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## Growth and mortality of spat, seed, and market-sized oysters (*Crassostrea virginica*) in low salinities and high temperatures

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GROWTH AND MORTALITY OF SPAT, SEED, AND MARKET-SIZED OYSTERS (*CRASSOSTREA VIRGINICA*)  
IN LOW SALINITIES AND HIGH TEMPERATURES

A Thesis

Submitted to the Graduate 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

Molly Marie Rybovich  
B.A., Florida Gulf Coast University, 2012  
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## **Abstract**

Changes in the timing and interaction of seasonal high temperatures and low salinity as predicted by climate change models could dramatically alter oyster population dynamics. Little is known explicitly about how low salinity and high temperature combinations affect spat (<25 mm), seed (26-75 mm), and market (>75 mm) oyster growth and mortality. Using lab and field studies, this project quantified the combined effects of extremely low salinities (< 5) and high temperatures (>30 °C) on growth and survival of spat, seed, and market-sized oysters. In 2012 and 2013, hatchery-produced oysters were placed in open and closed bags at three sites in Breton Sound, LA, along a salinity gradient which typically ranges from 5 to 20. Growth and mortality were recorded monthly. Regardless of size class, oysters at the lowest salinity site (annual mean = 4.8) experienced significantly higher mortality and lower growth than oysters located in higher salinity sites (annual means = 11.1 and 13.0); furthermore, all oysters in open bags at the two higher salinity sites experienced higher mortality, likely due to predation. To explicitly examine oyster responses to extreme low salinity and high temperature combinations, a series of laboratory experiments were conducted. Oysters were placed in 18 tanks in a fully crossed temperature (25°C, 32°C) by salinity (1, 5, 15) experiment with 3 replicates, and repeated twice for each oyster size class. Regardless of temperature, seed and market oysters held in low salinity tanks (salinity 1) experienced 100% mortality within seven days. In contrast, at salinity 5, temperature significantly affected the mortality rate; all size class oysters experienced > 50% mortality at 32 °C, and < 40% mortality at 25 °C. In higher salinity tanks (15), only market-sized oysters held at 32 °C experienced significant mortality (>60%). These experiments demonstrate that high water temperatures (> 30 °C) and low salinities (< 5) negatively impact oyster growth and survival, and that high temperatures alone may negatively impact market-sized oysters. It is critical to understand the potential impacts of climate and anthropogenic changes on oyster resources in order to better adapt and manage for long-term sustainability.

## Chapter 1: Literature Review

### Background

Populations in nature are dynamic and fluctuate with natural disasters, competition, as well as density dependent and independent factors. Specifically in marine communities, environmental cycles directly impact organisms' growth and survival. Climate fluctuations, which influence the environment in a predictable fashion through impacts on water temperature and salinity, may often explain and predict population dynamics.

This study focuses on eastern oyster (*Crassostrea virginica*) responses to environmental stressors. Oysters thrive in estuaries, which may be particularly vulnerable to changes in both terrestrial and oceanic environments. Furthermore, *C. virginica* is highly valued ecologically because they filter particulate matter in the water column, which increases light attenuation and improves water clarity (Coen et al., 1999). Oysters also form extensive reefs that help prevent erosion by holding down sediment and serving as a break-wall for storm surges (Meyer et al., 1997). Oyster reefs provide habitat and for many commercially valuable fish (Volety et al., 2009). Over 300 macrofaunal species associate with oyster reefs, and over 40 species may inhabit a single oyster bed at any given time (Wells, 1961). Especially in Louisiana, oysters are valued for their historical and cultural importance. Perhaps most importantly, the Louisiana oyster industry provides over one-third of all oysters consumed in the United States (Louisiana Department of Wildlife and Fisheries, 2012). Among Gulf of Mexico states, Louisiana consistently ranks first in landings, accounting for over 50% of all oysters harvested. In 2009, approximately  $6.4 \times 10^6$  kg of oysters were harvested with a total value of \$46.5 million. Preliminary data for 2010 indicate that oyster landings were the lowest on record since 1966, at approximately  $3.0 \times 10^6$  kg, with a value of slightly less than \$22

million (Lutz et al., 2012). Oyster fisheries continue to decline globally, and thus the continued sustainability of oyster populations is of high concern.

Salinity and temperature are the key factors controlling oyster physiology, and changes in historical regimes may have far reaching impacts. Natural and anthropogenic activities may influence water quality daily values, and greater seasonal differences have been observed in recent years (Allard and Keim, 2006). Ensuring future sustainability of oyster populations becomes problematic with changing environmental conditions. Understanding life history responses to environmental stressors is imperative for management decisions.

#### Effects of temperature and salinity on oyster growth and mortality

Temperature and salinity, which are controlled by climate and human activities, are the dominant factors influencing oyster biology and physiology. Changes in water quality may affect oysters differentially by size class, population, and location. Furthermore, the interactive effects of temperature and salinity are critically important because together they affect growth and mortality, which in turn determine production potential. In order to predict and manage population dynamics, there is a need for a better understanding of how changing temperature and salinity affect different oyster life history stages.

Temperature determines the solubility of oxygen in water. This indirectly affects oysters, as well as influences populations of organisms associated with oyster reefs – both beneficial and detrimental to the oyster (Owen, 1953a). Temperature directly affects the rate of water transport, feeding, respiration, gonad formation, and spawning (Galstoff, 1964). Oysters tolerate a wide range of temperatures and are commonly found in waters that range from 0 to 32 °C annually (Butler, 1954). Condition index and glycogen content tend to be higher in cooler water (Walne, 1970). Price et al (1976) reported increased shell height and meat weight associated with higher temperatures,



but this was off the coast of Maine where water does not commonly exceed 17 °C. Oyster heart rate generally increases with increasing temperature up to 32 °C, when they begin to decline (Dimock, 1967). Above 30 °C, valvular activity becomes abnormal – i.e., oysters may reduce pumping rates or close completely. This is exaggerated above 34 °C, and Loosanoff (1953) showed valves may remain completely closed 67% of the time at temperatures above 36 °C. Valve closures provide oysters protection from osmotic stress, but it affects feeding and gas exchange, and long-term closure may lead to mortality especially at high temperatures (Hand and Stickle, 1977).

In the Gulf of Mexico, temperatures occasionally reach 36 °C, but little is known about the prolonged effects of temperatures above 32 °C on oyster populations (Galstoff, 1964). Off the coast of Louisiana, significant decreases in oyster production were observed following prolonged periods of consecutive weeks when mean air temperature >27 °C and rainfall <7 cm; conversely, production increased when preceded by a relatively cool, wet summer (Owen, 1953a). Like temperature, salinity directly and indirectly affects the life of the oyster and associated organisms. Condition index is a direct function of gametogenesis and both decrease in low salinities. Loosanoff (1953) reports the minimum salinity required for normal gonadal development as 5-7.5, and according to Butler (1949), gametogenesis is totally inhibited below 6. Larval survival and recruitment are highly dependent upon salinity, and higher spat settlement is generally recorded down estuary in higher salinity waters (Davis, 1958; Kennedy, 1996; Volety, 2008). By interfering with the osmotic balance of oyster tissues, decreased salinities also inhibit enzymatic activity in the heart (Dimock, 1967).

Adult oysters can survive in a wide range of salinities (0 to 42), but normally occur at salinities between 10 and 30 (Gunter and Geyer, 1955). However, authors in different geographical regions report different salinity requirements for different size classes of oysters. Arnold (1868), Ryder (1885), Belding (1912), and Loosanoff (1932) report the lower range of salinity required for

oyster survival as 4-5. Gunter (1950) reports adult oysters in Texas surviving at 2 when temperatures were low (<25 °C). Amemiya (1926), Menzel (1966), and Breuer (1962) reported Gulf oysters surviving in salinities as low as 1.2. Populations in Pensacola, FL are able to survive in waters that range from 0.2-3.5 for five consecutive months annually (Butler 1952), but this is highly dependent upon temperature.

According to Newell (1985), oysters physiologically perform the best at salinities 15-18. At lower salinities, oysters exhibit partial or complete contraction of adductor muscles and a slowing or cessation of water current through the gills. For example, Loosanoff (1953) placed Long Island Sound oysters from salinity approximately 27 into separate tanks set at salinities 20, 15, 10, and 5. This led to pumping rate decreases of 24, 89, 91, and 99.6%, respectively. Loosanoff (1965) further confirmed feeding stops at salinities below 3, and the production of healthy pseudofeces stops at around 5. *C. gryphoides* (80-90 mm) and *C. cucullata* (30-35 mm) from salinity 32 (approximately 28-33 °C) off the west coast of India, were able to survive in tanks with salinities <13 for two weeks, though 100% mortality occurred after 75 days (Mane, 1978). The same experiment showed that in salinity 11.5, no mortality occurred within the first 10 days, but within 16 days, 34% of oysters were dead, after 24 days 42% of oysters died, and 100% mortality occurred by the end of the 75-day experimental period. Mortality was attributed to long periods of valve closure, as visible decreases in pseudofeces accumulation occurred in salinities below 13.

Although oysters tolerate salinities between 0 and 42, growth is best achieved at salinities 12-28. Excessive valve closures, poor spat production, and slower growth occur at salinities below 12 (summarized in Shumway, 1996). The minimum salinity required for adult oysters to grow at a normal rate is 7; no growth occurs below 5 (Chanley, 1958; Wells, 1961; Loosanoff, 1953). Similarly, larvae growth slows at salinities <10 (Calabrese, 1970). Oyster growth is rapid in warm waters such

as the Gulf of Mexico. Off the coast of Texas, minimum oyster growth rate was calculated as 7.8 mm/month, and in Barataria Bay as 8.4 mm mo<sup>-1</sup> (Gunter 1955). These rates are similar to those reported in Melancon (1990). Butler (1952) reported minimum growth rates for oysters in Apalachicola Bay, FL, as 4.1 mm mo<sup>-1</sup>; in 13 months, wild oysters reached a mean height of 52.6 mm, which was less than cultivated oysters (59.3 mm). However, these rates were calculated as if growth was constant between months. In Barataria Bay, large seed and small market-sized oysters grew approximately 20 mm from December to April (Owen 1953), and from January to September growth ranged from 14.7-31.4 mm (Mackin and Sparks, 1961). In Pensacola, FL, mean spat growth rates were reported as 22.7 mm mo<sup>-1</sup> and 14.2 mm mo<sup>-1</sup>, for May and June, respectively (Ingle and Dawson, 1952). There appear to be more data on the effects of temperature on oyster growth rate than the effects of salinity on growth rate or the combined effects of salinity and temperature on growth rates.

Most oyster production in the United States occurs at salinities between 5 and 15 because of excessive mortality from predation at salinities above 15, but the effect of salinity on mortality is highly dependent upon temperature (Shumway, 1996; Chanley, 1958; Loosanoff, 1953; Craig et al., 1989; Soniat, 1996; MacKenzie, 1977; Breithaupt and Dugas, 1979). Loosanoff (1955) described Long Island Sound adult oysters held in tanks of 23-27 °C experiencing 100% mortality in tanks of salinities 0 and 3 and approximately 75% mortality in salinities of 5. In contrast, at 17-20 °C oysters lived longer with higher survival at low salinities. Therefore, it is important to reference the temperatures existing at the time of exposure when discussing oyster mortality in relation to low salinities. The ranges of fluctuations, as well as the abruptness of change, in temperature and/or salinity influence the magnitude of effects (Hand and Stickle, 1977). For example, Feng and Van Winkle (1975) found that above 10 °C, heart rate is less inhibited after gradual decreases in salinity

from 5-10 than after abrupt decreases. The effects of temperature and salinity on oyster performance also vary geographically.

Specific combinations of temperatures and salinities for successful growth, reproduction, and development are population dependent (Heilmayer et al., 2008; Loosanoff, 1953; Shumway, 1996; Tolley et al., 2005). Oysters in different geographic regions have different salinity optima, including within individual bays across a gradient of freshwater influence (Wang et al., 2008). Biochemical mechanisms that control cellular osmolality following salinity stress may also differ geographically between conspecific oyster populations. For example, Pierce et al. (1992) described oysters in the Chesapeake Bay having narrower salinity tolerances than oysters from Atlantic coast sites. The amino acid pools of both gill and adductor muscles of Atlantic oysters were also larger than those of Bay oysters and different in composition. Furthermore, Pierce et al. (1992) found Atlantic oyster tissues rely primarily on taurine for salinity tolerance, while Bay oysters depend upon alanine, glycine, and proline. Additionally, large differences in hemolymph constituents between oysters from different locations have been attributed to population differences and/or long-term differences in environmental conditions (Fisher and Newell, 1986).

Besides salinity and temperature, oyster productivity is limited by predation. Predator abundance generally increases with temperature and salinity. For example, Hopkins (1962) reported increased boring sponge (*Cliona celata*) abundance with increased salinity. Mud crabs (*Scylla serrata*) and stone crabs (*Menippe mercenaria*), which commonly prey upon oyster spat, are also more common at higher salinities (Mackenzie, 1970). Other dominant oyster predators in the Gulf of Mexico include estuarine fish, parasites, and the oyster drill (*Urosalpinx cinerea*).

Oyster drills feed on oysters by drilling holes in shells, secreting enzymes that liquefy tissues, and pumping the tissues out. The shell is left “gaping” with the valves still attached at the umbo

(Brown et al., 2008). The lower limit of oyster drill feeding occurs at salinities of 12.5, and above that, predation upon spat increases with salinity (Manzi, 1970). Melancon (1990) observed in Barataria Bay increased drill abundances associated with increased salinities and, correspondingly, increased oyster mortalities.

Also in Barataria Bay, Brown et al. (2008) observed black drum (*Pogonias cromis*) abundance in the fall was higher at higher salinity sites. In the spring, black drum were common at both higher and lower salinity sites. Fine shell fragments were found in the spring in oyster experimental trays, indicating mortality was likely from black drum, rather than stone crabs or oyster drills, because black drum consume oysters by crushing the shell, swallowing the tissue, then regurgitate the fine shell fragments. Oyster mortality was significantly correlated to black drum abundance.

*Perkinsus marinus* is a well-documented oyster pathogen that thrives in the Gulf of Mexico. Warm, high salinity waters provide ideal conditions for *P. marinus* proliferation and propagation (Albright et al., 2007). Infection by *P. marinus* is more lethal at higher temperatures (Hewatt and Andrews, 1956). Lethality of *P. marinus* infection also increases at higher salinities. Lab studies have shown short-term decreases in salinity in early spring may significantly reduce *P. marinus* infection, with little or no oyster mortality (La Peyre et al., 2003). For this reason, freshet events have been suggested as a means to maintain productive and healthy oyster beds (Chatry et al., 1983; Wilber, 1992; La Peyre et al., 2003; La Peyre et al., 2009). However, the timing and duration of freshet events are crucial as oysters may not survive low salinities while temperatures are high.

