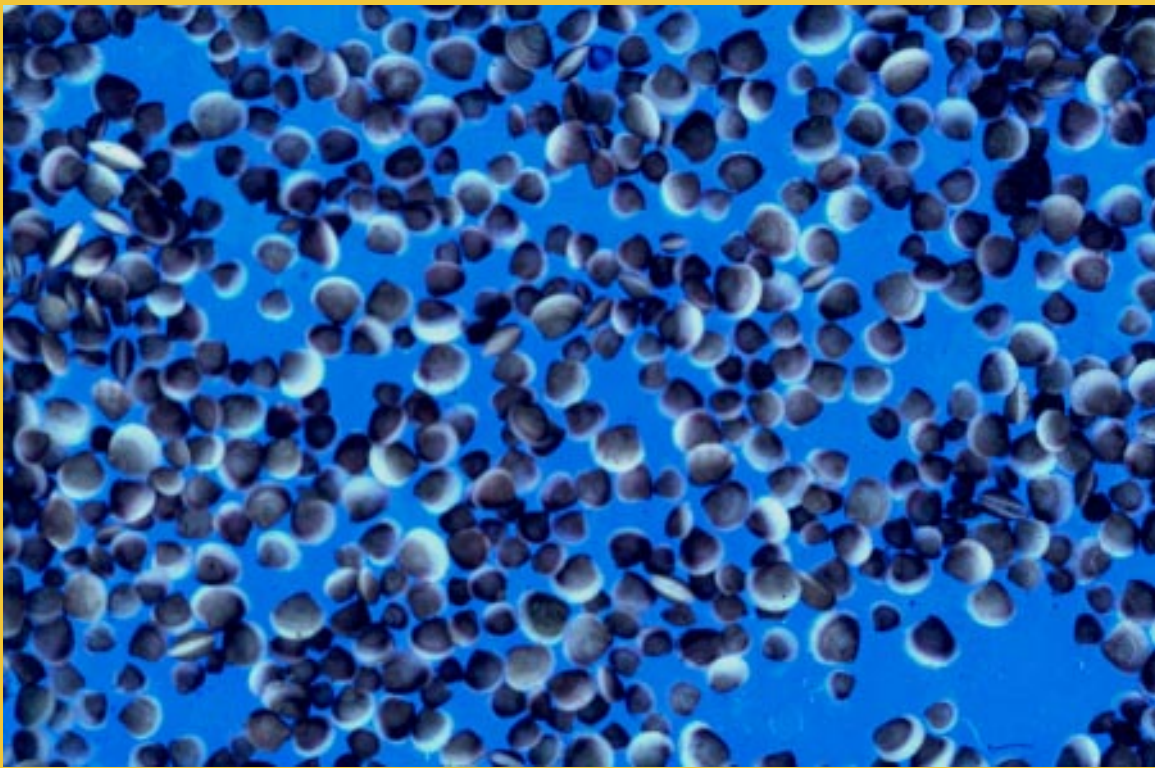

ENHANCING SEED AVAILABILITY

FOR THE HARD CLAM (*Mercenaria mercenaria*) AQUACULTURE INDUSTRY BY APPLYING REMOTE SETTING TECHNIQUES



April 2003

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Enhancing Seed Availability
For the Hard Clam (*Mercenaria mercenaria*) Aquaculture Industry
By Applying Remote Setting Techniques

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INTRODUCTION

Hard clam aquaculture has developed rapidly in Florida as well as in other states in the southeast. Adequate seed availability is a major concern to this industry and has, in recent years, faced critical shortages coupled with increased prices. Remote setting of hard clam, *Mercenaria mercenaria* (Linnaeus, 1758), seed would allow growers to become less dependent upon traditional seed sources, potentially reduce the cost of seed, and help ensure a greater chance of success by gaining control of and incorporating another step into their existing business. This would enhance the continued economic viability of clam farming, a significant and growing sector of Florida's aquaculture industry. Ultimately, the adoption of remote setting technologies will further local economic development by creating more infrastructure to support this industry.

The goal of this study was to test a technology that may help ensure a reliable and consistent supply of high quality and inexpensive clam seed to growers, thus fostering an emerging aquaculture industry by eliminating a seed shortage that limits sustainability. The overall objectives were to develop, test and demonstrate technical procedures and determine the financial feasibility of transferring remote setting technology from the Pacific Northwest molluscan shellfish industry to the hard clam aquaculture industry in Florida. Specific objectives of this project were to:

1. Develop harvesting, refrigeration, packaging and shipping protocols for clam pediveliger larvae;
2. Produce a minimum of 10 million pediveliger larvae, or pre-set clams, each project year for the large-scale evaluation of remote setting technology;
3. Test the survival and growth of pre-set clam larvae for rearing to a 1-mm seed size in identical remote setting systems at two different locations in Florida after overnight refrigeration storage and shipment;
4. Test two different management and feeding regimes (natural versus supplemental) at these nursery sites; and,
5. Compare the costs of producing 1-mm hard clam seed in a remote set nursery facility to the market price associated with purchasing 1-mm seed from a commercial clam hatchery.

BACKGROUND

Industry Development

The hard clam culture industry has a history extending back more than 20 years in Florida. Attempts to culture hard clams originated in the Indian River along the east central coast during the late 1970s as a means to create an alternative supply source to fluctuating wild stocks. The development of the industry on the Gulf coast of Florida began in the early 1990s, primarily through job retraining program efforts designed for displaced workers in the commercial fishing industry. Over 300 underemployed oyster harvesters and net fishermen were trained and placed into small-scale business enterprises. These technology transfer programs were comprehensive enough in scope to have launched a new industry for Florida's Gulf Coast (Chew 1999).

Currently in the state, about 450 shellfish growers farm over 1,700 acres of state-owned submerged lands in near-shore coastal waters. Production of hard clams has fast become established in areas where neither aquaculture nor a traditional fishery existed. This is reflected in the aquaculture surveys conducted biannually by the Florida Agricultural Statistics Service. Clam production reported since 1987 is presented in Figure 1. Over 140 million clams were produced statewide in 2001 (FASS 2002). Farm gate sales rose to \$15 million that year, representing an 11-fold increase over survey results just 10 years ago (FASS 1992).

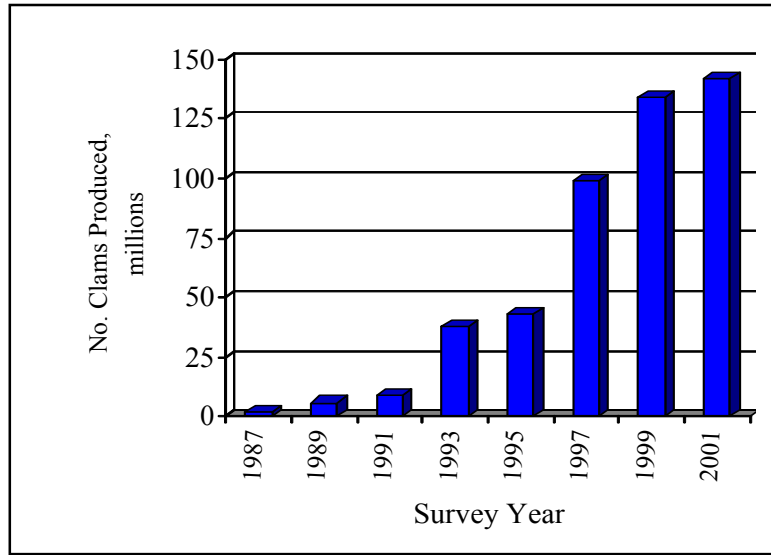


Figure 1. Florida hard clam aquaculture production, 1987-2001. Source: Florida Agricultural Statistics Service.

This industry represents a sizable economic contribution to the economy of Florida. A recent analysis assessed the economic impact to be \$34 million (Philippakos et al. 2001).

Clam Seed Availability

Efforts have moved from focusing on production to developing infrastructure support for this emergent industry (Sturmer and Vaughan 1997). One of the foremost concerns of growers has been a reliable and consistent supply of high quality seed. As this new industry has rapidly expanded, the availability and cost of field plantable seed has remained one of the major production concerns and expenses. The majority of growers incorporate a field nursery phase into their growout operations, in order to provide their own final plant seed of a size of 12 to 15 mm (shell length). The minimum sized seed that can be planted reliably, in terms of survival, in the field nursery phase is 5 mm (shell length). The larger the seed, the higher the price, with the price of 12-15 mm seed typically two times that of field nursery seed and five times that of hatchery (1-mm) seed. In an economic analysis of a small-scale, 2-acre clam culture operation, with assumptions applicable to the Cedar Key area, nursery clam seed for initial field planting represented the single most important annual variable cost (Adams and van Blokland 1998). The financial assumptions used in that study included 5-mm seed clams purchased at a price of one cent each, or \$10 per thousand, and a market price of 12 cents per littleneck clam (50 mm shell length). By planting a million clam seed per year, and assuming a 70% survival in the field nursery phase and 80% survival in the growout phase, a grower could realize an average annual net return to labor and capital of about \$31,000. In the same study, a single-variable sensitivity analysis was performed on the price of nursery seed. An increase from 1 to 1.5 cents per seed, increased total expenditures by 20% and decreased net returns by 12%.

Price increases for seed clams may not be unreasonable. In the past decade, both higher priced seed and seed shortages have been experienced by growers in Florida. Annual clam seed plantings have increased from an estimated 47 million in 1990 to over 400 million in 2000 (FASS 2000). Clam seed plantings reported since 1988 are presented in Figure 2. Shortages in the availability of clam seed are a result of continued increased demand as well as periodic events, such as unfavorable environmental conditions associated with the El Niño weather phenomenon in 1997. Seed availability in 1998 and 1999 reflected these occurrences. Concurrently, seed prices increased to a range of \$10-13 per thousand, compared to average market prices of \$8-10 per thousand.

