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Investigating the Response of the Eastern Oyster (Crassostrea virginica) to Low Dissolved Oxygen Conditions

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INVESTIGATING THE RESPONSE OF THE EASTERN OYSTER (*Crassostrea virginica*) TO LOW DISSOLVED OXYGEN CONDITIONS

by

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A Thesis
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ABSTRACT

The Eastern oyster *(Crassostrea virginica)* requires dissolved oxygen (DO) of about 4 mg/L to support proper physiological function. Hypoxia and/or microxia occur when DO decreases to ≤ 2 mg/L. Salinity and temperature changes resulting from large-scale freshwater influx have been identified as key factors influencing the oyster populations in the Mississippi Sound. It is important to investigate the impacts of DO conditions on oyster mortality, biomass, size, and condition during these freshening events. Under controlled laboratory experiments this thesis investigated 1) the response of oysters to microxic, hypoxic, and normoxic conditions 2) the response of oysters to combined hypoxia and low salinity conditions, and 3) the response curve of oysters at various DO concentrations (0.5mg/L, 1mg/L, 2mg/L, 3mg/L, 4mg/L, and 7mg/L) under normal temperature and salinity for 28 days. This study found the highest percent mortality in microxic conditions, with total mortality by day 14. Hypoxia negatively impacts oyster tissue quality and causes a decrease in oyster biomass. There was a significant increase in the change in biomass in the hypoxia treatment while the hypoxia combined with low salinity treatment saw no statistically significant change. This study illustrates the impact of DO concentrations and the combination of hypoxia with low salinity conditions during freshening events on the biology of oysters. The oyster-specific oxygen response curve showed an increase in survival as DO concentrations increased. The oyster response curve will complement an ecosystem model to assist in predicting changes in oyster biomass and mortality during freshwater inflow events in the Mississippi Sound.

ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Kim de Mutsert. It has been a great privilege to be under her selfless guidance. I learned something at every interaction, and this made me grow as a scientist. I also thank Dr. Chet Rakocinski for serving on my thesis committee and for his continuous support. I also appreciate Dr. Reginald Blaylock for being a member of my thesis committee. I would extend my gratitude to the faculty members Drs. Wei Wu, Zach Darnell, and Eric Saillant, all of whom have positively impacted my career. I thank my lab mates for assisting in varying capacities. I would like to show appreciation to Jeremy Johnson, Dr. Kevin Dillon, and Dr. Joe Griffitt for providing the facilities to complete the experiments.

Finally, thank you, Megan Gima, Benji Thames, and the whole Thad Cochran Marine Aquaculture Center for providing oysters for this experiment.

DEDICATION

I would like to dedicate this thesis to my family including my lovely parents, my adorable sister, and my beautiful spouse. Your invaluable support, motivation, and encouragement got me this far.

I am also dedicating this thesis to Maya – may her soul continue to rest in peace.

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LIST OF ILLUSTRATIONS

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
BCS	Bonnet Carré Spillway
CI	Condition index
DO	Dissolved Oxygen
EwE	Ecopath with Ecosim
GCRL	Gulf Coast Research Laboratory
H_2S	Hydrogen sulfide
mg/L	milligram per liter
N	Nitrogen
nGOM	Northern Gulf of Mexico
P	Phosphorus
pH	Potential Hydrogen
TCMAC	Thad Cochran Marine Aquaculture Center
USGS	United States Geological Survey
YSI	Yellow Springs instrument

GENERAL INTRODUCTION

Oysters are an important economic and ecological species that improve water quality benefit by filtering and consuming seston and other particulate material forms in the water column (Grizzle et al., 2008, Zu Ermgassen et al., 2013), thereby reducing the likelihood of algal blooms. Oysters also remove excess nutrients (nitrogen, N) from the coastal waters and limit eutrophication (Newell et al., 2005). Oyster reefs may be the only hard substrate in a predominantly soft-sediment environment (Lenihan 1999, Lenihan and Peterson 1998). This biogenic vertical structure serves as a refuge for dense assemblages of resident invertebrates as well as transient, foraging, and spawning species (Lenihan et al., 2001, Dillon et al., 2015). Oyster beds were once a dominant structural and ecological component of estuaries. Historically, intertidal reefs of the eastern oyster extended for miles, however, these reefs have largely disappeared (Woods et al., 2005).

Several reasons have been cited for the decline of oysters, including diseases, predation related to changes in salinity (Livingston et al., 2000), shortages of oyster clutch (Goulletquer et al., 1994), disturbance (e.g., dredging, boating, storms), natural and introduced predators (Mackenzie 2007), and alterations in freshwater inflow and salinity patterns (Gross and Smyth 1946; MacKenzie 1983, 1996a, 1996b; Rothschild et al., 1994; Kennedy et al., 1996; NOAA 1997; Lenihan and Peterson 1998). Impacts of stock decline and also caused by fishing activities such as the use of hydraulically driven tongs, negatively affect oyster reefs (Hargis and Haven 1995, MacKenzie 1996a, 1997b; Lenihan and Peterson 1998). Extreme water quality conditions resulting from freshening events also can cause a decrease in oyster biomass and death (Wilson et al., 2005).

In shallow coastal waters, which serve as habitat for natural oyster reefs and floating oyster cages or bags for aquaculture, an increase in runoff and water column stratification may create or extend low to no DO zones that result in oyster stress and mortality (Officer et al., 1984; Seliger et al., 1985). Oysters in estuaries are subjected to increasing low-oxygen stress as a result of increased eutrophication of rivers and estuaries caused by fertilizer runoff, animal and human wastes, increased nitrogen deposition, and degradation of riparian wetlands (Paerl 1985, Cooper and Brush 1991, Smith et al., 1992, Dyer and Orth 1994, Nixon 1995) all which increase the duration, frequency, and spatial scale of oxygen-depletion events.

In response to environmental changes during large scale freshwater inflow in coastal habitats, oysters reduce their aerobic respiration and overall growth (Widdows et al.,1979; Shick et al.,1983; Widdows and Shick 1985; Shick et al., 1986). Such freshwater inflow is one of the most influential landscape processes impacting community structure within coastal waters, estuaries, and floodplains (Sklar and Browder 1998). Inflowing freshwater reduces salinity and often contains high levels of sediment which when settled smothers invertebrates displaces fishes and alters aquatic community structure (Zedler and Onuf, 1984).

Nutrient enrichment from freshwater inflow is a major problem in the northern Gulf of Mexico (nGOM) where the coastal ecosystems receive sediment, nutrients, and pollutants from the Mississippi- Atchafalaya River drainages which encompasses 41% of the contiguous United States. The floodwaters from the Mississippi River and tributaries are regulated through river control structures such as the Bonnet Carré spillway (BCS) to

mitigate flooding in New Orleans and regulate water flow into the Mississippi Sound. Discharge from the BCS contributes to high levels of inorganic nutrients and vertical stratification in the Mississippi Sound. The role of freshwater inflow in the determination of coastal productivity and the effect on oyster populations is debated (La Peyre et al., 2009). Extended large-scale release of freshwater into coastal habitats causes a shift in the salinity gradient and water quality of the estuary ecosystem. Increased freshwater influx disrupts water circulation, salinity, and sedimentation and increases the likelihood of disease and parasites in the coastal waters (Shumway 1996). A study by Powell et al. (2003) suggests that freshwater diversion would negatively impact oyster yield due to the changes in water inflow patterns and salinity brought by freshwater diversion.

Efforts to predict organisms' responses to environmental stress in estuaries often focus on the impact of a single stress event and its implications for populations and communities (Kroeker et al., 2010). The inflow of freshwater into coastal waters creates a complex aquatic environment and imposes multiple stressors on marine resources. The stressors may act synergistically through various interconnected processes and interactions of environmental variables such as salinity, temperature, sediment load, turbidity, and so on, which, depending on the quantity and duration of the discharge, have a short- to long-term impact on the ecology of the estuary. Although, depending on their age, oysters are tolerant to broad ranges of environmental variables such as temperature and salinity (Shumway, 1996, Ingle and Dawson 1952, Van Sickle, 1976), complex interactions among multiple environmental stressors can cause high oyster mortality (Loosanoff 1953). Severe water quality changes can cause shifts in the biological

communities within the affected region (Das et al., 2012). Alderdice (1972) and Ponce-Palafox et al. (1997) concluded that changes in multiple environmental parameters critically impacted marine species' health. Similarly, Shumway (1996) maintains that multiple environmental stressors have synergistic effects on all aspects of oyster biology.

For this thesis, laboratory experiments investigated the response of oysters to dissolved oxygen concentrations. In Chapter 1, I investigated the response to microxic, hypoxic, and normoxic conditions under constant temperature and salinity in a 30-day experiment. Additionally, I investigated the survival, change in biomass, size, and condition of oysters to the combined impacts of hypoxia and low salinity. In Chapter 2, I conducted a 28-day laboratory experiment to quantify oyster seed response to six DO concentrations to include in an ecosystem model. In both chapters, the effects of these environmental conditions on oyster survival, biomass, size, and condition were quantified as response variables.

CHAPTER 1 - INVESTIGATING THE RESPONSE OF SEED OYSTERS IN MICROXIC, HYPOXIC, AND NORMOXIC CONDITIONS UNDER BRACKISH AND FRESHWATER CONDITIONS.

1.1 Introduction

Dissolved oxygen (DO) is vital to the health of aquatic ecosystems. Organisms in aquatic ecosystems require sufficient DO concentration for proper functioning, growth, and survival. Butler et al. (1978) documented that hypoxic conditions (~ 2mg/L) cause a slight decline in oyster functioning, reproduction, and physiology. Organisms exposed to hypoxic conditions experience reduced metabolism, oxygen consumption, and energy expenditure (Grieshaber et al., 1994). DO concentrations in aquatic ecosystems are affected by several water quality parameters like temperature and salinity that can limit the concentration available to support life. The introduction of non–point nutrient sources from agriculture, urban waste, and other anthropogenic sources contributes to high levels of nutrients in coastal waters. High nutrient loads in coastal ecosystems together with other environmental factors contribute to excess algal blooms which limits the amount of oxygen available for aquatic organisms. Hypoxia occurs mostly in aquatic ecosystems characterized by high productivity and stratification (Rabalais et al., 2010).

Oxygen depletion also can be caused by variations in water temperature and/or salinity which can lead to stratification. Resulting stratifications act as a barrier that hinders the vertical exchange of oxygen between shallow and deep water. Butler (1952) highlighted low salinity due to largescale freshwater can cause a negative effect on the oyster population. La Peyre et al. (2016) reported that low salinity and elevated temperature in summer reduce the success of oyster recruitment, survival, and growth.

Baker and Mann (1994) suggested both hypoxia and microxia have significantly negative effects on the growth of post-settlement oysters due to decreased or ceased feeding. Caddy (1993) reported hypoxia might reduce coastal production as well as the habitat's quality and quantity. Substantial impacts of these low oxygen conditions can either cause or contribute to the decline of oyster biomass in estuaries. During large scale freshwater release events reduced salinity together with the influx of nutrients might have combined negative effects on oysters. In addition to the low salinity conditions that occur during large freshwater influx, it is important to investigate how DO conditions that could happen during freshening events affect oysters.

1.1.1 Chapter Objectives

To facilitate a better understanding of how oxygen and salinity levels affect oysters, this study replicated the low dissolved oxygen and low salinity conditions that could happen during freshening events in the Mississippi Sound. To achieve this, two laboratory experiments were conducted with a clear set of objectives as follows:

1) Investigate the impacts of microxic, hypoxic, and normoxic conditions on oyster mortality, change in biomass, condition, and oyster size over time.
2) Investigate the combined impacts of hypoxia and low salinity on oyster mortality, change in biomass, condition, and oyster size over time.

