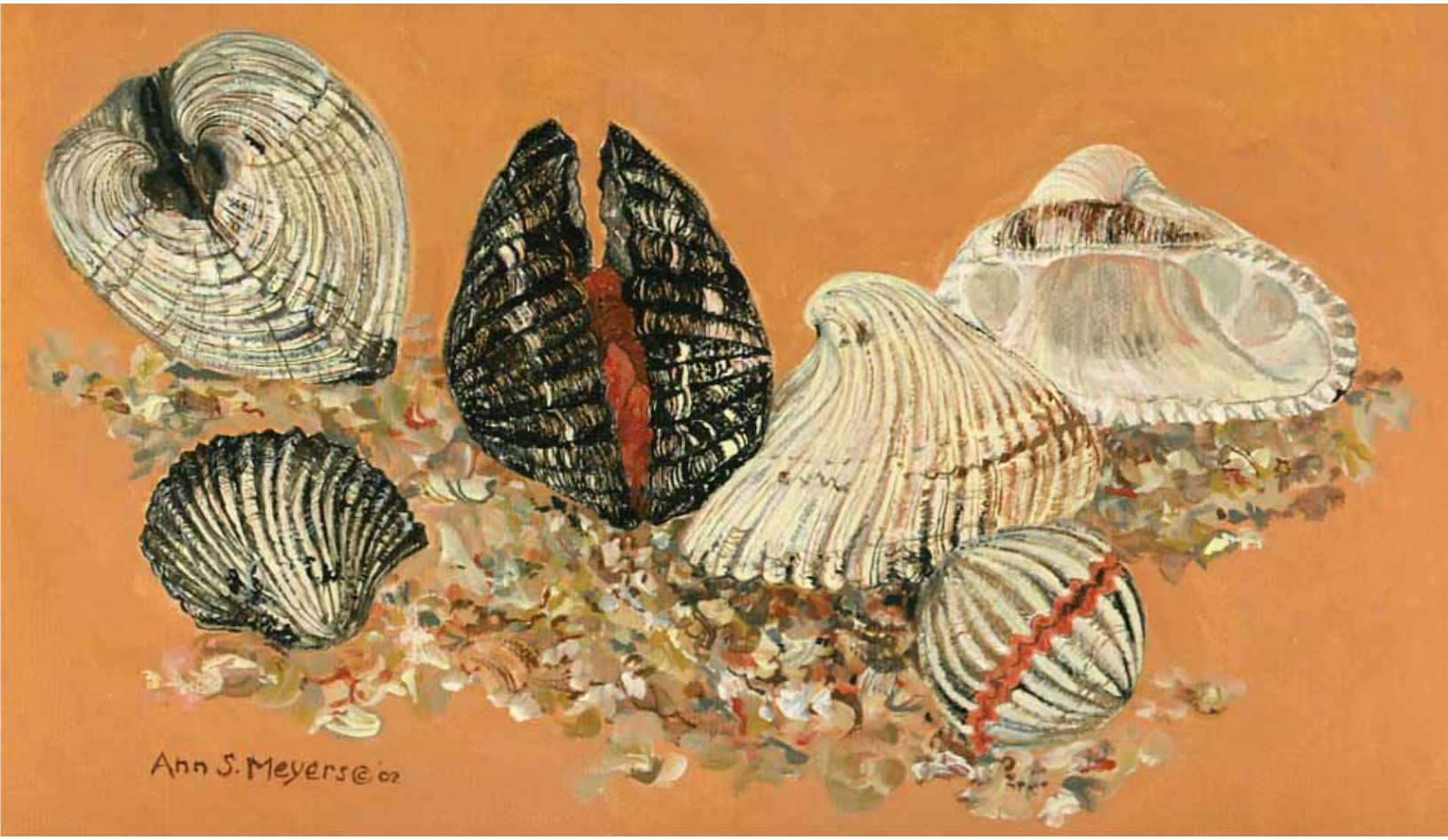
The Potential of Blood Ark and Ponderous Ark Aquaculture in Florida

Results of Spawning, Larval Rearing, Nursery and Growout Trials

Leslie N. Sturmer, Jose M. Nuñez, R. LeRoy Creswell, and Shirley M. Baker



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Preface

In November 1999, a workshop on New Molluscs for Aquaculture was conducted by the University of Florida Cooperative Extension Service, Florida Sea Grant, and the Florida Department of Agriculture and Consumer Services. The objective of the workshop was to characterize the culture and market potential of alternative molluscan shellfish species. Participants included aquaculturists, seed suppliers, researchers, biologists, marketers, economists, regulators, and resource managers. Workshop participants concluded that efforts should be undertaken to further determine the production feasibility and market demand for several mollusc species.

This document presents the results of research on two alternative species, the blood ark (*Anadara ovalis*) and the ponderous ark (*Noetia ponderosa*). The overarching goal of the project was to determine the feasibility of establishing a commercial aquaculture industry for producing blood and ponderous arks in Florida. Specific project aims reported here include: 1) developing spawning, larval rearing, and settlement techniques; and 2) evaluating production performance under nursery and growout conditions. The information presented is not a hatchery or growout manual, but is provided as a reference for shellfish hatchery operators and growers, resource managers, and others who may be considering the culture of blood or ponderous arks as alternative or additional species. The development of seed production techniques is presented in Chapter 2 and the evaluation of growth and survival from seed to market size is presented in Chapter 3.

Prior reports, published as journal articles or extension fact sheets, on other aspects of this project are provided in the appendices of this document. The gametogenic cycle and sex ratio for blood ark populations from the northwest coast of Florida (Appendix I) and for ponderous ark populations from Cedar Key (Appendix II) were determined by University of Georgia researchers Alan Power and Randal Walker. In a complementary study, John Baldwin with Florida Atlantic University documented the fertilization and early embryonic development of both ark species (Appendix III). Detailed knowledge of reproduction and development of ark clams is necessary for the advancement of successful hatchery spawning and rearing techniques. Finally, University of Florida extension faculty Robert Degner and Leslie Sturmer summarized research on the shellfish trade industry's awareness and acceptance of blood and ponderous arks (Appendix IV), as well as the nutritional composition and shelf life of these molluscs under commercial refrigeration (Appendix V). The latter two publications present information necessary for shellfish wholesalers in developing marketing strategies for these non-traditional molluscs.

In summary, this document demonstrates that spawning, larval rearing, nursery and growout, harvest, marketing, and distribution of blood and ponderous arks can be achieved. Specific areas that require further research and/or development by commercial interests are identified.

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Chapter 1: Introduction

Rationale

Hard clam, *Mercenaria mercenaria* (Linnaeus, 1758), aquaculture has become a major industry throughout the southeastern United States, including the Atlantic and Gulf coasts of Florida. From an industry that was virtually non-existent before the 1990s, Florida's hard clam farming industry sold 185 million hard clams to wholesale dealers, generating $19 million in producer revenues and an economic impact of $50 million in 2007 (Adams 2008). The growing and marketing of Florida farm-raised clams to wholesalers, restaurants, seafood shops, food service buyers, and direct retailing to consumers created a total impact which included $30.1 million in added revenue, $25 million in labor income, $3.7 million in property income (rentals, royalties, interest, etc.), $1.3 million in indirect business taxes, and 563 jobs (full and part-time). In spite of a fourteen-fold increase in clam production from 1991 to1999, dockside prices to the farmer remained fairly steady over the past decade. More recently, price declines associated with economic downturns have occurred after September 11, 2001 and in 2009.

Although clam farming has developed into a major enterprise in Florida, diversification from a single species product could help stabilize and expand the molluscan shellfish aquaculture industry. The rapid recruitment of fishermen into shellfish aquaculture, along with exceptional growth rates associated with the productive, subtropical waters of Florida, has encouraged growers to seek information on other bivalve species that would provide crop diversification and augment profit potential. Species diversification may be one alternative to mediate losses associated with a monoculture-based industry and to spread production risks. Native molluscan species which could be cultured and marketed along with hard clams are logical options.

Ark Clams

Ark clam, or arkshells, is the common name for a group of small- to large-sized edible marine bivalve molluscs in the order Arcoida. There are over 200 species worldwide found in many tropical, subtropical, and warm temperate areas (Broom 1985). Several ark species of the family Arcidae (*Scapharca*, *Arca*, and *Anadara* spp.) form the basis of economically significant molluscan fisheries and aquaculture operations throughout the world (Manzi and Castagna 1989). Species of *Anadara* are widely distributed and are harvested in China, Cuba, Colombia, Fiji, India, Indonesia, Japan, Korea, Malaysia, Mexico, the Philippines, Sri Lanka, Taiwan, Thailand, Venezuela, and West Africa (Broom 1985, Manzi and Castagna 1989). Ark clams, which are also referred to as cockles in south and southeast Asia, have been extensively cultured in China, Japan, and Malaysia (Nie 1990, Muthiah *et al.* 1992). In Thailand, cockles or arks have been cultured since 1900. About 3,600 acres were reported to be in production in 1980; however, seed shortages were noted to be a major obstacle in increasing production (Tookwinas 1983). The worldwide harvest of arks was 264,174 metric tons in 1993 (FAO 1995), declining to 97,296 metric tons in 1997 (FAO 1999).

Two ark clams native to the United States – the blood ark, *Anadara ovalis* (Bruguière, 1789), and the ponderous ark, *Noetia ponderosa* (Say, 1822) – are the focus of this study. The name blood ark is derived from the presence of hemoglobin pigment contained in the tissues of both species, resulting in an orange to red coloration. It is this characteristic that relegates the ark clams to a specific niche in the overall molluscan shellfish industry. Until recently, ark clam resources have been ignored by the fishing industry in the United States. A survey of ark populations in South Carolina,

undertaken in the 1980s, showed that arks could support a commercial fishery, but a marketing survey failed to produce viable markets (Anderson *et al*. 1984, Anderson and Eversole 1985). Since then, a small fishery for the blood ark and ponderous ark has developed mostly in Virginia, with live product primarily marketed as an ethnic food in Chicago, New York, Los Angeles, and Washington D.C., or exported to Mexico (McGraw *et al.* 1996, 1998). Unfortunately, limited stocks have prevented the development of a major fishery for these species. The demand for arks has outpaced the numbers that can be supplied by the fishery, particularly in Hispanic markets, which value the hemoglobin present in the clam meats. In addition, ark clams, referred to as *Akagai,* have become popular in the Japanese sushi markets.

Blood ark, *Anadara ovalis*

Ponderous ark, *Noetia ponderosa*

These two ark species have solid, strongly ribbed valves (shells) with a hinge line bearing numerous teeth arranged in a line on both valves (Abbott and Morris 1995). The shell of the blood ark is equivalved and oval in shape with about 35 radiating ribs, and is much thinner than that of the ponderous ark. The thick white shell of the ponderous ark is somewhat oblique in shape and has 27 to 31 well developed square ribs. The shell exterior of both species is covered with a hair-like periostracum (noncalcareous shell covering), ranging from brown to black in color. The blood ark lacks a well defined siphon with the inhalant and exhalant openings lying flush with the posterior margin of the shells; while the ponderous ark does not have any siphons (McGraw and Castagna 1994, Tookwinas 1983). Both the ponderous ark and blood ark, in their early stages of growth, use byssal threads (long, fine strong fibers) to attach themselves to a solid substrate so as not to be washed away by the tide or currents. Juvenile arks have been found attached to worm tubes, rocks, oysters, dead shells, adult arks, *etc*. (McGraw *et al.* 1998, Power and Walker 2002).

The blood ark, of the family Arcidae, occurs in estuarine waters from Cape Cod, Massachusetts to the Caribbean and Brazil (Abbott 1974). This species is found subtidally, ranging from below the low tide line down to a depth of 10 feet or more (Rehder 1981). It inhabits a variety of substrate types, but is most commonly found in sandy deposits (Alexander 1993, McGraw and Castagna 1994). Favoring salinities above 15 ppt, blood arks are often harvested as by-catch with hard clams in high salinity sounds from Virginia to Georgia (Castagna and Chanley 1973). Adult blood arks reach a shell length of 50 mm, on average, and are noted for early maturity and rapid growth rates (Abbott and Morris 1995, Power and Walker 2001, Power and Walker 2002). The bivalve is considered short-lived with a maximum lifespan of three to five years (McGraw *et al.* 1996, Walker 1998).

The ponderous ark of the family Noetiidae is common throughout the estuarine and nearshore waters of the southeastern Atlantic and Gulf of Mexico coastal areas of the United States (Abbott 1974). Shells of ponderous ark are found on beaches as far north as Cape Cod, Nantucket, and Long Island; but these shells are thought to be fossils. The species occurs at the substrate-water column interface in mud or sandy substrates at depths from just below the low tide line to 60 feet (McGraw and Castagna 1994, McGraw *et al.* 1998). Juveniles are most often found in shell debris or shell hash composition. The ponderous ark functions best in salinities above 18 ppt (Castagna and Chanley 1973). Adults can attain a length of 70 mm (Rehder 1981). Previously reported growth rates from Virginia are slow relative to the co-occurring blood ark, and the species can live for between 10 to 15 years (McGraw *et al*. 2001). The longevity and growth rate of the ponderous ark may have contributed to the over-fishing of wild stocks.

Until recently, life-history information for ark populations in the United States was limited to distribution, habitat preference, and anecdotal information from shell taxonomical guides and other popular literature. Loosanoff and Davis (1963), Chanley (1966), and Chanley and Andrews (1971) described the spawning and larval development of the ponderous ark. Studies were conducted in Virginia during the 1990s to obtain data on distribution, densities, growth rates, and size-age relationships of ark clams for purposes of fishery management and future aquaculture endeavors (McGraw and Castagna 1994). The blood ark was reported to grow twice as rapidly as the ponderous ark in the first two years after settlement, making it the principal aquacultural species of interest. Gametogenic cycles and condition indexes of both species were also studied, as well as predation rates by major predators (McGraw *et al.* 1998). For Virginia populations, the ponderous ark spawned in winter and summer while the blood ark spawned only during summer. In Georgia, an ark survey undertaken in the late 1990s found that most beds were limited in size and would not support a viable fishery (Walker and Gates 2001). As a result, interest mounted in developing an aquaculture industry. Growth rate studies of newly recruited blood arks in Georgia waters showed that it would grow to a possible market size of 40 mm shell length in 12 months (Walker 1998). The feasibility of culturing wild-caught blood ark juveniles in pearl nets and plastic bottom bags was assessed (Walker 1998, Power and Walker 2001). Reproductive studies indicated that some blood arks from Georgia are capable of spawning year around, but major spawning occurs from late spring through summer (Power and Walker 2002).

Natural recruitment (bivalve larvae metamorphosizing from free-swimmer to a juvenile benthos dweller) of blood and ponderous arks in newly planted clam culture bags has been observed by many growers in Florida (Sturmer *Pers. observ.*). On the east coast, both blood ark and ponderous ark recruitment occurs in the clam growing areas located in Matanzas River, Mosquito Lagoon, and Indian River; only the ponderous ark is found in the Alligator Harbor, Cedar Key, and Charlotte Harbor growing areas on the west coast. Our initial interest in arks for potential aquaculture was due to the high natural recruitment of the ponderous ark into clam culture bags during 1999 through 2001 in the Cedar Key area. Ponderous arks collected from harvested clam culture bags ranged from 30 to 40 mm in shell length, indicating that a commercial size could possibly be obtained in a year or so. Collected samples were replanted and reached an average size of 46-48 mm in an additional 6 to 8 months with little mortality. Growth results from these observations supported the proposal that ponderous arks may have potential for development into an aquaculture species in Florida. The transverse ark, *Anadara transversa* (Say, 1822), is also commonly found in clam bags; but this small ark only reaches a maximum length of approximately 30 mm (Walker and Power 2004).

Information is particularly scant on the biology and ecology of ark clams in the Gulf of Mexico region. Further, prior work has not been done with the aquaculture potential of the blood and ponderous

ark clams in Florida waters. The information on the hatchery and growout protocols of these bivalve species obtained by this study is necessary before clam growers and other individuals can be encouraged to consider the possibility of investing in ark clam culture. The fact that commercial fishing ventures have been unable to meet market demand for these candidate species means there is a high probability that the information obtained will have direct application to the development of new aquaculture products for Florida.

Project Objectives

A grant awarded to the University of Florida (UF) Agricultural Experiment Station by the U.S. Department of Agriculture (USDA) allowed for priority needs of the shellfish aquaculture industry in Florida to be addressed. The UF Shellfish Aquaculture Advisory Committee identified research areas of immediate concern and species diversification was ranked high. Through this support, an investigation of the aquacultural and market potential of blood ark and ponderous ark in Florida was launched in 2002. The specific objectives of this project were:

1. To develop reliable spawning and larval rearing techniques for the production of blood ark and ponderous ark clam seed.

2. To determine the embryonic and larval development of the two species of arks during hatchery production for documentation of protocol.

3. To monitor the production performance (survival and growth) of ark clams during the nursery and growout phases using culture techniques similar to those for hard clams.

4. To assess the magnitude of the potential domestic market for both ark clams.

5. To determine product attributes desired by the seafood trade, especially size, color, shell appearance, taste, nutritional analyses, and shelf life in customary refrigerated storage.

6. To educate shellfish aquaculture industry members, in particular hatchery operators, producers, and wholesalers, as to the rearing requirements and market attributes of these alternative species.

Production methods, similar to those used for hard clam culture, are described, and the results of our hatchery, nursery, and growout trials for blood arks and ponderous arks are presented in Chapters 2 and 3 that follow.

Chapter 2: Ark Clam Seed Production

Hatchery Description

An experimental molluscan shellfish hatchery was constructed at the University of Florida (UF) Whitney Laboratory for Marine Bioscience in St. Augustine, Florida. The 288 ft^2 hatchery (Figure 1) consisted of four 500-liter (133 gallon) Kalwall® tanks with conical bottoms (larval rearing tanks) and two 378-liter (100 gallon) fiberglass raceways (post-set rearing area). Two tanks contained larvae, while the remaining two were cleaned and allowed to dry; culture tanks were drained daily and the larvae transferred to the alternate tanks.

Fig. 1. Experimental molluscan shellfish hatchery located at the University of Florida Whitney Laboratory for Marine Bioscience.

Phytoplankton was cultured in six 170 liter (45 gallon) Kalwall® culture tubes and eight 19 liter (5 gallon) carboys, with fluorescent lights on a timer with a total algal production capacity of 1,172 liters (312 gallons). Seawater pumped from the Atlantic Ocean was filtered through a series of 20, 10, 5, and 1µm (micron) cartridge filters and two ultraviolet sterilizers (Lifeguard® QL-25 and QL-40, 25 and 40 watts, respectively) (Figure 2). Only treated seawater was used in the hatchery. Temperature was measured using a standard thermometer, and salinity was measured using a temperature-compensated refractometer.

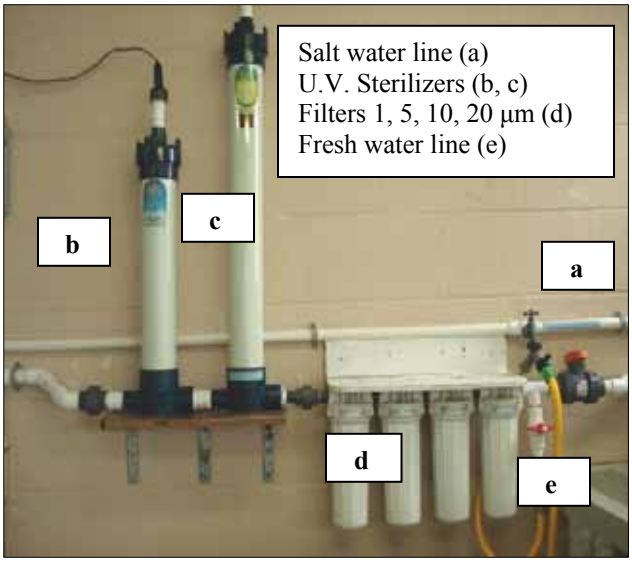


Fig. 2. Seawater filtration unit.

A spawning table constructed from a Rubbermaid® utility cart and a 75 liter (20-gallon) cement mixing tray was suitable for spawning both species of arks (Figure 3). White plastic strips placed in the bottom of the tray provided sufficient contrast to observe the pink/orange eggs of both species (Figure 4). A magnetic drive, submersible pump was placed inside a 19-liter (5-gallon) bucket and temperature-regulated water was pumped up to the spawning tray through a spray bar. The small volume of the bucket allowed for easy manipulation of water temperatures by adding chilled or hot water. A drain with 1- to 2-inch standpipe was used to control the water level in the tray. Placement of the tray on the cart allowed us to mobilize the spawning table and to store it when not in use.

Fig. 3. Spawning table.

Fig. 4. Blood ark clams on spawning table with white strips.

Broodstock Collection and Maintenance

Adult ark clams were collected from the wild between January 2002 and July 2005. Ponderous arks were collected from commercial clam aquaculture leases located in Cedar Key, New Smyrna, and St. Augustine, Florida. Blood arks were collected only from leases located on the east coast of Florida (New Smyrna and St. Augustine). In addition, both ponderous and blood arks were obtained from Georgia by University of Georgia partners.

Specimens collected from Cedar Key and Georgia were shipped overnight to the Whitney Laboratory in Styrofoam® containers with frozen gel packs. Animals collected from St. Augustine and New Smyrna were transported to the lab in coolers with gel packs, and kept covered overnight with moist towels. Overnight temperatures in the coolers ranged from 8 to12°C (46 to 54°F). Ark clams refrigerated overnight at 4°C (39°F) experienced high mortalities. With each shipment of adult arks, we attempted to induce spawning either the same day or the following day, depending upon arrival times (reproductively mature, or "ripe" bivalves often spawn in response to handling or other stresses).

Prior to spawning, the arks were thoroughly scrubbed to remove fouling organisms and placed on the spawning table in seawater at ambient temperature and salinity. Arks that failed to spawn on the first attempt were transferred to a broodstock conditioning system comprised of 40-liter (10-gallon) aquaria or coolers containing sand substrate, and held at 20°C (68°F) under static conditions. The adults were fed live algae (phytoplankton) according to feeding and maintenance protocols developed for hard clam broodstock (Hadley *et al*. 1997, Castagna and Kraeuter 1981), and received complete (100%) water exchanges twice weekly. Spawning was again attempted after six to eight weeks of conditioning or when seawater temperatures in the hatchery were conducive to larval rearing. Arks that spawned were returned to the natural environment for a refractory period prior to spawning again.

Spawning

Determination of ripeness — Prior to spawning, a few arks were checked for ripeness by dissection (Figure 5). The arks were carefully opened without tearing the visceral mass, and a small cut was made in the gonadal mass. Ripe males had a creamy white gonadal mass covering the gut area, while ripe females had a pinkish/orange gonadal mass covering the gut area. Gametes oozed out from very ripe animals. Samples were examined microscopically for sperm motility and egg development. Although mature sperm from ripe males actively swam upon addition of filtered sea water, smears of female gametes were not a reliable indicator of spawning success. Since the stage of the oocytes was difficult to assess, only a few ripe females were identified.

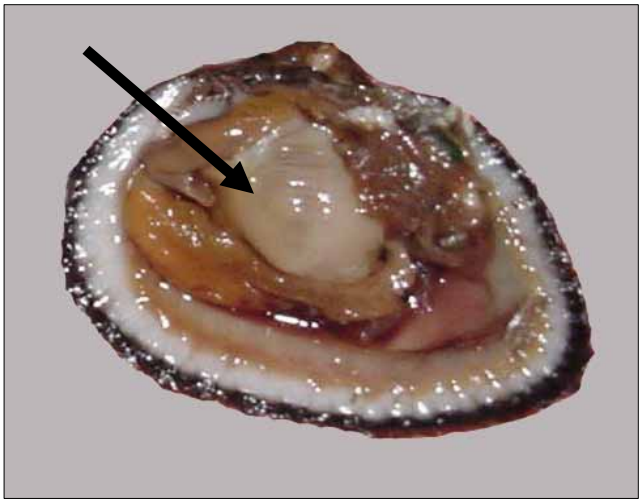


Fig.5. Ripe male ponderous ark with an arrow pointing to the creamy white gonadal mass covering the gut area.

Spawning induction — Ark clams were placed in filtered, UV-treated seawater at ambient temperatures, either individually in glass bowls (to separate species) or directly in the spawning tray (single species). Recently collected ark clams tended to release considerable amounts of detritus, sand, or mud once they started pumping. This material was flushed from the spawning tray or siphoned from individual beakers to minimize bacterial contamination. Algae (*Isochrysis galbana*, Tahitian strain) was added to the containers to stimulate pumping.

Once the arks opened their valves and began pumping, they were subjected to thermo-stimulation, a common technique used to induce spawning in hard clams and most other cultured

species of bivalves. Ark clams were induced to spawn by temperature cycling from 20°C (68°F) to 30°C (86°F) and held at these end temperatures for a minimum of 30 minutes. Temperatures were raised or lowered gradually over a period of 15 to 30 minutes. Most bivalve molluscs will spawn in response to an increase or decrease in temperature, depending on the species, their ripeness (gonadal index) or season. They often spawn on the first temperature increase, while others may require several cycles. In our experience with ark clams, the number of temperature cycles varied from two to six.

When ark clams began to spawn, they were segregated into individual containers where they continued to release gametes. When females stopped releasing eggs, they were removed from the container and fecundity was estimated using a Sedgewick-Rafter cell from a known volume of egg suspension. Sperm from several different males were combined to fertilize the eggs to ensure genetic diversity. Only a few drops of sperm suspension were required to fertilize thousands of eggs. The gametes were mixed and samples were microscopically examined to verify fertilization; extrusion of the polar body confirmed fertilization.

In addition to thermo-stimulated spawning attempts, we tried injecting serotonin in the abductor muscle (Gibbons and Castagna 1984). Although ark clams responded by active muscle contractions, this method was not successful in inducing spawning.

Spawning behavior — The spawning behavior of ark clams was similar to that of hard clams as described by Hadley *et al.* (1997). Males usually began spawning first, emitting a milky white stream of sperm which remained suspended in the water column (Figures 6a, 6b). Occasionally, some males released spermatocytes in clumps, which has similarly been described for hard clams when they are exposed to low salinity (Pline 1984); we were not able to determine if this was the case in ark clams. Sperm remained motile for up to two days if refrigerated. However, no unfertilized eggs were available to test the viability of refrigerated sperm.

Females released eggs for a few minutes to as long as an hour, depending on the size of the adult and ripening stage (Figures 6c, 6d). Eggs released into the water settled to the bottom of the container within a few minutes, and eggs released in clumps were typically of poor quality.

Blood ark spawning — Between June 11, 2002 and July 22, 2005, we conducted 29 spawning trials using a total of 1,151 blood arks. In ten of the trials, or 35% of the total, spawning was induced. Of the total number of broodstock used, 73 individuals, or 6%, spawned. Table 1 summarizes those trials resulting in spawns. The eight spawns, or 80%, which occurred during the months of April, June, and July were viable. The remaining two spawns, or 20%, which occurred in October and November, resulted in no or poor fertilization. These results corroborate the findings of Power *et al*. (2004) in which gonadal development of blood arks collected from the northeast coast of Florida peaked during the late spring-early summer months with a minor peak during the winter months (see Appendix I for more information).

The majority of animals spawned during the cooling cycle and it required 1 to 4 temperature cycles to induce spawning. Blood arks tended to spawn in pulses, dribbling a few eggs at a time for a period of 15 to 30 minutes, whereas males released sperm for a period of 15 to 60 minutes. More males than females spawned (56% versus 44%). In general, the number of ripe animals was low. The number of fertilized eggs from each spawning trial was estimated prior to stocking the larval tanks (Table 1). Similar numbers of eggs were obtained from wild-sourced broodstock from St. Augustine (18.49 million from 12 females – an average of 1.52 million/female) and conditioned broodstock (18.37 million from 9 females - an average of 2.05 million/female).

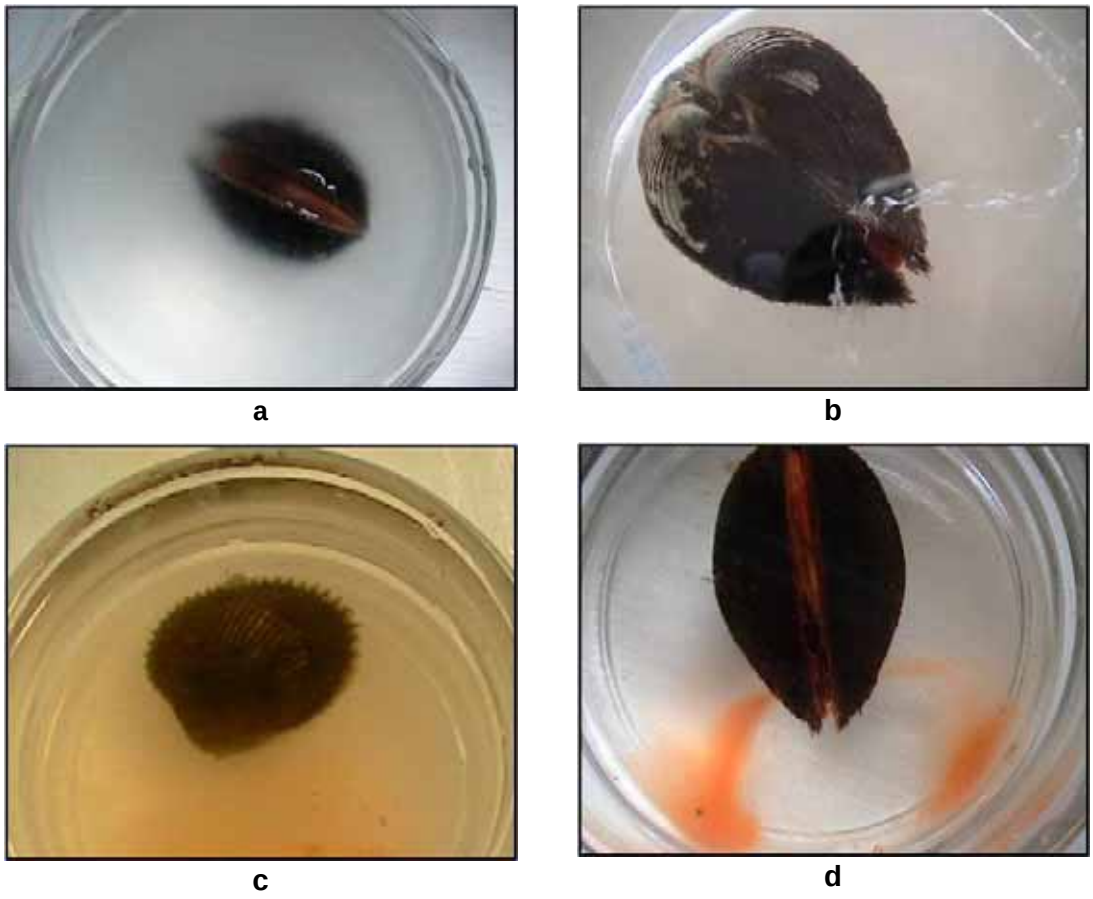


Fig. 6. Ark clam adults releasing gametes in individual containers during induced spawning: (a) male blood ark, (b) male ponderous ark, (c) female blood ark, (d) female ponderous ark.

