Induced Spawning Behavior and Larval Development of the Hard Clam, *Mercenaria mercenaria* (Linné, 1758) in Puerto Rico

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Abstract

The northern quahog, *Mercenaria mercenaria* was accidentally introduced to Puerto Rico in the early decades of the 20th century. Since then, a small-scale market has been established along the northeast coast of the island. The presence of hard clams or northern quahogs in localized areas in Puerto Rico and the possible revenues for the island economy present *Mercenaria* sp. as a suitable organism to be considered for local mariculture activities. Two attempts have been made to evaluate *Mercenaria* sp. as a possible candidate for mariculture. However, neither of them studied the reproductive response under controlled conditions in tropical waters. This experiment evaluated the response of two populations (Florida stock and Puerto Rico stock) of *M. mercenaria* to induced spawning by thermal stimuli. It also evaluated possible disease outbreaks during *Mercenaria mercenaria* rearing; growth and survival rates, and larval development. The results were compared with clam hatchery production in the southeastern region of United States. Relationships between clam responses of both populations to thermal stimuli, clam size and conditioning time were analyzed with a binomial non-parametric Kruskal-Wallis test. Although, the Florida stock displayed better performance, poor responses from both populations were obtained. Results revealed a negative effect of time spent by clams in the conditioning tank and their response to the induced spawning method ($P < 0.001$). Clam growth and survival rates were comparable with those reported for commercial hatcheries of the southeastern region of the United States. Presences of bacterial and ciliate infections were reduced with the implementation of better sanitary procedures.

Resumen

La almeja, *Mercenaria mercenaria* fue introducida accidentalmente en Puerto Rico para las primeras décadas del siglo veinte. Desde entonces, ha existido un mercado a pequeña escala para la venta de este producto en la costa noreste de Puerto Rico. Se considera una especie con potencial de cultivo local ya que hay poblaciones establecidas y por los posibles ingresos económicos que puede aportar a la economÍa isleña. En dos investigaciones previas se ha evaluado la producción de esta almeja. Sin embargo, ninguno de éstos ha estudiado su comportamiento reproductivo bajo condiciones controladas en agues tropicales. Este experimento evalua el comportamiento reproductivo de dos poblaciones de *M. mercenaria* (Florida y Puerto Rico) a estímulos térmicos utilizados para inducción de desove. Se analizó también el posible desarrollo de enfermedades durante el cultivo de las diferentes etapas larvales, la tasa de crecimiento, sobrevivencia y el desarrollo larval de la *M. mercenaria*. Los resultados se compararon con los datos de producción de viveros comerciales en la region sureste de Estados Unidos. La relación entre el comportamiento al método de desove de ambas poblaciones al estÍmulo térmico, tamaño de la almeja y el tiempo de acondicionamiento fue analizado utilizando una prueba no paramétrica binomial de Kruskal-Wallis. Aunque la población de Florida mostró una mejor respuesta al estÍmulo termal, ambas tuvieron un comportamiento pobre ante la inducción del desove. Los resultados indican que existe un efecto negativo en las almejas y éste afecta el comportamiento de ellas ante el estímulo térmico ($P < 0.001$). La tasa de crecimiento y la sobrevivencia de las almejas compara favorablemente con los datos de producción que se obtienen de los viveros comerciales en la región sureste de los Estados Unidos. La presencia de ciliados y bacterias en algunos de los tanques de cultivo se redujo con la implantación de un programa agresivo de higienización.

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In Memoriam of

Dr. Luis Almodovar
and
Cándido Corrada

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throughout by academic and professional career.

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Introduction

Most fish destined for human consumption come from the ocean; however, global fisheries are now undergoing dramatic changes. Despite the increasing demand, fisheries landings have diminished in the last decades. By 1995, there were 3.5 million vessels in the world fleet and, of the 17 main fishing grounds worldwide, four were commercially exhausted and the others had either reached or exceeded their natural limits (Elliot, 1996; Platt, 1994). Over exploitation of fish stocks has also taken place in the Caribbean where also, the islands have had to confront problems associated with pollution and urban development, and Puerto Rico is no exception. Based on fishery statistics from the Department of Natural and Environmental Resources in Puerto Rico (DNER), the maximum local landings ever reported occurred in 1979 when 7 212 million pounds of fish and shellfish were harvested (Collazo and Calderon, 1983). Since then, there has been a continual decrease in production with occasional minor increases mainly due to improvements in data collection efforts (Collazo and Calderón, 1983). Recent reports reveal the same trend over the last four years that have been published. Matos-Caraballo (2002) reported a total of 13 620 481 pounds (6 178 metric tons) of fish and shellfish landings in Puerto Rico between 1998 and 2001, with an average of 3 405 120 pounds per year.

New local and federal regulations have been implemented as a result of the over exploitation of the oceanic resources. In the last 20 years, the DNER and the U. S. Department of Commerce have placed legislative and management restrictions on the commercial and recreational fishermen regarding size, capture limits, seasonal limitations on fishing, bans, fisheries quotas restrictions, and closures of traditional fishing grounds to the commercial fishermen (Appendix A). Therefore, new ways must be considered to fulfill consumer needs, which may also serve as an alternative source of income for the fishermen, among other interested groups. The development of ecologically friendly fisheries that

considers the conservation and economic components of the existing natural resources is needed. One possible alternative to alleviate this situation is bivalve culture. Bivalves, such as clams, are filter feeders, therefore, they help to reduce water turbidity and do not require supplemental feeding. So there is no bioaccumulation of organic matter in the system with the proper technical management. They are hardy organisms and can tolerate wide ranges of water temperature and salinity.

Clams of the genus *Mercenaria* have a stable and profitable market in the eastern and southeastern zones of the United States. The commercial landings come mainly from *Mercenaria mercenaria* (Manzi and Castagna, 1989). However, commercial concentration of *Mercenaria campechiensis* can be found along the Atlantic coast of Florida and in the Gulf of Mexico (Manzi and Castagna, 1989). According to the Food and Agriculture Organization of the United Nations (FAO), in the year 2000 the United States produced 23 895 metric tons of *M. mercenaria*, which represented 6% of its total production, being surpassed only by catfish, Pacific cupped oysters, and rainbow trout (Anonymous, 2003).

Although its natural distribution occupies the eastern and southeastern coast of the United States, *M. mercenaria* have been introduced along the northwest coasts of the states of Washington and California in the United States (Hanna, 1966; Stanley and Dewitt, 1983; Manzi, 1985 and; Chew, 2001) and Europe (France and England) (Chew, 2001) because of their economic importance. *M. mercenaria* has been subject to a variety of studies, due to its commercial demand and economic importance. The life cycle of *M. mercenaria* is well known and it can spawn under controlled environments. Consequently, *M. mercenaria* is a suitable organism for mariculture.

M. mercenaria has been known by a variety of common names such as northern hard clam, hard shell clam, quahog and quahag depending on the area

where it is harvested (Kraeuter and Castagna, 2001). The same authors have established that hard clam is the most utilized common name for *M. mercenaria*. In 1988, the American Fisheries Society, as an attempt to standardized a common single name recommended northern quahog for this species. Therefore, in this study *M. mercenaria* will be mentioned as northern quahog.

Sometimes, *M. mercenaria* is confused with *M. campechiensis,* the southern quahog, because both species have numerous concentric lines of growth in the exterior of the valves and have a triagonal shape (Abbott, 1974). However, there are several external features that are used to differentiate these two species. The valves of *M. mercenaria* are thin and the exterior center has a smooth or glossy area, with purplish zones in the interior of the shell (Harte, 2001) and the shell is longer than wide (Menzel, 1971) (Figure 1). In *M. campechiensis*, the valves are inflated and rough (Abbott, 1974) and the valves are wide as long and in most cases white internally (Fischer, 1978). The form *notata* is a color variation of *M. mercenaria* and its valves are externally marked with zigzag brown blotches and exhibit no internal purple colored band in their posterior ends (Abbot, 1974; Harte, 2001).

The northern quahog (Linné, 1758) inhabits inshore embayments and estuaries throughout the Gulf of Saint Lawrence in Canada to the Gulf of Mexico and Florida (Abbott, 1974; Manzi, 1985). *M. campechiensis* (Gmelin, 1791), southern quahog, is found in near shore open ocean waters as far north as New Jersey and ranges southward to Florida and the Gulf of Mexico (Bert et al., 1993). However, natural hybridization between *M. mercenaria* and *M. campechiensis* may occur in areas where the two clams species are sympatric (Menzel and Menzel, 1965)

Northern quahogs can be found in large numbers in places below the low tide level and in intertidal zones at water depths of 1.1 (Manzi and Castagna, 1989) to

less than four meters (Anderson et al., 1978 and; Walker et al., 1980). These bivalves are abundant in sandy and sandy-mud bottoms but they demonstrate some preference for shell particulate bottom substrates and grass beds to avoid predators (Kraeuter and Castagna, 1989; Peterson and Beal, 1989).

Previous reports established the presence of two clam species of the genus *Mercenaria* in Puerto Rico and the Caribbean region: *M. mercenaria, M. mercenaria var. notata* and *M. campechiensis* (Menzel, 1971; Fischer, 1978; Juste, 1987; and Juste and Cortés, 1990). It is not clear, though, how and when these species of *Mercenaria* were introduced to Puerto Rico. Juste (1987) suggested a possible accidental introduction by military and commercial vessels landings along the main ports around the Island during the dawn of the 20th century.

Since their introduction they have been harvested by the local fishermen on a modest scale. However, no reports or derived estimates are currently available for commercial harvest or local consumption of clams in Puerto Rico. The Statistics Office of the Puerto Rico's Planning Board gathers information about fishery importation to the Island from United States and foreign countries. This governmental agency also reports fishery exportation commodities from Puerto Rico. Moreover, in the last two years fisheries data have been published more accurate, so detailed mollusks importation and exportation can be obtained from its records. Therefore, based on these data. (Puerto Rico Planning Board, 2003 and 2004), approximately 3 347 090 and 3 429 674 kilograms of fresh, frozen, salted, dried, canned and smoked mollusks were consumed in Puerto Rico in 2002 and 2003, respectively. This means an annual revenue of $9 837 734 and $10 625 475 for years 2002 and 2003, respectively (Table 1).

The regional market in Puerto Rico prefers to buy live fresh bivalves. Traditionally, small-scale vendors along the coastal regions of the Island offer live oysters and clams to costumers, mostly during weekends. Based on information

from these vendors, the cherrystone (2.5 to 3.25 inches shell length) and chowder (greater than 3.25 inches shell length) sizes are the most popular among the consumers. Because clam consumption is high vendors must buy imported product to meet their local market demand (Personal communication with regional vendors from Cataño and Dorado, 1998).

Its presence in localized areas around Puerto Rico and the possible revenues for the Puerto Rican economy present *M. mercenaria* as a potential organism to be considered for local mariculture activities. Despite this aquaculture prospective in the Caribbean region, only two attempts in US Virgin Islands and Puerto Rico have been made to evaluate the feasibility of northern quahog mariculture. Sunderlin et al. (1975) tried to raise *M. mercenaria* using cold water from an artificial upweller system in Saint Croix. In a preliminary study, Juste (1987) evaluated the feasibility to culture *M. mercenaria* in grow-out cages along the southwest coast of Puerto Rico using clam seed from Delaware. None of these studies, however, evaluated the reproductive response under controlled conditions of *M. mercenaria* in tropical waters. Therefore, investigations to evaluate if induced spawning of adult clams after an acclimation period in a conditioning tank and larval development of *M. mercenaria*, is viable under the tropical environmental conditions in Puerto Rico.

Therefore, the objectives of this research were:

- To compare spawning response of the *M. mercenaria*, local wild population with imported Florida animals used as brooders.

- To compare growth rates, survival rates and larval development of *M. mercenaria* raised in Puerto Rico with production data of commercial clam hatcheries in the United States.

- To monitor possible diseases outbreaks and the presence of pathogenic microorganisms in the culture vessels.

If the induced spawning technique protocol and larval growth rate of *M. mercenaria* indicate their biological feasibility, then clam culture, on a modest scale should be evaluated as an additional income for fishermen, satisfying local demand for hard clams, and provide a fresher, higher quality product to consumers.

Figure 1
The northern quahog, *Mercenaria mercenaria*

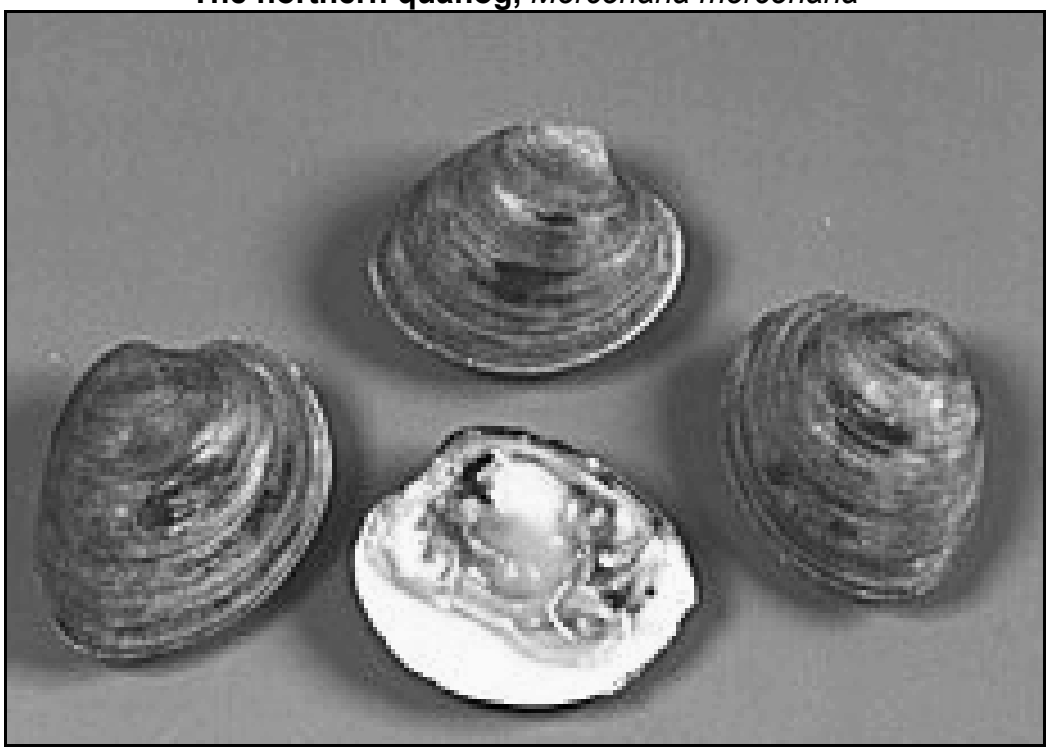


Table 1
Local consumption of mollusks in Puerto Rico for 2002 and 2003
(Fresh, frozen, salted, dried and smoked)

Year	Imports USA/ FC Kg (A)	Imports USA/ FC $ (B)	Exports USA/ FC Kg (C)	Exports USA/ FC $ (D)	Consumption Kg (A-C)	Consumption $ (B-D)
2002	3 361 624	9 940 344	14 534	102 610	3 347 090	9 837 734
2003	3 429 674	10 625 475	0	0	3 429 674	10 625 475

Source: Puerto Rico Planning Board, years 2003 and 2004. FC: Foreign countries

Literature Review

1. Life cycle of *Mercenaria* sp.

a. Sex determination

In the adult stage, *M. mercenaria* and *M. campechiensis* have separate sexes. However, their juveniles may exhibit some degree of consecutive hermaphroditism. Loosanoff (1937a) reported that 98% of sampled *M. mercenaria* contained ripe spermatozoa and function as males during their juvenile phase, although gonads contained both sperm cells and oocytes (immature eggs). Scientists have observed this juvenile sexual stage when these species of *Mercenaria* reach about 6 to 7 mm in shell length and spermatozoa surpass in number and mature faster than oocytes (Loosanoff, 1936; Menzel, 1971; Eversole et al., 1980 and; Dalton and Menzel, 1983:). Coe (1943) observed that during this phase, the juveniles could discharge sperm and function as males.

This juvenile male phase tendency was also, observed in reared stocks of *Mercenaria*. In 1980, Eversole et al. examined northern quahog specimens of less than 28 mm in shell length in a grow-out culture facility in a South Carolina estuary. These researchers observed that 90% of the sampled specimens were males.