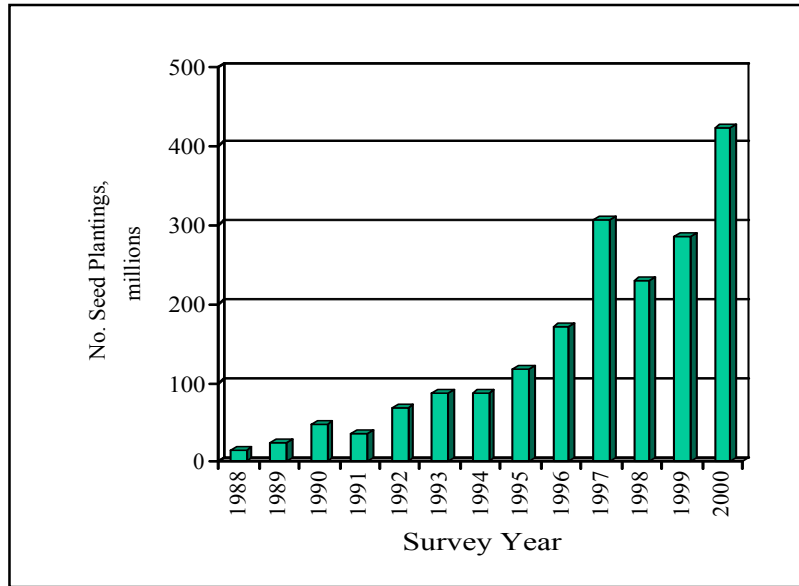


Figure 2. Florida hard clam seed plantings, 1988-2000. Source: Florida Agricultural Statistics Service.

Land-based Nursery Operations

Since buying seed is a significant cost associated with a clam culture operation, many growers have considered incorporating a land-based nursery facility into their business plan. The grower trades a lower initial seed cost for the additional costs of nursery equipment, and the cost of electricity and labor incurred during the operation of the nursery. Shortages of clam seed were a chronic problem to many new growout businesses during the years of 1994 through 1998. As a result, expansion was limited. Supply began to meet demand as increasing numbers of private-sector, land-based nurseries became operational. Currently, more than 90 private-sector, land-based nurseries are located in Florida. At these facilities, seed purchased from hatcheries at sizes as small as 1 mm (shell length) are nursed to a field plantable size of 5 mm in upwellers and raceways where natural seawater high in salinity and food (microalgae) is pumped through the system. Prices for these sized seed (1 mm) typically have ranged from \$3-4 per thousand with survivals of 70-80% achieved during the land-based nursery phase (Sturmer et al. 1995). However, clam farmers without waterfront access are at a disadvantage to operate land-based systems. Siting of a nursery facility requires access to high salinity water. Subsequently, several community-based endeavors have been undertaken in areas which support this type of culture activity and are used by growers lacking suitable coastal sites. Financial information regarding the integration of a land-based nursery phase into a grower's business operation is not available.

Hatchery Operations

Hard clam culture could not have advanced to its present state were it not for the development of hatchery techniques (Castagna and Manzi 1989). The techniques currently employed by most hatchery operations were defined in the mid-1950s and 1960s (Loosanoff and Davis 1963). A summary of these methods can be found in Castagna and Kraeuter (1981) and Castagna and Manzi (1989). A typical east coast commercial hatchery conditions broodstock (adult hard clams) and manipulates spawning by temperature cycling. The resulting free swimming larval stages are grown through metamorphosis (set) in fiberglass or plastic tanks, usually about 1000-gallon capacity, of filtered seawater. Production of monocultures of unicellular planktonic algae are needed to feed the larvae. Within 8 to 14 days, pediveliger larvae develop a foot and alternate between swimming and crawling. Metamorphosis, or settlement to a benthic stage, occurs naturally with the resulting early post-set clam about 200 to 300 microns (μm) in size. At this point, post-set clams are often kept in the hatchery and handled like larvae until they reach approximately 1 mm. Culture practices during this period fall into three basic methods - traditional, downweller, and upweller (Manzi and Castagna 1989). Each method entails adding cultured algae on a periodic basis and filtered seawater at increasing rates. These techniques make possible the production of large quantities of juvenile (seed) clams. Nonetheless, not many clam growers are encouraged to enter into this phase of production. The hatchery requires a substantial level of capital investment and technical expertise to achieve a consistently high quality seed supply (Adams et al. 1991, Adams and Pomeroy 1992). Consequently, most clam growers purchase seed from a commercial hatchery.

A study examining the financial characteristics of various hard clam hatchery, nursery and growout systems was conducted by Adams et al. (1991). The analysis indicated that a stand-alone hatchery system was not profitable given the operation size capable of an annual harvest capacity of 24 million 1-mm seed. To further examine how costs of production for a hard clam hatchery and nursery change as total output changes, Adams and Pomeroy (1992) determined at what size a stand-alone hatchery and nursery could become profitable. Results indicated most economies of scale were achieved at the respective 72 and 36 million clam output level. Significant cost reductions were achieved through vertical integration of the hatchery and nursery facilities at these output levels and beyond. For stand-alone nursery operations with an output capacity below 36 million clams, no cost incentive existed for vertical integration into hatchery operations. However, this study proposes to examine integration of the land-based nursery with the growout operation. The economic characteristics of this integration strategy have not been previously examined.

In Florida, there are about 14 private-sector hatcheries, most of which have developed as a result of vertical integration within a larger parent operation. The majority of these also sell surplus seed to land-based nursery operators and growers. This type of hatchery development produces redundancy of fixed investment and operational costs, which increases the overall cost of clam seed to all—the vertically integrated businesses and the growers who purchase seed from them. Since the profile of the industry is dominated by small businesses, the concept of larger hatcheries seems plausible by freeing existing hatcheries of laborious nursery maintenance through a division of labor. The problem of adequate clam seed availability can partially be solved by establishing more hatcheries and land-based nurseries. This project contributed to

solving the problem by testing and adapting remote setting technology, which had not been evaluated nor utilized by the hard clam aquaculture industry.

Remote Setting Technology

During the 1970s, the oyster industry in the Pacific Northwest also faced critical seed shortages. Remote setting of the Pacific oyster, *Crassostrea gigas*, was first investigated during 1972 in laboratory studies at Oregon State University (OSU) (Lund 1972). Larval handling and remote setting of this shellfish species was further refined by west coast oyster growers (Budge 1973) and additional OSU research (Henderson 1983). The method did not come into widespread practice until 1978 when the Whiskey Creek Oyster Hatchery in Oregon was started exclusively for the production of larvae for remote setting operations by Lee Hanson, who provided a history of remote setting at a 1991 workshop conducted by the Washington Sea Grant College Program (Nosho and Chew 1991). Prior to these efforts, oyster hatcheries had never been proven to be economically feasible due to the cost of cultch handling and growout problems. However, the advent of remote setting techniques provided a more efficient means of transferring oyster seed. As a result, the cultching process was transferred to the grower, thereby providing for a division of labor between the hatchery operator and the oyster farmer. Successful commercial remote setting methods have been well documented by Jones and Jones (1983, 1988) and subsequently applied to the production of Manila clam, *Venerupis japonica*, (Toba et al. 1992, Jones et al. 1993).

Remote setting intervenes at the life cycle stage when larvae are “competent,” or ready to settle, attach to cultch, and metamorphose to become “seed” or “spat” (Nosho and Chew 1991). This is the pediveliger or “eyed” stage at which the larvae can be safely removed from the culture medium for up to a week and shipped from the hatchery to the grower. The general process for oysters is as follows. Competent oyster pediveligers are hatchery produced, wrapped in moist nylon cloth and paper toweling, refrigerated, then shipped in an insulated container at 40° F via overnight delivery to growers. Upon arrival, the larvae are suspended in a covered tank of 85° F, filtered seawater containing washed and aged cultch (oyster shells). Larvae are usually introduced at a rate of 100 per shell, anticipating a 20-50% attachment, or set, to the shells during the 2-3 day settlement period. Partial changes of filtered seawater during this time provide natural food and maintain proper water quality. The cultch setting and spat condition are verified by microscope examination prior to placement in the growout area. A similar process is employed for the remote setting of Manila clams with the exception that cultch is not added to the setting tanks (Toba et al. 1992).

The remote setting technology revitalized the Pacific coast molluscan shellfish industry. Over the past two decades, it has become standard practice for most oyster and clam growers in Washington, Oregon and British Columbia to purchase pediveliger larvae at \$100-120 per million, rather than \$5-15 per thousand seed at 1-5 mm. As a result, west coast commercial hatcheries have evolved into high-volume larval rearing facilities utilizing 8,000 to 32,000 gallon tanks, capable of producing billions of Pacific oyster and Manila clam pediveligers annually. Further, the setting and nursery activities that once took place at a centralized hatchery are now dispersed about the region and are conducted by individual growers rather than hatchery operators.

METHODS

Pediveliger Production

The hatchery production of clam pediveliger larvae (pre-set seed) in this study was to be addressed at the Office of Sea Grant Development, Louisiana State University at their facility in Grand Isle, Louisiana. The facility's hatchery was designed for high volume production of oyster, *Crassostrea virginica*, larvae. A former pilot commercial facility, the hatchery was built as a prototype for transferring west coast remote setting technology to Louisiana and the Gulf of Mexico region, with a larval to algal rearing capacity ratio identical to Whiskey Creek Oyster Farm's hatchery in Oregon. Four 8,000-gallon larval rearing tanks have an estimated capacity of producing 60 million oyster pediveligers per week. However, the project was temporarily set back when the state's regulatory authority unexpectedly ruled that *Mercenaria mercenaria* was an exotic species, and could not be cultured in coastal Louisiana.

In lieu of using the remote set-style hatchery in Louisiana for producing pediveliger larvae, three commercial hard clam hatcheries participated in this project. Certified Florida clam broodstock were sent to a hatchery located out-of-state for production of pediveliger larvae in 2000. During 2001, two private-sector hatcheries located in Florida produced pediveligers. All of these facilities were large-scale operations, producing well over 100 million 1-mm seed annually. Standard hatchery rearing techniques for hard clams were employed. During larval culture, the clams were viewed daily under a microscope to monitor physical changes indicative of approaching metamorphosis. Competency of pediveliger larvae was determined by size, presence of foot, and amount of activity noted.

