Final Report

Species Diversification in Florida Shellfish Aquaculture: Nursery and Growout Culture of the Sunray Venus Clam

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PROJECT SUMMARY:

The goal of this research project was to evaluate, demonstrate, and develop aquaculture of the sunray venus clam *Macrocallista nimbosa* as a new species to diversify the bivalve culture industry in Florida. In our 2006-08 Florida Sea Grant project, we hypothesized that sunray venus clam seed could be obtained from a hatchery and reared to a harvestable size by shellfish growers using culture methods similar to those employed by the hard clam *Mercenaria mercenaria* industry. In that project, we successfully spawned and cultured larvae through metamorphosis and early nursery phases using techniques similar to those for hard clam hatcheries. In this project, we hypothesized that nursery and growout of the sunray clam to a harvestable size may be accomplished using culture methods similar to those employed by the Florida hard clam industry. Therefore, the objectives of this project were to: 1) establish methods for nursery and growout of sunray venus clams, 3) determine salinity and temperature preference of nursery and grow-out sized sunray venus seed clams, 4) evaluate shelf life and purging for grit removal ("degritting") of live product, and 5) examine alternative markets for a shucked meat (raw) product.

INTRODUCTION:

In the 2005 Florida Sea Grant (FSG) biennial competition, our research and extension team was funded to examine hatchery methods for the production of sunray venus clam seed and market acceptance of the product. Under the 2006-2008 project, we successfully collected a total of 291 wild sunray venus clam broodstock on seven excursions from the west coast of Florida, which were transported to Harbor Branch Oceanographic Institution (HBOI) on the east coast with less than 10% mortality after the first week in tanks. A portion of these clams were conditioned and spawned by thermal shock to produce two separate groups that were cultured through metamorphosis and early juvenile stage following standard hard clam techniques (our hypothesis for the project). Using standard hatchery protocols for post-set hard clam culture, both spawns produced healthy juveniles (46-63% return from pediveliger number) of approximately 2 mm shell length after two to three months of culture (~30 ppt, 24-28°C).

Approximately 150,000 of these clams were then cultured in land-based systems by three different nursery operators (project partners) to produce seed for field planting (occurred on 30 May 2007). These clams were used for consumer acceptance studies in that project. Unfortunately, funding was not available in the first FSG grant for experimental (versus general qualitative) field-nursery and grow-out trials. Therefore, in order to fully develop, test, and demonstrate technical methods to culture the sunray venus clam *Macrocallista nimbosa*, this work aimed to develop and define culture techniques for nursery and growout phases and explore alternative marketing opportunities.

Background: Over the past two decades, Florida has seen a dramatic increase in aquacultured shellfish production. The hard clam *Mercenaria mercenaria* industry grew from \$0.4 million in farm gate sales in 1987 to a high of \$18.3 million in 2001 (NASS 2002). In addition to the increase in the number of farming operations, many ancillary businesses, such as hatcheries,

culture equipment manufacturers, and shellfish wholesalers, have developed over the same time span. The total economic impact of the hard clam industry in Florida was calculated in 1999 to be \$33.9 million in output and \$9.0 million in labor income (Philippakos et al. 2001). However, the industry is built on a single clam species and the value fell to approximately \$13.0 million in 2003 as dockside prices plummeted from 13ϕ to 9ϕ per clam during the 2002-2003 economic downturn (NASS 2004), which was not reflected in some other bivalve species, such as oysters (R. Rheault, Pres. East Coast Shellfish Growers Assoc., pers. comm.). Further, hurricanes in 2004-2005 reduced output to 92 million clams valued at \$9.8 million (NASS 2006).

Although clam farming has developed into a major industry in Florida, diversification from a single species product may help stabilize and expand the molluscan shellfish aquaculture industry. The rapid recruitment of fishermen into shellfish aquaculture, along with the exceptional growth rates associated with the productive, subtropical waters of Florida, has encouraged producers 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 that could be cultured and marketed along with hard clams are logical options. Small clam farmers have neither the time nor resources to develop new product lines or investigate the feasibility of developing new aquaculture species.

Many shellfish aquaculturists and seed suppliers in Florida became aware of the need to diversify their businesses by participating in the New Marine Mollusks for Aquaculture Workshop in November 1999. The workshop allowed farmers and audience members, along with a selected panel of researchers, biologists, marketers, economists, regulators and resource managers, to characterize the culture and market potential of alternative molluscan species. It was concluded that efforts should be undertaken to further determine the production feasibility and market demand of the angel wing, bay scallop, blood ark, and sunray venus clam (Sturmer 2000).

The angel wing has been studied (Gustafson et al. 1991), but its thin shell and need for sand substrate during metamorphosis has delayed its use in an industry built on hard clam methods. Bay scallops can be successfully cultured in Florida (Blake et al. 2000), but new regulations may be needed for raft culture, which requires water column usage in Florida waters. Research on the culture and market potential of blood ark and ponderous ark in Florida has recently been completed (Power et al. 2005, Sturmer et al. 2007). Although these ark clams can be grown in polyethylene bottom cages, the market study revealed that they are not familiar to most American consumers. The sunray venus clam, of the four species identified as candidates, is most similar to a hard clam and was previously the target of a commercial fishery in Florida.

The sunray venus clam *Macrocallista nimbosa* (Lightfoot 1786) is an attractive venerid clam distributed from South Carolina through Florida and the Gulf states (Abbott 1974). Interest for commercialization of this species was noted by Akin and Humm (1960) when they found unusually dense populations at Alligator Harbor, Florida. From 1967 to 1972, two million pounds of clams were landed near Bell Shoal, St. Joseph Bay with a value estimated at \$0.25 million (Godcharles and Jaap 1973). Several processing plants were established in the area where clam meats were shucked and further processed into strips, minced or chopped pieces, or packaged whole (Stokes et al. 1968). During the same time period, processors engaged in an

education program to communicate to consumers features of their high quality clam meats (NOAA no date). Attributes promoted included grit-free meat as a result of clams being harvested from sandy bottoms, as well as a sweeter and more tender-tasting product than most other clams. Because of the potential economic value of the sunray venus fishery and the relatively small size of the productive area, the Florida Department of Natural Resources conducted surveys in the early 1970s to locate new clam beds of possible commercial significance along Florida's northwest coast (Jolley 1972). Although beds were located between Panama City and Cedar Key, these areas yielded smaller catches than those at Bell Shoal. Total landings of clam meat (289,000 pounds) from 1986 to 1992 were valued at \$183,031, however, insufficient natural stocks of clams, as well as the small size of fishing grounds, limited the development of the fishery (Stokes et al. 1968, Ritchie 1977).

Growth experiments using marked individuals in the wild suggested that these clams attain a length of three inches (40 g whole) within 12 months (Stokes et al. 1968), similar in production time to hard clams in Florida (Sturmer et al. 1995, Fernandez et al. 1999). This rapid growth rate, along with documentation on the high quality of its meats, could result in these clams being sold in the lucrative raw or steamer clam markets. Observations made by Stokes et al. (1968) on handling and processing of the sunray venus clam indicated that once refrigerated, clams maintained good closure and could be kept for a week. Shelf life is an important attribute when considering the marketing and distribution of molluscan shellfish as live shellstock. Although market and pricing information was obtained through the fishery for processed clam meats, there is a dearth of information about the potential of sunray venus as a shellstock product. The Florida Department of Agriculture and Consumer Service (FDACS), Bureau of Seafood and Aquaculture Marketing staff conducted a mollusk survey at the 2000 International Boston Seafood Show (FDACS 2000). Samples of sunray venus clams and other marine mollusks were collected from Florida waters by University of Florida extension agents, Leslie Sturmer and LeRoy Creswell, and provided for inspection by seafood wholesale buyers. The appearance of the sunray venus clams received the most interest by buyers with the product form preference for whole shellstock. According to survey results, wholesale prices suggested ranged from \$0.20 to \$0.25 each and \$1.50 to \$2.50 per pound. The small convenience sample of potential buyers revealed considerable interest in this clam for domestic and export markets.

Limited information pertaining to the life history of sunray venus clams exists (e.g., Futch 1967, Cake 1970). Haines (1975, 1976) performed the most comprehensive study for culture. In summary, sunray venus clams were found to be ripe year around, but peak spawning occurred in the fall (Haines 1976). Haines (1975) found that spawning this clam in the laboratory was difficult and attempts at inducing spawning by temperature shock, gonadal extracts, mechanical and chemical stimulations were all unsuccessful. Viable larvae were produced by "strip" spawning the clams, but strip spawning was only successful with animals collected in October and November (i.e., during natural spawning). Larvae were cultured in 2-4 L flasks and not in larger vessels, which may indicate culture method problems, such as using *Nannochloris oculata* as a primary food source. Some larvae were cultured through metamorphosis when using *Monochrysis* sp. and *Isochrysis* sp. in addition to *Nannochloris* sp., but no quantitative feed study was performed. Larvae developed in typical bivalve fashion from 90-120 µm D-stage larvae at 24-48 hours to 160 µm pediveligers and then metamorphosed at 180-200 µm after 14 days. These successful cultures occurred at 25-31 ppt and 23-25°C. Preliminary salinity tolerance

studies on embryos and larvae indicated optimal salinities of 25-35 ppt at 24°C for both stages, which was similar to the originating waters of the broodstock.

The prior fishery, market, and potential growth rate of the sunray venus clam, along with being a native species, makes it a logical choice as a new molluscan candidate species (Mann 1984) to diversify and expand the modern Florida hard clam industry. In the 2005 Florida Sea Grant biennial competition, our team was funded to examine hatchery methods for the production of sunray venus clam seed and market acceptance of the product. We were able to collect wild sunray venus clam broodstock from several sites along the west coast of Florida (Alligator Harbor, Cedar Key, and Longboat Key) and transport them to HBOI on the east coast with a survival of 90%. We were able to induce spawning of these broodstock by thermal shock (in contrast to that reported by Haines 1975) and produce two separate groups of larvae in 500 L tanks that were cultured through metamorphosis (7-10 days, 4-7 days earlier than reported by Haines [1975], probably better feeding techniques) and early juvenile stage following standard hard clam techniques (our hypothesis). Using standard hatchery protocols for post-set hard clam culture, both spawns produced healthy juveniles (46-63% return from pediveliger number, ~30K and ~400K respectively) of approximately 2 mm shell length after two to three months at ~30 ppt, 24-28°C (Figure 2). Approximately 150,000 of these clams were then cultured in land-based systems by three different land-based nursery operators (project partners) to produce seed for field planting, which occurred on 30 May 2007 in Alligator Harbor. Clams from this planting were used for consumer acceptance studies in the 2005 funded project.

Unfortunately, funding was not available in the 2006-08 grant for detailed experimental fieldnursery and growout trials. Therefore, in order to fully, test, demonstrate, and develop technical methods to culture the sunray venus clam *Macrocallista nimbosa*, this work aimed to further define culture techniques for nursery and growout phases and explore alternative marketing opportunities. Overall, the work aimed to introduce a new molluscan species to Florida shellfish producers, which would provide a different revenue source to small-scale hard clam culture enterprises, thus improving or stabilizing farm income.

GOAL AND OBJECTIVES:

The goal was to evaluate, demonstrate, and develop aquaculture of the sunray venus clam *Macrocallista nimbosa* as a new species to diversify the bivalve culture industry in Florida. In our 2006-08 Florida Sea Grant project, we successfully spawned and cultured larvae through metamorphosis and hatchery phase using techniques similar to those for hard clam hatcheries. In this project, we hypothesized that nursery and growout of the sunray clam to a harvestable size may be accomplished using culture methods similar to those employed by the Florida hard clam industry.

Therefore, the objectives of this project were:

- 1) To establish methods for land-based nursery culture of clams,
- 2) To compare field culture methods for nursery and growout of clams,
- 3) To determine salinity and temperature preference of nursery and growout sized seed clams,
- 4) To evaluate shelf life and purging for grit removal ("degritting") of live product, and

5) To examine alternative markets for a shucked meat product.