Population responses to variations in freshwater flow have been extensively studied, however results disagree on the degree or direction of influence on oyster productivity. Livingston et al. (2011) measured oyster growth and mortality in open and closed bags along a salinity gradient in Apalachicola Bay 1985-1986 (water temperature 16-33 °C, salinity 6-34). Growth was lowest in

low salinity sites, and a variety of factors influenced adult growth (density, river flow, current velocities), while young oyster growth was only significantly related to turbidity. Mortality was highest in higher salinity sites and in open bags. Further south, Volety (2008) measured oyster growth and *P. marinus* infection intensities along a salinity gradient in Caloosahatchee Estuary, Florida, 2001-2002 (temperature 18-32 °C, salinity upstream 20-23 and downstream 26-31). Predictably, *P. marinus* infection intensities increased downstream with salinity. Growth significantly differed between sampling months and, contrary to Livingston et al. (2011), juvenile oysters at upstream locations exhibited significantly higher growth than those at downstream locations. These studies, along with a large portion of field data on oyster growth and mortality in the Gulf, are from Florida which typically has higher salinity environments and *P. marinus* intensity than southeast Louisiana. A number of studies relating oyster mortality to freshwater inflows have been conducted in Texas as well (Pollack et al., 2011), and while instructive for a general purpose, difficulties may arise when extrapolating data to understudied Louisiana populations.

Early studies on Louisiana oysters were published before the construction of modern river diversions. Owen (1953a) reported excessive oyster mortalities during prolonged periods of high temperature and salinity. Optimum salinities for seed production in Louisiana were recommended by Chatry et al. (1983) but growth and mortality rates associated across different salinities were not specifically reported. Based on that recommendation, May salinities should occur between 6 and 8, June and July salinities should average 13, salinities should not increase to greater than 15 until late August, and September salinities should not average more than 20. However, these optimal salinity ranges are highly dependent upon temperature, which varies annually and with climate.

Over the last 25 years, large-scale restoration has sought to restore historic isohalines with the ultimate goal being to enhance productivity and support continued oyster production. The state

constructed the Caernarvon Freshwater Diversion to restore isohalines and moderate Breton Sound salinities. It is the second largest freshwater diversion in the Mississippi delta (maximum design discharge =  $250 \text{ m}^3 \text{ s}^{-1}$ ). Since CFD opened in 1991, Breton Sound has become one of the most studied field sites for the river diversion approach to coastal restoration. When CFD is opened to maximum capacity, high water discharge greatly reduces estuarine residence times, primarily by increasing down-estuary residual currents, but in the region > 30 km from the diversion, the impact of high water discharge is relatively small (Huang et al., 2011). Essentially when diversions open, salinity in the upper estuary drops to around 0-2, and these effects are most severe closest to diversion (i.e., <30km away).

Manipulation of isohaline location allows nutrient addition by freshwater inflow, and bottom-up increases in secondary productivity have been attributed to stimulation of primary productivity through increased freshwater flow (Kimmerer, 2002). Additionally, flow rate directly and indirectly affects many species. Specifically for oysters, increased flow rates can enhance vertical mixing, the transport of suspended food particles, potential nutritional quality, as well as reduce levels of parasitism. Flow rate also influences oyster mortality – primarily through its effect on sedimentation. For example, Lenihan (1999) found that oyster mortality was greatest where rates of sedimentation and burial of reef habitat were highest. While initially conceived to enhance oyster productivity, the operational objectives of the diversion projects were later expanded to add sediment and to help reverse the effects of saltwater intrusion into Breton and Barataria Basins. Therefore, freshwater diversion projects have potentially far reaching impacts on the marsh and associated flora and fauna.

The benefits of short term pulses of freshwater in winter and spring to enhance vertical mixing and reduce oyster predation are clear. However longer term freshets, especially during

summer months, appear detrimental as oyster mortalities associated with low salinity and high temperature have long been reported (Chatry et al., 1983; Soniat and Gauthier, 1989; La Peyre et al., 2003; La Peyre et al., 2009). Laboratory experiments have shown adult oyster mortality at 28 °C differs significantly between oysters exposed to a salinities of 1 (48%) than oysters exposed to a salinities of 5 (15%) and to control oysters kept in salinities of 25 (12%; La Peyre et al., 2009). Furthermore, field experiments in Breton Sound have demonstrated adult oyster growth rates differ significantly between low ( $4.3 \pm 2.3$ ), medium ( $8.3 \pm 3.3$ ), and high ( $12.0 \pm 4.4$ ) salinity sites. Oysters in the upper portion of the estuary demonstrated significantly less growth than oysters compared to other sites; in fact, growth in high salinity sites was four times higher than growth in low salinity sites (La Peyre et al., 2009). The same field study showed overall mortality was low and did not differ between sites, but oysters were held in predator-exclusion (closed) bags. In a subsequent study at the same sites, overall mortality was high and differed significantly between sites and type of bag (open or closed to predation – open bags had higher mortality; La Peyre et al., 2013). Overall growth was limited at the lowest salinity site and similar between other sites, and seed oyster growth ( $2.1 \pm 2.4 \text{ mm mo}^{-1}$ ) was significantly greater than market oyster growth ( $0.8 \pm 1.3 \text{ mm mo}^{-1}$ ).

Growth and mortality differ between oyster size classes, but surprisingly few have examined effects of low salinity on spat and seed oyster performance. According to Loosanoff (1953), spat in the Long Island Sound are able to resist reduced salinities as efficiently as adult oysters. Chanley (1958) reported Chesapeake oysters ranging 0.3-2.2 mm in shell height surviving in salinities as low as 5. Breuer (1962) also reported spat surviving off the Texas coast in 1.4-4.2 salinity waters. In Louisiana, previous studies state young oysters generally survive their first summer regardless of the temperature or salinity, provided that maximum extremes are not reached (Owen 1953a).



Furthermore, following prolonged periods when air temperatures  $\geq 27.8^{\circ}\text{C}$  and rainfall  $\leq 7$  cm, oyster mortalities in Breton Sound were significantly higher in larger ( $>65$  mm) oysters than oysters  $<60$  mm (Owen, 1953b). Similarly, Apalachicola oyster mortality in high salinity, high temperature conditions was higher in oysters  $>70$  mm than oysters 36-70 mm (Petes et al., 2012). Increased resilience to changes in salinity by smaller size classes is evidenced in other bivalves such as juvenile seed clams (*Mercenaria mercenaria*), which Baker et al. (2005) described as “surprisingly robust and resilient” to changes in salinity, experiencing less than 5% mortality after relatively abrupt reductions in salinity of 10 to 15. However, smaller oysters may be more vulnerable to predation because their shells are thinner and easier to penetrate. Annually, size class mortalities are not consistent and sometimes one size class has more than another. For example, most oyster mortality in Barataria Bay 1982-1985 was skewed toward smaller oysters, 20-40 mm in length which coincided with an increase in drill abundance, while deaths of larger oysters ( $>65$  mm) remained relatively low (Melancon, 1990).

Many studies relating salinity gradients to oyster mortality report highest mortality in high salinities or during low-rainfall years (mainly attributed to predation or disease). For example, modelled predictions of the introduction of a large ship canal in Galveston, TX, indicated oyster biomass inversely related to freshwater inflows, until salinity gets high enough for *P. marinus* to proliferate and populations decrease (Klinck et al., 2002). Few studies have specifically quantified the effects of prolonged periods of low salinity during flood-years or through human control of river flow. Oyster growers and managers have long reported oyster mortalities associated with low salinity and high temperature, signifying longer term freshets, especially in summer, may be detrimental to oyster populations. Despite these observations, there is a lack of data on the effects of prolonged periods of extremely low salinities, combined effects of low salinity and high

temperature on oyster growth and mortality, or differences between spat, seed, and market-sized oysters. Furthermore, much of the literature on *C. virginica* comes from the central and northeastern Atlantic coast and studies on oysters in the Gulf are largely based out of Texas and Florida. As previously noted, oysters' environmental tolerances are population-dependent and in order to effectively manage Louisiana oyster populations, their tolerances must specifically be investigated.

#### Climate variability

Extreme climate events may have significant consequences for fisheries production. Physiology, behavior, growth, development, reproductive capacity, mortality, and distribution may all be directly affected. Ultimate productivity, structure, and ecosystem composition may be indirectly affected. Specifically for oysters, population dynamics may be dramatically impacted by the timing of isohaline shifts, as well as the interaction of seasonal high temperatures and low salinity from freshwater inputs. Furthermore, changes in the amplitude of climate variability are likely to have greater consequences than changes in mean values. Predicted changes in seasonality, ocean pH, precipitation, temperature, and severe weather may negatively impact oyster population dynamics and distribution.

#### Projected Changes in Temperature and Salinity

Scenarios for CO<sub>2</sub> emissions indicate historical estuarine conditions will be significantly altered through increases in temperature and CO<sub>2[atm]</sub> (Brierley and Kingsford, 2009). When CO<sub>2</sub> enters the ocean, it dissolves forming carbonic acid (H<sub>2</sub>CO<sub>3</sub>), thereby decreasing ocean pH. The Royal Society (2005) estimates ocean pH has dropped by 0.1 (a 30% increase in hydrogen<sup>+</sup> ion concentration) in the last 200 years. As ocean pH and the saturation state of CaCO<sub>3</sub> decrease, shell mineralization becomes inhibited, particularly for early life history stages, which may cause

depressed growth rates, if not direct mortality (Ross et al., 2011; Waldbusser et al., 2010). For example in high CO<sub>2</sub> scenarios, only 5% of *C. gigas* developed into normal D-shaped veliger larvae, compared to 68% in a control group, although no difference was observed between the groups up to the trocophore stage (Kurihara et al., 2007). Furthermore as water temperatures increase, oyster vulnerability to disease may increase (Hofmann et al., 2001).

Average air temperature in the United States has increased approximately 1 °C over the past 50 years and is expected to increase 2-6 °C by 2100, depending on emission scenarios and climate sensitivity to emissions (Brierley and Kingsford, 2009; US Global Research Program). Along the Gulf Coast, historical data show an annual temperature pattern with high values in the 1920s–1940s, a drop in in late 1950s persisting through the 1970s, and an increase over the past two-plus decades, though recent temperatures have mostly not reached the highs of previous decades (Keim et al., 2011). The Hadley and Canadian general circulation models were used in the latest Intergovernmental Panel on Climate Change (IPCC) report, which suggest a warmer Gulf Coast region of about 2 °C ± 1 °C by 2050, but the Hadley model simulates less warming than the Canadian model (Ning and Abdollahi, 1999). Both models indicate exacerbated seasonal differences, with average July heat index increasing 4-11 °C above present levels.

As Earth's surface temperature increases, increased evaporation leads to more overall precipitation, but precipitation and stream flow projections are uncertain. Though climate models do not agree on the direction of future precipitation change, annual rainfall has generally increased since 1919, and that, combined with relatively cool temperatures, has led to a 36% increase in runoff (Keim et al., 2011). During the next 100 years in the Gulf Coast region, the Hadley model simulates increased annual rainfall, but the Canadian model simulates decreased annual rainfall. Differences in projections are mainly due to differences in simulated storm track locations.

According to the Hadley Model, the amount of precipitation Louisiana receives will not change in winter or spring, but will increase by approximately 10% in summer and fall (Swenson, 2013).

Models for climate variability in Mobile, Alabama, indicate under low emission scenarios, winter precipitation will significantly increase, both in the near-term and by mid-century, by about 4.2 cm; fall precipitation is projected to increase 5.6 cm by mid-century (US Department of Transportation, 2013). This suggests some disagreement between climate models in the magnitude and direction of projected changes in precipitation.

In general, precipitation changes are more notable at the seasonal and monthly scale. Though mean annual precipitation may not change dramatically, the timing of precipitation may shift. Perhaps the most important climate change consideration for Louisiana estuaries is how Mississippi River discharges will be affected. For the time period 2025-2034, the Hadley model predicts a MI River discharge increase of 5% and the Canadian Model predicts a decrease of ~35%; from 2090-2099, the Hadley model predicts an increase of ~50%, while the Canadian Model predicts a decrease of ~30% (Boesch et al., 2000).

Severe weather is also likely to increase during the next 100 years, as several models indicate increasing intensity of hurricane rainfall and wave height. The destructive energy of Atlantic hurricanes has intensified during the past 40-50 years, and this is expected to continue to increase, which may negatively impact flora and fauna. For example, in the Delaware River watershed following Hurricane Irene and Tropical Storm Lee in 2011, high oyster mortalities in the upper reaches of the Delaware Bay resulted from prolonged bay-wide low salinities. Monthly mortality exceeded the long term average at up-estuary locations and was attributed to low salinity (< 7) for greater than 20 days. Furthermore, model-based population recovery projections suggest that recovery will take up to 10 years for the uppermost beds (Munroe et al., 2013). Even if storms

do not increase in severity, storm surge and its effects will be intensified as sea level rises and natural coastal defenses deteriorate.

Increases in severe weather are predicted to increase coastal flooding and submergence of estuarine wetlands. Sea level has been gradually rising over the past 15,000 years and is regarded as one of the more certain consequences of increased global temperature (Burkett, 2013). Sea level increases of 30 cm are projected by 2100 (Boesch et al., 2000), and the current average rate of global sea-level rise ( $1\text{--}2\text{ mm yr}^{-1}$ ) is projected to accelerate two to four-fold over the next 100 years, though the Hadley model simulates a slower rate of sea-level rise than the Canadian model (IPCC, 2001). Furthermore, the relative or apparent rate of sea level rise during the 21st century will be greater in regions where the land surface is sinking or subsiding. For example, parts of the city of New Orleans are presently 2 m below sea level and may be 3 m or more below sea level by 2100 due to a combination of rising sea level and subsidence of the land surface (Burkett, 2013). Louisiana coast wide flood depths for the next 100 years are modeled to increase up to 4 m in less-optimistic scenarios, and up to 1 m even with the Master Plan in place (Johnson et al., 2013). The mid-range estimate of global sea level rise that will occur during the 21st century is 0.48 m (IPCC, 2001). This rate is accelerated along the Gulf due to a variety of factors besides subsidence such as gas, oil, and groundwater withdrawals, as well as drainage and dredge projects. The water level in Grand Isle exhibits a significant trend of increasing approximately  $1.2\text{ cm yr}^{-1}$  from 1955 through 2000 (Swenson, 2003). In south Louisiana, roughly 405,000 ha of coastal marsh have been converted to open water since 1940 (Burkett, 2013). Ultimately, these changes can negatively affect oyster population sustainability by altering environmental characteristics, such as salinity.

Climate conditions impact salinity forcing functions through a variety of ways. Potential salinity changes in different climate scenarios have been modeled for some Gulf of Mexico

estuaries. Along Florida and Texas coasts, predictions of climate-induced salinity changes show decreases of 2-5 for high freshwater input years, and increases of 5-7 for low freshwater input years (Orlando et al., 1993). Close to Breton Sound in Barataria Bay, the ranges of potential salinity changes have been calculated for different scenarios of river discharge, precipitation, and sea level rise. The worst case scenario (30% increase in Mississippi River discharge, 10% increase in precipitation) predicted decreases in salinity of 1-3 (Swenson, 2003). This simulation also indicated a change from low to high rainfall could shift the 5 isohaline 15 km south, and the 15 isohaline 8 km south. In contrast, a change from low to high Mississippi River discharge shifts the 5 isohaline 20 km south, and the 15 isohaline 10 km south. Effects of climate-induced salinity changes in Breton Sound may be comparable to those in Barataria Bay. However, Breton Sound is a much more dynamic estuary than Barataria Bay, and a negative change in one salinity forcing function may offset a positive change in another forcing. For example, a decrease in local precipitation could lead to an increase in estuarine salinity, but an increase in Mississippi River discharge occurring at the same time could offset this change.