Pediveliger Preparation

As clam pediveliger larvae approached 150 μm in size (shell length), daily samples were measured and placed into various sized sieves to determine optimum harvest screen size. A screen size of 130 μm was used to harvest pediveligers at the participating hatcheries. Once pediveliger larvae were removed from the rearing tanks, they were prepared for shipment using similar methods as for transporting Pacific oyster eyed larvae (Jones and Jones 1983) and Manila clam larvae (Jones et al. 1993).

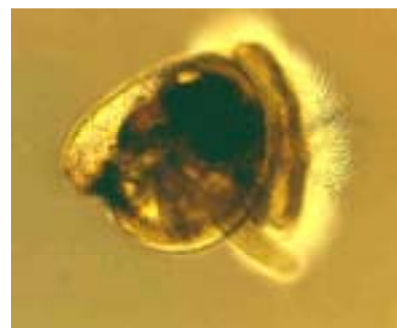


Figure 3. Hard clam pediveliger larva.

Counting - To count the harvested larvae, they were resuspended in a plastic bucket filled with 10 liters of filtered seawater. The larval suspension was then mixed well with a homemade plunger (a plastic graduated cylinder) for even distribution and a 10-ml sample was taken. The sample was diluted to 150 milliliters and three 1-ml subsamples were counted using a Sedwick-Rafter cell under a microscope. Counts were averaged and multiplied by the dilution factor (15X) and then by 10,000 (volume in bucket) to estimate the total number of larvae within the bucket. An alternative method was used in the second project year. By using a micropipette, three 200 μm subsamples were taken, counted, averaged, and multiplied by the total volume. The

amount of larvae to be shipped to each remote set nursery site could then be volumetrically determined by dividing the desired amount of larvae by the estimated number of larvae per liter in the bucket. The larval suspension in the bucket was mixed well with a plunger prior to removal of the desired sample volume.

Packaging - The designated amount of larval suspension was slowly poured through a paper coffee filter placed in a funnel (Figure 4). The larvae on the filter were rinsed with seawater from a wash bottle to accumulate them in a ball at the bottom of the filter. The ball was



Figure 4. Preparation of hard clam pediveliger larvae, including counting of subsample for estimation of numbers, filtering of larval suspension, and packaging of larval ball for shipment.

very lightly squeezed to remove excess water. The ball was then wrapped in more filters and several layers of paper towels moistened with seawater. The bundle was refrigerated for 2-3 hours at approximately 40-45° F before being placed in an insulated shipping box. Refrigerated gel packs were placed in the box, not in direct contact with the larvae. This procedure assured that the temperature of the larval ball would not exceed 50° F during shipping. Overnight delivery services limited the time out of water to 1 day or less for the larvae. No detrimental effect had been observed on the success of Manila clam larval set, maintained at this temperature, for this duration during shipping (Jones et al. 1993).

Remote Setting Unit Description

Participating land-based nurseries in this project were modified to incorporate a remote setting unit (Figure 5). These units consisted of a tank, downwellers and a mechanical filtration device.

Tank - A 250-gallon fiberglass tank 4 feet wide, 8 long, and 15 inches deep was used to hold wellers. The floor of the tank was formed to create shelves which elevated the wellers off the bottom by 2 inches. Two troughs ran the length of the tank to a centralized sump area at one end. This design aided in cleaning accumulated sediments, silt, uneaten algae, feces, etc. from the tank.



Figure 5. Remote setting unit including 250-gallon tank, sand and bag filtration, and 3 downwellers.

Downwellers - Each tank held a series of downwellers. Downwellers are screened chambers that are used to hold molluscan shellfish larvae or seed with a flow of water passing downward past the animals and through a screen at the bottom. Downwellers were constructed from 50-gallon polyethylene drums, resulting in a 24" diameter and 10" deep chamber. Up to six downwellers of this size could be held in the 250-gallon tank. A precision woven fabric mesh (Pecap) was used for the bottom screen material. Three screen sizes were used - 120, 200 and 425 microns (μm). Each downweller was fitted with an air-lift, constructed from 1"-diameter PVC pipe and a diffuser. The air-lift pumps were driven by a 115-volt air compressor.

Filtration - Adaptation of the water supply source included mechanical filtration to reduce the amount of particulate matter and suspended solids entering the unit. A sand filter (16"-diameter) was plumbed in-line. In the first year of the rearing trials, the filter medium used was silica sand with a particle size of 0.018" to 0.022" (No. 20). During the second year, a larger particle size of 0.036" (sieve size #620-1220) was used. In addition, polypropylene felt bag filters (32" length) were attached to the incoming line in each tank. Two filter bag sizes, 25 and 100 μm , were used during the rearing trials.

Remote Setting Procedures

Stocking - The pediveliger larvae were stocked in the remote set tanks as soon as possible upon receipt of the shipment. The condition of the larvae was evaluated by observing a small sample under a microscope. Observations on size, gut contents, general condition of the velum,

and presence or absence of ciliated protozoans were noted. For easier division of the larval ball, the larvae were added to a plastic bucket with 10 liters of seawater, mixed gently, and then evenly allocated among the downwellers per remote setting tank. The larvae were stocked at an initial density of about 350 per square centimeter of screen surface area, resulting in stocking about 1,000,000 per 24"-diameter weller. Three downwellers with 130 μm screening were initially placed in each remote setting tank. After the pediveligers settled onto the weller screens, the flow rate of each air-lift in the downwellers was adjusted to 1 to 2 liters per minute.

Cleaning - Regular maintenance of the seed, wellers, and tanks occurred on a daily and weekly basis. Daily activities included backflushing of the sand filter and switching out and cleaning the bag filters. Occasionally, when incoming waters were high in sediments, filter maintenance was performed twice daily. Seed in downwellers were rinsed daily to minimize fouling of the shell surface, clogging of the screens, and accumulation of silt, sediments, and feces. Saltwater was used for the smaller seed in the 120 and 220 μm wellers. When the seed reached 0.5 mm in shell length, freshwater was used for rinsing. (The larger seed are more tolerant to freshwater, which is more effective in reducing fouling.) The remote set tank was also rinsed with fresh water to remove as much silt, feces, and debris as possible. After the tanks were refilled, flow rates of the incoming water supply and air-lift pumps were adjusted. At least 1 to 2 times weekly, seed were placed into clean wellers. Frequency of this activity was dependent upon the degree of clogging of the weller screens and blockage of water flow across the screens.

Feeding - During the rearing trials, several feeding regimes were evaluated at the remote setting locations: 1) supplemental feeding with a commercial algal paste, 2) supplemental feeding with cultured live algae, and 3) no supplemental feeding. In the first regime, algal paste was added daily to the remote setting tank to provide a reliable food source. Algal paste, a commercially obtained concentrated culture of microalgae cells, contains a food grade preservative and is stored under refrigerated conditions. The formula used in the remote setting rearing trials contained several species (*Isochrysis galbana*, *Chaetoceros* sp., *Skeletonema* sp., and *Thalassiosira* sp.), with each milliliter of paste containing an equivalent of about 10 billion cells. Pediveligers in the "fed" setting tank were fed daily at a rate of between 20,000 and 50,000 algal cells per milliliter of tank water volume. During feeding, the water flow to the tank was stopped to make efficient use of the addition of supplemental algae. After 1-3 hours, water exchange was initiated and flows adjusted to allow for a rate of 2 to 5 gallons per minute. In the second feeding regime, cultured T-ISO (*Isochrysis galbana* Tahitian strain) was fed daily at approximately the same rate used for the algal paste. The "live" naked flagellate, an excellent food source for larval molluscan shellfish, was produced using standard algal culture techniques. In the third and "unfed" regime, a flow-through system was employed. The flow rate was adjusted between 2 to 5 gallons per minute, resulting in approximately 10 to 25 complete water changes per day. In this feeding regime, the nutrition of the animals was dependent upon the amount of naturally occurring algae present in the water supply source.

Sieving - Clam seed were examined regularly under a microscope to check for set, growth, development, and shell conditions (external fouling). Approximately 14 days after stocking, dependent upon observations and measurements of shell length, post-set clams from each remote set rearing unit were collectively sieved, or size-graded, on a 230 μm mesh screen. Those clams

retained on the sieve were volumetrically measured in a graduated cylinder using a wet packed method. Once sieved, the clams were placed in 200 μm mesh downwellers. Seed from each tank that fell through the sieve screen were volumetrically measured in a graduated cylinder and replaced in 120 μm downwellers for another 7 to 14 days. This process was repeated again during a similar time interval with clams being sieved on a 500 μm mesh screen and then being placed into 425 μm downwellers. The wellers were stocked so that the volume of seed per weller was maintained at less than 100 milliliters. By moving the clams to larger downweller screens as their increased size warranted, screen clogging problems were lessened, allowing for adequate flow of food and oxygen through the seed mass. In the second year of rearing trials, a routine schedule of weekly sieving was employed. In addition, two additional screen sizes were used during the sieving process, 400 and 600 μm mesh, to facilitate a better estimate of seed clams. Estimates were determined by counting three 1-milliliter subsamples (wet packed) per the following screen sizes: 230, 400, 500, and 600 μm mesh. These counts were then averaged with the estimates used by the participating hatcheries. The average counts used in estimating number of clam seed per sieve size are summarized in Appendix A.

Data Collection

Hydrological Conditions - Water temperature (minimum and maximum) and salinity in each remote setting tank were measured and recorded daily. To determine natural phytoplankton abundance, water samples were collected and filtered two or three times weekly from the tanks prior to feeding at each remote setting location. These samples were analyzed for chlorophyll *a* pigment at the University of Florida, Department of Fisheries and Aquatic Sciences using spectrophotometric determination. In addition, water samples were collected and preserved using Lugols solution. From these samples, general phytoplankton composition and abundance, in terms of total biovolume, were determined using light microscopy and the Utermohl method (Utermohl 1958).

