Effects of Hypoxia and Ploidy on Mortality of Eastern Oysters (*Crassostrea virginica*)

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Abstract

Hypoxia, defined as waters with a low oxygen concentration (<2 mg L^{-1} DO), is becoming more frequent and widespread in our coastal waters. This is problematic for some of our economically and ecologically important species, such as the eastern oyster, which is largely sessile. Off-bottom oyster culture is vulnerable to these hypoxic events, and the use of triploid and diploid oysters in the industry requires knowing if there are trade-offs in potential tolerance of 2N and 3N oysters to changing water quality conditions. In a controlled laboratory experiment (salinity = 20; temperature = 30^{o}C), cumulative mortality of diploid and triploid oysters was quantified under conditions of constant normoxia (5 mg L^{-1} DO) and hypoxia (1.0 mg L^{-1} DO). After 9 days, regardless of ploidy, oysters exposed to hypoxia had significantly greater cumulative mortality (67.7 ± 6.2%) as compared to those exposed to normoxia (21.3 ± 7.1%; p=0.004). Further research should be done to provide a better understanding of how the oyster industry can address potential effects of more frequent and extended hypoxic events in the future.

Introduction

The eastern oyster, *Crassostrea virginica*, is a foundation species common across the Gulf of Mexico that provides ecological support to its environment and supports an economically valuable production industry (LDWF 2020). Oyster growth, reproduction, and mortality are controlled by environmental variables such as salinity, temperature, and dissolved oxygen availability (Bayne 2017). However, with rising temperatures combined with coastal management in many regions affecting water quality, these environmental variables which critically affect oyster survival are experiencing rapid changes over important oyster areas (Bayne 2017). Maintenance of water quality variables within the oysters' tolerance range is especially important for oysters because oysters are sessile for the majority of their lives and are dependent on the conditions around them for survival.

While many studies have examined growth, reproduction, and mortality of oysters in response to changing salinity and temperature, few have explored oyster tolerance to hypoxia (Virtanen et al. 2019, Vaquer-Sunyer et al. 2008). Increased hypoxia events may have detrimental effects on oysters although only a few studies have specifically examined hypoxia events and oyster mortality (Keppel et al. 2016). The increased incidences of hypoxia are particularly detrimental to the aquaculture industry which is based on raising oysters in cages fixed in space. As temperatures rise, the amount of nutrients and hypoxic events are increasing in Louisiana estuaries and other oyster producing areas, negatively impacting the oyster industry (Levin et al. 2009). Understanding oyster tolerance to hypoxia would greatly inform oyster management, including off-bottom aquaculture (Breitburg et al. 2018).

Development of the off-bottom aquaculture across coastal Louisiana has involved increasing focus on the use of triploid (3N) oysters in addition to their grow-out of diploid (2N) oysters. Triploidy allows for the oyster to utilize energy for growth, rather than reproduction as occurs in diploid oysters, thus increasing growth rates and overall production. However, recent studies have shown higher mortality in triploid oysters compared to diploid (2N) oysters with some hypothesizing that this higher mortality may be a result of triploids stress response, which may make them more susceptible to changing water quality conditions, including increased hypoxic events (Lombardi et al. 2013).

Overall, across Louisiana's coast, oyster mortality from changes in environmental conditions are impacting the natural population and the aquaculture industry (LDWF 2020). In particular, estuaries, such as Calcasieu Lake, which supports one of the more productive oyster areas and is identified as a region to expand off bottom aquaculture (Figure 1), are experiencing such environmental changes from climate change and river management. As coastal areas continue to experience more widespread and frequent hypoxia events, understanding oyster response and tolerance to these changing environmental conditions, including increased frequency and duration of hypoxia, is important in the years to come. This study tested progeny of Calcasieu Lake 2N and 3N oysters to determine the effects of hypoxia on mortality in a salinity and temperature-controlled environment. Further research would provide a better understanding and identification of environmental stressors, such as hypoxia, on oyster mortality and would benefit the oyster producing industry.

