File Code: F2389-03-06-F

Project Title: Restoration of bay scallop (*Argopecten irradians*) populations on the west coast of Florida.

Authors: William S. Arnold[1], Stephen P. Geiger[1], Melanie L. Parker[1], Sarah P. Stephenson[1], Ami E. Wilbur[2]

[1]Florida FWCC Fish and Wildlife Research Institute, 100 Eighth Avenue SE, St. Petersburg, Florida 33701

[2]Center for Marine Science, University of North Carolina Wilmington, 1 Marvin Moss Lane, Wilmington, North Carolina 28409

Grant Number: NA03NMF4270093

Award Period: September 1, 2003 through August 31, 2006

Report Date: December 31, 2006

ABSTRACT

The goals of this project were to 1) continue implementing methods for restoration of bay scallop (*Argopecten irradians*) populations at four sites (Crystal River, Homosassa, Anclote, and Sarasota Bay) along the west coast of Florida, 2) select a restoration site in Sarasota Bay using the results of a physical oceanographic model as a guide, and 3) determine if cultured bay scallops deployed into cages in a field setting produced eggs of similar quality to that of their wild counterparts. To accomplish the first goal, adult scallops were collected from the field, induced to spawn, and their offspring raised to at least one-mm shell height (SH) in a commercial aquaculture facility. The resulting juvenile scallops were raised to 18mm SH in mesh bags that were deployed in local waters and then planted into cages at the chosen restoration sites during spring 2005. Their growth, mortality, and reproductive development were monitored through the fall 2005 spawning season. To accomplish the second goal, baseline data regarding water level, wind, river discharge, precipitation, and evaporation were collected from Sarasota Bay (see Appendix 1 for details) and used to calibrate a physical oceanographic model of that bay. From those model results, predictions of the dispersal patterns of particles representing passive bivalve larvae were obtained and used for selection of release sites for adult and larval scallops. To accomplish the third goal, various biochemical analyses were conducted on caged scallops and on wild scallops collected contemporaneously and within the vicinity of the scallop cages. The overall results of the study were mixed. Scallop production was compromised by the vagaries of hatchery production. Those scallops that were successfully planted into cages grew more slowly than their wild counterparts but survived at a higher rate and did appear to produce eggs of equivalent quantity and quality. In Sarasota Bay, the output from the hydrodynamic simulations provided useful predictions as to where offspring would be expected to settle, and assessment results suggest that those predictions were valid.

EXECUTIVE SUMMARY

Bay scallops (*Argopecten irradians*) support a culturally and economically important recreational fishery along the west coast of Florida, but many of the local scallop populations that support this fishery have been severely stressed in recent decades. This project was designed to implement and test aquaculture-based strategies to rebuild bay scallop populations that occupy coastal waters in or near Crystal River, Homosassa, Anclote, and Sarasota Bay, Florida.

Adult scallops were harvested from wild populations and induced to spawn in the laboratory. Offspring were raised in a commercial hatchery to at least one-mm SH. They were then planted into mesh bags that were deployed in local waters (generally Bayboro Harbor in downtown St. Petersburg or Anclote Harborage near Tarpon Springs) until they reached at least 18 mm SH, at which time they were planted into cages at the targeted restoration sites during spring 2005. Planted scallops grew to adult size throughout the following summer and spawned during fall. Growth, mortality, reproductive development, and biochemical composition were monitored during the growout phase, and those results were compared with similar data obtained from wild bay scallops that were harvested from the vicinity of the restoration sites. Following complete loss of the stock targeted for planting in Sarasota Bay, we implemented a novel restoration strategy that utilized the larval stage and did not require supervised laboratory or field growout.

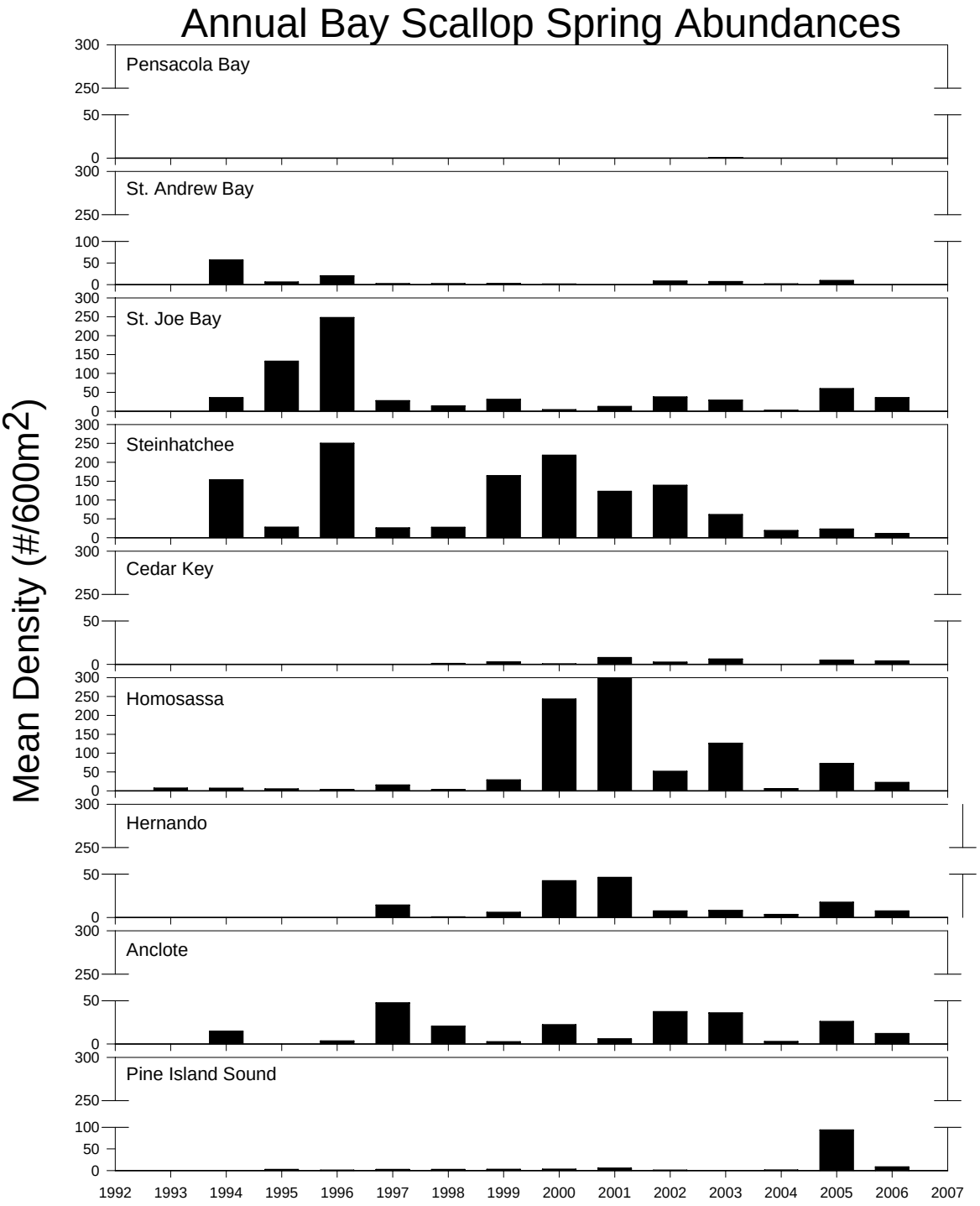
Scallops planted at the Anclote study site grew more slowly and never achieved a size as large as sympatric wild scallops, suggesting that fewer energetic reserves were available for gamete production. But, caged scallops experienced a lower rate of mortality than their wild conspecifics and may have experienced a more protracted spawning period. Reproductive analyses of both cultured and wild scallops indicate that the cultured scallops did successfully spawn, thus contributing larvae for replenishment of local populations. Planting of pediveligers in Sarasota Bay did not result in detectable quantities of adult bay scallops at the restoration site. We received anecdotal reports from bay shrimpers of increased scallop abundance in Sarasota Bay, and we also found concentrations of scallops in the bay where in previous years we were able to find few if any scallops. However, despite the apparent increase in scallop abundance in Sarasota Bay, no clear genetic link could be identified. It is apparent from the results of this study and our previous bay scallop restoration studies that additional work is needed to better understand and quantify the contribution of planted scallops to the success of future year classes.

PURPOSE

Bay scallop (*Argopecten irradians*) populations along the west coast of Florida once supported an active commercial (Murdock, 1955) and recreational (Arnold, 1994) fishery. In recent decades, however, many of the local populations that comprise the purported bay scallop metapopulation (*sensu* Hanski and Gilpin, 1996) in Florida have collapsed, with the result that the commercial fishery has been closed and recreational fishing severely curtailed. In 1997 the Florida Fish & Wildlife Research Institute (FWRI), in conjunction with the University of South Florida's College of Marine Science (USF), obtained funding to initiate restoration of bay scallop populations along the west coast of Florida between Tampa Bay and Crystal River. Those efforts constituted a continuation and expansion of restoration efforts being conducted by Dr. Norman J. Blake at USF and built upon basic research being conducted by FWRI (Marelli et al., 1997a; 1997b; 1999; Arnold et al., 1998; Arnold, 2001). The primary goals of that multi-institutional study were to determine the feasibility of utilizing laboratory cultured scallops as a means of enhancing recruitment success of bay scallops within the surrounding environment and to determine the potential for development of a commercial bay scallop aquaculture industry in Florida.

Both natural and anthropogenic factors, including hurricanes, red tide events, and overfishing, can act to deplete bay scallop populations. Bay scallops are well adapted to recover from such deleterious events because those events rarely impact bay scallops throughout the entire natural range of the species in Florida. Instead, only one or a few local populations would be affected. Under prehistoric conditions, a local population that was decimated by a natural "disaster" eventually would be replenished by larval supply from neighboring populations. However, development along peninsular west Florida may have interrupted natural linkages among local populations. For example, bay scallops that were once abundant in areas such as Pine Island Sound, Sarasota Bay, and Tampa Bay are now essentially non-existent, and similar loss without recovery appears to have occurred in the area between Tampa Bay and Crystal River (Figure 1). It therefore appears that anthropogenic alterations may have upset the natural relationship among bay scallop populations in Florida, and even improvements in water quality in important areas such as Tampa Bay (Blake et al., 1993) may not suffice to instigate natural recovery. Instead, recovery may require augmentation of depleted spawner stocks such that adult densities are adequate to ensure successful fertilization (Levitan, 1995) and effective production of larvae (Lewin, 1986). Otherwise, recruitment limitation (Peterson & Summerson, 1992) may prevent

Figure 1. Estimated density of bay scallops (*Argopecten irradians*) at various sites along the west coast of Florida during 1994-2006. Density estimates are derived from counting all scallop encountered along 20 randomly-located 600-m^2 transects (six transects at Cedar Key).

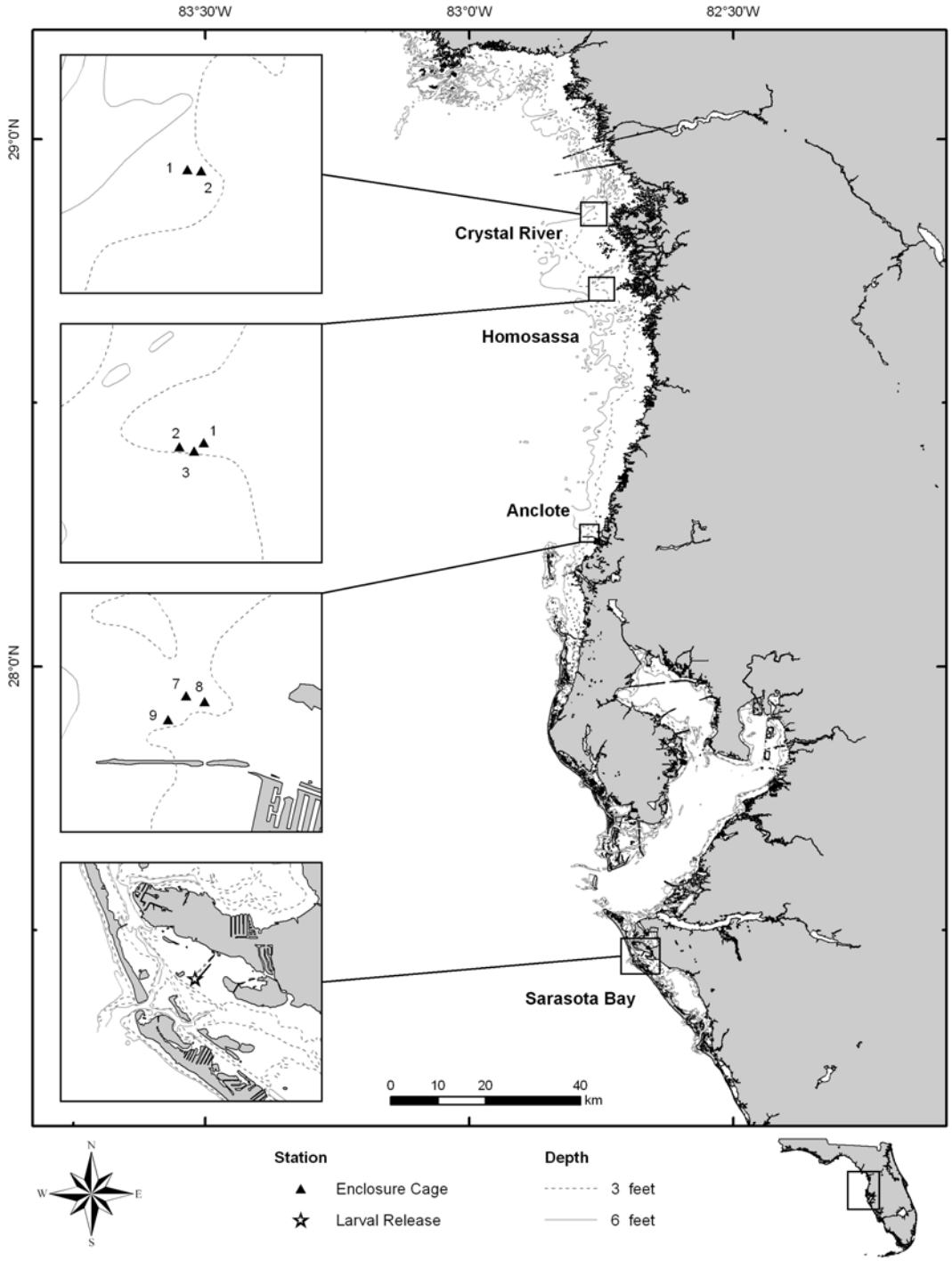


recovery of local bay scallop populations in Florida. Our research in Florida suggests that recruitment limitation has occurred in areas south of the Suwannee River (Arnold et al., 1998).

Initial attempts to restore bay scallops in the area between Tampa Bay and Crystal River appear to have been successful, but verifying that success has proven difficult despite an extensive and genetically based assessment program (Arnold et al., 2002). In the area of Tarpon Springs (i.e., Anclote) it is difficult to show strong gains in the population. The mean density increased from an average of 17.2 scallops per 600 m^{-2} during 1994-1998 to 18.1 per 600 m^{-2} during 1999-2006. In the Crystal River/Homosassa River region, bay scallop populations have experienced a substantial increase in abundance since restoration began in the late 1990s following ten to thirty years of almost complete collapse. The mean density increased from 6.6 per 600 m^{-2} during 1994-1998 to 106.0 per 600 m^{-2} during 1999-2006 (Figure 1). However, a genetic assessment was unable to unequivocally link this recovery to the restoration efforts (Wilbur et al., 2005). This lack of a detectable contribution from the restoration stocks may, in part, be attributed the potential recruitment of allocthonous larvae in the enhanced areas from populations in areas between Steinhatchee and St. Joseph Bay where scallops remain abundant. Additionally, although a state-wide survey shows that the mtDNA haplotypes exhibited by the restoration stock (and used to track the reproductive success of those scallops) are very rare in the wild population, it is possible that contributions from populations not included in the survey may have obscured the contribution from the hatchery-produced enhancement stock. Further, while the post-restoration assessment was extensive, the open nature of the bay scallop populations in north central Florida may have facilitated the dispersal of scallops away from the assessed areas. Nevertheless, it is undeniable that scallop abundance has increased considerably in the restoration target area and that this increase coincided with our restoration efforts. Members of the scientific and lay communities agree that it is extremely risky to terminate that restoration program in the face of such apparent success.

A second study continued our bay scallop population restoration efforts on the west coast of Florida (Arnold et al., 2005a). For that study we focused on two study topics. First, we continued our restoration efforts in the area between Anclote and Crystal River (Figure 2). Although scallops had become relatively abundant in that area, their status remains precarious because they are an annual species characterized by potentially extreme interannual variations in abundance. We therefore felt that it was imperative to continue to supplement

Figure 2. Location of bay scallop restoration study sites at Crystal River, Homosassa, Anclote, and Sarasota Bay on the Florida west coast.



larval supply in this area until we were sure that the populations had stabilized. Second, we initiated bay scallop restoration efforts in Sarasota Bay (Figure 2). Sarasota Bay was selected because it has been essentially devoid of scallops for several decades, it is enclosed and therefore should retain scallop larvae within a manageable area, and it supports large and healthy seagrass meadows that represent an essential habitat for bay scallops (Thayer and Stuart, 1974). Results of that study indicated that juvenile scallops planted at all sites grew almost as fast as their wild counterparts and, although mortality was high, large numbers of scallops did survive to successfully complete a fall spawning. However, as with the previous study (Arnold et al., 2002), results of genetic monitoring indicated no contribution from the caged scallops to the subsequent year class of wild scallops.

The present study was designed to continue our standard caging approach to bay scallop restoration at our Sarasota Bay, Anclote, Homosassa, and Crystal River study sites. We also planned several additional studies in Sarasota Bay, including the incorporation of the output from a physical oceanographic model to guide our site selection process, the biochemical analysis of both wild and planted scallops to determine relative egg quality from those two treatment groups, and (as a result of problems with hatchery production of juvenile scallops) to test the relative efficiency of releasing scallop larvae into enclosures as a means of establishing spawner patches. We have successfully applied the enclosure approach in Pine Island Sound (Leverone et al., 2004) and were anxious to test its value at another site.

APPROACH

Broodstock Collection, Conditioning and Spawning

All broodstock for this project were collected from either the targeted restoration population or, if a sufficient number of scallops could not be located in the target area, from the nearest available source population. In practice, all of the scallops we used as broodstock for successful rearing efforts were collected from the Anclote estuary, although many spawning efforts employed broodstock collected from the Homosassa/Crystal River area. For our fall 2004 and spring 2005 spawning efforts we collected adult scallops from both the Anclote and Crystal River areas. From the Anclote collection, 85 scallops were provided to the hatchery at USF and 119 were provided to a commercial shellfish hatchery in Palmetto, FL (Bay Shellfish, Inc.). From the Homosassa/Crystal River collection, 57 were provided to USF and 39 were provided to Bay Shellfish. Scallops were either spawned immediately (rarely) or conditioned for a period of several days to

several months. To condition the scallops, we either held them in cages hung from the FWRI dock located in Bayboro Harbor or we held them in tanks in the respective hatchery.

Additional scallop broodstock collection trips were made to Anclote during Fall 2005 and Spring 2006. Approximately 140 adult bay scallops were collected and transferred to Bay Shellfish Company in Palmetto, FL, where they were held in a flow through seawater system supplemented with cultured algae. The spawner stock was maintained under optimal conditions of temperature, salinity and food for several months until preparation for spawning.

Hatchery Propagation

Spawning procedures followed those described in Arnold et al. (2002). All spawning activities were conducted in the hatcheries using accepted cleaning and preparation techniques, and algal cultures required to feed the resultant offspring were initiated prior to each spawning event. Five-hundred-liter scallop culture tanks were filled with natural seawater that was first filtered through sand, diatomaceous earth, and charcoal, then treated with ultraviolet light, and finally filtered through a 1-µm mesh bag. Culture tanks were aerated with sterilized air stones connected to filtered, compressed air. Water temperature within the tanks was maintained at 26 ± 2°C.

For spawning, a group of six (University of South Florida) or more (20 - 30, Bay Shellfish) broodstock scallops were placed in clean buckets or trays filled with filtered and aerated seawater. Spawning was induced by temperature shock of no more than 5°C. Bay scallops are sequential hermaphrodites, first extruding male gametes and then female gametes during a spawning event. Following initiation of spawning, the water in the container within which the scallops were held would become clouded with sperm, at which time the scallops were moved to a clean container with fresh seawater. Once egg release was initiated, as indicated by clouds of orange particles, the mixture of sperm and eggs was allowed to sit for 15-20 minutes. Excess sperm were then rinsed away from the eggs through a 25-µm-mesh sieve, and the remaining fertilized eggs were transferred to a 500-L scallop culture tank. Whenever possible, the broodstock were then harvested, dissected, and a sample from the adductor muscle frozen at -80^{o} C for subsequent genetic analyses.

Scallop larvae were not fed during the first 24-48 hours (relying instead on endogenous yolk supplies) after which they were provided with a mixed phytoplankton diet for the remainder of the larval phase. At roughly 48 hours post-fertilization, the culture tank was partially drained and larvae that passed through a 53-

μm-mesh sieve (small larvae that were unlikely to survive) were discarded. The remaining larvae were then fed three times daily on a diet of mixed phytoplankton. The algal diet ration increased from 10,000 cells per ml to 50,000 cells per ml as the scallop larvae grew from a size of less than less than 110 μm to greater than 220 μm. Larval density was maintained at 10 scallops per ml during the first 48 hours, 4-5 scallops per ml for the next five days, and 1-2 scallops per ml thereafter. Simulated seagrass (clumps of thin plastic strips) or other suitable material was provided as a settlement substrate. Juveniles were fed at a rate of 50,000 algal cells per ml, three times daily. Water changes, usually 1/2 of the water in each tank each day, were conducted as needed to maintain proper water quality.

Hatchery propagation continued until the scallop spat were large enough to be transferred to 800 μm mesh bags, usually around a size of 1-2 mm shell height (SH = maximum distance from umbo to ventral margin). The bags of scallops were placed within 12.7-mm-mesh cages (described below) where they were allowed to feed on the natural phytoplankton assemblage. One half of the bags were hung from a dock located on Bayboro Harbor in St. Petersburg and one half of the bags were placed in cages planted at the Anclote restoration site. When water quality in Bayboro Harbor degraded to the point of threatening scallop survival, typically during early summer and typically in response to decreasing salinity, the juvenile scallops were transferred to either our Anclote or our Crystal River study site for final growout to planting size. In any case, growout bags were cleaned or replaced as often as necessary to maintain good water flow. Scallop density in the bags was maintained at levels which allowed roughly 1/3 surface cover or less within the bags. Scallops were maintained in the 800-μm-mesh bags until they reached a shell height of around 7 mm, at which time they were transferred to 2-mm-mesh bags at a density of 200 scallops per bag. The scallops were held in the 2-mm-mesh bags until they reached a minimum SH of 18 mm, at which time they were planted directly into the cages. The 18-mm-SH minimum size was chosen because that was the size at which the scallops could not fall through the diagonal of the 12.7-mm-mesh cages. During the rearing phase, periodic measurements of shell height were made by an intern from Florida State University. Shell height measurements were taken on three dates at Crystal Bay, four dates at Anclote, and thirteen dates at the Bayboro site (Appendix 1).

