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Effects of Dissolved Oxygen and pH on Eastern Oyster (Crassostrea virginica) Growth

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by

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ABSTRACT

Coastal ecosystems, known for their abundant natural resources and role in environmental processes, are facing challenges posed by climate change, pollution and anthropogenic activities. Among these challenges is the expansion of hypoxic zones, characterized by low dissolved oxygen (DO) levels, which poses a significant threat to benthic organisms and oyster fisheries. This research investigates the interaction between hypoxia and pH dynamics, intensified by freshwater input, and the biomineralization of Eastern oysters (*Crassostrea virginica)*. To explore these dynamics, experimental trials were conducted in 24 tanks, simulating normoxic, hypoxic, and fresh hypoxic conditions, to assess the impact of hypoxia on pH levels and the growth of juvenile oysters. The experimental setup included 30-day trials with controlled salinity, temperature, and DO levels, replicating conditions observed in the Mississippi Sound. Results revealed significant difference in dry shell weight during hypoxic conditions indicating shell erosion, although pH was not lower during hypoxia as expected. It can be concluded based on the data that dissolved shell material acted as a buffer and increased the pH. The analysis of biological parameters, including weight, size, and condition index, illustrates the potential ramifications of shell dissolution under hypoxic conditions. Significantly lower dry shell weight in hypoxic and fresh hypoxic conditions further substantiate the hypothesis of pH-driven shell erosion. This study illustrates the susceptibility of oysters to increased fluxes of freshwater, and the impacts events such as climate change and the opening of the Bonnet Carré Spillway can have on coastal ecosystems.

Keywords: hypoxia, Gulf of Mexico, Eastern oyster, coastal acidification, shell dissolution

DEDICATION

To my late grandmother, Linda “Nina” Fowler. None of this would have been possible without your constant encouragement and guidance that continues to be the driving force in everything I do.

ACKNOWLEDGMENTS

This project would not have been possible without the guidance of my advisor Dr. Kim de Mutsert, who allowed me to continue this project that started as a REU internship and was able to seamlessly transition into this Honor's Thesis. I would also like to thank unofficial advisor Dr. Alan Shiller, who gave me the initial encouragement and confidence I needed to pursue a path in research. Lastly, I would like to acknowledge the graduate student I worked with on this project, Abiola Obafemi who made me feel like a true equal in this project. Without his collaboration and support none of this would be possible.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BCS	Bonnet Carré Spillway
$CaCO_3$	Calcium carbonate
CI	Condition Index
CO_2	Carbon dioxide
DO	Dissolved Oxygen
MATLAB	Matrix Laboratory
pH	Potential Hydrogen
USM	The University of Southern Mississippi
YSI	Yellow Springs Instruments

INTRODUCTION

Coastal ecosystems serve as habitats for various marine species, making them components of global ecological and economic systems. These ecosystems face growing and multifaceted threats stemming from climate change, pollution, and other anthropogenic activities (Beniash et al. 2010). Among the numerous challenges coastal ecosystems encounter, one of the most significant is the expansion of hypoxic zones, which are characterized by low dissolved oxygen (DO) levels (David et al. 2005). Hypoxia can form when excessive nutrient runoff from rivers enters coastal waters. This runoff causes an increase in nutrients fueling phytoplankton blooms. When these phytoplankton die, they sink to the seafloor where bacteria begin decomposing them. This process consumes large amounts of oxygen leading to depletion of oxygen in the water column (Boesch et al. 2009). Another contributing factor to hypoxia caused by lack of mixing between freshwater discharge and denser salt water is stratification. Stratification prevents bottom waters from becoming reoxygenated (Obenour et al. 2012). Many coastal ecosystems are experiencing expanding hypoxic zones to the detriment of benthic organisms and oyster fisheries.

One of the consequences of hypoxia that has gained substantial attention in recent years is its potential to influence pH levels of coastal waters. Ocean acidification is the result of climate change which is driven by the uptake of atmospheric carbon dioxide (CO_2) and its conversion to carbonic acid in seawater. This process results in a reduction of pH, which has cascading effects on the geochemistry and biology of coastal ecosystems (Thomsen et al. 2015). Notably, calcifying organisms, such as oysters, play a crucial role in these ecosystems and are particularly susceptible to fluctuations in pH

(Cyronak and Eyre 2016). Oysters are not only important filter feeders that contribute to water quality improvement and nutrient cycling, but they also provide critical ecosystem services such as shoreline protection and support fisheries and aquaculture industries. This hypoxia driven acidification impacts coastal ecosystem and poses a threat to calcifying organisms.

Ocean acidification poses a significant threat to mollusk shell biomineralization by impacting carbonate availability and metabolic processes (Doney et al., 2009; Gazeau et al., 2007). The global temperature increase driven by climate change, approximately 0.5 °C, is particularly pronounced in coastal areas such as the northern Gulf of Mexico (Miller et al. 2009). This change in global temperature causes an increase in precipitation which influences the flow from the Mississippi River, resulting in heightened freshwater input into the northern Gulf of Mexico (Miller et al. 2009). This surge in freshwater, occurring seasonally from April to October, is closely linked to hypoxia, affecting estuarine ecosystems rich in mollusks (Justić et al. 1993).

In addition to hypoxia, reduced pH levels can impair the ability of oysters to form and repair their shells, making them more susceptible to predation, environmental stressors, and hampering their overall survival. The eastern oyster (*Crassostrea virginica*), a vital estuarine species, faces threats to both its biomineralization and ecosystem services due to these changing environmental conditions (Cressman et al., 2003;Gledhill et al., 2020).

This thesis aims to explore the complex interplay between hypoxia, pH, and biological parameters of oysters in coastal ecosystems. By synthesizing existing knowledge and presenting novel research findings, We intend to shed light on the mechanisms by which hypoxia influences pH and how these changes impact the health

and survival of eastern oysters. Understanding this interaction is essential for the conservation and management of coastal ecosystems, the sustainability of oyster populations, and the broader implications for ecosystem services and biodiversity conservation.

LITERATURE REVIEW

2.1 Freshwater Release Events from Bonne Carré Spillway

In 2019, the Mississippi River basin faced one of the wettest years on record, necessitating the activation of the Bonnet Carré Spillway (BCS) to mitigate flooding risks (NOAA National Centers for Environmental Information, 2019). Originating from the response to the "Great Mississippi Flood of 1927", The BCS, a flood control structure at the southern end of the Mississippi River, diverts a portion of the Mississippi River flow into Lake Pontchartrain, alleviating the threat of flooding in the New Orleans area (Lane et al. 2001). The frequency of BCS openings has increased in response to amplified precipitation, leading to heightened concerns for areas surrounding the lower Mississippi River (Gledhill et al. 2019). Unprecedented volumes of freshwater released through the BCS into Lake Pontchartrain and subsequently into the Mississippi Sound during extreme flooding events, such as those in 2019, have profound implications for coastal water quality (Gledhill et al., 2019). The economic impact is substantial, with estimates suggesting multimillion-dollar losses in the commercial oyster harvest following BCS openings (Posadas and Posadas 2017). Studies following previous BCS openings have identified the developments of bottom water hypoxia, a condition known to adversely affect marine organisms (Ho et al. 2019). The persistent threat of extended freshwater inflow events underscores the potential loss of critical oyster reef ecosystem services (Gledhill et al., 2019).

