DETERMINATION OF TEMPERATURE THRESHOLDS FOR THE NORTHERN HARD CLAM, AND EVALUATION OF BACKCROSSED F1 HYBRIDS (*MERCENARIA MERCENARIA* X *MERCENARIA CAMPECHIENSIS*)

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
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To my wonderful friends and family for their unwavering encouragement and faith in me

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DETERMINATION OF TEMPERATURE THRESHOLDS FOR THE NORTHERN HARD CLAM, AND EVALUATION OF BACKCROSSED F1 HYBRIDS (*Mercenaria mercenaria* X *Mercenaria campechiensis*)

By

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The northern hard clam, *Mercenaria mercenaria* (Linnaeus1758), is an important aquaculture species in Florida. Florida water temperatures are at the upper temperature range for *M. mercenaria* and high mortalities in the summer months may be a result of the combined stress of high temperatures, extreme salinities, and low dissolved oxygen. Basic breeding techniques, such as hybridization and polyploidy, may be useful to develop a more stress-tolerant hard clam suited for Florida waters. Basic aspects of thermal biology are not known for the northern hard clam grown by Florida shellfish farmers, nor have experimental hard clam lines, such as backcrossed hybrids, been evaluated in laboratory challenges. Therefore, the objectives of my research were to determine the upper acute temperature limit of cultured *M. mercenaria* and to evaluate the performance of backcrossed hybrids (♀ and ♂ *M. mercenaria* x (♀ and ♂ *M. mercenaria* x *M. campechiensis*) and reciprocal crosses) in laboratory challenges. The upper acute temperature limit was determined by exposing cultured *M. mercenaria* (n=40/trt) to four target temperatures (32, 34, 36, 38°C) and recording number of clam mortalities in four-hour intervals. All hard clams died within 28 hours of exposure in the 38°C treatment, whereas no clams died at any other treatment temperatures, indicating that the upper acute temperature is

near 38°C for Florida cultured *M. mercenaria*. Six-month-old and 12-month-old backcrossed hybrid hard clams and controls were challenged in mimicked summer stressor conditions in Florida: oxygen stress (<3 ppm), high temperature (32°C), and various salinities (15, 25, 35 ppt). Unfortunately, backcrossed hybrid hard clams exhibited no significant improvement in survival. Understanding stress limits in Florida strains of *M. mercenaria* and hybrids will contribute to potential management of summer mortality events and further development of hardier clam strains for Florida.

CHAPTER 1
INTRODUCTION

Background

Mercenaria mercenaria, the northern hard clam, is an important aquaculture species in Florida. The $19 million dollar industry (USDA 2007) grew rapidly over the last three decades and shows potential for further growth. *Mercenaria mercenaria* inhabit coastal waters ranging from Canada to the Gulf of Mexico with a difference in mean annual temperature of 25°C between its northern and southern distributions (Abbot 1974, Pickard and Emery 1982). This large geographic range indicates potential to survive in a wide array of climates. The waters in which the aquaculture industry of Florida operates, are at the upper temperature range of the northern hard clam. High mortalities of this species occur during the summer in Florida probably as a result of physiological stress of high temperatures combined with extreme salinities, low phytoplankton abundance, and low dissolved oxygen concentration (Scarpa et al. 2005). Efforts are being made to mitigate the effect of summer conditions on this valuable crop.

One means of reducing summer mortalities is to develop a clam stock that is more tolerant of high temperatures. Selective breeding and hybridization for improved cultured stocks is common practice in fin fish aquaculture and throughout agriculture. The Sunshine bass is a hybrid cross of the striped bass, *Morone saxatilis,* and the white bass, *Morone chrysops,* and is commonly aquacultured, because of the improved growth rate and culture characteristics compared to either parent species (Bartley 2001). Rainbow trout and char (*Oncorhynchus mykiss* x *Salvelinus sp*.) are hybridized for improved disease resistance along with many other salmonid species (Dorson et al., 1991, Bartley 2001). Many other freshwater fishes including carps, tilapias, loaches, catfishes, and drums are all hybridized to improve their performance as aquaculture species (Bartley 2001). The potential of breeding programs for bivalves is relatively

unexplored however, with only five well-known efforts world-wide (Haskin Shellfish Research Laboratory, New Jersey; Hatfield Marine Science Center, Oregon; Virginia Institute for Marine Science, Virginia; The French Research Institute for Exploration of the Sea (IFREMER), France; Commonwealth Scientific and Industrial Research Organization, Australia). Oyster breeding programs for disease resistance are successful at the Haskin Shellfish Research Laboratory, Hatfield Marine Science Center, and Virginia Institute for Marine Science.

Hybridization of the northern hard clam and southern quahog appears to produce a clam with improved thermal stress tolerance (Baker et al. 2011). The hybrids of *M. mercenaria* and *M. campechiensis* (produced from single-parent crosses with parents randomly selected from existing broodstock) have produced some families that consistently outperform pure *M. mercenaria* in survival when reared under commercial conditions (Sturmer et al. 2012). However, the hybrid clams gape excessively in refrigeration making them unacceptable for commercialization (Sturmer et al. 2012). Therefore, the next step is to examine the effectiveness of backcrossing hybrids with parental species to withstand summer conditions and tolerate refrigeration.

Aquaculture of *Mercenaria mercenaria* in Florida

From 2005 to 2008, the annual value of exported hard clams from the aquaculture industry in the United States grew from $34 million to $58 million, but between 2008 and 2010 declined to $45 million (USDA 2010). As many as 425 growers work on 593 ha off of Florida's coast (Bergquist et al. 2009). The United States Department of Agriculture census of aquaculture in 2005 reported the hard clam industry in Florida had $18 million in sales in 2001 and $9.8 million in sales in 2005 (USDA 2007); the decrease in 2005 was due to the hurricane season of

2004. Most of the growth of the hard clam aquaculture industry in Florida can be attributed to the increasing expansion of areas cultivated, rather than improved stocks or methods.

Culture methods for hard clams in Florida begin in the hatchery. Most growers purchase seed from hatcheries, where clams are spawned and raised until they are 1 mm or larger in shell length (Whetstone et al. 2005). Nursery systems serve as an intermediate holding place before clams are ready to be planted. Nurseries are usually weller or raceway systems, and hold clams until they are 5 to 6 mm in shell length, which is the usual minimum size to be field planted (Whetstone et al. 2005). Grow out takes place on lease areas, which support about 1,000,000 seed/acre (Whetstone et al. 2005). Most Florida growers use soft polyester mesh bags staked to the substrate to grow clams (Whetstone et al. 2005). These bags provide protection from predators and biofouling organisms, and aid the grower in organizing their crop, much like crop rows in traditional agriculture. The mesh size is increased after about 6 months of growth, when clams are sorted into their new bags to finish growing (Whetstone et al. 2005). The last stage of grow out takes 12 to 24 months, depending on environmental conditions and food availability (Whetstone et al. 2005). After the bags are collected for harvesting, the clams are brought to a wholesaler to be processed. Processing includes cleaning, size grading, counting, and packaging (Whetstone et al. 2005). The climate in Florida allows for almost year-round growth and continual harvesting (Bergquist et al. 2008).

Survival in summer months has recently been below the average of 50 to 70% (FAO), with 0% survival in the Big Bend (Dixie, Levy, and Citrus counties) area in 1998 (S. Maddox, USDA, Farm Service Agency, pers. comm). Summer water temperatures in Florida can exceed 30°C which is above the upper temperature threshold (27 to 30°C) of many phytoplankton species, limiting the amount of available food (Bergquist et al. 2009, Hoff and Snell 1987).

Elevated temperature also increases clam metabolism and energy demands at a time when food availability is low (Weber et al. 2007). Additional stress factors stem from lower dissolved oxygen and potentially lower salinites from the increased number of tropical storms in summer months. Salinity was almost 0 ppt in April 2003 and can be highly variable during summer months ranging between 10 and 30 ppt (Bergquist et al. 2009). Determination of the acute upper temperature limit may contribute to better managing the summer losses, which prevent Florida from achieving its full commercial clam aquaculture potential, by informing a selective breeding program.

Hard Clam Physiology and Environment

Geographically, *M. mercenaria* is native to the Gulf of St. Lawrence, Canada, to Indian River Lagoon, Florida (Abbot 1974). The population on Florida's Gulf Coast has likely been recently introduced by clam aquaculture (Arnold et al. 2004, Baker et al. 2008). This range encompasses huge extremes in temperatures, indicating the ability of the hard clam to acclimate and tolerate a wide range of conditions. Florida's sub-tropical climate allows for a long growing season. In recent years, however, temperatures in the summer have exceeded the upper range of optimal temperatures for hard clam growth (Baker et al. 2011, Sturmer et al. 2012).

An estuarine species, *M. mercenaria* is usually found in habitats with salinities between 20 to 30 ppt and optimum temperatures between 15 to 25°C (Grizzle et al. 2001). The effect of salinity on behavior and physiology is well documented for *M. mercenaria*. The limits for survival are between 12 and 46 ppt for adults and pumping rates are inhibited below 15 ppt and above 36 ppt (Castagna and Chanley 1973, Hamwi 1969). Behaviors indicating stress, including gaping and reduced burial activity, occur when salinities dip below 15 ppt clams will close their

valves to keep water of 12 ppt or less out of their internal tissues (Chanley 1957, Castagna and Chanley 1973).