Table 1. Summary of blood ark spawning trials from June 11, 2002 through July 22, 2005 which resulted in spawns.

Date	Broodstock origin *	Number of Animals	Number of Cycles	Cycle Phase	Number of Spawned Males	Number of Spawned Females	Percent Spawned (%)	Number of Fertilized Eggs
10/16/2002	Wild N.S.	8	2	Heating	0	3	38	0
3/27/2003	Wild S.A.	42	2	Cooling	1	3	10	Few
4/8/2003	Wild S.A.	102	2	Cooling	1	4	5	6,550,000
4/11/2003	Conditioned	15	1	Heating	4	3	47	8,200,000
4/18/2003	Wild S.A.	100	2	Cooling	5	2	7	3,200,000
4/23/2003	Wild S.A.	65	1.5	Heating	5	3	12	8,740,000
5/9/2003	Wild S.A.	74	3	Cooling	5	3	11	Few
6/26/2003	Conditioned	40	1	Cooling	8	4	30	3,920,000
6/26/2003	Wild N.S.	47	1	Cooling	1	1	4	Few
7/29/2003	Conditioned	40	4	Cooling	10	5	38	6,250,000
11/29/2004	Conditioned	48	2	Cooling	1	1	4	Few

* N.S. = New Smyrna, S.A. = St. Augustine, C.K. = Cedar Key

Fecundity was estimated for six females and varied among individual females (Table 2). The number of eggs ranged from 740,000 per female (43 mm length, 36 grams total weight) to 4 million per female (44 mm length, 27 grams total weight) with an average of 1.88 million per female. Total weight is defined as the combined weight of shell and meat, or the whole weight of the animal. The average zygote (fertilized egg) size was 55 µm length (Figure 7a), which was 15 µm, or about 20%, smaller than hard clam eggs which are ~70 µm in size (Hadley *et al*. 1997) (Figure 7c).

Table 2. Fecundity estimates for blood ark females.

	Female Size				
Date	Length (mm)	Height (mm)	Width (mm)	Total weight (g)	Number of eggs
4/18/2003	40	37	26	24.1	3,000,000
4/23/2003	44	42	30	33.9	1,278,333
4/23/2003	43	35	28	27.3	740,000
7/29/2003	50	39	35	52.4	750,000
7/29/2003	44	34	31	36.5	1,500,000
7/29/2003	44	37	31	36.7	4,000,000

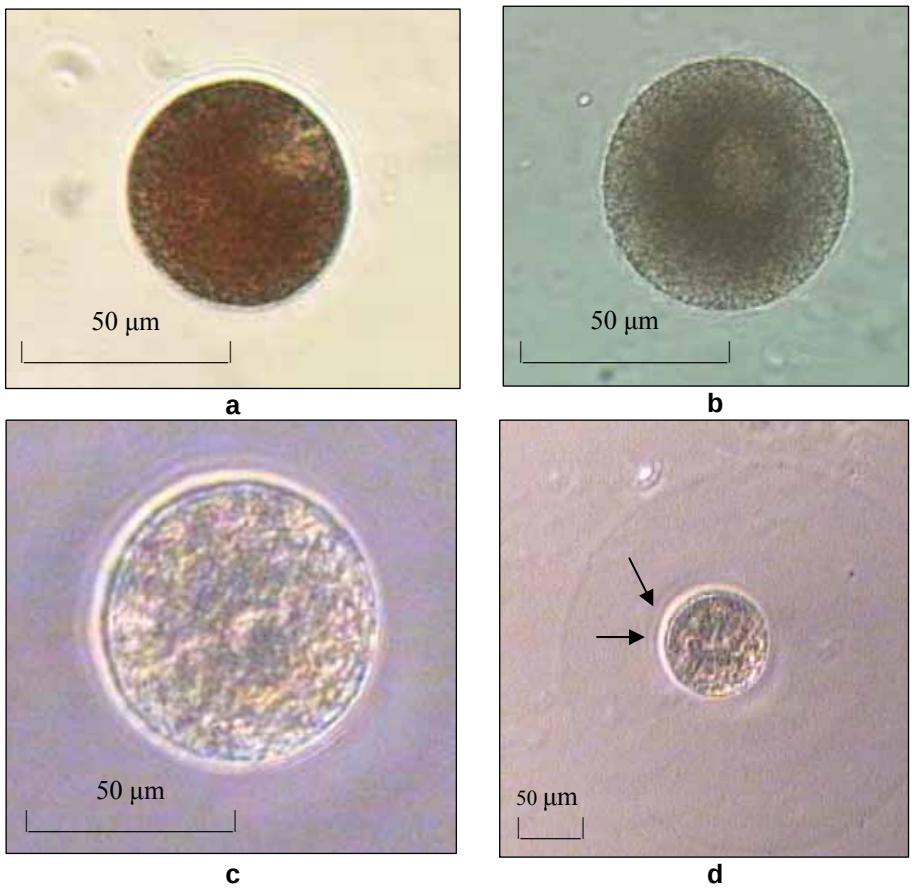


Fig. 7. Ark clam zygotes compared to a hard clam zygote, a) blood ark (55 µm), b) ponderous ark (65 µm), c) hard clam (70 µm). Note the gelatinous envelope (d), indicated by arrows, in the hard clam zygote which is not present in ark clam zygotes.

Ponderous ark spawning — Between May 13, 2002 and July 22, 2005, we conducted 51 spawning trials using a total of 2,267 ponderous arks. In nine of the trials, or 18% of the total, spawning was induced. Of the total number of broodstock used, 104 individuals, or 5%, spawned. Table 3 summarizes those trials resulting in spawns. The five spawns, or 55%, which occurred during the months of August, September, October, and November were viable. The remaining four spawns, or 44%, which occurred in March, April, and May resulted in no or poor fertilization. These results corroborate the findings of Power *et al.* (2005) in which gonadal development of ponderous arks collected from the Cedar Key area peaked during the summer and fall months with less spawning activity occurring during the spring when gametes were maturing (see Appendix II for more information).

Table 3. Summary of ponderous ark spawning trials from June 11, 2002 through July 22, 2005 which resulted in spawns.

Date	Broodstock origin *	Number of Animals	Number of Cycles	Cycle Phase	Number of Spawned Males	Number of Spawned Females	Percent Spawned (%)	Number of Fertilized Eggs
8/19/2002	Wild C.K.	79	3	Cooling	7	3	13	17,111,111
9/6/2002	Wild C.K., S.A.	47	4	Heating	2	2	9	Few
10/16/2002	Wild N.S.	48	2	Heating	19	9	58	**
11/7/2002	Wild N.S.	27	1st-2nd	Heating	6	3	33	**
9/26/2003	Wild N.S.	60	3	Heating	12	4	27	8,192,000
3/25/2004	Wild C.K.	106	3	Heating	0	3	3	Few
3/31/2004	Wild C.K.	94	3	Heating	5	3	9	Few
4/21/2005	Wild N.S.	35	2	Heating	5	0	14	0
5/18/2005	Wild C.K.	73	Spontaneous		16	5		Few

* N.S. = New Smyrna, S.A. = St. Augustine, C.K. = Cedar Key;
** Did not estimate numbers.

Thermo-stimulation for ponderous arks required 1 to 4 temperature cycles to induce spawning, with the majority of animals spawning during the heating cycle. Females released eggs for 20 to 90 minutes, whereas males released sperm for a period of 15 to greater than 60 minutes. More males than females spawned (69% versus 31%). In general, the number of ripe animals was low. Spawns resulting in the highest number of fertilized eggs were obtained from wild-sourced broodstock from Cedar Key (17.1 million obtained from three females – an average of 5.7 million/female).

Fecundity was estimated for five females and varied greatly among individuals (Table 4). The number of eggs ranged from 2.3 million per female (40 and 46 mm length) to 10.4 million per female (57 mm length) with an average of 5 million per female. Chanley (1966) reported two isolated female ponderous arks releasing 11 million eggs each. The average zygote (fertilized egg) size was 65 µm in length (Figure 7b), which was 10 µm larger than blood ark eggs and 5 µm, or 7%, smaller than hard clam eggs (Hadley *et al*. 1997).

Table 4. Fecundity estimates for ponderous ark females.

	Female Size				
Date	Length (mm)	Height (mm)	Width (mm)	Total weight (g)	Number of eggs
8/19/2002	57	49	52	n.a.	10,400,000
8/19/2002	53	42	36	n.a.	6,711,111
9/26/2003	46	33	32	n.a.	2,336,000
9/26/2003	50	39	34	n.a.	3,520,000
9/26/2003	40	31	28	n.a.	2,336,000

Larval Rearing

Larvae were reared from fertilization to setting following hatchery protocols similar to those used for hard clams (Hadley *et al.* 1997, Castagna and Kraeuter 1981); as a comparison, hard clam larvae were reared at the same time under identical hatchery conditions. Fertilized eggs (zygotes) were rinsed on a 20 µm screen with filtered sea water and placed in a 20 liter (5 gallon) bucket, where they were evenly suspended by gently mixing the water using a perforated plunger (Figure 8).

Fig. 8. Sampling ark clam eggs and larvae with a pipette while continuously and evenly suspending them in a bucket using a perforated plunger.

In order to estimate the number of eggs from the spawn, five aliquots were taken from the bucket using a volumetric pipette, counted with a dissecting microscope, and measured with a binocular microscope equipped with a calibrated ocular micrometer. Zygotes were stocked (7.8 – 16.4 zygotes/mL) into larval rearing tanks, containing 1 µm filtered, UV sterilized sea water. Light aeration (30 mL/minute) was provided by a single airstone per tank.

Larvae were kept in static cultures with daily water exchanges (100%) initiated 48 hours after fertilization. Daily maintenance also included population estimates, growth measurements, addition of algal food consistent with size and density of larvae, and video recording of larval development. Water exchanges consisted of draining the water from the tank through a sieve (or screen) placed in a 27 liter (7 gallon) container with a combined drain/standpipe. During draining, the sides of the tanks were rinsed with seawater to dislodge any attached larvae. A series of polyester (Pecap®) sieves (38, 54, 74, and 100 µm) were used to retain the larvae during draining. Once collected on the screens, larvae were rinsed with a gentle flow of filtered seawater and concentrated in a bucket filled with 10 liters of filtered seawater. Larvae were sampled using methods described for estimating egg numbers

(see above). At this time, they were also assessed for general health and normal behavior using a video camera for recording larval development. After estimating numbers, the larvae were stocked into cleaned larval rearing tanks which had been previously rinsed and disinfected using standard hatchery protocols (Hadley *et al*. 1997).

Phytoplankton species were cultured following standard methods of algal production using f/2 media (Guillard and Ryther 1962). When eggs were stocked into larval rearing tanks, a golden-brown flagellate (*Isochrysis galbana,* Tahitian strain) was added at approximately 10,000 cells/mL, and, again, on the following day. For the remainder of the larval period, densities of approximately 25,000 cells/mL (early stages) to 50,000 cells/mL (post set) were maintained on a daily basis. As larvae approached metamorphosis, a diatom (*Chaetoceros gracillis*) was added. Phytoplankton cell density in stock cultures was counted using a hemacytometer, and the volume to achieve the desired algal density in larval culture tanks was calculated.

Early Larval Development

The timing of early development for both species of arks was similar. For a complete description from fertilization to straight hinge veliger stage refer to the report in this publication by John Baldwin, Florida Atlantic University (Appendix III). Both arks reached the straight hinge veliger (D-shaped) stage around 24 hours after fertilization. These larvae had a well-developed velum and a long apical flagellum (a group of long cilia which adhere to one another to form a whip-like structure). Within a few days, the larvae reached the umbo stage and lost the apical flagellum. This is in contrast to hard clams, in which the apical flagellum is present through metamorphosis. Larvae were active swimmers and were dispersed throughout the water column. Prior to metamorphosis, behavior of larvae changed as they tended to congregate near the bottom of the tank. At that time, the appearance of a developing foot signifies the final larval stage - the pediveliger. This stage was prolonged for arks, and we did not observe the typical substrate exploration behavior that occurs prior to settlement, as is common for hard clams.

Blood ark larvae — Larval stages from straight hinge through pediveliger for the blood ark are illustrated in Figure 9. Of the 10 spawns, there were only two larval cohorts in which blood arks reached setting. These were spawned on April 8 and June 26, 2003. As we follow these two spawns, we will refer to them as BA1 and BA2, respectively. Water temperatures during the two larval rearing periods averaged 25.6^{o}C (78.1^{o}F) and 27.4^{o}C (81.3^{o}F), respectively. Salinities for both trials averaged 34-35 ppt (‰). Juveniles from the latter spawn (BA2) were used in the field nursery and growout trials. Table 5 summarizes both larval rearing periods. The size of the first veliger stage (straight hinge) measured 80-85 µm in length and 65-70 µm in height; this is about 25-30 µm (23-27%) smaller than a hard clam veliger at D-stage, which are ~110 µm in length. As larvae grew, the length to height ratio continued to increase. By the time of setting, larvae had acquired a distinctive oblong shape, 270-275 µm in length and 190-200 µm in height, 70-75 microns larger than hard clam pediveliger larvae, which are ~200 µm in length. Larvae were brown to reddish in color and became darker by the end of the larval period. The larval period lasted 17 days, about 5 to 7 days longer than what is typical for hard clams (9-12 days). Survival from straight hinge larvae to pediveliger was 8.7 and 3.5% in BA1 and BA2, respectively. Setting of blood arks was comparable to another ark clam (*Anadara granosa*), which took 18 days to set (Muthiah *et al.* 1992).

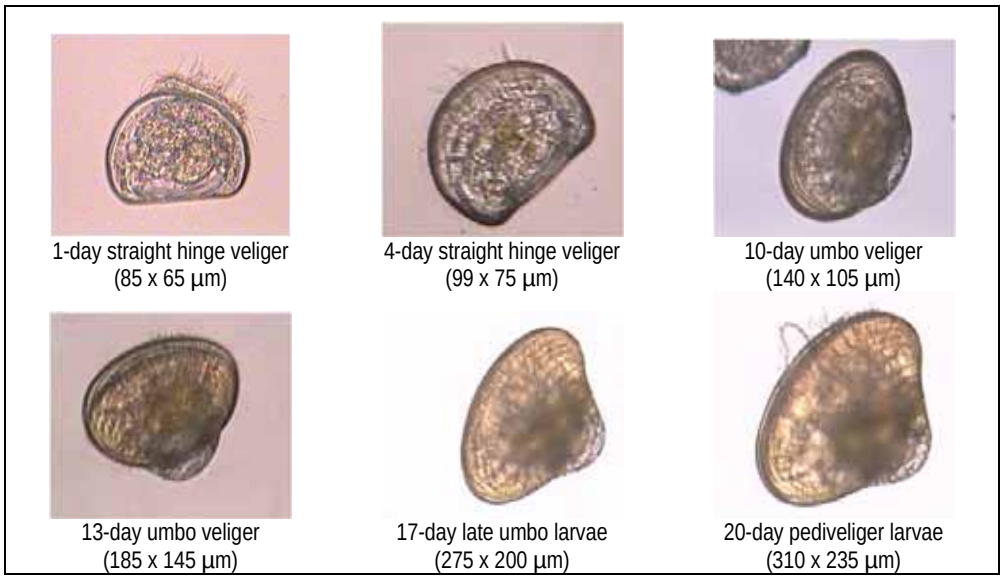


Fig. 9. Larval stages from straight hinge veliger through post-set for the blood ark.

Table 5. Larval development from fertilization (day 0) through setting (day 17) for two blood ark spawns.

Spawn date: April 8, 2003 (BA1) Average water temperature: 25.6°C					Spawn date: June 26, 2003 (BA2) Average water temperature: 27.4°C				
Day	Size (µm)	Screen size (µm)	Veligers per mL	Survival* (%)	Day	Size (µm)	Screen size (µm)	Veligers per mL	Survival* (%)
0	55		13.1		0	55		7.8	
1	85x65	34	4.6	100.0	1	80 x 65	34	4.3	100
2	90 x 70	34	4.6	100.0	2	85 x 70	34	4.1	94.4
3	105 x 80	34			3	90 x 75	34	3.2	73.1
4	120 x 95	34	3.5	76.1	4	90 x 75	34	2.6	59.3
5	120 x 95	54			5	100 x 75	34	2.3	53.2
6		54	2.2	47.8	6	110 x 90	34	2.2	51.9
7	130 x 95	54	1.4	31.3	7	125 x 100	34	1.7	38.9
8	130 x 95	54	1.4	29.3	8	125 x 100	54	0.7	15.7
9	135 x 105	54	1.3	28.7	9	125 x 100	54	0.5	12.0
10		54	1.2	27.0	10	140 x 105	54	0.3	7.9
11	145 x 135	54	1.0	22.2	11	150 x 115	54	0.3	6.4
12		74	0.7	15.7	12				
13	190 x 145	74	0.6	13.3	13	185 x 145	54	0.2	4.6
14	205 x 150	74	0.6	12.2	14				
15	235 x 170	74	0.5	10.4	15	185 x 145	74	0.2	3.5
16		110	0.4	13.0	16				
17	270 x 190	110	0.2	8.7	17	275 x 200	110	0.2	3.5

* Survival was estimated starting at day 1.

Ponderous ark larvae — Larval stages from straight hinge through pediveliger for the ponderous ark are illustrated in Figure 10. Of the nine spawns, there were only two larval cohorts in which ponderous arks reached setting. These were spawned on October 16, 2002 and September 26, 2003. As we follow these two spawns, we will refer to them as PA1 and PA2, respectively. Water temperatures during the two larval rearing periods averaged 26.1°C (79.0°F) and 26.5°C 9.7°F), respectively. Salinities for both trials averaged 34-35 ‰. Juveniles from the PA2 group were used in the field nursery and growout trials. Table 6 summarizes both larval rearing periods. The size of the first veliger stage (straight hinge) measured 90-95 µm in length and 70-75 µm in height, about 10 µm, or 9-11%, larger than a blood ark veliger. Like the blood ark larvae, the length to height ratio continued to increase as they grew. By the time of setting, larvae had acquired an oval shape, 210-235 µm in length and 155-175 µm in height, about 40-65 microns smaller than blood ark larvae and slightly larger (10-35 µm) than hard clam pediveliger larvae. Larvae were brown to reddish in color and became darker by the end of the larval period. The larval period of the two groups (PA1 and PA2) lasted 21 days, four days longer than that of the blood ark. The larval period was shorter in this study as compared to four to six weeks reported by Chanley (1966). Survival from straight hinge larvae to pediveliger in the PA2 group was estimated at 19%. The majority of the PA1 group died during larval rearing; we speculate that a defective UV sterilizer bulb contributed to mortalities. Despite the few numbers, we continued to maintain this group in a smaller tank (20 liter, 5 gallon).

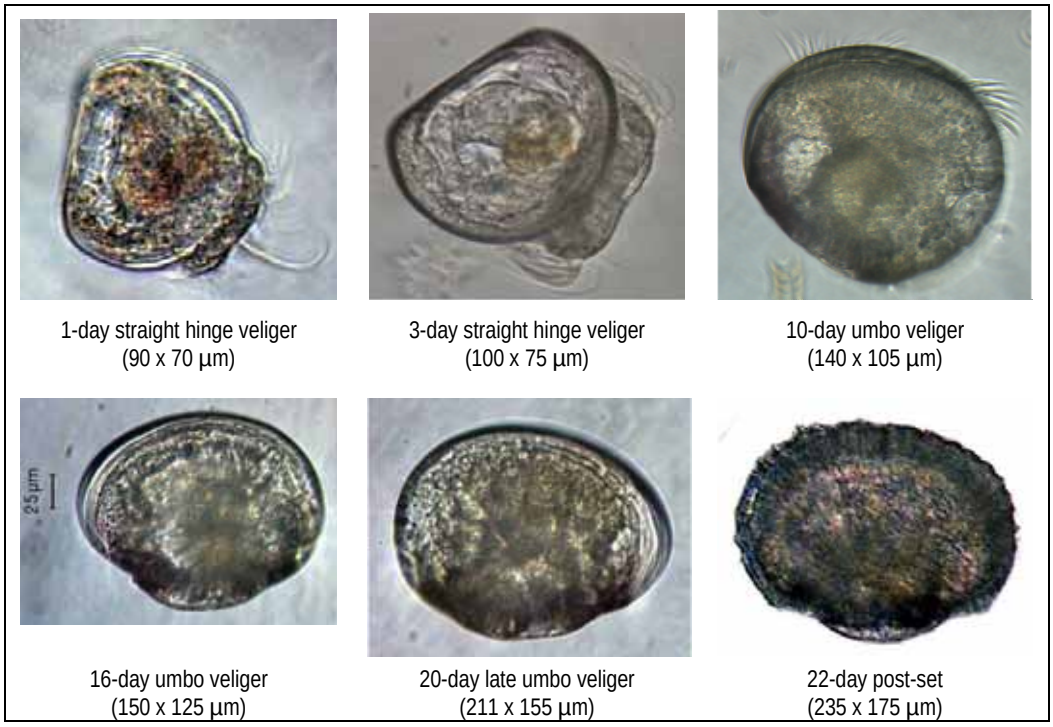


Fig. 10. Larval stages from straight hinge veliger to post-set for the ponderous ark.

Table 6. Larval development from fertilization (day 0) to setting (day 21) for two ponderous ark spawns.

Spawn date: October 16, 2002 (PA1) Average water temperature: 26.1ºC					Spawn date: September 26, 2003 (PA2) Average water temperature: 26.5ºC				
Day	Size (µm)	Screen Size (µm)	Veligers per mL	Survival (%)	Day	Size (µm)	Screen Size (µm)	Veligers per mL	Survival * (%)
0	65		n.a.	n.a.	0	65		16.4	
1	95 x 75		n.a.	n.a.	1	90 x 70		3.0	100
2	95 x 75	34	n.a.	n.a.	2	90 x 70	34	2.4	78.9
3	100 x 75	34	n.a.	n.a.	3	100 x 75	34	2.3	75.7
4	100 x 75	34	n.a.	n.a.	4	100 x 75	34	2.3	75.7
5	105 x 80	34	n.a.	n.a.	5	105 x 85	34	2.2	72.4
6	120 x 90	34	n.a.	n.a.	6	110 x 85	34	2.1	69.1
7	120 x 90	34	n.a.	n.a.	7	110 x 85	34	1.7	54.3
8	130 x 105	54	n.a.	n.a.	8	120 x 100	34	1.7	54.3
9	150 x 120	54	n.a.	n.a.	9	130 x 100	54	1.7	54.3
10	150 x 120	74	n.a.	n.a.	10	130 x 100	54	1.3	41.1
11	150 x 125	74	n.a.	n.a.	11	130 x 100	54	1.2	39.5
12	170 x 130	74	n.a.	n.a.	12	130 x 100	54	1.0	32.9
13	170 x 130	74	n.a.	n.a.	13	130 x 100	54	0.8	27.1
14	175 x 150	74	n.a.	n.a.	14	130 x 100	54	0.7	22.4
15	175 x 150	74	n.a.	n.a.	15	150 x 125	54	0.7	21.4
16	200 x 150	100	n.a.	n.a.	16	150 x 125	74	0.6	19.7
17	200 x 150	100	n.a.	n.a.	17	175 x 140	74	0.6	18.9
18	235 x 175	100	n.a.	n.a.	18	200 x 150	110	0.6	18.9
19	235 x 175	100	n.a.	n.a.	19	205 x 160	110	0.6	18.9
20	235 x 175	100	n.a.	n.a.	20	211 x 155	110	0.6	18.9
21	235 x 175	100	n.a.	n.a.	21	211 x 155	110	0.6	18.9

* Survival was estimated starting at day 1.

Setting and Post-set Rearing

When metamorphosis was first observed, defined as the absence of a velum and cessation of swimming, the pediveliger larvae were transferred from the larval tanks to downwelling cylinders. These downwellers (or silos) were constructed from 19 liter (5 gallon) buckets with a bottom screen of 120 µm mesh, and were suspended in 378 liter (100 gallon) raceway tanks (Figure 11). Water was air-lifted from the raceway tanks into the top of the downweller cylinders, thus directing the water flow through the arks and bottom screen.

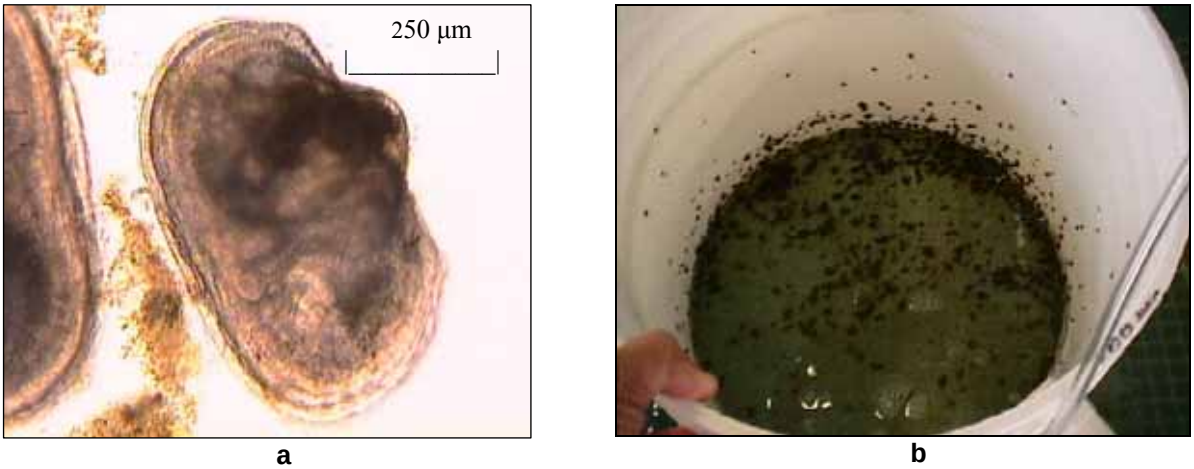
Metamorphosis, or setting, success was unpredictable; a large percentage of arks continued to swim in the downwellers and died without setting. Those that successfully set crawled using their foot with their shells upright. Set arks attached to the downwellers and each other by their byssal threads, making it difficult to dislodge them for counting or size sorting. Therefore, these activities were not conducted. Within a week after setting, some arks began to crawl up the sides of the downwellers. This upward migration of juvenile arks was noted by Power and Walker (2002) for blood arks and transverse arks, *Anadara transversa*. This behavior suggests that upwellers (upward flowing systems) should not be used for post-set rearing of ark clams. After a period of time, ark juveniles were transferred to the land-based nursery for further rearing.

During post-set rearing, arks were fed a 50:50 mixture of algae (*Isochrysis galbana,* Tahitian strain and *Chaetoceros gracillis*) daily at a cell density of approximately 50,000 cells/mL. The downweller tanks were static (not flow-through or recirculating) with complete water changes conducted on alternate days. During water changes, ark clams were rinsed with saltwater. Unlike hard clams, arks remained open most of the time and we avoided rinsing them with freshwater during the post-set culture period.

Fig.11. Downwellers in tanks used to rear post-set ark clams.

Blood ark setting and post-set rearing — Approximately 200,000 pediveliger larvae from the BA1 group were transferred to the setting downwellers on April 25, 2003. Setting survival could not be estimated because the strong byssal threads of the animals made it difficult to dislodge them from the sides of the downwellers. The post-set arks remained in downwellers for 34 and 38 days for the BA1 and BA2 groups, respectively. During this time, post-set arks grew to a length of 2.6 ± 0.35 mm and 3.2 ± 1.2 mm in the respective BA1 and BA2 groups, resulting in growth rates of 0.07-0.08 mm/day. Water temperatures during the two post-set rearing periods averaged 25.6°C (78.1°F) and 27.9°C (82.2°F), respectively. Salinities for both trials averaged 34-35 ‰.

A photomicrograph of blood arks in the downwellers prior to being moved to the land-based nursery is shown in Figure 12a. At this stage, juveniles were hardier; although they still attached to each other in clumps, arks were easier to handle and dislodge from the downwellers (Figure 12b). We could not estimate survival during this period because arks were difficult to count, unlike hard clams which separate easily allowing numbers to be estimated volumetrically.



a **b**

Fig. 12. Rearing juvenile blood arks a) photomicrograph of individual, b) post-set in downwellers.