#### Potential Effects on Oysters

Modifications in temperature, wind patterns, hydrologic cycles, and sea level rise influence nutrient input and behavior, which in turn effects stratification, flushing times, and phytoplankton productivity. Enhanced eutrophication is anticipated from both increased climate and human nutrient input (Statham, 2012). Reduced salinity, cyanobacterial blooms, and hypoxia/anoxia have been reported associated with massive freshwater discharges. For example, the 1997 opening of the Bonnet Carre Spillway deleteriously impacted benthos based on decreases in species diversity and abundance (Brammer et al., 2007). Furthermore, recent studies (summarized in Brander, 2007) suggest net primary production (NPP) has been declining globally, particularly in low latitudes.

Warming of surface layers increases stratification and reduces nutrient mixing depth, which could potentially lead to changes in species composition and ecosystem function (Piazza and La Peyre, 2011). However, predicting potential impacts of changing nutrient levels on marine communities is impeded by uncertainties over future nutrient additions and NPP changes.

The ability to prevent and mitigate potential deleterious effects on oyster populations is further challenged by the uncertainty of seasonal changes in precipitation. If current predictions of increased precipitation hold, particularly during the summer months, then decreases in salinity may directly cause oyster mortalities. Reduced salinities may also be indicative of a variety of other stressors, such as increased temperature and turbidity, or decreased phytoplankton concentration, which compound the effects of salinity on oyster health and survival. Furthermore, increases or decreases in precipitation may shift regions of low salinity and disease refuge away from optimal oyster bottom habitat, thereby negatively impacting reproduction and survival (Levinton et al., 2011).

The effects of changes in isohalines from freshwater inflows on the distribution and productivity of oyster reefs have been observed specifically in Breton Sound, LA. In July 1991, Louisiana broke rainfall records (>3 m) and Mississippi River discharge exceeded long-term averages. Significant oyster mortalities were recorded east of the Mississippi River during the heaviest rainfall months (April-May). In Cow Bayou, Bay Gardene, and Mozambique Point in Breton Sound, total oyster mortality reached 75%, 25%, and 50-75%, respectively (Dugas, 1991). Massive oyster mortalities were also observed in Breton Sound in 1973 when Mississippi River discharge increased, and water breached levees at numerous points on the east side of the river below Pointe a la Hache, Louisiana. The volume of this discharge is unknown, but Breton Sound was saturated with floodwaters. "Tremendous" freshwater volumes combined with warm temperatures had a

temporarily disastrous effect on oyster grounds within the influence of the discharges (Dugas, 1973). Mortalities were directly related to distance from the point of freshwater discharge and exceeded 90% in the upper reaches of the estuary. Additionally, mortality was not serious immediately after the Spillway opened in April, but increased alarmingly as depressed salinities continued into periods of elevated water temperatures in May and June (Dugas, 1973).

Reducing mortality is the principle feasible means of reducing the negative impacts of climate change (Brander, 2007). Therefore, it is imperative to understand what oysters can tolerate and how extreme temporal/seasonal changes may ultimately impact population dynamics. Variations in temperature and salinity are the most important factors influencing the biology and physiology of oysters. Many studies have quantified and modeled the effects of variations in salinity on adult (>76 mm) oysters but none, to our knowledge, have explicitly quantified the effects on spat (<25 mm) or seed (26-75 mm). As the survival and growth of spat represent a critical life stage for oysters, impacts at juvenile stages will affect future populations and recruitment years. Previous studies also have shown that optimal temperature and salinity combinations for oyster health and productivity are population-dependent. This study seeks to quantify the effects of varying salinity and temperature on Breton Sound stock spat, seed, and market-sized oysters in order to better understand and manage populations in southern Louisiana. Specifically, the objectives are to measure growth and mortality of spat, seed, and market-sized oysters in a variety of different salinities and temperatures with laboratory and field components. This project will be critical understanding the impacts of freshwater inflow on oyster resources and future populations.



## **Chapter 2: Field and lab studies on spat, seed, and market oyster tolerance to low salinity and high temperature**

### Introduction

Historical temperature and salinity regimes are changing with human control of rivers and climate, and these changes have increasing cascading effects on organisms (Ning et al., 2003; Piazza and La Peyre, 2011). Along the Gulf Coast, temperature is expected to increase  $2^{\circ}\text{C} \pm 1^{\circ}\text{C}$  by 2050 (Brierley and Kingsford, 2009; US Global Research Program). Of particular importance is the fact that models suggest exacerbated seasonal differences, with average July heat index increasing 4-11  $^{\circ}\text{C}$  above present levels (IPCC, 2001; Ning and Abdollahi, 1999). Though climate models do not agree on the magnitude and direction of future precipitation changes, annual rainfall has generally increased since 1919, and summer and fall precipitation are expected to increase significantly along the northern Gulf Coast (Swenson, 2013; US Department of Transportation, 2013; Ning et al., 2003). Of notable concern is how the timing of precipitation events may shift in relation to monthly or seasonal predicted temperature changes. Perhaps the most important climate change consideration for Louisiana estuaries is how timing of precipitation changes affects Mississippi River discharges, and ultimately estuarine salinities, which are critical controls on important estuarine resources, such as the economically important eastern oyster (*Crassostrea virginica*) (Boesch et al., 2000).

Changes in salinity and temperature are of high concern in Breton Sound, LA, as the region is the primary oyster producing region in coastal Louisiana (LDWF, 2012). Reducing mortality is the principal feasible means of reducing negative impacts of climate change (Brander, 2007). Therefore, it is imperative to understand what oysters can tolerate and how extreme temporal/seasonal changes may ultimately impact population dynamics. Temperature and salinity

are the most important factors influencing the biology and physiology of oysters, and oysters are well-known for their broad tolerance of temperature (0 to 32°C) and salinity (0 to 42) (Butler, 1954; Galstoff, 1964). However, oyster growers have long recognized that extended periods of low salinity during summer months are associated with significant increases in oyster mortality (Monroe et al., 2013; La Peyre et al., 2003, 2009, 2013). Besides causing direct mortality, prolonged periods of hot, low salinity environments may negatively affect oyster production by altering recruitment and growth rates, and changing population structure and sustainability (La Peyre et al., 2003; 2009; Levinton et al., 2011). It is critical to understand the potential impacts of climate and abiotic changes on oyster resources in order to better adapt and manage these resources for long-term sustainability.

Many studies have quantified and modeled the effects of salinity on adult (>75 mm) oysters but none, to our knowledge, have explicitly quantified the effects on spat (<25 mm) or seed (26-75 mm). Spat represent a critical life stage for oysters, and impacts on juvenile growth and survival will ultimately affect future recruitment years and populations. Furthermore, previous studies have shown optimal temperature and salinity combinations for oyster health and productivity are population-dependent, and most have been conducted on the central and northern Atlantic coasts (Heilmayer et al., 2008; Newkirk, 1978; Pierce et al., 1992; Dittman et al., 1998; Brown et al., 2005). While instructive for a general understanding, extrapolating the environmental preferences of previously studied oyster populations to specific unstudied populations, such as Breton Sound, can be problematic. Site-specific effects of different temperatures and salinities on Breton Sound oysters need to be specifically explored to properly understand, manage, and predict the responses of these populations.

This study seeks to quantify the effects of varying salinity and temperature on Breton Sound stock spat, seed, and market-sized oysters in order to better understand and manage populations in southern Louisiana. Specifically, the objectives are to quantify growth and mortality of spat, seed, and market-sized oysters in a variety of different salinities and temperatures with laboratory and field components. In the field, oyster growth and mortality was recorded monthly along a salinity gradient in Breton Sound. In the lab, oysters were exposed to extremely low salinities (1, 5, 15) and extremely high temperatures (25, 32 °C) and mortality was recorded every other day. By clarifying impacts of combined extreme salinity and temperatures on oysters at different life history stages, we will be able to better predict and manage sustainable oyster populations. This project will be critical to understanding the impacts of changing climate, and help inform the management of freshwater inflow on oyster resources.

## Methods

### Oysters

All oysters (eastern oyster; *Crassostrea virginica*) used in field and lab studies were spawned from Breton Sound stock oysters in May 2012 at the Louisiana Sea Grant Oyster Hatchery in Grand Isle, Louisiana. Lab experiments used 3600 total spat (<25 mm), 4050 total seed (26-75 mm), and 360 total market-sized (>75 mm) oysters; field experiments used 2,400 total spat, 960 total seed, and 480 total market-sized oysters.

All spat were maintained in aquaculture bags on a long line system at Louisiana Sea Grant Oyster Hatchery in Grand Isle, Louisiana, until deployment. Spat were deployed to field studies in July 2012 and lab studies in June and August 2012. Seed used in the first two lab studies were also stored in bags on Grand Isle, LA, and maintained until deployment. Seed were deployed in field and lab studies in September and October 2012. Mean salinity and temperature for Grand Isle from May

2012 to October 2012 were 14.9 and 28.2 °C, respectively. Market-sized oysters used in the second lab study were stored in bags in Grand Isle, LA, and maintained until deployment. Market-sized oysters were deployed for lab study 2 in July 2013. Mean salinity and temperature for Grand Isle from May 2012 to July 2013 were 13.8 and 23.2 °C, respectively (Table 2.1).

In March 2013, remaining oysters were moved to Louisiana Universities Marine Consortium in Chauvin, Louisiana, to better match ambient and experimental salinities, and maintained in bags on a long line system. These oysters were used for seed lab study 3 (June 2013), market and seed for field (deployed April 2013), and market-size lab study 1 (April 2013). Mean salinity and temperature for Chauvin from March 2013 to July 2013 were 18.2 and 24.2 °C, respectively (Table 2.1).

Table 2.1 Experiment timing and ambient salinity/temperature in Grand Isle where oysters were taken from at the start of each experiment. Asterisks denote oysters taken from LUMCON instead of Grand Isle.

Experiment	Dates	Temperature (°C)	Salinity
<u>Lab</u>			
Spat 1	June – July 2012	28.8	17.6
Spat 2	July – August 2012	29.9	14.6
Seed 1	September 2012	27.7	15.6
Seed 2	October 2012	22.2	18.5
Seed 3*	June 2013	30.0	16.1
Market 1*	April 2013	21.9	18.4
Market 2	July 2013	28.9	10.9
<u>Field</u>			
Spat	July – August 2012	29.9	14.6
Seed	September 2012 – March 2013	27.7	15.6
Seed and Market	April – November 2013	21.9	18.4

#### Lab Study

Experimental Design. Between July 2012 and July 2013, seven individual laboratory experiments were run to determine the effects of a range of salinity and temperature combinations on spat, seed, and market-sized oyster mortality. For each trial, oysters were placed in one of 18

(38 L) aquaria located at Louisiana State University. Each aquarium was randomly assigned one of six salinity by temperature combinations (salinity: 1, 5, 15; temperature: 25, 32 °C), with a total of 3 aquaria per treatment. A full factorial experiment was conducted twice for each oyster size class (3 salinities: 1, 5, 15; x 2 temperatures: 25, 32 °C; x 3 tanks x 2 trials), except for seed which was carried out three times. Oysters were not expected to grow significantly in the lab because their natural diet was altered. Prior to placement of oysters in each tank, water was passed through a filter, an air stone was placed in each tank, and salinity and temperature levels were adjusted to desired targets. Temperature was maintained with digital heaters, desired salinity was reached by adding Instant Ocean sea salt (Spectrum Brands) to filtered water, and both were checked daily. These temperatures represent average seasonal water temperatures for the coast of Louisiana, and these salinities correspond to levels commonly observed in Breton Sound.

At the beginning of each experiment, oysters were transported from either the Louisiana Sea Grant Oyster Hatchery in Grand Isle, Louisiana, or LUMCON in Chauvin, LA, to LSU. Spat were randomly assigned to treatment groups of 300 each and placed directly in separate aquaria adjusted to the treatment salinity and temperature (100 spat per tank; triplicate tanks per treatment). Spat were fed by adding 3 ml of marine algae (*Isochrysis galbani*) to each tank daily or until clearance. Oyster mortality was monitored every other day for two weeks by counting the number of live and dead oysters. Dead oysters were recorded and removed from tanks. Water quality (pH, NO<sub>2</sub>, NO<sub>3</sub>, NH<sub>4</sub>) was recorded at the end of each experiment to ensure that mortality was caused by salinity/temperature stress, rather than toxins. The procedure stated above was repeated for seed (n=75 per tank) and market-sized oysters (n=10 per tank).

Cumulative mortality on day 15 was analyzed separately by size class, using generalized linear mixed models (GLMMs) with temperature and salinity as factors and blocks placed on tank

and trial (SAS 9.3). All GLMMs were followed by LSMeans with a Tukey adjustment when significant differences were found ( $p < 0.05$ ), and all data are presented as mean  $\pm$  standard deviation, unless otherwise stated.

#### Field Study

**Study Area.** The Breton Sound estuary is part of the Plaquemines-St. Bernard delta complex, located approximately 20 km southeast of New Orleans. It is roughly 40 km long and 271,000 ha. Bound hydrologically to the west by the Mississippi River levee, Breton Sound is a geometrically complex estuary that consists of microtidal bays, bayous, channels, canals, and shallow lakes flanked by fresh, intermediate, brackish and salt marsh types. Tropic diurnal tides in Breton Sound may reach as high as 1 m at the estuary mouth, especially during wind-driven subtidal water level oscillations, but are fairly weak (0.4 m) and decrease in height up estuary. The Caernarvon Freshwater Diversion (CFD) empties into Breton Sound and it is the second largest river diversion in the state (up to  $250 \text{ m}^3 \text{ s}^{-1}$ ). Pulses release large fluxes of river water into the basin periodically and were originally designed to maintain appropriate salinities for oyster production over important grounds (US Army Corps of Engineers, 1984). Breton Sound contains a large portion of public oyster grounds, which are considered the backbone of Louisiana oyster resources, because they contribute to overall landings each year and supply seed for grow-out purposes on private leases (LDWF, 2012).

Three sites were established in Breton Sound near continuous data recorders which take hourly salinity and temperature readings – Cow Bayou (CB), Bay Gardene (BG), and Mozambique Point (MP); Figure 2.1). These sites occur along a natural salinity gradient, but are close enough to each other to have analogous water temperatures. All sites except Mozambique Point were located adjacent to USGS continuous data recorders, which provide hourly salinity and temperature

readings (USGS Recorders CB – 073745258 and BG – 07374527). No data recorders are located at Mozambique Point, but the Louisiana Department of Wildlife and Fisheries records weekly salinities there, and a YSI-650 Sonde was placed at the site to record hourly readings, which were downloaded monthly. The YSI-650 was calibrated once a month. Salinity, temperature, and dissolved oxygen were also recorded monthly during sampling with a YSI-85 handheld multimeter.