Methods

Oyster Stocks

Throughout December 2017 and January 2018, wild adult oysters were collected from the public Louisiana oyster ground located in Calcasieu Lake (CL; 29° 50′ 58″ N, 93° 17′ 1″ W) (Figure 1) (Marshall et al. 2021). The collected oysters were brought from Calcasieu Lake to the Michael C. Voisin Louisiana Sea Grant Oyster Research and Demonstration Farm in Grand Isle, Louisiana (29° 14′ 20″ N, 90° 0′ 11″ W) (Figure 1) where the oysters were conditioned for a period of approximately 8-9 months (Marshall et al. 2021). In the summer of 2019, the oysters were naturally induced to spawn by increasing water temperature. Gametes were collected, and diploid (2N) and triploid (3N) oysters produced. The diploid and triploid oysters were maintained in baskets suspended on adjustable long line system in Grand Isle until used for this experiment. In the summer of 2021, the progeny was collected and transported to the Louisiana State University Animal and Food Science building in Baton Rouge, Louisiana (LSU) to be used to test the effects of dissolved oxygen levels on oysters in a salinity and temperature-controlled environment. In total, 160 oysters were brought to LSU, half of which were diploid oysters, and the other half triploid oysters.

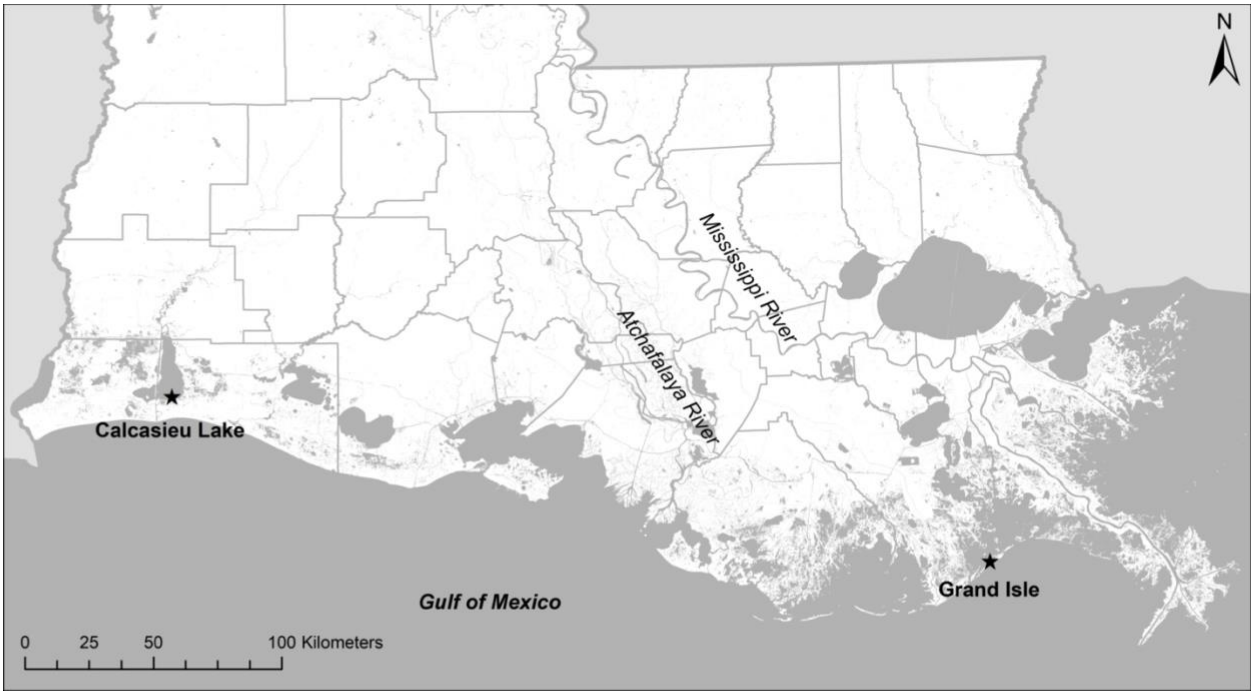

Figure 1: Locations of original oyster collection site (Calcasieu Lake, LA) and site of progeny grow out (Grand Isle, LA).

