An evaluation of oyster stocks, grow-out conditions, and off-bottom culture methods for increasing commercial production of eastern oysters (Crassostrea virginica) in the northern Gulf of Mexico

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AN EVALUATION OF OYSTER STOCKS, GROW-OUT CONDITIONS, AND OFF-BOTTOM CULTURE METHODS FOR INCREASING COMMERCIAL PRODUCTION OF EASTERN OYSTERS (Crassostrea virginica) IN THE NORTHERN GULF OF MEXICO

A Thesis

Submitted to the Graduate School Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Master of Science in The School of Renewable Natural Resources

by
Justin M. Leonhardt
B.S., University of Rhode Island, 2010
August 2013
Acknowledgements

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Abstract

This project examined the ecologically and economically valuable eastern oyster (*Crassotrea virginica*) in Louisiana with two field experiments. Little information has been gathered on the performance of local Louisiana oyster stocks and no study has focused on a comparison of stocks among variable estuarine conditions. Additionally, the use of alternative grow-out methods and intensive cage aquaculture has never been evaluated in Louisiana. For the first study, a dermo-resistant stock of oysters (LSU-OYS: ‘OBOY’) was compared to three wild oyster stocks along a salinity gradient. The objectives of this study were to determine the optimal oyster stock(s) and the ideal grow-out condition(s) for intensive oyster aquaculture production. The second experiment compared the efficiencies of three commercially used, off-bottom culture systems. The objective of this study was to suggest which off-bottom grow-out method(s) is most suitable for use in Louisiana estuaries. The results of the stock comparison suggested that the selected dermo-resistant stock had greater mortality than two of the three wild stocks in all the environmental conditions tested. Specifically, wild stocks taken from low salinity areas had greater performance in test areas with low salinities while wild stocks collected from high salinity areas had greater performance at high salinity sites. The results of the grow-out method comparison revealed that an adjustable long line system (ALS) was the most suitable culture system in both high and low salinity conditions, specifically due to overall higher survival, improved growth in shell height, and reduced effort in labor and handling time. For the first time, the performance of four oyster stocks and three intensive oyster culture methods were quantified, suggesting superior stocks, grow-out conditions, and culture systems for augmenting wild production and increasing total production in the Louisiana oyster industry.
Chapter 1: Introduction

Oyster Biology

Classification and Range

The eastern oyster (*Crassostrea virginica*) is classified under the Kingdom: Animalia, Phylum: Mollusca, Class: Bivalvia, Order: Ostreoida, and Family: Ostreidae. It is a sessile, filter feeding organism that can be found in estuarine and marine environments ranging from the Gulf of St. Lawrence, Canada down the eastern coast of the United States into the Gulf of Mexico (FAO 2013; Carriker and Gaffney 1996) (Figure 1.0).

Figure 1.1: Range for *Crassostrea virginica*. (From: The Oyster Fishery of the Gulf of Mexico, United States: A Regional Management Plan – 2012 Revision. Pub. No. 202, Gulf States Marine Fisheries Commission, Ocean Springs, Mississippi).

Life Cycle

Eastern oysters are broadcast spawners, such that sexually mature adult oysters reproduce by releasing gametes into the water column at salinities above 5 to 10 ppt and when water temperatures increase during the spring (Kennedy 1996; Medcof 1939; Butler 1956). After
fertilization and within the first 24 hours, the embryos develop into non-feeding, planktonic trocophore larvae. Twenty-four to 48 hours post fertilization, trocophore larvae become veliger (D-shape) larvae, where they grow in the water column for 2-3 weeks, feeding on phytoplankton, detritus, and bacteria (Kennedy 1996). Growing veliger larvae eventually develop an ‘eye spot’ and ‘foot’ and are classified as ‘eyed’ or ‘pediveliger’ larvae. These larvae then migrate to the benthos, where they attach to a hard surface known as ‘cultrh’ and metamorphose into ‘spat’ oysters (~1-24 mm). Once settled, spat oysters continue to feed on suspended particulate matter and grow into ‘seed’ oysters, where they reach sexual maturity. Previous studies show that first sexual maturity can be attained at 31 mm (~1 year of age) (Galtsoff 1964; Rothschild et. al 1990). Natural processes of larval development, settlement, and recruitment result in concentrations of adult oysters into sub-tidal and intertidal reef structures, which carry large ecological importance.

Figure 1.2: Life cycle of *Crassostrea virginica*. (From: The Oyster Fishery of the Gulf of Mexico, United States: A Regional Management Plan – 2012 Revision. Pub. No. 202, Gulf States Marine Fisheries Commission, Ocean Springs, Mississippi).
Adult Growth and Mortality

Eastern oysters are a eurythermic and euryhaline species capable of tolerating wide temperature and salinity ranges from 0-42°C and 0-42.5 ppt (Shumway 1996). Considering the eastern oyster is a poikilothermic species, faster metabolic and growth rates are generally observed during periods of elevated water temperatures. Salinity, however, and its synergistic effects with water temperature, ultimately determines oyster performance. The synergistic effects of temperature and salinity are known to have profound effects on oyster feeding, respiration, utilization of food reserves, parasite-disease interaction, predation rates, and growth (Shumway 1996). Specifically, low salinity levels (< 3 ppt), when accompanied by high water temperatures, induce valve closure and decrease feeding rates (Loosanoff 1953, 1965). These conditions (i.e., <2 ppt; > 25°C) sustained over several weeks can result in decreased oyster condition, reduced growth, and lead to oyster mortality (Heilmayer et. al 2008). Temperature and salinity also influence natural mortality from predation and disease, such that higher predator presence and interactions with disease occur at elevated salinity levels (i.e., >15 ppt) (Wells 1961; White and Wilson 1996). The eastern oyster can successfully recruit new individuals to populations, acclimate to and resist mortality from extreme abiotic conditions, and resist mortality from predation and disease. It is because of the resiliency of the species that the eastern oyster has provided a renewable resource for industry and commercial harvest worldwide.

Oyster Production

Global Production

In 2009, total global oyster production was estimated to be 4,303,401 metric tons (MT) of whole oyster product (meat and shell combined) (FAO 2009). The vast majority of this production came from China, where 3,503,782 MT were harvested. Much lower levels of total
harvest came from the Republic of Korea (240,911 MT) and Japan (210,188 MT) (FAO 2009). Behind these countries, the United States (128,910 MT) and France (104,641 MT) also had sufficient contributions to total production (FAO 2009). These top five producers of oysters contributed 98% of total world oyster production in 2009. The United States was also the leading world producer for *Crassostrea virginica*, producing 90,000 MT of the nation’s total harvest (FAO 2009) (Table 1.1).

Table 1.1: Total production of oysters in metric tons (MT) of whole oyster (meat + shell) from the top 5 producing countries in 2009. Note: Unknown species in China is due to a lack of species specification within the data set. (From: FAO Fisheries and Aquaculture Department-Yearbook for Fishery and Aquaculture Statistics-Aquaculture Production, 2009).

<table>
<thead>
<tr>
<th>Country</th>
<th>Total Production (MT)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>3,503,782</td>
<td>Unknown</td>
</tr>
<tr>
<td>Rep. of Korea</td>
<td>240,911</td>
<td><em>C. gigas</em></td>
</tr>
<tr>
<td>Japan</td>
<td>210,188</td>
<td><em>C. gigas</em></td>
</tr>
<tr>
<td>United States</td>
<td>128,910</td>
<td><em>C. virginica</em>; <em>C. gigas</em></td>
</tr>
<tr>
<td>France</td>
<td>104,641</td>
<td><em>C. gigas</em>; <em>C. eudilus</em></td>
</tr>
</tbody>
</table>

United States Production

In 2011, total national oyster production for the United States (U.S.) was estimated to be 31,332,947 pounds of oyster meat (NMFS 2011). *C. virginica* dominates total oyster production in the U.S, accounting for ~68% of the total national oyster harvest. The remaining percentage of production is of *C. gigas*, with the vast majority of this species being cultured in the state of Washington. The leading contributors to total oyster harvest in the U.S. can be seen in Table 1.1. These five leading contributors make up ~82% of total U.S. oyster production.
Table 1.2: Total oyster production in pounds (lbs.) of meat from the top 5 producing states in 2011. Other: refers to all remaining states that contributed to total national oyster production (From: National Marine Fisheries Service-NOAA: Annual Commercial Landing Statistics, 2011, Oysters by State).

<table>
<thead>
<tr>
<th>State</th>
<th>State Production (lbs)</th>
<th>State/Total (%)</th>
<th>Species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Louisiana</td>
<td>11,145,039</td>
<td>35.6</td>
<td>C. virginica</td>
</tr>
<tr>
<td>Washington</td>
<td>9,377,989</td>
<td>29.9</td>
<td>C. gigas</td>
</tr>
<tr>
<td>Texas</td>
<td>3,943,434</td>
<td>12.6</td>
<td>C. virginica</td>
</tr>
<tr>
<td>Florida</td>
<td>2,902,540</td>
<td>9.3</td>
<td>C. virginica</td>
</tr>
<tr>
<td>Virginia</td>
<td>1,389,139</td>
<td>4.4</td>
<td>C. virginica</td>
</tr>
<tr>
<td>Other</td>
<td>2,574,806</td>
<td>8.2</td>
<td>C. virginica; C. gigas</td>
</tr>
</tbody>
</table>

Louisiana Production

Historically, Louisiana leads the nation in annual oyster production, utilizing exclusively extensive, on-bottom culture methods (Wirth and Minton 2004; Supan 2002). Approximately 1.7 million acres of public oyster grounds are managed by the Louisiana Department of Wildlife and Fisheries (LDWF) (Figure 1.2). This management combined with the monitoring of commercial harvest from private leases (~400,000 privately leased acres) maintains Louisiana as the national leader in oyster production, with annual value typically exceeding $35 million in dockside sales (LDWF 2011; J. Supan, LA Sea Grant, pers. comm.). Interestingly, this level of commercial harvest is fully supported by the settlement and recruitment of natural, wild oyster populations within coastal Louisiana. These managed public oyster grounds are considered the backbone of the Louisiana oyster resource in terms of direct commercial harvest of market-sized oysters from these grounds as well as providing enough of the resource for seed bedding on private oyster leases (LDWF 2011). In order to maintain the integrity of these public grounds, LDWF deposits
cultch material in a process known as ‘cultch planting’, which aims to facilitate larval settlement and recruitment in these areas. Even with these reef building attempts by the state, LDWF has recently observed reductions in the oyster resource on these public seed grounds (LDWF 2011) (Figure 1.3).

Figure 1.2: Designated ~1.7 million acres of public oyster grounds managed by the LDWF (From: Louisiana Department of Wildlife and Fisheries website; Louisiana Public Oyster Areas Map http://www.wlf.louisiana.gov/fishing/oyster-program).
Problems in Louisiana

Natural Disasters and Freshwater Diversions

Recent large-scale events have contributed to the decline in oyster populations in Louisiana. Hurricanes Katrina and Rita in 2005 extensively damaged oyster beds by siltation and contamination while simultaneously destroying thousands of fishing vessels (CRS Report for Congress 2005). Based on the size and strength of Hurricane Katrina, LDWF estimated a direct loss of available resource valued at more than $205 million given an assumed 99% oyster mortality (CRS Report for Congress 2005). Hurricanes Gustav and Ike in 2008 and Isaac in 2012 likely imposed similar negative consequences to the resource and the industry. The BP Deep Water Horizon oil spill in spring and summer of 2010 and the record high levels of the Mississippi River in spring of 2011 prompted the opening of freshwater diversions which decreased salinity regimes within Louisiana estuaries for two consecutive years (Eberline 2012).
The extended time periods of low salinity accompanied by increasing water temperatures during the spring and summer months created stressful conditions (Heilmayer et. al 2008) and led to increased mortality and decreased growth and recruitment for oyster populations in Breton Sound, LA (Eberline 2012).

Overharvesting

The primary harvesting methods used for commercial oyster harvest in Louisiana are bottom dredging and hand-tonging. These traditional harvesting methods are known to decrease structural complexity on experimental oyster reefs (Lenihan and Peterson 2004). Additionally in the last 15 years, there has been a significant increase in the harvest of market-sized (sack) oysters, creating concern for reef degradation as a result of increasing fishing pressure (LDWF 2011). As shell removal exceeds shell replenishment, a net deficit of shell can occur and negatively impact the availability of seed oysters for private leases. This net deficit of cultch material from public oyster grounds has created concerns for long-term sustainability of the oyster resource (LDWF 2010). Availability of seed oysters is also affected by natural fluctuations in reproduction and recruitment of wild oysters, as well as unpredictable mortalities (as high as 50 to 85%) during grow-out on leases (Gulf States Marine Fisheries Commission 1991).

Predation and Disease

Predation and disease, which are mainly influenced by water temperature and salinity, are the primary contributors to natural mortality in the northern Gulf of Mexico, specifically in terms of excessive mortality due to protozoan parasite *Perkinsus marinus* (dermo) infections (Craig et al. 1989; Soniat 1996) and predation from southern oyster drills (*Stramonita haemastoma*) and black drum (*Pogonias cromis*) at salinities above 15 ppt (Breithaupt and Dugas 1979; George et
The yearly mortality rate due to *P. marinus* (dermo) has been estimated to be greater than 50% for market-sized oysters (La Peyre et al. 2003; Mackin 1962; Hofstetter 1977; Powell et. al 1996). While high annual mortality is generally offset by the almost continuous recruitment and rapid growth in the Gulf of Mexico, the economic impact of dermo and predation on the oyster industry has been substantial.

**Solutions**

**Intensive Oyster Aquaculture**

There are possible solutions to re-stabilize the industry and increase profitability. Intensive oyster aquaculture, which relies on hatchery-produced oyster seed and intensive grow-out methods, is a viable option for augmenting wild oyster production. Briefly, intensively reared oysters are spawned and grown as larvae in a protected hatchery setting. Pediveliger larvae are then set onto a desired cultch material (i.e., crushed oyster shell) and reared in a nursery system until they are seed oysters (>25 mm) and large enough to be grown in enclosed, off-bottom grow-out cages. One major advantage with using intensively cultured oysters is breeding programs can be established to select for beneficial characteristics (i.e., growth rate; disease-resistance) and produce superior oyster stocks.

**Importance of Stock**

The need to develop stocks of locally adapted oysters that are resistant to disease has long been recognized (Haskin and Ford 1979; Matthiessen et. al 1990). *P. marinus*, which is known to cause high mortality in adult oysters, inhibits production and profitability in the Louisiana oyster industry (Supan 2000). Recognizing that this disease has the greatest influence on oyster production in the state, a breeding program funded by NOAA’s Gulf Oyster Industry Program at Louisiana State University (LSU) has produced an oyster stock specifically selected for
resistance to the *Perkinsus* disease. This stock has been given the name ‘OBOYS’ from its origin of Oyster Bayou in Cameron Parish, LA. This stocks’ performance was quantified in this study.

A necessary step towards developing intensive oyster aquaculture in Louisiana is determining which stock of oysters to culture. Previous stock comparison studies revealed that growth, mortality, and reproductive success were due to the effects of site (i.e., location) and disease (Alphin et al. 2004; Sorabella and Luckenbach 2003). Due to the high prevalence of *P. marinus* in the northern Gulf of Mexico, it is necessary that selected OBOY stock is compared to wild oyster stocks to determine the most beneficial stock for hatchery seed production and stimulating overall production in the industry.