An additional spawn was conducted on December 14, 2005 to provide larvae for release into the Sarasota Bay enclosures. Approximately five million fertilized eggs were produced from this spawn. Resultant larvae were maintained according to standard hatchery procedures; growth and survival were checked daily.

After 12 days, the larvae had reached the "pediveliger" stage characterized by the development of a clearly visible foot that indicates that metamorphosis and settlement are imminent. At metamorphosis, pelagic (free-swimming) larvae change into small juvenile scallops that readily attach to suitable benthic substrates. Those larvae were then placed in bucket along with seawater collected from the rearing chamber and transferred to our field planting site.

Field Planting

Growout cages were 0.6-m L x 0.6-m W x 0.16-m H and were constructed of 12.7-mm-mesh plastic-coated wire mesh. To increase growth and survival, the cages were elevated 15 cm above the sediment-water interface by attaching PVC legs to each corner (Figure 3; Arnold et al., 2005a). Cages were deployed in various configurations, depending upon the number of scallops available for planting, at each of our Sarasota Bay, Anclote, Homosassa, and Crystal River study sites.

2005 Plantings

We were unsuccessful in producing scallops from our University of South Florida hatchery due to problems with water quality resulting from construction activities in Bayboro Harbor, the water body from which the hatchery water was drawn. Instead, we contracted with Bay Shellfish to provide juvenile bay scallops for our planting activities. Scallops produced by Bay Shellfish were initially moved from the hatchery to Bayboro Harbor on February 28, 2005 for final growout from the 1-2 mm SH stage to the 18 mm SH stage. A total of 240,000 scallops were planted at roughly 16,000 per bag. On March 4, 2005, approximately 96,000 juvenile scallops of Anclote parentage were transferred from Bayboro Harbor to the cages deployed at our Anclote study site. On May 18, 2005, approximately 5,000 juvenile scallops of Anclote parentage were transferred from Bayboro Harbor to the cages deployed at our Crystal River study site. As described above, these scallops were planted in bags within cages because they were less than 18 mm SH at the time of transfer. Those scallops suffered approximately 50% mortality prior to reaching 18 mm SH, leaving 2,400 scallops available for planting at our Crystal River and Homosassa study sites as described below.

By the first week of June 2005, roughly 23,000 scallops remained at Bayboro; those scallops were destined for planting in Sarasota Bay. At that time, mean SH of those scallops was approaching 10mm. Over the next three weeks the juvenile scallops held at Bayboro Harbor were graded and counted. Unfortunately, during the three day period June 27-29, 2005 an exceptionally strong red tide event entered Bayboro Harbor and

killed all of those juvenile scallops. This red tide persisted in Tampa Bay, and in Sarasota Bay, for the remainder of 2005 and into 2006 with only brief periods of relief, causing considerable problems with our restoration activities in Sarasota Bay.

On July 6, 2005, we transferred 1,400 juvenile scallops of Anclote parentage from the Anclote growout bags into cages at the Anclote study site (mean SH = 18.2 ± 3.3 mm S.D.), leaving approximately 400 undersized and relatively slow-growing scallops remaining in bags at that site. On July 13, 2005 we removed 2,300 juvenile scallops of Anclote parentage from the Crystal River growout bags and planted 1,000 into cages at the Crystal River study site (mean SH = 16.0 ± 3.1 mm S.D.) and 1,300 into cages at the Homosassa River study site (mean SH = 15.5 ± 2.1 mm S.D.). None of the scallops planted at the Homosassa study site remained alive on August 4, 2005. A datalogger placed near the Homosassa restoration site recorded salinities of below 14 ppt during this time period, although salinity at both the Anclote and Crystal Bay sites remained near or above 20 ppt.

Larval Release

Larval containment booms (Figure 4) were established in Sarasota Bay on December 27, 2005. Two booms were placed near Tidy Island in Sarasota Bay (Figure 2), a site chosen based upon the results from a particle dispersal model (see Appendix II). Five spat collectors were placed on the inside and five on the outside each boom. Meanwhile, scallop larvae were transported from Bay Shellfish that same morning in four separate 20-L buckets. The larvae were transferred to the boat, transported to the enclosure location, and approximately 50% of the larvae released into each boom later that afternoon. The booms were left undisturbed and the larvae were allowed to settle within each boom for 24 hours. On December 28, all spat collectors were retrieved, the booms were carefully recovered making sure that they were not allowed to drag over the newly set scallops, and the larvae were then allowed to grow and develop in a natural setting for the next several months.

A second larval release occurred in the spring of 2006. Larval containment booms were set up on April 6, 2006. Two booms were placed near Tidy Island in Sarasota Bay, on the identical spot where the December release had occurred. In fact, we left the anchors from the original enclosure deployment in place so that we could easily relocate the enclosure footprint for subsequent sampling, but those anchors also served as attachment points for the spring 2006 effort. Five spat collectors were placed inside each boom and five

collectors were placed outside each boom. Meanwhile, scallop larvae were transported from Bay Shellfish that same morning in four separate 20-L buckets. The larvae were transferred to the boat, transported to location and released into the booms later that afternoon. The booms were left undisturbed and the larvae were allowed to settle within each boom for 24 hours. On April 7, all spat collectors were retrieved and the booms carefully removed following the procedures described above. As noted above, the anchors were left in place and served to guide our subsequent sampling for scallop recruits. We sampled for recruits from both the December 2005 and April 2006 efforts on July 25, 2006.

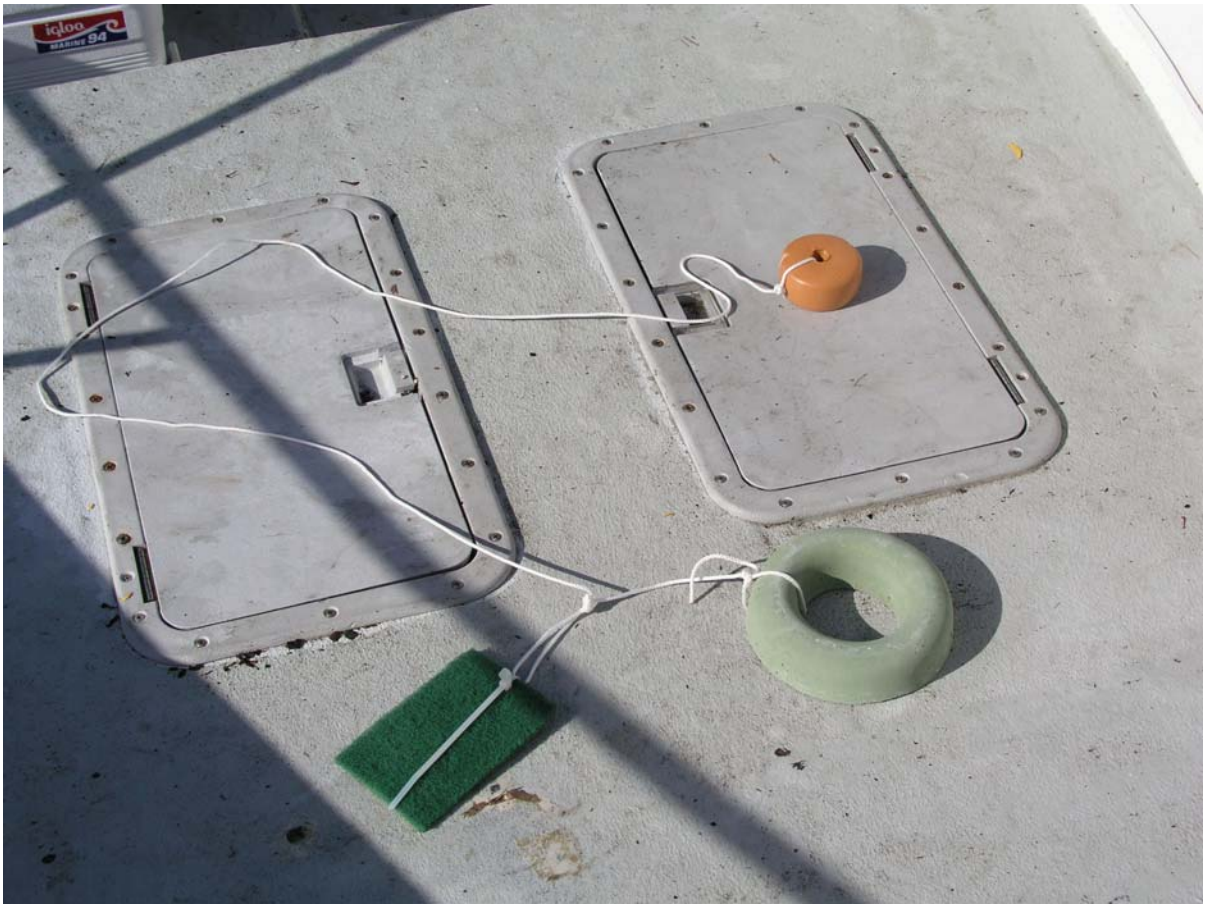
Figure 3. Cage used for field deployment of bay scallops along the west coast of Florida. Note PVC legs that raise the cage above the sediment-water interface, thereby increasing growth and survival of planted scallops (Arnold et al., 2005a)

Recruitment Monitoring

Spat collectors deployed both inside and outside of the enclosures were constructed from 4" x 6" scrub pads that provided numerous interstitial spaces for larval attachment (Figure 5). Each collector was positioned 0.5 m above the sediment-water interface and was attached to a bottom weight and a surface float. Collectors were deployed in the enclosures contemporaneous with larval release. When the booms were retrieved one day later, the spat collectors were retrieved, preserved in a 5% formalin solution and returned to the laboratory.

Figure 4. Enclosure booms constructed from sediment containment curtains and anchored to the substrate with hurricane tie-down anchors.

Figure 5. Recruit collectors used for monitoring the health and abundance of scallop larvae released into containment booms. Collectors also served to provide an index of relative recruitment of scallop larvae inside versus outside of the enclosure.



Before leaving the site, a buoy was attached to a line, anchored into the bottom, and left behind to mark the center point of each release point. In addition, the anchor screws that secured the bottom of the containment boom to the sediment surface were left in place when the booms were removed. A line was threaded through the loops of the anchor screws to mark the perimeter of the containment area. This allowed us to easily and accurately relocate each site when returning for subsequent monitoring efforts.

After seven days, the spat collectors were removed from the fixative, gently rinsed with tap water over a sieve and placed in a drying oven (40.5^{o} F) for 48 hours. After drying, each spat collector was gently brushed onto a sorting tray to dislodge attached scallop spat. The recruits were then identified and counted.

Field Assessment

Growth and survivorship were monitored at all sites approximately every six weeks. During each monitoring visit, the shell heights of ten scallops (or all remaining scallops if less than 10 were present) from each cage were measured and all live scallops from each cage were counted. These activities occurred at all restoration sites on each sample date with the exception of Sarasota Bay, where no juvenile scallops were planted.

At each visit to the Anclote restoration site, we attempted to collect 20 wild and 20 cultured scallops for assessment of reproductive state and various other biochemical indicators (see below for details). The scallops were returned to the lab where shell height was determined and each of the wild and cultured scallop groups split evenly into two batches, one of which was prepared for reproductive analyses and the other for egg quality analyses. The scallops used for reproductive analyses were dissected and the gonad preserved in 5% formalin in seawater for 24-48 hours then transferred to 95% ethanol. The scallops used for egg quality analyses were dissected into gonad, digestive gland, adductor muscle and remaining body components and the total and individual wet weights measured. Portions of the gonad, digestive gland, and adductor muscle were then frozen at -80^{o} C for subsequent biochemical analyses.

Preserved gonad tissues were embedded in JB-4 glycomethacrylate resin, sectioned, and stained with hematoxylin and eosin according to the procedures described in Arnold et al. (2005b). The stage of reproductive development (Table 1) was assessed according to the methods similarly described in Arnold et al. (2005b).

Frozen gonad and tissues were analyzed for lipid and protein content following Geiger et al. (2000) and Donnelly et al. (2004). Protein content, lactate dehydrogenase and malate dehydrogenase activities were determined for adductor muscles following Geiger et al. (2000). A 50-80 mg piece of the tissue was homogenized using a BioSystem™ bead beater set to low speed and run for two periods of 40 seconds, in between which the samples were cooled on ice for 5-10 minutes. A 10-ul subsample was frozen for protein analysis, 100-ul samples were frozen for the lipid analyses (gonad only), and the remainder of the sample was used for the enzyme analyses (muscle only).

Table 1. Qualitative reproductive staging criteria for bay scallops, *Argopecten irradians* (see Arnold et al., 2005b for a description of methods and analytical scheme).

Stage	Description
No Data	Unable to read slide
Inactive	Gonadal tissue undifferentiated
Developing	Follicle wall expanded and lumen contains spermatocytes or oocytes
Ripe	Follicular area filled with either dense or radiating bands of sperm or ova
Early Spawning	Central lumen of either male or female follicles beginning to empty or eggs or sperm visible in ducts
Late Spawning	Lumen of follicles extensively emptied
Spent	Follicle lumen emptied of ova and spermatids, many amoebocytes and cellular debris often present

Protein was determined by the modified Lowry et al. (1951) method, with bovine serine albumin as the standard. The proteins were reacted with cupric sulfate-tartrate and Folin-Ciocalteu's reagent. The amount of protein in the sample was estimated by comparing the absorbance of the samples to the absorbance of a known series of standards read at 750 nm on a spectrophotometer.

The lipid protocol was a modification of Reisenbichler and Bailey (1991). Lipids were extracted in two steps. In the first step, 1 ml of acetone was added to the 100-ul homogenate and the sample then thoroughly vortexed and centrifuged for 10 min at 2500 rpm. The lipid-bearing acetone fraction was removed and the pellet retained for additional lipid extraction. In the second step, the pellet was re-suspended in 800 µl of methanol and 400 µl of chloroform, after which an additional 400 µl of chloroform was added, and the entire sample was mixed again. Finally, 400 µl of deionized (DI) water was added to the sample and it was mixed a third time, covered with foil, and let stand for one hour. After one hour, the organic and aqueous phases had completely separated. The organic phase was pipetted off, added to the acetone fraction, and assayed for total lipid. Lipid analysis was accomplished by allowing the organic solvents to evaporate overnight, charring the residue with concentrated sulfuric acid at 200° C, allowing the lipid/acid mixture to cool, and reading the absorbance in a spectrophotometer at 375 nm. In most cases, the samples were diluted 10 fold (9 parts DI water: 1 part sample) to bring the absorbance within range. The amount of lipid in the sample was estimated by comparing the absorbance of the samples to the absorbance of a known sequence of stearic acid standards.

The activities of lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) were measured on 20-ul aliquots of freshly homogenized adductor muscle tissue prepared following a modification of Walsh et al. (1989) as described in Ikeda et al. (2000). To determine LDH activity, the homogenate was added to 1 ml of assay buffer (50 millimolar tris-buffer at pH 7.2) containing 0.115 mg ml^{-1} NADH and 0.55 mg ml^{-1} of Na-pyruvate. To determine MDH activity, the homogenate was added to 1 ml of assay buffer (50 millimolar tris buffer at pH 7.5) containing 0.115 mg ml^{-1} NADH and 5.3 mg ml^{-1} of oxaloacetate. Enzyme activity was calculated as the change in the quantity of NADH per minute per gram of wet mass, estimated by observing the change in absorbance at 340 nm measured on a spectrophotometer for one minute.

Genetic Assessment

DNA extraction

Several sources of DNA were used in the completion of this project. For broodstock scallops sacrificed after spawning we dissected small (8-10 mg) pieces of adductor muscle. Spat samples greater than 5mm were dissected and the adductor muscle removed for DNA extraction. Spat samples less than 5mm were processed in their entirety. Regardless of source, total DNA was extracted using the PureGene extraction kit (Gentra Systems Inc, Minneapolis, MN). Manufacturer's instructions were followed with the exception that reagent volumes were scaled down for the small tissue volumes.

mtDNAAnalysis

PCR amplification and sequencing: The resulting extracts served as a template for a polymerase chain reaction (PCR), using specific primers (Seyoum et al., 2003) designed to amplify 1049 base pairs (bp) of mitochondrial DNA (mtDNA) that includes a substantial portion of the ATP synthase subunit 6 coding region (SCAOPA-3) (Wilbur et al., 2005). Amplifications were performed in 50-µL volumes containing 1x PCR buffer, 2.5 mM of $MgCl_2$, 200 µM of each dNTP, 0.25 µM of each primer and 2.5 U of Taq polymerase. Each successful amplification was sequenced, using 2-4 sequencing primers (sca-1, sca-2; Seyoum at al., 2003 and/or sca-1rev: TAA GGA GTG AGG GTT ATA CC and sca-2rev: TAG CCA ACC TGC CCA AAC TC) as needed to obtain 930 bp of sequence. Sequencing was performed on an ABI 3100 Genetic Analyzer, using Big Dye ™ Terminator cycle-sequencing chemistry and standard protocols (Applied Biosystems, Foster City, CA).

Analysis: The sequences were edited and aligned in Sequencher (Gene Codes Co., Ann Arbor, MI). Individuals exhibiting identical haplotypes were identified using Arlequin (version 2, Schneider et al., 2000). Sequences

from broodstock that produced progeny for planting (restoration stocks) were compared to those generated from their offspring to confirm inheritance and clarify the composition of the restoration stocks. Sequences generated from wild spat samples were compared to the relevant restoration stock to identify spat that may have been the offspring of the restoration stock.

Microsatellite (SSR) development: Genomic DNA was extracted from the adductor muscle of five individuals from New York, North Carolina, and Florida as previously described. Ten µl of each of the best four extractions (quality assessed by agarose electrophoresis) per region (three for Florida) were combined to make two DNA cocktails, which were sent to Savannah River Ecology Laboratory, University of Georgia for a microsatellite "double" enrichment. The procedure for this involves digesting high molecular weight DNA with restriction enzymes, cloning and amplification of the DNA, enrichment via hybridization of DNA fragments to specific biotin labeled SSR oligos, and elution of "enriched" DNA. Three oligo mixtures (including different labeled SSRs) were used to produce three different enriched libraries (Ai02, Ai03, and Ai04).

The enriched libraries were amplified (1x Taq polymerase buffer, 2.0mM $MgCl_2$, 1 unit Taq polymerase, 25µg/ml bovine serum albumin, 200µM dNTPs, 0.5µM SNX-24f primer, 2 µL eluted DNA enrichment, sterile distilled water to a total volume of 25µL) and cloned using a pGEM-T Easy Vector System (Promega, Madison, WI). Each transformation was plated on 9 agar-ampicillin plates and then incubated overnight (~16 hours). For each enrichment, four-hundred eighty positive colonies identified by their white color, were picked using a sterile pipet tip, placed in 20 µl dH_2O, boiled for 5 minutes, and stored at -80^o C. Inserted fragments were amplified (1x Taq polymerase buffer, 1.5mM $MgCl_2$, 1 unit Taq polymerase, 150µM dNTPs, 0.4µM each SP6 and T7 primers, 0.5µL clone lysate, and sterile distilled water to a total volume of 25µl). Prior to amplification, clones were thawed to room temperature and centrifuged at 1000 rpm for 1 minute to produce a clone lysate suitable for PCR. Each amplified fragment was sequenced in one direction using BigDye® Terminator (Applied Biosystems, Foster City, CA) and standard cycle sequencing conditions. Sequences were analyzed on an ABI3100 automated sequncer (Applied Biosystems), edited in Sequencher 4.1.4 (Gene Codes Corp., Ann Arbor, MI) and screened for presence and quality of tandem repeated nucleotide sequences by eye. Uninterrupted di-, tri- and tetra-nucleotide repeats with consistent units were sequenced in the reverse direction to obtain suitable flanking sequence for primer design. Potential loci were compared to one another to prevent the development of the same locus from different clones. Primers were designed to flank

target regions as closely as possible using the Primer3 program (available online, http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi).

Primers were screened for consistent PCR amplification and then resynthesized with fluorescent label (either HEX or FAM, Applied Biosystems) on the forward primer and a 5' "pigtail" (Brownstein *et al.* 1996) on the reverse primer to promote adenylation and minimize stutter peaks. Fluorescently label amplification products were diluted 1:10-1:20 with sterile distilled water and 1 µL was added to 9 µL Hi-Di:Rox size standard solution (1025:25). Samples were visualized on an ABI PRISM 3100 Genetic Analyzer. Resulting chromatograms were analyzed using GeneScan 3.7 and Genotyper 3.7 software (Applied Biosystems).

Project Management

All field activities, including collection of broodstock, maintenance of cultured juvenile and adult scallops, site selection, field assessments, and collection of samples from scallop plantings, were conducted by Dr. William S. Arnold and staff at the Florida FWCC Fish and Wildlife Research Institute (FWRI; formerly the Florida Marine Research Institute). Staff from FWRI also analyzed slides to determine the stage of reproductive development. Scallop culture activities were performed under the auspices of Dr. Norman J. Blake at the University of South Florida College of Marine Science and Curt Hemmel at Bay Shellfish, Inc.'s hatchery in Palmetto, Florida. Histological preparation of samples for reproductive analysis was performed by FWRI's histology lab. Dr. Ami Wilbur of the University of North Carolina-Wilmington performed the genetic analyses. Dr. Peter Sheng at the University of Florida conducted the modeling exercises for Sarasota Bay.

FINDINGS

Hatchery Propagation

During the fall/winter of 2004/2005, ten sets of scallops (total N = 142) were delivered to USF for conditioning, resulting in no successful spawns. Five sets of scallops (total N = 158) were delivered to Bay Shellfish, resulting in one successful spawn that was utilized for this project's juvenile planting efforts. An additional five sets of scallops (Total N = approximately 140) delivered to Bay Shellfish during the fall/winter of 2005/2006 resulted in two successful spawns utilized in the larval release efforts.

For the cage-plating portion of our study, Bay Shellfish produced a single large spawn of scallops on February 7, 2005. The juvenile scallops (size range 0.8-1.2 mm SH) were initially placed in 800µm mesh bags inside standard restoration cages at our Bayboro and Anclote study sites. Scallops held at Bayboro were planted

at a density of roughly 8,000 per bag on February 28, 2005 (total N ~ 120,000) and scallops held at Anclote were planted at a density of 12,000-20,000 per bag on March 4, 2005 (total N ~ 120,000). As the scallops grew, additional cages were added and the density within each cage reduced to prevent overcrowding and food limitation. Bayboro bags/cages were cleaned roughly once per week and Anclote cages were cleaned roughly once every three weeks (see Appendix I).

For our restoration efforts in Sarasota Bay, Bay Shellfish produced two batches of scallop larvae. One batch was initiated on December 14 and was transferred to the field on December 27, 2005. We estimated a total of approximately one million larvae in that batch, of which one-half million larvae were released into each boom. The larvae were approximately 1-2 days post set. That late age may have contributed to lower than anticipated recruitment on the recruit collectors but also may have increased the likelihood of settlement at the restoration site. The second batch was initiated on March 27 and was transferred to the field on April 6, 2006. We estimated a total of approximately two million larvae in that batch, of which one million larvae were released into each boom.