Following, this sexual juvenile stage, these species of *Mercenaria* might undergo a sex change and thereafter, can only function as either males or females. Some investigations demonstrated that in *Mercenaria* specimens 2 years old or older there is some degree of sex change from male to female, until a 1:1 sex ratio is attained (Loosanoff, 1936 and 1937b; Bricelj and Malouf, 1980; Carriker, 2001*).* A study conducted by Walker and Heffernan (1994), after

examined 2604 specimens of *M. mercenaria* from 2 year-old and older classes, confirmed this 1:1 ratio. Based on measurements performed by Eversole (1989) on the available data of reproduction of clams in North America, these bivalves reach sexual maturity between one (males) and two (females) years of age and at a relatively small size of 30 to 35 mm shell length. Eversole et al. (1980) observed that males attain sexual maturity at a smaller size than females in *M. mercenaria*; however, this information cannot be used as an external indicator to distinguish between sexes.

b. Gametogenesis, spawning and fertilization

Gonadal size or gonadal-somatic index method has been widely used to determine gametogenic cycles in fishes and mollusks (Eversole, 1989). However, its application for clam gonadal studies is not frequently used because it is difficult to differenciate the gonad from the surrounding tissue in bivalves. Therefore, the primary method used to evaluate gonad maturation in northern quahogs is based on histological analysis. This analysis for describing *Mercenaria* gametogenic cycle involve categorizing its reproductive development into a series of subjecive stages. The number of stages and the criteria to classify them varies among researchers. The most extreme numbers of stages were used by Eversole et al. (1980) and Manzi et al. (1985) with only five stages while Porter (1964) utilized 14 stages to characterize seasonal reproductive cycles in clams. The main developmental stages to describe gametogenesis (spermatogenesis and oogenesis) are describe in Materials and Methods chapter (Histological Analysis section).

Histological gonad analysis in *M. Mercenaria* was first used by Loosanoff (1936, 1937a, 1937b and 1954) to describe spermatogenesis, juvenile male sexual phase and spawning patterns information. Gametogenic cycles of *M. Mercenaria,* based on histological analysis and their relation with environmental factors, have been also, described by Keck et al., 1975; Eversole et al, 1980;

Dalton and Menzel, 1983; Pline, 1984; and Manzi et al., 1985. However, no published study is known about detailed information of oogenesis in *M. Mercenaria* (Eversole, 2001). Therefore, histological gonad analysis in oogenesis in the northern quahog is based on investigations conducted with other bivalves (Jones, 1981; and Kent et al., 1998).

The geographical range of *M. mercenaria* influences natural reproductive cycles and spawning response due to seasonal changes in temperature. Spawning occurs when the water temperature reaches 24 to 25°C (Manzi, 1985). In northern zones of the United States, these temperatures are attained during the summer months. Thus, reproduction is limited to one season (Ropes, 1987). In temperate zones, spawning is synchronous and influenced by abrupt changes in water temperature (Loosanoff, 1937b and; Carriker, 1961). Hesselman et al. (1989) showed that in the Indian River in Florida the release of gametes was bimodal (September to December and March to June) but gametogenesis was less synchronous than in clam populations from northern areas. The same study presented gametogenic inactivity when temperatures exceeded 30°C in late summer. These studies demonstrated that gametogenesis and spawning peaks initiate earlier and last longer in lower latitudes.

Low salinity has a negative effect on spermatogenesis. A study conducted by Pline (1984) with five different salinities (30, 25, 20, 15, and 10 ppt) showed that gonads presented clumps of spermatocytes, which is a possible sign of gamete degeneration at lower salinities. Nonetheless, female gonads did not show harmful effects when subjected to the same salinities. In Puerto Rico, Juste and Cortés (1990) reported the presence of different size classes of clams in protected areas where temperatures ranged between 28.9 and 32°C and salinities between 15 and 34 ppt. The presence of different sizes of northern quahog suggested that gametogenesis and spawning cycles were occurring in these areas.

At present, there is no direct evidence that food is a limiting factor on the reproductive activity in northern quahogs. However, Eversole et al. (1984) observed a reduction in gonadal-somatic index in clams located in the intertidal zone and suggested that clams in this sector did not have sufficient time for feeding to invest energy in gametogenesis.

Reproduction and larval development under natural conditions have not been studied due to the difficulties in obtaining the data. The available information is based on research conducted under laboratory conditions. Various investigations reported the fecundity of a *M. mercenaria* female between 7.11 x 10^6 and 39.5 x 10^6 eggs, but the average production per female is considered around 7.39 x 10^6 eggs per clam per spawning cycle (Davis and Chaney, 1956; Bricelj and Malouf, 1980; Eversole, 2001). Northern quahog females required several spawning events in a spawning season to release all their gametes (Loosanoff, 1937a; Davis and Chanley, 1956). In a natural environment, external fertilization takes place when ripe northern quahog respond to environmental cues such as water temperature, salinity and other factors no get understood (Bricelj and Malouf, 1980; Hesselman, et al., 1989 and; Eversole, 2001).

c. Larval stages

Few field studies are available on the different *Mercenaria* larval stages on their natural environment. Most of these investigations examine lateral and vertical movements of larvae, their abundance and spatial and temporal changes of sampled northern quahog larvae in the field. Based on these data it is known that fertilized eggs undergo a series of morphological changes during their first three to 20 days of age (Losanoff, 1936) and develop into planktonic larval stages that are subjected to wave and tidal action, although they posses velum which enables them to maintain their vertical position in the water column (Carriker, 1961). Reproduction and fertilization under controlled laboratory

conditions and observations of larval development have established the different larval stages and their morphological characteristics. Most of these laboratory studies were conducted by Loosanoff and Davis (1950) and Carriker (1954 and 1961).

Based on these observations the first larval stage of the northern quahog is called the **trochophore** and it is a no shelled larvae with a total length between 50 and 60 µm. Its life span is short, less than 24 hours after fertilization have occurred (Carriker, 1961). Trochophores are very active swimmers due to their ciliated velum (Loosanoff and Davis, 1950). This larval stage does not eat and relay on their glycogen reserve to survive.

The **straight-hinged** or **D veliger** is the first shelled larval stage of *M. mercenaria.* During this time, the shell gland and mouth develop; thus, feeding begins (Carriker, 1961). It has a smooth shell secreted by the shell gland (Eyster and Morse, 1984) that is characterized by slightly asymmetrical shell valves. Manzi (1985) established that the average shell length at this stage is 105 µm and the age of D veliger larvae is between one to five days under controlled culture conditions. The ciliated velum is still present for locomotion (Loosanoff and Davis, 1950).

The previous stage is followed by the **umboned veliger** stage and its main characteristic is the smooth arc near the hinge line at the point where the umbo forms (Carriker, 1961). Information about age and shell length varies among researchers. Carriker (1961) observed in umboned veliger, shell length and age of larvae of 140 to 220 µm and 3 to 20 days, respectively. Whereas, Hardley et al. (1997) obtained shell length of 90 to 140 µm at an age of 5 to 8 days old.

In the **pediveliger stage**, larvae have a velum but, also, a foot is developed so they have the ability to swim and crawl on the sediment (Carriker, 1954). The pediveliger stage is considered a transitional form between its planktonic and

benthic existence and can be observed in six to 20 day old cultures; with sizes between 170 to 220 µm long (Carriker, 1961).

Once the larva undergoes metamorphosis, it reaches the b**yssal plantigrade stage** and measures between seven to nine millimeters in shell length (Eversole, 1987). In its early benthic plantigrade stage, it is unable to swim but has the ability to crawl and attach to an object by means of byssus threads. The byssal plantigrade is very active and can disattach, crawl around and reattach in another location with better environmental conditions.

Later, this organism develops into a **juvenile plantigrade**, which is characterized by the presence of a strong and large foot; its byssus thread becomes nonfunctional and, finally, burrows itself into the sediment to become mostly, a sessile organism (Eversole, 2001). Roberts et al. (1989) demonstrated that clams exhibit a mean vertical movement of 1.5 to 2 cm over the tidal cycle. They are closest to the sediment surface at high to early ebb tide and are deepest at low to early flood tide. Lateral movement is also displayed by clams but it is not clear if the displacement is the result of active movement or passive dispersal due to natural causes, such as, wave action, storms, etc. (Roberts et al., 1989; and Fegley, 2001).

Manzi and Castagna (1989) reported that northern quahogs could exceed 25 years in age in temperate zones. However, other studies indicated maximum ages of 28 years in Florida (Jones et al., 1990), 31 years in North Carolina (Peterson et al., 1983), 36 years in New Jersey (Lutz and Haskin, 1985) and, 40 years in Rhode Island (Jones et al., 1990). Their fast shell growth occurs during the first four years of life, where they attain most of their total adult size (Manzi, 1985; Fegley, 2001). Researchers found specimens between 70 and 80 mm in shell length, in unexploited clam populations in Virginia and South Carolina, but, Anderson et al. (1978) and Walker et al. (1980) reported northern quahogs greater than 100 mm long in Georgia.

2. Feeding

Northern quahogs are filter feeders and have an anatomical advantage over other bivalves because they possess two siphons fused to the body base and other structures that enable them to sort and sieve food particles effectively (Malouf and Bricelj, 1989; Grizzle et al., 2001). Clams obtain their food both by filtering suspended particulate matter and by absorbing dissolved organics directly from the water through the inhalant siphon. In the surface of the gills or ctenidia, water currents are produced by ciliary action, and the gills retain certain particles. Later, food particles are transferred to the labial palps and finally to the mouth (Eversole, 1987). Particles rejected by the labial palps are bound in mucus and eliminated as pseudofeces through the inhalant siphon. Grizzle et al. (2001) suggested that clams control the total amount of food ingested using pseudofeces as a mechanism to reject excess or unwanted material filtered by the gills. This feeding mechanism allows larval and adult clams to regulate the quality and quantity of food ingested. The exhalant siphon is used to expel fecal pellets.

Food habits vary at different life stages as northern quahogs of increasing size ingest proportionally larger food particles. Clam larvae feed mostly on phytoplankton (small flagellates and diatoms) while adult clams prefer bigger phytoplankton species and also ingest suspended detritus and bacteria to meet their nutritional demands (Manzi and Castagna, 1989). Loosanoff and Davis (1963) demonstrated that larval stages and adults of bivalves are capable of selecting particular phytoplankton species from a mixture of algal cells.

3. Effects of environmental factors on *Mercenaria mercenaria* physiology

It was previously established that environmental factors such as, temperature, salinity, and the quality and quantity of food have a direct impact on gametogenic

activity and spawning cycles in bivalves. However, these parameters also, affect other physiological activities in bivalves like *M. mercenaria*. Temperature and salinity are the two major physical factors that are highly correlated with clam physiology. Lough (1975) reported that shell growth is influenced not only by genetic factors but also by environmental parameters, such as, temperature and salinity. An investigation by Ansell (1968) concluded that the optimum temperature for shell growth ranges between 15 to 25ºC and observed a decline in shell growth when temperature readings were above of below these parameters. Other researchers obtained similar results and demonstrated no shell growth in juveniles and adults at temperatures below 9ºC or above 31ºC (Bardach et al., 1972; Manzi and Castagna, 1989). Investigations by Ansell (1964) determined that in temperatures less than 6ºC, northern quahogs cease pumping. Other investigations demonstrated that salinities below 15 ppt inhibit feeding rate, pumping, growth, and long-term survival in juveniles and adults *M. mercenaria* (Chanley, 1958; Castagna and Chanley, 1973). As a general rule, these marine bivalves prefer salinities not less than 20 ppt (Manzi, 1985).

4. Hybridization of *M. mercenaria* and *M. campechiensis*

Natural hybridization between *M. mercenaria* and *M. campechiensis* may occur in areas where the two clam species are sympatric (Menzel and Menzel, 1965). Various investigations have demonstrated that these two species can also hybridize under laboratory conditions (Loosanoff, 1954; Haven and Andrews, 1957). Investigation by Menzel and Menzel (1965) showed no chromosome abnormalities or infertility in F_1 and F_2 hybrids. Sunderlin et al. (1975) obtained better growth rates with *M. mercenaria* and *M. campechiensis* hybrids in studies conducted in Saint Croix.

However, a recent investigation in Florida discovered an increase in the frequency of gonadal neoplasia in the hybrids of these two species (Bert et al., 1993). Other investigations conducted by Arnold et al. (1996) showed that

growth rates in Florida were not always better in hybrids than in *M. mercenaria*. They concluded that the relative growth of the two *Mercenaria* species and their hybrids had a tendency to be habitat-dependent.

5. Predators and diseases

In their natural environment clams are subjected to a series of predators, which seem related to predator-prey sizes. Although the list is extensive, Arnold (1984) presented juvenile and adult sizes of blue crabs (*Callinectes sapidus*), as a major predator on northern quahogs at different life stages. Rays, drills, starfishes and conch or whelks are the best-known organisms that prey on adult northern quahogs (Kraeuter, 2001). However, newly set northern quahogs are vulnerable to a variety of predators such as, protozoans, flatworms, annelids, gastropods, echinoids, and crustaceans (Gibbons and Blogoslowski, 1989; Kraeuter, 2001). Some disease infections have also been reported, caused by Rickettsia-like organisms, digenean metacercaria, and the protistan parasite "QPX". The "QPX" is capable of infecting both juvenile and adult clams as well (Whyte et al., 1994).

6. Hatchery techniques

Due to the increasing demand for clams and undependable natural stocks, *Mercenaria* sp. has become an important subject of biological research. Results of these investigations were used to establish hatchery techniques to provide a continuous and reliable source of seedstock. Some of these methodologies included: broodstock conditioning, spawning induction, and the development of mechanisms to overcome possible pathogenic organisms in the culture systems.

The first successful attempt to reproduce and culture different clam larval stages under laboratory conditions was achieved by Wells between 1920 and 1926 (cited by Manzi and Castagna, 1989). Almost 30 years later, Loosanoff and

Davis (1963) established some basic techniques for clam seed production. They obtained the pediveliger stage clams within 7 to 14 days.

New studies to improve survival and reducing the time between larval stages were conducted to increase predictability in larvae culture system. The use of a broodstock conditioning tank was one innovation that enables better control of physical condition. Thus allowing the manipulation of the gonadal cycles and spawning period, to extend the availability of larvae throughout the year (Castagna, 2001). In the conditioning tanks, water temperature is maintained between 18 to 20º C for a gradual conditioning and to maintain clams with ripe gonads for longer periods (Hadley, et al, 1997). The conditioning tank includes an aeration system and salinity between 25 to 30 ppt (Hadley, et al., 1997). Under these conditions, northern quahogs develop ripe gonads in four to six weeks.

Thermal stimulation is the most widely used technique in hatcheries to induce rapid and synchronous spawning and improves seed production in northern quahogs. It consists of gradual changes in water temperature from 22 or 24º C to 28º C, and back to 22º C until spawning occurs. A modification of this method is the addition of sperm in the inhalant siphon to stimulate other clams. Several chemical methods have been evaluated for their spawning induction potential. However, only serotonin proved to be efficient but not as much as the thermal stimulation technique. Gibbons and Castagna (1985) evaluated the use of serotonin, a molluscan neurotransmitter, to induce spawning in clams. Their results demonstrated that the injection of 0.2 to 20 mM of serotonin in the anterior adductor muscle of ripe male clams, greater than 36.4 mm, stimulated spawning. Hydrogen peroxide is another inexpensive chemical method that is used for spawning induction in bivalves. The introduction of 50 ml of 6 % solution (dilution from 30% of hydrogen reagent grade) to 12 liters of seawater

stimulates the synthesis of hormone-like prostaglandin molecules, thus enhancing bivalve spawning (Morse et al, 1983).

Another hatchery advancement is feeding broodstock and larvae stages with mass-produced phytoplankton grown under controlled conditions. Presently, there are two hatchery methods for larval culture differing only in the techniques for algal production. The Wells-Glancy method, developed by Wells and later refined by Glancy, relies on blooms of phytoplankton concentrated by filtration or centrifugation (Manzi, 1985). The Milford method, developed by Loosanoff, consists of a controlled culture of a specific phytoplankton as food for clam larvae (Loosanoff and Davis, 1963) and is widely used in commercial hatcheries (Manzi, 1985).

Studies performed by several investigators showed that dietary protein and lipid must be present in phytoplankton used as food to maintain rapid growth and survival of different life stages in northern quahogs (Gallager and Mann, 1986; Helm and Laing, 1987; Wikfors et al., 1992; Fidalgo et al., 1998). After hatching occurs, the first larval stage (trochophore), as well as pediveliger, during metamorphosis to the plantigrade stage, larvae do not feed and depend on their nutritional reserves. Therefore, lipids are the major essential factor for larval development during the metamorphosis process of pediveliger larvae.

Although a variety of dinoflagelates and diatoms are used in clam culture, starting with the veliger stage, *Isochrysis galbana* and *Chaetoceros calcitrans* are used the most for feeding these bivalves (Hadley et al., 1997). Both phytoplankton species have good nutritional value and they are high in lipid (Wikfors et al., 1992; and Fidalgo, et al., 1998).

Diseases under hatchery conditions are uncommon and are mostly related to high clam stocking density, high temperature and poor sanitation of the facilities (Leibovitz, 1978). In the hatchery phase, vibrosis and larval mycosis may cause

mass mortalities: (Kraeuter and Castagna, 1984; Ford, 2001). Observations of mass mortalities due to *Pseudomonas* and *Vibrio* have been reported in larvae and newly set clams (Leibovitz, 1978; Elston, 1990). Larval rearing containers in bivalve cultures may suffer from ciliate infestations; however, they are mainly an indication of high bacterial concentration. Investigations by Plunket and Hidu (1978) demonstrated that the marine ciliate, *Uronema marinun*, fed on the bacteria in highly infected tanks rather than on tissue of the American oyster, *Crassostrea virginica*.