Biological Conditions - Observations on clam growth, development, and shell conditions were noted daily for each remote setting tank at each remote setting nursery location. During the weekly or biweekly sieving activities, packed volumes of seed clams from the various sieve sizes were measured. The number and screen size of wellers stocked after each sieve were recorded. Other biological features documented included survival and time to reach a 1-mm shell length clam, the typical seed size presently obtained by land-based nursery operators from a hatchery for land-based nursing. This seed size was determined by using a 780 μm sieve for grading. To assess 1-mm seed production, the total volume of seed sieved and retained on the 780 μm screen was measured in a graduated cylinder per remote setting tank. Three 1-milliliter subsamples (wet packed) were counted to estimate the number of seed produced in the remote setting nursery phase. The count used in estimating the number of 1-mm seed sieved is included in Appendix A.

Operation and Maintenance - Operation and maintenance of the remote setting unit were documented in terms of man-hours. Standardized data sheets were developed and used during the rearing trials to insure that information was recorded in a similar manner at each remote set location (Appendix B).

REMOTE SETTING REARING TRIALS

Over a two-year period, a commercial assessment of remote setting hard clam pediveliger larvae was conducted for evaluation of technique, site location, and seasonal and annual variations. Rearing trials in the field were not designed as replicated experiments but did serve as a large-scale demonstration of remote setting. Thus, the statistical significance between trial run differences was not tested. Results from these trials were used to provide operational guidelines for remote setting of hard clam seed.

Trial Locations

The remote setting rearing trials were performed by three industry project partners in Florida in conjunction with the University of Florida Shellfish Aquaculture Extension Program. One site was located at New Smyrna Beach in Volusia County on the east coast and two sites were located at Cedar Key in Levy County on the west coast. The partners were selected because of the location of their operations with access to high quality seawater. Two remote setting units were installed at the New Smyrna Beach site (NSB) and one of the Cedar Key sites (CK1) allowing for comparison of selected management regimes during the rearing trials. Only one unit was installed at the second Cedar Key site (CK2). These commercial land-based nursery operators were trained to participate in the field trials and further cooperated in the project by devoting a portion of their facilities, equipment, and labor in rearing the clam seed. It must be noted that these operators also exercised their own management styles during the rearing trials.

Spring 2000 Rearing Trials

Pediveliger larvae, or pre-set seed, were shipped from an out-of-state hatchery on May 11, 2000 to New Smyrna Beach. The shipment contained approximately 12 million larvae equally divided into four larval balls. A packed volume measurement of 3 million larvae, ranging in size from 150 to 225 μm shell length, was 17 milliliters, or an equivalent of about 176,500 larvae per ml. On the following day, May 12, two remote setting tanks, each containing three 120 μm downwellers, were stocked with 3 million larvae. The time in shipment, from packaging of larvae at the hatchery to stocking at the NSB remote set site, was 22 hours. The other half of the shipment was then driven to Cedar Key. An equal number of larvae was stocked in two tanks at the CK1 site, resulting in a shipment time of 26 hours. Two feeding regimes were evaluated at both sites (Table 1). Algal paste was used as a supplemental feed in one tank, whereas the other tank operated solely as a flow-through system. The trial was terminated on June 22 at the NSB site, resulting in a 41-day rearing period. At the CK1 site, the trial ran for 57 days, terminating on July 8.

Water quality conditions recorded during stocking of pediveligers were 80° F for water temperatures at both remote setting locations and 34 ppt and 27 ppt for salinities at the NSB and CK1 sites, respectively. Means and standard deviation values of water quality parameters measured during the rearing trials are presented in Table 2. Whereas the mean minimum water temperature (76° F) was similar at both locations, the mean maximum water temperatures differed by 3.6° F. The NSB site averaged 85.3° F over a 41-day period; and, the CK1 site averaged 81.7° F over a 57-day period. Salinities also differed greatly between locations with a

mean of 35.2 ppt obtained at the NSB site and a mean of 29.3 ppt at the CK1 site. Mean chlorophyll *a* values for the NSB site were about half those of the CK1 site, 5.7 µg/l versus 11.7 µg/l, respectively. The total phytoplankton biovolume, in terms of µmeters³ per milliliter, and the top three dominant taxa of phytoplankton for 11 sampling dates at the CK1 site and 1 sampling date at the NSB site are identified in Appendix C (Note: Only one sample at NSB site was properly preserved).

Table 1. Management regimes evaluated during the Spring 2000 remote setting rearing trials.

Site	Dates	Days	Management	
			Ship (hrs)	Feed
NSB	May 12- June 22	41	22	Algal paste
				None
CK1	May 12-Jul 8	57	26	Algal paste
				None

Table 2. Water quality means ± standard deviation values for the Spring 2000 remote setting rearing trials.

Site	Min Temp (°F)	Max Temp (°F)	Salinity (ppt)	Chlor <i>a</i> (:g/l)
NSB	76.5 ±2.3	85.3 ±2.9	35.2 ±1.7	5.7 ±3.8
CK1	76.7 ±2.6	81.7 ±2.6	29.3 ±2.9	11.7 ±6.0

Sieving of post-set clams was initiated on the third and fourth weeks after stocking at the NSB and CK1 trial locations, respectively. Observations at both locations indicated a large percentage of clams died prior to the first sieve. The number of 1- mm seed retained on a 780 µm screen during the first sieve and those sieves conducted on a biweekly basis thereafter is illustrated in Figure 6. At the NSB site, 187,000 1-mm seed were produced after 4 weeks from the tank in which algal paste was added. No additional seed was obtained from this tank with the remaining smaller seed experiencing 100% mortality prior to the next sieve. In the tank which received no supplemental feed, there was no production of 1-mm seed. Again, a large die-off occurred between the fourth and sixth week sieves. At the CK1 site, 18% of the 1-mm seed produced from the tank receiving supplemental feed was sieved up on the fourth week, 57% on the sixth week, and 25% on the eighth week. A total of 501,000 1-mm seed was obtained over this period. Although the distribution of 1-mm seed production from the unfed tank was similar (22% in week 4, 52% in week 6, and 26% in week 8), only about half the amount of 1-mm seed was obtained for a total of 281,000 1-mm seed produced.

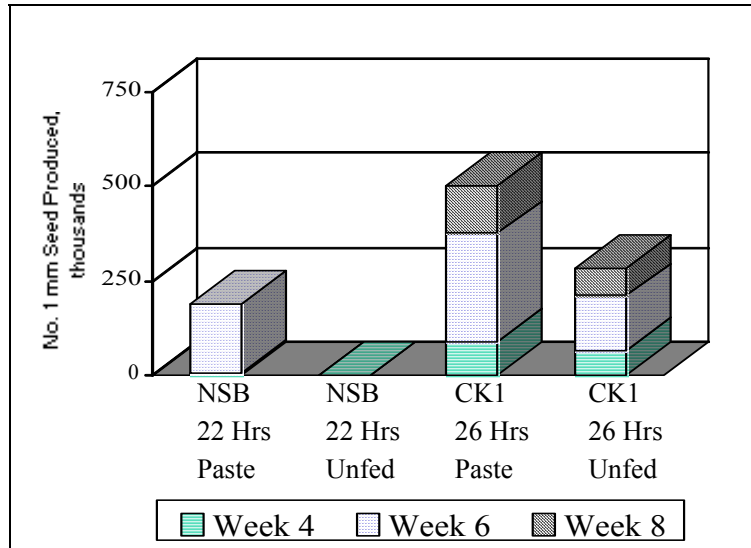


Figure 6. Sieving results and number of 1-mm seed produced during the Spring 2000 remote setting rearing trails.

The production results for the Spring 2000 rearing trial are summarized in Table 3. Survival of clams at setting was not fully determined, but survival estimated at first sieve provided an indication of setting success. This estimate based on clam counts obtained for each sieve size used. At the NSB site, survival to first sieve did not differ greatly between feeding regimes with an average of about 43% estimated on day 21 (week 3). Survival to first sieve was estimated at the CK1 site on day 28 with 50% obtained for post-set clams that were not supplementally fed and 77% for those clams fed with algal paste. Production of 1-mm seed at the NSB site was negligible with only a 6% rate obtained from the tank in which algal paste was used. This production rate was determined by dividing the total number of 1-mm seed sieved during the trial by the number of pediveligers stocked. In the unfed tank, no production was achieved. A 17% production rate was obtained at the CK1 location from the tank supplementally fed with algal paste. About half of that production, or 9%, was obtained from the tank receiving no supplemental feed.

Site differences may have contributed to production discrepancies. Higher water temperatures and salinities, coupled with lower chlorophyll *a* values, experienced at the east coast location could have been stressful to small clam seed. Water temperatures of 92° F and salinities of 37 ppt were recorded during the last 2 weeks of the rearing trials at the NSB site. Regardless of site location, relatively poor performance of the clam seed in the unfed tanks was documented. The addition of algal paste increased seed production by 78%.

Table 3. Production results for the Spring 2000 remote setting rearing trials.

Site	Management		Survival to 1 st Sieve* (%)	1-mm Seed Production (%)
	Ship (hrs)	Feed		
NSB	22	Algal Paste	47	6
		None	39	0
CK1	26	Algal Paste	77	17
		None	50	9

*NSB=Day 21, CK1 = Day 28

Spring 2001 Rearing Trials

A commercial hatchery located on the east central coast of Florida provided clam pediveliger larvae for the Spring 2001 field rearing trails. Approximately 12 million larvae were prepared for shipping to the remote setting nursery locations on April 25, 2001. A packed volume measurement of 3 million larvae, ranging in size from 150 to 210 μm shell length, was 12 milliliters, or an equivalent of about 250,000 larvae per ml. The proximity of this hatchery as well as other hatcheries in Florida to nursery locations prompted an evaluation of shipment times to determine if this variable had an effect on pediveliger survival and seed production. Many nursery operators are within several hours of a hatchery thus reducing delivery time. Two shipping times, 2 hours versus 20 hours, were tested at the NSB site (Table 4). Pediveliger larvae were shipped overnight to Cedar Key resulting in 20 hours from packaging of larvae at the hatchery to stocking at the CK1 site.