Field Assessment

Crystal River

The mean SH of the cultured scallop planted at Crystal River increased from 16.0 ± 3.1 mm on July 13, 2005 to 47.6 ± 5.8 mm on June 16, 2006 (Figure 6). At that time, only 21 of the original 1000 scallops remained alive at the Crystal River site, and those scallops were released to the wild. Growth of cultured scallops was slow and steady at the Crystal River site, but terminal SH never achieved the size typical of wild scallops at the same location during the same time of year. Wild scallops in the Crystal River area usually are at least 50 mm SH during fall. The cultured scallops that were planted on July 13, 2005 suffered 90% mortality during the first three weeks post-planting, but after that mortality rates decreased to between zero and two percent per sampling date. A small number of cultured scallops remained alive in the cages through June 16, 2006, at which time the surviving scallops were released to the wild.

Homosassa

The cultured scallops planted into cages at our Homosassa study site suffered 100% mortality by the second assessment date (Figure 7). Their initial planting size was similar to their counterparts planted at the Crystal River site (SH = 15.5 ± 2.1), but salinity conditions apparently were not favorable for scallop survival.

Figure 6. Growth of cultured bay scallops hung off Bayboro docks (first two data points), and growth and mortality of cultured bay scallops planted into cages at the Crystal River study site.

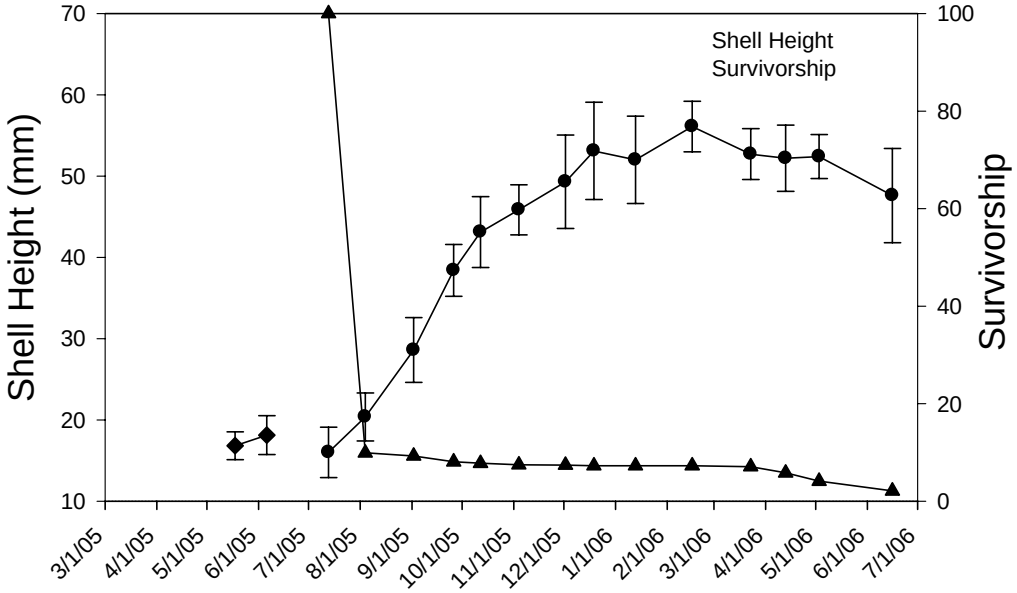


Figure 7. Growth and mortality of cultured bay scallops planted into cages at the Homosassa study site.

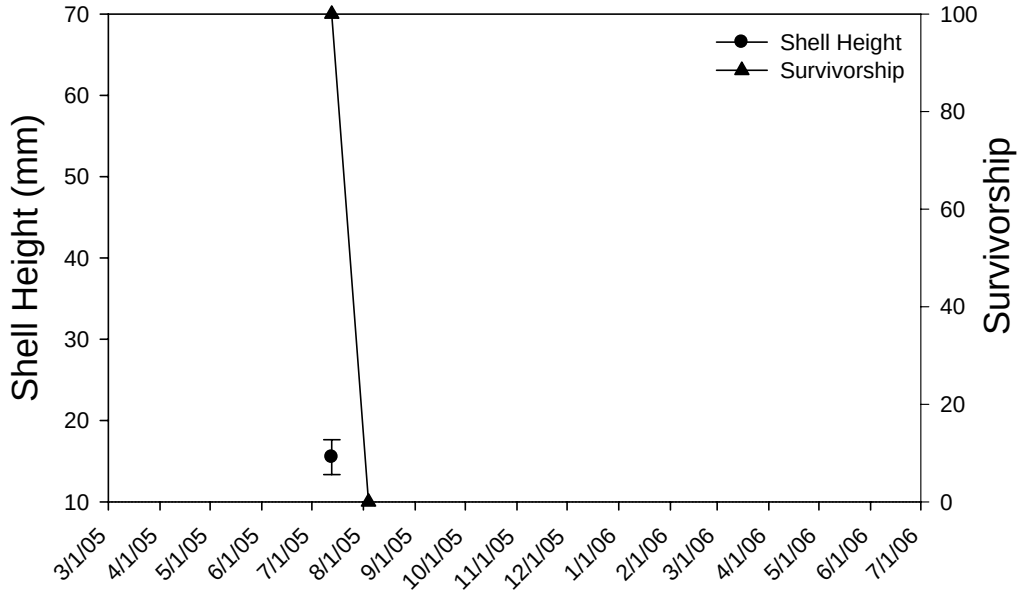
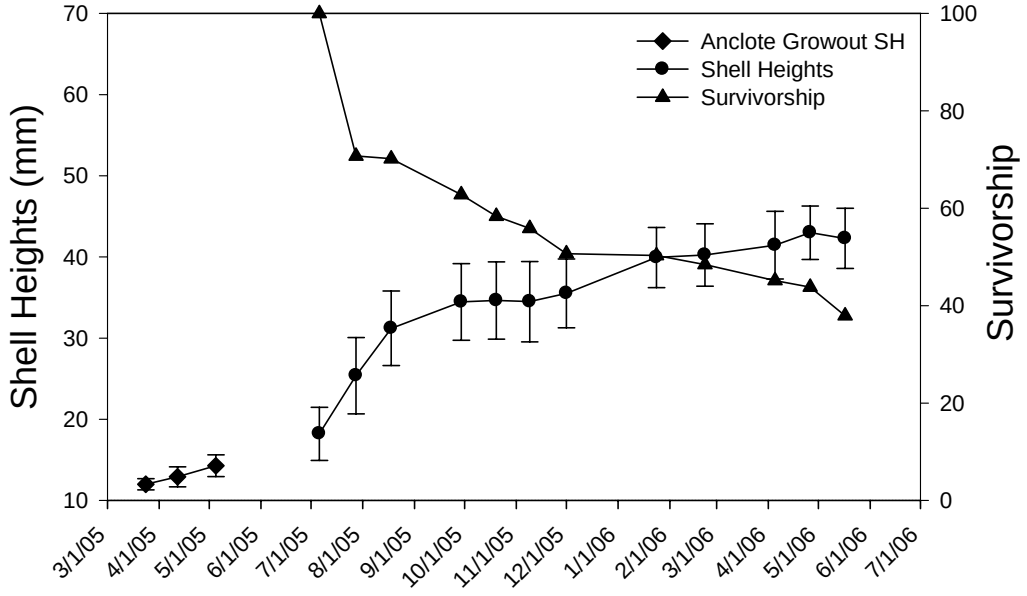


Figure 8. Growth of cultured bay scallops hung off Bayboro docks (first three data points), and growth and mortality of cultured scallops planted into cages at the Anclote study site.



Anclote

Cultured scallops planted at our Anclote study site grew reasonably well but suffered considerable post-planting mortality, with almost 30% of them dying within three weeks following planting (Figure 8). Mortality then slowed to a more gradual pace and remained relatively low until the final sample date. Scallops that were originally planted at about 18 mm SH in July 2005 reached a final SH of 42 mm by May 17, 2006. Approximately 40% of the scallops originally planted were still alive by the final assessment date and it is likely that they spawned successfully. These remaining scallops were released to the wild.

Sarasota Bay

No juvenile scallops were planted in Sarasota Bay. Additionally, recruit collectors deployed during both the December and April larval releases had very few scallop spat (Table 2). Finally, no juveniles or adults were collected at our Sarasota Bay restoration site during our July 2006 survey. However, at a nearby site we did collect 35 adult scallops for genetic assessment, which is far more scallops than we have ever been able to collect in Sarasota Bay. We also received reports from commercial shrimper Gary Fulford of exceptional scallop catches in Sarasota Bay during spring and summer 2006.

Table 2. Number of scallop spat per recruitment collector for larvae released into containment booms in Sarasota Bay. For both the December and April enclosure releases, five collectors were placed inside and five outside each enclosure boom.

	12/27-28/2005				4/6-7/2006			
Replicate	Outside		Inside		Outside		Inside	
1	0	0	2	1	0	0	0	0
2	0	0	0	2	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	1	2	3	0	0	0	0
5	0	0	0	1	0	0	0	0
Mean	0	0.2	0.8	1.4	0	0	0	0

Histology and Biochemistry

Samples for histological and biochemical analyses were only collected at our Anclote restoration site during the 2005-2006 study year. We collected samples of planted scallops for histological and biochemical analyses on ten dates, and we collected wild scallops for comparative purposes on nine dates that were somewhat contemporaneous with our collection of planted scallops (Table 3). However, on the January 24, 2006 collection date we could not find any wild scallops.

Gonad development and spawning

The timing of gonad development and spawning of scallops collected from our Anclote study site was similar between caged and wild cohorts (Figure 9) although some differences are noteworthy. Both female and male scallops collected from the wild exhibited more advanced gonadal development in late summer 2005. At that time, males and females from the caged group were in either the undifferentiated or developing phase, whereas most of the male and female scallops collected from the wild were ripe or even spawning. However, by late October the males and females of both groups were spawning although more of the wild scallops were in the spent stage. Wild scallops continued spawning into November whereas most of the caged scallops were spent or redeveloping. This trend continued into December. We were unable to collect any wild scallops during our late January sampling effort, but the caged scallops were either ripe or had initiated spawning once again. In late

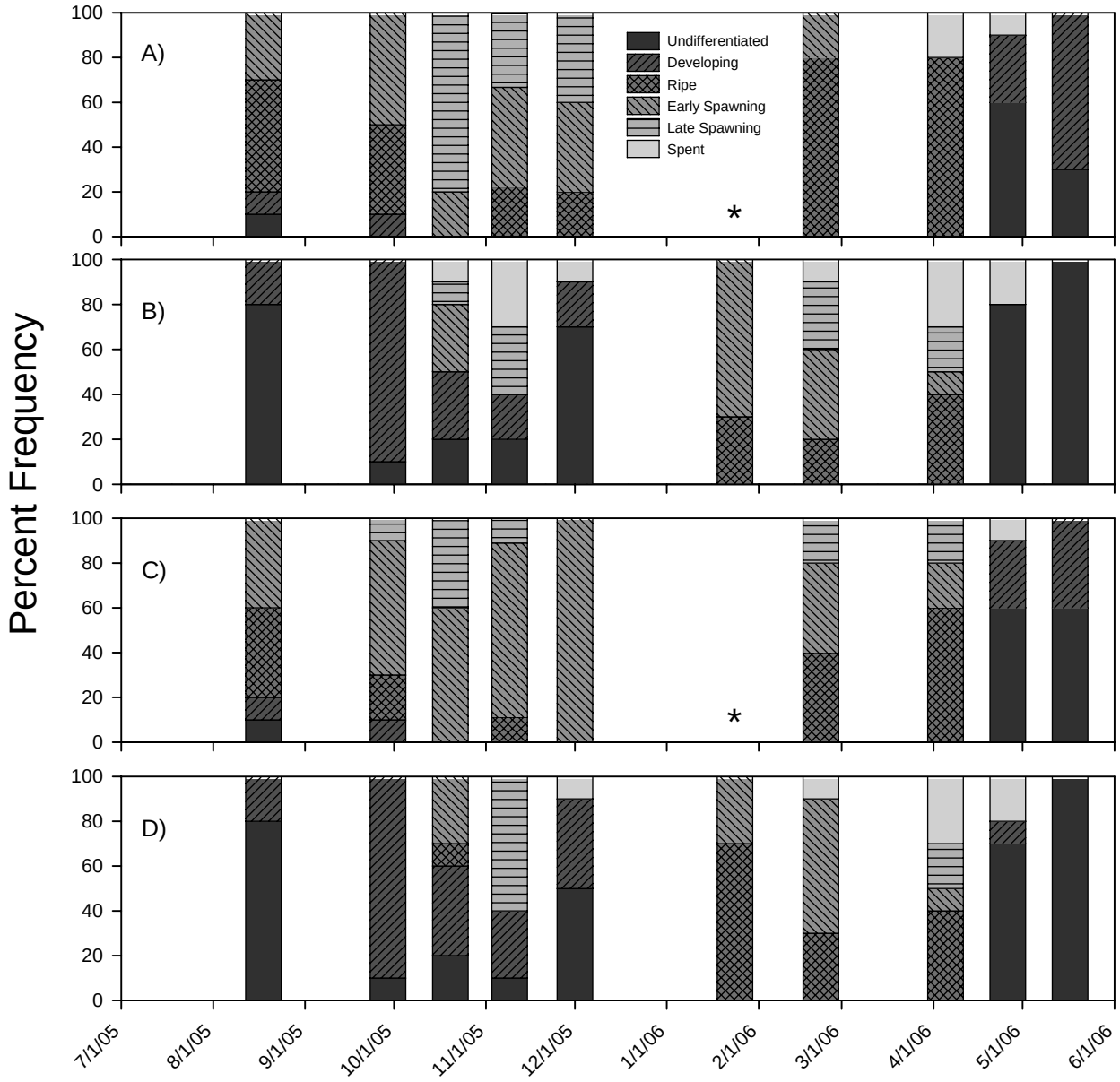
February, scallops from both groups were spawning once again; the only distinct difference at this time was that females from the wild lagged behind the other three groups in their spawning activities.

Caged scallops did not recover as quickly from the late winter spawning activity as did the wild scallops. As a result, during spring 2006 the caged scallops lagged behind the wild scallops in their development and spawning patterns. For example, during early April most of the wild scallops were fully ripe, spawning, or spent, whereas more of the cages scallops were in the early spawning stage of reproductive activity. This difference was small, however, and by the May 2006 termination of our study all four groups of scallops exhibited either undifferentiated gonads or were in the early stages of redevelopment. Results from out gonadal analyses are generally consistent with the results from the tissue biochemical analyses described below.

Table 3. Sample size for histological and biochemical assessment of bay scallop reproduction. For each collection date half of the total number scallops collected were used for histological assessment and the other half for biochemical assessment. Histological samples were dissected and the gonad was preserved in a 5% formalin-seawater solution. Biochemical samples were dissected and divided into gonad, digestive, muscle and remaining visceral tissues. A total, gonad, digestive, and muscle wet weight was recorded for each sample and a portion of the gonad, muscle, and mantle were frozen. The exceptions include Anclote wild scallops from January 24, 2006, when no wild scallops were collected.

	Histological Assessment		Biochemical Assessment	
Date	Anclote Wild	Anclote Restoration	Anclote Wild	Anclote Restoration
8/18/2005	10	10	9	10
9/29/2005	10	10	10	10
10/20/2005	5	10	5	10
11/9/2005	9	10	9	10
12/1/2005	5	10	5	9
1/24/2006	-	10	-	11
2/22/2006	5	10	5	10
4/5/2006	5	10	5	10
4/26/2006	10	10	9	10
5/17/2006	10	10	11	10
Total	**69**	**100**	**68**	**100**

Figure 9. Gonadal development and spawning patterns of the female (A-B) and male (C-D) component of: wild bay scallops collected from the vicinity of the Anclote study site (panels A and C); cultured scallops planted into cages at the Anclote study site (panels B and D). As indicated by the asterisks on this and subsequent plots, no wild scallops were collected on the January 24, 2006 date.



Tissue chemical composition

The total tissue weight of Anclote wild scallops tended to increase from fall 2005 through spring 2006 (Figure 10). Gonad wet weight of wild scallops collected from Anclote was relatively stable at approximately 1g, with the exception of a marked peak in February and early March. Adductor muscle wet weight and viscera wet weight tended to increase slightly during spring but were essentially unchanged. High variability in August and November may be partially explained by, in each case, a single individual in a smaller size class (Figure 11). Although cultured scallops planted at our Anclote site had a protracted period of very slow growth in tissue wet weight, those scallops began the study with less tissue wet weight than the wild scallops collected from Anclote (Figure 10). They never fully recovered from this size difference, having smaller adductor muscle, gonad, and remaining viscera throughout the study (Figures 11, 13 & 14). With the exception of late spring 2006, we observed a protracted but gradual increase in wet weight of each tissue component in the cultured scallops planted at Anclote. The tissue weights of cultured scallops were always much smaller than their wild counterparts.

Changes in tissue total biochemical content closely followed changes in tissue wet weights. Tissues from the Anclote restoration stock had less total protein, carbohydrate, and lipid than wild scallops. Wild scallops had larger tissues and therefore had more total protein and lipid. The most noticeable changes in the tissues of the wild scallops were that adductor muscle total protein generally declined (Figure 15) while gonad total protein peaked during February (Figure 16). Lipid content in the gonads of wild scallops also peaked during February (Figures 17). In cultured scallops planted at Anclote, adductor muscle total protein increased gradually until April 2006. Both lipid and protein in the gonad peaked in the winter months (Figures 15-17).

The biochemical composition was more similar between populations and over time when considered on a percent composition basis (Table 4). Adductor muscles decreased from about 15-20% protein at the beginning of the study to about 10% at the conclusion (Figure 18). Gonad protein content of both wild and caged scallops began to increase in December (Figure 19). In caged animals it peaked at around 10% in January (no wild scallops were collected in January). In both wild and caged scallops, gonad protein declined again to around 4% by May. Gonad lipid content followed an

identical pattern (Figures 20). These findings suggest that scallops have certain optimal levels of biochemical content in their tissues and, regardless of tissue size or total tissue content of each constituent, the ratio between biochemical constituents is held within a narrow range.

Lactate Dehydrogenase (LDH) as a measure of anaerobic activity declined in the adductor muscle of scallops collected from both populations throughout the course of the study (Figure 21). By November and December, scallops had lost almost all of their anaerobic ability. This indicates that scallops would have little or no ability to swim away from predators or to maintain shell closure by the time they were in condition to spawn (e.g. Winter and Hamilton, 1985). Malate Dehydrogenase

Table 4. Chemical composition of tissues of wild (AN) and caged restoration (ANR) scallops from our Anclote restoration site.

Site	Number	Date	Gonad % Protein	Gonad % Lipid	Muscle % Protein
AN	1-10	8/18/05	6.10 ± 1.36	3.43 ± 1.14	13.36 ± 4.44
AN	11-20	9/29/05	5.46 ± 0.98	2.67 ± 1.01	14.86 ± 5.78
AN	21-25	10/20/05	4.08 ± 1.42	2.93 ± 0.52	18.31 ± 2.86
AN	26-34	11/9/05	5.55 ± 0.76	4.20 ± 0.89	13.57 ± 3.75
AN	35-39	12/1/05	6.69 ± 1.31	4.43 ± 0.85	14.88 ± 2.61
AN	*-nd-*	1/24/06			
AN	40-44	2/22/06	6.71 ± 1.05	3.85 ± 1.77	13.16 ± 1.65
AN	45-49	4/5/06	5.65 ± 0.75	3.19 ± 0.55	11.51 ± 3.38
AN	50-59	4/26/06	4.01 ± 1.58	1.37 ± 0.81	6.71 ±3.98
AN	60-69	5/17/06	3.68 ± 0.89	2.24 ± 1.12	10.03 ± 3.80
ANR	1-10	8/18/05	5.80 ± 1.17	1.79 ± 0.45	19.84 ± 3.63
ANR	11-20	9/29/05	5.47 ± 0.78	1.99 ± 0.62	13.63 ± 4.47
ANR	21-30	10/20/05	6.20 ± 1.09	1.09 ± 0.40	17.99 ± 5.13
ANR	31-40	11/9/05	5.59 ± 0.44	1.78 ± 1.05	12.28 ± 3.95
ANR	41-49	12/1/05	5.89 ± 1.10	1.91 ± 0.43	12.24 ± 0.77
ANR	50-60	1/24/06	9.73 ± 1.87	4.93 ± 1.29	11.89 ± 4.74
ANR	61-70	2/22/06	6.68 ± 0.96	3.77 ± 1.17	13.22 ± 1.83
ANR	71-80	4/5/06	5.84 ± 1.40	3.06 ± 1.36	10.56 ± 2.36
ANR	81-90	4/26/06	4.14 ± 1.18	2.13 ± 0.93	12.18 ± 5.89
ANR	91-100	5/17/06	4.15 ± 1.17	1.69 ± 0.63	10.26 ± 4.09

(MDH) activity peaked in either April (Anclote caged) or December (Anclote wild) (Figure 22). LDH activities more closely tracked the general pattern of declining muscle protein, while MDH activities were more independent. As seen in previous studies, MDH activities may be related to energy transfer

during production of eggs, but the pattern is not direct. In general, enzyme activities were more variable and appear less informative than more basic chemical composition analyses (Table 5).

Table 5. Enzyme activities in adductor muscle of wild (AN) and caged restoration (ANR) scallops from our Anclote restoration site.

Site	Number	Date	Muscle LDH	Muscle MDH
AN	1-10	8/18/05	0.58 ± 0.81	0.09 ± 0.06
AN	11-20	9/29/05	0.72 ± 0.72	0.31 ± 0.20
AN	21-25	10/20/05	0.47 ± 0.43	0.15 ± 0.10
AN	26-34	11/9/05	0.20 ± 0.09	0.13 ± 0.07
AN	35-39	12/1/05	0.12 ± 0.12	0.30 ± 0.21
AN	*-nd-*	1/24/06		
AN	40-44	2/22/06	0.40 ± 0.30	1.28 ± 0.24
AN	45-49	4/5/06	0.35 ± 0.29	1.71 ± 0.61
AN	50-59	4/26/06	0.33 ± 0.35	0.76 ± 0.24
AN	60-69	5/17/06	0.30 ± 0.17	1.12 ± 0.54
ANR	1-10	8/18/05	0.46 ± 0.51	1.08 ± 0.57
ANR	11-20	9/29/05	0.63 ± 0.29	0.75 ± 0.51
ANR	21-30	10/20/05	0.72 ± 0.29	0.58 ± 0.29
ANR	31-40	11/9/05	0.52 ± 0.43	0.64 ± 0.41
ANR	41-49	12/1/05	0.30 ± 0.27	1.94 ± 0.71
ANR	50-60	1/24/06	0.25 ± 0.16	1.03 ± 0.59
ANR	61-70	2/22/06	0.18 ± 0.14	0.72 ± 0.31
ANR	71-80	4/5/06	0.34 ± 0.21	0.20 ± 0.30
ANR	81-90	4/26/06	0.16 ± 0.19	1.18 ± 0.39
ANR	91-100	5/17/06	0.37 ± 0.21	0.95 ± 0.70

Figure 10. Box-and-whisker plot of total tissue wet weight for (A) wild scallops collected within the vicinity of the Anclote study site, (B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10^{th} – 90^{th} percentile range, the whiskers represent the 10^{th} and 90^{th} percentiles, the filled gray box represents the 25^{th} and 75^{th} percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.