To achieve these objectives, this study investigated the response of seed oysters to microxic, hypoxic, and normoxic conditions under optimal temperature and salinity. This was done by evaluating how long seed oysters can withstand hypoxic and microxic conditions as compared to normoxic conditions, as well as how sublethal DO conditions impact oyster biomass, size, and condition. When microxic conditions proved to be lethal

pretty quickly, the additive effects of low salinity were just investigated under hypoxic conditions as the second objective.

1.2 Materials and Methods

1.2.1 Experimental design

Two experiments were performed: The first experiment utilized manipulative laboratory experiments to investigate oysters' response to normoxic (control), hypoxic, and microxic treatments over time. In this experiment, 7mg/L, 2mg/L, and 0.5mg/L represent normoxic, hypoxic, and microxic treatments respectively. Based on average spring data from the United States Geological Survey (USGS), a salinity of 23 and a temperature of 23°C were maintained for all treatments.

I intended to conduct two low salinity and DO treatments to examine their combined effects on oysters; however, I was unable to complete both treatments. For the completed hypoxia combined with low salinity treatment, the salinity of 3, 2mg/L, and 23°C were maintained to replicate hypoxia combined with low salinity conditions (fig 1.4) like those experienced during a prolonged BCS opening in the Mississippi Sound in 2019.

A total of 480 seed oysters between 8 - 23 mm were obtained from the Thad Cochran Marine Aquaculture Center (TCMAC), Deer Island farm site, for use in each treatment. After retrieval from Deer Island, the oysters were thoroughly washed in untreated seawater in the nursery holding tank to get rid of mud and barnacles from the shell surface. The oysters were placed individually in marked petri dishes in twenty-gallon glass chambers running on a flow-through filtration system at the Gulf Coast Research Laboratory (GCRL) Toxicology laboratory. Prior to the start of the experiments, the oysters were acclimated for about two weeks in twenty-four glass chambers (20 oysters in a chamber) filled with artificial seawater, organized in six arrays,

with four chambers in each array. All treatments in this chapter were 30–day experiments. The term “take-out days” refers to times when oysters in an array were completely removed. On take–out days, individual oysters in the four glass chambers (an array) were removed and metrics were recorded using their petri dish numbers.

There were six take-out days at 5-day intervals for each treatment (normoxic treatment, figure 1.1 and hypoxic treatment, figure 1.2), except for cases of total mortality, in which the experiment was discontinued (microxic treatment, figure 1.3). The experiment to investigate the combined effects of hypoxia and low salinity also had six take-out days at 5-day intervals (hypoxia combined with low salinity treatment, figure 1.4). Day 1 of the experiment was the day following acclimation and after the set of desired water conditions (temperature, salinity, and DO) had been achieved.

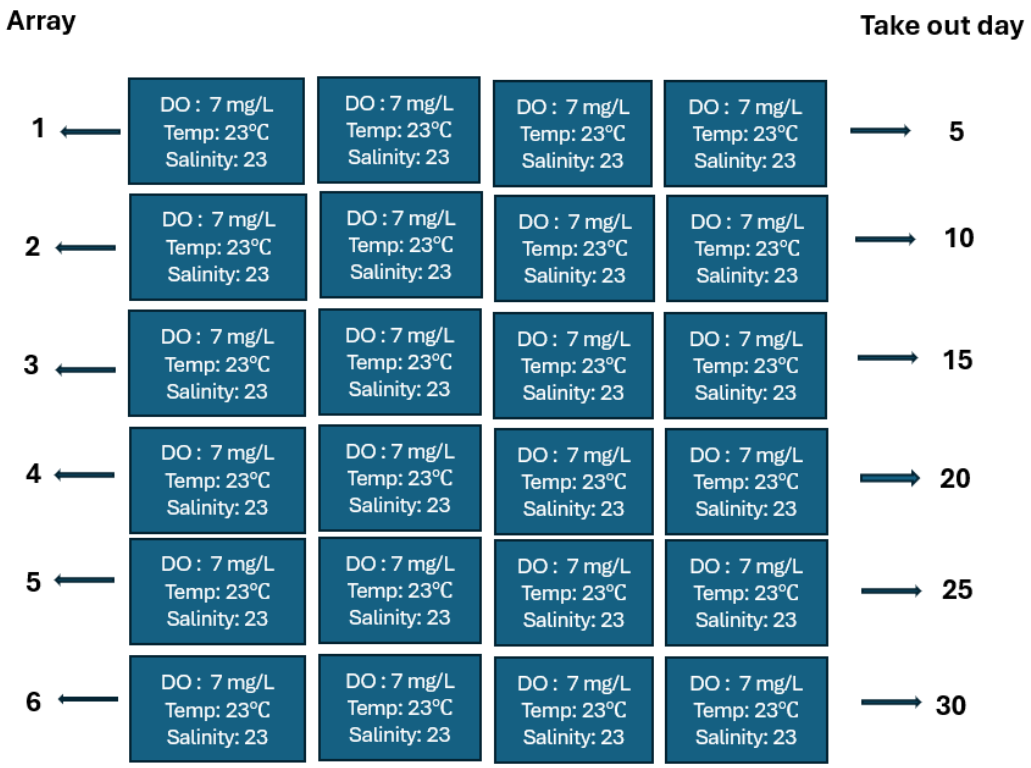

Figure 1.1 *Diagram of the normoxic treatment set-up showing the array, take–out days, and desired water parameters: A square box represents a 20-gallon glass chamber with 20 oysters each (n = 480).*

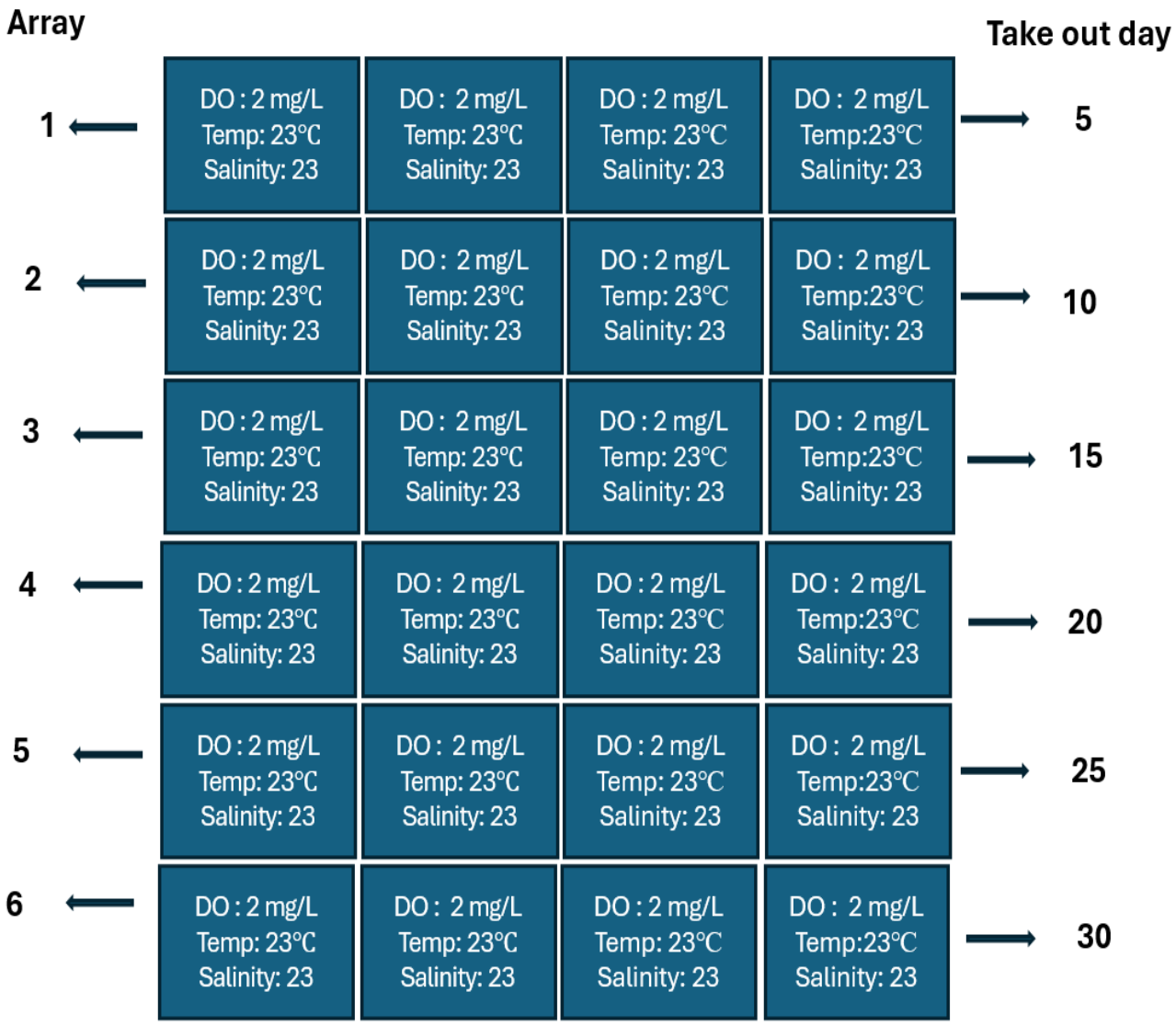

Figure 1.2 *Diagram of the hypoxic treatment set-up showing the array, take–out days, and desired water parameter. A square box represents a 20-gallon glass chamber with 20 oysters each (n = 480).*

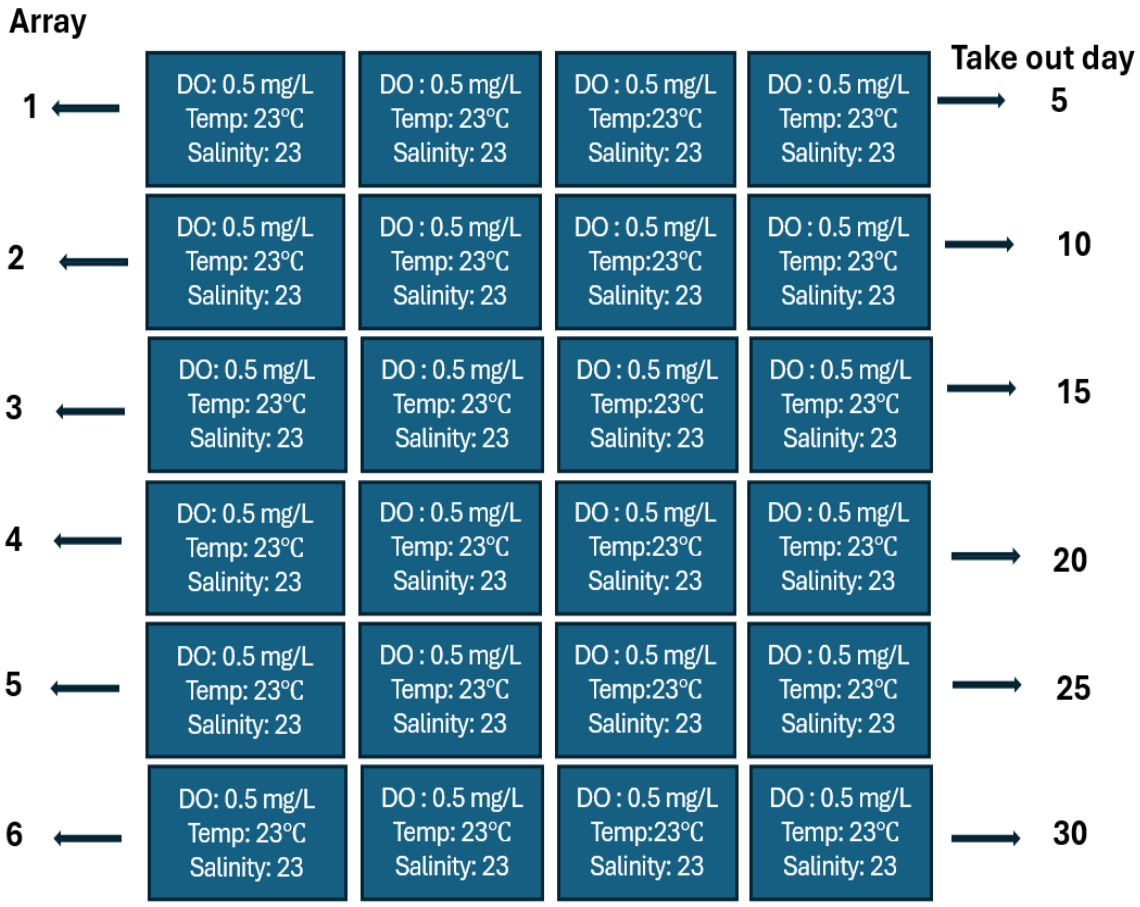

Figure 1.3 *Diagram of the microxic treatment set-up showing the arrays, take–out days, and desired water parameters. A square box represents a 20-gallon glass chamber with 20 oysters each (n = 480).*