Experimental Design

Prior to the oysters arriving at LSU, four, 400-L recirculating tanks were prepared for use for the experiments. Tanks were cleaned, rinsed, and filled with water adjusted to a salinity of 20 using artificial seawater (Crystal Sea Marinemix, Marine Enterprises International, Baltimore, Maryland, USA). Four submersible Hygger temperature controller HG-902 heaters were attached to each of the tanks in order to maintain water temperature at 30°C in all the tanks throughout the experiment. One to two air stones were also added to each tank and air flowmeters (Brooks Instruments) allowing the control of the amount of aeration supplied to each tank to control dissolved oxygen (DO, mg L^{-1}) levels within the water. Plastic tubing and air pumps supplied air to the stones for all of the tanks. This design allowed for measurement of cumulative oyster mortality in diploid and triploid oysters, when exposed to normoxia (5 mg L^{-1} DO) or hypoxia (1 mg L^{-1} DO). The experiment was repeated 4 times, but this paper will only go into detail about the second trial.

In June 2021, 160 oysters were brought from Grand Isle, LA to LSU, 80 of which were diploid oysters, and the other 80 were triploid oysters. In the lab, oysters were further divided and placed into trays in each of the tanks, each tray holding 20 diploid and 20 triploid oysters. The oysters were distributed randomly across the four tanks (N=20 oysters per ploidy per tank). The size of the oysters ranged from 67.5 to 115.0 mm shell height. The oysters were acclimated to 30°C for approximately 13 days where they were fed approximately 10 mL of Shellfish Diet 1800 (Reed Mariculture Inc. Campbell, California, USA) every other day and maintained in normoxic conditions. The four tanks were randomly assigned to hypoxia or normoxia treatments.

The two treatments were created using the air stones controlled by flow meters and adjusted to either normoxia (5 mg L^{-1} DO) or hypoxia (1 mg L^{-1} DO).

Environmental Variables

DO, salinity, and temperature were measured twice a day (morning, afternoon) using a YSI PRo2030 probe. Conditions were adjusted as needed to maintain target conditions for DO, salinity and temperature. After acclimation, DO levels were adjusted to target hypoxia conditions (1 mg L^{-1} DO) or maintained at normoxic levels (5 mg L^{-1} DO) to begin the experiment. Both hypoxic tanks reached their target DO levels by Day 2 of the experiment. The number of live and dead oysters from each tank and ploidy was recorded twice daily and dead oysters removed. Cumulative mortality was calculated for each ploidy group within each tank following standard methods (Ragone Calvo et al. 2003).

Statistical analyses

The data was analyzed using R (R Core Team 2020). A two-way analysis of variance (ANOVA) was used to test if ploidy (2N, 3N) and/or treatment (hypoxia, normoxia) affected cumulative oyster mortality. ANOVA was also used to test if there was a significant interaction between treatment and ploidy. Tukey's HSD was used when significant differences ($p < 0.05$) were found.

Results

Cumulative Oyster Mortality

ANOVA indicated a significant effect of treatment (p=0.004) but not ploidy (p=0.105) on mortality, or the interaction of the two factors. Cumulative mortality was higher under hypoxia conditions, with a mean cumulative mortality at 67.2 ± 6.2% after 9 days (Figure 2). Cumulative mortality was lower under normoxia conditions, with a cumulative mortality at 21.3 ± 7.1% after 9 days (Figure 2).

