IMPROVING TOLERANCE TO HYPOXIA IN THE EASTERN OYSTER,

CRASSOSTREA VIRGINICA

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IMPROVING TOLERANCE TO HYPOXIA IN THE EASTERN OYSTER, *CRASSOSTREA VIRGINICA*

Courtney B. Ford

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IMPROVING TOLERANCE TO HYPOXIA IN THE EASTERN OYSTER,

CRASSOSTREA VIRGINICA

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VITA

Courtney B. Ford, daughter of Charles Ford and Rhonda (Gaskin) Ford, was born November 7, 1981, in Fort Belvore, Virginia. She graduated from Grissom High School in Huntsville, Alabama in 1999. She attended Auburn University in Auburn, Alabama, and graduated with a Bachelor of Science degree in Zoology in August, 2003. After working as a Laboratory Technician in Auburn University's Department of Biological Sciences for one semester, she entered Graduate School, Auburn University, in January, 2004.

THESIS ABSTRACT

IMPROVING TOLERANCE TO HYPOXIA IN THE EASTERN OYSTER,

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The loss of oyster reefs is attributed to a variety of causes including over fishing, disease, and periodic hypoxia. Hypoxia, as well as brief periods of anoxia, is a problem in Mobile Bay, in particular. Development of hypoxia-tolerant oysters through selective breeding could assist in oyster reef restoration efforts. Eastern oysters, *Crassostrea virginica,* collected from a suspected hypoxic reef and from a normoxic reef, both within Mobile Bay, were subjected to anoxic conditions (<0.05mg/L) in the laboratory and their mean LT-50s and LT-90s determined. Oysters from the hypoxic reef (LT-50=102.5 h and LT-90=122.0 h) showed significantly greater tolerance to anoxia (α=0.05) than those from the normoxic reef (LT-50 = 94.0 h and

LT-90 = 109.7 h). Survivors from each tested group along with controls which were not subjected to laboratory anoxia, were spawned and the LT-50s and LT-90s of the offspring (mean height 13.6 mm) determined. Mean LT- 50s ranged from 103.0 h (offspring of survivors from the hypoxic reef) to 126.6 h (offspring of survivors from the normoxic reef). Mean LT-90s ranged from 125.0 h (offspring of survivors from the hypoxic reef) to 150.8 h (offspring of survivors from the normoxic reef). The offspring of the survivors from the normoxic reef were found to be significantly more tolerant to hypoxia than the offspring of the survivors from the hypoxic reef. Although the hypoxic reef survivors did not produce the expected increase in mean LT-50 and LT-90 between generations, results from the normoxic reef survivors (LT-50=126.6 h and LT-90=150.8 h) as well as the hypoxic reef controls (LT-50 =124.1 h and LT-90 =143.9 h) suggest future selection and breeding trials.

ACKNOWLEDGEMENTS

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I. INTRODUCTION

The eastern oyster, *Crassostrea virginica*, is widespread in the Gulf of Mexico and along the eastern seaboard of North America. It is an important commercial species throughout its range. Not only does the eastern oyster form reefs which support a variety of economically and ecologically important marine species (Coen et al. 1999), but oysters also help improve water quality by filtering substantial volumes of water (Newell et al. 2002; Ulanowicz and Tuttle 1992). Because of overfishing, disease, and water quality, oyster reefs have been declining and as a result their significance in maintaining healthy estuaries has diminished (Rothschild et al. 1994). Recently it has been found that hypoxia may be preventing the recovery of damaged reefs (Lenihan and Peterson 1998). Hypoxic conditions are continuing to become more widespread and may be affecting the survival of existing oyster reefs and efforts to restore already damaged reefs (Kuo and Nelson 1987).

In Mobile Bay, Alabama in particular, several areas including Bon Secour Bay have historically been affected by low oxygen conditions and have not been harvested since 1967 (May 1973). Reef restortation research at Fish River Reef in Bon Secour Bay documented a total loss of planted oysters and recently set spat due to low oxygen conditions (Saoud et al. 2000). In the northeastern area of the bay, there is recorded evidence of jubilees caused by low oxygen going back two centuries (May 1973). All

together, the affected areas currently make up 39% of the natural reef area of Alabama and have significant spat sets only once a decade (May 1973).

The eastern oyster is the dominant species of Mobile Bay and is of great economic importance to the Alabama coastal community as well as the Gulf Coast community in general. In 2003 the Gulf Coast region alone produced 29.2 million pounds of oyster meats, accounting for 72 percent of the national total (NOAA 2004). Because of its environmental importance throughout its vast range, as well as its economic importance in the Gulf region, studies on how to increase productivity of the eastern oyster in these areas would be very beneficial.

Salinity and Temperature as factors involved in Hypoxia

Estuaries, along with other coastal bodies of water, are prone to hypoxic conditions as a result of variations in physical parameters and stratification (Breitburg 1990; Turner et al. 1987). Salinity stratification along with increased temperatures create the conditions for low dissolved oxygen levels in the bottom waters of estuaries. Since oysters live on the bottom and are sessile they are susceptible to hypoxia and sometimes anoxia (Kennedy et al. 1996). In general, the further dissolved oxygen deviates from saturation values, the greater the stress is on the eastern oyster (Kuo and Neilson 1987).

Stratification in estuaries occurs when river discharge pushes low-density fresh water over higher density salt water, resulting in a top layer that is low in salinity and a bottom layer that is high in salinity (Lenihan and Peterson 1998). Organic materials settling to the bottom are decomposed by bacteria which consume oxygen. Salinity stratification prevents well-oxygenated surface waters from mixing with the bottom

waters, resulting in the bottom layer becoming deficient in oxygen (Lenihan and Peterson 1998). In very shallow estuaries ranging from 3 to 6 meters deep, wind currents are able to homogenize and mix the waters both laterally and longitudinally (May 1973; Schroeder et al. 1992). Although Mobile Bay is considered a shallow estuary, there are areas 20 to 25 m in depth which are formed from natural as well as manmade causes (May 1973; Turner et al. 1987). Since wind currents are unable to mix deep areas, they remain stratified, and act as pockets for low oxygen waters. Under certain conditions, wind stress can flood shallower areas of the bay where oysters reside with deep hypoxic waters (Widdows et al. 1989).

Hypoxia often corresponds with an increase in temperature and is more common in estuaries during the summertime than any other time of the year (May 1973; Widdows et al. 1989). High temperatures reduce the solubility of oxygen in water (Ebbing 1996) and increase the rate of oxygen consumption by bacteria engaged in decomposition of organic matter (Turner et al. 1987). With high temperatures less salinity stratification is needed to induce depleted oxygen levels (Buzzelli et al. 2002; Ebbing 1996).

Effect of Hypoxia on the Eastern Oyster

Low oxygen conditions, which characterize hypoxia, are not the only cause of oyster mortalities but other secondary factors are involved as well. Oyster diseases, such as larval vibriosis, juvenile oyster disease, and "dermo" cause high mortalities in oysters (Kennedy et al. 1996). These diseases are mostly prevalent during the summer when hypoxia is more common (Boyd and Burnett 1999). Hypoxia, combined with its accompanying low pH and high CO_2 levels, increases stress which results in weakening

the immune system. Hypoxia reduces the reactive oxygen intermediate and the production of hemocytes in the blood, which both aid in immunity against diseases and other foreign substances (Boyd and Burnett 1999).

