EFFECT OF TEMPERATURE ON THE METABOLIC RATE OF DIPLOID AND TRIPLOID *Mercenaria mercenaria*

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL
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To my family and friends who have been there since the beginning. . .

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EFFECT OF TEMPERATURE ON THE METABOLIC RATE OF DIPLOID AND TRIPLOID *Mercenaria mercenaria*

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Chair: Shirley Baker
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Hard clam, *Mercenaria mercenaria*, production in Florida experiences high mortalities during summer months due to physiological stressors, such as low salinities and high water temperatures. Triploid clams may offer improved stress resistance and production over diploids. The current study characterized and compared the metabolic efficiency of diploid and triploid clams at typical water temperatures in Florida. Oxygen uptake rates were determined at 20, 25, 27, 30 and 32°C at 25 ppt for clams of similar sizes ($\bar{x}$ = 54.61 and 51.2 mm shell length for diploids and triploids, respectively). Attempted acclimation to 35°C at 25 ppt resulted in 100% mortality. Oxygen uptake rate varied significantly as a function of temperature ($p < 0.000$). Oxygen consumption rate increased with temperature from 481 $\mu g\ g^{-1}\ hr^{-1}$ at 20°C to 1479 $\mu g\ g^{-1}\ hr^{-1}$ at 27°C and did not change significantly above 27°C. These results suggest that 27°C is a temperature threshold, beyond which there may be an onset of partial anaerobic metabolism. Oxygen uptake rates of triploid and diploid clams were not statistically different ($p = 0.694$). Oxygen uptake rates were determined at 27°C at 25 ppt and 15 ppt for clams of similar sizes ($\bar{x}$ = 59.5 and 62.1 mm shell length for diploids and triploids, respectively). Salinity had no affect on diploid oxygen consumption rate ($p > 0.05$). Triploid clams had significantly lower oxygen

consumption rates ($p = 0.012$) at 15 ppt than at 25 ppt and significantly lower ($p = 0.035$) oxygen consumption rates than diploids at 15 ppt. These results suggest that triploid clams may have a metabolic advantage over diploid clams at lower salinities, but whether this offers any significant physiological advantage over diploids during fluctuating and sometimes extreme environmental conditions in the field is unknown.

CHAPTER 1
INTRODUCTION

The northern quahog (hard clam), *Mercenaria mercenaria* (Linnaeus, 1758), is an important aquaculture species for the state of Florida, particularly in the Cedar Key and Charlotte Harbor areas along the west coast. In 2003 and 2005, there was a decrease in the number of hard clams produced and sold (USDA 2004, USDA 2006). During the summer, high water temperatures (as high as 35°C), in conjunction with fluctuating salinities (12 ppt to 36 ppt) and low dissolved oxygen (as low as 0 mg L^{-1}), coincide with massive mortalities (Scarpa et al. 2005).

Prior to experiencing these summer stressors, sexually mature hard clams spawn, releasing a significant quantity of energy in the form of gametes (Hesselman et al. 1989, Eversole 2001). It is believed that energy depletion through spawning events, combined with environmental stressors in the summer, are the leading cause of the mortalities (Sturmer, personal communication). Culture of triploid hard clams, which are sterile and do not lose energy to reproduction, may prevent mortalities. In addition to being sterile, triploid hard clams may have a lower metabolic rate (Zorous et al. 1980, Koehn & Gaffney 1984, Hawkins et al. 1986, Gosling 1992, Mitton 1993, Tremblay et al. 1998) as a result of increased heterozygosity, which may also contribute to survival of summer environmental stressors.

Much of the research to date on *M. mercenaria* has been conducted in the northeastern part of the United States on diploid hard clams. Little to no research has been conducted on the physiology of triploid *M. mercenaria* (Buzzi 1990), with none occurring in Florida until recently (Hoover 2007). To determine if there is physiological support for the hypothesis that triploid hard clams are better suited to surviving summer stressors than diploids, the metabolic rates of

triploid and diploid *M. mercenaria* were determined in a laboratory setting at different temperatures and salinities.

Mercenaria mercenaria: An Important Aquaculture Species in Florida

The production and sales of the hard clam have experienced tremendous growth since harvesting of wild clams first began in the 1880s (Zajicek & Zimet 1998). During the mid-1980s, a shift in interest from harvesting wild clams to farm-raised hard clams began to grow (Zajicek & Zimet 1998). In 1989, the sale of hard clams cultured in Florida totaled $1 million, which represented less than 1% of the total aquaculture market in Florida (USDA 1990). The farm-gate value of cultured clams has fluctuated over the years, but on the whole has increased since the 1980s (Figure 1-1). By 1997, the number of farm-raised hard clams harvested exceeded the number of wild clams landed in Florida (Zajicek & Zimet 1998).

The overall increase in farm-gate value of cultured hard clams through the late 1990s and early 2000s can be attributed to an increase in the number of growers, an increase in the number of growing areas, and new educational efforts put forth during the mid-1990s for displaced fishermen (Philippakos et al. 2001). In 1989, there were only 41 clam growers in the state, concentrated in the Indian River Lagoon area on the east central coast. By 2007, this number had increased to over 400 growers on 1800 acres of lease area (Sturmer, personal communication) located across the state from the "Big Bend" counties of Dixie, Levy, and Citrus to the southern west coast counties of Charlotte and Lee to the central east coast counties of Volusia, Brevard, and Indian River (USDA 2006). In 2005, the sale of cultured hard clams reached $10.7 million, which represented approximately 14% of the total sales of aquaculture products produced in Florida (USDA 2006). Sales in 2005 were lower than expected due to the severe losses inflicted by hurricanes in 2004 and 2005. In general, however, the production of cultured hard clams has primarily increased due to an increase in lease sites with little

improvement in culturing techniques or strain development (For technique improvements see Menzel & Sims 1962; for strain development see Scarpa et al. 2005).

Environmental Effects on Hard Clam Physiology

Temperature Effects

Mercenaria mercenaria is found naturally from the Gulf of St. Lawrence to the Indian River Lagoon, Florida, and along the Gulf Coast of Florida to Texas (Abbot 1974). This wide distribution of the hard clam indicates that this species is able to tolerate a wide range of temperatures; there is a 25ºC mean temperature difference between the Gulf of St. Lawrence habitat and the Florida habitat due to the Gulf Stream and Labrador Current (Pickard & Emery 1982).

Growth of juvenile and adult *M. mercenaria* is greatly influenced by temperature, with optimal growth occurring between 15 and 25°C (Grizzle et al. 2001) and ceasing above 31°C and below 9°C (Ansell 1968, Rice & Pechenik 1992). At about 4°C, juveniles and adults become inactive (Loosanoff 1939). Shell growth of the clam can only occur during aerobic respiration (Whetstone et al. 2005). When growth is slow, or there are adverse growing conditions, the shell will grow laterally (i.e., in thickness) and produce a dark band; therefore, faster growing individuals have thinner shells with lighter bands (Jones et al. 1990, Arnold et al. 1991, Whetstone et al. 2005). In Florida during the summer and the fall, clams form dark, slow-growth bands that indicate suboptimal growing conditions, presumably due to excessively high water temperatures; light, rapid growth bands form during the winter and the spring that indicate optimal growing conditions due to cooler water temperatures (Jones et al. 1990, Arnold et al. 1991).

The optimal pumping rate for food and oxygen consumption by *M. mercenaria* occurs between 20 and 25°C (Hamwi 1969). At lower temperatures, adult *M. mercenaria* show

discontinuous feeding activity (Loosanoff 1939) with pumping rate no longer detectable below 6°C, and similarly ceasing above 32°C (Hamwi 1969).

Coastal water temperatures are variable throughout Florida and depend on day length, season, water depth, and weather. Shallow water areas may experience temperatures as high as 38ºC in the summer and a temperature fluctuation of more than 31ºC over the course of a year (Weber et al. 2007, Figure 1-2, Appendix). Lease sites where clams are cultured may experience temperature fluctuations of 11ºC within a 24-hour period (Weber et al. 2007, Appendix). These large fluctuations in temperature affect not only the physiology of the clams but the water quality as well. *Mercenaria mercenaria* is at its thermal extreme in Florida, as indicated by high mortality rates during the summer.

Salinity Effects

Mercenaria mercenaria is an estuarine species living in a salinity range of 20 to 30 ppt (Grizzle et al. 2001). The tolerance of the hard clam to salinity extremes increases with age, but is inversely related to temperature. Adults are able to survive salinities as low as 12.5 ppt and as high as 35 ppt, with a limited ability to survive up to 46 ppt with valve closure (Castagna & Chanley 1973). Maximum pumping rates of the hard clam occur between 23 and 27 ppt with inhibition occurring below 15 ppt and above 36 ppt (Hamwi 1969). Salinities below 10 ppt generally have a negative effect on hard clams by inhibiting feeding, burrowing, growth, and long-term survival of both juveniles and adults (Chanley 1958; Castagna & Chanley 1973). Salinity fluctuations on the scale of hours may affect clam physiology by disrupting enzyme function (Page & Di Cera 2006).

Salinity in coastal waters, which can range from 0.5 to 29 ppt, is affected by precipitation, run-off and evaporation (Baker et al. 2007, Figure 1-3). Lease sites in Florida reach salinities as

high as 40 ppt and as low as 5 ppt (Baker et al. 2007). This variation may occur on the time scale of hours to years.

Van Winkle (1968) discovered that metabolic rates of hard clams are to be high in salinities below 20 ppt, while Hamwi (1969) determined that maximum oxygen consumption occurs between 21.5 and 25.5 ppt. Hamwi (1969) stated that oxygen consumption of *M. mercenaria* is more sensitive to salinity than to temperature changes.

Dissolved Oxygen Effects

Mercenaria mercenaria is tolerant of low dissolved oxygen levels. Hartman et al. (1974 in Eversole 1987) determined that dissolved oxygen concentrations necessary for successful culture of the hard clam range from 6.8 to 7.4 mg L^{-1}. However, Savage (1976 in Eversole 1987) determined that low dissolved oxygen levels do not severely affect hard clams and that the associated stresses have no long-lasting effect. *Mercenaria mercenaria* is able to maintain normal oxygen consumption rates down to 5 mg L^{-1} dissolved oxygen and above by increasing the efficiency of oxygen extraction from the water column (Hamwi 1969). At dissolved oxygen levels below 5 mg L^{-1}, hard clams reduce their oxygen consumption rate and begin to resort to anaerobic respiration (Hammen 1980). At extreme low dissolved oxygen levels, clams will close their valves and depend on anaerobic respiration completely (Hamwi 1969). In this state, *M. mercenaria* is able to survive for up to 18 days at 1 to 6°C (Loosanoff 1939). Under these types of extreme conditions in which the clam is deprived of oxygen, *M. mercenaria* will incur an oxygen debt which must be replenished upon re-opening (Hamwi 1969). While low dissolved oxygen levels alone may not be deleterious to the hard clam, low dissolved oxygen levels combined with high water temperatures can lead to reduced survival (Baker et al. 2002).

Dissolved oxygen levels typically remain above 2 mg L^{-1} in Florida estuarine waters (Berquist et al. 2008 in press, Figure 1-4). However, the dissolved oxygen concentration can

become anoxic, reaching 0 mg L^{-1} (FDACS 2003-2008). While these low levels are atypical, they occur during the summer months when water temperatures are higher. The combination of low dissolved oxygen and warm water temperatures physiologically stress the clams and may ultimately lead to their death (Hoover 2007).

Reproduction in Hard Clams

Sexual maturity in *Mercenaria mercenaria* is believed to be a function of size (Belding 1931, Quayle & Bourne 1972, Bricelj & Malouf 1980, Loosanoff 1937a); clams reach sexual maturity at a shell length of approximately 33 mm (Eversole 1986). Hard clams are protandric hermaphrodites in which approximately 98% of the young clams undergo a juvenile male phase (Loosanoff 1936, 1937a). As maturation progresses, about half of the individuals will change sex to female, resulting in a sex ratio of about 1:1 (Loosanoff 1936, 1942, Walker & Heffernan 1995). It is thought that the sex ratio favors males during early development because female gonads take longer to mature and reach fully functional status. Little development of the gonads occurs in juveniles during their first winter, with rapid spermatogenesis occurring in the spring with an increase in water temperatures (Loosanoff 1937a). Loosanoff (1936, 1937a) observed that in populations of *M. mercenaria* in northern latitudes the sex change occurs after spermatozoa has been discharged in early fall of the second year. *Mercenaria mercenaria* does not experience reproductive senescence with age (Bricelj & Malouf 1980, Peterson 1983, 1986).

Gametogenesis in the hard clam begins when water temperatures reach approximately 10°C as long as there is an adequate food supply (Eversole 1987). Spawning occurs between 23 and 25°C (Loosanoff 1937b, Porter 1964, Keck et al. 1975, Eversole et al. 1980, Kassner & Malouf 1982, Dalton & Menzel 1983, Manzie et al. 1985). This reproductive temperature range results in two spawning events in Florida with a large spawn in the spring and a small spawning event in the fall (Heffernan et al. 1989, Hesselman et al. 1989). Each spawning event represents

a massive loss of energy to the hard clam with approximately 52% of the total organic production released as spawn (Ansell et al. 1964). Eversole (2001) concurred with the amount of energy lost as spawn, concluding that 26 to 46% of the annual growth for both males and females is tied up in annual reproductive efforts. These reproductive efforts are of interest in the clam industry, as it represents an energy loss that the clams could otherwise put into somatic growth, allowing them to reach market size sooner or allow them to survive summer stressors.

Triploidy

Triploid and Diploid Comparison

Triploid clams may represent a solution to diploid mortalities due to energy loss problems. Triploid clams contain 3 sets of chromosomes instead of the typical 2 sets of chromosomes in diploid organisms. Due to their extra chromosomes, triploid clams are sterile and have reduced gametogenesis, which may result in improved survival and somatic growth (Barber & Mann 1991). Triploid clams may also have energy stores to help survive summer stressors that are not available to the spawning diploids.

Research has shown that triploid European flat oysters, *Ostrea edulis*, and *M. mercenaria* have faster growth rates than their diploid counterparts (Hawkins et al. 1994, Eversole et al. 1996); this faster growth of triploids would allow them to reach market size sooner than slower-growing clams, thus avoiding summer stressors. There are several hypotheses as to why triploid organisms grow faster than diploids, including polyploidy gigantism, energy reallocation and increased heterozygosity.

Growth Hypotheses

According to the hypothesis of polyploidy gigantism, triploids are thought to grow faster than diploids because they have an increase in cell volume that is not counter-balanced by fewer cells (Guo & Allen 1994). Triploid cells have 50% more DNA than diploids (Guo & Allen

1994); they may therefore require more cytoplasm to maintain a given cytoplasm/nucleus ratio and have larger cells than diploids. Larger triploid cells may need more nutrients for growth. Therefore, in good growing conditions where food is unlimited, triploids should be larger than diploids before sexual maturation. In adverse growing conditions where food is limited, triploids may not be larger than diploids before sexual maturation.