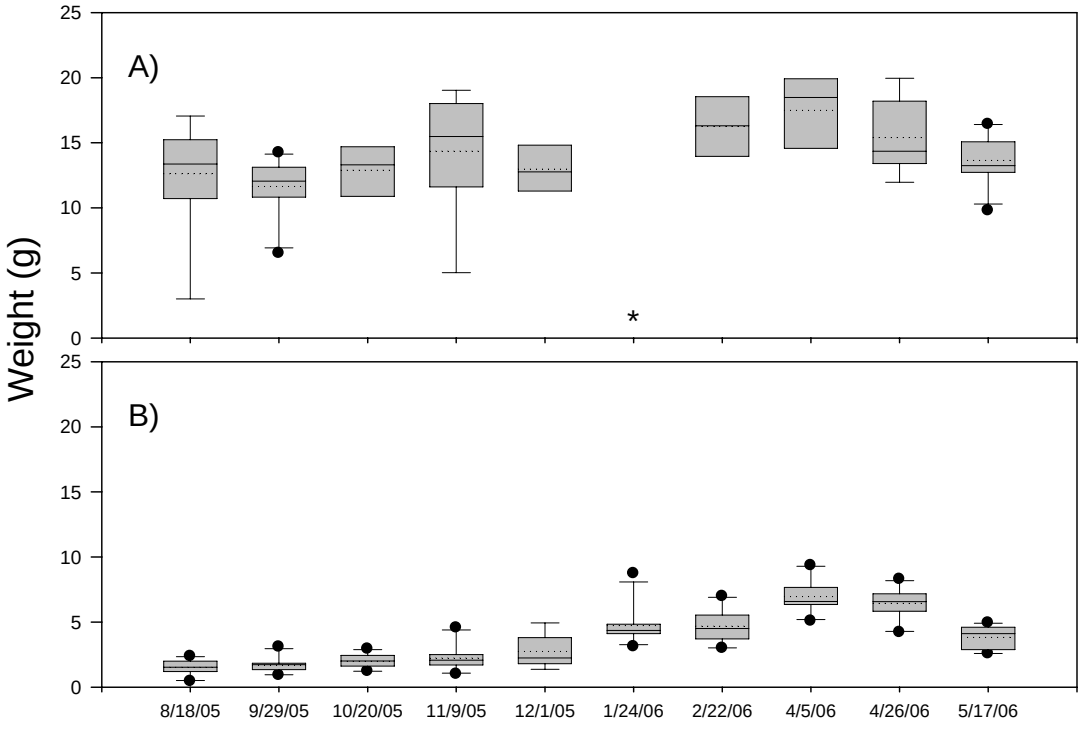


Figure 11. Box-and-whisker plot of adductor muscle tissue wet weight for (A) wild scallops collected within the vicinity of the Anclote study site, (B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10^{th} – 90^{th} percentile range, the whiskers represent the 10^{th} and 90^{th} percentiles, the filled gray box represents the 25^{th} and 75^{th} percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.

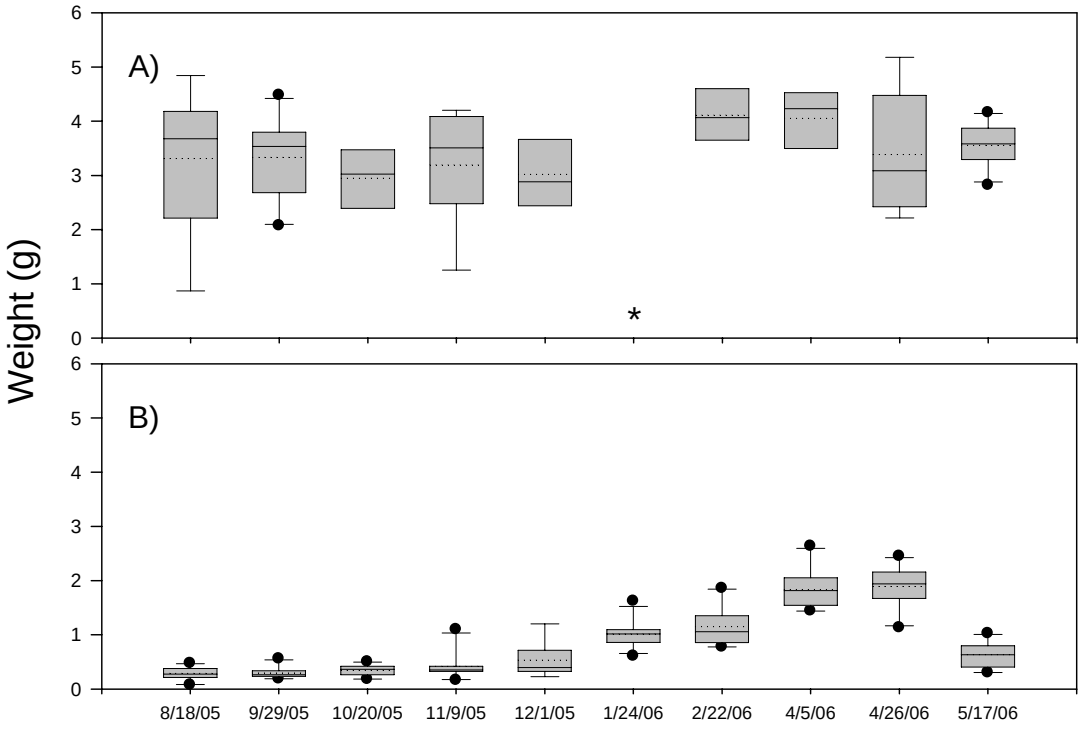


Figure 12. Box-and-whisker plot of total shell height for (A) wild scallops collected within the vicinity of the Anclote study site, (B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10^{th} – 90^{th} percentile range, the whiskers represent the 10^{th} and 90^{th} percentiles, the filled gray box represents the 25^{th} and 75^{th} percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.

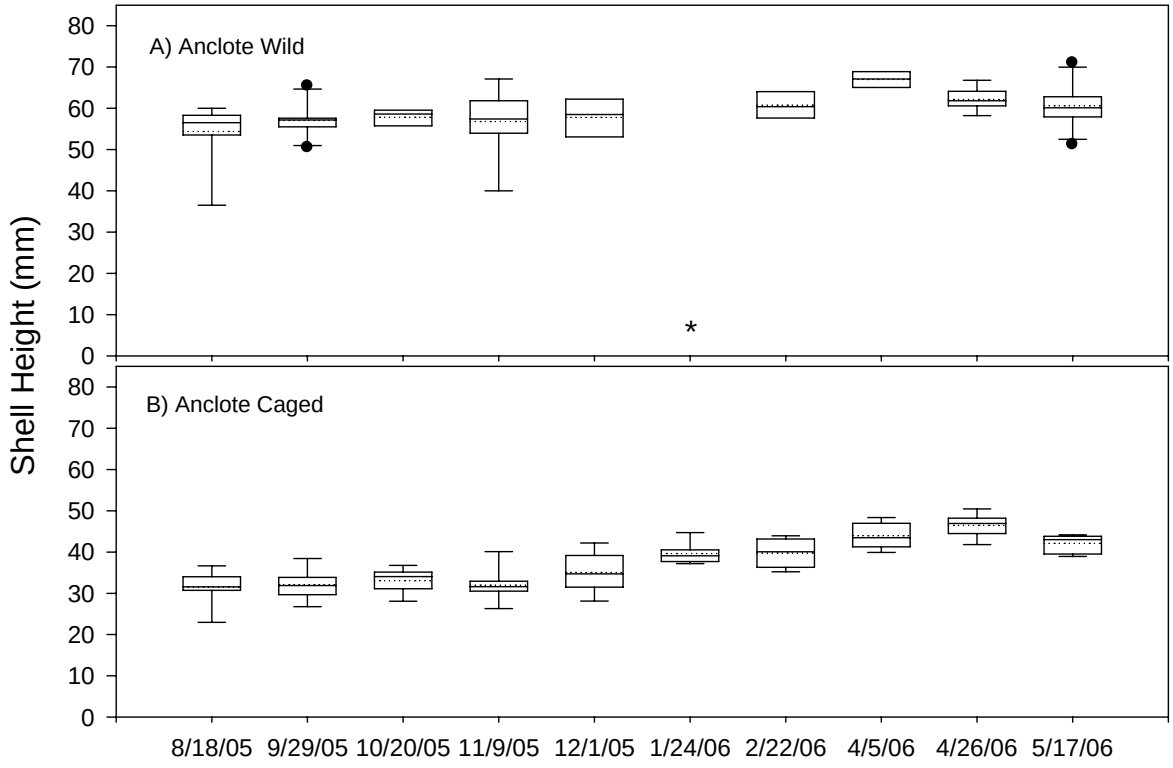


Figure 13. Box-and-whisker plot of gonad tissue wet weight for (A) wild scallops collected within the vicinity of the Anclote study site, (B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10^{th} – 90^{th} percentile range, the whiskers represent the 10^{th} and 90^{th} percentiles, the filled gray box represents the 25^{th} and 75^{th} percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.

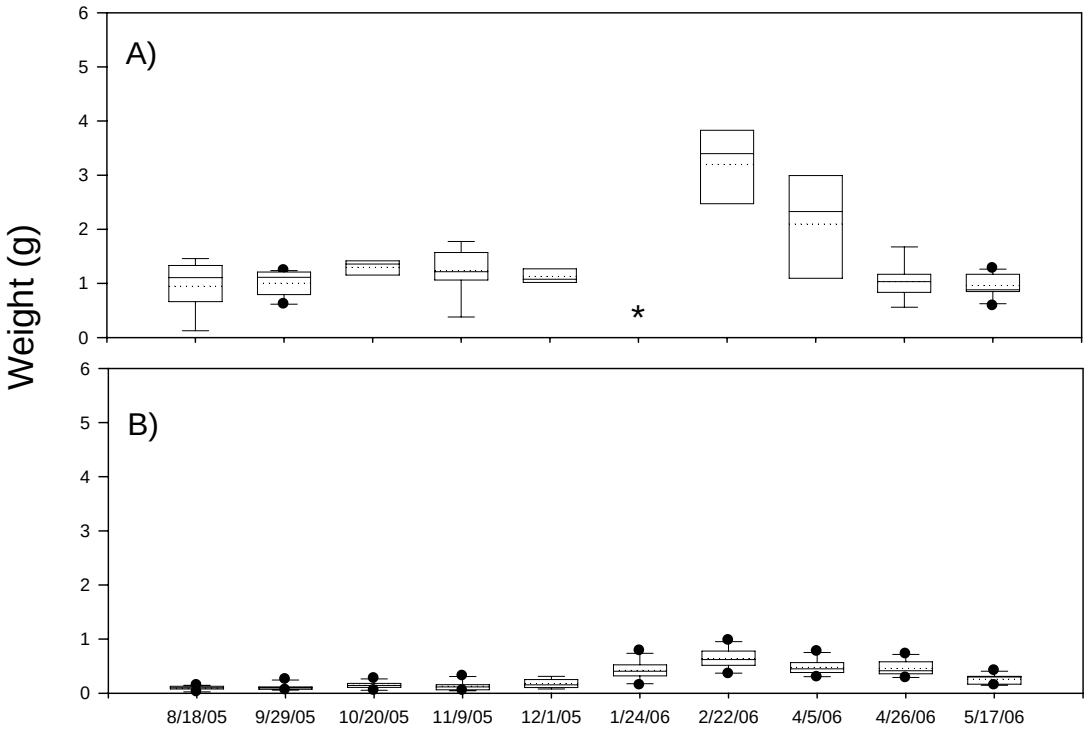


Figure 14. Box-and-whisker plot of visceral tissue wet weight for (A) wild scallops collected within the vicinity of the Anclote study site, (B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10^{th} – 90^{th} percentile range, the whiskers represent the 10^{th} and 90^{th} percentiles, the filled gray box represents the 25^{th} and 75^{th} percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.

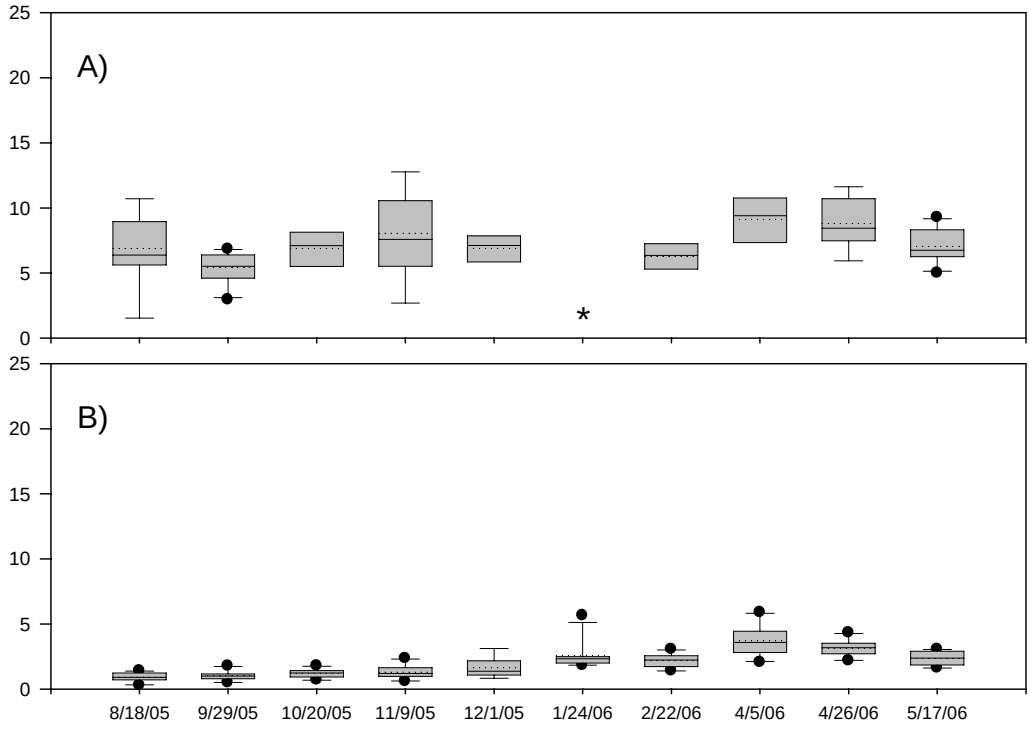


Figure 15. Box-and-whisker plot of adductor muscle total protein content for (A) wild scallops collected within the vicinity of the Anclote study site and,(B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10^{th} – 90^{th} percentile range, the whiskers represent the 10^{th} and 90^{th} percentiles, the filled gray box represents the 25^{th} and 75^{th} percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.

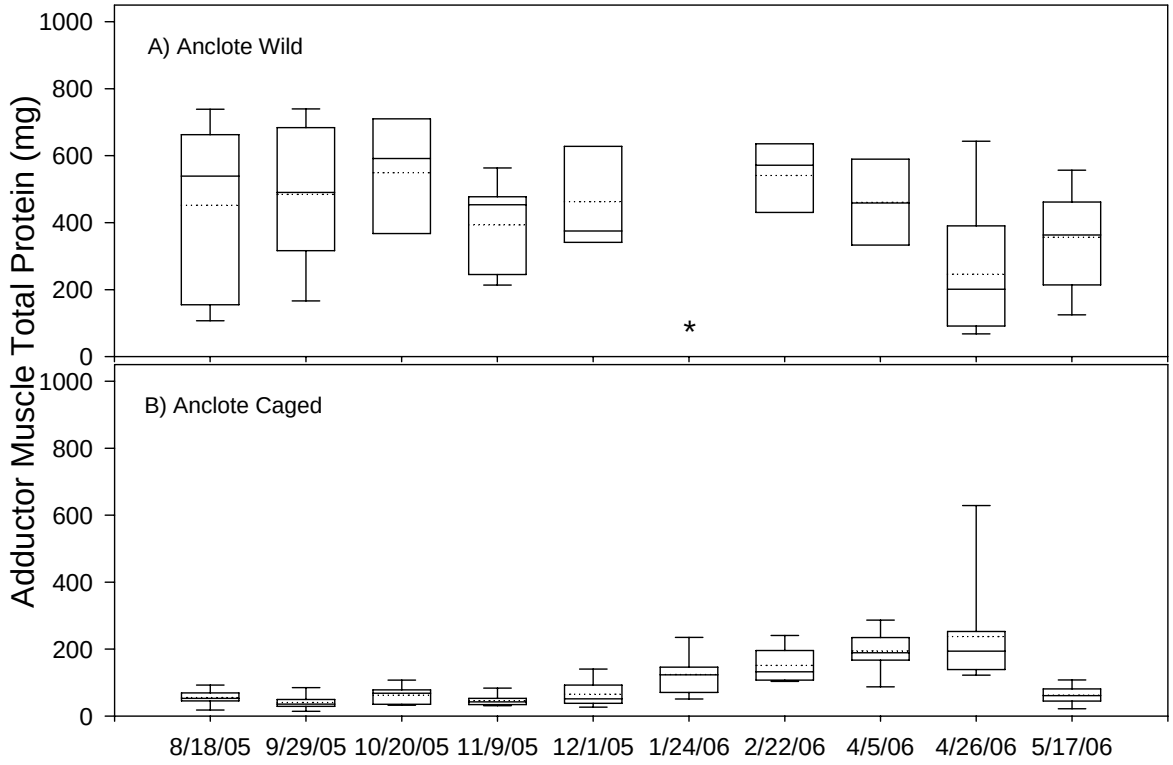


Figure 16. Box-and-whisker plot of gonad total protein content for (A) wild scallops collected within the vicinity of the Anclote study site and (B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10^{th} – 90^{th} percentile range, the whiskers represent the 10^{th} and 90^{th} percentiles, the filled gray box represents the 25^{th} and 75^{th} percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.

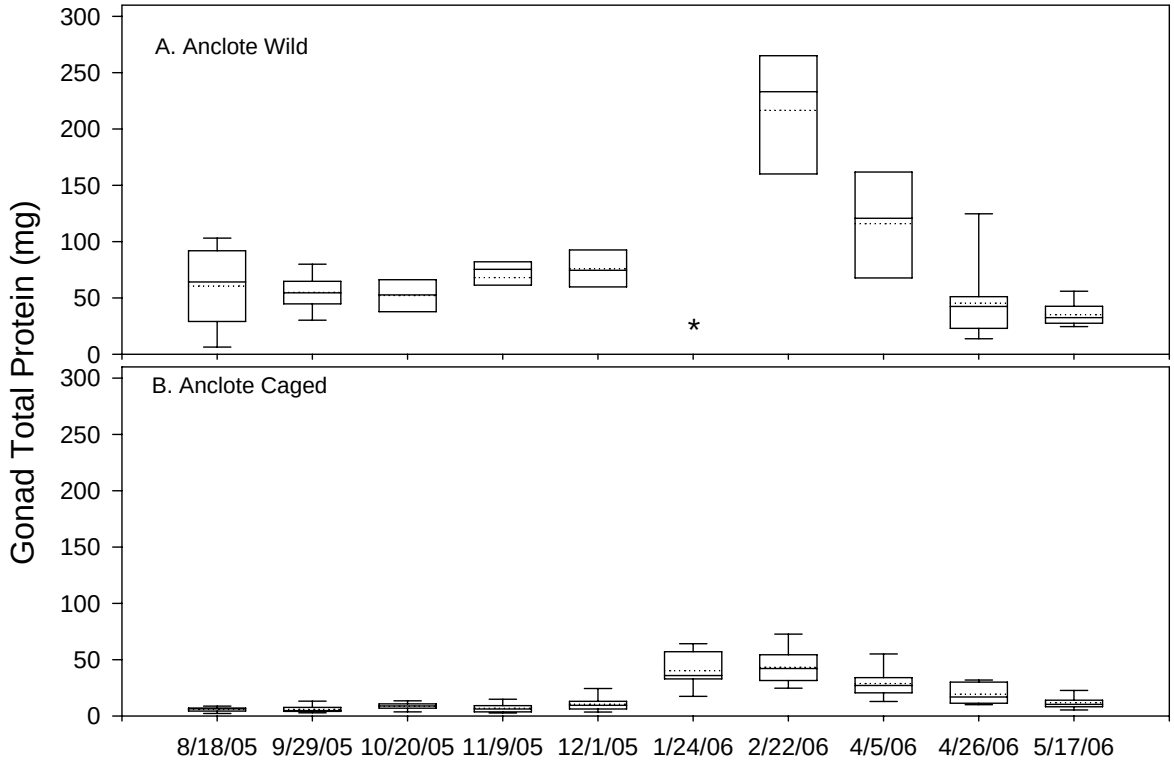


Figure 17. Box-and-whisker plot of gonad total lipid content for (A) wild scallops collected within the vicinity of the Anclote study site and (B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10^{th} – 90^{th} percentile range, the whiskers represent the 10^{th} and 90^{th} percentiles, the filled gray box represents the 25^{th} and 75^{th} percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.

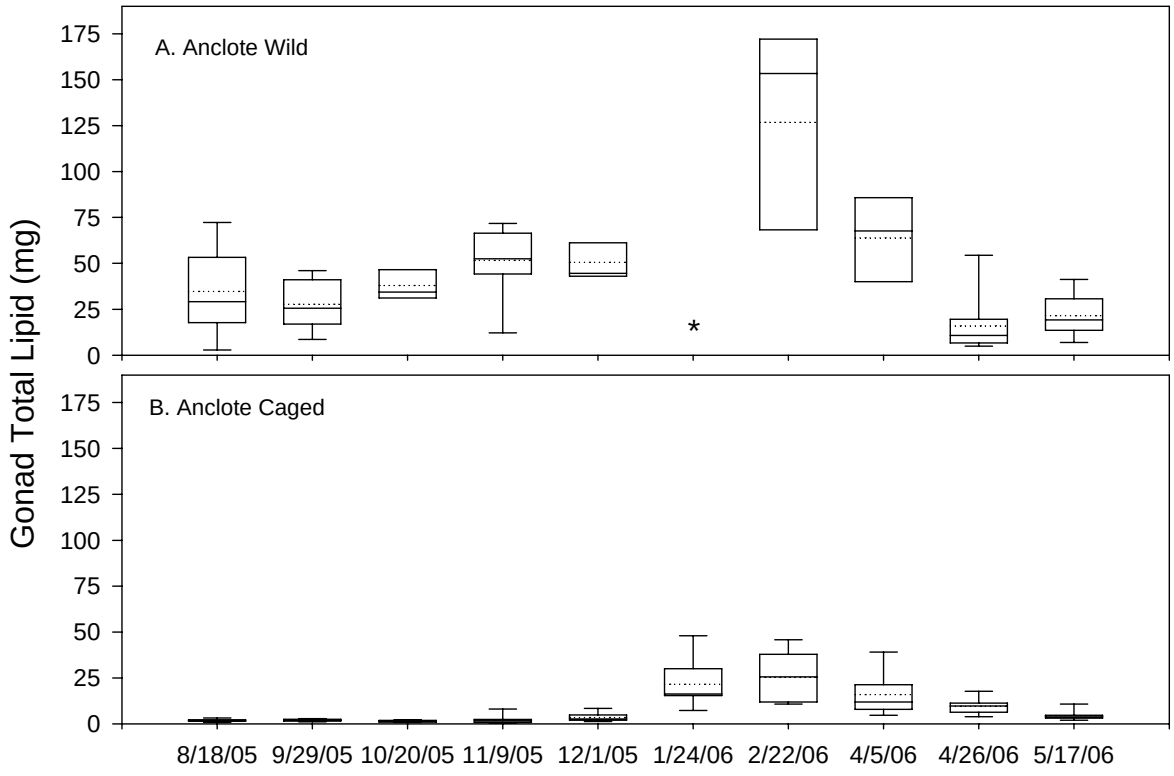


Figure 18. Box-and-whisker plot of adductor muscle percent protein content for (A) wild scallops collected within the vicinity of the Anclote study site and (B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10^{th} – 90^{th} percentile range, the whiskers represent the 10^{th} and 90^{th} percentiles, the filled gray box represents the 25^{th} and 75^{th} percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.