In the Caribbean region only three known studies had been conducted with *M. mercenaria* and *M. campechiensis.* Sunderlin et al. (1975) compared the growth of these two species and their hybrids in Saint Croix (U.S. Virgin Islands) using cold water from an artificial upwelling mariculture system. Results showed that clams attained marketable sizes in 13 months and the best survival rates were obtained with the hybrids. However, high costs in pumping cold water from depths of 870 m made this culture method impractical and uneconomical.

Juste (1987) evaluated the technical feasibility of clam culture in cages in the southwest coast of Puerto Rico using imported clam seed. These cages were placed in wave protected areas with sandy to sandy-gavel bottom near cays in La Parguera, Puerto Rico. Her results were discouraging due to high mortalities from predation and high temperatures during the experiment. Genetic studies compared local populations of *M. mercenaria* with specimen from the northeast coast of the United States of *M. Mercenaria* to evaluate their electrophoretic patterns similarities (Juste, 1992). This study demostrated a high degree of genetic similarity between populations from the east coast of the United States and local clam populations.

The present study was the first attempt to raise seedstock from induced spawning of *M. mercenaria,* the northern quahog in a tropical environment, Puerto Rico.

Materials and Methods

A two-year study was conducted in the laboratories of the marine station operated by the Department of Marine Sciences, University of Puerto Rico, in Magueyes Island, Lajas, Puerto Rico. The experiment was carried out on a four-stage culture protocol: 1) broodstock conditioning, 2) induced spawning, 3) larval rearing tanks and, 4) clam larvae setting in a downweller system. The purpose of this investigation was to determine the feasibility to produce 1 mm-size seeds as a result of artificial spawning induction of *M. mercenaria* in Puerto Rico. It was also the intention of this study to obtain data on survival and larval growth rates of these organisms and compare them with clam production from commercial hatcheries in the United States. Spawning trials were repeated 15 times; while, the larval rearing phase and clam larvae setting were each evaluated only once.

1. Specimen collections

A first batch of 36 *M. mercenaria*, between 55 and 80 mm in shell length, collected in three locations along the Esperanza's Peninsula at Cataño, Puerto Rico (Figure 2), was used for broodstock individuals. Subsequently, four more batches of ten to 12 northern quahogs each, were obtained from the same sites of Esperanza's Peninsula at Cataño but at different time intervals during the duration of the experiment to replace dead clams. All northern quahog population from Puerto Rico was obtained from this location in Cataño.

Meanwhile, three additional batches of 80 *M. mercenaria*, between 50 to 81 mm in shell length, were donated from the University of South Florida and Harbor Branch Oceanographic Institution, both research facilities are located in Florida. These batches were sent to Puerto Rico at different times, also, to replace dead organisms during the experiment. All batches from both universities were considered at a single Florida population of northern quahog. The stocks from Florida were held in quarantine in Puerto Rico prior to their use as brooders.

Both of these populations were transported to the laboratory on a wet cloth and maintained in a cool environment with gel packs to lower their metabolic activity and to avoid spontaneous spawning. All animals were scrubbed clean of debris and were examined for possible external parasites prior to their introduction into their respective acclimation tanks. Ten clams of each population were killed to evaluate for possible presence of pathological conditions present in the tissues or inside and outside their shells. Northern quahogs were measured to the nearest 0.01 cm with a vernier caliper for shell length (anterior-posterior measurement). All individuals were marked with a magic marker to identify each population (Florida or Puerto Rico) and had a record of the collection date or arrival date. These information were used to identify the sex of spawning organisms, their gamete production in females and to evaluate the possible effect of time spent in the acclimation tank on the spawning behavior in *M. mercenaria*.

2. Histological analysis

Ten to 16 northern quahogs of each population were used for histological analysis of the gonads, based on the methodology used by other researchers (Blake and Hesselman, 1986; Hesselman et al., 1989 and; Morales-Alamo and Man, 1989). Each clam was opened and the total wet weight of the animal without the shell was recorded. Gonadal tissue of approximately four millimeters thick was removed. Each gonad segment was placed in Davidson's fixative for 24 hours, dehydrated in alcohol, cleared with xylene and embedded in paraffin. Later, sections of seven micrometers each were cut with a microtome, mounted and stained with hematoxylin and eosin and observed with a compound microscope.

Northern quahogs were sampled before and after the spawning induction trials. Gametogenic stages were identifyed following a modification of Jones

(1981), Blake and Hesselman (1986) and Kent et al. (1998) techniques. The characteristics used for each stage were:

Males

Early Active Phase: Thickened of alveolar walls, with spermatogonia, primary spermatocytes proliferating into lumen.

Late Active Phase: Secondary spermatocytes abundant, spermatids massing into lumen.

Ripe: Mature sperm form dense masses in alveoli.

Spawned: Partially or totally devoid of sperm, primary spermatogonia developing in thickening alveolar walls.

Females

Early Active Phase: Oogonia embedded in alveolar walls, early oocytes still attached to the membrane.

Late Active Phase: Enlarging oocytes fill lumina, some still attached to membrane.

Ripe Phase: Large and rounded oocytes fill lumina of follicle.

Spawned Phase: Partially or complete elimination of eggs, gonad appears flaccid.

Undeterminable: No visible signs of oogonia or spermatogonia.

3. Broodstock conditioning

Northern quahogs were transferred to an acclimation tank (Figure 3) prior to their spawning induction trials to prepare them for gametogenesis (Landers, 1971; Muranka and Lannan, 1984; Goodsell and Eversole, 1991; and Hadley et al., 1997). *M. mercenaria* individuals were placed in trays inside a 1500L-circular cement tank with a semiclosed, recirculating water system. Seawater was filtered through both, 1 and 5-micrometer mechanical filters and sterilized with an

ultraviolet lamp. The tank had complete of water exchange every 24 hours. To avoid toxic levels of ammonia, the water was aerated with an airlift system to maintain vertical circulation inside the tank. The acclimation tank was kept at 24 to 28ºC, with continuous water circulation from the one-micrometer filtered seawater from the algal feeding tanks described below (Figure 4). After 8 months, a chiller system was added at the bottom of the conditioning tank to maintain water temperature at 18±2 ºC (Figure 5). This procedure eliminated unwanted spontaneous spawning previously experienced in the conditioning tank (Landers, 1971) and reduced bacterial infections.

4. Phytoplankton feeding

Brooders larvae, and post-set stages of *M. mercenaria* were fed with live phytoplankton produced in a phytoplankton culture room prepared for this experiment. According to Goldstein and Roels (1980), Riisgard (1988), and Manzi and Castagna (1989) clam broodstock must be fed one time 1 to 3 billion algal cells/clam/day to produce ripe gonads under laboratory conditions. Brooders were fed with live phytoplankton.

The feeding regime consisted of a combination of the golden brown dinoflagelate, *Isochrysis galbana*, and the diatom, *Chaetoceros gracilis* cultivated in Guillard's F/2 medium (Guillard, 1983). Three months before the end of the experiment, the phytoplankton concentration in the conditioning tank was increased to 5 billion algal cells/clam/day. To attain this phytoplankton concentration, northern quahogs were fed with a combination of an algal paste of *I. galbana* and *Thalassiosira weissflogii*, another diatom that is widely used in shellfish hatcheries in order to obtain good gonad condition in less than one month. This algal paste, commercialy known as Instant Algae®, consists of a high concentration of phytoplankton which is mixed with water and it is subjected to a proprietary process to prevent bacteria growth (Reed Mariculture, 2004). To

concentrate the phytoplankton, the company use a large-scale centrifuge, which also, disrupts the internal mechanisms of this organism and prevent it from reproducing (Reed Mariculture, 2004). Based on this procedure, the company sells each 1L-package of *I. galbana* and *T. weissflogii* with a phytoplankton density of 3.1 x 10^9 and 1.8 x 10^9 per mililiter, respectively. No algal paste was used in larval rearing cultures to avoid water quality problems within the larval culture tanks.

Early veliger larval stages were fed only *I. galbana* at a density of 1,000 to 15,000 cells per clam/day (Hadley et al., 1997). Once the animals attained the late veliger, pediveliger and post-set stages the ration was adjusted to a minimum of 30,000 to 150,000-cells/clam/day of *I. galbana* and *C. gracilis* (Hadley et al., 1997)

5. Spawning induction procedure and reproduction

One day before spawning induction occurred, one or two northern quahogs were dissected to observe gonadal state. *M. mercenaria* from each population were scrubbed and rinsed with a solution of freshwater and ten percent of white cooking vinegar to remove algae and other fouling organisms. Afterwards, they were placed on a spawning table and left overnight in a controlled environment with 18ºC seawater at 29 ppt salinity (Figure 6). The spawning table was a wooden trough, six-inches deep with recirculation system and drainage. Sides and bottom were painted black to provide a contrasting background to the color the eggs and sperm. Clams were left overnight on this table before spawning induction trials began.

Attemps to spawn broodstock clams were made by thermal stimulation. This method uses temperature changes from 18 to 30ºC. The temperature was regulated placing ice in zip-lock bags to cool the water or by pouring boiling seawater into the entrance pipe of the recirculation system to warm the water.

Each spawning trial consisted of three consecutives cold-hot cycles which was a modification of various procedures presented in previous works (Loosanoff and Davis, 1950; Castagna and Kraeuter, 1981; Castagna, 2001: and Blake, 2002, personal communication). The protocol was as follows:

1. Phytoplankton feed was added to the spawning table before the induced spawning commenced to ensure that around 40% of the clams were open and siphoning. This step lasted 15 to 30 minutes.

2. Water temperature was raised from 18 to 20ºC and after 5 minutes the, water temperature was then raised to 28ºC in between 30 to 45 minutes. The temperature was then kept at 28ºC for 30 to 45 minutes.

3. If no spawning occurred, then the water temperature was dropped to 18ºC slowly to begin another cycle. Tempeature reduction lasted approximately 30 to 45 minutes. When water temperature reached 18ºC, it was held for 30 to 45 minutes before restarting.

4. At the end of the third cold-hot cycle, water temperature was reduced at a faster pace, but avoiding changes of more than 1ºC per minute. This step lasted approximately 13 to 15 minutes.

5. If no spawning occured after 3 cold-hot cycles the procedure stopped and it was reiniciated the next day. In some cases, the spawning trial was resume after two to four weeks, if more than 50% of the clams displayed poor behavior during the thermal stimulation.

6. If spawning occurred, sperm suspension extracted from a male spawner was applied to the inhalant siphon of the other clams as an additional stimulation for brooders (Castagna and Kraeuter, 1981).

7. Each spawning clam (Figure 7) was transferred to a container with seawater at the same temperature at which spawning commenced.

Gametes from spawning clams were examined with a compound microscope to determine the sex of the spawner and a hemocytometer slide to determine the number of female gametes or immature animals). A 0.01 ml of water containing gametes of each female spawner was placed on a hemocytometer to obtain an estimate of the total number of the number of eggs per female. The gametes were washed and rinsed through a 150 μm sieve to eliminate feces and other particulates.

Sperm were then added to the egg 2 L container at a rate of 1000 to 2000 sperms for each egg (Hadley et al., 1997). Once the eggs were fertilized, unused sperm were removed by straining the all eggs on a 33 μm-sieve and then rinsed with them with a 1 μm filtered seawater using a spray nozzle plastic bottle. Later zygotes were placed in 38 L culture plastic containers.

6. *Mercenaria mercenaria* response during spawning trials

The behavior patterns of northern quahog individuals from each population were observed during each trial. Changes in clam behavior at each cool or warm cycle were recorded and three categories were developed to quantify and evaluate the data of the each clam. The categories used were as follows:

0: No reaction of the clam or siphon out for feeding.
1: Clam heavily siphoning and about to spawn.
2: Clam spawned.

7. Larval rearing tanks

Fertilized eggs were distributed into 38 L conical polyethylene tanks at a stocking density of 10 zygotes per ml of *M. mercenaria*, with 16 replicates. Each 38 L plastic cultture container was labeled with the spawning date and the initial stocking density. Culture tanks were slightly aerated to avoid the settlement of early veliger larvae. These larval rearing tanks were placed in a semiclosed one story room; therefore, each of the 38 L plastic culture containers had a loose cover to avoid airborne contamination. Each tank had a daily seawater exchange at a rate of 50% with the same temperature and salinity as its culture water. During water exchanges, clam larvae were rinsed through a 33µ plastic mesh sieve to remove dead individuals and debris and to retain healthy larvae. Salinity was maintained at 29 ppt during all the larval rearing culture procedure. A complete drain down was performed weekly in each of the 38 L plastic culture containers. During this procedure, every culture container was scrubbed with a 10 % iodine solution and tap water. If pink spots were visible, then the culture container was scrubbed with solution of 10 ml household chlorine (5.25 % active ingredient) in 1 L of tap water. To avoid chlorine residues in the culture containers, they were thoroughly rinse with water and a solution of 20% of sodium thiosulfate

Every seven days, samples of 25 individuals were evaluated with a compound or inverted microscope to observe their larval development, larval behavior, the presence of pathogenic organisms and measured with an ocular micrometer (Manzi and Castagna, 1989; Hadley et al., 1997). A Sedgewick-Rafter counting cell and a graduated cylinder were used to count the number of larvae per container to calculate survival rates and feeding rations.

No food was added until larvae reached D-veliger stage (Gallager and Mann, 1986; Hadley et al, 1997). Once the larvae were one day old, they were fed with

Isochrysis galbana at a density of 1000 algal cells per larvae for about eight to ten days. By the time larvae attained pediveliger stage, 170 to 230 µm shell length, a velum and a visible foot, they were transferred to the next culture system.

8. Downwellers

Pediveliger clams were held in 9.5 L plastic buckets or cylinders (15 cm diameter x 25 cm deep) with a 150-micron mesh screen attached to the bottom, which supported the clams but it had a removable safety ring so the mesh screen could be replace as larvae grew, to hold only the healthy animals and to remove the weak or dead ones. Each cylinder was suspended inside a 38 liter-plastic tank with an airlift installed on the outside of the bucket (Figure 8). The airlift consisted of a 1.25 inch-PVC elbow and a 14 inch in length 1.25 inch-PVC pipe with an airline and an airstone for tank aeration. This action pumped the water from beneath the mesh screen back to the clams, consequently, creating a downwelling effect**.**

Clams at pediveliger stage were fed a single batch of algae at a rate of 30,000 to 50,000 cells/larvae/day (Manzi and Castagna, 1989; and Hadley et al, 1997). The procedure for water changes and clam examinations were the same as those performed for larval rearing tanks. Dead or moribund clam larvae were discarded at each weekly sampling.

Once the clam larvae attained 500 µm, they metamorphosed into post-set organisms. This stage was evident at eight to 14 days post-fertilization. Food concentration was around 75,000 to 150,000 of live algal cells/clam/day. Half the water volume of each tank was changed on a daily basis. During this time clams were counted using volumetric procedures, their survival and growth rates were also determined.

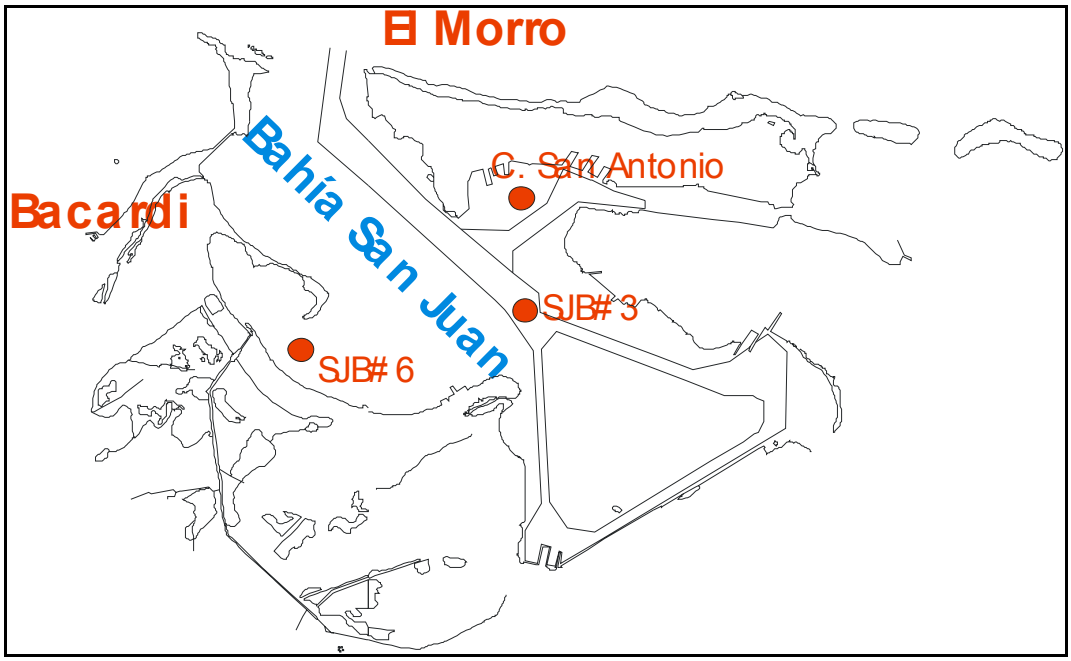
9. Physical parameters

Seawater flowed through a sand filter and ultraviolet lamp and eventually was screened through a one-micron mesh bag for use in the conditioning tank, spawning phase, larval rearing tanks, and downweller silos. At each stage of the experiment, temperature, salinity, dissolved oxygen concentration and pH were monitored. Temperature (±0.5ºC) and dissolved oxygen concentration (±0.2 ppm) were recorded using a portable YSI oxygen meter. Salinity readings (±1 ppt) were recorded with a hand refractometer and the pH (±0.01unit) with a pH meter. Every three days, water samples from the conditioning tank were obtained for ammonia and nitrite analysis using LaMotte colorimeter kits.