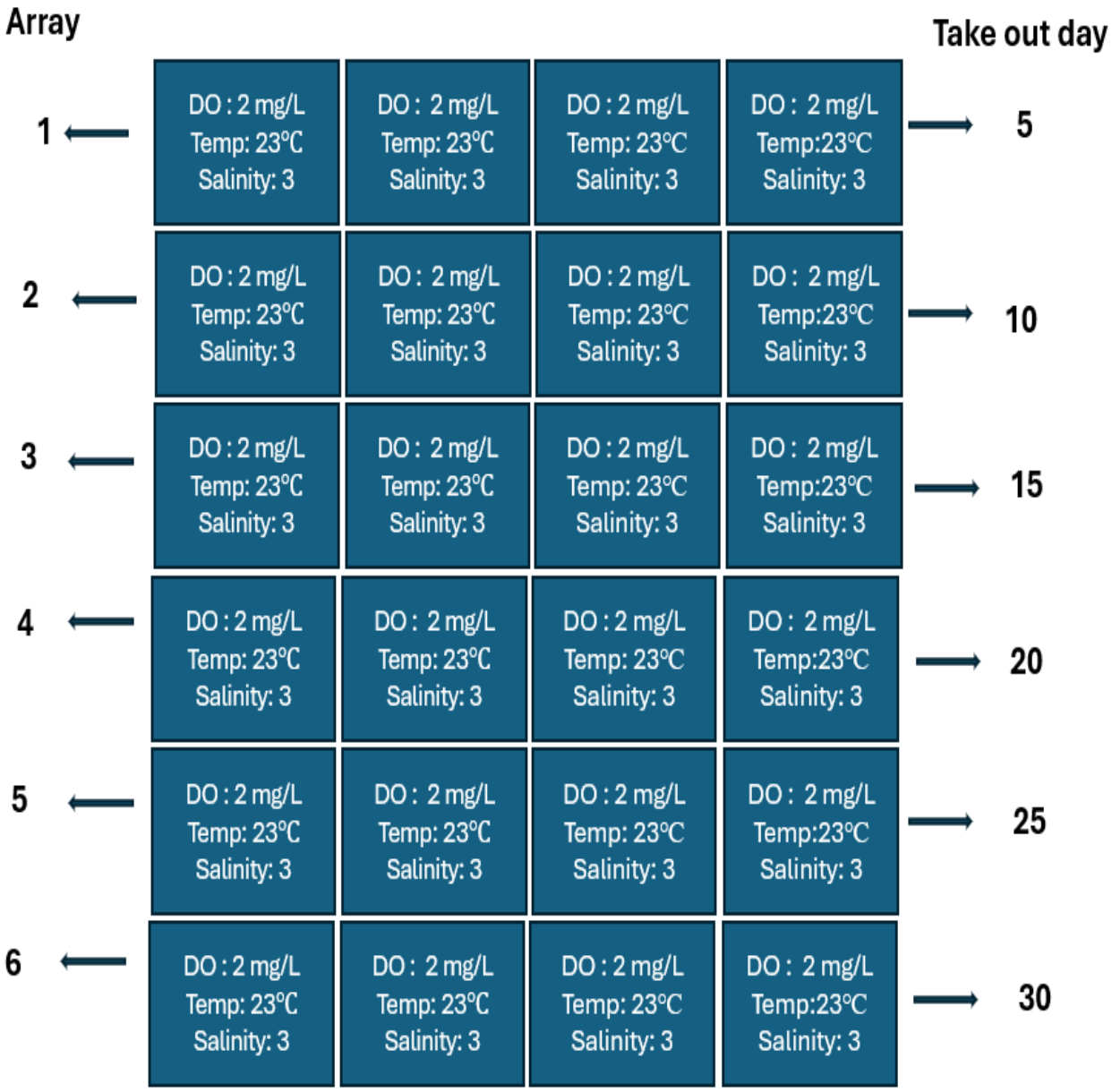

Figure 1.4 *Diagram of the hypoxia combined with low salinity treatment set-up showing the arrays, take–out days, and desired water parameters: A square box represents a 20-gallon glass chamber with 20 oysters each (n = 480).*

On the first and the take-out days, the oysters' shell height and length were recorded in millimeters using an electronic dial caliper. Halves of the total shell length (a) and shell height (b) were calculated and multiplied with a mathematical constant to estimate the size of the oyster (fig. 1.5).

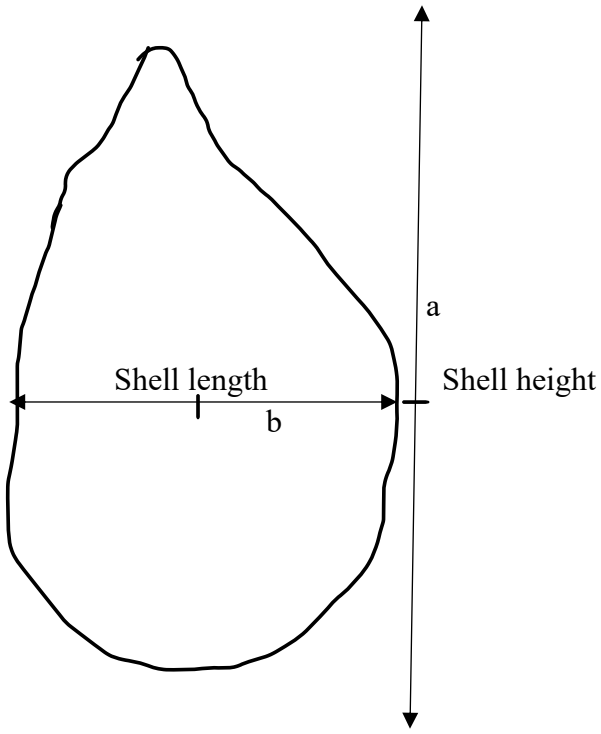

Figure 1.5 *Diagrammatic representation of oyster shell dimension showing shell length and shell height. Letter (a) signifies half of the oyster shell height and letter (b) signifies half of the oyster shell length.*

The size of the oysters at the start and take-out days was calculated with the equation for the area of an ellipse:

$$\text{size of the oysters} = \pi\, ab, \qquad \text{where, } a = \text{half shell height, } b = \text{half shell length},$$

$$\pi = \text{mathematical constant } (3.14)$$

Percent change in the size of the oysters on take-out days was analyzed with the equation:

$$Percent\ change\ in\ size = New\ size - Original\ size / Original\ size * 100$$

Also, for the first day and the take-out days, the oysters were weighed on a scale to the nearest 0.1 g. Change in biomass for the treatments was estimated as the average weight difference between Day 1 and take–out days. I counted the number of oysters with gaped shells in their chambers daily to determine the percent mortality. On each take-out day, oysters from the first and subsequent arrays were collected, and metrics were recorded. Oyster wet meat was freeze-dried for 48 hours, and oyster shells were placed in a laboratory oven at 25°C for 15 minutes to evaporate any water. CI was analyzed with Walne and Mann's (1975) relationship analysis formula:

$$Condition\ index: (Dry\ meat\ weight\ (g))/(Dry\ shell\ weight\ (g)\ ) * 100$$

The oysters were fed Shellfish Diet 1800® (Reed Mariculture, Campbell, CA, USA). Daily feed quantity was determined by multiplying 0.35ml of the feed by the oysters' average start weight in the chambers. Residual feed and dirt were siphoned from the chambers to prevent nitrogenous waste accumulation. Water temperature was controlled by submerging the chambers in water baths. A YSI ProDSS Multiparameter Water Quality Meter® was deployed daily to record water temperature (°C), DO (mg/L and %), and salinity. pH was measured with an EcoSense® pH 10A pen tester.

1.2.2 Statistical analysis

I performed an analysis of variance (ANOVA) in R statistical software (RStudio 2023.12.0+369 "Ocean Storm"). I used Rmisc, tidyr, and agricolae packages for statistical analysis, data manipulation, and other miscellaneous utilities like summarize, plots, and upper and lower bound intervals. Dependent variables for this treatment are mortality, biomass, condition, and size while independent variables are the DO conditions as well as hypoxia combined with low salinity conditions.

One-way ANOVA followed by post hoc Tukey test was conducted to compare microxic, hypoxic, and normoxic treatments for significant differences. I conducted a t-test to compare the means of hypoxia and hypoxia combined with low salinity treatments for significant differences. I used a generalized linear regression model to analyze changes (increase or decrease) over time in each treatment. An alpha level of < 0.05 was considered significant for all analyses.

1.3 Results

The 0.5 mg/L treatment was discontinued after day fourteen due to the total mortality of oysters in all chambers. Surprisingly, quite a few mortalities were recorded in the 7 mg/L treatment, while the 2 mg/L treatment saw the lowest mortality (fig.1.6).

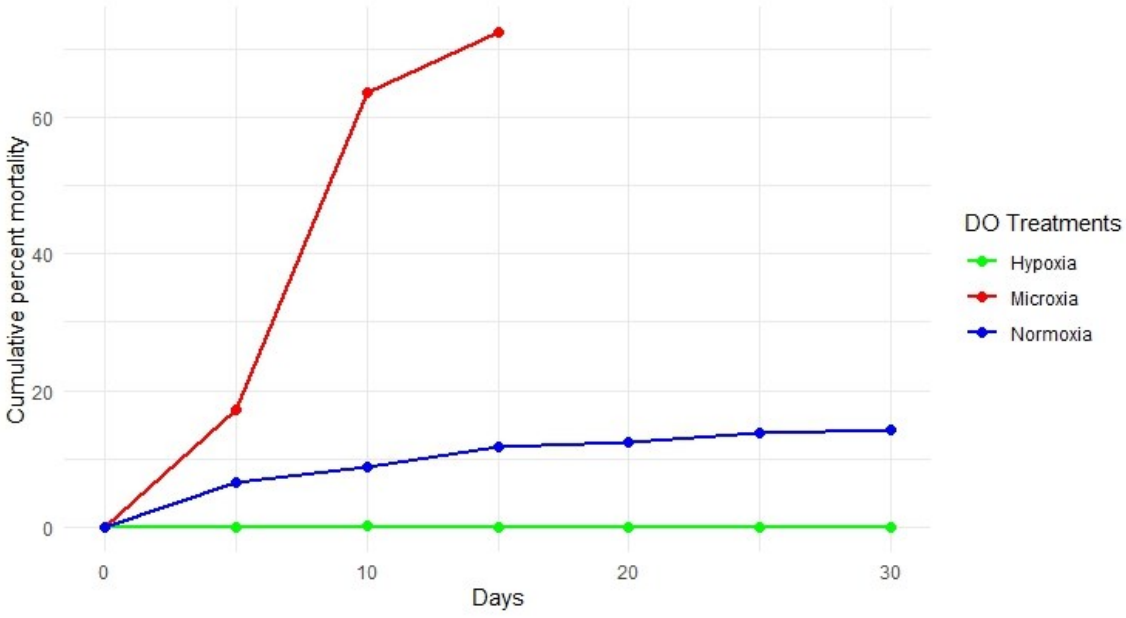

Figure 1.6 *Cumulative percent mortality of oysters in DO treatments.*

There was a significant difference between the change in biomass of the treatments ($p < 0.001$, $F = 23.08$). The Tukey test indicated that the mean change in biomass was significantly higher in 7mg/L than in 0.5mg/L and 2mg/L. There was no significant difference in the mean change in biomass of the 2mg/L and 0.5mg/L treatments. Over time, there was a significant increase in the 2mg/L treatment but no statistically significant changes in the 7mg/L and 0.5mg/L treatments (fig1.7).