Selective Breeding and Genetics of the Eastern Oyster

Selective breeding involves picking individuals that share a desired phenotype to contribute to the genotypes of the next generation (Allen et al. 1993). Selective breeding of *C. virginica* has been very successful in the past when traits have been used that were both commercially important and "amenable to selection" (Sheridan 1997). Traits that have been selected for include resistance to diseases such as MSX and "dermo", accelerated growth rates, liveweights, shell shape and shell prismatic layer in a few breeds of oysters (Allen et al. 1993; Gaffney et al. 1992; Longwell 1974). Studies on female chromosomes in eastern oysters indicate some crossing over of the genes resulting in the necessary genetic variability which makes selective breeding in the species possible (Longwell 1974).

Commercially favorable traits of *C. virginica* are primarily controlled by either additive or non-additive genes. Additive genes respond to the effects of selective breeding (Longwell 1974). Heritability, which displays the amount of phenotypic variance within a trait that is derived from the effects of additive genes, can be used to determine how much selective breeding actually contributes to the genotype of the resulting population under study (Longwell 1974; Rawson and Hilbish 1990).

Before a particular desirable trait can be selected for in a species under study, there must be natural variation for the trait among individuals in the population that broodstock are gathered from. Most genetic variation studies of the eastern oyster have

focused on variation of allozyme frequencies due to geography. Very little variability has been found at the allozyme level between oyster populations along the Atlantic and Gulf coasts of North America (Hare and Avise 1996; Karl and Avise 1992; Kennedy et al. 1996). In contrast, populations to the extreme north, such as Canada, and to the extreme south, such as Mexico, differ greatly from each other in terms of allelic frequencies (Hoover and Gaffney 2005; Kennedy et al. 1996).

More importantly, studies have been done on the difference in allozyme frequencies between selective breeding populations, such as those raised in a hatchery, and the wild populations that they were derived from. While hatchery production makes it possible to produce genetically superior oysters when compared to their counterparts in the wild, it can also cause a loss of genetic diversity due to inbreeding resulting in negative outcomes (Gaffney et al. 1992; Longwell 1974). It is important to retrieve the progeny of the hatchery larvae from multiple families within the wild population. This reduces the chance of a nonrandom union of gametes and will result in a broodstock that has an allelic frequency quite similar in variation to that of the wild population (Gaffney et al. 1992). Some studies have even found that achieving heterozygosity by breeding different geographically separated populations together results in favorable traits (Mallet and Haley 1983).

Research Objectives

The objectives of this study were to determine if there was a difference in tolerance to hypoxia as measured by time to death under anoxic conditions, between oysters from a potentially hypoxic reef of Mobile Bay and oysters from a normoxic reef of Mobile Bay, and then to see if similar differences could be seen in the first

generation of the survivors and controls after exposure to anoxia. Because oysters have sufficient genetic variability for selection studies dealing with resistance to diseases, accelerated growth rates, liveweights, shell shape and shell prismatic layer, it is possible that tolerance to hypoxic conditions can be improved through selective breeding (Allen et al. 1993; Gaffney et al. 1992; Longwell 1974). Hatchery produced, hypoxia-tolerant oysters could contribute to oyster restoration efforts where periodic low oxygen is a limiting factor.

II. MATERIALS AND METHODS

Phase I

Oysters were collected from 2 sites within Mobile Bay. Three hundred oysters were taken from White House, a reef thought to experience periodic hypoxia, and a total of 300 from Cedar Point, a normoxic reef (Fig. 1). Once brought to the lab, the oysters were separated from each other and cleaned of fouling organisms. The 300 oysters from each site were measured (mean height) with calipers, randomly divided into 150 controls and 150 experimentals, and then randomly divided into 5 exposure groups (48-hour, 72-hour, 96-hour, 120-hour and 144-hour) with 3 replicates of 10 oysters each. The number of hours in each exposure group represented how long that particular group would be kept under anoxic conditions (for the experimental oysters) and under normoxic conditions (for the control oysters). Each replicate of 10 oysters was placed in a mesh bag and labeled according to its site, exposure group, replicate, and whether it was experimental or control.

One of two insulated acclimation tanks (2.4x0.9x0.8 m) was filled with filtered (1µm) seawater (18.6˚C, 22.9ppt and 7.6 mg/l DO) treated with 341 L/min UV lights and aerated with two air stones. Oysters were scrubbed again right before they were placed in the acclimation tank and over the course of one week, acclimated to 28.0˚C by raising the temperature 1.0˚C every 12-hour period until reaching 28.0˚C. They were

fed 50.5 ml of 100,000 cell/ml *Pavlova lutheri* algae daily while they were acclimating. The empty acclimation tank was filled with seawater treated as above each day, and the oysters transferred from the other tank. A Hydrolab MiniSonde 4 ® was programmed to measure dissolved oxygen (DO) and temperature (˚C), placed in the acclimation tank with the oysters, and moved with the oysters each time they were switched to the other tank.

While oysters were acclimating, two insulated, airtight covered tanks that were the same size as the acclimation tanks, were filled with seawater (20.0˚C, 24.0 ppt and 6.5 mg/l) treated as above. In the experimental tank, nitrogen was bubbled through two airstones to reduce the DO concentration (Widdows et al. 1989) to anoxic conditions (< 0.05 mg/l). The control tank was supplied with air through two airstones to maintain the DO at normoxic conditions (3.5-6.0 mg/l). Temperatures in each of these two tanks were established at 28.0˚C before any oysters were put in. A 350 gpm magnetic drive pump recirculated seawater through a mesh bag containing Purigen to remove proteins, nitrates, ammonia and other organics. After the oysters were acclimated and both the experimental and control tanks were ready, the 600 experimental and control oysters were checked for mortalities. The experimental oysters were placed within groups according to the times they would be taken out (their exposure group times), and then randomly placed within the experimental tank. The control oysters were grouped and placed in the control tank in the same way. A Hydrolab MiniSonde 4 ® was programmed and placed in each of the two tanks before the oysters were placed inside.