METHODS AND RESULTS:

OBJECTIVE 1. Establish methods for land-based nursery culture of sunray venus clams.

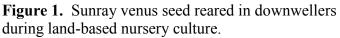
Land-based nursery trials were conducted at the UF Shellfish Aquaculture Research and Education Facility in Cedar Key. Four 200-gallon fiberglass tanks (4' wide, 8' long, and 10" deep) and four 140-gallon tanks (3.7' wide, 7.6' long, and 8" deep) were used in these trials. The larger tanks held up to 10 wellers each and the smaller tanks held 6 wellers each. The wellers were constructed from plastic bins, 19.5" by 13.5" in size or 1.8 square feet in bottom area. Fiberglass screening (900 micron mesh) was used for the bottom screen material (Figure 1). No substrate was added to the bins.



2008 Land-based Field Nursery Trial 1

In the first trial, unfiltered saltwater was distributed to each downweller within a tank via a spray bar, resulting in a flow rate of about 6 liters per minute per bin. Water temperature and salinity were measured daily using a maximum/minimum thermometer and refractometer, respectively. Seed were initially rinsed daily with freshwater to remove accumulated silt, sediments and feces, and to minimize clogging of the bottom screens. After 3-4 weeks, rinsing was reduced to every other day. After cleaning, the bins were rotated within the tank to minimize differences of bin location. In addition every two weeks, the bins were switched amongst the tanks. The seed were supplementary fed daily with a commercially available algal paste at a rate to achieve 50,000 cells/ml. These procedures were based on those previously used to nurse hard clam seed in this facility for other projects.





On July 10, 2008, about 63,000 sunray venus seed from three replicate families (single parent crosses) spawned on January 23, 2008 were shipped from the Experimental Molluscan Hatchery of HBOI. The seed were enclosed in nylon mesh bags and packed inside a foam shipping

container with ice packs and a Tidbit® temperature data logger. The seed were unpacked the following morning in Cedar Key, resulting in a shipment time of about 24 hours. Hard clam seed are often shipped via overnight courier from commercial hatcheries to other land-based nurseries for further culture. To determine if shipping had a negative effect on sunray venus seed, a burial trial was initiated immediately. One hundred seed (>3.0 mm sieve size) from female groups # 55 and 61 were placed into replicate small trays (12" by 6"); each tray filled 1" deep with a liter of beach sand. The trays were submerged in a fiberglass tank filled with saltwater and gently aerated. After 1, 2, 4, and 8 hours, the bins were inspected and assessed to determine whether the seed had completely buried, partially buried, or not buried. The project team felt that this test should give an indication of the health of the seed after shipping. Table 1 provides a summary of the results of the burying test for sunray venus seed in the first nursery trial. After 4 hours, 95% of the seed in both groups had buried. Data could not be retrieved from the TidBit logger, so air temperatures during shipping could not be determined. Although the gel packs had thawed after 24 hours, the seed bags were still cool to the touch during unpacking of the shipping box.

Table 1. Summary of burying test for sunray venus seed shipped overnight (10 July 2008) fromHBOI hatchery.

Group	Sieve Ave SL Size (mm)				Percentage (%) Buried After					
Group	(mm)	(mm) (<u>+</u> SD)	(mm) (<u>+</u> SD)	(mm) (<u>+</u> SD)	1 hr	2 hrs	4 hrs	8 hrs		
55	>3.0	6.5 (0.5)	2.4 (0.2)	4.6 (0.4)	64	87	95	96		
61	>3.0	6.8 (0.6)	2.5 (0.2)	4.8 (0.5)	76	84	95	95		

There were adequate numbers of >3.0 sieve seed from two female groups (55 and 61) to evaluate three stocking densities. Seed from female group 55 (average size: 6.5 mm SL, 2.4 mm SW, 4.6 mm SH) were stocked into triplicate bins at a density of 1,000 (low) and 2,000 (medium) per square foot of bottom area; whereas seed from female group 61 (average size: 6.8 mm SL, 2.5 mm SW, 4.8 mm SH) were stocked into triplicate bins at a density of 2,000 (medium) and 3,656 (high) per square foot. Each replicate bin was randomly placed into a different tank. Remaining seed (>5.0 mm sieve size) from the two female groups were stocked into two replicate bins each at densities of $2,560-2,737/\text{ft}^2$; whereas seed (>4.0 mm sieve size) from the two female groups were stocked into 2 replicate bins each at densities of $1.315-1.500/\text{ft}^2$. Within a week mortalities were observed in all bins with higher mortalities noted in bins stocked with smaller (>3.0 mm) seed. It is uncertain whether shipping stress or other stressors may have attributed to these mortalities. However, water temperatures were high, exceeding 90°F several afternoons and reaching 94°F on July 26. After 4 weeks it was decided to terminate the trial. Maximum and minimum temperatures during this period averaged $89 + 4.4^{\circ}F$ and $82 + 2.3^{\circ}F$, respectively. Salinities averaged 30.0 + 1.5 ppt during this period. Because of the amount of shell, seed were sieved on a 5.0 mm screen regardless of female group or initial stocking sieve size. Effect of stocking density could not be determined.

2008 Land-based Nursery Trial 2

A second land-based nursery trial was initiated on October 2, 2008. About 30,600 sunray venus seed that had been sieved on a 3.0 mm screen and another 125,000 seed sieved on a 2.0 mm from three replicate families (single parent crosses) spawned on May 8, 2008 were shipped from HBOI the previous day. The seed were packaged as in the previous seed shipment. A burial trial using the protocol described previously was initiated. One hundred seed from each female group (#1, 3 and 5) and each sieve size (>3.0 and >2.0 mm) were assessed after 1, 4, and 24 hours. Table 2 provides a summary of the results of the burying test for sunray venus seed in the second nursery trial. After 4 hours, 60% of the seed in the larger sieve group had buried; whereas, 47% of the seed in the smaller sieve group were buried. After 24 hours, 79% of the seed in the larger sieve group had buried; whereas, 83% of the seed in the smaller sieve group were buried. There were differences in burying response among the families. Air temperatures in the shipping box which contained the larger seed averaged $67.4 \pm 5.1^{\circ}$ F during transport time; whereas, air temperatures in the shipping box which contained the smaller seed averaged $50.0 \pm 2.6^{\circ}$ F during transport time.

Female	Sieve	Ave SL	Ave SW	Ave SH	Percenta	age (%) Bi	uried After
Group	Size (mm)	(mm) (<u>+</u> SD)	(mm) (<u>+</u> SD)	(mm) (<u>+</u> SD)	1 hr	4 hrs	24 hrs
1	>3.0	6.1 (0.7)	2.3 (0.2)	4.3 (0.4)	66	89	93
3	>3.0	6.1 (0.7)	2.3 (0.2)	4.2 (0.5)	40	39	83
5	>3.0	5.9 (0.6)	2.2 (0.2)	4.1 (0.5)	38	54	62
1	>2.0	4.5 (0.4)	1.8 (0.2)	3.2 (0.3)	50	67	93
3	>2.0	4.4 (0.5)	1.7 (0.2)	3.1 (0.3)	58	41	73
5	>2.0	4.6 (0.5)	1.8 (0.2)	3.2 (0.4)	41	32	83

Table 2. Summary of burying test for sunray venus seed shipped overnight (2 October 2008)

 from HBOI hatchery.

There were adequate numbers of seed from three female groups (1, 3, and 5) to evaluate three stocking densities. The larger seed (>3.0 mm sieve) were stocked at 3,000/ft² in two downwellers per female group. The smaller seed (>2.0 mm sieve) were stocked at 2,000 and 4,000/ft² in four downwellers per density and per female group. Each replicate downweller, or bin, was randomly placed into a different tank. Flow rates, cleaning, feeding rates and other procedures were the same as those described in the first nursery trial. After two weeks, the feeding rate of the supplemental algal paste was increased to 75,000cells/ml/day.

After three weeks, the larger seed (>3.0 mm) were sieved on a 4.0 mm screen. The percentage of seed that sieved up in female groups 1, 3, and 5 averaged 11.7, 14.8, and 14.9%, respectively. The >4.0 mm sieved seed were placed into separate wellers per female group. After five weeks, the smaller seed (>2.0 mm) were sieved on a 3.3 mm screen. The percentage of seed that sieved

up in female groups 1, 3, and 5 stocked at 2,000/ft² averaged 13.5, 9.1, and 16.7%, respectively; whereas, female groups 1, 3, and 5 stocked at 4,000/ft² averaged 10.0, 5.3, and 15.2%. The >3.3 mm sieve seed were placed into separate wellers per female group. Over this 5-week period, daily maximum and minimum water temperatures averaged $73.5 \pm 6.4^{\circ}$ F and $68.6 \pm 6.1^{\circ}$ F, respectively. Salinities averaged 29.6 ± 0.9 ppt. These hydrological conditions should have supported good growth. However, sieve results indicated slow growth. In addition, we observed a small amount of shell in the wellers.

At the initiation of this nursery trial, there was excess seed (~6,000) remaining after stocking the density study at the UF facility. These seed were placed in a raceway at a commercial facility and stocked into two shallow trays (40" by 20", or 5.5 ft²) at about $1,100/ft^2$. Further, unfiltered saltwater was distributed to these trays by laminar flow from the head of the raceway tank. After five weeks, over 80% of these seed were retained on 3.3 mm screen. Further, seed in these trays were observed to continuously have their siphons out, while this was not so for the sunray venus in the downwellers at the UF facility. The agitation of the water delivery in the downwellers may have hindered filtration and feeding.

Based on these observations, the project team decided to modify the stocking density study ongoing at the UF facility. One downweller from each female group was maintained at each of the original stocking densities of 2,000 and 4,000/ft². The remaining seed (<3.3 mm) were restocked at $1,000/\text{ft}^2$ into both downwellers and trays, the same size as those used in the commercial facility (Figure 2). Further, a distributor bar was submerged at the head of each tank, and provided 60 liters of water per minute per tank. After an additional 6 weeks, the trial was terminated. During this time period, daily maximum and minimum water temperatures averaged 67.7 + 5.1 and $57.5 + 5.6^{\circ}$ F, respectively. Salinities averaged 29.7 + 1.3 ppt. Seed from each female group (1, 3, and 5), each original size group, each stocking density (1,000, 2,000, 3,000, and $4,000/\text{ft}^2$), and each nursery system (weller versus tray) were separately sieved on 3.3 and 4.0 mm screens during December 16-18, 2008. Volumes for each sieve size (>4.0, >3.3, and <3.3) mm) were determined and a subsample volume of 5-20 milliliters was counted to estimate numbers and survival. Due to the low number of replications per variable it was difficult to assess differences due to original size group, density, or system. Rather data were summarized by female group with survivals ranging from 66.9 to 69.4% over the 11-week nursery period. Increase in volume over this period per female group 1, 3, and 5 was 145, 130, and 120%, respectively. The lower temperatures experienced over the last six weeks of the nursery trial may have limited growth during this period. After sieving and counting, the seed were combined by female group and by sieve size to initiate a field nursery trial (see Objective 2).

2009 Land-based Nursery Trial 1

In our 2008 land-based nursery trials, we observed slow growth (5-15% were retained on a 3.3 mm sieve after 5 weeks) and poor survival (66-69%) of sunray venus seed nursed in downwellers at the UF Shellfish Aquaculture Research and Education Facility in Cedar Key. Stocking densities evaluated varied from 2,000 to 4,000/ft², based on area of the weller screen. Conversely, over 80% of the seed from the same cohort nursed at a commercial facility were retained on a 3.3 mm sieve after 5 weeks. In the latter system, seed were nursed in shallow trays at a density of 1,000/ft² with saltwater distributed to the trays via laminar flow from one end of



Figure 2. Sunray venus seed nursed in trays in a 200-gal tank.

the tank. Seed in trays were observed to continuously have their siphons out, while this was not so for the seed nursed in the downwellers. The agitation caused by the water delivery to the downwellers may have hindered filtration and subsequently feeding.