Outside the optimal temperature range of 15 to 25°C, clams become stressed and growth can be adversely affected (Grizzle et al. 2001). Under stress, shell growth of the clam will become lateral or thicker, and produce dark growth bands when growth is slow or suboptimal; these bands are laid down in both summer and fall in Florida (Arnold 1991). Clams investing energy in lateral shell growth may be diverting energy from possible tissue or meat growth. Growth ceases when temperatures exceed 31°C (Ansell 1968) as they did in June 2004, August 2005, August 2007, June 2008, July 2009, and July 2010 (Gulf Jackson Lease Area, Levy County, water quality data, FDACS data, 2004, 2007-2010). Water temperatures in Florida are also highly variable and can fluctuate by as much as 11°C in a 24-hour period, preventing time required for acclimation (Weber et al. 2007).

Temperature affects metabolism and water pumping rates. Because clams are ectotherms, increased metabolism from high water temperatures results in high energy demand. Pumping rates are maximally efficient at temperatures between 20 to 25°C, but cease to undetectable levels around 32°C (Hamwi 1969). Clams respond to high temperatures by closing their valves and resorting to anaerobic metabolism (Hamwi 1969). Other signs of metabolic stress include rising to the surface of the sediment and gaping, or opening the valves. At some unknown critical high temperature, even anaerobic metabolism ceases, stopping growth, reducing immune response, and causing death (Weber et al. 2007). High water temperatures are also correlated with low phytoplankton densities, causing further physiological stress from low food availability at a time when energy needs are increased (Bergquist et al. 2009).

Dissolved oxygen level is a compounding environmental factor affecting hard clam behavior and survival. While low dissolved oxygen, or hypoxic conditions (dissolved oxygen less than 2 mg/L) alone may not be stressful to hard clams, reduced survival is noted when combined with high temperatures (Baker et al. 2002). Under hypoxic conditions, oxygen uptake is maintained by increasing the efficiency by which oxygen is pulled from the water column, but under extreme environmental stress, the valves close leaving the clam to metabolize anaerobically (Hamwi 1969), which can be sustained for up to 18 days, but only at low temperatures (1 to 6°C) (Loosanoff 1939).

Hard clam physiology with regard to environmental limits is a relevant contemporary topic of research, because of the implications for the species in regard to climate change. This paradigm links an increased cellular demand for oxygen with increasing temperature to the environmental availability of dissolved oxygen. Warmer water is typically lower in dissolved oxygen. When oxygen availability is limited, an animal switches to anaerobic metabolism and protects its cellular functions with emergency mechanisms. An animal sustained in this state is not a healthy and actively growing animal.

Given these recent developments in understanding the effects of exceeding optimum temperature ranges, it is prudent to pursue the development of a hard clam strain for the Florida aquaculture industry with high thermal tolerance. Determining the acute upper temperature limit in *M. mercenaria* will contribute to understanding and potentially managing summer mortality events, which are problematic for clam growers in Florida. When water temperatures are expected to be in the upper critical range, a grower could for example harvest larger clams, rather than risk losing them. It is clear that, for Florida to remain competitive in the clam aquaculture industry, a hardier strain of *Mercenaria mercenaria* is needed.

Bivalve Breeding Methods

Stock improvement is common practice in aquaculture through hybridization and selective breeding for various traits including disease resistance, high growth rates, improved shelf life, improved flesh quality, sterility, and improved environmental tolerance (Bartley 1997). Quantitative trait loci (QTL) are phenotypic traits that vary in degree, like skin or hair color, and contribute to polygenetic effects. Multiple genes contribute to an animal's taste, growth, environmental tolerance, or other commercially valuable characteristics (Newkirk 1979). Heat tolerance, or degree of heat tolerance is likely a QTL (Newkirk 1979). Improving environmental tolerance in a cultured population helps to keep it healthy in possibly unpredictable conditions (Bartley 2001). This would be especially true of clam and other bivalve culture which is mostly subject to the natural conditions of the body of water and stochastic events versus, a tightly controlled fin fish recirculating facility, for example.

Research has been done on selective breeding of oysters for disease resistance and improved flesh quality in a handful of programs world-wide. The Haskin Shellfish Research Laboratory of Rutgers University has breeding programs in place to produce fast growing Eastern oysters, *Crassostrea virginica*, resistant to Juvenile Oyster Disease, MSX, and Dermo, for mid-Atlantic restoration projects (Allen et al. 1993). The Coastal Oregon Marine Experiment Station at Oregon State University has a Molluscan Broodstock Program that aims to improve Pacific oyster *Crassostrea gigas* and Asian oyster *Crassostrea sikamea* broodstock to enhance commercial yields, create a broodstock management program for industry for sustainable commercial production, and maintain a repository for selected top-performing oyster families (Langdon et al. 2003, Brake et al. 2003, Evans et al. 2003). College of William and Mary Virginia Institute of Marine Science details their extensive selective breeding program for

disease resistance, meat yield, and growth in depth in the 2009 report of The Aquaculture Genetics and Breeding Technology's Oyster Breeding Program (VIMS 2009). Australia's national scientific organization, Commonwealth Scientific and Industrial Research Organisation, has breeding programs for the Pacific oyster and hybridization programs for abalone. The French Research Institute for Exploration of the Sea (IFREMER) program is devoted to selective breeding of commercially important bivalves including oysters and blue mussels. Although these programs have been successful at producing genetically superior shellfish for aquaculture, their limited number demonstrates the untapped potential for growth in using breeding methods for improving shellfish aquaculture, particularly that of hard clams.

Hard clams are diploid, having two sets of chromosomes in each somatic cell. During reproduction, haploid (one set of chromosomes) gametes combine to create diploid (two sets of chromosomes) offspring with a set of chromosomes from each parent. Triploid animals have three sets of chromosomes and usually cannot reproduce. Triploids are often favorable in aquaculture, because they are, for the most part, sterile and often larger (Beaumont and Fairbrother 1991, Guo and Allen 1994, Eversole et al. 1996). The increase of genetic material in each cell is theorized to create an additive effect of a larger animal, known as polyploidy gigantism (Guo and Allen 1994). It has also been suggested that energy diverted from reproduction is allocated into tissue or meat growth (Beaumont and Fairbrother 1991, Eversole et al. 1996). The hypothesis of increased heterozygosity states fitness of an animal is increased with genetic diversity, therefore the third set of chromosomes improves fitness and general growth (Beaumont and Fairbrother 1991).

Triploidy is commonly used in Pacific oyster aquaculture; several methods have been developed for inducing triploidy. The most reliable method is to breed a tetraploid (four sets of

chromosomes) with a diploid, which results in 100% triploidy (Guo et al. 1996). Other methods include interfering with polar body development in meiosis I and II by introduction of chemical, high pressure, or temperature stress (Beaumont and Fairbrother 1991). Of these methods suppression of the second polar body with cytochalasin B produces the highest percent of triploid larvae, but is dangerous for the technician because of toxicity (Guo et al. 1996).

Triploid hard clams have been tested for stress resistance. Triploid hard clams were challenged in a laboratory under salinity (10, 25, 40 ppt) and oxygen stress (D.O. < 2 mg/L). Their survival and burial was compared to diploid hard clam siblings. Triploids had better survival in the 25 ppt hypoxic treatment, but overall were not found to survive significantly better than diploid hard clams (Hoover 2007).

Hybridization is the breeding of two individuals within or between species, and aims to specifically highlight an advantageous trait or generally improve the hardiness of the stock for culture conditions (Bartley et al. 2001). Research has been conducted on hybridizing *M. mercenaria* with the southern hard clam, *M. campechiensis*, which both occur and hybridize naturally on Florida's east coast in the Indian River area (Dillion and Manzi 1989). *M. campechiensis* is not favorable for culture because of its limited shelf life, gaping only days after harvest, but tolerates high summer water temperatures more readily than *M. mercenaria* (Menzel 1989). *M. campechiensis* has a natural range extending as far north as southern New Jersey, but is most common from North Carolina to the Gulf of Mexico, and has been reported in the Caribbean (Baker et al. 2008). Although these two species will readily hybridize in the wild, they usually remain geographically separate, preferring different environments (Menzel 1989). *M. campechiensis* prefers near shore environments with salinities above 30 ppt (Menzel 1989). *M. mercenaria* occurs in estuarine areas with salinities ranging from 20 to 30 ppt (Grizzle et al.

2001). It has also been suggested that although the Indian River area contains naturally occurring hybrids, the species remain discrete in other geographic locations where they co-occur because of increased seasonality providing different reproductive cues for each species that prevents hybridization (Dillion and Manzi 1989, Dillon 1992). Hybrids of northern hard clams and southern hard clams have been successfully produced and reared in hatchery systems that resulted in a viable and fertile first generation (F1), second generation (F2), third generation (F3), and backcross generations with either parent (Menzel 1989). Hybrids have outperformed pure *M. mercenaria* for growth and total production in field trials, but had reduced shelf life (Scarpa et al. 2011).

