EFFECTS OF TEMPERATURE, SALINITY AND DISSOLVED OXYGEN ON SURVIVAL OF TRIPLOID AND DIPLOID HARD CLAMS, *Mercenaria mercenaria*

By

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To my family for all their patience, support, and understanding.

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High mortalities of cultured *Mercenaria mercenaria*, the northern hard clam, have occurred along the west coast of Florida during the summer months and are thought to be caused by low salinities, high temperatures, and low levels of dissolved oxygen. *M. mercenaria* also lose a substantial amount of energy through a spring spawning event. It is evident that an improvement in hard clam aquaculture is needed, and triploidy may be a potential solution. Based on the energy reallocation hypothesis, triploids will divert energy from reproduction to somatic growth resulting in a larger and potentially hardier clam. Triploids were produced using cytochalasin B during meiosis II division and ploidy analysis confirmed triploid state. Triploids and sibling diploids were exposed to salinities of 10, 25, and 40 ppt and dissolved oxygen levels of < 2 mg·L^{-1} and > 5 mg·L^{-1} with temperature constant at 32^{o}C for 21 days. Observations of clam mortality, siphon extension, and burial were conducted at 12 hour intervals to assess lethal and sublethal treatment effects. Survival analysis was performed using the Lifereg procedure available on SAS to test the difference in survival between triploids and diploids in each treatment. Survival of diploid and triploid *M. mercenaria* differed significantly in two of the treatments: 25 ppt hypoxia ($P=0.0159$) and 10 ppt hypoxia ($P=0.0387$). Burial rates decreased over the duration of the experiment in the 25 ppt hypoxia and 40 ppt hypoxia treatments,

indicating a sublethal effect of hypoxia. There was an advantage of triploidy in the 25 ppt hypoxia treatment, a range seen in the field; however the overall results of this experiment suggest that triploidy does not confer an advantage for survival of *M. mercenaria*.

CHAPTER 1
INTRODUCTION

In Florida, the hard clam, *Mercenaria mercenaria* is an important aquaculture species for coastal residents in rural areas on the east and west coasts (Figure 1-1), including Cedar Key and Charlotte Harbor (USDA 2004). In recent years, high clam mortalities have occurred along the west coast of Florida during the summer months and are thought to be caused by low salinities, high temperatures, and low levels of dissolved oxygen (Scarpa et al. 2005). Prior to summer stressors, mature *M. mercenaria* are releasing a substantial amount of energy through an intense spring spawning event (Hesselman et al. 1989, Eversole 2001). It is evident that an improvement in hard clam stock is needed, and triploidy is a potential solution. Based on the energy reallocation hypothesis, triploids should allocate little or no energy towards reproduction and will instead allocate the energy to somatic growth resulting in a larger and potentially hardier clam (Beaumont & Fairbrother 1991). Since Florida already has fast growing clam populations, I examined whether triploidy would relate to a hardier clam, one with additional resources available with which to survive summer stressor events. Triploid and diploid *M. mercenaria* were challenged in a laboratory setting to determine differences in survival.

Aquaculture of *Mercenaria mercenaria* in Florida

Aquaculture of *Mercenaria mercenaria* is a multimillion dollar industry in the state of Florida, bringing in 13 million in net sales in 2003, and 10.7 million in 2005 (USDA 2004, USDA 2006). There are three regions of Florida where hard clam aquaculture is concentrated, the "Big Bend" region of Dixie, Levy and Citrus counties; the south west region of Charlotte and Lee counties, and the central east region, of Volusia, Brevard and Indian River counties (USDA 2002, USDA 2004). Cedar Key, in the "Big Bend" region and Charlotte Harbor in the southwest region are the main areas of clam aquaculture on the west coast of Florida.

Techniques to improve hard clam aquaculture in the state of Florida were investigated by Menzel in the 1950s and 1960s (1962). Hard clam aquaculture became more prevalent in the 1970s when attempts were made to supplement wild stock of *M. mercenaria* in the Indian River Lagoon (Philippakos et al. 2001). In 1991, as part of a job-retraining program funded by the state, shellfish aquaculture was introduced to the west coast of Florida and is now a primary source of income for its rural coastal residents (Colson & Sturmer 2000). In recent years, *M. mercenaria* has become one of the most economically important aquaculture species in Florida's marine environment (Arnold et al. 2000).

Description of Cedar Key and Environmental Factors

Cedar Key, in the "Big Bend" region of Florida is one of the main areas of clam aquaculture on the west coast of Florida. The Cedar Key Estuary is open to the Gulf of Mexico but water quality over the clam beds can be significantly influenced by freshwater inputs from the Suwannee River system (Colson & Sturmer 2000). Water quality monitoring stations were deployed on several clam leases and data on temperature, salinity and dissolved oxygen were collected every 30 minutes. Reported water quality values and additional archived data are available from the Florida Department of Agriculture and Consumer Services (FDACS 2007), Division of Aquaculture, http://sondes.floridaaquaculture.com/sondes/archiveintro.htm. Gulf Jackson, one of the most productive clam leases on the west coast, frequently reaches a low of 15 parts per thousand (ppt), and reaches a low of 2 to 12 ppt at least once every year (Figure 1-2, A) (FDACS 2007). In addition to annual fluctuations in salinity, hard clams in Cedar Key are exposed to regular tidal fluctuations of 5 ppt, and can experience extreme fluctuations of 24 ppt over 24 hours (Baker et al. 2005). Summer water temperatures range from 26^{o}C to 32^{o}C, with extremes of 34^{o}C to 35^{o}C in June and July (Figure 1-2, E). Temperatures of 32^{o}C can continue into October (FDACS 2007). Dissolved oxygen levels are extremely variable on the Gulf

Jackson clam lease in Cedar Key (Figure 1-2, C). Dissolved oxygen concentrations can fall below 5 milligrams per liter ($mg \cdot L^{-1}$), every year, April through October. In 2002, short duration anoxic (<1 $mg \cdot L^{-1}$) events occurred in both April and June (FDACS 2007).

Description of Charlotte Harbor and Environmental Factors

Charlotte Harbor is located on the southwest coast of Florida, and is a more recent area than Cedar Key for *Mercenaria mercenaria* aquaculture. The Charlotte Harbor Estuary is separated from the Gulf of Mexico by barrier islands, causing reduced mixing, increasing residence time within the estuary and influencing water quality (McPherson et al. 1996). Little water quality data are available for Charlotte Harbor. We know however that salinity in Charlotte Harbor fluctuates as a result of freshwater runoff from the Caloosahatchee, Myakka, and Peace Rivers. Incomplete data sets from 2002 and 2003, which do not include the rainy season, show that in May of 2002 salinity remained above 35 ppt (Figure 1-2, B), while the lowest salinity (17 ppt) was recorded in July of 2003 (FDACS 2007). Charlotte Harbor temperatures range from 26°C to 32°C in May through October. In July of 2002 (Figure 1-2, F) and June of 2003, water temperatures stayed between 30°C and 32°C for three weeks (FDACS 2007). Charlotte Harbor shows similar variability in dissolved oxygen levels as Cedar Key, with several hypoxic events occurring May through August. In July of 2002 (Figure 1-2, D), dissolved oxygen concentrations remained below 5 $mg \cdot L^{-1}$ for seventeen days, and frequently approached 0 $mg \cdot L^{-1}$ for short periods of time.

Anatomy and Behavior of *Mercenaria mercenaria*

Mercenaria mercenaria are important members of the benthic community. They burrow into the sediment which protects them from predation, stabilizes their movement and facilitates feeding (Doering 1982). They are suspension feeders and use specialized cilia on their gills to pump water through their siphons and into the mantle cavity which facilitates feeding, respiration

and waste removal (Hamwi 1969, Roegner & Mann 1991). The rate of water movement through the mantle cavity can be measured directly or indirectly. Rate of water movement is measured directly by measuring actual pumping rate, the volume of water pumped in a given time. Rate of water movement may also be measured indirectly by measuring clearance rate, the amount of particles removed from the water column in a given time (Grizzle 2001). Changes in activity, such as a decrease in pumping or burial, may be useful indicators of adverse environmental conditions. For a more detailed review of *M. mercenaria* anatomy see Eble (2001).

Physiology, Distribution and Survival of *Mercenaria mercenaria*

The physiology, distribution and survival of *Mercenaria mercenaria* in the field can be influenced by many factors including water quality, food availability, physical disturbances, biological disturbances, pollution and planting parameters. We know that these factors can affect survival, but it is difficult to understand to what extent their individual and combined effects are responsible for field mortalities (Kraeuter & Castagna 1989). Reduction in physiological and behavioral activities, such as burial, food consumption and oxygen uptake, has been noted in several studies in response to suboptimal ranges of temperature, salinity and dissolved oxygen (Chanley 1957, Hamwi 1969, Castagna & Chanley 1973, Van Winkle 1976). Roegner and Mann (1991), Eversole (1987), and Grizzle (2001) have reviewed the biology, reproduction and physiology of *M. mercenaria*.

M. mercenaria can tolerate a wide range of temperatures, as indicated by its native range extending from the Gulf of St. Lawrence in Canada to the Indian River Lagoon, Florida (Abbott 1974). *M. mercenaria* are also present along the Gulf Coast to Texas (Abbott 1974). There was a significant population increase along the west coast of Florida in the 1990s after it became an important aquaculture species (Arnold et al. 2004). Growth of juvenile and adult *M. mercenaria* is greatly influenced by temperature, with maximum growth occurring at 20^{o}C and shell growth

ceasing at temperatures above 31°C (Ansell 1968, Rice & Pechenik 1992). In the southeastern United States, slow growth bands occur in the summer and fall indicating sub optimal growth, likely due to high temperatures, while fast growth bands occur during winter and spring when water temperatures are cooler (Jones et al. 1990, Arnold et al. 1991). Maximum pumping rates for food and oxygen consumption occur between 20 to 25°C (Hamwi 1969). Temperatures of 6°C and 32°C represent the low and high limits at which pumping rates are no longer detected; although some clams may remain open (Hamwi 1969). *M .mercenaria* may be able to handle rapid changes in water temperatures within its tolerance limits, as demonstrated by a return to pumping within a few hours following a shift from cold water at 5°C to 24°C (Hamwi 1969). *M. mercenaria* is living at its thermal tolerance limit in Florida, as indicated by poor growth during summer months and decreased pumping rates at temperatures near 32°C. This indicates that *M. mercenaria* could be negatively affected by summers with higher than average temperatures.