Importance of Environment

Identifying the ideal grow-out conditions for intensive oyster aquaculture is of great importance. In Louisiana, most oyster production occurs between 5 and 15 ppt due to excessive mortality from *P. marinus* infections (Craig et al. 1989, Soniat 1996) and predation from southern oyster drills (*Stramonita haematoma*) and black drum (*Pogonias cromis*) at salinities above 15 ppt at which oyster growth is greatest (Galtsoff 1964; MacKenzie 1977; Breithaupt and Dugas 1979; Brown et al. 2008). Based on the performances of the OBOY stock and wild oyster stocks in different conditions, the most optimal stock and grow-out conditions for this stock can be determined for facilitating industry production.

Importance of Off-Bottom Cage Culture

Advantages in using intensive culturing methods, specifically off-bottom cage culture, include improvements in growth, reductions in predator-related mortality, and opportunities to control bio-fouling. Cage culture permits intertidal placement of oysters, such that oyster culture can take place in areas where the natural bottom is unsuitable for traditional, on-bottom
cultivation (i.e., mud bottom and intertidal zones). Also, oysters cultured in intertidal zones, where they have been uniquely adapted to survive, have been reported to have accelerated growth, improved survival, and greater marketability through improved shell and meat quality when compared to extensively grown oysters (Ogasawara et al. 1962; Gillmor 1982; Ventilla 1984; Littlewood 1988; Crosby et al. 1991; Littlewood et al. 1992; O’Beirn et al. 1994; Handley 1997; Moroney and Walker 1999; Leggett 2000; Swartzenberg et al. 1997). Furthermore, considering off-bottom cage culture methods are known to decrease predator-driven mortality, higher salinity areas (>15 ppt) would become more viable areas for oyster production. Overall, intensive cage grow-out methods could increase the amount of total area for oyster production and facilitate oyster production in higher salinity areas.

Goals and Objectives

The goal of the first study was to identify the most suitable oyster stock(s) for hatchery production and intensive grow-out based on the grow-out of adult oysters of different geographic origin along a salinity gradient. The objective of this study was to evaluate the stock selected for dermo-resistance (OBOY) against the progeny of wild oysters collected from Louisiana major oyster seed grounds to see which stock is best suited for hatchery seed production and grow-out. The goal of our second study was to identify the most suitable off-bottom grow-out system(s) for facilitating commercial production of oysters in Louisiana estuaries. The objective of this study was to evaluate three off-bottom cage methods in two different environments and suggest the most optimal off-bottom grow-out system(s) in response to these variable environmental conditions.
Chapter 2: An evaluation of oyster stocks and grow-out conditions for eastern oysters (*Crassostrea virginica*)

Introduction

In the coastal waters of the northern Gulf of Mexico, the eastern oyster (*Crassostrea virginica*) plays an important ecological role and has high economic value (La Peyre et al 2009; Coen and Grizzle 2007; Plunket and La Peyre 2005; Dame 1996). Historically, Louisiana leads the nation in annual oyster production, accounting for ~35% of the total annual oyster harvest from 1999 to 2009 (LDWF 2011; Wirth and Minton 2004). In recent years, the Louisiana Department of Wildlife and Fisheries (LDWF) has observed reductions in the oyster resource on their public seed grounds (LDWF 2011). Additionally in the last 15 years, there has been a significant increase in the harvest of market-sized (sack) oysters, creating concern for potential net deficits of shell on these grounds and lease degradation (LDWF 2011).

Availability of wild seed is also affected by natural fluctuations in reproduction and recruitment of wild oysters as well as unpredictable mortalities (as high as 50 to 85%) during grow-out on leases (Gulf States Marine Fisheries Commission 1991; Supan 2002). Predation and disease are the primary contributors to natural mortality in the Gulf of Mexico, specifically in terms of excessive mortality from the protozoan parasite dermo (*Perkinsus marinus*) (Ray and Anderson 1988; Craig et. al 1989; Soniat 1996) and predation from southern oyster drills (*Stramonita haemastoma*) and black drum (*Pogonias cromis*) at salinities above 15 ppt (Breithaupt and Dugas 1979; George et al. 2008). It has been estimated that the yearly mortality rate due to *P. marinus* has been greater than 50% for market-sized oysters (La Peyre et. al 2003; Mackin 1962; Hofstetter 1977; Powell et al. 1996). While these high annual mortality rates are generally offset by the almost continuous recruitment and rapid growth in the Gulf of Mexico, the economic impact of dermo and predation on the oyster industry has been substantial.
Natural and anthropogenic events have also contributed to the decline in Louisiana oyster populations. Hurricanes Katrina and Rita in 2005 extensively damaged oyster beds by siltation and contamination (CRS Report to Congress 2005). Hurricanes Gustav and Ike in 2008 and Isaac in 2012 likely imposed similar negative consequences to the resource and the industry. In response to the BP Deep Water Horizon oil spill in spring and summer of 2010 induced large scale freshwater releases from the river systems to prevent oil from reaching the estuaries along the Louisiana coast. The sudden decrease in salinity, combined with elevated water temperatures during the summer months of 2010, created stressful conditions leading to high mortality and minimal growth and recruitment in Breton Sound, LA (Eberline 2012). More recently in 2011, record high levels of the Mississippi River created similar environmental conditions, challenging oyster survival and recruitment for a second consecutive year (Eberline 2012). The combined effects of harvesting and lease destruction, predation and disease, natural disasters, and anthropogenic influences have driven the oyster populations across southern Louisiana to historically low levels (LDWF 2011).

Intensive oyster aquaculture, which relies typically on hatchery-produced oyster seed and intensive grow-out methods, is a viable option for augmenting wild oyster production and potentially increasing profitability in the industry. A major advantage with using intensively cultured oysters is that selective breeding programs can be established in hatcheries to produce superior (i.e., disease-resistant) oyster seed for cultivation. The need to develop stocks of locally adapted oysters that are resistant to disease has long been recognized (Haskin and Ford 1979; Matthiessen et al. 1990). Previous research studies in the Chesapeake and Delaware Bays have successfully bred disease-resistant strains of oysters resistant to the effects of *Haplospiridium nelsoni* (MSX) (Haskin and Ford 1979; Carnegie and Burreson 2011) as well as strains resistant
to both MSX and dermo (Ragone-Calvo et al. 2003). Due to the high prevalence of *P. marinus* in the northern Gulf of Mexico, a breeding program funded by NOAA’s Gulf Oyster Industry Program at Louisiana State University (LSU) has produced an oyster stock specifically selected for resistance to *P. marinus*. This stock has been given the name ‘OBOY’ from its origin of Oyster Bayou in Cameron Parish, LA.

A necessary step towards developing intensive oyster aquaculture in Louisiana is determining which stock of oysters to culture. Previous stock comparison studies revealed that growth, mortality, and reproductive success were due to the effects of site (i.e., location) and disease (Alphin et al. 2004; Sorabella and Luckenbach 2003). It is essential that the selected OBOY stock be compared to wild oyster stocks to determine the most beneficial stock for hatchery seed production and promote profitability the industry. The objective for this study was to evaluate the selected OBOY stock against the progeny of wild oyster stocks collected from major Louisiana oyster seed grounds and quantify each stock’s performance, using the following hypotheses:

\[ H_0: \mu_{OB} = \mu_{CAL} = \mu_{SL} = \mu_{BS} \]

\[ H_A: \mu_{OB} \neq \mu_{CAL} \neq \mu_{SL} \neq \mu_{BS} \]

This comparative study aimed to determine whether the OBOYs have an advantage over the wild stocks in terms of growth, mortality, and susceptibility to *P. marinus*.

Identifying the ideal grow-out conditions for intensive oyster aquaculture is also of great importance. In Louisiana, most oyster production occurs between 5 and 15 ppt due to excessive mortality from *P. marinus* infections (Craig et al. 1989, Soniat 1996) and predation from southern oyster drills (*S. haemastoma*) and black drum (*P. cromis*) at salinities above 15 ppt at which oyster growth is greatest (Galtsoff 1964, MacKenzie 1977, Breithaupt and Dugas 1979;
Brown et al. 2008). For this study, a salinity gradient in Breton Sound, LA (3 sites) and a high salinity site in Grand Isle, LA were used to represent variable environmental conditions and determine which grow-out conditions were the most advantageous for improving growth and survival for increasing industry profitability.

In identifying the most suitable oyster stock(s) and ideal grow-out condition(s), this project aims to ultimately promote sustainability by augmenting wild harvest and improve industry profitability through selecting superior stocks based on their performance in different conditions.

Methods

Oysters

The dermo-resistant oysters (i.e., OBOYs) are the descendants of large oysters, collected in 1999 from dermo endemic areas (i.e., Oyster Bayou, Cameron Parish). Their progeny has been challenged in the field (F0) and in the laboratory (F1 and F2) with *P. marinus* for two subsequent generations. These oysters are considered to have acquired enhanced resistance against dermo because; 1) they show decreased mortality (<20% mortality over three years) (J. La Peyre pers. comm.) and grown to a large size despite the presence of *P. marinus* in field testing off Grand Isle; and, 2) they have exhibited significant delayed progression of infection and mortality compared to ‘control’ oysters collected in areas where *P. marinus* prevalence and intensity have historically been low (J. La Peyre pers. comm.). The oysters that were used in this study were the F4 generation of the OBOY stock.

Two of the three wild stocks used in the study were collected in October and November 2010 from two public seed grounds from Sister (Caillou) Lake (29.234171°N; 90.917221°W) and Breton Sound (Bay Gardene) (29.5910°N 89.6425°W). Lake Calcasieu (29.0003°N;
90.2323°W) provided the third wild stock for the study. Historically, these grounds are known to have different salinity characteristics due to their differences in geographical location. The monthly mean salinities (i.e., 10 years) for each of these areas are provided below (Figure 2.1). All oysters collected were brought to the Sea Grant Oyster Research and Demonstration Farm in Grand Isle, LA (29.2278° N, 90.0122° W), where they were placed in labeled mesh bags held in an adjustable long line system (ALS) prior to spawning.

Figure 2.1: Historical (mean±SE) monthly salinity conditions for Sister (Caillou Lake), Lake Calcasieu, and Breton Sound (Bay Gardene) over the past 10 years.

All four stocks were spawned at the Louisiana Sea Grant Oyster Hatchery in Grand Isle, LA in May 2011 to produce an F0 generation for the wild stocks and an F4 generation for the OBOY stock. Each oyster stock (4 separate spawning events) was mass spawned (~150 oysters) by temperature induction to collect eggs and sperm from individual males and females.
Fertilization involved combining pooled eggs and pooled sperm from each stock to ensure genetic contributions from many individuals. The pooled eggs were placed in 1 µm-filtered ambient seawater (~15 ppt) for thirty minutes to allow for hydration. The pooled sperm were observed to ensure motility before pooling the gametes for fertilization. A target ratio of 10 sperm per egg (equatorial plane) was used to ensure successful fertilization. Zygotes were placed in 4,650L grow-out tanks after cleavage was observed in >80% of the brood. Larvae were maintained in these 4,650L grow-out tanks and fed a combination of *Isochrysis* aff. *galbana* Clones TISO and/or CISO, *Chaetoceros* aff. *muelleri* Clone CGRA, and *Chaetoceros* aff. *calcitrans* Clone CCAL during rearing. Pediveliger (~280µm) larvae were set on micro-cultch material (~500µm in size) to produce single oysters. After 48 hours, the resulting spat were transferred to an upwell nursery system, where they were grown to 25 mm in shell height (i.e., seed oysters) prior to placement in the ALS at the Sea Grant Oyster Research and Demonstration Farm in Grand Isle, LA. Seed oysters were held in the ALS until final deployment in November 2011. The mean shell heights (mm±SD) for the four stocks were 41.7±7.1 for the OBOYS (OB), 46.6±6.9 for Lake Calcasieu (LC), 47.6±6.7 for Sister Lake (SL), and 48.6±8.0 for Breton Sound (BS).

**Study Areas**

The oysters were deployed at three different sites along a low to intermediate salinity gradient in the Breton Sound estuary in Southeast Louisiana and at a high salinity site at the Sea Grant Oyster Research and Demonstration Farm in Grand Isle, LA.

Breton Sound is a 271,000 ha estuary in the Mississippi River deltaic plain. The estuary consists of microtidal bays, bayous, and canals that contain various marsh types including fresh, intermediate, brackish, and salt marshes. The area is subject to flooding from the Caenarvon
Freshwater Diversion (CFD), which was designed to moderate salinities and reintroduce controlled river inflows to the estuary. Pulses from this diversion can deliver substantial amounts of freshwater and have a significant impact on salinity regimes within the estuary (Snedden et al. 2007; La Peyre et al. 2009; Eberline 2012; La Peyre 2013 submitted). Other freshwater diversions that influence salinity regimes in the estuary include White Ditch, Bayou Lamoque, and Bohemia (P. Banks pers. comm.).

Grand Isle is a barrier island located between the Gulf of Mexico and the Barataria estuary. The area generally exhibits higher salinity conditions from saltwater influx from the Gulf of Mexico.

The sites were chosen based on historical data (Figure 2.2) from USGS real-time monitoring stations located in Breton Sound and Barataria Pass, which is adjacent to Grand Isle. In Breton Sound, Cow Bayou (CB) (29.5768°N 89.7103°W) was the low salinity site (~5 ppt), Bay Gardene (BG) (29.5910°N 89.6425°W) was the low-intermediate salinity site (~10 ppt), and Mozambique Point (MP) (29.3718°N 89.2912°W) was the intermediate salinity site (~15 ppt). Grand Isle (GI) (29.2278° N, 90.0122° W), which is a barrier island bordering the Gulf of Mexico, was the high salinity site (~20+ ppt) (Figure 2.3).

Experimental Design

At each of the four sites, 4 ALS culture bags containing 75 oysters per bag were deployed for each stock, or three hundred oysters per stock per site, for a total of 1,200 oysters deployed at each site. A total of 4,800 oysters (1,200 per stock) were needed for the experiment along with 64 ALS culture bags. All bags used for the study were fully enclosed to prevent the risk of predation mortality. Since predation was largely removed, mortality could be more readily attributed to stressful abiotic conditions and *P. marinus*. Oysters deployed at the three sites in
Breton Sound were held off-bottom by fitting the ALS culture bags with 1’ PVC legs to elevate the bags off the bottom (Figure 2.4). The bags were arranged in four different orientations at each site as to eliminate a placement bias (Figure 2.5). Oysters deployed at the Sea Grant Oyster Research and Demonstration Farm in Grand Isle were placed in ALS culture bags and suspended beneath the surface in the ALS system. These bags were not air dried to maintain consistency with the bags deployed in Breton Sound. Each line of deployed bags were arranged differently to eliminate a placement bias. At all four sites, oyster growth (shell height) and mortality (counts of live/dead) data were collected bimonthly, starting in November 2011 and ending in November 2012, for a total of seven sampling periods.

Figure 2.2: Historical (mean±SE) monthly salinity conditions for Breton Sound sites: Cow Bayou, Bay Gardene, Mozambique Point, and Grand Isle over the past 10 years. Note: Mozambique Point historical data only represents data since January 2010.
Morphometrics, weights, condition index, and *P. marinus* infection intensities data were collected at the time of deployment to establish pre-deployment baselines for each stock (n=15 for each stock). Subsequent samplings were performed during the March, July, and September 2012 samplings to quantify any changes in these parameters over time. For each of these samplings, 15 oysters from each stock at each site were removed for laboratory analyses, with an attempt to select oysters without bias, for a total of 15 oysters per stock x 4 stocks per site x 4 sites per sampling period, or 240 oysters (60 oysters per stock) removed for each sampling period.
Figure 2.4: ALS longline bags fitted with 1’ PVC legs for deployment in Breton Sound. Bags used for growth, mortality, and lab analyses.