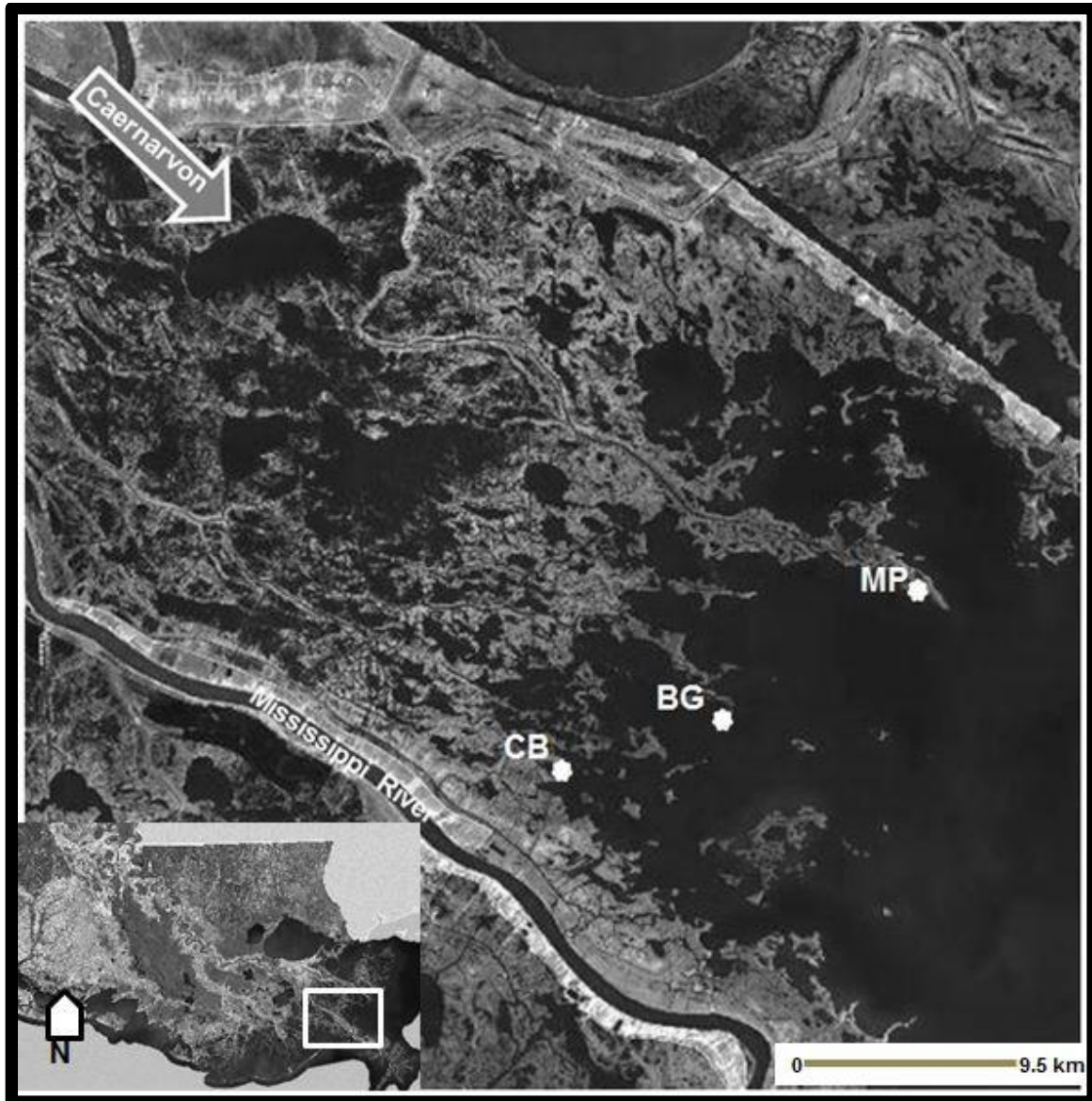


Figure 2.1 Map of study area in Breton Sound, LA. CB = Cow Bayou, BG = Bay Gardene, MP = Mozambique Point.

Experimental Design. To quantify size class growth and mortality in the field, the same stock oysters used in the laboratory experiment (spat, seed, and market) were placed in predator-exclusion (closed bags) and predator-inclusion (open bags) enclosures at the selected sites. As the project focused on using the same stock of oysters (spawned in May 2012), spat were deployed in July 2012, seed were deployed in September 2012, and seed and market were deployed in April 2013.

Four predator-inclusion aquaculture bags and four predator-exclusion aquaculture bags were deployed at each site. Each bag was held just off bottom by one-foot PVC legs. Predator-inclusion bags were constructed by removing the top of an ALS bag with scissors and securing the bag top-up to PVC poles. Unmodified aquaculture bags were used for predator-exclusion bags. All size classes were sampled approximately monthly for growth and mortality, following protocols listed below.

Approximately 100 spat-on-shell ( $11.4 \pm 3.7$  mm) were placed in each bag at each site July – August 2012 and sampled at 3 and 7 weeks post-deployment. Spat reached seed ( $>25$  mm) size on August 17, 2012 and were discarded. All bags were brought back to LSU and cleaned before deployment of other oysters. Clusters of 75 seed ( $42.2 \pm 9.8$  mm) were placed in each bag at each site September 2012 – March 2013. In March 2013, seed were discarded because several open bags were lost, and remaining bags were brought back to LSU and cleaned. In April 2013, 40 seed oysters ( $49.8 \pm 4.7$  mm) were placed in each of eight ALS bags (four open and four closed) at each site along with 20 market oysters. Approximately 20 market-sized oysters ( $111.6 \pm 3.9$  mm) were placed in the same bags as seed at each site in April 2013. Prior to this deployment, condition index was calculated for 50 seed and 50 market as the ratio of dry tissue weight to dry shell weight multiplied by 100 (Mann, 1978; Lucas and Beninger, 1985). Condition index was  $4.4 \pm 1.1$ , which is considered relatively high.



Interval percent mortality (IM) was calculated by dividing the number of dead oysters by the total number of oysters in each bag at each sampling. Cumulative mortality was then calculated by multiplying interval percent mortality by the proportion of surviving oysters from the previous sampling period and adding to the cumulative mortality from the previous sampling period.

$$\text{Cumulative Mortality} = \text{CM}_{t-1} + \text{IM}_t * (1 - \text{CM}_{t-1})$$

Where  $\text{CM}_{t-1}$  is last month's cumulative mortality and  $\text{IM}_t$  is interval mortality (Ford et al., 2006). Shell height was recorded for 25 oysters per bag. Monthly growth rates ( $\text{mm mo}^{-1}$ ) were calculated by dividing the change in mean oyster height (mm) for each time period by the number of elapsed days then multiplying by 28.

$$\text{Monthly Growth} = [H_t - H_{t-1}] / t - (t-1) * 28$$

Where H is mean oyster height (mm), t is the current sampling date, and t-1 is the last sampling date (La Peyre et al., 2013).

Statistical Analyses. Cumulative mortality, interval mortality, and interval growth rates ( $\text{mm mo}^{-1}$ ) were analyzed using generalized linear mixed models (GLMMs). Data were examined separately by size class, examining site and month and their interaction, and mortality was examined with bag type (open, closed) as a factor. All GLMMs were followed by LSMeans with a Tukey adjustment when significant differences were found ( $p < 0.05$ ) and all data are presented as mean standard deviation.

To examine the relationship between interval growth and mortality to interval temperature and salinity, a forward stepwise multiple regression was run separately by size class (spat, seed, market) with interval growth and mortality as the dependent variables, and interval salinity and temperature as independent variables. Regression models were selected in Excel by the trendlines that yielded highest  $R^2$  values.

## Results

### Lab Study

**Water Quality.** Throughout lab studies, aquarium salinity remained at desired levels.

Temperature in 25 °C tanks ranged 22 – 27 °C, and temperature in 32 °C tanks ranged 28 – 34 °C

(Appendix E, F). Water quality parameters such as pH, NO<sub>2</sub>, NO<sub>3</sub>, NH<sub>4</sub>, alkalinity, and hardness, were all within normal, non-toxic ranges (Appendix E).

**Mortality. Spat:** Cumulative mortality at day 15 was significantly affected by the interaction of temperature and salinity ( $p=.0005$ ; Figure 2.2). High mortality (>60%) occurred in all tanks at salinity 1, but mortality was achieved faster in tanks with higher temperatures. At salinity 5, high mortality (>60%) occurred in tanks of 32°C, but low mortality (<20%) occurred in tanks of 25°C. Low mortality (<10%) occurred in tanks of salinity 15 regardless of temperature.

**Seed:** Cumulative mortality at day 15 was significantly affected by salinity ( $p=.0106$ ; Figure 2.2), and not temperature, with higher cumulative mortality in tanks of salinity 1 (>90%), and lower mortality (<35%) in tanks of salinity 15, regardless of temperature.

**Market:** Total cumulative mortality was not significantly affected by salinity, temperature, or the interaction of temperature and salinity (Figure 2.2), with mortality >40% in all treatments except for 25°C, salinity 15, which had 0% mortality. This indicates temperatures of 32 °C, or salinity of 5 regardless of temperature, may be detrimental to market oysters.

### Field Study

**Water Quality.** From July 2012 to November 2013, water temperature ranged 10.0 – 32.8 °C (mean  $24.3 \pm 6.1$ ; Figure 2.3) and did not differ between sites ( $p=.5743$ ). Site had a significant effect on dissolved oxygen ( $p<.0001$ ; range 5.4 – 11.3 mg L<sup>-1</sup>; mean  $8.1 \pm 1.7$ ; Table 2.2), though actual differences were small and all sites were within normoxic ranges.

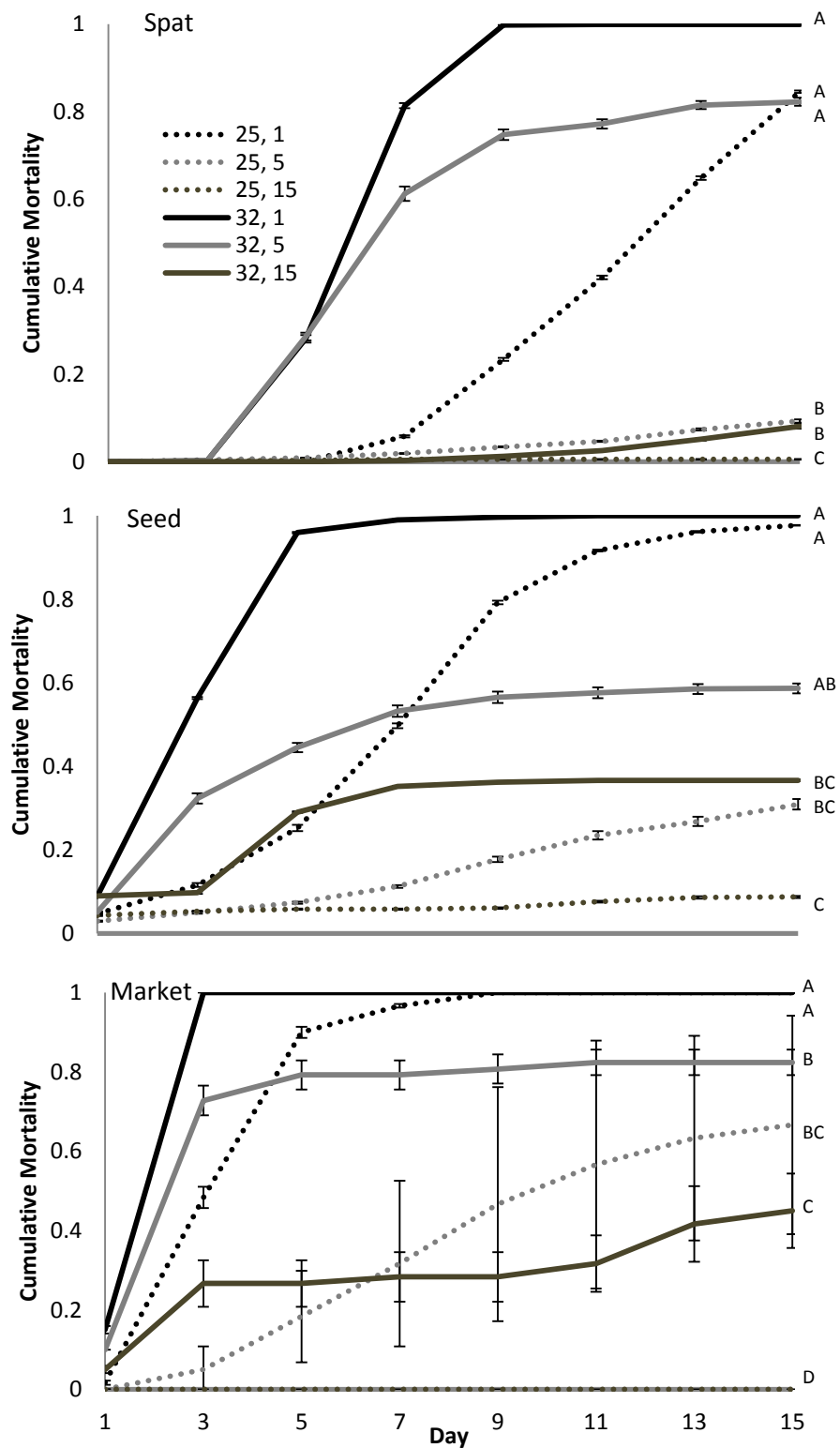


Figure 2.2 Mean cumulative mortality  $\pm$  standard error for all tanks and trials of spat, seed, and market-sized Breton Sound, LA, stock oysters recorded every other day. Solid lines represent treatments in 32 °C and dotted lines represent treatments of 25 °C. Letters denote significance for salinity by temperature interaction ( $p < 0.05$ ).

Salinity differed significantly by site ( $p < .0001$ ), following the expected gradient from low to high: Cow Bayou, Bay Gardene, Mozambique Point (Table 2.2; Figure 2.3). During this period, at Cow Bayou, there were 24 days where salinity was less than 5 and temperature was greater than 31 °C. At Mozambique Point, only one day was recorded when salinity was less than 5 and temperature was greater than 31 °C (June 13, 2013). There were 48 days at Bay Gardene where salinity was below 5, and 21 days where temperature was greater than 31 °C, but 0 days where these conditions coincided.

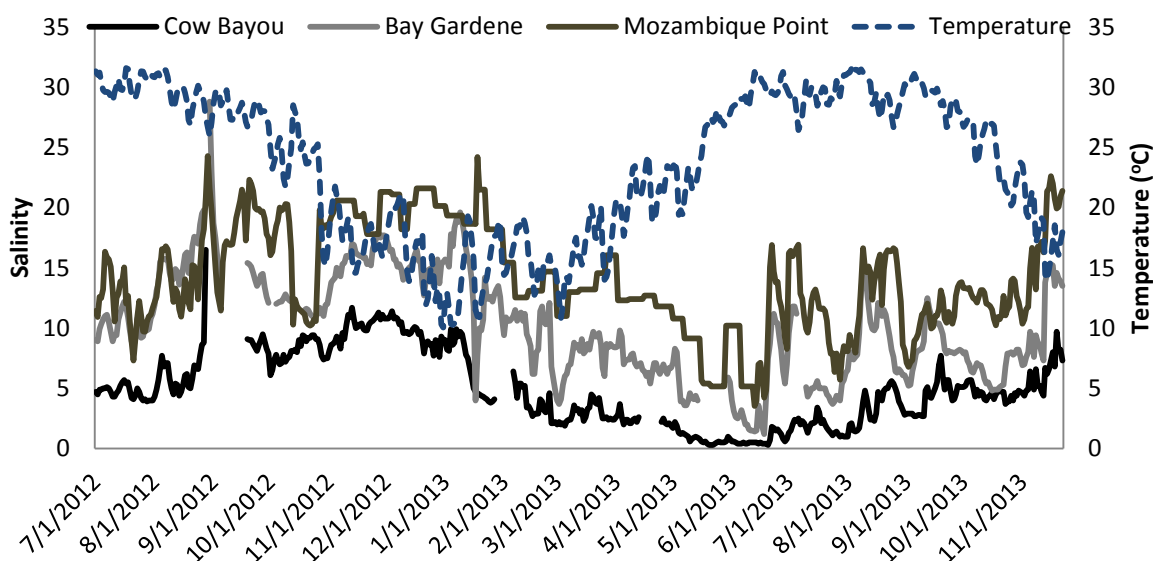


Figure 2.3 Temperature (°C) and salinity at three field sites were recorded daily throughout the duration of the field experiment July 2012 – November 2013. Solid lines represent salinity and the dotted line represents temperature.