M. mercenaria is an estuarine species and is typically found in regions with salinities ranging from 20 to 30 ppt (Grizzle et al. 2001). Castagna and Chanley (1973) report the lower salinity limit for survival to be 12.5 ppt for adult *M. mercenaria*, with an upper range of 35 ppt, with limited survival possible up to 46 ppt. Wells (1957) noted a correlation between areas of low salinity, near tributaries and river inlets, and an absence or lowered density of *M. mercenaria*, suggesting that salinity structures the population distribution within an estuary. Salinities between 23 to 27 ppt are optimal for maximum food and oxygen consumption in *M. mercenaria*. Pumping rates are inhibited below 15 ppt and above 36 ppt (Hamwi 1969) and Van Winkle (1976) noted decreased activity at salinities below 14 to 21 ppt. Castagna and Chanley (1973) found that burial activity for some bivalves was reduced or completely inhibited by salinities below 15 ppt. In addition, some bivalves exhibited valve gaping behavior and a lack of

response to repeated tactile stimuli, appearing dead, but eventually closing when removed from the water. When exposed to low salinities (<12.5 ppt), *M. mercenaria* closes its shell, creating a water tight seal that protects the body tissues from unfavorable conditions (Chanley 1957). Survival of *M. mercenaria* at lower or higher salinities can be affected by several factors including individual size, water temperature, salinity fluctuations, genetic history and the source of freshwater (Chanley 1957).

M. mercenaria are also affected by dissolved oxygen levels. At dissolved oxygen levels of 5 $mg \cdot L^{-1}$ or more, *M. mercenaria* are able to maintain oxygen uptake rates by increasing the efficiency with which they remove oxygen from the water column (Hamwi 1969). However, below 5 $mg \cdot L^{-1}$ oxygen uptake is reduced and anaerobic and aerobic respiration may occur simultaneously (Hammen 1980). Under extreme environmental conditions, below their activity threshold, *M. mercenaria* will close their valves and resort to anaerobic respiration (Hamwi 1969), they are able to sustain complete valve closure for up to 18 days at 1 to 6^{o}C (Loosanoff 1939). Dissolved oxygen levels less than 5 $mg \cdot L^{-1}$ (hypoxia) are not thought to be detrimental on their own, but can lead to reduced survival when coupled with higher temperatures (Baker et al. 2002).

Reproduction in *Mercenaria mercenaria*

As juveniles, hard clams have both male and female gonads present, although spermatogenesis dominates this life stage. Following the juvenile stage, hard clams develop into adults with separate sexes, although males appear to retain the ability to change into females at a later time (Loosanoff 1936). Reproduction or spawning events in *M. mercenaria* are primarily influenced by temperature, as demonstrated by the latitudinal variability in gametogenic cycles (Hesselman et al. 1989). Spawning typically occurs in water temperatures ranging from 20 to 25^{o}C throughout the geographical range (Hesselman et al. 1989). In southern populations,

spawning begins earlier in the year with bimodal or polymodal peaks, two or more spawning events, due to temperatures reaching the critical range sooner, and for longer periods of time (Heffernan et al. 1989, Hesselman et al. 1989). In Florida and Georgia, the spring spawn is typically the largest (Heffernan et al. 1989, Hesselman et al. 1989). *M. mercenaria* can reach sexual maturity in as little as one year in southern populations, at a shell length of 20 to 35 mm. (Eversole 1980, Hesselman et al. 1989, and Walker & Heffernan 1994).

In clams, reproduction is an important aspect of the overall energy budget. Typically, non-respired energy is partitioned into shell growth, somatic growth, and reproduction (Grizzle et al. 2001). Ansell et al. (1964) found that roughly 52% of annual organic production was released as gametes and Eversole (2001) indicated that annual reproductive effort based on several studies represents 26 to 46% of annual growth for both male and female clams. Eversole (2001) has reviewed reproduction in *M. mercenaria*.

Induction of Triploidy in *Mercenaria mercenaria*

Mercenaria mercenaria are diploid in naturally occurring populations, meaning they have two sets of chromosomes per cell. During reproduction one set from the maternal gamete (egg) and one set from the paternal gamete (sperm) combine to create another diploid organism. Triploids are organisms with three sets of chromosomes per cell. In free-spawning bivalve mollusks, the egg is released just prior to the first meiotic division, which allows researchers to induce triploids by suppressing either meiosis I or II. Suppression of a meiotic division results in a diploid egg, which is then fertilized by a haploid sperm, forming a triploid organism (Beaumont & Fairbrother 1991). Triploidy can be induced using a variety of methods, including pressure, thermal, or chemical shock, or by mating tetraploid organisms with diploid organisms. In this study cytochalasin B (CB) was used as a chemical shock to suppress meiosis II. CB, one

of a class of fungal metabolites, is thought to inhibit micro-filament formation in cells (Copeland 1974) and is commonly used to induce triploidy in bivalves (Beaumont & Fairbrother 1991).

Several studies have shown an increase in growth of triploid bivalves as compared to diploids, especially following diploid spawning events (Guo & Allen 1994, Eversole et al. 1996, Hand et al. 1999, Brake et al. 2004). These differences have been attributed to several hypotheses including increased heterozygosity, polyploidy gigantism, and energy reallocation. Increased heterozygosity suggests that greater genetic diversity should manifest in higher fitness and therefore higher growth of triploid organisms due to an extra allele at each loci (Beaumont & Fairbrother 1991, Garnier-Gere et al. 2002). The hypothesis for polyploidy gigantism states that increased growth in triploids is a result of increased cell size due to an additional chromosome without a reduction in cell number per organ that is typically seen in higher organisms including fish (Guo & Allen 1994, Nell 2002). A third hypothesis says that triploids are sterile and as a result put little or no energy towards reproduction, instead diverting this energy to somatic growth (Beaumont & Fairbrother 1991, Eversole et al. 1996). Triploids are supposed to be sterile as a result of the two homologous chromosomes from the female being unable to synapse at meiosis (Beaumont & Fairbrother 1991).

Triploidy has been induced in *M. mercenaria* in two previous studies with contrasting results. Hidu et al. (1988) found no difference in size between diploids and triploids over three growing seasons in Maine. In contrast, Eversole et al. (1996) found that triploid hard clams were significantly larger than diploids in South Carolina waters, but only after 47 months and two spawning events. The triploids in their study did not respond to spawning stimuli and showed reduced and abnormal production of gametes upon histological examination (Eversole et al. 1996). The results of these two studies may have differed because the diploid clams in the

Eversole study had experienced two spawning seasons, while the diploid clams in the Maine study had not yet spawned. These two observations lend support to the energy reallocation hypothesis, which states that sterile triploids will divert energy from reproductive growth into somatic growth.

Methods of Investigation

This study is a subset of a larger study to examine the potential for improvement of Florida *Mercenaria mercenaria* aquaculture using triploid induction. Clam farmers have reported lower than average survival (~53% on average down from 67% in 1999) during the summer months (USDA 2004), with a complete loss of newly planted seed for Cedar Key farmers in 2002 (Baker et al. 2005). The objective of this portion of the study was to determine, in a laboratory setting, if triploid hard clams have a better survival rate than diploids when subjected to environmentally stressful conditions. Conditions for this experiment were based on results from previous experiments and observed water quality parameters in both Cedar Key and Charlotte Harbor. *M. mercenaria* were challenged with salinities of 10 ppt, 25 ppt and 40 ppt, to reflect both low and high extremes found in both locations. Dissolved oxygen was maintained at normoxic (>5 $mg \cdot L^{-1}$) and hypoxic (<5 $mg \cdot L^{-1}$) levels. Since temperature apparently exacerbates the effects of salinity and dissolved oxygen, experiments were conducted at 32^{o}C, which represents a stressful temperature but one that is frequently observed in both estuaries. These experimental factors were chosen to cause physiological stress in order to determine possible causes of mortalities in the field and to test the hypothesis that triploids differ from diploids in their tolerance of extreme environmental conditions.

Figure 1-1. Map of shellfish harvest areas for clams, mussels and oysters. Clam farming is centered on the west coast in areas 25 to 37, surrounding Cedar Key, and 56, 58 and 62 near Charlotte Harbor. Clam harvesting of natural populations occurs along areas of the west coast and the entire east coast (FDACS 2007).