Figure 2.5: Bag orientations at each site in Breton Sound (different orientations to eliminate a placement bias).

Water Quality

Hourly water temperature and salinity data were collected from real-time monitoring stations over the course of one year (November 2011 to November 2012) to monitor the effects of water temperature and salinity at each site. USGS station 073745258 (Cow Bayou at American Bay) was used for temperature and salinity parameters for the low salinity site, USGS station 07374527 (Northeast Bay Gardene) was used for the low-intermediate salinity site, and USGS station 073802516 (Barataria Pass at Grand Isle) was used for the high salinity site (Figure 2.6). The intermediate site, Mozambique Point, lacks a USGS real-time monitoring station, so a continuous data recorder (YSI Incorporated, 600 OMS V2 SONDE, Yellow Springs,
OH, USA) was obtained from USGS in order to gather hourly temperature and salinity data at this site (Figure 2.7). A transportable display unit (YSI Incorporated, 650 Multiparameter Display System, Yellow Springs, OH, USA) was used to upload and sort the data for Mozambique Point. The temperatures and salinities for all four sites were compiled to form daily means for temperature and salinity parameters for the sampling year. Mean water temperatures and salinities were also calculated for each sampling interval, allowing for direct correlations to be made between each stock’s performance and changes in environmental conditions over the course of the study. All water quality parameters are expressed as a mean ± standard deviation per month unless otherwise stated.

Figure 2.6: USGS real-time monitoring station for collecting hourly temperature and salinity data.

Figure 2.7: YSI SONDE 600 OMS data recorder and YSI 650 MDS Display for collecting hourly temperature and salinity data at Mozambique Point.
Mortality and Growth

Growth was assessed by haphazardly selecting twenty-five oysters from each bag (100 per stock at each site) and measuring them with digital vernier calipers (level of precision: 0.01mm) (Mituyoto Corporation, ABS Coolant Proof Calipers, Kawasaki-shi, Kanagawa 213-8533, Japan) from the umbo to the furthest point in which shell is present (shell height). Monthly shell height growth rates were calculated with the formula:

\[
\frac{(H_t - H_{t-1})}{t-(t-1)} \times 30
\]

where:
- \( H_t \): average shell height
- \( t \): current sampling time
- \( t-1 \): previous sampling time
- 30: number of days/month

Mortality was assessed by recording the number of dead and live oysters in each bag for each sampling period. Interval mortality (IM) data was obtained by taking the mortality counts of the four bags containing each stock and averaging them together at each site. This provided a total stock mortality proportion (dead/total) for each stock within each of the four sites (one mortality proportion for each stock within each site). Interval mortality proportions were multiplied by 100 to obtain an interval mortality percentage for each sampling period. Since 15 oysters of each stock at each site were removed for laboratory analysis in March, July, and September of 2012, adjusted interval mortalities were obtained to account for these removed oyster samples. Adjusted interval mortalities (AIM) were calculated by taking the interval mortality proportion and multiplying it by (100-previous cumulative mortality). Cumulative mortality (CM) was then calculated by adding the previous cumulative mortality to the adjusted interval mortality for each sampling period using the formulas provided below. Dead oysters were discarded after each sampling to avoid double counting.

\[
IM_t = \frac{\text{dead}}{\text{total}} \times 100
\]
AIMₜ=(dead/total) x (100-CMₜ₋₁)
CMₜ=AIMₜ+CMₜ₋₁

t: current sampling time
t-1: previous sampling time

Disease and Condition Index

*P. marinus* infection intensity (i.e., parasites per gram of tissue) and condition index were determined as described by La Peyre et al. (2009) by sampling three to four oysters from each bag (15 oysters per stock per site). The number of parasites per gram of oyster wet tissue was determined using the whole-oyster procedure as described by Fisher and Oliver (1996) and modified by Nickens et al. (2002). Dermo infection intensity was represented using three levels of infection: Low (less than 10,000); Moderate (between 10,000 and 500,000); and, High (greater than 500,000 hypnospores (parasites) per gram of tissue). Oysters having high levels of infection were susceptible to mortality from the disease (Encomio et. al 2005). Median values were used to express infection intensity due to the possibility of obtaining skewed means from a few oysters having extreme levels of infection.

Each whole oyster sample was initially weighed and then shucked to access the soft tissue. The meat was removed and blotted to remove residual oyster liquor and the adductor muscle was removed and discarded. Wet shells of each sample were weighted at this time. Each meat sample was then added to 20 mL of sterile artificial seawater (SAS) at 15 ppt in a 50 mL test tube and weighed. Wet meat weights were then calculated by subtracting the weight of the meat, SAS, and 50 mL test tube from the weight of the SAS and 50 mL test tube, which was previously recorded. Meat and SAS were then homogenized using a BioSpec Bio-Homogenizer (BioSpec Products Inc., Bio-Homogenizer M 133/1281-0, Bartlesville, OK, USA). One milliliter (mL) of each homogenized sample was transferred to a 15 mL test tube containing 9 mL of
alternate Ray’s fluid thioglycollate medium (ARFTM) supplemented with lipid and chloramphenicol and mixed by vortexing. A .05 mL volume of nystatin suspension was gently layered over each sample to prevent fungal growth during hypnospore (parasite) expansion. After one week, the parasites were isolated by centrifuging at 1,500 x g for 10 minutes. The ARFTM supernatant was discarded and the pellet was resuspended by vortexing. Samples were then incubated in 10 mL of 2 N NaOH at 60°C for 4-6 hours to digest remaining tissue and leaving the hypnospores intact. Samples were then washed three times (centrifuging and discarding supernatant each time), first in 10 mL of deionized water and then twice again in 10 mL of 0.1 M phosphate buffer with 0.5 mg/mL of bovine serum albumin (BSA) to prevent hypnospore clumping. Samples were then serially diluted in 96 well plates, stained with Lugol’s Iodine working solution, and counted using a light microscope at 200x.

Condition index was determined by taking 10-mL aliquots of each oyster tissue homogenate (oyster + SAS) and drying them at 65°C for 48h. The weights of the aliquots were determined by subtracting the weight of the dry tissue and pan minus the weight of the pan. The dry weight for the whole oyster tissue was calculated based on the weight of the dried 10 mL aliquots x total volume of homogenized tissue in SAS, divided by 10.

\[ A_d \times V_t \div 10 \]

\( A_d \): weight of dried 10 mL aliquot
\( V_t \): volume of homogenized tissue in SAS

Final condition indices of each sample were calculated by taking the ratio of dry weight of tissue to the wet whole weight of the whole oyster minus the wet shell weight and multiplied by 100 to determine oyster condition index as recommended by Lucas and Beninger (1985) (See
All condition indices are reported as mean ± standard deviation unless otherwise stated.

\[ CI = \frac{\text{dry tissue weight}}{\text{wet (whole weight } – \text{ shell weight})} \times 100 \]

Statistical Analyses

SAS version 9.3 (SAS Institute Inc., SAS 9.3, Software, Cary, North Carolina, USA) and SigmaStat version 3.5 (Systat Software Inc. SigmaStat 3.5, San Jose, California, USA) were used to analyze the data. All figures were constructed using SigmaPlot version 9.0 (Systat Software Inc. SigmaPlot 9.0, San Jose, California, USA).

Shell heights and monthly growth rates of each stock were analyzed using a three-way ANOVA with stock, site, and time (i.e., month) as the main effects in the statistical model. Two-way and three-way interactions between these treatments were also determined. The three-way ANOVAs (Lenihan et al. 2001) were followed by post-hoc Tukey-Kramer pairwise comparisons (\(\alpha=.05\)) (Chu and La Peyre 1993), which were used to suggest areas of significance. All growth data is expressed as mean ± standard deviation unless otherwise stated.

Interval mortalities were calculated for each sampling period by taking the number of dead oysters and dividing by the total number of oysters for each stock at each site. A logistic regression analysis was performed on these proportions of dead/total to obtain predicted probabilities of mortality for each interval under a logit transformation (Sokal and Rohlf 1995). Cumulative mortality was compared and analyzed using a Chi-Square Analysis with a significance value of \(\alpha=.05\) (Dowdy and Wearden 1991).

Significant differences in condition for each stock were identified by using two and three-way ANOVAs (two-way: La Peyre et al. 2009; three-way: Lenihan et al. 2001) using the same treatment variables as in the growth ANOVAs (site and stock only for the two-way analysis).
Data was log transformed to satisfy the assumption of homogeneity of variance. The ANOVA tests were followed by post-hoc Tukey-Kramer pairwise comparisons ($\alpha=.05$) (Chu and La Peyre 1993) to identify areas of significance.

Infection intensities of each stock were analyzed by log transforming the data and using a Kruskal-Wallis one-way ANOVA on Ranks (Chan and Walmsley 1997). Paired Dunn’s tests ($\alpha=.05$) were used to find any areas of significance.

Results

Water Quality

Monthly mean water temperatures were not significantly different among sites from November 2011 to November 2012 (Table 2.1; Figure 2.8). Salinity, however, did vary among sites with Grand Isle (21.6 ± 5.8) having significantly higher salinity than Mozambique Point (13.0 ± 5.28), Bay Gardene (10.4 ± 4.9), and Cow Bayou (4.9 ± 3.0) (ANOVA, $p=.0001$). Salinity for Mozambique Point and Bay Gardene differed slightly between sites, but were not significantly different from each other ($p=.7497$). Cow Bayou has significantly lower salinity the all other sites (ANOVA, $p=.0001$) (Table 2.2; Figure 2.9).

Table 2.1: Temperature (°C) at field sites based on hourly daily means taken from continuous data recorders located at each field station. Mean (SD) cumulative oyster mortality (%) for each stock at each site is also presented. The number of days that temperature was recorded was based on daily means for each site. Data are from continuous recorders from Nov. 4, 2011 to Oct. 26, 2012 for sites in Breton Sound (CB, BG, and MP) and from Oct. 12, 2011 to Nov. 28, 2012 for the site in Grand Isle (GI). Sites with less than 358 days for Breton Sound sites and 412 days for Grand Isle are due to days when the recorders failed to collect the data.

<table>
<thead>
<tr>
<th>Site</th>
<th>Temperature (°C)</th>
<th>No. of days temp. level recorded</th>
<th>Approximate Cum. Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min/max</td>
<td>Mean(SD)</td>
<td>&lt;12°C</td>
</tr>
<tr>
<td>CB</td>
<td>9.3/32.0</td>
<td>23.0 (6.0)</td>
<td>13</td>
</tr>
<tr>
<td>BG</td>
<td>10.4/31.7</td>
<td>23.1 (6.0)</td>
<td>12</td>
</tr>
<tr>
<td>MP</td>
<td>10.0/34.3</td>
<td>23.6 (6.3)</td>
<td>14</td>
</tr>
<tr>
<td>GI</td>
<td>12.5/32.2</td>
<td>23.6 (5.4)</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 2.8: Mean daily water temperatures and water temperature ranges for Cow Bayou (CB), Bay Gardene (BG), Mozambique Point (MP), and Grand Isle (GI).

Table 2.2: Salinity (ppt) at field sites based on hourly daily means taken from continuous data recorders located at each field station. Mean (SD) cumulative oyster mortality (%) for each stock at each site is also presented. The number of days that salinity was recorded was based on daily means for each site. Data are from continuous recorders from Nov. 4, 2011 to Oct. 26, 2012 for sites in Breton Sound (CB, BG, and MP) and from Oct. 12, 2011 to Nov. 28, 2012 for the site in Grand Isle (GI). Sites with less than 358 days for Breton Sound sites and 412 days for Grand Isle are due to days when the recorders failed to collect the data.

<table>
<thead>
<tr>
<th>Site</th>
<th>Salinity (ppt)</th>
<th>No. of days salinity level recorded</th>
<th>Approximate Cum. Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min/max Mean(SD)</td>
<td>&lt;2 ppt</td>
<td>&lt;3 ppt</td>
</tr>
<tr>
<td>CB</td>
<td>1.0/16.5 4.9 (3.0)</td>
<td>72</td>
<td>42</td>
</tr>
<tr>
<td>BG</td>
<td>1.6/26.2 10.4 (4.9)</td>
<td>3</td>
<td>21</td>
</tr>
<tr>
<td>MP</td>
<td>3.2/24.9 13.0 (5.3)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>GI</td>
<td>8.9/36.3 21.6 (5.8)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Figure 2.9: Mean daily salinity (ppt) and salinity ranges for Cow Bayou (CB), Bay Gardene (BG), Mozambique Point (MP), and Grand Isle (GI).

Figure 2.10: Mean water temperatures and salinities for each sampling interval at the low salinity site: Cow Bayou (CB).
Figure 2.11: Mean water temperatures and salinities for each sampling interval at the low-intermediate salinity site: Bay Gardene (BG).

Figure 2.12: Mean water temperatures and salinities for each sampling interval at the intermediate salinity site: Mozambique Point (MP).
Mortality

In low salinity, the OBOY stock had significantly higher cumulative mortality (83.5%) than the Sister Lake (p=.001), Lake Calcasieu (p=.002), and Breton Sound stocks (p=.016). The Sister Lake stock (59.3%) had significantly lower cumulative mortality than the Lake Calcasieu (p=.001) and Breton Sound stocks (p=.001) (Figure 2.14).

In low-intermediate salinity, the Breton Sound stock (17.2%) had significantly higher cumulative mortality than both the Sister Lake (4.7%) (p=.001) and Lake Calcasieu stocks (8.7%) (p=.001). The OBOY stock (15.3%) had significantly higher mortality than the Sister Lake (p=.001) and Lake Calcasieu stocks (p=.005), but was not significantly different from the Breton Sound stock (p=.581) (Figure 2.15).
In intermediate salinity, the OBOY stock (15.1%) had significantly higher cumulative mortality than the Lake Calcasieu (7.5%) (p=.004) and Sister Lake stocks (6.9%) (p=.002), but was not significantly different from the Breton Sound stock (p=.701). The Breton Sound stock (13.6%) had significantly higher cumulative mortality than the Sister Lake (p=.008) and Lake Calcasieu stocks (p=.018) (Figure 2.16).

In high salinity, the Breton Sound stock (26.4%) had significantly higher mortality than the Sister Lake (18.7%) (p=.039) and Lake Calcasieu stocks (13.4%) (p=.001). The OBOY stock (23.3%) had significantly higher mortality than the Lake Calcasieu stock (p=.003), but did not differ from the Sister Lake stock (p=.211). In this high salinity site, a steady increase in mortality was observed in all of the four stocks. A trend was observed such that the Breton Sound and OBOY stocks had consistently higher mortalities than the Sister Lake and Lake Calcasieu stocks (Figure 2.17).

Figures 2.14: Cumulative mortalities of the four stocks (OB, LC, SL, and BS) at the low salinity site: Cow Bayou (CB).
Figures 2.15: Cumulative mortalities of the four stocks (OB, LC, SL, and BS) at the low-intermediate salinity site: Bay Gardene (BG).