Table 2.2 Breton Sound salinity, temperature (°C), and dissolved oxygen ( $\text{mg L}^{-1}$ ) ranges are displayed on top of means  $\pm$  standard deviations for all sites July 2012 – November 2013 recorded daily by USGS monitoring stations and YSI-650 Sonde. Salinity and dissolved oxygen differed between each site ( $p < .0001$ ), but no differences were detected regarding temperature between sites.

Site	Salinity	Temperature	Dissolved Oxygen
Cow Bayou	0.3 – 16.5	10.0 – 32.3	8.6 – 11.3
	5.6 $\pm$ 3.4	21.7 $\pm$ 6.2	10.1 $\pm$ 1.4
Bay Gardene	1.2 – 28.8	10.1 – 31.7	5.8 – 10.1
	10.5 $\pm$ 4.5	23.4 $\pm$ 6.2	8.0 $\pm$ 1.5
Mozambique Point	3.3 – 24.3	11.9 – 32.8	5.4 – 8.9
	13.7 $\pm$ 4.1	26.4 $\pm$ 5.0	7.3 $\pm$ 1.4

Mortality and Growth. Spat: Two open bags at Bay Gardene and one open bag at Mozambique Point were lost during Hurricane Isaac and not used in data analyses.

Multiple stepwise regression for interval growth rates showed a significant positive relationship with salinity ( $R^2=.88$ ;  $p=.0054$ ; Figure 2.4), but no significant relationships between temperature and growth were detected. Generalized linear mixed models revealed interval growth rates differed significantly by site ( $p<.0001$ ; Figure 2.5). Growth was generally lower at Cow Bayou (4.9 mm/month) and similar at Bay Gardene (8.9 mm/month) and Mozambique Point (9.4 mm/month).

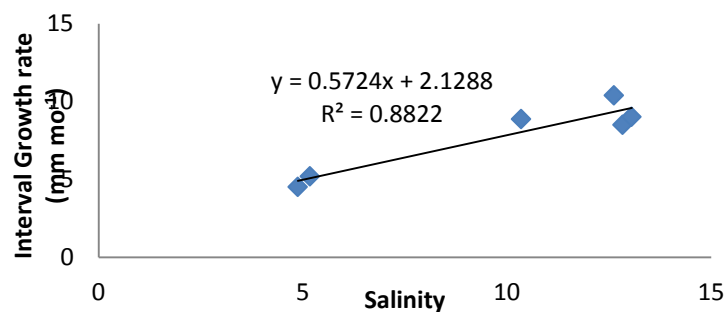


Figure 2.4 Spat interval growth from July – August 2012 regressed linearly on interval salinity.

Generalized linear mixed models revealed there was a significant effect of site ( $p=.0397$ ) and bag type ( $p=.0237$ ), but not the interaction, on cumulative mortality (Figure 2.6). Mortality in open bags was higher than in closed at all sites. In closed bags, highest cumulative mortality (52.5%) was recorded at Cow Bayou and lowest cumulative mortality (28.7%) was recorded at Mozambique Point. Cumulative mortality in closed bags at Bay Gardene was 34.3%. In open bags, highest cumulative mortality was recorded at Cow Bayou (81.6%) and lowest cumulative mortality (49.4%) was recorded at Mozambique Point. Cumulative mortality in open bags at Bay Gardene was 50.3%. Multiple stepwise regression for interval mortality did not reveal any significant relationships between mortality and interval salinity and temperature.

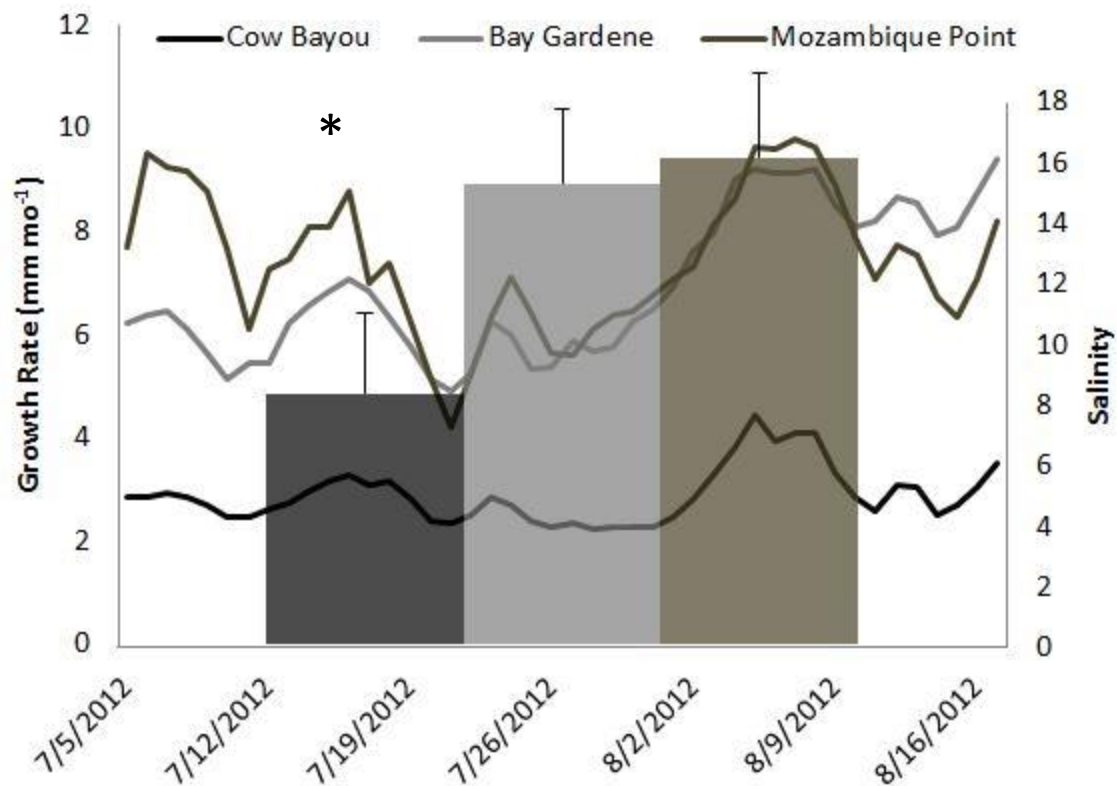


Figure 2.5 Mean interval growth rate (mm mo<sup>-1</sup>)  $\pm$  standard deviation and daily salinity for spat in all bags at three sites recorded July – August 2012. Lines represent salinity and bars represent growth rate. Asterisk denotes significance ( $p<0.05$ ).

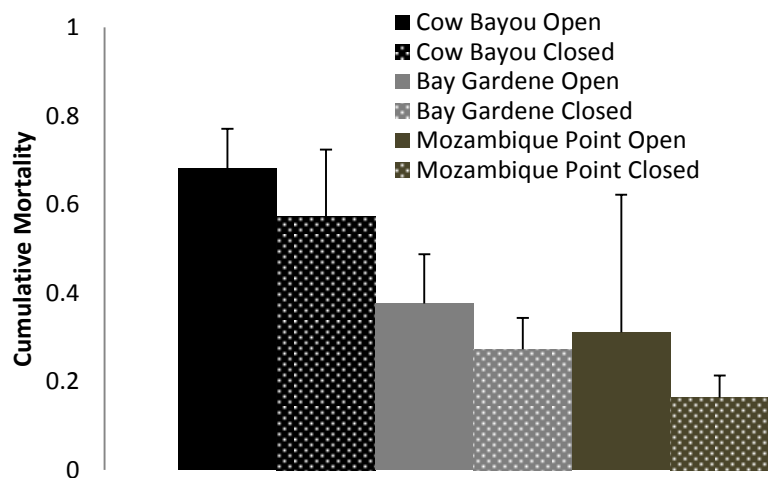


Figure 2.6 Overall spat cumulative mortality recorded in open and closed bags at three sites July – August 2012. Solid bars represent mortality in open bags and dotted bars represent mortality in closed bags.

Seed: While seed were deployed September 2012 – March 2013, all open bags and one closed bag at Mozambique Point were lost and not included in mortality analyses. Because of the replacement of bags and stocks, cumulative mortality is calculated separately for seed 1 (Sept-Mar), and seed 2 (Apr-Nov). After remaining oysters were discarded and replaced in April 2013, two closed bags at Mozambique Point were buried in July 2013 and were not included in mortality analyses. All remaining bags were included in growth analyses.

Multiple stepwise regression for interval growth rates showed a significant positive relationship with salinity ( $R^2=.13$ ;  $p=.0469$ ; Figure 2.7), but no significant relationships between temperature and growth were detected. Generalized linear mixed models revealed there was a significant interactive effect of site and month ( $p=.0001$ ) on interval growth rate (Figure 2.8). Overall growth rates were similar at Bay Gardene (3.9 mm/month per week), Mozambique Point (3.8 mm/month), and Cow Bayou (3.6 mm per week).

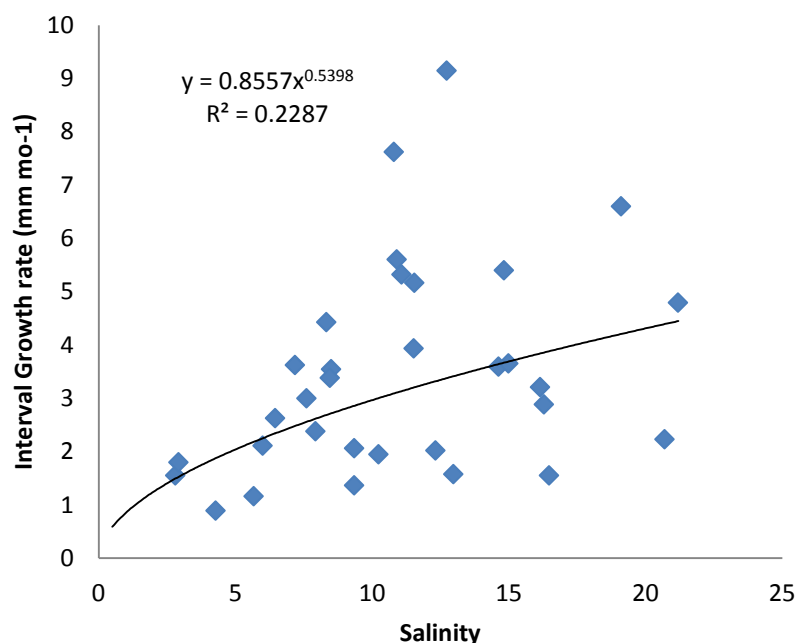


Figure 2.7 Seed interval growth from September 2012 – November 2013 regressed on interval salinity. A power trendline was selected in Excel as the model with the highest  $R^2$  value.

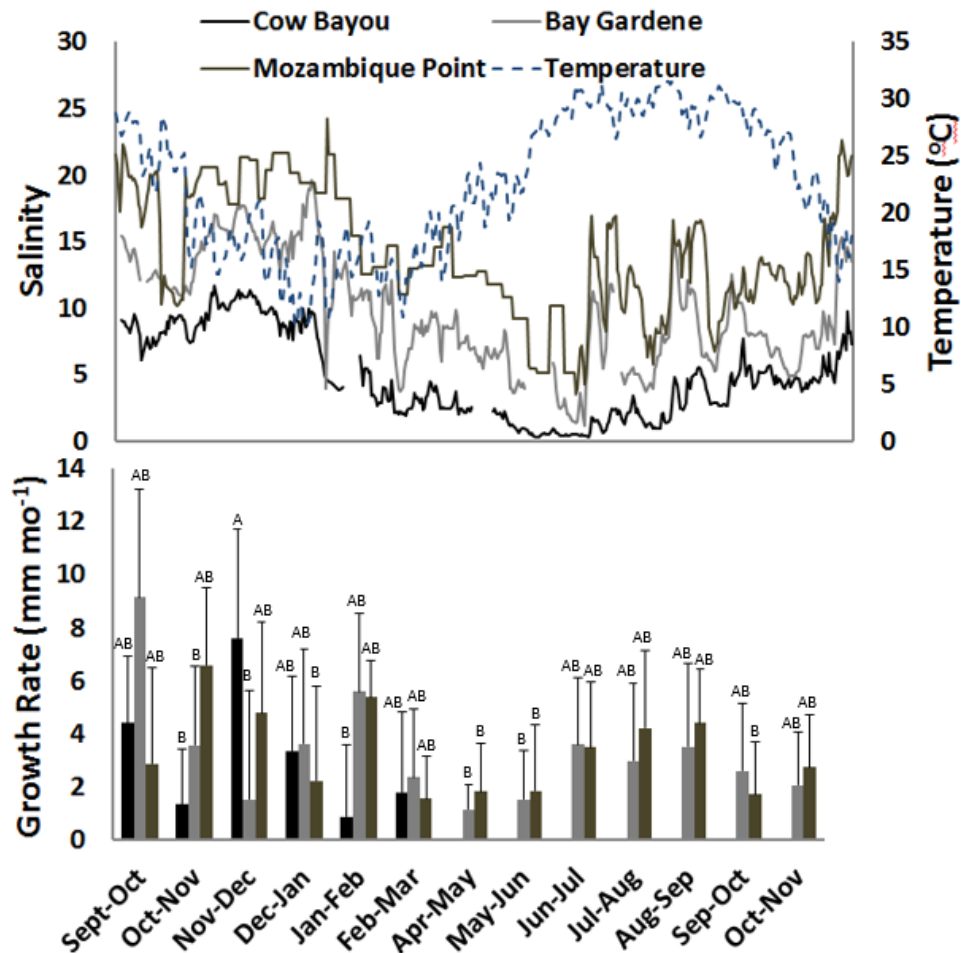


Figure 2.8 Mean interval growth rates ( $\text{mm mo}^{-1}$ )  $\pm$  standard error, and daily salinities and temperatures, for seed in all bags at three sites recorded September 2012 – November 2013. After initial deployment in September 2012, remaining oysters were discarded in March 2013 and replaced the following month. All seed oysters at Cow Bayou died by May 2013. Bars represent growth rates for the previous month (i.e., October = growth from September-October), solid lines represent salinity, and the dotted line represents water temperature. Letters denote significance for the month by site interaction ( $p < 0.05$ ).

Growth at Cow Bayou in December was significantly different than growth at Bay Gardene in November 2012, December, May 2013, and June, as well as at Mozambique Point in October 2012, June 2013, May 2013, and Cow Bayou in November 2012 and February 2013.

Multiple stepwise regression for interval mortality showed a significant negative relationship with salinity ( $R^2 = .15$ ,  $p = .0258$ ; Figure 2.9), but no significant relationships between temperature and mortality were detected.