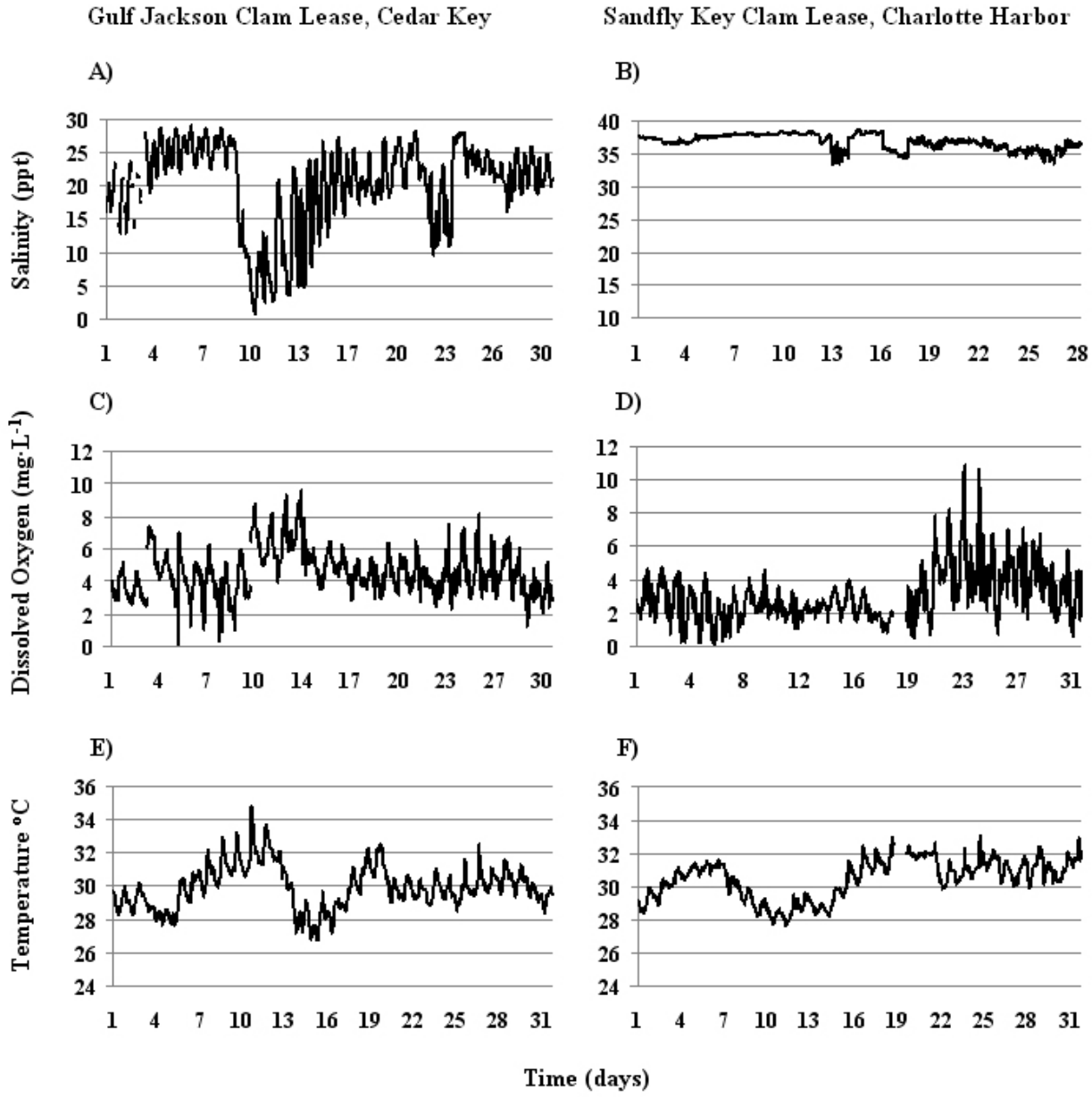

Figure 1-2. Extreme water quality values recorded at the Gulf Jackson clam lease in Cedar Key, FL and the Sandfly Key clam lease in Charlotte Harbor, FL: A, April 2003 B, May 2002 C, June 2002 D, July 2002 E, July 2003 F, July 2002 (FDACS 2007).

CHAPTER 2
MATERIALS AND METHODS

Production and Grow-out History of Triploid and Diploid *Mercenaria mecenaria*

Triploid and diploid *Mercenaria mercenaria* were produced by Dr. John Scarpa of Harbor Branch Oceanographic Institution (HBOI) during the spring of 2004. Triploidy was induced using cytochalasin B, a chemical that prevents the formation of polar bodies and, thus, retention of a set of chromosomes. Cytochalasin B was applied to fertilized *M. mercenaria* eggs during meiosis I (MI) and meiosis II (MII) development phases. Spawn from a single set of parents were separated into three groups to which the following treatments were applied: 1) triploidy was induced during meiosis I, 2) triploidy was induced during meiosis II, or 3) no treatment was applied, leaving the clams diploid. For a more detailed description of triploid production, see methods by Allen and Bushek (1992). This process was repeated to create four separate families, each with diploid, triploid MI, and triploid MII sibling groups. *M. mercenaria* were raised in the HBOI laboratory to a size typically used in the field by clam farmers and planted in summer of 2004 on two clam leases in Charlotte Harbor, Florida and on one clam lease in Cedar Key. Field studies were disrupted during the 2004 hurricane season with the loss of all *M. mercenaria* planted on clam leases in Charlotte Harbor. As a result, all remaining *M. mercenaria*, comprising two families were relocated from Cedar Key, FL to Charlotte Harbor, FL in the fall of 2004. *M. mercenaria* from two families were sampled from Charlotte Harbor during November 2005 and 125 clams from each group were sent to HBOI for analysis of condition index, histology, and ploidy.

Group C (diploid control) and group D (triploid MII) from the November sampling event were reserved for use in laboratory challenges and represented a single family. Group D had a total of 278 clams and prior ploidy analyses indicated that this group was 80% triploid, while

other family groups were estimated to be 20 to 30% triploid. Group D was transported to the University of Florida laboratory facilities and individually sampled to determine ploidy state, ensuring 100% triploidy for experimental use. Meanwhile, group C remained at HBOI in a flow-through system.

Tissue Sampling and Ploidy Determination of Triploid *Mercenaria mercenaria*

Mercenaria mercenaria from group D were placed in a 38 liter aquarium with the ventral margin facing up, on a grid platform of a styrene lighting panel. When an individual clam opened, a plastic coffee stirrer was inserted between the valve edges to keep the clam slightly open. The clam was then removed from the water and a BD Tuberculin Syringe with a 26 gauge needle was inserted into the anterior adductor muscle and a minute amount of hemolymph was withdrawn. Hemolymph was ejected into a 1.5 mL plastic microcentrifuge tube and 0.5 mL of DAPI/detergent/DMSO solution was added (Allen & Bushek 1992). Samples were placed on dry ice, if available, or on regular ice and then moved to an ultra cold freezer (-72^{o}F) within 8 hr or 30 min, respectively. *M. mercenaria* were individually numbered to correspond to hemolymph sample number and returned to a holding tank. Hemolymph samples were packed with dry ice pellets and shipped priority overnight to Dr. John Scarpa at HBOI. At HBOI, hemolymph samples were assessed for ploidy using flow cytometry (Allen & Bushek 1992). Following ploidy sampling, group D was returned to HBOI and maintained in a flow-through system with group C until the laboratory challenge. Temperatures in the flow-through system fluctuated around 21^{o}C and salinities around 25 ppt. Phytoplankton was added to the flow through system to ensure excess food was available for suspension feeding by *M. mercenaria*. Of the 278 *M. mercenaria* in group D, 215 were found to be triploid. Only those individuals known to be triploids were subsequently included in group D and used in laboratory challenges.

Experimental Aquarium System Design

There were a total of twenty four experimental systems, each system was independent and consisted of up to three individual tanks with a single sump. Measurements for aquaria system are reported in English units to facilitate future use of the design and purchase of standard parts.

Individual Tanks

Large Kritter Keepers (model # 20025, Lee, San Marcos, CA) were used for individual tanks; they were rectangular (14.5" long by 8.75" wide by 9.25" high) and capable of holding up to 19 liters of water. A 1" hole was drilled on one of the narrow sides of the tank, the center being 1 ½" from the top, and equidistant from the sides. This hole was fitted with a ½" tee eliminator and ½" mnpt, male national pipe thread, by barb fitting, reducing tank capacity to 17 liters. To prevent leaking, a rubber washer of 1" inner diameter, 2" outer diameter was glued with silicon around the tee eliminator on the inside of the tank. This served as a passive drain with ½" return tubing, keeping water levels from overflowing tank sides. Return tubing entered a slanted drainage manifold with three upturned 1" pvc tee's, one slip elbow and one slip by fnpt female national pipe thread, elbow connected to a barb with 1" tubing which returned water to the main sump.

In order to create a sand bed for the clams to bury in, platforms (Figure 2-1) were constructed using Plaskolite white, egg-crate style, styrene plastic lighting panels. Platforms were cut to fit inside the tanks and were raised off the bottom and supported by 1" sections of ½" pvc connected to the platform by cable ties. Nylon mesh of 125µm was used to keep the sand above the platform and was secured using ¼" tubing split lengthwise on long sides. Silicone glue was used along all sides to secure platforms to tanks and to ensure water up-welled through the sand bed, rather than around. Tubing (1/4"), which was used deliver water below the sand bed, was inserted through the mesh and held in place with rubber washers (which also kept sand

above the platform). The ¼" tubing extended below the platform and formed a circular loop containing three equally spaced ¼" tees which caused the water to circulate underneath the platform, removing potential anoxic spots, and creating even up-welling through the sand. Premium grade play sand was used for the sediment substrate. Prior to use, it was rinsed at least four times with tap water until the water ran clear, followed by a rinse with reverse-osmosis water.

Sump Tanks

Glass aquaria (38 liters) were used as sump tanks. The sump housed equipment and allowed for water changes with minimal effect on individual tanks. Temperature was controlled within the sump using a 200 watt Rena Cal Top Light submersible heater (Aquarium Pharmaceuticals, Inc., Chalfont, PA). A "Rid-Volt" titanium grounding probe (Taam, Inc., CA) prevented electrical currents from developing in the system. Water was pumped from the sump to the individual tank by a Quiet One Pump (model number 1200, Pentair Aquatics, El Monte, CA) with a ½" mntp port from the pump connected to a ½" fntp to ½" barb. Tubing (½") connected to a second ½" barb with a ¾" mntp connector attached to a ¾" check valve to prevent back flow from the tank to the sump in case of power loss. The check valve connected to a ¾" pvc manifold containing tee fittings for ½" ball valves. The ½" ball valve had a ½" mntp x ¼" barb connected to the ¼" tubing for the individual tanks. Water return to the sump is described in the individual tank section.

To minimize fluctuations in temperature due to external room conditions, outsides (sides and bottom) of the individual tanks and sumps were fitted with ¾" insulation, including a removable lid. The lids aided in preventing evaporation and contamination of water in the tanks. All external pvc pipes were insulated using ¼" thick pipe insulation (Figure 2-2).

Air stones delivering air or nitrogen, depending on the treatment, were placed in both the tank and the sump. Dissolved oxygen levels were recorded in $mg \cdot L^{-1}$ and percent saturation as indicated by an YSI (Yellow Springs Instrument, Ohio) 600 QS sonde and 650 MDS data logger.