Backcrossing has been suggested as a method of genetic improvement in other forms of aquaculture.Backcrossing involves breeding a hybrid with a favorable trait, like heat tolerance, back to a parental species (Bartley 1997). Backcrossed families of *M. mercenaria* x *M. campechiensis* hybrids have been developed with the goal of preserving the improved heat tolerance of the hybrids and emphasizing the shelf life of the northern hard clams (Sturmer et al. 2012).

Summer temperatures in Florida are thought to be a major factor in summer mortality events, and data on acute upper temperatures will inform growers when their crop is at risk. An effort should be made to improve hard clam stocks through breeding techniques to reduce summer-related mortality and increase the maximum aquaculture production in Florida. Backcrossed hard clams could potentially provide farmers with a superior animal for culturing; however more information on their performance under exposure to various stresses is necessary. If ocean temperatures continue to rise, as expected due to climate change, there could be serious implications for clam growers in Florida, where temperatures are already stressful, emphasizing

the need for stock development. The objectives of my research were to 1) determine the acute upper temperature limit for cultured hard clams *M. mercenaria,* and 2) examine the performance of backcrossed hybrid hard clams in laboratory challenges against hard clam controls. It was hypothesized that backcross hybrid hard clams would survive longer in the challenge conditions than pure hard clams.

CHAPTER 2
MATERIALS AND METHODS

Upper Acute Temperature Limit

The northern hard clams, *M.ercenaria mercenaria* (Lennaeus 1758), used in the acute upper temperature limit experiment, were harvested from culture bags on a Dog Island clam lease in the Gulf of Mexico near Cedar Key, Florida in September 2011. Ambient water temperature was 26°C and clams were held overnight at 26°C and 25 ppt in the University of Florida Shellfish Aquaculture Research and Education Facility (SAREF) in Cedar Key. Clams were transported in a cooler to the University of Florida Fisheries and Aquatic Sciences laboratory in Gainesville, Florida, whereupon they were placed in acclimation tanks. Clams were acclimated in 30 gallon tanks of 25 ppt 26°C water. During acclimation temperature was increased 2°C per day until 32°C was reached. Water changes of 50% were conducted daily during acclimation, and clams were observed for mortality. Any dead animals were removed. Salinity was kept the same throughout the experiment, 25 ppt. Shell lengths were taken from a subsample (n = 25) while clams were acclimating

The acute upper temperature limit of *Mercenaria mercenaria* was determined by exposing clams to various target temperatures after being acclimated at 32°C and 25 ppt for four days. There were two tanks of each temperature for duplication (Figure 2-1A). The animals were spilt haphazardly into eight groups of 20 each and stocked into eight 38-L tanks (50.8 x 25.4 x 31.75 cm). Each tank was equipped with a Fisher Scientific memory monitoring temperature probe for reading the temperature, at least one Marineland visi-therm 200 or 300-W aquarium heater, and one air stone. The temperature was raised 1°C every 30 minutes at the start of the experiment until target temperatures of 32, 34, 36, and 38°C were established in each duplicate tank. Tank temperatures were increased in a staggered order so that all tanks reached intended

test temperatures at the same time. Temperatures were raised by use of Marineland visi-therm 200 or 300-W aquarium heaters (model number ML90443-00, Marineland Aquarium Products, Cincinnati, Ohio); heaters could not be set above 32°C, therefore additional heaters were added to some tanks to reach target temperatures. Once intended temperatures were reached, clam viability was monitored every 4 hours until 100% mortality was reached in a single temperature treatment. The number of dead animals was recorded every 4 hours and mortalities removed. Mortality was determined by visually inspecting the tanks for gaping clams. Gaping clams were prodded at the mantle's edge. If they were unresponsive or unable to hold their valves closed after manual compression, they were considered dead and removed from the tank. Water changes were conducted every 8 hours; dissolved oxygen was monitored every 12 hours with a 650 MDS YSI meter (Yellow Springs Instruments, Yellow Springs, Ohio).

Laboratory Challenges of Backcross Hybrids (*M. mercenaria* X *M. campechiensis*)

Backcrossed hybrid hard clams were produced at Harbor Branch Oceanographic Institute at Florida Atlantic University, in Fort Pierce, Florida. Backcrossed families were produced by breeding previously developed hybrid hard clam (*M. mercenaria* x *M. campechiensis* and reciprocal cross) families to *M. mercenaria* stocks. Each clam "family" had four groups; *Mercenaria mercenaria* pure-breds as a control group, two backcross groups where the mother of the hybrid animal was *M. mercenaria*, and a single backcross group where the mother of the hybrid animal was *M. campechiensis*. Group numbers (31 – 42) were used to code for the specific crosses and the heritage of each family and its previous generations. For example, in families D and E, a hybrid female were backcrossed to a pure hard clam male. Within each family, there were control hard clam groups of pure hard clam females crossed with the same male used to produce the backcrosses (Table 2-1). For family F, the reciprocal hybrid cross was

used for backcrossing. That is, a pure hard clam female was crossed with a hybrid male, and the control hard clam group used the same female (Table 2-1).

All clams were first reared the SAREF in Cedar Key, Florida, before being planted into commercial lease sites in the Gulf of Mexico near Cedar Key. Backcross hybrid hard clams were harvested after 6 months (April 2011) and 12 months (October 2011) and held no longer than two weeks in the SAREF until they were delivered to the University of Florida Fisheries and Aquatic Sciences laboratory Gainesville, Florida. Once clams were received, subsamples of shell length from 30 individuals in each group were measured. Clams were also labeled on both valves with their group numbers using a Sharpie marker before being placed in to acclimation tanks.

Experimental design

Acclimation of clams was conducted in 30 gallon aquaria for each set of environmental parameters for one week by, increasing salinity at about 2 ppt/day and temperature at about 2°C/day (Figure 2-1B). After acclimation to particular challenge conditions, clams were sorted into experimental aquaria with corresponding conditions as described above. Each clam group was haphazardly divided into sets of 10 to a replicate aquaria resulting in 40 clams per aquaria, a group of 10 individuals from each of 4 groups in a family and 2 families per system.

Backcross hybrid clam families E and F were tested at 6 months of age (April 2011), and families D and F at 12 months (October 2011). Laboratory challenges of backcross hybrids were conducted in systems as described in Hoover (2007) and described briefly as follows. Each system consisted of two 17-L aquaria each connected to a 38-L sump, (Figure 2-2A). Each sump contained a 200-W heater and a 120-V 60-Hz Quietone pump (model number 1200, Pentair Aquatics, El Monte, CA) that pumped water back into the two associated aquaria. The aquaria overflowed back into the sump, thus creating a recirculating system. Each system was replicated

16 times resulting in four blocks of treatments with 32 total aquaria. Temperature was maintained at 32°C in all systems. In each of the four blocks, each system was randomly assigned a salinity treatment of 15, 25 (control), or 35 ppt at normoxia (D.O. > 5 mg/L), or 25 ppt hypoxia (D.O. < 2 mg/L). Hypoxic conditions were created by bubbling N_2 into the sumps with air tubing and an air stone from a source liquid N_2 tank. All tanks were insulated to minimize fluctuation in water temperature. Seawater was stored in a series of 378.5-L tanks, mixed to the appropriate salinities with Oceanic natural sea salt mix (Aquarium Systems, Ohio) and well water. Enough sea water was prepared for daily 30% water changes (Figure 2-2B).

Procedure

Observations of mortality and gaping behavior were conducted every 24 hours (Figure 2-3A). Mortality was assessed in two ways: failure to remain closed when valves were manually compressed and unresponsiveness when probed at the mantle edge (Figure 2-3B). Gaping behavior is often indicative of death. If a gaping animal was responsive and able to hold itself closed, it was considered to be alive, but possibly stressed or close to death. Dead clams were removed. All tanks were monitored for a minimum of 24 days or until all clams in at least one treatment expired.

Water quality parameters were also monitored daily to ensure temperature, salinity, and dissolved oxygen levels were within acceptable ranges. Tank temperatures were monitored continuously with Fisher Scientific digital thermometers (model number 15-077-8D, Pittsburgh, Pennsylvania), which stored the minimum and maximum temperatures over the last 24-hour period. Sump temperature was also monitored daily before water changes with a 650 MDS YSI sonde (Yellow Springs Instrument, Yellow Springs, Ohio) Salinity was checked daily using a refractometer.

Data Analysis

The acute upper limit experiment mortality data was entered in a Microsoft Excel spreadsheet. The data where plotted in a graph to show mean (n=2) percent mortality over time in hours at each experimental temperature.