According to the energy reallocation hypothesis, triploids may, in part, grow faster than diploids due to the opportunity to reallocate energy from gametogensis to somatic growth (Allen & Downing 1986, Barber & Mann 1991, Hawkins et al. 1994, Eversole et al. 1996). The difference in growth becomes more pronounced between triploids and diploids once the diploids reach sexual maturity, which is typically after one year (Stanley et al. 1984, Barber & Mann 1991, Eversole et al. 1996). Research conducted by Eversole et al. (1996) on *M. mercenaria* showed that the gonads of triploid clams contain some oogenic and spermatogenic stages, but that gametogenesis is greatly retarded and none of the triploids spawn. While the gonadal lumen areas are similar in diploids and triploids, the sex cells occupy 2 to 4 times as much area in diploids compared to triploids (Eversole et al. 1996).

The final, and possibly most important, hypothesis is increased heterozygosity or an increase in allelic diversity. In MI (i.e., triploids produced by stopping polar body formation during meiosis I) and MII (i.e., triploids produced by stopping polar body formation during meiosis II) triploids, their increased heterozygosity, due to three rather than two copies of the same locus (i.e., progressive haploidization), may contribute to their faster growth (Allendorf & Leary 1984, Stanley et al. 1984). Research suggests that triploid organisms have a lower probability of being exposed to a recessive deleterious mutation than their diploid counterparts because of this extra chromosome, and thus cells are not deprived of gene activity (Zorous et al.

1996). Triploids that are produced from mating tetraploids with diploids are expected to be more heterozygous than induced MI or MII triploids (Wang et al. 2002), while triploids with MI induction are more heterozygous than MII triploids (Stanley et al. 1984, Hawkins et al. 1994).

Heterozygosity levels in MII triploids vary among bivalves. Stanley et al. (1984) and Hawkins et al. (1994) found that in the oysters, *Crassostrea virginica* and *O. edulis* MII triploids are not more heterozygous than diploids. In contrast, Garnier-Géré et al. (2002) found that in the Pacific oyster, *Crassostrea gigas*, MII triploids have a significantly higher average allozyme and microsatellite diversity than their diploid counterparts, indicating that they are indeed more heterozygous. Hawkins et al. (2000) determined that both MI and MII triploid Pacific oysters are more heterozygous than diploids while MI triploids are the most heterozygous.

Bivalves with increased heterozygosity also have greater efficiency of protein synthesis (Hawkins et al. 1986) and lower protein turnover and therefore greater growth efficiencies. Heterozygosity reduces the level of protein synthesis needed for standard metabolism, thus reducing the cost of maintenance (Tremblay et al. 1998). The energy saved may then be used for somatic growth or to facilitate a response to stress (Zorous et al. 1980, Koehn & Gaffney 1984, Gosling 1992, Mitton 1993). Protein turnover, which is the continuous breakdown and replacement of cellular proteins, plays an important role in the energy budget and physiology of organisms (Hawkins & Day 1999) and represents a general index of the energy requirements for maintenance (Hawkins 1991). Reduced protein turnover is correlated with lower energy expenditure and higher growth efficiencies (for review see Hawkins & Day 1996, Hedgecock et al. 1996, Bayne & Hawkins 1997). This slower protein turnover, in conjunction with its associated lower energy expenditure and higher growth efficiencies, represent the metabolic basis for the positive correlation between growth and increased heterozygosity (Koehn &

Shumway 1982, Diehl et al. 1986, Hawkins et al. 1986, 1989, 1994, Hedgecock et al. 1996, Toro et al. 1996).

While heterozygosity is positively correlated with growth, viability and fertility in several species of bivalve mollusks (Mitton & Grant 1984, Zousos & Foltz 1987, Volcakaert & Zouros 1989, Koehn 1990, Gosling 1992, Mitton 1993), standard oxygen consumption has been found to be negatively correlated with heterozygosity (Koehn & Shumway 1982, Mitton et al. 1986), which may result in a lower energetic cost of maintenance (Hawkins et al. 1986). Potentially, the energy saved from maintenance can be allocated to somatic growth (Hawkins & Bayne 1992, Toro & Vergara 1998).

Total Energy Budget

Energy Budget Scheme

The total energy budget for a heterotrophic organism (Equation 1-1 from Warren and Davis 1967) can be broken down into "energy acquisition" processes, including C, and "energy expenditure processes", including $M_{r,a}$,SDA, F, U and $G_{s,r}$.

$$C = (M_r + M_a + SDA) + (F + U) + (G_s + G_r); \quad (1\text{-}1)$$

C = Rate of energy consumption;
M_r = Standard metabolic rate;
M_a = Metabolic rate increased due to activity;
SDA = Metabolic rate increased due to specific dynamic action;
F = Waste loss due to egestion (i.e., feces);
U = Waste loss due to excretion (i.e,. urine);
G_s = Somatic growth and storage;
and, G_r = Reproductive growth

In general, *M. mercenaria* will spend energy pumping water and sorting particles to be ingested or rejected as pseudofeces. After ingestion, energy will be lost through egested feces and excretion, and expended through metabolic heat loss (i.e., specific dynamic action), activity, somatic growth, and reproductive products.

This study will focus only on the standard metabolic rate (i.e., the energy required to maintain life while fasting) of diploid and triploid *M. mercenaria* on an acute timescale (i.e., within seven days of acclimation). Certain assumptions can be made about the remaining portions of the energy budget in order to eliminate them and greatly simplify the comparison of diploid and triploid clams. The experimental clams will be fasted for 48 to 72 hours in filtered water (0.35 μm) before measurements begin. Therefore, energy gained through food consumption and lost through egestion can be eliminated from the equation. Specific dynamic action or SDA is the metabolic cost of processing ingested food. Since the clams will be fasted and all food sources eliminated, SDA will no longer be a factor within the energy budget. Active metabolic rate (M_a) is the level of energy required to perform specific levels of exercising activities. Clams in general are sedentary organisms that bury when given the opportunity. The clams in this experiment will not be given such an opportunity to bury and thus, active metabolic rate is a minor factor in the energy budget in this case. Research by Bayne and Newell (1983) concluded that excretion of ammonia contributed to less than 10% of energy losses in the energy budget of fed bivalves. Thus, we can also assume that energy lost through urine in fasted clams is minimal and can therefore be ignored. Due to the short time scale of the measurements, growth and reproduction of the clams will play a very minor role within the energy budget and can also be ignored. This leaves standard metabolic rate as the primary focus.

Metabolic Rate

A common approach to examining the influence of the environment on an organism as a whole is to study its metabolism, which is the energy an organism uses to carry out various life functions through the oxidation of fuel. An organism's oxygen consumption rate can be measured as a proxy for metabolic rate, as it represents the organism's overall aerobic energy demand.

The environmental factors that influence the metabolic rate of bivalves include temperature, salinity, food availability, pH, and dissolved oxygen. Temperature is an important factor in the metabolic activity of ectotherms, which do not regulate their body temperature. As temperature increases, the rate of enzyme reactions, the basis of all metabolic pathways, increases due to an increase in molecular motion, resulting in a greater number of collisions between enzymes and their substrate. An increase in temperature results in an increase in the number of enzyme-substrate collisions necessary to overcome the activation energy required for the reaction to occur and therefore, the reaction rates involved in the different portions of the energy budget will increase, including standard metabolic rate. In general, metabolic rate will double or triple with a 10°C increase in temperature.

In *M. mercenaria*, oxygen consumption rate and feeding increase markedly with an increase in acclimation or seasonal temperatures between 10 and 27°C (Grizzle et al. 2001). Hamwi (1969) determined that oxygen consumption rate of *M. mercenaria* increases up to approximately 25°C (Figure 1-5), with maximum oxygen consumption rate within the range of 21 to 25°C (Hamwi 1969). Oxygen consumption rate in hard clams apparently decreases at temperatures above 25°C (Hamwi 1969). This temperature may represent the upper thermal limit at which enzyme damage begins to occur and results in a decline in metabolic activity and oxygen consumption. At this point, the animal may begin to depend on anaerobic respiration for essential metabolic processes.

Research Goals

Most research conducted on *M. mercenaria* has been done on diploid clams from the northeastern part of the United States with none conducted on triploid *M. mercenaria* until recently. No research has been performed to determine the metabolic rate of triploid *M. mercenaria*.

The principal objective of this project is to determine the standard metabolic rate (i.e., the level of metabolic activity in a fasted organism) of triploid and diploid *M. mercenaria* over a range of temperatures. The null hypothesis is that the metabolic rate of triploid and diploid clams will not differ when the clams are exposed to temperatures ranging from 20 to 32°C, representing the range of temperatures hard clams are exposed to in natural Florida environments. If the null hypothesis is not rejected, it suggests that MII triploid clams are not more heterozygous than diploid clams and require the same amount of energy for survival. The alternative hypothesis is that the metabolic rates of triploids will be different from their diploid counterparts at the various temperatures.

The second objective of this project is to determine the standard metabolic rate (i.e., the metabolic activity in a fasted organism) of triploid and diploid *M. mercenaria* at the temperature where oxygen consumption is greatest and at a stressful salinity. The null hypothesis is that the metabolic rate of triploid and diploid clams will not differ when the clams are exposed to a stressful temperature and salinity. If the null hypothesis is not rejected, it suggests that triploid and diploid clams require the same amount of energy for survival. The alternative hypothesis is that the metabolic rates of triploids will be different from their diploid counterparts at the high water temperature and decreased salinity.

The final objective of this project is to compare the oxygen consumption rates from the temperature experiment (present study) to the oxygen consumption rates obtained from Hamwi (1969) (Figure 1-5). Hamwi's dissertation (1969) is cited throughout the literature concerning the physiology of *M. mercenaria*. The oxygen consumption rate of *M. mercenaria* being equal to zero at 32°C was of particular interest as this is an experimental temperature for this research.

The comparison to Hamwi (1969) is in the hopes to further elucidate the physiological constraints of the species at various temperatures.

Figure 1-1. Florida production and sales of cultured clams (Taken from Sturmer 2006).

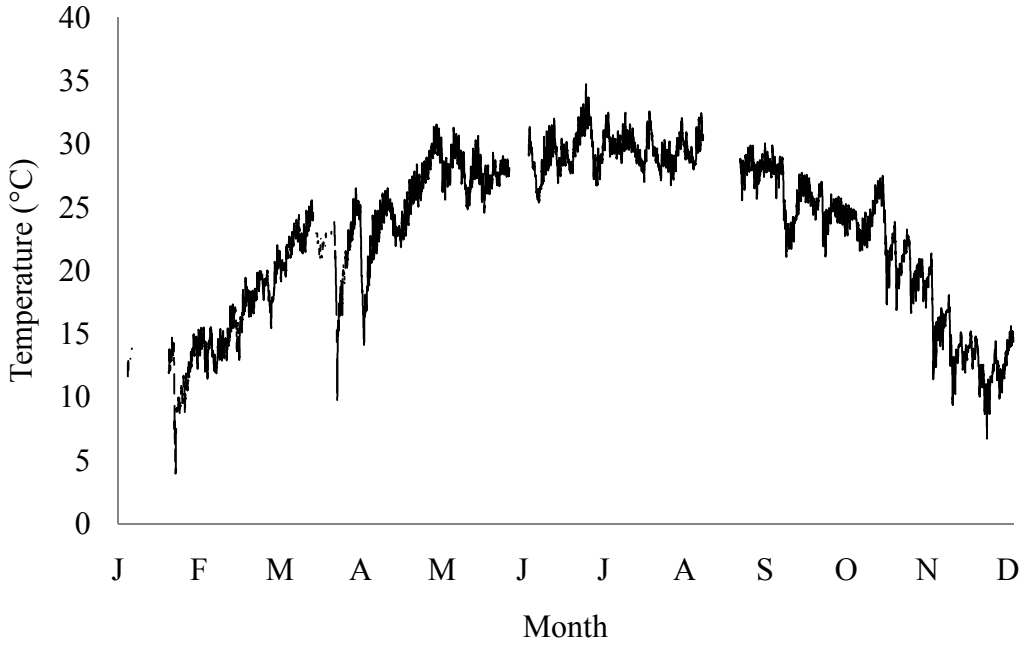

Figure 1-2. Temperature at the Gulf Jackson lease area during 2003 (FDACS 2003).

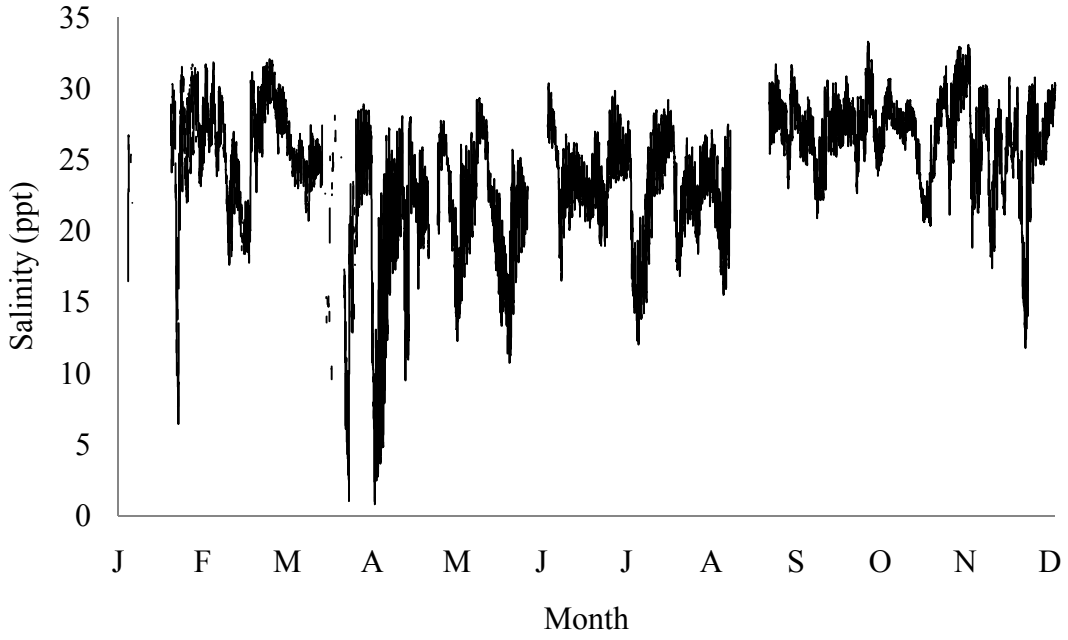

Figure 1-3. Salinity at the Gulf Jackson lease area during 2003 (FDACS 2003).