Ponderous ark setting and post-set rearing — A small number of pediveliger larvae from the PA1 group were transferred to the setting downwellers on November 6, 2002. Approximately 287,000 pediveliger larvae from the PA2 group were transferred to the setting downwellers on October 17, 2003. As with the blood arks, setting survival could not be estimated because the strong byssal threads of the animals made it difficult to dislodge them from the wellers and from other arks without damage. Water temperature in the tanks during the PA1 and PA2 post-set rearing periods were maintained with aquarium heaters and averaged 24.6 ± 0.95ºC (76ºF) and 24.9 ± 0.58ºC (77ºF), respectively. Salinities for both periods averaged 34-35 ‰.

Post-set arks from the PA1 group remained in downwellers at the Whitney Lab hatchery/nursery over the winter for approximately 5.5 months. They reached 4.2 ± 0.39 mm in length, attaining a growth rate of 0.7 mm per month. On April 22, 2003 about 3,300 arks were moved to a commercial land-based nursery nearby in St. Augustine. Figure 13 shows a photomicrograph of ponderous arks during the post-set rearing period. Post-set arks from the PA2 group remained in downwellers for 31 days, at which time they had reached an average length of 0.44 ± 0.14 mm.

Fig. 13. Photomicrograph of juvenile ponderous ark, 650 µm in length.

Settlement (Metamorphosis) Experiments

Metamorphosis is the transformation from the final larval stage to a juvenile, entailing dramatic change in morphology, physiology, and behavior. In marine bivalve molluscs, settlement (or metamorphosis) involves a transition from a swimming, planktonic veliger larva to an essentially sedentary juvenile. It should be noted that marine invertebrate larvae tend to fall into two categories related to larval development and metamorphosis. “Determinant” larvae will metamorphose at a pre-determined point in their larval cycle (i.e. at “competency to metamorphose”) and will live or die depending on the environmental conditions at that time. Hard clam larvae are in the determinant category. “Indeterminant” larvae will not metamorphose until the environmental conditions (e.g. substrate, chemical cues) are appropriate. Therefore, the larval phase may be extremely protracted in the absence of suitable substrate or environmental cues. Ark clams appear to be in the indeterminant category.

Larval settlement and metamorphosis define a period of high stress, and hatcheries frequently experience significant mortalities during this portion of the production cycle. Several factors may contribute to mortality at metamorphosis in bivalves. These include 1) insufficient nutrition during the larval phase, 2) sub-optimal salinity or temperature, 3) poor water quality contributing to high levels of heterotrophic bacteria (i.e. *Vibriosis*), 4) lack of appropriate substrate for settlement, and 5) lack of chemical inducers for settlement. The first three items are rarely problems for hatchery production if proper hygiene and management protocols are emphasized. However, the lack of appropriate substrate or chemical inducers can be a significant problem.

Determining the appropriate substrate or chemical inducer for settlement and metamorphosis is not straightforward, particularly for bivalve species for which there is little documentation. Substrates are important for successful metamorphosis in some bivalves. While some do not require substrate (e.g. hard clams), others require sediment (e.g. pholad clams), and several species require a hard surface (e.g. mussels and oysters). Since ark clams have byssal threads at post-set, the availability of a hard substrate may be important. Some molluscs require chemical cues (dissolved organic compounds) to induce metamorphosis. Chemical cues may include hydrogen peroxide (H_2O_2), potassium chloride (KCl), epinephrine, and nor-epinephrin. Identification of substrates and chemical cues necessary for ark clam settlement and metamorphosis, if any, should significantly improve survival through this stressful process.

In May 2003, experiments were conducted to evaluate the potential for physical substrates and chemical cues to increase successful settlement (metamorphosis) of blood ark larvae. To evaluate substrate-type cues, one hundred milliliters of UV sterilized seawater were placed in glass containers and 20 blood ark larvae (near setting stage) were added to each glass container. Seven treatments (three replicates each) were evaluated:

1) seawater only (control),
2) seawater and sand substrate (sand < 250 μm),
3) seawater and sand/mud substrate,
4) polypropylene strands (one foot of aged monofilament line) suspended in the water column,
5) sand substrate and seawater containing exudates from adult ark clams,
6) sand/mud substrate and seawater containing exudates from adult ark clams, and
7) polypropylene strands and seawater containing exudates from adult ark clams.

To evaluate other chemical cues, 100 milliliters of UV sterilized seawater were placed in glass containers and approximately 20 blood ark pediveligers were added to each. Three treatments (3 replicates each) were evaluated:

1) hydrogen peroxide (H_2O_2) at a concentration of 60 ppm,
2) potassium chloride (KCl) at a concentration of 15 mM above ambient seawater, and
3) nor-epinepherin at a concentration of 10^{-4}molar.

Larvae were fed *Isochrysis* at a concentration of about 2,500 cell/mL. For both substrate and chemical cue evaluations, glass containers were checked daily for settled larvae. After five days all larvae were either dead or we were unable to find them in the large volume of water and substrate. For this reason, further experiments were conducted in containers with smaller volumes.

In an attempt to improve setting of ponderous ark clams, a preliminary trial was conducted in April 2004 using various algal species (*Isochrysis, Pavlova, Chaetocerous*, a blue green, and a mixture of benthic algae collected from the land-based nursery and sieved through a 35 μm sieve). Tissue culture plates (6-well) were used in which 10 mL of seawater [water temperature, 21ºC (70°F)] was added to each well. Each algal species was replicated two times and two wells served as controls with no food. Ten ponderous ark pediveliger larvae were stocked into each well. The initial algal cell densities were about 10,000 cell/mL. Setting was assessed daily for six days. Metamorphosis only occurred in the benthic algae treatment with 60% of the ponderous larvae set by Day 6 (Table 7). This suggests that arks may rely on pedal feeding during early post-set, and this approach for inducing metamorphosis should be explored further.

Table 7. Results of ponderous ark setting experiment evaluating several algal species after six days.

	Percentage (%)		
Algal Treatment	**Live Veligers**	**Dead Veligers**	**Set**
No Food	0	100	0
Isochrysis	30	70	0
Pavlova	45	55	0
Chaetocerous	25	75	0
Blue greens	0	100	0
Benthic diatoms	15	25	60

Land-based Nursery

A land-based nursery consisting of two 1,650-liter (440-gallon) shallow raceways (4 x 8 x 2 feet deep) were located at the Whitney Lab (Figure 14). Water was supplied to the raceways from the Intracoastal Waterway using a 1-Hp submersible pump. The upper raceway served as a sediment settling tank, and two screens (200 and 500 μm), were used to filter incoming water. Water from the upper tank drained through a standpipe into the bottom tank which housed the clams in downwellers. This minimized the introduction of fouling organisms, predators, and silt to the juvenile ark clams. Downwellers (24-inch diameter, with 200 and 400 μm screens) were placed inside the bottom tank. Arks were rinsed on alternate days, or as needed, if excessive siltation was observed. Water temperature and salinity of incoming water were monitored every 30 minutes by a YSI 6600 data logger.

Fig. 14. Shallow raceways and cylindrical downwellers comprised the land-based nursery at the Whitney Laboratory used to rear ark juveniles.

Blood ark land-based nursery — BA1 and BA2 ark juveniles were transferred to 200 µm downwellers in the land-based nursery 34 and 38 days after post-set, respectively, on May 29, 2003 and August 20, 2003. Again, numbers were not estimated because clams still had heavy byssal threads. One month later, arks were moved to larger mesh downwellers (400 µm). At this time, arks were still attached to each other and the downwellers but were easy to separate and remove from the downwellers (Figure 15).

Fig. 15. Blood ark juveniles reared in downwellers during land-based nursery stage.

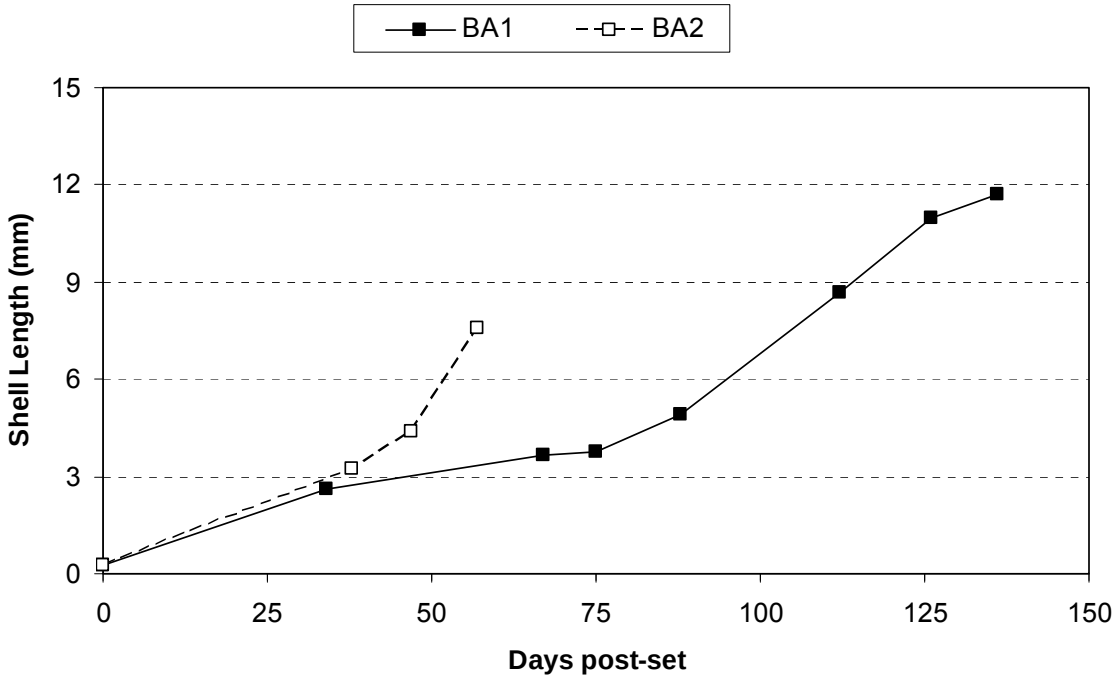


Fig. 16. Growth (shell length, millimeters) of blood arks (BA1 and BA2) from post-set through land-based nursery rearing. Shell length at the time of set was 0.27 mm for BA1 and BA2.

Growth of BA1 and BA2 arks from post-set through land-based nursing is presented in Figure 16. Water temperatures during the land-based rearing periods averaged 24.6 ± 1.8ºC (76ºF) and 23.8 ± 1.5ºC (75ºF) for the BA1 and BA2 groups, respectively. Salinities for the respective trials averaged

30.2 ± 3.2 ‰ and 29.1 ± 3.1 ‰. BA1 arks reached an average length of 11.7 ± 1.7 mm in 102 days, achieving a growth rate of 2.7 mm/month. BA2 arks reached an average length of 7.6 ± 1.0 mm in 19 days, achieving a growth rate of 6.8 mm/month. Given the small number of BA1 arks (< 100), only BA2 arks (~14,000) were sieved and sorted by size prior to being moved to the field for further culture. Results of field trials for blood arks are reported in the next section and compared with hard clams following similar culture protocol.

Ponderous ark land-based nursery — Arks in the PA1 group over-wintered at the Whitney Laboratory were transferred after 167 days to a commercial land-based nursery nearby (about 3,300 clams) on April 22, 2003. PA1 arks reared in downwellers for 48 days reached an average shell length of 10.0 + 1.4 mm, achieving a growth rate of 3.7 mm/month. Water temperatures during this rearing period averaged 24.1 ± 1.7ºC (76°F) . Salinities averaged 31.1 ± 1.4 ‰. The nursery operator closed the facility for the summer; ponderous arks were transported to another land-based nursery located in Cedar Key on June 9, 2003 for further rearing. After a month (July 9, 2003) of being reared in 1-mm mesh trays held in raceways, arks reached an average length of 13.1 + 1.4 mm, achieving a growth rate of 3.1 mm/month. Water temperatures and salinities during this period averaged 28.9 + 1.5ºC (84.1 + 2.8°F) and 23.7 + 2.4 ‰, respectively. In early August, mortalities were observed; it was estimated that over 80% of the ponderous arks died within a week. High water temperatures (>32ºC [90°F]), coupled with a decline in salinity (17 ‰) at that time, may have attributed to the mortalities. Hard clams nursed in the same system were also stressed, but mortalities were estimated at less than 50%. Growth of PA1 arks from post-set through land-based nursery rearing is presented in Figure 17.

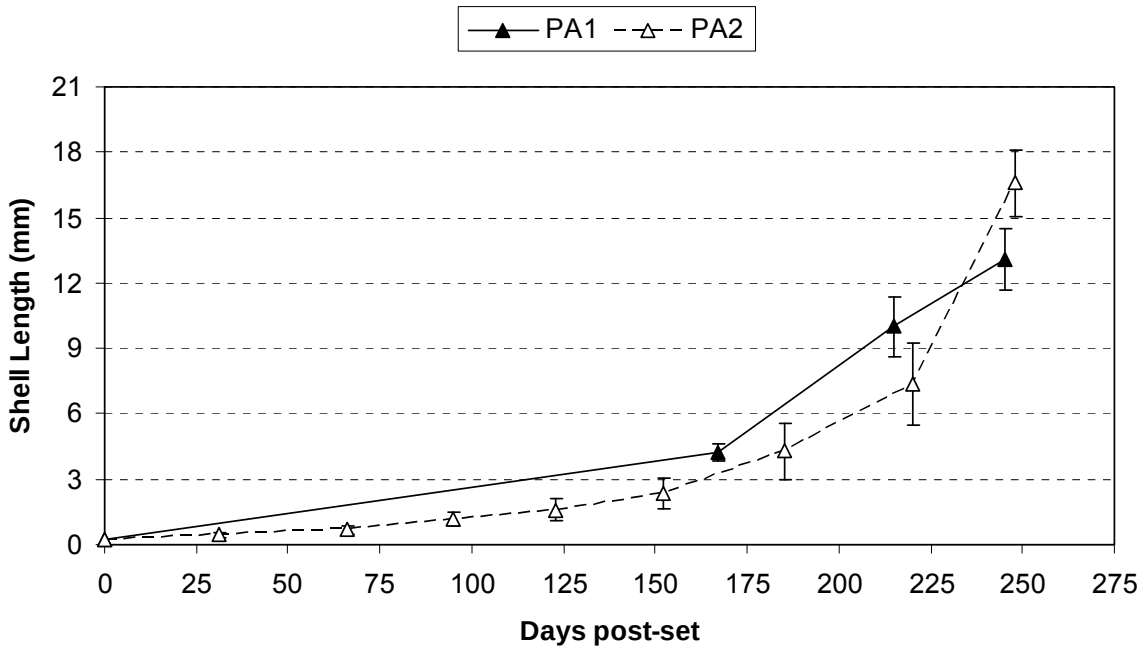


Fig. 17. Growth (shell length, millimeters) of ponderous arks (PA1 and PA2) from post-set through land-based nursery rearing. Shell length at the time of set was 0.24 mm (PA1) and 0.21 mm (PA2). Error bars reflect the standard deviation from the averages.

PA2 post-set arks were transferred to a commercial hatchery/nursery in Cedar Key on November 17, 2003 for over-wintering. An estimate of the number of arks was not made; average shell length and width was 0.44 + 0.14 mm and 0.31 mm + 0.09, respectively. Arks were reared in 210 μm mesh

downwellers held in a recirculating tank system. Methods used for over-wintering hard clams in the facility were also employed for the ponderous arks. These included supplemental feeding with cultured algae twice daily and exchanging water once daily. As the arks grew they were transferred to downwellers with larger mesh sizes (500 and 710 µm). It was noted this was difficult due to the strong byssal threads of the ark juveniles. In April, the system was switched to flow-through; unfiltered, ambient seawater was provided to the wellers. On June 22, 2004 about 1,200 arks at an average size of 16.6 + 1.6 mm in length and 8.6 + 0.9 mm in width were transferred to the field nursery. From November through April, growth was slow with an average rate of 1.9 mm/month in length obtained as water temperatures averaged 16.8 + 4.0oC (62.2 + 7.1oF) over this time period. As water temperatures increased to a monthly average of 27.2 + 2.3 oC (80.9 + 3.3 oF) in May and 30.6 + 1.0 oC (87.0 + 1.8 oF) in June, respective growth rates increased to 3.1 mm/month (May) and 9.22 mm/month (June) (Figure 17). Salinities averaged 25.0 + 4.4 ‰ over the 7-month nursery rearing period. Information on continued field nursing and growout of this group of ponderous arks can be found in the next section.

Comparison with Hard Clam Seed Production

Two groups of hard clams were spawned and reared at the Whitney Lab experimental hatchery to compare with the production of ark clam seed. Broodstock obtained from a domestic source were spawned on June 27, 2003 (HC1) and broodstock collected from the wild were spawned on March 17, 2004 (HC2). Both groups of hard clams were spawned and cultured using the same hatchery conditions as for the ark clams. The HC1 group was reared simultaneously in the hatchery with the BA2 group. The results are presented in Table 8.

Table 8. Larval development from fertilization (day 0) to setting (day 9 to 12) for two hard clam spawns.

Spawn date: June 27, 2003 (HC1) Average water temperature = 27.4ºC					Spawn date: March 17, 2004 (HC2) Average water temperature = 25.6ºC				
Day	Size (µm)	Screen size (µm)	Veligers per mL	Survival * (%)	Day	Size (µm)	Screen Size (µm)	Veligers per mL	Survival* (%)
0	70				0	85			
1	105 x 90	34	7.2	100.0	1	100 x 85	34	8	100.0
2	120 x 95	34	6.9	95.6	2	125 x 100	54	8	100.0
3	125 x 105	34	6.7	93.3	3	135 x 100	54		
4	155 x 135	54	5.6	77.2	4	145 x 120	54	7	91.8
5	160 x 130	74	5.2	72.8	5	150 x 130	54		
6	170 x 155	74	5.2	71.7	6	160 x 140	74	7	88.6
7	200 x 180	100	4.8	66.7	7	160 x 140	74	5	69.0
8		100	4.5	62.4	8	175 x 155	100	3	41.3
9	225 x 215	100	4.5	62.2	9	175 x 155	100		
					10	175 x 155	100	3	38.5
					11	185 x 200	100		
					12	210 x 195	100	3	38.0

The duration of the larval stage for the two hard clam groups ranged from 9 to 12 days in contrast to 17 and 21 days for the blood and ponderous arks. Comparative growth rates of blood arks (BA 1 and BA2), ponderous arks (PA1 and PA2), and hard clams (HC1 and HC2) from straight hinge veliger (day 1) to metamorphosis (set) is illustrated in Figure 18.

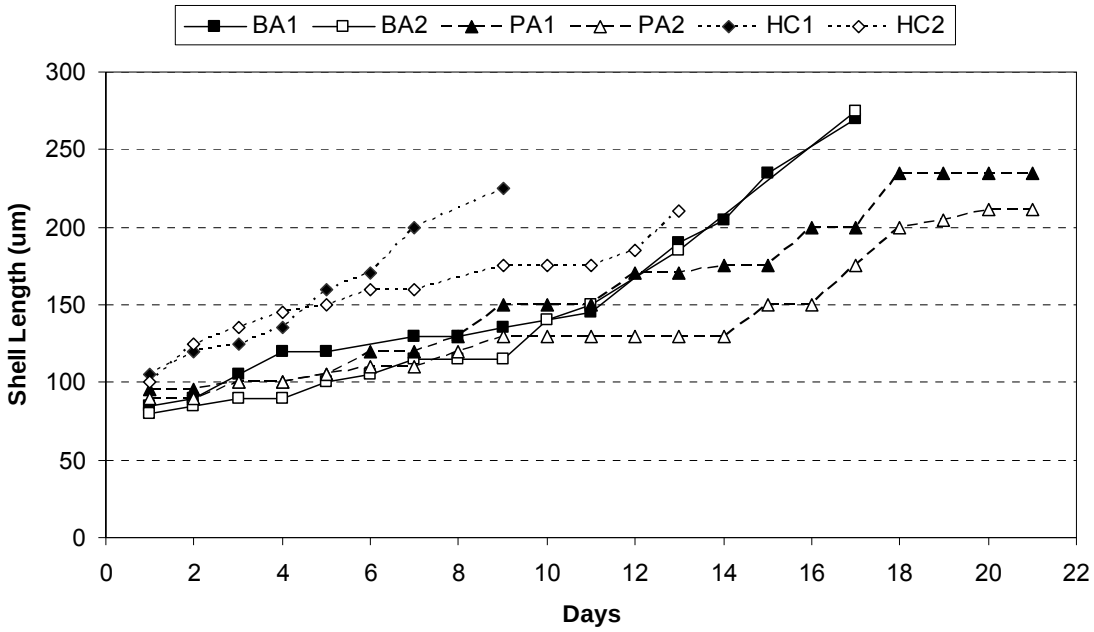


Fig. 18. Comparative growth (shell length, microns) of blood arks (BA1 and BA2), ponderous arks (PA1 and PA2), and hard clams (HC1 and HC2) from straight hinge veliger (day 1) to metamorphosis (set).

Survival to set was 62% and 38% for HC1 and HC2, respectively; whereas survival to set was 9% and 3.5% for the blood ark groups (BA1 and BA2) and 19% for the ponderous ark group (PA2). Differences in larval sizes (zygote, straight hinge, and pediveliger) are noted in the previous sections. Hard clam pediveligers completed metamorphosis within a few days (2 - 4) after being transferred to the downwellers with a few pediveligers remaining in the tanks. Hard clams exhibited a distinctive and short pediveliger stage followed by commercially acceptable survival to post-set, in contrast to problems encountered with setting of both ark clam species during our trials. Based on our observations, production of ark clam seed will not be as straightforward or as reliable as the culture of hard clam seed. The hatchery operator must be prepared to handle smaller sizes during the larval stages, a longer larval rearing period, and unpredictability during setting. In addition, the strong byssal threads of the post-set and juvenile ark clams will limit handling and sieving during the nursery culture period. Further research on ark clam seed production and technology improvement are encouraged to support development of an emergent ark clam culture industry.

Summary

These seed production trials document the first time blood arks and ponderous arks were spawned and reared under hatchery conditions. Although the number of ark clam juveniles produced was low, we were able to culture one group each of blood and ponderous arks through to field-plantable size. The following results summarized from these trials and our experiences in spawning, larval rearing, and setting of ark clams provide guidelines for future efforts of shellfish hatchery managers.

1. Low numbers of adult ark clams were ripe. Only 6% of the blood ark adults and 5% of the ponderous ark adults spawned during a total of 70 trials over a 4-year period.

2. The highest percentage of spawns (45%) for wild collected blood ark adults occurred during the months of March, April, and May.

3. Laboratory conditioning of blood arks greatly increased spawning success during those spring months and extended the spawning period through June and July. This suggests the need for establishing domestic broodstock so ark clams can be conditioned and spawned when needed, reducing the problems associated with the logistics and unpredictability of collecting ripe adults from the wild.

4. The highest percentage (55%) of spawns for wild collected ponderous ark adults occurred during the months of August, September, October, and November. We did not attempt to condition ponderous ark broodstock in the hatchery.

5. Both ark clams responded positively to thermo-stimulation techniques used for spawning hard clams. Thus, no changes are needed in current hatchery methodology to induce spawning of blood and ponderous arks.

6. The early development of both ark clams is similar to that of hard clams, although the sizes of the larval stages do vary among the clam species. For example, ark clams have smaller straight hinge veliger larvae. Hatchery managers will need to use smaller mesh sieves, or screens, during water exchanges; 35 μm sieves (screens) are recommended during the early larval rearing period. With minimal changes to equipment and logistics, rearing of ark clam larvae can be accomplished in hard clam hatcheries.

7. The larval rearing period for both ark clams is longer than that for hard clams. Extending the culture period by 5 to 10 days in the hatchery will most likely result in higher production costs and ark clam seed prices.

8. Determining competency for setting was difficult for both ark clam species. Further, metamorphosis and post-set survival was low in all of our trials. Behavior (probing of bottom) and size of umbo veliger may be better indicators of competency. It is recommended that when veliger larvae reach a length of 270-275 μm for blood ark and 210-235 μm for ponderous ark they be transferred to setting downwellers. Some success of adding benthic algae to the post-set culture system indicates that pedal feeding may be likely. Further evaluation is warranted.

9. The strong byssal threads of post-set and juvenile ark clams resulted in “clumping” of animals and adhesion to the bottom screens and surfaces of wellers. Sorting, sieving, and handling of ark clams during post-set and land-based nursery culture are difficult.

10. For ark clam seed production to become commercially viable, improvements in broodstock conditioning and spawning, and increased survival during larval rearing and setting, is necessary.

Chapter 3: Ark Clam Field Nursery And Growout Production Trials

Juvenile ark clams reared at land-based nursery sites described in the previous chapter were planted in the natural environment (field) for continued evaluation of growth and survival under culture conditions. Both the field nursery and growout of ark clams were conducted on open-water hard clam aquaculture leases. Evaluation of culture gear was dependent on the number of ark clam juveniles available. Likewise, the number of replications in the field was limited to seed availability. For both the blood ark and ponderous ark, methods used during handling, stocking, and planting followed those considered standard by the hard clam culture industry in Florida (Whetstone *et al*. 2005). Some modifications, which are noted, were based on the biological characteristics of ark clams.

Blood Ark Production Trials

Growing area — Blood ark juveniles nursed at the UF Whitney Laboratory for Marine Bioscience, located south of St. Augustine in St. Johns County, were planted on a shellfish aquaculture lease located within 1,000 feet of the laboratory. The commercial lease, sited in the Matanzas River south of the Matanzas Inlet off of the Intercoastal Waterway, had a unique feature in that much of the area became exposed on a typical low tide. The ark clams were planted in a deeper portion of the lease that remained submerged. The bottom substrate of the lease was characterized by hard sand while the deeper portion consisted of a combination of sand and mud. Blood arks were not evaluated at shellfish growing areas on the west coast of Florida. This ark species has not been reported to naturally occur in the Gulf of Mexico, nor have growers on the west coast noted recruitment of this species in their culture bags.

Water quality conditions — Water quality conditions were monitored using a YSI 6600 data sonde which was deployed at the Whitney Laboratory's floating dock. Hourly measurements were recorded at six to twelve inches above the bottom. Monthly averages and standard deviations for water temperature and salinity values from September 2003 through September 2004 are shown in Figure 19. Water temperatures ranged from a monthly average of 52.7 ^{o}F (11.6^{o}C) to 75.4 ^{o}F (24.1^{o}C) between the winter (January and February) and summer (August) months. Salinities showed less of an annual pattern and varied little during the first 11 months of the field culture period. During this period, monthly averages ranged from 24.7 ‰ in October to 34.2 ‰ in January. The lease site is influenced by an exchange of Atlantic Ocean waters at the Matanzas Inlet; as such, salinity values are generally high. However, rainfall associated with Tropical Storm Charley lowered salinities during August 2004; the monthly average was 25.2 ‰. On September 5, 2004, another tropical disturbance, Tropical Storm Frances, passed through the area. Maximum sustained winds of 42 mph and maximum gusts of 63 mph were recorded by the Princess Place Preserve weather station located at the St. Johns-Flagler county line. Total rainfall over a 2-day period measured 7.9 inches resulting in coastal flooding. While salinity for the month of September (2004) averaged 19.8 ‰, values dropped below 10 ‰ for 6 days after the passing of the tropical storm. Measurements of food availability, or phytoplankton abundance, were not determined in this study.

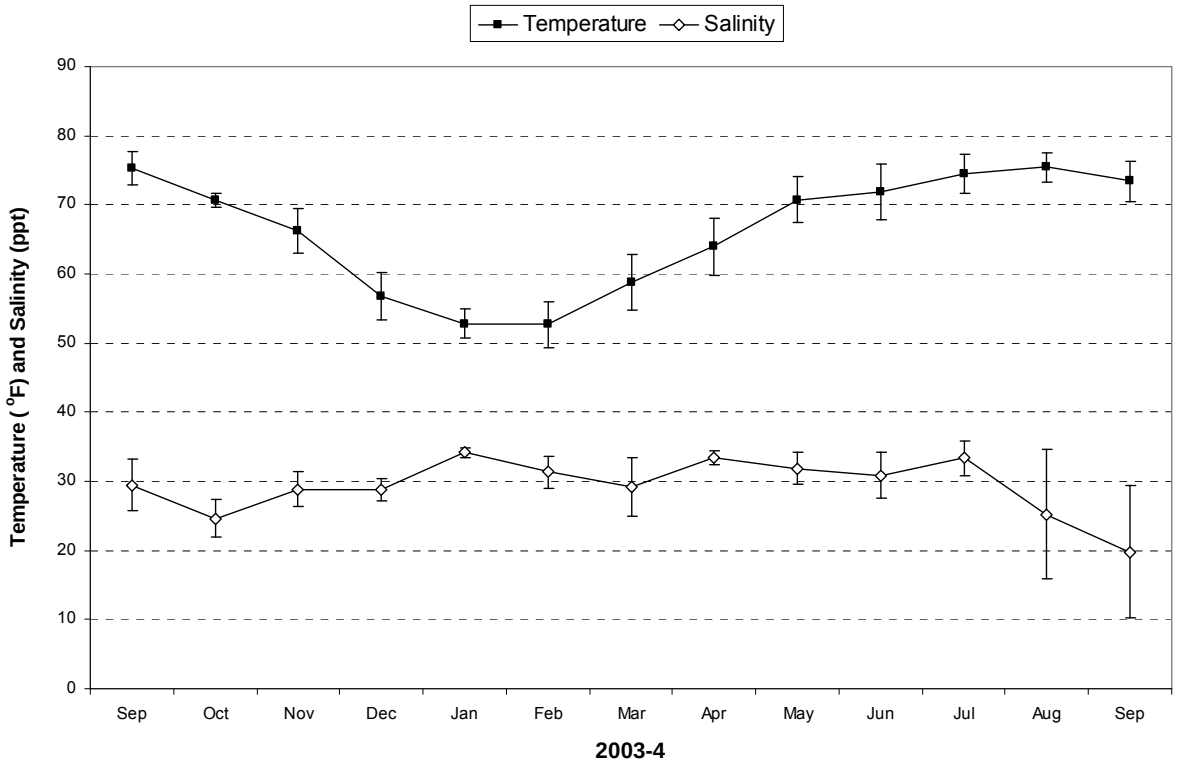


Fig. 19. Monthly averages of bottom water temperature (°F) and salinity (‰) values taken at the UF Whitney Laboratory between September 2003 and September 2004. Error bars reflect the standard deviation of the monthly averages.