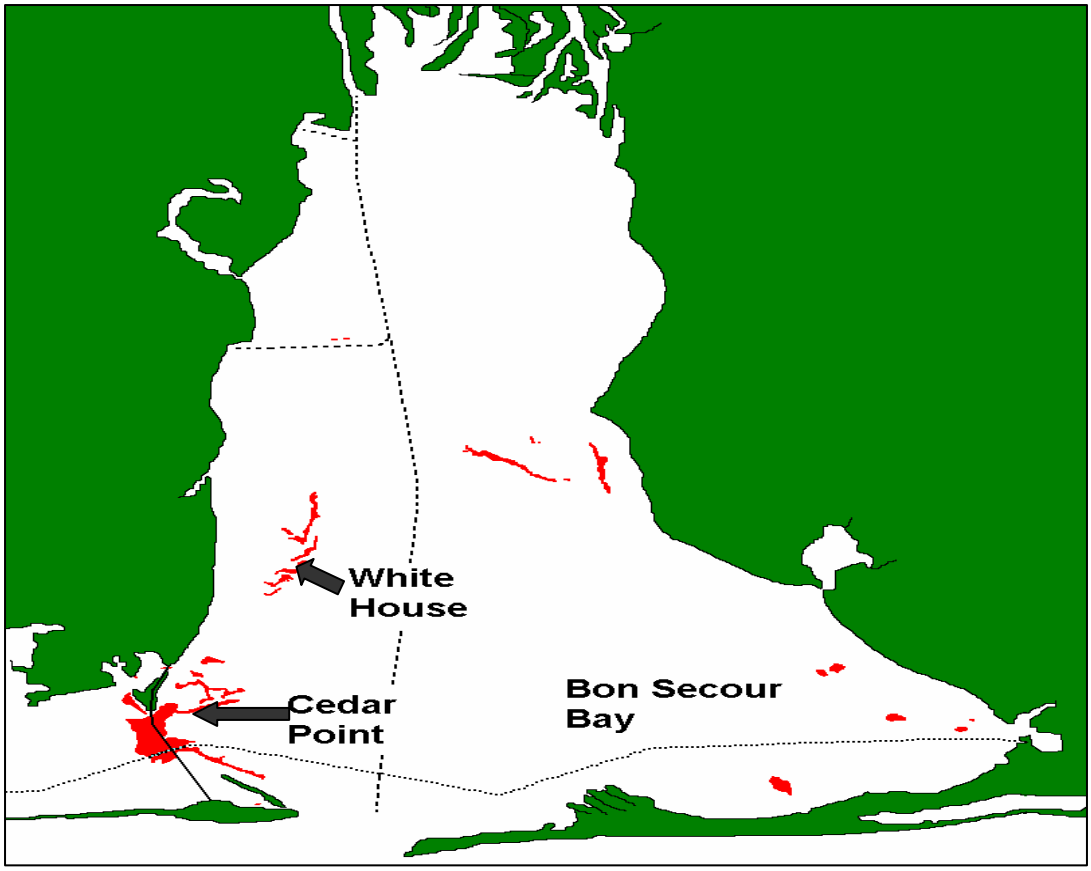

Figure 1. Location of White House Reef (potentially hypoxic) and Cedar Point Reef (normoxic) in Mobile Bay, Alabama.

After a period of 48 hours all bags were removed from the experimental tank and the oysters were checked for mortality. Dead oysters were removed, their numbers recorded, and the 48-hour bags were placed in one of two recovery tanks the same size as the acclimation, experimental, and control tanks, filled with seawater (24.0 ppt and 4.9 mg/l) treated as above and maintained at 26.0˚C. The rest of the bags were placed back in the experimental tank. The same procedure was repeated for each of the other 4 exposure groups, (72-hour, 96-hour, 120-hour, and 144-hour) at their appropriate times. Control oysters were checked using the same procedures. While in the recovery tank, oysters were fed *Pavlova lutheri* algae daily just as in the acclimation phase. The oysters were switched from each tank daily with a programmed Hydrolab MiniSonde 4 ® as was done above. Each bag in the recovery tank was removed after 48 hours. Dead oysters from each bag were removed and counted.

Phase II

In Phase II the objectives were to determine and compare the times to death of the offspring (F1 generation) of oysters that survived 96 hours or more and controls from Phase I. Since the number of survivors from Phase I was potentially too low for a successful spawn, additional survivors were created using the same methods as in Phase I and the oysters that survived more than 101 hours were retained for spawning. Survivors from the two anoxic exposures were kept separate and were labeled CPE 1 or CPE 2 (Cedar Point Experimental first survivor group or second survivor group) and WHE 1 or WHE 2 for the respective White House survivors. Controls (oysters not exposed to anoxia) were labeled either CPC or WHC. All of the survivors and controls were kept in flow through unfiltered seawater (19.1-25.2˚C and 18.0-27.2 ppt) for

9 days (for the additional survivor oysters) to 24 days (for the 96-hour oysters) prior to spawning. Four males and four females from each survivor and control group were strip spawned (with the exception of the CPE1 group which had 3 males strip spawned) and the eggs and sperm mixed for fertilization (Creswell et al. 1990).

Fertilized eggs were stocked in 113.6 liter tanks (3 per group, 1,000,000 per tank) and in 946.4 liter tanks (1 per group), at numbers ranging from 6,530,000 for the CPE2 group to 9,000,000 for the rest of the groups. The tanks were filled with seawater (25.0˚C, 25.0 ppt, and 6.5 mg/l) treated as above. Larvae were maintained in the tanks (Creswell et al. 1990; Dupuy et al. 1977) until they reached 250 μm in diameter, set on micro-cultch (250 μm) (Creswell et al. 1990), and grown for two months in flow through unfiltered seawater (23.0-31.0˚C and 6.0-30.1ppt).

The CPE2 larvae had high mortality and no oysters were produced. The remaining five groups (CPE1, CPC, WHE1, WHE2, and WHC) of the F1 generation were randomly divided (3 replicates each) as in Phase I, measured (mean height) and acclimated to 28.0˚C over a period of two days (ambient temperature= 26.5˚C, 17.0 ppt and 5.5 mg/l) in seawater treated as above in one of two aquaria (113.6 liters) which contained a programmed Hydrolab MiniSonde 4 ®. Replicates were placed in one of two aquaria (113.6 liters), either an anoxic aquarium (< 0.05 mg/l) or a normoxic aquarium (6.0- 6.5 mg/l) each with seawater (17.0 ppt) treated as above and maintained at 28.0˚C. The same procedures regarding checking for mortality and recovery tanks (113.6 liters, ambient temperature=26.0˚C, 18.0 ppt , 7.0 mg/l, Hydrolab MiniSonde 4 ®) were followed as in Phase I. Water was changed every day in all aquaria.

Data Analysis

In both Phase I and Phase II mortality was analyzed using the SAS probit procedure (SAS Institute Inc. 1989) to estimate the mean time it took for 50 % (LT-50) and 90% (LT-90) of the oysters in each group to die. Mortalities were adjusted when mortality occurred in the corresponding control (Robertson and Preisler 1992). Mean LT-50s and LT-90s were compared among groups to determine significant differences ($\alpha = 0.05$) using the lethal dose ratio test (Robertson and Preisler 1992) (Appendix, Tables 2, 3 and 4).

III. RESULTS

In Phase I, White House (hypoxic) oysters had a significantly higher mean LT-50 (102.5 h) and LT-90 (122.0 h) than Cedar Point (normoxic) oysters (94.0 h and 109.7 h) (Figs. 2 and 3). There were two mortalities found in the Cedar Point control oysters while none were found in the White House control oysters. Dissolved oxygen in the experimental tank remained below 0.05 mg/l except for a brief (3 hour) spike to 0.17 mg/l and temperature was near 28˚C (Fig. 4) while the control tank was 3.5-6.0 mg/l except for a brief (30 minute) spike to 7.5 mg/l and 28˚C except for a brief (30 minute) spike to 23.5˚C (Fig. 5). Mean oyster heights were 71.7 mm (range = 51.3mm-114.8mm) for White House and 81.4 mm (range = 45.5mm-121.6mm) Cedar Point.

In Phase II the mean LT-50s of the F1 generation were 126.6h for the CPE1 oysters, 116.8 h (CPC), 103.0 h (WHE1), 108.0 h (WHE2), and 124.1 h (WHC). The mean LT-90s were 150.8 h for the CPE1 oysters, 140.9 h (CPC) ,130.5 h (WHE1) , 125.0 h (WHE2), and 143.9 h (WHC) (Figs. 6, 7, 8, and 9). There were no mortalities found in the control oysters. The CPE1 oysters had a significantly higher mean LT-50 than the CPC, WHE1 and WHE2 oysters. The WHC oysters had a significantly higher mean LT-50 and LT-90 than both the WHE1 and WHE2 oysters. There were no significant differences found between the WHC and CPC oysters or between

the WHC and CPE1 oysters (Figs. 7 and 9). Dissolved oxygen in the experimental aquarium remained below 0.05 mg/l except for a few brief (2 hour) spikes (the highest going up to 0.4 mg/l) and temperature was near 28˚C (Fig. 10) while the control aquarium was 6.0-6.5 mg/l and 28˚C (Fig. 11). Mean heights were similar among the 5 groups (Appendix, Table 1).