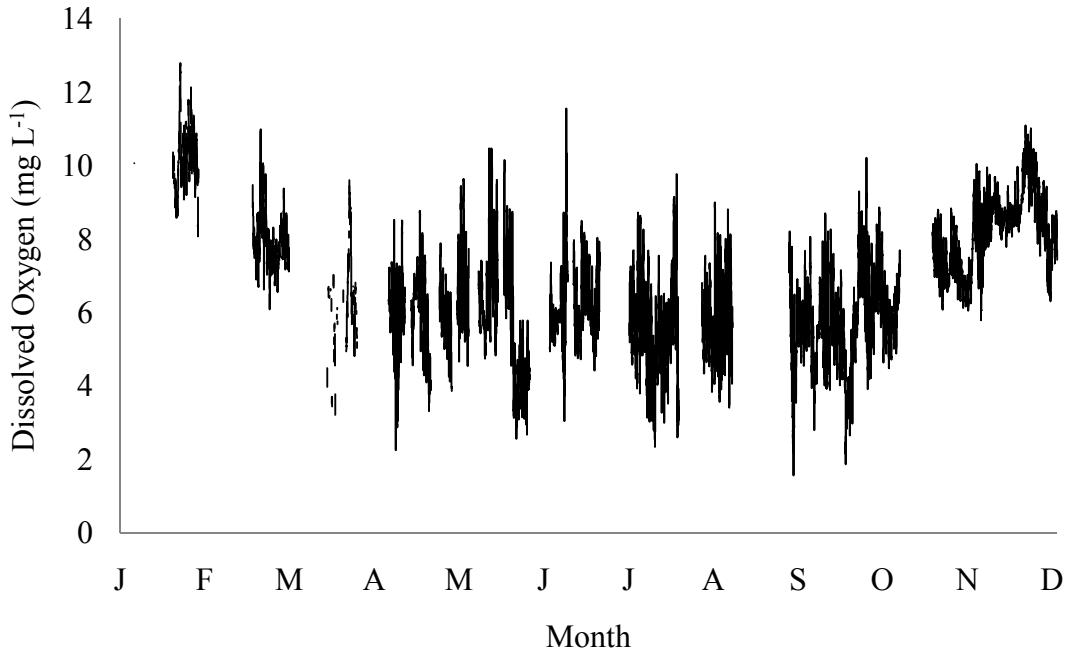

Figure 1-4. Dissolved oxygen at the Gulf Jackson lease area during 2003 (FDACS 2003).

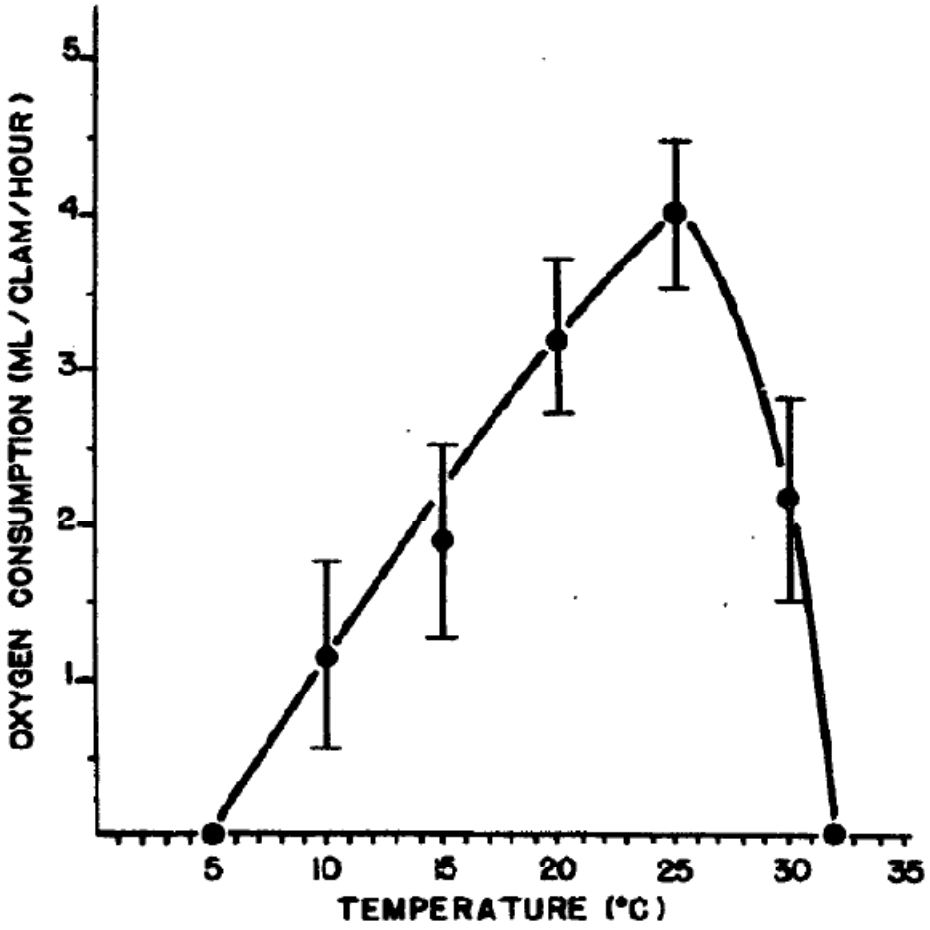

Figure 1-5. Oxygen consumption rate as a function of temperature for diploid *Mercenaria mercenaria* (Taken from Hamwi 1969).

CHAPTER 2
METHODS AND MATERIALS

Triploidy Induction

Mercenaria mercenaria are naturally diploid and therefore triploidy must be induced by suppressing polar body formation during meiosis I (MI) or meiosis II (MII) resulting in a diploid egg, which is then fertilized by a haploid sperm (Beaumont and Fairbrother 1991). Suppression of MI or MII can be induced by pressure, temperature, chemical shock, or by mating a tetraploid individual with a diploid individual.

Triploid clams used in this study were produced at Harbor Branch Oceanographic Institution at Florida Atlantic University in Fort Pierce, Florida by Dr. John Scarpa. Cytochalasin B (CB), a member of a class of fungal metabolites, was used to suppress MII polar body formation (Allen & Bushek 1992). Cytochalasin B is thought to inhibit the micro-filament formation in cells (Copeland 1974) and is often used to induce triploidy in bivalves (Beaumont & Fairbrother 1991). The heterozygosities of the MII triploid clams and the diploid clams in this project were unknown.

Grow-out and Collection of Clams

All diploid and triploid clams, produced from one set of parents, were out-planted in April 2006 to commercial clam lease sites at Cedar Key, Florida (Latitude and longitude for the corners of the high-density lease area are as follows: NE – 29°10'56" N 83°04'31" W; NW – 29°10'55" N 83°04'43" W; SE – 29°09'17" N 83°04'36" W; SW – 29°09'21" N 83°04'54" W). Clams used to measure the oxygen consumption rate at different temperatures were collected in May 2007, while clams used to measure the oxygen consumption rate at two salinities were collected in November 2007. The temperature experiment was conducted from August 2007 to November 2007. The salinity experiment was conducted in January and February 2008.

Ploidy Determination

Triploid production is not 100% successful, with only 20 to 80% of individual clams being triploid (Hoover 2007). In this project, triploid induction was found to be 83% successful. Therefore, the actual ploidy of all "triploid" clams was determined. Following collection from Cedar Key grow-out sites, the clams were rinsed with freshwater for approximately 1 hour and scrubbed to eliminate fouling organisms. They were measured for wet weight, volume, shell length, shell width, and shell height. As the clams were measured, the shells were dried and individually numbered with a black Sharpie®. The number on the triploids corresponded with their hemolymph sample number. The "triploid" clams were placed into a grid platform in seawater to open. Once a clam opened, a plastic coffee stirrer was placed between the valve edges to prevent the clam from closing. A 1 ml BD® tuberculin syringe with a 26 gauge needle was inserted into the anterior adductor muscle to remove approximately 100 µl of hemolymph. The hemolymph sample was ejected into a 1.5 mL plastic centrifuge tube into which 0.5 mL of DAPI/detergent/DMSO solution was added (Allen & Bushek 1992). The true ploidy of the "triploid" clams was determined using flow cytometry (Allen & Bushek 1992) at Harbor Branch Oceanographic Institution at Florida Atlantic University in Fort Pierce, Florida by Dr. John Scarpa's lab. Only individuals determined to be truly triploid were used in the oxygen consumption experiments.

Clam Maintenance

Before oxygen consumption measurements were conducted, the clams were maintained in a re-circulating seawater system consisting of six 227 L cylindrical tanks. One tank held diploid clams while another tank held the triploid clams. The four tanks without clams had a high turnover rate while the two clam tanks had a slower turnover rate to allow for feeding.

The re-circulating system was designed to maintain water quality with an in-line bio-ball filter, a fluidized bed, and a bead filter. Water quality parameters were maintained within optimal ranges for hard clams, according to Epifanio and Sma (1975), including: ammonia (0 to 0.0004 M), nitrite (0 to 0.04 M), and nitrate (0 to 0.16 M). A titanium heater placed in the sump tank (503 L) maintained the water temperature of all the tanks, and covers were placed over each tank to reduce evaporation. Temperature was monitored daily with a digital thermometer while salinity was monitored daily with a refractometer. Initially, the temperature in the system was 25°C, similar to the temperature at the collection site for the clams (~24°C). Salinity was maintained at 25 ppt, the salinity at which the clams were collected from the field. Salinity was adjusted as needed by adding well water to the sump. Air stones were placed in the sump and the two clam tanks to provide aeration and water movement.

The clams were continuously fed Instant Algae Shellfish Diet 1800® (Reed Mariculture) which is a mix of *Isochrysis* (25%), *Pavlova* (20%), *Tetraselmis* (20%), *Thalassiosira weissflogii* (30%) and *Nannochloropsis* (5%). Clams were fed the algae at a rate of 2% of their combined dry weight every day. Feed estimate was based on the average shell-free dry weight of ten sacrificial clams. The feed was placed in an inverted one-gallon milk jug with an airstone and allowed to drip into the two holding tanks containing the clams. The milk jug was checked daily for clogging. Prior to the oxygen consumption measurements, feeding was stopped and all of the clams were not fed for at least 48 hours before Day 1 (i.e., the clams were fasted for approximately 168 hours by the end of the oxygen consumption measurements).

Temperature and Salinity Acclimation

A 37.9 L glass aquarium insulated with 19.0 mm Styrofoam was set up to maintain and acclimate the clams prior to the oxygen consumption measurements. This system contained a Top-Fin® Power Filter with cleansing pads to trap large particles (i.e., mechanical filtration) and

Hagen® Fluval Biomax filter media to allow for nitrifying bacterial growth (i.e., biological filtration). The temperature of this system was maintained by a water bath heater (Finnex Model HC-0800 Thermo-Controller with Deluxe Titanium Heater Model TH-0500) or a water bath chiller (TECO® Model CA 200 and CURRENT Model 2635 1/10th HP Prime Mini-ChillerTM). Salinity was maintained at 25 ppt as described previously, with the exception of the salinity experiment, as described later. All other water quality parameters were monitored on a daily basis to ensure clam health. The water temperature and salinity initially reflected the temperature and salinity in the large holding tanks. Once the clams were added to the 37.9 L aquarium, the temperature and/or salinity was adjusted so the clams were slowly acclimated to experimental parameters (by approximately 2ºC day^{-1} or 1 ppt day^{-1}): 20, 25, 27, 30 and 32°C at 25 ppt or 27ºC at 15 ppt.

The clams were acclimated for 7 days after the target temperature or salinity was reached. Research on the mussel *Mytilus edulis* indicates that the shift from an acute temperature response to a new steady state after continuous exposure is slow (7 to 30 days) (Newell & Pye 1970, Bayne 1971, Widdows & Bayne 1971). Acclimation time is even longer for the queen scallop, *Chlamys opercularis*, which requires 35 days (McLusky 1973). Acclimation time of the hard clam to these temperatures is unknown. Therefore, to assess the success of acclimation, the oxygen consumption rate of five diploid clams was measured 3 times over the 7 day acclimation period (i.e., Day 1, Day 3, and Day 6). In other words, oxygen consumption rates were measured for the same individual clams during acclimation to determine if the oxygen consumption rates obtained on Day 7 of the experiment were acclimation oxygen consumption rates or stress induced oxygen consumption rates.

A separate 37.9 L glass aquarium insulated with 19.0 mm Styrofoam was set up as a clean (i.e., this water did not contain clams and was filtered to 0.35 μm) source of water for the oxygen consumption measurements (i.e., this water was used in the respiration chambers). A submersible pump (Pentair Aquatics Quiet One 1200) and air stones circulated the water. The temperature of this system was maintained by a water bath heater (Finnex Model HC-0800 Thermo-Controller with Deluxe Titanium Heater Model TH-0500) or a water bath chiller (TECO® Model CA 200 and CURRENT Model 2635 1/10th HP Prime Mini-ChillerTM). Salinity was maintained at 25 ppt as described previously, with the exception of the salinity experiment, as described later.

Oxygen Consumption Measurements

Temperature Effects

The oxygen consumption of 15 diploid and 15 triploid hard clams was measured at each temperature and salinity combination. To control for the allometric relationship between oxygen consumption and body size in clams, the clams were of uniform shell length ($\bar{x}$ = 54.5 mm SL, SE = 0.5 for diploids and $\bar{x}$ = 51.8 mm SL, SE = 0.5 SL for triploids). These sizes represent diploid clams that have spawned at least once (Eversole 1986).

Two water baths, each containing approximately 12.0 L of tap water, were insulated with 19.0 mm Styrofoam. Each water bath contained a submersible stir plate (Variomag® TELEMODUL 40 S control unit with Variomag® TELESYSTEM HP 6 stirring plates). A submersible pump (Pentair Aquatics Quiet One 1200) with a 12.7 mm I.D. tube circulated the water. The water temperature was maintained by a water bath heater (Finnex Model HC-0800 Thermo-Controller with Deluxe Titanium Heater Model TH-0500) or a water bath chiller (TECO® Model CA 200 and CURRENT Model 2635 1/10th HP Prime Mini-ChillerTM). A YSI

(Yellow Springs Inc.) Sonde 650 MDS was used to measure the temperature of the holding tanks, the clean water source tank, and the water baths on a daily basis.

Acrylic respiration chambers (Strathkelvin RC 400; approximately 730 mL) contained a stir bar and a platform on which to place the clam. At the start of each oxygen consumption measurement, clams that had extended siphons in the acclimation aquarium were placed into a respiration chamber (i.e., one clam per respiration chamber) with 0.35 µm filtered seawater from the clean water source. Care was taken to make sure no air bubbles were present in the chambers. Each chamber was placed on top of the submersed magnetic stir plate. There were a total of eight experimental chambers and two control chambers (i.e., chambers without clams).

Oxygen concentration in each chamber was measured with a Strathkelvin oxygen electrode (1302 microcathode oxygen electrode). The microelectrode was connected to the interface of the Strathkelvin Respirometry system (Model 928) and oxygen concentration was recorded on a computer with Strathkelvin software (version 2.2). The electrodes were calibrated daily for 100% oxygen. Oxygen concentration was continuously monitored using the Strathkelvin software while clam behavior was continuously monitored by observing the clams throughout the experiment. When a clam opened, defined as a clam with its siphon visible (Figure 2-1), the oxygen consumption measurement for that clam began. The measurement continued until the clam either closed its valve (i.e., the siphon was no longer visible) or the oxygen concentration in the respiration chamber declined to 5 mg L^{-1}or 60% oxygen saturation.

Once an experiment endpoint was reached, clam volume (mL) was determined by displacement in a 500 mL (clams < 40 mm SL) or 1000 mL (clams > 40 mm SL) graduated cylinder. The clam was then frozen for a period no less than 24 hours after which the clam was removed from the freezer and allowed to reach room temperature. The clam was pried open and

inverted to drain most residual water. The clam tissue was removed from the shell using a scalpel, placed onto a pre-weighed aluminum weigh boat, weighed for shell-free wet weight (g), and placed into an oven (Precision Economy Oven) at 80ºC until dry (≤ 48 hours). The clams were removed from the oven, allowed to cool to room temperature in a sealed container with desiccant, and weighed for shell-free dry weight (g). For each of these measurements, the weight of the pre-weighed aluminum weigh boat was subtracted to determine the weight of the clam tissue.