10. Statistical analysis

A descriptive analysis was used to compare larval growth rate, survival rate and time for settlement against data from the experiment and the available information from commercial clam hatcheries. A generalized estimating equation for regressions in time (SAS,version 8.0, 2003) was used to evaluate clam growth and survival with water quality parameters. Differences in clam responses of Florida and Puerto Rico stocks to the thermal stimuli, clam size, and conditioning time were analyzed with a binomial non-parametric Kruskal-Wallis test (R program, version 8.0, 2004). All statistical differences were determined at 95% confidence interval (Ott, 1993).

Figure 2
Map of the *Mercenaria mercenaria* collection sites of Puerto Rico stock



Courtesy of United States Geological Survey
Red dots identify collector sites of the northern quahog, *Mercenaria mercenaria*.

Figure 3
Acclimation tank airlift system for *Mercenaria mercenaria* broodstock conditioning.

Figure 4
Phytoplankton feeding tank for *Mercenaria mercenaria* broodstocks

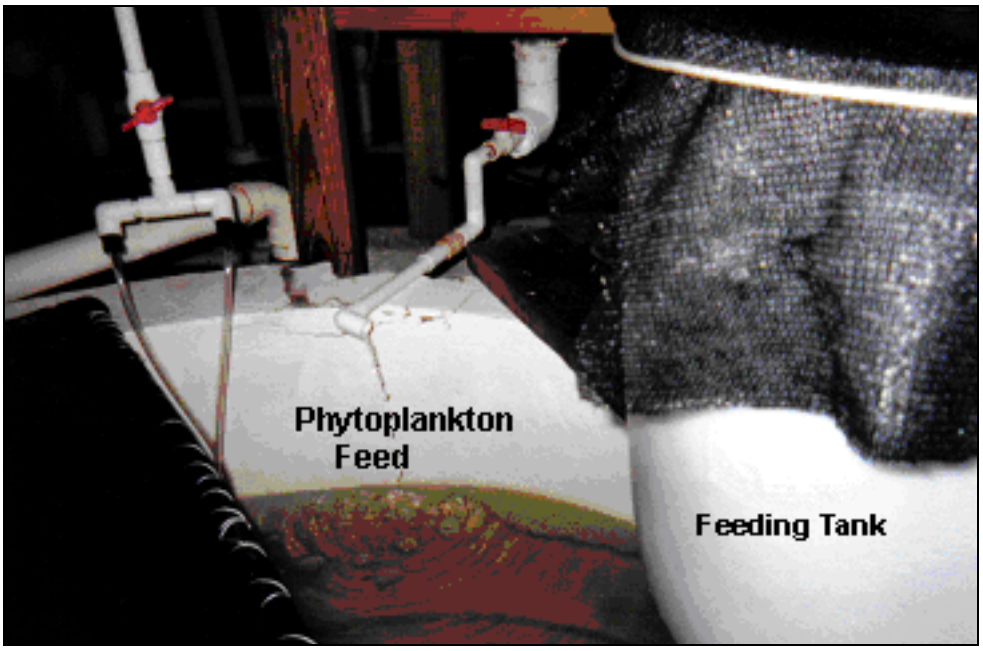


Figure 5
Chiller unit to maintained seawater temperature at 18± 2º C in acclimation tank

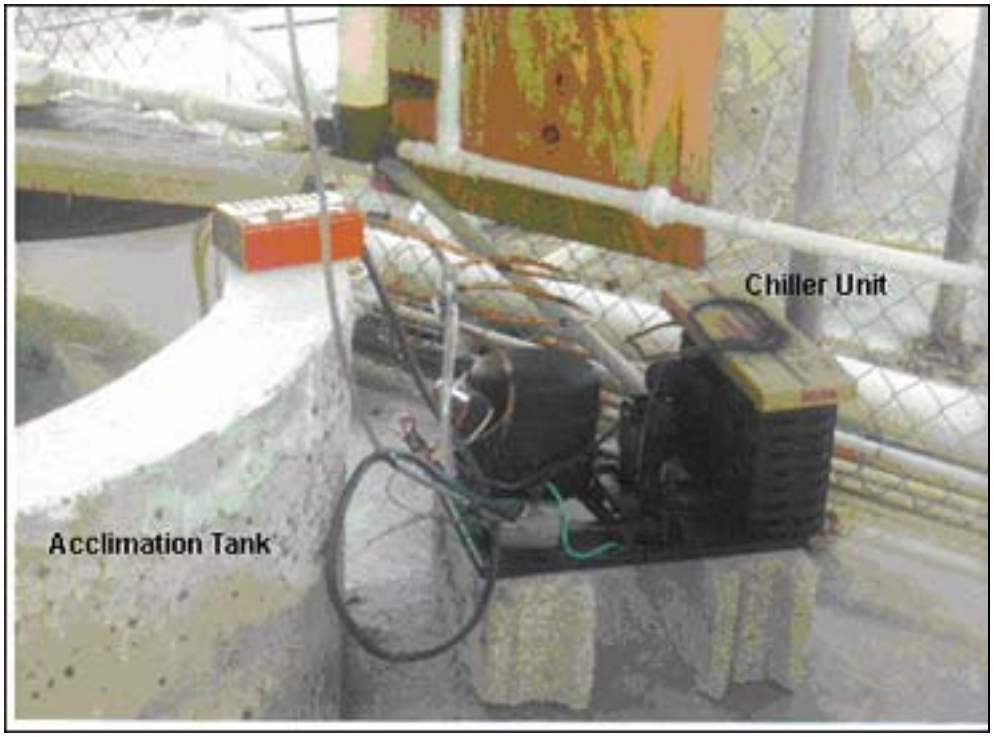


Figure 6
Spawning table with water recirculation system

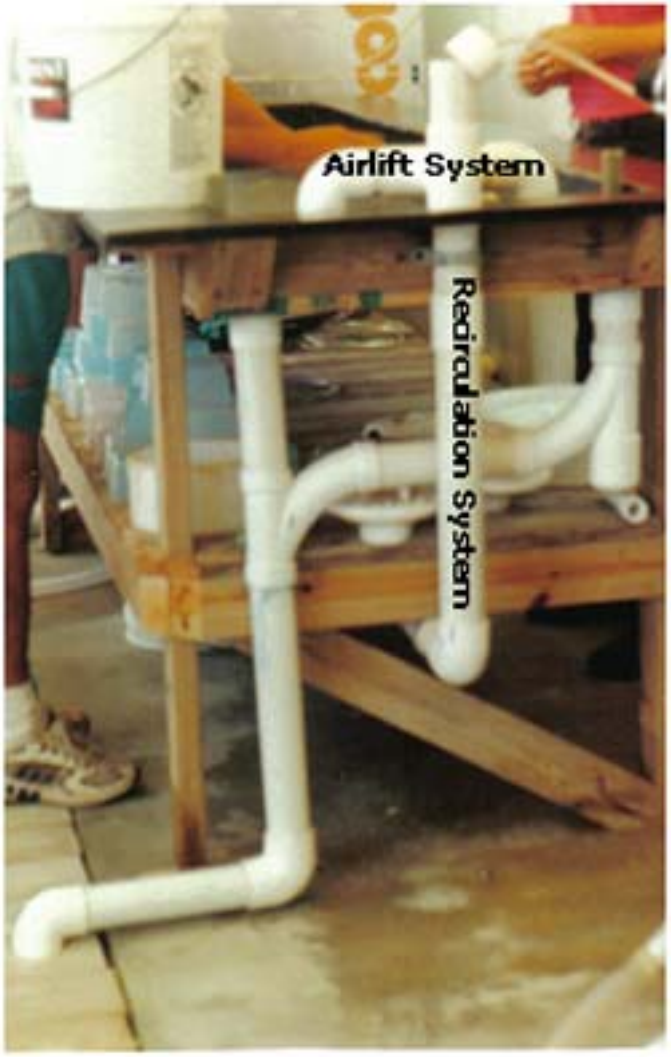


Figure 7
Spawning *Mercenaria mercenaria* using thermal stimuli technique

A. Spawning Female

B. Spawning Male

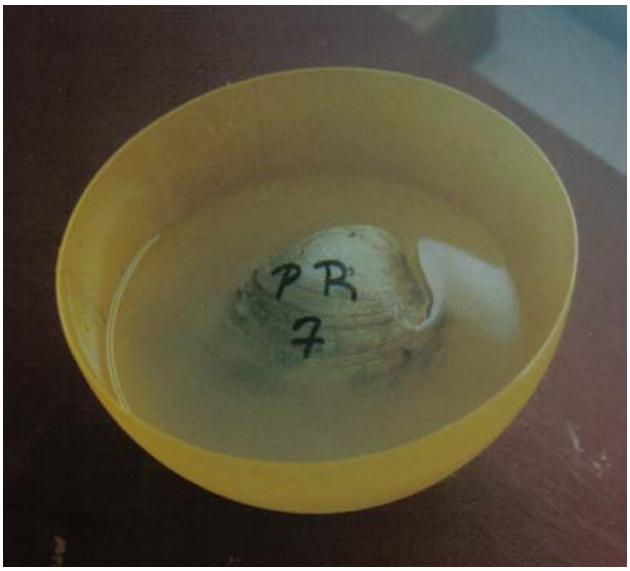
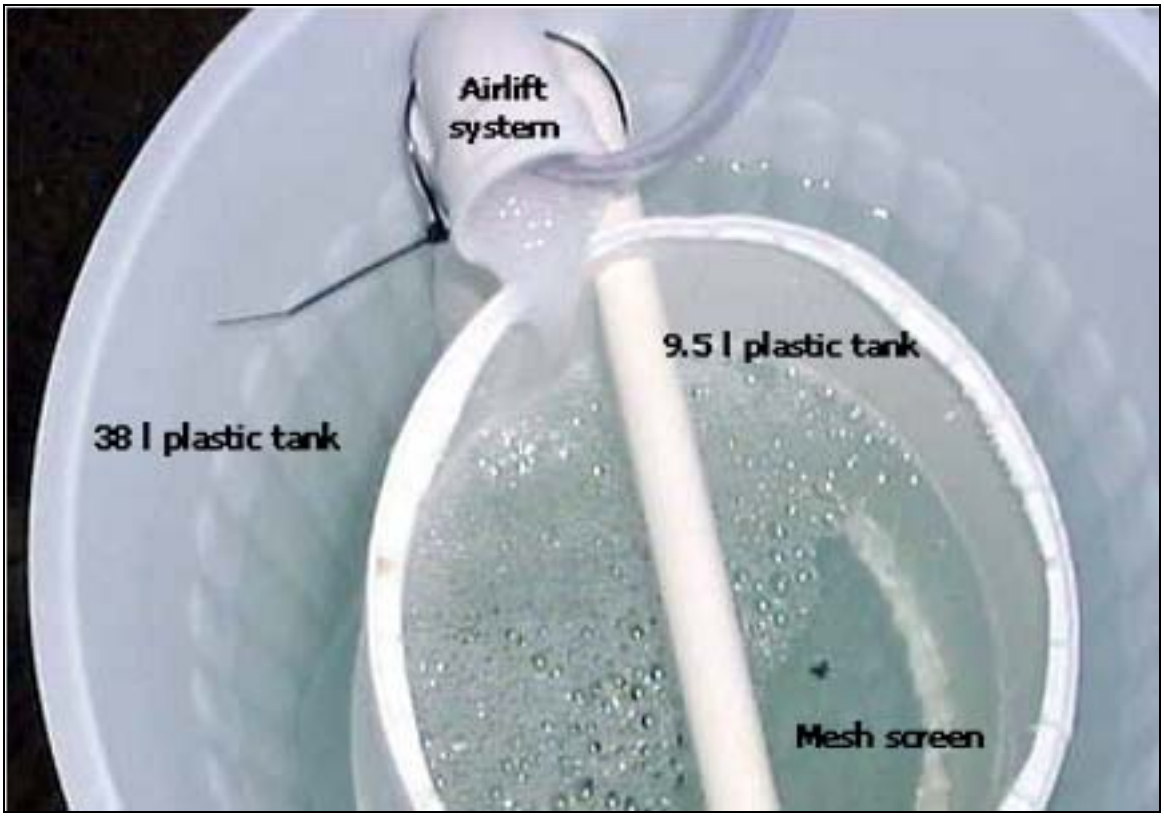


Figure 8
Downwellers used to rear early and post-set *Mercenaria mercenaria*



Results

1. Induced spawning: response of *Mercenaria mercenaria* from Florida and Puerto Rico stocks.

A. Determination of gonadal development of both stocks before and after their conditioning protocol.

Gonadal histological preparations of both populations of *Mercenaria mercenaria* were analyzed to evaluate their reproductive potential before their introduction into the conditioning tank. The same procedure was also performed at the end of the experiment in order to evaluate possible changes in gametogenic stages of these specimens. Clams with a shell length greater than 46 mm were used for histological analysis to avoid immature animals. The results of this analysis are summarized in Table 2.

Histological examinations from 16 clams from Florida population prior to their transference to the conditioning tank revealed that nine were males; their gonads were characterized by the abundance of sperms in the late active development and ripe stages (Figure 9b and 9c). In this Florida population, three males (Figure 9a) and two females (Figure 10a) showed an early active development stage. Also, two of the females were partially spawned (Figure 10c), which were probably due to a response to the stress caused by the airplane trip and their manipulation upon arrival. These clams were preconditioned in an acclimation tank to develop ripe gonads before their shipment to Puerto Rico, so they could be used as a baseline to compare spawning response of local stocks. No pathological conditions were observed in the population from Florida.

In contrast, the local stock with no previous conditioning under laboratory conditions, presented a different gonadal tissue pattern. Gonad samples from these 10 clams indicated that four of the individuals were reproductively

undeveloped with neither visible signs of oogonia or spermatogonia in the follicle area (undeterminable stage) (Figure 9e). Three northern quahogs were in their spawned phase (Figure 9d) (Figure 10c) and one male presented an early active development phase (Figure 9a). Although no previous work has been performed on the gametogenic stages of *M. mercenaria* in Puerto Rico, these data suggest that they might have been in the resting stage prior to commencing gametogenesis. Pathological conditions in one of the specimens from the Puerto Rico populations were also noticed. Northern quahog with this condition showed abnormal germ cells arising form the germinal epithelium and filling the lumen of the follicles (Figure 10d). It was observed the occurrence of this pathological condition in northern quahogs above 70 mm in shell length. Based on this information, there is a possible relation between the condition observed in some specimens of *M. mercenaira* and a PCB 1260 spill that occurred in San Juan Bay in September 1985. Juste (1987) observed abnormal gonadal growth and no reproductive activity in clams collected from the San Juan Bay after the spill. Abnormal cell growth in the gonadal tissues of northern quahog, as observed in this study, might arrest or eliminate gametogenesis in affected organisms (Hesselman et al., 1989).

Histological preparations after conditioning, presented more than 50% of both populations of northern quahogs in a spawned stage. Three clams of the Florida stock displayed pathological conditions in their gonads. This is unusual in organisms raised under controlled conditions. However, these clams as a normal procedure were introduced in a dilute muriatic acid (10%) bath, prior to their arrival to Puerto Rico to avoid possible introduction of ectoparasites. It is possible that some of these northern quahogs opened their valves during this procedure, thus, affecting their gonads. Local stocks presented five clams in their ripe phase and another five in their spawned phase.

B. Responses of *M. mercenaria* to spawning induction by thermal stimuli: Florida and Puerto Rico stocks.

In a three-year study, 15 spawning induction trials using the thermal stimuli method were performed to evaluate and compare clam responses between the Puerto Rico and Florida stocks. The results of these trials are summarized in Table 3. Overall, three of the 15 induced spawning trials resulted in a positive response from the clams. In the successful cases, spawned clams were obtained in the third thermal cycle of these trials. However, these data are not consistent with spawning responses to the thermal shock method presented by other researchers. Previous researches have reported that in any induced spawning event between 15 to 20% of mature clams spawn during the first cycle (Ansell, 1967; Bricelj and Malouf, 1980).

