227-017-3144-7

Bartol, I. K., Mann, R., & Luckenbach, M. (1999). Growth and mortality of oysters (*Crassostrea virginica*) on constructed intertidal reefs: Effects of tidal height and substrate level. *Journal of Experimental Marine Biology and Ecology*, *237*(2), 157–184. https://doi.org/10.1016/S0022-0981(98)00175-0

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*, 1–48. https://doi.org/10.18637/jss.v067.i01

Bayne, B. L. (2000). Relations between variable rates of growth, metabolic costs and growth efficiencies in individual Sydney rock oysters (*Saccostrea commercialis*). *Journal of Experimental Marine Biology and Ecology*, *251*(2), 185–203. https://doi.org/10.1016/S0022-0981(00)00211-2

Beck, M. W., Brumbaugh, R. D., Airoldi, L., Carranza, A., Coen, L. D., Crawford, C., Defeo, O., Edgar, G. J., Hancock, B., Kay, M. C., Lenihan, H. S., Luckenbach, M. W., Toropova, C. L., Zhang, G., & Guo, X. (2011). Oyster reefs at risk and recommendations for conservation, restoration, and management. *BioScience*, *61*(2), 107–116. https://doi.org/10.1525/bio.2011.61.2.5

Bible, J. M., Cheng, B. S., Chang, A. L., Ferner, M. C., Wasson, K., Zabin, C. J., Latta, M., Sanford, E., Deck, A., & Grosholz, E. D. (2017). Timing of stressors alters interactive effects on a coastal foundation species. *Ecology*, *98*(9), 2468–2478. https://doi.org/10.1002/ecy.1943

Bilkovic, D. M., Mitchell, M., Mason, P., & Duhring, K. (2016). The role of living shorelines as estuarine habitat conservation strategies. *Coastal Management*, *44*(3), 161–174. https://doi.org/10.1080/08920753.2016.1160201

Blake, B., & Bradbury, A. (2012). Washington Department of Fish and Wildlife plan for rebuilding Olympia oyster (*Ostrea lurida*) populations in Puget Sound with a historical and contemporary overview. *Washington Department Fish & Wildlife*.

Borchers, H. (2022). *Pracma: Practical numerical math functions_*. (Version 2.4.2) [Computer software package]. R package. https://CRAN.R-project.org/package=pracma

Bozek, C. M., & Burdick, D. M. (2005). Impacts of seawalls on saltmarsh plant communities in the Great Bay Estuary, New Hampshire USA. *Wetlands Ecology and Management*, *13*(5), 553–568. https://doi.org/10.1007/s11273-004-5543-z

Braby, C. E., & Somero, G. N. (2006). Following the heart: Temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). *Journal of Experimental Biology*, *209*(13), 2554–2566. https://doi.org/10.1242/jeb.02259

Brown, H. M., Briden, A., Stokell, T., Griffin, F. J., & Cherr, G. N. (2004). Thermotolerance and Hsp70 profiles in adult and embryonic California native oysters, *Ostreola conchaphila* (Carpenter, 1857). *Journal of Shellfish Research*, *23*(1), 135–142.

Buhle, E. R., & Ruesink, J. L. (2009). Impacts of invasive oyster drills on Olympia oyster (*Ostrea lurida* Carpenter 1864) recovery in Willapa Bay, Washington, United States. *Journal of Shellfish Research*, *28*(1), 87–96. https://doi.org/10.2983/035.028.0115

Bulseco, A. (2012). A synopsis of the Olympia oyster (*Ostrea lurida*). *Aquaculture*, *262*.

Byrne, R. A., Gnaiger, E., McMahon, R. F., & Dietz, T. H. (1990). Behavioral and metabolic responses to emersion and subsequent reimmersion in the freshwater bivalve, *Corbicula fluminea*. *The Biological Bulletin*, *178*(3), 251–259. https://doi.org/10.2307/1541826

California Department of Fish & Wildlife. (2008). *Culture of oysters (Status of the Fisheries)*. https://wildlife.ca.gov/Conservation/Marine/Status#28027678-status-of-the-fisheries-report-through-2008

California Department of Fish & Wildlife. (2023). *Upper Newport Bay State Marine Conservation Area*. https://wildlife.ca.gov/Conservation/Marine/MPAs/Upper-Newport-Bay

Campbell, M. D., & Hall, S. G. (2019). Hydrodynamic effects on oyster aquaculture systems: A review. *Reviews in Aquaculture*, *11*(3), 896–906. https://doi.org/10.1111/raq.12271

Casas, S. M., Filgueira, R., Lavaud, R., Comeau, L. A., La Peyre, M. K., & La Peyre, J. F. (2018). Combined effects of temperature and salinity on the physiology of two geographically-distant eastern oyster populations. *Journal of Experimental Marine Biology and Ecology*, *506*, 82–90. https://doi.org/10.1016/j.jembe.2018.06.001

Chestnut, A., & Fahy, W. (1953). *Studies on the vertical distribution of setting of oysters in North Carolina*. University of North Carolina, Institute of Fisheries Research. https://aquadocs.org/bitstream/handle/1834/27664/gcfi_5-21.pdf?sequence=1&isAllowed=y

Crosby, S. C., Sax, D. F., Palmer, M. E., Booth, H. S., Deegan, L. A., Bertness, M. D., & Leslie, H. M. (2016). Salt marsh persistence is threatened by predicted sea-level rise. *Estuarine, Coastal and Shelf Science*, *181*, 93–99. https://doi.org/10.1016/j.ecss.2016.08.018

Davis, J. (2008). Evaluating ecological impacts of living shorelines and shoreline habitat elements: An example from the Upper Western Chesapeake Bay. *Management, Policy, Science and Engineering of Nonstructural Erosion Control in the Chesapeake Bay: Proceedings of the 2006 Living Shoreline Summit, CRC Publ. No. 08-164*.

de Zwart, D., Kramer, K. J. M., & Jenner, H. A. (1995). Practical experiences with the biological early warning system “mosselmonitor.” *Environmental Toxicology and Water Quality*, *10*(4), 237–247. https://doi.org/10.1002/tox.2530100403

Deck, A. K. (2011). *Effects of interspecific competition and coastal oceanography on population dynamics of the Olympia oyster, Ostrea lurida, along estuarine gradients* [Master’s thesis, University of California, Davis]. eScholarship. https://escholarship.org/uc/item/6xq0z4fs

Dinnel, P. A., Peabody, B., & Peter-Contesse, T. (2009). Rebuilding Olympia oysters, *Ostrea lurida* Carpenter 1864, in Fidalgo Bay, Washington. *Journal of Shellfish Research*, *28*(1), 79–85. https://doi.org/10.2983/035.028.0114

Dowd, W. W., & Somero, G. N. (2013). Behavior and survival of *Mytilus congeners* following episodes of elevated body temperature in air and seawater. *Journal of Experimental Biology*, *216*(3), 502–514. https://doi.org/10.1242/jeb.076620

Emery, M. (2022). *Not so shellfish after all: How native oyster (Ostrea lurida) may aid eelgrass (Zostera marina) restoration by nitrogen fixation* [Master’s thesis, California State University, Fullerton]. Zenodo. https://zenodo.org/record/6955010

Feng, S. Y. (1965). Heart rate and leucocyte circulation in *Crassostrea virginica* (gmelin). *The Biological Bulletin*, *128*(2), 198–210. https://doi.org/10.2307/1539549

Fox, J., & Weisberg, S. (2011). *An R companion to applied regression* (2nd ed.). Sage Publishers.