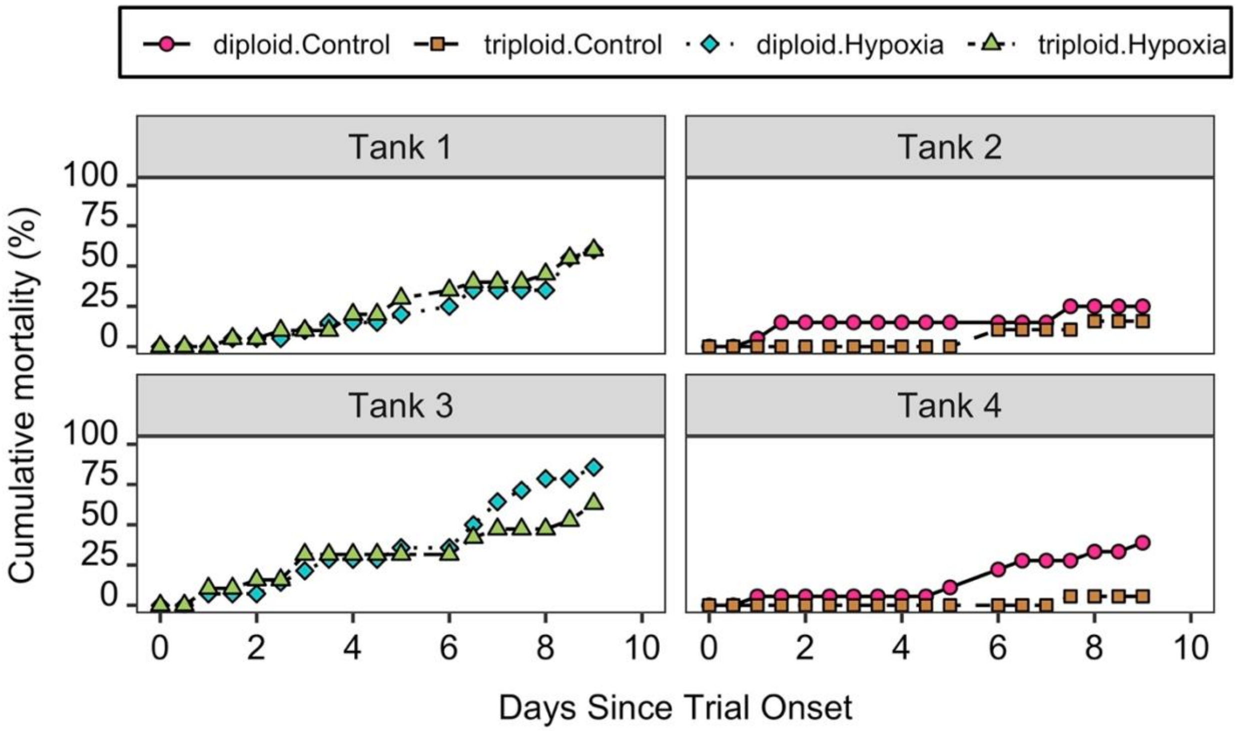

Figure 2: Cumulative mortality (%) of oysters by tank, treatment (normoxia, hypoxia), and ploidy (2N, 3N) throughout the 9-day trial. Each tank contained 20 diploid (2N) oysters and 20 triploid (3N) oysters.

Environmental Variables

Experimental tanks were maintained at target salinity of 20 (range: 20-21.1) and temperature of 30°C (range: 27.4-30.8°C) (Figure 3). On day 3 of the trial the power went out causing the heaters to temporarily shut off. The drop in temperature is noted in Figure 3 and explains the lower range of the temperature range reported. Tanks undergoing hypoxia were maintained at a target dissolved oxygen approximately 1 mg L^{-1} (range: 0.5-1.6 mg L^{-1} DO) (Figure 3). Day 0-1 DO values were excluded from the DO range since the tanks were being adjusted to the target DO. Tanks undergoing normoxia were maintained at a target DO of 5 mg L^{-1} DO (range: 5.2 – 5.8 mg L^{-1} DO) (Figure 3). The pH of the tanks was measured intermittently to ensure the tanks were not accumulating excess amounts of ammonia or nitrate from dying oysters (range: 7.29-8.04) (Figure 3).