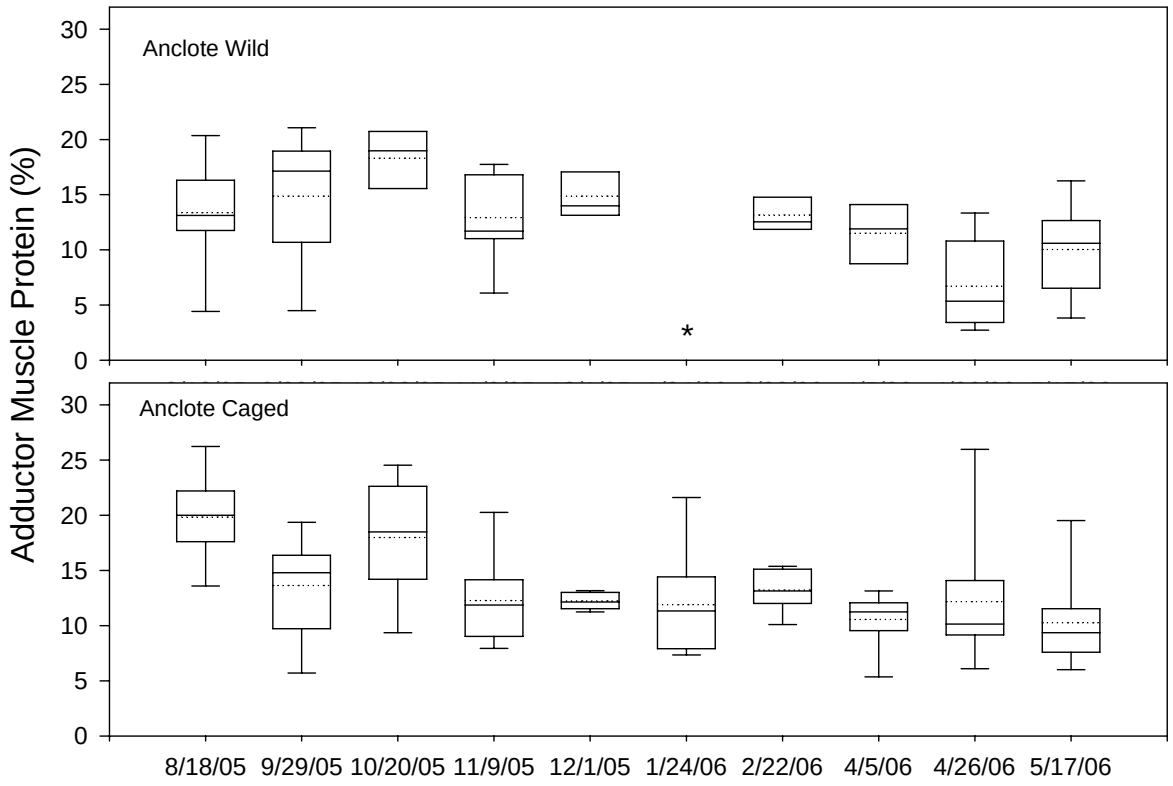


Figure 19. Box-and-whisker plot of gonad percent protein content for (A) wild scallops collected within the vicinity of the Anclote study site and (B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10^{th} – 90^{th} percentile range, the whiskers represent the 10^{th} and 90^{th} percentiles, the filled gray box represents the 25^{th} and 75^{th} percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.

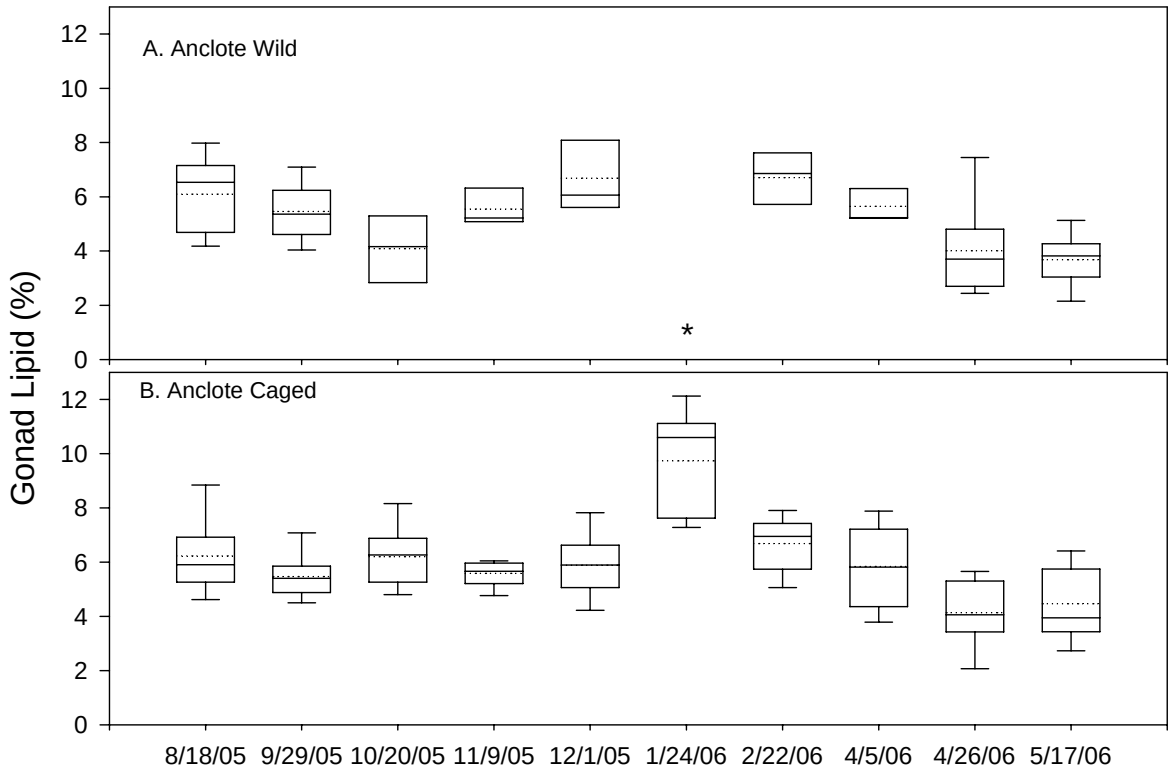


Figure 20. Box-and-whisker plot of gonad percent lipid content for (A) wild scallops collected within the vicinity of the Anclote study site and (B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10^{th} – 90^{th} percentile range, the whiskers represent the 10^{th} and 90^{th} percentiles, the filled gray box represents the 25^{th} and 75^{th} percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.

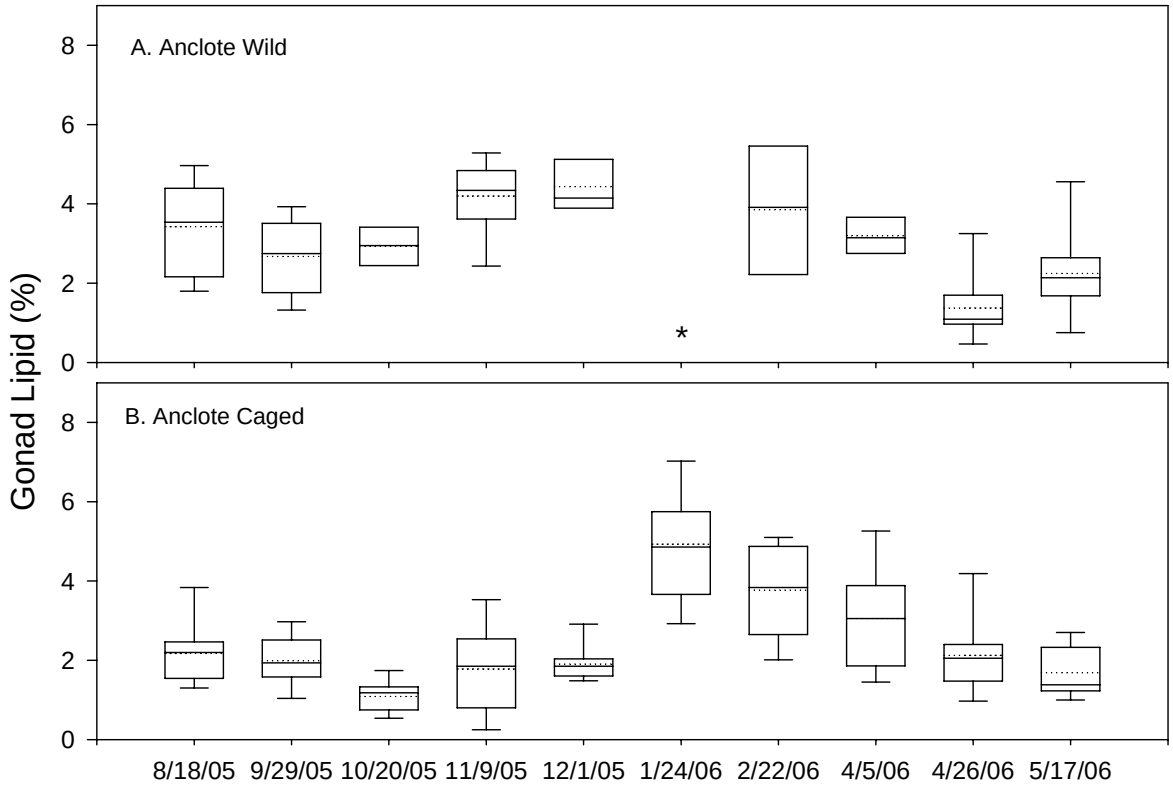


Figure 21. Box-and-whisker plot of adductor muscle lactate dehydrogenase (LDH) activity for (A) wild scallops collected within the vicinity of the Anclote study site and (B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10^{th} – 90^{th} percentile range, the whiskers represent the 10^{th} and 90^{th} percentiles, the filled gray box represents the 25^{th} and 75^{th} percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.

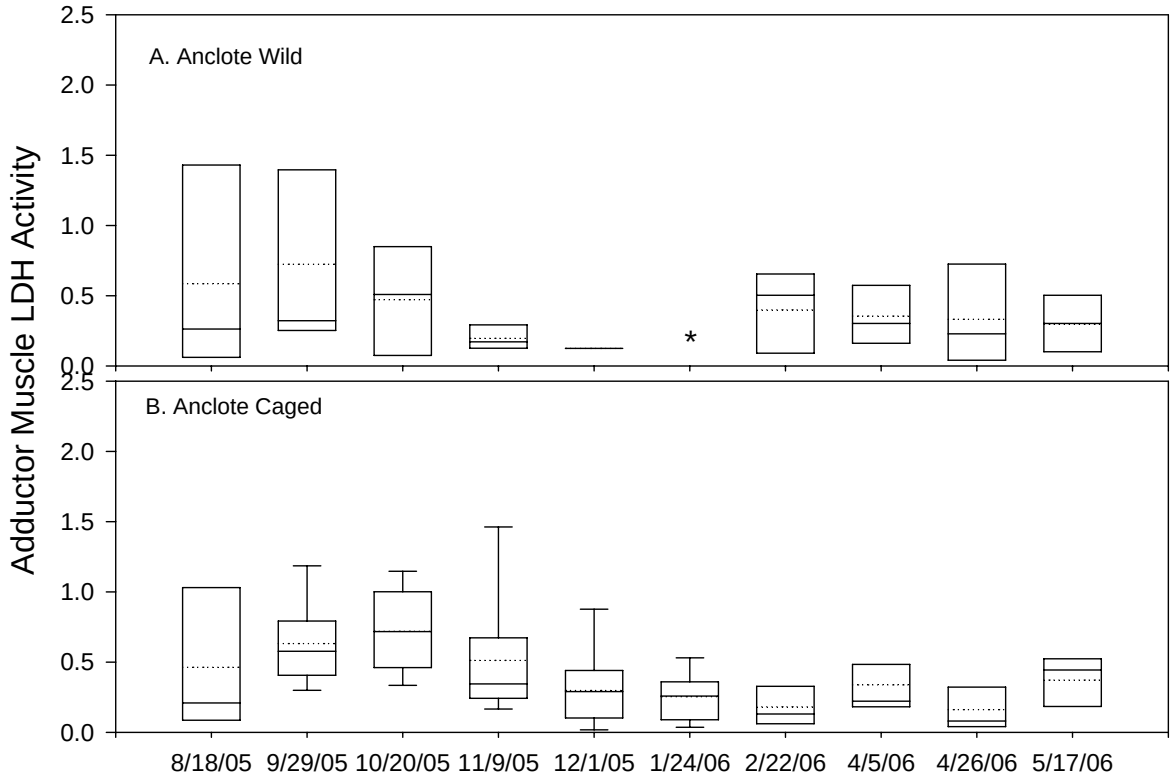
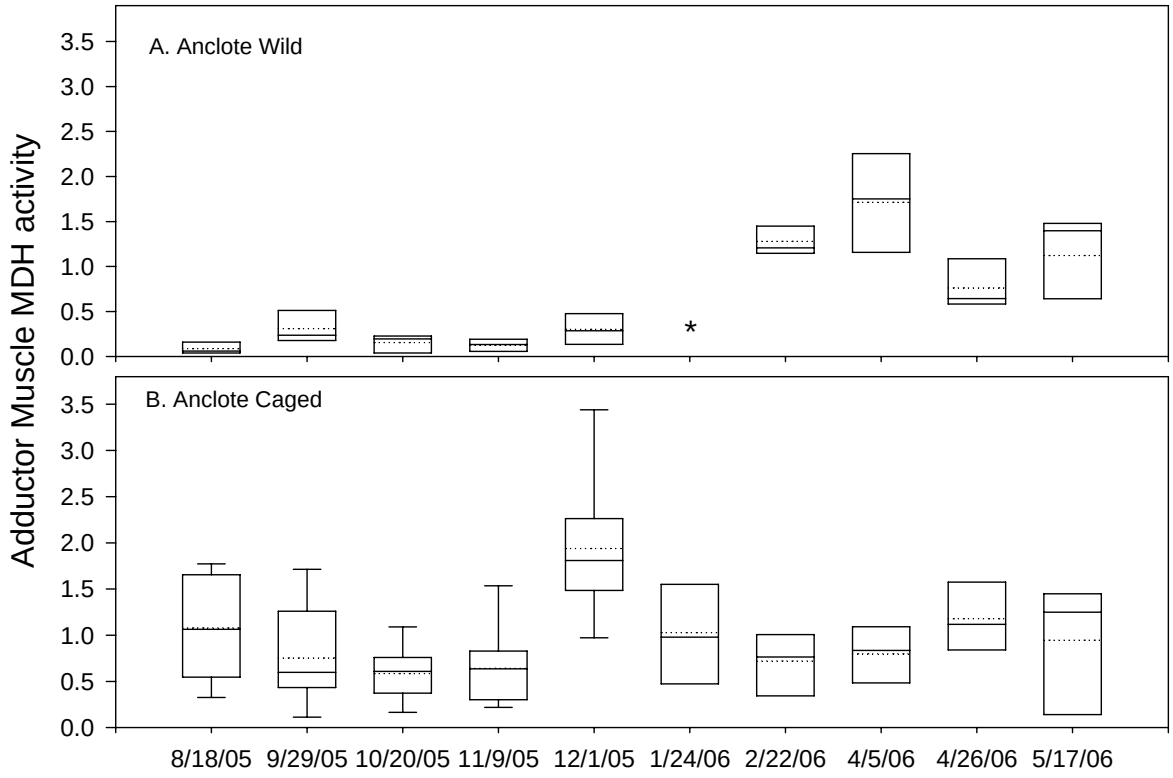


Figure 22. Box-and-whisker plot of adductor muscle malate dehydrogenase (MDH) activity for (A) wild scallops collected within the vicinity of the Anclote study site and (B) cultured scallops planted into cages at the Anclote study site. The filled circles represent values that fall outside of the 10th – 90th percentile range, the whiskers represent the 10th and 90th percentiles, the filled gray box represents the 25th and 75th percentiles, the dotted line represents the mean, and the solid line within each gray box represents the median. Asterisk indicates date on which no wild scallops could be collected.



Genetic Analyses

MtDNA analysis

Two broodstock collections were characterized using the SCAOPA3 mtDNA marker. One set (December 2005) included 27 scallops, which generated larvae that were released into Sarasota Bay in December 2005 for the first enclosure effort. The other set (April 2006) included 21 scallops that were spawned in the hatchery, and whose larvae were subsequently released into Sarasota Bay for the second enclosure effort. The absence of a pre-release sample of scallops from Sarasota Bay (due to the absence of scallops in Sarasota Bay) prevents the specific evaluation of the distinctiveness of the larvae generated from these broodstock groups. The haplotype composition of the broodstock, however, was dominated by unique haplotypes. In the December group, 84% of the scallops characterized (N = 25) exhibited unique haplotypes (haplotype diversity = 0.999928) and in the April group, 90% (N = 19; haplotype diversity = 0.999966) were new when compared to a haplotype database including the sequences of ~950 scallops from Florida. The novelty of the broodstock composition suggests that a contribution to the wild population resulting from the releases should be detectable.

The assessment samples for these releases involved the analysis of spat (N = 132) collected in Tampa and Sarasota Bays in 2006, as well as the sampling of adults in Sarasota Bay (N = 35). Of the 132 spat samples collected, 11 were determined to be calico scallops (*Argopecten gibbus*), 42 failed to yield amplifiable DNA or exhibited weak sequencing, and 79 were completely analyzed. Seventy-three distinct haplotypes were observed in the spat (haplotype diversity = 0.999939) and 70 were new based on comparison with the database. There were 5 haplotype matches between the broodstock and the collected spat (Table 6). The majority of these matches, however, involve spat collected before the releases (06spat 18, 31, 35) or in Tampa Bay (06spat 14, 15, 64, 96, 97 105, 108) which was quite distant but not completely isolated from the release site.

Comparison of the broodstock haplotypes to the sample of adults collected 3-6 months after the releases did not reveal any matches. The 35 scallops collected from Sarasota Bay exhibited 35 haplotypes, including 33 new sequences (haplotype diversity = 0.999957).

Microsatellite (SSR) development: Seventy-four percent (477/648) of the colonies selected for amplification and sequencing yielded useable sequences. Evaluation of the sequence data revealed 19.5% (93) of the sequenced inserts to contain potential microsatellite loci: 41 contained tetranucleotide repeats, 19 contained

trinucleotide repeats, and 25 contained dinucleotide repeats. The remaining 8 were compound microsatellites. Of the 93 potential microsatellite loci, we were able to design primers for 77 loci. The remaining potentials either had repeat segments that were too long for automated analysis (>500 base pairs), had highly irregular repeat units, or did not have sufficient flanking regions for primers to be designed. Of the 77 sets of primers, 34 sets have been synthesized and tested for utility. Of the 34 sets tested, six failed to amplify and 11 sets were inconsistent or yielded too many or the wrong sized fragment. Thirteen primer pairs performed well initially as unlabeled primers, but once resynthesized with a fluorescent label were found to be problematic either because of inconsistent amplification, null (non-amplifying) alleles or lack of variation. Five loci were deemed suitable for routine use in genetic assessments (Table 7). Preliminary statistics and allele frequency distributions for 3 populations in Florida (Steinhatchee, Anclote and Pine Island) can bee seen in Table 8, and Figures 23-27, respectively.

Table 6: Haplotype matches between broodstock and wild spat based on SCOPA3 sequence. All spat were collected from Tampa Bay in March, 2006.

Broodstock #	Haplotype #	Spat #
Dec		
1	455	97
7	614	105
17	53	14, 15, 64, 96, 108
Apr		
10	129	31
18	33	18, 35

Table 7. Primer sequence, expected size, annealing temperature and repeat motif for 5 microsatellite loci.

Locus	Primers	Size (bp)	Annealing Temp. (^{o}C)	Repeat Motif
AICL 112	F: TGCCAAATCCATTTGCATATTA R: [GT]TTCCCTGTTCACTTGACAGACC	214	56	$(GACA)_1GATG(GACA)_{12}$
AICL 115	F: TGCGGTATTTGAGTCCCCTA R: [GT]TTGACCTTTTGACCCCAAAT	201	56	$(GTCT)_{10}$
AICL 131	F: CCCTATGGCTTCCTCAACCT R: [GT]TTAACTTTCTGTGCCGTGGA	250	50	$(CAA)_9$
AICL 271	F: CCTTACATGACCCTGGCTGT R: [GT]TTCATCTAATTTATCAACCGACCA	91	50	$(CAAA)_8$
AICL 327	F:GCAAAATCCACCCATCAGTT R:[GTTT]ACCGGAGGGGACTAGTGTTT	103	58	$(CAGA)_6$

Table 8. Summary statistics for Anclote (AN), Pine Island (PI) and Steinhatchee (ST) scallops populations genotyped at 5 microsatellite loci. N=number of individuals genotyped, Na=number of alleles observed, Ho= observed heterozygosity, He=expected heterozygosity, and P-values associated with a test for confirmation to expectations under the assumption of Hardy-Weinberg equilibrium (FSTAT, Goudet, 1995). Significant deviations are indicated by the asterisks.

Locus		N	Na	Ho	He	P-value
AICL112	AN	99	25	0.758	0.873	0.0015*
	PI	98	28	0.765	0.839	0.2496
	ST	25	13	0.800	0.844	0.3109
AICL115	AN	97	14	0.619	0.734	0.0255
	PI	92	11	0.609	0.694	0.1234
	ST	25	7	0.400	0.614	0.0126*
AICL131	AN	90	16	0.778	0.811	0.0334
	PI	87	15	0.828	0.853	0.2624
	ST	24	11	0.958	0.836	0.0500
AICL271	AN	99	10	0.828	0.804	0.4055
	PI	95	10	0.779	0.770	0.3154
	ST	25	7	0.800	0.714	0.3381
AICL327	AN	50	6	0.300	0.303	0.5333
	PI	50	6	0.280	0.271	0.7157
	ST	25	5	0.240	0.222	1.0000

Figure 23. Microsatellite locus 112: Allele frequencies for scallops collected from Steinhatchee (ST), Anclote (AN), and Pine Island (PI). Bubble size reflects frequency in the population.