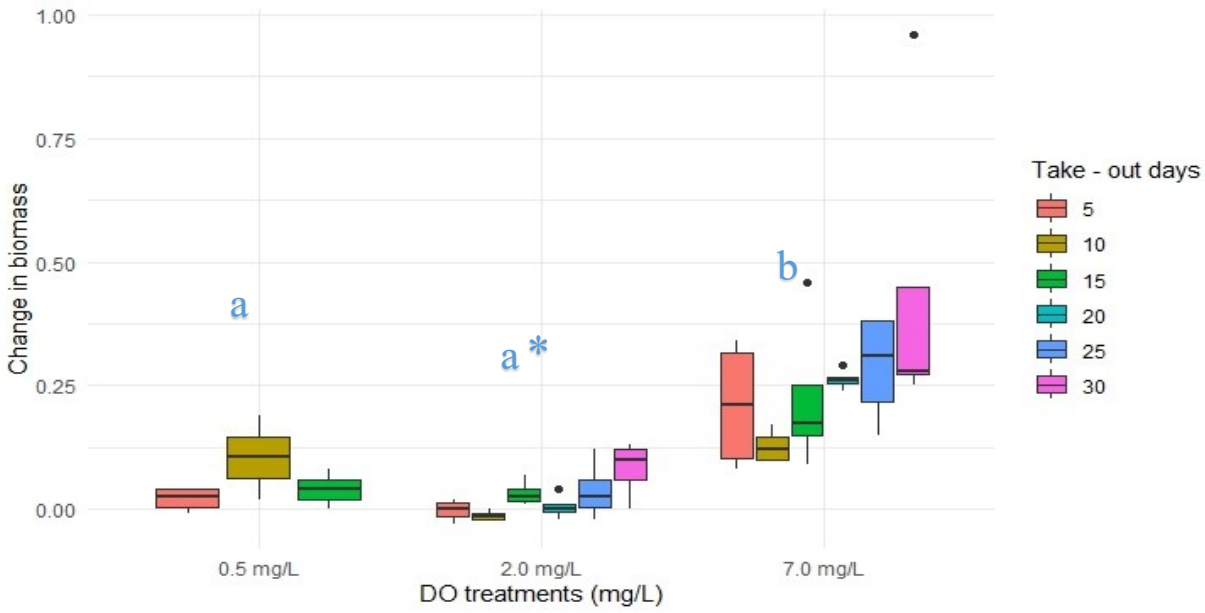

Figure 1.7 *Change in biomass in DO treatments. Significant differences between treatments are indicated with letters. The asterisk (*) denotes treatments with significant change over time.*

There was a significant difference between the CI of the treatments ($p < 0.001$, $F = 94.7$). The Tukey test indicated that the mean CI was significantly higher in 2mg/L than in 0.5mg/L and 7mg/L. Over time, there was a significant decrease in the CI in the 2 mg/L treatment but no statistically significant change in the 0.5mg/L and 7mg/L treatments (fig. 1.8).

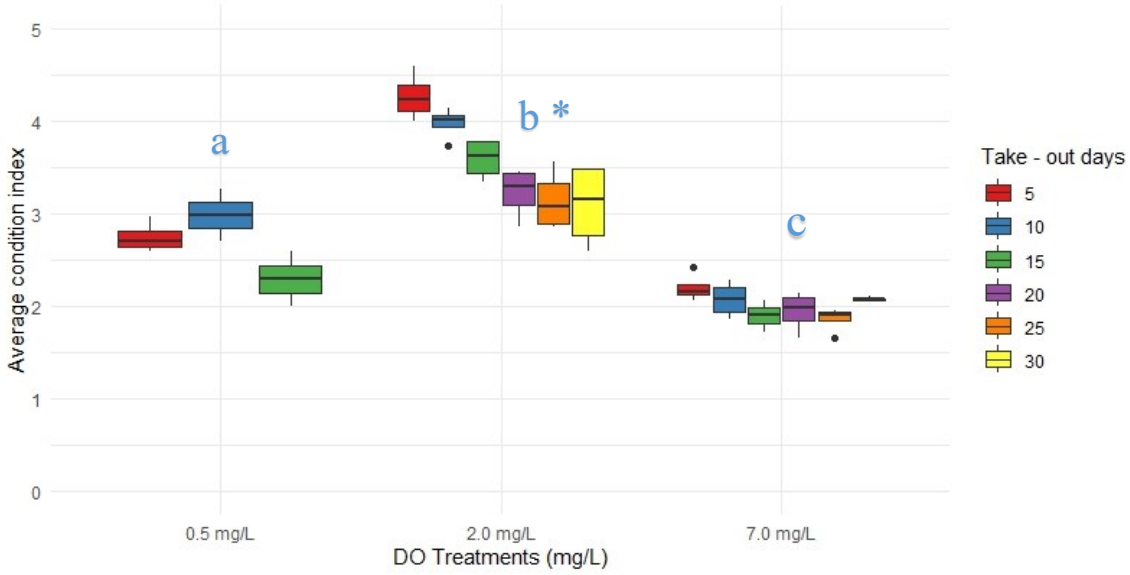

Figure 1.8 *Condition index of the oysters in DO treatments. Significant differences between treatments are indicated with letters. The asterisk (*) denotes treatments with significant change over time.*

There was a significant difference between the oyster size in the treatments ($p < 0.001$, $F = 39.7$). The Tukey test indicated that the mean percent change in size is significantly higher in 7mg/L than in 2mg/L and 0.5mg/L. Over time, there was a significant increase in oyster size in the 7mg/L and 2mg/L treatments and a significant decrease in the 0.5mg/L treatment (fig 1.9).

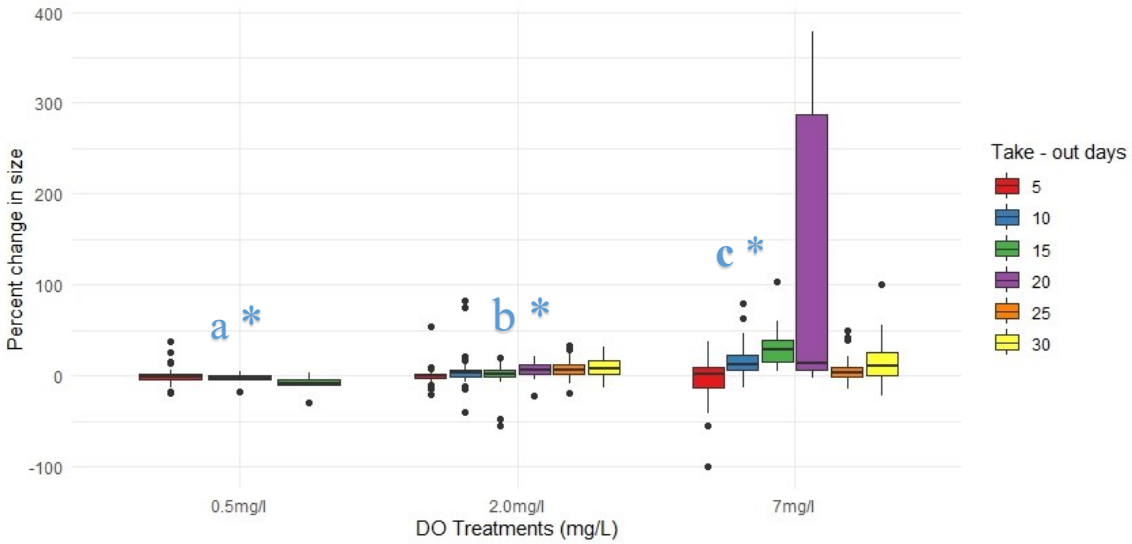

Figure 1.9 *Percent change in the size of the oysters in DO treatments. Significant differences between treatments are indicated with letters. The asterisk (*) denotes treatments with significant change over time.*

Figures 1.10 – 1.13 showed the findings from the experiment that investigated the combined effects of hypoxia and hypoxia combined with low salinity on seed oyster. The cumulative number of mortalities steadily rose until day eleven and stabilized till the end of the experiment. The hypoxic treatment had only very low mortalities compared to the hypoxia combined with low salinity treatment (fig 1.10).

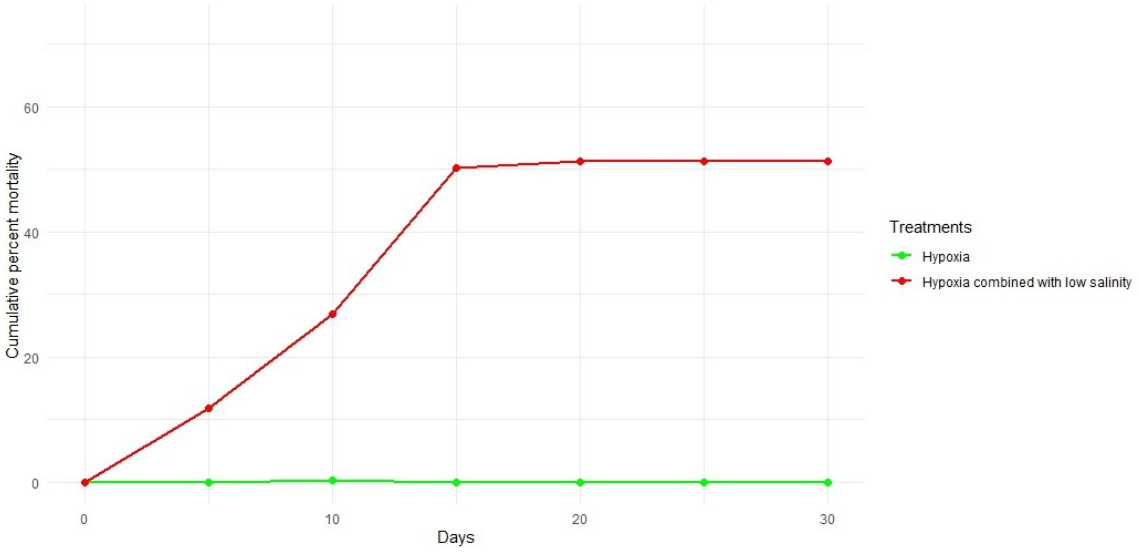

Figure 1.10 *Percent mortality of oysters in the hypoxia and hypoxia combined with low salinity treatments.*

There was no statistically significant difference in change in biomass between the treatments ($p = 0.86$, $t = 0.17$). Over time, there was a significant increase in change in biomass in hypoxia treatment but no statistically significant change in biomass in the hypoxia combined with low salinity treatment (fig 1.11).