In September 2009, we conducted another land-based nursery trial to determine the influence of system design and stocking density on growth and survival of sunray venus seed. Four 140-gallon tanks (3.6' wide, 7.5' long, and 8" deep) and three 200-gallon fiberglass tanks (4' wide, 8' long, and 10" deep) were used. The bottom of the larger tank was molded to support four trays off the bottom. Each tray was constructed from 1" plastic coated wire with fiberglass screening (900 micron mesh) attached (Figure 2). The dimensions of the trays were 1.8' wide, 3.3' long, and 2" deep. The bottom area of the 140-gallon tank and tray was 27 ft² and 5.9 ft², respectively.

On September 3, about 380,000 sunray venus seed spawned on July 9 were shipped from the

Experimental Molluscan Hatchery of HBOI-FAU. The seed were enclosed in nylon mesh bags and packed inside two foam shipping containers, each with two gel packs and a Tidbit® temperature data logger. The seed were unpacked the following afternoon in Cedar Key, resulting in a shipment time of approximately 24 hours. Hard clam seed are often shipped via overnight courier from commercial hatcheries to other land-based nurseries for further culture. To determine if shipping had a negative effect on sunray venus seed, a burying test was initiated immediately. One hundred seed (>1.2 mm sieve size) from each shipment box were placed into two replicate small trays (12" by 6"); each tray filled 1" deep with a liter of beach sand. The travs were submerged in a fiberglass tank filled with saltwater and gently aerated. After 2, 18, and 24 hours, the bins were inspected and assessed to determine whether the seed had completely buried, partially buried, or not buried. The project team felt that this test should give an indication of the health of the seed after shipping. Table 3 provides a summary of the results of the burying test for sunray venus seed. After 24 hours, 84-94% of the seed from both boxes had buried. Temperatures in the shipping boxes averaged 43.2+5.0°F in box 1 and 53.1+4.8°F in box 2. Although the gel packs had partially thawed after 24 hours, the seed bags were still cool to the touch during unpacking of the shipping box.

Box	Shipping	Ave Shipping	Percent	tage (%) Buried	d After:
	Time (hrs)	Temperature, °F (<u>+</u> SD)	2 hr	18 hrs	24 hrs
1	22.5	43.2 (5.0)	4.5	55.5	84.0
2	22.5	53.1 (4.8)	22.5	72.0	94.5

Table 3. Summary of burying test for sunray venus seed shipped overnight from HBOI hatchery.

There were adequate numbers of >1.2 mm sieve seed (average number per milliliter, 294/ml; average shell length, 3.7 mm) to evaluate two stocking densities in the 27 ft² tanks and four stocking densities in the 5.9 ft² trays. Seed were stocked into two replicate tanks at $1000/\text{ft}^2$ (27,000/tank, 92 ml/tank) and 2000/ft² (54,000/tank; 184 ml/tank). In addition, seed were stocked into triplicate trays at a density of 1,000/ft² (5,890/tray, 20ml/tray), 1,500/ft² (8,835/tray, 30ml/tray), 2,000/ft² (11,780/tray, 30ml/tray), and 2,500/ft² (14,725/tray, 50ml/tray). One tray of each stocking density was placed inside a 200-gallon tank. Unfiltered saltwater was distributed to each tank by laminar flow via a distributor pipe at one end of the tank, resulting in a flow rate of 50-65 liters per minute. Seed in the tanks and travs were rinsed every 1-2 days with freshwater to remove accumulated silt, sediment, and feces. This was easily accomplished in the tanks with travs by pulling the standpipe and allowing the water to drain prior to rinsing the seed. After cleaning, the trays were rotated within the tank to minimize differences of tray location. In addition, every two weeks, the trays were switched amongst the tanks. In the tanks without trays, seed were rinsed out of the tank with the sediments, collected in a small mesh sieve, and then restocked into the cleaned tank. Water temperature was recorded hourly with a Tidbit® temperature data logger; salinity was measured daily using a refractometer.

After 53 days, the stocking density trial was terminated. Typically, it takes about 8 weeks for hard clam seed (>1.2 mm sieve) to reach a size in which the majority can be retained on a 3.3 mm mesh sieve (the smallest sieve size used in grading seed for the field nursery). During this time period, water temperatures and salinities averaged $81.4\pm7.0^{\circ}$ F and 27.2 ± 1.3 ppt, respectively. Seed from each stocking density and each nursery system (tank versus tray) were separately sieved on 3.3 and 4.0 mm screens during October 26-28. Volumes for each sieve size (>4.0, >3.3, and <3.3 mm) were determined and two subsamples (5-50 milliliters) were counted to estimate numbers and survival. Prior to sieving, 30 animals from each group were measured for shell length (SL). Results from this nursery trial are presented in Table 4.

System	Stocking Density (# / ft ²)	# Reps	Ave SL mm (±SD)	Ave Survival % (±SD)	Ave >4.0 mm Sieve % (±SD)	Ave >3.3 mm Sieve % (±SD)
Tank	1000	2	7.4 (0.4)	84.6 (1.2)	53.2 (1.7)	26.3 (2.7)
I allk	2000	2	7.3 (0.1)	89.0 (2.0)	46.9 (6.3)	25.2 (1.8)
	1000	3	8.9 (0.6)	88.9 (1.6)	79.1 (7.6)	14.1 (6.4)
Trov	1500	3	8.4 (0.2)	91.0 (6.7)	75.0 (9.1)	15.4 (5.2)
Tray	2000	3	8.3 (0.7)	87.8 (2.7)	74.7 (4.1)	15.3 (3.4)
	2500	3	8.1 (0.5)	88.3 (2.7)	69.9 (5.6)	17.5 (3.2)

Table 4. Summary of harvest data for sunray venus land-based nursery trial, Cedar Key.

The harvest data were statistically analyzed with SAS version 9.2 software. Percentage data were arc-sine-square-root transformed prior to analysis. Treatment means of all dependent variables

were subjected to either a t-test or a one-way analysis of variance according to the General Linear Model procedures of SAS. A Tukey's studentized range test was used to compare treatment means when the ANOVA was significant. All statistical tests were considered significant when $p \le 0.05$.

Within the tank nursery system, no significant differences were detected between the 1,000 and 2,000/ft² stocking densities in SL (p=0.87), survival (p=0.12), >4.0 mm seed (p=0.30), or >3.3 mm seed (p=0.68). Furthermore, in the tray system, no significant differences were detected between the 1,000, 1,500, 2,000, and 2,500/ft² stocking densities in SL (p=0.34), survival (p=0.66), >4.0 mm seed (p=0.47), or >3.3 mm seed (p=0.82). However, when comparing nursery rearing systems stocked at identical densities, significant differences were detected. At 1,000/ft², the sunray venus nursed in the tray system had significantly greater SL (p=0.05) and >4.0 mm seed (p=0.026). Similarly, at 2,000/ft², the sunray venus nursed in the tray system had significantly less >3.3 mm seed (p=0.04).

These results are commercially acceptable as survival over the 7.5-week nursery period ranged from 85-91% and seed greater than a 3.3 mm sieve size at harvest ranged from 72-93% (Figure 3). Using a laminar flow of water distributed from one end of the tank, as opposed to delivering water via a spray bar extending the entire length of the tank, resulted in less agitation and disturbance to the sunray venus seed. Although the amount of seed greater than a 3.3 mm sieve size ranged from 93% in trays stocked at 1,000/ft² to 87% in trays stocked at 2,500/ft², these values were not significantly different. Given the system modifications, it may be that densities typically used in



Figure 3. Sunray venus seed after 7.5 weeks in nursery culture trays.

nursing hard clam seed (i.e., 3000-3,500/ft²) could be used for sunray venus. The significant differences in sunray venus growth observed in the tray versus tank systems may have reflected the differences in handling the seed during rinsing. Further, seed in trays were routinely rotated to minimize affects associated with distance from the water outflow.

2009 Land-based Nursery Trial 2

An additional 375,000 seed from the July spawn was shipped to Cedar Key from HBOI-FAU on October 7, 2009. Seed had been sieved on a 1.6 mm sieve and were estimated at 206/ml. The seed were stocked at a commercial nursery facility and reared in trays similar to those previously described. Initial stocking densities were high (3,500-4,000/ft²). These seed were handled in a manner similar to that used by commercial nursery operators. Seed were sieved on 4.0 and 3.0 mm screens at regular intervals (November 10-11, November 30-December 3, and December 17). The larger seed (>4.0 and >3.3 mm) were then field planted, and the remaining seed (<3.3 mm) were restocked at lower densities in the trays. Further, as the volume of seed increased, a portion was transferred to the UF facility so that final densities were maintained at approximately 2,500/ft². The seed were supplementary fed daily with a commercially available algal paste at a density of 50,000-75,000 cells/ml. After 10 weeks (December 17), the trial was terminated. At that time, the seed were sieved on 4.0, 3.3, 3.0 and 2.3 mm screens. During this period, water

temperatures and salinities averaged $69.8\pm8.9^{\circ}$ F and 27.1 ± 1.5 ppt, respectively. The total number of seed sieved on multiple occasions is as follows: >4.0 mm - 45,480 (14% of live seed); >3.3mm - 162,772 (50%); >3.0 mm - 41,192 (12%); and >2.3 mm - 74,800 (23%). The overall survival was estimated at 86.5%. Growth was slower than that observed in the previous trial, which was most likely due to the lower water temperatures.

OBJECTIVE 2. Compare field culture methods for nursery and growout of sunray venus clams.

2008 Field Nursery Trial

Over 115,000 sunray venus seed, previously land-based nursed at the UF Shellfish Aquaculture Research and Aquaculture Facility in Cedar Key, were planted on a UF research lease located within a commercial shellfish aquaculture high-density lease area (known as Dog Island) in the Gulf of Mexico, east of Cedar Key in Levy County. Table 5 summarizes planting data for two field nursery trials (August 8 and December 18, 2008). Three replicate families (single parent crosses) were evaluated in both trials. Sunray venus seed were stocked into polyester mesh (3-4 mm) bottom bags and covered with a layer of polyethylene netting at similar densities (311-326 per square foot, first trial; 325-403 per square foot, second trial). These densities are about 45-65% of those typically used in hard clam field nurseries (625/ft²). Differences in these trials were the size of seed planted, as three sizes (3.3, 4.0, and 5.0 mm) were used to sort/grade seed at completion of the land-based nursery stages.

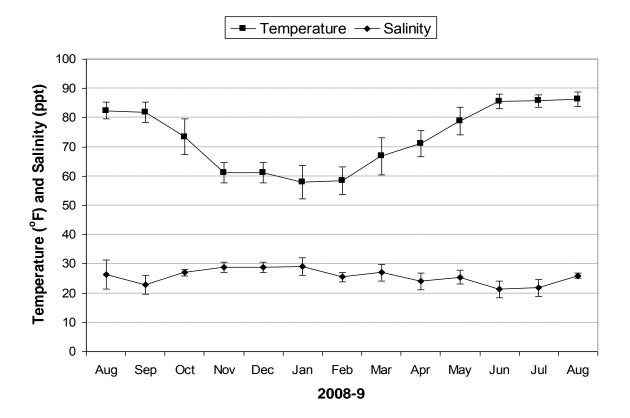
Plant Date	Female Rep	Sieve Size (mm)	Ave # per ml	# Bags	# per Unit	Density (# per sq.ft.)	Ave SL (mm) (<u>+</u> SD)	Ave SW (mm) (<u>+</u> SD)	Ave SH (mm) (<u>+</u> SD)
	55	>5.0	7.1	2	4977	311	10.3 (1.8)	3.6 (0.6)	7.1 (1.1)
08.08.08	61	>5.0	6.8	3	5700	356	10.3 (2.0)	3.6 (0.6)	7.0 (1.2)
	85	>5.0	5.2	2	5220	326	10.3 (1.7)	3.5 (0.5)	7.0 (1.0)
	1	>4.0	8.9	3	5983	374	7.8 (0.6)	2.9 (0.5)	5.5 (0.5)
	1	>3.3	17.3	2	6055	378	6.3 (0.3)	2.3 (0.1)	4.5 (0.3)
10 10 00	3	>4.0	10.9	2	5213	374	7.6 (1.0)	2.7 (0.3)	5.4 (0.6)
12.18.08	3	>3.3	14.4	2	6457	404	6.5 (0.6)	2.4 (0.2)	4.6 (0.4)
	5	>4.0	10.7	3	5362	335	8.0 (1.2)	2.8 (0.3)	5.5 (0.6)
	5	>3.3	17.5	2	5338	333	6.5 (0.3)	2.3 (0.1)	4.6 (0.2)

Table 5.	Summary	of planting	data for sunra	y venus field nurser	v trials Cedar Kev
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Water quality conditions at the Dog Island High-density Lease Area (HDLA) during field nursery trials (August 2008 - August 2009) were monitored using a YSI 6600 data sonde, which

was deployed at a piling less than 100' from the site where sunray venus were reared. Measurements, every 30 minutes, were recorded at six inches above the bottom. Monthly averages and standard deviations for water temperature and salinity values during the field nursery trials are shown in Figure 4. Water temperatures ranged from a low of $57.9\pm5.7^{\circ}$ F in January 2009 to a high of $86.3\pm2.5^{\circ}$ F in August 2009. Salinities showed less of an annual pattern ranging from 21.3 ± 2.8 ppt in June 2009 to 29.1 ± 3.0 ppt in January 2009.