Figures 2.16: Cumulative mortalities of the four stocks (OB, LC, SL, and BS) at the intermediate salinity site: Mozambique Point (MP).
Figures 2.7: Cumulative mortalities of the four stocks (OB, LC, SL, and BS) at the high salinity site: Grand Isle (GI)

In low salinity, predicted probabilities of mortality were low from November 2011 to March 2012. The highest levels of predicted mortality occurred from March to May and ranged from the Sister Lake stock having the lowest predicted mortality of 46.1% to the OBOY stock having the highest predicted mortality of 59.3%. Predicted mortalities were lower from May to July, but then escalated to higher levels from July to September, with the Sister Lake stock having the lowest predicted mortality of 22.1% and the OBOY stock having the highest predicted mortality of 34.6% (Figure 2.18).

In low-intermediate salinity, predicted probabilities of mortality were low from November 2011 to March 2012. The highest levels of predicted mortality occurred from March to May and ranged from the Sister Lake stock having the lowest predicted mortality of 4.5% to the OBOY stock having the highest predicted mortality of 8.0%. Predicted mortalities were lower from May to July, but then rose to higher levels from July to September with the Sister
Lake stock again having the lowest predicted mortality of 1.5% and the OBOY stock having the highest predicted mortality of 2.8%. Mortality for all sampling intervals was lower at the low-intermediate salinity site when compared to the low salinity site (Figure 2.19).

The intermediate salinity site had comparable interval mortalities to that of the low-intermediate salinity site. The predicted probabilities of mortality were low from November 2011 to March 2012. The highest levels of predicted mortality occurred from March to May and ranged from the Sister Lake stock having the lowest predicted mortality of 3.5% to the OBOY stock having the highest predicted mortality of 6.3%. Predicted mortalities were lower from May to July, but then slightly rose to higher levels from July to September with the Sister Lake stock again having the lowest predicted mortality of 1.2% and the OBOY stock having the highest predicted mortality of 2.2% (Figure 2.20).

Figures 2.18: Predicted interval mortalities of the four stocks (OB, LC, SL, and BS) at the low salinity site: Cow Bayou (CB).
Figures 2.19: Predicted interval mortalities of the four stocks (OB, LC, SL, and BS) at the low-intermediate salinity site: Bay Gardene (BG).

Figures 2.20: Predicted interval mortalities of the four stocks (OB, LC, SL, and BS) at the intermediate salinity site: Mozambique Point (MP).
In high salinity, the predicted probabilities of mortality were low from November 2011 to March 2012. The highest levels of predicted mortality occurred from March to May and ranged from the Sister Lake stock having the lowest predicted mortality of 8.4% to the OBOY stock having the highest predicted mortality of 14.6%. Predicted mortalities were lower from May to July, but then increased to higher levels from July to September with the Sister Lake stock again having the lowest predicted mortality of 3.0% and the OBOY stock having the highest predicted mortality of 5.4% (Figure 2.21).

Figures 2.21: Predicted interval mortalities of the four stocks (OB, LC, SL, and BS) at the high salinity site: Grand Isle (GI).

In regards to the observed mortalities, the Sister Lake and Lake Calcasieu stocks were consistently lower than that of the OBOY and Breton Sound stocks for all sampling intervals, suggesting that the Sister Lake and Lake Calcasieu stocks are more likely to survive, regardless of the sites in which they were reared (Figures 2.22, 2.23, 2.24, and 2.25).
Figures 2.22: Observed interval mortalities of the four stocks (OB, LC, SL, and BS) at the low salinity site: Cow Bayou (CB).

Figures 2.23: Observed interval mortalities of the four stocks (OB, LC, SL, and BS) at the low-intermediate salinity site: Bay Gardene (BG).
Figures 2.24: Observed interval mortalities of the four stocks (OB, LC, SL, and BS) at the intermediate salinity site: Mozambique Point (MP).

Figures 2.25: Observed interval mortalities of the four stocks (OB, LC, SL, and BS) at the high salinity site: Grand Isle (GI).
Growth

There were a few factors that may have influenced significance levels for shell heights and interval growth rates. A reduced number of oysters from each stock were measured at the low salinity site after May 2012, due to the high mortality levels that occurred from March to May 2012. Hurricane Isaac in late August 2012 influenced the experiment and removed ALS culture bag replicates from the low-intermediate and intermediate salinity sites. Measurements from May to November 2012 at the low salinity site and September to November 2012 at the low-intermediate and intermediate salinity sites are from reduced sample sizes and any significant differences found at these intervals were reviewed cautiously.

There were significant interactions between site*stock (ANOVA, p=.0001), site*month (ANOVA, p=.0001), and stock*month (ANOVA, p=.0038) in the analysis. There were significant differences found for all six sampling periods (January to November 2012) (ANOVA, p=.0001), such that growth in shell height was positively correlated with time. All sites (CB, BG, MP, and GI) were also significantly different (ANOVA, p=.0001). Growth in shell height was positively correlated with average site salinity, such that oysters in high salinity had the largest shell heights for all sampling periods. Oysters placed in intermediate salinity (MP) had smaller shell heights than oysters placed in high salinity. Oysters placed in low-intermediate salinity (BG) had even smaller shell heights than oysters placed in high and intermediate salinity conditions for each corresponding sampling time. Oysters placed in low salinity (CB) had the smallest shell heights of all four sites for the entirety of the study (Figures 2.26, 2.27, 2.28, and 2.29).
Figures 2.26: Observed shell heights of the four stocks (OB, LC, SL, and BS) at the low salinity site: Cow Bayou (CB). Note: horizontal line at 75 mm in shell height indicates commercial-size.

Figures 2.27: Observed shell heights of the four stocks (OB, LC, SL, and BS) at the low-intermediate salinity site: Bay Gardene (BG). Note: horizontal line at 75 mm in shell height indicates commercial-size.
Figures 2.28: Observed shell heights of the four stocks (OB, LC, SL, and BS) at the intermediate salinity site: Mozambique Point (MP). Note: horizontal line at 75 mm in shell height indicates commercial-size.

Figures 2.29: Observed shell heights of the four stocks (OB, LC, SL, and BS) at the high salinity site: Grand Isle (GI). Note: horizontal line at 75 mm in shell height indicates commercial-size.
In low salinity, there were a few significant differences found among stocks. For the January, March, and May 2012 samplings, the Breton Sound stock was significantly larger than the OBOY stock (Tukey-Kramer, p=.0001). The Sister Lake stock was also significantly larger than the OBOY stock in March and May 2012 (Tukey-Kramer, p=.0033). Despite these significant differences, the Breton Sound stock had a mean deployment size of 50.7±8.0 mm while the OBOY stock had a mean deployment size of 46.1±6.3 mm when first deployed at the low salinity site in November 2011. Significant differences that were found in January were maintained through until May, with minimal growth in all stocks occurring from January to July 2012. Significant differences in shell height growth between the Breton Sound and OBOY stocks at Cow Bayou are likely not biologically meaningful. The stocks placed in the low-intermediate, intermediate, and high salinities lacked any significant differences in shell height over the course of the study.

There was a significant interaction between site*month (ANOVA, p=.0001). Growth rates were positively correlated with average site salinity. In low salinity (Figure 2.30), growth rates were the lowest compared to the other three sites (ANOVA, p=.0001). Growth rates were low from March until September 2012 with mean growth rates for all stocks below 2 mm/month until July 2012. The low growth rates from March to July coincided with these intervals of low salinity conditions (2.2±1.7 ppt from March to May and 4.4±1.7 ppt from May to July). The highest growth rates occurred from September to November 2012 when salinity rose to 8.3±0.9 ppt for that interval. Growth rates in low-intermediate salinity were overall higher than the growth rates in low salinity (ANOVA, p=.0001). Growth rates were lowest from March to May 2012 with no stocks surpassing 2 mm/month. Growth rates did however increase from May to July 2012 and remained at these higher levels (3.3±0.6 mm/month) until November 2012. During
that time, growth rates slightly decreased from July to September 2012 (2.8±0.9 mm/month), but increasing to higher rates from September to November 2012 (3.9±0.8 mm/month) (Figure 2.31). In intermediate salinity, trends in growth rate were similar to the observed growth rates in low-intermediate salinity, with the overall growth rates being slightly higher, but not significantly different from the growth rates in low-intermediate salinity conditions (ANOVA, p=.7497) (Figure 2.32). Oysters in high salinity had the highest growth rates for each interval sampled (ANOVA, p=.0001) (Figure 2.33).

![Growth Rate Graph](image_url)

**Figures 2.30**: Observed growth rates of the four stocks (OB, LC, SL, and BS) at the low salinity site: Cow Bayou (CB).
Figures 2.31: Observed growth rates of the four stocks (OB, LC, SL, and BS) at the low-intermediate salinity site: Bay Gardene (BG).

Figures 2.32: Observed growth rates of the four stocks (OB, LC, SL, and BS) at the intermediate salinity site: Mozambique Point (MP).
Figures 2.33: Observed growth rates of the four stocks (OB, LC, SL, and BS) at the high salinity site: Grand Isle (GI).

Time (i.e., seasonal variation) was influential on growth rates (ANOVA, p=0.0001). For all the sites, growth rates followed a similar trend throughout the year. The highest growth rates generally were observed from November to January and the lowest growth rates were observed from March to May 2012. Only the high salinity area (GI) had elevated growth rates in March to May 2012 compared to the lower salinity areas in Breton Sound. The lowest growth rates in high salinity occurred in July to September 2012.

Among all four sites, the four stocks failed to have any significant differences in growth rates (ANOVA, p=0.2184). Given the lack of significant differences in the shell height growth analysis, all four stocks have similar growth performances in relation to the salinities (i.e., sites) in which they were placed.
Condition Index

The initial two-way ANOVA (stock and site) suggested that there were no significant differences in condition index among the oyster stocks prior to deployment in November 2011 (ANOVA, p=.1542).

The three-way ANOVA revealed significant interactions between site*stock (ANOVA, p=.0001), site*month (ANOVA, p=.0001), and stock*month (ANOVA, p=.0001). The analysis also suggested that there were significant differences in condition index regarding sampling times. Specifically, March 2012 had significantly higher condition indices than July and September (Tukey-Kramer, p=.0001). July 2012 was significantly different from September 2012 with condition indices being significantly higher in July (Tukey-Kramer, p=.0001).

There were significant differences in condition index among the four sites. Oysters in low salinity had significantly higher condition index than oysters in low-intermediate, intermediate, and high salinity conditions for both July and September 2012 (Tukey-Kramer, p=.0001). Oysters in low-intermediate and intermediate salinities lacked significant differences for all sampling times (Tukey-Kramer, p=.9870). Oysters in high salinity (GI) had significantly lower condition index than all sites having lower salinity conditions (MP, BG, and CB) for all sampling times (Tukey-Kramer, p=.0001).

Significant differences in condition index among stocks were found in July and September 2012. In low salinity during July 2012, the OBOY stock (14.6±2.3) had significantly higher condition index than the Breton Sound (11.2±1.6) (Tukey-Kramer, p=.0001), Lake Calcasieu (11.2±1.8) (Tukey-Kramer, p=.0054), and Sister Lake (11.1±2.2) stocks (Tukey-Kramer, p=.0002). Similarly in intermediate salinity during July, the OBOY stock (10.2±2.1) had significantly higher condition index than the Breton Sound stock (8.0±2.4) (Tukey-Kramer,
In low salinity during September 2012, the OBOY stock (12.9±1.8) had significantly higher condition index than the Breton Sound (10.3±2.2) (Tukey-Kramer, p=.0081), Lake Calcasieu (10.8±1.5) (Tukey-Kramer, p=.0016), and Sister Lake (10.0±2.1) stocks (Tukey-Kramer, p=.0001). Similarly in intermediate salinity during September 2012, the OBOY stock (7.8±1.9) had significantly higher condition index than the Breton Sound stock (6.4±1.3) (Tukey-Kramer, p=.0157) (Figure 2.36). No other significant differences were found among stocks for any other sites or time periods. Given these results, there is an apparent trend in condition index for the sampling months of July and September 2012, such that the OBOY stock generally had higher condition index than all the other stocks.

Figures 2.34: Condition index for the four stocks (OB, LC, SL, and BS) at the four sites: CB, BG, MP, and GI in March 2012.
Figures 2.35: Condition index for the four stocks (OB, LC, SL, and BS) at the four sites: CB, BG, MP, and GI in July 2012.

Figures 2.36: Condition index for the four stocks (OB, LC, SL, and BS) at the four sites: CB, BG, MP, and GI in September 2012.
Disease

There were no significant differences in infection intensity among the four stocks prior to deployment (Kruskal-Wallis, p > .05) (Figure 2.35). There were no significant differences in *P. marinus* infection intensity among stocks at each site for all sampling periods (November 2011, March, July, and September 2012) (Kruskal-Wallis, p > .05). There were however a few trends that need to be considered.

The time of year, and seasonal variation associated with time, was the largest influential factor in terms of *P. marinus* infection intensity. A trend in infection intensity was observed such that the lowest levels of infection were observed in March 2012 (Figure: 2.37). Infection intensity escalated to higher levels in July 2012 (Figure: 2.38) and increased to the highest observed levels in September 2012 (Figure 2.39). For this study, signs of *P. marinus* proliferation and increases in infection intensity began to occur in July and continued to occur through August and September 2012. Given that elevated mean monthly temperatures were observed during these summer months, *P. marinus* proliferation during these months can be attributed to these changes in environmental conditions.

A trend was also observed regarding salinity. Specifically, the salinities in which the oysters were placed influenced infection intensity, where higher salinity sites had greater infection levels. Oysters in low salinity had overall low infection intensities with no oyster samples having a moderate or high level of infection for any of the sampling times. Oysters in low-intermediate and intermediate salinities had higher infection intensities than oysters in low salinity. Specifically, oysters in low-intermediate salinity had low infection intensities in March 2012. In July 2012, the majority of the oysters maintained a low level of infection, except for the OBOY stock, which 13.3% had moderate infection levels. In September 2012, the majority of
the stocks maintained low infection levels except for the OBOY stock, which had 13.3% moderate infection and 33.3% heavy infection levels. The Sister Lake (13.3%) and Breton Sound (26.7%) stocks also exhibited moderate infection levels in September 2012. Oysters in intermediate salinity exhibited a trend, where slightly higher levels of infection were observed when compared to oysters in low-intermediate salinity at all sampling times. Specifically, oysters in intermediate salinity had low infection levels with the exception of the Sister Lake (6.7%) and Lake Calcasieu (6.7%) stocks having moderate infection levels in March 2012. Low infection intensities for the oyster in these salinities continued through to July 2012, where only the OBOY stock (13.3%) had heavy levels of infection. Infection intensity increased through September 2012, where the OBOY stock had 13.3% moderate infection and 33.3% heavy infection. Infection intensity also increased for the Sister Lake (13.3%) and Breton Sound (26.7%) stocks with both having moderate infection levels in September 2012. Oysters in high salinity exhibited a trend in having the highest infection intensity of all salinity conditions. Infection intensity was primarily low in March 2012 and only the OBOY (21.4%) and Sister Lake (13.3%) stocks had moderate infection levels. Infection intensity increased in all stocks in July 2012. Specifically, the OBOY stock had 21.4% moderate and 21.4% heavy infection, the Sister Lake stock had 20.0% moderate and 6.67% heavy infection, the Lake Calcasieu stock had 13.3% moderate infection, and the Breton Sound stock had 33.3% moderate and 20.0% heavy infection. Infection intensity continued to increase into September 2012 with all stocks having mainly moderate and heavy infections. For the OBOY stock, only 20.0% had low infection levels, while 26.7% had moderate infection and 53.3% had heavy infection. The Breton Sound stock had 20.0% low infection intensity, but primarily moderate and high infection levels of 53.3% and 26.7% were observed. The Lake Calcasieu stock had the lowest levels of infection
compared to the other three stocks. Specifically, 33.3% had low infection, 46.7% had moderate infection, and only 20.0% had high infection levels. The Sister Lake stock had the highest levels of infection for September 2012 with 46.7% having moderate infection and 53.3% having high infection intensity. In high salinity, the majority of each stock had either moderate to high infection intensity in September 2012. Though not significantly different, the Lake Calcasieu stock showed a trend of having the lowest levels of infection intensity, where *P. marinus* had its greatest influence. The OBOY and Breton Sound stocks had similarly higher levels of infection, while the Sister Lake stock had the highest level of infection when compared to the other three stocks in high salinity conditions.