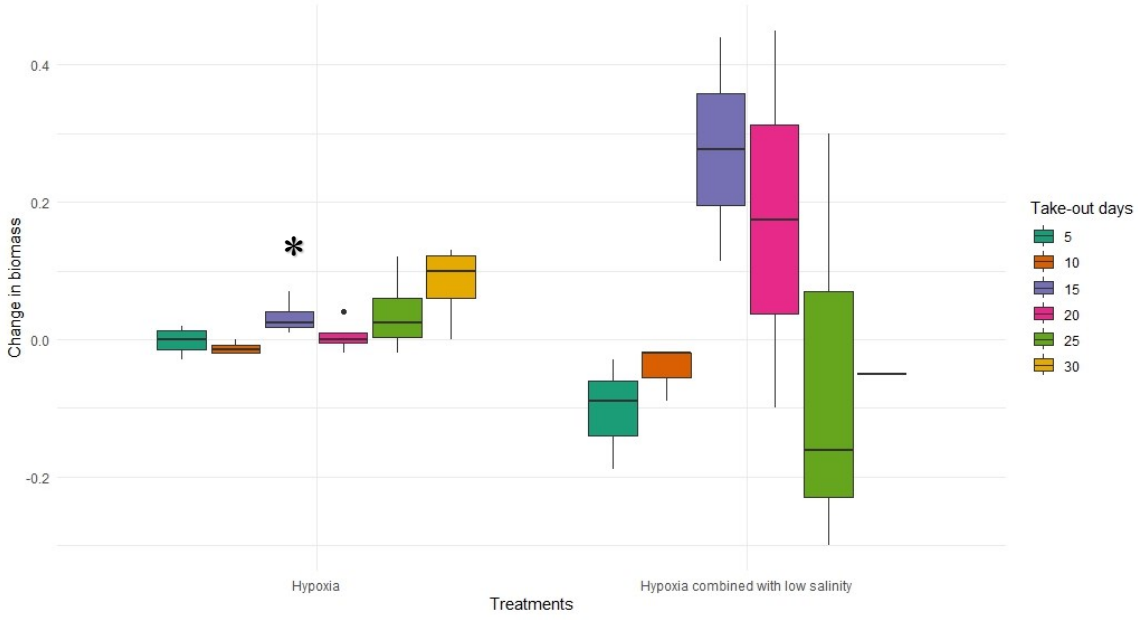

Figure 1.11 *Change in biomass in the hypoxia and hypoxia combined with low salinity treatments. The asterisk (*) denotes treatments with significant change over time.*

There was a significant difference in the CI between the treatments ($p < 0.001$, $t = 13.24$. The hypoxia treatment had a significantly higher CI than the hypoxia combined with low salinity treatment. Over time, there was a significant decrease in the CI in the hypoxia but no statistically significant change in the hypoxia combined with low salinity treatment (fig 1.12).

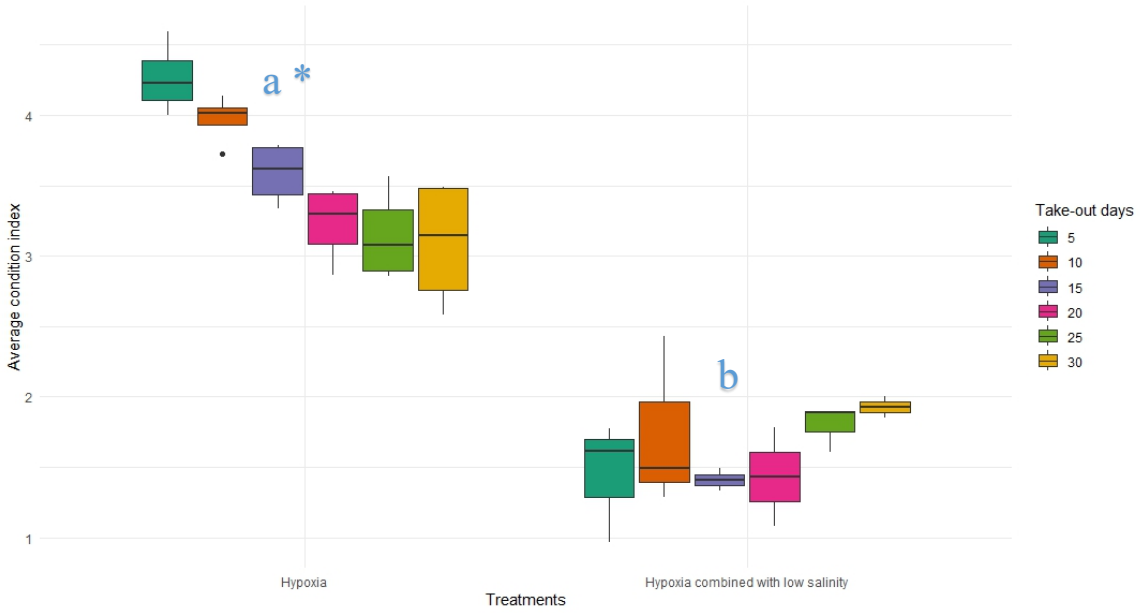

Figure 1.12 *Condition index of the oysters in the hypoxia and hypoxia combined with low salinity treatments. Significant differences between treatments are indicated with a letter. The asterisk (*) denotes treatments with significant change over time.*

There was a significant difference in percent change in size between the hypoxia and hypoxia combined with low salinity treatments ($p < 0.001$, $t = 9.23$). The hypoxia treatment had a significantly higher percent change in size compared to the hypoxia combined with low salinity treatment. Over time, there was a significant increase in size in the hypoxia treatment but no statistically significant change in the hypoxia combined with low salinity treatment (fig 1.13).

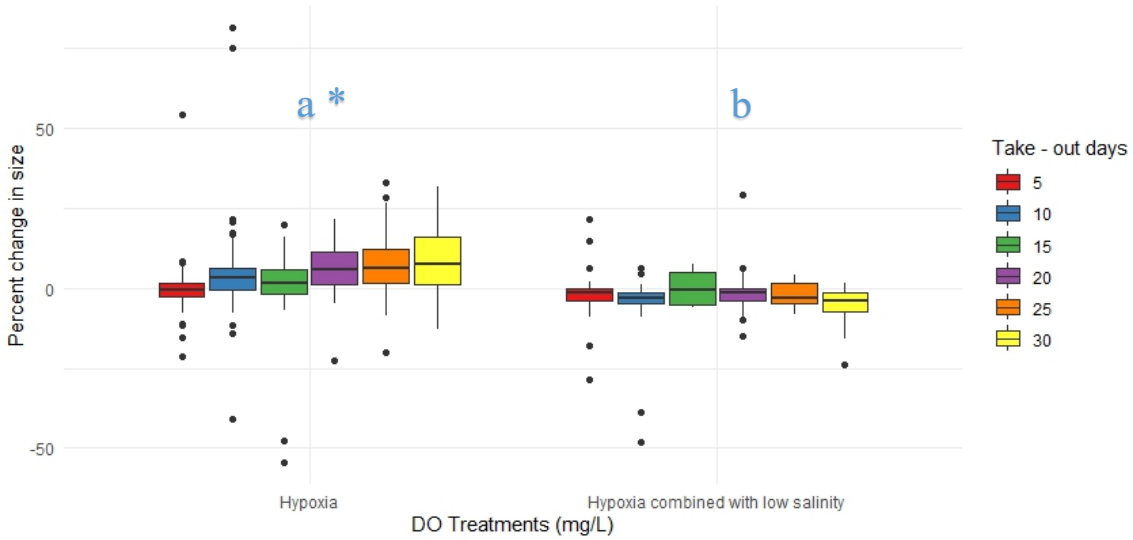

Figure 1.13 *Oyster size in the hypoxia and hypoxia combined with low salinity treatments. Significant differences between treatments are indicated with a letter. The asterisk (*) denotes treatments with significant change over time*

There were physical differences between oyster meat in the normoxic and hypoxic treatments. Oysters in the hypoxic treatment had cloudy and blistered oyster meat along with a bad odor and discolored shells (fig 1.14).

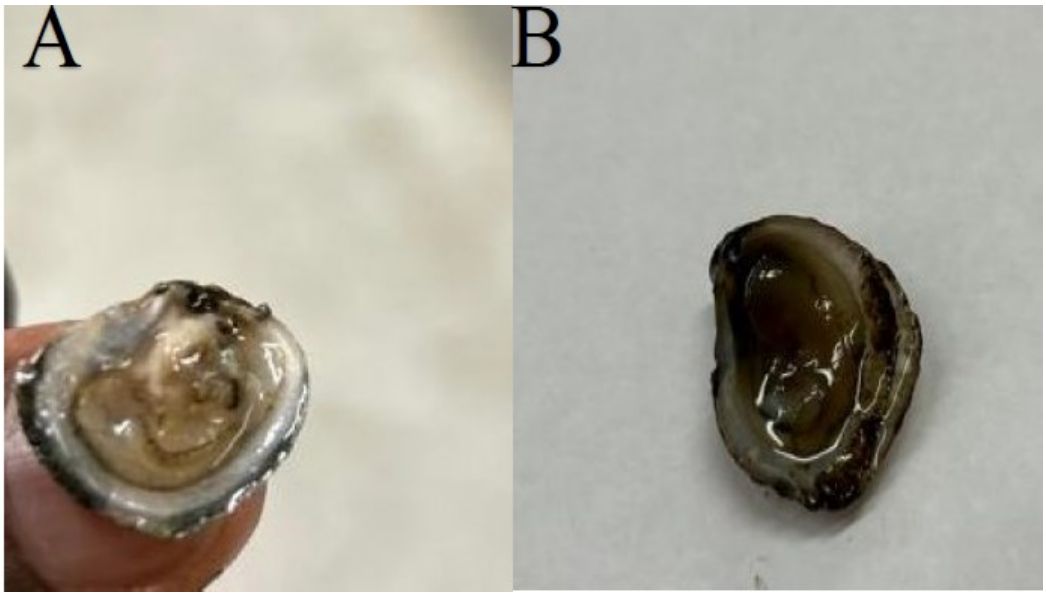

Figure 1.14 *Oyster meat in (A) normoxic and (B) hypoxic treatments.*

1.4 Discussion

The results of this study found that oysters are unable to survive in prolonged microxic conditions (0.5mg/l). Despite the oysters' ability to adjust their oxygen demand, all oysters in the microxic (0.5mg/L) were dead within fourteen days. Oyster tolerance can be determined as the sublethal and/or lethal response to unfavorable water quality conditions. It is important to note oyster tolerance in lethal oxygen deficiency is dependent on age and other environmental parameters such as temperature and salinity. Studies by Sparks et. al. (1958), and NOAA (2007) found lethal effects of microxic conditions on the oysters after five days. Stickle et al. (1989) reported that 50% of adult oysters died at days 5, 19, and 28 at 30℃, 20℃, and 10℃ respectively, while 100% of juvenile oysters were dead in 6 days.

There was a significant difference in the oysters' biomass between the treatments. Over time, only the 2mg/L treatment saw a significant increase. Low mortality in the hypoxic treatment suggests this condition does not cause death, at least over the short term, but may inhibit growth. This finding is consistent with that of Le Moullac et al. (2007), who stated bivalves are adapted to survive periodic hypoxia, the occurrence of which increases in estuaries, bays, and lagoons. Oysters regulate sublethal DO stress through adaptation and adjustment of their energy metabolism. Usuki (1962) found that cilia utilized for feeding ceased movement in low oxygen conditions. The common way bivalves respond to low DO is to only narrowly open their valves, thereby resulting in reduced water, nutrient, and food intake (Newell and Langdon 1996, Frank et al., 2007). Oysters exposed to oxygen deficiencies switch to anaerobic respiration and feed less. Oyster adaptation to poor water conditions inhibits growth (Baker and Mann 1994). Over

time, there was a significant increase in the size of the oysters in the 7mg/L treatment which suggests a suitable oxygen requirement needed to support oyster growth. Before total mortality of the oysters in the microxic treatment was recorded, there was a significant decrease in the size of the oysters in the 0.5mg/L treatment over time, this might suggest a negative impact of extremely low DO conditions on oyster growth even at optimal temperature and salinity. My hypoxic treatment suggests oysters may adjust to sublethal oxygen shortages while other factors such as temperature and salinity stay in the optimal range, however, this might result in decreased energy metabolism and production of oyster meat with a lower market value.