2.2 How Freshwater Affects Estuary Water Quality

Estuaries are dynamic ecosystems where freshwater from rivers meets and mixes with saltwater from the ocean. These environments are particularly susceptible to changing

environmental conditions, including alterations in dissolved oxygen, temperature, salinity, and nutrient levels (Nixon 1995). Proximity to land and natural freshwater sources makes estuarine ecosystems particularly vulnerable to sea level rise and changes in environmental conditions such as dissolved oxygen (DO), temperature, and salinity (Ko et al. 2016).

2.3 Dissolved Oxygen (DO) and its Relevant Effect on pH

Eutrophication-induced acidification serves as an additional stressor in stratified coastal ecosystems, with the Mississippi/Atchafalaya River system, one of the world's largest river basins, contributing to high phytoplankton production on the continental shelf of the northern Gulf of Mexico (Laurent et al. 2017). Respiration-induced acidification, exacerbated by reduced buffering capacity, significantly affects pH in this region, with pH values less than or equal to 7.85 considered acidified (Sunda and Cai, 2012; Laurent et al., 2017). The strong stratification resulting from large freshwater inputs emerges as a critical driver of bottom water acidification in these coastal areas (Laurent et al., 2017). The interaction between CO_2 diffusion into seawater, carbonic acid formation, and subsequent shifts in carbonate ion concentrations significantly contributes to the acidification process (Melzner et al., 2013; Brewer, 1997). As both hypoxia and acidification are promoted by climate change and eutrophication, a parallel increase in CO_2 and decrease in pH and aragonite saturation states is observed (Stevens and Gobler 2018). The intensification of acidification effects with rising temperatures places marine invertebrates under prolonged stress, resulting in lower growth rates and reduced sizes (Talmage and Gobler, 2011; Sokolva, 2013). Concurrent acidification and low oxygen

conditions have been shown to independently depress growth and survival rates of estuarine bivalves (Gobler et al. 2014).

2.4 Importance of Calcium Carbonate Equilibrium to Calcifiers

In nearly all ocean surface waters, the thermodynamically favored formation of $CaCO_3$ relies on the abundance of dissolved calcium (Ca^{2+}) and carbonate (CO^{3-}) ions (Barton et al. 2012). The decrease in oceanic (CO_3^{2-}) as atmospheric CO_2 rises has far-reaching implications for the various phases of calcium carbonate, including calcite and aragonite (Morse and Mackenzie, 1990). Studies indicate a significant decrease in the growth rate of *C. virginica* under lower pH conditions, emphasizing the sensitivity of this species to changing ocean chemistry (Miller et al. 2009). Larval growth reduction, observed under reduced pH, is accompanied by negative effects on survival and metamorphosis (Talmage and Gobler, 2009). Acidification, through the titration of carbonate ions to bicarbonate, diminishes the availability of carbonate ions critical for calcifying organisms (Kleypas et al. 2006). The dissolution of atmospheric CO_2 into seawater, leading to carbonic acid formation, underscores the delicate balance between carbonate and bicarbonate ions, ultimately contributing to seawater pH reduction (Leung, Zhang, and Connell 2022). Marine calcifiers face heightened sensitivity to CO_2-driven changes in ocean chemistry, resulting in decreased pH and declining $CaCO_3$ saturation states (Ries, Cohen, and McCorkle 2009).

2.5 How Decreased pH can Impact Calcium Carbonate Equilibrium

Experimental acidification studies consistently report adverse impacts on shell growth in oysters, affecting shell thickness and integrity (Ries 2011). Estuarine acidification, influenced by reduced salinity, pH, and total alkalinity, disrupts the complex and

biologically controlled process of calcium carbonate deposition in oyster shells (Smith et al. 2003; Waldbusser et al. 2011). Changes in carbonate chemistry inhibit the ability of calcifying organisms to produce an exoskeleton, further challenging their adaptability to changing environmental conditions (Waldbusser et al. 2013). Confronted with reduced environmental pH and carbonate ion concentration, oysters allocate more energy to maintaining optimal pH levels at calcification sites, countering unfavorable concentration gradients. In such lowered pH environments, oysters may encounter difficulties assembling the mineralized foliated layer correctly, leading to reductions in shell hardness and stiffness (Meng et al. 2019). The stress induced by ocean acidification compels oysters to regulate physiological functions, potentially impacting their overall health and survival (Lemasson et al. 2017).

The intricate relationship between decreased pH and biocalcification highlights the vulnerability of oysters to changing environmental conditions (Hoffman and Todgham, 2010). The following equation illustrates the reaction CO_2 undergoes when dissolving in water.

$$CO_{2\,(g)} + H_2O_{(l)} \rightleftharpoons H_2CO_{3\,(aq)} \rightleftharpoons H^+_{(aq)} + HCO_3^-{}_{(aq)} \rightleftharpoons 2H^+_{(aq)} + CO_3^{2-}{}_{(aq)}$$

As the concentration of CO_2 increases, free H^+ ions also rise, subsequently decreasing the pH. This reduced pH and abundance of free H^+ ions lead to the following reaction with $CaCO_3$ occurring more frequently:

$$H^+_{(aq)} + CaCO_{3\,(s)} \rightarrow HCO_3^-{}_{(aq)} + Ca^{2+}{}_{(aq)}$$

When an excess of hydrogen ions is present (lower pH), the free hydrogen ions begin to attach to the carbonate ions in shells, causing them to dissolve.

The primary objectives of this study are to explore the interactions between hypoxia, pH dynamics, and the growth of eastern oysters. Specifically, this research aims to investigate hypoxic conditions influence on pH levels and the subsequent effect this has on the biomineralization of eastern oysters. We hypothesize that hypoxic conditions, influenced by freshwater release events, will lead to a decrease in pH. This anticipated decrease in pH would affect the biomineralization process of eastern oysters. Changes in other biological variables that could be affected by both low dissolved oxygen and pH, including overall weight, size, shell weight, meat weight, condition index, and mortalities will be analyzed as well. By synthesizing existing knowledge and conducting experimental trials, this study seeks to contribute to a comprehensive understanding of how changing environmental conditions, particularly those related to hypoxia and pH, affect the health and survival of oyster populations.