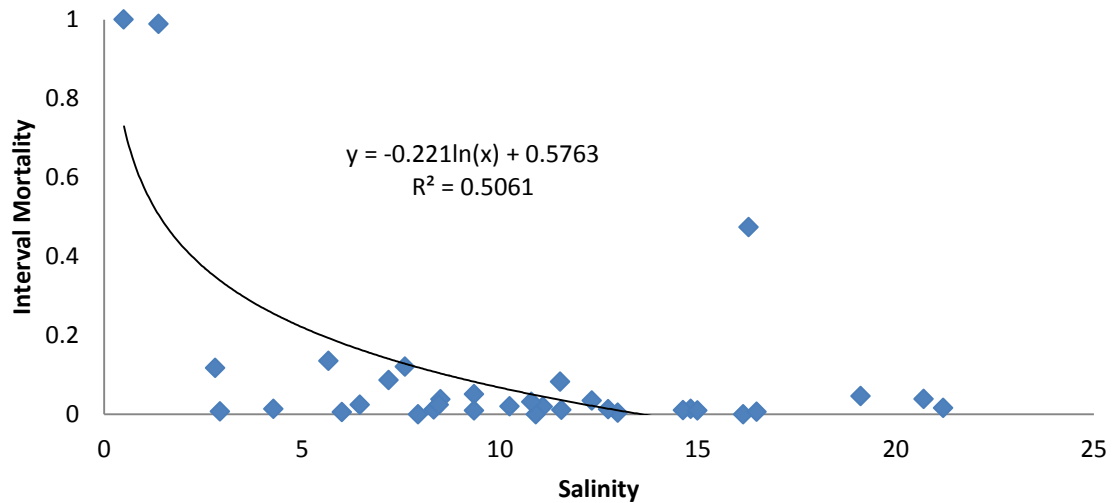


Figure 2.9 Seed interval mortality in closed bags from September 2012 – November 2013 regressed on interval salinity. A polynomial trendline was selected in Excel as the model with the highest  $R^2$  value.

Generalized linear mixed models revealed there was a significant interactive effect of site, cage type, and month ( $p=.0118$ ) on interval mortality (Figure 2.10), while cumulative mortality was significantly affected by site ( $p=.0218$ ) and bag type ( $p<.0001$ ), but not their interaction. Mortality in closed bags during the first deployment, September 2012 – March 2013, was highest at Mozambique Point (11%) and lowest at Bay Gardene (4%; Figure 2.11).

During the second deployment, April – November 2013, mortality was highest at Cow Bayou in both open and closed bags, which sustained 100% mortality by June 2013 (Figure 2.12). At Bay Gardene, mortality was highest in open bags during December 2012 and August – November 2013. At Mozambique Point, mortality was highest in open bags May – October 2013. At all sites, higher mortality was recorded in open bags than in closed bags. By November 2013, lowest mortality occurred at Mozambique Point (35.3%), which was not significantly different from Bay Gardene (35.9%).

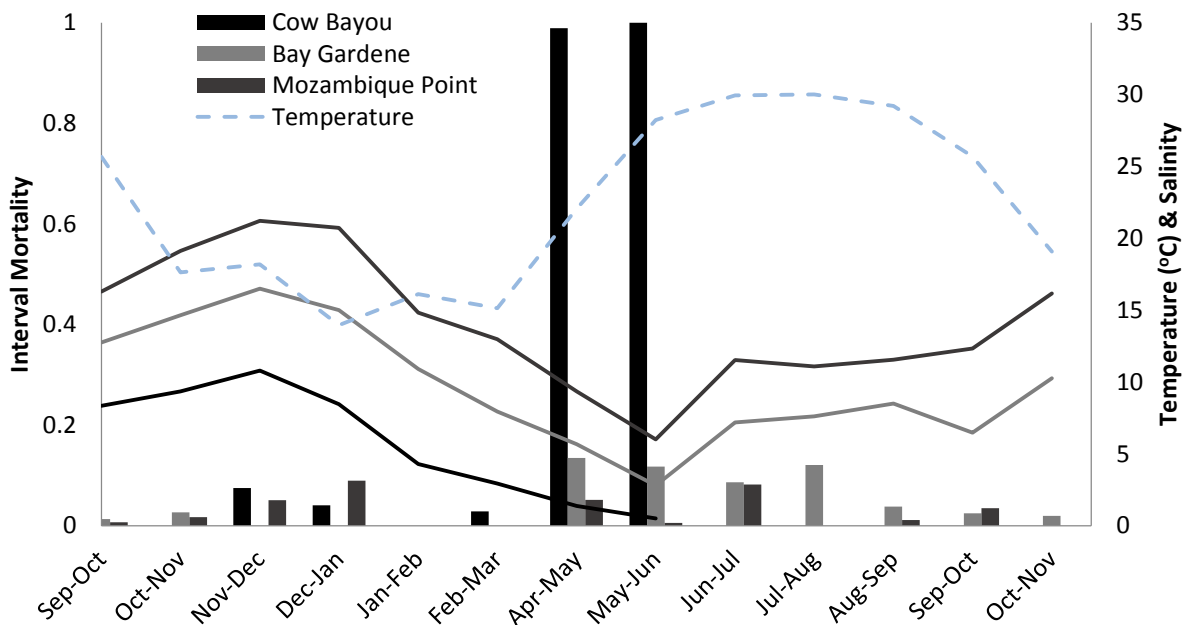


Figure 2.10 Seed closed bag interval mortality, and salinity and temperature recorded on sample dates, for three sites recorded October 2012 – November 2013. After initial deployment in September 2012, remaining oysters were discarded in March 2013 and replaced the following month. Bars represent interval mortality, solid lines represent interval salinity, and the dotted line represents interval temperature. Zero mortality occurred where there are no bars. All oysters died at Cow Bayou by June 2013.

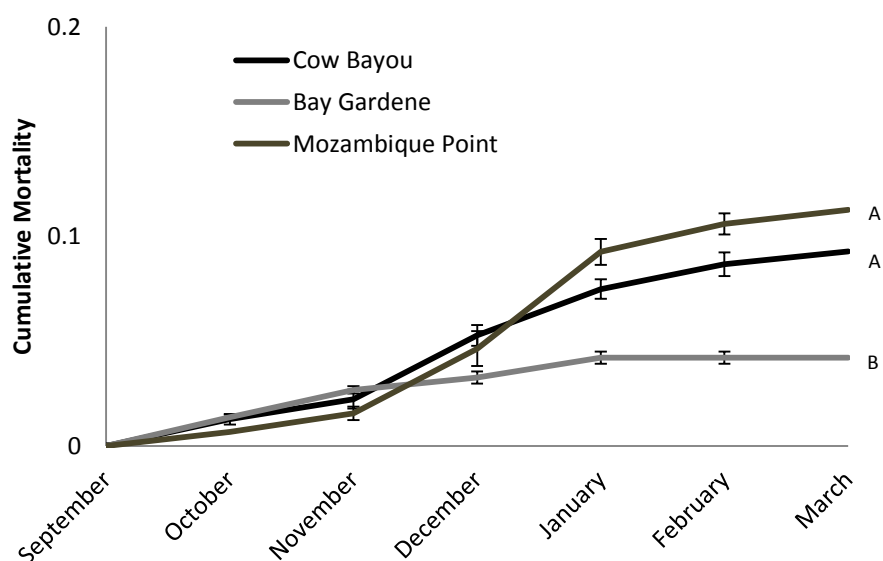


Figure 2.11 Seed cumulative mortality in closed bags recorded monthly September 2012 – March 2013. Letters denote significance for month by site interaction. Open bags were lost or buried during this trial.

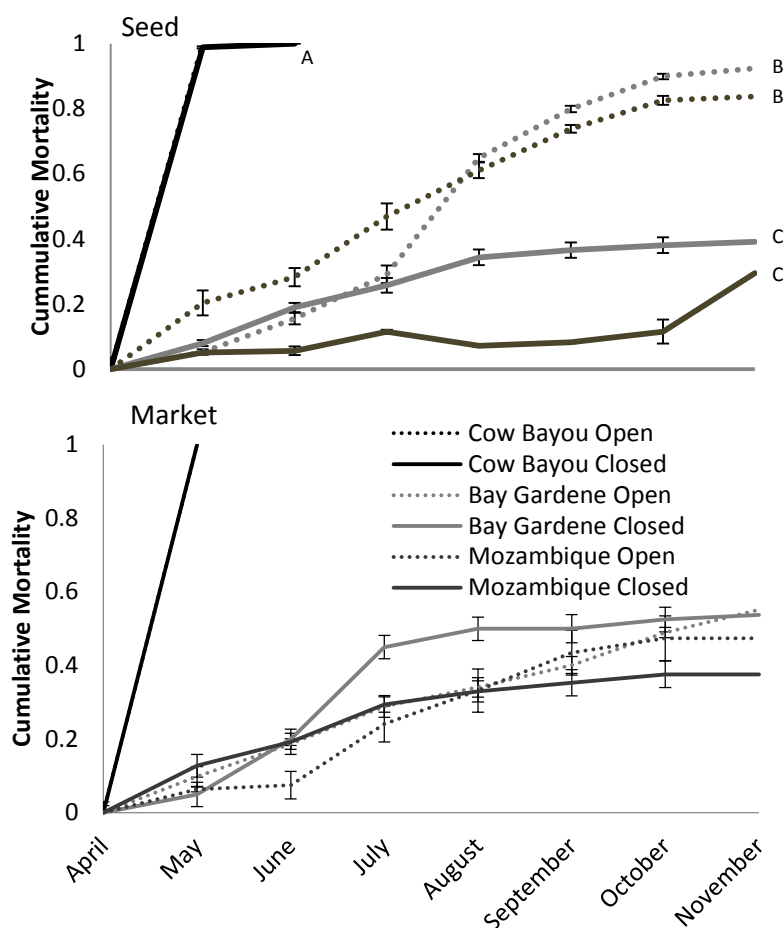


Figure 2.12 Market and seed cumulative mortality recorded monthly in open and closed bags at all sites April – November 2013. Solid lines represent closed bags and dotted lines represent open bags. All oysters at Cow Bayou died by June 2013. Letters denote significance for the month by site interaction ( $p < 0.05$ ).

Market: Two closed bags at Mozambique Point were buried in July 2013 and were not included in mortality analyses. All market oysters at Cow Bayou died by May 2013.

There was a significant interactive effect of site and month ( $p = .0379$ ) on interval growth rate, though overall, very little growth was observed (Figure 2.13) – Bay Gardene ( $1.3 \pm 0.5 \text{ mm mo}^{-1}$ ) and Mozambique Point ( $1.5 \pm 1.3 \text{ mm mo}^{-1}$ ). Growth at Mozambique Point in July 2013 was significantly different from growth at Bay Gardene in October and at Mozambique Point in May and

November. Multiple stepwise regression for interval growth did not reveal any significant relationships between growth and interval salinity and temperature.

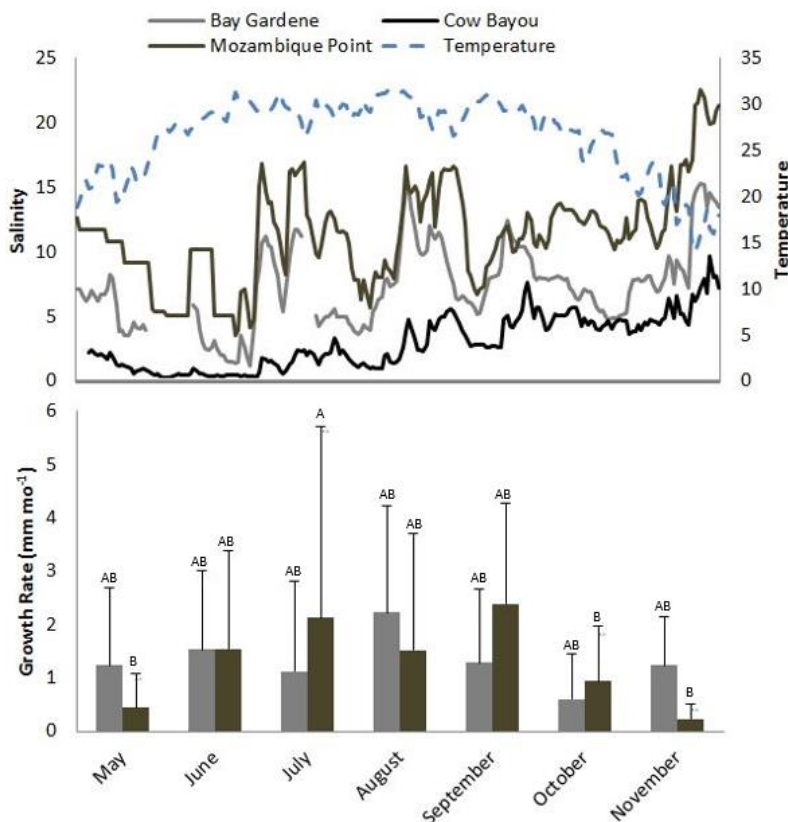


Figure 2.13 Mean interval growth rate (mm)  $\pm$  standard deviation, and daily salinities and temperatures, for market-sized oysters in all bags at three sites recorded April – November 2013. All oysters at Cow Bayou died in May 2013. Letters denote significance for the month by site interaction ( $p < 0.05$ ).

Interval mortality was only significantly affected by month ( $p = .0056$ ; Figure 2.14). High mortality, in both open and closed bags, occurred May – July (Figures 2.12 and 2.14) when temperatures were high (Figure 2.3), and this was especially evident at the lowest salinity site. Total cumulative mortality was not significantly affected by site or bag type (Figure 2.12) although mortality followed the general trend with highest cumulative mortality recorded at Cow Bayou (100% by May 2013) and lowest recorded at Mozambique Point (53.5%); mortality at Bay Gardene

was similar to Mozambique Point (53.8%). Multiple stepwise regression for interval mortality did not reveal any significant relationships between mortality and interval salinity and temperature.

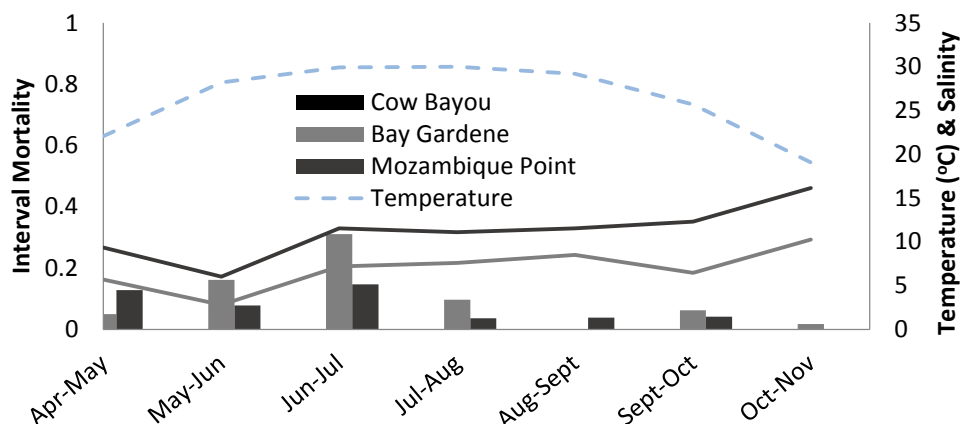


Figure 2.14 Market closed bag interval mortality, and salinity and temperature recorded on sample dates, for three sites recorded April – November 2013. Bars represent interval mortality, solid lines represent interval salinity, and the dotted line represents interval temperature. Zero mortality occurred where there are no bars. All oysters died at Cow Bayou by June 2013.