Figures 2.37: *P. marinus* infection intensities for the four stocks (OB, LC, SL, and BS) at each site (CB, BG, MP, and GI) in March 2012.
Figures 2.38: *P. marinus* infection intensities for the four stocks (OB, LC, SL, and BS) at each site (CB, BG, MP, and GI) in July 2012.

Figures 2.39: *P. marinus* infection intensities for the four stocks (OB, LC, SL, and BS) at each site (CB, BG, MP, and GI) in September 2012.
Discussion

This study provides data on the performance of different oyster stocks under different salinity conditions. The data indicates that the use of different stocks in different salinity conditions can promote oyster survival, reduce *P. marinus* infection intensity, and potentially increase oyster production.

Mortality and Growth

In this study, the most influential factor in terms of oyster survival and growth was salinity, provided the uniform temperatures found among all sites throughout the sampling year. Previous findings from Heilmayer et al. (2008) suggested that the synergistic effects of low salinities (< 2 ppt) accompanied by elevated water temperatures (> 25°C) reduce growth and induce mortality. It is likely that oysters placed in low salinity had the highest cumulative mortality and lowest growth rates due to the low salinity conditions throughout the year, especially from March to May 2012 which was dominated by stressful temperature (25.5±2.5°C) and salinity (2.3±1.7 ppt) conditions. Previous studies by Loosanoff (1953, 1965) and Shumway (1996) also observed that feeding decreased when oysters are maintained at <3 ppt, resulting in mortality especially when coupled with high water temperatures. Valve closure and decreased feeding rates due to these salinity extremes could also suggest the insufficient growth and high mortality observed at the low salinity site. Furthermore, oysters placed in low-intermediate salinity, when compared to oysters placed in low salinity, experienced lower levels of mortality and elevated growth rates due to slightly higher salinity levels from March to May 2012 (25.3±2.4°C; 4.4±2.3 ppt), while oysters placed in intermediate salinity had slightly lower mortality and slightly higher growth rates to that of oysters in low-intermediate salinity for the same interval. Considering that there were drastic differences in cumulative mortality between
oysters placed in low salinity and low-intermediate salinity, there may be a distinct low salinity threshold for oyster survival in low salinity estuaries. Oysters placed in low-intermediate and intermediate salinities had the lowest cumulative mortalities of the four sites, while oysters placed in high salinity had the second highest levels of cumulative mortality after the oysters exposed to low salinity. Growth rates in high salinity, however, exceeded the growth rates in low-intermediate and intermediate salinities. Previous findings from Brown and Hartwick (1988) suggest high growth rates are associated with elevated levels of phytoplankton, increased water temperatures, and non-stressful salinity regimes. Additionally, a growth rate analysis by Kraeuter et al. (2007) in Delaware Bay, NJ suggested that growth rates at low salinity was much lower than growth rates in high salinity, despite the greater presence and intensity of *P. marinus* in high salinity areas. In this study, it is likely that the higher salinity conditions at Grand Isle accelerated growth rates though out the sampling year, however it has been reported that high salinities accompanied with high water temperatures can affect oyster growth and survival (Shumway 1996). High water temperatures and salinities from May to September 2012 at the Grand Isle site could explain the mortalities and decreased growth rates observed during the summer months. Alternatively, optimal conditions for *P. marinus* proliferation could have also contributed to these decreased growth rates, as described by Paynter and Burreson (1991), as well as increased levels in mortality during these intervals. When growth rates from this study were compared to the growth rates of oysters from the Chesapeake Bay north, higher growth rates appeared to occur in the Gulf of Mexico. This is not unexpected, given the extended growth season within the southern latitudes (Kraeuter et al. 2007).

In regards to cumulative mortality among stocks, the Sister Lake and Lake Calcasieu stocks had lower levels of mortality than the OBOY and Breton Sound stocks in all salinity
conditions over the course of the sampling year. Furthermore, the predicted probabilities of mortality for each stock were strongly correlated to the observed mortalities in low, low-intermediate, and intermediate salinity sites (CB, BG, and MP) for each sampling interval. This was not the case, however, for the high salinity site (GI), in which predicted mortalities were greatest from March to May 2012, but there were greater observed mortalities from July to September 2012 and September to November 2012. The logistic model may not be sufficient in suggesting accurate predicted mortalities when sites have different environmental conditions or are located in different geographical areas. Never the less, the results of the interval mortality analysis supported the findings in cumulative mortalities, such that the Sister Lake and Lake Calcasieu stocks had lower mortality than the OBOY and Breton Sound stocks, regardless of location (i.e. salinity). Interestingly, there were no significant differences in shell height or growth rate among any of the stocks over the course of the sampling year, revealing that significant differences in mortality ultimately determined stock performance in the different salinities being evaluated. It was expected that the selected OBOY stock would out-perform the three wild stocks. It is possible that inbreeding depression in the selected stock may have inhibited its ability to survive when compared to the unselected stocks. Previous research comparing select and unselected stocks have shown unselected stocks can out-perform selected stocks (Paynter and Burreson 1991; Burreson 1991), however, it is difficult to determine if these mortalities are from inbreeding, genetic trade-offs associated with selection (Gaffney and Bushek 1996) or other environmental factors. Out-breeding the OBOY stock with the Lake Calcasieu stock will increase genetic diversity and may improve the OBOY stock’s performance. Also, considering the experiment was conducted in the Breton Sound estuary, the Breton Sound stock used in the study was expected to have an advantage given that it was reared in the same
conditions from which the parent stock was taken. Results from this study suggest that the Breton Sound stock may be an inferior stock when compared to other Louisiana oyster stocks. This may also provide an explanation for the current lack of wild oyster production in the Breton Sound estuary, however, heavy fishing effort and freshwater influx to the region (LDWF 2010), siltation of the estuary from the CFD, and the multiple hurricanes over the past decade are more likely to be contributors to this lack in oyster productivity in this particular estuary.

In regards to overall stock performance, the Sister Lake stock had the lowest cumulative mortality in low to intermediate salinities, while the Lake Calcasieu stock had the lowest cumulative mortality in high salinity. Interestingly, cumulative mortalities between the Sister Lake and Lake Calcasieu stocks appeared to converge in intermediate salinity (MP) from September to November 2012. Given the convergence in performance among these two stocks and the improved survival of each stock in different salinity conditions, the Lake Calcasieu stock may have improved survival over the Sister Lake stock in areas of higher salinity, while the Sister Lake stock may have improved survival over the Lake Calcasieu stock in areas of lower salinity. Historical data from USGS real-time monitoring station 08017118 in Lake Calcasieu exhibited mean salinity conditions of 19.6±5.7 ppt from November 2002 to 2012 while historical data from USGS real-time data monitoring station 07381349 in Sister (Caillou) Lake revealed that mean salinity conditions from November 2002 to 2012 were 11.8±5.3 ppt. Given the observed mortalities and their correlations to the historical data, it is likely that each stock’s performance is a reflection of the environmental conditions of its origin. A genetics review by Gaffney and Bushek (1996) suggested that oyster performance (i.e., physiological variation) can be a function of environmental conditions, genotype, or a synergistic effect of both factors. Bushek (1994) and Varney et al. (2009) also revealed that different geographical regions show
distinct differences in their response to susceptibility to disease. These differences, however, are among stocks separated by large distances, which exhibit differences among populations and sub-populations between different regions of the Atlantic and Gulf of Mexico coasts. Rose et al. (2006) examined spatial and temporal population structures with microsatellite loci on a smaller scale within the Chesapeake Bay. Their results indicated that though subtle, genetic differentiation was evident due to differences in geographical distance (spatially). A subtle pattern in isolation by distance was also observed (Rose et al. 2006). Given the results of these previous studies, it is apparent that environmental conditions have a great influence on oyster performance, but the effects of genotype should not be overlooked within unselected stocks or wild populations. The results of this study suggest local stock adaptation may be possible on smaller scales (i.e., within state waters) given the differences in mortality for each stock in different salinity conditions in relation to the historically different salinity conditions the parent stocks originated.

**Condition Index**

The OBOY stock exhibited a trend of higher condition index than all other stocks, specifically in July and September 2012 in low salinity. Similarly in intermediate salinity, the OBOY stock exhibited higher condition index than the Breton Sound stock in July and September 2012. Higher condition indices generally indicate greater health (Heilmayer et al. 2008), but the OBOY stock at in low salinity had the highest cumulative mortalities of all stocks at that site. Due to this finding, condition index parameters (dry meat weights and cavity volumes) were reviewed. In general, the OBOY stock had a smaller cavity volume and either equal or greater dry meat weights than the other wild stocks. The larger meat mass contained in a smaller volume elevated the condition index values for the OBOY stock when compared to the
wild stocks. Therefore, the OBOY stock was not necessarily ‘healthier’ than the other stocks in low or intermediate salinity given the stock’s elevated mortality levels at these sites.

Oyster condition is known to be affected by spawning (Mann 1978). It is also possible that the OBOY stock failed to spawn during the spring months (i.e., March to May) due to stress from low salinity. This would explain why the OBOY stock maintained its higher condition index throughout the summer months, where condition is generally lowered from spawning in the spring (Supan and Wilson 2001). Histology analyses could help define variations in condition index in terms of glycogen levels and gamete production for future studies.

Disease

There were no significant differences among stocks at any salinity condition (low-high) for each sampling time (March, July, and September 2012). *P. marinus* infection intensity remained low at low salinity throughout the study and was not a contributing factor to mortality at Cow Bayou. A study performed by La Peyre et al. in 2003 suggested that *P. marinus* proliferation accelerates at water temperatures of 25°C and at salinities greater than 20 ppt. Even though temperatures were optimal from April to September 2012, the sub-optimal salinity conditions limited *P. marinus* proliferation and infection at this site. It is also unlikely that *P. marinus* had an effect on mortality in low-intermediate to intermediate salinities given the low to moderate levels of infection intensity found at these sites. The lack of heavy *P. marinus* infection intensity found in the oysters placed in the Breton Sound estuary (CB, BG, and MP) suggests that they were not subject to *P. marinus* induced mortality.

*P. marinus* had the greatest effects on the oyster stocks placed in high salinity. A study by Brown et al. (2005) compared the performance (growth, mortality, and *P. marinus* infection) of different oyster stock from the Chesapeake Bay, North Carolina, South Carolina, and Louisiana,
as well as hybrids stocks derived from the Chesapeake Bay stock crossed with each of the other
stocks by location. Their results suggested that \textit{P. marinus} had the greatest effect on mortality at
the high salinity site and mortality from \textit{P. marinus} varied depending on oyster stock, suggesting
genetic variations among stocks regarding disease-resistance (Brown et al. 2005). In this study,
heavy levels of infection were found in all stocks in July 2012 with the Lake Calcasieu and Sister
Lake stocks having lower percentages of heavy infection than the OBOY and Breton Sound
stocks. Infection intensity only increased from July to September 2012 with the Lake Calcasieu
stock continuing to have the lowest percentage of heavy infection and Sister Lake and OBOY
stocks having the highest percentage of heavy infection. When correlated with mortality from
September to November 2012, the Lake Calcasieu stock had the lowest observed mortality
(2.46\%) while the Sister Lake stock had the highest observed mortality (8.86\%) for this interval.
Considering the elevated levels of heavy infection in the Sister Lake stock from November to
September 2012, \textit{P. marinus} may have a greater effect on mortality for the Sister Lake stock in
areas where \textit{P. marinus} is more prominent (i.e., higher salinities). Considering the lower
percentage of heavy \textit{P. marinus} infection and lower mortality for Lake Calcasieu stock from
September to November 2012, this stock exhibits signs of having a natural-resistance to the \textit{P.
marinus} disease. The results of this study and the results of Brown et al. (2005) suggest that
disease-resistance is a natural-selection process that can occur in oysters when pathogen presence
and exposure is high. Given its generally lower infection levels compared to the other three
stocks tested, it is recommended the Lake Calcasieu stock be used for hatchery seed production
in higher salinity conditions where \textit{P. marinus} becomes a more influential factor in oyster
mortality.
Chapter 3: An evaluation of off-bottom culture methods for eastern oysters (*Crassostrea virginica*)

**Introduction**

In the coastal waters of the northern Gulf of Mexico, the eastern oyster (*Crassostrea virginica*) plays an important ecological role and has high economic value (La Peyre et al. 2009; Coen and Grizzle 2007; Plunket and La Peyre 2005; Dame 1996). Historically, oyster populations formed large, structurally complex, intertidal and sub-tidal reefs off the coast of Louisiana (Bartol and Mann 1997; Hargis 1999). The harvest of these abundant and productive reefs has allowed for the Louisiana oyster industry to lead the nation in annual oyster production, accounting for 35% of the total annual oyster harvest in the nation from 1999 to 2009 (LDWF 2011; Wirth and Minton 2004). In recent years however, LDWF has observed reductions in the oyster resource on their public seed grounds (LDWF 2011). Additionally in the last 15 years, there has been a significant increase in the harvest of market-sized (sack) oysters from these reefs (LDWF 2011). Traditional harvesting methods, including hand tongs and dredges, are known to decrease structural complexity on oyster reefs (Lenihan and Peterson 2004) and have created concern for potential net deficits of shell on these grounds and lease degradation (LDWF 2011).

Currently, the Louisiana oyster industry relies fully on wild oyster populations for both seed and market-sized oyster production. The availability of wild seed, however, is inconsistent and affected by natural fluctuations in reproduction and recruitment of wild oysters as well as unpredictable mortalities (as high as 50 to 85%) during grow-out on leases (Gulf States Marine Fisheries Commission 1991). Predation and disease are also contributors to oyster mortality in the Gulf of Mexico, specifically in terms of excessive mortality from the protozoan parasite dermo (*P. marinus*) (Craig et al. 1989, Soniat 1996) and predation from southern oyster drills.
and black drum (*Pogonias cromis*) at salinities above 15 ppt (Breithaupt and Dugas 1979; George et al. 2008). While these high annual mortality rates are generally offset by the almost continuous recruitment and rapid growth in the Gulf of Mexico, the economic impact of dermo and predation on the oyster industry has been substantial.