Frank, D. M., Hamilton, J. F., Ward, J. E., & Shumway, S. E. (2007). A fiber optic sensor for high resolution measurement and continuous monitoring of valve gape in bivalve molluscs. *Journal of Shellfish Research*, *26*(2), 575–580. https://doi.org/10.2983/0730-8000(2007)26[575:AFOSFH]2.0.CO;2

Fuentes, C. M., Whitcraft, C. R., & Zacherl, D. C. (2020). Adaptive restoration reveals potential effect of tidal elevation on oyster restoration outcomes. *Wetlands*, *40*(1), 93–99. https://doi.org/10.1007/s13157-019-01166-7

Gedan, K. B., Kirwan, M. L., Wolanski, E., Barbier, E. B., & Silliman, B. R. (2011). The present and future role of coastal wetland vegetation in protecting shorelines: Answering recent challenges to the paradigm. *Climatic Change*, *106*(1), 7–29. https://doi.org/10.1007/s10584-010-0003-7

Gillespie, G. E. (2009). Status of the Olympia oyster, *Ostrea lurida* Carpenter 1864, in British Columbia, Canada. *Journal of Shellfish Research*, *28*(1), 59–68. https://doi.org/10.2983/035.028.0112

Gillmor, R. (1982). Assessment of intertidal growth and capacity adaptations in suspension-feeding bivalves. *Marine Biology*, *68*, 277–286.

Gray, M. W., & Langdon, C. (2019). Particle processing by Olympia oysters *Ostrea lurida* and Pacific oysters *Crassostrea gigas*. *Estuaries and Coasts*, *42*(3), 779–791.

Griffith, K. (2018). *Edge effects in estuarine habitat mosaics: An experimental test using restored eelgrass beds* [Master's thesis, San Diego State University]. Pro Quest.

Grizzle, R. E., Langan, R., & Huntting Howell, W. (1992). Growth responses of suspension-feeding bivalve molluscs to changes in water flow: Differences between siphonate and nonsiphonate taxa. *Journal of Experimental Marine Biology and Ecology*, *162*(2), 213–228. https://doi.org/10.1016/0022-0981(92)90202-L

Grosholz, E. D., Zabina, C., & Obernolte, R. (2008). *Planning for native oyster restoration in San Francisco Bay* (Agreement 05–134). California Coastal Conservancy. https://www.researchgate.net/publication/242211277_Planning_for_Native_Oyster_Restoration_in_San_Francisco_Bay_Final_Report_to_California_Coastal_Conservancy_Agreement_05-134

Ha Park, K., Kim, Y.-S., Chung, E.-Y., Choe, S.-N., & Choo, J.-J. (2004). Cardiac responses of Pacific oyster *Crassostrea gigas* to agents modulating cholinergic function. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *139*(4), 303–308. https://doi.org/10.1016/j.cca.2004.12.009

Hartig, F. (2022). *DHARMa: Residual diagnostics for hierarchical (multi-level/mixed) regression models* (Version 0.4.6) [Computer software package]. R package. https://cran.r-project.org/web/packages/DHARMa/vignettes/DHARMa.html

Heck, K., Hays, G., & Orth, R. (2003). Critical evaluation of the nursery role hypothesis for seagrass meadows. *Marine Ecology Progress Series*, *253*, 123–136. https://doi.org/10.3354/meps253123

Heide, T., Nes, E. H. van, Geerling, G. W., Smolders, A. J. P., Bouma, T. J., & Katwijk, M. M. van. (2007). Positive feedbacks in seagrass ecosystems—Implications for success in conservation and restoration. *Ecosystems*, *10*(8), Article 8. https://doi.org/10.1007/s10021-007-9099-7

Hellicar, A. D., Rahman, A., Smith, D. V., Smith, G., McCulloch, J., Andrewartha, S., & Morash, A. (2015). An algorithm for the automatic analysis of signals from an oyster heart rate sensor. *IEEE Sensors Journal*, *15*(8), 4480–4487. https://doi.org/10.1109/JSEN.2015.2422375

Helmuth, B. S. T. (1998). Intertidal mussel microclimates: Predicting the body temperature of a sessile invertebrate. *Ecological Monographs*, *68*(1), 51–74. https://doi.org/10.1890/0012-9615(1998)068[0051:IMMPTB]2.0.CO;2

Hendriks, I., Olsen, Y., Ramajo, L., Basso, L., Steckbauer, A., Moore, T., Howard, J., & Duarte, C. (2014). Photosynthetic activity buffers ocean acidification in seagrass meadows. *Biogeosciences*, *11*. https://doi.org/10.5194/bg-11-333-2014

Hettinger, A., Sanford, E., Hill, T. M., Lenz, E. A., Russell, A. D., & Gaylord, B. (2013). Larval carry-over effects from ocean acidification persist in the natural environment. *Global Change Biology*, *19*(11), 3317–3326. https://doi.org/10.1111/gcb.12307

Howard, M. (2019). *Impacts of paired Olympia oyster (Ostrea lurida) and Eelgrass (Zostera marina) restoration on fish and infaunal communities in Upper Newport Bay, California* [Master’s thesis, California State University, Long Beach]. Pro Quest.

Hui, T. Y., Dong, Y., Han, G., Lau, S. L. Y., Cheng, M. C. F., Meepoka, C., Ganmanee, M., & Williams, G. A. (2020). Timing metabolic depression: Predicting thermal stress in extreme intertidal environments. *The American Naturalist*, *196*(4), 501–511. https://doi.org/10.1086/710339

Hyndman, R., Athanasopoulos, G., Bergmeir, C., Caceres, G., Chhay, L., O’Hara-Wild, M., Petropoulos, F., Razbash, S., Wang, E., & Yasmeen, F. (2023). *Forecast: Forecasting functions for time series and linear models* (Version 8.21.1) [Computer software package]. R package. https://pkg.robjhyndman.com/forecast/

Jou, L., Lin, S., Chen, B., Chen, W., & Liao, C. (2013). Synthesis and measurement of valve activities by an improved online clam-based behavioral monitoring system. *Computers and Electronics in Agriculture*, *90*, 106–118. https://doi.org/10.1016/j.compag.2012.09.008

Ko, G., Dineshram, R., Campanati, C., Chan, V. B. S., Havenhand, J., & Thiyagarajan, V. (2014). Interactive effects of ocean acidification, elevated temperature, and reduced salinity on early-life stages of the Pacific oyster. *Environmental Science & Technology*, *48*(17), 10079–10088. https://doi.org/10.1021/es501611u

Koivisto, M. E., & Westerbom, M. (2010). Habitat structure and complexity as determinants of biodiversity in blue mussel beds on sublittoral rocky shores. *Marine Biology*, *157*(7), 1463–1474. https://doi.org/10.1007/s00227-010-1421-9

Kraus, N., & McDougal, W. (1996). The effects of seawalls on the beach: Part I, an updated literature review. *Journal of Coastal Research*, *12*(3).