A surprising result in the hypoxic treatment was the cloudy and blistered appearance of oyster meat, sometimes accompanied by a foul smell and discolored shells. A physical blemish on oyster shell and meat could cause a decrease in the market value of shellfish. The quality of an oyster's shell is a defining characteristic, as noted by Mizuta and Wikfors (2019) and low quality shells can prevent sale. The impacts of water quality conditions on oyster meat are understudied, therefore, a further study with more focus on the impacts of oxygen deficiency on oyster meat is suggested.

Another significant aspect of the oyster response to oxygen deficiency is the CI. The ratio of oyster meat to oyster does not yield detailed information concerning amounts of proteins, carbohydrates, and fats, an increase in oyster CI is generally associated with increased glycogen content stored within the tissue of the animal or the reproductive state (Galtsoff 1964). The CI in the 2mg/L treatment saw a significant decrease over time which might be due to the reduced health status of the oysters under this DO deficiency. A possible explanation for the CI decrease might be due to the periodic switch to

prolonged anaerobic metabolism and the inability to take in food and nutrients (Newell and Langdon 1996). The low CI in the 7mg/L treatment might be due to the poor health status of the oyster batch used for the normoxic treatment.

There was no significant difference in change in biomass between hypoxia and hypoxia combined with low salinity treatment while the hypoxia combined with low salinity treatment saw no significant changes over time. La Peyre (2020) reported that oysters exposed to low salinity conditions closed their valves, ceased feeding, and grew less. Response to environmental conditions may be specific to life stages as oyster juveniles' responses to environmental stressors might be different in adult oysters (Pruett et al., 2021). In my experiment, high mortality under hypoxia combined with low salinity treatment suggested that this condition has lethal effects on oysters. This could be attributed to the combined effects of multiple stressors on oysters.

In conclusion, these experiments provided insight into the response of juvenile oysters in different combinations of oxygen and salinity conditions. The mortality rate was highest in the microxic treatment while the highest growth was in the normoxic treatment. Oysters can survive hypoxic conditions but suffer sublethal effects like a decrease in biomass, slowed growth, and decreased tissue quality. Oysters exposed to low dissolved oxygen showed a decline in tissue quality and might fall short of marketable quality in the aquaculture industry. The findings showed that oxygen conditions played a significant role in determining oyster mortality, biomass, condition, and growth. Further investigation of oyster response to DO levels will provide additional information on how varying DO concentrations impact their survival, biomass, size, and condition in the Mississippi Sound.

CHAPTER 2 - INVESTIGATING OYSTER RESPONSE TO DISSOLVED OXYGEN CONCENTRATIONS CAUSED BY FRESHWATER INFLUX AND ITS APPLICATION IN AN ECOSYSTEM MODEL

2.1 Introduction

Coastal wetlands and their water quality are significantly affected by the discharge of nitrogen (N), phosphorus (P), and dissolved organic constituents into the ecosystem. Oxygen levels in coastal waters can become totally deficient through 1) freshwater influx from rivers and flood control structures, 2) stratification, 3) an increase in temperature, and 4) excessive growth of primary producers (Andrews 1982). Disruption of the flow regime in natural landscapes like upstream watershed modifications, and diversion of overland flows influence coastal watersheds (Childers et al., 2006; Koch et al., 2015). Poor water quality negatively impacts essential shallow habitats such as oyster beds and mangroves (Orth et al., 2006; Duarte et al., 2008; Beck et al., 2011; Koch et al., 2015).

The mixing of influxes from flood control structures in coastal waters often leads to variations in water quality. These inflows can form a warm surface epilimnion layer that overlays a cold, and dense hypolimnion. Coastal hypolimnion layers hold little to no oxygen to support the aerobic respiration of aquatic life. Oxygen-deficient layers can be formed either during freshwater influx, summertime, or the presence of nutrient-rich waters in deeper layers. Dissolved organic nutrients (N, P) and sunlight in coastal waters contribute to excessive growth of phytoplankton and buildup of hydrogen sulfide (H_2S) resulting in an anoxic environment (Lampert and Sommer 2007). Organisms in oxygen-deficient environments deal with physiologically perturbed conditions that limit their

growth, biomass, and survival. For instance, high mortality of coastal and estuarine organisms is common in areas with extended physicochemical changes due to extreme flooding during heavy rainfall, freshwater influx (Munroe et al., 2013, Konecny and Harley 2019), elevated temperature, hypoxia, hurricanes, and so on. Depending on the severity and duration of the event, pelagic fishes can migrate, whereas sessile species must switch to anaerobic respiration or otherwise reduce their oxygen demand in response to poor water quality. Additionally, water quality disruptions impact oysters physiological condition over time. Lucas and Beninger (1985) highlighted that condition indices (CI) can measure the effect of environmental factors on oyster shells and tissue and can estimate how these changes impact oysters' fitness, health, and productivity.

The decline of oyster biomass in estuaries is due to a variety of environmental stress factors (La Peyre et al., 2009), with severe variations in DO concentration having a significant role. Increased precipitation and sea level has facilitated the need to re-engineer flood control structures (Houghton et al., 2001, Parkinson and Cavaleri, 2022, IPCC 2007), and this has contributed to an increase in the construction of water management structures to mitigate flooding and restore wetlands (Poff et al., 2007). Human–related activities have significantly altered the quantity and frequency of freshwater inflow into the Gulf of Mexico coastal habitats.

The Bonnet Carré Spillway (BCS) was built as a flood control project to divert the Mississippi River into Lake Pontchartrain to protect the city of New Orleans (Allison et al., 2013) The BCS is usually opened in the spring and early summer. Periodic openings of the BCS affect biological, chemical, and physical conditions of surrounding coastal

waters (Mize and Demcheck, 2009; Lane et al., 2001; Bargu et al., 2011; Roy and White, 2012; Kolic et al., 2014; Adebayo and Amer, 2017; Roy et al., 2017).

Changes in inflow regimes characterized by quantity and timing directly or indirectly affect oyster population in the GoM. These changes impact oysters' growth and oyster settlement to form reefs thereby contributing to the decline of the oyster population in the Mississippi Sound. Since 2005, oyster reefs in Mississippi Sound have been severely degraded by the frequent intrusion of freshwater from the BCS (MDMR 2011). Armstrong et al. (2021) reported that the 2011 prolonged freshwater inflow through the BCS resulted in about 86 percent of oyster mortalities This significant loss resulted in stock depletion and economic hardships in the oyster fisheries industry and its closed sectors. As a consequence, oyster recovery efforts were halted resulting in low landings and economic loss (MDMR 2011a).

Despite significant coastal changes caused by human activities and climate change factors, the estuary along the GOM remains an important source of oyster production (Livingston et al., 1999, La Peyre et al., 2003, La Peyre et al.,2009, Beseres Pollack et al., 2011, McCrea-Strub et al., 2011). There is considerable debate about the use of flood control structures for water management purposes due to their effects on the physico-chemical and biological characteristics of aquatic ecosystems. It is important to investigate species-specific localized responses and identify the threshold and tolerance of these species in the face of freshening events, summer hypoxia, and elevated coastal temperature events. Previous studies by Baker and Mann (1975) investigated the effects of hypoxia on post-settlement oyster feeding and ingestion rate. Their study suggested that post-settlement oysters suffered reduced ingestion rates after exposure to microxic

conditions and this might reduce oyster biomass. Their study used a controlled experiment to investigate the oysters' response to oxygen conditions and found that hypoxic conditions might limit recruitment, growth rate, and survival. La Peyre et al. (2013) used laboratory experiments to investigate how environmental parameters impact the different life stages of oysters. Their study quantifies the response of oysters to extreme changes in environmental parameters. They found that interactive effects of environmental parameters might limit oyster recruitment. Their findings provide a valuable understanding of how extreme events may affect oyster populations and assist in building accurate models, especially in the face of climate change and variations.

Some studies have used Ecopath and Ecosim (EwE) model to predict the impacts of large-scale water influx on marine resources (de Mutsert et al., 2017). The ecosystem modeling software evaluated management practices and policy decisions (Sinnickson et al., 2021). Additionally, simulations in EwE address aquatic ecological questions in different scenarios (Colléter et al., 2013). The time step of the model is one month, and models developed in EwE have been deployed in the past and presently as support tools for decision-makers. de Mutsert et al. (2017) developed species-specific response curves to different environmental parameters and highlighted species tolerance ranges. Species-specific DO response curve is still missing in the model, therefore a controlled laboratory experiment investigating species response to DO concentrations is needed to complement temperature and salinity curves. A month-long oyster laboratory exposure experiment is locally relevant as it gives insight into the response of a species representative of the local environment to DO concentrations and can assist predict how freshening events will impact their populations.

2.1.1 Chapter Objectives

The goal of this study is to update the ecosystem model developed by de Mutsert et al., (2017) with locally relevant findings from this 28-day laboratory experiment which serves as a model time step. The objectives of this study are to;

1) Quantify the oyster percent survival, change in biomass, condition, and oyster size in a gradient of DO concentrations.

2) Create a response curve to update an ecosystem model.

The results from this study will assist in predicting the effects of changes in dissolved oxygen on oyster reefs in the Mississippi Sound. The findings from this study will also provide answers on how DO concentrations affect oyster survival, biomass, size, and CI.

The findings provided much-needed information on oyster-specific responses to environmental factors and will be included in a decision-making tool for policymakers to ensure effective assessment of management plans.

2.2 Materials and Methods

2.2.1 Laboratory Exposure Treatment

Seed oysters (n = 480) from the Thad Cochran Marine Aquaculture (TCMAC) Deer Island oyster farm site were shipped in shaded bags to nursery holding tanks at the Gulf Coast Research Laboratory (GCRL). Once on land, barnacles or overset were removed and mud was sprayed off the oysters. The seed oysters were transferred to experimental chambers at the GCRL Toxicology Unit. Oysters were tagged and randomly selected into the exposure chambers. Six DO concentrations (0.5mg/L, 1mg/L, 2mg/L, 3mg/L, 4mg/L, and7 mg/L) in quadruplicate were maintained at a temperature of 30°C and a salinity of 18 (fig 2.1). The selected DO treatments represent microxic to normoxic conditions. The temperature and salinity values for this exposure experiment were selected based on data from the United States Geological Survey (USGS) average conditions from July to August in the Western Mississippi Sound from 2019 to date.

The selected values replicate constant suitable temperature and salinity conditions in the Mississippi Sound in summer. At the toxicology building, seed oysters (height 7 – 23 mm) were kept in glass chambers under aerated artificial seawater from acclimation till the end of the treatment. Upon arrival, oyster seeds were fed Shellfish diet 1800® (Reed Mariculture, Campbell, GA, USA). Feed ration was determined by multiplying the average weight at the start of the experiment by 0.35ml of the feed. For the exposure treatment, 20 seed oysters were kept in 24 glass chambers of 20-gallon size for 28 days to reflect the time step in the EwE.

A digital YSI ProDSS Multiparameter Water Quality Meter® was deployed daily to monitor temperature, salinity, and DO while an EcoSense® pH 10A pen tester was

used to monitor pH values. The oysters' change in biomass was analyzed as the average weight difference at the start and end of the experiments.