The total number of clams used during all of the induced spawning trials was 375, but only 23 successfully spawned; seven females and 16 males. Males spawned before than females at temperatures above 24ºC, but females displayed a better spawning behavior at temperatures between 26 and 28ºC. The average number of eggs per spawned female was 1.37×10^6.

Due to the low percentage of successful spawnings, the thermal stimuli method as a clam spawning induction technique was evaluated. An exact binomial test was used to analyze if northern quahogs reacted to sudden changes in water temperature. Heavy siphoning activity (#1) or spawning (#2) were considered positive reactions and closed or feeding clams (#0) were an indication of no reaction to the spawning stimuli. There was a highly significant difference ($P<0.001$) between thermal stimuli method and clam response in all three thermal cycles of each spawning trial (Appendix B).

It was also evaluated the possible relation of clamshell sizes and the clam response to the thermal stimuli. A non-parametric Kruskal-Wallis test was used to analyze the data. No significant differences ($P>0.05$) were observed, thus, there is not a clamshell size effect on the spawning response of the specimens used in this experiment (Appendix C).

However, there was an effect on the number of days clams were conditioned in the acclimation tank and clam behavioral response to thermal stimuli based on the three cycles of each trial. Figure 11 shows a negative effect of time spent by clams in the conditioning tank and their response to the spawning induction method for cycles two ($P<0.001$) and three ($P<0.001$) of each induced spawning trial (Appendix D). These results indicate that the longer the time northern quahogs were in the conditioning tank, prior to thermal stimuli, the less positive clam response (heavy siphoning and spawn) were obtained to the induced spawning method. Clams that spent between one and 14 days in the acclimation tank had a better behavioral response than individuals with more conditioning time. Furthermore, clams did not display any positive behavioral response during cycle number one in any of the induced spawning trials. These findings do not coincide with reports by other authors (Utting and Millican, 1997; Eversole, 2001). Based on these results brooders properly conditioned do spawn within one hour of spawning stimulus. Therefore, a detailed evaluation of the techniques used for conditioning of brooders in this study may present a possible explanation to the low spawning performance of northern quahogs.

C. Acclimation tank parameters.

Hatchery conditioning of bivalve broodstocks has been implemented in commercial clam culture as a mechanism to improve their spawning efficiency and to obtain better egg survival and larval viability (Manzi, 1985; Utting and Millican, 1997; Eversole, 2001). Moreover, under proper management, clam

conditioning might prolong natural spawning season in these organisms. To attain this goal, it is necessary to maintain control over some environmental and biological requirements that control the gametogenesis cycle in clams.

The rate of broodstock conditioning is regulated by three important factors: temperature, salinity, and food availability. It is known that temperatures between 23 and 28ºC increase the rate of maturation in spermatogenesis and ovogenesis. However, temperatures above 30ºC or below 15ºC have a direct negative effect on gametogenesis in northern quahogs (Manzi, 1985; Hesselman et al., 1989; Eversole, 2001). Salinities under 25 ppt can affect oocytes development and ova maturation (Hadley et al., 1997; Utting and Millican, 1997). In addition, food quantity and the nutritional value of the algae fed must meet clam requirements to produce healthy gametes and, consequently, the production of high quality eggs.

Water quality parameters were recorded every three days in the conditioning tank for 30 months. Table 3 shows monthly average readings of water temperature, salinity, pH, ammonia, and nitrite levels. Dissolved oxygen levels, salinity, pH, nitrite, and ammonia concentrations were below relative safety ranges for clam conditioning. However, there were problems with water temperature and food availability of algae during the conditioning period of brooders.

The conditioning tank was located in a semi-closed laboratory; therefore it was subjected mainly to bacterial contamination. Consequently, a chiller and a recirculation water system were placed inside the conditioning tank. At lower temperatures bacterial diseases are reduced. The temperature was maintained at 18±2°C for gradual conditioning of northern quahogs. At the end of the first year, problems with the temperature sensor of the chiller resulted in temperature changes from 15 to 22°C inside the acclimation tank. Hadley et al. (1997)

demonstrated that for gametogenesis to occur, steady temperatures must be achieved.

Furthermore, clams were fed with a combination of *I. galbana* and *C. gracilis* (or *C. calcitrans*), both phytoplankton species highly recommended for broodstock conditioning due to their high lipid and essential fatty acids components needed for a proper gonadal development (Hadley et al., 1997, Fidalgo et al., 1998; Castagna, 2001). Due to logistical problems, algae culture stocks were not at times sufficient to maintain proper daily feeding levels. Also, brooders were fed with algae cultured in outdoor 200-liter cylindrical tanks and as the culture ages, its biochemical composition, mainly lipids and fatty acids undergoes chemical changes (Fidalgo et al., 1998) which might delay or arrest gametogenesis to attain ripe gonads. Several authors (Manzi, 1985; Eversole, 1989; Castagna, 2001) have mentioned that for clams to spawn gonads must reach a certain degree of ripeness.

2. Larval rearing of *Mercenaria mercenaria*

A. General Information

Larval culture of northern quahogs lasted 62 days, following the same culture procedures used in commercial hatcheries. This protocol included five components: northern quahog zygote transfer to each of the 16 larval rearing tanks; their relocation to the downwellers; daily partial water drain down (50% of total water volume) and algae feeding in all experimental units; water quality measurements; and weekly counts and measurements of larvae to determine survival rates and shell growth in all tanks.

During the experiment, high mortalities occurred in two of the rearing tanks. First, a persistent bacterial infection in one tank killed 98.6 % of the larvae. In the second unexpected heavy showers caused rainwater to run along the treated wooden beam supporting the roof and dripped into one of the tanks, the day when zygotes were stocked into the rearing tanks. Treated wood has copper chemicals, which is very toxic to clam larvae and juveniles (Castagna and Kraeuter, 1981). Consequently, low survival rates (0.19%) and poor growth performance suggest that this contaminated water entered the tank, having a negative effect on clam larvae development.

At the beginning of the experiment, each rearing tank was stocked with 283 500 fertilized eggs; by the end of this study, the production per culture vessel varied from 12 871 to 35 998 clams of one mm size. The box-plot illustrated in Fig. 12 shows the average distribution of individuals during the nine weeks of the experiment. This distribution is asymmetrical based on the position of the median (horizontal line inside the box) near to the upper line or quartile. Therefore, the number of individuals per rearing tank at any given week was very similar. The high percentage of clam larvae losses during the first three weeks of the experiment is also obvious.

B. Comparative data analysis of larval stages raised under controlled conditions in Puerto Rico and the southeastern region of the United States.

Table 5.shows the data collected from of the average shell length growth rate of the 14 38 L culture tanks. Table 5 also, includes the percentage of larvae retained in different sized mesh sieves were used as the measure of larval survival. However, there are no previous scientific studies in the Caribbean region to compare and evaluate northern quahog larval development under tropical environmental conditions. Therefore, the information used to evaluate and analyze the data obtained in this experiment was based on successful larval rearing under laboratory conditions in places with well-established culture techniques in the southeastern region of the United States.

The information was compiled mainly from commercial hatcheries located in Florida and South Carolina, and from applied research studies from Harbor Branch Oceanographic Institution and South Carolina Sea Grant Consortium. Techniques utilized in this research project were based mainly on the information obtained from the aforementioned institutions. Therefore, the data summarized in Table 6 may serve as a guideline to analyze the results of this study.

i. Planktonic (free swimming) life stage

The first two days after fertilization, a 5 ml sample of every tank was examined microscopically to evaluate the general condition of the larvae. They were actively swimming and their ciliated veli were visible. Early or D-veliger, umboned and late veliger clam stages were observed during the first two weeks of the experiment. Average survival rates in all containers for the first and second week were 47.47 and 61.89%, with a median shell length of 168 and 196 µm, respectively (Table 5). The first larval stages of the experiment compare favorably with the data obtained from commercial hatcheries presented in Table 6.

However, survival rate for the first week was lower than estimates from Table 6. This situation may be related to the presence of some ciliates in the bottom and walls of seven of the rearing containers, eventhough water exchanges were performed daily. Ciliates are indicators of bacterial contamination. Based on these findings, clam hatcheries lower their culture densities to 1-2 larvae per ml by the end of the first week to avoid overcrowding, debris and feces accumulation, and bacterial contamination (Manzi, 1985; Hadley et al., 1997).

Such practice was not considered for this study. As a matter of fact, average stocking densities of culture vessels for the first and second week of the experiment were 4.75 and 2.90/ml, respectively (Table. 7). A possible explanation for lower survival rates for the first week may be attributed to overcrowding. Moreover, the second week data indicates an increase in survival rate with the lower stocking density than during the first week.

Pediveliger, a very sensitive and short-lived stage, was observed during the third week of the study. Some pediveliger larvae were observed crawling on the bottom of the culture containers, while others, were swimming in the water column at the beginning of the third week. The presence of the velum and the foot was also evident. During this transitional stage, larvae metamorphosed and changed their life style from planktonic to a benthic. Larval survival and average shell length of the study (Table 5) are very similar to the values presented in Table 6.

The presence of ciliates during the third week was obvious although sanitary procedures were improved. Investigations by several authors discovered that during this stage, the larvae seldom eat and mainly depend on their food reserves (Davis and Calabrese, 1964; Manzi, 1985). Therefore, it is suggested that the presence of ciliates is a result of an increase in bacteria concentration due to the uneaten algae in the culture tanks. Pediveliger larvae were retained in

the larval rearing tanks until they reached around 300 μm in shell length to assure that the majority of the larvae were at that stage. At the end of the third week, larvae were transferred to the downwellers.

ii. Plantigrade (dissoconch) stages

Early and mid-post set individuals characterized the appearance of an adult shell or dissoconch for weeks 4 and 5 of the experiment. The presence of different clam length sizes was noticed; consequently, two or three different mesh screen were used to obtain more reliable average measurement data. Average survival rates and average shell length for weeks 4 and 5 show similar results to data from Table 5. Daily shell growth rate for the first five weeks of the experiment ranges between 4 and 17 μm (Table 8). Hadley et al. (1997) established the daily shell growth of *M. mercenaria* between 10 to 20 μm under laboratory conditions. Culture containers for the experiment presented a similar trend, except for week 2.

Survival rates for the last weeks of the study (six, seven and eight) surpassed 90% (Table 5). Therefore, the experiment obtained better survival rates than those found for commercial hatcheries (Table 6). Survival rates and average shell growth of these late post-set clams obtained in the study are very similar to the results for commercial hatcheries (Table 6). In all cases, the majority of the clams were retained in the highest mesh screen sizes (500 and 710 μm). Highest daily growth rates of these clams were obtained for weeks 6 and 7, with values of 32 to 44, and, 36 to 44, respectively. These findings surpass the data presented by Manzi (1985). He observed that late post-set clams could grow at a rate of 20 to 30 μm per day.

Under hatchery conditions, the larval rearing phase ended when the clams attained 1 mm shell length or can be retained in a 710-mesh screen; for the most part attained by week 8. In the present experiment, some culture containers still

had 16% of the clams below 1 mm in size by the end of the eighth week. Therefore, the experiment was extended for another week to determine if these clams would reach the target size in few days. Although 84% (Table 5) finally attained 1 mm size, cost production and the additional time would make it uneconomical.

iii. Water quality parameters

Water parameters were monitored before noon because the experimental area received indirect sunlight during morning hours. Water quality values recorded during the experiment were similar to the ones recommended for larval rearing culture (Manzi, 1985; Hadley et al, 1997; Aquaculture Center for Training, Education and Demostration (ACTED), 1997). There were no significant differences ($P>0.05$) between clam survival rates and water physicochemical parameters (Appendix E).

Average temperature readings in all tanks ranged between 25 and 32ºC. Temperature was slightly higher in some rearing tanks because of their proximity to the walls in the experimental area. However, it did not affect survival rates in the experimental units**.** Figure 13 shows the distribution of average temperature readings throughout the experiment.

Dissolved oxygen concentrations in the tanks were controlled with an aeration system to avoid high mortalities of the clam larvae. Figure 14 presents a graphical representation of oxygen readings during the experiment. These readings oscillated between 4.0 and 5.8 mg/l. The distribution of pH readings during the experiment is presented in Figure 15. No dramatic changes were recorded and pH distribution fluctuated between 7.9 and 8.2. Freshwater was used to maintain salinity concentration at 29 ppt.

Average survival rate for all 14 38 L rearing tanks was 9.32±2.37 (Table 5) and 82% of those northern quahogs attained 1 mm size by the eighth week, which is a slightly lower value than the average survival rate for commercial hatcheries (Table 6). In spite of this, the data obtained in this experiment in relation to survival and larval growth rates, compare favorably, and in some cases surpass, the published production data obtained from hatcheries in the southeastern zone of the United States. As such, strict care must be observed with sanitary procedures to avoid high loses due to the high concentration of bacteria, mainly when water temperatures are above 30^0C.

3. Diseases of hatchery cultured *Mercenaria mercenaria*

Aquaculture reared organisms are susceptible to pathogenic infections because they are grown in high densities. Hatchery techniques also culture clam larvae and juvenile at high concentrations, thus providing opportunities for the development of infections by pathogens. The most common pathogenic agents found in clam of larvae and juveniles' clam hatcheries are bacteria, fungi and protozoa (Gibbons and Blogoslawski, 1989; Ford, 2001).

Although, good sanitary procedures were practiced in this study, bacterial infection was observed in six of the culture vessels, during the first 3 weeks of larval rearing. High ambient air temperatures, limited control of airborne dust in the experimental area and clam overcrowding may be as causal agents for these infections. Although phytoplankton feeding could be considered as a possible infectious agent, it was discarded due to the strict sanitary procedures performed in the algae culture room.

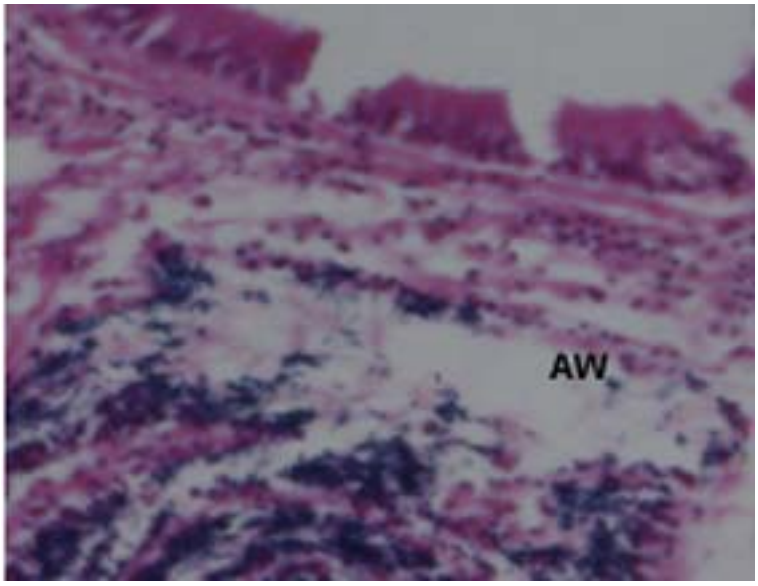
Pink stains were visible on the bottom and walls of these tanks, which are characteristic of *Pseudomonas* sp. (Gibbons and Blogoslawski, 1989; Hadley et al., 1997; Ford, 2001). These pink stains were also observed in the conditioning tank when temperature exceeded 20^0C. Brown (in Gibbons and Blogoslawski, 1989) discovered that concentrations of 1 x 10^6 to 1 x 10^7 cells/mL of *Pseudomonas* sp. might cause between 10 to 100 percent of mortality in clam larvae and juveniles. Although no conclusive statements can be considered, these bacteria may be related to the high mortalities observed during the first two weeks of the experiment in culture vessels. In fact, one of the larval rearing tanks had 98% of mortality.

During weekly measurements, the presence of some ciliates was observed in the water and inside dead clams. These protozoans are mostly associated with

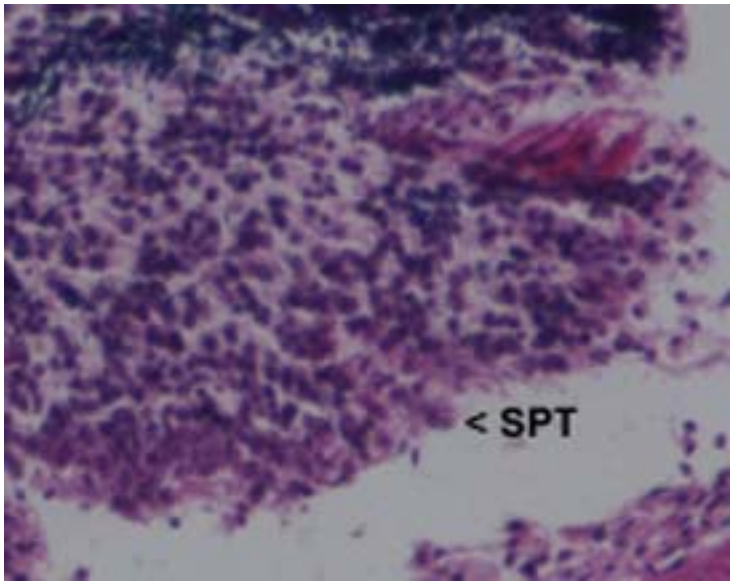
bacterial blooms but do not harm clam larvae or juveniles. However, Loosanoff (1959) found that the ciliate, *Condylostoma* sp. may ingest larval clams in laboratory cultures. These ciliates were also observed in outdoors algae culture and in the acclimation tank. The use of chlorine instead of iodine solution during bacterial outbreaks was implemented during the experiment to overcome this problem. These procedures controlled ciliate populations within the larval culture rearing tanks and conditioning tank. However, in outdoor phytoplankton culture their concentration was reduced but not eliminated.