Statistical analyses of the data from the backcross hybrid hard clam experiments were using JMP and Statsdirect. Statsdirect formatted the daily mortality data binomially into days of mean survival by backcross hybrid group number by assigning each individual with a 0 or 1, respectively, for alive or dead on a particular day. This data output was then transferred to JMP as the continuous response variable Mean Days of Survival for each group in each treatment, e.g., Mean Days of Survival for group 42 in 15 ppt. A two-way factorial ANOVA was performed using group number and treatment as independent variables for the dependent variable Mean Days of Survival. The interaction was not significant in either challenge ($p=0.99$), so the model was run again with the removal of the interaction as an additive model. Dunnett's method was used to examine the differences between the control (25 ppt normoxia) and the three treatments (15 ppt normoxia, 35 ppt normoxia and 25 ppt hypoxia). Differences within families, or between groups, were examined with Wilcoxon rank sums tests for a difference. Results were considered significantly different if $p < 0.05$.

Table 2-1. The group number, parental cross, and families used in the backcross experiments;M=*M. mercenaria* C=*M. campechiensis*. In parental cross, the first two letters represent the cross of the female parent, and the second two letters represent the cross of the male parent. Families D & F were used at 12 months of age and families E & F were used at six months of age.

Group number	**Parental cross (♀ x ♂)**	**Family**
31	MM X MM	D
32	MC X MM	D
33	MC X MM	D
34	CM X MM	D
35	MM X MM	E
36	MC X MM	E
37	MC X MM	E
38	CM X MM	E
39	MM X MM	F
40	MM X MC	F
41	MM X MC	F
42	MM X CM	F

A) **B)**

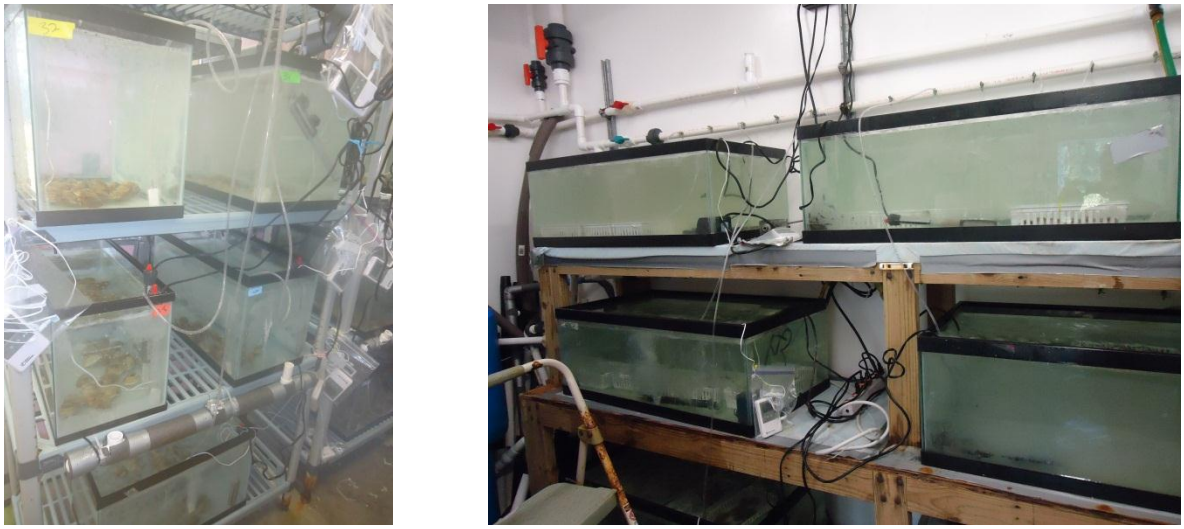


Figure 2-1. Experimental aquaria and the acclimation process. A) The acute upper thermal limit experiment system. Five 38-liter aquaria equipped with multiple heaters to reach target temperatures. B) View of acclimation tanks used for all experiments. Photos courtesy of Melissa Broderick.

A) **B)**

Figure 2-2. Experimental systems. A) One block of the system used to challenge backcrosses. The 38-liter sumps were equipped with a pump that moved water into the two 17-liter aquaria above where clams were placed. B) View of the wet lab with large sea water reservoirs on the right, acclimation tanks in the back, and experimental challenge systems on the left. Photos courtesy of Melissa Broderick.

A) **B)**

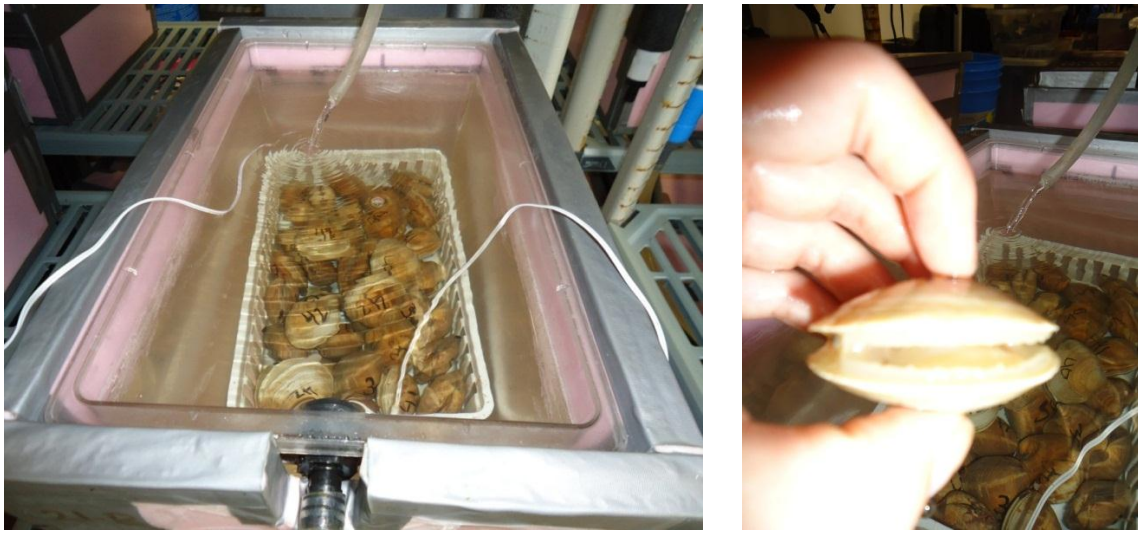


Figure 2-3. Backcross experiment daily checks. A) Example of a gaping clam. B) The inside of a aquaria holding all four groups family of backcross hybrid clams in the challenge experiment. Photos courtesy of Melissa Broderick.

CHAPTER 3
RESULTS

Water quality

All water quality parameters stayed near expected values for each experiment, with the exception of N_2 (Figure 3-1). During the challenge of the six-month-old backcross hybrids and controls there was some difficulty maintaining gradual temperature increase during acclimation, because of issues with electrical wiring causing frequent power loss. Fuses in the laboratory blew continuously during the acclimation process. The fuses would be reset and the electronics redistributed, but it took several arrangements to find a balance. During this process, clams experienced large fluxes in temperature from heaters powering on and off overnight; mortality was considerable and probably related to the stress of this unstable temperature. In other words, clams were slowly being adjusted to experimental temperature of 32°C at a rate of 2°C/day, but failing power would shut off heaters, and the water would cool down by as much as 6°C overnight. Deceased clams were removed multiple times a day, and 50% water changes were performed daily. During challenge of 12-month-old backcross hybrids and controls, dissolved oxygen in hypoxic tanks peaked above 5 mg/L at times, because of a leak in the nitrogen delivery line (Figure 3-2). New leaks were found throughout the experiment resulting in intermittent hypoxia for those tanks. In an attempt to keep dissolved oxygen low for those tanks, no water changes were made until N_2 flow was restored.

Upper temperature limit

Target temperatures (32, 34, 36, 38°C) were successfully reached in 1-degree increments within the planned acclimation time (1°C/30 mins). The mean shell length of a subsample of 25 clams was 55.1 mm (S.D. = 3.6 mm). The first mortalities were observed at 16 hours of exposure to 38°C. Both replicates at 38°C experienced similar mortality; 4 deaths and 6 deaths, out of 20

clams/tank for an average of 25% mortality. Over the next four hours, (i.e., after 20 hours of exposure), in the 38°C treatment had another 7 and 10 deaths for a total mortality average of 68%. By 28 hours, 100% mortality occurred in both replicates. No mortality was experienced at any other temperature during the 28 hours (Figure 3-3).

Backcross hybrid challenges

Six-month-old backcross hybrids

Six-month-old hard clams had a mean shell length of 38.3 mm (S.D. = 1.8 mm n=240). There was no significant difference ($p = 0.96$) in mean days of survival between backcross hybrid hard clam groups and pure bred hard clams under the conditions tested. Treatment had a significant effect ($p<0.001$) on mean days of survival for six-month-old backcross hybrid and pure bred hard clams. Mean days of survival was significantly lower in 15 ppt normoxic and in 25 ppt hypoxic conditions ($p<0.001$) as compared to the control treatment of 25 ppt normoxia (Figure 3-4A). The 35 ppt normoxia treatment was not significantly different ($p=0.11$) from the control 25 ppt normoxia treatment. The median is often a more appropriate statistic when distributions are non-normal, as the Mean Days of Survival for this experiment. The median for the distribution of mean days of survival in all six-month-old clams were 2.7 days. Wilcoxon's rank sums test was used to evaluate differences between groups in mean days of survival, because the distribution of mean days of survival was non-normal. There was no significant relationship between mean days of survival and group number ($p=0.96$, Figure 3-5A) using the Wilcoxon's rank sums test. This indicates that backcross hybrids and hard clam controls performed similarly; and that there were no group differences, because a family was made up of four specific groups.