Seawater Storage

Seawater (approximately 35 ppt) was obtained from University of Florida Whitney Marine Laboratory, located at Marineland, Florida on the East coast of the state, using a portable 550 gallon container. Seawater was kept in a 3000 gallon holding tank adjacent to the laboratory at Department of Fisheries and Aquatic Sciences in Gainesville until needed for experimental use. Water was pumped to the lab and through a filtration series which included cartridge filters, media filters and U/V sterilizer, and into six 105 gallon (397.5 liters) storage tanks. The cartridge filtration included a 100 μm pre-filter and three 20 μm filters that combined to create 75 ft^2 (6.97 m^2) of surface area. Cartridge filtration was followed by 2 media filters containing 2072 in^3 (0.03 m^3) of carbon media. The U/V sterilizer had a 40 W bulb and processed water at 25 gpm @ 15,000 $\mu Ws/cm^2$.

Water of 0 ppt was produced by reverse osmosis (R/O) using a Kent Marine R/O 200 HiF (Franklin, WI) system and stored in a 180 gallon storage tank. Water in the storage tanks was maintained at the salinities and temperatures (Finnex HC-0800 and TH-500 Titanium Heater) used in the experiment, allowing fast and accurate water changes.

Experimental Design of Tolerance Challenge

This experiment was designed as a two by three factorial, with temperature held constant in all treatments at 32°C. Salinity was maintained at 10 ppt, 25 ppt, and 40 ppt and oxygen was maintained at normoxic levels above 5 $mg \cdot L^{-1}$ (72 to 85% saturation, depending on salinity) or at hypoxic levels of less than 2 $mg \cdot L^{-1}$ (28 to 34% saturation). At 32°C the expected dissolved

oxygen levels for 100% saturation for 10 ppt, 25 ppt and 40 ppt are 6.92 $mg \cdot L^{-1}$, 6.38 $mg \cdot L^{-1}$, and 5.88 $mg \cdot L^{-1}$ respectively. These values were chosen to reflect summer conditions in SW Florida estuaries. Treatments were blocked to minimize potential room effect on temperature, for a total of four blocks, with one treatment replicate randomly assigned within each block. *M. mercenaria* were randomly assigned, with 9 triploid clams, group D and 9 diploid clams, group C in each replicate.

Acclimation of Triploid and Diploid *Mercenaria mercenaria*

Mercenaria mercenaria were acclimated in three 30-gallon tanks to 32°C and 10 ppt, 25 ppt or 40 ppt. Temperature was raised by 2°C per day from a starting temperature of 21°C until the experimental temperature of 32°C was reached. Salinity was adjusted 3 ppt per day from a starting value of 25 ppt until experimental salinities of 10 ppt and 40 ppt were reached. Lower salinities were adjusted by adding R/O water to acclimation tanks. Higher salinities were adjusted by slowly adding a stock solution of 100 ppt to acclimation tanks. The stock solution was made by adding Instant Ocean synthetic sea salt (Aquarium Systems, Ohio) to R/O water and stirring until thoroughly dissolved. Acclimation schedules were staggered so that each acclimation tank reached experimental values simultaneously; tanks with starting conditions furthest from the intended experimental values were begun first.

Health Assessment of Triploid and Diploid *Mercenaria mercenaria*

Observations of *Mercenaria mercenaria* mortality, siphon extension, and burial were conducted at 12 hour intervals. For each tank, a rough count of siphon extension was made to assess the presence/absence of filtering activity (Figure 2-3). *M. mercenaria* were considered buried when they were completely or partially buried into the substrate in a natural position, i.e. posterior margin of the shell extending upwards from the sediment (Figure 2-4). Gaping behavior, if any, was noted. Gaping *M. mercenaria* had their shells open, mantle covering or

partially covering the opening, and no siphon extension behavior (Figure 2-5). *M. mercenaria* on the sediment surface were assessed for mortality, counted and temporarily removed to a holding basket. *M. mercenaria* within the sediment were uncovered, placed in a holding basket, assessed for mortality, and counted. Sediment was thoroughly mixed and evenly distributed within the tank.

Mortality was assessed in two ways: failure to respond when touched by a blunt probe along the mantle edge, or inability to remain closed when manually compressed. Dead individuals were not returned to the system. The observation time was recorded and the shell length, height, and width of dead individuals were measured to the nearest 0.1 mm using digital calipers. All non-gaping *M. mercenaria* were considered alive and were returned to the sediment surface of the tank. Tanks were monitored for 21 days or until all individuals within a treatment had died.

Water Quality Parameters in Aquarium Systems

Temperature, salinity and dissolved oxygen levels were monitored at 12 hour intervals with an YSI (Yellow Springs Instrument, Ohio) 600 QS sonde and 650 MDS data logger. Prior to each sampling event, the YSI was calibrated to 100% oxygen saturation following standard protocol. Salinity was calibrated using a typical marine standard of 55.91 μS/cm held at 32°C. Salinity measurements were randomly verified using a refractometer. In addition to measurements taken every 12 hours, temperature was monitored continuously with Fisherbrand digital thermometers (model number 15-077-8D, Pittsburgh, PA). Digital thermometers stored the minimum and maximum temperature, providing a rough estimate of the daily temperature variation in each tank. Salinity was adjusted with R/O or hypersaline water, as needed. Ammonia and nitrite were checked daily with ion specific colorimeters (model # HI93700 and

#HI93707, Hanna, Woonsocket, RI). A 2 ml sample of tank water was placed into a Hanna cuvet and diluted to 8 ml with distilled water. The cuvet was gently swirled until the sample was completely mixed, wiped clean, and a zero reading was taken. Ammonia or nitrite reagents were added to the cuvet, following the manufacturer's instructions for seawater analysis, and values were recorded. Readings were multiplied by five to correct for initial dilution.

Data Analysis

Water quality data were summarized by calculating the means for each individual tank, and the means for the treatment (n=4 tanks). Burial data was calculated as the number of *Mercenaria mercenaria* that buried or attempted to bury during a 12 hour period over the total number of *M. mercenaria* present in the tank during that time period. Burial data was analyzed using a linear regression in SAS 9.1.

To compare survival of triploid and diploid *M. mercenaria*, survival analysis was performed, using the Lifereg procedure available on SAS Version 9.1. Survival analysis is designed to evaluate the occurrence of events over time. In this study, survival was assessed every twelve hours for twenty one days. *M. mercenaria* alive at the end of the experiment are considered right censored data, in that they did not experience an event during the given timeframe, which is taken into account in survival analysis (Allison 1995). Survival data is binomially distributed; each individual is either alive (0) or dead (1) at each given time. Therefore, a logit regression is performed using the maximum likelihood method to fit a regression line (Sokal & Rohlf 1995). Survival rates were calculated from the slope of the regression line (β), using the equation $100(e^{\beta} - 1)$. Further details on survival analysis using SAS are provided in Allison (1995).

Figure 2-1. Experimental tanks. A, View of sand platform from below showing ¼" tees used for circulating water beneath sand bed. B, Platform glued to sides of tank. C, Side view of the tank, showing the platform supporting a sand bed and clams.

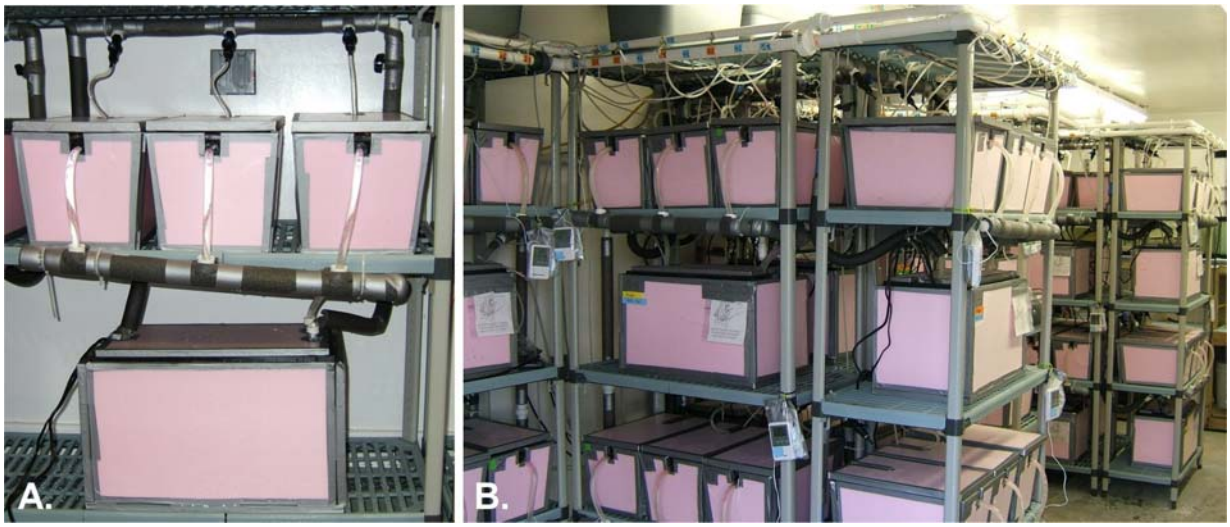

Figure 2-2. Experimental system. A, Close up of one system, including three replicate tanks and one sump. B, View of wet lab set-up during experiment.

Figure 2-3. Examples of *M. mercenaria* siphon extension.

Figure 2-4. *M. mercenaria* burial positions. A, Surface (not counted as buried). B, Partially buried. C, Completely buried.

Figure 2-5. A, Example of gaping behavior. B, Example of a dead clam.

CHAPTER 3
RESULTS

Water Quality

Most water quality parameters remained near the target values throughout the experiment (Table 1-6, Appendix 1). The only parameter with significant excursions from the target value was dissolved oxygen in the hypoxia treatments (Table 3-1). Periodic spikes of dissolved oxygen above 5 $mg \cdot L^{-1}$ in the hypoxic treatment were a result of a loss of nitrogen gas flow when a gas cylinder ran empty. These periods of normoxia (> 5 $mg \cdot L^{-1}$) did not last for long periods of time, as they were either anticipated, or occurred at a mortality check. After a new cylinder of nitrogen was started, dissolved oxygen levels returned to below 2 $mg \cdot L^{-1}$ within 30 minutes.