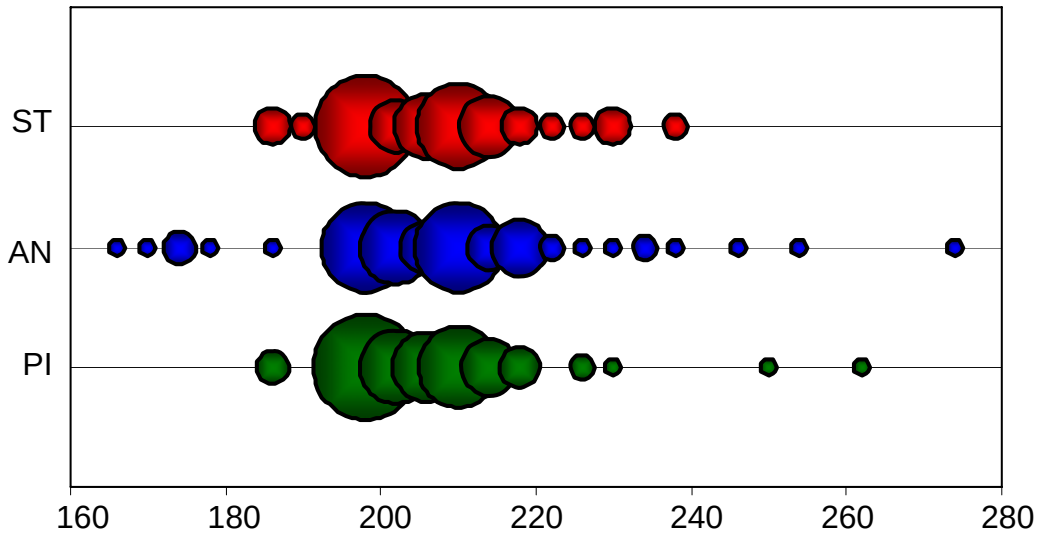


Figure 24. Microsatellite locus 115: Allele frequencies for scallops collected from Steinhatchee (ST), Anclote (AN), and Pine Island (PI). Bubble size reflects frequency in the population.

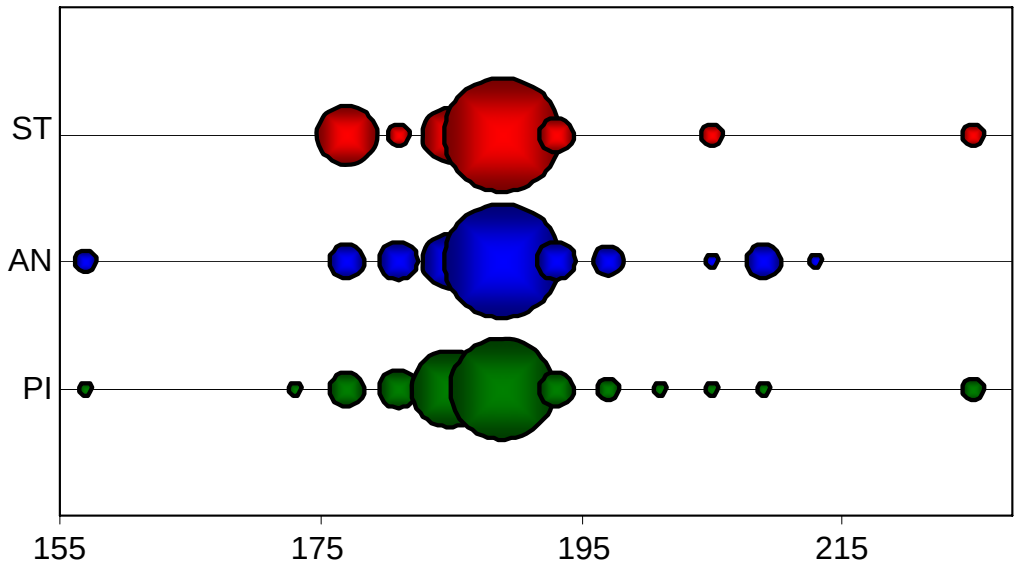


Figure 25. Microsatellite locus 131: Allele frequencies for scallops collected from Steinhatchee (ST), Anclote (AN), and Pine Island (PI). Alleles 335 and 301 each present at a frequency of 0.01 in the Anclote sample was excluded from graph for clarity. Bubble size reflects frequency in the population.

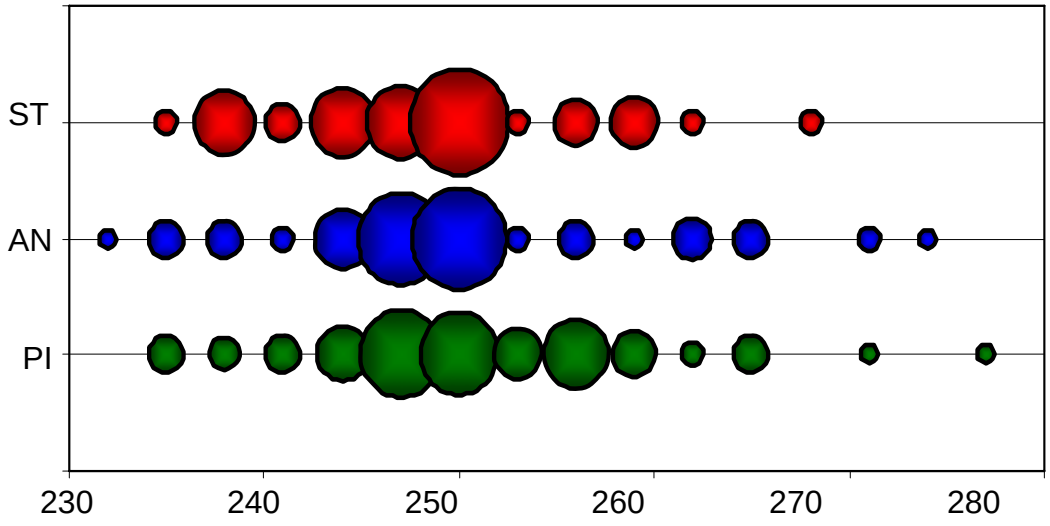


Figure 26. Microsatellite locus 271: Allele frequencies for scallops collected from Steinhatchee (ST), Anclote (AN), and Pine Island (PI). Bubble size reflects frequency in the population.

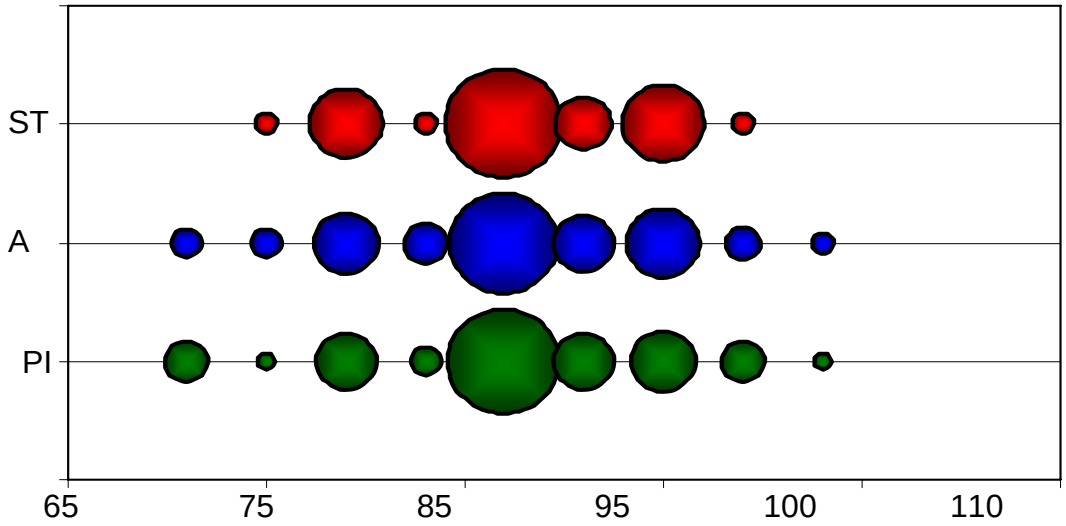
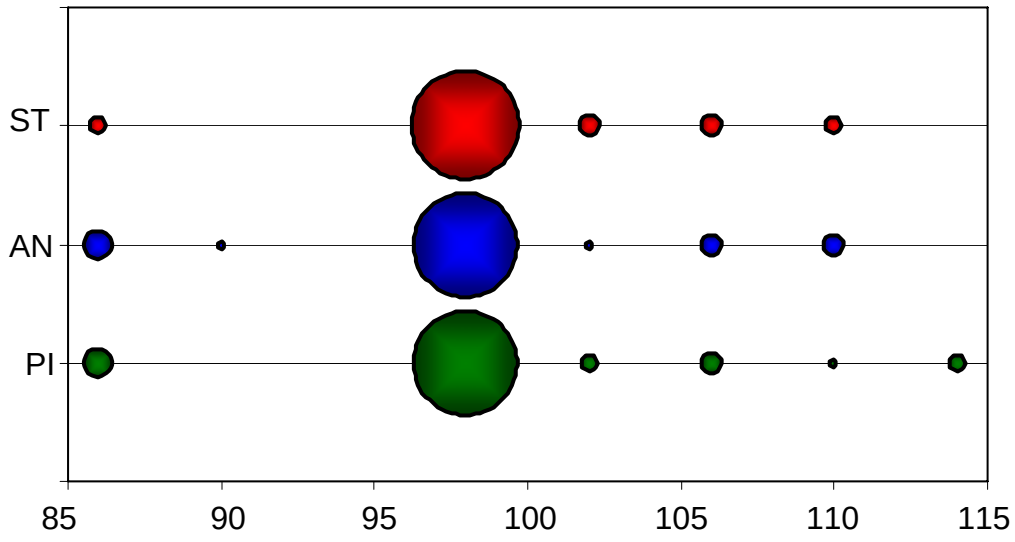


Figure 27. Microsatellite locus 327: Allele frequencies for scallops collected from Steinhatchee (ST), Anclote (AN), and Pine Island (PI). Bubble size reflects frequency in the population.



Problems Encountered

The most persistent problem associated with the execution of this project was our inability to raise adequate numbers of cultured scallops to the desired planting size of 18 mm SH. Successful bay scallop restoration efforts are fundamentally tied to the availability of adequate numbers of scallop seed to support the project (Wenczel et al., 1993). Results from this study and from other studies (e.g., Wenczel et al., 1993) clearly indicate that producing juvenile bay scallops is not a trivial matter, and success during one year does not assure success during subsequent years. Refinement of scallop culture methods and the identification or development of a hatchery or group of hatcheries that can be relied upon to accomplish production tasks are necessary precursors to the continuation of bay scallop population restoration efforts in Florida and in other United States coastal waters.

A second problem relates to the persistent and insidious blooms of red tide (*Karenia brevis*) that are inimical to bay scallop survival and that severly compromised the success of our restoration efforts. Red tide is a natural phenomenon along the west coast of Florida, but the debate continues as to whether red tide events have become more frequent or more intense in recent decades. Regardless, overcoming the impacts of red tide events particularly in coastal waters from Tampa Bay south may

require frequent restoration efforts that respond to red tide induced mortality events. Such frequent efforts are best supported by the larval release approach because that approach allows for a rapid and targeted response at relatively low cost. Similar considerations apply in areas such as North Carolina (Summerson and Peterson, 1990) and New York (Cosper et al., 1987) where harmful algal bloom events also have severely impacted bay scallop populations.

Additional Work

A recent socioeconomic study by Dr. Charles Adams at the University of Florida indicated that the recreational bay scallop season in Florida generated a minimum of at least $1 million in economic activity to each of the associated communities. Bay scallops also function as a critical component of coastal seagrass beds, providing trophic transfer from primary producers to upper level consumers. Thus, the maintenance of a healthy bay scallop metapopulation in Florida waters is a desirable and worthwhile goal. To accomplish that goal requires that multiple local populations remain healthy, including populations in areas that are targeted by the recreational fishery (e.g., Crystal River, Homosassa) and populations in areas that are outside of the recreational fishing zone (e.g., Anclote, Sarasota Bay).

The need to maintain many local populations derives from the structure and function of the metapopulation. Bay scallops only live about one year, so local populations fluctuate in abundance (Figure 1) dependent upon the vagaries of the natural environment and resultant variations in year-class strength. With respect to reproductive viability, local populations come and go on a roughly annual basis, so the more local populations that are extant the more stable is the metapopulation. The restoration efforts that we describe in this report were designed to rebuild and maintain local populations of bay scallops in four areas along the west Florida shelf. Although the genetic evidence generated from this and previous studies (Seyoum et al., 2003; Wilbur et al., 2005) suggests that our restoration efforts have not contributed offspring to the following year-class, this and previous studies (Arnold et al., 2002; 2005a; 2005b) indicate that bay scallop population abundance is generally higher in areas where restoration has been conducted and in the appropriate time frame following those activities, than in those same areas when restoration did not occur. This may be coincidental, and more work is needed to verify this relationship. In this study, we chose Sarasota Bay as a test site to better

define the relationship between restoration activities and subsequent population abundance, but red tide has proven to be a substantial impediment to the success of our experimental efforts. We therefore suggest that similar work should be undertaken in Florida panhandle estuaries such as St. Andrew Bay and Pensacola Bay where bay scallop populations may benefit from restoration efforts and resultant increased larval supply but where red tide has historically been less of a problem. To better evaluate the success of our bay scallop restoration efforts, more specific and unequivocal genetic identification techniques must be developed and applied. Finally, we need to better understand the physical oceanographic processes that link local scallop populations along the west coast of Florida. This latter need provides an ideal linkage between applied biological processes and the developing Coastal Ocean Observing System (COOS).

EVALUATION

Project Evaluation

In general, the goals of this project were achieved but it took two years to realize success. During the first year of the project, and for reasons described above, we were not able to produce adequate numbers of scallops to meet the obligations of the grant. During the second year of the project, production of cultured scallops was much more successful and we were able to plant cultured scallops at all sites, to monitor their growth, mortality, reproductive development, and biochemical composition, and to apply the predictions of a physical oceanographic model in the selection of appropriate sites for scallop restoration efforts.

We made a few modifications to the original research plan. One change involved raising juvenile scallops at our Anclote study site rather than in Bayboro Harbor in downtown St. Petersburg. However, this was not a fundamental change because we had pursued a similar strategy during previous scallop restoration efforts. In both instances, the switch from Bayboro to Anclote occurred in early summer. At that time, increased rainfall decreases salinity within the confines of Bayboro Harbor to levels that are not suitable for bay scallop survival and growth. Additionally, as rainfall is flushed into Bayboro Harbor it brings along a host of potentially detrimental contaminants that further stress the cultured scallops. Growing the scallops to planting size (18 mm SH) at the Ancote site rather

than in Bayboro Harbor does increase effort, labor costs, and travel costs, but it is a necessary solution to the problems that we encounter in Bayboro Harbor beginning in June of each year.

A second and more promising change involved the use of the larval phase to rebuild patches of scallops rather than using juvenile scallops planted into cages for that purpose. This approach considerably reduced the cost of our culture efforts, allowed us to choose our restoration sites with less lead-time, and also removed the scallops from the cages that reduced the flux of food and oxygen to the scallops and also prevented them from swimming and other natural behaviors. We continue to test this approach to scallop restoration at various sites in Florida and we are aware that the larval release also is being tested in other areas along the eastern seaboard of the United States (Murphy et al., 2005).

Dissemination of Results

Much of the work conducted for this project was a continuation of bay scallop restoration activities that have been ongoing along the west coast of Florida since the mid-1990s. Those research efforts have been described in publications by Arnold et al. (2002), Arnold et al. (2005b), Seyoum et al. (2003), and Wilbur et al. (2005). Additionally, we are continuing this work under various grants from federal and state entities. We have not yet identified any additional publications that will derive from the present study, but we do anticipate that upon termination of our NMFS-funded bay scallop restoration efforts in fall 2006 we will prepare additional manuscripts for publication in peer-reviewed journals. We also anticipate continued communications with the public and with a multitude of media outlets throughout Florida to discuss bay scallop restoration and the status of bay scallop populations in Florida waters.

ACKNOWLEDGMENTS

Numerous individuals are not included on the author list for this final report but nevertheless contributed valuable assistance in the execution of this project. Commercial fisherman Gary Fulford provided information concerning scallops captured during his Sarasota Bay trawling activities. University of South Florida College of Marine Science graduate student Noland Elsaesser conducted scallop spawnings and initial hatchery rearing. FWRI Molluscan Fisheries research staff who participated in some aspect of this study include Janessa Cobb, Carla Beals, Michael Hofmann, Brett Pittinger, Mark Gambordella, and Kristina Ramer. Curt Hemmel, owner of Bay Shellfish

Incorporated, provided scallop larvae and seed and was indispensable to the completion of this project. Jay Leverone of Mote Marine Laboratory assisted with the enclosure experiments in Sarasota Bay. We express our sincere gratitude to the National Marine Fisheries Service for their willingness to financially support this project.

REFERENCES

Arnold, W.S. (1994). Bay scallops: summary report to the Florida Marine Fisheries Commission. Florida Department of Environmental Protection, Florida Marine Research Institute, 13 pp.

Arnold, W.S. (2001). Bivalve restoration and enhancement strategies in Florida, USA. Hydrobiologia 465: 7-19.

Arnold, W.S., T.M. Bert, N.J. Blake, C. Crawford, M. Harrison, J. Landsberg, D. Marelli, M.Parker, S.Peters, R. Reese, S. Seyoum, G. Vermeer, C.Way, A. Wilbur & C. Young (2002). Restoration of the bay scallop (*Argopecten irradians* Say) in Florida: An integrated approach to stock enhancement and evaluation of benefit. Final Report, Grant NA76FK0426 to the National Marine Fisheries Service.

Arnold, W.S., N.J. Blake, S.P. Geiger, M.L. Parker, S.C. Peters & A.E. Wilbur (2005a). Bay scallop (Argopecten irradians) population restoration on the west coast of Florida. Final report, NOAA National Marine Fisheries Service, Grant NA17FD2368, 89 pp.

Arnold, W.S., N.J. Blake, M.M. Harrison, D.C. Marelli, M.L. Parker, S.C. Peters & D.E. Sweat (2005b). Restoration of bay scallop (*Argopecten irradians* (Lamarck)) populations in Florida coastal waters: Planting techniques and the growth, mortality, and reproductive development of planted scallops. J. Shellfish Res. 24: 883-904.

Arnold, W.S., D.C. Marelli, C.P. Bray & M.M. Harrison (1998). Recruitment of bay scallops *Argopecten irradians* in Floridan Gulf of Mexico waters: scales of coherence. Mar. Ecol. Prog. Ser. 170: 143-157.

Blake, N.J., Y. Lu & M. Moyer (1993). Evaluation of Tampa Bay waters for the survival and growth of southern bay scallop larvae and juveniles. Final Report, Tampa Bay National Estuary Program, Technical Publication #04-93, 18 pp.

Brownstein, M.J., J.D. Carpten & J.R. Smith (1996). Modulation of non-templated nucleotide addition by Taq DNA polymerase: Primer modifications that facilitate genotyping. Biotechniques 20: 1004-1006

Cosper, E.M., W.S. Dennison, E.J. Carpenter, V.M. Bricelj, J.G. Mitchell, S.H. Kuenstner, D. Colflesh & M. Dewey (1987). Recurrent and persistent brown tide blooms perturb coastal marine ecosystems. Estuaries 10: 284-290.

Donnelly, J., H. Kawall, S.P. Geiger & J.J. Torres (2004). Metabolism of Antarctic micronektonic crustacea across a summer ice-edge bloom: respiration, composition, and enzymatic activity. Deep-Sea Research II 51: 2225-2245.

Geiger, S.P., J. Donnelly & J.J. Torres (2000). Effect of the receding ice-edge on the condition of mid-water fishes in the northwestern Weddell Sea: results from biochemical assays with notes on diet. Mar. Biol. 137: 1091-1104.

Goudet J. (1995). fstat V1.2. A computer program to calculate *F*-statistics. J. Hered. 86: 485-486.

Hanski, I.A. & M.E. Gilpin, eds. (1996). Metapopulation Biology: Ecology, Genetics, and Evolution. Academic Press, San Diego, 512 pp.

Ikeda, T., J. J. Torres, S. Hernandez-Leon & S. P. Geiger (2000). Metabolism. In: R.P. Harris, P.W. Weibe, J. Lenz, H.R. Skjoldal & M.E. Huntley (eds.), Zooplankton Methodology Manual. Academic Press, San Diego, pp. 455-532.

Leverone, J.R., W.S. Arnold, S.P. Geiger & J. Greenawalt (2004). Restoration of bay scallop populations in Pine Island Sound: Competent larval release strategy. Final report to the Charlotte Harbor National Estuary Program. Mote Marine Laboratory Technical Report #974, 13 pp.

Levitan, D.R. (1995). The ecology of fertilization in free-spawning invertebrates. In: L. McEdward (ed.), Ecology of Marine Invertebrate Larvae. CRC Press, Boca Raton, pp. 123-156.

Lewin, R. (1986). Supply-side ecology. Science 234: 25-27.

Lowry, O.H., N.J. Rosenbaugh, N.L.Farr & R.J.Randall (1951). Protein measurements with the Folin phenol reagent. J. Biol. Chem. 193: 265-275.

Marelli, D.C., W.S. Arnold & C. Bray (1999). Levels of recruitment and adult abundance in a collapsed population of bay scallops (*Argopecten irradians*) in Florida. J. Shellfish Res. 18: 393-399.

Marelli, D.C., M.K. Krause, W.S. Arnold & W.G. Lyons (1997a). Systematic relationships among Florida populations of *Argopecten irradians* (Lamarck, 1819) (Bivalvia: Pectinidae). The Nautilus 110: 31-41.

Marelli, D.C., W.G. Lyons, W.S. Arnold & M.K. Krause (1997b). Subspecific status of *Argopecten irradians concentricus* (Say, 1822) and of the bay scallops of Florida. The Nautilus 110: 42-44.

Murdock, J.F. (1955). Investigation of the Lee County bay scallop fishery. University of Miami Marine Laboratory, Report ML 9469, 10 pp.

Murphy, D.C., W.C. Walton, S. Roberts & B.A. Walton (2005). Experimental use of bay scallop, *Argopecten irradians irradians*, pediveligers in restoration. J. Shellfish Res. 24: 668-669.

Peterson, C.H. & H.C. Summerson (1992). Basin-scale coherence of population dynamics of an exploited marine invertebrate, the bay scallop: implications of recruitment limitation. Mar. Ecol. Prog. Ser. 90: 257-272.

Reisenbichler, K.R. & T.G.Bailey (1991). Microextraction of total lipid from mesopelagic animals. Deep-Sea Research 38: 1331-1339.

Schneider, S., D. Roessli & L. Excoffier (2000). Arlequin version 2.0: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Seyoum, S., T.M. Bert, A. Wilbur, C. Crawford & W.S. Arnold (2003). Development, assessment, and application of a mitochondrial DNA genetic tag for the bay scallop (*Argopecten irradians*). J. Shellfish Res. 22: 11-117.

Summerson, H.C. & C.H. Peterson (1990). Recruitment failure of the bay scallop, *Argopecten irradians concentricus*, during the first red tide, Ptychodiscus brevis, outbreak recorded in North Carolina. Estuaries 13: 322-331.

Thayer, G.W. & H.H. Stuart (1974). The bay scallop makes its bed of seagrass. Mar. Fish. Rev. 36: 27-30.

Walsh, P.J., C. Bedolla & T.P. Mommsen (1989). Scaling and sex-related differences in toadfish (*Opsanus beta*) sonic muscle enzyme activities. Bull. Mar. Sci. 45: 68-75.