Twelve-month-old backcross hybrids

Twelve-month-old hard clams had a mean shell length of 47.1 mm (S.D. = 2.6 mm, n=240). Survival during acclimation was noticeably improved in 12-month-old backcross hybrids, and losses were minimal when compared to 6-month-old backcross hybrids. The median for the distribution of mean days of survival was 19.7 days for the length of the challenge experiment. Wilcoxon's rank sums test showed groups were not significantly different in days of mean survival ($p=0.95$, Figure 3-4B). Dunnett's method was then used to compare the treatments to the control (25 ppt normoxia, Figure 3-4B). The 25 ppt intermittent hypoxia treatment was not significantly different ($p=0.343$) from the control. However, mean days of survival in 35 ppt and 15 ppt were found to be significantly different ($p<0.001$) from the control treatment; with backcross hybrids in 35 ppt having higher mean days of survival than the control, and backcross hybrids in 15 ppt having lower mean days of survival than the control (Figure 3-4B). There was no significant relationship between mean days of survival and group number ($p=0.95$, Figure 3-5B).

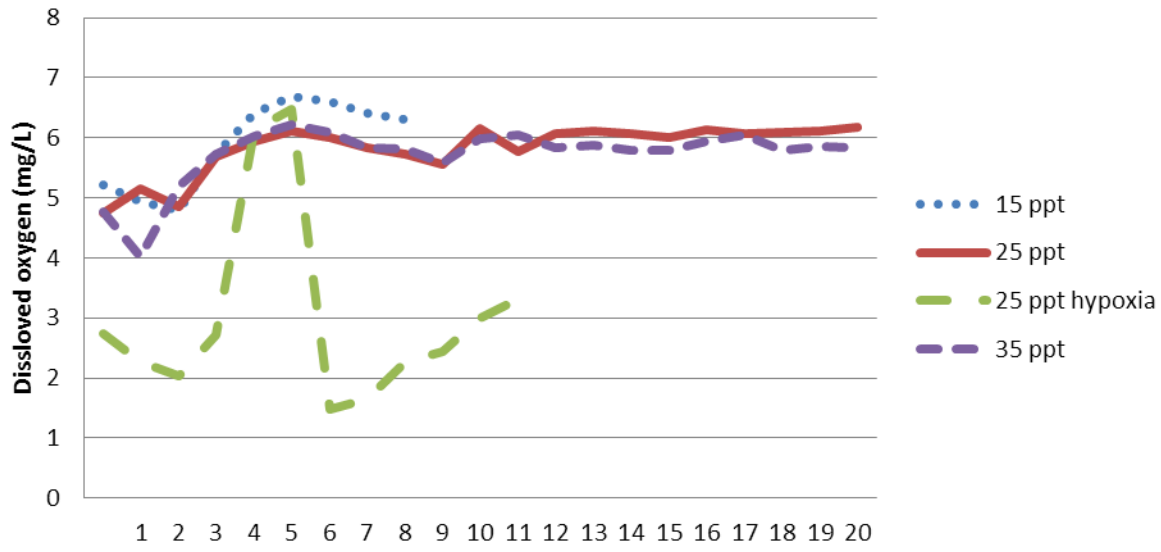


Figure 3-1. The daily dissolved oxygen concentration (mg/L) averaged for each treatment in the six-month-old backcross hybrid challenge. Dissolved oxygen data was not collected once all clams had died in a treatment.

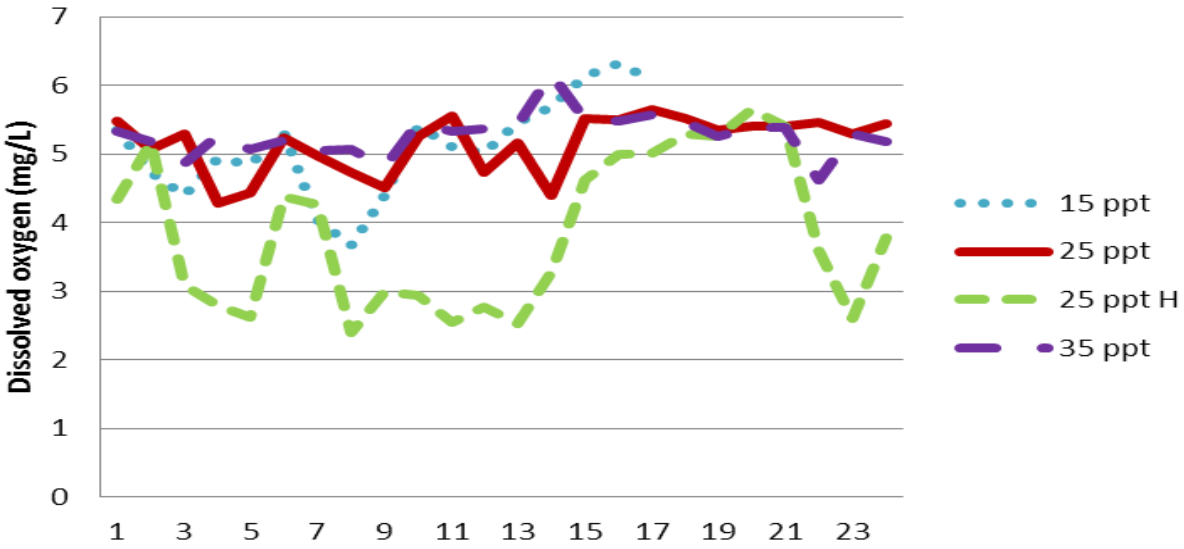


Figure 3-2. The daily dissolved oxygen concentration (mg/L) averaged for each treatment in the 12-month-old backcross hybrid challenge. Disssolved oxygen data was not collected once all clams had died in a treatment.

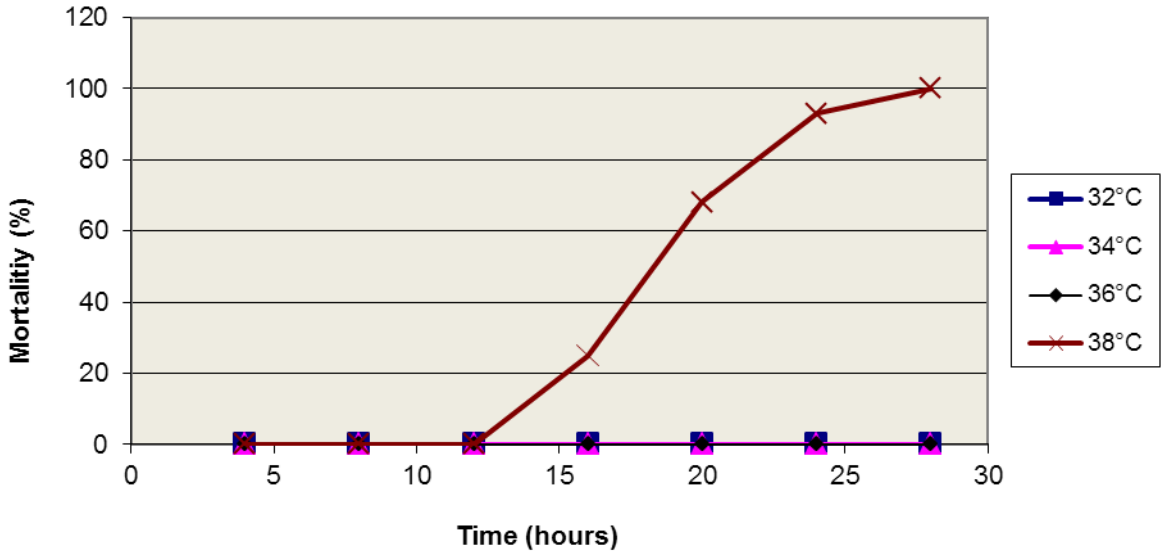
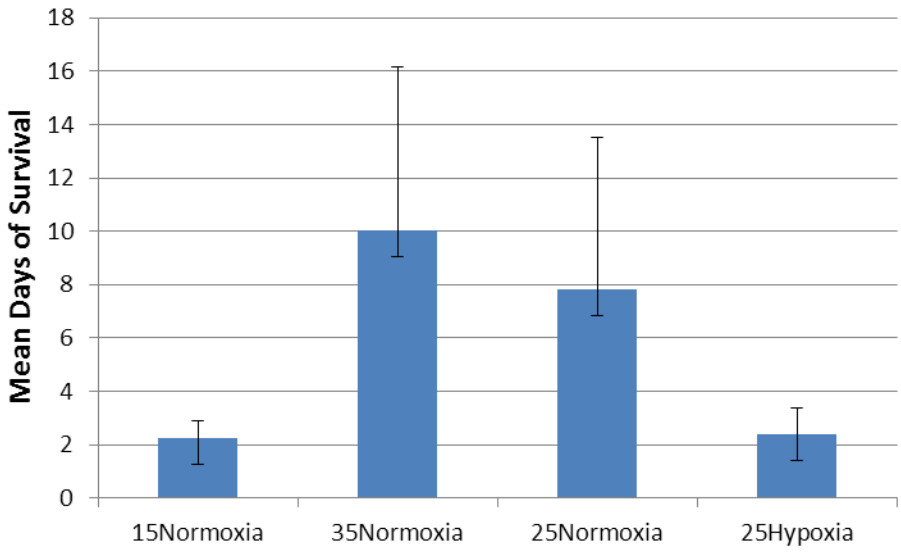


Figure 3-3. Upper acute temperature limit of *M. mercenaria*. Mean (n = 2) mortality for Florida cultured *M. mercenaria* (55.1 mm shell length S.D.= 3.6 mm, n = 160) exposed to four temperatures to determine upper acute temperature limit.