METHODS

3.1 Study Species and Collection

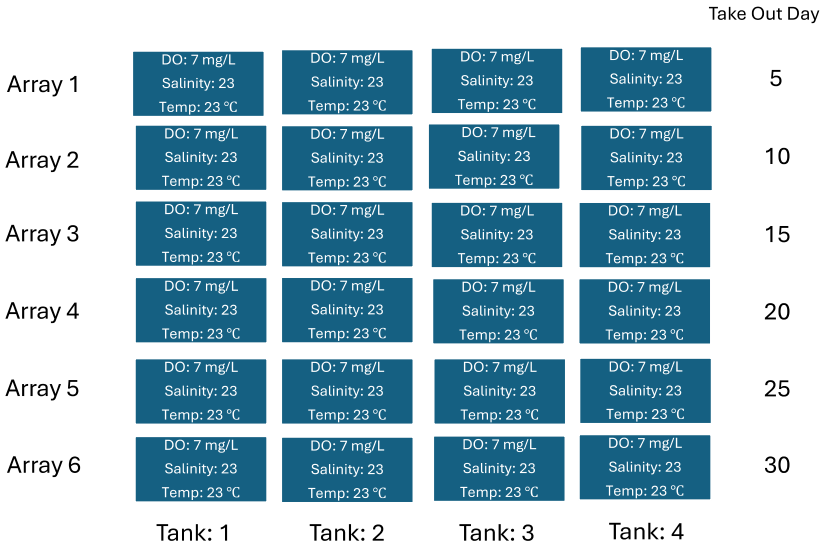
The oysters used in this study (*C. virginica*) were collected at the Deer Island Aquaculture Park oyster farm and acclimated at Thad Cochran Marine Aquaculture Center at the Gulf Coast Research Laboratory in Ocean Springs, MS. The preferred oyster sizes (9 mm-20 mm in length) were selected using sieves at the beginning of a trial. They were then counted and placed into a separate container while being checked to make sure they were visibly alive. Once the desired amount was collected, the oysters were rinsed thoroughly with water and quickly transported to the Toxicology Laboratory at USM's Cedar Point campus where the experiments would take place to be placed into tanks to get acclimated.

3.2 Experimental Setup

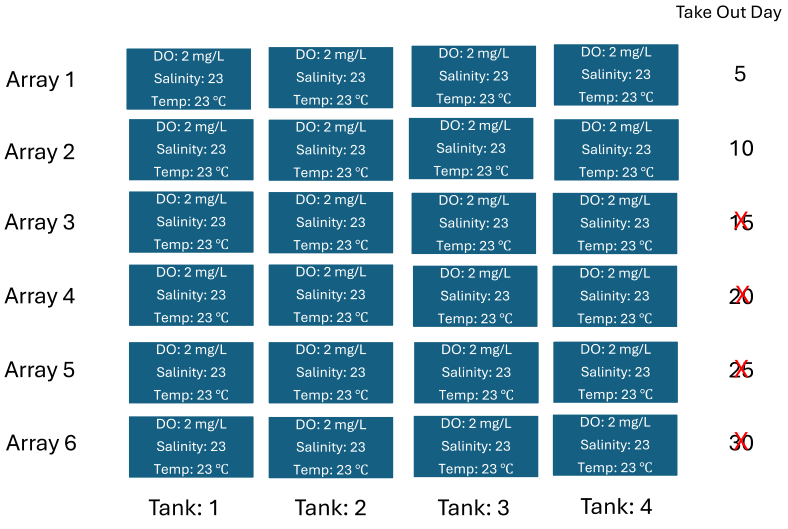
For the setup for this experiment, 24 tanks (20 gallons each) were utilized and divided into 6 arrays, with 4 tanks in each array (see Fig. 1). Each tank had 20 labeled Petri dishes to keep track of each oyster's individual weight, height, and length. The system used allows for salinity, temperature, and target DO to be manually set per tank and then regulates the values set. This ensures all the tanks stay within the appropriate range of the predetermined values. Prior to conducting experiments, the tanks were cleaned, and new tubing was installed to reduce any contamination. Freshwater and saltwater entered a large drum (1 per array) at ratios determined by the set salinity for the array. This water was periodically mixed to avoid layering and flowed into the 4 tanks in the corresponding array. Tanks were acclimated to parameters that match that of the nursery's before adjusting to the experimental values.

Three treatments were conducted at USM's Toxicology Laboratory between June of 2023 and February of 2024. Normoxic and hypoxic treatments were kept at consistent temperatures ranging from 22-24°C with a target temperature of 23°C, while the target temperature for fresh hypoxic was 30°C to replicate summer temperatures. Similarly, salinity for normoxia (treatment 1) and hypoxia (treatment 2) trials were kept close to 23 ppm to mimic the mean salinity value observed in the Mississippi Sound. The last treatment, fresh hypoxic (treatment 3) was set to 3 ppm to replicate freshwater influx from the Bonnet Carré Spillway. For treatment 1 (normoxia), DO was kept around the target of 7 g/mL for 30 days. In treatment 2 (hypoxia) DO was kept < 2 g/mL for 11 days before the trial ended due to high mortality of oysters. This was decided due to one tank in Array 3, two tanks in Array 4, and all four tanks in Array 5 experiencing total mortality. Lastly, treatment 3 (fresh hypoxia) had a target DO of < 2 g/mL and lasted for 30 days. The following figure (Fig. 1) shows the set DO, salinity, and temperature for each tank during each treatment.

Normoxic Treatment



Hypoxic Treatment



Fresh Hypoxic Treatment

Fig. 1. Illustration of experimental set up for each treatment. Includes arrays, tanks, target DO, salinity and temperature. For the hypoxic treatment, even though 4 more takeout days were expected, treatment ended on day 11 due to high mortalities.

3.3 Initial Measurements, Daily Measurements, and Clearance Days

At the beginning of each trial, each of the 480 oysters had their weight (g) measured with an electric scale, as well as length and width measured with digital calipers (mm). Each oyster was placed on a Petri dish at random which were labeled from 1-20 and 20 at a time were placed into a tank. The oysters were allowed to acclimate for approximately a week depending on the length of time the tanks took to reach desired parameter values. Once the treatment reached the desired values, day 1 began and daily measurements continued to be recorded for the duration of the trial.

Daily measurements were recorded by a handheld YSI (626870-1 ProDSS) to determine temperature, salinity, DO (%), DO (mg/L), and pH was determined using a pH meter (EcoSense pH10A ± 0.02) that were calibrated per manufacturer instructions before starting the trials. This process was done for each tank every day of the trial. After water quality was tested and recorded, manual checks in each tank were completed to determine daily oyster mortalities per tank. An oyster was considered deceased if the mouth was ajar and did not close with exposure to air. If any were deceased, it was recorded with the daily water quality data. Lastly, oysters were fed a shellfish diet and a daily amount of 3.5 mL multiplied by the average weight of oysters per tank. The average weight per tank was determined by averaging the individual weights of the oysters initially taken and multiplying that average by the amount of living oysters in each tank. This provided us with the amount to feed each tank every day. The formula for feeding is explained below:

$$v_f = (m_a \times 3.5\,mL)(n) \qquad \textit{Eq.1}$$

Where v_f is the volume of food, m_a is the initial average mass of all oysters, and n is the number of oysters alive in a specific tank.