Figure 4. Monthly averages of water temperature (°F) and salinity (ppt) values measured at the Dog Island High-density Lease Area near Cedar Key between August 2008 and August 2009. Error bars reflect the standard deviation from the monthly averages.



The seven nursery bags planted on August 8, 2008 were retrieved from the field on April 2, 2009. The bags were buried. Further, there was no evidence of predator holes on the recovered bags. However, very few sunray venus were alive (average 28/bag, Female 55; average 25/bag, Female 61; average 3/bag, Female 85). Although the shells were of various sizes, the majority were small, indicating that mortalities occurred very early in the nursery period. In reviewing the water quality conditions during this nursery period, we noted that heavy rains and coastal flooding associated with Tropical Storm Faye occurred during August 2008. Salinities dropped from 33 ppt at planting to 17 ppt on August 25. It may be environmental stressors associated with a rapid decrease in salinity coupled with high water temperatures (86°F) resulted in seed mortalities. Further, it must be noted that high mortalities were observed in these groups during the land-based nursery phase. It is interesting to note that hard clam seed planted by growers on other leases within the Dog Island HDLA were not affected by the drop in salinity in August

2008, suggesting that sunray venus seed may be more vulnerable than hard clams to lower salinities.

The 14 nursery bags planted on December 18, 2008 at the Dog Island site were retrieved on July 27, 2009. In this trial, we evaluated three female groups (#1, 3, 5) and two seed sizes (>4.0 mm sieved seed versus >3.3 mm sieved seed). Again, high mortalities were noted with the majority of shell the size when planted. There were 100% mortalities noted in bags stocked with Females 1, 3 & 5 at the >3.3 mm sieve size. At the >4.0 mm sieve size, average survival for the three female groups was $17.4\pm2.4\%$ (Female 1), 0% (Female 3), and $2.1\pm0.02\%$ (Female 5). Average size of the two female groups (Females 1, 5) was 24.4 ± 3.1 mm SL, 8.6 ± 0.9 mm shell width (SW), and 14.6 ± 1.6 mm shell height (SH). These seed were planted at water temperatures of 62-65°F and salinities of 30 ppt, which should not have been problematic. Again, it must be noted that mortalities and slow growth were observed in these groups during the land-based nursery phase.

2009 Field Nursery Trial

Due to the limited number of field nursery seed available to initiate growout trials, we received seed from HBOI-FAU on May 28, 2009, which had been spawned on May 8, 2008. Approximately 35,000 seed from three replicate female groups (#1, 3, 5) at >6.9 mm sieve size were planted in nursery bags at 8,092-9,180/bag, or at a rate of 505-575/ft² (see Table 6 for seed sizes at planting). After 11 weeks, the four nursery bags were harvested on August 13 (Table 6). Survival over the nursery period ranged from 54 (Female 3) to 81% (Female 5). Growth was similar among the three groups, ranging from 0.9 to 1.0 mm per week. The seed were sieved on a 9.0 mm screen and used in growout trials.

			g Data	0	Harvesting Data						
Female Group	# Bags	Ave SL (mm) (<u>+</u> SD)	Ave SW (mm) (<u>+</u> SD)	Ave SH (mm) (<u>+</u> SD)	Ave SL (mm) (<u>+</u> SD)	Ave SW (mm) (<u>+</u> SD)	Ave SH (mm) (<u>+</u> SD)	Ave Survival (%)	Ave Growth (mm, SL) per week		
1	1	13.7 (1.2)	4.7 (0.4)	9.0 (0.8)	20.1 (3.2)	7.1 (1.1)	12.2 (1.7)	63.6	0.91		
2	2	13.7 (1.4)	4.6 (0.5)	8.8 (0.9)	20.2 (3.1)	7.0 (1.2)	12.1 (1.5)	53.6	0.91		
3	1	11.8 (1.5)	4.2 (0.5)	7.8 (0.8)	18.6 (3.2)	6.4 (1.1)	11.3 (1.7)	81.2	0.97		

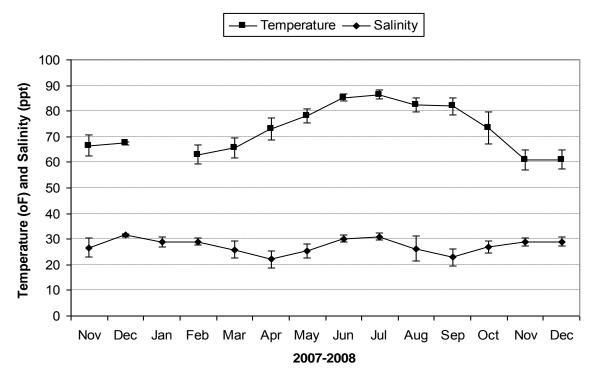
Table 6. Summary of	planting and ha	rvesting data for su	inray venus field nurser	v trial.
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2007-8 Growout Trials- Cedar Key

Sunray venus growout trials were conducted at the UF research lease off of Cedar Key as described in the field nursery trials above. The site is basically a submerged sand spit with the soil substrate characterized by a mixture of sand with some mud. Water quality conditions at the Dog Island High-density Lease Area during November 2007 through December 2008 were

monitored using a YSI 6600 data sonde, which was deployed at a piling less than 100' from the site where the sunray venus were reared. Measurements, every 30 minutes, were recorded at six inches above the bottom. Monthly averages and standard deviations for water temperature and salinity values are shown in Figure 5. Water temperatures ranged from a low of 61.0°F in November 2008 to a high of 86.5°F in July 2008. Salinities showed less of an annual pattern ranging from 22.1 ppt in April 2008 to 31.5 ppt in December 2007

Figure 5. Monthly averages of water temperature (°F) and salinity (ppt) values taken at the Dog Island High-density Lease Area near Cedar Key between November 2007 and December 2008. Error bars reflect the standard deviation from the monthly averages.



Stocking Density Evaluation About 24,000 sunray venus juveniles (23.8 mm SL, 8.5 mm SW, 14.3 mm SH), previously field nursed in bottom bags, were used in a growout trial evaluating stocking densities (Table 7). Three densities (38/ft², low; 50/ft², medium; and, high, 63/ft²) were tested in 10 replicate bottom bags. In comparison, stocking densities typically used by hard clam growers in Florida range from 50-75 per square foot. The bottom bags were constructed of flexible polyester mesh (9 mm) and were 4 feet by 4 feet in dimension, or 16 square feet. The bags were secured and attached to the substrate by wire or PVC pipe stakes and covered with a single layer of polyethylene netting for additional predator protection. In this trial, sunray venus were sampled every four months. Three replicate bags were pulled each sample period. Although these bags were replanted to produce product for market evaluations, they were not re-sampled because the disturbance of pulling and replacing could possibly have a negative effect on production characteristics. At sampling, a minimum of 50 clams per replicate were measured for growth (shell length, SL; shell width, SW; shell height, SH; and, whole-weight) and survival. In addition, a minimum of 15 clams were shipped to HBOI to measure meat weight and condition index, which was calculated using the ratio of dry meat: dry shell x 100. Presence of predators and fouling organisms were noted during field sampling.

Plant Date	Reps	# per Unit	Density (# per sq.ft.)	Total # Planted	Ave SL (mm) (<u>+</u> SD)	Ave SW (mm) (<u>+</u> SD)	Ave SH (mm) (<u>+</u> SD)
	10	600	38	6000	23.8 (2.7)	8.5 (1.0)	14.3 (1.6)
11.27.07	10	800	50	8000	23.8 (2.7)	8.5 (1.0)	14.3 (1.6)
	10	1000	63	10000	23.8 (2.7)	8.5 (1.0)	14.3 (1.6)

 Table 7. Summary of planting data for sunray venus growout density trials, Cedar Key.

The growout trial was terminated after 12 months, which is within the culture period (10-18 months) required for hard clams to reach a marketable size of ~50 mm SL, 25 mm SW (i.e., littleneck size) (Table 8). Statistical analysis of growth and survival data from the two sample periods (March 25, 2008 [4 months]; July 23, 2008 [8 months]) and completion of the trial (December 3, 2008 [12 months]) was conducted using a one-way analysis of variance (ANOVA). A Tukey's honestly significant difference test was used to compare treatment means when the analysis was significant (p>0.05).

		2			5	0		2	,		
Sample/ Harvest Date	Reps	# per Unit	# Days	Ave. Survival (%) (<u>+</u> SD)	Ave SL (mm) (<u>+</u> SD)	Ave SW (mm) (<u>+</u> SD)	Ave SH (mm) (<u>+</u> SD)	Ave TWt (g) (<u>+</u> SD)	Ave Dry Mt Wt (g) (<u>+</u> SD)	Ave CI (<u>+</u> SD)	Ave Growth (mm,SL) / month
	3	600	119	87.6 (9.1)	28.8 (1.61)	10.9 (0.51)	17.6 (0.68)	3.75 (0.45)	0.21 (0.03)	10.6 (0.21)	1.28
03.25.08	3	800	119	83.7 (2.6)	27.9 (0.84)	10.6 (0.29)	17.1 (0.36)	3.51 (0.24)	0.21 (0.02)	10.4 (0.29)	1.05
	3	100	119	92.1 (3.5)	28.4 (1.31)	10.7 (0.49)	17.2 (0.59)	3.55 (0.40)	0.21 (0.02)	10.3 (0.14)	1.18
	3	600	239	78.6 (3.6)	45.4 (0.69)	18.0 (0.30)	26.5 (0.20)	13.6 (0.44)	0.80 (0.04)	11.0 (0.58)	2.75
07.23.08	3	800	239	83.3 (8.8)	45.6 (0.43)	17.9 (0.33)	26.3 (0.10)	13.6 (0.41)	0.83 (0.05)	10.8 (0.11)	2.77
	3	1000	239	83.6 (3.6)	42.5 (1.83)	17.4 (0.57)	25.0 (0.68)	11.6 (0.98)	0.70 (0.07)	10.6 (0.21)	2.38
	3	600	372	73.1 (16.4)	54.4 (5.47)	22.0 (0.97)	30.4 (2.88)	24.1 (4.93)	1.35 (0.27)	11.3 (0.71)	2.50
12.03.08	3	800	372	67.2 (22.2)	55.3 (4.54)	22.1 (1.06)	31.1 (2.44)	24.9 (4.54)	1.43 (0.25)	11.1 (0.55)	2.56
	3	1000	372	74.5 (14.2)	50.3 (5.21)	21.0 (2.08)	25.4 (2.68)	19.9 (4.71)	1.14 (0.14)	10.8 (0.62)	2.16

Table 8. Summary of harvest data for sunray venus growout density trials, Cedar Key.