Field nursery — About 14,000 juvenile blood arks from the June 26, 2003 spawn group (BA2, see previous chapter) were transferred to the field nursery site on September 8, 2003. At that time, their average size was 7.6 ± 1.0 mm in shell length (SL). Arks were stocked into nine high-density polyethylene bags (Durethene®, OBC-1 cage), referred to as "hard" bags, which are typically used in oyster culture (Figure 20). To seal the bags, which were open on both ends, the ends were folded and a slotted 1-inch PVC pipe was slid over the fold. The dimensions of the folded bag were 34-inch length by 19-inch width, or 4.5 square feet, with a mesh size of 3/16 inch. Stocking densities varied from 250 to 500 per square foot. The bags were secured to the bottom of the lease by using 1-inch PVC pipe stakes. The bags were inspected periodically. Very little fouling was noted, probably due to the cooler water temperatures encountered during the field nursery period.

Fig.20. Planting blood ark juveniles in a polyethylene (hard) bottom bag for evaluation of the field nursery stage.

On December 7, the hard bags were recovered from the lease site. A pooled sample of 100 arks was measured to the nearest 0.1 mm using calipers. After three months, average shell length was 15.4 + 2.8 mm, resulting in a growth rate of 2.6 mm/month in SL. This growth rate was similar to those reported for hard clam seed nursed in polyester mesh bags

at a nearby location in Oak Hill (2.8 mm/month in SL) (Fernandez *et al.* 1999) and at Cedar Key on the west coast of Florida (2.4 mm/month in SL during winter, 4.2 mm/month in SL during summer (Sturmer *et al.* 1995). Although no predators were found in the bags, overall survival was estimated at 66%. Several bags had silted over and high mortalities were noted in these bags. Unlike most clams, arks do not have siphons and filter water by opening their valves (shells). Excessive burial of the culture gear could most likely result in suffocation of the arks. Hard bags that did not bury supported ark survival exceeding 80%. The survival of field-nursed ark clams was within the range of 59.0 to 94.5% found for hard clams in a 13-week field nursery study by Fernandez *et al*. (1999) and within the range of 58 to 88% found by Sturmer *et al*. (1995) in a 3-month field nursery study. This is the first documentation of growth and survival rates for hatchery-produced blood ark juveniles.

Growout — A growout study was initiated on December 8, 2003 with 9,225 field-nursed blood arks (average size:15.4 $\pm$ 2.8 mm shell length [SL], 10.6 $\pm$ 2.0 mm shell width [SW], 0.44 per milliliter) (Figure 21). About a third of the arks, or 2,925, were stocked into hard bags (Durethene®, OBC-2 cage) with a mesh size of 3/8 inches. The bags were sealed as in the field nursery and the dimensions of the folded bag were the same (4.5 ft^2). Each hard bag was stocked using volumetric measuring methods with 225 ark clams, resulting in a density of 50 per square foot (Figure 22). It is interesting to note that at this size, byssal threads were not prevalent and handling of juveniles was similar to that of hard clams. The planting density tested was at the lower end of the range (50-75 ft^2) used by clam growers in Florida. The remaining arks, about 6,300, were stocked into polyester mesh bottom bags, henceforth, referred as "soft" bags, with the dimensions of 3 feet by 3 feet, or 9 square feet, and a mesh opening of 9 mm (Figure 22). These bags are typically used for clam culture in Florida, although they are usually 16 square feet in size. Ark clam juveniles were stocked at the same density used for the hard bags, or 50/ft^2, resulting in 450 ark clams stocked per soft bag. A total of 13 hard bags and 14 soft bags were planted on the commercial shellfish aquaculture lease. Bags were secured to the bottom of the lease by using 1-inch PVC pipe stakes for the hard bags and 1/8-inch galvanized wire stakes for the soft bags.

Fig. 21. Stocking blood ark juveniles (left) using volumetric measuring techniques (right) for evaluation of the growout stage

Fig.22. Stocking (left) and planting (right) blood ark juveniles in polyester mesh (soft) bottom bags for evaluation of the growout stage.

The bags were inspected periodically and sampled every three months for estimates of growth and survival during the growout period. Samples were taken March 16, June 16, and September 10, 2004. Three replicates each of hard bags and soft bags were pulled during each sample period. From each replicate bag, 50 ark clams were measured for shell length and shell width. Survival was determined by counting the number of live arks in each bag. Fouling organisms both on the bags and arks, as well as presence or absence of predators, were noted. Once sampled, the bags were planted back on the lease but not used in subsequent sample dates; the disturbance of pulling culture bags for sampling and then replacing them could possibly have a negative effect on growth and other production characteristics of benthic (bottom dwelling) infaunal (buried in substrate) molluscs.

Growth in shell length (SL) of blood arks in hard and soft bags over a 9-month growout period is illustrated in Figure 23. On March16, blood arks from soft bags averaged 21.6 $\pm$ 0.7 mm in SL, while ark clams in hard bags averaged 23.2 $\pm$ 0.8 mm in SL. Growth rates during the coolest period of the year were 2.1 and 2.6 mm/month, respectively. Three months later on June 16, blood arks in soft bags averaged 31.1 $\pm$ 0.4 mm in SL, while arks in hard bags averaged 29.1 $\pm$ 0.9 mm in SL. The spring growing period resulted in the highest growth with rates of 2.0 and 3.2 mm/month, respectively. On September 10, blood arks in soft bags averaged 34.3 $\pm$ 1.2 mm in SL, while ark clams in hard bags averaged 35.3 $\pm$ 1.6 mm in SL.

The summer growing period resulted in the lowest growth with rates of 1.1 and 2.1 mm/month in SL, respectively. Overall growth rates of arks cultured in soft and hard bags during the 9-month period were 2.1 and 2.2 mm/month in SL, respectively. Growth in shell width (SW) of blood arks in hard and soft bags over a 9-month period is illustrated in Figure 24. On March 16, blood arks sampled from soft bags averaged 12.9 $\pm$ 0.5 mm in SW, while ark clams sampled from hard bags averaged 14.2 $\pm$ 0.3 mm in SW, resulting in respective growth rates during this period of 0.8 and 1.2 mm/month. On June 16, blood arks in soft bags averaged 19.7 $\pm$ 0.2 mm in SW, while arks in hard bags averaged 19.3 $\pm$ 1.2 mm in SW, resulting in respective growth rates of 1.7 and 2.2 mm/month. On September 10, blood arks in soft bags averaged 21.9 $\pm$ 1.0 mm in SW, while ark clams in hard bags averaged 23.2 $\pm$ 0.9 mm in SW, resulting in respective growth rates of 0.7 and 1.3 mm/month. Overall growth rates of blood arks cultured in soft and hard bags during the 9-month period were 1.2 and 1.4 mm/month in SW, respectively. As blood arks grew, the length to width ratio of the valves (shells) decreased, resulting in a thicker shape.

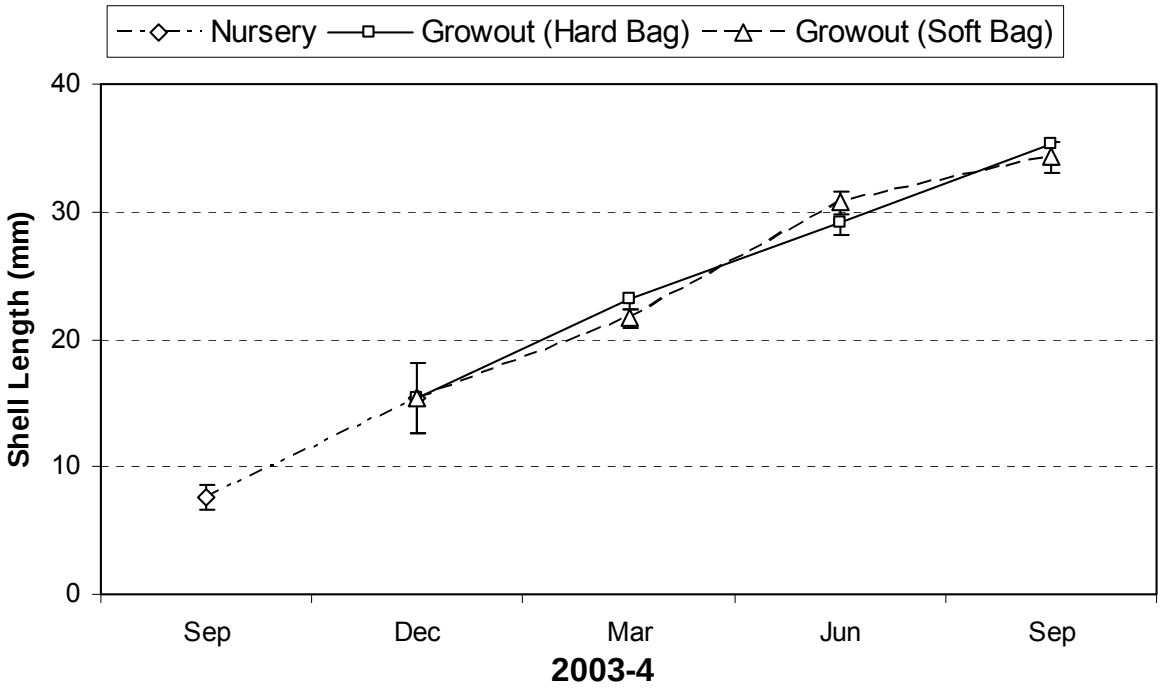


Fig. 23. Growth in shell length (SL) of blood arks in hard bags over a 3-month field nursery period and in hard and soft bottom bags over a 9-month growout period. Error bars reflect the standard deviation from the averages.

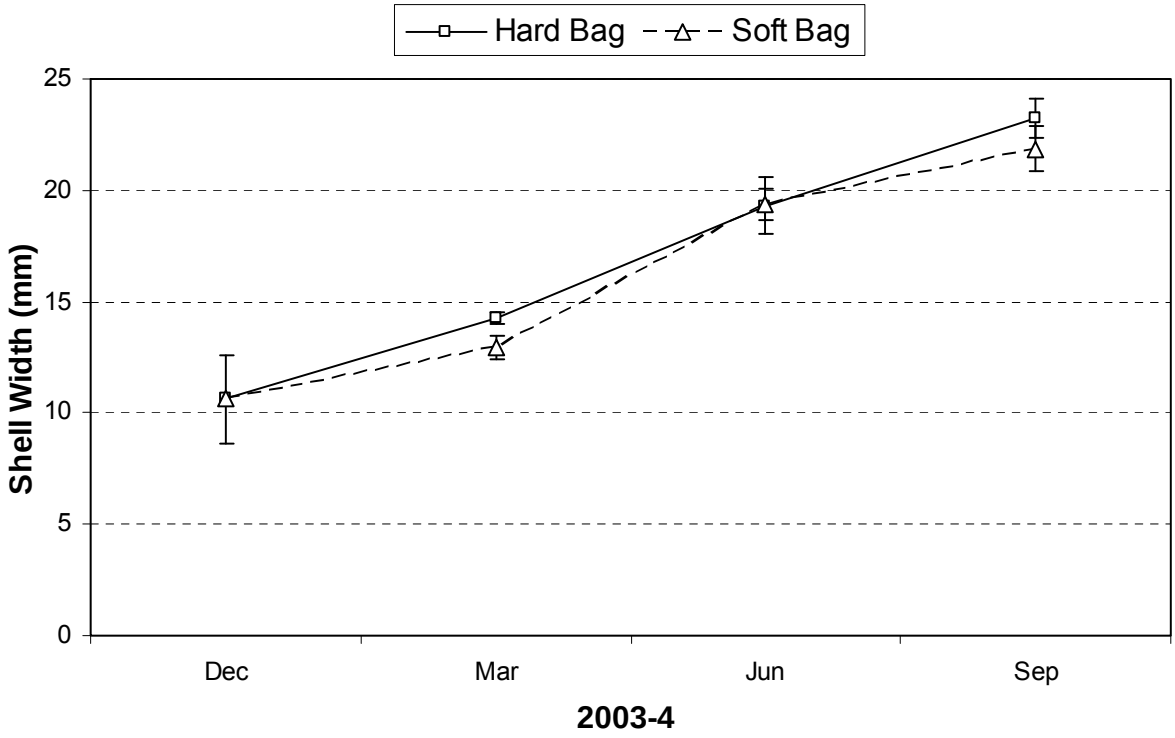


Fig. 24. Growth in shell width (SW) of blood arks in hard and soft bottom bags over a 9-month growout period. Error bars reflect the standard deviation from the averages.

Filamentous green algae (Enteromorpha sp.) was found attached to the hard bags and on some ark clams in the March sample. In June, fouling on some of the arks in the hard bags consisted of oyster spat (Crassostrea virginica) and slipper shells (Crepidula sp.). Fouling was limited to the umbo (beak) or dorsal (top) margin of the ark shells, where the thick, fibrous periostracum (non-calcareous shell covering) had eroded. McGraw et al. (1998) reported that fouling was not a significant problem in Virginia populations due to the arks' periostracum. There were no fouling organisms observed on arks planted in soft bags, as the polyester material allowed the bags and clams to bury or settle into the bottom sediments. Two of the replicate soft bags sampled in June were excessively buried, and meats were found in some of the gaping ark shells indicating recent mortalities.

Comparison with hard clam production — The intent of the growout study was to rear blood arks a minimum of 12 months (the typical growout period for hard clams planted at 12-15 mm in SL) or until they reached at least 1 inch (25 mm) in shell width, the minimum size that was found to be acceptable by wholesalers in the market study conducted by the UF Agriculture Market Center (Degner *et al*. 2005). The September sampling was conducted as soon as possible after the passing of Tropical Storm Francis. Unfortunately, the majority of arks in the sample bags had recently died with meat still remaining in the shells. Arks with meats were counted as "live" to assess survival over the sample period. The remaining culture bags were harvested and the study terminated. Mortalities were assumed to be the result of low salinities (<10 ‰) due to coastal flooding. The crop of hard clams on the commercial lease was also affected by this climatic event, with mortalities assessed at 100% by the grower. Survival over the 9-month growout period is illustrated in Figure 25. Survival of arks in soft bags was estimated at 100, 80.8, and 80.0% for the respective March, June, and September sample periods; whereas survival of arks in hard bags was estimated at 100, 93.8, and 92.8% for the same sample periods. The higher mortality of arks found in the soft bags was probably due to suffocation, as the polyester mesh allowed the clams to bury deeper. The hard bags generally kept the arks at the surface sediments. There was no evidence that predation was a factor in blood ark mortalities; no predators were found in the bags, nor were shell fragments or chipped shells.

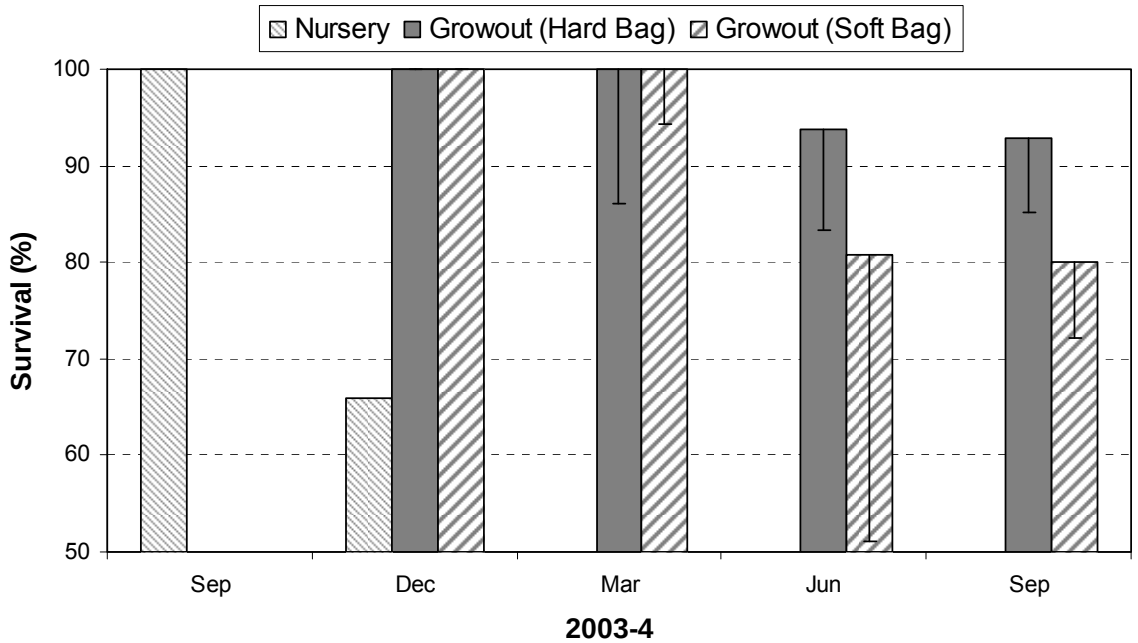


Fig. 25. Survival rates of blood arks in hard bags over a 3-month field nursery period and in hard and soft bottom bags over a 9-month growout period. Error bars reflect the standard deviation from the averages.

Two-factor analyses of variance (ANOVA) were performed on the production data to determine statistically significant effects of sample date, bag type, or interaction on shell length, shell width, and survival. Survival proportions were arcsine transformed before analyses. Both shell length ($p < 0.0001$) and shell width ($p < 0.0001$) increased significantly with sampling date, as expected. The two-factor ANOVA indicated a significant interaction effect of date and bag type on shell length ($p = 0.0394$); but Tukey multiple comparison tests did not reveal significant effects of bag type on shell length on any of the three sample dates. However, bag type had a significant effect on shell width ($p = 0.0405$) as blood arks grown in hard bags had greater width than those grown in soft bags. Although survival was different between culture bag types in June and September, sampling date ($p = 0.2279$), bag type ($p = 0.2116$), and their interaction ($p = 0.7618$) had no significant effects on survival.

Although the study was terminated early, results indicated that blood arks could potentially reach market size (minimum 25 mm in SW) in a growout period of 12 months or less. Blood arks cultured in hard bags had obtained an average width of 23 mm in nine months (see Figure 24). Assuming an average growth rate of 1.4 mm/month in SW, it would require another one to two months for blood arks to reach the desired harvest size.

The growth rate of blood arks has been previously documented for natural populations occurring in coastal waters of the southeastern United States. This was first reported by McGraw *et al.* (1996) in Virginia, where an average shell length of 23.7 mm was attained in the first year and 40.1 mm in the second year. In Georgia, an initial study on the growth of three size categories (<10 mm, 10-20 mm, >20 mm) of wild-collected blood arks held in suspended pearl nets was conducted (Walker 1998). Larger sizes of 38.3 to 43.4 mm in SL were recorded after one year, with growth rates ranging from 1.26 to 2.58 mm/month. In the second year, the largest sizes reached were 48.4 to 52.6 mm, but growth rates decreased to 0.72 to 0.89 mm/month. In that study, rapid growth occurred in the spring with good growth continuing into the fall and minimal growth in the winter; whereas in Florida, growth was good in the fall and continued into the winter, with the greatest growth occurring in the spring and least growth in the summer. Annual growth rates were also measured in Georgia for wild-collected blood arks (32 mm average shell length) cultured in plastic bottom bags from September 1999 through August 2000 at stocking densities of 35 and 74/ft^2 (Power and Walker 2001). Arks reached 45 mm in SL and growth rates averaged 1.1 mm/month for both densities. Shell width was not measured in any of these studies. Natural mortality rates of blood arks for the first year class were also noted in these studies. In Virginia, McGraw *et al.* (1996) observed that survival of ark stocks declined to 13.6%. Walker (1998) determined a large variation in mortality rates held in pearl nets in Georgia, ranging from 36.8 to 42.9% in the first year and from 8.3 to 73.9% in the second year. It was observed that predation was a contributing factor. Survival of arks planted at low (35/ft^2) and high (74/ft^2) densities in bottom bags was 42.9 and 40.2% (mortalities of 57.1 and 59.8%), respectively (Power and Walker 2001).

Growth rates of hatchery-produced blood arks obtained in this study are higher than those observed previously for this species. To directly compare, blood arks (14 mm in SL) grew to 17 mm over an 8-month period from October to June in Virginia. In Georgia, the same size arks (14 mm in SL) reached 17 mm over a 3-month period from October to January. By June, these blood arks had grown to 30 mm in SL (8-month period) and reached approximately 40 mm by September (11-month period). In Florida, blood arks (15 mm in SL) were planted in December and had reached 29 to 31 mm in June (6-month period) and by September had obtained 34-35 mm in SL (9-month period). These differences are most likely due to warmer temperatures and a longer growing season. Mortality of blood arks cultured in polyethylene (plastic) bottom bags was substantially lower in this study (8%) than in the Georgia study. These results support the conclusion of previous investigators that

blood arks may have excellent potential for development into an aquaculture species in the southeastern United States.

Ponderous Ark Production Trials

Growing area — Ponderous ark juveniles nursed at a commercial hatchery/nursery in Cedar Key were planted on a shellfish aquaculture lease located within the Gulf Jackson High-Density Lease Area. The commercial lease area, sited in the Gulf of Mexico west of Cedar Key, is widely known within the industry as a productive area for culturing hard clams. Many growers at this lease area had observed recruitment of ponderous arks in their culture bags. Broodstock used in spawning trials in this study were collected from culture bags harvested from this site. The bottom substrate of the lease used in these field trials was characterized by a combination of sand and mud, with the sediment much softer than that found at the site used for blood ark trials. Although this ark species is reported to naturally occur along both coasts of Florida, there were not a sufficient number of juveniles to evaluate ponderous arks at a shellfish growing area on the east coast of Florida.

Water quality conditions — Water quality conditions during July 2004 through May 2005 were monitored using a YSI 6600 data sonde which was deployed at a piling marking the perimeter of the Gulf Jackson High-density Lease Area, less than 500' from the site where the ponderous arks were cultured. Measurements, every 30 minutes, were recorded at six inches above the bottom. Funding to maintain the monitoring station was curtailed in May 2005. For the remainder of the culture period, water quality data were obtained from two stations located adjacent to the Gulf Jackson High-density Lease Area sampled periodically by personnel in the Shellfish Environmental Assessment Section of the Florida Department of Agriculture and Consumer Services using a hand-held YSI 650 data sonde.

Monthly averages and standard deviations for water temperature and salinity values from July 2004 through June 2006 are shown in Figure 26. Monthly averages of water temperature ranged from a low of 59.4^{o}F (15.2^{o}C) in January 2005 and December 2005 to a high of 87.5^{o}F (30.8^{o}C) in July 2004 and 85.9^{o}F (29.9^{o}C) in July 2005 and June 2006. Salinities showed less of an annual pattern and varied little during the majority of the field culture period. With the exception of two months, monthly averages remained within the range tolerated by hard clams. Monthly averages of salinity ranged from a high of 26.9, 26.3, and 26.0 ‰ during the respective months of March 2006, July 2004, and June 2006 to a low of 17.7 and 18.6 ‰ obtained in the respective months of May 2006 and April 2005. The lease area is located about nine miles south of the Suwannee River and is influenced by its discharge. The river typically reaches crest conditions during the spring. In 2005, the Suwannee River Basin was influenced by rainfall and runoff associated with the 2004 hurricane season resulting in flood stages. Although the monthly average in March 2005 was 22.3 + 3.6 ‰, salinities were recorded below 20 ‰ on 16 days and below 10 ‰ on 5 days, with the lowest value of 8.7 ‰ recorded on March 30. During the following month (April 2005), the average was 18.6 + 5.1 ‰ with salinities recorded below 20 ‰ on 27 days and below 10 ‰ on 8 days. The lowest value of 3.1 ‰ was recorded on April 4. Measurements of food availability, or phytoplankton abundance, were not determined in this study.

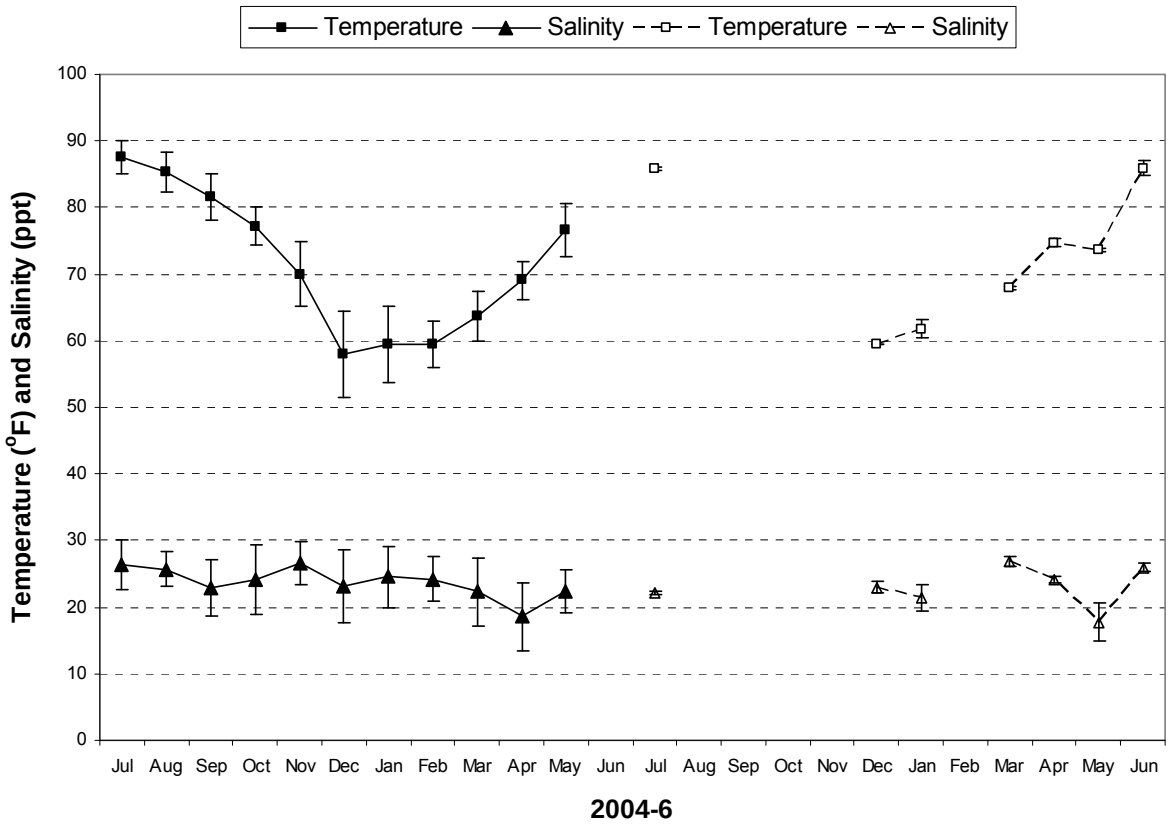


Fig. 26. Monthly averages of bottom water temperature (°F) and salinity (‰) values taken at the Gulf Jackson High-density Lease Area near Cedar Key between July 2004 and June 2006. Error bars reflect the standard deviation of the monthly averages.

Field nursery — About 1,200 juvenile ponderous arks from the September 26, 2003 spawn group (PA2, see previous chapter) were transferred to the field nursery on June 22, 2004. At that time, their average size was 16.6 ± 1.6 mm in shell length (SL), 8.6 ± 0.9 mm in shell width (SW), and 1.8 ± 0.6 grams in total weight. Arks were stocked into three high-density polyethylene bags, previously referred to as "hard" bags, resulting in a stocking density of 85 per square foot. Hard bag dimensions, preparation, and deployment were the same as described in the field nursery section for blood arks. Fouling, consisting primarily of tunicates, was greater in the ponderous ark field nursery than observed in the blood ark field nursery, probably due to the warmer water temperatures encountered. The surface of the bags was cleaned during periodic inspections. The number of juveniles available limited the evaluation of polyester mesh ("soft") bags for field nursery culture. Because of the muddier (softer) substrate at this lease area, it was decided to use the hard bag rather the soft bag typically used by hard clam growers.

On December 14, the hard bags were recovered from the lease site. A sample of 50 arks from each bag was measured to the nearest 0.1 mm using calipers and to the nearest 0.1 gram using an electronic scale. After six months, average shell length was 29.2 ± 1.6 mm, resulting in a growth rate of 2.2 mm/month in SL; average shell width was 18.8 ± 1.0, resulting in a growth rate of 1.8 mm/month in SW. The growth rate of field-nursed ponderous arks was slower than that observed for bloods arks (2.6 mm/month in SL); however, a smaller seed was stocked and higher stocking densities were used in the blood ark field nursery trial. Average total weight of ponderous arks during

the nursery period increased from 1.8 to 9.2 ± 2.1 grams. Although no predators were found in the bags, overall survival was estimated at 75.6%. One of the bags was excessively buried and a mortality of 59.2% was noted in that bag. Like the blood ark, ponderous arks do not have siphons, as most clams do, and filter water by opening their valves (shells). Excessive burial of the culture gear could result in suffocation of the arks. Survival of ponderous arks in hard bags that did not bury exceeded 90%. Although the field nursery culture period for the ponderous ark juveniles was twice that of the blood arks (six months versus three months), survival of field-nursed ponderous arks was higher than that obtained for blood arks (75.6% versus 66.0%) and within the ranges reported for hard clams field-nursed in Florida waters (Fernandez *et al*. 1999, Sturmer *et al*. 1995).