Tentative removal days were to occur on day 5, 10, 15, 20, 25, and 30 of each treatment. Removal days involved 4 tanks of the same row (1 array) to have the oysters taken out one tank at a time. Each individual oyster was then weighed and measured again with data recorded for each oyster based on the number on the Petri dish.

3.4 Determining Condition Index and Data Analysis

During clearance days after weight and measurements were recorded, each surviving oyster was shucked and had the meat separated from the shell. The meat was placed in a test tube by tank number and placed in a laboratory freezer at - 30°C for 48h. The meat was then transferred to a freeze dryer for another 48h then weighed once again. The shells from the oysters were also separated by tank and dried out in a laboratory oven at 50°C for 15m to evaporate any water. The dry weight of the shells could then be measured by tank. Dry meat and shell weight was used to calculate condition index per tank using the following formula:

$$CI = \frac{dry\ meat\ weight\ (g)}{dry\ shell\ weight\ (g)} \times 100 \qquad \textit{Eq.2}$$

In order to determine size for analysis of shell loss, the following equation for area of an oval was used:

$$A = length \times height \times \pi \qquad \textit{Eq.3}$$

Data was analyzed with MATLAB to compare results from treatment 1 (normoxia), treatment 2 (hypoxia), and treatment 3 (fresh hypoxia) using ANOVA and *post hoc* Tukey tests to determine significance at an alpha level of 0.05.

RESULTS

4.1 pH Results in Normoxic, Hypoxic, and Fresh Hypoxic Conditions

The average DO and pH over the course of each treatment are shown in Table 1 and Fig. 2 (a & b) respectively. The average DO (x ± SEM) for the normoxic treatment was 6.71 ± 0.07 mg/L, while the average DO for the hypoxic treatment was 1.62 ± 0.06 mg/L. The average DO observed in the fresh hypoxic treatment was 3.15 ± 0.09 mg/L which was a bit higher that the target DO of 2 mg/L. The post hoc Tukey tests for DO (Table 2) indicated that all treatments average DO values were significantly different. The normoxic treatment had an average pH of 7.63 ± 0.006, the hypoxic treatment average pH was 7.79 ± 0.01, and the fresh hypoxic treatment had an average pH of 7.65 ± 0.02. All three treatments illustrated trends of decreasing that would shift to increasing consistently throughout the timeframe of the treatment (Fig 2c). Overall, pH between treatment did not results in significant differences between based on results from the post hoc Tukey tests, although ANOVA test did result in significance ($p < 0.05$)(Table 2).

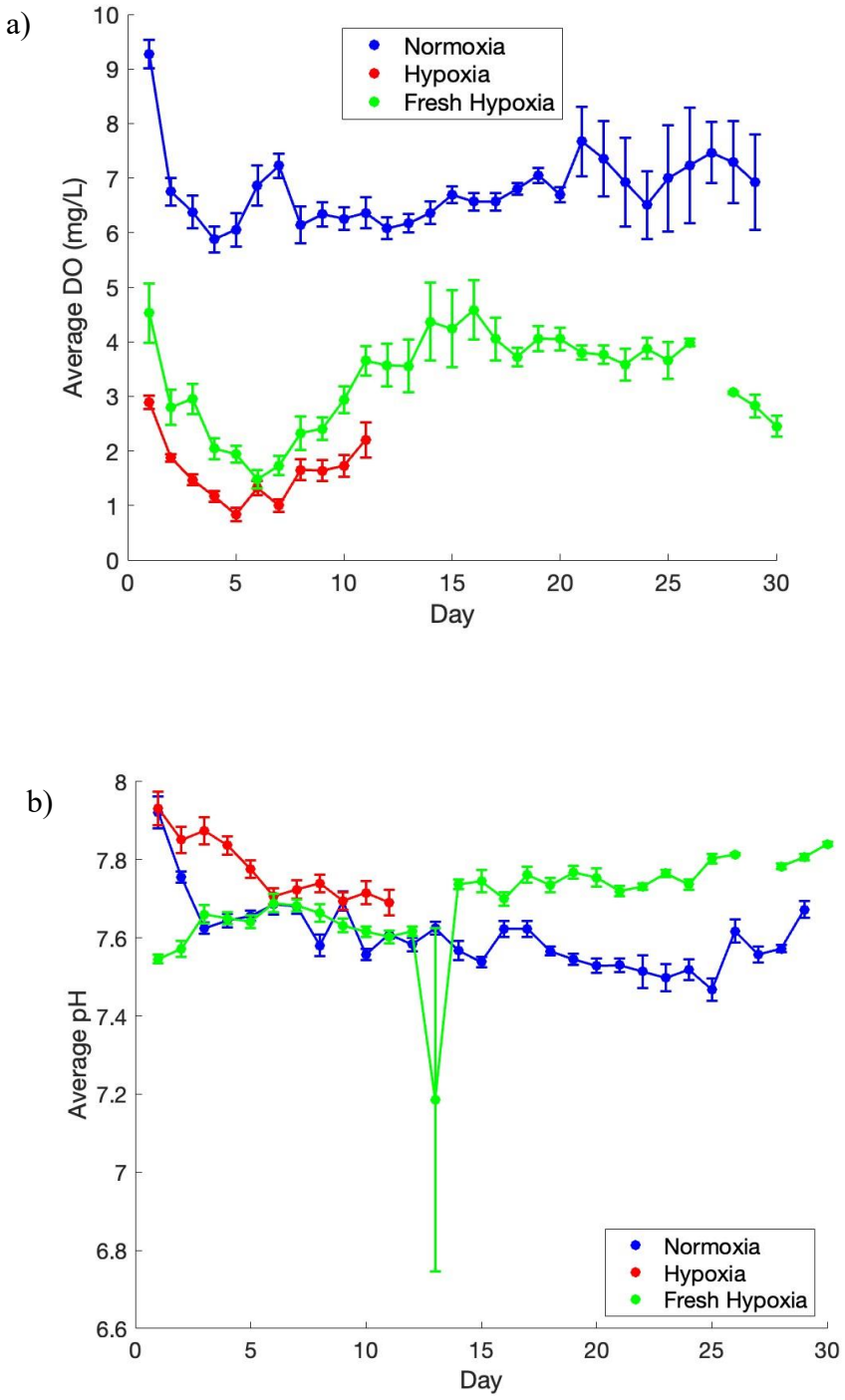


Fig. 2. (a) Average DO (mg/L) for treatment 1 (normoxia), treatment 2 (hypoxia), and treatment 3 (fresh hypoxia). (b) Average pH for all treatments over time. Duration of the normoxic treatment

and the fresh hypoxic treatment was 30 days, while the hypoxic treatment lasted 11 days. Error bars represent standard error (SEM).