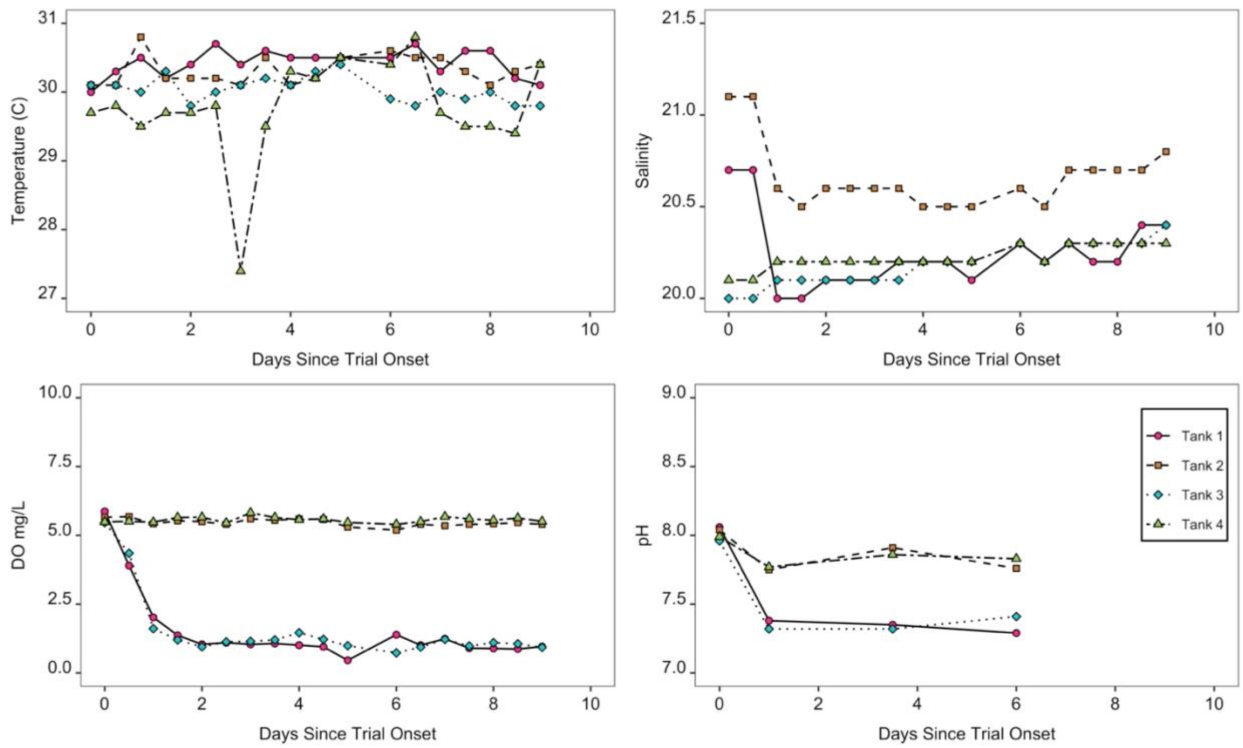

Figure 3: Water quality (temperature (°C), salinity, DO (mg L^{-1}), pH) by replicate tanks throughout the 9-day trial. Oysters in tanks 1 and 3 were exposed to hypoxia conditions while oysters in tanks 2 and 4 were exposed to normoxia conditions.

Discussion

Progeny of Calcasieu Lake 2N and 3N oysters were assessed to determine the effects of low dissolved oxygen levels on cumulative mortality in a salinity and temperature-controlled environment. The most important finding was that hypoxia conditions led to a significantly higher cumulative mortality within a 7-day period, regardless of oyster ploidy. Given the increasing prevalence of hypoxic events across our coastal systems, better understanding of oyster tolerances and thresholds (DO levels, duration) remains critical to inform oyster production and restoration activities. Further, development of broodstock to support triploid production for enhanced growth may benefit by identifying oyster populations more tolerant to variable environmental conditions, or hypoxic events.