Langevin, J. (2019). *The distribution and demography of the invasive Pacific oyster, Crassostrea gigas, and native Olympia oyster, Ostrea lurida, in the San Diego River* [Master’s thesis, University of San Diego]. Theses. https://doi.org/10.22371/02.2019.003

Lefcheck, J. S., Marion, S. R., & Orth, R. J. (2017). Restored eelgrass (*Zostera marina* L.) as a refuge for epifaunal biodiversity in mid-western Atlantic coastal bays. *Estuaries and Coasts*, *40*(1), 200–212. https://doi.org/10.1007/s12237-016-0141-x

Lenth, R. (2018). *Lsmeans: Least-squares means* (Version 2.30-0) [Computer software package]. R package. https://cran.r-project.org/web/packages/lsmeans/index.html

Lin, C. (2022). *How low can you go? Expanding oyster tidal niche with predator induction* [Master's thesis, University of South Alabama]. Pro Quest. https://www.proquest.com/docview/2746991286/abstract/5038264742414AB6PQ/1

Livingston, R. J., Lewis, F. G., Woodsum, G. C., Niu, X.-F., Galperin, B., Huang, W., Christensen, J. D., Monaco, M. E., Battista, T. A., Klein, C. J., Howell, R. L., & Ray, G. L. (2000). Modelling oyster population response to variation in freshwater input. *Estuarine, Coastal and Shelf Science*, *50*(5), 655–672. https://doi.org/10.1006/ecss.1999.0597

Lowe, A., Kobelt, J., Horwith, M., & Ruesink, J. (2018). Ability of eelgrass to alter oyster growth and physiology is spatially limited and offset by increasing predation risk. *Estuaries and Coasts*, *42*, 743–754. https://doi.org/10.1007/s12237-018-00488-9

Lowe, G. (1974). Effect of temperature change on the heart rate of *Crassostrea gigas* and *Mya arenaria* (Bivalvia). *Proceedings of the Malacological Society*, *41*(29), 29–36.

Lucas, J. S. (2012). Bivalve molluscs. In *Aquaculture: Farming aquatic animals and plants* (2nd ed., pp. 541–566). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781118687932.ch23

Luković, J., Chiang, J. C. H., Blagojević, D., & Sekulić, A. (2021). A later onset of the rainy season in California. *Geophysical Research Letters*, *48*(4), e2020GL090350. https://doi.org/10.1029/2020GL090350

McDevitt-Irwin, J. M., Iacarella, J. C., & Baum, J. K. (2016). Reassessing the nursery role of seagrass habitats from temperate to tropical regions: A meta-analysis. *Marine Ecology Progress Series*, *557*, 133–143. https://doi.org/10.3354/meps11848

Miller, L. P. (2022). Monitoring bivalve behavior and physiology in the laboratory and field using open-source tools. *Integrative and Comparative Biology*, *62*(4), 1096–1110. https://doi.org/10.1093/icb/icac046

Miller, L. P., & Dowd, W. W. (2017). Multimodal in situ datalogging quantifies inter-individual variation in thermal experience and persistent origin effects on gaping behavior among intertidal mussels (*Mytilus californianus*). *Journal of Experimental Biology*, *220*(22), 4305–4319. https://doi.org/10.1242/jeb.164020

Moore, K. A. (2004). Influence of seagrasses on water quality in shallow regions of the lower Chesapeake Bay. *Journal of Coastal Research*, *10045*, 162–178. https://doi.org/10.2112/SI45-162.1

Narayan, S., Beck, M. W., Reguero, B. G., Losada, I. J., Wesenbeeck, B. van, Pontee, N., Sanchirico, J. N., Ingram, J. C., Lange, G.-M., & Burks-Copes, K. A. (2016). The effectiveness, costs and coastal protection benefits of natural and nature-based defences. *PLOS ONE*, *11*(5), e0154735. https://doi.org/10.1371/journal.pone.0154735

National Oceanic and Atmospheric Administration. (2023). *Past Weather | National Centers for Environmental Information (NCEI)*. https://www.ncei.noaa.gov/access/past-weather/92657

Nezlin, N. P., Kamer, K., Hyde, J., & Stein, E. D. (2009). Dissolved oxygen dynamics in a eutrophic estuary, Upper Newport Bay, California. *Estuarine, Coastal and Shelf Science*, *82*(1), 139–151. https://doi.org/10.1016/j.ecss.2009.01.004

Nichols, K., Zacherl, D., & Whitcraft, C. R. (2019). *Upper Newport Bay Living Shorelines Project*. https://www.coastkeeper.org/wp-content/uploads/2022/02/UNBLSP-SCC-Final-Report-1.pdf

Oates, M. S. (2013). *Observations of gonad structure and gametogenic timing in a recovering population of Ostrea lurida (Carpenter 1864)* [Master's thesis, University of Oregon]. University of Oregon Scholars' Bank.

O'Donnell, J. E. D. (2017). Living shorelines: A review of literature relevant to New England coasts. *Journal of Coastal Research*, *332*, 435–451. https://doi.org/10.2112/JCOASTRES-D-15-00184.1

Patrick, C. J., Weller, D. E., Li, X., & Ryder, M. (2014). Effects of shoreline alteration and other stressors on submerged aquatic vegetation in subestuaries of Chesapeake Bay and the mid-atlantic coastal bays. *Estuaries and Coasts*, *37*(6), 1516–1531. https://doi.org/10.1007/s12237-014-9768-7

Peter-Contesse, T., & Peabody, B. (2005). *Reestablishing Olympia oyster populations in Puget Sound, Washington*. University of Washington. https://wsg.washington.edu/wordpress/wp-content/uploads/Reestablishing-Olympia-Oyster.pdf

Porter, E. T., & Breitburg, D. L. (2016). Eastern oyster, *Crassostrea virginica*, valve gape behavior under diel-cycling hypoxia. *Marine Biology*, *163*(10), 218. https://doi.org/10.1007/s00227-016-2980-1

Pritchard, C., Shanks, A., Rimler, R., Oates, M., & Rumrill, S. (2015). The Olympia oyster *Ostrea lurida*: Recent advances in natural history, ecology, and restoration. *Journal of Shellfish Research*, *34*(2), 259–271. https://doi.org/10.2983/035.034.0207

R Development Core Team. (2023). *R: A language and environment for statistical computing* (Version 4.3.1) [Computer software package]. R Core Team.

Ricart, A. M., Ward, M., Hill, T. M., Sanford, E., Kroeker, K. J., Takeshita, Y., Merolla, S., Shukla, P., Ninokawa, A. T., Elsmore, K., & Gaylord, B. (2021). Coast-wide evidence of low pH amelioration by seagrass ecosystems. *Global Change Biology*, *27*(11), 2580–2591. https://doi.org/10.1111/gcb.15594

Riisgård, H. U., Lassen, J., & Kittner, C. (2006). Valve-gape response times in mussels (*Mytilus edulis*)- effects of laboratory preceding-feeding conditions and in situ tidally induced variation in phytoplankton biomass. *Journal of Shellfish Research*, *25*(3), 901–911. https://doi.org/10.2983/0730-8000(2006)25[901:VRTIMM]2.0.CO;2

Rimler, R. N. (2014). *Larval supply, settlement, and post-settlement performance as determinants of the spatial distribution of Olympia oysters (Ostrea lurida) in Coos*

Bay, OR [Master's thesis, University of Oregon]. University of Oregon Scholars' Bank.