The only statistical differences associated with stocking densities were observed at eight months as the high density treatment ($63/ft^2$) had lower growth than the low ($38/ft^2$) and medium ($50/ft^2$) stocking densities for shell length (p=0.028), shell height (p=0.008), and total weight (p=0.017). Survival after 12 months ranged from 67.2% (medium treatment) to 74.5% (high treatment), which would be considered commercially acceptable for hard clam culture. Evidence of predation was noted as several of the replicate bags in the medium treatment had holes. Also the plastic cover netting had been dislodged from several of the bags. Fouling on the bags was minimal. Growth of sunray venus in the low ($38/ft^2$) and medium ($50/ft^2$) treatments was similar and averaged 54-55 mm in SL, 22 mm in SW, 30-31 in SH, 24 g in whole weight, 1.4 g in dry meat weight, 11.1-11.3 in condition index, and 2.5 mm (SL) in monthly growth. A density effect was evident as sunray venus clams harvested from the high treatment ($63/ft^2$) were smaller and averaged 50 mm in SL, 21 mm in SW, 25 mm in SH, 20 g in whole weight, 1.1 g in dry meat weight, 10.8 in condition index, and 2.2 mm (SL) in monthly growth. Growth rates were comparable to those documented for hard clams.

Culture Gear Evaluation About 25,000 sunray venus juveniles (26 mm SL, 9 mm SW, 15 mm SH), previously field nursed in either cages or bottom bags, were used in two growout trials evaluating several culture systems (Table 9). In both trials, bottom bags were utilized and compared with bags modified with internal frames constructed of 1" and 1.5" PVC pipe. This bag modification is generally not used by the hard clam industry, but was evaluated for culturing the angel wing clam in Florida waters. The height of the frame allowed for sediment to accumulate within the bag while also allowing space for angel wings to grow. The project team felt this may also offer an advantage for rearing sunray venus. The bags with no frames were covered with an additional single layer of polyethylene netting. For bags with frames, the netting was attached to the frames. Stocking densities of 44 and 53/ft² were used in the respective gear trials. Also, in the latter trial seed were planted directly on the bottom and covered by a polyester mesh netting (4'x8' or 32 ft²) edged with lead line. Bottom plants, typically used in other Atlantic coastal states culturing hard clams consist of a single layer of protective cover netting. This culture system allows the clams to burrow directly into the substrate and requires hand or rake harvesting. The stocking density (53/ft²) used was the same as that for the bags.

Due to the limited number of replications in these trials, bags were not sampled periodically. Growth and survival of sunray venus clams using the various culture gear were determined after 12 months (Table 10). Statistical analysis of the November 21, 2007 plant data (n=3) was conducted using the same methods as was used for the stocking density study. There were no statistical differences observed. Survival ranged from 64.7% (1"frame) to 76.3% (no frame) in the first trial and from 78.2% (1.5" frame) to 82% (no frame and 1" frame) in the second trial. One bottom net was harvested and survival was 53.0%. With survival rates commercially acceptable in all gear treatments, there seemed to be no advantage of using bottom plants or frames over the bag method (Figure 6). However, growth data indicated there may be an advantage as larger clams were harvested from bags with frames (1.61-1.72 g dry meat weight, first trial; 1.5-1.59 g dry meat weight, second trial) versus bags with no frames (1.61 g dry meat weight, first trial; 1.17 g dry meat weight, second trial). There was little difference between the frame heights. Sunray venus clams harvested from the bottom plant averaged 2.11 g in dry meat weight. Growth rates of sunray venus in bags with frames (2.5-2.6 mm SL/month) were comparable to those obtained in the low and medium stocking density trials (2.5-2.6 mm SL/month). The highest growth rate was obtained in the bottom plant (3.1 mm SL/month). The

remaining gear replicates of the December 13, 2007 plant were harvested intermittently as product was required for other studies; thus, additional harvest data is not available.

	· · · · ·	1		-	-			
Plant Date	Gear	Reps	# per Unit	Density (# per sq.ft.)	Total # Planted	Ave SL (mm) (<u>+</u> SD)	Ave SW (mm) (<u>+</u> SD)	Ave SH (mm) (<u>+</u> SD)
	Bags - No frame	3	702	44	2106	26.4 (2.4)	9.3 (1.0)	15.9 (1.4)
11.21.07	Bags - 1" frame	3	702	44	2106	26.4 (2.4)	9.3 (1.0)	15.9 (1.4)
	Bags - 1½" frame	3	702	44	2106	26.4 (2.4)	9.3 (1.0)	15.9 (1.4)
	Bags - No frame	6	844	53	5064	26.1 (3.0)	9.3 (1.1)	15.5 (1.8)
10 12 07	Bags - 1" frame	6	844	53	5064	26.1 (3.0)	9.3 (1.1)	15.5 (1.8)
12.13.07	Bags - 1½" frame	6	844	53	5064	26.1 (3.0)	9.3 (1.1)	15.5 (1.8)
	Bottom Plant	2	1688	53	3376	26.1 (3.0)	9.3 (1.1)	15.5 (1.8)

Table 9. Summary of planting data for sunray venus growout equipment trials, Cedar Key.

Figure 6. Sunray venus clams harvested from a polyester mesh bottom bag (left) and a bag with an internal PVC frame (right).





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Harvest Date	Gear	Reps	# Days	Ave Survival (%) (<u>+</u> SD)	Ave SL (mm) (<u>+</u> SD)	Ave SW (mm) (<u>+</u> SD)	Ave SH (mm) (<u>+</u> SD)	Ave TWt (g) (<u>+</u> SD)	Ave Dry Mt Wt (g) (<u>+</u> SD)	Ave CI (<u>+</u> SD)	Ave Growth (mm,SL) / month
	Bags - No frame	3	377	76.3 (9.1)	56.1 (1.88)	22.7 (0.44)	31.5 (1.10)	26.9 (2.12)	1.61 (0.30)	11.2 (0.52)	2.40
12.02.08	Bags - 1" frame	3	377	64.7 (8.3)	58.2 (2.49)	22.3 (0.13)	33.5 (1.50)	29.3 (1.76)	1.72 (0.13)	11.0 (0.46)	2.56
	Bags - 1.5" frame	3	377	75.1 (7.4)	58.7 (0.73)	22.1 (0.13)	33.7 (0.39)	29.2 (0.74)	1.61 (0.05)	10.4 (0.23)	2.60
	Bags - No frame	2	363	82.4 (3.1)	48.9 (4.04)	20.4 (1.15)	27.6 (2.05)	18.9 (3.62)	1.17 (0.17)	10.9 (0.32)	1.91
12.10.08	Bags - 1" frame	2	363	82.2 (10.8)	55.9 (0.41)	21.8 (0.05)	31.8 (0.23)	26.0 (0.69)	1.59 (0.04)	11.0 (0.21)	2.50
	Bags - 1.5" frame	2	363	78.2 (1.7)	55.7 (1.81)	21.5 (0.24)	31.8 (0.76)	25.5 (1.47)	1.50 (0.14)	11.1 (0.66)	2.48
12.12.08	Bottom Plant	1	365	53.0	62.9	22.7	34.8	32.6	2.11	11.6	3.07

Table 10. Summary of harvest data for sunray venus growout equipment trials, Cedar Key.

Shell deformities or irregularities had been observed for sunray venus cultured in bottom bags at a lease area in Franklin County during the previous Florida Sea Grant-funded project. The deformities were limited to the ventral margin (shell lip) with one valve (shell) usually having excessive curvature resulting in a depression (or indentation) of the shell (Figure 7). This caused the shell lips not to meet in some of the clams, thus leaving a visible opening. Deformities were observed in some of the bags from the gear trial harvested on December 10, 2008 and were quantified. Of the live sunray venus clams harvested from bags with frames, the number of deformities ranged from 0.5-3.7% (1.5" frames) to 1.7-2.3% (1" frames). In bags without frames, the number of deformities increased to 19.2-22.0%.

Figure 7. Sunray venus at harvest (left) and with shell deformities (right).



2009-10 Growout Trial – Cedar Key

About 6,500 sunray venus juveniles $(24.7\pm2.7 \text{ mm SL}, 8.9\pm0.8 \text{ mm SW}, 14.9\pm1.4 \text{ mm SH})$, previously field nursed in bottom bags and harvested on July 27, were planted on July 29, 2009 at the UF research lease off of Cedar Key. Three replicate growout bags were planted (4'x4', 9 mm mesh) with and without an internal 1" PVC pipe frame at densities of 50-52/ft². About 5,000 sunray venus juveniles $(24.9\pm4.2 \text{ mm SL}, 9.0\pm1.7 \text{ mm SW}, 14.7\pm2.3 \text{ mm SH})$, previously field nursed in bottom bags and harvested on August 13-24, were planted on August 18-25, 2009 at the UF research lease. Two culture systems were evaluated (bottom bag and bottom plant) in triplicate at stocking densities of 53 to $62/\text{ft}^2$. The bottom plants were small in size, 4' by 6-8', or 24-32 ft². A summary of the planting data for growout trials conducted at Cedar Key during 2009-10 is summarized in Table 11.

Culture System	Reps	Stocking Density (#/ft²)	Ave SL mm (±SD)	Ave SW mm (±SD)	Ave SH mm (±SD)
Bottom bag Bottom bag - 1" PVC frame	3	50-52	24.7 (2.7)	8.9 (0.8)	14.9 (1.4)
Bottom bag Bottom plant	3	53-62	24.9 (4.2)	9.0 (1.7)	14.7 (2.3)
	System Bottom bag Bottom bag - 1" PVC frame Bottom bag	SystemRepsBottom bag3Bottom bag - 1" PVC frame3Bottom bag3	Culture SystemRepsDensity (#/ft²)Bottom bag350-521" PVC frame353-62	Culture SystemRepsDensity (#/ft²)Ave SL mm (±SD)Bottom bag 1" PVC frame350-5224.7 (2.7)Bottom bag Bottom bag353-6224.9 (2.7)	Culture SystemRepsDensity (#/ft2)Ave SL mm (±SD)Ave SW mm (±SD)Bottom bag 1" PVC frame350-5224.7 (2.7)8.9 (0.8)Bottom bag Bottom bag350-5224.9 (2.7)9.0

Table 11. Summary of planting data for sunray venus growout trials in Cedar Key.

The culture units were harvested over multiple dates, beginning on June 15 and ending on December 6, 2010, with culture days varying from 322 (10.5 months) to 476 days (15.5 months) (Table 12). This temporal variation did not allow statistical analysis to be performed on the data; however, additional information on production performance was provided. Survival of sunray venus clams cultured in bottom bags was acceptable at 10 months (60%), but declined to 48% at 15 months, during which growth (shell length) increased from 48.9 to 55.4 mm. Like the previous trial, bags with internal frames and bottom plants did not perform as well as the bags.

Tuble 12. Summary of harvesting data for summy venus growout mars in ocdar Rey.									
Harvest Dates	Culture System	Reps	# Days	Ave Survival % (±SD)	Ave SL mm (±SD)	Ave SW mm (±SD)	Ave SH mm (±SD)	Ave TW g (±SD)	
6.15 - 21.10	Bottom bag	2	322-328	60.0 (4.5)	48.9 (0.9)	20.2 (0.2)	28.2 (0.1)	18.0 (0.6)	
11.08.10	Bottom bag – 1" PVC frame	3	468	41.7 (10.6)	58.7 (0.4)	23.8 (0.3)	35.4 (0.1)	31.3 (0.4)	
11.08 - 12.06.10	Bottom bag	3	441-476	48.0 (7.8)	55.4 (9.7)	21.6 (2.3)	32.5 (5.8)	26.5 (10.5)	
11.08 - 12.06.10	Bottom plant	3	441-476	32.7 (12.3)	62.4 (1.8)	23.0 (0.3)	36.9 (1.8)	33.5 (3.0)	

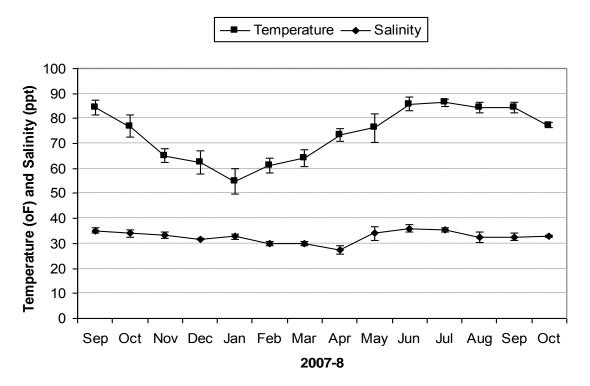
Table 12. Summary of harvesting data for sunray venus growout trials in Cedar Key.