Histological examination of the gonads did not show the presence of parasites, trematodes or necrotic tissue. However, pathological tissues were observed from some clams of the Florida and Puerto Rico stocks. Clams from Florida came from a hatchery that as a normal procedure used muriatic acid bath for sanitation purposes prior to shipping them to Puerto Rico. Therefore, the presence of pathogenic tissues could be a response to this chemical stress.

Figure 9
Sections of gonadal tissue from male *Mercenaria mercenaria*

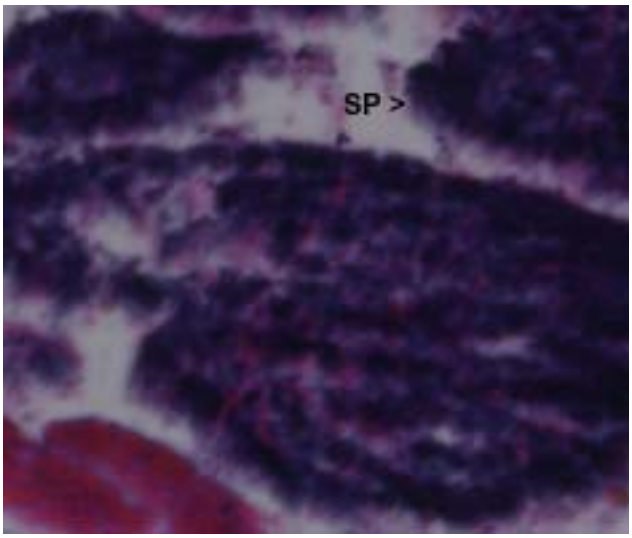


a. Early active development AW: alveolar walls

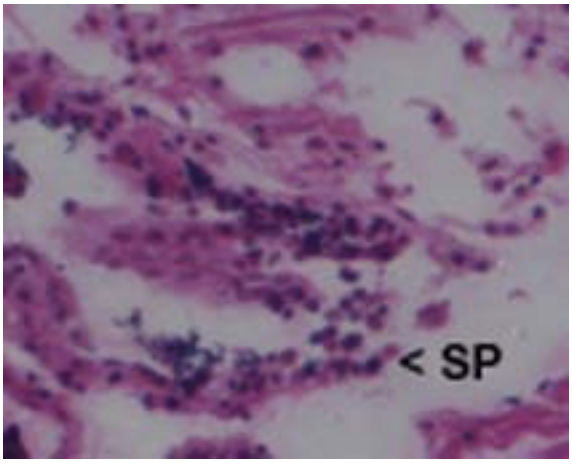


b. Late active development SPT: spermatids

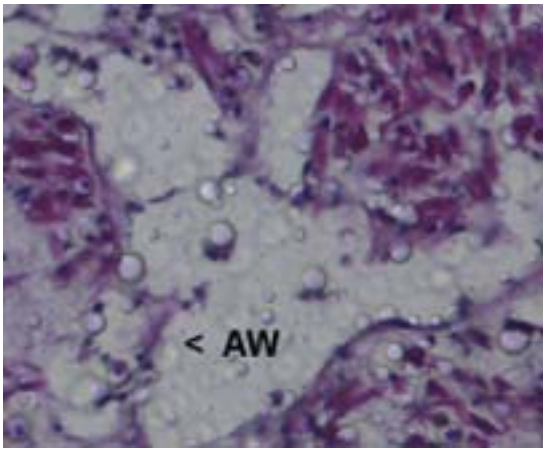
Figure 9 cont.



c. Ripe gonad SP:: sperms fill lumina

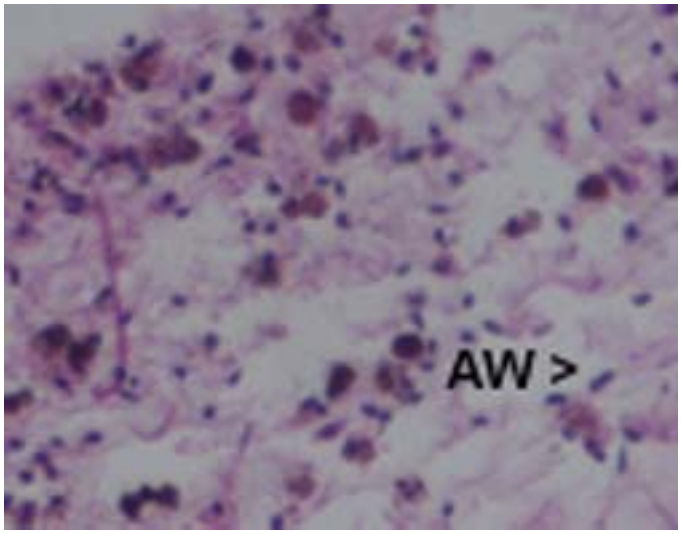


d. Spawned clam AW: thicken alveolar walls

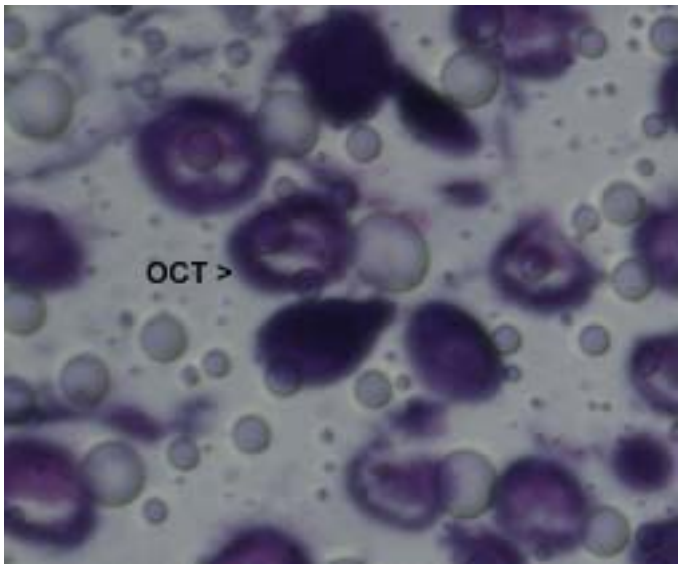


e. Undeterminable AW: alveolar walls without oogonia or spermatogonia

Figure 10
Sections of gonadal tissue from a female *Mercenaria mercenaria*

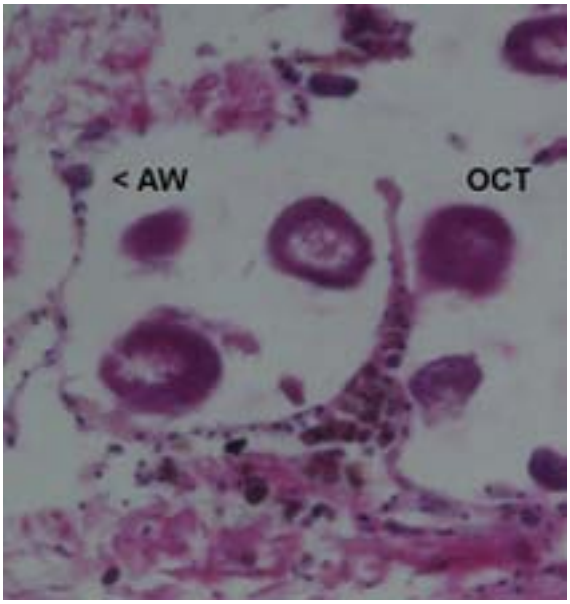


a. Early active development AW: alveolar walls

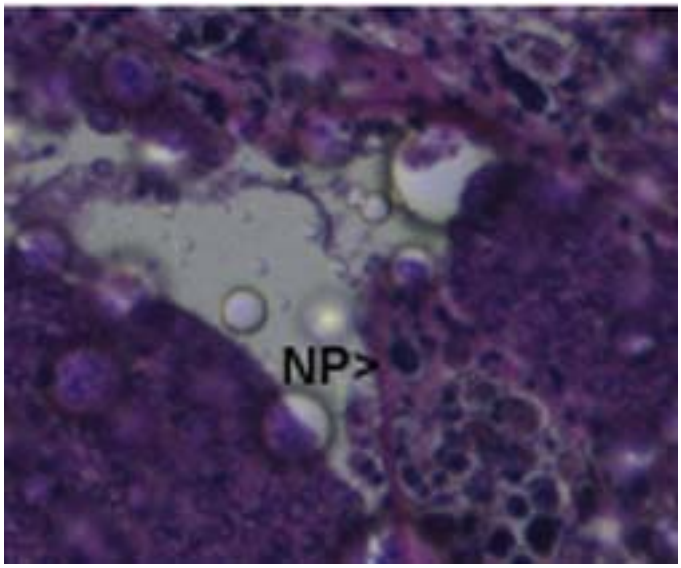


b. Ripe gonad OCT: oocytes in lumina

Figure 10
Sections of gonadal tissue from a female *Mercenaria mercenaria*, cont.

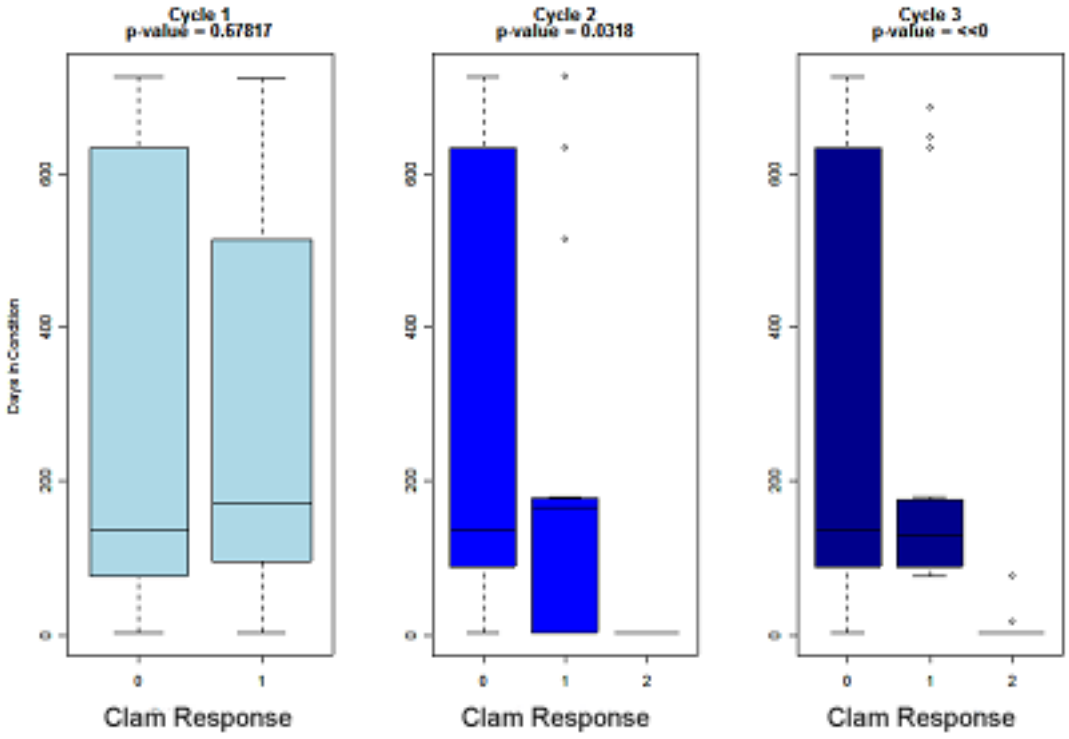


c. Spawned clam OCT: oocytes
AW: flaccid alveolar walls



d. Neoplasma (NP) from a female

Figure 11
Effect of days in conditioning and *Mercenaria mercenaria* (Florida and Puerto Rico stocks) response to spawning induction by thermal stimuli



0: No clam reaction 1: Heavy Siphoning; its about to spawn 2: Spawned

Figure 12
Average distribution of Mercenaria. *mercenaria* larvae in 14 38 L culture tanks per week