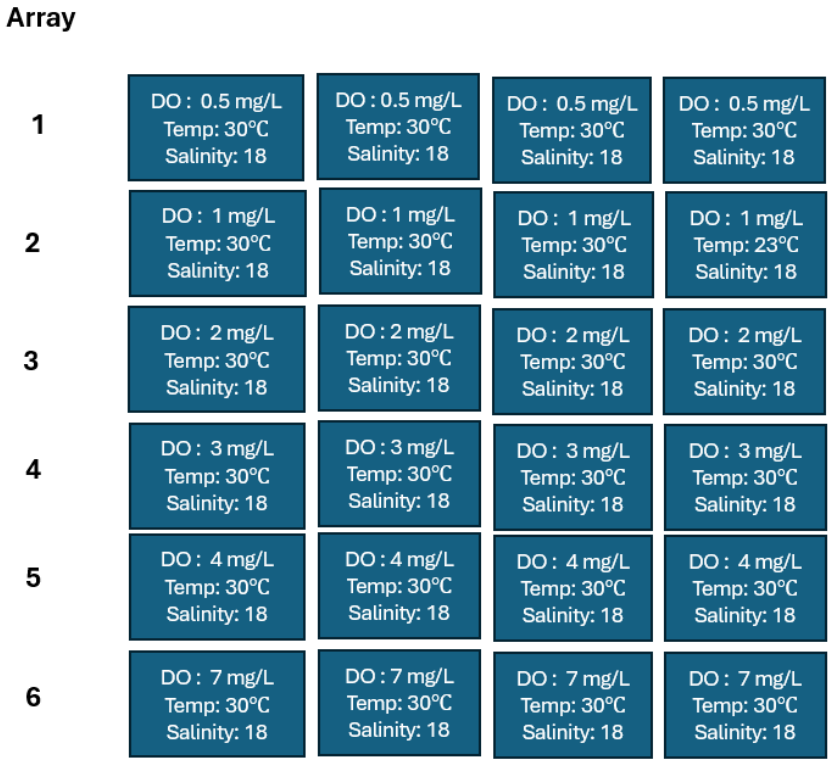

Figure 2.1 *Diagram of the 28-day experiment set-up showing the arrays and varying DO concentration: A square box represents a 20–gallon glass chamber with 20 oysters each (n = 480).*

The size of the size was analyzed with the equation an area of an ellipse:

$$Oyster\ size : \pi ab$$

$$where, \quad a = half\ shell\ height \quad b = half\ shell\ length$$

$$\pi = mathematical\ constant\ (3.14)$$

Percent change in size at the end of the experiment was analyzed with the equation:

$$Percent\ change\ in\ size = New\ size - Original\ size / Original\ size * 100$$

The condition index (CI) was calculated using the arithmetic equation of Walne and Mann (1975):

$$Condition\ index\ (CI):\ (Dry\ meat\ weight\ (g)) / (Dry\ shell\ weight\ (g)\ ) * 100$$

After 28 days, to determine the CI in DO concentrations, oyster meat, and shell in all treatments were pooled separately. Oyster wet meat was freeze-dried for 48 hours while oyster shells were placed in a laboratory oven at 25℃ for 15 minutes to evaporate any water. The total number surviving in DO treatments was calculated as oyster percent survival in treatments. A sigmoid curve was created with percent survival data to inform an ecosystem model.

2.2.2 Statistical analysis

Statistical analysis was performed in R statistical software (RStudio 2023.12.0+369 "Ocean Storm"). At the end of the experiment, Analysis of variance ANOVA followed by the Tukey test was used to compare the means of the DO treatments for significant differences, and a p-value < 0.05 was considered significant. The dependent variables for the experiment are survival, biomass, size, as well as condition while the independent variables are the six DO levels. A sigmoid curve was developed from the percent survival figure. The intercept was forced to zero to create the best fit for the curve. The sigmoid curve created will be used to complement an ecosystem model.

2.3 Results

The 0.5 mg/L treatment had the lowest percent survival with 0% on day seven followed by the 1 mg/L treatment. The 4 mg/L and 7 mg/L treatments respectively had the highest percent survival (fig. 2.2).

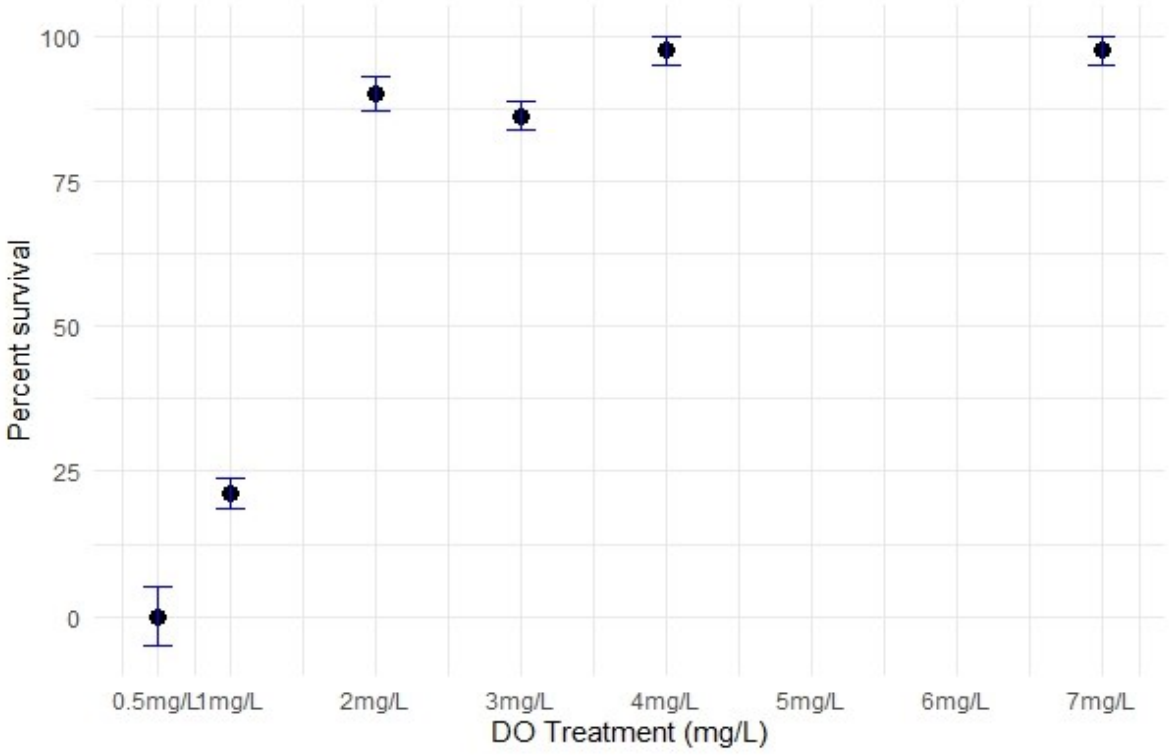

Figure 2.2 *Oyster percent survival curve in DO treatments for the 28-day exposure experiments. Error bars represent standard error.*

There was a significant difference in change in biomass between the treatments ($p < 0.001$, $F = 9.6$). The Tukey test indicated that the change in biomass is significantly higher in 3mg/L, 4mg/L, and 7mg/L than in 2mg/L but no significant change in 1mg/L. There was no change in biomass in the 0.5.mg/L treatment because all the oysters were dead (fig 2.3).

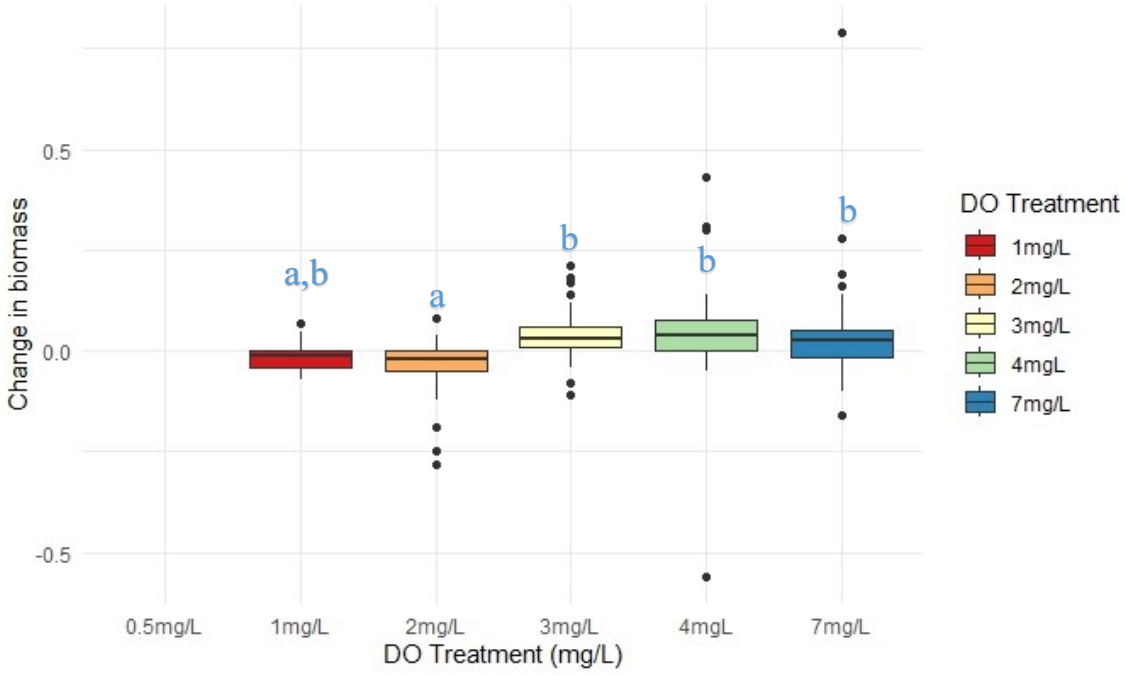

Figure. 2.3 *Change in biomass in DO treatments. Significant differences between treatments are indicated with letters.*

There was no significant difference in the CI between the treatments (p = 041, F = 1.05). There was no CI in the 0.5mg/L treatment due to zero survival (fig 2.4).

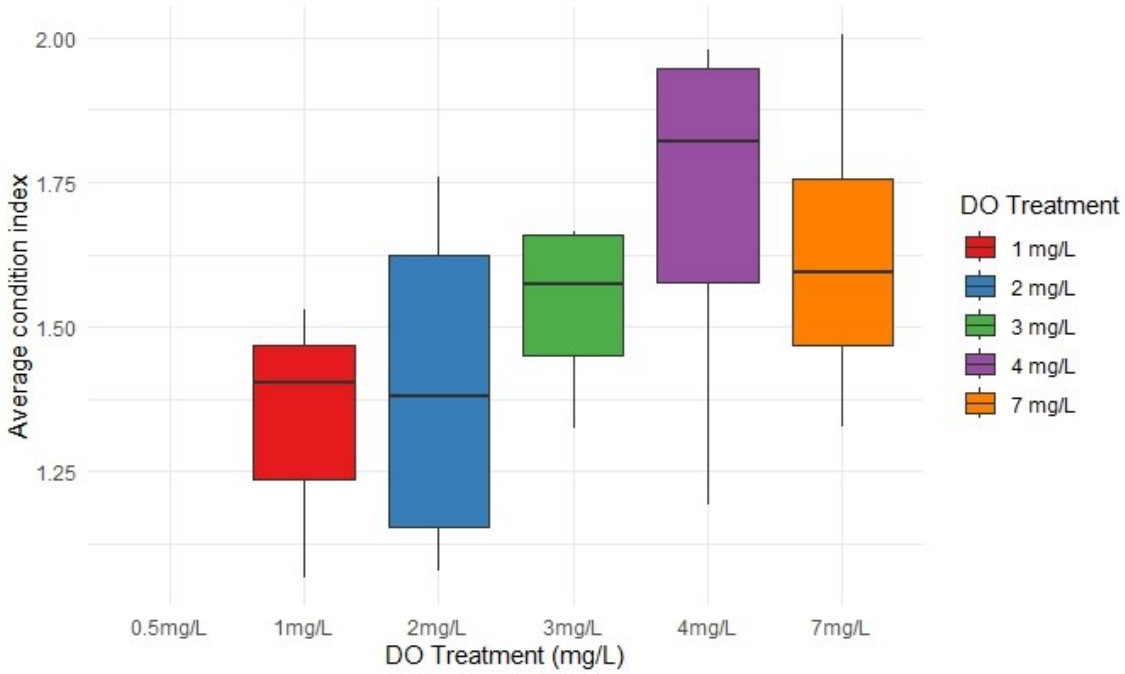

Figure 2.4 *Condition index of the oysters in DO treatments.*

There was no significant difference in percent change in size between the treatments ($p = 0.184$, $F = 1.56$). There was no oyster size measurable in the 0.5 mg/L treatment owing to total mortality (fig 2.5).