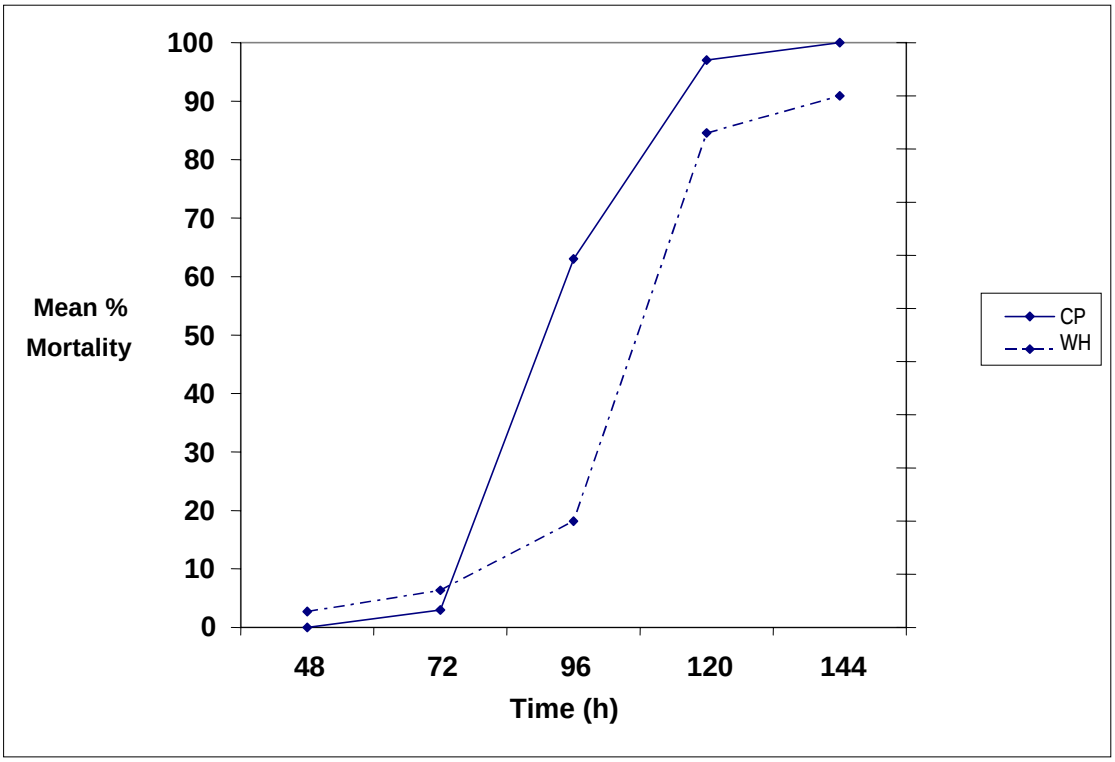

Figure 2. Mean percentage mortalities for Cedar Point (CP) and White House (WH) oysters at 48, 72, 96, 120 and 144 hours when held in anoxic seawater.

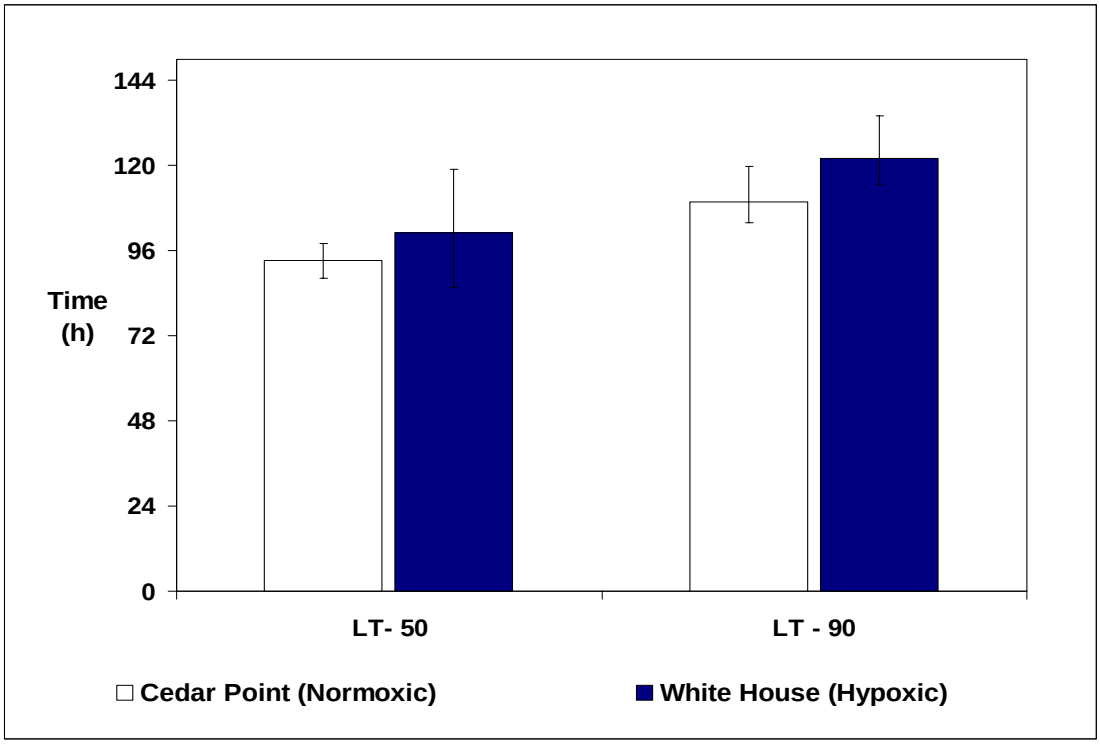

Figure 3. Mean LT-50s and LT-90s for Cedar Point (CP) and White House (WH) oysters. The mean LT-50s and LT-90s for WH oysters are significantly higher (α=0.05).

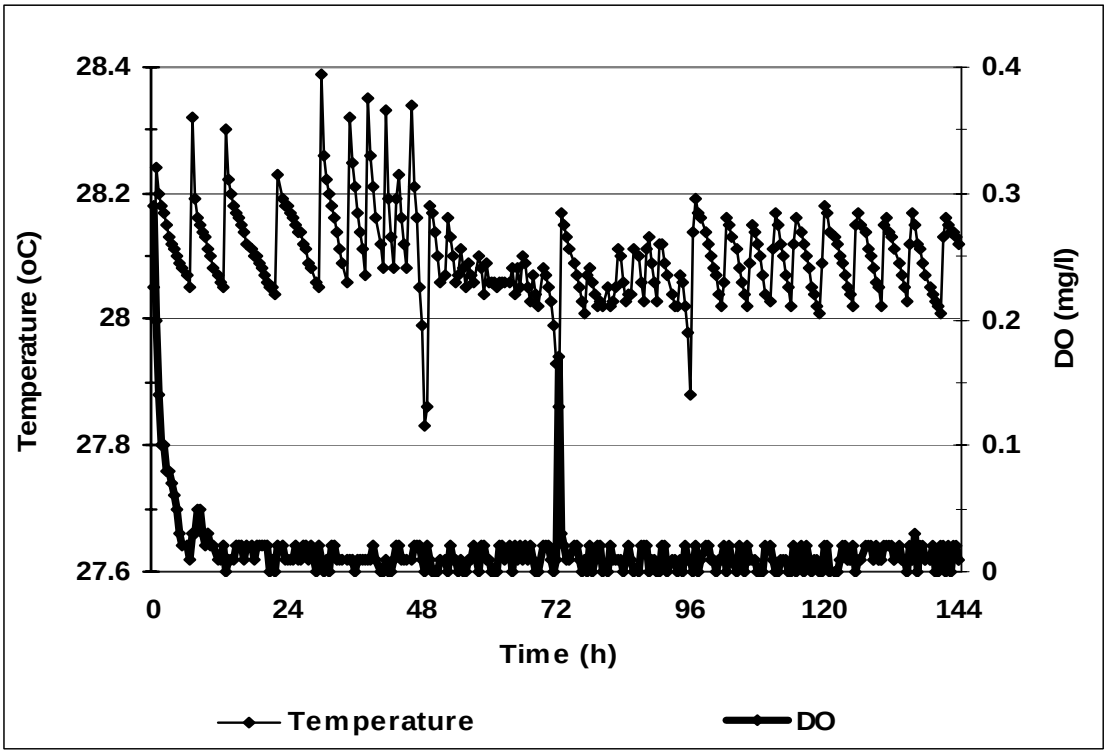

Figure 4. DO (dissolved oxygen) and temperature for experimental tank in Phase I (0 to 144 h).