Poor performance at the NSB site during the 2000 rearing trials warranted supplemental feeding both remote setting tanks with algal paste. The two feeding regimes, unfed versus supplemental feeding with algal paste, were evaluated again at the CK1 site. The trial was terminated on June 14 at the NSB site, resulting in a 49 to 50-day rearing

period. At the CK1 site, the trial ran longer for 72 days, terminating on July 6. The hatchery operator also participated in these rearing trials with an additional 6 million pediveliger larvae maintained at the hatchery site (HAT). Half of these larvae were stocked directly in downwellers after drain down, resulting in a shipping time of 0 hours. This served as a “quasi” control to determine if handling, refrigeration, and shipping had a deleterious effect on pediveligers. The other half were prepared for shipment and a 20-hour shipping time was simulated. Both batches were maintained until July 5 by the hatchery operator using standard operating procedures. Cultured, or “live,” algae was fed to both batches during this period.

Water quality conditions recorded during stocking of pediveligers were 70° F for water temperatures at both remote setting locations and 34 ppt and 29 ppt for salinities at the NSB and CK1 sites, respectively. Stocking conditions were similar to those of the previous year. Means and standard deviation values of water quality parameters measured during the rearing trials are presented in Table 5. Both mean minimum water temperatures and mean maximum water temperatures differed between sites by 3.6° F and 6.0°F, respectively. Salinities, were, again,

Table 4. Management regimes evaluated during the Spring 2001 remote setting rearing trials.

Site	Dates	Days	Management	
			Ship (hrs)	Feed
HAT	Apr 25 – Jul 5	72	0	Live Algae
	Apr 26 – Jul 5	71	20	
NSB	Apr 26 – Jun 14	50	2	Algal Paste
	Apr 26 – Jun 14	49	20	
CK1	Apr 26 – Jul 6	72	20	Algal Paste
				None

higher at the east coast location with a mean of 37.6 ppt obtained at the NSB site versus a mean of 28.0 ppt at the CK1 site. Chlorophyll *a* values were similar at both sites with means of 8.4 µg/l and 9.4 µg/l obtained. The total phytoplankton biovolume, in terms of µmeters³ per milliliter, and the top three dominant taxa of phytoplankton for 7 sampling dates at the CK1 site and 6 sampling dates at the NSB site are identified in Appendix C. Hydrological conditions were not monitored at the hatchery (HAT) site.

Table 5. Water quality means ± standard deviation values for the Spring 2001 remote setting rearing trials.

Site	Min Temp (°F)	Max Temp (°F)	Salinity (ppt)	Chlor <i>a</i> (:g/l)
NSB	71.9 ±1.8	77.4 ±5.7	37.6 ±2.5	8.4 ±6.0
CK1	75.5 ±5.7	83.4 ±5.3	28.0 ±2.3	9.4 ±3.0

Sieving of post-set clams was initiated earlier in these trials than in the previous year, with the first sieve conducted two weeks after stocking at all sites. One-mm seed were retained on a 780 µm screen during the fourth week for both east coast sites (NSB, HAT) and during the sixth week at the west coast site (CK1). The number of 1- mm seed produced during weeks 4 through 10 is

illustrated in Figure 7. At the NSB site, results from the two remote setting tanks in which shipping times varied from 2 to 20 hours were similar. Over 80% of the 1-mm seed produced were sieved during the sixth week. A total of 532,000 and 436,000 1-mm seed were obtained over a 7-week period for the 2-hour and 20-hour shipping regimes, respectively. At the CK1 site, 16% of the 1-mm seed produced from the tank

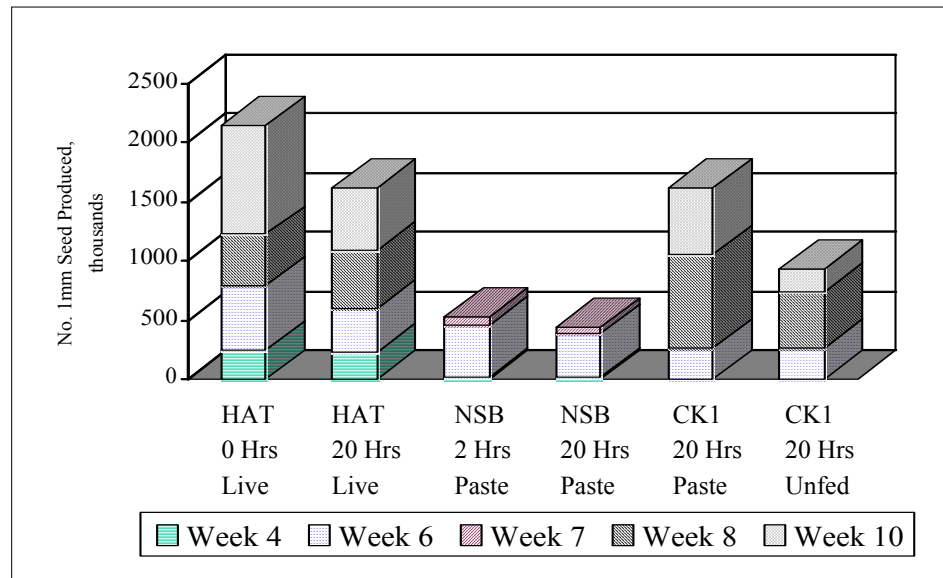


Figure 7. Sieving results and number of 1-mm seed produced during the Spring 2001 remote setting rearing trials.

receiving supplemental feed was sieved up on the sixth week, 49% on the eighth week, and 35% on the tenth week. A total of 1,621,000 1-mm seed was obtained over this period. Although the distribution of 1-mm seed production from the unfed tank was similar (28% in week 6, 51% in week 8, and 21% in week 10), only about half the amount of 1-mm seed was obtained for a total of 933,000 1-mm seed produced. At the hatchery (HAT) site, over 2 million 1-mm seed were sieved up during a 10-week period. The distribution was unlike that observed at other sites with

12% of the seed sieving up on the fourth week, 25% on the sixth week, 20% on the eighth week, and 43% on the tenth week. About 25% less, or 1,613,000 1-mm seed, were obtained during the same time period from the batch of clams that underwent refrigeration and simulated shipment.

The production results for the Spring 2001 rearing trials are summarized in Table 6. At all sites observations of clam pediveliger larvae indicated a high degree of set after stocking with little mortality. Estimates of clam survival to first sieve were similar for remote setting nursery locations and management regimes evaluated, ranging from 50 to 67% on day 16. This estimation of survival was not determined at the hatchery site. Production of 1-mm seed at the NSB site was more than twice that achieved in the first year trials, yet production continued to lag behind the CK1 site. No differences in seed production rates, 14% versus 18%, were associated with duration of shipping times (2 versus 20 hours, respectively). Production of 1-mm seed at the CK1 site was more than three times that achieved in the first year trials. A 54% production rate was obtained from the tank supplementally fed with algal paste. About half of that production, or 25%, was obtained from the “unfed” tank. Site differences may have again contributed to production discrepancies. Salinities of 40 ppt and greater were recorded at the NSB location during the last two weeks of the rearing trial. Nonetheless, experience gained by nursery operators during the previous trials, as well as different sources of seed stocks, may have influenced the overall higher production rates attained. At the hatchery (HAT), 71% of those pediveligers stocked and reared using standard hatchery techniques reached a 1-mm seed size. The production rate for the batch of seed that underwent refrigeration and simulated shipment was 25% lower, or 54%. This was the same rate (54%) obtained from the supplementally fed tank at the CK1 nursery site indicating that both algal paste and natural phytoplankton composition at that site provided adequate nutrition to pre- and post-set clams.

Table 6. Production results for the Spring 2001 remote setting rearing trials.

Site	Management		Survival to 1 st Sieve* (%)	1-mm Seed Production (%)
	Ship (hrs)	Feed		
HAT	0	Live Algae	---	71
	20		---	54
NSB	2	Algal Paste	67	18
	20		50	15
CK1	20	Algal Paste	65	54
		None	58	25

*NBS, CK1=Day 16

Fall 2001 Rearing Trials

Another commercial hatchery located on the east central coast of Florida provided clam pediveliger larvae for the Fall 2001 field rearing trials. Approximately 9 million larvae were prepared for shipping to the remote setting nursery locations on September 7, 2001. A packed volume measurement of 3 million larvae, ranging in size from 215 to 235 µm shell length, was 10 milliliters. This was equivalent to about 300,000 larvae per ml. Six million pediveliger larvae

were delivered to New Smyrna Beach and stocked into two remote setting tanks the following day, September 8, after 20 hours of shipping time (Table 7). The remaining larvae were stocked the same time (20 hours shipment) at a second Cedar Key site (CK2). The nursery operator participating in the Spring trials did not run his facility in the fall so another remote setting unit was set up at CK2.

Table 7. Management regimes evaluated during the Fall 2001 remote setting trials.

Site	Dates	Days	Management	
			Ship (hrs)	Feed
NSB	Sep 8 – Oct 15	37	20	Algal Paste
			20	Algal Paste
CK2	Sep 8 – Nov 3	56	20	Algal Paste

Another feeding regime was evaluated during this rearing trial. The nursery operator in New Smyrna Beach had set up an algal culture rearing system at his facility during the summer. Hence, the use of “live” cultured algae, specifically T-ISO (*Isochrysis galbana* Tahitian strain), was compared as a supplemental feed with algal paste. Only the supplemental feeding regime with algal paste was evaluated at the CK2 site. The trial was terminated on October 15 at the NSB site, resulting in a 37-day rearing period. At the CK2 site, the trial ran longer, terminating on November 3 after 56 days.