Growout — A growout study was initiated on December 14, 2003 with field-nursed ponderous arks (average size: 29.2 ± 1.6 mm SL, 18.8 ± 1.0 mm SW, 9.2 ± 2.1 g total weight). About 285 arks were stocked into each of three 4.5 ft^2 hard bags (Durethene®, OBC-2 cage) with a mesh size of 3/8 inches, resulting in a density of 62 per square foot (Figure 27). This planting density was higher than that used in the blood ark growout trial (50/ft^2) and within the mid-range of densities (50-75/ft^2) used by hard clam growers in Florida. Again, because of the softer sediment found on the leases in this area, it was decided to use hard bags rather than soft mesh bags. Bags were secured to the bottom of the lease by using 1-inch PVC pipe stakes.

Fig. 27. Planting ponderous ark juveniles in a polyethylene (hard) bottom bag for evaluation of the growout stage.

The bags were inspected periodically and sampled every six months (June 27, 2005; December 14, 2005) for estimates of growth and survival during the growout period. After 18 months, the bags were harvested and the trial terminated on June 21, 2006 (Figure 28). Because of the limited number of hard bags, or replicates, the same three bags were pulled each sample period. The disturbance of pulling these culture bags for sampling, and then replacing them, could possibly have had a negative effect on growth and survival. From each replicate bag, 50 ark clams were measured for shell length, shell width, and total weight, and then returned to the bag. Survival was determined by counting the number of live arks in each bag. Observations of fouling organisms on the bags and arks, as well as presence or absence of predators, were noted.

Fig. 28. Harvesting ponderous arks from polyethylene (hard) bottom bags after 18 months in the growout stage.

Growth in shell length (SL) and shell width (SW) of ponderous arks in hard bags over the 18-month growout period is illustrated in Figure 29. On June 27, 2005, ponderous arks averaged 36.7 ± 0.5 mm in SL and 23.8 ± 0.1 mm in SW. This 6-month period resulted in the highest growth with rates of 1.25 and 0.83 mm/month, respectively. Six months later on December 14, 2005, ponderous arks averaged 39.9 ± 0.8 mm in SL and 27.3 ± 0.2 mm in SW. This period resulted in the lowest growth with rates of 0.5 and 0.58 mm/month, respectively. The hard bags were harvested six months latter on June 21, 2006. At that time, ponderous arks had reached 44.1 ± 0.6 mm in SL and 30.0 mm ± 0.3 mm in SW, resulting in growth rates of 0.7 mm and 0.45 mm/month, respectively, over the 6-month period. Overall growth rates of ponderous arks cultured in hard bags during an 18-month period were 0.83 and 0.62 mm/month in SL and SW, respectively. These rates were considerably lower (265% for SL, 225% for SW) than those reported in the previous chapter for blood arks grown in hard bags (2.2 mm/month in SL, 1.4 mm/month in SW). This concurs with growth studies conducted by McGraw and Castagna (1994) of natural populations of ark clams in Virginia. Their findings indicated that blood arks grew about twice as fast as ponderous arks during the first two years after settlement.

Growth in total (whole) weight of ponderous arks in hard bags over the 18-month growout period is illustrated in Figure 30. Ponderous arks increased in weight from an average of 16.9 ± 0.5 grams on July 27, 2005 to 24.7 ± 0.7 grams on December 14, 2005. At harvest on June 21, 2006, ponderous arks had reached an average whole weight of 31.3 ± 1.2 grams. Over the 18-month period, ponderous arks increased in weight an average of 1.2 grams per month. Whereas growth rates of shell length and shell width decreased over the growout period, the increase in total weight of ponderous arks remained relatively constant varying from 1.1 to 1.3 grams/month over the 6-month sample periods. The number of clams per pound is an important commercial parameter used by the shellfish industry to define various size grades at harvest. For example, a one inch (25 mm) SW hard clam, referred to as a "littleneck" grade, measures 12 to 15 per pound. At harvest, ponderous arks averaged 14.5 per pound at an average shell width of 30 mm (1.2 inches).

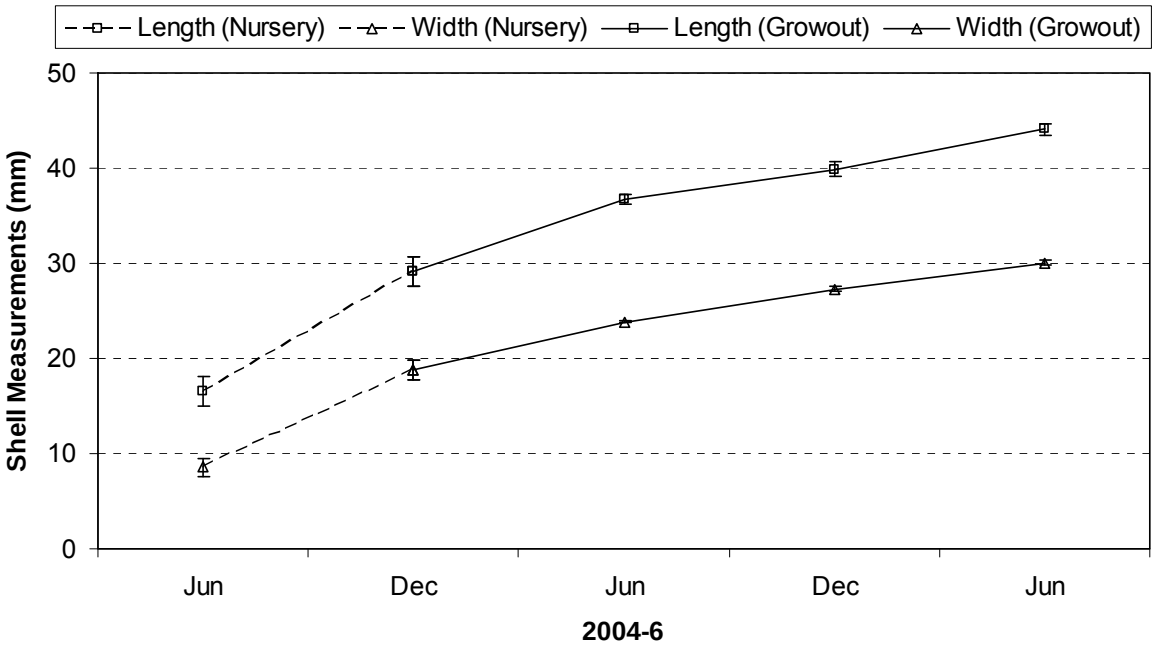


Fig. 29. Growth in shell length and shell width of ponderous arks in hard bottom bags over a 6-month field nursery period and an 18-month growout period. Error bars reflect the standard deviation from the averages.

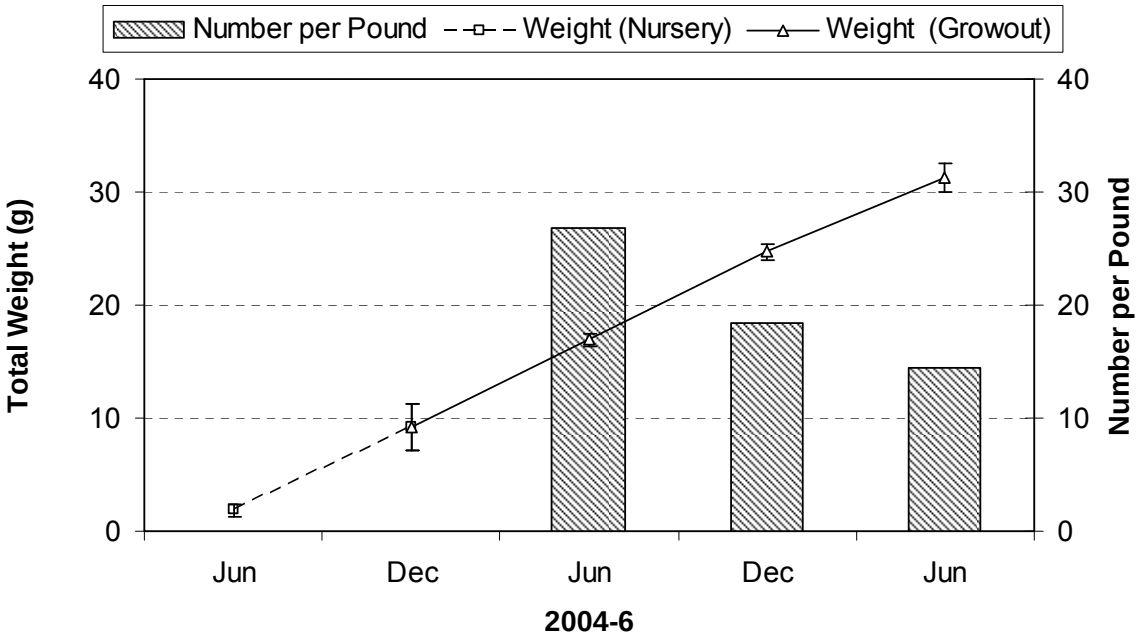


Fig. 30. Growth in total weight of ponderous arks in hard bottom bags over a 6-month field nursery period and an 18-month growout period. Error bars reflect the standard deviation from the averages. The number per pound for average total weights of ponderous arks in June 2005, December 2005, and June 2006 is also shown.

Fouling organisms consisted primarily of sea squirts (*Molgula manhattensis, Styela plicata*) and oyster spat (*Crassostrea virginica*) with some barnacles (*Balanus eburneus*) and mussels (*Brachidontes exustus*) set on the hard bags. For the most part, the bags were fairly clean. At harvest in June 2006, fouling on some of the arks consisted of oyster spat (*C. virginica*) and slipper shells (*Crepidula* sp.). As was observed for the blood arks grown in hard bags, fouling was limited to the umbo or dorsal margin of the ark shells, where the thick, fibrous periostracum had eroded.

The intent of this growout study was to rear ponderous arks a minimum of 12 months or until they reached at least 1¼ inches (31 mm) in shell width, the minimum size found acceptable by wholesalers in the market study conducted by the UF Agriculture Market Center (Degner *et al*. 2005). After 12 months, ponderous arks averaged 27 mm in SW. Since comments from wholesalers indicated that a larger ponderous ark may be preferable, the growout trial was extended another 6 months; at which time, ponderous arks averaged 30 mm in SW and 44 mm in SL (Figure 31).

Fig. 31. Sampling ponderous arks after 18 months in growout bags.

Survival over the 18-month growout period is illustrated in Figure 32. Survival of ponderous arks in hard bags was estimated at 93.0 $\pm$ 5.4% and 81.6 $\pm$ 9.3% for the respective June and December 2005 sample periods. At harvest in June 2006, survival of ponderous arks was 71.7 $\pm$ 6.9% over an 18 month growing cycle. The hard bags generally kept the arks at the substrate surface. One of the hard bags harvested in June 2006 was partially buried and a few meats were found in some of the opened ark shells. Although predators were found inside the bags, in particular stone crabs (*Menippe mercenaria*), there was no evidence that predation was a factor in ponderous ark mortalities, as shell fragments or chipped shells were not found. It is possible shell fragments could have washed out of the mesh openings of the bags. McGraw and Castagna (1994) observed that natural populations of ponderous arks in Virginia had fewer predators than blood arks because of their thicker shells. It also appears that the thick periostracum covering the shell provides some protection from predators. Other organisms commonly found inside the hard bags during sampling were mud crabs (*Panopeus herbstii*), oyster toadfish (*Opsanus beta*), and gobies (Family Gobiidae). It was noted in laboratory and field predation experiments conducted in Virginia that xanthid crabs, in particular *Panopeus herbstii*, were the primary predators of ark clams less than about 25 mm in SL (McGraw *et al.* 1998).

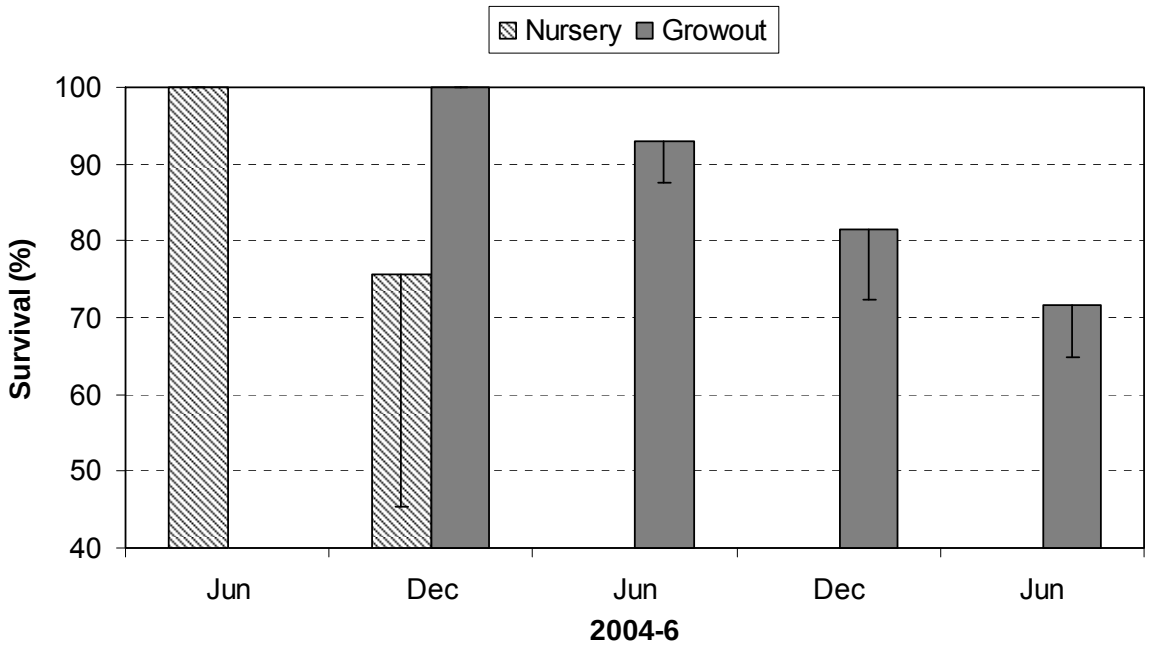


Fig. 32. Survival rates of ponderous arks in hard bottom bags over a 6-month field nursery period and an 18-month growout period. Error bars reflect the standard deviation from the averages.

The growout period in this trial was twice that of the blood ark trial (18 months versus 9 months), making it difficult to compare survival of these two arks under culture conditions. However, survival of ponderous arks sampled after six months was estimated at 93.0%, the same as that for blood arks in hard bags (93.8%) after the same time period. Like the blood arks, ponderous arks also experienced episodic decreases in salinity (less than 10 ‰) during growout as a result of heavy rains and runoff associated with tropical storms. Yet, ponderous arks seemed to be fairly resilient as mortalities of only 7% were noted during the June 2005 sample period following rapid salinity changes in March and April. Only juvenile hard clams (seed), particularly those that had been recently planted at the Gulf Jackson lease area, experienced high mortalities during this same time period. One explanation for these different physiological responses may be that water temperatures in the spring were lower (Cedar Key: 63.7 $\pm$ 3.6°F, March 2005; 69.0 $\pm$ 2.9°F, April 2005) than those in late summer (St. Augustine: 75.4 $\pm$ 2.1°F, August 2004; 73.4 $\pm$ 2.9°F, September 2004). Blood arks likely faced multiple simultaneous environmental stressors resulting in high mortalities; whereas ponderous arks did not.

There is very little information on the growth rates of natural populations of ponderous arks occurring in coastal waters of the southeastern United States and none on cultured stocks. This study is the first documentation of growth and survival rates for hatchery-produced ponderous arks under culture conditions. A study was conducted in Virginia to provide some basic information on ark clams for management of a fishery that developed on the Eastern shore in the 1990s (McGraw and Castagna 1994). Although the only measurements provided were of shell height (SH, the distance from the umbo to the ventral margin of the shell), a regression analysis provided of height on length, with a high degree of correlation (r^2=0.94, p=0.03), allowed for predicting shell length. Wild-caught ponderous arks (initial average size of 6.3 mm SH, or approximately 10 mm SL) were placed in trays in October 1992 and held through June 1993 at which time growth was checked. After eight months, an average shell height of 9.0 mm (approximately 12.5 mm SL) was obtained. Although ponderous

arks grew very little over this time period, most growth took place during the winter months. In the same study, mean height of ponderous arks sampled from retail markets in the area was 55 mm (SH), or approximately 68 mm in SL, a considerably larger animal than that harvested in this study (44 mm in SL). It was suggested that ponderous arks could be cultured as an alternative to inconsistent fishery supplies year-round, but would take four to six years to reach minimal market size.

Comparison with hard clam production — Growth rates of hatchery-produced blood arks and ponderous arks obtained in this study are comparable to those obtained for cultured hard clams in Florida. In 1988, Vaughan *et al*. determined that hard clams cultured in the Indian River (east central Florida) reached commercial "littleneck" size (25 mm in SW) in approximately 12 to 18 months from a seed size of 12-15 mm in SL. The best growth of hard clams was noted to occur during the fall and spring in the Florida panhandle (Menzel 1961). In Oak Hill (northeast Florida), clams grew from 21 to 33 mm in SL during an 8-month study (Fernandez *et al*. 1999). Seasonal growth rates for hard clams cultured in Cedar Key (northwest Florida) were first documented by Sturmer *et al*. in 1995. In that study, growout-size seed clams (14 mm in SL) planted during the winter (January) obtained an average growth rate of 2.8 mm in SL per month and reached 48 mm in SL ("littleneck" size) in 12 months; rapid growth was observed during late summer and early fall (3.7 mm/month) with minimal growth during the winter (1.0 mm/month). In the same study, growout-size seed clams (15 mm in SL) planted during the summer (July) reached 55 mm in SL (about 27-28 mm in SW) in 12 months resulting in an average growth rate of 3.3 mm in SL per month. Seasonal growth rates were higher than those observed for the winter-plant, but the pattern of growth was similar with faster growth (4.1 mm/month) occurring during late summer and early fall and slower growth (2.2 mm/month) occurring during the winter.

In the present study, blood arks of a seed size (15 mm, SL) similar to that reported above for hard clams, reached 22-23 mm in SW in about 9 months, a significantly shorter growout period than that documented for hard clams cultured in Florida. A larger size seed (29 mm, SL) was planted in the ponderous ark growout trial; yet, it took 12 months to reach 27 mm in SW.

Although this is almost twice the growout period as that for the blood ark, it is within the range reported for hard clam growout in Florida. Using growth data reported on post-set rearing and land-based nursery trials in the previous chapter and on field nursery and growout trials in this chapter, it would take a minimum of 14 months for post-set blood arks (less than 1 mm, SL) to reach "littleneck" size (25 mm, SW) and 25 months for ponderous arks to do so. In comparison, hard clams require 18 to 24 months, depending on the growing location in Florida and season planted.

As blood and ponderous arks grew, the length to width ratio of their valves (shells) remained at 1.5, resulting in a thicker shape than hard clams which typically grow at a ratio of 2 to 1 (SL:SW). A regression analysis of shell length on shell width for ponderous arks ($y = 0.7536x-3.2764$), blood arks ($y = 0.6491x + 0.0533$), and hard clams ($y = 0.5548x - 1.3264$) showed a high degree of correlation (ponderous ark, $r^2 = 0.9693$; blood ark, $r^2 = 0.9476$; hard clam, $r^2 = 0.9847$) (Figure 33). For example, at a shell length of 40 mm, the corresponding shell width for ponderous arks, blood arks, and hard clams would be 27, 26, and 21 mm, respectively. At a shell length of 50 mm, the corresponding shell width for these clam species would be 34, 33, and 26 mm, respectively. These morphometric data suggest that shell width, rather than shell length, may be a more useful measurement in documenting and comparing ark clam growth in future studies.

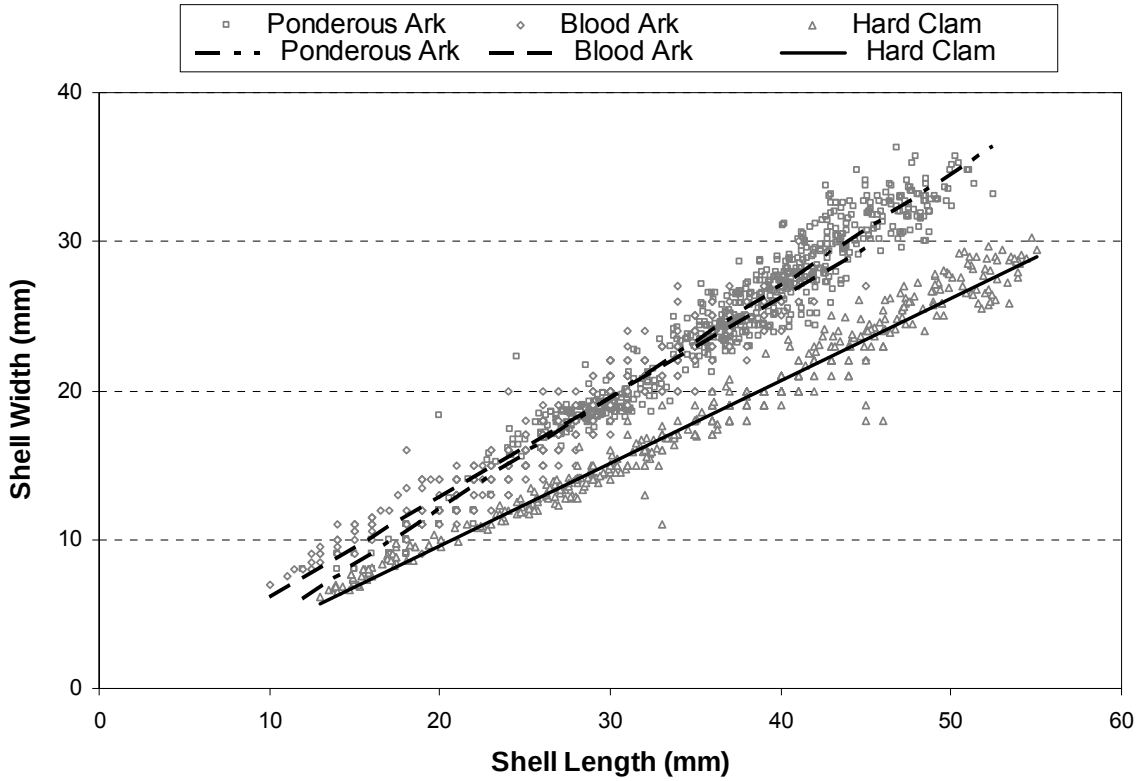


Fig. 33. Regression of shell length on shell width of ponderous arks, blood arks, and hard clams from cultured samples (n = 500).

Krauter *et al*. (1998) suggested that survival of planted clams should be more than 40 to 50% for commercial culture to be profitable. In Florida, commercially acceptable survival rates for hard clams in the field nursery (3 months) and growout (12-18 months) are considered to be about 70 and 80%, respectively, resulting in an overall survival of 56% (Adams and Sturmer 2005). In this study, survival of blood arks cultured in hard bags during a 3-month field nursery and 9-month growout period was 66 and 93%, respectively, resulting in an overall survival of 61%. Survival of ponderous arks also cultured in hard bags during a 6-month field nursery and 18-month growout period was 76 and 72%, respectively, resulting in an overall survival of 55%.

Summary

These production trials represent the first documentation of hatchery-produced blood arks and ponderous arks grown under culture conditions in open-water environments. Although the number of ark clam juveniles produced was low, one group each of blood and ponderous arks were cultured through to potential market size. The following results summarized from these trials and our experiences in nursing and growing ark clams provide guidelines for future efforts of shellfish growers.

1. Blood arks and ponderous arks are naturally found in waters of higher salinity and at the sediment surface in both mud-sand and shell substrates. Based on these criteria, many of the shellfish aquaculture lease areas in Florida would potentially be suitable for ark clam culture.

2. In these trials, arks were grown on commercial hard clam aquaculture leases. Blood arks were reared on the east coast (St. Augustine) and ponderous arks on the west coast (Cedar Key). Since blood arks do not naturally occur in the Gulf of Mexico, they could not be cultured on leases located on the west coast. The natural range of ponderous arks is from Virginia to Texas; therefore, this ark species could be cultured on both the Atlantic and Gulf of Mexico coasts. However, insufficient number of ponderous ark juveniles in this study limited culturing this species to one location.

3. At the seed sizes used in the field nursery trials (blood ark, 8 mm in SL; ponderous ark, 16 mm in SL), ark clams no longer had prominent byssal threads. Thus, handling procedures used in hard clam culture for estimating numbers and stocking culture bags were suitable for both ark species.

4. Because both ark species lack siphons and filter by opening their valves at the substrate surface, it was decided to evaluate polyethylene (hard) bags for rearing arks in the field nursery and growout. These bags are constructed from a hard plastic and provide predator protection as well as minimize excessive subsidence of the culture gear and organisms. Polyester (soft) bags that are used to culture hard clams in Florida were also evaluated in the blood ark growout trial as the sediments at the St. Augustine lease site consisted of a sand-mud mixture. Both of these bottom bags were secured by using either PVC pipes or galvanized wire stakes.

5. Although salinities varied little during the majority of these rearing trials, arks in each growout trial experienced episodic decreases in salinities (less than 10 ‰) as a result of tropical storm influences. Blood arks (34-35 mm, SL) experienced 100% mortality in late summer (2004), as did the hard clam crop on the same lease. Ponderous arks were of similar size (37 mm, SL) when exposed to low salinities in early summer (2005). Yet, mortality over the sample period was only 7%. Further, only small hard clam seed just recently planted on the lease area were subjected to mortalities during the same time period.

6. The number of ark clam juveniles available for the field nursery trials determined densities used. Blood arks were stocked at rates ranging from 100 to 520/ft^2, and ponderous arks were stocked at 85/ft^2. In comparison, hard clam juveniles are typically stocked at a density of 625/ft^2. In the growout trials, blood arks were planted at a density of 50/ft^2 in both polyethylene (hard) and polyester (soft) bottom bags and ponderous arks were planted at 62/ft^2 in hard bags only. These densities fall within the range used in hard clam growout (50-75/ft^2).

7. Average growth rates for blood arks during a 3-month field nursery and 9-month growout in hard bags were 2.6 and 2.2 mm in SL per month, respectively. Blood arks cultured in soft bags over the same growout period grew at a similar rate of 2.1 mm in SL per month. Over the 12-month rearing period, blood arks (initial size of 7.6 mm, SL) reached an average size of 22 and 23 mm in SW, respectively, in soft and hard bottom bags.

8. Average growth rates for ponderous arks during a 6-month field nursery and 18-month growout in hard bags were 2.2 and 0.83 mm in SL per month, respectively. Over the 24-month rearing period, ponderous arks (initial size of 16.6 mm, SL; 8.6 mm, SW) reached an average size of 30 mm in SW. However, after six months in the growout phase, or a total of

12 months in the field, ponderous arks had reached an average size of 23.8 mm in SW, resulting in a growth rate during that period of 1.2 mm in SL per month.

9. Survival of blood arks cultured in hard bags during a 3-month field nursery and 9-month growout period was 66 and 93%, respectively, resulting in an overall survival of 61%. Survival of blood arks cultured in soft bags during the same growout period was 80%.

10. Survival of ponderous arks cultured in hard bags during a 6-month field nursery and 18-month growout period was 76 and 72%, respectively, resulting in an overall survival of 55%. For both ark species, mortality seemed to be associated with excessive burial of some of the culture bags, possibly resulting in suffocation of the arks. Although it might be expected that hard bags would minimize this risk, it may be that use of sandier (firmer) substrates and periodic inspection of the bags are necessary.

11. Predation did not seem to be a contributing factor to mortality. Predators were not found in the culture bags of blood arks, nor were shell fragments or chipped shells. During sampling of the ponderous arks, stone crabs and mud crabs were observed, but again, there was no evidence of predation. The thick shells of these molluscs, in particular the ponderous ark, as well as their fibrous periostracum, may provide some protection from these predators.

12. Fouling of culture bags was minimal at the St. Augustine growing location; whereas at the Cedar Key growing location, tunicates and oyster spat were found on the surface of the hard bags during sampling. Very little fouling was noted on the arks themselves, again, most likely because of their periostracum. Some oyster spat were observed on ponderous arks at harvest, but this was limited to the umbo area of the shell, where the periostracum had eroded.

13. In Florida, market-size hard clams (25 mm, SW) can be obtained in 18-24 months from post-set seed (<1 mm SL). Using growth data from these rearing trials, we estimate that it would take a minimum of 14 and 25 months for post-set blood arks and ponderous arks, respectively, to reach a similar size. These growth rates are higher than those previously reported from Virginia and Georgia, probably due to Florida's warmer water temperatures and longer growing season.

14. Field nursery and growout procedures used for ark clams in these trials are not beyond the capacities of most shellfish growers. Seed availability and market demand will determine if hard clam growers diversify their businesses by culturing blood arks and ponderous arks.

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Appendix I:

Baldwin, J. 2003. Diversification for the Hard Clam Aquaculture Industry Through Investigation of the Blood Ark, Anadara ovalis, and Ponderous Ark, Noetia ponderosa, Reproduction and Development. Final Report R/LR-A-37, Florida Sea Grant College Program, Gainesville, Florida USA. 10 pp.

Diversification for the Hard Clam Aquaculture Industry Through Investigation of the Blood Ark, *Anadara ovalis*, and Ponderous Ark, *Noetia ponderosa*, Reproduction and Development

Final Report of Grant Number R/LR-A-37
Florida Sea Grant College Program

John Baldwin, Department of Biological Sciences, Florida Atlantic University

Abstract

Early development of the blood ark, *Anadara ovalis* (Bruguiere, 1789), and the ponderous ark, *Noetia ponderosa* (Say, 1822) was analyzed with an emphasis on the processes of meiotic maturation and early embryogenesis through gastrulation, using light and fluorescence microscopy. Both *A. ovalis* and *N. ponderosa* oocytes were released at the metaphase I stage of meiosis and shared similar schedules of early development through initial veliger stage. The first polar body is produced 10 min post-fertilization, completing meiosis I, and the second polar body is produced at 20 min post-fertilization, resulting in meiotic completion. At approximately 30 min post-fertilization, a partial polar lobe is produced which is followed by first cleavage. At 40 min post-fertilization, the embryo undergoes first cleavage which is holoblastic and divides the embryo into two unequal halves. The second cell division occurs at approximately one hour and twenty minutes, producing a four-celled embryo consisting of a single large macromere and three smaller macromeres. Holoblastic cell divisions continue every twenty minutes with a spiral cleavage arrangement typical of bivalve mollusks. By five hours, the embryo has rounded in shape and is now a blastula stage embryo and begins to spin rapidly. Gastrulation occurs at approximately eight hours by epiboly followed by invagination. By 15 hours post-fertilization, the developing embryo has reached the trochophore larval stage and by 21 hours post-fertilization has developed into a D-shaped veliger. Differences in the timing of development between species were negligible. In addition, the behavior and developmental timeline of the ark clams was very similar to that of *Mercenaria mercenaria*, which also reaches the D-shaped veliger stage in less than 24 hours.