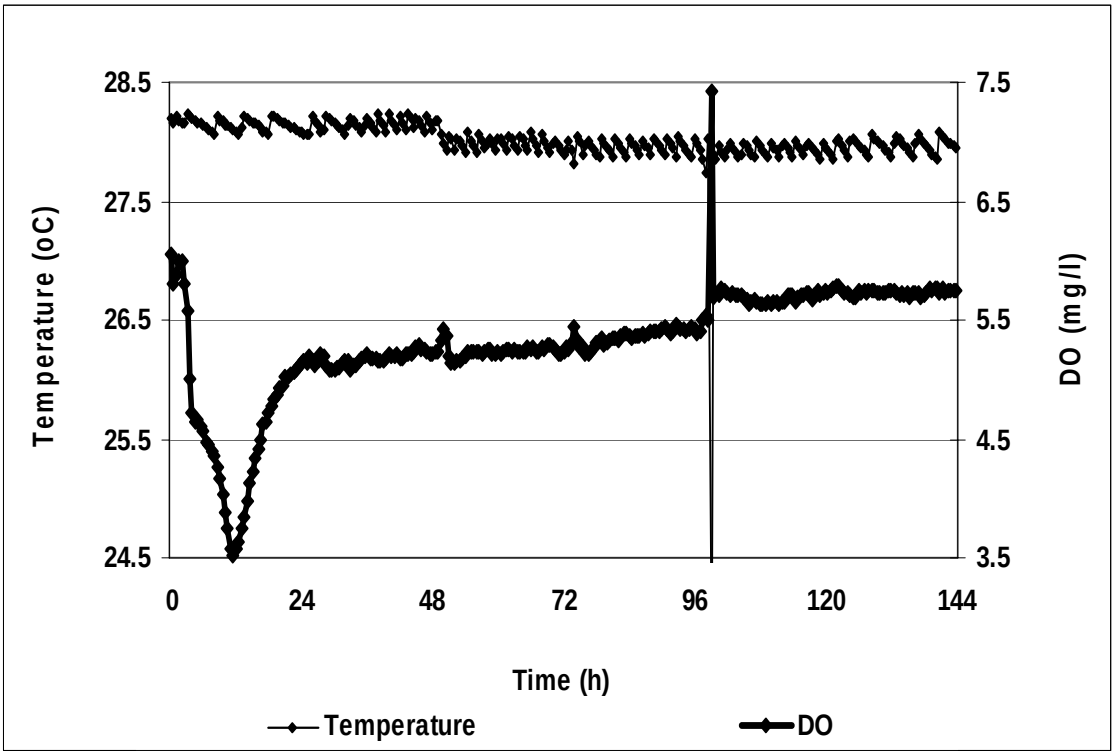

Figure 5. DO and temperature for control tank in Phase I (0 to 144 h).

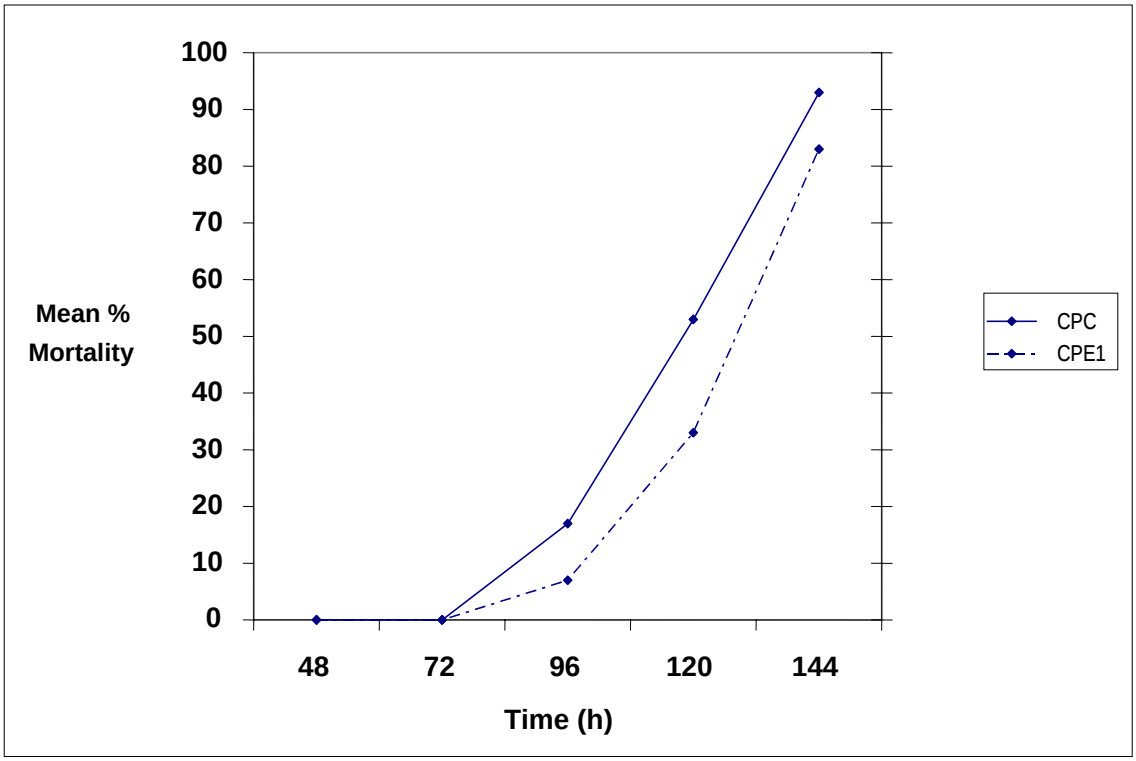

Figure 6. Mean percentage mortalities for Cedar Point Control (CPC) and Cedar Point Experimental 1 (CPE1) oysters at 48, 72, 96, 120 and 144 hours when held in anoxic seawater.