Survival Analysis

Survival analysis indicated that salinity ($df = 2$, $\chi^2 = 3144.5$, $p < 0.0001$) and dissolved oxygen ($df = 1$, $\chi^2 = 67.9$, $p < 0.0001$) had significant effects on *Mercenaria mercenaria* survival. Survival of diploid and triploid *M. mercenaria* differed significantly in two of the treatments, 25 ppt hypoxia and 10 ppt hypoxia. In the 25 ppt hypoxia treatment (Figure 3-1, C), diploid *M. mercenaria* began dying on day 14, with 55% surviving to the final day (day 21). Triploid *M. mercenaria* began dying on day 17, with 89% surviving to the final day (day 21). Triploids had a 16.4% higher survival rate than did diploids between day 14 and day 21, the time period in which mortalities occurred ($\log y = 4.028 - 0.179[\text{diploid}]$, $df = 1$, $\chi^2 = 5.82$, $p = 0.0159$). In the 10 ppt hypoxia treatment, triploid and diploid *M. mercenaria* began dying on days 3 and 4, respectively (Figure 3-1, A), with 0% survival on day 7. Diploid *M. mercenaria* had a 7.6% higher survival rate than did triploids between day 3 and 7, the time period in which mortalities occurred ($\log y = 2.44 + 0.0736[\text{diploid}]$, $df = 1$, $\chi^2 = 4.28$, $p = 0.0387$).

Survival of diploid and triploid *M. mercenaria* did not differ for the remaining treatments: 10 ppt normoxia, 25 ppt normoxia, 40 ppt hypoxia and 40 ppt normoxia. In the 10 ppt normoxia treatment (Figure 3-1, B) diploid and triploid *M. mercenaria* began dying on day 3 and mortalities of both occurred at regular intervals until all the individuals in the treatment were dead, on day 7. No mortalities of diploid or triploid *M. mercenaria* occurred in the 25 ppt normoxia treatment (Figure 3-1, D). Both diploid and triploid *M. mercenaria* in the 40 ppt normoxia treatment (Figure 3-1, F) had 97% survival. In the 40 ppt hypoxia treatments (Figure 3-1, E), diploid *M. mercenaria* began dying on day 4, with 22% surviving to the final day (day 21), while triploid *M. mercenaria* began dying on day 8, with 31% surviving to the final day.

Reburial Rates

Reburial rates refer to the proportion of *M. mercenaria* in a tank re-burying in 12 hours following excavation from the sand. *M. mercenaria* in the 25 ppt normoxia treatment had an average reburial rate of 66.5% (Figure 3-2, B). Although average reburial rate ranged from 39.6% to 87.5%, there was no significant change in reburial rate over the course of the experiment ($r^2 = 0.0074$). Some individuals, both buried and unburied, were open and siphoning at each 12 hour interval in the 25 ppt normoxia treatment for the duration of the experiment. Clams in the 25 ppt hypoxia treatment had an average reburial rate of 20.8% over the course of the experiment, with a range from 0% to 65.3% (Figure 3-2, A). Reburial rates declined over the course of the experiment ($r^2 = 0.5287$, $p < 0.0001$). Some individuals in the 25 ppt hypoxia treatment were open and siphoning at each 12 hour interval for the duration of the experiment.

No clams in the 10 ppt normoxia or 10 ppt hypoxia treatments buried during the experiment and no siphon activity was noted. Clams began to gape prior to mortalities.

Clams in the 40 ppt normoxia treatment had an average reburial rate of 55.3%, with a range of 29.5% to 86.9% (Figure 3-2, D). Reburial rate increased slightly over the course of the

experiment ($r^2 = 0.1336$, $p < 0.0001$). Some individuals were open and siphoning at each 12 hour interval in the 40 ppt normoxia treatment for the duration of the experiment. Clams in the 40 ppt hypoxia treatment had an average reburial rate of 14.2%, with a range of 0% to 56.9% (Figure 3-2, C). Reburial rates declined over the course of the experiment ($r^2 = 0.4732$, $p < 0.0001$). Some individuals in the 40 ppt hypoxia treatment were open and siphoning at each 12 hour interval for the duration of the experiment.

Table 3-1. Water quality values averaged across treatments.

Treatment	Water Quality Value	Average	Minimum	Maximum	Standard Error
10 ppt, Normoxia	Temperature (^{o}C)	32.3	31.4	33.1	0.148
10 ppt, Normoxia	Salinity (ppt)	10.8	10.2	11.3	0.087
10 ppt, Normoxia	Dissolved Oxygen (mg·L^{-1})	6.33	5.36	6.96	0.109
10 ppt, Normoxia	% Dissolved Oxygen	89.4	78.2	95.6	2.12
10 ppt, Normoxia	Ammonia (ppm)	2.03	0.53	4.59	0.526
10 ppt, Normoxia	Nitrite (ppm)	0.16	0.03	0.40	0.050
25 ppt, Normoxia	Temperature (^{o}C)	32.2	31.1	33.4	0.075
25 ppt, Normoxia	Salinity (ppt)	25.3	23.5	26.9	0.105
25 ppt, Normoxia	Dissolved Oxygen (mg·L^{-1})	6.04	5.55	6.52	0.036
25 ppt, Normoxia	% Dissolved Oxygen	95.3	87.6	101.8	0.58
25 ppt, Normoxia	Ammonia (ppm)	0.76	0.00	2.29	0.137
25 ppt, Normoxia	Nitrite (ppm)	0.14	0.01	0.29	0.015
40 ppt, Normoxia	Temperature (^{o}C)	32.2	30.7	33.4	0.089
40 ppt, Normoxia	Salinity (ppt)	40.3	37.6	42.4	0.171
40 ppt, Normoxia	Dissolved Oxygen (mg·L^{-1})	5.56	5.03	6.05	0.033
40 ppt, Normoxia	% Dissolved Oxygen	95.0	84.9	102.8	0.60
40 ppt, Normoxia	Ammonia (ppm)	0.79	0.00	2.44	0.148
40 ppt, Normoxia	Nitrite (ppm)	0.11	0.01	0.23	0.011
10 ppt, Hypoxia	Temperature (^{o}C)	32.1	31.1	33.1	0.168
10 ppt, Hypoxia	Salinity (ppt)	10.4	10.0	10.8	0.073
10 ppt, Hypoxia	Dissolved Oxygen (mg·L^{-1})	1.56	0.22	5.21	0.408
10 ppt, Hypoxia	% Dissolved Oxygen	11.5	3.5	20.9	2.53
10 ppt, Hypoxia	Ammonia (ppm)	2.19	0.34	4.36	0.568
10 ppt, Hypoxia	Nitrite (ppm)	0.13	0.04	0.29	0.032
25 ppt, Hypoxia	Temperature (^{o}C)	32.2	30.6	33.5	0.089
25 ppt, Hypoxia	Salinity (ppt)	25.5	24.5	26.9	0.080
25 ppt, Hypoxia	Dissolved Oxygen (mg·L^{-1})	0.75	0.24	4.31	0.114
25 ppt, Hypoxia	% Dissolved Oxygen	9.0	3.8	31.5	0.94
25 ppt, Hypoxia	Ammonia (ppm)	0.79	0.00	2.38	0.153
25 ppt, Hypoxia	Nitrite (ppm)	0.11	0.00	0.23	0.013
40 ppt, Hypoxia	Temperature (^{o}C)	32.2	30.4	33.3	0.087
40 ppt, Hypoxia	Salinity (ppt)	40.3	37.6	42.2	0.160
40 ppt, Hypoxia	Dissolved Oxygen (mg·L^{-1})	0.76	0.20	4.12	0.113
40 ppt, Hypoxia	% Dissolved Oxygen	10.0	3.4	35.1	1.09
40 ppt, Hypoxia	Ammonia (ppm)	0.96	0.00	2.48	0.152
40 ppt, Hypoxia	Nitrite (ppm)	0.10	0.00	0.26	0.014