Natural and anthropogenic events have also contributed to the decline in Louisiana oyster populations. Hurricanes Katrina and Rita in 2005 extensively damaged oyster beds by siltation and contamination while simultaneously destroying thousands of fishing vessels (CRS Report for Congress 2005). Hurricanes Gustav and Ike in 2008 and Isaac in 2012 likely imposed similar negative consequences to the resource and the industry. The BP Deep Water Horizon oil spill in spring and summer of 2010 induced large scale freshwater releases from the Mississippi River to prevent oil from reaching the estuaries along the Louisiana coast. The sudden decrease in salinity combined with elevated water temperatures during summer months of 2010 created stressful conditions leading to high mortality and minimal growth and recruitment in Breton Sound, LA (Eberline 2012). More recently in 2011, record high levels of the Mississippi River created similar environmental conditions, challenging oyster survival and recruitment for another subsequent year (Eberline 2012). The combined effects of over-harvesting and lease destruction, predation and disease, natural disasters, and anthropogenic influences have driven the oyster populations across southern Louisiana to historically low levels (LDWF 2010).

In Louisiana, extensive aquaculture (i.e., seed bedding and dredging) is currently the only method used for commercial production. Industry profitability is currently limited due to a lack of technological options for oyster aquaculture (Supan 2000). Using intensive oyster aquaculture, which relies on hatchery-produced oyster seed and intensive grow-out methods, is a viable option for augmenting wild oyster production and potentially increasing profitability in the
industry. Advantages in using intensive culturing methods, specifically off-bottom cage culture, include improvements in growth, reductions in predator-related mortality, and opportunities to control bio-fouling. Cage culture permits intertidal placement of oysters such that oyster culture can take place in areas where the natural bottom is unsuitable for traditional, on-bottom cultivation (i.e., mud bottom and intertidal zones). Also, oysters cultured in intertidal zones, where they have been uniquely adapted to survive, have been reported to have accelerated growth, improved survival, and greater marketability through improved shell and meat quality when compared to extensively grown oysters (Ogasawara et al. 1962, Gillmor 1982, Ventilla 1984; Littlewood 1988; Crosby et al. 1991; Littlewood et al. 1992; O’Beirn et al. 1994; Handley 1997; Moroney and Walker 1999; Leggett 2000; Swartzenberg et al. 1997).

The goal of this study was to identify the most suitable off-bottom grow-out system for stimulating oyster production within the oyster industry. The three intensive culture methods that were reviewed in this study are currently being used for commercial oyster production outside of the Gulf of Mexico: adjustable longline system (ALS), the OysterGro™ system, and the floating bag system. The objective of this study was to evaluate these three off-bottom cage methods in two different environments to suggest the most optimal off-bottom grow-out system(s) in response to the differences in environmental conditions, using the following hypotheses.

\[ H_0: \mu_{ALS} = \mu_{OysGro} = \mu_{Floating Bags} \]

\[ H_A: \mu_{ALS} \neq \mu_{OysGro} \neq \mu_{Floating Bags} \]

In identifying the most effective intensive grow-out system(s), this project aims to ultimately promote sustainability by augmenting wild harvest and improve industry profitability through greater quality and marketability of the oysters produced from intensive aquaculture methods.
Methods

Oysters

The dermo-resistant oysters (OBOYs) are the descendants of large oysters, collected in 1999 from dermo endemic areas (i.e., Oyster Bayou, Cameron Parish). Their progeny has been challenged in the field (F0) and in the laboratory (F1 and F2) with *P. marinus* for two subsequent generations. These oysters are considered to have acquired enhanced resistance against dermo because 1) they show decreased mortality (<20% mortality over three years) (J. La Peyre pers. comm.) and grown to a large size despite the presence of *P. marinus* in field testing off Grand Isle - and - 2) they have exhibited significant delayed progression of infection and mortality compared to ‘control’ oysters collected in areas where *P. marinus* prevalence and intensity have historically been low (J. La Peyre pers. comm.). The oysters that were used in this study were the F4 generation of the OBOY stock.

The wild stock used in the study was collected from a Louisiana public oyster reef in Lake Calcasieu in Oyster Bayou, Cameron Parish. All oysters collected were brought to the Sea Grant Oyster Research and Demonstration Farm in Grand Isle, LA where they were placed in labeled bags held in an adjustable longline system (ALS) prior to spawning.

The two stocks were spawned at the Louisiana Sea Grant Oyster Hatchery in Grand Isle, LA in May 2011 to produce an F0 generation for the Lake Calcasieu stock and an F4 generation for the OBOY stock. Each oyster stock (2 separate spawning events) was mass spawned (~150 oysters) by temperature induction to collect eggs and sperm from individual males and females. Fertilization involved combining pooled eggs and pooled sperm from each stock to ensure genetic contributions from many individuals. The pooled eggs were placed in 1 µm-filtered ambient seawater for thirty minutes to allow for hydration. The pooled sperm were observed to
ensure motility before pooling the gametes for fertilization. A target ratio of 10 sperm per egg (equatorial plane) was used to ensure successful fertilization. Zygotes were placed in 4,650L grow-out tanks after cleavage was observed in >80% of the brood. Larvae were maintained in these 4,650L grow-out tanks and fed a combination of Isochrysis aff. galbana Clones TISO and/or CISO, Chaetoceros aff. muelleri Clone CGRA, and Chaetoceros aff. calcitrans Clone CCAL during rearing. Pediveliger (~280µm) larvae were set on micro-cultch material (~500µm in size) to produce single oysters. After 48 hours, the resulting spat were transferred to an upwell nursery system, where they were grown to 25 mm in shell height (i.e., seed oysters) prior to placement in the ALS at the Sea Grant Oyster Research and Demonstration Farm in Grand Isle, LA. Seed oysters were held in the ALS until final deployment in November 2011. The mean shell heights (mm) for the two stocks prior to deployment were 41.7±7.1 for the OBOY (OB) stock and 46.6±6.9 for Lake Calcasieu stock (LC).

Study Areas

The off-bottom grow-out cages were deployed at two sites, each having different salinity conditions. The first site was an intermediate salinity site located at the Louisiana Universities Marine Consortium (LUMCON) in Cocodrie, LA (29.2469° N, 90.6614° W). The second site was a high salinity site located at the Sea Grant Oyster Research and Demonstration Farm in Grand Isle, LA (29.2278° N, 90.0122° W) (Figure 3.1). The sites were chosen based on historical data from a real-time monitoring data collecting meter at the LUMCON facility and the USGS real-time monitoring station 073802516: Barataria Pass at Grand Isle, which is adjacent to Grand Isle.
Figure 3.1: Study area map of south central Louisiana indicating the two study sites at LUMCON (LCN) in Cocodrie, LA and Grand Isle (GI) in Grand Isle, LA.

Water Quality

Hourly water temperature and salinity data were collected from real-time monitoring stations over the course of one year (November 2011 to November 2012) to monitor the effects of water temperature and salinity at each site. A privately owned, real-time monitoring station at LUMCON was used for water temperature and salinity data for the LUMCON site. Unfortunately, there were time intervals where this station did not record water temperature or salinity parameters, specifically from deployment in November 2011 to the first week in January 2012 and again from late March to mid July 2012. The nearby USGS real-time monitoring station 07381324 Bayou Grand Caillou at Dulac, LA (~12 miles north of the study site), therefore, was used to compare the limited LUMCON data. Simple linear regressions on water temperature and salinity were used to extrapolate these missing values. For water temperature, the two datasets (Dulac and LUMCON) were highly correlated ($r^2=.969$) and the missing temperature values were extrapolated using the equation:
Y=0.933 + (.957*X)

X: temperatures in Dulac, LA
Y: extrapolated values for water temperature in LUMCON

For salinity, the two datasets were correlated ($r^2=0.783$) and the missing LUMCON salinity data was extrapolated using the equation:

Y=4.391 + (0.888*X)

X: salinities in Dulac, LA
Y: extrapolated values for the salinity in LUMCON

USGS real-time monitoring station 073802516: Barataria Pass at Grand Isle was used to collect temperature and salinity data for the Grand Isle site. The temperatures and salinities for the two sites were compiled to form daily means for temperature and salinity parameters for the sampling year. Mean water temperatures and salinities were also calculated for each sampling interval, allowing for direct correlations to be made between the performance of each grow-out method and the changes in environmental conditions over the course of the study. All water quality parameters are expressed as mean ± standard deviation per month unless otherwise stated.

Gear Types

Three cages types were compared in the study. The adjustable longline system (ALS) is widely used by commercial oyster farmers in Australia and has been field tested at the Louisiana Sea Grant Oyster Research and Demonstration Farm off Grand Isle, LA for the past ten years. It consists of a series of mesh bags, attached to a long line cable, and supported by posts placed about 3 meters apart. The cable can be moved vertically to adjust the height at which the oyster bags are suspended. The bags can be suspended above the surface of the water to control bio-fouling (Figure 3.2).
Figure 3.2: Oyster bags hanging on an adjustable long-line purchased from BST Oyster Co., Cowell, South Australia and set up near shore the Louisiana Sea Grant Oyster Research and Demonstration Farm in Grand Isle, LA.

The OysterGro™ floating cages are being used successfully by oyster farmers along the Atlantic coast of Canada and are currently being introduced in the Chesapeake Bay. This wire mesh cage can hold up to six oyster bags and is suspended just below the surface by two air-tight floats (Figure 3.3). The cage can be flipped manually to expose the oysters to air and control bio-fouling.

Figure 3.3: OysterGro™ floating cage grow-out system (exposed and submerged).
Like the OysterGro™, floating bag systems are widely used for commercial grow-out on the Atlantic coast of Canada down to Chesapeake Bay. This simple design, which attaches air-tight floats to an oyster culture bag, suspends the oysters at the surface (Figure 3.4). Considering portions of this system cannot be air-dried during use, fouling can become problematic and lead to increases in de-fouling efforts during grow-out.

Figure 3.4: Floating bag grow-out system.

Experimental Design

Each of the two sites consisted of eight bags on an ALS longline system, two OysterGro™ floating cages: each containing four bags, and eight floating bags tethered with gangions to a mainline. Seventy-five oysters were placed in each bag, while six hundred oysters (300 of each stock) were used in each grow-out method, for a total of 2,400 oysters deployed at each site. Oysters deployed in the ALS were suspended beneath the surface to maintain consistency with the bags deployed in the other grow-out methods such that all bags were continuously submerged between samplings. The bags containing the two stocks were alternately arranged for each grow-out method to eliminate a placement bias (Figures: 3.5-3.7). All bags for each grow-out system were pressure washed at each sampling to remove fouling organisms, prior to oyster removal for counting and measuring. Oyster growth and mortality data were collected bimonthly from November 2011 to November 2012. Morphometrics, weights, condition index,
and *P. marinus* infection intensities were collected at the time of deployment to establish pre-deployment baselines for the two stocks and three grow-out systems (n=15 for each stock). Subsequent samplings in March, July, and September 2012 were performed to quantify any changes in these parameters over time. For each of these samplings, 15 oysters from each stock, in each grow-out method, at each site were removed for laboratory analysis. A total of 180 unbiased selected oysters (30 oysters per grow-out method at each site) were removed for each sampling period.

Figure 3.5: Stock orientation for the Adjustable Longline System (ALS) at LUMCON and Grand Isle.

Figure 3.6: Stock orientation for the Floating Bag System (Fl. Bags) at LUMCON and Grand Isle.

Figures 3.7: Stock orientation for the OysterGro™ system (OysGro) at LUMCON and Grand Isle.
Mortality and Growth

Growth was assessed by haphazardly selecting twenty-five oysters from each bag (100 per stock at each site) and measuring them with digital vernier calipers (level of precision: 0.01mm) (Mituyoto Corporation, ABS Coolant Proof Calipers, Kawasaki-shi, Kanagawa 213-8533, Japan) from the umbo to the furthest point in which shell is present (shell height). Monthly shell height growth rates were calculated with the formula:

\[
\left[ \frac{H_t - H_{t-1}}{t-(t-1)} \right] \times 30
\]

H: average shell height
t: current sampling time
t-1: previous sampling time
30: number of days/month

Mortality was assessed by recording the number of dead and live oysters in each bag for each sampling period. Interval mortality (IM) data was obtained by taking the mortality counts of the four bags containing each stock and averaging them together at each site. This provided a total stock mortality proportion (dead/total) for each stock within each of the four sites (one mortality proportion for each stock within each site). Interval mortality proportions were multiplied by 100 to obtain an interval mortality percentage for each sampling period. Since 15 oysters of each stock at each site were removed for laboratory analysis in March, July, and September of 2012, adjusted interval mortalities were obtained to account for these removed oyster samples. Adjusted interval mortalities (AIM) were calculated by taking the interval mortality proportion and multiplying it by (100-previous cumulative mortality). Cumulative mortality (CM) was then calculated by adding the previous cumulative mortality to the adjusted interval mortality for each sampling period using the formula provided below. Dead oysters were discarded after each sampling to avoid double counting.

\[
IM_t = \frac{\text{dead}}{\text{total}} \times 100
\]
\[ AIM_t = \frac{\text{dead/total}}{t} \times (100 - CM_{t-1}) \]
\[ CM_t = AIM_t + CM_{t-1} \]

\( t \): current sampling time  
\( t-1 \): previous sampling time

**Disease and Condition Index**

*P. marinus* infection intensity (i.e., parasites per gram of tissue) and condition index were determined as described by La Peyre et al. (2009) by sampling three to four oysters from each bag (15 oysters per stock per site). The number of parasites per gram of oyster wet tissue was determined using the whole-oyster procedure as described by Fisher and Oliver (1996) and modified by Nickens et al. (2002). Dermo infection intensity was represented using three levels of infection: Low (less than 10,000), Moderate (between 10,000 and 500,000), and High (greater than 500,000 hypnospores (parasites) per gram of tissue). Oysters having high levels of infection were susceptible to mortality from the disease (Encomio et al. 2005). Median values were used to express infection intensity due to the possibility of obtaining skewed means from a few oysters having extreme levels of infection.

Each whole oyster sample was initially weighed and then shucked to access the soft tissue. The meat was removed and blotted to remove residual oyster liquor and the adductor muscle was removed and discarded. Wet shells of each sample were weighted at this time. Each meat sample was then added to 20 mL of sterile artificial seawater (SAS) at 15 ppt in a 50 mL test tube and weighed. Wet meat weights were then calculated by subtracting the weight of the meat, SAS, and 50 mL test tube from the weight of the SAS and 50 mL test tube, which was previously recorded. Meat and SAS were then homogenized using a Biospec biohomogenizer (BioSpec Products Inc., Bio-Homogenizer M 133/1281-0, Bartlesville, OK, USA). One mL of each homogenized sample was transferred to a 15 mL test tube containing 9 mL of alternate
Ray’s fluid thioglycollate medium (ARFTM) supplemented with lipid and chloramphenicol and mixed by vortexing. A .05 mL volume of nystatin suspension was gently layered over each sample to prevent fungal growth during hypnospore (parasite) expansion. After one week, the parasites were isolated by centrifuging at 1,500 x g for 10 minutes. The ARFTM supernatant was discarded and the pellet was resuspended by vortexing. Samples were then incubated in 10 mL of 2 N NaOH at 60 °C for 4-6 hours to digest remaining tissue and leaving the hypnospores intact. Samples were then washed three times (centrifuging and discarding supernatant each time), first in 10 mL of deionized water and then twice again in 10 mL of 0.1 M phosphate buffer with 0.5 mg/mL of bovine serum albumin (BSA) to prevent hypnospore clumping. Samples were then serially diluted in 96 well plates, stained with Lugol’s Iodine working solution, and counted using a light microscope at 200x.