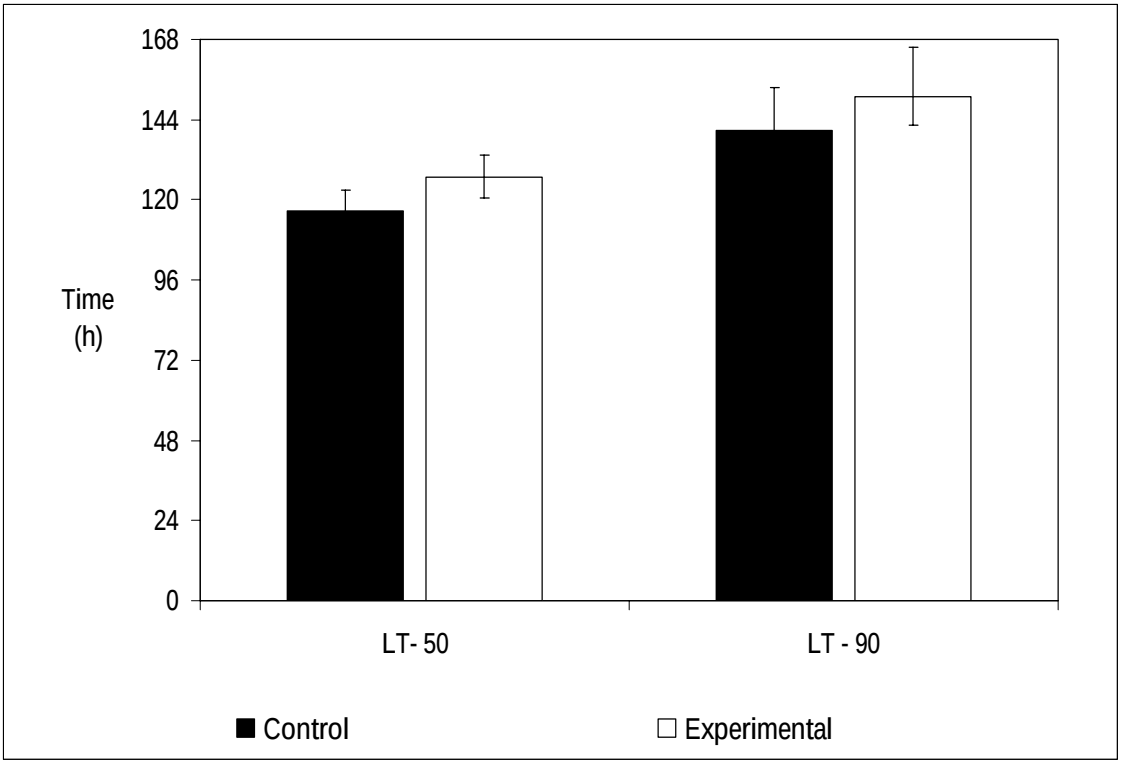

Figure 7. Mean LT-50s and LT-90s for Cedar Point Control (CPC) and Cedar Point Experimental 1 (CPE1) oysters. The mean LT-50s for CPE1 oysters are significantly higher (α=0.05).

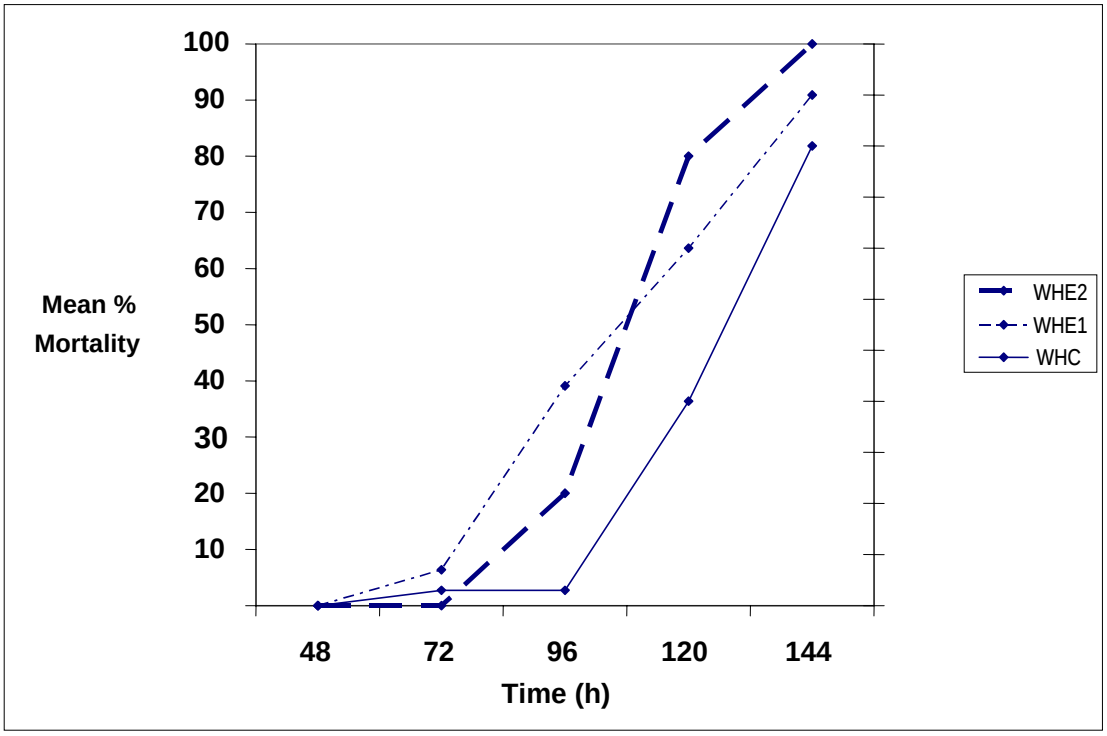

Figure 8. Mean percentage mortalities for White House Experimental 2 (WHE2), White House Experimental 1 (WHE1), and White House Control (WHC) oysters at 48, 72, 96, 120 and 144 hours when held in anoxic seawater.

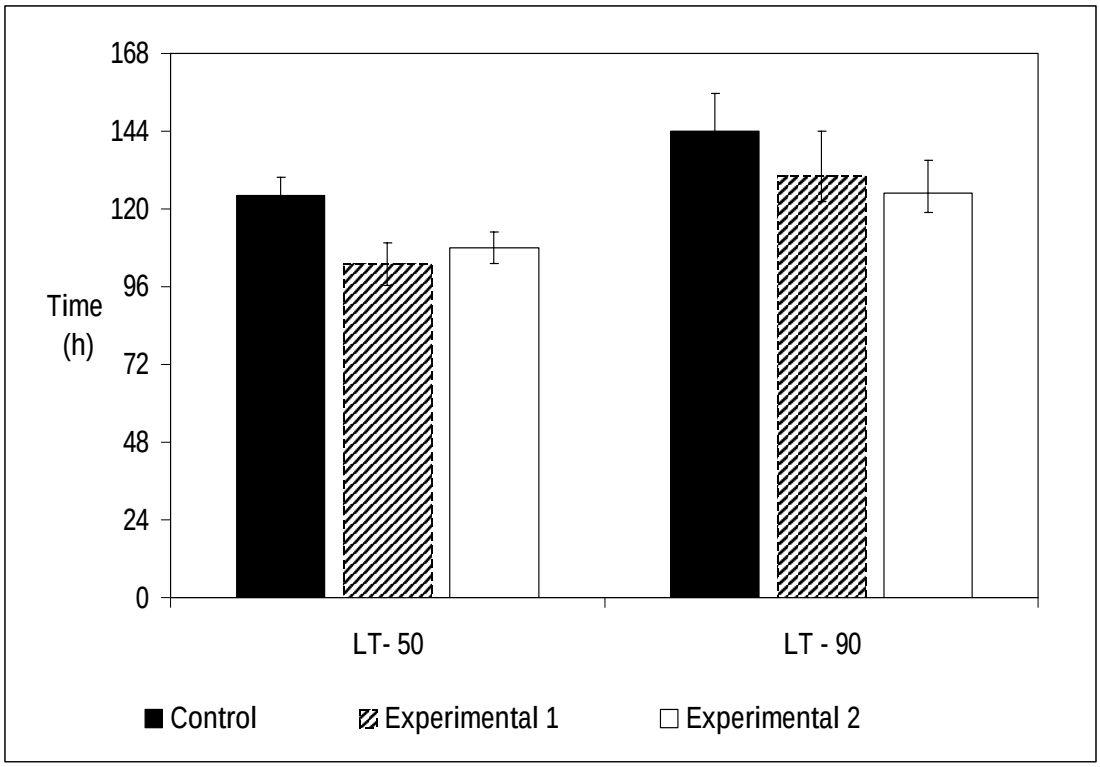

Figure 9. Mean LT-50s and LT-90s for White House Control (WHC), White House Experimental 1 (WHE1) and White House Experimental 2 (WHE2) oysters. The mean LT-50s and LT-90s for WHC oysters are significantly higher (α=0.05).