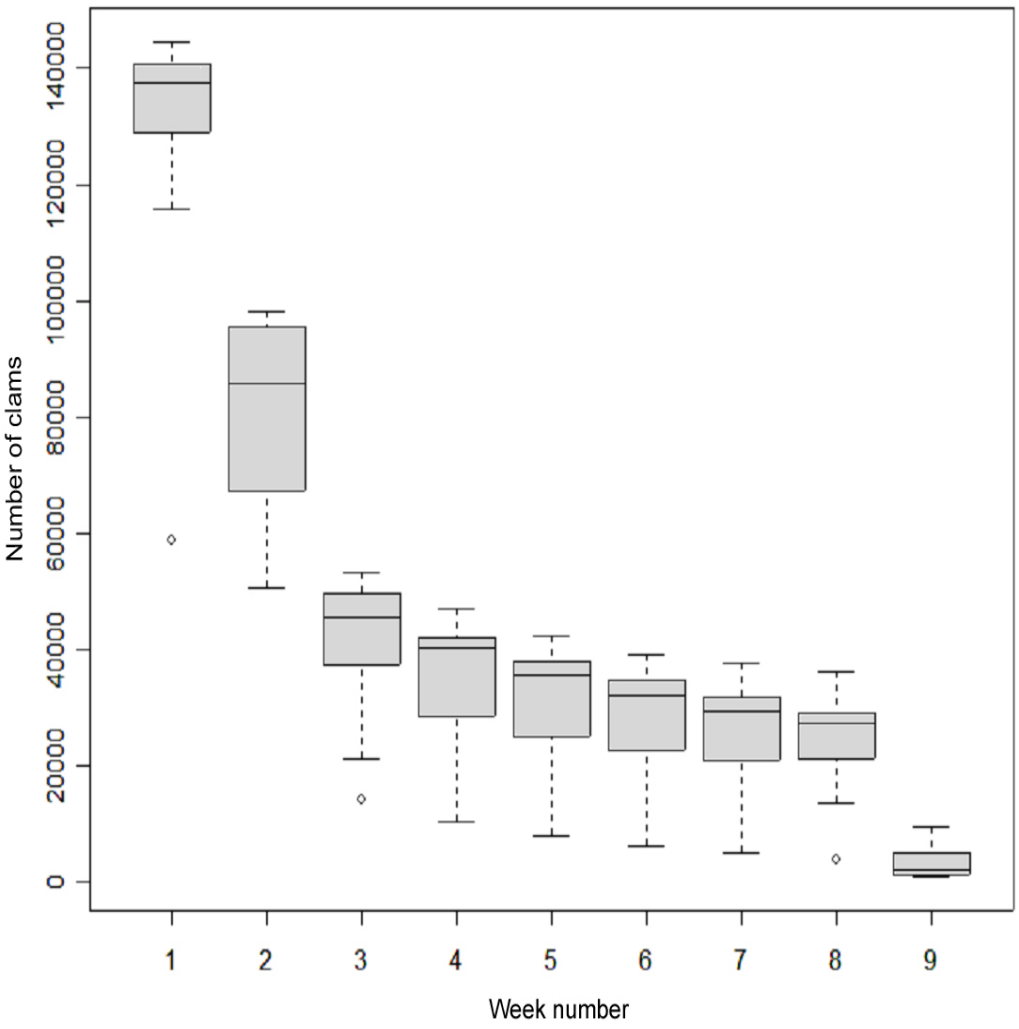


Figure 13
Weekly average temperature variations in 14 38 L rearing tanks

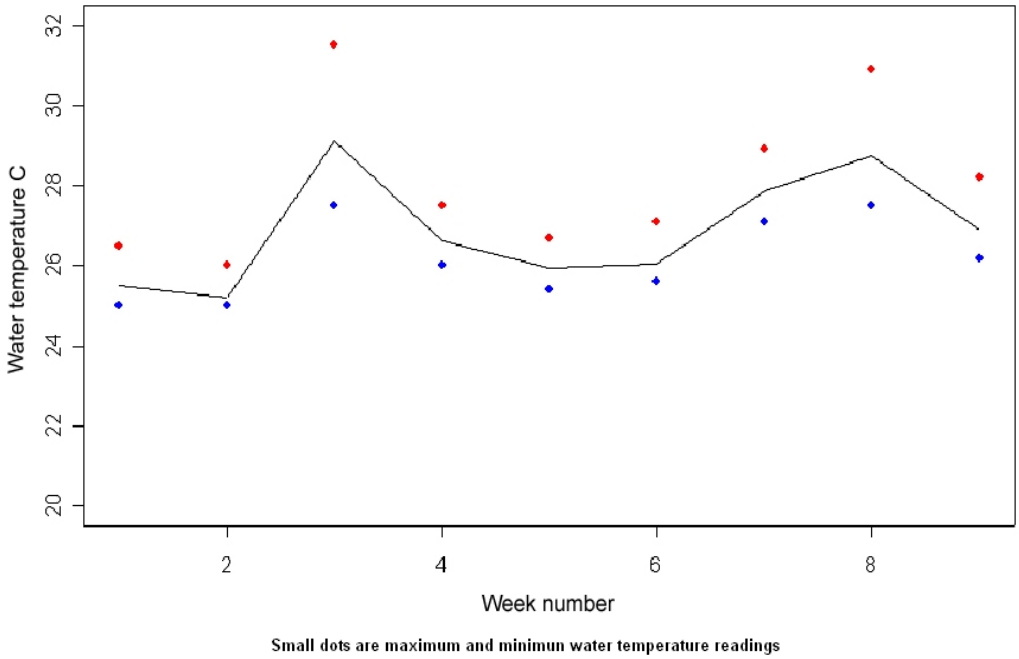


Figure 14
Weekly average dissolved oxygen variation in 14 38-L rearing tanks

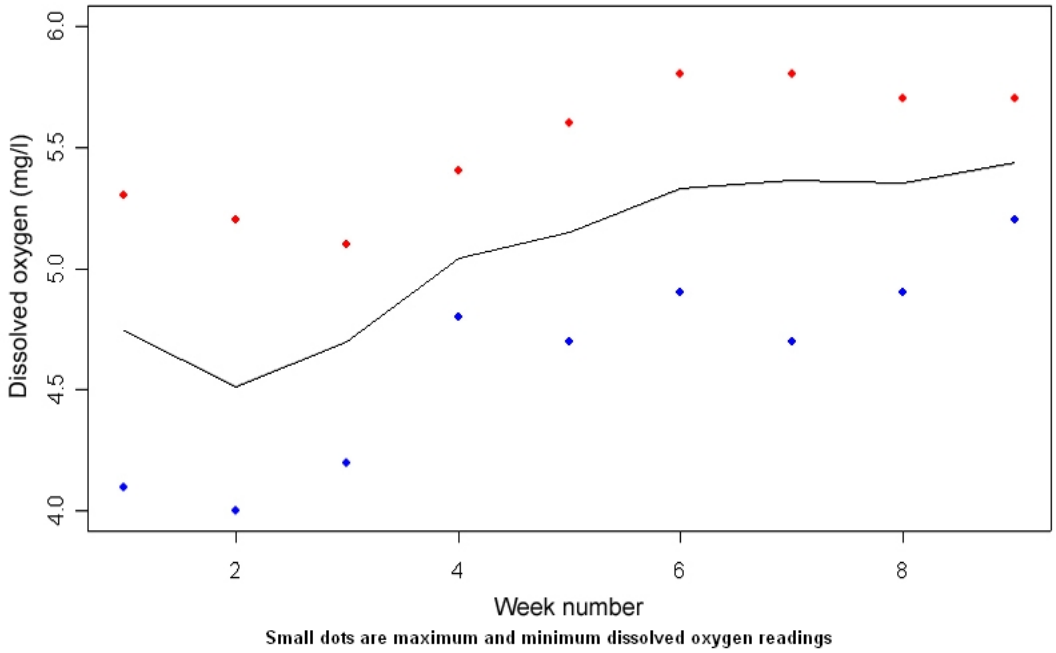


Figure. 15
Weekly average pH variations of 14 38-L-rearing tanks

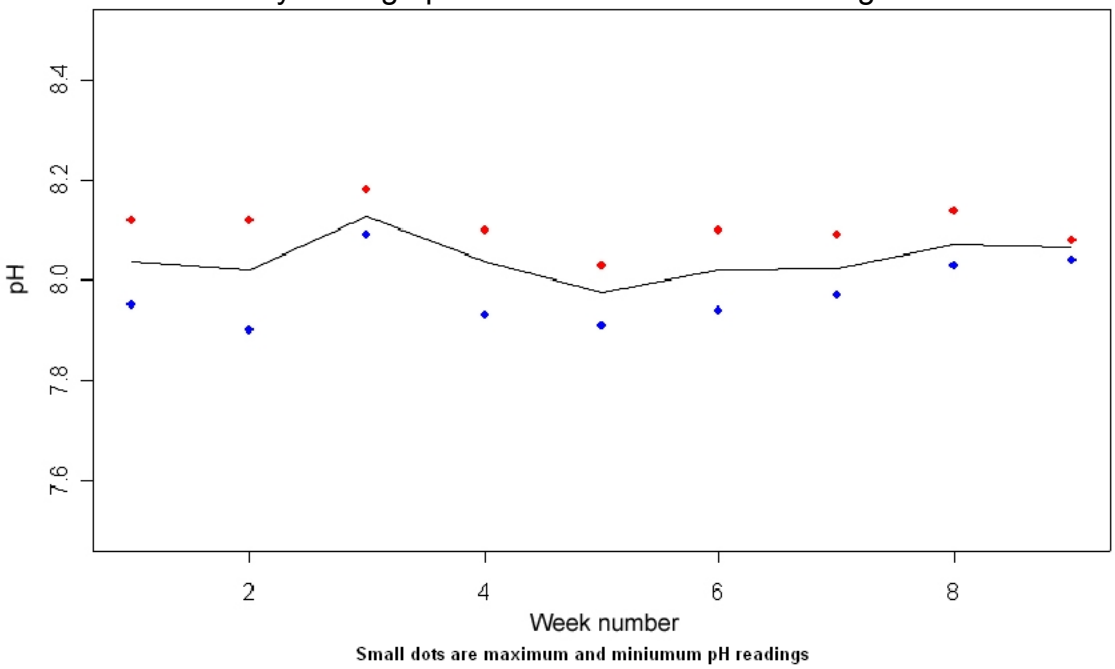


Table 2
Gonadal development of both populations of *Mercenaria mercenaria* before and after the conditioning phase.

Before the Conditioning Phase						
Population	**Early Active Stage (#)**	**Late Active Stage (#))**	**Ripe (#)**	**Spawned Stage (#))**	**Pathological Condition (#)**	**Undeterminable (#)**
PR (males)	1			1	1	4
PR(females)			1	2		
FL (males)	3	2	7			
FL(females)	2			2		
After the Conditioning Phase						
Population	**Early Active Stage (#)**	**Late Active Stage (#)**	**Ripe (#))**	**Spawned Stage (#))**	**Pathological Condition (#))**	**Undeterminable (#))**
PR (males)			5			
PR(females)				5		
FL (males)				4	2	1
FL(females)			1	7	1	

Table 3.
Mercenaria mercenaria responses to induced spawning trials by thermal stimuli

Trial	Spawned Clams			
Number	PR female	PR male	FI female	FI male
1	- [a]	- [a]	1 [b]	2
2	- [a]	- [a]	4 [c]	8
3	0	3	2	3
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0

[a] No clams from Puerto Rico were used for this trial. [b] 2.1×10^6 eggs/female.
[c] 1.0×10^6 eggs/female (average).

Table 4.
Average water quality parameters of the *Mercenaria mercenaria* acclimation tank.

Month/Yr.	Water Temp. ºC	OD mg/L	Salinity ppt	pH	Nitrite	Ammonia
March 01	18.06 ±1.72	6.53 ±0.41	33.43 ±2.37	N/A	0.10 ±0.04	0.86 ±0.10
April 01	18.67 ±1.53	6.13 ±0.42	37.00 ±0.76	8.05 ±0.03	0.24 ±0.15	0.85 ±0.09
May 01	18.27 ±0.46	6.64 ±0.51	35.67 ±0.98	N/A	0.08 ±0.00	0.80 ±0.00
June 01	18.21 ±1.35	6.58 ±0.52	35.71 ±0.83	N/A	0.08 ±0.00	0.81 ±0.05
July 01	18.33 ±0.77	6.30 ±0.38	34.93 ±1.53	N/A	0.08 ±0.00	0.80 ±0.00
Aug. 01	17.67 ±0.59	6.51 ±0.31	33.77 ±0.86	N/A	0.11 ±0.09	0.81 ±0.05
Sept. 01	17.55 ±0.50	6.69 ±0.38	32.70 ±1.34	7.84 ±0.07	0.08 ±0.00	0.83 ±0.07
Oct. 01	18.58 ±1.27	6.57 ±0.38	32.62 ±1.39	7.68 ±0.28	0.15 ±0.08	0.84 ±0.09
Nov. 01	18.96 ±2.55	6.49 ±0.69	33.00 ±1.70	7.99 ±0.10	0.09 ±0.07	0.74 ±0.33
Dec. 01	15.00 ±1.46	7.23 ±0.59	32.17 ±1.70	7.88 ±0.04	0.26 ±0.16	0.83 ±0.28
Jan. 02	17.21 ±2.49	6.92 ±0.64	32.00 ±1.28	N/A	0.08 ±0.00	0.82 ±0.06
Feb. 02	15.21 ±1.89	7.20 ±0.42	31.29 ±1.70	N/A	0.20 ±0.13	0.86 ±0.10
March 02	18.67 ±2.06	6.52 ±0.41	32.00 ±2.40	N/A	0.11 ±0.05	0.84 ±0.09
April 02	17.63 ±1.11	6.98 ±0.26	32.50 ±1.29	N/A	0.08 ±0.00	0.80 ±0.00
May 02	18.27 ±0.61	6.66 ±0.58	35.91 ±0.97	N/A	0.08 ±0.01	0.82 ±0.06
June 02	18.35 ±0.97	6.60 ±0.51	29.70 ±0.82	N/A	0.08 ±0.00	0.82 ±0.06
July 02	18.15 ±0.47	6.29 ±0.32	28.20 ±1.14	7.95 ±0.10	0.08 ±0.00	0.82 ±0.06
Aug. 02	17.58 ±0.73	6.57 ±0.26	29.44 ±0.73	7.75 ±0.07	0.15 ±0.08	0.80 ±0.00
Sept. 02	21.00 ±1.83	5.58 ±0.49	27.75 ±2.06	8.03 ±0.07	N/A	0.80 ±0.00

Table 4 cont.

Month/Yr.	Water Temp. ºC	OD mg/L	Salinity ppt	pH	Nitrite mg/l	Ammonia mgl
Oct. 02	19.29 ±0.76	6.43 ±0.51	29.57 ±0.98	7.75 ±0.09	0.20 ±0.08	0.80 ±0.00
Nov. 02	18.50 ±0.87	6.54 ±0.34	29.50 ±1.12	7.94 ±0.07	0.07 ±0.03	0.78 ±0.31
Dec. 02	17.75 ±0.96	7.08 ±0.22	30.25 ±0.50	7.83 ±0.03	0.10 ±0.01	0.95 ±0.10
Jan. 03	17.31 ±1.94	6.90 ±0.56	30.00 ±0.76	N/A	0.08 ±0.00	0.80 ±0.00
Feb. 03	15.75 ±1.37	7.27 ±0.41	29.67 ±0.52	N/A	0.22 ±0.13	0.87 ±0.10
March 03	18.00 ±1.85	6.53 ±0.41	29.57 ±0.53	N/A	0.10 ±0.04	0.83 ±0.08
April 03	18.81 ±1.65	5.98 ±0.42	29.75 ±0.89	8.05 ±0.04	0.25 ±0.13	0.88 ±0.10
May 03	18.20 ±0.35	6.73 ±0.60	29.60 ±0.84	N/A	0.08 ±0.00	0.80 ±0.00
June 03	18.40 ±1.63	6.42 ±0.32	29.60 ±0.84	N/A	0.08 ±0.01	0.84 ±0.08
July 03	18.28 ±0.51	6.30 ±0.35	28.00 ±1.32	7.91 ±0.13	0.08 ±0.00	0.82 ±0.07
Aug. 03	17.56 ±0.73	6.57 ±0.26	29.33 ±0.50	7.74 ±0.07	0.14 ±0.06	0.84 ±0.09
Sept. 03	20.88 ±1.36	6.21 ±0.23	29.38 ±0.92	8.02 ±0.11	0.08 ±0.01	0.85 ±0.09
Oct. 03	19.50 ±0.93	6.50 ±0.52	29.63 ±0.92	7.79 ±0.11	0.19 ±0.08	0.83 ±0.07
Nov. 03	18.75 ±0.66	6.68 ±0.19	29.50 ±1.12	7.91 ±0.08	0.09 ±0.05	0.80 ±0.32
Dec. 03	17.75 ±0.89	7.08 ±0.21	30.25 ±0.46	7.81 ±0.06	0.10 ±0.01	0.95 ±0.09

Table 5.
Weekly average larval growth rate and survival of the 14 38L-culture tanks

Week No.	Mesh Screen Size (µm)	Average Shell Length (µm) + SD	Median (µm)	Average Survival % per screen mesh per week	Average Survival % per week*
1	150	163.34±12.27	168	47.47	47.47
2	150	193.25±15.78	196	61.89	61.89
3	150	316.31±89.02	306	55.52	55.52
4 4	150 210	256.81±36.81 448.05±115.89	252 427	8.09 91.91	85.45*
5 5	210 300	352.04±83.24 563.69±133.22	364 532	2.69 97.31	90.70*
6 6	300 500	580.91±65.79 851.42±161.48	590.5 844	5.49 94.51	90.99*
7 7 7	300 500 710	611.27±14.34 847.48±109.80 1215.13±300.79	619 844 1153	4.37 62.44 33.19	91.96*
8 8 8	300 500 710	620.21±41.60 913±106.62 1218.63±165.20	619 900 1181	1.44 16.59 81.97	91.36*
9 9 9	300 500 710	615.28±44.17 913.32±81.75 1409±245.51	619 928 1406	1.05 14.96 84.00	94.00*
Overall Survival Rate: 9.32±2.37					

* Average Clam Larvae Survival rate in each of the 14 38 L cultured tanks.

Table 6
Larval development of *Mercenaria mercenaria* raised in commercial and research hatcheries.

Larval Stage	Age of Larvae (Days)	Shell Length (µm)	Survival (%)	Source
Trochophore	Less than 24hrs	50-60	50-75	Loosanoff and Davis, 1963; Hadley et al., 1997
Early and Mid-Veliger or Umboned (LRT)	1-5 5-8	105 90-140	25-90	Manzi, 1985 Hadley et al., 1997
Late Veliger (LRT)	3-15 8-14	 140-220	25 25-50 50	Aiken, 1993; Adams et al., 1991 Hadley, et al., 1997
Pediveliger (LRT to DWL)	8-12 14-21	180-200 170-280	50	Aiken, 1993; Castagna and Kraeuter, 1981; Hadley et al., 1997
Early Post Set (DWL)	21-35	300-400	25-90 50	HBOI, 1997 Hadley et al., 1997
Mid-Post Set (DWL)	30-40	400-600	25-90	HBOI, 1997 Hadley, et al., 1997
Late Post Set	45-56	600-1000	25-90	HBOI, 1997 Hadley, et al., 1997
Overall Survival Rate from Zygote to 1 mm Clam		**10 10-15 10-20**	Hardman, 1999 Aiken, 1993 HBOI, 1997	

Table 7
Average stocking densities of 38L 14 culture containers by week

Week Number	Recommended Stocking Densities in Culture Containers	Stocking Densities in Cultured Containers
1	1-2 clams/mL[a]	4.75 clams/mL
2	1-2 clams/mL[a]	2.90 clams/mL
3	1-2 clams/mL[a]	1.60 clams/mL
4	50 clams/cm^2 [b]	27.24 clams/cm^2
5	25 clams/cm^2 [b]	24.50 clams/cm^2
6	25 clams/cm^2 [b]	22.19 clams/cm^2
7	25 clams/cm^2 [b]	20.32 clams/cm^2
8	25 clams/cm^2 [b]	18.70 clams/cm^2
9	25 clams/cm^2 [b]	1.08 clams/cm^2

[a] Hadley et al., 1997 [b] HBOI, 1997

Table 8
Mercenaria mercenaria larvae daily shell growth rate per week.

Week Number	Mesh Screen Size (µm)	Daily Clam Growth Rate (µm)
1	150	10.40
2	150	4.00
3	150	16.00
4	210	17.00
5	210 300	16.00 15.00
6	300 500	32.00 44.00
7	300 500 710	4.00 36.00 44.00
8	300 500 710	- [a] 8.00 4.00
9	300 500 710	- [a] 4.00 32.00

[a]Empty shells

Discussion

Northern quahog farming in Puerto Rico to be considered as a possible additional activity for fishermen relies on three fundamentals: identification of grow-out culture areas, evaluation of local stocks as brooders and a reliable source of clam seed. This study evaluated if induced spawning of adult clams after an acclimation time in a conditioning tank and larval development of *M. mercenaria*, a temperate climate species, is viable under the tropical environmental conditions in Puerto Rico.