4.2 Biomass and Growth Differences

To keep data consistent due to the hypoxic treatment being shorter than the normoxic treatment and fresh hypoxic treatment, day 10 averages for biological parameters were used when comparing metrics. The normoxic treatment experienced a net increase of 0.125 g ± 0.0365 in biomass, while the hypoxic treatment had a net decrease of -0.095 g ± 0.0335 and the fresh hypoxic treatment also saw a decrease of -0.0865 ± 0.0215 (Fig. 3a). The biomass consistently increased for the normoxic treatment and decreased for the hypoxic and fresh hypoxic treatment. The hypoxic treatment saw the biggest decrease in weight compared to normoxic and fresh hypoxic conditions. The Tukey tests determined that the difference in biomass was significant between the normoxic and hypoxic treatments (Table 2).

Size (mm^2) was also significantly different between the three treatments. The difference in size for the normoxic treatment was 133.02 mm^2 ± 34.01, in the hypoxic treatment the difference in size was -33.97 mm^2 ± 31.20, and in the fresh hypoxic the difference in size was -78.26 ± 40.60 (Fig 3b). There is a significant difference between the normoxic and hypoxic treatments, as well as normoxic and fresh hypoxic treatments (Table 2).

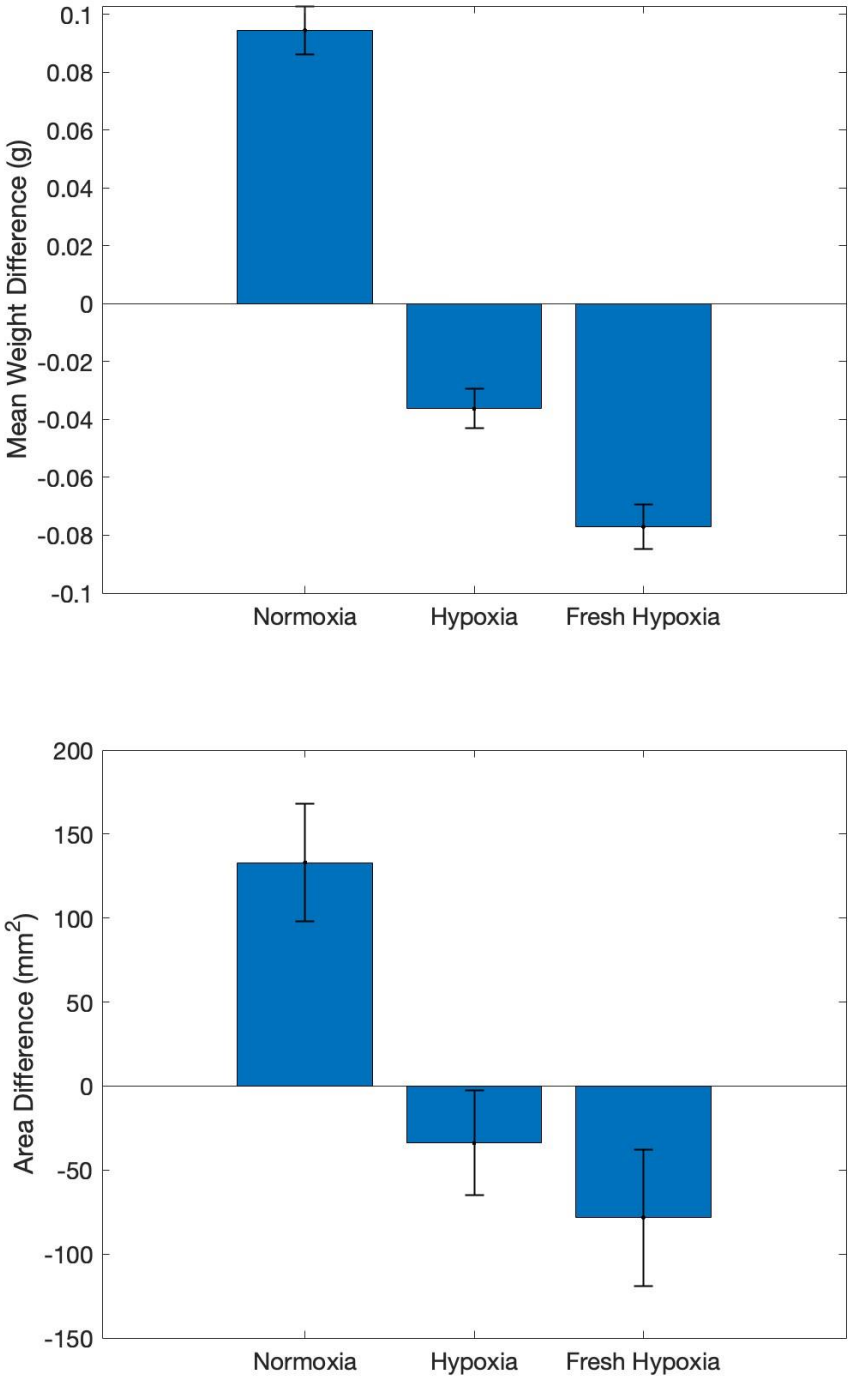
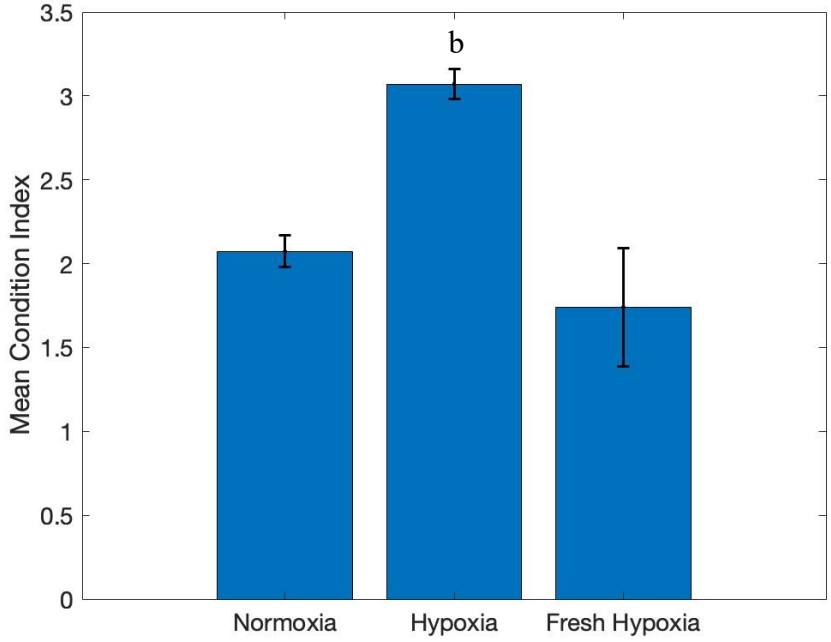