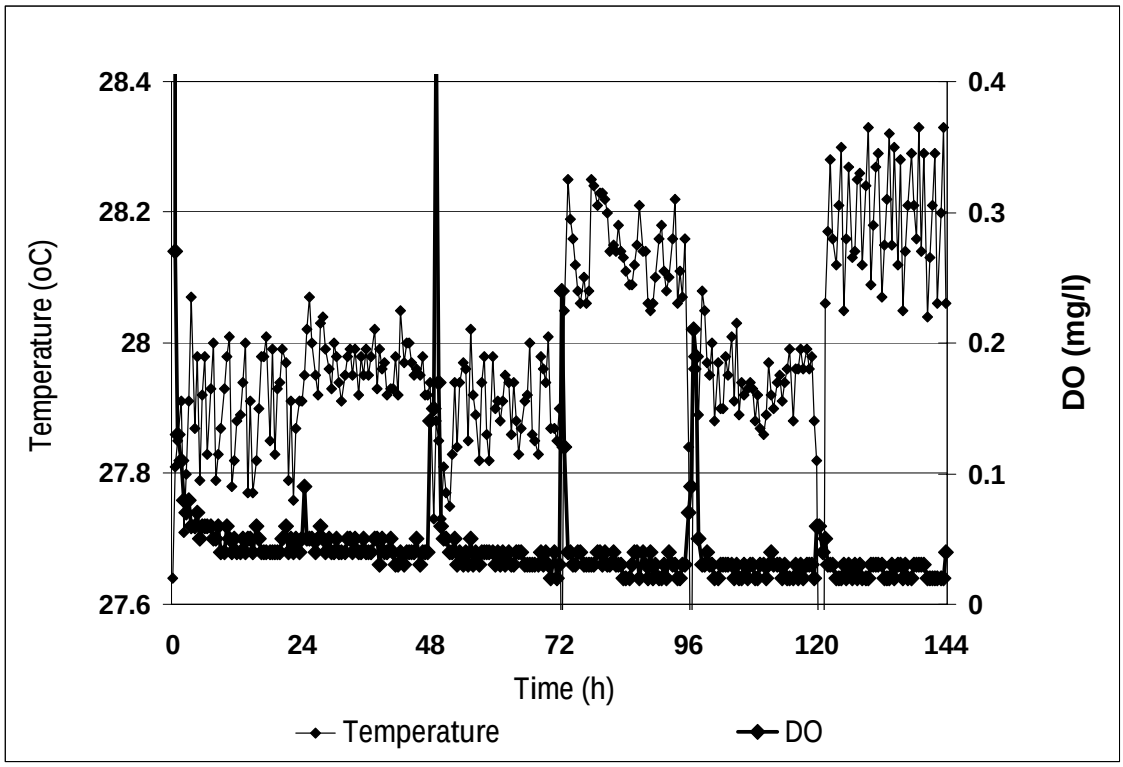

Figure 10. DO (dissolved oxygen) and temperature for experimental aquarium in Phase II (0 to 144 h).

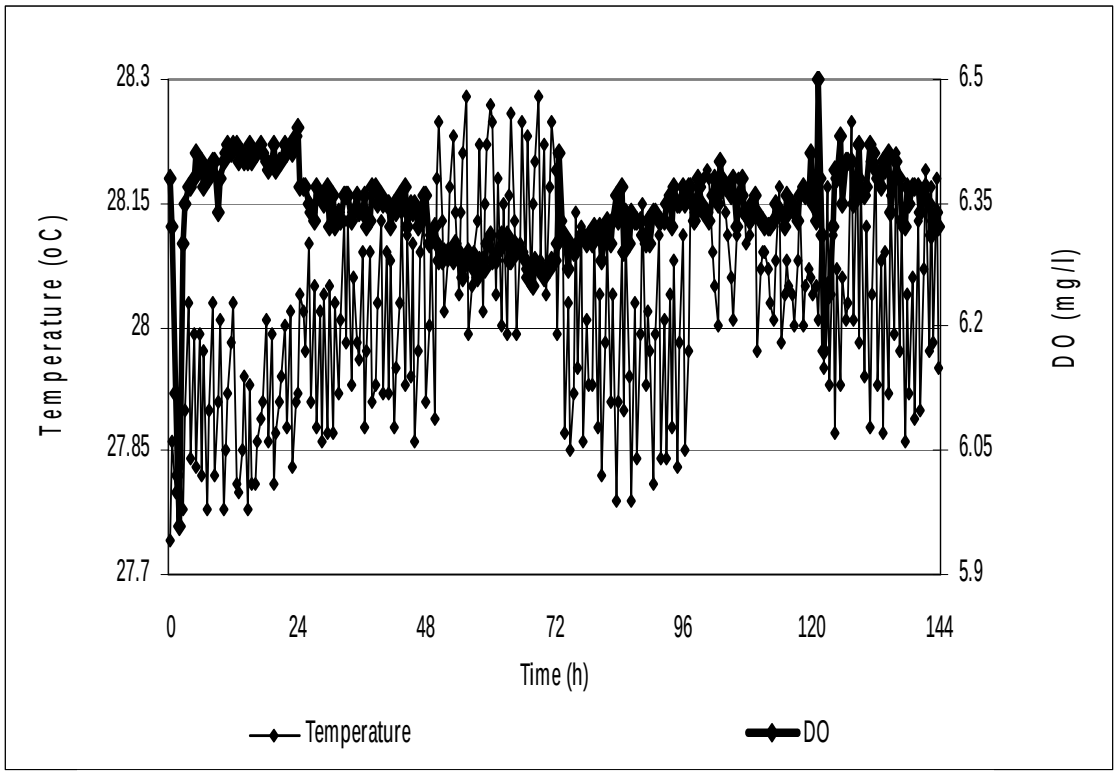

Figure 11. DO and temperature for control aquarium in Phase II (0 to 144 h).

IV. DISCUSSION

In Phase I of this study oysters from a reef thought to be experiencing hypoxia in Mobile Bay (White House) were significantly more tolerant to anoxia than oysters from a normoxic reef (Cedar Point). Tolerance to anoxia by a particular group of oysters is dependant on the conditions of the natural environment in which they were found (Stickle et al. 1989; Van Winkle 1967). Groups of bivalves that survive sporadic periods of hypoxia in their natural environments show more heterozygosity than other groups (Borsa et al. 1992; Myrand et al. 2002). The more heterozygous individuals have lower basal metabolic rates than homozygous individuals and are therefore more physiologically homeostatic (Myrand et al. 2002). The more physiologically stable an individual is, the more it is able to cope with stress induced upon it by anoxia (Myrand et al. 2002). White House (WH) oysters, which came from a reef that experienced sporadic periods of hypoxia, were potentially more heterozygous than the Cedar Point (CP) oysters which were not exposed to hypoxia.