## Discussion

Predicted climate change patterns for the northern Gulf of Mexico may have profound cascading effects on resident organisms. Results from this study indicate that high water temperatures ( $> 30^{\circ}\text{C}$ ) combined with low salinities ( $< 5$ ) negatively impact spat, seed, and market-sized oyster growth and survival, and that high temperatures alone may negatively impact market-sized oysters. If current climate model predictions, which suggest an average increase in July of  $4\text{--}11^{\circ}\text{C}$  (IPCC 2001), coincide with increased precipitation, the oyster industry, in particular, will face significant challenges in managing adult oyster mortality.

It is critical to understand the potential impacts of climate and abiotic changes on oyster resources in order to better adapt and manage these resources for long-term sustainability. Past lab experiments demonstrated that generally, oyster mortality increases as salinity decreases and temperature increases, but the exact limits and effects on different size class oysters were not

elucidated (Loosanoff, 1953; Galstoff, 1964; Loosanoff, 1958). In controlled lab experiments using local oysters of three size classes, we found that market-sized oysters were most sensitive to high temperatures and low salinity (both alone and together). Spat were the least sensitive, only experiencing high mortality at extreme low salinity (1), and at the high temperature, low salinity (5) combination.

Results from our field study indicate similar differences in tolerances. In our field deployed spat, closed bags experienced greater than 50% mortality at the lowest salinity site (Cow Bayou), where salinity ranged 3.9 – 7.7 and temperatures ranged 27.3 – 32 °C. This is likely due to the fact that for the most of the trial, salinity remained below 5 with high temperatures. Higher mortality in open bags is likely due to predation. At the same low salinity field site, 100% of seed and market oysters died with similar salinity (0.3 – 9.7) and temperature ranges (13 – 32.3 °C). It is possible that the lower salinity range of 0.3 (compared to 3.9 for spat) may explain differences in survival, although the lab study suggests that at high temperatures, any salinity below 5 would be detrimental. Andrews et al. (1959) and Kennedy (1991) suggested that oysters could survive periods of low salinities by inducing a state of metabolic depression through prolonged valve closure, providing corresponding low ambient temperatures, but when temperatures reach levels >30 °C, the detrimental effects of low salinity are exacerbated. Defining “low” salinity remains critical, and may be population and location specific.

Increased resilience to changes in salinity by smaller size classes has been found in other bivalves such as juvenile seed clams (*Mercenaria mercenaria*), which Baker et al. (2005) described as “surprisingly robust and resilient” to changes in salinity, experiencing less than 5% mortality after relatively abrupt reductions in salinity of 10 to 15. Furthermore, following prolonged periods when air temperatures  $\geq 27.8$  °C and rainfall  $\leq 7$  cm, oyster mortalities in Breton Sound were significantly higher in larger (>65 mm) oysters than oysters <60 mm (Owen, 1953b). These differences in mortality by size class follow well-established patterns, however size class mortalities have not been consistent annually.

For example, most oyster mortality in Barataria Bay 1982-1985 (salinity range 15 – 25, temperature range 12 – 30 °C) was skewed toward smaller oysters, 20-40 mm in length, which coincided with an increase in drill abundance, while deaths of larger oysters (>65 mm) remained relatively low (Melancon, 1990).

Market oysters appear to be more sensitive to high temperatures than spat or seed, as mortality was high in all salinity treatments (1, 5, 15) at 32 °C. This is in agreement with Shumway (1996) who reported 30 °C as the upper temperature range adult oysters can tolerate. Lab experiments on Florida oysters showed adults can survive in salinity 2 at tanks of 15 °C and 30 °C for 14 days (Heilmayer et al., 2008). However, our data show market oysters experienced high mortality (~80%) in salinity ≤ 5 at 32 °C. Furthermore, market oyster mortality reached 100% at Cow Bayou within one month of deployment (salinity < 5, 100% of time; temperature > 20 °C). Previous studies have also shown market oysters experiencing 100% mortality at Cow Bayou in 2010 and 2011 (La Peyre et al., 2013). This finding is particularly disturbing given predictions of increased summer temperatures (IPCC 201; Brierley and Kingsford, 2009; Ning and Abdollahi, 1999).

Higher temperatures increase metabolic activities such as pumping rate, heart rate and respiration, while low salinities (< 5) interfere with the osmotic balance of somatic tissues, disrupting whole system functioning (Heilmayer et al., 2008; Anderson and Anderson, 1975; Shumway, 1996). A number of lab studies have demonstrated that oysters subjected to salinity treatments below 5 ceased feeding, produced no feces or pseudofeces, and maintained their valves in a closed position. During the closed-valve stage, increased anaerobic metabolism conserves energy (Michaelidis et al., 2005). However, this leads to accumulation of anaerobic end products and a nearly complete cessation of gas exchange between oyster and ambient environment, resulting in the buildup of carbon dioxide in tissues, which is described as one of the main reasons for summer mortalities in oyster populations (Heilmayer et al., 2008; Michaelidis et al., 2005; Lannig et al., 2008). With limited market survival,

decreased spawning and thus recruitment, as well as availability of hard substrate, can be expected, and can have cascading population effects.

Market-size oyster survival is important when considering population sustainability, as they are responsible for spawning necessary for recruitment. While not measured in this study, several studies have reported limited or no recruitment when salinity is below 10 (La Peyre et al., 2013; Cake, 1983; Chatry et al., 1983; Pollack et al., 2011). Limited recruitment may affect the future abundance of oysters that reach reproductive size, substrate accretion and availability, and thus future recruitment levels (Southworth and Mann, 2004; Powell et al., 2009). Furthermore, successful oyster populations are represented by multi-year classes because it reflects the accumulation of many spat falls over many years (He et al., 2012). One poor recruitment year may not be detrimental to a population unless it is also associated with high mortality of reproductive oysters, which this study indicates may happen in high temperatures regardless of salinity.

Predator and parasite abundance may also affect oyster survival. Parasite abundance was not measured in this study, but past studies have not found it to be lethal in this region due to low salinities (<25) (La Peyre et al. 2009, 2013). Predation, however, may be an issue, as field mortality was higher in open bags than closed, indicating predation. Predation was likely from blue crabs (*Callinectes sapidus*), stone crabs (*Menippe adina*) and black drum (*Pogonias cromis*) due to observed crab and drum and shell fragments in bags. While oyster drills (*Stramonita haemastoma*) are typically a main predator of oysters in the Gulf (St Amant, 1938; Brown and Richardson, 1987), they require sustained salinities greater than 15 (Garton and Stickle, 1980), and were only observed at Mozambique Point in August. Mortality in open bags was predicted to be higher at higher salinity sites, because oyster predator abundance increase with increasing salinities (Melancon, 1991; Manzi, 1970; Hopkins, 1962; Mackenzie, 1970). However, mortality in open bags was higher at the low salinity site (Cow Bayou) than the high salinity



site (Mozambique Point; Figure 2.4), which may indicate that under current salinity conditions in Breton Sound, salinity determines oyster mortality more so than predation.

Currently, the Louisiana industry has market-size oysters for harvest within less than 18 months and changes in growth rates, assuming survival, could significantly affect production rates.

Growth rates usually increase with increasing temperature, as well as with increasing salinity (Dekshenieks et al., 1993; Harding, 2007; Hofmann et al., 2004). As a result, growth rates are typically held to be higher in warmer months, and in higher salinity sites. Interestingly, our monthly results indicate a different trend with higher salinity and lower temperatures supporting greater growth. In fact, lowest growth rates occurred in seed and market-size oysters during summer months during this study. Specifically, there was a general trend of decreasing growth rates from February through August, and higher growth after September. In Breton Sound, low salinity generally also coincides with the warmest months, and may be contributing to the difference in trends observed from other regions. Typically, growth rates decrease as size increases (Kraeuter et al., 2007), and this was the pattern in Breton Sound oysters 2012-2013. Spat grew faster than seed and market sized oysters, and seed sized oysters grew faster than market oysters.

Our data support previous research that the minimum salinity required for oysters to grow is 7, and minimal growth occurs below 5 (Chanley, 1958; Wells, 1961; Loosanoff, 1953), as low growth ( $0.9 \pm 2.7$  mm) was observed at Cow Bayou in February 2012 when monthly average salinity was  $4.3 \pm 1.0$ . Furthermore, low growth ( $2.3 \pm 1.2$  mm) was observed at Bay Gardene May – August 2013 when salinity averaged  $5.8 \pm 2.1$ . Gunter (1955) reported minimum oyster growth rates in Barataria Bay as  $8.4$  mm  $\text{mo}^{-1}$ , however this rate was calculated as if growth was constant between months. Our data show seed growth ranged from  $0.9$  mm  $\text{mo}^{-1}$  at Cow Bayou in February 2013 to  $9.1$  mm  $\text{mo}^{-1}$  at Bay Gardene in October 2012 (Appendix H).

Understanding differences in life-history stage tolerances, and the potential effects of predicted climate changes with increased temperatures disproportionately occurring in summer months when salinities are low, are critical to predicting population dynamics and success. Models for precipitation remain highly variable making it difficult to plan, but worst case scenarios for the Breton Sound predict decreases in salinity of 1-3 (Swenson, 2003), which could significantly shift isohalines south and directly cause oyster mortalities if this occurs during hot summer months, regardless potential temperature increases. Although not measured in this study, reduced salinities may also be indicative of a variety of other stressors, such as increased turbidity, changed or decreased phytoplankton communities and concentrations, all of which may further compound the effects of salinity on oyster health and survival. Furthermore, if isohalines shift, regions of low salinity, disease and predation refuge, and optimal oyster bottom habitat may no longer align thus negatively impacting reproduction and survival (Levinton et al., 2011).

These results suggest low growth and mass mortalities are likely to occur if salinity drops below 2, regardless of the time of year, but oysters may survive longer if low salinity events occur during fall or winter, when average temperatures are below 25 °C. The contrasting effects of salinity on natural mortality versus recruitment and growth, combined with the seasonal timing of high and low salinity, complicate efforts to predict and respond to different freshwater inflow management regimes. With the ultimate goal of maximizing oyster production over multiple years, predicting upcoming oyster seasons to develop effective management strategies requires being able to understand and adjust for differences in salinities, which can be controlled to some extent, by management of freshwater inflows on an annual or seasonal basis (La Peyre et al., 2009; La Peyre et al., 2013; Gauthier et al., 1990). If model predictions regarding increased July heat and summer precipitation hold true, however, managers may need to seriously consider other options for oyster production such as off-bottom aquaculture.

### Chapter 3: Conclusion

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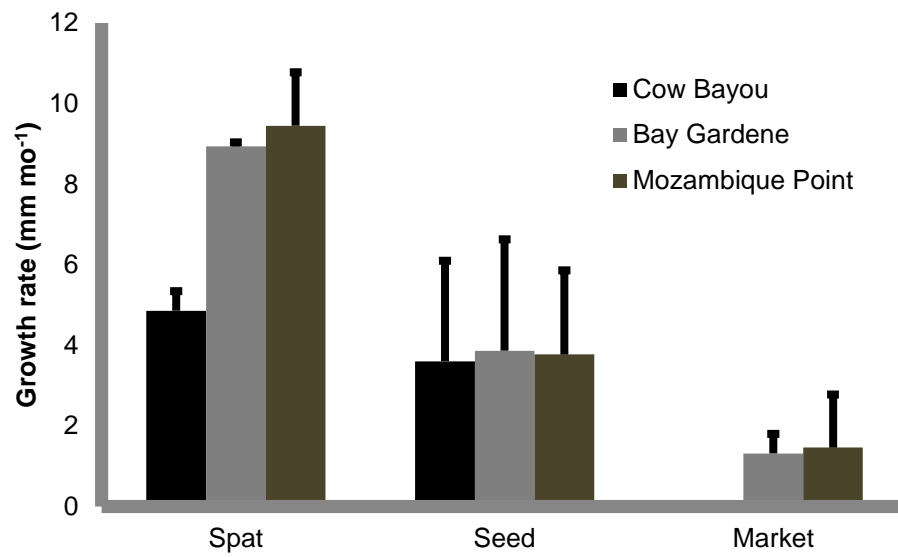
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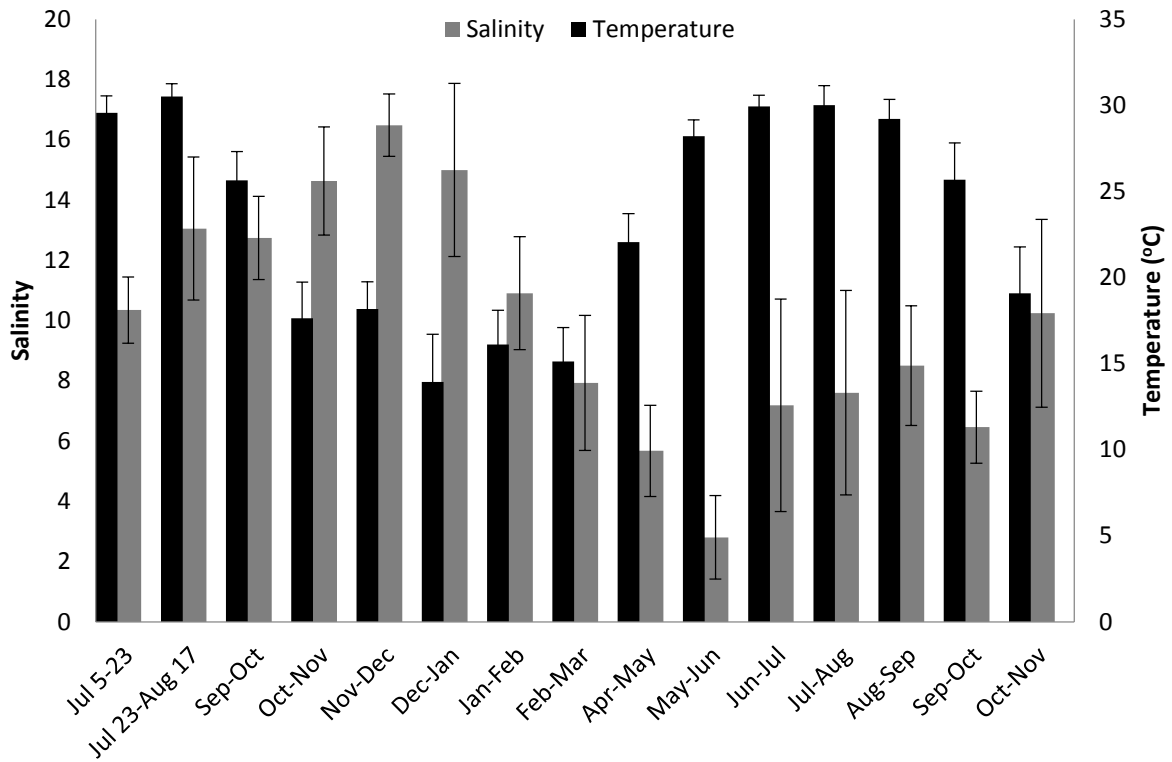
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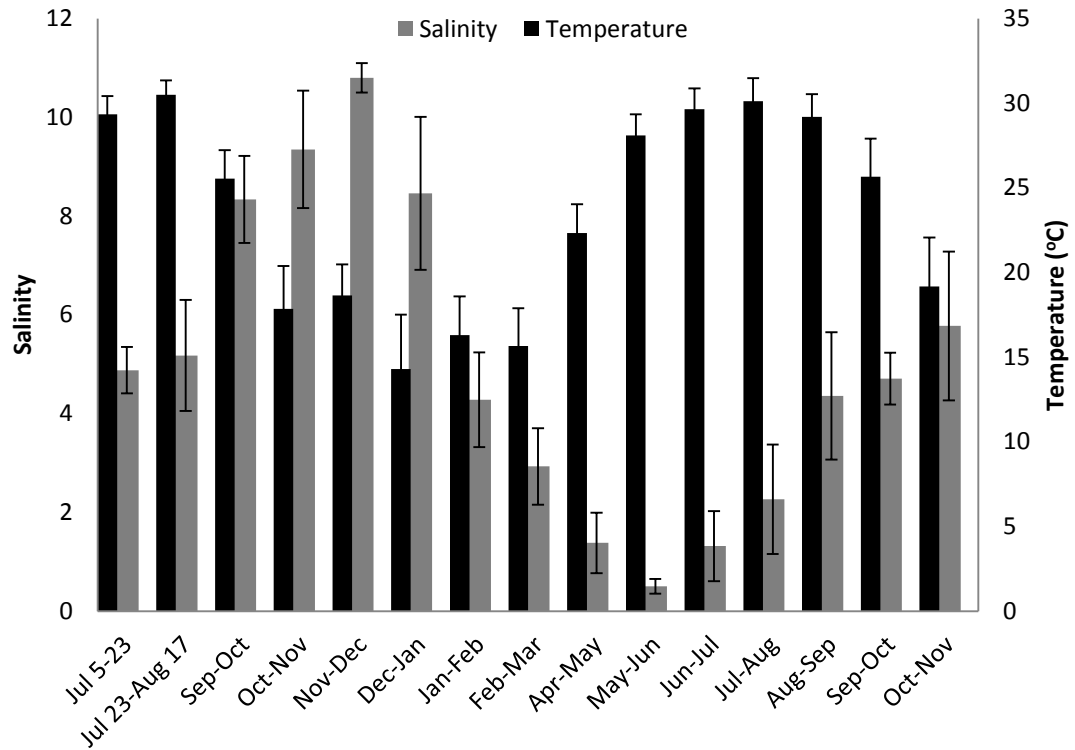
**Appendix A: Overall mean growth rate ( $\text{mm mo}^{-1}$ )  $\pm$  standard deviation for spat, seed, and market oysters at 3 sites recorded July 2012 – November 2013.**