The difference in mortality after 9 days between the normoxic and hypoxic conditions indicate limited tolerance to a deficiency of oxygen of both diploid and triploid oysters. Studies show that the median lethal oxygen concentration for a majority of benthic organisms, such as oysters, is an oxygen concentration below 4.59 mg L^{-1} DO (Vaquer-Sunyer et al. 2008). Lower levels of oxygen available means that oysters cannot perform physiologic processes normally, which can ultimately lead to mortality. This explains why the experimental tanks where oysters were subjected to hypoxia resulted in an increase in mortality compared to those subjected to normoxia. Other studies with oysters have demonstrated that even diel-cycling hypoxia (as opposed to constant hypoxia tested here) resulted in reduced growth, although not necessarily mortality (Keppel et al. 2016). The occurrence of hypoxia, its duration, and level may all influence oyster responses.

With recent introduction of new technologies (hatchery production of seeds, selected seedstocks, and improved off-bottom grow-out methods), aquaculture operations are developing around the use of triploid oysters in order to increase their production (Wadsworth et al. 2019). However, studies show unexplainable triploid oyster mortality along the northern Gulf of Mexico coast (nGoM; Wadsworth et al. 2019). Studies suggest triploid oysters potentially have a lower tolerance to extreme environmental stressors, but the results of this study here fails to identify a difference in 2N and 3N mortality when exposed to constant hypoxic conditions. A similar study which tested against different oyster species and ploidies generated similar results, where eastern oyster ploidy had no effect on time to mortality (Lombardi et al. 2013). As aquaculture increasingly depends on triploid oysters to enhance production, this finding indicates that triploids are equally susceptible to extended (>7 days) exposure to hypoxic conditions, suggesting that differences in mortality between triploid and diploid aquaculture are likely due to other factors. Further comparisons of triploid and diploid performance when exposed to adverse conditions would help identify any potential differences related to mortality in triploid and diploid oysters. Alternatively, testing different oyster population’s tolerance of hypoxia may help identify more tolerant phenotypes or genotypes to use as broodstock for future oyster production.

In coastal waters, studies have shown that the dissolved oxygen levels have gradually decreased over time, therefore leading to the widespread occurrence of hypoxia in areas where oysters are heavily present (Vaquer-Sunyer et al. 2008). This decreasing trend of oxygen concentration can be attributed to rising global temperatures due to climate change (Breitburg et al. 2018). Due to the rising trend of insufficient oxygen in coastal areas due to increasing temperatures, populations of not only oysters, but other benthic organisms are expected to face more, “sublethal stress such as reduced growth and reproduction, physiologic stress, forced migration, reduction of suitable habitat, increased vulnerability to predation, and disruption of life cycles,” (Vaquer-Sunyer et al. 2008). Furthermore, studies also show that areas with rising temperatures are expected to be the most susceptible to hypoxia in the future (Virtanen et al. 2019). Since hypoxia already occurs naturally during the late summer months, rising temperatures are assumed to only worsen widespread and lengthen the duration of hypoxic events in the coast and create an inhabitable environment for oysters and other benthic organism in the future (Virtanen et al. 2019).

Calcasieu Lake represents a typical Louisiana estuarine system and is an important oyster growing area. Calcasieu also exemplifies a typical seasonally hypoxic coastal area meaning oysters will encounter hypoxia conditions sometime during maturity. If conditions conductive to hypoxia continue to increase as temperatures continue to rise in future decades, oyster production activities will need to consider different strategies to avoid mortality due to seasonal or episodic hypoxia events, or more coastal work (eutrophication) may be necessary to reduce widespread hypoxia.

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