2007-9 Growout Trial – Alligator Harbor

Sunray venus growout trials were conducted in cooperation with three growers whose leases were located within the Alligator Harbor Aquaculture Use Area east of Carrabelle in Franklin County. The lease area is located about a mile east of the beach where adult sunray venus were collected for brood stock. The substrate at this lease area is characterized by hard-packed sand. Water quality conditions during September 2007 through October 2008 were monitored using a YSI 6600 data sonde which was deployed at a piling marking the perimeter of the lease area. Measurements, every 30 minutes, were recorded at six inches above the bottom. Monthly averages and standard deviations for water temperature and salinity values are shown in Figure 8. Water temperatures ranged from a low of 55.0°F in January 2008 to a high of 86.3°F in July 2008. Salinities were consistently high and higher than those measured at the Cedar Key growing site, ranging from 27.4 ppt in April 2008 to 35.9 ppt in June 2007.

Over 13,000 sunray venus juveniles (27-33 mm SL, 9-12 mm SW, 16-19 mm SH), previously field nursed in either cages or bottom bags, were planted on three grower's leases (Table 13). Polyester mesh bottom bags were used by all growers, but the dimensions of the bag varied (ie., 3' x 4' bags were used by Grower A, 4' x 4" bags were used by Growers B and C). In addition, Grower A used polyethylene cover netting, Grower B used galvanized wire, and Grower C did not use any cover netting. Stocking densities ranged from 50 to 70 per square foot.

Figure 8. Monthly averages of water temperature (°F) and salinity (ppt) values taken at the Alligator Harbor Aquaculture Use Area near Carrabelle between September 2007 and October 2008. Error bars reflect the standard deviation from the monthly averages.



Plant Date	Lease	Reps	# per Unit	Density (# per sq. ft.)	Total # Planted	Ave SL (mm) (<u>+</u> SD)	Ave SW (mm) (<u>+</u> SD)	Ave SH (mm) (<u>+</u> SD)	Ave T Wt (g) (+SD)
00 12 07	A	1	632	53	632	32.9 (2.9)	12.4 (1.2)	18.7 (1.6)	5.2 (1.3)
09.13.07	А	3	508	42	1523	27.1 (4.0)	10.0 (1.3)	16.0 (2.0)	2.8 (1.0)
00 12 07	В	4	890	56	3560	26.9 (3.3)	9.5 (1.2)	15.9 (1.7)	-
09.13.07	В	4	1115	70	4460	26.9 (3.3)	9.5 (1.2)	15.9 (1.7)	-
09.29.07	С	4	800	50	3200	-	-	-	-

Table 13. Summary of planting data for sunray venus growout trials, Alligator Harbor.

After 13 months, half of the bags were harvested on October 29, 2008 (Table 14). Due to the limited number of replications in this trial, production data was not be statistically analyzed. Survival ranged from 38.4 to 66.4%. The lower survival was associated with bags that had not been covered with netting. Holes and crushed shell in all the bags were evidence that predation (most likely stone crabs and cownose rays) was the cause of mortality. Mortality was higher than that observed in bags harvested from the Cedar Key site. Sunray venus clams averaged 48-56 mm in SL, 22 mm in SW, 27-31 mm in SH, 19-23 g in total weight, 0.9-1.1 g in dry meat weight, and 9.5-10.3 in condition index. Growth rates (1.6 mm SL/month) were lower than those obtained in the Cedar Key trials using similar gear and stocking densities (1.9-2.6 mm SL/month). In this trial, the number of shell deformities was quantified. Deformities ranged from 32.9 to 47.6% of the live sunray venus harvested from five bags on Grower B's lease; whereas the percentage of deformities was lower (7.9-15.5%) in the three bags harvested from Grower's C lease.

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Harvest Date	Lease	Reps	# Days	Ave Survival (%) (<u>+</u> SD)	Ave SL (mm) (<u>+</u> SD)	Ave SW (mm) (<u>+</u> SD)	Ave SH (mm) (<u>+</u> SD)	Ave TWt (g) (<u>+</u> SD)	Ave Dry Mt Wt (g) (<u>+</u> SD)	Ave CI (<u>+</u> SD)	Ave Growth (mm,SL) / month
10.29.08	В	1	412	66.4	48.2	21.6	27.2	19.0	0.87 (0.31)	9.5 (1.0)	1.57
10.29.08	В	4	412	58.3 (26.7)	48.9 (2.12)	21.4 (0.51)	31.0 (0.49)	23.4 (0.99)	1.02 (0.07)	10.3 (0.59)	1.62
10.29.08	С	3	396	38.4 (14.0)	56.2 (0.79)	21.0 (0.22)	31.0 (0.49)	23.4 (0.99)	1.18 (0.12)	9.85 (0.44)	-

Table 14. Summary of harvest data for sunray venus growout trials after 13 months, Alligator
Harbor.

The remaining growout bags were left with the intent of obtaining information after 18 months of growout. On April 7, 2009 (18.8 months), three bags were harvested each from Grower A's and Grower B's leases (Table 15). Survival ranged from 50.6 to 55.1% and was within the range of survival obtained after 13 months of growout. Holes and crushed shell in all bags were evidence that predation (most likely stone crabs and cownose rays) was the cause of mortality. Some fouling was observed on shells. Sunray venus clams averaged 50-54 mm in SL, 22-23 mm in SW, 28-30 mm in SH, 20-23 g in total weight, 0.9-1.1 g in dry meat weight, and 7.8-9.0 in condition index. Growth rates (1.2-1.4 mm SL/month) were lower than those obtained during the first 13 months of growout (1.6 mm/month). The additional 5 months of growout coincided with the coldest period of the year (November-March). Water temperatures averaged 60.7 ± 5.9 °F over this time period; salinities averaged 30.7 ± 4.7 ppt. Shell deformities ranged from 2-14% of the live sunray venus harvested per bag; whereas deformities observed after 13 months were higher, ranging from 8-48% per bag.

I ubic I	able 13. Summary of harvest data for sumay venus trais after 16 months, 7 migator flaroor.									
			Ave	Ave	Ave	Ave	Ave	Ave Dry	Ave	Ave
	Bono	#	Survival	SL	SW	SH	TWt	Mt Wt	CI	Growth
Lease	Reps	Days	(%)	(mm)	(mm)	(mm)	(g)	(g)		(mm, SL)
			(<u>+</u> SD)	per month						
^	2	570	55.1	53.6	22.8	29.7	23.2	1.12	9.0	1 1 1
A	3	570	(10.5)	(3.6)	(0.6)	(2.1)	(3.9)	(0.18)	(0.3)	1.41
В	3	570	50.6	49.8	21.7	27.7	20.3	0.87	7.8	1.22
	5	570	(9.6)	(2.5)	(0.6)	(1.5)	(2.8)	(0.16)	(0.7)	1.22

Table 15. Summary of harvest data for sunray	venus trials after 18 months, Alligator Harbor.
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2009-10 Growout Trial – Alligator Harbor

Over 17,000 sunray venus juveniles (21.2-21.5+2.9-3.1 mm SL, 7.3-7.6+1.0-1.2 mm SW, 12.8-12.9+1.5-1.6 mm SH), previously field nursed in bottom bags and harvested on August 13, were planted on August 18, 2009 at a grower's lease (Grower A). The objective of this growout trial was to evaluate systems and methods that may reduce shell deformities observed during harvest of previous trials at this location (Table 16). Two sites within this lease were planted - the shallow end where sunray venus had previously been cultured and the deeper end. Characteristics of the bottom substrates at these sites differed. The soil on the shallow end was sandy and hard packed; whereas the soil at the deeper end consisted of more mud and was softer. At each site, three culture systems were evaluated in triplicate. The systems consisted of a) 3'x4' 9 mm mesh polyester bottom bag covered with plastic netting, b) 3'x4' 9 mm mesh polyester bottom bag with an internal 1" PVC pipe frame covered with plastic netting (Figure 9), and c) 4'x6'



Figure 9. Planting sunray venus in a bottom bag with PVC pipe frame.

bottom plant covered with a single layer of 9 mm mesh polyester netting and plastic netting. In addition, 3 bottom bags were planted at the shallow end and buried using a pump. This method has been used by clam growers in the Indian River to accelerate the burial of clams in bags. All culture systems were stocked at $53/\text{ft}^2$. This trial was terminated in January-February 2011.

Plant Date	Site	Culture System	Reps	Stocking Density	Ave SL mm	Ave SW mm	Ave SH mm
Duio				(#/ft²)	(±SD)	(±SD)	(±SD)
		Bottom bag	3				
	8.18.09 AH - Shallow	Bottom bag with 1" PVC frame	3		21.5 (3.1)	7.6 (1.2)	12.9 (1.6)
8.18.09		Bottom plant	3	53			
		Bottom bag using pump	3		21.2 (2.9)	7.3 (1.0)	12.8 (1.5)
		Bottom bag	3				
8.18.09	AH- Deep	Bottom bag with 1" PVC frame	3	53	21.5 (3.1)	7.6 (1.2)	12.9 (1.6)
		Bottom plant	3				

Table 16. Summary of planting data for sunray venus growout trial in Alligator Harbor (AH).

After 17-18 months, sunray venus clams cultured in a variety of bottom gear at the Alligator Harbor lease site were harvested. One of the replicate bottom bags in which a pump with a diffuser was used to assist in planting the sunray venus was harvested prior on the first of September to assess growth. At 12.5 months, survival of sunray venus in the replicate bag was 74.4% and growth was 52.4 mm for shell length, 20.4 mm for shell width, and 21.0 grams for total weight. Survival and growth data for all other culture gear are summarized in Table 17. Due to unequal number of replicates, treatment means were subjected to a one-way analysis of variance according to the General Linear Model procedure of SAS 9.2. A Tukey's honestly significant difference test was used to compare treatment means when the analysis was significant ($p \le 0.05$). Survival of sunray venus planted in bags using the pump method in the shallow portion of the lease was the highest observed and was statistically different from other cultured methods with the exception of the bottom bags (without using a pump at plant), which averaged 49.3%. The lowest survival observed was for sunray venus cultured in bags with internal PVC frames (average 26.6% in shallow area and 18.7% in deeper area). Mortality was associated with predation, most likely stone crabs Menippe mercenaria, as evidenced by bags with holes and presence of shell fragments. Survival of sunray venus reared using the bottom plant method was 41.0% in the shallow portion and 31.2% in the deeper portion of the lease. It seems that location on the lease did not influence survival or growth. Average shell length, width, height, and total weight were similar for sunray venus cultured in the various bottom bag treatments (although statistical differences were determined among treatment means). The exception is growth observed of sunray venus clams reared using the bottom plant method as shell length averaged 62-63 mm, shell width averaged was 23.7 mm, and total weight averaged 34.7 grams.