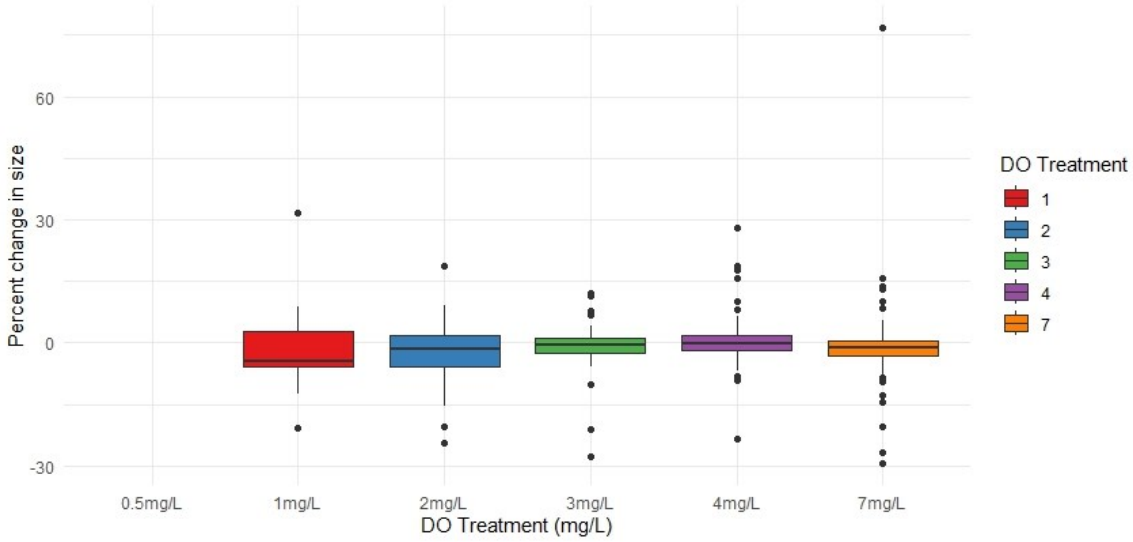

Figure 2.5 Percent change in the size of the oysters in DO treatments.

Survival was selected from the response variables in the experiment to develop a sigmoidal curve to identify oyster DO tolerance and thresholds (fig 2.6). The sigmoidal response curve will complement the EwE model to provide insight into how dissolved oxygen impacts oysters in the Mississippi Sound.

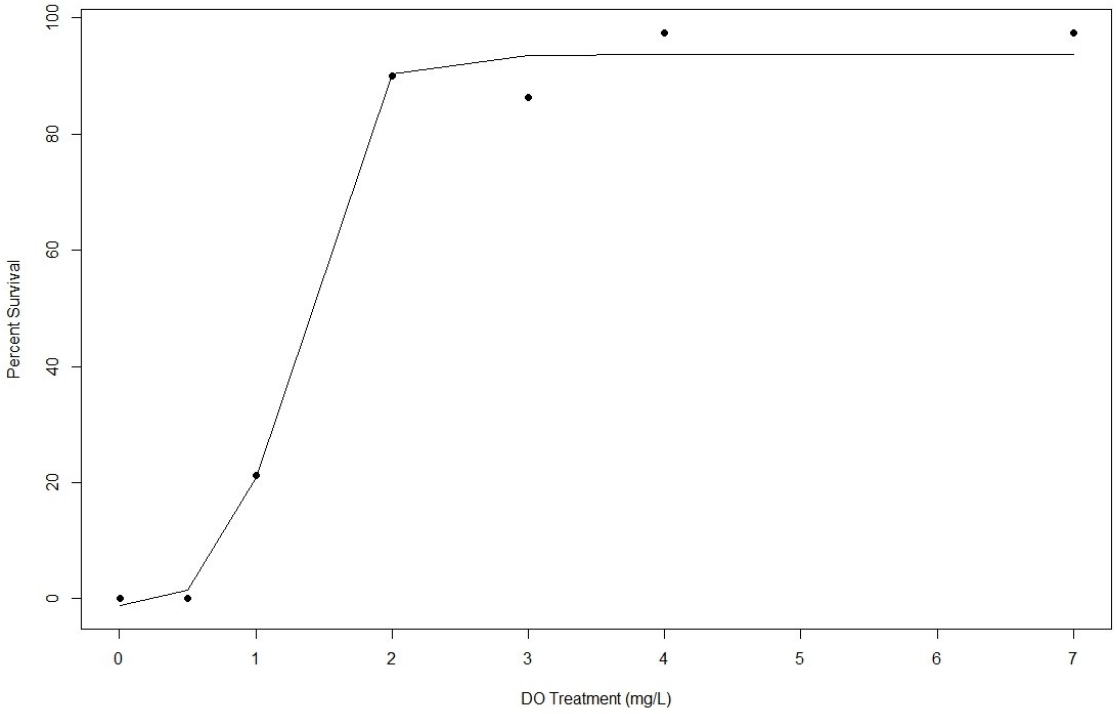

Figure 2.6 *Sigmoidal curve for oyster percent survival in DO treatments for 28-day exposure experiments.*

2.4 Discussion

This study set out to assess the response of seed oysters to a range of DO concentrations at a constant temperature of 30℃ and salinity of 18. The experiment showed the highest percent survival in the 7 mg/L and 4 mg/L DO concentrations at 97.5%. This is consistent with Marshall et al. (2021b) who reported low mortality in oysters exposed to continuously aerated water with constant temperature and salinity. After 28 days, no survival was recorded in 0.5 mg/L, which represents microxic conditions, and low survival in 1 mg/L, and 2 mg/L, which represents hypoxic conditions (11.25% and 67.5% survival respectively). A possible explanation for this is that bivalves are unable to maintain their aerobic metabolism at concentrations below 2 mg/L (Tang and Riisgard 2018) which leads to an increase in mortality after a few days. Sparks et al., (1958) found total mortality at about five days in water containing < 1 mg/L DO, also signifying a lethal effect on the oyster biomass and population. Oysters in DO concentrations of 1 mg/L and 2 mg/L saw a decrease in biomass after 28 days. The 1 mg/L and 2 mg/L concentrations inhibited the lowest biomass respectively. This is similar to findings from Pruett et al. (2021) who reported that an oxygen concentration of about 2 mg/L limits the recovery and resilience of oyster population and restoration. Oysters in 4 mg/L DO saw the highest increase in biomass followed by oysters in 7mg/L concentration, this highlighted an ideal oxygen concentration for biomass increase.

Our experiment showed changes in DO concentrations have a significant effect on oyster growth. The 7 mg/L DO concentration saw the highest increase in oyster growth which can be due to the high metabolic rate and food intake to support body development (Casas et al., 2018). Oysters in the 1 mg/L and 2 mg/L DO concentrations saw a

reduction in growth. Widdows et al. (1989) reported oyster larvae' response to low oxygen conditions was a reduction in feeding and ingestion activity reducing their growth rate. All oysters in the 0.5 mg/L concentration were dead and growth could not be determined. The CI in the 0.5 mg/L concentration could not be determined because all oysters were dead. There was no statistically significant difference in the CI of the treatment and the significant change could not be determined. In the sigmoid curve, the survival was lowest at the 0.5mg/L concentration, a slight increase was observed in the 1mg/L concentration. There was a sharp increase in the 2mg/L concentration. The survival rate slightly decreased at 3mg/L while the survival was highest at 4mg/L and 7mg/L. The curve showed an increase in survival as the DO concentrations increased and a more stable trend at higher DO concentrations.

Long-term changes in weather patterns such as increased flooding, surface runoff, and extreme precipitation will continue to impact oyster reefs in the Mississippi Sound. Such climate change events and increasing urban run-off have resulted in an increase in the construction of flood control structures to mitigate flooding which in turn can cause hypoxic conditions in coastal waters. This thesis investigated one environmental factor (DO), oysters' response to multiple factors may vary in natural reefs relative to other environmental parameters like salinity, temperature, and suspended sediments. These findings will fill an important knowledge gap about oysters' response to hypoxic conditions.

Findings from this study will complement the available salinity and temperature response curve in an ecosystem model created by de Mutsert et al. (2017) by providing information about oysters' response to multiple environmental conditions. Model

simulations will assist in evaluating the response of oysters in freshwater inflow events. The experiments may not fully capture environmental gradients during large scale freshwater influx as climate change intensifies. Further studies should investigate how suspended sediment and a combination of environmental variations which are common during large scale freshwater influx affect the biology of oysters. Further studies could investigate the response of important economic and ecological species to different environmental changes in estuaries. The results from this study will serve as a decision support tool for policymakers to make informed decisions about the operations of the water management structure.

2.5 Conclusion

The main goal of this chapter is to create the needed information to update an ecosystem model with locally relevant information. Oyster responses to a gradient of DO concentrations were investigated through percent survival, changes in oyster biomass, size, and CI. Oyster biomass decreased in the 2mg/L, 1mg/L, and 0.5mg/L concentrations compared to higher DO concentrations. The 0.5mg/L concentration was lethal, as total mortality was recorded after seven days. These results were recorded under controlled laboratory conditions with constant benign temperature and salinity representative of average summer conditions in the Mississippi Sound.

After evaluation of four output metrics, oyster survival was deemed most appropriate to create an oyster-specific response curve for DO. The response curve created from this exposure experiment allows for an improved capability to evaluate the effects of dissolved oxygen reductions on important living marine resources with an ecosystem model.

The ecosystem model simulations will assist policymakers in decision-making processes involved in the operations of coastal restoration projects in the Mississippi Sound, thereby supporting decisions that could result in mitigating the effects of large-scale freshwater influx on important marine resources.

GENERAL CONCLUSIONS

Before this study, oyster-specific response to dissolved oxygen concentrations was understudied. Chapter 1 of this study investigated oyster response to microxic, hypoxic, and normoxic conditions. I identified lethal and sublethal dissolved oxygen conditions for oyster seed. Oysters exposed to a DO concentration of 0.5 mg/L experienced total mortality. The 2 mg/L concentration inhibited oyster seed growth, resulting in reduced CI, and reduced tissue and shell quality. The 7 mg/L concentration saw the highest increase in oyster seed growth. The investigation on oyster response to hypoxia combined with low salinity revealed significantly higher mortality in hypoxic waters with low salinity compared to hypoxic waters alone. This showed the lethal effects of combined multiple stressors on oyster survival. Building on this knowledge, chapter 2 quantifies oyster percent survival, change in biomass, growth, and CI in a gradient of DO concentrations. This experiment showed a significant increase in biomass in 3 mg/L, 4 mg/L, and 7 mg/L concentrations. The sigmoidal curve was created from the percent survival figure at the end of the experiment. The curve showed zero (lowest) percent survival in the 0.5 mg/L concentration, a slight increase in the 1 mg/L concentration followed by a steep increase in percent survival in the 2 mg/L concentration. After 2 mg/L the curve leveled off and stabilized with similar survival in 3mg/L, 4mg/L, and 7 mg/L.

This study fills a knowledge gap about the oysters' response to dissolved oxygen levels thereby identifying lethal and sublethal dissolved oxygen concentrations for the oyster's population. Furthermore, findings from this experiment will update an ecosystem model to assist in running different scenarios of freshwater inflow events in the

Mississippi Sound. The findings will be useful in predicting oysters' response to dissolved oxygen concentrations and combined environmental conditions in EwE and can be deployed as an important decision-making tool for policymakers on the management of water control structures on the Mississippi River. In conclusion, this thesis provided insight into oysters' response to dissolved oxygen concentrations common during freshwater inflow events.

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