1. Induced spawning: response of *Mercenaria mercenaria* from Florida and Puerto Rico stocks.

A. Determination of gonadal development of both populations before starting the conditioning phase.

Before starting the conditioning phase, a histological examination of individuals from both stocks revealed that their gonads exhibited different gametogenic phases. Clams from the Florida stock displayed a better reproductive capacity than local individuals because they were preconditioned prior to their shipment to Puerto Rico. These clams were raised under laboratory conditions to be used as brooders.

Clams that were collected from the municipality of Cataño in February, 2001, showed low gametogenic activity. In fact, 40% percent displayed an undeterminable stage and other 40% were either spawned or in early developing stage. In April 1985, Juste (1987) performed a histological analysis of clams from the same area and she reported that the sampled female clams were in early and late active developing stages, while the males exhibited late active developing stage or ripe gonads. Additionally, male clams collected on June,

2001, exhibited ripe gonads. Although no determination of the reproductive cycle has been performed on local *M. mercenaria* stocks, these findings suggest that local clams from Cataño area might commence their gametogenic cycle between February and April, and around May or June the clams might have ripe gonads.

Larger sized individuals (10%) from the local stock of *M. mercenaria* presented some pathological conditions in their gonads that may hamper their reproductive capacities. Juste (1987), also, observed some degeneration of the connective gonadal tissue in clams from this population. Similar cellular disorders were also detected in the largest sized classes of clams in the Indian River, Florida population. Hesselman et al. (1988) reported that this inflammatory reaction is an internal defense mechanism to foreign substances and, as a result, gametogenesis is inhibited. It seems that, the accidental PCB spill that occurred in 1985 is still affecting larger sized clam class from the Cataño area. Although, juvenile clams of about 25 mm in shell length were observed during the collection of the clams, it is necessary to consider other places with better physicochemical environmental conditions to transplant these organisms and evaluate the recovery potential of these northern quahogs. It is suggested the Piñones Lagoon area in Loiza as a possible site to be considered because in the 20th century, it was considered a highly productive zone with adequate environmental conditions for clams.

B. Responses of *M. mercenaria* to spawning induction by thermal stimuli: Florida and Puerto Rico stocks.

After, 15 trials, both stocks of *M. mercenaria* displayed poor behavioral response to induced spawning by thermal stimuli. Moreover, spawning activity from both populations were less than the number of spawned clams obtained by thermal shock in the US hatcheries. On two occasions despite this erratic behavior preconditioned clams from the Florida stock spawned inside the acclimation tank, after the thermal stimulation trial to induce spawning failed. It is

evident that the acclimation time of Florida stocks to local conditions was insufficient and they did not response to the thermal stimuli.

Only three specimens of the local population displayed a response to the thermal stimuli. These difficulties may have arisen from the gametogenic variability of *M. mercenaria* broodstock obtained in the wild and the lack of knowledge of the spawning peaks of the PR population. The capability to spawn, number of spawned eggs and egg diameter were evaluated in *M. mercenaria* from the Massachusetts and South Carolina regions, and a cross of these two different stocks. Knaub and Eversole (1988) found statistically differences in the average number of eggs released by female, spawning attempts, and egg sizes. These findings can be considered as evidences for the Bricelj and Malouf (1980) hypothesis that genetic differences might be explained by differences in reproduction behavior among northern quahogs of different regions. It is recommended to evaluate other non-traditional methods to induce spawning in local stocks, other than thermal stimuli.

An increase in number of clams that failed to spawn with increasing time spent in the conditioning tank was noted. It may have been a result of inadequate broodstock conditioning. Reproductive activity after clams were conditioned in the tank did show a high percentage of partially spawned or undetermined specimens. Therefore, it can be speculated that many of the immature clams at the beginning of the experiment failed to reach the desired level of maturity.

C. Acclimation tank parameters.

It is expected that broodstock conditioning improved spawning response to thermal stimulus, better egg size and higher survival rates in early larval stages. However, the conditioning to bring broodstock to their optimum stage depends upon the stage of gonadal development at the beginning of the conditioning cycle

and the rate of conditioning (Muranka and Lannan, 1984). Both conditions are related to the knowledge of annual gametogenesis cycle of clams and the conditioning environment such as, temperature, salinity, and supplemental feeding (Loosanoff, 1937; Giese and Pearse, 1974; and Pline, 1984), which are still unknown for the local Puerto Rico stock.

Northern quahogs from the Florida and Puerto Rico stocks displayed a positive response to induced spawning by thermal stimuli only during the first three spawning trials. Afterwards, there were no more spawnings. The local stock did not attain the adequate gonadal ripeness in the broodstock conditioning system. Additionally, ripe northern quahogs from Florida stock held in seawater of temperature between 15 and 19ºC did not maintain adequate ripeness to release their gametes during the induced spawning trials. Therefore, subsequent retention of northern quahogs from both populations in the conditioning system did not improve or extend their gonadal development.

Physical and chemical seawater parameters in the conditioning tank were those recommended for Florida and South Carolina stocks of northern quahogs. However, isolated geographically communities, such as Puerto Rico population, in a tropical environment, would have a different condition pattern and different responses to induced spawning cues (Knaub and Eversole, 1988). Temperatures inside the acclimation tank, ranged between 15 and 22ºC. Therefore, low temperature might decrease metabolic activity in clams and reduce their gametogenesis activity thus, reducing their positive behavioral response to induced spawning. Moreover, seasonal temperature changes are relatively low in Puerto Rico. Gamete development and spawning patterns in tropical areas for bivalves might be related to the abundance of food rather than differences in the temperature of their environment.

The algal feeding regime is another important factor needed to produce mature clams. The quantity and quality of algal feed used for broodstock conditioning may promote gametogenesis; achieve gonadal maturation, and spawning (Giese and Pearse, 1974). The presence of sufficient quantities of essential fatty acids is a very important controlling factor in gametogenesis (Navarro et al., 2000). These researchers, a study with the scallop, A*rgopecten purpuratus* demonstrated that the highest percentage of ripe scallops occurred in individuals fed with a diet of microalgae supplemented with lipids.

Previous studies showed a positive relationship between the polyunsaturated fatty acids in phytoplankton, such as *I. galbana*, and broodstock spawning performance, egg survival and viability of larvae (Gallager and Mann, 1986; and Utting and Millican, 1997). However, biochemical composition of this phytoplankton changed with changes in temperature (Brown et al., 1993 in Zhu et al., 1997). A negative relationship between lipid concentration and temperature was found, affecting the nutritional value of the phytoplankton.

Phytoplankton mass culture tanks for growing *I. galbana and C. gracilis* to feed broodstock northern quahogs were located outdoors, so high sunlight concentrations and high temperatures up to 40ºC were reached during daytime. Therefore, the nutritional value of the algae used for feeding brooders might have been negatively affected. However, nutritional studies should be conducted to evaluate this assumption.

Based on these results, it is suggested that frequent water temperature fluctuations had a negative effect on gonad development in brooders. Poor gametogenic condition of the clams after conditioning and poor spawning behavioral response to thermal stimuli support this hypothesis.

Clam conditioning as conducted in this study, is not necessarily the best way to acclimatized local stocks of *M. mercenaria*. It is suggested that it would be best to leave northern quahogs in their natural environment until they are about to spawn, approximately two weeks, then placing them in a indoor conditioning tanks to phytoplankton feeding regime that would permit gonadal maturation. However, a preliminary study should be conducted to evaluate the gametogenic and spawning cycles of local stocks under natural environmental conditions before any other attempt to induce spawning.

2. Larval rearing of *Mercenaria mercenaria*

The results of this project on *M. mercenaria* under controlled conditions reveal that larval production and the development of different larval stages are similar to these and within the same time intervals of larval production found in Florida and South Carolina hatcheries. These findings suggest that northern quahog larval stages can be reared in tropical laboratories adopting similar techniques from commercial facilities.

Larvae development and settlement was achieved within the same time intervals as commercial facilities from Florida and South Carolina. Growth rates during the last three weeks of the present study were similar to those reported for commercial hatcheries. Loosanoff and Davis (1963) concluded that there is a growth rate increment as temperature increases up to 26 to 28°C.

However, overall survival rates were lower than the lowest obtained for US hatcheries. Bacterial and ciliate problems need to be overcome to avoid high mortalities during the first larval stages in a culture system in a tropical environment. The lowest survival rates were observed the first weeks of the larval rearing experiment. During the same time, problems with ciliates and

bacterial infections were present. It is recommended to avoid places with semiclosed or open spaces to perform larval rearing cultures.

No chemical inducers were used in the present study to accelerate clam larvae settlement as the one used in oysters and abalones such as GABA-mimetic polypeptides (Morse, 1984). Time for settlement (between the third and four week of culture), and survival rates, (55.52 and 91.91) showed that there is no need to use costly inducers.

Results from this experiment demonstrated that northern quahog larvae can be cultured in quantities comparable to clam seed production in commercial hatcheries in Florida and South Carolina, if the of temperature and phytoplankton feeding are accomplish.

3. Diseases of Hatchery Cultured *Mercenaria mercenaria*

Cultivated northern quahogs are kept at higher densities than in wild populations, therefore the occurrence and prevalence of parasites and tissue pathology is greater than in their natural environment. In larval rearing there are two important diseases caused by *Vibrosis* sp and *Sirolpidium zoophtlorum* (larval mycosis). These infecting agents can cause rapid mass mortalities in larval cultures. Larvae and juveniles are more prone to be affected by bacteria, viruses, protozoans, helminths and parasitic crustaceans than adults (Manzi, 1985 and; Gibbons and Blogoslawski, 1989). Larval culture disease problems have been associated with bacterial infestations due to fouling and debris. (Ford, 2001).

Preventive measurements and effective treatments avoided serious problems with larvae survival and growth during the experiment. However, due to high temperatures, bacterial concentrations were elevated in the experimental units, especially during the first three weeks of the study. This agent exhibited pink stains on the walls and bottom of some of larval rearing tanks. Ciliates in the broodstock conditioning tank, outdoors algae culture tanks, and in the experimental units were also observed. Ciliates are common symbionts in northern quahog larval culture. Its presence is an indication of a high bacterial infestation. Cleaning procedures and filtered water avoided the introduction of other organisms during the water drain downs and increase survival rates in the larval culture tanks. No other foreign agent was observed during this experiment. Therefore, to avoid possible accidental introduction of pathogenic agents such as QPX, haplosporidian (*Perkinsus marinus*) and other infectious organisms it is not recommended that new stocks of northern quahogs be introduced to Puerto Rico as brooders.

Final statement

Findings of the study are significant. It was demonstrated that thermal stimuli as a spawning inducer for local populations of northern quahog in Puerto Rico is not an appropriate method. However, larval stages, survival, and growth rates of *M. mercenaria* raised under controlled conditions in Puerto Rico compared favorably with clam production rates from commercial hatcheries and research facilities in the southeastern coast of the United States.

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APPENDICES

Appendix A
Governmental regulations impacting fisheries activities in Puerto Rico

Local Regulations

Fisheries Act Number 278, November 1998

Federal Regulations

Consolidation of Regulations for Spiny Lobster Fishing in the US Caribbean EEZ, Puerto Rico and the US Virgin Islands 50 CFR Part 622 1984

Regulation for Reef Fish Fishing in the US Caribbean EEZ, Puerto Rico and US Virgin Islands, as amended Federal Register 61, no. 235 1996

Regulation for Queen Conch Fishing in the US Caribbean EEZ, Puerto Rico and US Virgin Islands, as amended Federal Register 61, no. 241 1996

Regulation for Fishing Corals and Reef Associated Plants and Invertebrates in the US Caribbean EEZ, Puerto Rico and US Virgin Islands. CFR Part 902 and CFR Part 670 1995

Fishing Gear Requirements in the US Caribbean EEZ, Puerto Rico and Virgin Islands 50 CFR Part 622 2003

Fishing Regulations for Atlantic Sharks

Marine Sanctuaries Title 16, Chapter 32

Regulations for Highly Migratory Species: Billfish, Swordfish, Tuna and Shark

Appendix B.
Statistical analysis of *Mercenaria mercenaria* spawning response to the three thermal cycles in all spawning trials.

# Cycle	No reaction	Reaction	
		Heavy Siphoning	Spawned
Cycle 1	375	30	0
Cycle 2	355	49	1
Cycle 3	361	24	19

Test 1:

Ho: No Reacton in all three cycles= 0
Ha: No Reaction in all three cycles> 0

```
sample estimates: probability of success 0.1013180
number of successes = 123, number of trials = 1214
p-value = <<0.001
```

Test 2:

Ho: No Reacton in the first cycle = 0
Ha: No Reacton in the first cycle > 0

```
sample estimates: probability of success 0.07407407
number of successes = 30, number of trials = 405
p-value = <<0.001
```

Test 3:

Ho: No Reacton in the second cycle = 0
Ha: No Reacton in the second cycle > 0

```
sample estimates: probability of success 0.1234568
number of successes = 50, number of trials = 405
p-value = <<0.001
```

Test 4:

Ho: No Reacton in the third cycle = 0
Ha: No Reacton in the third cycle > 0

```
sample estimates: probability of success 0.1064356
number of successes = 43, number of trials = 404
p-value = <<0.001
```

Appendix C
Statistical analysis of the spawning response of *Mercenaria mercenaria* in relation to clamshell sizes

```
        Kruskal-Wallis rank sum test

Clamshell sizes and thermal cycle 1
Kruskal-Wallis chi-squared = 0.4849, df = 1, p-value = 0.4862

        Kruskal-Wallis rank sum test

Clamshell sizes and thermal cycle 2
Kruskal-Wallis chi-squared = 0.3778, df = 2, p-value = 0.8279


        Kruskal-Wallis rank sum test

Clamshell sizes and thermal cycle 3
Kruskal-Wallis chi-squared = 1.86, df = 2, p-value = 0.3946
```

Appendix D

Statistical analysis of the relation between *Mercenaria mercenaria* response to induced spawning and time spend in the acclimation tank.

Kruskal-Wallis rank sum test

Time in Condition by Cycle1

Kruskal-Wallis chi-squared = 0.1722, df = 1, **p-value = 0.6782**

Kruskal-Wallis rank sum test

Time in Condition by Cycle2

Kruskal-Wallis chi-squared = 6.8965, df = 2, **p-value = 0.0318**

kruskal.test(TimeinCond~Cycle3, data=ClamBehavior)

Kruskal-Wallis rank sum test

Time in Condition by Cycle3

Kruskal-Wallis chi-squared = 40.9306, df = 2, **p-value = 1.294e-09**

Appendix E

Statistical analysis of the relation between *Mercenaria mercenaria* survival rates in rearing tanks and water physiological parameters.

The GENMOD Procedure

Model Information

Data Set	WORK.CLAMS
Distribution	Negative Binomial
Link Function	Log
Dependent Variable	nclam
Observations Used	133

Class Level Information

Class	Levels	Values
weeks	9	1 2 3 4 5 6 7 8 9
tank	16	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Parameter Information

Parameter	Effect	weeks
Prm1	Intercept	
Prm2	weeks	1
Prm3	weeks	2
Prm4	weeks	3
Prm5	weeks	4
Prm6	weeks	5
Prm7	weeks	6
Prm8	weeks	7
Prm9	weeks	8
Prm10	weeks	9
Prm11	WTempC	
Prm12	OD	
Prm13	pH	

Criteria For Assessing Goodness Of Fit

Criterion	DF	Value	Value/DF
Deviance	121	136.7665	1.1303
Scaled Deviance	121	136.7665	1.1303
Pearson Chi-Square	121	92.5725	0.7651
Scaled Pearson X2	121	92.5725	0.7651
Log Likelihood		64041005.145	

Analysis Of Initial Parameter Estimates

Parameter		DF	Estimate	Standard Error	Wald 95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept		1	10.0077	2.2486	5.6005	14.4149	19.81	<.0001
weeks	1	1	3.5674	0.2402	3.0966	4.0383	220.51	<.0001
weeks	2	1	3.0905	0.2574	2.5860	3.5950	144.17	<.0001
weeks	3	1	2.5750	0.2279	2.1284	3.0216	127.71	<.0001
weeks	4	1	2.2937	0.2071	1.8877	2.6997	122.62	<.0001
weeks	5	1	2.1267	0.2133	1.7086	2.5447	99.42	<.0001
weeks	6	1	2.0203	0.2084	1.6118	2.4288	93.96	<.0001
weeks	7	1	1.9879	0.1992	1.5975	2.3782	99.63	<.0001
weeks	8	1	1.9909	0.2120	1.5755	2.4063	88.23	<.0001
weeks	9	0	0.0000	0.0000	0.0000	0.0000	.	.
WTempC		1	-0.0335	0.0540	-0.1392	0.0723	0.38	0.5353
OD		1	0.0622	0.1262	-0.1851	0.3095	0.24	0.6222
pH		1	-0.1544	0.2107	-0.5673	0.2585	0.54	0.4636