	Table 17. Summary of narvest data for sunray venus growout trial in Alligator Harbor (AH).									
Harvest Dates	Site	Culture System	Reps	# Days	Ave Survival % (±SD)	Ave SL mm (±SD)	Ave SW mm (±SD)	Ave SH mm (±SD)	Ave TW g (±SD)	
		Bottom bag	3		49.3 (7.9) ^{ab}	57.0 (2.9) ^{abc}	22.8 (0.5) ª	33.5 (1.9) ^{ab}	27.1 (2.7) ^b	
1.19.11 &	AH -	Bottom bag with 1" PVC frame	³ 520 54	520 & 549	26.6 (6.9) ^{de}	54.0 (2.4) ^{cd}	21.6 (1.0) ^{ab}	32.2 (2.2) ^{ab}	23.6 (2.5) ^{cb}	
2.17.11	2.17.11 Snallow Botton using p Botto	Bottom bag using pump	2	040	62.5 (1.1) ª	53.0 (3.6) ^{cd}	21.2 ^{ab}	31.1 ^b	22.2 ^{cb}	
		Bottom plant	3		41.0 (2.9) ^{bcd}	62.4 (0.3) ^{ab}	23.8 (0.2) ^a	37.3 (0.4) ^a	34.7 (0.4) ^a	
		Bottom bag	3		45.5 (8.0) ^{abc}	55.8 (1.2) ^{bcd}	22.1 (0.8) ^{ab}	32.9 (1.0) ^{ab}	25.1 (1.5) ^{cb}	
1.19.11 & 2.17.11	AH- with 1" F	Bottom bag with 1" PVC frame	3	520 & 549	18.7 (7.7) ^{de}	48.8 (4.2) ^d	19.9 (1.7) ^b	29.5 (2.7) ^b	18.2 (4.0) °	
	Bottom plant	3		31.2 (0.2) ^{cde}	63.3 (1.2) ^a	23.6 (0.2) ^a	37.3 (1.4) ^a	34.7 (1.9) ^a		
	<i>p</i> -value					0.0001	0.0025	0.0010	<0.0001	

Table 17. Summary of harvest data for sunray venus growout trial in Alligator Harbor (AH).

It must be noted that the bottom plants evaluated in all of these trials at both growing locations were small in size, with 32 ft² being the largest size. This was due to limited availability of seed to stock a more commercial-size plant (e.g., 100 ft²). Thus, the ratio of the net perimeter to the surface area was large. This feature most likely affected survival as there was more access by predators, particularly the moon snail *Polinices duplicatus*, which buries and moves through the substrate. Presence of moon snails and evidence of predation on shells (holes near umbo) were observed during the harvest of all bottom nets. Further, it is apparent that our lack of knowledge regarding bottom plant methodology also influenced the outcomes. The use of bottom plant methods for sunray venus clam growout seems promising and needs to be further evaluated on a commercial scale.

OBJECTIVE 3. Determine salinity and temperature preference of nursery and grow-out sized sunray venus seed clams.

The salinity tolerance of nursery seed was examined. Three separate families (55, 61 & 85) of sunray nursery seed clams were utilized as replicates and exposed to salinities of 10, 20, 30 or 40 ppt in triplicate 4-L beakers. Twelve individuals from each family (avg initial whole wt = 19 ± 3 mg, avg initial length = 4.7 ± 0.3 mm) were utilized in each beaker (n=36 clams/beaker). Individual clams were placed in separate openings of a plastic grid (1.2 x 1.2 cm openings) with screen (1.0 mm openings) on the bottom. The trays were suspended approximately 8 cm from the bottom in 3 L of treatment water, which was gently aerated. Clams were fed twice per day with the microalgae *Isochrysis* sp. at a density of 100,000 cells/mL; water was changed completely every other day. Clams were examined daily for mortality and weighed weekly for three weeks. Temperature ranged from 25-28.5°C.

All clams in the 10 ppt treatment died by the end of week 1 (Figure 10). After three weeks, survival at 30 ppt (81%) was significantly greater than at 40 ppt (55%), but not from 20 ppt (69%). Survival was not significantly different between 40 ppt and 20 ppt. Interestingly, family 85 exhibited significantly lower survival (50%) as compared to family 61 (74%) and 55 (81%), which were not different from each other (Figure 11).

Growth (% whole weight change) for surviving clams was significantly affected by salinity. After three weeks (Figure 12), clams at 30 ppt grew significantly more (270%) than those at 40 (208%), which grew significantly more that those at 20 ppt (125%). Family 85 exhibited significantly lower growth (171%) as compared to family 61 (225%), but was not different from 55 (207%) (Figure 13). Family 61 and 55 were not different from each other.

Figure 10. Mean (n=3) survival of sunray venus clams exposed to various salinities for three weeks.

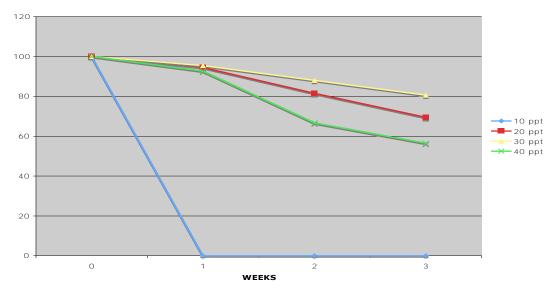


Figure 11. Mean (n=3) survival of sunray venus clam families over three weeks.

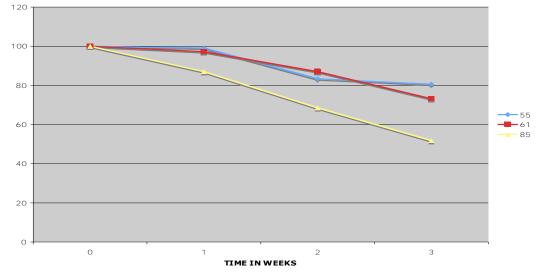
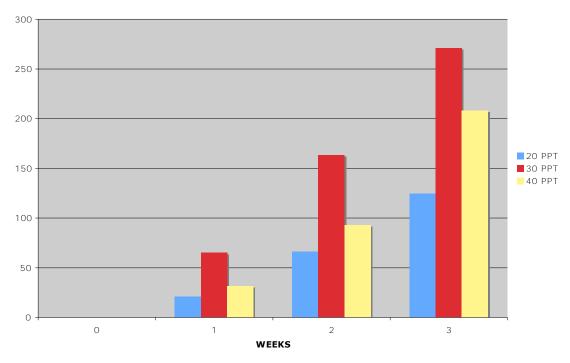
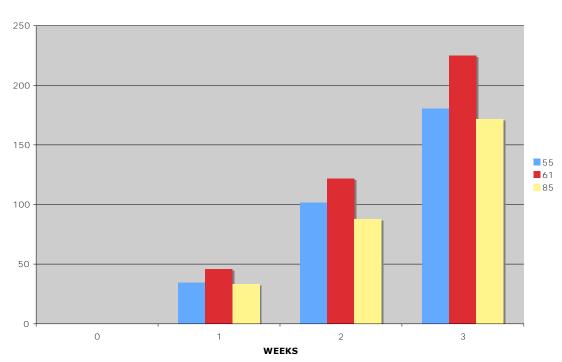


Figure 12. Mean (n=3) cumulative growth (% whole weight change) of sunray venus clams exposed to various salinities for three weeks.



PERCENTAGE GROWTH BY SALINITY

Figure 13. Mean (n=3) cumulative growth (% whole weight change) of families of sunray venus clams over three weeks.



PERCENTAGE GROWTH OF FAMILIES

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Optimum Salinity for Juvenile Sunray Venus Clams

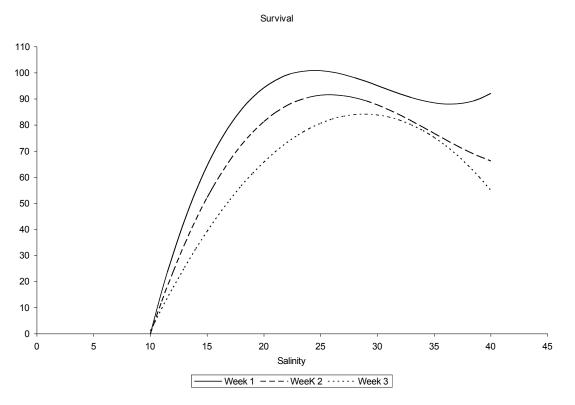
Using the SAS stepwise regression analysis, formulas were used to show the optimum salinity for the highest proportion of survival in each of the three weeks.

Week 1: $(-2.77778+(0.39938*salinity)+(-0.01366*salinity^2)+(0.00014969*salinity^3))*100$ Week 2: $(-1.84815+(0.2412*salinity)+(-0.00572*salinity^2)+(7.87037*10^{-7}*salinity^4))*100$ The cubed salinity value was zero in this equation, hence it was removed from the equation. Week 3: $(-1.10417+(0.13495*salinity)+(-0.00234*salinity^2))*100$ The numbers are a parameter estimate from a stepwise regression analysis of the proportion of

survival in the clams during the third week.

The longer the time clams were exposed to the salinity treatments, the optimum salinity for survival gradually increases (Figure 14) from approximately 23 ppt after one week of exposure to approximately 28 ppt after three weeks of exposure. These results indicate that the optimum salinity for growth and survival of sunray venus clam nursery seed is near 30 ppt. Additionally, the differences found between families imply potential for increasing growth and survival through selective breeding.

Figure 14. Stepwise regression analysis (n=3) to predict survival of sunray venus clams exposed to various salinities for three weeks.



OBJECTIVE 4. Evaluate shelf life and purging to remove grit ("degritting") of live product.

Shelf Life Trials – Winter 2008

To determine the survival of sunray venus clams under typical commercial refrigeration, an evaluation of shelf life was initiated. The first trial was conducted with sunray venus harvested from cages at the Dog Island lease area in Cedar Key on October 22, 2008. Fifty sunray venus that held on a 7/8" grader and 12 sunray venus that held on a 1" grader were tested (Figure 15). Each size group was placed into a polyethylene tubular netting, which is typically used in bagging and transporting hard clams after harvesting. The two bags were placed inside a refrigerator along with a Tidbit® temperature data logger. The sunray venus clams were checked daily for survival. Gaped clams were determined to be dead when they did not respond by closing their valves to specified agitation, or tapping, after the clams were held for a short time at room temperature. Dead sunray venus were counted and removed from the sample bags. The trial was conducted for a 14-day period. There were no dead clams noted during the first week. After 7 days, 2 dead sunray venus were noted in the 7/8" group. Continued mortalities occurred in this group. After 14 days, a total of 5 clams were dead, resulting in 10% mortality, or 90% survival in refrigerated storage. There were no mortalities in the larger clams (1" SW), resulting in 100% survival. Air temperatures averaged 37.0 \pm 2.4°F over this period.

Another trial was conducted on December 2, 2008 with sunray venus clams harvested from bottom bags at the Dog Island site. Fifty sunray venus that held on a 7/8" grader were placed inside a refrigerator. Two days later, a second group of fifty 7/8" sunray venus clams harvested again from bottom bags were tested. Procedures were the same as those described in the first trial. After 14 days survival of the first and second groups of sunray venus was 92 and 94%, respectively. Air temperatures averaged $37.4 + 1.9^{\circ}$ F over this period. In both trials, the majority

of the sunray venus remained closed throughout the evaluation with little liquid accumulating on the bottom of the tray holding the bags. If the clams were allowed to warm up, they would gape and lose liquor. No odors were detected. Air temperatures in both trials were below the minimum required for storage of molluscan shellfish (\leq 45°F) and for storage of food products in restaurants (\leq 40°F).

Figure 15. Sunray venus clams harvested from bottom bags being sorted on a hand grader.



These results are similar to those for hard clams harvested during the winter at cooler water temperatures. However, survival of hard clams in refrigerated storage is reduced during the warmer summer months. Therefore, additional shelf life trials were conducted with "market" size sunray venus harvested during the summer of 2009. During the harvest of sunray venus clams in October 2008 from the Alligator Harbor growout trials and in November 2008 from the Dog Island growout trials, a portion were shucked and evaluated for the presence of a grit pocket. In all samples, we could not detect grit by taste testing.