Oxygen concentration of the water during the experiment was measured in μg O_2 L^{-1}. The weight-specific oxygen consumption rates of the clams were calculated based on the following equation (Modified from Cech 1990):

$$Q_{O_2} = \frac{\{([O_2]_I - [O_2]_F)*V\} \div SFDW}{T}; \quad (2\text{-}1)$$

Q_{O_2} = Weight-specific O_2 consumption rate (μg O_2 g^{-1} h^{-1});
$[O_2]_I$ = O_2 concentration in water (μg O_2 L^{-1}) at the start of the experiment;
$[O_2]_F$ = O_2 concentration in water (μg O_2 L^{-1}) at the end of the experiment;
V = Total volume of respirometer – Volume of Clam (L);
SFDW = Shell free dry weight of the clam (g)
and T = Time that clam was open (h).

The oxygen consumption rate of the control chamber was determined for the corresponding time a clam was open. This measurement was then subtracted from the clam oxygen consumption rate as a correction factor to eliminate the oxygen consumption of the oxygen probe. When a clam opened and closed multiple times throughout the oxygen consumption measurements, only the time period in which the clam was open the longest was used for analysis.

Salinity Effects

Based on the temperature data, with 27°C being the temperature at which a physiological change occurs and the clams first become stressed, the oxygen consumption rates of diploid (n =

5) and triploid (n = 5) clams was measured at 15 and 25 ppt at 27°C. The methods for this experiment were similar to those of the temperature experiment, with a few exceptions. Clams in the salinity experiment were adjusted to 15 ppt or 25 ppt at a rate of 1 ppt per day from 27 ppt. The temperature was then adjusted to 27ºC by 1 degree per day from 25ºC. Oxygen consumption was assessed after 7 days of temperature and salinity acclimation. The clams used for this experiment were of uniform shell length ($\bar{x}$ = 59.5 mm SL, SE = 1.4 for diploids and $\bar{x}$ = 62.1 mm, SE = 2.3 SL for triploids).

Statistical Analyses

Data distributions of the oxygen consumption rates were tested for normality using the skewness and Kurtosis tests, which indicated that the data was non-normal. The corrected oxygen consumption rates were therefore log transformed (Zar 1984). Data transformation of the oxygen consumption rates only partially corrected for non-normality of the data. Analysis of variance, however, is robust to violations of normality (Olejnik & Algina 1984). All statistical analyses were conducted using SPSS statistical software (SPSS 16.0 for Windows).

A two-way repeated measures analysis of variance was performed on the oxygen consumption data at 20, 25, and 30°C to test the null hypotheses and to determine if there were effects of acclimation duration, acclimation temperature, or their interaction on oxygen consumption rates of diploid hard clams. Based on those results, a second two-way repeated measures analysis of variance was performed on the oxygen consumption data from Days 3 and 6 at 20, 25, and 30°C to determine where there was interaction within the data. Due to a lack of data on Day 3 for 27 and 32°C, a separate two-way repeated measures analysis of variance was performed on the oxygen consumption data from these temperatures.

A two-way analysis of variance and Tukey post hoc analysis was performed to test the null hypotheses that there were no effects of temperature, ploidy, or their interaction on the final oxygen consumption rates recorded on Day 7.

The temperature experiment data were then examined using the Arrhneius plot to determine if a physiological change occurred within the clams. The Arrhenius plot, which in this case is the log of oxygen consumption rate versus the reciprocal of the absolute temperature, is used to examine discontinuity in the Q_{10} or temperature coefficient value of organisms (Hochachka and Somero 2002). Temperature coefficient values are often used to describe the effect temperature has on oxygen consumption rate and typically doubles for every 10°C increase in temperature for cold-blooded animals. The Arrhenius plot should be a linear relationship that increases with an increase in temperature. Discontinuities in Q_{10} values or deviations from linear in the Arrhenius plot signify a disturbance in the molecular structures that support the oxygen consumption rate (Hochachka & Somero 2002) and suggest the upper thermal tolerance limit of the organism has been reached. A change in slope, or E_a, at the Arrhenius Break Temperature (ABT), suggests a qualitative and/or quantitative change of enzymes (Bartholomew 1982). Therefore, by examining the Arrhenius plot of the oxygen consumption rates of the hard clam, we can further elucidate if and when a physiological change occurs.

To verify that the physiology of the clams used in the salinity experiments was not different from the clams in the temperature experiments, a two-way analysis of variance was performed to test the null hypothesis that there were no effects of treatment (i.e., clam batch) or ploidy on the oxygen consumption rates of clams at 27°C and 25 ppt. To test the direct effect of

salinity on the oxygen consumption rate of clams at 27°C, a two-way analysis of variance was conducted with salinity (15 versus 25 ppt) and ploidy (diploids versus triploids) as main effects.

Hamwi (1969) versus Weber (Present Study)

Hamwi (1969) reports his oxygen consumption rates as mL O_2 hr^{-1} on an individual basis while the present study reports oxygen consumption rates as μg hr^{-1} on a weight-specific basis using shell-free dry weight. Hamwi gives clam sizes used for his oxygen consumption rates from Figure 1-5 (present study) as approximately 200 g wet weight. Therefore, a linear regression using data from Table 10A in Hamwi's dissertation was used to determine the shell-free dry weight of clams based on a 200 g wet weight (i.e., 200 g wet weight = 4.2 g shell-free dry weight). The data from Hamwi's dissertation (Figure 1-5) was thus converted to μg g^{-1} hr^{-1}. The two data sets (i.e., Hamwi's converted data and the present study data) were plotted to examine trends.

Further comparison of data from Hamwi and the present study was conducted by examining Q_{10} values. Cold-acclimated animals, such as Hamwi's clams from New Jersey, and warm-acclimated animals, such as the present study's clams from Florida, may have different Q_{10} values due to differences in enzyme efficiency and mitochondrial proton leakage. Thus, Q_{10} values were determined for oxygen consumption rates from 20 to 30°C for Hamwi's converted data and the present study using equation 2-2.

$$Q_{10} = \left(\frac{k_1}{k_2}\right)^{\frac{10}{t1-t2}}; \qquad (2\text{-}2)$$

Q_{10} = Temperature coefficient value
k_1 = Oxygen consumption rate at t_1
k_2 = Oxygen consumption rate at t_2
t_1 = Higher temperature of interest
t_2 = Lower temperature of interest

The calculated Q_{10} values were then further compared to Q_{10} values obtained from Shumway and Koehn (1982) as they studied cold- versus warm-acclimated Eastern oysters. Based on the Arrhenius plot, Q_{10} values were calculated for the temperature ranges of 20 to 27°C and 27°C to 32°C for the present study.

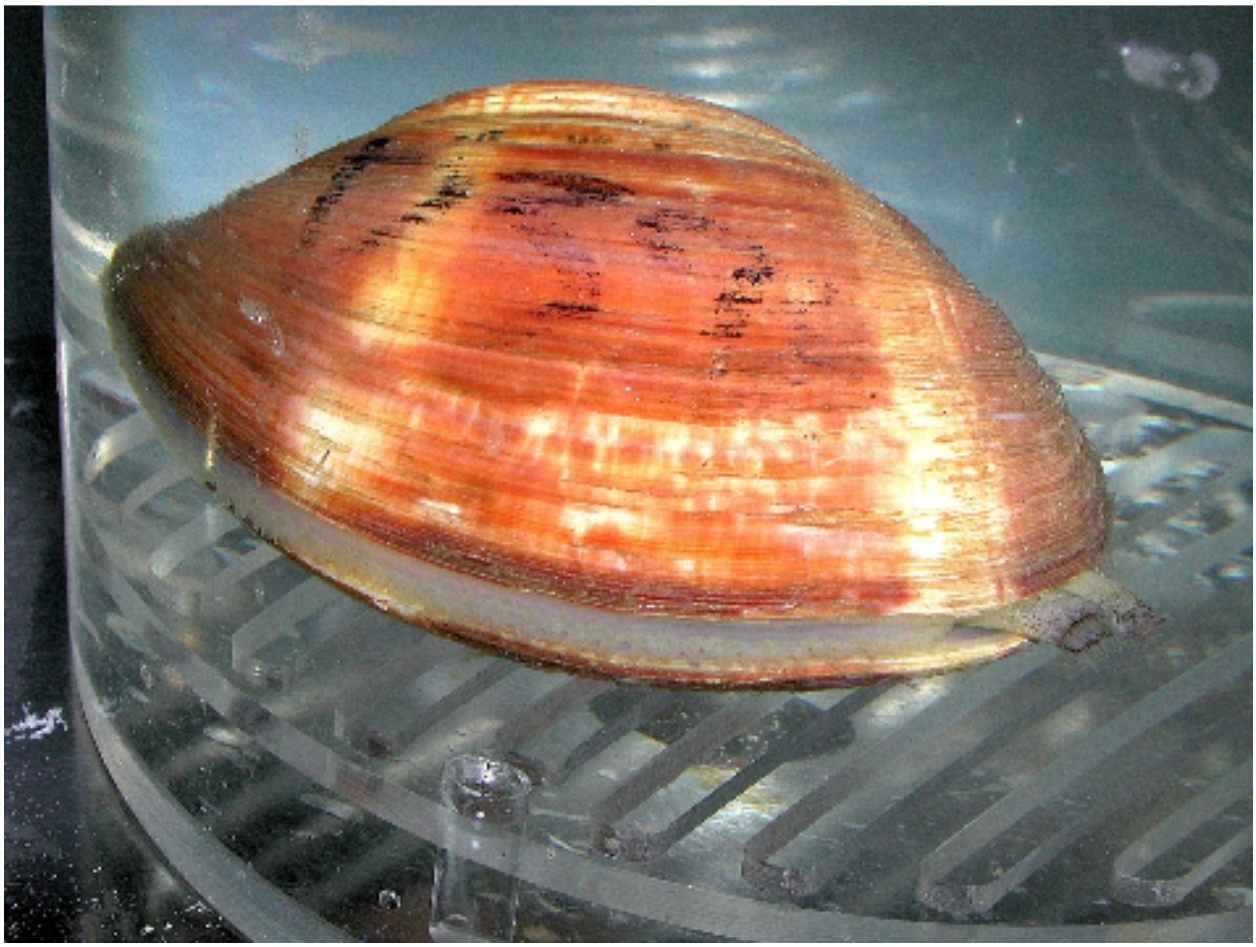

Figure 2-1. Example of *M. mercenaria* siphon extension.

CHAPTER 3
RESULTS

Temperature and Salinity Acclimation

Acclimation duration and temperature had a significant interaction ($p \leq 0.000$) on weight-specific oxygen consumption rates of five diploid hard clams for Days 1, 3 and 6 at 20, 25, and 30ºC (Figure 3-1). For Days 3 versus 6 at 20, 25, and 30°C, acclimation duration*temperature interactions ($p = 0.679$) was not significant. The main effect of acclimation duration ($p = 0.133$) was also not significant while temperature ($p = 0.050$) was significant (Figure 3-1). For 27 and 32 ºC, acclimation duration ($p \leq 0.125$), temperature ($p \leq 0.435$), and their interaction ($p \leq 0.230$) did not have significant effects on weight-specific oxygen consumption rates of diploid hard clams at 27 and 32ºC (Figure 3-1). Based on these results and Figure 3-1, an acclimation duration of 7 days is appropriate for this experiment as the clams appear to be acclimated by Day 6.

Oxygen Consumption Measurements

Temperature Effects

Ploidy had no significant effect ($p = 0.591$) on oxygen consumption rate. While ploidy did not have an effect on oxygen consumption rates, temperature had a highly significant effect ($p \leq 0.000$; Figure 3-2). Oxygen consumption rates increased from 498.3 ± 27.1 μg g^{-1} hr^{-1} at 20ºC to 1527.0 ± 139.0 μg g^{-1} hr^{-1} at 27ºC for diploids. Oxygen consumption rates also increase for triploids from 464.0 ± 34.6 μg g^{-1} hr^{-1} at 20ºC to 1431.6 ± 104.0 μg g^{-1} hr^{-1} at 27ºC. Beyond 27ºC, the oxygen consumption rates of both diploid and triploid clams plateaued.

Oxygen consumption rates at 20ºC were significantly lower than oxygen consumption rates at all other temperatures (Tukey's post-hoc multiple comparison for $p < 0.05$). Oxygen consumption rates at 25ºC were significantly lower than oxygen consumption rates at 27 and

32ºC, but were not different than oxygen consumption rates at 30ºC. Oxygen consumption rates at 27, 30 and 32°C were not significantly different from each other.

Several interesting behavioral observations were made during these experiments that have physiological implications. Clams at 30 and 32°C were open less time than clams at all other temperatures (Figure 3-3). At 32°C, the clams took longer to open and extend their siphons than clams at all other temperatures. Many clams in the 32°C acclimation tank did not open after 13 days, resulting in a lower sample size for this temperature. A few clams at each temperature would explore their surroundings with their foot for a brief amount of time, but only at 32°C did they appeared lethargic. At 32°C, foot movement was slower than at lower temperatures.

Salinity Effects

For the temperature and salinity experiments that were conducted at different times with different batches of clams, treatment (i.e., clam batch), ploidy, and their interaction were not significant (all $p > 0.05$). The oxygen consumption rate for diploids was 1527.0 ± 139.0 μg g^{-1} hr^{-1} for the temperature experiment at 27ºC, 25 ppt and 1464.2 ± 126.0 μg g^{-1} hr^{-1} for the salinity experiment at 27ºC, 25 ppt. The oxygen consumption rate for triploids was 1431.6 ± 104.0 μg g^{-1} hr^{-1} for the temperature experiment at 27ºC, 25 ppt and 1802.7 ± 533.9 μg g^{-1} hr^{-1} for the salinity experiment at 27ºC, 25 ppt.

Salinity treatment ($p = 0.012$) and ploidy ($p = 0.035$) were significant while their interaction ($p = 0.057$) did not have a significant effect on weight-specific oxygen consumption rates of the hard clams at 27°C. The weight-specific oxygen consumption rate from the 25 ppt salinity experiment was 1464.2 ± 126.0 μg g^{-1} hr^{-1} for diploids and 1802.7 ± 533.9 μg g^{-1} hr^{-1} for triploids. The oxygen consumption rate of diploid clams from the 15 ppt salinity experiment was 1011.9 ± 201.8 μg g^{-1} hr^{-1} while the oxygen consumption rates for triploid clams from the same

experiment was 1129.1 ± 257.0 μg g^{-1} hr^{-1}. Triploid clams had significantly lower oxygen consumption rates at 15 ppt than they did at 25 ppt (Figure 3-4).

Arrhenius Model

Since ploidy had no significant effect on oxygen consumption rates, diploid and triploid data were combined for the Arrhenius plot (Figure 3-5). An Arrhenius Break Temperature occurred at approximately 27°C, as indicated in the graph by a change in slope. Below 27°C, the oxygen consumption rate increased with an increase in temperature, with a slope that was significantly different from 0 ($p \leq 0.000$). Above 27°C the oxygen consumption rates plateaued, with a slope not significantly different from 0 ($p = 0.947$).