Water quality conditions recorded during stocking of pediveligers were 28.5 ppt salinity at both remote setting locations and 75° F and 72° F water temperatures at the NSB and CK2 sites, respectively. Means and standard deviation values of water quality parameters measured during the rearing trials are presented in Table 8. Minimum and maximum water temperatures at the NSB site were within the range of those means obtained previously during the Spring rearing trials. Water temperatures at the CK2 site were substantially lower than those in the Spring trials, with means differing by 3 to 7.4° F. During the last week of the rearing trial at CK2, water temperatures dropped below 60° F. Salinities at the NSB site were the lowest experienced throughout the 2-year study and were similar to those of the CK2 location (mean of 26.6 ppt versus 27.4 ppt). Chlorophyll *a* values at the CK2 site were 30 to 60% higher than values obtained during the Spring trials. Water samples were not collected at the NSB site for chlorophyll *a* determinations.

Table 8. Water quality means ± standard deviation values for the Fall 2001 remote setting rearing trials.

Site	Min Temp (°F)	Max Temp (°F)	Salinity (ppt)	Chlor <i>a</i> (:g/l)
NSB	73.7 ±3.4	80.1 ±4.0	26.6 ±1.6	---
CK2	69.3 ±6.0	78.7 ±6.1	27.4 ±2.3	15.2 ±5.4

Sieving of post-set clams was initiated two weeks after stocking at both remote setting sites. One-mm seed were retained on a 780 µm screen beginning the fifth week at the NSB site and the fourth week at the CK2. The number of 1-mm seed produced during weeks 4 through 8 is illustrated in Figure 8.

At the NSB site, about half of the 1-mm seed production (49%) from the tank that was supplementally fed with algal paste was sieved on week 5. The other half (51%) sieved up the following week for a total of 396,000.

Although sieving results from the tank that was supplementally fed with “live” algae were similar (61% in week 5 and 39% in week 6), the number of 1-mm seed

produced almost doubled with 669,000 obtained in that time period. Additional seed may have been produced if the nursery operator extended the rearing trials beyond 6 weeks, but the facility was in the process of closing for the winter and the study was terminated. At the CK2 site, 26% of the 1-mm seed produced from the tank receiving algal paste was sieved on the fourth week, 18% on the fifth week, 30% on the sixth week, and 26% on the eighth week. A total of 1,218,000 1-mm seed was obtained over this period.

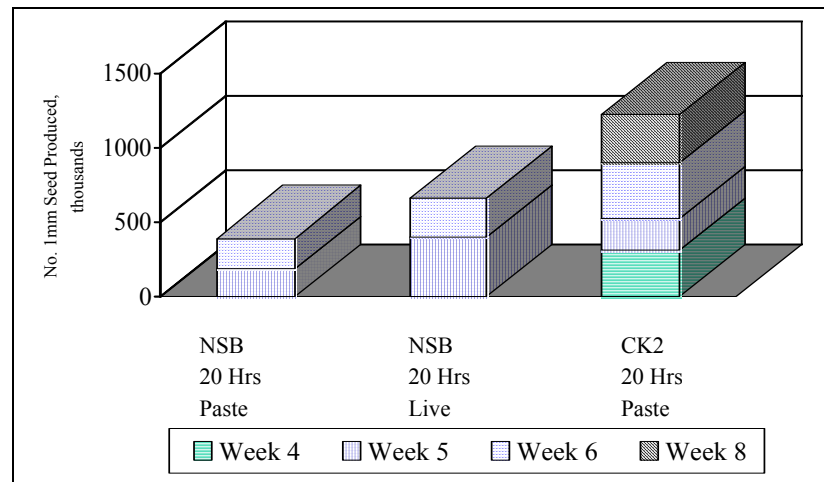


Figure 8. Sieving results and number of 1-mm seed produced during the Fall 2001 remote setting rearing trials.

The production results for the Fall 2001 rearing trials are summarized in Table 9. Observations after stocking indicated all pediveligers had set with no mortality. Survival was estimated on day 23

at the NSB site and on day 12 at the CK2 site. Survival of post-set clams was higher than in previous trials, ranging from 70 to 88%. Production of 1-mm seed, based on a percentage of pre-set clams stocked, was 13% for the

Table 9. Production results for the Fall 2001 remote setting rearing trials.

Site	Management		Survival to 1 st Sieve* (%)	1-mm Seed Production (%)
	Ship (hrs)	Feed		
NSB	20	Algal Paste	70	13
		Live Algae	83	22
CK2	20	Algal Paste	88	41

*NSB=Day 23, CK2=Day 12

algal paste-fed tank at the NSB site. This production rate (15%) was similar to that obtained during the Spring trial at the same site. Supplemental feeding with cultured “live” algae increased production by 70% with a rate of 22% obtained. Production at the CK2 site using algal

paste was over twice that obtained for the same feeding regime on the east coast and 80% greater than the cultured algae feeding regime (41%). Even though salinities were similar during the fall trials, site differences still prevailed. Management styles may have also contributed to these continuing differences. For example, the east coast nursery operator employed an aggressive culling practice during sieving, discarding clams from the smaller sieve sizes on a routine basis.

FINANCIAL ANALYSIS

To date there are no studies that provide an assessment of the financial characteristics of using remote setting technology for hard clams. Previous studies have addressed the use of remote setting techniques for oysters (Supan et al. 1999; Bohn et al. 1995). This analysis provides hard clam nursery operators with the basic information with which to assess the financial merit of adopting remote setting technology for the production of hard clam seed.

In this analysis, the financial characteristics of the remote setting facility are described. The initial investment required to construct a pilot-scale system are estimated. The operational costs, such as supplies, variable, and overhead expenses, are estimated. These costs are then combined into a total cost estimate that is expressed on a per unit of production basis. For the purposes of this analysis, the production unit is assumed to be 1,000 post-set, 1-mm seed clams. The production unit is an industry standard by which post-set seed clams are purchased on the commercial market. The estimated per unit production cost is compared to the current unit market price, providing an assessment of the financial feasibility of investing in the remote setting technology as opposed to purchasing seed on the open market. The risk associated with seed production and the clam seed market are not explicitly included in the analysis.

Production Assumptions

The remote setting production facility addressed in the financial analysis is assumed to consist of a single-tank system. This system is assumed to be utilized for either one or two production runs during a given year. This system is assumed to be stocked with approximately 3,000,000 pediveliger clam larvae during each production run. The larvae are to receive a supplemental feeding with a commercially prepared algal paste. Given the results of the trial runs completed during the course of this study, the production rate (i.e., from pediveliger larvae to 1-mm post-set clam seed) during a run is assumed to be an average of 37%. This was the average production rate obtained from the Cedar Key trials (n=3) in which algal paste was used. The quality of the 1-mm clam seed produced by the remote set system is assumed to be equivalent to that of clam seed purchased from existing commercial hatcheries. Future grow-out studies that track remote setting-derived clam seed with commercially purchased clam seed are needed to support or refute this assumption.

Initial Investment Requirements

The remote setting system described by the financial analysis is of a size thought to be sufficient for a hard clam grower of average size in Florida (i.e., an average size grower in Florida may require from 1 to 2 million seed of a field plantable size per year). In addition, the remote setting system is assumed to be incorporated into a pre-existing shore-based nursery

operation, common to hard clam growers in Florida. This pre-existing nursery operation is assumed to possess some excess capacity in terms of space and pumping capacity. The relatively minimal space (4' X 8' X 2') and pumping requirements (2-5 gpm) of the proposed one-tank remote setting system are thus assumed to be available to most existing nursery operations. Many existing hard clam nursery systems in Florida consist of fully plumbed, multi-tiered trays, which may be altered to accommodate a single remote-setting tank. Thus, the costs associated with space and water pumping are not included in the analysis. If excess capacity for space and pumping does not exist within the existing nursery operation, or if a grower wishes to invest in a remote setting facility but does not already own a shore-based nursery operation, then the investment costs associated with a small pole shed, electrical hookups, pump, plumbing and other items would need to be included. However, these costs are not included in the following financial analysis. The total initial investment requirement for the one-tank remote setting system is \$3,007 (Table 10). The single largest cost category is the wellers (approximately 1/3 of the total cost). The 250-gallon tank, with wooden support frame and plumbing, is \$475. The analysis assume that the initial cost is paid by the operator without the use of investor capital or a bank loan.

Operational Expenses

A number of expenses were incurred in conducting the trial runs with the remote setting system. These trial runs were conducted in a manner similar to what would be done by a commercial nursery while adopting the remote setting technology. Thus, the expenses are anticipated to be similar to what should be expected during the commercial operation of a one-tank, remote setting facility.

Table 10. Initial investment requirements for one-tank remote setting system.

Item	Cost
Fiberglass tank (250 gallon) w/support and plumbing	\$475
Sand filter	\$200
Bag filters and adaptor	\$122
Air compressor (w/plumbing & accessories)	\$360
Wellers	\$1,020
Sieves	\$120
Dissecting scope	\$400
Refractometer	\$165
Miscellaneous supplies	\$145
	\$3,007

The expenses are separated into two categories: supplies and variable/overhead expenses. Supplies are initially described in Table 11.

Table 11. Supplies for one-tank remote setting system: one and two runs per year.

Item	Units/Run	\$/Unit	Runs per Year	
			One	Two
Algae paste	1 liter	\$145/liter	\$145	\$290
Filter sand	100 lbs	\$5/50 lbs	\$10	\$20
Filter gravel	25 lbs	\$6/50 lbs	\$3	\$6
Air valves	15	\$1	\$15	\$30
Air stones	50	\$0.55	\$28	\$56
Airline tubing	100' coil	\$13/coil	\$13	\$26
TOTAL			\$214	\$428

The variable costs (i.e., those costs that vary directly with the number of pediveliger larvae stocked) are described along with the overhead expenses in Table 12.

Table 12. Cost budget for one-tank remote setting system.