Phase II was carried out in order to determine if differences in tolerance to anoxia between the two groups would appear in the F1 generation as well. The offspring of challenged CP oysters (CPE 1) were found to be significantly more tolerant to anoxia than the offspring of unchallenged CP oysters (CPC). The parent CP oysters that survived the anoxia challenge in Phase I may have been the most heterozygous

individuals from the group, and therefore more physiologically able to deal with the stress induced by the anoxic conditions (Borsa et al. 1992; Myrand et al. 2002). This could explain why in the F1 generation the CPE1 oysters showed a greater tolerance to anoxia than the CPC oysters whose parents did not get selected under any anoxia challenge.

Within the White House group, the WHC oysters were found to be significantly more tolerant to anoxia than the WHE 1 and WHE 2 oysters. WHE1 and WHE2 oysters were expected to be more tolerant to anoxia than the WHC oysters just as the CPE 1 oysters were more tolerant than the CPC oysters. The low resistance to anoxia displayed by the WHE1 and WHE2 oysters could have resulted from a loss in heterozygosity and allelic diversity after these groups were spawned. It has been found in another study that mass selection at commercial hatcheries can result in a great deal of inbreeding and loss of genetic diversity (Gaffney et al. 1992). However, usually a loss of heterozygosity in the first generation hatchery population is only expected to occur if the parent group possessed either skewed allelic frequencies or allelic frequencies that were not uniform due to previous generations of selection in the environment it came from (Gaffney et al. 1992). In this case, the WH parental group that survived the anoxia challenge may have had the most skewed allelic frequencies of all the other parental groups since this group was presumably selected for hypoxia tolerance in the wild and then underwent an additional selection at the laboratory (Gaffney et al. 1992). As a result, the WHE1 and WHE2 oysters possibly possessed a much lower level of heterozygosity than their parents and other groups in the F1 generation and in turn had a lower tolerance to anoxia than the WHC oysters.

The CPE1 oysters were found to be significantly more tolerant to anoxia than both the WHE1 oysters and the WHE2 oysters. Again, this could be explained by the potentially lower heterozygosity present in the WHE1 and WHE2 oysters. Since the parents of the CPE1 oysters did not come from a hypoxic environment in which a great deal of selection went on in previous generations there was not much of a loss in heterozygosity after spawning the first generation at the laboratory (Gaffney et al. 1992).

White House control oysters, although nominally more tolerant to anoxia than CPC oysters, were not significantly more tolerant. This contradicts the findings in Phase I and could be due to other factors such as unexplained variability in the experiment. Since the difference in tolerance found in Phase I was part of the basis for work in Phase II this apparent discrepancy should be examined in future research.

Tolerance to anoxia in the progeny of selected oysters as measured by mean LT-50s ranged from 103.0 h to 126.6 h while LT-90s were from 125.0 to 150.8. Saoud et al. (2000) reported a series of low oxygen events in Mobile Bay including one lasting over a 6 day period (130 h) which resulted in the total mortality of planted oysters and recently set spat. Results of the current study suggest that some oysters would have the capability of surviving anoxia up to 150 h. Efforts to restore marginally viable reefs affected by low oxygen may be enhanced through the use of hatchery produced oysters that are more tolerant to low oxygen conditions.

Oysters have adequate genetic variability for selective breeding procedures to be effective. Oysters have been the subject of breeding programs that selected for shell height, resistance to disease and accelerated growth rates (Sheridan 1997). While the

current study was inconclusive because of mixed results perhaps due to reliance on a single selected generation, there was enough evidence to suggest further selection and testing for tolerance to hypoxia. Future research should focus on intense selection of the existing breeding lines with adequate time between challenges and spawning, outcrossings, and the addition of genetic stock from other hypoxic sites.

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VI. APPENDIX

Appendix, Table 1.

Mean heights (mm) and height ranges (mm) for Cedar Point Experimental 1 (CPE1), Cedar Point Control (CPC), White House Experimental 1 (WHE1), White House Experimental 2 (WHE2) and White House Control (WHC) oysters.

	CPE1	CPC	WHE1	WHE2	WHC
Mean height	11.9	13.7	13.9	13.4	12.9
Height range	(10.0 - 15.3)	(11.5 - 16.7)	(10.5 - 18.2)	(11.5 - 16.8)	(10.4 - 18.5)

Appendix, Table 2.

Comparison of lethal times (LT-50, LT-90), fiducial limits (95% FL), lethal dose ratios (LDR), and 95% confidence intervals (95% CI) of the ratios for White House Reef (WH) and Cedar Point Reef (CP) oysters. An asterick (*) indicates a significant difference ($\alpha = 0.05$) based on the confidence limits of the ratio excluding 1.

	WH	CP
LT-50 (95% FL)	102.5 (97.2 - 108.3)	94.0 (88.5 – 99.0)
WH LDR (95% CI)		* 3.52E-9 (1.62E-16 - 0.08)
LT-90 (95% FL)	122.0 (114.9 - 134.1)	109.7 (104.0 - 119.9)
WH LDR (95% CI)		* 7.20E-13 (2.28E-24 - 0.23)

Appendix, Table 3.

Comparison of lethal times (LT-50), fiducial limits (95% FL), lethal dose ratios (LDR), and 95% confidence intervals (95% CI) of the ratios for F1 generation oysters. An asterick (*) indicates a significant difference ($\alpha = 0.05$) based on the confidence limits of the ratio excluding 1.

	CPE1	CPC	WHE1	WHE2	WHC
LT-50	126.6	116.8	103.0	108.0	124.1
(95% FL)	(120.7 – 133.3)	(111.0 - 122.9)	(96.5 – 109.3)	(103.0 – 113.0)	(118.6 – 129.6)
CPE1					
LDR		*1.64E-10	*6.82E+23	*6.14E+18	0.00
(95% CI)		(5.83E-19 - 0.05)	(1.24E+15 - 3.76E+32)	(8.32E-10 - 4.54E+26)	(1.86E-11 – 3.18E+5)
CPC					
LDR			*1.12E+14	*1.01E+9	6.75E-8
(95% CI)			(3.10E+5 - 4.10E+22)	(21.50 – 4.72E+16)	(8.01E-16 – 5.68)
WHE1					
LDR				9.01E-6	*1.66E+21
(95% CI)				(9.19E-14 – 8.83E+2)	(9.62E+12 - 2.86E+29)
WHE2					
LDR					*1.49E+16
(95% CI)					(7.43E+8 - 3.00E+23)

Appendix, Table 4.

Comparison of lethal times (LT-90), fiducial limits (95% FL), lethal dose ratios (LDR), and 95% confidence intervals (95% CI) of the ratios for F1 generation oysters. An asterick (*) indicates a significant difference ($\alpha = 0.05$) based on the confidence limits of the ratio excluding 1.

	CPE1	CPC	WHE1	WHE2	WHC
LT-90	150.8	140.9	130.5	125.0	143.9
(95% FL)	(142.3 - 165.7)	(133.2 - 153.4)	(122.3 – 143.8)	(119.0 – 135.1)	(137.0 – 155.6)
CPE1					
LDR		1.48E-10	*2.96E+20	*8.28E+25	1.16E-7
(95% CI)		(3.78E-25 - 5.77E+4)	(3.62E+5 - 2.43E+35)	(4.17E+12 - 1.64E+39)	(1.24E-21 - 1.08E+7)
CPC					
LDR			4.38E+10	*1.22E+16	0.00
(95% CI)			(0.00 - 4.51E+24)	(6.42E+3 - 2.33E+28)	(1.26E-16 – 12.93E+9)
WHE1					
LDR				2.79E+5	*3.43E+13
(95% CI)				(6.11E-8 – 1.28E+18)	(1.48 - 7.96E+26)
WHE2					
LDR					*9.58E+18
(95% CI)					(2.79E+7 - 3.29E+30)