Roegner, G., & Mann, R. (1995). Early recruitment and growth of the American oyster *Crassostrea virginica* (Bivalvia: Ostreidae) with respect to tidal zonation and season. *Marine Ecology Progress Series*, *117*, 91–101. https://doi.org/10.3354/meps117091

Seale, E. M., & Zacherl, D. C. (2009). Seasonal settlement of Olympia oyster larvae, *Ostrea lurida* Carpenter 1864 and its relationship to seawater temperature in two southern California estuaries. *Journal of Shellfish Research*, *28*(1), 113–120. https://doi.org/10.2983/035.028.0103

Sellers, M. A., & Stanley, J. G. (1984). *Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (North Atlantic): American oyster*. The Service.

Sharma, S., Goff, J., Moody, R. M., Byron, D., Heck Jr., K. L., Powers, S. P., Ferraro, C., & Cebrian, J. (2016). Do restored oyster reefs benefit seagrasses? An experimental study in the Northern Gulf of Mexico. *Restoration Ecology*, *24*(3), 306–313. https://doi.org/10.1111/rec.12329

Shumway, S. E. (1977). Effect of salinity fluctuation on the osmotic pressure and Na+, Ca2+ and Mg2+ ion concentrations in the hemolymph of bivalve molluscs. *Marine Biology*, *41*(2), 153–177. https://doi.org/10.1007/BF00394023

Signal Developers. (2013). *signal: Signal processing*. http://r-forge.r-project.org/projects/signal/

Smith, C. S., Puckett, B., Gittman, R. K., & Peterson, C. H. (2018). Living shorelines enhanced the resilience of saltmarshes to Hurricane Matthew. *Ecological Applications*, *28*(4), 871–877. https://doi.org/10.1002/eap.1722

Sumner, C. E. (1981). Growth of Pacific oysters, *Crassostrea gigas* Thunberg, cultivated in Tasmania. II. Subtidal culture. *Marine and Freshwater Research*, *32*(3), 411–416. https://doi.org/10.1071/mf9810411

Sutula, M., Kamer, K., Cable, J., Collis, H., Berelson, W., & Mendez, J. (2006). Sediments as an internal source of nutrients to Upper Newport Bay, California. *Santa Ana Regional Water Quality Control Board*.

Tate-Pulliam, E. (2021). *The effects of restoration methodology using Olympia oysters (Ostrea lurida) and eelgrass (Zostera marina) on infaunal invertebrate community composition in Newport Bay, California* [Master's thesis, California State University, Long Beach]. Pro Quest. https://www.proquest.com/docview/2642239137/abstract/4ECCD5E758FC4D8CPQ/1

Timmins-Schiffman, E., Coffey, W. D., Hua, W., Nunn, B. L., Dickinson, G. H., & Roberts, S. B. (2014). Shotgun proteomics reveals physiological response to ocean acidification in *Crassostrea gigas*. *BMC Genomics*, *15*(1), 951. https://doi.org/10.1186/1471-2164-15-951

Trimble, A. C., Ruesink, J. L., & Dumbauld, B. R. (2009). Factors preventing the recovery of a historically overexploited shellfish species, *Ostrea lurida* Carpenter 1864. *Journal of Shellfish Research*, *28*(1), 97–106. https://doi.org/10.2983/035.028.0116

Tronske, N. B., Parker, T. A., Henderson, H. D., Burnaford, J. L., & Zacherl, D. C. (2018). Densities and zonation patterns of native and non-indigenous oysters in southern California bays. *Wetlands*, *38*(6), 1313–1326. https://doi.org/10.1007/s13157-018-1055-0

Trueman, E. R. (1967). Activity and heart rate of bivalve molluscs in their natural habitat. *Nature*, *214*(5090), Article 5090. https://doi.org/10.1038/214832a0

Valdez, S. R., Peabody, B., Allen, B., Blake, B., & Ruesink, J. L. (2017). Experimental test of oyster restoration within eelgrass: Oyster restoration in eelgrass. *Aquatic Conservation: Marine and Freshwater Ecosystems*, *27*(3), 578–587. https://doi.org/10.1002/aqc.2722

van der Heide, T., Govers, L. L., de Fouw, J., Olff, H., van der Geest, M., van Katwijk, M. M., Piersma, T., van de Koppel, J., Silliman, B. R., Smolders, A. J. P., & van Gils, J. A. (2012). A three-stage symbiosis forms the foundation of seagrass ecosystems. *Science (New York, N.Y.)*, *336*(6087), 1432–1434. https://doi.org/10.1126/science.1219973

van Wesenbeeck, B. K., Griffin, J. N., van Koningsveld, M., Gedan, K. B., McCoy, M. W., & Silliman, B. R. (2013). Nature-based coastal defenses: Can biodiversity help? In S. A. Levin (Ed.), *Encyclopedia of Biodiversity (Second Edition)* (pp. 451–458). Academic Press. https://doi.org/10.1016/B978-0-12-384719-5.00323-3

Wagner, E., Dumbauld, B., Hacker, S., Trimble, A., Wisehart, L., & Ruesink, J. (2012). Density-dependent effects of an introduced oyster, *Crassostrea gigas*, on a native intertidal seagrass, *Zostera marina*. *Marine Ecology Progress Series*, *468*, 149–160. https://doi.org/10.3354/meps09952

Wallace, D. H. (1966). Oysters in the estuarine environment. *American Fisheries Society Special Publication*, *3*, 68–73.

Walne, P. R. (1958). Growth of oysters (*Ostrea edulis* L.). *Journal of the Marine Biological Association of the United Kingdom*, *37*(3), 591–602. https://doi.org/10.1017/S0025315400005634

Wasson, K. (2010). Informing Olympia oyster restoration: Evaluation of factors that limit populations in a California estuary. *Wetlands*, *30*(3), 449–459. https://doi.org/10.1007/s13157-010-0056-4

Wasson, K., Zabin, C., Bible, J., Briley, S., Ceballos, E., Chang, A., Cheng, B., Deck, A., Grosholz, T., Helms, A., Latta, M., Yednock, B., Zacherl, D., & Ferner, M. (2015). A guide to Olympia oyster restoration and conservation. *Elkhorn Slough National Estuarine Research Reserve*.

Widdows, J. (1973). Effect of temperature and food on the heart beat, ventilation rate and oxygen uptake of *Mytilus edulis*. *Marine Biology*, *20*(4), 269–276. https://doi.org/10.1007/BF00354270

Wilson, R., Reuter, P., & Wahl, M. (2005). Muscling in on mussels: New insights into bivalve behaviour using vertebrate remote-sensing technology. *Marine Biology*, *147*, 1165–1172. https://doi.org/10.1007/s00227-005-0021-6

Wood, V. (2018). *The effect of sedimentation on oysters (Ostrea lurida) adjacent to eelgrass beds (Zostera marina)* [Master's thesis, California State University, Fullerton]. ScholarWorks.