Introduction

With funding from the Florida Sea Grant College Program, an investigation into the diversification of the hard clam aquaculture industry was launched in the late spring of 2002. For this investigation we looked at two native Florida species of molluscan shellfish, commonly known as "ark" clams: the blood ark, *Anadara ovalis* (Bruguiere, 1789), and the ponderous ark, *Noetia ponderosa* (Say, 1822). A special research grant was also awarded to the University of Florida Agricultural Experiment Station by the U.S. Department of Agriculture in 2002. These non-recurring federal funds were allocated to address priority needs of the food fish and shellfish aquaculture industries in Florida. The UF Shellfish Aquaculture Advisory Committee identified research areas of immediate concern and species diversification was ranked high. Through this funding, a preliminary investigation of blood ark and ponderous ark culture in Florida is currently

being conducted by several UF investigators (Baker, Creswell, Nunez, and Sturmer). This USDA-funded work is investigating the biological feasibility of developing hatchery and field rearing techniques for these two marine bivalve species. Despite the stimulated interest in Florida ark clam aquaculture, several aspects of their reproductive biology were poorly understood. In this study, with funding from Florida Sea Grant College Program, documentation of fertilization and early embryonic development of *A. ovalis* and *N. ponderosa*, was conducted in cooperation and collaboration with the above UF researchers at the UF Whitney Marine Lab. Should a commercial aquaculture fishery develop for ark clams, dependence on natural recruitment of seed stocks may not be economically sustainable. Since there is no documentation of egg activation, meiotic maturation, and early embryological development for these species, careful studies were initiated. Detailed knowledge of fertilization and early development in embryos of ark clams is necessary for the development of hatchery techniques and aquaculture management.

Obtaining Gametes

Reproductively mature individuals of the blood ark, *A. ovalis*, and the ponderous ark, *N. ponderosa* were collected from wild stocks for spawning induction at the University of Florida Whitney Laboratory bivalve hatchery. Arks can be checked for ripeness by sacrificing a few of them to determine their gonadal state (Figure 2). The ark should be carefully opened, making sure not to tear the body, and then peeling the mantle back. An ark that is unripe will have a grayish or tan coloration in its gut region. Ripe clams will have a creamy white gonadal mass covering the gut area (Figure 2A). If the clam is very ripe, puncturing the gonadal mass will cause gametes to ooze out (Figure 2B).

Broodstock animals were induced to spawn by alternating the water temperature between 20^{o}C to 30^{o}C with a 10 minute ramp period and holding at the end range temperature for 30min. This alternating hot and cold cycle was repeated until spawning occurred and is a commonly used method of mollusk spawning. Reproductively ripe arks of both species would typically spawn on the second to fourth warm cycle. At that time individuals were removed from the spawning tray, rinsed with fresh ambient seawater (24-26 ^{o}C, 27-30 ppt salinity) and placed in individual dishes where they would continue to spawn. Sperm and eggs were collected from these individual dishes and used for *in vitro* fertilization assays. In addition, attempts to spawn arks by injection of serotonin into the abductor muscle, which is used routinely to stimulate bivalves to spawn (Gibbons and Castagna, 1984), did not lend itself as a more advantageous method in its ability to induce spawning. This spawning method was dropped in favor of temperature cycling as a more reliable and simpler method.

Once spawning took place, male and female clams were identified and spawning individuals were removed from the spawning table and separated into small bowls (Figure 3). Spawning females were identified as their oocytes were released from their siphon and sunk to the bottom of the water column. The oocytes have a granular appearance, orange/pink in *A. ovalis* and *N. ponderosa*. Spawning males expelled their sperm, through their siphons, but instead of settling on the bottom, the sperm remained suspended in the water column and have a milky white appearance. Typical female

fecundity was estimated to be between 800,000 and 1 million oocytes for the blood ark, *A. ovalis* and between 7 and 12 million for the ponderous ark, *N. ponderosa* for animals of similar size (45 mm).

A normal oocyte was approximately 70µm in diameter. Under the microscope the yolk had a coarse orange/pink, granular appearance and filled the oocyte entirely. No jelly coat was detected around oocytes from any spawnings. Oocytes are viable for at least one hour after release. Oocytes which are released prematurely from clams that are not fully ripe had a cylindrical form and were not completely filled with yolk. While oocytes in this state may be fertilized, they will not fully develop. Prior to fertilization attempts a small sample of oocytes should be observed under the microscope to ascertain if the gametes appear normal. Unfertilized oocytes should have a nice round appearance a few minutes after release.

Normal spermatozoon, viewed under a microscope, were observed swimming rapidly. It is best to use the sperm for fertilization as quickly as possible, but the sperm can be kept cool (4-10 ^{o}C, in a refrigerator) for two to three hours and still remain viable. The heads of the spermatozoa have a blunt triangular structure measuring approximately 3.5 - 4.5µm long. The sperm's flagellum (tail) measured as long as 60µm.

Fertilization and Early Development

Gametes from spawning males and females were collected from individual dishes and mixed in a 1 L beaker with an approximate sperm to oocyte ratio of 50-100 sperm per oocyte. Only spawning trials with greater than 90% of the embryos reaching the four cell stage were used. Microscopic examinations of samples allowed us to determine the developmental stage of oocytes and embryos at specific times pre- and post-fertilization. To monitor development of meiotic and mitotic stages, samples were taken periodically and fixed with 3% gluteraldehyde in seawater for conventional light microscopy. In addition immunofluorescent microscopy was used for early developmental studies (meiosis). For immunofluorescent microscopy, zygotes and embryos were taken and fixed in cold 90% methanol-50 mM EGTA, pH 6.0 for one hour, washed in Tris-buffered saline (TBS) treated with TBS + 1% bovine serum albumin (BSA) and incubated with monoclonal antibody to β-tubulin antibodies for one hour at room temperature (Harris, 1987). After rinsing in TBS and blocking as described above, embryos were then incubated with goat anti-mouse IgG antibodies conjugated to tetramethyl rhodamine. Samples were then dehydrated through a graded ethanol series and mounted in methyl salicylate (Hertzler and Clark, 1992, Summers *et al*., 1991) for viewing on a Bio Rad MRC-1024 ES laser scanning confocal microscope coupled to an Olympus inverted microscope or a Nikon epifluorescent compound microscope.

Unfertilized oocytes of both species, *A. ovalis* and *N. ponderosa*, were released from the female during spawning in metaphase I arrest. Oocytes remained at this meiotic stage until fertilization. The following schedule of development is at 24-26^{o}C and 27-30 ppt (Figures 5 and 6). Upon fertilization (time zero), the zygote resumes meiosis. The first polar body is produced 10 min post-fertilization, completing meiosis I, and with the

second polar body is produced at 20 min post-fertilization, resulting in meiotic completion (Figure 4). At approximately 30 min post-fertilization, a partial polar lobe is produced which is followed by first cleavage. At 40 min post-fertilization the embryo undergoes first cleavage which is holoblastic and divides the embryo into two unequal halves. The second cell division occurs at approximately one hour and twenty minutes, producing a four-celled embryo consisting of a single large macromere and three smaller macromeres. Holoblastic cell divisions continue every twenty minutes with a spiral cleavage arrangement typical of bivalve mollusks. By five hours, the embryo has rounded in shape and is now a blastula stage embryo and will begin to spin rapidly. Gastrulation occurs at approximately eight hours by epiboly followed by invagination. By 15 hours post-fertilization, the developing embryo has reached the trochophore larval stage and by 21 hours post-fertilization has developed into a D-shaped veliger. Differences in the timing of development between species were negligible (Figures 5 and 6). In addition the developmental timeline of the ark clams was very similar to that of *M. mercenaria* which also reaches the D-shaped veliger stage in less than 24 hours under similar conditions.

Conclusions and Future Directions

Our findings indicate that typical spawning techniques used in the hard clam hatcheries are successful when applied to ark clams and that the early embryonic developmental events of the blood ark, *A. ovalis*, and ponderous ark, *N. ponderosa* are congruent with that of M. *mercenaria*. As a result we would expect that these two Florida native ark species could supplement current hard clam production with minimal operational changes in the early hatchery stages. The availability of ripe broodstock of both species made procedures very challenging, providing short windows of opportunity to take advantage of reproductive wild arks from different areas throughout the season. As a result efforts were made to condition arks at the Whitney Lab for spawning trials. For future work, with both *A. ovalis* and *N. ponderosa*, the establishment of domestic broodstock is highly recommended for hatchery production due to the unpredictability and logistics of obtaining reproductively ripe wild arks. Additional studies to improve hatchery technology, specifically broodstock conditioning, larval rearing and growout, and the effects of temperature, salinity, and nutrition on larval growth, are also required and are ongoing at the Whitney Lab.

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Figure 1. Broodstock Adults.

A. *Anadara ovalis*

B. *Anadara ovalis*

Figure 2. Checking Ripeness. Clams can be checked for ripeness by sacrificing a few of them to determine their gonadal state. The clam should be carefully opened, making sure not to tear the body, and the mantle is then peeled back. A clam that is unripe will have a grayish or tan coloration in its gut region. Ripe clams will have a creamy white gonadal mass covering the gut area (A). If the clam is very ripe, puncturing the gonadal mass will cause gametes to ooze out (B).

A

B

Figure 3. Released Gametes. *Anadara ovalis* female (A) and male (B) releasing gametes in individual culture dishes during artifical spawning.

A. Female

B. Male

Figure 4. Meiosis in *Anadara ovalis.* (A) Unfertilized oocytes released from female in the metaphase I stage of meiosis, **(B)** At five minutes after fertilization we see the formation of the first polar body during telophase - 1. **(C)** Ten minutes we see the extrusion of the first polar body. **(D)** 15 minutes metaphase - 2 is is visible. **(E)** Twenty minutes anaphase - 2 which will result in the formation of the second polar body. *Noetia ponderosa* demonstrated a similar set of images with the same timing.

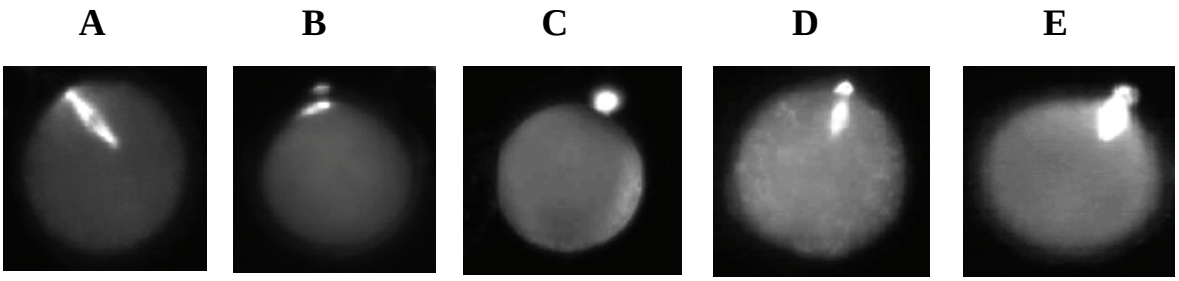
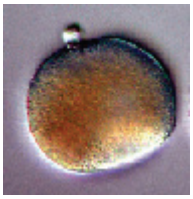
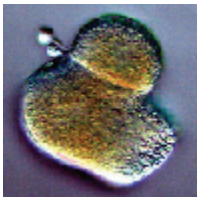


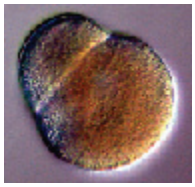
Figure 5. Early Development of *Anadara ovalis*



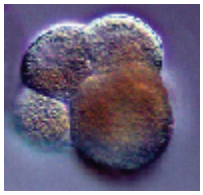
A. 10-15 minutes



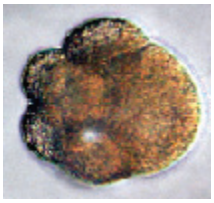
B. 40 minutes



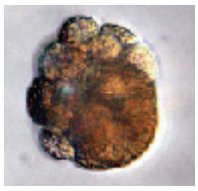
C. 1hr 5 minutes



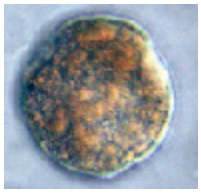
D. 1hr 20 minutes



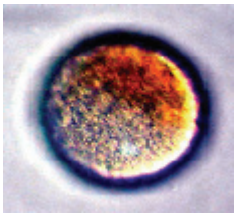
E. 1hr 40 minutes



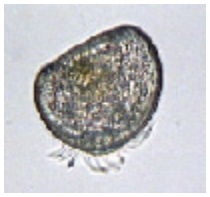
F. 2 hrs



G. 4hrs - 5hrs



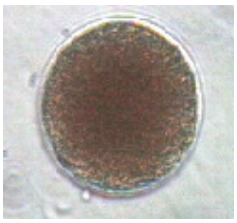
H. 7hrs – 8 hrs



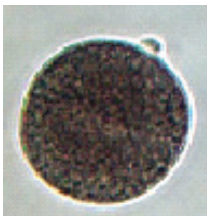
I. 19hrs - 21hrs

Stage	**Time**
Fertilization	**0**
First polar body	**10-15 min**
Second polar body	**20-30 min**
Polar lobe formation	**40-50 min**
First cleavage	**1 hour**
Second cleavage	**1 hr 20 min**
Third cleavage	**1 hr 40 min**
Ciliated blastula	**4-5 hrs**
Early gastrula	**7-8 hrs**
Trochophore	**12-15 hrs**
D-shape veliger	**19-21 hrs**

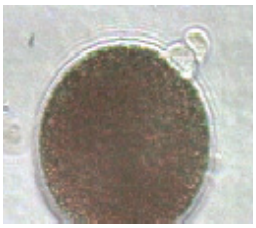
Figure 6. Early Development of *Noetia ponderosa*



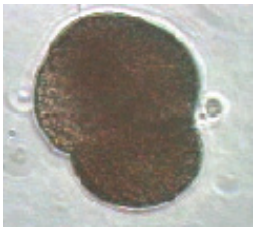
A. 0 minutes



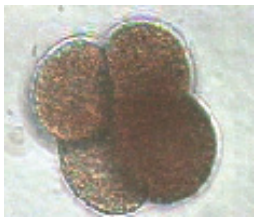
B. 10-15 minutes



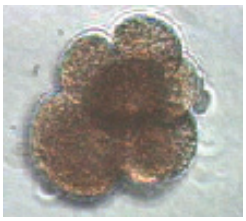
C. 40 minutes



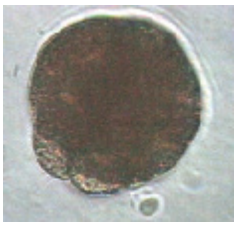
D. 60 minutes



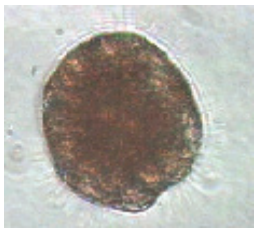
E. 1hr 20 minutes



F. 1hr 40 minutes



G. 4hrs - 5hrs



H. 8hrs – 9 hrs

I. 21hrs

Stage	**Time**
Fertilization	**0**
First polar body	**10-15 min**
Second polar body	**20-30 min**
Polar lobe formation	**40-50 min**
First cleavage	**1 hour**
Second cleavage	**1 hr 20 min**
Third cleavage	**1 hr 40 min**
Ciliated blastula	**5 hrs**
Early gastrula	**8-9 hrs**
Trochophore	**12-15 hrs**
D-shape veliger	**21 hrs**

Appendix II:

Power, A.J., J. Nunez, M. Mitchell, R.L. Walker, and L. Sturmer. 2004. Reproductive pattern of the blood ark, *Anadara ovalis*, from the northeast coast of Florida. *Journal of Shellfish Research* 23(1):173-178.

Journal of Shellfish Research, Vol. 23, No. 1, 173-178, 2004.

REPRODUCTIVE PATTERN OF THE BLOOD ARK, *ANADARA OVALIS* FROM THE NORTHEAST COAST OF FLORIDA

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ABSTRACT In blood arks, the sexes are separate; however, a low incidence of hermaphrodites was observed in the current study (2.17%). Males occurred more frequently than females (m/f = 2.68). Ripe arks were present year round outside of the period from August to November 2002. A bimodal reproductive pattern was apparent with a peak in gonadal development during the late spring-early summer months (45% ripe in May), followed by a quiescent period in the late summer-fall, and followed in turn by a minor peak during the winter months (21% ripe in December). Spawning was apparent in all months with the exception of September, and individuals in this phase were most numerous in June (50%) and July (64.29%). Dribble spawning is likely used as a strategy to extend its spawning period and increase its reproductive success. A low incidence (3.33%) of parasitic infection by digenetic trematodes resulting in castration was also noted. The implications of these findings on the aquaculture potential of this species are discussed.

KEY WORDS: ark, gametogenesis, reproduction, sex ratio, spawning, *Anadara avails*

INTRODUCTION

Several ark species *(Scapharca, Area*, and *Anadara* spp.) form the basis of economically significant molluscan fisheries and extensive culture operations (Baqueiro et al. 1982, Baqueiro 1989, Broom 1985, MacKenzie 2001, Manzi & Castagna 1989, Nie 1990, Umezawa 1992). A member of the ark shell family Arcidae, the blood ark, *Anadara ovalis* (Bruguiere 1789) occurs from Cape Cod, Massachusetts, to the West Indies and Brazil, at depths ranging from the low-tide line to >3 m (Abbott 1974, Anderson et al. 1984, Rehder 1981, Walker & Gates 2001). The species favors sandy deposits (Alexander 1993) and salinities above 15 ppt (Chanley & Andrews 1971). With a short lifespan (<5 y), early maturity (10-12 mm/~8 mo), rapid growth rates, and established markets in the Northeast, this bivalve has shown tremendous potential as an aquaculture species to diversify hard clam operations in Southeastern coastal waters (McGraw et al. 1996, McGraw et al. 1998, Power & Walker 2001, Power & Walker 2002, Walker 1998).

In Florida, *A. ovalis* occurs mainly on the eastern coast whereas the related ponderous ark *(Noetia ponderosa*, Say 1822) also inhabits the Gulf Coast. A concerted effort is currently underway among researchers and extension agents on both coasts of Florida and on the Georgia coast to investigate the hatchery-rearing protocol for both species. A systematic histologic examination of the gonads is a critical step providing information on the gametogenic cycle and sex ratios. By knowing the natural gametogenic cycle of a species, hatchery operators will know when to collect brood stock in the field while they are at their optimum ripe stage prior to their spawning. Histologic analysis of gonadal tissue also allows hatchery operators to know if animals from the natural population can be spawned more than once per year. This can save time and money in conditioning the bivalves to an optimum ripe stage within the hatchery. Knowledge of the sex ratio of a species determines the number of individuals that must be held for conditioning and spawning.

The reproductive biology of the blood ark has not previously been investigated for populations anywhere in Florida. In Virginia, blood arks are reported to spawn in the summer months when water temperatures reach above 17°C (Chanley & Andrews 1971, McGraw et al. 1998). In Georgia, blood arks spawn earlier, from late spring through summer (Power & Walker, 2002). In general, spawning periods and gametogenesis in marine bivalves start earlier and last longer in southern geographical areas than in northern ones (Eversole 1989, Thompson et al. 1996), and therefore the gametogenic cycles from Florida can be expected to be unique and clearly warrant investigation.

MATERIALS AND METHODS

Blood arks were collected from harvested clam bags during processing at certified shellfish wholesalers (Sturmer et al. 1995) and subsequently replanted in a commercial clam lease on the Matanzas River near "Marineland" south of St. Augustine, Florida, for holding and continued growout over the annual study period. The salinity and temperature of the river were monitored at the Whitney Laboratory floating dock every 30 min using a YSI 6600 data sonde.

Each month between May 2002 and April 2003, approximately 30 specimens were shipped to the Shellfish Research Laboratory in Savannah, Georgia. Immediately upon arrival, each individual was measured for shell length, width, height, and total weight (McGraw et al. 1996), and a mid-lateral gonadal sample (ca. 1 cm^2) was dissected for histologic analysis. Notes on the gonad color were recorded. Gonadal tissue was fixed in Davidson's solution, refrigerated for 48 h, washed with 50% ethanol, and preserved in 70% ethanol until processing. We processed tissues according to procedures outlined in Howard and Smith (1983). The examination of prepared gonadal slides was conducted with a Zeiss Standard 20 microscope (20x). Each animal was sexed and assigned to a developmental stage as described by Walker and Heffernan (1994) and Spruck et al. (1994). A staging criteria of 0 to 5 was used for early active (EA = 3), late active (LA = 4), ripe (R = 5), partially spawned (PS = 2), spent (SP = 1), and inactive (IA = 0). The determination of monthly gonadal index (G.I.) values was obtained by averaging the number of specimens ascribed to each category score. We tested sex ratios against a 1:1 ratio with chi-square (x^2) statistics (Elliott 1977).

RESULTS

Monthly mean water temperature and salinity data for the Matanzas River are shown in Figure 1. Water temperatures ranged from 12 to 28°C between the winter and summer months. Salinities showed less of an annual pattern, and values ranged from 22

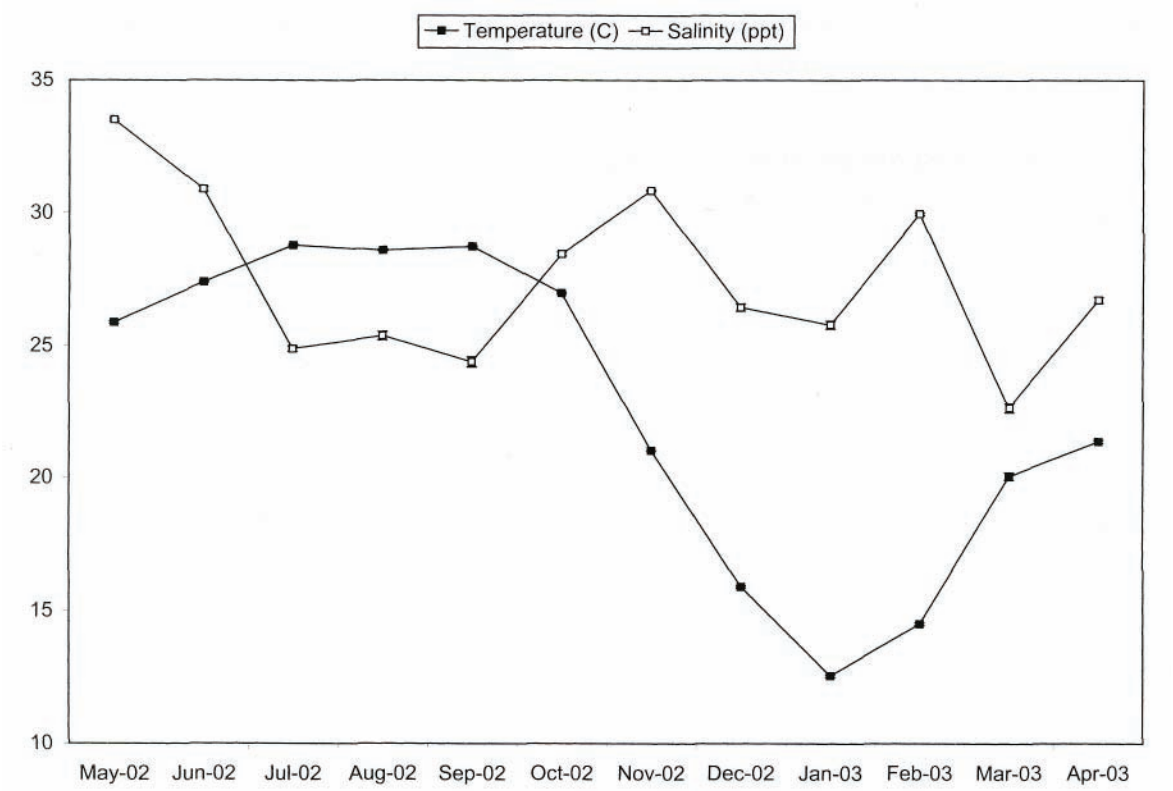

Figure 1. The mean monthly water temperature and salinity of the Matanzas River, Florida, from May 2002 to April 2003.

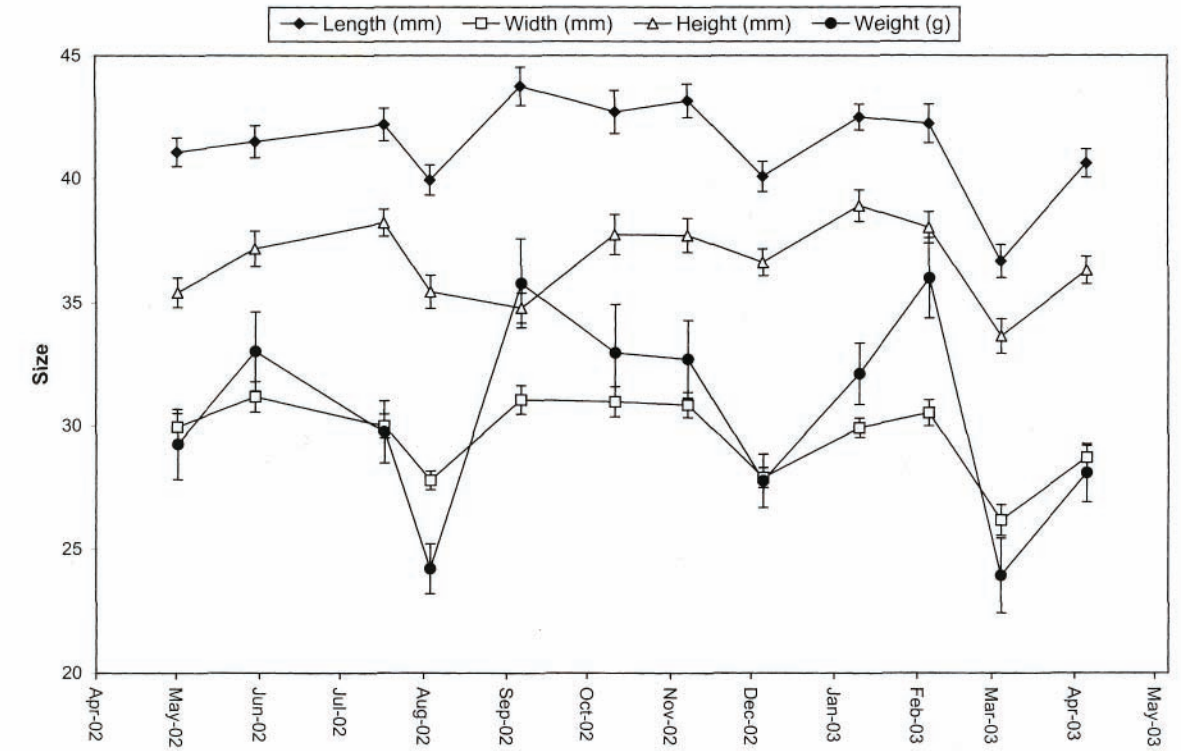

Figure 2. The mean monthly shell length, shell width, shell height (mm ± standard error) and total wet weight (g ± standard error) of the blood arks, *Anadara ovalis*, sacrificed for the histologic analysis between May 2002 and April 2003.

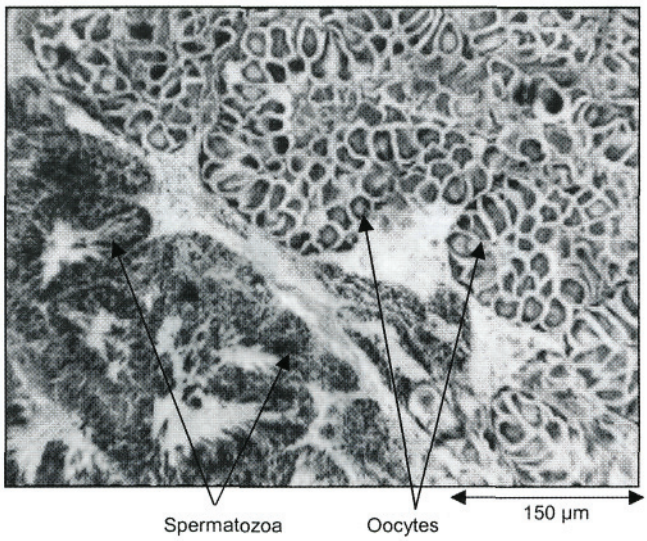

Figure 3. A histologic section through the gonad of a hermaphroditic blood ark, *A. avails,* **showing ripe oocytes and spermatozoa.**

to 33 ppt. Figure 2 displays the mean size parameters (length, width, height, and weight) of monthly samples. The average length of blood arks from all samples combined was 41.34 mm (±0.22 SE). The largest and smallest ark observed in terms of shell length, width, height, and weight measured 52.59 mm, 39.58 mm, 41.15 mm, 58.90 g, and 29.50 mm, 21.00 mm, 88.10 mm, and 13.10 g, respectively.