Item	Units/Run	\$/Unit	Runs per Year	
			One	Two
Larvae	3 million	\$125/million	\$375	\$750
Supplies			\$214	\$428
Labor	104 hours	\$5.15/hr	\$536	\$1,072
Elec. Utilities	403 KwH	\$0.085/KwH	\$34	\$68
TOTAL COST			\$2,097	\$3,256
1-mm seed produced (37% survival)			1,110,000	2,220,000
Cost/1000 seed (w/labor)			\$1.88	\$1.47
Cost/1000 seed (w/o labor)			\$1.41	\$0.97

Supplies - These expenses are computed on the basis of one and two runs per year (Table 11). The expense categories include algae paste, filter sand, filter gravel, air valves, air stones, and airline tubing. The single largest supply cost is algae paste. A single liter of the paste is utilized, at a cost of \$145 per run. The algae paste accounts for approximately two-thirds of the supply costs. The supply costs for a single run is \$214, while the supply costs for the two run system is double that cost estimate.

Variable / Overhead Expenses - The variable expenses estimated for the system include pediveliger larvae, labor and electrical utilities (Table 12). The larvae are assumed to be purchased on the commercial market for \$125 per million. This price was derived by contacting several commercial molluscan shellfish hatcheries on the east and west US coasts. Currently, there is no commercial hatchery consistently producing hard clam (*Mercenaria mercenaria*) pediveliger larvae in large quantities for commercial sale to remote set facilities. However, some of these hatcheries are producing manila clam and oyster larvae. The hatchery techniques are similar, thus the production costs are assumed to be similar. Interviews with hatchery operators provided an estimate of the projected market price of hard clam pediveliger larvae per million. This price may change as the market for larvae develops. However, the price of \$125 per million is felt to be appropriate.

Given that the tank would be stocked at a density of three million larvae, the larvae cost per run is estimated to be \$375. Records kept during the project trial runs provided an estimate of the labor requirements associated with the various tasks performed during a remote setting run. These records suggest that a remote setting run would require approximately 104 hours of labor. This analysis assumes that labor can be hired for \$5.15 per hour at the minimum wage. Thus, the labor cost per run is estimated to be \$536. The electrical utility cost is associated with the use of the air compressor and lighting. The total electrical usage for a single run is estimated to be 403 kilowatt hours (KwH). The cost per KwH is determined to be \$0.085. Thus, the electrical expense associated with a single remote setting run is estimated to be \$34. The single “overhead” cost is depreciation. Given the economic life associated with each of the capital

investment items in Table 10, the annual depreciation is assumed to be \$938 (assuming straight-line depreciation and a zero salvage value). This value is held as a constant regardless of whether one or two runs per year are attempted. Thus, the total variable/overhead cost is estimated to be \$2,097 and \$3,256 for one and two runs, respectively.

Cost Per Unit of Production

The initial stocking density in the single remote setting tank was 3,000,000 pediveliger larvae. Assuming a 37% production rate from pediveliger larvae to 1-mm seed clams, approximately 1,110,000 seed were produced during a single run (2,220,000 during two runs). Given that total costs for a single run was \$2,097, the cost per 1,000 seed clams is estimated to be \$1.88 (Table 12). An estimate of the single-run cost per 1,000 clams is also generated excluding labor costs (\$1.41) for those nursery operators who may wish to either not hire extra labor or not count their own labor costs. However, excluding any labor costs will lead to an underestimation of the true costs of operating a remote set facility. The costs per 1,000 seed clams for two runs per year is estimated to be \$1.47 (including labor) and \$0.97 (excluding labor).

Financial Feasibility

Cost Savings - The current expected market price for purchasing 1-mm seed clams is \$3 per 1,000. Given the cost estimates of producing 1-mm seed clams with the remote setting technology, a cost savings can be achieved. For example, the cost (labor included) of producing 1,000 1-mm seed clams in a single run system is estimated to be \$1.88. This represents a savings of 37%. The cost savings associated with a two-run system is estimated to be 51%. The total annual seed cost savings (labor included) associated with the remote setting system for a one and two-run production year is \$1,243 and \$3,397, respectively. These values were computed by multiplying the 1,000 seed clam units produced in the one (1,110) and two-run (2,220) options by the respective cost savings. For example, the cost savings associated with the two-run option is \$1.53 (\$3.00 minus \$1.47). Given that the initial cost of the constructing the one-tank system is \$3,007, the two-run option allows the initial investment cost to be totally recovered by savings during the first year of operation.

Break-Even Considerations - The potential investor in a remote set facility will also want to know at what point the cost of pediveliger larvae become cost prohibitive. The larvae cost, while not the largest single expense, may well be the most volatile in the near future. This is due to the current absence of a viable source and market for mass produced hard clam larvae. If the larvae cost increases, at what point will the remote setting technology no longer be financially viable? In other words, at what price per million for pediveliger larvae does a potential investor become indifferent to using the remote setting technology if the market price for 1-mm hard clam seed is \$3 per thousand? Under the assumptions utilized in the preceding analysis, the break-even cost per million for pediveliger larvae is \$536 and \$692 for one and two runs, respectively, when the cost of labor is included. Thus, if the cost per million of pediveliger larvae increased to over \$692, then there would be no cost savings associated with producing 1-mm seed clams with the remote setting system. Similarly, the break-even cost per million for pediveliger larvae is \$715 and \$871 for one and two runs, respectively, when the cost of labor is excluded.

Minimum Production Rate - The analysis utilized an average production rate of 37%. In this case, “production” is defined as the estimated percentage of pediveliger larvae that survive to 1-mm seed size. At this level of production, the system generated cost savings. However, if the production rate was inadvertently reduced, these savings also would be reduced. At some lower level of production, the cost savings would be reduced to zero. The break-even production rate would be defined as that rate of survival (from pediveliger larvae to 1-mm seed clams) for which the cost of raising 1,000 1-mm seed clams in the remote set system would just equal the current market price of 1,000 1-mm seed clams purchased from a commercial hatchery. The break-even production rate for the one-tank system with one and two runs per year would be 23% and 18%, respectively (labor cost included). The break-even production rate for the one and two runs per year without labor included would be 17% and 12%, respectively.

Summary of Financial Analysis

The financial analysis suggests that the remote setting technology when employed at a relatively modest scale (e.g., a one-tank system) generates a seed cost savings compared to purchasing 1-mm seed clams from a commercial hatchery. When operating the system for two runs, the system produces 2.22 million 1-mm seed clams at a cost of \$1.47 per 1,000. This represents a cost savings of \$1.53 per 1,000 seed when compared to the current market price of \$3.00 per 1,000 1-mm clam seed if purchased from a commercial hatchery. In addition, the initial cost is recovered from these cost savings during the first year of operation. The survival rate in the remote setting system would have to fall from the assumed level of 37% to below 18% for the system to not be able to provide cost savings. However, a risk does exist in that no commercial source of hard clam pediveliger larvae is currently available on a consistent basis. The analysis assumes that 1 million pediveliger larvae would be available from a commercial hatchery at a cost of \$125. This price may change as this market develops. However, the price per 1 million pediveliger larvae would have to increase to over \$692 for the operator to be indifferent to using the remote setting technology versus buying the 1-mm seed at the current price of \$3.00 per 1,000.

SUMMARY

Until the work conducted at the National Marine Fisheries Service center in Milford, Connecticut during the 1960s (Loosanoff and Davis 1963), mass culture of hard clam larvae and juveniles was not possible. Since then, hatchery techniques have become standardized. Increased numbers of commercial hatcheries producing seed have enabled clam aquaculture to become established in every state along the east coast of the United States. Recent efforts in shellfish aquaculture technology transfer programs focusing on economic development in fishery dependant communities in Florida and other states have furthered the expansion of this industry. These successful training programs have resulted in creating a demand for seed which exceeds the available supply from existing hatcheries. Given the increased demand for seed, the information on alternative hatchery and nursery protocols obtained by this study is necessary for the future growth of this industry.

Technical procedures were developed and demonstrated during 2000-01 to determine the feasibility of transferring remote setting technology from the Pacific Northwest molluscan

shellfish industry to the hard clam culture industry in Florida. Competent pediveliger larvae obtained from commercial hatcheries were refrigerated, stored overnight, and delivered chilled to remote setting locations for evaluation of technique, site, seasonal and annual variations involved in growing these to 1-mm seed size. Participating land-based nurseries were modified to incorporate mechanical filtration of water supply, remote setting tanks, and downwellers. Management regimes evaluated in large-scale, field rearing trials included supplemental feeding with a commercial algal paste or cultured algae versus none, and duration of shipping times. Biological features documented included survival and time to reach a 1-mm seed, the minimum size presently obtained by nursery operators. The following results, summarized from these rearing trials, provide for operational procedures and guidelines for remote setting of hard clam seed (Figure 9).



Figure 9. Production of 1-mm hard clam seed from pediveliger larvae in a remote setting tank unit.

1. In selecting a location for a remote setting facility, the site would require a water supply source in which salinities were stable, optimally ranging from 25 to 30 ppt, with natural phytoplankton abundance reaching 10 $\mu\text{g/l}$ of chlorophyll *a* pigment.
2. Procedures for harvesting, handling, and packaging hard clam pediveliger larvae for shipment were developed. Pediveligers, or pre-set clams, were refrigerated and shipped up to 26 hours without detrimental effects to setting.
3. Varying shipping times from 2 hours to overnight delivery (20 hours) in rearing trials ($n=2$) resulted in negligible differences (2%-17%) in estimated survival of post-set clams to first sieve.