Wright, J. M., Parker, L. M., O'Connor, W. A., Williams, M., Kube, P., & Ross, P. M. (2014). Populations of Pacific oysters *Crassostrea gigas* respond variably to elevated CO2 and predation by *Morula marginalba*. *The Biological Bulletin*, *226*(3), 269–281. https://doi.org/10.1086/BBLv226n3p269

Zacherl, D., Moreno, A., & Crossen, S. (2015). Exploring restoration methods for the Olympia oyster *Ostrea lurida* Carpenter, 1864: Effects of shell bed thickness and shell deployment methods on shell cover, oyster recruitment, and oyster density. *Journal of Shellfish Research*, *34*(3), 819–830. https://doi.org/10.2983/035.034.0311

zu Ermgassen, P. S. E., Spalding, M. D., Grizzle, R. E., & Brumbaugh, R. D. (2013). Quantifying the loss of a marine ecosystem service: Filtration by the Eastern oyster in US estuaries. *Estuaries and Coasts*, *36*(1), 36–43. https://doi.org/10.1007/s12237-012-9559-y

APPENDIX

SUPPLEMENTARY TABLES AND FIGURES

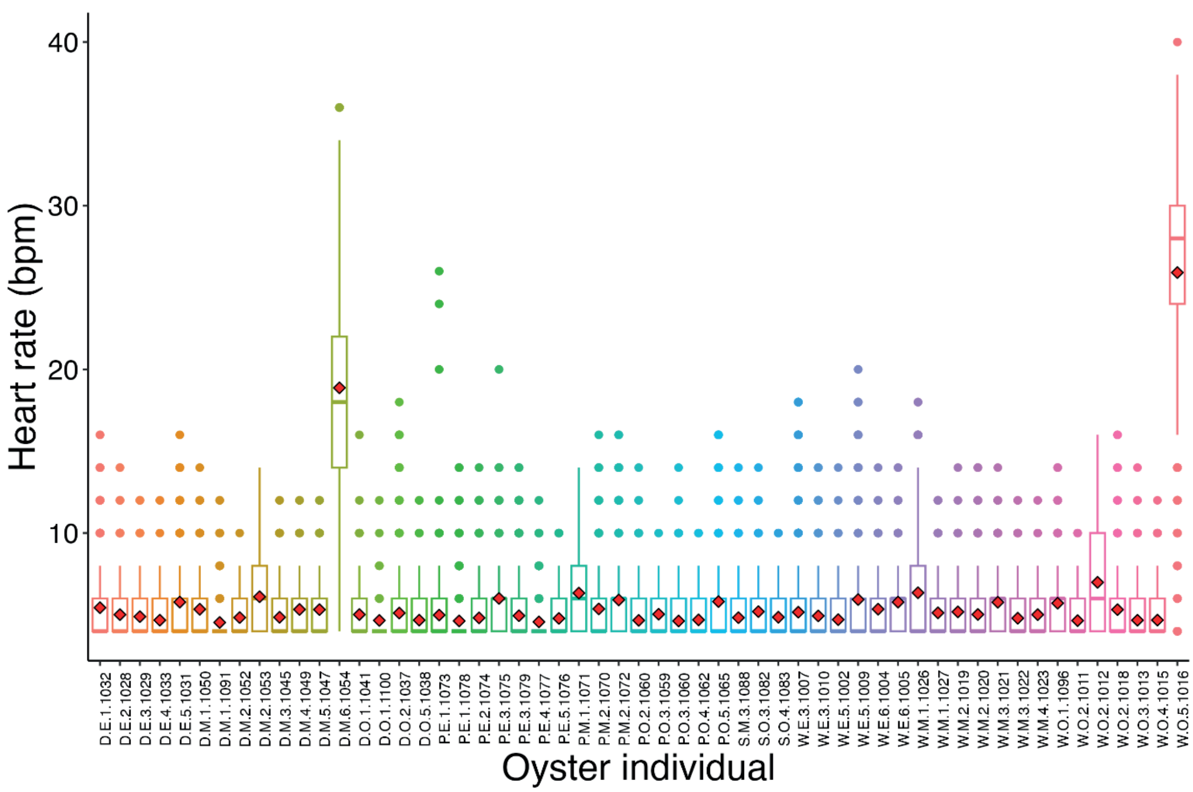

Figure A.1. Range of heart rates of all individual oysters across their entire deployment. Individuals are separated by color. Red diamonds represent averages and black bars represent medians.

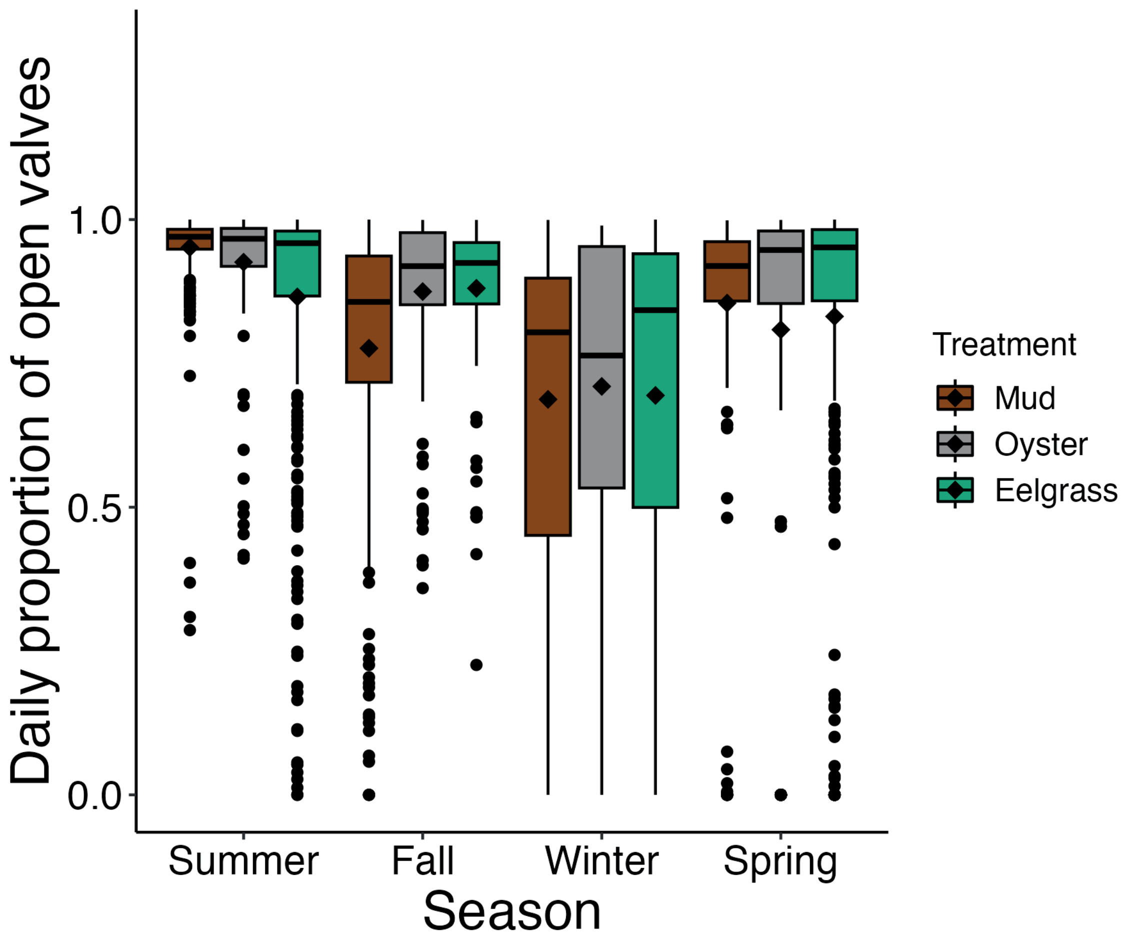

Figure A.2. The proportion of open valves separated by season, colored by treatment type. Black diamonds represent averages and black bars represent medians.