Hamwi (1969) versus Weber (Present Study)

Hamwi's converted data shows that clams increase their oxygen consumption rates until 25°C. At temperatures higher than 25ºC, the clams' decrease their oxygen consumption rates significantly. According to Hamwi's converted data, the oxygen consumption rates at 5º and 32ºC are 0 μg g^{-1} hr^{-1} (Figure 3-6). Contrary to Hamwi, the oxygen consumption rates from the present study increase from 20 to 27°C and then plateau from 27 to 32°C (Figure 3-6). Comparing the two data sets indicates a similar trend at lower temperatures, but divergence at the higher water temperatures (Figure 3-6).

The Q_{10} values determined from Hamwi's converted data were lower than Q_{10} values from the present study (Table 3-1). Shumway and Koehn (1982) also found that warm-acclimated Eastern oysters had higher Q_{10} values than cold-acclimated individuals (Table 3-1).

Table 3-1. Q_{10} values from Hamwi (1969), present study, and Shumway and Koehn (1982).

Temperature Range (°C)	Salinity (ppt)	Q_{10} Value	Study
20 to 30	20.2 to 22.2	0.68	Hamwi (1969)
20 to 30	25	2.63	Present study
20 to 27	25	5.0	Present study
27 to 32	25	1.1	Present study
20 to 30 (acclimated to 10)	28	1.80	Shumway & Koehn (1982)
20 to 30 (acclimated to 20)	28	2.28	Shumway & Koehn (1982)
20 to 30 (acclimated to 30)	28	3.00	Shumway & Koehn (1982)

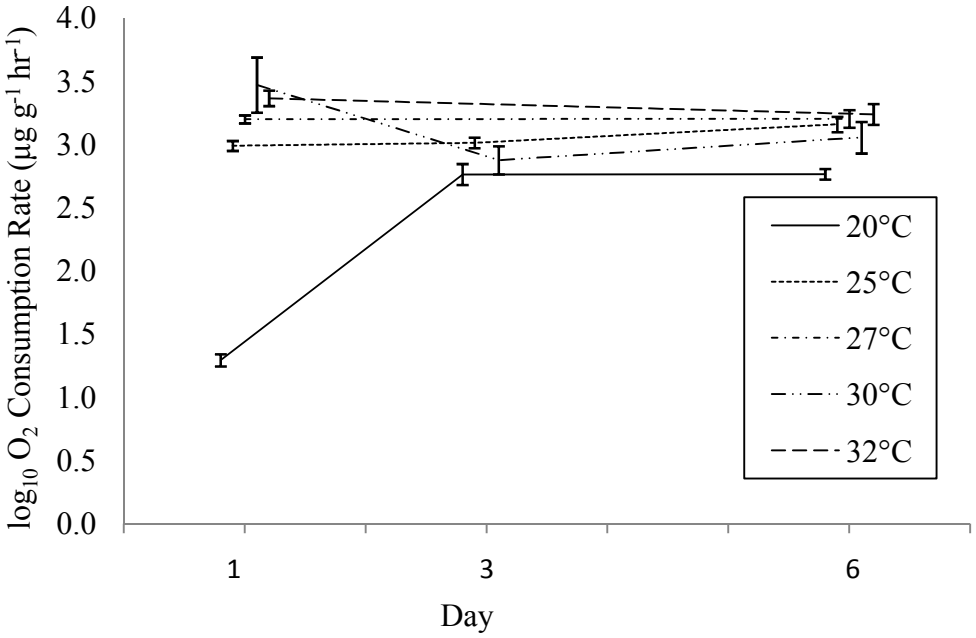

Figure 3-1. Mean ± 1 SE log-transformed weight-specific oxygen consumption (µg g^{-1} hr^{-1}) of diploid clams during acclimation for the temperature experiment. Data is offset on the day axis. For all data points, n = 4, except Day 3 for 27 and 32°C which are missing due to the clams not opening within the 24 hour period.

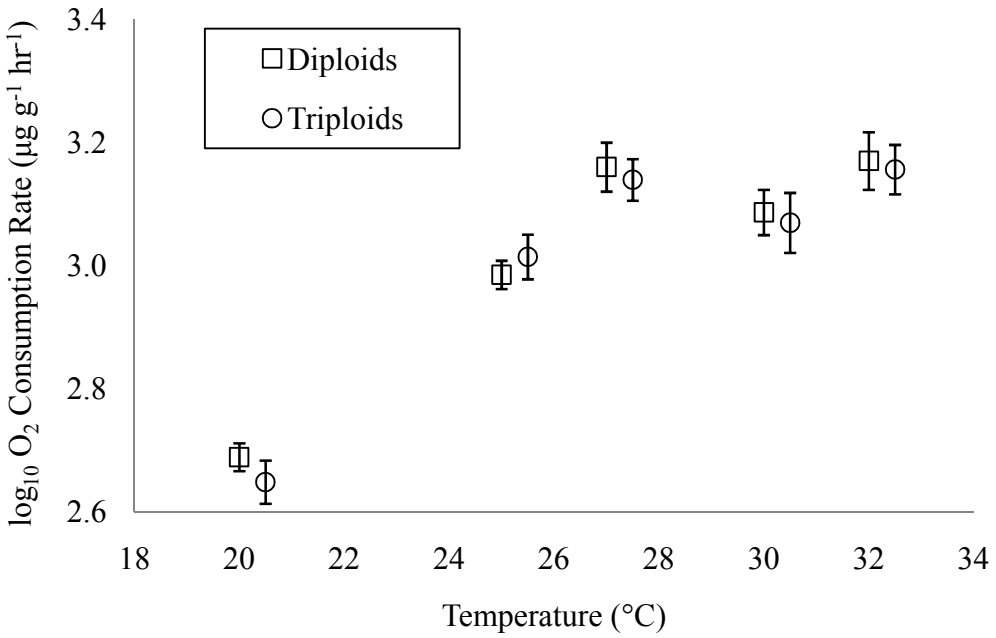

Figure 3-2. Mean ± 1 SE log-transformed weight-specific oxygen consumption (µg g^{-1} hr^{-1}) for individual clams on Day 7 at temperatures of 20, 25, 27, 30 and 32°C. Data is offset on the temperature axis for clarity. For all data points from 20 to 30°C, n = 13 to 15; for data points at 32°C, n = 8 to 12.

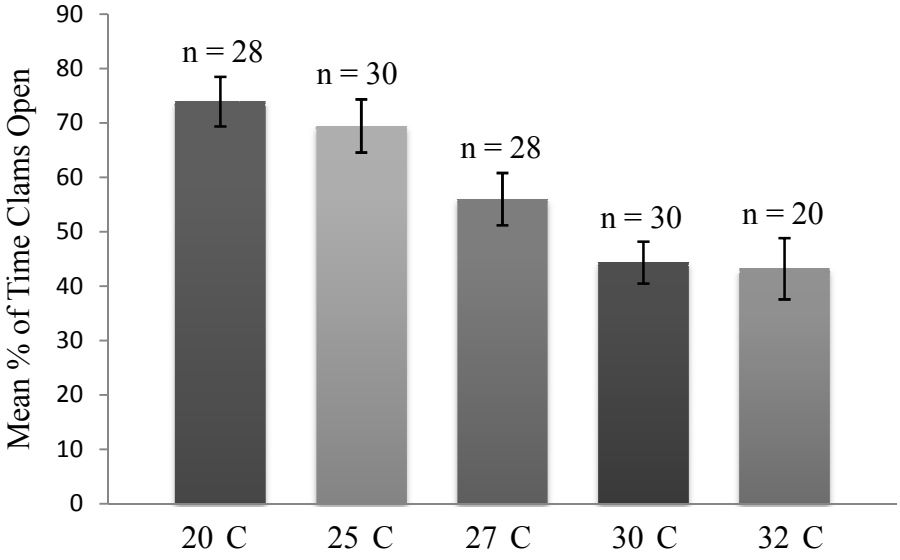

Figure 3-3. Mean ± 1 SE percent time clams open in respiration chambers for 20, 25, 27, 30 and 32°C past Day 7 of temperature experiments.

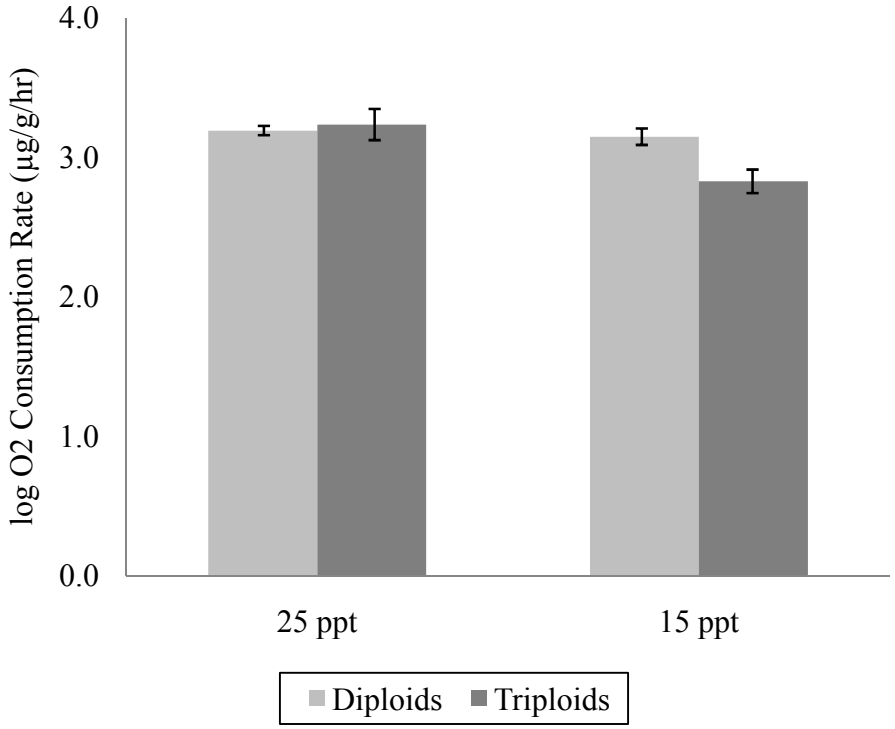

Figure 3-4. Mean ± 1 SE log-transformed weight-specific oxygen consumption (µg g^{-1} hr^{-1}) for clams in salinity experiments (27°C, 25 ppt and 27°C, 15 ppt). For all data points, n = 5 to 6.

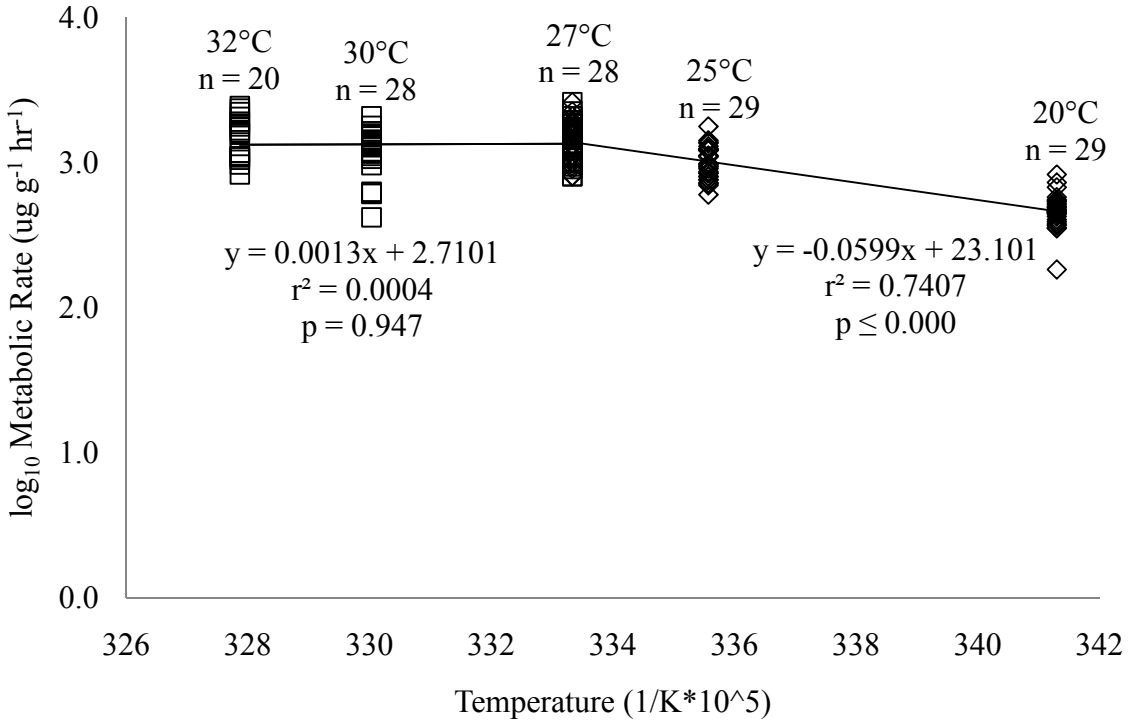

Figure 3-5. Arrhenius plot of log-transformed weight-specific oxygen consumption measurements for *M. mercenaria* from 32 to 20°C with an ABT at 27°C, n = 30.

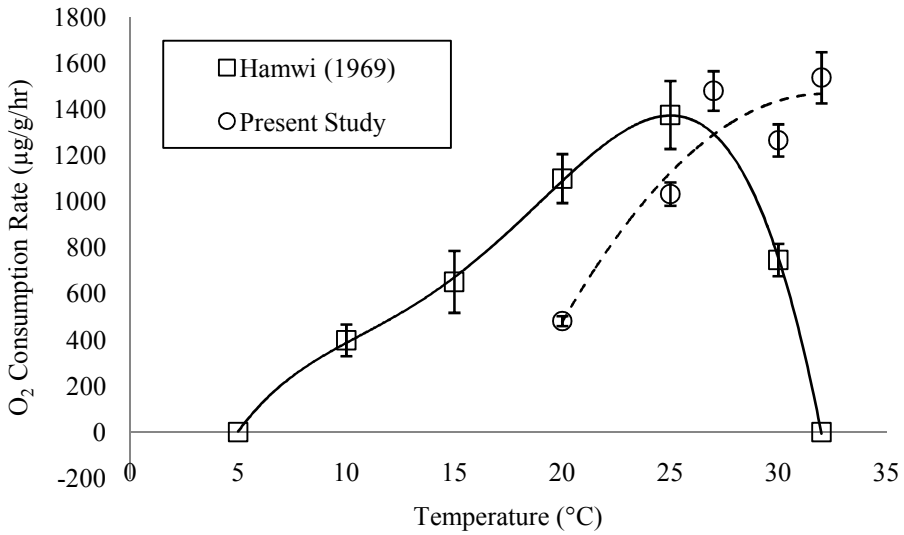

Figure 3-6. Mean ± 1 SE weight-specific oxygen consumption (µg g^{-1} hr^{-1}) for New Jersey acclimated clams (Hamwi 1969) and Florida acclimated clams (Weber this study) at various temperatures. It should be noted that Hamwi's data presented here is only an approximation, as he did not publish actual clam weights.