Shelf Life Trial – Summer 2009

The shelf life of sunray venus clams under typical commercial refrigeration was evaluated in October and December 2008. After 14 days, survival of sunray venus clams ranged from 90-94%. These results were similar to those for hard clams harvested during the winter at cooler water temperatures. However, survival of hard clams in refrigerated storage is reduced during the warmer summer months. Therefore, a shelf life trial was conducted with "market" size sunray venus harvested during the summer of 2009.

This trial was initiated with sunray venus obtained from a "replant" bag on August 17, 2009 (water temperatures over a 24-hour period on that day ranged from 81.7-84.9°F). These clams had been harvested in December 2008 and replanted at the Dog Island lease area. A sample of 50 sunray venus clams were measured and averaged 52.7+4.7 mm in shell length, 21.7+1.1 mm in shell width, 30.3+2.0 mm in shell height, and 23.3+3.7 g in total weight. Two hundred sunray venus were placed into two bags (100/bag), made from polyethylene tubular nettings, which is typically used in bagging and transporting hard clams after harvesting. The bags were first placed in an air conditioned room for 12 hours along with a Tidbit® temperature data logger, prior to being placed inside a refrigerator. Tempering is a post-harvest treatment employed by many clam wholesalers in Florida during the summer months, which allows the product harvested from warm waters to acclimate prior to placement in a refrigerated cooler at the required storage temperature of \leq 45°F. The sunray venus clams were checked every other day for survival. Gaped clams were determined to be dead when they did not respond by closing their valves to specified agitation, or tapping, after the clams were held for a short time at room temperature. Dead sunray venus were counted and removed from the sample bags. The trial was planned for a 14-day period. There were no dead clams noted during the first week. After 9 days, 12 dead sunray venus were noted in one bag and 5 in the other for an average mortality of 8.5%, or survival of 91.5%. However, 21 sunray venus were gaping in the first group and 31 in the second group. On day 11, another two clams were dead in the first bag and three in the second for an overall mortality of 11%, or survival of 89%. The trial was terminated as the smell from the dead and gaping clams would have prevented the clams from being marketed. Air temperatures during tempering averaged 71.8+1.6°F; air temperatures during refrigerated storage averaged 42.2+3.0°F. These results indicate that shelf life of sunray venus clams harvested in the summer months may be limited to 7-8 days, which is about the same time period found for hard clams.

Shel Life and Purging Trials 2010

Shelf Life Another shelf life trial was conducted on May 21, 2010 with sunray venus harvested from the growout density studies. In this trial, 100 sunray venus were tempered at an air temperature of \sim 65°F for six hours prior to placing clams in refrigerated storage in an effort to extend survival. At seven days, there was no gaping, death, or smells observed. At 11 days, there were two dead sunray venus clams for a 98% survival and two clams that were gaping, which closed when agitated.

Purging to Remove Grit According to a NOAA (no date) report, one of the attributes of sunray venus clam meat was that it was "grit-free." However, observations of others (e.g., Stokes et al. 1968) suggested that grittiness was problematic in sunray venus clams. Two sources of grit may be found. The first source of grit is commonly found in filter-feeding, infaunal (bottom-dwelling) bivalves. It is associated with materials (e.g., sand, sediments) found in the mantle or shell cavity of the animal, typically accumulated during the harvest process. The second source, which is referred to as a "grit pocket," holds deposits known as renal calculi, or kidney stones. These are composed of calcium phosphate and may range in size from <0.1 to 2.5 mm (Tiffany III 1979). The grit pocket can be observed as a dark mass in the dorsal portion of the meat near the kidney and siphon retractor muscle (Figure 16). Renal calculi are found in all stages of the life history of the sunray venus clam, although no known reason has been given for the existence of this physiological adaptation. The individual stones continue to grow and aggregate until harvest or death (Tiffany III et al. 1980).

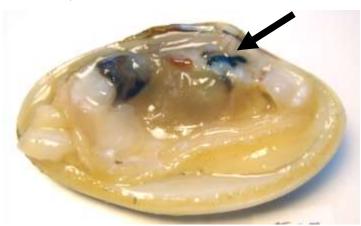


Figure 16. Internal anatomy of a sunray venus clam with an arrow pointing to the grit pocket.

During the 2006-8 field trials conducted by UF, 10 sunray clams of nursery seed size (~8-10 mm SL) were examined histologically for any sign of renal calculosis, or "grit pocket"; but, no signs were evident. Tiffany (1979) indicated that all of the animals he tested were greater than one year of age. Therefore, this may not form or be noticeable until a certain life stage or age. The age of cultured sunray venus clams at harvest may range from 18 to 24 months, depending on the targeted market size and culture conditions. To evaluate the efficacy of purging harvested sunray venus clams in reducing the grit pocket size and presence, sunray venus clams from replants of the stocking density study completed in December 2008 were harvested in May 2010. The sunray venus clams were placed in tanks with running saltwater (the water source was from conditionally approved shellfish harvesting waters) for seven days post-harvest. Fifteen sunray

venus clams were collected at one, three, and seven days post-harvest and measured for morphological (shell width, shell length, total weight, and meat weight) and grit pocket characteristics (height, length, area, and weight). An analysis of variance was performed to identify statistical differences (p < 0.05) between parameters for sunray venus clams collected on different days post-harvest. Morphological characteristics (values ranging from 23.7 to 24.4 mm SW, 57.2 to 58.8 mm SL, 29.6 to 31.9 g TW, and 5.19 to 6.05 g MW) were statistically similar (p > 0.05) for the three post-harvest samples (Table 18). Although statistical differences did exist for grit pocket height (p = 0.007), no significant variation was detected for grit pocket length (p =0.429), grit pocket area (p = 0.066), or grit pocket weight (p = 0.3182). Grit pocket height was statistically similar for clams measured on days one (6.05 ± 0.69 mm) and three (5.97 ± 0.68 mm), but by day seven grit pocket height (6.83 ± 0.94 mm) was significantly higher from the two previous days. This could be associated with a different sized sample of sunray venus clams collected on day seven as compared to those collected on days one and three post-harvest. Although the grit pocket size did not diminish over time due to purging, average grit pocket weight amounted to only 3.3% of the total weight of sunray venus clams.

Table 18. Means and standard deviations (\pm SD) of the morphological characteristics of cultured sunray venus clams and grit pocket measurements during a purging study conducted in 2010.

Days Post- Harvest	Shell Width (mm±SD)	Shell Length (mm±SD)	Total Weight (g±SD)	Wet Meat Weight (g±SD)	Grit Pocket Height (mm±SD)	Grit Pocket Length (mm±SD)	Grit Pocket Area (mm ² ±SD)	Grit Pocket Weight (g±SD)
1	23.7	57.2	29.6	5.19	6.05	6.51	39.7	0.167
	± 2.3 ª	± 5.6 ª	± 6.7 ª	± 1.54 ª	± 0.69 ^b	± 1.16 ª	± 9.8 ª	± 0.062 ª
3	24.4	58.4	31.3	5.53	5.97	6.89	41.3	0.200
	± 1.4 ª	± 3.0 ª	± 3.5 ª	± 0.84 ª	± 0.68 ^b	± 1.11 ª	± 9.2 ª	± 0.053 ª
7	24.4	58.8	31.9	6.05	6.83	7.00	48.1	0.187
	± 1.8 ª	± 5.3 ª	± 7.3 ª	± 1.57 ª	± 0.94 ª	± 0.91 ª	± 11.4 ª	± 0.064 ª

Different superscript letters following SD denote significant differences (p < 0.05) between means.

OBJECTIVE 5. Examine alternative markets for a shucked meat (raw) product.

To obtain a larger size sunray venus for evaluating the potential of sushi markets, about 5,000 clams harvested from the stocking density and gear trials in December 2008 were replanted into bottom cages and 15 mm polyester mesh bottom bags at densities of 30-48/ft² at the Dog Island site. These were harvested in the fall of 2010 for the product evaluation test. Table 19 summarizes the harvest data obtained in October and November 2009 and sizes of sunray venus clams used in this evaluation.

Harvested sunray venus were wet stored for 1-2 days at the UF Shellfish Aquaculture Research and Education Facility prior to delivery to purge the clams of grit (Figure 17a). Incoming waters for this facility are classified conditionally approved for shellfish harvesting and were open for harvesting during this time period. After harvest, the sunray venus were counted, bagged in polyethylene tubular netting, and tagged as live shellstock by a participating certified shellfish wholesaler in accordance with state standards (Comprehensive Shellfish Control Code, Chapter 5L-1, Florida Administrative Code). The bagged clams were placed into either insulated shipping boxes or coolers chilled with gel packs to maintain air temperatures below 45°F during delivery to the sushi restaurants (Figure 17b).

Harvest Date	# Harvested	Ave SL mm (<u>+</u> SD)	Ave SW mm (<u>+</u> SD)	Ave SH mm (<u>+</u> SD)	Ave TWt g (<u>+</u> SD)
10.21.09	437	66.5 (3.6)	25.0 (1.1)	38.7 (2.0)	40.1 (5.5)
10.28.09	339	67.6 (3.2)	25.5 (0.9)	39.0 (1.9)	41.4 (4.6)
11.04.09	324	68.0 (4.2)	25.9 (2.1)	38.8 (2.2)	42.6 (6.7)
11.09.09	490	68.1 (3.8)	26.4 (1.1)	38.9 (2.6)	43.7 (6.3)

Table 19. Summary of sunray venus clams harvested for the alternative market evaluation.

Figure 17. Sunray venus clams a) held in wet storage, and b) bagged and tagged for delivery to a restaurant in the alternative market evaluation.



Approximately 1,200 cultured sunray venus were delivered to four sushi restaurants in the north Florida region. Sunray venus were served raw in the traditional sushi or sashimi manner or prepared in a variety of other ways, including ceviche, on the half-shell, etc. Within those restaurants, 101 patrons tried the sunray venus clams and completed surveys. Most respondents were only slightly hesitant to try raw sunray venus clams. Nineteen per cent (19%) of the respondents detected grittiness in the clams. For all respondents, 83% were willing to order the product again and 91% were willing to recommend the product. The acceptance rate was lower in traditional Asian sushi restaurants with 63% willing to order again and 64% willing to recommend. The product attribute ratings for the respondents are summarized in Table 18.

In summary, the overall assessment of the survey respondents was favorable. The survey findings suggested that sunray venus clams, prepared as a raw product, would be an acceptable product if served in restaurants within the north Florida region. The survey did find that Asian consumers, though most consumers rated the product Overall either Excellent or Very Good, were more willing to rate the product either Good, Fair, or Poor.

	Excellent	Very Good	Good, Fair, or Poor
	% of all responde	nts for each attribute	
Appearance	60	24	16
Taste	44	31	25
Texture	45	25	30
Tenderness	43	32	25
Value	53	20	27
Size	40	29	31
Color	56	24	20
Overall	43	33	24
Anglo consumers	51	30	19
Asian consumers	32	27	32

Table 18. The product attribute ratings of raw (uncooked) sunray venus clams provided by patrons of four sushi restaurants.

OUTCOMES:

The basic premise that hard clam culture methods are suitable for the sunray venus clam *Macrocallista nimbosa* held up to testing. The sunray venus clam can be cultured in the land-based nursery, field nursery, and growout stages and is accepted by consumers, which may help diversify and expand the Florida clam culture industry. The different aspects of culture tested to date (i.e., nursery and growout culture) indicate some differences between methods used for hard clams and those needed for this species. Density may need to be lower for all aspects of culture as compared to the hard clam, but this is still being evaluated, and laminar flow versus spray bars in nursery culture may be preferred. Additionally, bottom culture methods may be more suitable for this bivalve than the use of bottom bags. If product diversification occurs, the potential for this clam to help expand the Florida clam culture industry is great.

PROBLEMS ENCOUNTERED:

Shell deformities were first observed and reported in the 2009 Interim Report. The project team continued to address this problem by evaluating various growout systems and deployment methods. The development of deformities may also be related to substrate, which still needs investigation.

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