**Appendix B: Mean water temperatures and salinities  $\pm$  standard deviation for each sampling interval at Bay Gardene.**

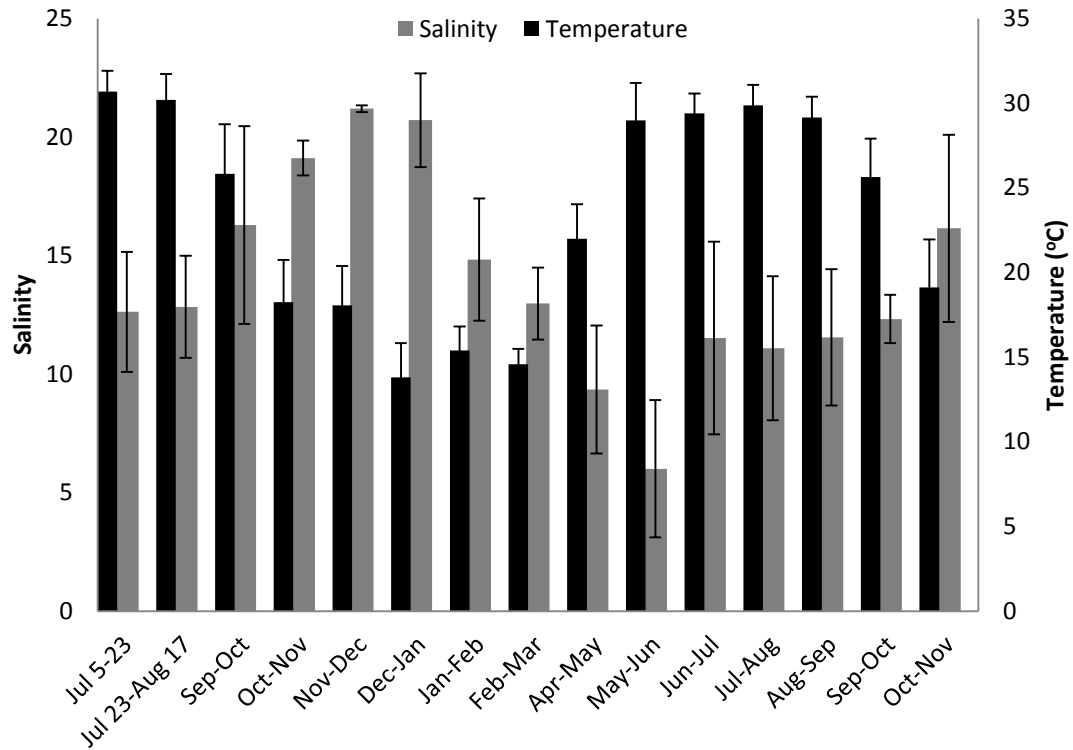


**Appendix C: Mean water temperatures and salinities  $\pm$  standard deviation for each sampling interval at Cow Bayou.**





**Appendix D: Mean water temperatures and salinities  $\pm$  standard deviation for each sampling interval at Mozambique Point.**



**Appendix E: Water quality parameter ranges, means, and standard deviations recorded for each lab experiment. NO<sub>2</sub>, NO<sub>3</sub>, NH<sub>4</sub>, hardness, and alkalinity are reported as parts per million.**

Trial	pH	NO <sub>2</sub>	NO <sub>3</sub>	NH <sub>4</sub>	Alkalinity	Hardness
Spat 1	8.0 – 8.5	0.0 – 0.0	0.0 – 0.0	0.0 – 0.0		
	8.5 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
Spat 2	8.0 – 8.4	0.0 – 1.0	0.0 – 0.0	0.0 – 0.0		
	8.4 ± 0.1	0.7 ± 0.4	0.0 ± 0.0	0.0 ± 0.0		
Seed 1	8.0 – 8.4	0.0 – 0.5	0.0 – 0.0	0.0 – 0.0		
	8.1 ± 0.2	0.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0		
Seed 2	7.8 – 8.0	0.0 – 0.0	0.0 – 0.0	0.0 – 0.0		
	7.9 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0		
Seed 3	7.5 – 8.3	0.0 – 0.5	0.0 – 5.0	0.0 – 0.5	240 – 240	250 – 425
	8.1 ± 0.2	0.1 ± 0.1	0.1 ± 2.3	0.0 ± 0.1	240 ± 0.0	420 ± 29.0
Market 1	7.2 – 8.4	0.0 – 1.0	0.0 – 0.0	0.5 – 3.0	180 – 240	250 – 425
	8.2 ± 0.4	0.5 ± 0.4	0.0 ± 0.0	2.2 ± 1.0	238 ± 10.0	376 ± 79.5
Market 2	8.0 – 8.0	0.2 – 0.3	0.0 – 0.0	0.6 – 0.6	240 – 240	425 – 425
	8.2 ± 0.4	0.1 ± 0.2	0.0 ± 0.0	0.6 ± 0.0	240 ± 0.0	425 ± 0.0

**Appendix F: Water temperature (°C) ranges are displayed on top of means  $\pm$  standard deviations for target treatment temperatures recorded in each lab experiment.**

Trial	25°C	32°C
Spat 1	25.0 – 27.0	30.0 – 34.0
	26.0 $\pm$ 1.4	32.0 $\pm$ 2.1
Spat 2	24.8 – 27.6	32.2 – 34.6
	26.6 $\pm$ 0.8	33.2 $\pm$ 0.9
Seed 1	22.8 – 28.6	30.6 – 34.4
	24.8 $\pm$ 1.6	32.3 $\pm$ 0.9
Seed 2	23.2 – 26.4	29.6 – 33.4
	25.0 $\pm$ 0.6	31.4 $\pm$ 0.8
Seed 3	23.4 – 26.1	24.1 – 32.0
	24.5 $\pm$ 0.7	30.8 $\pm$ 1.4
Market 1	22.8 – 27.2	28.0 – 33.2
	24.2 $\pm$ 1.0	31.6 $\pm$ 0.9
Market 2	23.3 – 26.6	30.2 – 32.5
	24.5 $\pm$ 0.8	31.5 $\pm$ 0.5

**Appendix G: Mean interval growth rates  $\pm$  standard deviation recorded at each site. May to November 2013 seed and market values at Cow Bayou are not available due to 100% mortality.**

	Growth (mm mo <sup>-1</sup> )		
<u>Spat</u>	Cow Bayou	Bay Gardene	Mozambique Point
July 2012	4.5 $\pm$ 1.8	8.9 $\pm$ 1.8	8.5 $\pm$ 1.5
August 2012	5.2 $\pm$ 1.3	9.0 $\pm$ 1.0	10.4 $\pm$ 1.8
<u>Seed</u>			
October 2012	4.4 $\pm$ 2.5	9.1 $\pm$ 4.1	2.9 $\pm$ 3.6
November 2012	1.4 $\pm$ 2.0	3.6 $\pm$ 3.0	6.6 $\pm$ 2.9
December 2012	7.6 $\pm$ 4.1	1.5 $\pm$ 4.1	4.8 $\pm$ 3.4
January 2013	3.4 $\pm$ 2.8	3.6 $\pm$ 3.5	2.2 $\pm$ 3.4
February 2013	0.8 $\pm$ 2.7	5.6 $\pm$ 2.9	5.4 $\pm$ 1.4
March 2013	1.8 $\pm$ 3.0	2.4 $\pm$ 2.6	1.6 $\pm$ 1.6
May 2013	.	1.2 $\pm$ 0.9	1.8 $\pm$ 1.8
June 2013	.	1.5 $\pm$ 1.8	1.8 $\pm$ 2.5
July 2013	.	3.6 $\pm$ 2.5	3.5 $\pm$ 2.4
August 2013	.	3.0 $\pm$ 2.9	4.2 $\pm$ 2.9
September 2013	.	3.5 $\pm$ 3.1	4.4 $\pm$ 2.0
October 2013	.	2.6 $\pm$ 2.5	1.7 $\pm$ 2.0
November 2013	.	2.0 $\pm$ 2.0	2.7 $\pm$ 2.0
<u>Market</u>			
May 2013	.	1.2 $\pm$ 1.5	0.4 $\pm$ 0.6
June 2013	.	1.5 $\pm$ 1.5	1.5 $\pm$ 1.8
July 2013	.	1.1 $\pm$ 1.7	2.1 $\pm$ 3.6
August 2013	.	2.2 $\pm$ 2.0	1.5 $\pm$ 2.2
September 2013	.	1.3 $\pm$ 1.4	2.4 $\pm$ 1.9
October 2013	.	0.6 $\pm$ 0.9	0.9 $\pm$ 1.0
November 2013	.	1.2 $\pm$ 0.9	0.2 $\pm$ 0.3

**Appendix H: Mean interval salinity  $\pm$  standard deviation recorded at each site and mean interval temperature  $\pm$  standard deviation for all sites. Temperature did not differ by site.**

Trial	Salinity			Temperature
<u>Spat</u>	Cow Bayou	Bay Gardene	Mozambique Point	
July 2012	4.9 $\pm$ 0.5	10.3 $\pm$ 1.1	12.6 $\pm$ 2.5	29.3 $\pm$ 1.1
August 2012	5.2 $\pm$ 1.1	13.1 $\pm$ 2.4	12.8 $\pm$ 2.2	30.5 $\pm$ 0.8
<u>Seed</u>				
October 2012	8.3 $\pm$ 0.9	12.7 $\pm$ 1.4	16.3 $\pm$ 4.2	25.5 $\pm$ 1.7
November 2012	9.4 $\pm$ 1.2	14.6 $\pm$ 1.8	19.1 $\pm$ 0.7	17.8 $\pm$ 2.5
December 2012	10.8 $\pm$ 0.3	16.5 $\pm$ 1.0	21.2 $\pm$ 0.1	18.7 $\pm$ 1.7
January 2013	8.5 $\pm$ 1.5	15 $\pm$ 2.9	20.7 $\pm$ 2.0	14.3 $\pm$ 3.2
February 2013	4.3 $\pm$ 1.0	10.9 $\pm$ 1.9	14.8 $\pm$ 2.6	16.3 $\pm$ 2.3
March 2013	2.9 $\pm$ 0.8	7.9 $\pm$ 2.2	13 $\pm$ 1.5	15.7 $\pm$ 2.2
<u>Seed and Market</u>				
May 2013	1.4 $\pm$ 0.6	5.7 $\pm$ 1.5	9.4 $\pm$ 2.7	22.3 $\pm$ 1.7
June 2013	0.5 $\pm$ 0.2	2.8 $\pm$ 1.4	6 $\pm$ 2.9	28.1 $\pm$ 1.2
July 2013	1.3 $\pm$ 0.7	7.2 $\pm$ 3.5	11.5 $\pm$ 4.1	29.6 $\pm$ 1.2
August 2013	2.3 $\pm$ 1.1	7.6 $\pm$ 3.4	11.1 $\pm$ 3.0	30.1 $\pm$ 1.3
September 2013	4.4 $\pm$ 1.3	8.5 $\pm$ 2.0	11.6 $\pm$ 3.0	29.2 $\pm$ 1.3
October 2013	4.7 $\pm$ 0.5	6.5 $\pm$ 1.2	12.3 $\pm$ 1.0	25.6 $\pm$ 2.3
November 2013	5.8 $\pm$ 1.5	10.2 $\pm$ 3.1	16.4 $\pm$ 4.0	19.2 $\pm$ 2.9

**Appendix I: Mean interval growth and salinity ( $\pm$ SD) for all oysters recorded July 2012 – November 2013. Data are for spat oysters (7/5 – 8/17/2012), seed oysters 1 (9/16/2012 – 3/17/2013), seed 2 and market (4/21 – 11/23/2013). Growth for seed 2 is displayed to the right of growth for market.**

Site	Oyster growth (mm mo <sup>-1</sup> )		Salinity					Mean (±SD)
	Mean (±SD)		# days					
			<1	<3	<5	<15	>15	
Spat								
CB	4.9(±1.6)		0	0	26	48	0	5
BG	9.0(±1.4)		0	0	0	37	6	11.9(±2.4)
MP	9.5(±1.7)		0	0	0	34	10	12.7(±2.4)
Seed 1								
CB	3.2(±2.5)		0	19	51	177	0	7.3(±2.9)
BG	4.3(±2.7)		0	0	5	116	58	12.7(±3.5)
MP	3.9(±2.0)		0	0	0	20	49	17.1(±3.8)
Seed 2 & Market								
CB	.	.	44	119	174	212	0	3.0(±2.1)
BG	2.5(±1.0)	1.3(±0.5)	0	14	43	193	3	7.4(±3.2)
MP	3.4(±1.4)	1.3(±0.8)	0	0	4	136	34	12.2(±3.7)

**Vita**

Molly Rybovich was born and raised in Palm Beach County, Florida. She loves her cat, Mr. Buttons.