CHAPTER 4
DISCUSSION

While Hamwi (1969) extensively examined the oxygen consumption rates of diploid *Mercenaria mercenaria*, my study was the first to investigate the metabolic rates of diploid and triploid *M. mercenaria* in the state of Florida. I found that triploids had similar metabolic rates to diploid clams at high temperature and typical salinity (i.e., 27°C, 25 ppt), but had slightly lower metabolic rates at high temperature and low salinity (i.e., 27°C, 15 ppt). As expected, temperature had a significant effect on the oxygen consumption rates of both diploid and triploid clams. Contrary to Hamwi (1969), however, oxygen consumption rate did not decline above 25°C, but appeared to plateau at 27°C suggesting a physiological change at this temperature.

Ploidy Effects

There are several explanations for the lack of difference in oxygen consumption rate between triploid and diploid clams in the temperature experiment, including lack of triploid heterozygosity. First, it is unclear whether the MII triploids used in this study had greater heterozygosity compared to the diploids. For example, in oysters, only MI triploids are consistently more heterozygous than their diploid counterparts (Stanley et al. 1984, Hawkins et al. 1994, Hawkins et al. 2000); MII triploid Pacific oysters are more heterozygous than diploids (Granier-Géré et al. 2002) while MII triploid Eastern oysters are equal in heterozygosity to their diploid counterparts (Stanley et al. 1984, Hawkins et al. 1994). The triploid clams used for this project were the result of induction at meiosis II (MII clams) and no heterozygosity analysis was conducted. Therefore, the MII triploid clams used in this study may not have been more heterozygous than their diploid counterparts. This assertion is consistent with the similar oxygen consumption rates for the diploids and triploids at all of the temperatures examined. Testing the heterozygosity and comparing the oxygen consumption rates of diploids and MI and MII

triploids, as well as triploids produced by mating tetraploids with diploids, will provide a better understanding of the correlation between heterozygosity and metabolic rate.

The results of the salinity experiment, however, suggest that there is some physiological difference between triploid and diploid clams at higher temperatures (>27°C) and low salinities (<15 ppt). It is unclear if the lower oxygen consumption rate for triploids at 27°C and 15 ppt is a metabolic advantage or disadvantage. The difference in metabolism may only be perceptible under specific conditions. In laboratory experiments, "environmental conditions" are static and thus may be less stressful then fluctuating field environmental conditions. The metabolic differences discovered here may be more or less important in the field. However, the small acute changes in metabolic rate discovered here could accumulate over the span of the triploid's life and result in a metabolic advantage allowing them to survive high water temperatures and low salinity levels.

Temperature Effects

Based on the principle of enzyme kinetics, the metabolic rate and oxygen consumption rate of an ectotherm will increase with an increase in temperature (Hochachka and Somero 2002). I found that oxygen consumption rates of *M. mercenaria* increased with temperature, as expected, to 27°C but plateaued with further increase in temperature. Hamwi (1969) determined that the oxygen consumption rate of *M. mercenaria* increases with an increase in temperature until approximately 25°C, at which point the oxygen consumption rate of hard clams rapidly decreases.

My data indicate that a physiological or biochemical change took place at 27°C. Although anaerobic metabolism was not measured, which would require measurement of the total heat production or metabolic end-products, two observations suggest the clams began to resort to anaerobic metabolism at temperatures above 27°C. First, my observations suggest that

clams held at the higher water temperatures, particularly at 32°C, may have entered a heat coma as they took longer to open and were lethargic when they did open. Heat coma has been described in the snail *Littorina littorea* as curling of the lateral foot area and loss of the ability to contract the foot (Clarke et al. 2000a, b). In *L. saxatilis*, they experienced heat coma and a significant decrease in the oxygen consumption rate occur at 32°C, which was determined to be this species' critical temperature (Sokolova & Pörtner 2003). *Crassostrea virginica* is observed to open slowly between 27 and 30°C, suggesting a heat coma (Shumway & Koehn 1982). These observations suggest that stressful temperatures alter an organism's physiology as well as behavior.

Second, examining my data as an Arrhenius plot supports a transition to anaerobic metabolism at high temperatures; an Arrhenius Break Temperature (i.e., a change in slope) occurred at 27°C. There are several explanations for such a transition. First, Bartholomew (1982) interpreted a change in slope of the Arrhenius equation as a qualitative and/or quantitative change in metabolic enzymes. Secondly, the transition to anaerobiosis may occur as a result of insufficient capacity of the circulatory and ventilatory systems and suggests that survival is limited beyond the critical temperatures at which transition occurs (Hughes 1973, Pörtner 2001). In addition, temperatures near the upper thermal limit of an organism cause damage to the mitochondria. Enzymes in the ATP-generating pathways may lose activity at higher water temperatures, impairing mitochondrial respiration (Hochachka & Somero 2002). Lastly, Pörtner et al. (1999) claim that higher temperatures cause an increase in the oxygen demand, possibly due to proton leakage. Therefore, at higher temperatures, the limited dissolved oxygen is used less efficiently by the mitochondria, causing an energy deficit and leading to the use of anaerobic

metabolism. At high temperatures, metabolic function becomes strained causing an increase in energetic demand for survival.

Salinity Effects

Salinity, in general, has an effect on the survival, activity, pumping, and oxygen consumption rates of many bivalve species. *Mercenaria mercenaria* may be unable to tolerate low salinities; they are typically not found in locations where salinity is below 20 ppt (Wells 1957). Van Winkle et al. (1976) determined that the activity (i.e., extended and open siphons) of the hard clam is a function of salinity, with approximately 20% of the clams active at salinities below 14 ppt at 15°C and 21 ppt at 3°C and 27°C.

Pumping rate has a strong, positive linear relationship with oxygen consumption rate from < 1 to 5 mg O_2 L^{-1} in *M. mercenaria* (Hamwi 1969), and is especially affected by low salinities. For example, the Japanese oyster, *Crassostrea gigas*, maintains a normal pumping rate between 25 and 39 ppt, but stops pumping below 13 ppt salinity (Hopkins 1936). Even with acclimation, *C. gigas* has decreased pumping rates at lower salinities (Loosanoff and Smith 1949). *Mercenaria mercenaria* is more efficient at adjusting to higher salinities than to lower salinities, which is supported by the reduction of ciliary activity of the gills in *M. mercenaria* (Hopkins 1949, Van Winkle 1967) and other bivalve species (Wells et al. 1940, Van Winkle 1967) at decreased salinities. Hamwi (1969) determined the maximum pumping rate of *M. mercenaria* to be between 23 and 27 ppt. The pumping rate of non-acclimated *M. mercenaria* is inhibited below 15 ppt and above 32 ppt at 25°C while acclimated *M. mercenaria* are able to increase their upper salinity limit to approximately 36 ppt but the lower salinity limit remains unchanged at 15 ppt (Hamwi 1969).

The effect of salinity on oxygen consumption rate varies among bivalve species. As acclimation temperature increases, the effect of exposure salinity decreases the rate of oxygen

consumption in the Eastern oyster, *Crassostrea virginica* (Shumway & Koehn 1982). In the Eastern oyster, low salinities have no effect on oxygen consumption rates (Galtsoff 1947). *Mytilus edulis* maintains constant oxygen consumption rates between 25 ppt and 32 ppt, with decreased oxygen consumption rates at lower salinities (Bouxin 1931). Hamwi (1969) determined that salinities between 21.5 and 25.5 ppt allow for maximum oxygen consumption rates in the hard clam; beyond this range the oxygen consumption rate declines (Hamwi 1969).

These different effects of salinity on oxygen consumption may be attributed to osmoregulation abilities, salinity adaption, health, condition, or size of the various species. The cost of osmoregulation may account for both the increased oxygen consumption rates in hypoosmotic media and decreased oxygen consumption rates in hyperosmotic media (Hiscock 1953). However, Potts (1954) and Potts and Parry (1964) determined that only a small fraction of energy is used for osmotic regulation. No other satisfactory explanation has been found in the literature.

I found that, while salinity had no effect on the oxygen consumption rate of diploid clams, triploid clams had lower oxygen consumption rates at 15 ppt than their diploid counterparts, and thus may be better able to survive low salinity events. Based on the polyploidy gigantism hypothesis, triploid clams have larger cells, which mean that triploid cells have a smaller surface area to volume ratio. The smaller surface area to volume ratio may make osmoregulation easier and/or more efficient for triploids than for diploids.

Combined with the increased efficiency or ability to osmoregulate, triploid clams may have extra energy stores available for osmoregulation. This concept, based on the energy reallocation hypothesis, may allow triploids to divert energy that diploid clams would use for reproduction into energy for cell maintenance, including osmoregulation.

Hamwi (1969) versus Weber (Present Study)

A dissertation by Hamwi (1969) on the oxygen consumption and pumping rates of hard clams is highly cited throughout the literature on *M. mercenaria*. The differences between Hamwi (1969) and my data may be explained in three ways. First, I took care to observe the clams during the experiment to ensure the siphon was out and the clams were respiring. I found that at 30 and 32ºC clams stayed closed for longer periods of time (Figure 3-3), making data collection difficult. Hamwi does not allude to clam observations during his experiments, therefore his clams may have been closed during portions of the experiments, potentially explaining values of 0 mLO_2 $clam^{-1}$ hr^{-1} at 32°C.

Second, Hamwi (1969) examined the oxygen consumption rate of clams of approximately 200 g wet weight while the present study investigated the oxygen consumption rate of clams of approximately 50 g wet weight. Smaller organisms consume more oxygen per gram of mass than larger animals, but larger organisms consume more oxygen overall than smaller animals. To compare Hamwi's results to the present study, I expressed Hamwi's data on a per gram shell-free dry weight basis on the estimation that his clams were, on average, 200 g wet weight. Even if his clams were smaller, the resulting weight-specific oxygen consumption would be even greater than what was calculated and given in Figure 3-6. Therefore, although the exact elevation of Hamwi's oxygen consumption curve given in Figure 3-6 may require adjustment with additional information on his clam weights, it would most likely still be as great, or greater, then the oxygen consumption curve for the present study in Florida.

Finally, Hamwi used hard clams acclimated to New Jersey temperatures, while we examined clams acclimated to warmer Florida waters. Heat resistance is greater in organisms living in warmer water than those living in colder water (Henderson 1929, Reshoft 1961, Zhirmunsky 1967, Kennedy & Mihursky 1972). Cold-acclimated bivalves have higher oxygen

consumption rates than their warm-acclimated counterparts within their normal temperature range (Kennedy & Mihursky 1972). Thus, clams living in Florida may have lower oxygen consumption rates at similar temperatures than those clams examined in New Jersey. This may explain the lower oxygen consumption rates of my Florida clams, particularly at 20°C.

Several factors may contribute to differences in Q_{10} in cold- and warm-acclimated animals. First, cold-acclimated organisms have greater enzyme efficiency than warm-acclimated organisms, allowing for greater temperature compensation in metabolism as temperature increases (Hochachka & Somero 2002). Second, mitochondrial proton leakiness is lower in cold-acclimated organisms than in temperate organisms (Hardewig et al. 1999). As temperature increases, the amount of saturated acyl chains in the mitochondria lipid layer increases. In other words, the lipid membrane of the mitochondria becomes less rigid at higher temperatures due to fewer double bonds. A decrease in the number of double bonds in the mitochondrial membranes decreases the amount of proton leakage of the mitochondria (Hulbert & Else 2000). Rolfe and Brown (1997) found that up to 20% of oxygen consumption by mitochondria in mammals is due to proton leakage and does not result in energy (ATP) synthesis. Therefore, at low temperatures, an increase in temperature causes a greater increase in oxygen consumption than might be expected (i.e. Q_{10} greater than 2) because not all of the oxygen is going to energy synthesis. The high Q_{10} value of 5.0 determined for the temperature range of 20 to 27°C may be explained by this phenomenon. Within this temperature range, I suggest that mitochondrial leakiness is relatively greater in the warm-acclimated Florida clams than in Hamwi's clams acclimated to colder New Jersey shores. As a result, my clams have higher Q_{10} values than Hamwi's clams.

Implications for Hard Clam Culture in Florida

Based on this research, other laboratory studies (Hoover 2007) and field data (Scarpa et al. 2005, 2007, 2008), culturing of triploid clams will probably not significanly reduce summer

mortalities or improve clam production and sales in Florida. The lower oxygen consumption rate of triploid clams compared to diploid clams at 15 ppt and 27°C could be considered a metabolic advantage or disadvantage, depending on other unexamined physiological parameters. If the triploid clams do in fact have extra energy stores (based on the energy reallocation hypothesis), than their lower oxygen consumption rate at 15 ppt could be considered advantageous. The triploid clams would be using less energy for maintenance than the diploid clams under these conditions and would be able to reserve energy stores. The lower metabolic rate of the triploid clams at high temperatures and low salinities may mean that triploid clams are better suited to survive summer stresses. Over the course of a year, the lower metabolic rate and potentially higher survival rate of triploids could result in increased productivity for the clam farmers. If, on the other hand, the lower oxygen consumption rate indicates that triploid clams have reached their maximum aerobic metabolism at 15 ppt and are beginning to resort to anaerobic metabolism, than their lower oxygen consumption rate could be considered disadvantageous. The triploid clams would be using energy stores to survive and may die sooner than diploid clams under these conditions. Data were not collected on differences in energy stores between diploid and triploid clams or on metabolic end-products indicative of anaerobic metabolism. Therefore, it is unclear if the lower metabolic rate of the triploids at 15 ppt is a metabolic advantage or disadvantage. Further research to determine the oxygen consumption rate of diploid and triploid clams acclimated to 15 ppt over the entire temperature range of 20 to 32°C would clarify if the decrease in oxygen consumption rate of triploids is consistent over the entire range or if maximum aerobic metabolism is reached.

While the outcome of this experiment does not offer a simple solution for the clam farmers of Florida, there are still options to examine. For example, experiments are beginning to

determine if hybridization of *M. mercenaria* with *Mercenaria campechiensis* will help alleviate summer mortality issues while maintaining an acceptable shelf-life at grocery stores before gaping. *M. campechiensis*, the southern quahog, is typically found along the Atlantic coast from North Carolina to Florida and within the Gulf of Mexico (Abbot 1974). This means that Florida is near the middle of the thermal range for *M. campechiensis* compared to the extreme thermal range for *M. mercenaria*, which may allow *M. campechiensis* to better survive summer water temperatures. However, past research has shown that *M. campechiensis* is unable to survive on ice as long as *M. mercenaria*, which means that *M. campechiensis* has a shorter shelf-life at grocery stores. It is hoped that hybrids of the two clam species will have superior summer survival rates and an acceptable shelf-life.

Another option is to culture alternative or additional species, such as the sunray venus clam, *Macrocallista nimbosa*. Diversification of the industry from a monoculture to a multi-species industry will allow it to be less dependent on *M. mercenaria*. Sunray venus clams have been found to be fast growing with meat of good quality (Stokes et al. 1968). This clam is found from South Carolina to Florida and along the Gulf states (Abbot 1974). Massive mortality events are not expected to be an issue, as Florida is within the middle of the species temperature range. An easy transition from *M. mercenaria* production to *M. nimbosa* production is expected (Scarpa, personal communication).