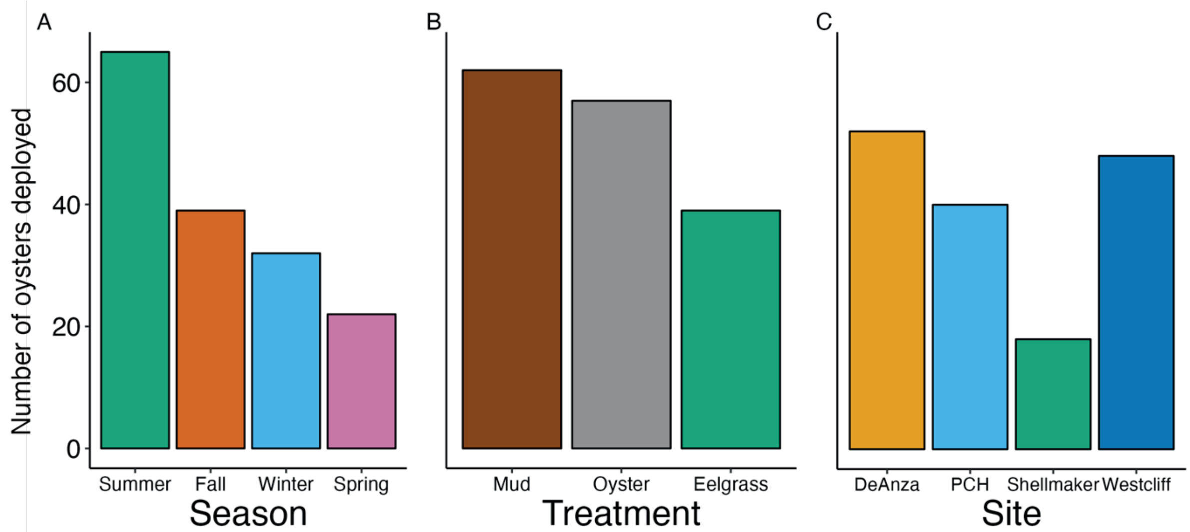

Figure A.3. Total number of oysters deployed across A) Season, B) Treatment, and C) Site. Summer, Fall, Winter, and Spring season represent data collected from July 15, 2022- September 30, 2022, October 1, 2022- December 31, 2022, January 1, 2023-March 31, 2023, and April 1, 2023-June 19, 2023, respectively.

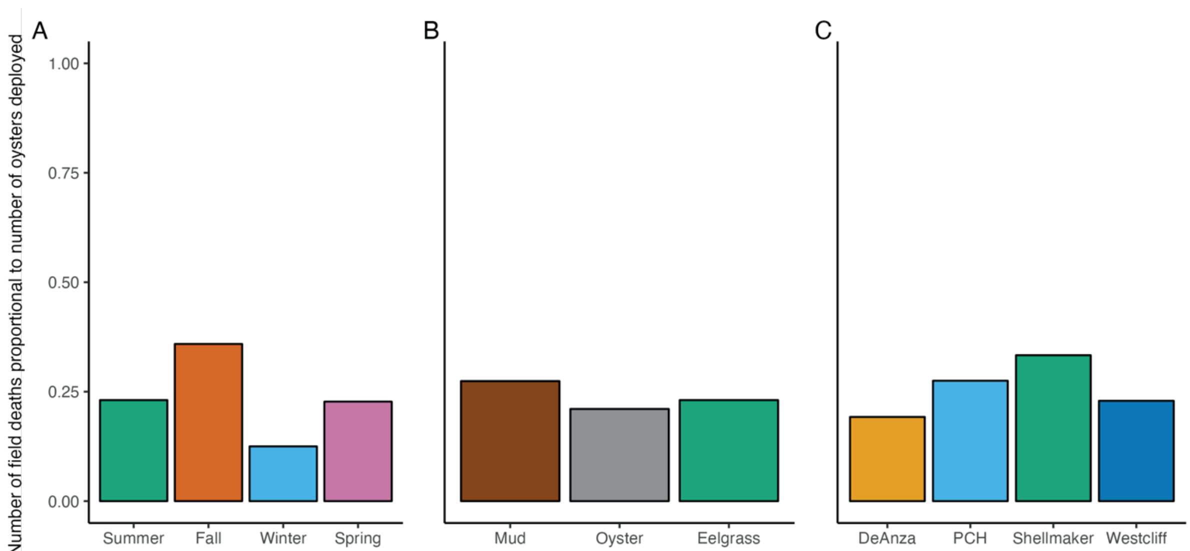

Figure A.4. The number of oyster deaths divided by the number of oysters deployed across A) Season, B) Treatment, and C) Site. Summer, Fall, Winter, and Spring season represent data collected from July 15, 2022- September 30, 2022, October 1, 2022- December 31, 2022, January 1, 2023-March 31, 2023, and April 1, 2023-June 19, 2023, respectively.