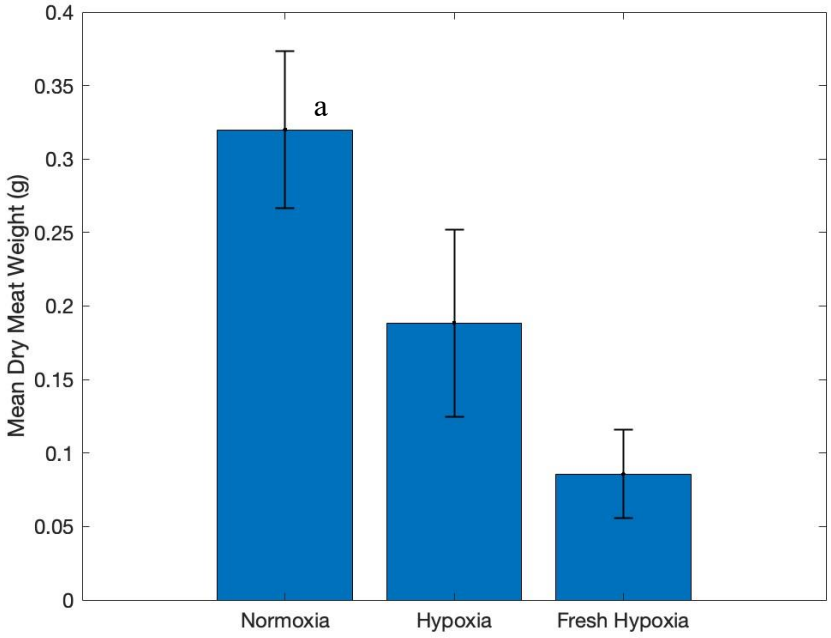
Fig. 3. (a) Difference in initial and final wet weight (g) for normoxic, hypoxic, and fresh hypoxic treatments on day 10. (b) Difference in size (area in mm^2) for all treatments on day 10.

Treatments with the same letter (ex. a & a) are not significantly different, while treatments with different letters (ex. b & c) are significantly different.

4.3 Dry Shell Weight, Dry Meat Weight, and Condition Index Differences

Condition index (CI) was 2.07 ± 0.10, 3.11 ± 0.09 and 1.44 ± 0.35 for the normoxic, hypoxic and fresh hypoxic treatment respectively (Fig 4a). For mean dry meat weight, the normoxic treatment averaged at 0.32 ± 0.11. In comparison, hypoxic dry meat weight averaged at 0.19 ± 0.13 and fresh hypoxic dry meat weight averaged at 0.086 ± 0.030. This was the only variable in the treatments where a significant difference was not present ($p > 0.05$, Fig. 4b). On the other hand, dry shell weight between treatments was significantly different (Table 2). The dry shell weight for the normoxic treatment was 15.46 ± 2.38, which was significantly higher compared to the other treatments. The hypoxic and fresh hypoxic treatment were lower with averages of 6.05 ± 2.09 and 5.95 ± 2.61 respectively, and did differ significantly from one another (Fig. 4c, Table 2).


Mean Condition Index
3.5
3
2.5
2
1.5
1
0.5
0
b
Normoxia
Hypoxia
Fresh Hypoxia


Mean Dry Meat Weight (g)
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
a
Normoxia
Hypoxia
Fresh Hypoxia

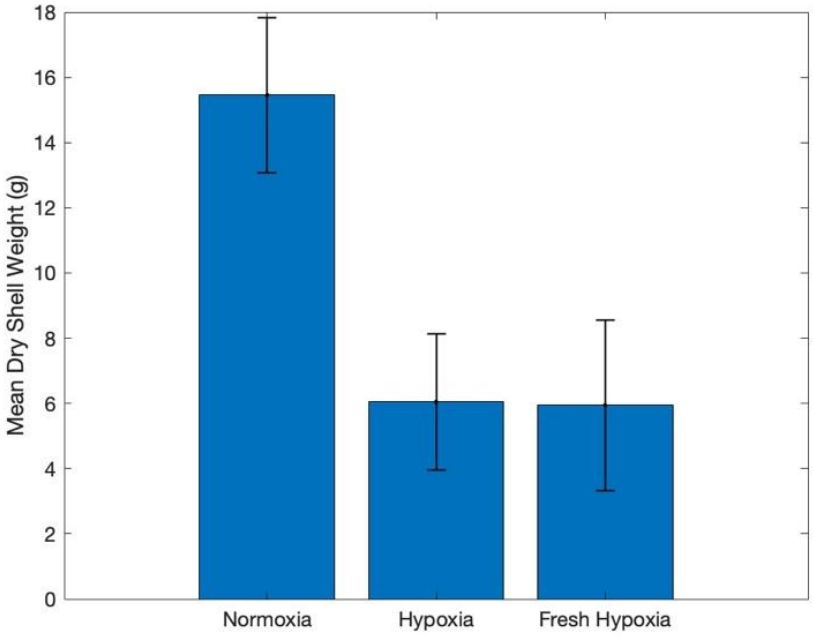


Fig. 4. (a) Condition index comparison for all treatments. (b) Dry meat weight (g) normoxia, hypoxia, and fresh hypoxia for day 10. (c) Dry shell weight (g) for normoxia, hypoxia, and fresh hypoxia treatments for day 10. Treatments with the same letter (ex. a & a) are not significantly different, while treatments with different letters (ex. b & c) are significantly different.

4.4 Mortality Differences

Average mortality was measured up to day 10 in all treatments to keep data consistent due to total mortality in the hypoxic treatment by day 11. By day 10, the normoxic treatment had an average mortality total of 4.75 ± 0.85 oysters per tank, the hypoxic treatment had an average mortality of 9 ± 3.72 oysters per tank, and the fresh hypoxic treatment had an average mortality 6.5 ± 3.97 oysters per tank. There was no significant difference in average mortality when the treatments were compared, but this was likely due to the high variability per tank. Additional information is provided with % survival per treatment for the length of each treatment (Fig. 5b), which shows a complete picture

of survival rate under the three treatments. The hypoxic treatment was stopped at day 11 because the entire Array 5 (4 tanks) saw complete mortality before its scheduled take out day of day 15. The normoxic and fresh hypoxic treatments did not see mass mortalities in full arrays like those observed during the hypoxic treatment.