This research has contributed to a more complete understanding of *M. mercenaria* physiology, especially at high water temperatures. Historically, Hamwi (1969) is widely cited as indicating that the oxygen consumption rate of *M. mercenaria* is drastically reduced above 25°C. In contrast, my research shows that the metabolic rate of the hard clam dramatically increases with temperature to 27°C and remains high to at least 32°C. I hypothesize that 27°C is the

thermal threshold for the hard clam and that they either enter a heat coma or begin to resort to anaerobic metabolism at this temperature.

The results of this study help explain why there are high summer mortalities. Increased energy requirements are further exacerbated by insufficient phytoplankton availability during the summer months. In Florida, particularly in the Cedar Key and Charlotte Harbor areas, phytoplankton is low during the summer (FDACS 2008). Ansell (1963, 1969), Ansell et al. (1964) and Mihursky (1967) determined that a loss of biomass will ensue if insufficient amounts of phytoplankton are available for the clams. Thus, algal abundances may be too low for the clams to maintain their metabolic rate and cost of maintenance. Future experiments to determine annual glycogen stores or condition index would clarify if metabolic rate and costs associated with maintenance are being supported by the summer algal abundances or if energy stores are being depleted.

In Florida coastal waters, temperatures are typically above 27°C from May until September with temperature peaking at about 32°C in July (FDACS 2008). At 27°C, the onset of anaerobic metabolism will result in physiological challenges in addition to increased metabolism. Energy stores will be depleted, toxic end products will accumulate, and an oxygen debt will be incurred that must be paid back (for review see Hochachka and Somero 2002). This means that from May until July, the clams will become increasingly stressed while having smaller energy resources. Follow-up research on anaerobic metabolism, including metabolic end-products and heat production, along with testing for mitochondrial proton leakage would clarify if 27°C is the thermal limit of *M. mercenaria* and if a biochemical change occurs at this temperature.

Future research on triploid and diploid *M. mercenaria* should focus on the total energy budget to determine where triploids actually reallocate the energy saved from gametogenesis.

My data shows that triploids are not allocating the energy saved from gametogenesis into metabolism. Field data shows that triploids do not grow faster than diploids and therefore are not putting the energy saved from gametogenesis into growth (Scarpa et al. 2005, Scarpa et al. 2007, Scarpa et al. 2008). It is unclear where the extra energy is being allocated or if triploids do not take in as much energy as diploids.

APPENDIX
EDIS PUBLICATION (FA151): THE ROLE OF WATER TEMPERATURE IN HARD CLAM AQUACULTURE

FA151

UF UNIVERSITY of FLORIDA
IFAS Extension

The Role of Water Temperature in Hard Clam Aquaculture[1]

Kerry Weber, Leslie Sturmer, Elise Hoover, and Shirley Baker[2]

Introduction

This document describes the affects of water temperature in hard clam production in Florida. A glossary of terms is provided at the end of the document.

What is water temperature?

Temperature is the measurement of heat in a material and is related to the motion of the particles that make up the material. Many physical properties of materials depend on temperature, including phase (solid, liquid or gas), density, and solubility. Temperature is one of the more important parameters collected with water-quality data because data such as conductivity, pH, and dissolved oxygen concentrations are dependent upon water temperatures.

Temperature also plays an important role in biology by determining the rate of biochemical reactions. Aquatic organisms have a range of water temperatures in which they function best. Outside this range, organisms do not function as well. Organisms also have upper and lower temperature tolerances that are incompatible with life.

How is water temperature measured?

Many methods have been developed for measuring temperature. Thermometers and thermistors are used most frequently to measure the temperature of liquids such as sea water.

Thermometer: Water temperature is easily measured using a thermometer. A thermometer contains a liquid that expands as its heat increases and contracts as its heat decreases. Therefore, the length of the liquid in the thermometer's tube varies with temperature. Temperature is determined by observing the length of the liquid and reading the calibrated scale printed on the side of the thermometer.

Maximum-minimum thermometer: One type of thermometer is the maximum and minimum (max-min) thermometer that records the highest and lowest temperatures during a given time and is a simple method by which to determine the extremes of

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temperature at a given location. The thermometer consists of a U-shaped tube filled with mercury. One arm contains alcohol and records the minimum temperature; the other arm conains a vacuum and records the maximum temperature reached. As the mercury is pushed around the tube by the expansion or contraction of the alcholol, it pushes two small markers that record the furtherest point reached by the mercury in each arm of the tube. The markers are reset by gravity or with a small magnet.

Thermistor: A thermistor is a temperature-sensitive electrical resistor; when water temperature changes, the resistance of the thermistor changes in a predictable way, allowing for temperature to be measured. Monitoring probes, installed at several lease areas in Florida, contain thermistors that measure water temperature (see http://shellfish.ifas.ufl.edu/clammrs.htm).

Scales: Several temperature scales are in use. The Fahrenheit (°F) and Celsius (°C) scales are most frequently encountered. Throughout most of the world, and the entire scientific world, the Celsius scale is used for measuring temperature. However, people in the United States are most familiar with and use the Fahrenheit scale. Celsius and Fahrenheit measurements can be converted using Equations 1 and 2.

Equation 1. Convert from Celsius (i.e., temperature measured in Celsius) to Fahrenheit

$$\text{Fahrenheit Temp} = [\text{Celsius Temp} \times (9/5)] + 32$$

Example: 22°C → °F = [22°C x (9/5)] + 32 = 71.6°F

Equation 2. Convert from Fahrenheit (i.e., temperature measured in Fahrenheit) to Celsius

$$\text{Celsius Temp} = (\text{Fahrenheit Temp} - 32) \times (5/9)$$

Example: 81°F → °C = (81°F – 32) x (5/9) = 27.2°C

Why is water temperature variable?

Water temperature in coastal areas is regulated by many environmental variables including daily and seasonal meteorological cycles; water depth; amount of mixing due to wind, storms and tides; and incoming water sources (e.g., precipitation, tributaries, man-made canals). Coastal water temperature fluctuates on a daily and seasonal basis. During daylight hours, energy from the sun warms the water, while heat is lost to the cooler atmosphere at night. In areas of Florida where hard clams are cultured, temperatures may fluctuate by more than 20°F (11°C) during a 24-hour period. Consider the following example from the Gulf Jackson High Density Lease Area located in the Gulf of Mexico at Levy County. On March 30, 2003, at 8:00 a.m. the water temperature was 72.3°F (22.4°C). At 7:30 a.m. the next day, the water temperature was 49.6°F (9.8°C), having fallen by 22.7°F (12.6°C) within a 24-hour period as a cold front moved through the area.

Seasonal water temperatures are also regulated by the amount of sunlight. Daylight hours are shorter and the sun is less intense (lower on the horizon) in the winter than in the summer, resulting in a net loss of energy to the atmosphere in the winter. Temperatures in shallow waters may fluctuate by more than 55°F (31°C) over the course of a year. For example, in 2003 at Gulf Jackson High Density Lease Area, temperatures reached a low of 39°F (4°C) in January and a high of 95°F (35°C) in July. For more examples of yearly, monthly, and daily water temperature fluctuation, see Figure 1, Figure 2, and Figure 3.

Water depth influences water temperature. In shallow bodies of water, energy from the sun is able to penetrate to the bottom and heat the entire water column; water in shallow tidal areas may reach temperatures near 100°F (38°C). Deep bodies of water may become stratified, with warmer, less dense, water floating on top of colder, denser, water near the bottom. At relatively shallow lease areas (< 6 feet at mean high water), such as the Gulf Jackson High Density Lease Area, there may be little difference in temperature between the top and bottom layers. For example, in 2003, there was an average difference between surface and bottom temperatures of only 0.5°F (0.3°C) (Shellfish Environmental Assessment Section, Florida Department of Agriculture and Consumer Services, Division of Aquaculture (personal communication)). However, at deeper lease areas, such as the Sand Fly Key High Density Lease Area in Charlotte Harbor, growers report a difference of over 5°F (2.8°C) between the surface and bottom layers.

Wind, storms, and tides can have a significant impact on water temperature. Wind and storms primarily affect temperature by breaking up stratification, mixing the water, and equally distributing the heat throughout the water column. Tides also affect temperature; during high tides, cooler marine waters intrude into warmer coastal areas, the waters mix, and the temperature is lowered. The opposite happens during low tides; warm terrestrial waters (i.e., rivers and streams) flowing into estuaries have a greater influence than they do during high tide, causing the water temperature to increase. It should also be noted that tidally-induced temperature fluctuations may be greater during spring tides (new and full moons) than during neap tides (first and fourth quarter moons). In areas of Florida where hard clams are cultured, water temperature may vary by 5°F (2.8°C), or more, over a single tidal cycle. For example, at the Gulf Jackson High Density Lease Area in 2003, the temperature recorded at high tide (12:11 p.m., +3.5 feet) was 85.3°F (29.6°C), while the temperature recorded at low tide (7:44 p.m., +0.1 feet) was 90.1°F (32.3°C) (Figure 3).

The freezing point of seawater varies with salinity; seawater at 35 ppt freezes at 28.6°F (-1.9°C), while brackish water freezes at higher temperatures and freshwater freezes at 32.0°F (0.0°C). The estuarine waters of Florida rarely, if ever, freeze. However, clams may be exposed to freezing air temperatures if there is an extremely low or blowout tide during which the clams are not covered by water.

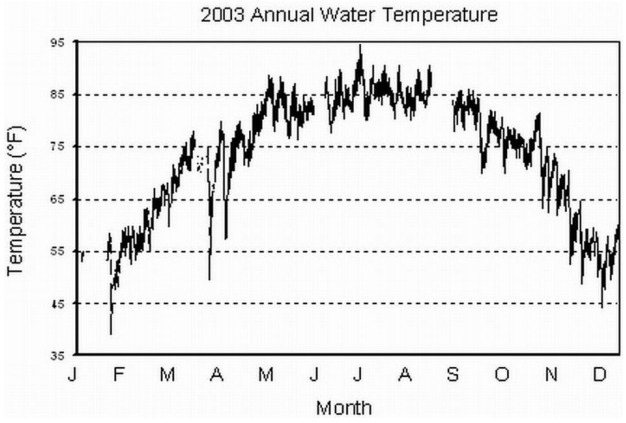

Figure 1. Water temperature fluctuation at the Gulf Jackson lease area, Levy County, Florida, in 2003. Credits: University of Florida, 2003

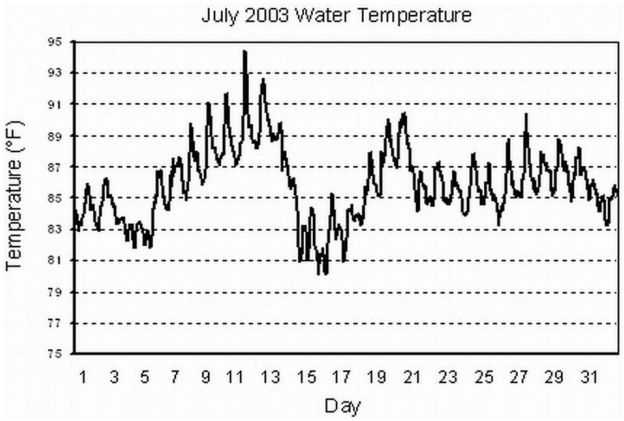

Figure 2. Water temperature fluctuation at the Gulf Jackson lease area, Levy County, Florida, in July 2003. Note the phases of the moon for the month were first quarter, July 6; full moon, July 13; third quarter, July 20; and new moon, July 28. Credits: University of Florida, 2003.

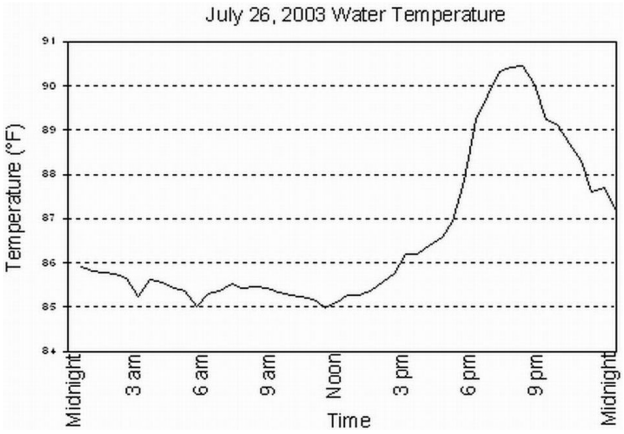

Figure 3. Water temperature fluctuation at the Gulf Jackson lease area, Levy County, Florida, on July 26, 2003. Note high tides were predicted at 1:37 a.m. (+3.0 feet) and 12:11 p.m. (+3.5 feet); whereas low tides were predicted at 6:10 a.m. (+2.1 feet) and 7:44 p.m. (+0.1 feet). Credits: University of Florida, 2003.

How does water temperature affect the physiology of hard clams?

Temperature plays an important role in biology by determining the rate of biochemical reactions; as temperature increases, biochemical reactions become faster. Metabolism is the biochemical breakdown of food to energy and is temperature dependent.

Like all other invertebrates, clams are cold-blooded organisms (poikilothermic); their body temperature fluctuates with that of the environment and their metabolism is directly influenced by water temperature. Increasing water temperature increases

metabolic rate, while decreasing temperatures will decrease metabolic rate, affecting both growth and reproduction of clams. At the upper and lower extremes of temperature tolerance, these biochemical processes will cease, resulting in diminished growth, poor health, or death.

The limits of temperature tolerance are changeable. Frequently, the range of temperature tolerance is different in summer and in winter for the same species. An organism that is acclimated to winter temperatures may tolerate and be active at a temperature so low that it would kill an organism acclimated to summer temperatures. A winter-acclimated organism is less tolerant of high temperatures than a summer-acclimated organism.

Temperature also affects water quality. For example, the solubility of gases decreases with increasing temperature. Therefore, the amount of oxygen dissolved in water decreases by about half as the temperature is raised from 32°F (0°C) to 78°F (30°C). Since oxygen is a requirement for aerobic metabolism, at high temperatures it becomes a challenge for clams to obtain sufficient quantities

What are signs of temperature stress?

Clams subject to temperature stress may exhibit valve, or shell, closure. Although clams can keep their valves closed for several days, they must obtain their energy through anaerobic metabolism. Clams may also exhibit shell gaping, especially following longer-term exposure to high temperatures. Signs of adverse environmental conditions in juvenile or adult hard clams may go unnoticed because they are infaunal, living buried in the sediment. However, stressed clams may rise to the surface of the sediment or fail to bury, which may be indications of temperature stress or other adverse environmental conditions, such as suboptimal salinities.

How does water temperature affect hard clam production?

Hard clams inhabit coastal waters over a very wide geographic range, from Canada to Florida. This natural distribution is evidence of the adaptability of this species to a broad range of water temperatures, both as larvae and adults. Florida represents the southernmost limit of the hard clam, where subtropical temperatures allow for a long growing season. However, water temperatures in Florida may also exceed the optimum temperature range for hard clams during the summer months. A temperature range from 60 to 80°F (16-27°C) is considered optimal for hard clams. Over this range, pumping rates, feeding rates, growth, and other activities are at their maximum. Above and below this range, the clams will begin to show signs of stress. Growth ceases below 48°F (8°C) and above 88°F (31°C). Clams remain closed at temperatures below 37°F (3°C), and pumping rates decline sharply above 80°F (27°C), declining to zero at 90°F (32°C). It is difficult to determine an exact temperature that is lethal because duration of exposure is very important. A high temperature, that can be tolerated for several hours, may be lethal if continued for several days. As discussed below, other environmental conditions are important as well.