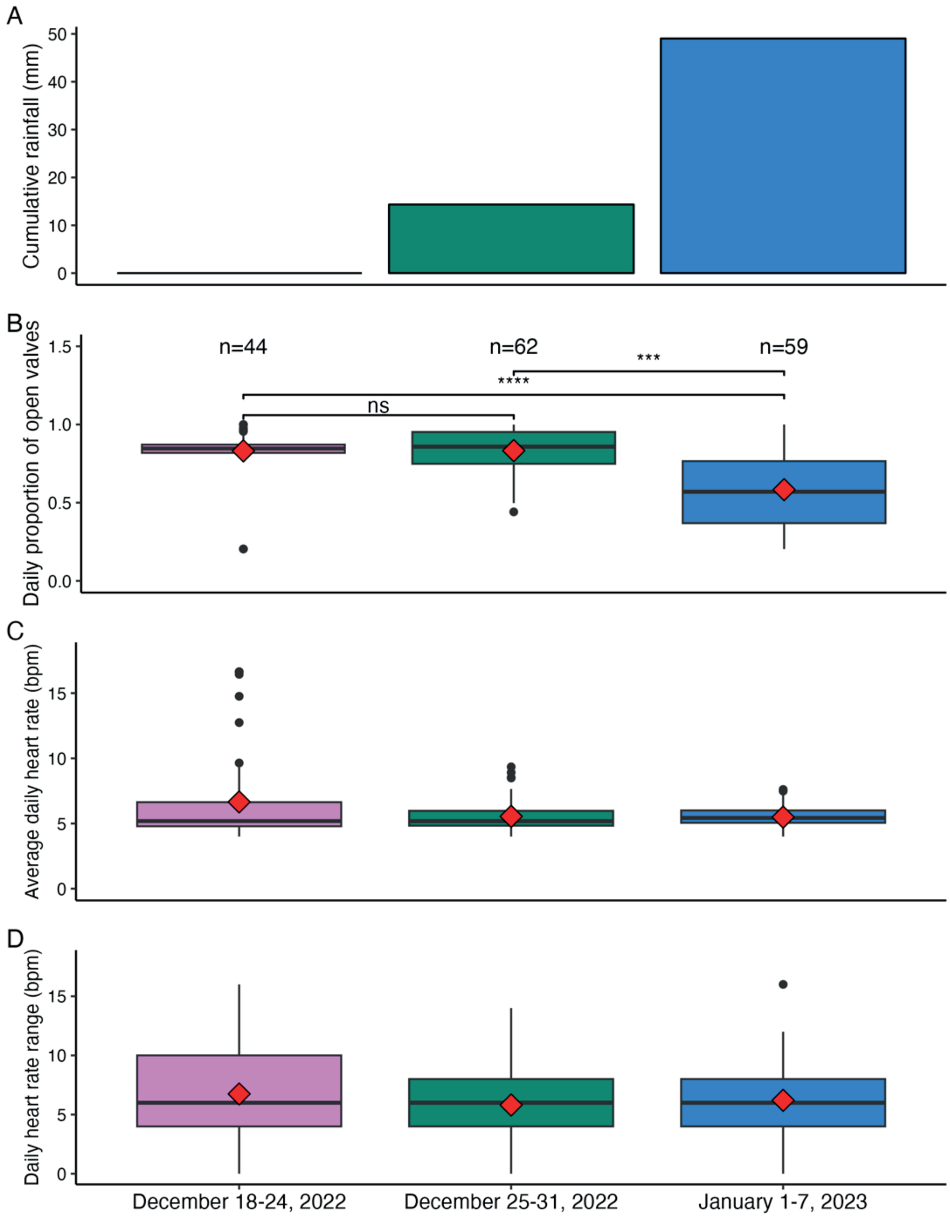

Figure A.5. Plot of A) cumulative rainfall (mm), B) daily proportion of open valves, C) average daily heart rate (bpm), and D) daily heart rate range (bpm) of all oysters from December 18-24, 2022 (n=44 oysters), December 25-31, 2022 (n=62 oysters) and January 1-7, 2023 (n=59 oysters). Red diamonds represent averages and black bars represent medians. Significant differences as determined by Dunn's post-hoc comparisons, ***= p<0.001, ****= p<0.0001.

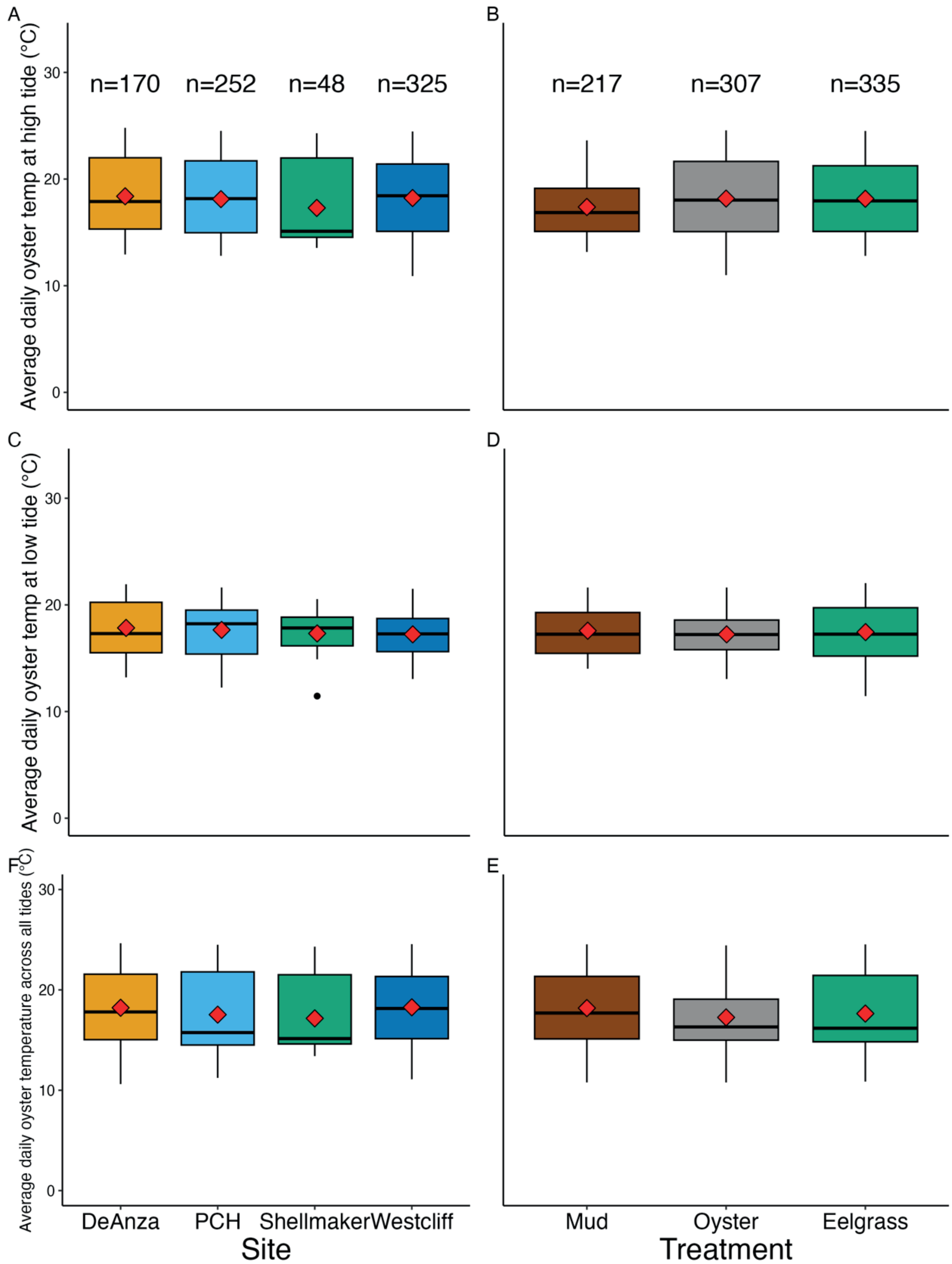

Figure A.6. Plot of oyster temperature at high tide separated by A) Treatment and B) Site, at low tide separated by A) Treatment and B) Site, and at all tides separated by A) Treatment and B) Site. Red diamonds represent averages and black bars represent medians. N=number of days.

Table A.1. Dry tissue mass, length, and width of oysters collected at the end of experiment.

Individual oyster	Dry tissue mass (g)	Length (mm)	Width (mm)
P.E.1.1078	0.1897	31	35
P.E.2.1074	0.1416	36	22
P.E.3.1079	0.303	40	27
P.O.3.1060	0.2057	36	38
P.O.5.1065	0.4193	45	29
W.E.3.1010	0.3286	43	26
W.E.5.1009	0.2806	49	30
W.E.3.1007	0.2232	36	24
W.O.3.1099	0.1328	33	23
W.O.2.1012	0.2918	35	37
W.O.1.1096	0.1832	33	25
W.E.3.1007	0.2193	36	24
W.M.5.1020	0.238	34	23
W.M.3.1022	0.1879	28	24
W.M.4.1023	0.3295	40	28
W.M.2.1019	0.3643	39	32
P.M.2.1098	0.0469	34	22
D.O.2.1037	0.2042	35	33
D.O.1.1100	0.2699	30	24
D.M.1.1102	0.2044	30	25
D.E.2.1030	0.363	37	37
D.E.3.1033	0.2971	36	30
S.M.2.1090	0.2741	27	24

Table A.2. Post-hoc pairwise lsmeans results for the generalized linear mixed effects model on the interaction between season and treatment on proportion of open valves. Results are given on the log (not the response) scale. Tukey p value adjustment method used.