Wenczel, P., C. Smith & S. Tettelbach (1993). Planting bay scallops: Results of reseeding bay scallops in the Peconic Bay New York 1986 to 1992. Final report submitted to the New York State Urban Development Corporation, the New York State Department of Environmental Conservation, and the County of Suffolk, 135 pp.

Wilbur, A.E., S.Seyoum, T.M. Bert & W.S. Arnold. (2005). A genetic assessment of bay scallop (*Argopecten irradians*) restoration efforts in Florida's Gulf of Mexico coastal waters (USA). Cons. Gen. 6: 111-122.

Winter, M.A. & P.V. Hamilton (1985). Factors influencing swimming in bay scallops, *Argopecten irradians* (Lamarck, 1819). J. Exp. Mar. Biol. Ecol. 88: 227-242.

Appendix I

Monitoring growth and mortality of the bay scallop, *Argopecten irradians* (Lamark), on the Gulf Coast of Florida.

Tina Ramer, Florida State University, Tallahassee, Florida.

Introduction

Bay scallop (*Argopecten irradians*) are suspension-feeding bivalves that are often found in seagrasses, most commonly in the species *Thalassia*. They recruit to coastal bays, sounds, estuaries, and inshore sides of barrier islands where waters are less than 12 m in depth. The bay scallop is thought to be a semelparous, or a short- lived, species, with a life span of one to two and a half years (Gutsell, 1930; Barber and Blake, 1981) depending on area. *Argopecten irradians* can be found along the Atlantic coast from Cape Cod, Massachusetts, to South Carolina and on the Gulf Coast, from Florida to Texas (Clark, 1965).

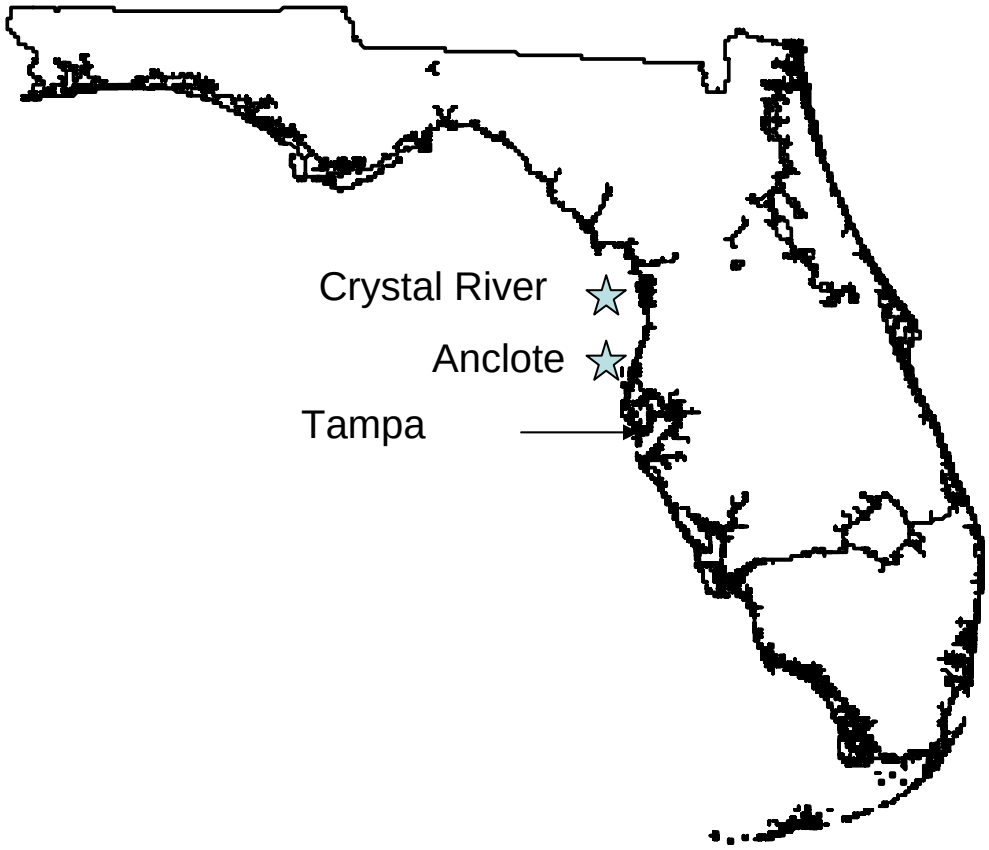
Argopecten irradians is important both ecologically and economically. Ecologically, they are important because as suspension feeders, they help maintain water clarity in the environments within which they occur. The bay scallop, as a food source, is important because it supports large recreational activities, which in turn benefits the Florida economy. The commercial fishery for bay scallops in Florida has not been in effect since 1994 due to the declining bay scallop populations (Carlisle, 2000).

The objective of my study was to monitor the growth and mortality of the juvenile bay scallop in estuaries on the Gulf Coast of Florida. There have been few, if any, studies on the growth of juvenile bay scallop in Florida. To accomplish this, I compared the growth patterns of scallops from three different sites along the west coast of Florida: Bayboro Harbor, Anclote, and Crystal River. Results from this study will help contribute information to ongoing restoration projects.

Study sites

There are three sites where we are studying the growth and mortality of bay scallops. The first site is the Bayboro dock in front of FWRI. The conditions at this site are the worst of the three sites. The dock is in a bay where there is a high amount of runoff, pollutants, and boat fluids. The cages are hung off the dock so that they are not sitting in the muddy bottom but rather suspended in the water column. Bayboro also has a higher frequency of red tide, which is extremely lethal to bay scallops. The Anclote and Crystal Bay study sites are

Figure1. Map of Florida indicating the three field sites where we monitored the growth of the juvenile bay scallops.



very similar to each other. In Anclote the cages are placed in *Thalassia* with six to eight inch pvc pipes on the four corners of the cages to keep them off the bottom as a means of increasing survival. The water quality is very good at Anclote, with few toxins and little variability in salinity compared to the other two sites. Anclote does suffer from harmful algal blooms. Crystal River cages are also placed in *Thalassia* and the cages are raised as well. The difference between Crystal River and Anclote is that Crystal River has high river runoff, which in turn lowers the salinity and may introduce more pollutants.

Methods

Bay scallop growth was monitored over the course of a single growing season. Juvenile scallops were provided by a commercial hatchery in Palmetto, Florida (Bay Shellfish Inc.) owned and operated by Curt Hemmel. The juvenile scallops, sized 0.8-1.2 mm shell height (SH), were initially placed in 800μm mesh bags in two sites, Bayboro and Anclote. Bayboro was planted at a density of roughly 8,000 per bag on 2/28/05 and Anclote was planted at a density of 12,000-20,000 per bag on 3/4/05. At each site roughly 1/3 of the bottom cage space was covered by scallops. As the scallops grew, additional cages were added to maintain a consistent density to prevent overcrowding and food limitation.

The Bayboro dock cages and bags were cleaned twice a week, whereas the Anclote and Crystal River cages were not checked as frequently due to the distance from FWRI. At Bayboro, the 800 μm mesh bags were taken out of the cages every other week and brought to the lab so the scallops could be sorted. Scallops smaller than 7 mm SH were returned to clean 800-μm-mesh bags. Scallops larger than 7 mm SH were placed in 4-mm-mesh bags. The shell height of each of ten scallops from each bag was measured to the nearest 0.1 mm with vernier calipers. Two hundred of the larger scallops were placed in each 4-mm-mesh bag to reduce overcrowding. To estimate the number of smaller scallops, we counted the number required to fill a 10 ml beaker. The remaining small scallops were put into a 100 ml beaker and their volume measured to the nearest 10 ml. The total number of small scallops was then calculated by multiplying the amount it took to fill 10 ml by the total number of milliliters they filled. Growth and mortality of the Anclote scallops was estimated similarly once per month. Beginning on 5/18/05, presorted large spat (> 7 mm SH) from the Bayboro dock were planted at the Crystal River site (200 per bag, 2-3 bags per cage). Thereafter, their growth and mortality were also monitored on an approximately once monthly schedule.

Data from the counts in each bag were used to estimate mortality in each cage. After each bag was sorted a new estimate of abundance was determined. This allowed us to estimate mortality for only those scallops in each cage, not those removed via sorting. Loss was estimated by dividing the number of scallops alive on each date by the number of scallops alive on the previous date (% Loss) or on the initial date (Total Loss). Shell height measurements were used to estimate growth over the duration of the study. Similar estimates from field planted scallops were used to compare growth and mortality at each of the three sites. Growth curves were calculated by observing changes in the length-frequency curve measured during each sampling period (c.f. McBride et al., 1995). Several bags of larger, sorted scallops were maintained at the FWRI dock site to reduce the bias of only transporting faster growing scallops to the other sites.

Results

Shell height measurements were taken on three dates at Crystal River, four dates at Anclote, and thirteen dates at the Bayboro site (Table 1). At each site, ten scallops were measured from each planting cage on each date. The Bayboro site had no measurements taken in July because there was 100 % mortality due to a strong bloom of the red tide organism, *Karenia brevis*, which occurred in the bay.

Table 1. Samples taken at our three study sites on different sampling dates. This portrays the number of scallops measured and how many sets were sorted.

Crystal River

Date	Sets	Measured Per Set	Total Measured
5/18/2005	10	10	100
6/6/2005	10	10	100
7/13/2005	23	10	130

Anclote

Date	Sets	Measured Per Set	Total Measured
3/24/2005	4	10	40
4/12/2005	4	10	40
5/5/2005	14	10	140
7/6/2005	14	10	140

Bayboro

Date	Sets	Measured Per Set	Total Measured
3/21/2005	4	10	40
5/5/2005	2	10	30
5/6/2005	3	10	30
5/16/2005	5	10	50
5/17/2005	6	10	60
5/19/2005	6	10	60
5/31/2005	2	10	20
6/1/2005	6	10	60
6/2/2005	3	10	30
6/9/2005	2	10	20
6/13/2005	4	10	40
6/14/2005	2	10	20
6/27/2005	5	10	50

The shell height of the juvenile scallops increased significantly with time at each of our three study sites (Table 2). The fastest growth occurred at Anclote. Similar growth was recorded at the Crystal River and Bayboro sites. Crystal River scallops reached a mean SH of 14-21 mm by July 13 (Figure 2). Anclote scallops reached a mean shell height of 16-20 mm by July 6 (Figure 3). Bayboro scallops achieved a mean shell height of 8-14 by June 13-14 (Figure 4).

To compare growth rates between the sites even more closely we re-analyzed the data so that similarly aged scallops (two to four months) from each site were compared. The results still showed that Anclote scallops grew faster than their Crystal River and Bayboro counterparts. Anclote scallops averaged about 0.1783 mm/day compared to Bayboro's 0.0527 mm/day, and Crystal Bays 0.0711 mm/day (Table 2).

Table.2. Data from each of the three sites. R square value represents how fast the scallops grow. Each site has its own shell height formula for their environment, and also for different days. The df of degrees of freedom indicates how many scallops were observed.

Site	**R Square**	**F Value**	**P Value**	***df***	**Shell Height**
Crystal Bay (all)	0.387	25.8888	0.0000084 4	42	SH=(0.0711*Age)+1.901
Anclote (all)	0.9529	687.7561	3.85E-24	35	SH=(0.156*Age)-2.218
Anclote (Day 66-128)	0.9615	648.6957	6.52E-20	27	SH=(0.1783*Age)-4.649
Bayboro (all)	0.6468	87.917	2E-12	49	SH=(0.0735*Age)+5.867
Bayboro (Day 66-119)	0.2388	12.238	.001186	40	SH=(0.0527*Age)+3.627

Fig.2. Shell height (mm) vs. age (days) of the juvenile scallops at Crystal River. Growth was monitored over a four month period.

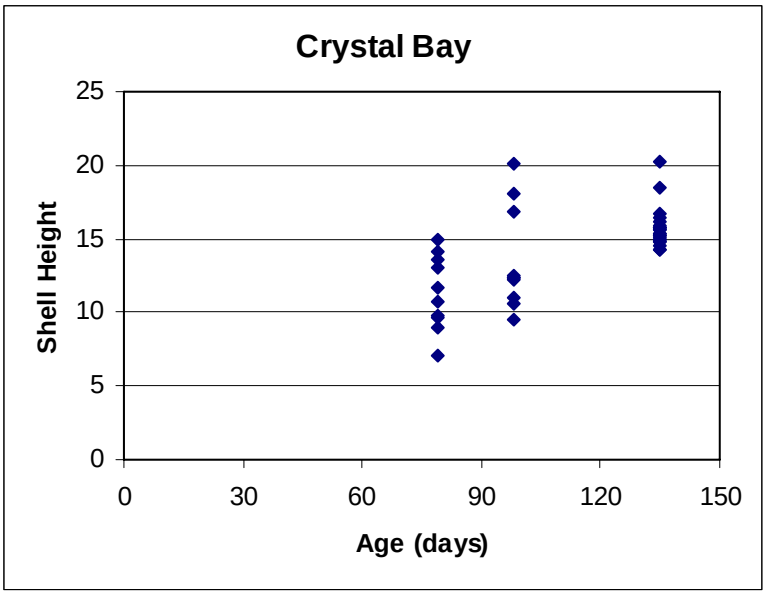


Fig.3. Shell Height (mm) vs. Age (days) of the juvenile scallops at Anclote. Growth was monitored over a four month period.

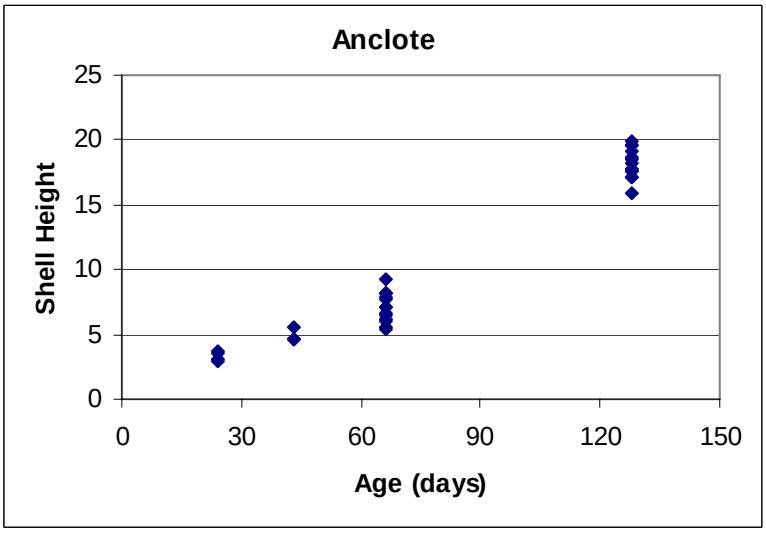
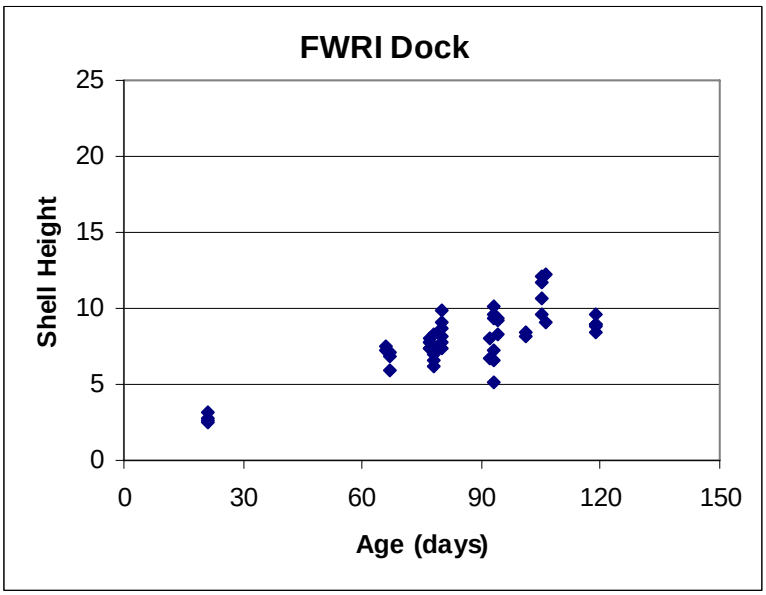


Fig. 4. Shell Height (mm) vs. Age (days) of the juvenile scallops in Bayboro. Growth over three month period was monitored.



Counts of live scallops were conducted on 5/18/05 and 7/14/05 at our Crystal River site, on 3/24/05, 4/12/05, and 7/14/05 at our Anclote site, and on 2/28/05, 3/21/05, 5/6-20/05, 6/1/05, and 6/29/02 at our Bayboro site. Loss was estimated by dividing the number of scallops alive on one date by the number of scallops alive on the previous sample date (% Loss) or by the initial date (Total Loss).

Anclote and Bayboro sites had high initial loss when scallops were still small, but as scallops size increased mortality decreased at the Bayboro site until complete mortality occurred on June 29 when all of the scallops were destroyed by red tide. Mortality continued to increase at the Anclote site. Crystal River scallops, which were planted at a larger size, had the lowest mortality.

Discussion

The growth of the bay scallop, *Argopecten irradians,* was monitored to determine which environment produced better growth and survival. The results indicate that at each site there was significant growth (p-values at each site < 0.001). Barber and Blake (1983) conducted a similar experiment on the Florida bay scallop and found that the mean shell height increased from early May to August concomitant with increasing water temperature. Our results also show that the scallops' growth increased throughout the summer months as

the water temperature increased. In St Joseph Bay, Florida bay scallops also show high growth rates during the summer months (Bologna, 1998).

Table 3. Mortality at each of the three study sites. Indicates the total percent lost from the previous date that the scallops were monitored.

Date	Crystal River	% Loss	Total loss
5/18/2005	3800		
7/14/2005	2300	39.47	39.47

Date	Anclote	% Loss	total loss
3/24/2005	113,000		
4/12/2005	42336	62.53	62.53
7/14/2005	1900	95.51	98.32

Date	Bayboro	% Loss	total loss
2/28/2005	120,000		
3/21/2005	38,804	67.66	
5/6-20/2005	31,665	18.40	73.6125
6/1-6/27	22,627	28.54	81.1442
6/29/2005	0	100.00	100

The Bayboro site had the slowest growth rate and relatively low mortality, but at this site the large scallops (> 7mm) were removed and transferred to the other growout sites. Anclote showed the best growth of all the sight but showed the highest mortality. The Bayboro site is in an industrial bay which is constantly experiencing road runoff and boat pollutants that the scallops are filtering into their systems and which could be affecting their growth. Crystal River also experiences some runoff brought in by the river, which can shock the

scallops and reduce their growing capability. Due to the conditions at these two sites there is often a change in the salinity of the water which may negatively affect scallop growth and mortality.

Red tide appears to have had a substantial impact on the scallops in Bayboro. Around June 29th, the red tide made its way into the bay and killed all the scallops on the dock. In general, harmful algal blooms such as *Karenia brevis* affect marine organisms by paralyzing their respiratory systems, which means that the scallops suffocate because of the mass amount of phytoplankton (Carlisle, 2000). In Bogue Sound, North Carolina between 1987-1988 a HAB event stemming from a bloom of *Gymnodinium breve* killed all the scallops in the sound (David 1998). Red tide is a very important factor for scallop populations so this should be taken into concern when trying to find environments for scallop restoration.

References

Barber,B.J & N.J. Blake (1981). Energy storage and utilization in relation to gametogenesis in *Argopecten irradians concentricus* (Say). J. Exp. Mar. Biol.Ecol.52: 121-134.

Barber, B.J. & N.J. Blake (1983). Growth and reproduction of the bay scallop, *Argopecten irradians* (Lamarck) and its southern distributional limit. J.Exp. Mar. Biol. Ecol. 66: 247-256.

Bologna, P. (1998). Growth, production, and reproduction in bay scallops *Argopecten irradians concentricus* (Say) from the northern Gulf of Mexico. J. of Shellfish Res. 17: 911-917.

Bricelj, V.M. & S. Shumway (1991). Physiology: Energy acquisition and utilization. *In:* S. Shumway (ed.), Scallops: Biology, Ecology and Agriculture. Elsevier, New York, pp. 305-346.

Carlisle, E. (2000). The Gulf Of Mexico dead zone and red tides. The Louisiana Environment. 28 July, 2005. http://www.tulane.edu/~bfleury/Web%20Projects/Elisabeth%20Carlisle/nrabalais@lumcon.edu

Clarke, A.H.,Jr. (1965). The scallop superspecies *Aequipecten irradians* (Lamarck). Malacologia 2: 161-188.

Gutsell, J.S. (1930). Natural history of the bay scallop. U.S. Bur. Fish. Bull. 46: 569-632.

Appendix II

3-D Sarasota Bay Modeling to Support Restoration of Bay Scallop Populations

Y. Peter Sheng and Yanfeng Zhang
Civil & Coastal Engineering Department
University of Florida
Gainesville, FL 32611-6580

Executive Summary

Funded by Florida Fish and Wildlife Commission, this study in undertaken to support the restoration of bay scallop population in the Sarasota Bay estuarine system. After reviewing the historical wind and precipitation data in the region, it was decided that 2001 represents a "typical" year. Using a 3-dimensional circulation model CH3D, one-year simulation of the Sarasota Bay system in 2001 was conducted. Results of the one-year simulation, including simulated vs. measured water level and simulated residual flow and salinity fields for each month of 2001, are first presented in this report. The CH3D model was then coupled to a 3-dimensional particle tracking model to simulate the fate and dispersion of particles released from three locations in Sarasota Bay during November 1 to November 14, 2004. Based on detailed comparison of model results, release site #3 is found to be the best since most of the particles remained in the estuary after 14 days. Digital results of the model runs will be provided to Florida Fish and Wildlife Commission soon.

Introduction

The Sarasota Bay System is a barrier island-lagoon located along the Southwest coast of Florida. Circulation in the Sarasota Bay is primarily driven by tide, and to a lesser extent by wind. river discharges, and precipitation. Sheng and Peene (1995) previously conducted a modeling and monitoring study to quantify the three-dimensional circulation in the Sarasota Bay estuarine system. CH3D, developed originally by Sheng (1986, 1989) was used in that study. This study uses an enhanced version of CH3D with a larger model domain and a finer model grid to conduct long-term simulations of Sarasota Bay circulation. In addition, to support Florida Fish and Wildlife Commission's effort to restore bay scallop population in the Sarasota Bay, we conducted three-dimensional particle tracking modeling to simulate the trajectories of particles (which are supposed to represent scallop larvae) released from three candidate sites within the Sarasota Bay. The purpose of the simulation is to determine the best release site (among the three sites) and the best release time (flood tide, high tide, ebb tide, and low tide), based on comparison of model results to one another.

Measured data used in the simulation.

Measured data used in the simulation include water level, wind, river discharge, precipitation and evaporation. Plots of measured data can be found in Appendix 1.

Sarasota Bay numerical grid and simulation setup

The simulation was made using a newly developed 230x64 grid in UTM coordinate system as shown in Figure 1. A zoom-in plot of the Sarasota Bay grid is shown in Figure 2. In order to minimize the open boundary effect on model results, the open boundaries along north, west and south were extended at least 10 km away from the coastline. Bathymetry data from GEODAS were

interpolated onto the entire grid. Locations of data measurement stations are shown in Figure 3.