Our laboratory studies indicate at a salinity of 25 ppt, growout-size clam seed (10-15 mm shell length) and pasta-size clams (25-30 mm shell length) tolerate 90°F (32°C) for longer than 15 days, experiencing mortalities of only 1% and 4%, respectively. However, high temperature apparently increases the effects of salinity stress. At 10 ppt, pasta-size clams begin dying after four days of exposure to 90°F (32°C), with a total of 12% mortality by day 15, while growout-size clam seed begin dying by day 6 with a final mortality of 4.5%. At 40 ppt, both pasta-size clams and growout-size clam seed begin dying within the first day of exposure, with a total of 98% and 96% mortality by day 12 of exposure. These data are derived from laboratory experiments and should be viewed only as rough approximations of what may occur under more complex field conditions.

Other environmental conditions affect the ability of clams to survive adverse temperature conditions, including salinity and dissolved oxygen. For example, low salinity ($\leq$ 10 ppt), high salinity ($\geq$40 ppt), and low dissolved oxygen concentrations will intensify the effects of stressful temperatures. Furthermore, physiological conditions (e.g., energy stores and spawning stage), age, size, and acclimation

Box 1: Overview of Hard Clam Production in Florida

Hard clam production has three culture stages - production of small seed in a hatchery, growth of larger seed in a land-based nursery and/or field nursery, and growout to marketable size on an open water lease.

Hatchery - Clam culture begins in the hatchery with the production of seed. In the hatchery, adult clams are induced to spawn by altering the temperature of the water. Fertilized eggs and resulting free-swimming larval stages are reared under controlled conditions in large, cylindrical tanks filled with filtered, sterilized seawater. Larvae are fed cultured phytoplankton (microscopic marine algae) during a 10 to 14-day larval culture phase. After approximately 2 weeks, the larvae begin to settle out of the water column and metamorphose into juvenile clams. Even though a true shell is formed at this time, post-set seed are still microscopic and vulnerable to fluctuating environmental conditions. Thus, they are maintained in downwellers at the hatchery for another 30 to 60 days until they reach about 1 mm in size.

Nursery - The land-based nursery protects small seed until they are ready to be planted out onto the lease for growout. Nursery systems built on land usually consist of weller systems or raceways. Water, pumped from an adjacent saltwater source, provides naturally occurring phytoplankton and oxygen to the clam seed. Depending on water temperatures, 1-2 mm seed, obtained from the hatchery, require from 8 to 12 weeks to reach 5-6 mm in shell length, the minimum size planted in the field.

Growout - Clams are primarily grown on estuarine or coastal submerged lands leased from the State of Florida. Since clams are bottom-dwelling animals, growout systems are designed to place the clam seed on the bottom and provide protection from predators. Most clam growers in the state use a soft bag of polyester mesh material. The bag is staked to the bottom and naturally occurring sediments serve as the bottom substrate. Bag culture usually involves a 2-step process. The first step entails field nursing seed with shell lengths of 5-6 mm (1/4 inch) in a small-mesh bag. After about 3-6 months, the seed reach a growout size of 12-15 mm shell length (1/2 inch) and they are transferred to a bag of larger mesh size. A crop of littleneck clams (25 mm or 1-inch shell width) can be grown in 12-18 months.

history also determine the tolerance of a clam to temperature. The rate of temperature change is also important; clams will be more likely to show signs of stress if the temperature changes rapidly (i.e., hours to days), than if the temperature changes relatively slowly (i.e., days to weeks), allowing acclimation to occur.

How can I manage my crop in response to water temperature?

Consider temperature regime in selecting a lease site

In the northeastern United States, the major temperature-related concerns for clam growers are cold water temperatures and ice. However, in Florida, we have few days in which the water temperature falls below 48°F (8°C), the temperature below which clam growth ceases. For example, in 2003 at the Gulf Jackson High Density Lease Area, only seven days had temperatures below 48°F (8°C). High temperatures, rather than low temperatures, are of greater concern in Florida. Again, taking Gulf Jackson High Density Lease Area in 2003 as an example, there were 30 days on which temperatures exceeded 88°F (31°C), the temperature above which clam growth ceases.

When considering a nursery or growout location, salinity regime should be the primary environmental factor in site selection. However, water temperature also plays an important role in the growth and survival of hard clams. Therefore, it is important to take temperature into account when selecting nursery and growout sites. In addition, two physical factors, depth and water flow, can either contribute to or offset temperature problems and should be considered in site selection. For example, shallow water (3 feet or less) will rapidly warm in the sun, and may reach temperatures near 100°F (38°C) in the summer. Such shallow water depths may occur periodically at some sites during spring tides or other extremely low (blowout) tides. Growers might consider sites located in deeper water to avoid such extreme temperatures. On the other hand, deep sites may periodically experience stratification. Water, below the thermocline, may have too little oxygen or phytoplankton to support optimal clam growth.

Water currents should also be considered when selecting a site. High temperatures will be of greater concern in areas protected from currents by a landmass (for example, in the lee of an island), or that are stagnant; these areas are more likely to reach high temperatures on hot summer days. Water currents and tidal exchange allow for mixing and

flushing of shallow warm water with cooler water and also helps aerate the water, preventing hypoxia.

Understand the temperature regime at your site

To manage a clam crop proactively, it is important to understand the temperature regime at a given nursery or growout lease site. To better understand and respond to daily, seasonal, and annual variations in water temperature, growers should take frequent temperature measurements, as well as record their activities and subsequent crop performance.

A maximum and minimum (max-min) thermometer, which records the highest and lowest water temperatures reached during a given time period, is inexpensive and easy to use. A max-min thermometer should be placed near the bottom on the site where the clams are planted, not near the surface. Stratification of the water column can occur, resulting in warmer water on the top and cooler water on the bottom.

Taking temperature measurements over diurnal (daily) and tidal cycles will allow the grower to better understand the temperature fluctuations at a site. For example, temperature measurements taken in the summer months will help the grower determine how hot the water gets during a low tide that coincides with the heat of the day. Temperature measurements taken over a 24-hour period in the summer will allow the grower to determine when the coolest water temperatures occur and plan daily activities, such as harvest, accordingly.

Historical temperature records may also prove useful. Monthly water quality data can be obtained for shellfish harvesting areas in Florida by contacting a Shellfish Environmental Assessment Section (SEAS) field office of the Florida Department of Agriculture and Consumer Services, Division of Aquaculture (see http://www.floridaaquaculture.com/seas/seas_mngmt.htm). Archived water quality data collected during 2002-5 at selected aquaculture lease areas in 6 coastal counties can be found at http://shellfish.ifas.ufl.edu/clammrs_archives.htm

Nurse clam seed at compatible water temperatures

Winter water temperatures in the Cedar Key area and panhandle of Florida become cold enough to reduce or stop the growth of seed clams. Therefore, land-based nurseries in these areas typically do not operate during the winter. However, land-based nurseries in southwest and east central Florida experience warmer winter water temperatures and nurse seed clams during the winter.

High summer temperatures are of primary concern, especially on the southwest coast and central east coast of Florida, where land-based nurseries typically close for the summer. In the Cedar Key area and panhandle of Florida, land-based nurseries can continue to nurse seed clams throughout the summer if maintenance is conducted daily. To prevent bacterial contamination, tanks or raceways should be rinsed daily with freshwater to control marine bacteria and prevent accumulation of sediment.

Conduct farm activities with water temperature in mind

In the subtropical climate of Florida, seed clams can be purchased, planted, and transferred throughout the year. However, both water and air temperatures should be considered when scheduling these activities. In the winter, seed can be stressed or killed by exposure to cold air. Therefore, it is suggested that growers do not buy, plant, or transfer seed clams immediately before or during a winter cold front. Rather, growers should pay attention to local weather forecasts and schedule these activities after a cold front has passed, during warming trends. When transporting seed clams, contact with cold air can be minimized by covering the bags of clams with an insulating layer, such as empty growout bags or an old blanket.

Seed clams can be successfully purchased, planted, and transferred throughout the summer if extreme caution is taken in their handling. To minimize exposure to high air temperatures during transfer of growout-size seed to larger mesh bags, this activity could be conducted on a boat at the lease site, preferably under shade. If growout-size seed clams

are transported to an upland facility to be sieved, transferred, and rebagged, these activities should be conducted in a shaded area and the growout bags should be transported back to the lease site immediately. Alternatively, the growout bags could be held overnight in an air conditioned location but care should be taken to prevent the clams from drying out or getting too cold. If a grower leases multiple sites or has a site that varies in depth, deeper areas that may not get as hot as shallower areas could be reserved for summer use.

When harvesting clams during the summer, growers must be aware of the effects of elevated temperature on product quality. When water and air temperatures are high, survival in refrigerated storage (shelf-life) decreases, and the maximum allowable hours from harvest to refrigeration (time-temperature matrix) is reduced in accordance with shellfish harvesting standards (Comprehensive Shellfish Control Code, Chapter 5L-1, Florida Administrative Code), to ensure product safety.

Both growers and shellfish wholesalers can minimize the effects of elevated temperature on product quality. First, growers can reduce stocking density of clams that are to be harvested in the summer. Reduced stocking density will decrease temperature stress by increasing the availability of food and oxygen to individual clams. Second, if growers examine the diurnal temperature cycle at their site, they will most likely note that both water and air temperatures are coolest in the early morning. It is therefore preferable to harvest in the early morning hours when temperatures are lower. Finally, growers must shade a product from the point of harvest until delivery to the wholesaler to keep the clams as cool as possible. Wholesalers are allowed to dry temper a product, a process by which clams are acclimated by a step-down process to the final storage temperature of 45°F (7°C) (see http://shellfish.ifas.ufl.edu/temp_acclimation.htm). Dry tempering increases shelf-life during the summer months and minimizes microbial growth.

Summary

Water temperature in clam leases is an environmental factor that affects clam survival and growth. Since clam growers cannot control temperature on their leases, it should be a consideration for selecting sites and developing appropriate management strategies. The essential first step is temperature monitoring; with this information the clam grower can evaluate lease quality, determine optimal seed clam nursing periods, and plan daily farm activities. To minimize the potential economic impact to the industry, it is prudent to be aware of environmental conditions and to note any instances of mortality. Assistance from UF/IFAS extension shellfish and aquatic animal health specialists is available.

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Glossary of terms used

Acclimation – The process of physiological adjustment to changes in conditions

Aeration – The process by which air is mixed with or dissolved into water

Aerobic metabolism – Cellular reactions requiring oxygen to produce energy from food molecules

Anaerobic metabolism – Cellular reactions producing energy from food molecules in the absence of oxygen. Anaerobic metabolism produces far less energy per food molecule than does aerobic metabolism

Biochemical reactions – Chemical reactions converting a substrate to an end product, aided by an enzyme, and forming the basis of metabolism

Blowout tide – An unusually low tide as a result of a low tide combined with a weather front, usually a cold front

Conductivity – The ability of a solution to carry an electrical current; often used to determine salinity

Diurnal – A daily cycle recurring every 24 hours; refers to the variation in temperature that occurs from the highs of the day to the lows of the night

Downweller – An open-ended cylinder in which clam seed are suspended on a screen and water flows down over the clams

Enzyme – A protein that catalyzes, or accelerates, biochemical reactions

Growout-size clam seed – Refers to clams greater than 10 mm in shell length that are grown on open-water leases in large mesh bags

Hypoxia – Reduced or inadequate concentration of dissolved oxygen in water

Infaunal – Aquatic organisms that live in the substrate, usually a soft sediment

Larva – Immature state of an organism that differs markedly in structure from the adult

Metabolism – The complete set of biochemical reactions that takes place in cells, allowing organisms to grow, reproduce, and respond to their environment

Metabolic rate – The rate at which food is converted to energy; the amount of energy expended in a given period; or the rate at which oxygen is used in aerobic metabolism

Metamorphosis – The marked and rapid transformation of a larva into an adult form

Neap tide – Tides that occur around the time of the first quarter and fourth quarter of the moon. At these points in the lunar cycle, the tide's range is minimum; high waters are lower than average, low waters are higher than average, slack water is present longer than average, and tidal currents are weaker than average

Phytoplankton – Freely-floating microscopic aquatic plants (algae)

Poikilotherm – An organism whose body temperature varies with the temperature of the surrounding environment

Proteins – Complex molecules participating in every cellular process and having structural, mechanical or enzymatic functions

Raceway – Shallow tank or tray with horizontal flow of seawater

Salinity – The concentration of salts dissolved in water

Seed – Refers to clams less than 10 mm in shell length

Shelf-life – Length of time that food remains suitable for sale or consumption; for clams, length of time shellstock remains alive in refrigerated storage

Signs – Objective evidences of disease

Solubility – The ability of a substance (e.g., salt) to dissolve into a solvent (e.g., water)

Spring tide – Tides that occur around the time of the new moon or full moon. At these points in the lunar cycle, the tide's range is maximum; high waters are higher than average, low waters are lower than average, slack water is shorter in duration than average, and tidal currents are stronger than average

Stratification – Cold (near the bottom) and warm (near the surface) waters form layers that act as barriers to mixing

Thermocline – An area of rapid change in temperature with depth

Tidal cycle – The cyclic rising and falling of the ocean surface, caused by tidal forces of the moon and sun acting on the oceans, and resulting in changes in depth and oscillating currents

Time-temperature matrix – Regulatory requirement for harvesting molluscan shellfish (clams) in which the maximum allowed time from harvest to refrigeration is based on month of the year (water temperature)

Upweller – An open-ended cylinder in which clam seed are suspended on a screen and water flows up between the clams

Weller system – Consists of open-ended cylinders suspended in a water reservoir or tank. Seawater circulates among the seed clams (either up or down), which are supported on a screen at the bottom of the cylinder

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BIOGRAPHICAL SKETCH

Kerry Weber grew up in northern New York with a love for the ocean. As a high school student, she volunteered at the St. Lawrence Aquarium and Ecological Center every summer and participated in the Sea Education Association's Oceanography of the Gulf of Maine. Kerry attended the University of New England (UNE) in Biddeford, Maine, where she received her Bachelor of Science with a degree in marine biology and a minor in chemistry. While at the UNE, she interned at Bigelow Laboratory for Ocean Sciences during her summers researching phytoplankton and participating in research cruises. During the school year, Kerry was a lab assistant studying the osmoregulation of green crabs and rock crabs as it pertained to salinity. On weekends, she volunteered at the Marine Animal Rehabilitation Center to nurse seals for release back to the wild. As a senior, she conducted an honors thesis project examining the feeding habit of starfish upon mussels as a function of seasonal temperature variations. After graduation, Kerry moved to California to participate in the AmeriCorps/Los Angeles Conservation Corps/U.S. Forest Service program to help create a fire break for towns in the San Bernardino National Forest. She then became a teaching assistant at her high school to help students with minor learning disabilities graduate from high school. It was here that Kerry realized she missed the ocean and research and thus decided to go back to school for her master's degree. She received her Master of Science from the University of Florida in the summer of 2008.