Of the 323 arks examined histologically, 73 (22.60%) were sexually indeterminate, 177 (54.80%) were male, 66 (20.43%) were female, and 7 (2.17%) were hermaphroditic (Fig. 3). Figure 4 reveals the size distribution of arks according to these categories. Both male and female arks were most abundant in the 42-mm shell-length class and represented in similar proportions in the smaller and larger size classes. Hermaphroditic arks occurred between 38 and 48 mm. Males dominated in all monthly samples with the exception of September 2002, where there were two with four females; however, a large percentage (75%) of individuals in this sample did not display any gonadal activity and therefore were sexually indeterminate (Fig. 5). The \$^2$ test revealed that the overall male/female ratio of 2.68 was significantly different from parity ($X^2 = 7.89$; $P < 0.01$). Histologic examination and visual observations of the gonads revealed that all orange-red colored gonads were females whereas those showing white coloration were typically males.

Ripe arks were present in most samples, with the exception of those taken from August to November 2002 (Fig. 6). Similarly, partially spawned individuals were observed throughout the year with the notable exception of September 2002, when all gonads were completely spent. While an extended spawning period from May to September was observed, a bimodal annual pattern was apparent in the G.I. plot. Gonadal development peaked during the late spring-early summer months (45% ripe in May), followed by a quiescent period in the late summer-fall, and followed in turn by a minor peak during the winter months (21% ripe in December). The largest coordinated spawning effort was in June and July, where 50% and 64.2% of individuals had partially spawned gonads. Partially spawned gonads often had smaller areas of ripe and late active follicles.

A low incidence (3.33%) of parasitic infection by digenetic trematodes resulting in castration was also noted. No seasonal

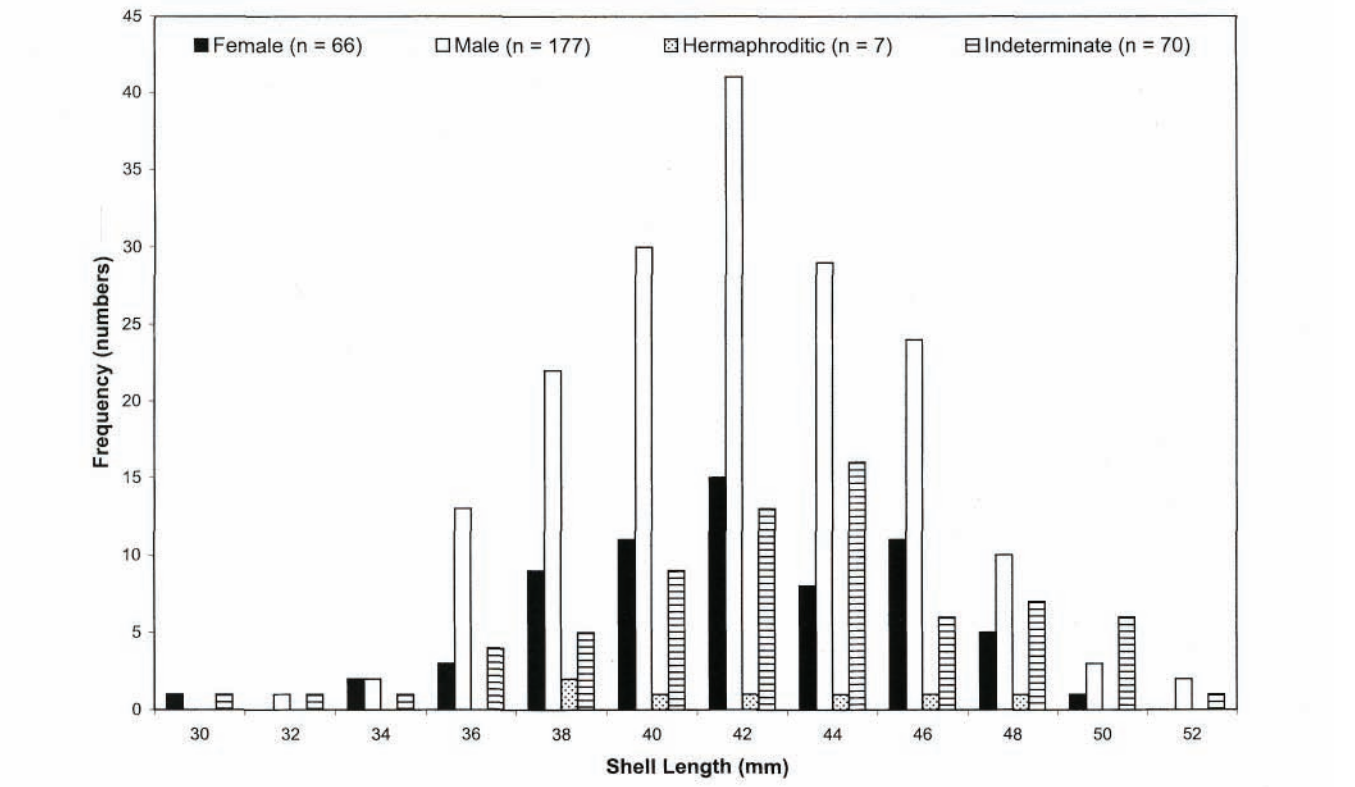

Figure 4. The frequency size distribution (2-mm shell length classes) of blood arks, *A. ovalis,* **identified as male, female, hermaphrodite, or indeterminate during the course of the annual study.**

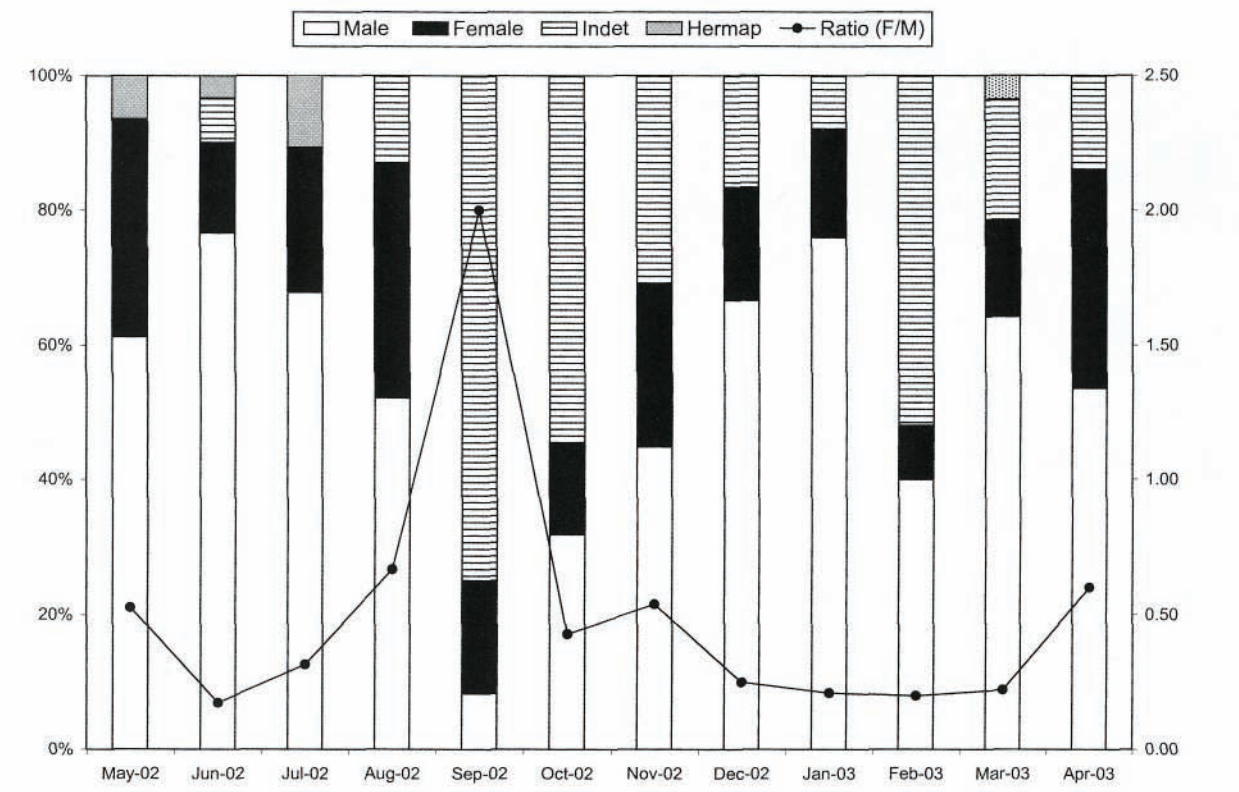

Figure 5. Variations in the female:male ratio of blood arks, *A. avails*, in monthly samples taken from St. Augustine, Florida, between May 2002 and April 2003. Shown also are the percentage of individuals each month labeled as male, female, hermaphrodite, and indeterminate.

pattern in parasitic infection was evident, with one specimen being infected in the months of May, June, July, September, November, December 2001, and March 2002, and two specimens in the months of January and February 2002. Spearman's rank correlation coefficients detected a significant correlation between mean water salinity levels and the mean monthly G.I. (r_s = 0.67, t = 2.07, df = 11, a = 0.05).

DISCUSSION

In Long Island Sound, New York, the transverse ark, *Anadara transversa* (Say, 1822), is reported to spawn between May and August (Loosanoff & Davis 1963). Further south in the Chesapeake, this species spawns from May to September (Chanley & Andrews 1971). In Georgia, a more extended period is observed for the transverse ark with major spawning occurring between April and July, and some spawning also occurring in the winter months of December and January (Walker & Power, 2003). Similarly, for the related blood arks, a spawning period from May to September has been identified for the Chesapeake area (McGraw et al. 1998) whereas a more extended period over most of the year but peaking in the summer has been reported for Georgia (Power & Walker 2002). Further south in Cost Rica, ripe *Anadara tuberculosa* (Sowerby, 1883) are present throughout the year with a peak in spawning activity between May and September (Cruz 1984a). Additionally, *Anadara similis* (Adams, 1852) and *Anadara grandis* (Broderip & Sowerby, 1829) from these waters are also ripe year round but have peaks during the winter months (Cruz 1984b, Cruz 1987). From this synopsis, it would appear that the spawning pattern of the Arcidae family becomes less confined to a single narrow season with decreasing latitude and the associated reduction in temporal fluctuations in temperature and coastal phytoplankton abundance. Based on the current study, it is apparent that blood arks from the northeast of Florida can also remain in the ripe and spawning phases for most of the year and that salinity may be a controlling factor in the gametogenic cycle.

A prolonged spawning period has distinct advantages and disadvantages for producing the species in a commercial hatchery. During the microscopic analysis of histologic slides, partially spawned gonads with patches of ripe and late active follicles were frequently observed. It is therefore likely that this species achieves a long spawning season by maturing different sections of its gonad at different times. This would give rise to a "dribble" spawning reproductive strategy, wherein gametes are not released synchronously as a massive event within the population, but instead small amounts are periodically released over an extended time period. In a hatchery, such a strategy would reduce the number of viable gametes available through a single induced spawning effort. As a result, either more individuals or repeated spawning attempts during several weeks and months might be necessary to generate sufficient numbers of larvae.

A clear advantage is that broodstock can be obtained and spawned almost anytime outside of the fall months, allowing for multiple spawnings per year. Of course, the optimal time for spawning is the early summer based on the percentage of individuals synchronously in the desirable late active, ripe, and partially spent gonadal stages (>80%). During the winter reproductive peak, less than 50% of individuals display gonads at these stages. Therefore, spawning in the early summer months would reduce the number of individuals required for conditioning.

A reduction in the number of broodstock required is important

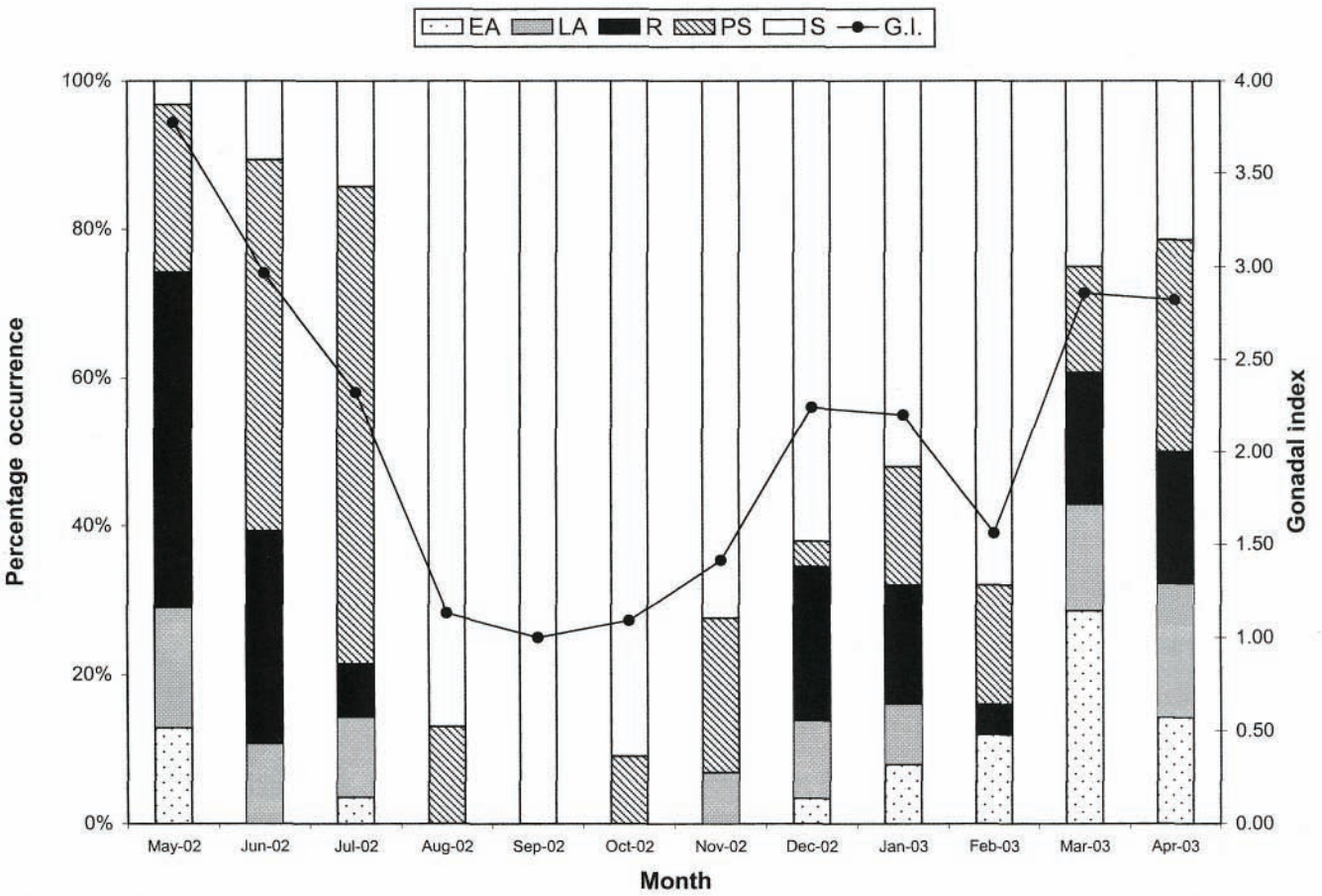

Figure 6. The relative frequency (percentage) of each gonadal developmental phase (EA = early active, LA = late active, R = ripe, PS = partially spawned, and S = spent) of *A. ovalis* between May 2002 and April 2003. Monthly gonadal index (G.I.) values are also shown and were determined by averaging the number of specimens ascribed to each category score (EA = 3, LA = 4, R = 5, PS = 2, SP = 1).

because only one in three to four blood arks are likely to be female (M:F = 2.68:1.00). The male domination reported here is in general agreement with previously reported sex ratios for the species in Virginia (1.98:1.00 M:F; McGraw et al. 1998) and in Georgia (2.44:1.00 M:F; Power & Walker 2002). This is, however, the highest male to female ratio recorded for a member of the Arcidae family (see review in Power & Walker 2002). Hermaphroditism was observed for the species in the current study for the first time, albeit at a low incidence. One individual displayed a large area of mature oocytes sandwiched between two similarly sized areas of spermatozoa whereas the other six had only a few follicles of the extra gamete. Hemaphroditism is rare in the whole Arcidae family. A low incidence has been reported for *Anadara granosa* (0.003%) *and Anadara senilis* (0.004%) Broom (1983, 1985, respectively). It has been suggested that these might be protandric hermphrodites. Males typically dominate protandric bivalve species in the first year whereas older age classes are generally equal. However, the specimens examined in the current study were generally of the larger and older size classes and were still dominated by males. Males and females were also represented in similar ratios throughout the entire shell length size distribution analyzed (i.e., 30-52 mm). In addition, one male ark maintained in a conditioning tank with 39 others (both sexes) at the Whitney Laboratory was induced to spawn female gametes 2 mo after having last released spermatozoa. This does not support a protandric hermaphroditic life cycle, and the observed phenomenon cannot be explained.

ACKNOWLEDGMENTS

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Appendix III:

Power, A.J., L. Sturmer, C. Lucas, R.L. Walker, and J. Manly. 2005. Gametogenic cycle of the ponderous ark, *Noetia ponderosa* (Say, 1822), from Cedar Key, Florida. *Journal of Shellfish Research* 24(1):69-73.

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GAMETOGENIC CYCLE OF THE PONDEROUS ARK, *NOETIA PONDEROSA* (SAY, 1822), FROM CEDAR KEY, FLORIDA

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ABSTRACT The gametogenic cycle of the ponderous ark, *Noetia ponderosa* (Say, 1822), was studied in a Cedar Key, Florida population between March 2001 and January 2003. Ponderous arks are dioecious, and no hermaphrodites were found in this study (n = 592). The sex ratio of females to males was 0.84:1.00, but was not significantly different from parity. On the Gulf coast of Florida, the ponderous ark dribble spawns over most of the year, peaking in the summer and fall months and with the least spawning activity occurring during the spring when gametes are maturing. A small percentage (5.2%) was found infested with an undescribed digenetic trematode. The Cedar Key area has an important commercial hard clam *(Mercenaria mercenaria)* aquaculture industry, and the implications of these findings on the potential for its diversification based on this species are discussed.

KEY WORDS: ark, gametogenesis, growth, reproduction, spawning, sex ratio

INTRODUCTION

A member of the ark shell family (Arcidae), the ponderous ark, *Noetia ponderosa* (Say, 1822), is common throughout the estuarine and nearshore waters of the southeastern Atlantic and Gulf of Mexico coastal areas of the United States (Abbott 1974, Anderson et al. 1984, Anderson & Eversole 1985, McGraw & Castagna 1994, Walker & Gates 2001). The species favor shelly substrates at depths from the low water line to 18 m and salinities above 17.5 ppt (Abbott 1974, Chanley & Andrews 1971, Rehder 1981, McGraw et al. 1996). Adult shells can attain a length of 7 cm (Rehder 1981). Other than a few reproductive and population dynamics studies from Virginia (Chanley 1966, Chanley & Andrews 1971, McGraw & Castagna 1994, McGraw et al. 1996, McGraw et al. 1998, McGraw et al. 2001) little is known about the species. Information is particularly scant on the arks biology and ecology in the Gulf of Mexico region.

At the northern geographical limit in the Chesapeake, ponderous arks are reported to spawn in late spring and summer, with additional activity albeit on a lesser scale in the fall and early winter (McGraw et al. 1998). Previously reported growth rates from Virginia are slow relative to the co-occurring blood ark, *Anadara ovalis* (Bruguiere, 1789), and the species can live for between 10 to 15 y (McGraw et al. 2001). Ponderous arks were commercially harvested from the Eastern Shore of Virginia during the 1990s to supply ethnic markets in the northeast region (McGraw & Castagna 1994). Given the species longevity and growth rates, it is not surprising that wild stocks were quickly overfished. In Georgia, an ark survey undertaken in the late 1990s found that most beds were limited in size and would not support a viable fishery (Walker & Gates 2001). Consequently, interest is mounting throughout the southeastern states in developing an ark based aquaculture industry (Power et al. 2004). This study, funded through the United States Department of Agriculture's Cooperative State Research, Education, and Extension Service (USDA CSREES), is part of an overall evaluation of this species to provide diversification to the hard clam aquaculture industry.

The culture of hard clams *(Mercenaria mercenaria,* Linnaeus. 1791) represents the fastest growing segment of the aquaculture industry in Florida, with an economic impact of approximately $34 million to the state (Philippakos et al. 2001). The industry supports over 400 growers, 14 hatcheries, 90 land-based nurseries, and 55 certified shellfish wholesalers (Ruth et al. 2003). During the early 1990s displaced workers from the commercial fishing industry on the Gulf coast of Florida were retrained in clam farming, and today the Cedar Key area has some of the most productive shellfish leases in the nation (Colson & Sturmer 2000). Ponderous arks in this area often naturally recruit into the clam culture bags, and they grow and survive well under these conditions (authors observations). It is believed that this species could represent an opportunity for diversification to small-scale hard clam culture enterprises using similar culture methods and thus improve farm incomes. The availability of ark seed is dependent on the development of hatchery protocol for this species. Because other ark species have displayed marked latitudinal differences in gametogenic patterns (e.g., Power et al. 2004, McGraw et al. 1998) a critical first step is to comprehend the reproductive cycle of the species at Cedar Key on the Gulf Coast of Florida.

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MATERIALS AND METHODS

Arks were collected on an ongoing basis from harvested hard clam bags during processing at certified shellfish wholesalers (Sturmer et al. 1995) and subsequently replanted at a commercial clam lease in Cedar Key for holding during the study period. Stocking densities maintained were <538/m^2 (50/ft^2). Bottom water temperature and salinity data were taken every 30 min by a YSI sonde (Model 6600) data recorder deployed on site at the clam lease area.

Each month between March 2001 and January 2003, excluding April 2002, 19 to 33 arks were randomly collected and shipped alive to the Shellfish Research Laboratory in Savannah, GA. Immediately upon arrival each individual was measured for shell length and total weight (McGraw et al. 1996), and a midlateral gonadal sample (ca. 1 cm^2) was dissected for histologic analysis. Gonadal tissue was fixed in 10% formalin buffered with seawater for 48 h, washed with 50% ethanol, and preserved in 70% ethanol until processing. Tissues were processed according to procedures outlined in Howard and Smith (1983). The examination of pre-

pared gonadal slides was conducted with a Zeiss Standard 20 microscope (x20). Each animal was sexed, and ratios were tested against a 1:1 ratio with x^2 statistics (Elliott 1977). Individuals were assigned to 1 of 6 developmental stages: early active (EA), late active (LA), ripe (R), partially spawned (PS), spent (S), and inactive (IA). Stages in gonadal development are assigned based on gonadal appearance, the presence or absence of different sexual products, and evidence of gamete release. EA and LA are based upon descriptions of "early" and "later development" provided by Kennedy & Krantz (1982). Individuals at full maturity in their "later development" stage were treated as a separate stage, R for the present study. Kennedy and Krantz's "spawning" and "advanced spawning and regressing" describe our PS and S stages. IA is best described by Eversole & Minchener's (1980) "undifferentiated stage". A semiquantitative numerical assignment (0-5) was then used to rank these stages from 0 to 5: EA = 3; LA = 4; R = 5; PS = 2; SP = 1; IA = 0 (Walker & Heffernan 1994, Spruck et al. 1994). In reproductive studies, the expression of gonadal tissue as a percentage of somatic tissue is often used as an index of gonadal function. However, gonadal growth can be allometric, and therefore we selected the relative proportion of individuals in each gametogenic stage as our gonadal index (i.e., independent of body size). The determination of monthly gonadal index (GI) values was obtained by multiplying the number of specimens ascribed to each ranked score by the respective score, summing all such values, and dividing this figure by the total number of males or females analyzed. Image analysis of ovarian sections was also carried out with a Motic digital microscope (B3 Professional Series) and the Motic Images 2000 Version 1.3 software. For each female, the mean of two oocyte diameter measures (taken at right angles to each other) was calculated for 30 randomly selected oocytes and used to calculate mean monthly oocyte size.

RESULTS

Water temperature and salinity at Cedar Key are given in Figure 1. Water temperature followed an annual cycle where maximum temperature occurred in July (29.8°C) to August (29.7°C) in 2001 and in August (29.6°C) to September (29.3°C) in 2002 and lowest temperatures occurred in January 2002 and 2003 (16.8°C and 14.3°C, respectively). Between the 2 y sampled, water temperatures broadly followed the same pattern, however a more rapid increase was observed during the spring of 2002, and temperatures later that year also reached lower than those previously recorded. Salinity remained high throughout the study period ranging from 22 ppt in June 2001 to 34 ppt in December 2001, however the summer months of 2002 experienced higher salinities than those recorded in the previous summer.

Arks ranged in shell length from a mean of 40.7 mm (April 2001) to 48.6 mm (January 2003). Mean total wet weight ranged from 23.9 g to 45.3 g. Of the 592 specimens histologically processed, 310 (52.4%) were male, 259 (43.8%) were female, and 23 (3.9%) were sexually indeterminate. The sex ratio was 0.84 females: 1.00 males and not significantly different was parity (x^2 = 1.32). A small percentage of the animals (5.2%) were infested with one to two undescribed digenetic trematodes. No hermaphrodite arks were observed.

Noetia ponderosa appears to ripen rapidly during the spring and to spawn for a prolonged time period that peaks in the summer and fall months (Fig. 2). Spawning was however noted year round. In both years, 3 discrete peaks were noted in mean oocyte size (2001: April, August, November; 2002: May, August, December), again supporting an extended spawning cycle (Fig. 3). In the first year these peaks decreased successively, however this trend was not apparent in the second year (Fig. 3). On the whole, combined male and female gonadal indices and ovarian oocyte size increased in both years through the spring when early and late active arks dominated the reproductive stages (Fig. 2, Fig. 3). In 2001, mean oocyte diameter peaked in April (45.93 |xm) and remained high through the summer months (gonadal index peaking in July at 3.97) with varying levels of late active, ripe, and partially spent individuals then subsequently decreased through the fall (gonadal index dropping to 2.04 in August) to a mean of 21.94 |xm in

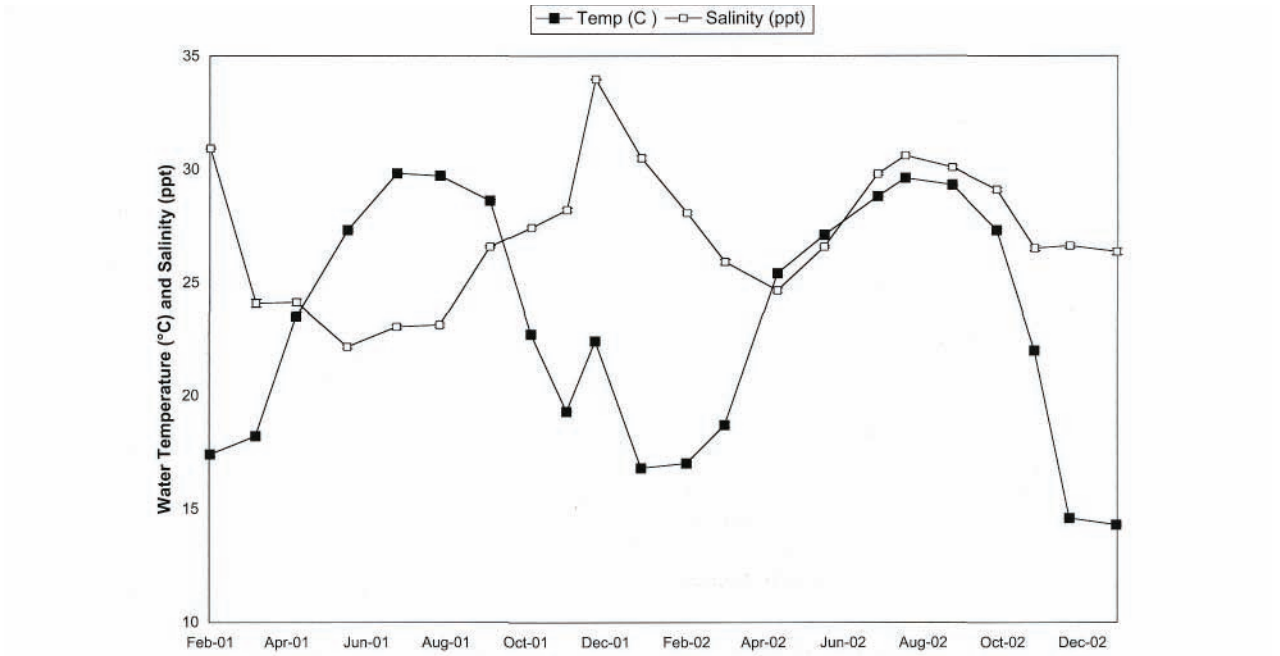

Figure 1. The monthly mean bottom water temperatures (°C) and salinity (ppt) of Cedar Key, FL from March 2001 to January 2003.