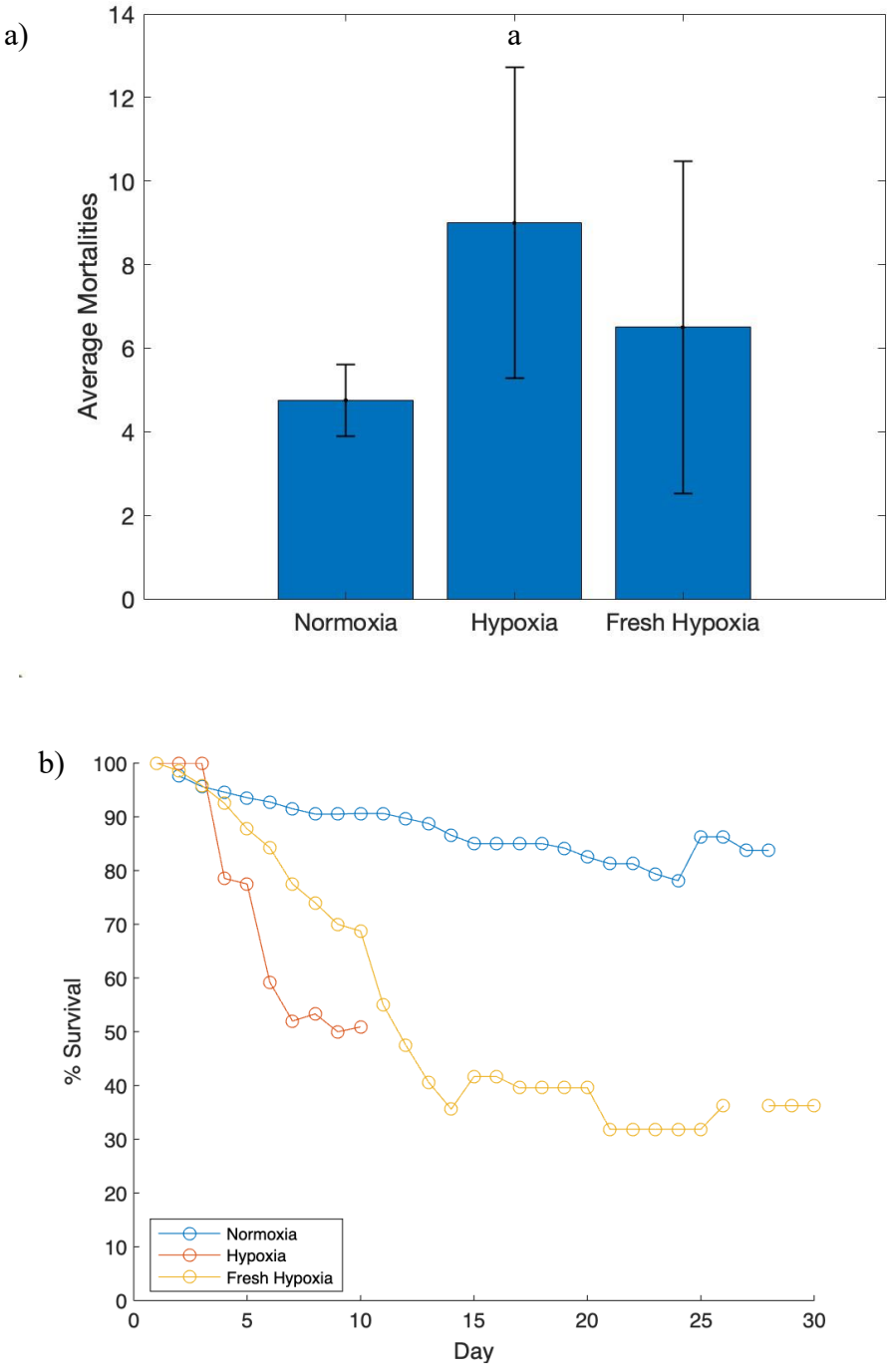


Fig. 5. (a) Average mortalities and standard error from mean (SEM) for Day 10 in normoxia, hypoxia, and fresh hypoxia treatments. Treatments with the same letter (ex. a & a) are

not significantly different, while treatments with different letters (ex. b & c) are significantly different. (b) Percent survival of oysters over time for each treatment.

Table 1. Table of mean ± standard error of mean (SEM) values for all variables in previous graphs. Asterisk (*) signifies ANOVA test found a significant different in variable.

Treatment	Avg DO (mg/L)*	Avg pH*	Avg Weight Diff (g)*	Avg Size Diff (mm^2)*	Avg Mortality*	Dry Meat Weight (g)*	Dry Shell Weight (g)*	Condition Index*
1 (normoxia)	6.71 ± 1.43	7.63 ± 0.13	0.125 ± 0.037	133.02 ± 35.01	4.75 ± 0.85	0.320 ± 0.053	15.46 ± 2.38	2.07 ± 0.10
2 (hypoxia)	1.60 ± 0.89	7.79 ± 0.16	-0.095 ± 0.08	-33.97 ± 31.20	9 ± 3.72	0.189 ± 0.064	6.05 ± 2.09	3.11 ± 0.09
3 (fresh hypoxia)	3.15 ± 0.09	7.65 ± 0.02	-0.087 ± 0.022	-78.26 ± 40.60	6.5 ± 3.97	0.086 ± 0.030	5.95 ± 2.61	1.44 ± 0.35

Table 2. Table of post hoc Tukey test p-values comparing all treatments. T1 = normoxic treatment, T2 = hypoxic treatment, and T3 = fresh hypoxic treatment. Differences are significant where $p < 0.05$.

Tukey tests	Avg DO (mg/L)	Avg pH	Avg Weight Diff (g)	Avg Size Diff (mm^2)	Avg Mortality	Dry Meat Weight (g)	Dry Shell Weight (g)	Condition Index
T1 vs. T2	0.00	0.078	0.00	0.0195	0.622	0.245	$1.52\ E^{-5}$	$1.25\ E^{-11}$
T1 vs. T3	0.00	0.67	0.00	0.0085	0.952	0.0475	$2.51\ E^{-8}$	$2.92\ E^{-4}$
T2 vs. T3	0.00	1.71	0.0452	0.678	0.491	0.449	0.876	$5.48\ E^{-15}$

DISCUSSION

The results of this study provide valuable insights into the impact of varying dissolved oxygen (DO) levels on oyster health and the evidence for shell dissolution due to decreasing pH. The pH results, in conjunction with other parameters, shed light on the complex interactions between environmental conditions and oyster physiology.