A)



B)

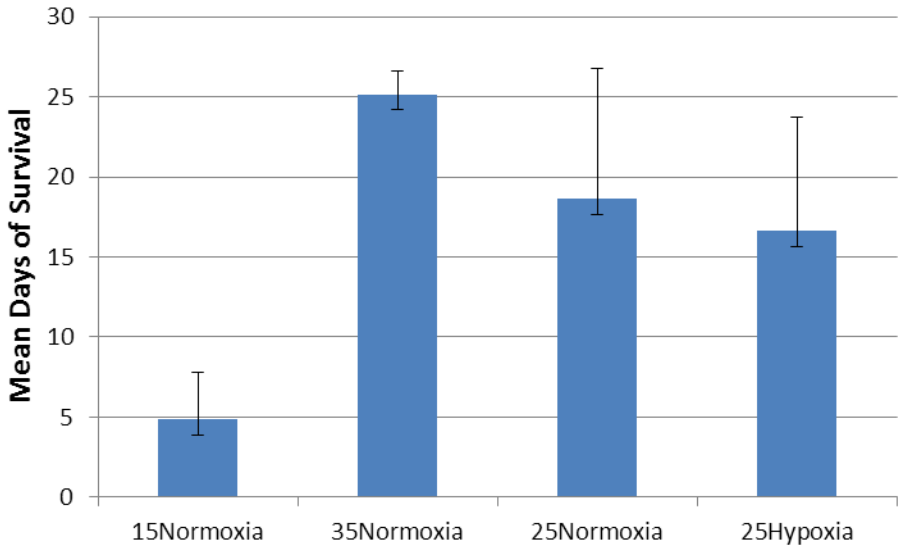
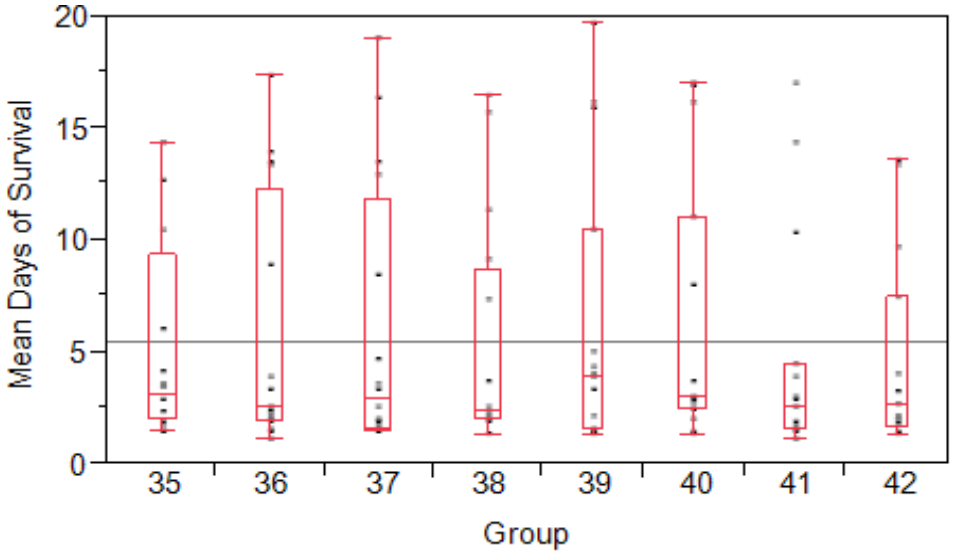


Figure 3-4. Mean days of survival across all groups by treatment with standard deviations A) Six-month-old hard clams; 15 ppt and 25 ppt hypoxia were significantly lower than the control, 25 ppt normoxia. B) Twelve-month-old clams; 35 ppt was significantly higher days of mean survival than the control (25 ppt normoxia) and 15 ppt was significantly lower days mean survival than the control (25 ppt normoxia).

A)



B)

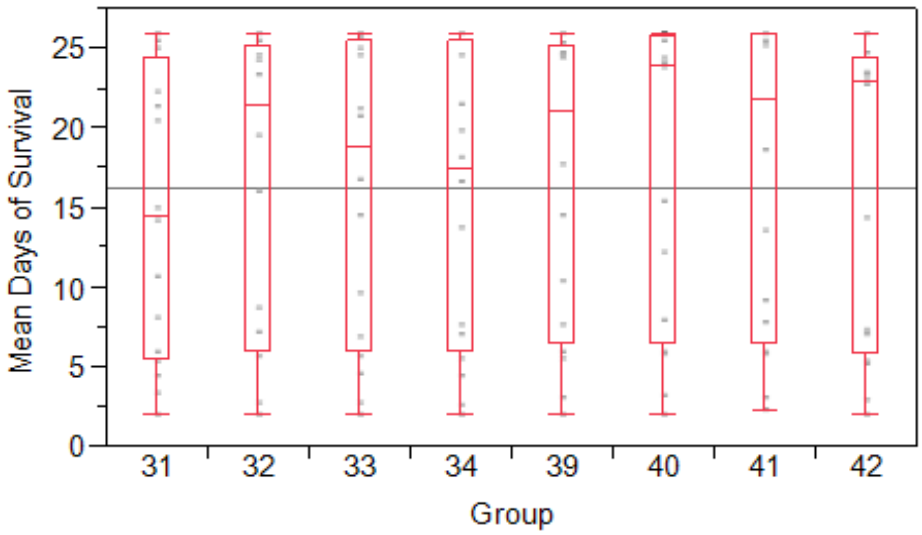


Figure 3-5. Box and whisker plot of mean days of survival by group with quantiles to display variability in data A) Six-month-old backcross hybrids, days of mean survival was not found to be statistically different among groups. Midline represents mean days of survival across all groups. B) Twelve-month-old backcross hybrids, mean survival was not found to be statistically different among groups. Midline represents mean days of survival across all groups.

CHAPTER 4
DISCUSSION

Farmers in Florida have been suffering heavy losses of hard clams in the summer when environmental conditions are stressful. Water temperatures are high, phytoplankton abundance is low, salinity is variable, and dissolved oxygen can be low. Breeding a hard clam with improved environmental tolerance is vital for overcoming these summer challenges.

In this study, I found that 1) I found that the upper acute temperature for Florida cultured *M. mercenaria* was 38°C, 2) that an increase of only 2°C from 36°C to 38°C increased mortality from 0 to100%, 3) treatments were a significant factor affecting days of mean survival for backcross hybrid hard clams and controls, 4) backcross hybrid hard clams did not survive challenge conditions differently than control clams.

I found that 38°C was the acute upper thermal limit for cultured *M. mercenaria*. All animals at 38°C died within 28 hours while at 36°C and lower temperatures, all other clams survived during this interval. Clams exposed to 36°C were gaping, indicating stress at the conclusion of the experiment, but upon manual compression were able to hold themselves closed; making 2°C the difference between 0 and 100% mortality within 28 hours. The influence of just 1°C on mortality was noted by Kennedy and Mihursky (1972) who examined critical upper temperatures for bivalves in the Patuxent estuary. Kennedy and Mihursky (1972) also found *Gemma gemma* acclimated at 25°C had an upper critical temperature of 35.6°C, and *Mulinia lateralis* acclimated at 25°C had an upper critical temperature of 33.5°C. Hicks and McMahon (2002) acclimated the brown mussel, *Perna perna*, at 15, 20, 25, and 30°C and found the upper thermal limit to be 30°C in long-term experiments and 44°C during acute thermal stress. Environmental limits were investigated in the penshell, *Atrina maura*, which had an upper

temperature of 33.2°C (Leyva-Valencia et al. 2001). In a study of the critical thermal maximum of the sea cucumber, *Apostichopus japonicas*, acclimated at 16, 21 and 26°C, critical thermal maximums were 33.1, 34.1 and 36.6°C, respectively (Wang et al. 2012). Compared to these other aquatic poikilotherms, Florida cultured *M. mercenaria* have a fairly high thermal limit. It is possible that clam culturists have been unknowingly selecting temperature tolerant animals.