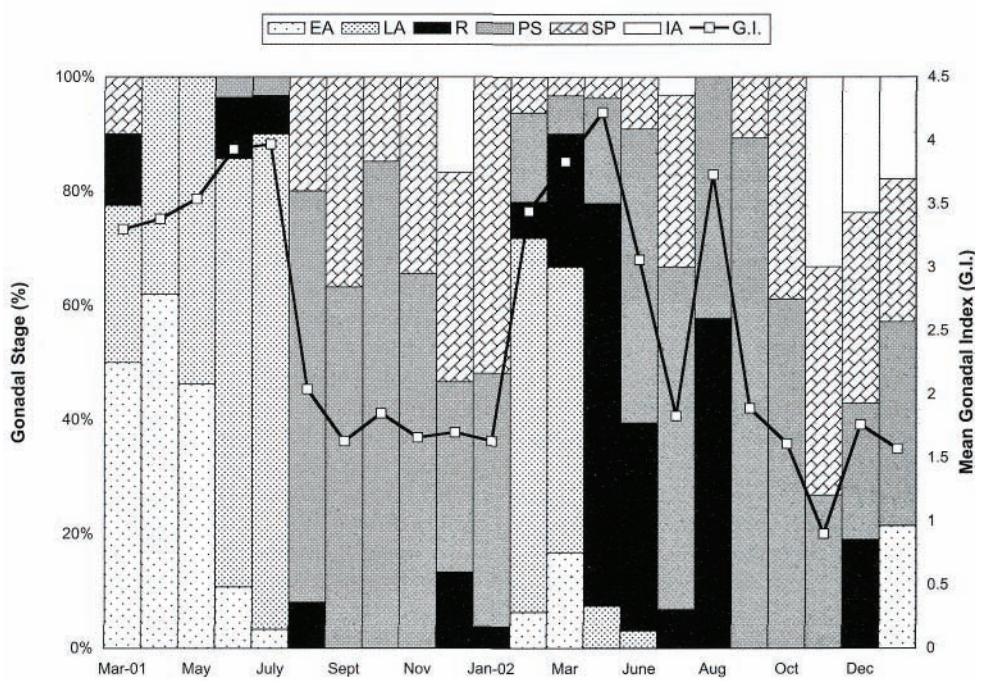

Figure 2. The relative frequency of each gonadal phase for the ponderous ark, *Noetia ponderosa,* in Cedar Key, FL from March 2001 to January 2003 (EA = early active, LA = late active, R = ripe, PS = partially spawned, SP = spent, IA = inactive). Monthly gonadal index (GI) values are also shown and were determined by averaging the number of specimens ascribed to each category score (EA = 3, LA = 4, R = 5, PS = 2, SP = 1).

December (Fig. 3). Through the winter months indices and oocytes remained small with spent and inactive individuals observed together with many partially spawned and some ripe individuals (Fig. 2). In the ensuing spring, oocytes matured and steadily increased to 39.86 pLm in May (gonadal index peak of 4.22) and remained large through the end of the year (41.51 jxm in December). During this period two minor decreases in oocyte size were observed (30.21 (jim in July and 33.40 |xm in September) prior to a substantial drop in January 2003 (21.87 |xm). Combined male and female gonadal indices for the same period exhibited a similar trend fluctuating, remaining until water temperatures begin to decrease in late summer and fall (Fig. 1} with indices dropping to 1.83 in July and 0.9 in November (Fig. 2).

DISCUSSION

Ponderous arks from Cedar Key started at an unknown age but were 40 mm in shell length. In Virginia, these would have been

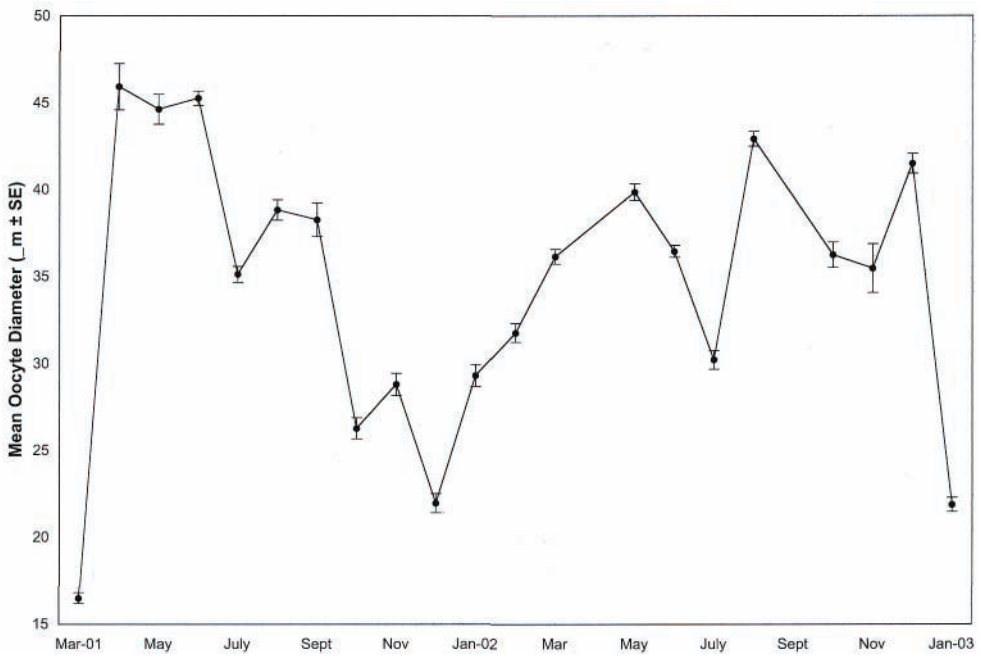

Figure 3. The mean oocyte diameter (um ± SE) of female ponderous arks, *Noetia ponderosa* in Cedar Key, FL from March 2001 to January 2003.

4 y old (McGraw et al. 2001) and in Georgia about 3 y (Walker & Gates 2001). It is assumed that the natural recruitment of arks in newly planted hard clam bags in Cedar Key occurred when the pediveliger larvae metamorphosized to a postset benthos dweller. The first arks were collected from bags that were harvested after 12 to 20 mo, implying that growth rates on the Gulf coast of Florida may exceed those previously reported for Virginia and Georgia. Because monthly samples were randomly selected from arks pooled from harvested clam bags on an ongoing basis, we cannot be certain that we were dealing with a single cohort, and therefore growth data was not a focus of the present investigation and is being acquired by another aspect of this USDA CSREES funded project. However, raising these clams with hard clam gear and techniques does appear to be a viable option and growth to market size in these waters could potentially be reduced to half of what it may take in Virginia.

On the Gulf coast of Florida the ponderous ark appears to be a dribble spawner that releases gametes over most of the summer and fall and even into the winter months. Early active and late active stages tend to occur in the spring when water temperatures are recovering after the winter months. There appears to be a rapid change from late active to spawning stages. Female gonads were rarely observed so packed with eggs that eggs appear box shape, as is seen in the ripe stages of many marine bivalves (Loosanoff et al. 1966), however large oocytes were present in most samples. The transition from spent to early active also appears to be short as few inactive stages of reproduction were observed. In addition in some spent stage females, early active stages were observed in the same gonads.

The gametogenic cycle between consecutive years was not identical but quite similar and indicates a dribble spawning strategy, with spawning occurring over most of the year and peaking in the summer and fall months. The prolonged spawning period of *Noetia* in Cedar Key is similar to the pattern of spawning that was observed in Virginia (Chanley & Andrews 1971, McGraw et al. 1998). It was their conclusion that *Noetia* spawns about 8 mo of the year, with ripening occurring in spring when water temperatures increased above 17°C. The spawning pattern of the family Arcidae typically becomes broader with decreasing latitude, and many species including *Anadara ovalis, Anadara similis* (Adams, 1852), *Anadara grandis* (Broderip & Sowerby, 1829), and *Anadara tuberculosa* (Sowerby, 1883) have ripe individuals year round in the warm waters of Florida and Central America (Power et al. 2004, Cruz 1984a, Cruz 1984b, Cruz 1987). The advantage of a prolonged spawning period in terms of hatchery production is that operations can continue year round. However, it is worth examining the quality of gametes released after the initial major spawning in the summer/fall period. While three peaks in oocyte size were observed in 2001, each successive peak diminished as the year progressed (April 45.93, August 38.82, November 28.78). This trend was not apparent in the subsequent year with very little difference observed between peaks (May 39.86, August 42.92, December 41.51). It is worth noting that local waters warmed earlier and that salinities were quite different during the second year, and the mean monthly shell lengths observed in year two were larger and significantly different from the first (ANOVA, $P = 0.03$), possibly indicating differences in reproductive fecundity with age and size.

Members of the family Arcidae tend to have equal sex ratios and are dioecious: *Anadara antiquata* (Toral-Barza & Gomez 1985), *Anadara granosa* (Pathansali 1966, Broom 1983), *Anadara senilis* (Broom 1985), *Anadara subcrenata* (Broom 1985), *Anadara trapezia* (Broom, 1985), and *Anadara tuberculosa* (Cruz 1984a, Dzyuba & Maslennikova 1982). However males dominated in populations of *Anadara ovalis* in Florida (Power et al. 2004), Georgia (Power & Walker, 2002), and Virginia (McGraw et al., 1998). Females dominated in a population *of Anadara transversa* in Georgia (Walker & Power, in press) and in *Anadara senilis* in Africa (Yoloye 1974). Hermaphroditism occurs very rarely: 0.003% in *Anadara granosa* (Broom 1983), 0.004% in *Anadara senilis* (Broom 1985), and 2.17% in *Anadara ovalis* (Power et al. 2004). The ponderous ark appears to follow the typical pattern: an equal sex ratio and no hermaphrodites (this study; McGraw et al. 1998). Again this is favorable for hatchery production, reducing the number of broodstock required to attain adequate gametes from both sexes.

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Appendix IV:

Degner, R.L., T.B. Southwell, L.N. Sturmer, and K.L. Morgan. 2005. Marketing opportunities for blood ark clams and ponderous ark clams. University of Florida/IFAS EDIS Publication FE478. Gainesville, Florida USA. 3 pp.

FE478

Marketing Opportunities for Blood Ark Clams and Ponderous Ark Clams[1]

Robert L. Degner, Tiffany B. Southwell, Leslie N. Sturmer, and Kimberly L. Morgan[2]

Significant growth in Florida's hard clam industry has motivated aquaculturists to explore alternative clam species to reduce potential production risks and to augment market expansion. Diversity of species could also provide some degree of protection against catastrophic losses.

Two alternative clam species with commercial potential are the Blood Ark (*Anadara ovalis*) and the Ponderous Ark (*Noetia ponderosa*). In the United States, wild stocks of Blood Arks range from Massachusetts to Texas. They are also found in the Caribbean and along the coast of Brazil. The Ponderous Ark is found from Virginia to Texas. Wild stocks of the Blood Ark and Ponderous Ark clams are currently harvested in North Carolina and Virginia in limited quantities for ethnic markets in the United States.

The development of a major fishery for these species has been limited by a variety of factors, including dispersed wild clam populations; minimal understanding of clam reproduction; and relatively small, isolated ethnic markets. Until recently, these clams have been largely overlooked by the shellfish and fishing industries.

The basic objective of this study was to determine the present market potential of Blood Ark and Ponderous Ark clams in the United States. This paper summarizes research that attempted to quantify the shellfish trade industry's present awareness and acceptance of the Blood Ark and Ponderous Ark clams as a first step in evaluating the economic feasibility of producing these clams in commercial quantities using commercially feasible aquacultural production techniques by the existing hard clam industry.

This study was conducted in three phases. Phase I was a nationwide census of all shellfish dealers certified by the Food and Drug Administration. Questionnaires were mailed to 2,133 firms to determine the current market situation for the two selected species of clams. In Phase II, firms responding to the initial survey that had expressed interest in possibly selling the two species of clams were sent live samples for evaluation. Phase III determined shelf life (life expectancy under typical

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commerical refrigeration) and nutritional composition. Results of Phase III are reported in IR-05-1, published by the Florida Agricultural Market Research Center (http://www.agmarketing.ifas.ufl.edu) and summarized in EDIS publication FE568 (http://edis.ifas.ufl.edu/FE568).

In Phase I, about 92 percent of the responding shellfish firms had no experience with Blood Ark clams. Only one percent of respondent firms sold Blood Ark clams during 2001. Among certified shellfish shippers that responded to the questionnaire, over 90 percent had no experience with Ponderous Ark clams. Less than two percent had seen them at trade shows, and only one percent had sold them in the past. With only one respondent currently selling Ponderous Arks, market exposure is presently extremely limited. It appears that the current market for the Blood Ark and Ponderous Ark clams is extremely limited due to lack of consistent supplies, limited market outlets, and virtually nonexistent consumer demand.

On a positive note, some shellfish shippers indicated an interest in learning more about these clam types. Out of 309 respondents, 97 provided mailing address information and requested Blood Ark clam samples, and 96 gave contact information to receive Ponderous Ark clam samples. However, when samples became available, only 83 firms agreed to accept and evaluate them.

In Phase II, 83 firms received live samples of the two types of clams. They were asked to evaluate a number of basic product characteristics, including appearance, taste, aroma, and textural properties. These respondents were also asked to estimate potential sales through their respective firms.

Samples of the two types of clams were harvested from approved waters in mid-November of 2003, and were held overnight in a refrigerated facility owned by a certified shellfish dealer in Cedar Key, Florida. Approximately eight clams of each type were placed in separate small, color-coded plastic mesh bags with official shipper tags affixed to each bag. The two bags of clams were placed in an insulated shipping container along with refrigerated gel packs, evaluation instructions, and a one-page questionnaire for each type of clams. The samples were shipped via a major courier service within several hours of packaging, and were delivered to all recipients before noon the next day.

Nearly one-half of the 83 samples were sent to firms in the Northeast, and nearly one-third went to firms in the South. Only two firms in the Midwest agreed to evaluate samples; both were in the Chicago area. Approximately one-fifth of the samples were shipped to firms on the West coast. Despite the care and diligence exercised in gaining the cooperation of the shellfish dealers receiving samples, only 52 of the 83 provided completed product evaluations, even after several follow-up telephone calls.

The majority of the sample recipients indicated that the samples arrived in excellent condition. However, several said their samples were slightly too warm for optimum shellfish storage conditions. Respondents were asked whether or not they could detect an odor from the samples. Sixty percent said they could not detect an odor. Of those who said they could detect an odor, only a small minority described the odor as “slightly unpleasant.”

Respondents were asked to rate the appearance of the shells using a zero-to-ten rating scale where zero represented “very unattractive” and ten represented “very attractive.” Both species of clams received mediocre evaluations, with average ratings of approximately five. Respondents were also to comment on appearance; critics complained of the black color and “fuzzy” or “furry” appearance. There was concern that cleaning would be time consuming and costly. There were very few positive comments about the clams' appearance.

Meat color was evaluated using the same zero-to-ten rating scale. The attractiveness ratings for meat color fared worse than those for shell appearance, with average ratings of 4.2 for Blood Arks and 3.6 for Ponderous Arks. A paired t-test indicated that the meat color rating differences between the two species were statistically significant. As the mean ratings suggest, meat color evaluations were heavily skewed towards the “very unattractive” end of the rating scale; nearly 15 percent of the respondents gave a zero rating to meat color of Blood Arks, and 22 percent gave a zero rating

to meat color of Ponderous Arks. Most comments made with respect to meat color were negative, criticizing the bloody appearance, but some were positive, stating the clam meat was “colorful” and “normal after cooking.”

Respondents rated “taste” using a similar scale of zero to ten, where zero represented “very poor” and ten represented “extremely good.” Respondents were asked to rate taste if eaten raw or if eaten cooked. Mean ratings were just under five for taste if eaten raw, and approximately five if eaten cooked. Taste ratings for whether eaten raw or eaten cooked were not statistically different for the two types of clams.

Texture was evaluated using a five-point semantic differential scale ranging from “much too tough” to “much too soft,” with the mid-point being “just right.” Respondents said that both types of clams were “slightly” or “much too tough” (55 percent for Blood Arks and 60 percent for Ponderous Arks). Texture differences were not statistically significant for the two species of clams.

When questioned about the number of clams of each type they could sell per week, just over one-half of the dealers said they could not sell any of the Blood Arks. About one-fifth of all respondents would not project sales, stating that they simply did not know how many, if any, they could sell. Eleven firms made positive sales projections, but these estimates were extremely variable, ranging from 30 to 170,000 clams per week.

Sixty percent of the respondents felt they could not sell any Ponderous Ark clams. About one-fifth said they did not know how many they could sell. Only eight firms provided weekly sales estimates; their responses ranged from 30 to 120,000 clams.

When asked for general comments about the clam samples, numerous respondents said they had trouble cooking them. Comments were similar for both types of clams. Many said it took too long for them to cook; some said they never opened and others said they were difficult to open. Respondents also mentioned marketability issues. Although many of the comments were negative, some were positive. Most of the positive comments reflected the opinion that both types of clams would appeal to ethnic markets, primarily Asian consumers.

In summary, the current market for both Blood Ark and Ponderous Ark clams is very limited in the United States. There is widespread lack of familiarity with these species, and very few shellfish dealers, probably less than one percent, are currently selling them.

As to the potential marketability of these species, the product evaluations and many of the respondents' comments indicate that these two species of clams are perceived as being too different from clams currently available on the market. These findings, coupled with the low response rates and shellfish dealers' unwillingness to evaluate free samples, leads us to conclude that it is unlikely that there will be a widespread, mainstream demand for them.

Despite the overall pessimistic tone of these findings, market development proponents should recognize the importance of ethnic markets in target locations on both the East and West coasts. Targeting seafood dealers in these areas with large Asian and Hispanic populations could result in profitable niche markets.

Additionally, producer groups should work with the Aquaculture Division of the Florida Department of Agriculture and Consumer Services (FDACS) to provide information about Blood Ark and Ponderous Ark clams to potential dealers at national seafood trade shows. Such information could also be incorporated into the FDACS website to foster greater knowledge in the trade and to arouse dealers' curiosity. These promotional methods could serve as relatively inexpensive promotional tools.

The complete report can be found on the website of the Florida Agricultural Market Research Center under “Publications”, Market Research Publications, 2000-present (Marketing Opportunties for Two Ark Clam Species). The online address is http://www.agmarketing.ifas.ufl.edu.

Appendix V:

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FE568

Nutritional Composition and Marketable Shelf-Life of Blood Ark Clams and Ponderous Ark Clams[1]

Leslie N. Sturmer, Kimberly L. Morgan, and Robert L. Degner[2]

Introduction

The rapid growth of Florida's hard clam industry over the past decade has motivated aquaculturists to explore alternative molluscan species, which could reduce exposure to production risks and simultaneously promote market expansion. Species diversification could possibly provide some degree of protection against potential disastrous losses associated with a monoculture-based industry. Further, production of other molluscan species could potentially increase sales and profitability, expanding clam consumers' options. The Blood Ark (*Anadara ovalis)* and the Ponderous Ark (*Noetia ponderosa*) clams represent two possible production opportunities.

The overall objective of this study was to determine the market potential of Blood Ark and Ponderous Ark clams in the United States. The study was conducted in three phases during 2003 and 2004. The major focus of Phase I was to assess the market situation for the two types of clams, specifically to determine the trade's knowledge about them and attitudes toward handling them if adequate supplies were available. In Phase II, firms identified in Phase I as potential marketers of these clams were asked to evaluate basic product characteristics of live samples, including appearance, taste, aroma, and textural properties, and to estimate potential sales through their respective firms. Detailed results concerning Phase I and Phase II procedures, findings, and conclusions can be found in EDIS publication FE478, Marketing Opportunity for Blood Ark Clams and Ponderous Ark Clams (http://edis.ifas.ufl.edu/FE478), and the complete report can be found on the website of the Florida Agricultural Market Research Center under Publications, Market Research Publications, 2000-present (Marketing Opportunties for Two Ark Clam Species). The online address is http://www.agmarketing.ifas.ufl.edu.

Phase III determined the nutritional composition of each species and the shelf life under typical commercial refrigeration. Comparisons were also made to the cultured hard clam, *Mercenaria mercenaria*.

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Phase III Findings: Nutritional Composition of Ark Clams

Comprehensive nutritional analyses were conducted for the Blood Ark and Ponderous Ark clam species and compared with the cultured hard clam. Samples of cultured ark clams were collected from their respective growing areas in St. Augustine (east coast of Florida) and Cedar Key (west coast of Florida). One hundred gram samples of meat (wet weight) were shucked for each ark clam species and delivered in coolers to an accredited private food-testing laboratory in Gainesville, Florida. Official methods were used following the AOAC (Association of Official Analytical Chemists) Official Methods of Analysis (Horwitz, 2002).

The nutrition facts and labeling for cultured Blood Ark clams were determined for a serving size of 100 grams of raw, edible portion. This serving is low in calories (35) and total fat (0.5 g), and high in protein (7 g). Cholesterol is relatively low (35 mg), with the serving portion representing 12 percent of the daily value. There is no detectable carbohydrate (0 g) in this portion. In terms of percent daily value, a serving of Blood Ark clams provides six percent of Vitamin A, two percent of Vitamin C, and six percent of calcium. Blood Ark meats are high in iron (70 percent of daily value), which is most likely associated with the presence of hemoglobin and erythrocytes in the clam meats. Of note is the high sodium content (740 mg), which represents 31 percent of the daily value. Even for a saltwater mollusk, this level is particularly high (Table 1). One plausible explanation for this may be that the meat sample (wet weight) contained fluids from within the shell. These trapped fluids may have abnormally increased the sodium content.

The nutrition facts and labeling for cultured Ponderous Ark clams were also determined for a serving size of 100 grams of raw, edible portion. Like the Blood Ark clams, this serving is low in calories (50) and total fat (1 g), and even higher in protein (11 g). The cholesterol level for Ponderous Ark clams (55 mg) is higher than in the Blood Ark sample, representing 18 percent of the daily value. There is little carbohydrate (1 g). In terms of percent daily value, a serving of Ponderous Ark clams provides six percent of Vitamin A, four percent of Vitamin C, and ten percent of calcium. Ponderous Ark meats are also high in iron (50 percent of the daily value), again most likely associated with the blood pigment content. Although the sodium content (480 mg) is lower than that for the Blood Ark clams, it is still relatively high, representing 20 percent of the daily value (Table 1).

A nutritional analysis for the hard clam was obtained from the Florida Department of Agriculture and Consumer Services, Bureau of Seafood and Aquaculture Marketing. Approximate nutritional values for four ounces (114 g) of raw, edible portion were converted to 100-gram equivalents, and resulting values are as follows: Calories (70), Calories from fat (9), Total Fat (0.9 g), Saturated Fat (0 g), Cholesterol (40 mg), Sodium (57 mg, or 2 percent of the Recommended Daily Intake (RDI)), Carbohydrate (0 g), Protein (16 g), Calcium (4 percent of RDI), and Iron (20 percent of RDI). Information on Vitamin A and Vitamin C values was unavailable (Table 1).

In comparison to the two species of ark clams, the hard clam is slightly higher in calories and protein, but similar in total fats, cholesterol, and total carbohydrate. Greater differences between the hard clam and ark clams are found in the iron and sodium values. Ark clams provide two to three times the daily percent values for iron than do hard clams, whereas ark clams contain ten times the amount of sodium than do hard clams.

Phase III Findings: Shelf Life of Ark Clams

Molluscan shellfish are typically shipped as live shellstock and adequate shelf life is an important product attribute. Federal regulations require that live mollusks be placed in refrigerated storage (<45°F) within a predetermined time/temperature harvest matrix to reduce probable levels of *Vibrio* bacteria (FDA, 2003). For these reasons, the shelf life of live Blood Ark and Ponderous Ark clams was investigated to assure product quality and safety.

To determine the survival of these two ark clam species in refrigerated storage, an evaluation of shelf life was conducted in April 2004. Procedures

followed those developed by Applewhite et al. (1996) and Otwell (1998) for determining shelf life of hard clams. Blood Ark clams were harvested from a commercial shellfish aquaculture lease located in the Intercoastal Waterway on the east coast of Florida at

8:00 AM on March 31, 2004. Ponderous Ark clams were harvested from a commercial lease in the Gulf of Mexico on the west coast of Florida at 9:00 AM on the same day. Bottom water temperatures at the time of harvest were recorded. Immediately post-harvest, ark clams were transported in coolers under ambient conditions to the Florida Fish and Wildlife Conservation Commission's marine laboratory in Cedar Key. At 1:00 PM ark clams were received at the laboratory and placed under tempering conditions at 68°F following protocols defined in the National Shellfish Sanitation Program, Model Ordinance, Guide for the Control of Molluscan Shellfish, VIII@.03 OPTION 1.E (FDA, 2003) and the Comprehensive Shellfish Control Code, Rule Chapter 5L-1.013(3)(b), Florida Administrative Code (FAC). During tempering, 100 ark clams were randomly selected from each species, of which a sub-sample of 25 was measured and weighed. Each ark clam sample of 100 was then placed into polyethylene tubular netting, which is typically used by shellfish dealers in bagging and transporting hard clams.

As defined in the harvest time/temperature matrix per Rule Chapter 5L-1.008(5), FAC, molluscan shellfish must be placed into refrigeration within 12 hours of the time of harvest during the month of April. If tempering is included as an alternative post-harvest process, then the time to refrigeration can be extended up to 16 hours from the time of harvest. Ark clams were placed into a thermostat-controlled refrigerator set at the standard storage temperature of 45°F on March 31 at 7:30 PM. Air temperatures were recorded inside the refrigerator using a minimum/maximum thermometer. The ark clams were checked daily for survival with the exception of two days during the evaluation period. Gaped ark clams were determined to be “commercially dead” when they did not respond by closing their shell to specified agitation, or tapping, after the ark clams were held for a short time at room temperature. Dead ark clams were counted and removed from the sample bags. The general conditions of the ark clams during storage were also noted. The evaluation was conducted until 50 percent of the Blood Ark clams died. At that time, percent survival was also determined for Ponderous Ark clams.

Water temperature at the time of harvest of the Blood Ark clams was 67°F. The clams used in the Blood Ark sample averaged 1 7/8” in shell length, 1 1/4” in shell width and 12.6 clams per pound. During the shelf-life evaluation, minimum air temperatures in the refrigerator averaged 41.4°F (+/-3.3°F) and maximum air temperatures averaged 52.4°F (+/-2.5°F). The overall average daily temperature was 46.9°F (+/-2.7°F). The noncommercial refrigerator used in this study was not able to maintain air temperatures consistently below 45°F.

The first Blood Ark mortality occurred on the fourth day of the evaluation. Mortalities were not noted again until the tenth day when five Blood Ark clams did not respond to agitation. Mortalities then occurred almost daily, with the number of mortalities increasing rapidly after the 19th day. On Day 23, the cumulative number of dead Blood Ark clams was 57 and the shelf-life evaluation was terminated.

Survival of Blood Ark clams in refrigerated storage was 99 percent or greater during the first nine days of the evaluation. Survivals dropped below 90 percent and 50 percent after Days 13 and 23, respectively. After the first week of the evaluation, liquid began accumulating in the bottom of the tray holding the Blood Ark bag. The tray was wiped clean daily thereafter. In addition, gaping occurred frequently in the live Blood Ark clams and agitation was required before they would close. The remaining live ark clams at the end of the evaluation sounded “hollow” and a strong odor was detected. A commercial mortality of greater than five percent would be considered unacceptable by the shellstock shipper industry, thus the shelf life of Blood Ark clams harvested under spring conditions may be limited to ten days. During warmer water temperatures experienced in summer months, shelf life may be further reduced, thus limiting shipment of live Blood Ark clams during that time period.

Water temperature at the time of harvest of the Ponderous Ark clams was 68°F. The clams used in the Ponderous Ark sample averaged 2” in shell

length, 1 3/8” in shell width and 10.5 clams per pound. The shelf life evaluation for the Ponderous Ark clams was conducted simultaneously and in the same non-commercial refrigerator that was used for the Blood Ark clams. Thus the temperatures for the Ponderous Ark shelf life evaluation were identical to those reported above for the Blood Ark clams.

The first Ponderous Ark mortality occurred on Day 23 of the evaluation. This was the same day that the Blood Ark evaluation was terminated because over 50 percent of the Blood Ark clams had died. It was decided to end the Ponderous Ark evaluation on the same day.

Survival of Ponderous Ark clams in refrigerated storage was 100 percent during the first 22 days of the evaluation. On Day 23, the first mortality occurred, dropping the survival rate to 99 percent. The majority of the Ponderous Ark clams remained tightly closed throughout the evaluation with no liquid accumulating on the bottom of the tray holding the Ponderous Ark bag. There was no gaping observed in the remaining live ark clams and no odors were detected.

A baseline for the survival of Florida farm-raised hard clams during refrigeration was obtained for product harvested from commercial aquaculture leases in the Gulf of Mexico during April through October 1997 (Otwell, 1998). In April, 100 percent of the hard clams survived for seven days after placement in refrigerated storage. After ten days, the survival of hard clams dropped to 90 percent. In this study, the Blood Ark clams showed similar responses to refrigerated storage as hard clams. It may be that like hard clams, survival of Blood Arks may be reduced when harvested during the warmer summer months. However, extending the tempering protocol to its full duration of 16 hours may help prolong the refrigerated shelf life of Blood Arks as it has with hard clams. The tolerance of live Ponderous Ark clams to refrigerated temperatures exceeds that observed for hard clams and other molluscan shellfish, such as oysters.

Summary

In summary, like the hard clam and other molluscan shellfish, ark clams are an excellent source of protein and especially good source of iron and calcium. However, based on these results consumers who must restrict their intake of sodium should take these nutritional facts into consideration or reduce their portion size. These results demonstrate that commercial distribution of live shellstock of both ark clam species is achievable. Alternative harvesting, handling and storage techniques, such as tempering, used to increase survival of hard clams in refrigerated storage when harvest water temperatures exceed 80°F should also be considered for the Blood Ark clam.

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Table 1. Nutritional composition of Blood Ark, Ponderous Ark, and Hard Clams.

Nutritional Parameter	**Type of Clam** Blood Ark Clam (*Anadara ovalis*) -----	Ponderous Ark Clam (*Noetia ponderosa*) -	Hard Clam (*Mercenaria mercenaria*) -Values per 100-gram portion[a] ------
Calories	35	50	70
Calories from fat	N/A	N/A	9
Total fat (grams)	0.5 1		0.9
Saturated fat (grams)	N/A	N/A	0
Protein (grams) Cholesterol (milligrams)	7 35	11 55	16 40
Carbohydrates (grams)	0	1	0
Sodium (milligrams)	740	480	57
Percent of RDI[b]			
Cholesterol	12	18	13
Vitamin A	6	6	N/A
Vitamin C Calcium	2 6	4 10	N/A 4
Iron	70	50	20
Sodium [b]	31	20	2

[a] Values for hard clams were derived from 114-gram (4-ounce) portions (Florida Department of Agriculture) RDI = Recommended Daily Intake.
N/A = Not available