The recorded pH values in this study indicate differences between normoxic conditions and hypoxic conditions that while not statistically significant, still affected other results significantly. While fresh hypoxia vs. hypoxia and normoxia vs. fresh hypoxia also did not exhibit much difference from one another, they still corelate appear to have a correlation to DO values of their respective treatments. These differences in pH can be attributed to the contrasting dissolved oxygen (DO) levels in the three treatments. The normoxic treatment maintained an average DO of 6.79 ± 0.089 mg/L, which is characteristic of well-oxygenated environments. In contrast, the hypoxic treatment exhibited severely reduced DO levels with an average of 1.62 ± 0.082 mg/L, consistent with hypoxic conditions. The fresh hypoxic treatment was meant to have hypoxic conditions similar to the hypoxic treatment, but the average DO was higher than what is considered hypoxic; 3.15 ± 0.09 mg/L. This average DO was in the middle of the other two treatments which coincides with the average pH of the fresh hypoxic treatment which is also in the middle of the normoxic treatment and hypoxic treatment.

As ocean temperatures rise, hypoxia and acidification in coastal zones has become a more common occurrence (Rabalais and Turner 2019; Diaz and Rosenberg 2008). The variations in pH observed in response to changes in DO levels provide evidence of the sensitivity of oyster habitats to altered oxygen concentrations. In hypoxic conditions, the

lack of oxygen causes oysters to resort to anaerobic respiration, resulting in lower pH (Coxe et al. 2023). This consistent fluctuation in pH reflects the influence of hypoxia on the carbonate system and its potential consequences for the oysters' calcareous shells.

The decreases in total biomass observed in the hypoxic treatment and the fresh hypoxic treatment are particularly significant and highlight the potential for multi-stressor effects. Oysters in freshwater hypoxic conditions exhibited a notable decrease in biomass compared to the those experiencing hypoxia alone. Since the average DO for the fresh hypoxic treatment ended up being a bit higher than hypoxia (3.15 ± 0.09 mg/L), it could suggest that the combination of freshwater exposure and hypoxia may exacerbate the negative impacts on oyster biomass. Hypoxia alone exhibited a decrease in biomass that was significantly different than both treatments, but not as much of a decrease as seen in fresh hypoxic conditions. This decline in biomass could be associated with reduced energy availability due to hypoxia, but it may also be connected to shell dissolution, as a portion of the oyster's energy and resources may have been diverted to repair and maintain the eroded shells. Similar results were noticed in Gobler et al. 2014, where hypoxia and acidification caused a decrease in growth of juvenile bivalves.

Furthermore, the differences in size between the treatments also point towards the potential impacts of shell dissolution. Oysters in the hypoxic treatment and fresh hypoxic treatment conditions displayed a decrease in size, indicating that their shells may have become more fragile and eroded under these conditions. In a study by Waldbusser et al. (2011), eastern oysters significant shell dissolution was recorded in pH averages as high as 7.67, which is similar to from the values measured in the hypoxic and fresh hypoxic treatments. Hypoxic and fresh hypoxic conditions did not have a significant difference

from one another indicating that the decrease in salinity from 23 to 3 did not have as much of an effect on the oyster's size than the decrease in dissolved oxygen alone did.

To further understand the potential impact of pH on oyster shells, we can examine the results regarding dry shell weight, as well as dry meat weight and condition index. In the hypoxic and fresh hypoxic treatments, the dry shell weight was significantly lower than in the normoxic conditions. This decline in dry shell weight in hypoxic conditions suggests that the oysters in the hypoxic and fresh hypoxic treatments might have experienced shell dissolution and a loss in shell. The higher pH levels observed in the hypoxic treatment could have been due to the dissolution of calcium carbonate, a major component of oyster shells. The dissolution of the shells under lower pH would act as a buffer and cause the pH to increase again. This is why we see a sawtooth pattern in pH measurements throughout the experiment, which portrays the constant decrease and subsequent increase in pH over time. This evidence aligns with the notion that lower pH (acidic conditions) can lead to the erosion of calcium carbonate-based shells.

The higher condition index in the hypoxic treatment compared the normoxic treatment may be a consequence of the oysters in hypoxic conditions losing shell at a more rapid rate than can be produced which is caused by more acidic conditions, while the meat of the oyster was less affected by change in pH. This resulted in a higher meat to shell ratio for the hypoxic oysters resulting in a higher CI than normoxic oysters. This effect was not seen in the freshwater hypoxic treatment, because both shell weight and meat weight were lowered. The combined stress of freshwater conditions and low oxygen appears to hinder oyster growth in fresh hypoxic conditions, extending beyond only shell

dissolution hypothesized for hypoxic conditions. This multi-stressor effect warrants further exploration to better understand its implications.

Mortality differences between the 3 treatments were not significantly different based on comparing average mortality per tank at a time when this measurement was still taken in all three treatments (day 10). There were dramatic differences when analyzing the percent survival per day over all oysters per treatment for the full length of the experiments. Oysters in normoxia exhibit a gradual but small decline in survival rate over time and maintained a higher percentage of survival in comparison to hypoxic and fresh hypoxic conditions (lowest around 80%). However, the hypoxic treatment experienced a steep decline in survival early into the experiment until day 11 when total mortality occurred. This caused a more rapid mortality rate than the other treatments suggesting that hypoxia has a negative impact on oyster survival. The fresh hypoxic treatment shows a similar trend to hypoxic treatment with the difference being the delayed and slower decline in mortality rate in comparison; however, the lowest percent survival of 30% was observed around day 21. The delay in the rapid decline of mortalities could be to the slightly higher DO average in the fresh hypoxic treatment than in the hypoxic treatment. It could be useful to duplicate the fresh hypoxic treatment at a DO closer to the hypoxic treatment to better determine if multi-stressor effects would increase the speed of mortality compared to hypoxia alone.

CONCLUSION

In conclusion, the results presented in this study provide evidence that hypoxic conditions may lead to shell dissolution in oysters. The noticeable differences in both pH

and dry shell weight between treatments suggest a connection between lower pH levels and the degradation of shells. The pH levels were higher during the hypoxic treatment, highlighting a possible relationship between increased acidity and the calcium carbonate oyster shells acting as a buffer.

While shell dissolution is a major component influencing oyster health in hypoxic conditions, it is not the sole factor. Oysters in hypoxic conditions likely exhibit altered behavior, such as keeping their shells closed more often preventing them from eating as often. The loss of biomass is a result of the reduced feeding rates and due to anaerobic respiration. The combination effect of hypoxia reducing growth and pH dissolving shells is likely the cause of reduced and even negative growth during these conditions.

These findings emphasize the importance of monitoring and understanding the impacts of changing environmental conditions, such as hypoxia and decreasing pH, on the health and survival of oyster populations. Further research could involve more detailed biochemical analysis to investigate changes in shell composition, such as quantifying calcium carbonate content. These measurements along with pH would provide more evidence of shell dissolution and provide more context for pH variability during treatments.

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