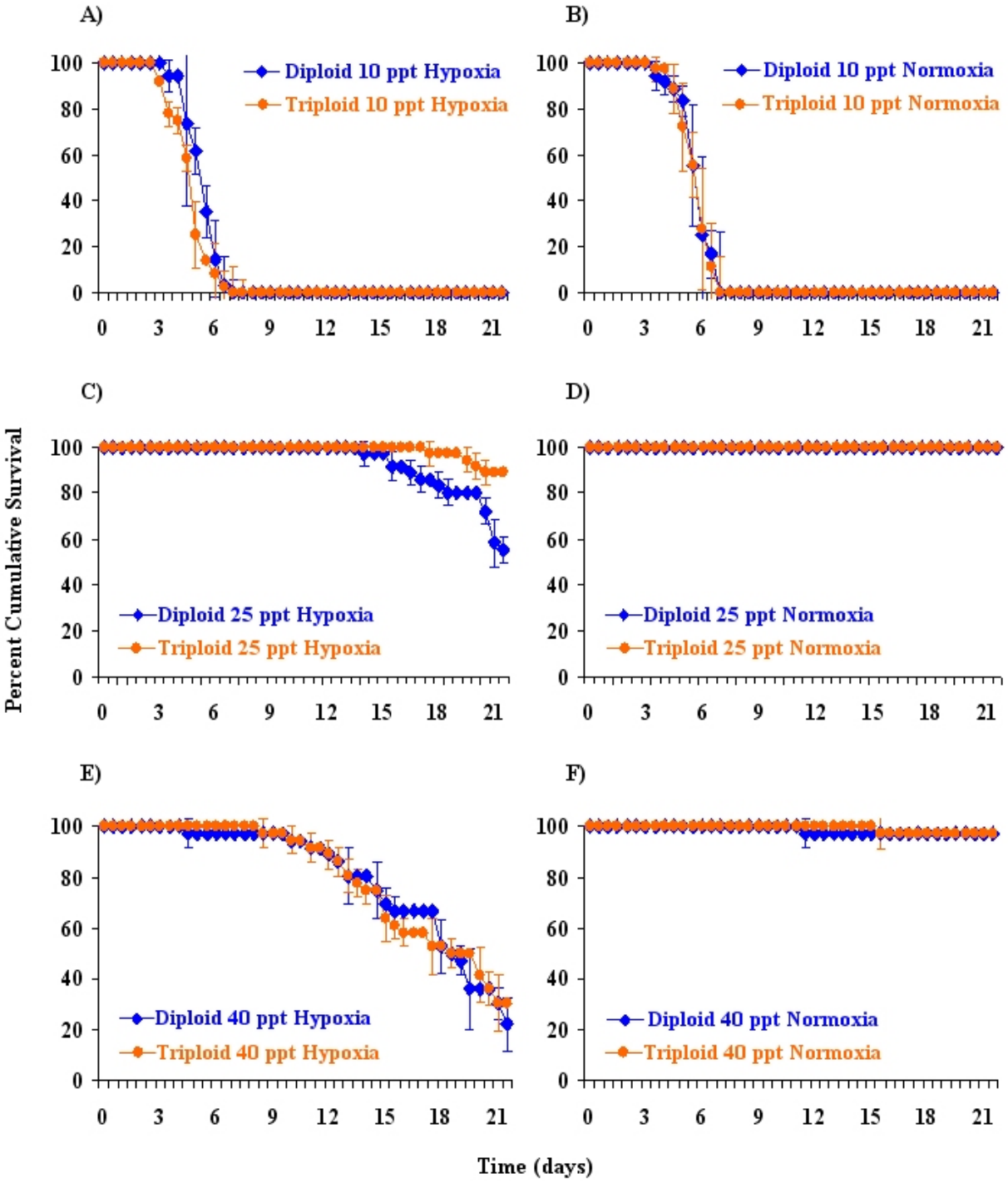

Figure 3-1. Percent cumulative survival of diploid and triploid hard clams, *M. mercenaria*: A, 10 ppt hypoxia B, 10 ppt normoxia C, 25 ppt hypoxia D, 25 ppt normoxia E, 40 ppt hypoxia F, 40 ppt normoxia.

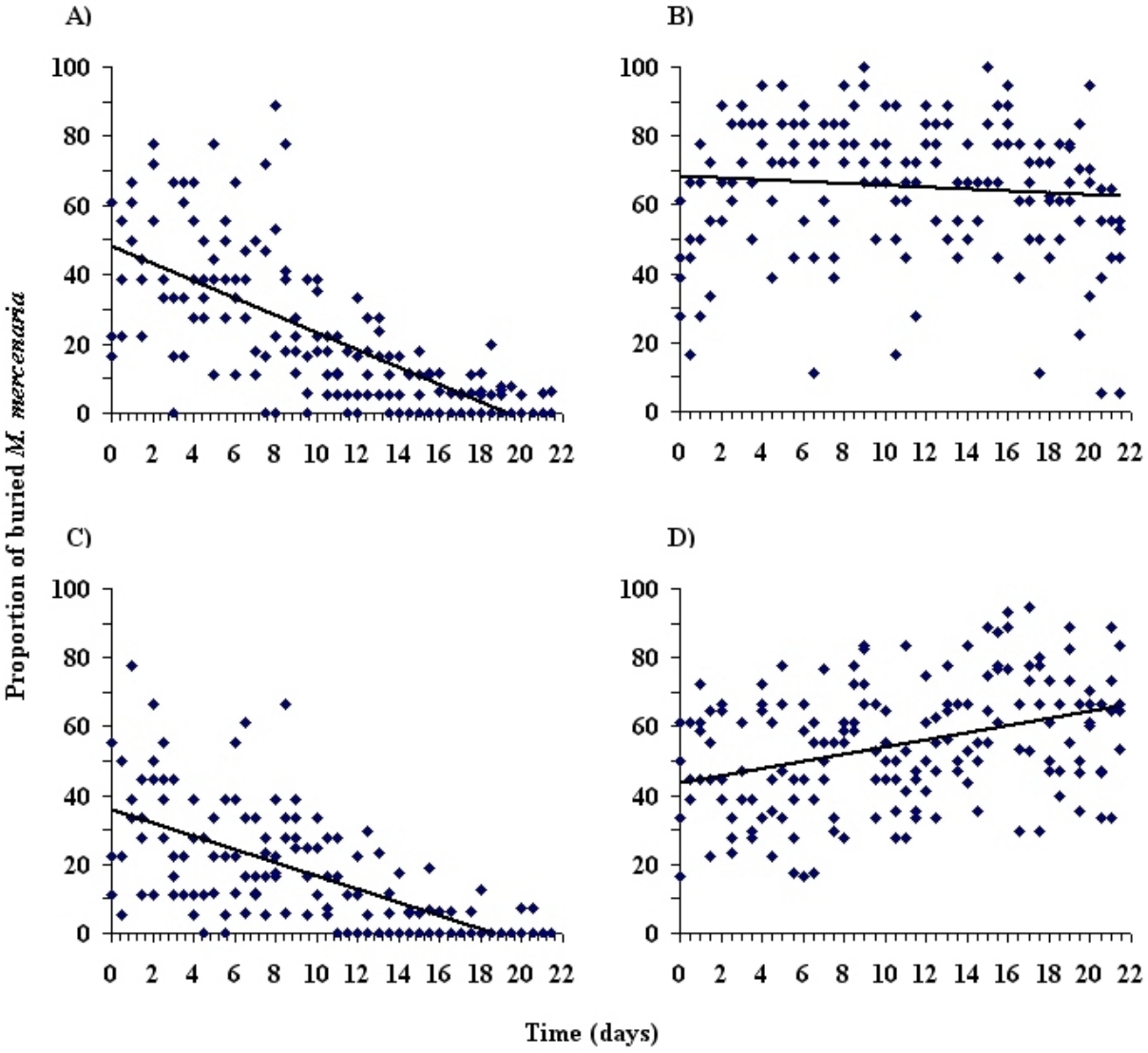

Figure 3-2. Scatter plot of the proportion of diploid and triploid hard clams, *M. mercenaria*, that buried within 12 hours following placement on the surface: A, 25 ppt hypoxia B, 25 ppt normoxia, C, 40 ppt hypoxia D, 40 ppt normoxia.

CHAPTER 4
DISCUSSION

This is the first study to look at survival differences between diploid and triploid *Mercenaria mercenaria* in a controlled laboratory setting. I found that triploids had no advantage over diploids under conditions of extreme salinity stress, or salinity stress combined with hypoxia. Triploids did have a significant advantage over diploids when hypoxia was combined with a non-stressful salinity of 25 ppt. However, the advantage seen in triploids did not occur until day 15. Diploid *M. mercenaria* had a higher survival rate than triploid clams in the 10 ppt hypoxia treatment. While the survival curves for diploids and triploids were statistically significant, they were nearly identical to the survival curves for 10 ppt, normoxia treatment in which no difference was seen.

There are several potential reasons a difference in survival between diploids and triploids was not more apparent. These include 1) inadequate sample size, 2) the hypothesis of energy reallocation in triploids does not occur, and 3) the hypothesis of energy reallocation was not realized in this study because diploid clams had not experienced a spawning event.

First, my sample sizes were relatively small. During the 2004 hurricane season, a significant portion of experimental clams were lost, and remaining families had low percentages of triploidy. As a result, *M. mercenaria* used in this experiment were from one family, and only a MII triploid group and sibling diploid group was available for experimental use. Ploidy analysis was performed and yielded only enough triploid *M. mercenaria* to have nine individuals per tank or a total of thirty-six triploids and thirty-six diploids exposed to a particular treatment. A higher sample size may help minimize individual variability, while more than one family and triploid type would have increased the genetic diversity, resulting in a better estimation of what

could happen on a population level. Despite the lack of a larger sample size, a difference was detected and it is uncertain if a larger sample size would have improved our results.

Second, while triploid clams used in this study had greatly reduced gametogenic development when compared to diploids (S. Laramore, Harbor Branch Oceanogr. Inst., pers. comm.), it is unclear if or how additional energy is stored, and whether or not that energy can be readily accessed during stressor events. Several hypotheses have been proposed to account for the often faster growth and larger size of polyploid organisms. The energy reallocation hypothesis suggests that the growth advantage triploids have over diploids is due to the extra energy that should be available when gametes are not formed. This hypothesis has not been directly tested for the hard clam *Mercenaria mercenaria*. However, Eversole et al. (1996) found that triploid hard clams exhibited reduced gametogenesis and did not release gametes when exposed to spawning stimuli, which supports the energy reallocation hypothesis. In addition, they found that triploids were significantly larger than diploids, following two spawning events that occurred over 47 months (Eversole et al. 1996). In Florida, *M. mercenaria* typically reaches market size following 10 to 18 months of growth in the field (Sturmer 2004) while in South Carolina *M. mercenaria* reaches market size after 24 months (Eldridge et.al. 1976). If a growth advantage is not seen until 47 months in triploid *M. mercenaria*, triploidy may not benefit the aquaculture industry. This is in contrast to the success seen in oyster research, where triploid oysters are typically bigger than their diploid cohorts at time of harvest (Nell 2002).