Pairwise comparisons	Estimate	SE	Df	Z ratio	P value
Mud Winter - Oyster Winter	0.39757	0.1894	Inf	2.099	0.6237
Mud Winter - Eelgrass Winter	-0.04305	0.1732	Inf	-0.249	1.0000
Mud Winter - Mud Fall	-0.01466	0.0722	Inf	-0.203	1.0000
Mud Winter - Oyster Fall	-0.00387	0.1938	Inf	-0.020	1.0000
Mud Winter - Eelgrass Fall	0.15584	0.1766	Inf	0.882	0.9993
Mud Winter - Mud Spring	0.36262	0.0654	Inf	5.545	<.0001
Mud Winter - Oyster Spring	0.05689	0.1855	Inf	0.307	1.0000
Mud Winter - Eelgrass Spring	-0.14427	0.1745	Inf	-0.827	0.9996
Mud Winter - Mud Summer	-0.28053	0.0831	Inf	-3.377	0.0354
Mud Winter - Oyster Summer	-0.15928	0.1782	Inf	-0.894	0.9992
Mud Winter - Eelgrass Summer	0.02015	0.1689	Inf	0.119	1.0000
Oyster Winter - Eelgrass Winter	-0.44062	0.1899	Inf	-2.320	0.4621
Oyster Winter - Mud Fall	-0.41223	0.1873	Inf	-2.201	0.5491
Oyster Winter - Oyster Fall	-0.40144	0.0946	Inf	-4.241	0.0013
Oyster Winter - Eelgrass Fall	-0.24173	0.1930	Inf	-1.253	0.9846
Oyster Winter - Mud Spring	-0.03495	0.1909	Inf	-0.183	1.0000
Oyster Winter - Oyster Spring	-0.34068	0.0742	Inf	-4.588	0.0003
Oyster Winter - Eelgrass Spring	-0.54184	0.1912	Inf	-2.834	0.1657
Oyster Winter - Mud Summer	-0.67810	0.1842	Inf	-3.681	0.0124
Oyster Winter - Oyster Summer	-0.55685	0.1443	Inf	-3.859	0.0064
Oyster Winter - Eelgrass Summer	-0.37742	0.1858	Inf	-2.031	0.6718
Eelgrass Winter - Mud Fall	0.02839	0.1711	Inf	0.166	1.0000
Eelgrass Winter - Oyster Fall	0.03918	0.1944	Inf	0.202	1.0000
Eelgrass Winter - Eelgrass Fall	0.19889	0.0605	Inf	3.287	0.0472
Eelgrass Winter - Mud Spring	0.40566	0.1747	Inf	2.322	0.4605
Eelgrass Winter - Oyster Spring	0.09994	0.1859	Inf	0.538	1.0000

Pairwise comparisons	Estimate	SE	Df	Z ratio	P value
Eelgrass Winter - Eelgrass Spring	-0.10123	0.0484	Inf	-2.093	0.6278
Eelgrass Winter - Mud Summer	-0.23749	0.1672	Inf	-1.421	0.9598
Eelgrass Winter - Oyster Summer	-0.11623	0.1782	Inf	-0.652	1.0000
Eelgrass Winter - Eelgrass Summer	0.06320	0.0734	Inf	0.861	0.9994
Mud Fall - Oyster Fall	0.01079	0.1918	Inf	0.056	1.0000
Mud Fall - Eelgrass Fall	0.17050	0.1745	Inf	0.977	0.9982
Mud Fall - Mud Spring	0.37728	0.0793	Inf	4.760	0.0001
Mud Fall - Oyster Spring	0.07155	0.1833	Inf	0.390	1.0000
Mud Fall - Eelgrass Spring	-0.12961	0.1725	Inf	-0.751	0.9998
Mud Fall - Mud Summer	-0.26587	0.0714	Inf	-3.725	0.0106
Mud Fall - Oyster Summer	-0.14461	0.1762	Inf	-0.821	0.9996
Mud Fall - Eelgrass Summer	0.03481	0.1667	Inf	0.209	1.0000
Oyster Fall - Eelgrass Fall	0.15971	0.1974	Inf	0.809	0.9997
Oyster Fall - Mud Spring	0.36648	0.1953	Inf	1.876	0.7742
Oyster Fall - Oyster Spring	0.06076	0.0919	Inf	0.661	1.0000
Oyster Fall - Eelgrass Spring	-0.14041	0.1957	Inf	-0.718	0.9999
Oyster Fall - Mud Summer	-0.27667	0.1888	Inf	-1.466	0.9498
Oyster Fall - Oyster Summer	-0.15541	0.1518	Inf	-1.024	0.9972
Oyster Fall - Eelgrass Summer	0.02402	0.1903	Inf	0.126	1.0000
Eelgrass Fall - Mud Spring	0.20678	0.1781	Inf	1.161	0.9917
Eelgrass Fall - Oyster Spring	-0.09895	0.1891	Inf	-0.523	1.0000
Eelgrass Fall - Eelgrass Spring	-0.30011	0.0650	Inf	-4.615	0.0002
Eelgrass Fall - Mud Summer	-0.43637	0.1707	Inf	-2.556	0.3049
Eelgrass Fall - Oyster Summer	-0.31511	0.1816	Inf	-1.736	0.8518
Eelgrass Fall - Eelgrass Summer	-0.13569	0.0791	Inf	-1.716	0.8612
Mud Spring - Oyster Spring	-0.30573	0.1870	Inf	-1.635	0.8963
Mud Spring - Eelgrass Spring	-0.50689	0.1760	Inf	-2.880	0.1480
Mud Spring - Mud Summer	-0.64315	0.0840	Inf	-7.659	<.0001
Mud Spring - Oyster Summer	-0.52189	0.1797	Inf	-2.904	0.1392

Pairwise comparisons	Estimate	SE	Df	Z ratio	P value
Mud Spring - Eelgrass Summer	-0.34247	0.1705	Inf	-2.009	0.6878
Oyster Spring - Eelgrass Spring	-0.20116	0.1872	Inf	-1.074	0.9957
Oyster Spring - Mud Summer	-0.33742	0.1801	Inf	-1.874	0.7759
Oyster Spring - Oyster Summer	-0.21617	0.1395	Inf	-1.550	0.9265
Oyster Spring - Eelgrass Summer	-0.03674	0.1817	Inf	-0.202	1.0000
Eelgrass Spring - Mud Summer	-0.13626	0.1685	Inf	-0.808	0.9997
Eelgrass Spring - Oyster Summer	-0.01500	0.1795	Inf	-0.084	1.0000
Eelgrass Spring - Eelgrass Summer	0.16443	0.0786	Inf	2.092	0.6287
Mud Summer - Oyster Summer	0.12126	0.1724	Inf	0.703	0.9999
Mud Summer - Eelgrass Summer	0.30068	0.1629	Inf	1.846	0.7922
Oyster Summer - Eelgrass Summer	0.17943	0.1743	Inf	1.030	0.9971