Condition index was determined by taking 10-mL aliquots of each oyster tissue homogenate (oyster + SAS) and drying them at 65°C for 48h. The weights of the aliquots were determined by subtracting the weight of the dry tissue and pan minus the weight of the pan. The dry weight for the whole oyster tissue was calculated based on the weight of the dried 10 mL aliquots x total volume of homogenized tissue in SAS, divided by 10.

$$A_d \times V_t / 10$$

$A_d$: weight of dried 10 mL aliquot
$V_t$: volume of homogenized tissue in SAS

Final condition indices of each sample was calculated by taking the ratio of dry weight of tissue to the wet whole weight of the whole oyster minus the wet shell weight and multiplied by 100 to determine oyster condition index as recommended by Lucas and Beninger (1985). All condition indices are reported as mean ± error unless otherwise stated.

$$CI = \frac{\text{dry tissue weight}}{\text{wet (whole weight – shell weight)}} \times 100$$
Statistical Analyses

SAS version 9.3 (SAS Institute Inc., SAS 9.3, Software, Cary, North Carolina, USA) and SigmaStat version 3.5 (Systat Software Inc. SigmaStat 3.5, San Jose, California, USA) were used to analyze the data. All figures were constructed using SigmaPlot version 9.0 (Systat Software Inc. SigmaPlot 9.0, San Jose, California, USA).

Two simple linear regressions were used to extrapolate water quality parameters in LUMCON, where the dependent variable was the limited LUMCON data and the independent variable was the more complete USGS real-time monitoring station data at Dulac, LA. The regressions yielded equations that were used to extrapolate mean values for water temperature and salinity parameters.

Shell heights and monthly growth rates of each stock were analyzed using a three-way ANOVA with stock, site, and time (i.e., month) as the main effects in the statistical model. Two-way and three-way interactions between these treatments were also determined. The three-way ANOVA (Lenihan et al. 2001) were followed by post-hoc Tukey-Kramer pairwise comparisons ($\alpha=.05$) (Chu and La Peyre 1993), which were used to suggest areas of significance. All growth data is expressed as mean ± standard deviation unless otherwise stated.

Interval mortalities were calculated for each sampling period by taking the number of dead oysters and dividing by the total number of oysters for each stock for each grow-out method. A logistic regression analysis was performed on these proportions of dead/total to obtain predicted probabilities of mortality for each interval under a logit transformation (Sokal and Rohlf 1995). Cumulative mortality was compared and analyzed using a Chi-Square Analysis with a significance value of $\alpha=.05$ (Dowdy and Wearden 1991).
Significant differences in condition for each stock were identified by using two and three-way ANOVAs (two-way: La Peyre et al. 2009; three-way: Lenihan et al. 2001) using the same treatment variables as in the growth ANOVAs (site and stock only for the two-way analysis). Data was log transformed to satisfy the assumption of homogeneity of variance. The ANOVA tests were followed by post-hoc Tukey-Kramer pairwise comparisons (α=.05) (Chu and La Peyre 1993) to identify areas of significance.

Infection intensities of each stock were analyzed by log transforming the data and using a Kruskal-Wallis one-way ANOVA on Ranks (Chan and Walmsley 1997). Paired Tukey tests (α=.05) were used to find any areas of significance.

Results

Water Quality

Water temperatures were not significantly different among sites from November 2011 to November 2012 (Table 3.1; Figure 3.9). Salinity, however, did vary among sites with Grand Isle (22.1±5.8 ppt) exhibiting higher salinity conditions than LUMCON (12.6±4.6 ppt) (Table 3.2; Figure 3.10).

Table 3.1: Temperature (°C) at field sites is based on hourly daily means taken from continuous data recorders located at each field station. Mean (SD) cumulative oyster mortality (%) for each stock at each grow-out method is also presented. The number of days that temperature was recorded was based on daily means for each site. Data are from continuous recorders from Nov. 4, 2011 to Oct. 26, 2012 for sites in Breton Sound (CB, BG, and MP) and from Oct. 12, 2011 to Nov. 28, 2012 for the site in Grand Isle (GI). Sites with less than 358 days for Breton Sound sites and 412 days for Grand Isle are due to days when the recorders failed to collect the data.

<table>
<thead>
<tr>
<th>Site</th>
<th>Temperature (°C) Min/max (left)</th>
<th>Mean(SD) (right)</th>
<th>No. of days temp. level recorded</th>
<th>Cum. Mortality (%)</th>
<th>Site</th>
<th>ALS</th>
<th>OysGro</th>
<th>Fl. Bags</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCN</td>
<td>10.4/32.3</td>
<td>23.2 (5.7)</td>
<td>6 &lt;15 23 &lt;20 104 135 46</td>
<td>15.8 (1.5)</td>
<td>GI</td>
<td>12.5/32.2</td>
<td>23.6 (5.4)</td>
<td>0 &lt;8 105 96 132 42</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.0</td>
<td>22.0</td>
<td>8.7</td>
</tr>
</tbody>
</table>

Table 3.2: Salinity (ppt) at field sites is based on hourly daily means taken from continuous data recorders located at each field station. Data are from continuous recorders from Nov. 4, 2011 to Oct. 26, 2012 for sites in Breton Sound (CB, BG, and MP) and from Oct. 12, 2011 to Nov. 28, 2012 for the site in Grand Isle (GI). Sites with less than 358 days for Breton Sound sites and 412 days for Grand Isle are due to days when the recorders failed to collect the data.

<table>
<thead>
<tr>
<th>Site</th>
<th>Salinity (ppt) Min/max (left)</th>
<th>Mean(SD) (right)</th>
<th>No. of days salinity level recorded</th>
<th>Cum. Mortality (%)</th>
<th>Site</th>
<th>ALS</th>
<th>OysGro</th>
<th>Fl. Bags</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCN</td>
<td>22.1±5.8</td>
<td>23.6±4.6</td>
<td>6 &lt;15 20 &lt;25 130 185 46</td>
<td>15.8±1.5</td>
<td>GI</td>
<td>12.6±4.6</td>
<td>20.8±0.8</td>
<td>18.6 (0.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>12.0</td>
<td>22.0</td>
<td>8.7</td>
</tr>
</tbody>
</table>
Table 3.2: Salinity (ppt) at field sites is based on hourly daily means taken from continuous data recorders located at each field station. Mean (SD) cumulative oyster mortality (%) for each stock at each grow-out method is also presented (Mean (SD) of both stocks in each method). The number of days that salinity was recorded was based on daily means for each site. Data are from continuous recorders from Nov. 4, 2011 to Oct. 26, 2012 for sites in Breton Sound (CB, BG, and MP) and from Oct. 12, 2011 to Nov. 28, 2012 for the site in Grand Isle (GI). Sites with less than 358 days for Breton Sound sites and 412 days for Grand Isle are due to days when the recorders failed to collect the data.

<table>
<thead>
<tr>
<th>Site</th>
<th>Salinity (ppt)</th>
<th>No. of days salinity level recorded</th>
<th>Cum. Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min/max (left)</td>
<td>&lt;5</td>
<td>&lt;10</td>
</tr>
<tr>
<td></td>
<td>Mean(SD) (right)</td>
<td>ppt</td>
<td>ppt</td>
</tr>
<tr>
<td>LCN</td>
<td>4.6/5.0 (4.6)</td>
<td>0</td>
<td>159</td>
</tr>
<tr>
<td>GI</td>
<td>7.8/36.3 (5.8)</td>
<td>0</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 3.9: Mean daily water temperatures and water temperature ranges for LUMCON (LCN) and Grand Isle (GI).
Figure 3.10: Mean daily water salinities and salinity ranges (ppt) for LUMCON (LCN: solid line) and Grand Isle (GI: dashed line).

Figure 3.11: Mean water temperatures (°C) and salinities (ppt) for each sampling interval for LUMCON (LCN).
Mortality

In intermediate salinity (LCN), there were significant differences found among the two stocks and among the three grow out methods. Specifically, the Lake Calcasieu stock had 117 cumulative mortalities while the OBOY stock had 182, suggesting that Lake Calcasieu had significantly lower mortality than OBOY (p=.001) in intermediate salinity. The OysterGro™ (131 mortalities) had significantly higher cumulative mortality than the ALS (95 mortalities) (p=.008) and the floating bags system (73 mortalities) (p=.001). The floating bags system and ALS were not significantly different (p=.085), but the floating bags system had slightly lower mortality than the ALS. The floating bag system had the lowest cumulative mortality compared to the ALS and OysterGro™ in intermediate salinity (Figure 3.13).
Figures 3.13: Cumulative mortality of each grow-out method for each stock at the intermediate salinity site: LUMCON (LCN).

In high salinity (GI), there were also significant differences found among the two stocks and among the three grow-out methods. Similar to the results in intermediate salinity conditions, the Lake Calcasieu stock (162) had significantly fewer mortalities than the OBOY stock (200) (p=.026). These differences in stock influenced the performance of the grow-out methods and made it difficult to define which grow-out method had superior performance over the others. All three grow-out systems that were analyzed with the effect of the Lake Calcasieu stock lacked any significant differences between each other. Specifically, the ALS (49 mortalities) did not differ from the floating bag system (54 mortalities) (p=.706), the ALS did not differ from the OysterGro™ (59 mortalities) (p=.411), and the floating bag system did not differ from the OysterGro™ system (p=.734). There was a significant difference among the grow-out methods that were analyzed with the effect of the OBOY stock. Specifically, the OysterGro™ (54 mortalities) had significantly lower cumulative mortality than the ALS (75 mortalities) (p=.047).
The floating bag system (71 mortalities) did not differ significantly from either the ALS (p=.775) or the OysterGro™ (p=.108) (Figure 3.14).

Figures 3.14: Cumulative mortality of each grow-out method for each stock at the high salinity site: Grand Isle (GI).

There were other significant differences found among the different stocks and the different methods, but these differences reflected variations in stock performance and were unrelated in terms of suggesting differences in grow-out method performance. For example, ALS containing Lake Calcasieu oysters had 83.6% survival while ALS containing OBOY oysters had 75% survival, suggesting that the effect of stock greatly influenced the performance of the ALS grow-out method for this site.

Interval mortalities in intermediate salinity were consistent with the cumulative mortalities for each stock and each grow-out method. Specifically, there were slightly higher observed and predicted probabilities of mortality for the OBOY stock as opposed to the Lake
Calcasieu stock for all sampling intervals in each grow-out method (November 2011 to November 2012) (Figures 3.15 and 3.16). The floating bag system had the lowest predicted probabilities of mortality, followed by the ALS, and then the OysterGro™, which had the highest levels of predicted mortality in intermediate salinity (Figure 3.15).

Figures 3.15: Predicted probabilities of mortality for the grow-out methods for each stock at the intermediate salinity site: LUMCON (LCN).

In high salinity, there were slightly higher observed and predicted probabilities of mortality for the OBOY stock versus the Lake Calcasieu stock for all sampling intervals in each grow-out method. The predicted probabilities of mortality did not differ between grow-out methods at this high salinity site (Figures 3.17 and 3.18).
Figures 3.16: Observed mortality at each interval for the grow-out methods for each stock at the intermediate salinity site: LUMCON (LCN).

Figures 3.17: Predicted probabilities of mortality for the grow-out methods for each stock at the high salinity site: Grand Isle (GI).
Figures 3.18: Observed mortality at each interval for the grow-out methods for each stock at the high salinity site: Grand Isle (GI).

Growth

There were no significant differences in shell height among the two stocks in intermediate salinity (ANOVA, p=.2414). With this lack of differentiation among stocks, differences between each grow-out method were more clearly defined. Significant interactions were found between stock*method (ANOVA, p=.0477), stock*month (ANOVA, p=.0488), and method*month (ANOVA, p=.0002). There were significant differences found among the grow-out methods (ANOVA, p=.0001). Specifically, the ALS and floating bags system were not significantly different and had similar shell heights for each method at each sampling time (Tukey-Kramer, p=.8542). Both systems however had significantly greater growth in shell height than the OysterGro™ (Tukey-Kramer, p=.0001) over the course of the study (Figure 3.19).
Figures 3.19: Cumulative growth in shell height for the grow-out methods for each stock at the intermediate salinity site: LUMCON (LCN). Note: horizontal line at 75 mm in shell height indicates commercial-size.

In high salinity, significant interactions were found between method*month (ANOVA, p=.0001). The two oyster stocks were significantly different such that the OBOY stock had greater growth in shell height than the Lake Calcasieu stock (ANOVA, p=.0005). The ALS had significantly greater growth in shell height for all sampling periods (Tukey-Kramer, p=.0001).

For sampling periods in April, October, and November 2012, the ALS had significantly greater growth in shell height than the floating bag system (Tukey-Kramer, p=.0378), while the floating bag system was not significantly different from the OysterGro™ for those same sampling periods (Tukey-Kramer, p=.1125). For sampling periods January, May, and July 2012, the ALS was not significantly different from the floating bag system. The floating bag system was not significantly different from the OysterGro™, except in July 2012, where the floating bags were significantly greater than the OysterGro™ (Tukey-Kramer, p=.0014). Given these results, the
ALS had the greatest shell heights of the three grow-out methods. Specifically, the ALS had significantly greater shell heights than the floating bag and OysterGro™ systems in April, October, and November 2012. As for January, May, and July 2012, the ALS had significantly greater shell heights than only the OysterGro™ (Figure 3.20).

Figures 3.20: Cumulative growth in shell height for the grow-out methods for each stock at the high salinity site: Grand Isle (GI). Note: horizontal line at 75 mm in shell height indicates commercial-size.

There were no significant differences in growth rate between stocks (ANOVA, p=.2732) or grow-out methods (ANOVA, p=.5526) for each sampling interval at the intermediate salinity site. There was however a significant interaction found between method*month (ANOVA, p=.0007) Likewise, there were no significant differences in growth rate between stocks (ANOVA, p=.7654) or grow-out methods (ANOVA, p=.0778) for each sampling interval at the high salinity site, but a significant interaction was found between method*month (ANOVA, p=.0197) (Figures 3.21 and 3.22).
Figures 3.21: Interval growth rates for the grow-out methods for each stock at the intermediate salinity site: LUMCON (LCN).

Figures 3.22: Interval growth rates for the grow-out methods for each stock at the high salinity site: Grand Isle (GI).
Condition Index

The initial two-way ANOVA (stock and site) suggested that there were no significant differences in condition index between the stocks prior to deployment in November 2011 (ANOVA, p=.9983).

The three-way ANOVA suggested that there were significant differences in condition index regarding sampling times for each site. Significant interactions were found between stock*method (ANOVA, p=.0067), stock*month (ANOVA, p=.0011), method*month (ANOVA, p=.0001), and stock*method*month (ANOVA, p=.0001) for the intermediate salinity site. For this site, March 2012 had significantly higher condition indices than August and October (Tukey-Kramer, p=.0001). August 2012 was significantly different from October 2012 with condition indices being significantly higher in August (Tukey-Kramer, p=.0002). Likewise in high salinity, March 2012 had significantly higher condition indices than August and October (Tukey-Kramer, p=.0001). Oysters sampled in August 2012 had significantly higher condition index than oysters sampled in October 2012 (Tukey-Kramer, p=.0001). Significant interactions for the high salinity site were found between stock*month (ANOVA, p=.0006) and among stock*method*month (ANOVA, p=.0074).