Two other hypotheses have been proposed to explain increased triploid growth; polyploidy gigantism and increased heterozygosity. Polyploid gigantism suggests that cell volume increases to accommodate an additional chromosome, without a reduction in cell number typically seen in higher organisms (Guo & Allen 1994). This hypothesis has not been tested for *M. mercenaria*

and would not necessarily translate into higher survival rates. Triploid *Mulinia lateralis*, the dwarf surfclam, were significantly larger than diploids after 30 days, prior to sexual maturation and continued to be larger after three months, following sexual maturation, but without undergoing a spawning event, suggesting growth was not related to energy reallocation. While the authors consider increased heterozygosity a possibility, they assert that the 72% increase in size of triploids over diploids cannot be adequately explained by that hypothesis and suggest instead that polyploidy gigantism is the primary cause of the increased growth of *Mulinia lateralis* (Guo & Allen 1994). Increased heterozygosity, or increased genetic diversity, sometimes referred to as "hybrid vigor", may lead to increased fitness, including faster growth rates (Beaumont & Fairbrother 1991). Hawkins et. al. found overall that meiosis I triploids had greater multi-locus enzyme heterozygosity and greater growth than either meiosis II triploids or diploids in both the European flat oyster, *Ostrea edulis* (1994) and the Pacific oyster, *Crassostrea gigas* (2000). This suggests that at least part of the higher growth seen in meiosis I triploids is a result of increased allelic variation (Hawkins 2000). This phenomenon may not have played a role in this study, since the triploids were created by suppressing meiosis II division. In addition, it is uncertain if increased hybrid vigor would enhance survival during stressor events. No hypothesis has been proven for all triploids and, in fact, all three hypotheses may contribute to increased size of triploid mollusks (Guo & Allen 1994, Hawkins et. al. 2000).

Third, while diploid clams used in this study apparently spawned in the fall prior to or shortly after collection from the field, it is uncertain if diploid clams used in this study had undergone a spring spawning event. Sexual maturity in hard clams is typically associated with reaching 20 to 35 mm in shell length (Knaub & Eversole 1988). Diploid clams in this study had an average shell length of 44.5 mm and a minimum and maximum of 28.9 mm and 56.4 mm,

respectively. Spawning events can be influenced by many factors, including gamete ripeness, food supply and water conditions, but is typically triggered by seasonal changes in temperature and can differ throughout the hard clam's geographical range (Eversole 2001) and between brood stock strains (Knaub & Eversole 1988). Histology was performed on sixteen diploid clams from group C at the time that they were collected in November 2006. Two of unknown sex were either spent or in stage 0 to 1, six were in stage five or just prior to spawning and eight were in active emission, stages 6 to 8 (S. Laramore, Harbor Branch Oceanogr. Inst., pers. comm.). There was a three month period between when the histology was performed and the start of the experiment, so the histological state of the clams just prior to the start of the experiment was unknown.

Eversole et al. (1996) found that triploids do not have a significant growth advantage over diploids until after the diploids have undergone two spawning events. If that is the case, and the diploid clams used in my study had spawned once, at most, then there may be no basis for expecting greater triploid survival in my experiment. Considering the timing of spring spawning events, followed by summer stressor events, it would be beneficial to try to induce spawning for both diploid and triploid groups immediately prior to running an experiment.

In addition to examining differences between triploid and diploid clams, this is the first laboratory study to examine the effects of multiple stressors of salinity and dissolved oxygen on *M. mercenaria* survival when combined with the high temperatures reached during Florida summers. At higher temperatures, mortalities do not occur unless there is at least on additional stressor present, such as low salinity or low dissolved oxygen levels. High and normal salinities and high temperature at normal dissolved oxygen levels are probably not causing mortalities in the field.

I also made observations of clam behavior that have important implications. For example, while high temperature and low dissolved oxygen treatments did not appear to affect valve movement, clams closed their valves in response to salinities of 10 ppt. Clams at this salinity did not appear to be siphoning, and no burial activity occurred, suggesting that they were undergoing anaerobic respiration. Clams remained closed between four and eight days and eventually died. As a result of valve closure, hypoxia would not have had an effect until after clams began gaping prior to mortality, which typically occurred within 24 hours of first observed gaping behavior. The only study to look at complete and sustained valve closure, found that hard clams could remain closed for up to 18 days at temperatures of 1 to 6^{o}C (Loosanoff 1939). At higher temperatures of 15 to 22^{o}C, Chanley (1957) found that juvenile hard clams (10 to 21.5 mm SL) exposed to 10 ppt salinity could survive for 28 days with no mortalities, with 50% mortality occurring on day 41, while seed clams (1.8 to 3.6 mm SL) exhibited 100% mortality within 15 days. This study indicates that at high summer temperatures, sustained low salinities can be lethal to adult hard clams within 4 days with a potential for complete crop losses within 8 days. This may be important for future management of freshwater inputs into these estuaries during summer months.

There is limited information in the literature on the effects of higher salinities on juvenile and adult hard clams (Grizzle et al. 2001). Hamwi (1969) found the upper salinity limit for a detectable pumping rate of acclimated clams to be 36 ppt at 25^{o}C. In a larval study, Davis (1958) found that hard clam larvae did not develop in salinities above 32.5 ppt. This study found that high salinity of 40 ppt, and normoxia had little or no impact on adult clam survival, siphon extension or burial activity, it did not address the physiological aspects of pumping rates or oxygen uptake.

Burial rates decreased over the duration of the experiment in the 25 ppt hypoxia and 40 ppt hypoxia treatments, treatment in which high subsequent mortalities occurred, indicating a sub-lethal effect of hypoxia. This suggests that clams planted during poor conditions may take longer to bury in the sediment leaving them vulnerable to predation (Doering 1982).

Results of my experiments do not adequately explain summer mortalities in the field. It may be that fluctuations in the environment, particularly of salinity, may be more physiologically stressful than static conditions due to the amount of adjustment in physiological functions, particularly osmotic regulation. Compounding the factors that I examined, there may be an effect of phytoplankton availability and feeding rates during stressor events. If pumping rates decrease outside of optimal physiological ranges, hard clam feeding rates will decrease as well (Hamwi 1969). In addition, Charlotte Harbor typically experiences several red tide events (*Karina brevia*) during the summer months, which may further increase the physiological stress of clams already experiencing high temperatures, fluctuating salinities and low dissolved oxygen events.

Does triploidy have the potential to improve hard clam culture in the state of Florida? Results of this experiment suggest that triploidy does not increase the overall survival of *M. mercenaria* during extreme environmental conditions. However there was an advantage of triploidy in the 25 ppt hypoxia treatment, a scenario that is more likely than others to occur on a regular basis in the field. Additional avenues of research could help to clarify the benefit of triploidy. Other experiments within this study will address survival under aquaculture conditions in two field experiments, survival of seed clams during extreme environmental conditions in a laboratory challenge, and differences in scope for growth of diploid and triploid hard clams.

These additional experiments may help clarify the potential of triploidy to improve hard clam culture on the West coast of Florida.

There are other potential benefits to triploidy, aside from enhanced growth and survival, including aquaculture of non-native species and an increased glycogen content. Sterility, in addition to potentially enhancing growth, can ensure that non-native organisms are not reproducing. Non-native species can affect natural populations of bivalves in two ways; 1) through competition with other species for resources and 2) through hybridization with native congeneric species (Arnold 2004, Beaumont and Fairbrother 1991). Sterility may also result in enhanced taste. Bivalves, including *M. mercenaria* store their energy as glycogen, which becomes depleted during the spawning season. Triploids divert little or no energy to spawning, potentially resulting in a sweeter tasting clam since glycogen supplies are not depleted. In a study by Allen and Downing (1991), consumers and oyster growers rated triploid oysters higher in taste, texture and overall preference over control diploids, which they attribute to an increase in glycogen storage in triploids along with lack of gonadal material, which negatively affects texture and firmness.

APPENDIX
WATER QUALITY DATA

Table A-1. Average water quality values for tanks in treatment 10 ppt, hypoxia.

Tank #	Water Quality Value	Average	Minimum	Maximum	Standard Error
1	Temperature (^{o}C)	31.9	31.4	32.7	0.10
8	Temperature (^{o}C)	32.0	30.7	32.9	0.17
18	Temperature (^{o}C)	31.9	30.7	33.3	0.21
23	Temperature (^{o}C)	32.8	31.6	33.7	0.19
1	Salinity (ppt)	10.5	10.1	10.9	0.06
8	Salinity (ppt)	10.5	10.0	11.0	0.07
18	Salinity (ppt)	10.4	9.8	10.9	0.10
23	Salinity (ppt)	10.3	10.0	10.5	0.06
1	Dissolved Oxygen ($mg \cdot L^{-1}$)	1.7	0.2	5.2	0.40
8	Dissolved Oxygen ($mg \cdot L^{-1}$)	1.6	0.4	4.8	0.28
18	Dissolved Oxygen ($mg \cdot L^{-1}$)	2.0	0.2	5.8	0.53
23	Dissolved Oxygen ($mg \cdot L^{-1}$)	1.0	0.1	5.1	0.42
1	% Dissolved Oxygen	16.6	3.7	29.8	4.16
8	% Dissolved Oxygen	17.2	5.4	28.7	2.46
18	% Dissolved Oxygen	6.1	2.7	11.4	1.37
23	% Dissolved Oxygen	6.1	2.0	13.5	2.14
1	Ammonia (ppm)	1.7	0.1	4.3	0.46
8	Ammonia (ppm)	2.4	0.1	4.1	0.49
18	Ammonia (ppm)	1.9	0.7	3.0	0.43
23	Ammonia (ppm)	2.8	0.5	6.1	0.89
1	Nitrite (ppm)	0.1	0.1	0.2	0.02
8	Nitrite (ppm)	0.2	0.0	0.4	0.04
18	Nitrite (ppm)	0.1	0.1	0.3	0.03
23	Nitrite (ppm)	0.2	0.1	0.4	0.04

Table A-2. Average water quality values for tanks in treatment 10 ppt, normoxia.