Although the Florida coast of the Gulf of Mexico does reach peak water temperatures of 35°C (August 2011 Dog Island, Cedar Key, Florida), and can experience large fluxes in temperature of up to 3°C/30 minutes (August 2008 Dog Island, Cedar Key, Florida), there is a cooling period at night. The natural increase in temperature in the Gulf of Mexico is not as rapid as manipulated in the laboratory. The rapid increase of temperature in the laboratory was used to specifically initiate stress response in the clams. When allowed to acclimate, animals can typically survive much higher temperatures; it has been shown that animals living in warmer waters have higher heat resistance (Henderson 1929, Kennedy and Mihursky 1972). Increasing the temperature as quickly as in the present experiment prevents further physiological adjustment; therefore, isolating the effect of temperature stress. There is no consensus in the literature on the appropriate rate of temperature increase; experiments have increased temperature in acclimated animals from a range of 1°C/5 minutes to 1°C/day (Kennedy and Mihursky 1972, Hicks and McMahon 2002, Alexander and McMahon 2003, Peck et al. 2009)

Thermal biology is a field of growing interest, because of the implications for global climate change. As water temperatures in the Gulf of Mexico annually increase, thermal tolerance on clams and other invertebrates will be an important economic issue for Florida. Work on fruit flies, *Drosophilla melanogaste*r, confirms that the protocol of upper temperature limit experiments influences the results, and in some cases can create a bias (Santos et al. 2011).

Santos et al. (2011) modeled the critical temperature of *Drosophilla* and compared the results to published protocols revealing inconsistency and bias in results. Santos et al. (2011) conclude that short-term acclimatory responses have a confounding effect in long-term critical temperature studies in *Drosophilla*, and that acute studies are preferred. These findings may extend to other ectotherms including bivalves. The procedure followed in the upper acute temperature limit experiment on Florida cultured hard clams aimed to increase the temperature of acclimated animals by a rate that was perhaps reasonable on the hottest and most stressful day in the Gulf of Mexico to a set of target temperatures that are observed in the summer months (Florida Department of Agriculture and Consumer Services, Division of Aquaculture, Dog Island, Cedar Key, Florida sonde data). However, in the field, temperatures this extreme would not be sustained, because of diurnal warming and cooling. If this experiment is repeated, it may be improved by extending the experiment to determine how much longer it would take for clams to experience death at the lower temperatures, and adding a dirurnal cooling period. I would also suggest taking tissue samples and examining histological changes in those animals dying from heat stress.

In the laboratory challenge of six-month-old backcross hybrid hard clams, differences in mean days of survival were found among treatments. Days of mean survival in the control treatment (mean = 6.6 days) was significantly different from the hypoxic treatment and the 15 ppt treatment (mean = 2.2 days). Hard clams are generally found in coastal areas where salinities range from 12 to 30 ppt, with most populations occurring >15 ppt (FAO 2012), yet in both experiments full strength sea water treatments (35 ppt) had the highest mean days of survival. It was also surprising that in both challenges, clams survived poorest in the 15 ppt normoxic treatment. It was expected that hypoxia would be the most stressful treatment, because hard

clams resistance to periods of hypoxia is diminished in combination with heat stress (Baker et al. 2002). My results did not reflect this effect.

The N_2 flux problem in the 12-month-old backcross hybrid hard clam challenge should not have had any effect on clams in the other treatment aquaria, yet survival differences remained unapparent between backcrosses and hard clam controls across treatments. The improved survival (mean = 19.7 days) in the 12-month-old backcross hybrids may have been the result of the increased size and age of animals. Additionally, 12-month-old backcross hybrids were held in Cedar Key for only one night after harvest compared to a few weeks for the six-month-old animals. During this holding time, it is possible that clams were nutritionally stressed. Although the holding facility was flow through, clams may not have been receiving enough food to reach satiation.

The possibility that older, larger animals handle stress better than younger, smaller individuals has been examined. Yuan et al. (2010) found that in the mussel *Mytella charruana*, larger individuals (20 to 24 mm) survived salinity stress better overall than smaller (3 to 19 mm) mussels, but that smaller mussels tolerated a wider range of salinities (2 to 40 ppt) than larger mussels. However, in a study of temperature tolerance in Arctic marine phyla, Peck et al. (2009) found that across 14 species from six phyla, small animals survived to warmer temperatures than did larger ones; the top 10% largest animals in the size distribution for each species failed to survive to the highest temperature of exposure. I found; however, that larger clams, survived longer than smaller clams under stressful conditions.

Indirectly related to size and age is reproductive status, which affects an animal's energy stores, with up to 52% of the total organic production released during spawning events in hard clams (Ansell et al. 1964). An animal has a budget of energy based on how much food and

oxygen are available, if energy is being allocated to developing gametes then less energy is available for handling environmental stress. It follows that, if an animal has stored a great deal of energy in reproductive tissues, it may be vulnerable to environmental stress after spawning, until energy stores are replenished in the somatic tissues. Upon histological review (courtesy of Dr. Susan Laramore, Harbor Branch Oceanographic Institute, Ft. Pierce, Florida) of a subsample (n = 40) of six-month-old backcross hybrid hard clams from families E and F, it was determined that some individuals were in early gonadal development (25%) or a post spawn state (33%). It may be that the capacity to tolerate stress was reduced in these animals from the start, because of their reproductive status.

No differences in mean days of survival were found among groups or between families in either challenge. High mortalities occurred during the acclimation process for six-month-old backcross hybrid hard clams due to power issues. Clams continued to expire in high numbers until a few days after starting the experiment. This stress was likely exacerbated by deteriorating water quality as animals died, despite daily 50% water changes.

In the past, this recirculating laboratory challenge setup has been successfully used to delineate survival differences between hybrid and pure hard clam families validating the system's ability to detect differences (Baker et al. 2011). It is possible that backcross hybrids are more similar to the pure hard clams than their hybrid relatives in a way which makes their differences undetectable by this system. If a hybrid is genetically 50% *M. campechiensis* and 50% *M. mercenaria*, then it follows that backcross hybrids should be 75% *M. mercenaria* and 25% *M. campechiensis*. Therefore, I may not have detected any advantages of backcross hybridization, because backcross hybrids have genetic composition that is very similar to the pure hard clams to which they are being compared.

Field data indicated that the same backcross hybrid families used in this experiment, did have greater survival in the field nursery and grow out than did pure hard clam controls (Sturmer et al. 2012). In the field nursery, survival was improved in backcross hybrids (71 to 82%) compared to control hard clam group within a family (65%) and, at harvest, 65% of backcrossed families yielded higher survival (81 to 91%) compared to hard clams (79%) (Sturmer et al. 2012). From the standpoint of a clam farmer, the trends being described in the field concerning backcross hybrids are much more relatable; these are the conditions to which farmers will actually be exposing their crop. The laboratory challenge design was intended to tease apart differences in families exposed to a single particular stressor, but perhaps the families are not yet different enough in one generation of backcrossing for this subtle difference to be significant. I hypothesize that, in the field, backcrosses are continually exposed to stressful conditions, and any slight genetic advantage makes a difference in survival. Whereas, the laboratory challenge was not stressful enough to illustrate these subtly emerging family differences. Generally, one generation is enough time to see differences when hybridizing two species (Bartley 2001). Hybrids did produce families that outperformed control hard clams in both the field and the laboratory challenges (Baker et al. 2011). However, Newkirk (1983) notes in his review of shellfish breeding programs that gains are made with each generation, and that real success of a breeding program may take several generations.

Backcross hybrids of *M. mercenaria* and *M. campechiensis* should continue to be explored as a product to improve the efficiency and longevity of hard clam aquaculture in Florida. Although, the results of this research effort indicated that there were no differences in backcross hybrid hard clams compared to control hard clams when exposed to stressful environmental conditions, field data is promising. Developing a breeding program is crucial to

the continuation of a promising and economically valuable industry in Florida. Further consideration of the breeding program and future efforts may include research in marker-assisted selection efforts which are becoming more widespread in aquaculture. Developing broodstock that survive heat challenges may also produce genetically superior stock.

In conclusion, I found that the acute upper temperature limit for Florida cultured *M. mercenaria* was 38°C and that as little as 2°C differential in water temperature can result in mortality. Identifying the acute upper temperature limit can inform the effort to breed heat tolerant hard clams for the aquaculture industry. The acute upper temperature limit of Florida cultured northern hard clams can be compared to the acute upper temperature limit of northern hard clams along the U.S. east coast to assess if Florida culture efforts have unknowingly been selecting for heat tolerance. Additionally, I examined survival of backcross hybrid hard clams compared to pure hard clams in potentially stressful summer conditions in laboratory challenges. I found that treatment conditions had a significant effect on mean days of survival for all clams. These indicate that both salinity and dissolved oxygen levels, under summer temperature (32°C) have a significant effect on the survival of hard clams. There were no advantages of backcross hybrid hard clams compared to pure hard clams, suggesting that backcross hybrids and pure hard clams have similar stress tolerance thresholds. Future investigation of backcross hybrids and hard clams should test at more extreme conditions. The hard clam aquaculture industry of Florida may benefit from continuing to explore other methods of selective breeding.

APPENDIX
WATER QUALITY DATA

Table A-1. Mean water quality values in each replicate of treatment 15 ppt normoxia for 12-month-old hard clams.