In intermediate salinity, the condition indices between stocks did not differ significantly (ANOVA, p=.6906), but there were significant differences among grow-out methods (ANOVA, p=.0001). For the grow-out methods containing the Lake Calcasieu stock, the ALS had significantly lower condition index than the floating bag system (Tukey-Kramer, p=.0017). Likewise, the ALS had significantly lower condition index than the OysterGro™ (Tukey-Kramer, p=.0018), while the floating bag and OysterGro™ systems did not differ statistically (p=1.000). For the grow-out methods containing the OBOY stock, the ALS (Tukey-Kramer,
p=.0001) and the floating bag system (Tukey-Kramer, p=.0082) had significantly lower condition than the OysterGro™ while the ALS and floating bag system did not differ significantly (Tukey-Kramer, p=1.000). In terms of stock, the Lake Calcasieu stock had significantly lower condition indices in the ALS when compared to the floating bag and OysterGro™ systems. Similarly with the OBOY stock, the adjustable long line and the floating bag systems had significantly lower condition indices than the OysterGro™, suggesting the OysterGro™ produces oysters yielding higher condition indices compared to the other two grow-out methods (Figure 3.23).

Both stocks reared in high salinity revealed different results for condition index. Specifically, the two stocks were significantly different such that the OBOY stock had higher condition index than the Lake Calcasieu stock (ANOVA, p=.0001). There were no significant differences, however, among grow-out methods found in high salinity (ANOVA, p=.4104) (Figure 3.24).

Figures 3.23: Condition Index for the grow-out methods for each stock for March, August, and October 2012 at the intermediate salinity site: LUMCON (LCN).
Disease

There were no significant differences in infection intensity among the two stocks prior to deployment (Kruskal-Wallis, p>.05). There were no significant differences in *P. marinus* infection intensity among stocks at either site for all sampling periods (November 2011, March, August and October 2012) (Kruskal-Wallis, p>.05). There were however a few trends that need to be considered.

The time of year, and seasonal variation associated with time, was the largest influential factor in terms of *P. marinus* infection intensity in high salinity (GI). At this site, infection intensity in March 2012 was low, but escalated to higher levels of infection in August 2012. Infection intensity continued to increase to even higher levels in September and October 2012. For this study, signs of *P. marinus* proliferation and increases in infection intensity began to occur in August and continued to occur through September and October 2012. In contrast, the
two stocks deployed in intermediate salinity, within each grow-out method, had relatively low infection levels for all three sampling periods. The data suggest that salinity level (i.e., site) had a large influence on *P. marinus* infection intensity (Figure 3.26).

There were no significant differences (Kruskal-Wallis, p > 0.05) in infection intensity among the two stocks or among the three grow-out methods at each site, but there were a few trends that need to be considered. In high salinity during August and October 2012, the grow-out methods all had relatively similar proportions of low, moderate, and heavy levels of infection. However the level of infection between the two stocks diverged. Specifically, the OBOY stock had higher levels of infection when compared to the Lake Calcasieu stock regardless of the grow-out method (except in the OysterGro™ in October 2012). This suggests that the OBOY stock may be more susceptible to the disease when conditions are ideal for *P. marinus* to proliferate.

![Graph showing infection levels for each stock and grow-out method in March, August, and October 2012 at the intermediate salinity site: LUMCON (LCN).](image)

Figure 3.25: *P. marinus* infection levels for the grow-out methods for each stock in March, August, and October 2012 at the intermediate salinity site: LUMCON (LCN).
Discussion

This study provides data on the performance of three difference off-bottom cage methods in different environmental conditions within two Louisiana estuaries. The results of this study indicate that the use of different intensive culture gears can have an effect on oyster performance, which can also vary, depending on the environmental conditions in which the oysters are deployed.

Mortality

The Lake Calcasieu stock outperformed the OBOY stock with less total mortality in both LUMCON and Grand Isle. These results concur with the results of the previous chapter. In some instances, primarily in Grand Isle, there was a significant interaction between oyster stock and grow-out method. These interactions were carefully reviewed in a manner that addressed the
effects of stock in order to suggest the performances of each grow-out method. At LUMCON, the floating bag and adjustable longline systems were advantageous over the OysterGro™ due to their significantly lower mortality. Similar results were found by Coddington et al. (2013) in an Alabama estuary, such that the OysterGro™ had significantly higher cumulative mortality (~50%) when compared to the ALS, floating bag, and LoPro™ cage systems. Coddington et al. (2013) also quantified bio-fouling removal time among the four methods. Despite the only significant difference found between the ALS and the OysterGro™, the differences may not have a significant economic impact. Given these finding by Coddington et al. (2013) and the results of this study, it is recommended that the ALS and the floating bag system be used over the OysterGro™ in intermediate salinity due to their significantly lower mortality levels and nominal differences in the time to remove the bio-fouling from the oysters. In Grand Isle, it was more difficult to suggest optimal grow-out methods due to the significant interactions between stock and method. The OBOY stock deployed in the OysterGro™ had significantly lower total mortality (54 mortalities) than the OBOY stock deployed in the ALS (75 mortalities). The Lake Calcasieu stock in the OysterGro™ (59 mortalities), though not significantly different, had slightly higher total mortality than the Lake Calcasieu stock in the floating bag (54) and ALS systems (49). Since the OBOY stock had greater survival in the OysterGro™ and the Lake Calcasieu stock had greater survival in the ALS and floating bag systems, the performance of the three grow-out methods in high salinity conditions are dependent on the oyster stock being used within each system. A study by Walton et al. (2013 submitted) suggested similar results such that there were significant differences among grow-out methods depending on the ploidy (2n or 3n) of the oysters within each grow-out method. When looking at only the results of the Lake Calcasieu stock in all three grow-out methods in this study, however, there are no significant
differences in cumulative mortality among the three different grow-out methods. Aside from the
significant differences among methods due to the effects of oyster stock, it is suggested that the
ALS, floating bag system, and the OysterGro™ are all suitable for use in high salinity areas. As
previously stated, Coddington et al. (2013) suggested that the OysterGro™ had significantly
higher mortality than the ALS, floating bag, and LoPro™ systems in an Alabama estuary. The
results of this study and the study by Coddington et al. (2013) have conflicting results regarding
the viability of the OysterGro™ in elevated salinity conditions. The effects of oyster stock and
the differences in environmental conditions between the two study areas may be more influential
than anticipated and must be considered when selecting a grow-out method for commercial
aquaculture.

Growth

Both the OBOY stock and the Lake Calcasieu stock lacked significant differences in shell
height growth at the intermediate salinity site. Significant differences in grow-out methods were
found, such that the adjustable longline and floating bag systems had significantly greater growth
in shell height over the OysterGro™. Specifically, oysters deployed in the ALS reached a mean
commercial-size in approximately 12 months after being spawned in the hatchery, where mean
commercial-size was the time at which both the OBOY and Lake Calcasieu stocks reached mean
market-size in the same grow-out method. Likewise, oysters in the floating bag system also
reached a mean commercial-size in around 12 months. The OysterGro™ produced mean
commercial-size oysters in 16 months, 4 months longer than the previous two culture systems.
Additionally, there were no significant differences in growth rates among the three grow-out
methods, however, this lack of significance could be a result of comparing the mean growth rates
of only four bags per stock (n=4), for each culture method, at each sampling period. In terms of
growth in shell height in intermediate salinity, it is recommended that the ALS and floating bag system be used over the OysterGro™. Similar results were found by Coddington et al. (2013) such that the ALS and floating bag system had significantly greater growth in shell height than the OysterGro™ and LoPro™ systems. Additionally, a study by Mallet et al. (2013) also revealed that different surface floating gears (floating bags and horizontal ropes) can produce different growth trajectories at the same culture sites. At the high salinity site, the OBOY stock had significantly greater growth in shell height over the Lake Calcasieu stock. More importantly, the ALS system appears to be the preferred grow-out method in high salinity conditions. For all seven sampling periods from November 2011 to November 2012, the ALS had significantly greater growth in shell height when compared to the OysterGro™. Sampling periods in March, September, and November 2012 suggested that the ALS also had significantly greater growth in shell height over the floating bags system for these periods. Specifically, oysters reared in the ALS reached mean commercial-size in a mere 10 months, while oysters reared in the floating bag and OysterGro™ systems reached mean commercial-size in 12 months, 2 months longer than oysters grown in the ALS. It is important to mention, however, that the oysters used in this study were only handled bimonthly for sampling and a handling treatment was not tested. In commercial aquaculture operations, optimizing husbandry methods (i.e. handling) is a critical element for increasing farm productivity (Robert et al. 1993; Handley 2002; Louro et al. 2007). Coddington et al. (2013) did test a handling treatment (i.e., tumbling) and found that tumbling significantly reduced shell height, but can had a positive effect on shell morphology metrics (i.e., creating oysters for the half-shell market). In regards to this study, it is expected that tumbling would have also increased the time in which it took the oysters to reach commercial-size, thus increasing the time to sell the final product. The degree of time invested in handling should be
further examined in order to identify an ‘optimized handling level’, similar to that of a study by Mallet et al. (2009) in New Brunswick, Canada, for improving potential intensive aquaculture operations in the northern Gulf of Mexico.

Condition Index

At LUMCON, the condition index of the OBOY and Lake Calcasieu stocks were not significantly different. There were significant differences in condition index, however, found between the three grow-out methods. The OysterGro™ had significantly greater condition index than the ALS, regardless of the effect of stock. The performance of the floating bag system varied depending on the stock of oysters being cultured. For the OBOY stock, the floating bag system had significantly lower condition index than the OBOY stock in the OysterGro™ and had a similar mean condition index to that of the ALS. For the Lake Calcasieu stock, the floating bag system had significantly greater condition index than the Lake Calcasieu stock in the ALS and was not significantly different from the OysterGro™. Given these results, the OysterGro™ yields the greatest condition index for oysters exposed to low salinity conditions. At Grand Isle, there were no significant differences in condition index between the three grow-out methods, but there was a significant difference among stocks. Specifically, the OBOY stock had higher condition index than the Lake Calcasieu stock.

Disease

There were no significant differences in P. marinus infection intensity between the two stocks or the three grow-out methods for any of the three sampling periods (March, July, and September 2012). It is likely that P. marinus infection was not a factor in variations in the performance of the three grow-out methods. An overall trend was observed in July and
September 2012 in Grand Isle. Specifically, the OBOY oysters in all three grow-out methods had higher levels of infection when compared to Lake Calcasieu oysters.
Chapter 4: Conclusion

In both studies, salinity was most influential in terms of growth, mortality, condition index, and *P. marinus* infection intensity. Low salinity conditions (i.e., <5 ppt) are not recommended for intensive oyster aquaculture. At Cow Bayou, elevated water temperatures and sustained low salinity conditions limited growth and induced high levels of mortality. Oysters cultured in these areas could take upwards of 19 to 21 months mean market size (75 mm), which is not ideal for grow-out in Louisiana estuaries. Low-intermediate salinity conditions, similar to that of Bay Gardene, are ideal for intensive culture. Regardless of stressful, low salinity conditions that occurred at this site, the duration of these conditions were reduced, resulting in reduced mortality while sustaining ideal growth rates. Oysters placed in these conditions can expect to reach market size in 14 months. At that time, only ~5% mortality can be expected if the optimal stocks are used for culture (i.e., the Sister Lake stock). Areas with similar conditions to that of Mozambique Point are optimal for intensive culture. This intermediate salinity site had lowest cumulative mortality of all sites and had optimal growth. Oysters deployed there reach mean market size in 12 months and only ~2% mortality can be expected by the time with the use of the optimal stocks (i.e., Sister Lake or Lake Calcasieu). Areas with high salinity, like Grand Isle, are also optimal for intensive culture. Survival was not as high as Bay Gardene or Mozambique Point by the end of the study, but market size in was reached in 11 months with only ~4.5% mortality when they reach market size. Since these oysters reach market size in less than one year, mortality from *P. marinus* would be obsolete and the oysters would go to market before the disease has time to employ its effects on its oyster host.

Recommendations for this study are to intensively culture oysters in areas of elevated salinities (>15 ppt). Intensive culture in these salinity conditions will generate optimal oyster
growth rates such that market-sizes are attained before summer stressors can take effect (i.e., high water temperatures and *P. marinus* infection). Intensive culture can take place in salinity conditions below 15 ppt, but culture is not recommended in areas where the mean salinities are below 5 ppt. Oysters placed in these conditions are expected to have reduced growth rates and higher probabilities of mortality when compared to oysters cultured at higher salinities. Different stocks should be used depending on the environmental conditions provided by different geographical areas and the environmental conditions of each stock's origin. Specifically for Louisiana, the Sister Lake stock should be used in areas of historically low salinity while the Lake Calcasieu stock should be used in areas exhibiting historically high salinity conditions. It is recommended that these two stocks be the primary stocks cultured in hatchery settings to supply seed production for both high and low salinity conditions and facilitating commercial intensive aquaculture in Louisiana estuaries.

For grow-out methods, the ALS and floating bag system are the recommended methods for intensive oyster aquaculture in low salinity conditions. Over the course of the study, oysters deployed in the ALS had the least cumulative mortality and had significantly faster growth in shell height than the OysterGro™ system. The floating bag system also has comparable growth in shell height and low cumulative mortality levels to that of the ALS. Although the OysterGro™ had the highest condition index of the three grow-out methods, its performance in cumulative mortality and growth in shell height were inferior to the other two grow-out methods. Areas that have similar salinity conditions to LUMCON can expect little to no effect of *P. marinus* due to the lack of heavy infection found throughout the study at this particular site.

The ALS, OysterGro™, and floating bag systems are all recommended methods for intensive oyster aquaculture in high salinity conditions. There was a lack of significant
differences in cumulative mortality among the methods that contained the superior Lake Calcasieu stock. While the ALS and floating bag system had significantly greater growth in shell height over the OysterGro™, the growth of the oysters in the OysterGro™ was still sufficient in producing commercial-sized oysters in a 12 month period. *P. marinus* was more influential in these higher salinity conditions. However, given the oysters grown in either the ALS or OysterGro™ reach commercial-size in about one year, the oysters would be brought to market before the summer, when *P. marinus* has the greatest effect. Even if the oysters took longer to reach market size in the first season, they would reach market-size before the following season, where *P. marinus* has the greatest effect on mortality. Though all three of these grow-out methods are viable for use in high salinity areas, the oyster stock deployed in the system will ultimately determine the performance of these grow-out systems. Also, given the inferior performance of the OysterGro™ at LUMCON, it is evident that environmental conditions also influence the performance of each grow-out method.

Applications for any of these grow-out systems will ultimately depend on state and federal permitting agencies, specifically in whether or not permanent structures (i.e., pilings) can be legally placed on private leases. The ALS system requires permanent structures for operation. If permitting for fixed structures is forbidden, the use of other methods that do not require these fixed structures will dominate intensive production, regardless of their advantages or disadvantages. Interestingly, the ALS at the Sea Grant Oyster Research and Demonstration Farm has performed exceedingly well over the past nine years, particularly in its resilience to severe hurricanes that have come ashore. Obtaining permits to deploy the ALS, however, will continue to be a major challenge for this culture system in Louisiana and other Gulf states (Maxwell 2007). It is also important to mention that these culture methods, regardless of method, can
interact with other commercial fisheries and regulations therein. Even if regulations are implemented to protect the property of oyster growers, mobile and static gear types will continue to interact and affect multiple industries.
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Vita

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