4. Setting success was not fully determined in this study, but survival at first sieve averaged 63% in all rearing trials (n=11), ranging from 40 to 88% per trial.
5. Production to a 1-mm seed size, based on a percentage of pediveligers stocked, ranged from 0 to 17% over a 6 to 8-week period during the Spring 2000 trials. During the Spring 2001 trials, production to a 1-mm seed size ranged from 15 to 54% over a 7 to 10-week period. In the Fall 2001 trials, production of 1-mm seed ranged from 13 to 41% over a 5 to 8-week period.
6. Production rates to a 1-mm seed size averaged 20% for all field rearing trials (n=11), ranging from 0 to 54% per trial.
7. Variability of results was due to several factors in this study including seed source, site location, and management practices. For example, overall production attained in trials conducted at the west coast sites (n=5) in Cedar Key, was 142% greater than that attained at the east coast site (n=6) in New Smyrna Beach, or an average production rate of 29% versus 12%. In addition, the culling practices conducted by the east coast nursery operator during sieving contributed to shorter nursery periods than corresponding Cedar Key trials. Duration of trials differed from 2 to 3 weeks per location. Certainly this management practice could have contributed as well to the lower production of 1-mm seed at the east coast site.
8. Variability of results was not due to seasonal differences in this study. During 2001, the overall production rate attained in the Spring trials (n=4) averaged 28%; whereas, production in the Fall trials (n=3) averaged 25%.
9. Addition of food was necessary to achieve adequate survival to a 1-mm seed size. Production was increased by 89% to 116% in those trials (n=3) in which the use of algal paste was compared to no supplemental feeding regime. In trials (n=3) which "live" cultured algae was compared to algal paste as a supplemental food, production was increased from 0 to 260%. These differences may also have been influenced by site location.
10. Technical procedures developed for remote setting of hard clam pediveliger larvae are not beyond the capabilities of most nursery operators.
11. Given current and assumed market conditions, the adoption of remote setting technology generates cost savings when compared to the purchase of 1-mm seed from commercial hatcheries.

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Appendix A

Estimated Counts of Hard Clam Post-set Seed

Sieve Size (:m)	Number/milliliter (packed wet volume)
>230	25,000
>400	7,000
>500	4,500
>600	3,000
>780	1,500
>1,000	1,200

Appendix B

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REMOTE SETTING CHECKLIST

Daily - AM:

1. Measure and record maximum/minimum water temperatures from tank.
2. Measure and record salinity from tank.
3. Backflush sand filter.
4. Turn off water flow to tanks and drain. Do one tank at a time.
5. Rinse seed in wellers on a daily basis. Use saltwater for clams in 120 μm and 200 μm wellers and freshwater in 425 μm and 710 μm wellers.
6. Clean tanks with freshwater.
7. Replace filter bags with clean ones.
8. Refill tanks.
9. Adjust airlift flows.
10. Adjust water flow (2-5 gpm) to unfed tank. Stop flow to fed tank once filled.
11. Add algal paste and cultured algae to the fed tank(s).

Daily - PM:

1. Check seed under microscope and record observations.
2. Check airlift flows.
3. Backflush sand filter if needed. Change bag filter on unfed tank if needed.
4. Adjust water flow (2-5 gpm) to fed tank(s).

Weekly:

1. Two to three times weekly, collect water sample from unfed tank or fed tank prior to feeding, preserve with Lugols solution. Store in dark place.
2. Two to three times weekly, collect water sample and filter. Store filter in desiccant bottle and freeze.
3. Two to three times weekly, switch wellers and airlifts with clean ones.

Remote Set Trial _____ Week of _____ to _____, 20__

MONITORING	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Daily:							
Water Temperature (min – °F)							
Water Temperature (max – °F)							
Salinity (ppt)							
Weekly:							
Volume water filtered for chlorophyll (ml)							
Time (minutes)							
INITIALS:							
MAINTENANCE	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Daily:							
Sand filter backflushed / Filter bags switched							
Wellers rinsed / Tanks rinsed and cleaned							
Check airlift flows and water flow in unfed tank							
Algal paste fed (volume)							
Time (minutes)							
INITIALS:							
Weekly:							
Wellers and airlifts changed - 2 to 3X/week							
Clean dirty weller and airlift systems – 2 to 3X/week							
Time (minutes)							
INITIALS:							

Remote Set Trial _____ Week of _____ to _____, 20__

INSPECT SEED – DAILY OBSERVATIONS:

Day 1

Day 2

Day 3

Day 4

Day 5

Day 6

Day 7

OTHER OBSERVATIONS:

Remote Set Location _____ Tank Regime _____

Week _____ Date _____

BIWEEKLY SIEVE DATA:			
Sieve Size (microns)	Volume (mls)	Number of Wellers	Comments
< 230 μ		Weller Size: 120 μ	
> 230 μ		Weller Size: 200 μ	
> 400 μ		Weller Size: 200 μ	
> 500 μ		Weller Size: 425 μ	
> 600 μ		Weller Size: 425 μ	
> 780 μ		Weller Size: 710 μ	
TOTAL			
OTHER OBSERVATIONS:			

Appendix C

Phytoplankton Analyses Spring 2000 Rearing Trials

Site: Cedar Key (CK1)

Sample Date	Biovolume, $\mu\text{meters}^3/\text{ml}$	Top Phytoplankton Taxa, species
6/1/00	989,598	<i>Licmophora gracilis</i> <i>Thalassionema nitzchoides</i> <30 μ Centric diatom 30 μ
6/4/00	818,401	<i>Protoperidinium quinquecorne</i> <i>Licmophora gracilis</i> <i>Nitzchia</i> spp.
6/10/00	2,582,964	<i>Thalassionema nitzchoides</i> <30 μ <i>Amphiprora</i> spp. Centric diatom 10 μ
6/12/00	764,948	<i>Thalassionema nitzchoides</i> <30 μ Pennate diatom >5 μ <15 μ <i>Amphiprora</i> spp.
6/14/00	844,278	<i>Amphiprora</i> spp. Centric diatom 10 μ <i>Asterionellopsis glacialis</i>
6/17/00	829,212	<i>Amphiprora</i> spp. <i>Asterionellopsis glacialis</i> Pennate diatom >15 μ <25 μ
6/19/00	853,733	<i>Pleurosigma</i> / <i>Gyrosigma</i> <i>Navicula</i> spp. <i>Thalassionema nitzchoides</i> <30 μ
6/20/00	241,175	<i>Thalassionema nitzchoides</i> <30 μ Centric diatom 10 μ <i>Nitzchia closterium</i>
6/24/00	4,770,783	<i>Amphiprora</i> spp. Centric diatom 10 μ <i>Thalassionema nitzchoides</i> <30 μ

**Phytoplankton Analyses
Spring 2000 Rearing Trials (continued)**

Site: Cedar Key (CK1)

Sample Date	Biovolume, $\mu\text{meters}^3/\text{ml}$	Top Phytoplankton Taxa, species
7/01/00	317,019	Pennated diatom $>5\mu <15\mu$ Centric diatom 10μ <i>Thalassiosira</i> 10μ
7/04/00	1,760,811	<i>Thalassionema nitzchoides</i> $<30\mu$ Centric diatom 40μ <i>Thalassiosira</i> spp.

Site: New Smyrna Beach (NSB)

Sample Date	Biovolume, $\mu\text{meters}^3/\text{ml}$	Top Phytoplankton Taxa, species
6/19/00	3,685,043	<i>Chaetoceros</i> sp. 15μ <i>Chaetoceros</i> sp. 10μ <i>Protoperidinium quinquecorne</i>

**Phytoplankton Analyses
Spring 2001 Rearing Trials**

Site: Cedar Key (CK1)

Sample Date	Biovolume $\mu\text{meters}^3/\text{ml}$	Top Phytoplankton Taxa, species
4/28/01	593,378	<i>Leptocylindrus danicus</i> Centric diatom 25 μ <i>Thalassiosira</i> sp.
5/5/01	1,823,734	<i>Amphiprora</i> c.f. * <i>Thalassiothrix faunenfeldii</i> <i>Navicula</i>
5/12/01	3,072,507	<i>Amphiprora</i> c.f.* Centric diatom 10 μ <i>Paralia sulcata</i>
5/17/01	4,215,804	<i>Amphiprora</i> c.f. * <i>Thalassiosira</i> Centric diatom 10 μ
5/24/01	352,595	<i>Protoperidinium quinquecorne</i> ¹ <i>Amphiprora</i> c.f. <i>Scrippsiella trochoidea</i>
6/5/01	752,201	<i>Protoperidinium quinquecorne</i> ¹ <i>Paralia sulcata</i> <i>Navicula</i> sp.
6/18/01	3,781,809	<i>Protoperidinium quinquecorne</i> * ¹ <i>Thalassiosira nitzchoides</i> <i>Heterocapsa niei</i>

* Denotes individual taxa that have a biovolume greater than $10^6 \mu\text{m}^3/\text{ml}$.

¹ Species included on Florida Marine Research Institute Harmful Algal Blooms Species of Interest.

**Phytoplankton Analyses
Spring 2001 Rearing Trials (continued)**

Site: New Smyrna Beach (NSB)

Sample Date	Biovolume $\mu\text{meters}^3/\text{ml}$	Top Phytoplankton Taxa, species
4/30/01	4,733,532	<i>Gymnodinium</i> sp. <10 μ * <i>Navicula</i> sp. <i>Melosira</i> sp.
5/3/01	292,285	<i>Gymnodinium</i> sp. <10 μ <i>Protoperidinium</i> sp. <i>Protoperidinium quinquecorne</i> ¹
5/6/01	224,847	<i>Protoperidinium quinquecorne</i> ¹ <i>Protoperidinium</i> sp. <i>Gymnodinium</i> sp.10 μ
5/9/01	448,011	<i>Protoperidinium</i> sp. <i>Pleurosigma/Gyrosigma</i> <i>Gymnodinium</i> sp.10 μ
5/15/01	1,298,219	<i>Protoperidinium quinquecorne</i> ¹ Pennate diatom >5 μ <25 μ <i>Navicula</i> sp.
5/23/01	1,178,253	<i>Scrippsiela trochoidea</i> Pennate diatom >25 μ <50 μ <i>Protoperidinium quinquecorne</i> ¹

* Denotes individual taxa that have a biovolume greater than 10⁶ $\mu\text{m}^3/\text{ml}$.

¹ Species included on Florida Marine Research Institute Harmful Algal Blooms Species of Interest.



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