Tank #	Water Quality Value	Average	Minimum	Maximum	Standard Error
3	Temperature (^{o}C)	32.2	31.5	32.7	0.12
7	Temperature (^{o}C)	32.3	31.5	32.9	0.11
17	Temperature (^{o}C)	32.7	31.4	33.3	0.18
19	Temperature (^{o}C)	32.2	31.2	33.6	0.18
3	Salinity (ppt)	10.9	10.3	11.6	0.10
7	Salinity (ppt)	10.4	9.6	10.9	0.08
17	Salinity (ppt)	11.0	10.5	11.6	0.10
19	Salinity (ppt)	10.7	10.3	11.2	0.07
3	Dissolved Oxygen ($mg \cdot L^{-1}$)	6.4	5.1	7.0	0.13
7	Dissolved Oxygen ($mg \cdot L^{-1}$)	6.4	5.2	7.4	0.13
17	Dissolved Oxygen ($mg \cdot L^{-1}$)	6.2	5.8	6.6	0.06
19	Dissolved Oxygen ($mg \cdot L^{-1}$)	6.3	5.3	6.8	0.11
3	% Dissolved Oxygen	89.5	74.3	98.7	3.22
7	% Dissolved Oxygen	88.7	74.3	93.7	2.24
17	% Dissolved Oxygen	90.2	86.6	93.5	0.93
19	% Dissolved Oxygen	89.2	77.5	96.3	2.10
3	Ammonia (ppm)	1.7	0.2	4.2	0.45
7	Ammonia (ppm)	2.1	0.8	4.8	0.48
17	Ammonia (ppm)	2.2	0.9	4.6	0.55
19	Ammonia (ppm)	2.2	0.3	4.9	0.63
3	Nitrite (ppm)	0.1	0.0	0.3	0.03
7	Nitrite (ppm)	0.2	0.1	0.3	0.03
17	Nitrite (ppm)	0.2	0.0	0.7	0.10
19	Nitrite (ppm)	0.2	0.1	0.4	0.04

Table A-3. Average water quality values for tanks in treatment 25 ppt, hypoxia.

Tank #	Water Quality Value	Average	Minimum	Maximum	Standard Error
4	Temperature (^{o}C)	31.9	29.9	33.0	0.09
11	Temperature (^{o}C)	32.6	31.3	33.7	0.10
14	Temperature (^{o}C)	32.2	31.1	33.7	0.06
24	Temperature (^{o}C)	32.0	30.0	33.5	0.11
4	Salinity (ppt)	25.4	24.2	26.8	0.08
11	Salinity (ppt)	25.2	24.4	26.7	0.08
14	Salinity (ppt)	25.6	24.9	27.0	0.07
24	Salinity (ppt)	25.6	24.5	27.2	0.09
4	Dissolved Oxygen ($mg \cdot L^{-1}$)	0.7	0.3	2.5	0.06
11	Dissolved Oxygen ($mg \cdot L^{-1}$)	0.8	0.2	5.2	0.14
14	Dissolved Oxygen ($mg \cdot L^{-1}$)	0.7	0.2	4.7	0.13
24	Dissolved Oxygen ($mg \cdot L^{-1}$)	0.8	0.2	4.8	0.12
4	% Dissolved Oxygen	9.5	4.9	18.6	0.61
11	% Dissolved Oxygen	9.6	3.5	42.0	1.17
14	% Dissolved Oxygen	7.6	3.3	32.6	0.83
24	% Dissolved Oxygen	9.3	3.4	32.6	1.15
4	Ammonia (ppm)	0.7	0.0	2.4	0.15
11	Ammonia (ppm)	0.8	0.0	2.0	0.13
14	Ammonia (ppm)	1.0	0.0	2.3	0.17
24	Ammonia (ppm)	0.7	0.0	2.9	0.16
4	Nitrite (ppm)	0.1	0.0	0.3	0.01
11	Nitrite (ppm)	0.1	0.0	0.2	0.01
14	Nitrite (ppm)	0.1	0.0	0.2	0.01
24	Nitrite (ppm)	0.1	0.0	0.3	0.01

Table A-4. Average water quality values for tanks in treatment 25 ppt, normoxia.

Tank #	Water Quality Value	Average	Minimum	Maximum	Standard Error
5	Temperature (^{o}C)	32.3	30.7	33.9	0.10
9	Temperature (^{o}C)	32.3	31.5	33.0	0.05
13	Temperature (^{o}C)	32.4	31.2	34.2	0.10
20	Temperature (^{o}C)	31.9	30.9	32.5	0.05
5	Salinity (ppt)	25.2	23.1	26.5	0.10
9	Salinity (ppt)	25.3	24.5	26.8	0.08
13	Salinity (ppt)	25.3	22.5	27.2	0.13
20	Salinity (ppt)	25.6	24.0	27.2	0.11
5	Dissolved Oxygen ($mg \cdot L^{-1}$)	6.0	5.5	6.5	0.04
9	Dissolved Oxygen ($mg \cdot L^{-1}$)	6.1	5.6	6.5	0.03
13	Dissolved Oxygen ($mg \cdot L^{-1}$)	6.0	5.6	6.5	0.03
20	Dissolved Oxygen ($mg \cdot L^{-1}$)	6.1	5.5	6.5	0.04
5	% Dissolved Oxygen	95.1	85.9	100.8	0.60
9	% Dissolved Oxygen	95.2	88.7	101.8	0.56
13	% Dissolved Oxygen	95.5	89.7	102.3	0.53
20	% Dissolved Oxygen	95.2	86.2	102.1	0.62
5	Ammonia (ppm)	0.6	0.0	2.1	0.12
9	Ammonia (ppm)	1.0	0.0	2.4	0.13
13	Ammonia (ppm)	0.8	0.0	2.9	0.15
20	Ammonia (ppm)	0.7	0.0	1.9	0.14
5	Nitrite (ppm)	0.2	0.1	0.3	0.01
9	Nitrite (ppm)	0.1	0.0	0.3	0.01
13	Nitrite (ppm)	0.1	0.0	0.4	0.02
20	Nitrite (ppm)	0.1	0.0	0.3	0.02

Table A-5. Average water quality values for tanks in treatment 40 ppt, hypoxia.

Tank #	Water Quality Value	Average	Minimum	Maximum	Standard Error
6	Temperature (^{o}C)	32.0	29.8	33.3	0.09
10	Temperature (^{o}C)	32.0	29.5	32.9	0.09
16	Temperature (^{o}C)	32.7	31.3	33.7	0.09
22	Temperature (^{o}C)	32.2	31.2	33.4	0.07
6	Salinity (ppt)	40.2	37.7	42.0	0.15
10	Salinity (ppt)	40.1	37.6	41.9	0.14
16	Salinity (ppt)	40.4	37.9	42.4	0.19
22	Salinity (ppt)	40.5	37.3	42.5	0.17
6	Dissolved Oxygen ($mg \cdot L^{-1}$)	0.8	0.2	3.8	0.12
10	Dissolved Oxygen ($mg \cdot L^{-1}$)	1.0	0.3	4.5	0.13
16	Dissolved Oxygen ($mg \cdot L^{-1}$)	0.6	0.1	3.7	0.10
22	Dissolved Oxygen ($mg \cdot L^{-1}$)	0.7	0.2	4.5	0.10
6	% Dissolved Oxygen	9.7	3.7	34.6	1.20
10	% Dissolved Oxygen	13.1	4.3	47.9	1.36
16	% Dissolved Oxygen	6.9	2.4	25.8	0.85
22	% Dissolved Oxygen	10.5	3.2	31.9	0.95
6	Ammonia (ppm)	0.9	0.0	2.4	0.16
10	Ammonia (ppm)	0.9	0.0	1.7	0.11
16	Ammonia (ppm)	1.2	0.0	3.4	0.19
22	Ammonia (ppm)	0.9	0.0	2.5	0.15
6	Nitrite (ppm)	0.1	0.0	0.3	0.01
10	Nitrite (ppm)	0.1	0.0	0.3	0.01
16	Nitrite (ppm)	0.1	0.0	0.3	0.02
22	Nitrite (ppm)	0.1	0.0	0.3	0.01

Table A-6. Average water quality values for tanks in treatment 40 ppt, normoxia.

Tank #	Water Quality Value	Average	Minimum	Maximum	Standard Error
2	Temperature (^{o}C)	31.9	30.4	32.8	0.07
12	Temperature (^{o}C)	32.0	30.5	32.6	0.08
15	Temperature (^{o}C)	32.5	30.8	34.2	0.10
21	Temperature (^{o}C)	32.5	31.3	34.0	0.10
2	Salinity (ppt)	40.1	37.5	41.6	0.14
12	Salinity (ppt)	40.6	38.1	42.6	0.15
15	Salinity (ppt)	40.3	38.3	42.2	0.14
21	Salinity (ppt)	40.2	36.4	43.3	0.25
2	Dissolved Oxygen ($mg \cdot L^{-1}$)	5.6	5.1	6.1	0.03
12	Dissolved Oxygen ($mg \cdot L^{-1}$)	5.6	5.0	5.9	0.03
15	Dissolved Oxygen ($mg \cdot L^{-1}$)	5.6	5.1	6.1	0.03
21	Dissolved Oxygen ($mg \cdot L^{-1}$)	5.5	4.9	6.1	0.04
2	% Dissolved Oxygen	94.3	87.4	103.8	0.60
12	% Dissolved Oxygen	95.3	79.8	102.0	0.64
15	% Dissolved Oxygen	95.6	88.7	102.8	0.52
21	% Dissolved Oxygen	94.8	83.7	102.7	0.63
2	Ammonia (ppm)	0.7	0.0	1.8	0.12
12	Ammonia (ppm)	0.8	0.0	2.2	0.13
15	Ammonia (ppm)	0.8	0.0	2.3	0.14
21	Ammonia (ppm)	0.9	0.0	3.5	0.19
2	Nitrite (ppm)	0.1	0.1	0.3	0.01
12	Nitrite (ppm)	0.1	0.0	0.2	0.01
15	Nitrite (ppm)	0.1	0.0	0.2	0.01
21	Nitrite (ppm)	0.1	0.0	0.3	0.01

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BIOGRAPHICAL SKETCH

Elise Ann Hoover was born in Janesville, Wisconsin, on March 7th 1977. She moved with her parents to Gainesville, Florida, in 1981 and has been a resident since that time. Yearly trips to Crescent Beach on the east coast of Florida and an introduction to scuba diving cemented her love of the ocean and a desire to study in a related field. She received her BS in environmental science from the University of Florida in May of 2000. Elise went on to teach laboratory sections for Invertebrate Zoology and Marine Biology with Dr. Frank Maturo at the University of Florida, followed by identification of invertebrates with an environmental consulting firm. She returned to the University of Florida to pursue a master's degree in the spring of 2004 and will receive her degree in the spring of 2007.