Tank #	Water Quality Value	Mean (n=26)	Standard Deviation	Maximum	Minimum
4	Salinity (ppt)	15.0	0.0	15	15
6	Salinity (ppt)	15.0	0.0	15	15
9	Salinity (ppt)	14.8	0.8	15	12
14	Salinity (ppt)	15.0	0.0	15	15
4	Temperature (°C)	32.8	0.3	33.1	32.24
6	Temperature (°C)	33.2	2.3	35.8	31.2
9	Temperature (°C)	30.6	1.2	33.1	28.5
14	Temperature (°C)	32.5	2.0	34.8	31.1
4	Dissolved Oxygen (mg/L)	5.0	0.6	5.7	4.3
6	Dissolved Oxygen (mg/L)	4.0	2.3	5.3	1.3
9	Dissolved Oxygen (mg/L)	5.3	0.7	6.3	3.7
14	Dissolved Oxygen (mg/L)	4.2	1.7	5.5	2.3

Table A-2. Mean water quality values in each replicate of treatment 25 ppt, normoxia for 12-month-old hard clams.

Tank #	Water Quality Value	Mean (n=26)	Standard Deviation	Maximum	Minimum
3	Salinity (ppt)	24.6	1.6	27	20
5	Salinity (ppt)	23.5	2.3	25	20
11	Salinity (ppt)	20.0	0.0	20	20
15	Salinity (ppt)	24.0	2.0	25	20
3	Temperature (°C)	31.6	1.0	34.1	31.0
5	Temperature (°C)	31.1	0.6	33.3	30.0
11	Temperature (°C)	33.5	1.5	35.3	31.2
15	Temperature (°C)	32.2	1.7	34.2	26.6
3	Dissolved Oxygen (mg/L)	5.1	0.6	5.8	3.4
5	Dissolved Oxygen (mg/L)	5.4	0.3	6.3	4.8
11	Dissolved Oxygen (mg/L)	3.9	2.0	5.6	1.4
15	Dissolved Oxygen (mg/L)	5.2	0.6	6.0	2.6

Table A-3. Mean water quality values in each replicate of treatment 25 ppt, hypoxia for 12-month-old hard clams.

Tank #	Water Quality Value	Mean (n=26)	Standard Deviation	Maximum	Minimum
2	Salinity (ppt)	23.9	1.9	25	20
8	Salinity (ppt)	24.1	2.3	25	15
12	Salinity (ppt)	21.6	2.1	25	20
16	Salinity (ppt)	24.8	1.0	25	20
2	Temperature (°C)	33.0	0.6	33.3	30.3
8	Temperature (°C)	31.1	0.5	32.4	29.5
12	Temperature (°C)	33.0	2.0	34.7	28.7
16	Temperature (°C)	32.8	0.8	33.3	29.3
2	Dissolved Oxygen (mg/L)	4.1	1.2	5.7	2.6
8	Dissolved Oxygen (mg/L)	4.4	1.3	6.5	1.8
12	Dissolved Oxygen (mg/L)	2.9	1.6	5.1	1.1
16	Dissolved Oxygen (mg/L)	3.3	1.6	5.1	1.0

Table A-4. Mean water quality values in each replicate of treatment 35 ppt 12-month-old hard clams.

Tank #	Water Quality Value	Mean (n=26)	Standard Deviation	Maximum	Minimum
1	Salinity (ppt)	32.3	2.2	35	30
7	Salinity (ppt)	31.7	2.1	35	30
10	Salinity (ppt)	32.2	2.2	35	30
13	Salinity (ppt)	32.0	2.1	35	30
1	Temperature (°C)	32.1	0.8	34.0	29.6
7	Temperature (°C)	30.8	1.8	35.4	28.3
10	Temperature (°C)	32.6	0.6	33.5	30.1
13	Temperature (°C)	31.2	1.1	34.6	29.2
1	Dissolved Oxygen (mg/L)	5.6	0.5	6.4	3.4
7	Dissolved Oxygen (mg/L)	5.3	0.3	5.8	4.5
10	Dissolved Oxygen (mg/L)	5.0	0.8	8.3	3.7
13	Dissolved Oxygen (mg/L)	5.2	0.3	5.5	4.5

Table A-5. Mean water quality values in each replicate of treatment 15 ppt for six-month-old hard clams.

Tank #	Water Quality Value	Mean (n=21)	Standard Deviation	Maximum	Minimum
1	Salinity (ppt)	15.0	0.0	15	15
5	Salinity (ppt)	15.3	0.5	16	15
11	Salinity (ppt)	15.7	0.6	16	15
14	Salinity (ppt)	15.0	0.0	15	15
1	Temperature (°C)	31.3	0.3	31.6	31.0
5	Temperature (°C)	32.5	1.3	34.1	29.9
11	Temperature (°C)	34.3	0.5	34.7	33.8
14	Temperature (°C)	32.4	0.5	32.9	31.7
1	Dissolved Oxygen (mg/L)	6.1	0.7	6.8	4.9
5	Dissolved Oxygen (mg/L)	5.8	0.9	6.7	4.6
11	Dissolved Oxygen (mg/L)	4.5	0.4	4.8	4.1
14	Dissolved Oxygen (mg/L)	5.3	0.7	6.4	4.5

Table A-6. Mean water quality values in each replicate of treatment 25 ppt normoxia for six-month-old hard clams.

Tank #	Water Quality Value	Mean (n=21)	Standard Deviation	Maximum	Minimum
3	Salinity (ppt)	25.2	0.7	27	24
7	Salinity (ppt)	24.9	0.3	25	24
9	Salinity (ppt)	23.9	1.8	25	20
15	Salinity (ppt)	23.4	0.9	25	23
3	Temperature (°C)	32.5	1.1	33.3	29.9
7	Temperature (°C)	33.1	0.8	33.8	29.9
9	Temperature (°C)	33.6	0.4	34.1	32.8
15	Temperature (°C)	33.8	1.3	35.0	32.3
3	Dissolved Oxygen (mg/L)	6.0	0.3	6.4	5.1
7	Dissolved Oxygen (mg/L)	5.8	0.5	6.2	4.5
9	Dissolved Oxygen (mg/L)	5.7	0.6	6.2	4.5
15	Dissolved Oxygen (mg/L)	4.7	0.6	5.6	4.2

Table A-7. Mean water quality values in each replicate of treatment 25 ppt hypoxia for six-month-old hard clams.

Tank #	Water Quality Value	Mean (n=21)	Standard Deviation	Maximum	Minimum
4	Salinity (ppt)	23.8	0.8	25	23
8	Salinity (ppt)	24.9	0.3	25	24
12	Salinity (ppt)	23.6	1.7	25	20
13	Salinity (ppt)	24.2	1.1	25	22
4	Temperature (°C)	31.7	1.5	33.3	29.6
8	Temperature (°C)	31.5	1.4	32.7	28.1
12	Temperature (°C)	32.5	0.9	33.0	30.5
13	Temperature (°C)	33.0	0.6	33.5	32.0
4	Dissolved Oxygen (mg/L)	3.4	1.9	6.5	1.7
8	Dissolved Oxygen (mg/L)	2.7	2.0	6.4	0.8
12	Dissolved Oxygen (mg/L)	3.0	1.9	6.5	1.1
13	Dissolved Oxygen (mg/L)	4.1	1.5	6.5	2.1

Table A-8. Mean water quality values in each replicate of treatment 35 ppt for six-month-old hard clams.

Tank #	Water Quality Value	Mean (n=21)	Standard Deviation	Maximum	Minimum
2	Salinity (ppt)	34.5	1.3	37	32
6	Salinity (ppt)	34.4	1.3	37	32
10	Salinity (ppt)	33.7	1.7	36	30
16	Salinity (ppt)	30.3	0.6	31	30
2	Temperature (°C)	31.7	1.6	33.7	27.7
6	Temperature (°C)	31.9	1.8	34.4	28.0
10	Temperature (°C)	32.8	1.0	33.6	29.4
16	Temperature (°C)	33.6	1.0	34.2	32.4
2	Dissolved Oxygen (mg/L)	5.9	0.4	6.5	4.7
6	Dissolved Oxygen (mg/L)	5.8	0.4	6.4	5.1
10	Dissolved Oxygen (mg/L)	5.6	0.3	6.0	4.8
16	Dissolved Oxygen (mg/L)	3.5	2.6	5.7	0.6

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BIOGRAPHICAL SKETCH

Melissa Ann Broderick was born in the naval base hospital of Bremerton, Washington. After many years of moving, her family settled in Cape May, New Jersey. Melissa has always wanted to be a marine biologist. Growing up on the beach deeply connected her with marine life and a fascination for conservation grew. She received her B.S. in marine sciences from Rutgers University in 2009. After working in an oyster hatchery as a technician at The Haskin Shellfish Research Laboratory Cape Shore facility for a summer, Melissa moved to Gainesville to pursue a M.S. at the University of Florida. No funding was available, and an aquaculture/agriculture internship opportunity in Walt Disney World strengthened her passion for aquaculture. She began her graduate work at the University of Florida in the summer of 2010, and received her M.S. in the summer of 2012. She left Gainesville to study freshwater bivalve conservation in San Antonio, Texas as a field biologist.