The water level along the open boundaries were generated by combining harmonic tidal constituents (obtained from the ADCIRC model) with long-period (longer than tidal period) water level generated by a filtering process which removes tidal signals from measured water level in the region. The procedures for generating the open boundary condition is explained next.

(A) Tidal constituents from ADCIRC tidal databases were extracted to produce the water level along the open boundaries. A total of seven constituents were considered using version ec2001_v2d database, which includes: M2, S2, N2, K2, O1, K1 and Q1. Details of the ADCIRC tidal database can be found at http://www.marine.unc.edu/C_CATS/tides/tides.htm.

(B) Measured water level at the NOAA St. Petersburg station within Tampa Bay was processed with the Doodson and Warburg 39-hourly weighted average tidal filter to obtain the long term filtered water level during 2001 (Figure 4), which was then used at all three open boundaries.

(C) Mean water level at the open sea was then adjusted to account for the local vertical datum. Since vertical datum NAVD88 is used for the bathymetry and water level data, the Mean Sea Level (MSL) used in the ADCIRC tidal databases needs to be adjusted first before applied to CH3D simulation. Although there is no known datum information available for the open boundaries of this study, a few isolated stations in the vicinity were found with MSL to NAVD88 datum adjustment. These stations include Clear Water station & Port Manatee station from National Ocean Survey and AG7424 station & AG5037 station from Nation Geodetic Survey (http://www.ngs.noaa.gov/cgi-bin/ngs_opsd.prl). As shown in Figure 5, the vertical datum difference between NAVD88 and MSL can be approximately represented by a linear function along the western open boundary. Along the northern and southern open boundaries, constant datum adjustment is used.

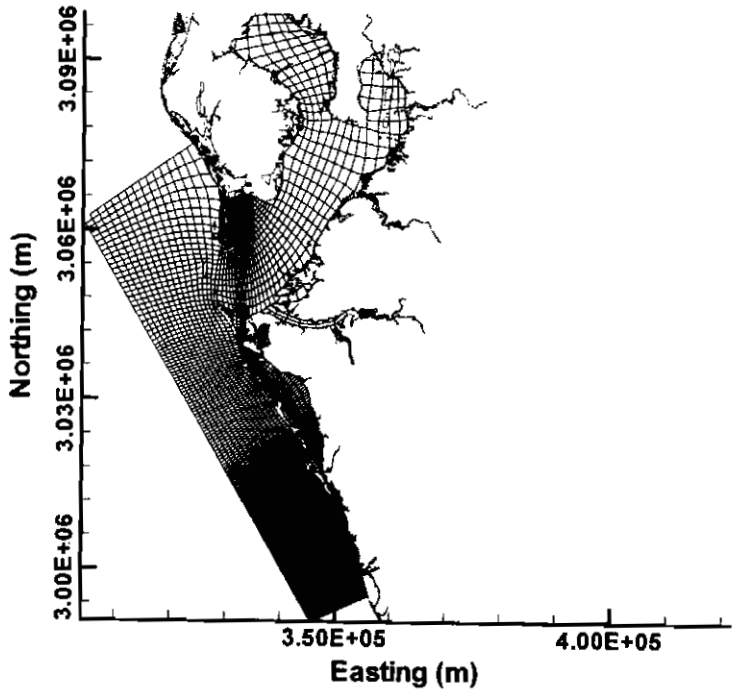


Figure 1 The Sarasota Bay 230x64 grid system in UTM coordinate system.

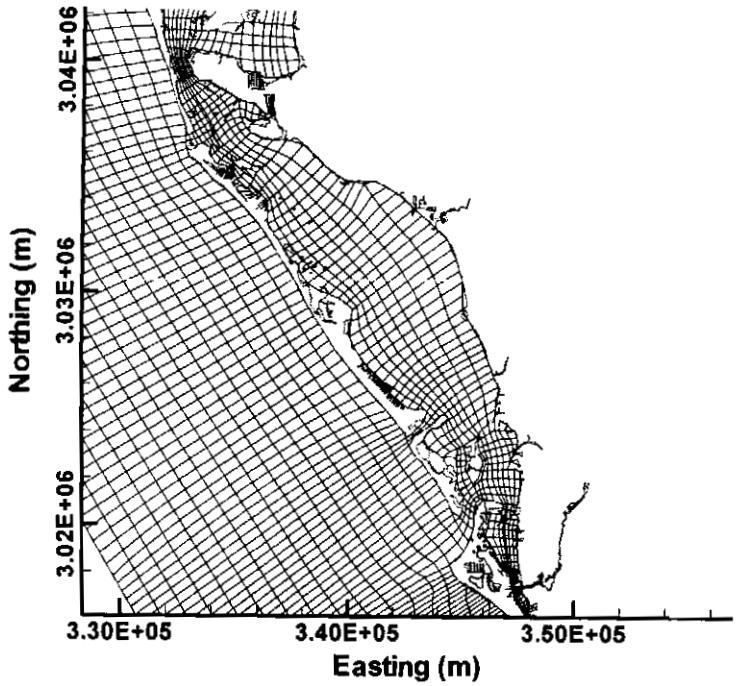


Figure 2 A zoom-in plot of the Sarasota Bay grid system in UTM coordinate system..

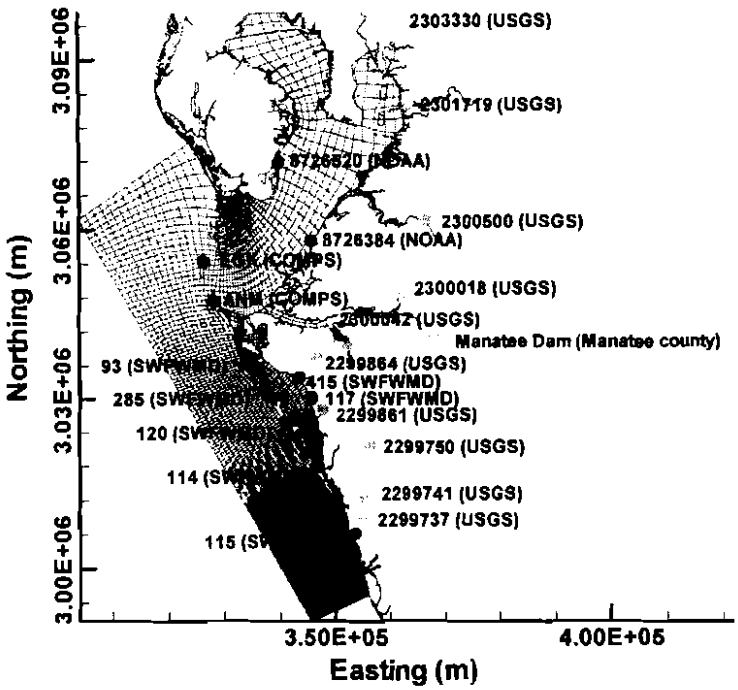


Figure 3. Locations of the data measurement stations within the Sarasota Bay grid system.

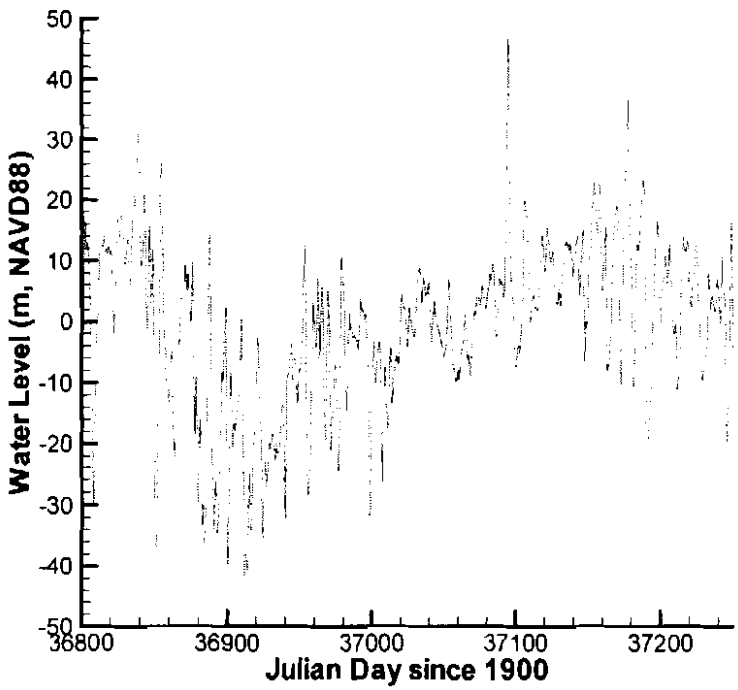


Figure 4. Filtered water level at St. Petersburg during year 2001. The filter removes signals with periods od 39 hours or less.

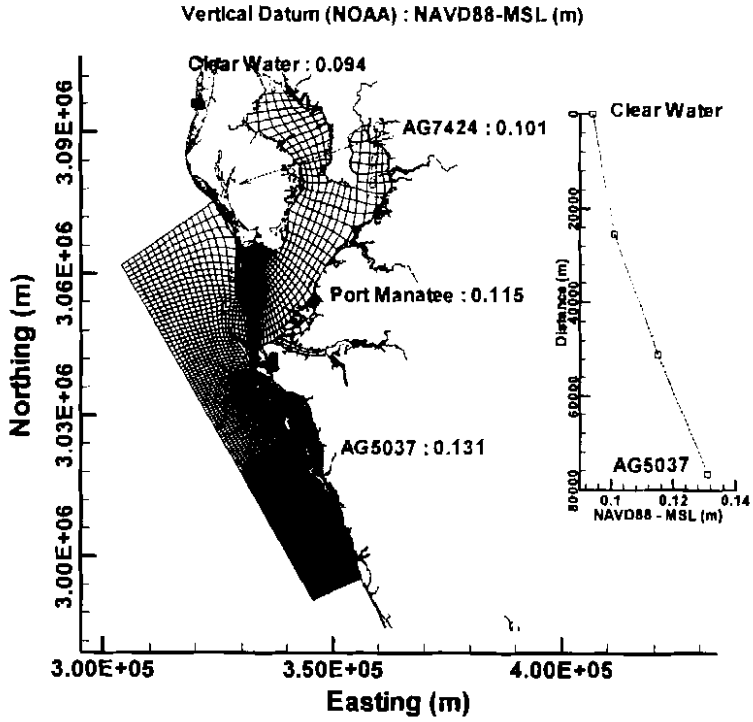


Figure 5 Datum adjustment for Sarasota Bay simulation.

Simulation results.

First a three-month from Oct.2000 to Dec.2000 was carried out to allow the model to spin up, i.e., to eliminate the transient effect. Then a one-year simulation of 2001 was made using the initial condition generated by the spin up simulation. The model output includes water level and residual flow and salinity fields for each month. The projection used in the output is UTM NAD83.

Water level

The water levels at four monitoring stations during 2001 are shown in Figure 6. Good agreement with data are achieved for all stations,as evidenced by the error analysis of simulated water level shown in Table 1. The overall Root Mean Square (RMS) error for all stations is 6.716 cm, which corresponds to a 4% normalized error after normalization by the range of water

level. The overall relative error (absolute difference) is 5.41 cm. A detailed time series comparison of water level during January, 2001 is shown in Figure 7.

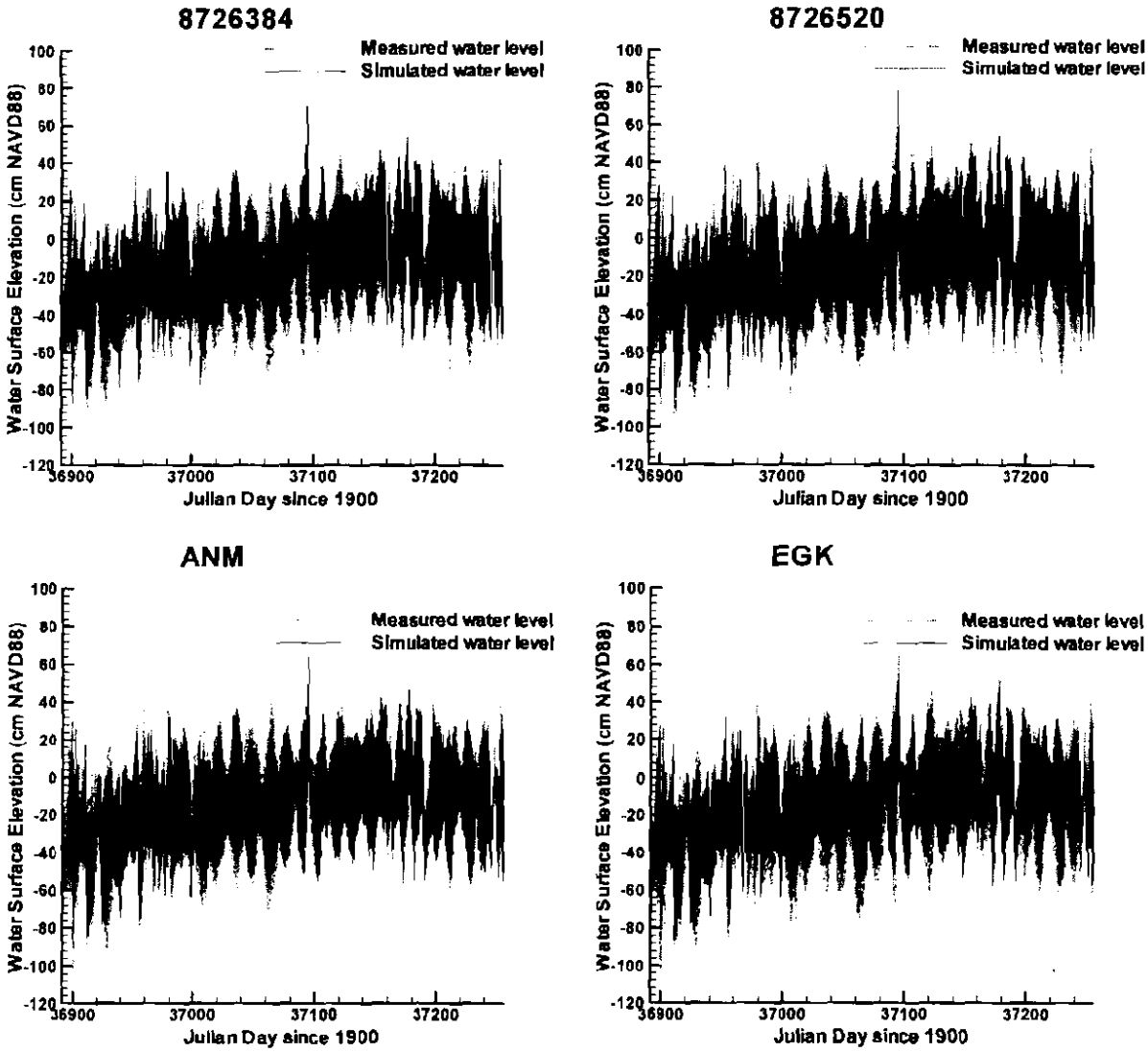


Figure 6 Simulated vs. measured water levels at four monitoring stations during 2001

Table 1 Error analysis of simulated water levels of Sarasota Bay 2001 simulation

Station Name	RMS error (cm)	Normalized RMS	Relative Error (cm)
COMPS-EGK	6.862	0.043	5.488
COMPS-ANM	7.199	0.049	5.901
NOAA-8726520	6.248	0.033	4.993
NOAA-8726384	6.556	0.036	5.257
Overall	6.716	0.04	5.410

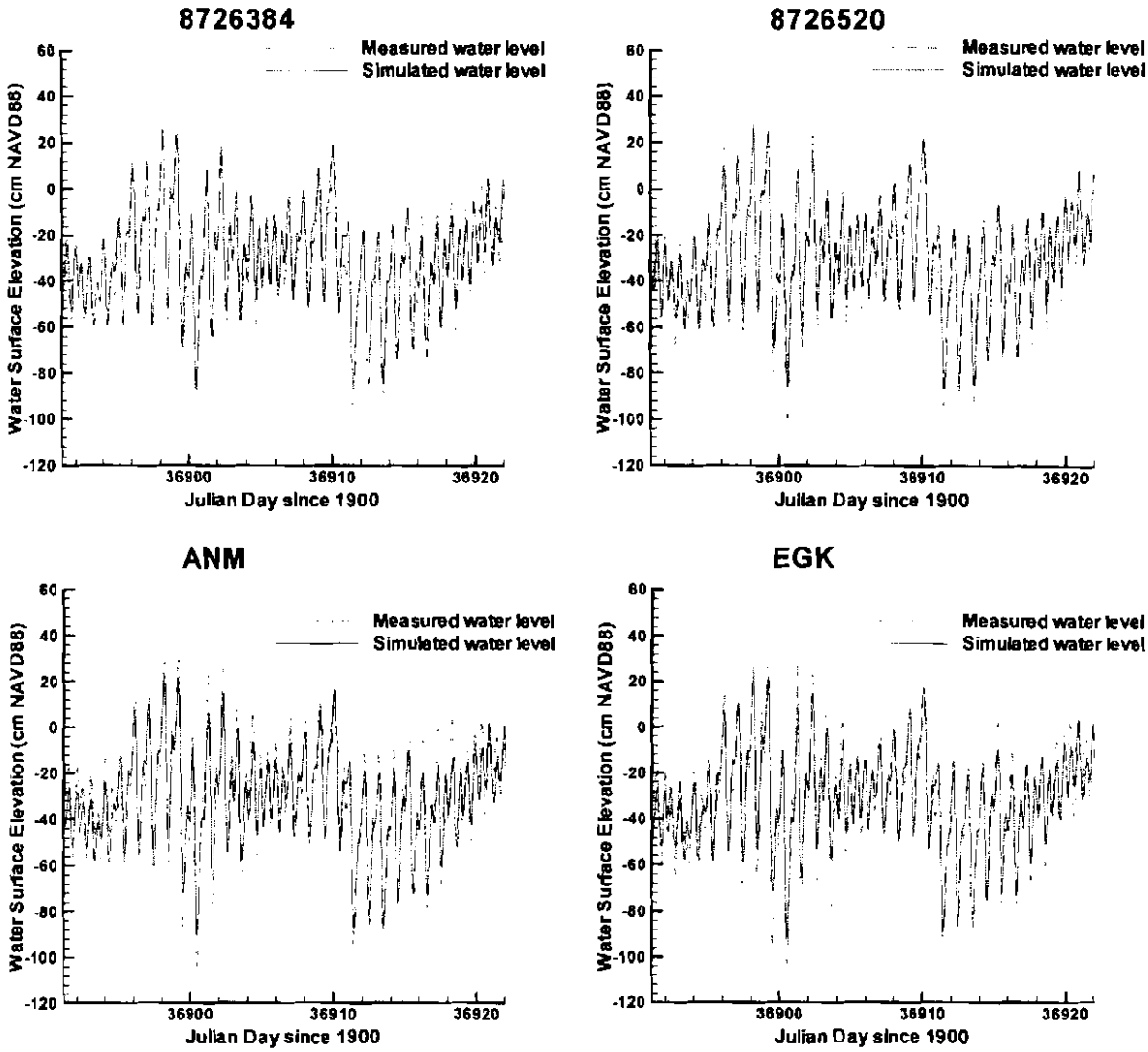


Figure 7. Simulated vs. measured water levels at four monitoring stations in Sarasota Bay during January, 2001.

Residual flow

The monthly residual flow within Sarasota Bay is shown from Figures 8 through 19. The monthly variation in mean water level is primarily caused by the seasonal water level variation along the offshore open boundary, and to a lesser extent the variation in the mean wind field.

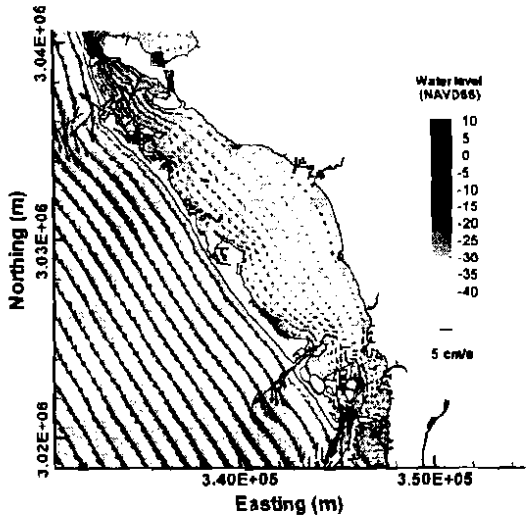


Figure 8 Sarasota Bay residual flow during January, 2001

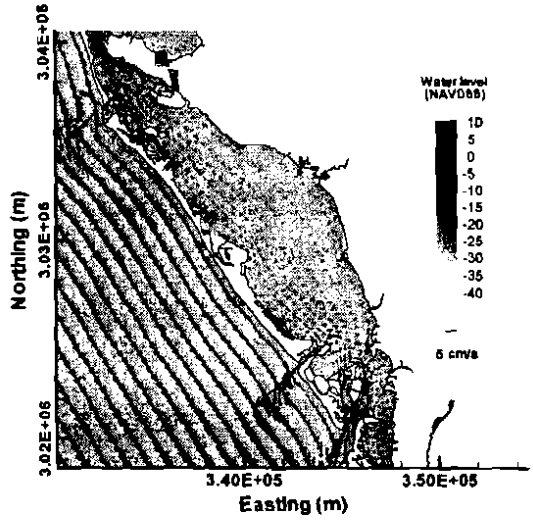


Figure 9 Sarasota Bay residual flow during February, 2001

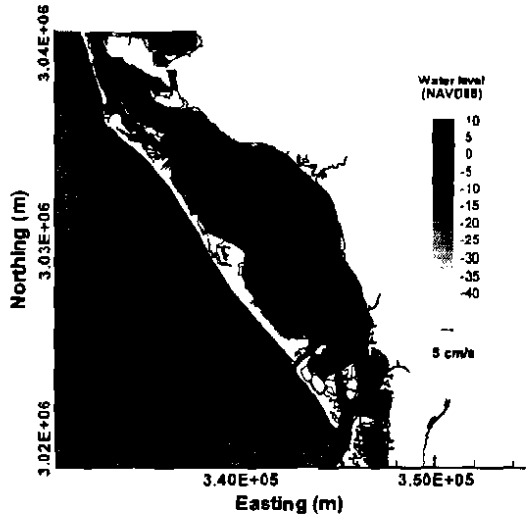


Figure 10 Sarasota Bay residual flow during March, 2001

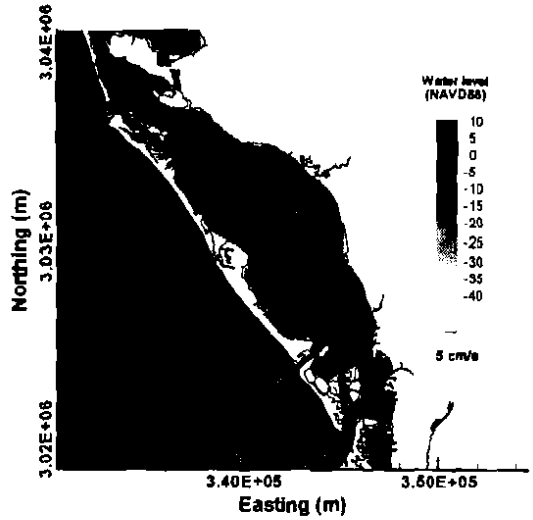


Figure 11 Sarasota Bay residual flow during April, 2001

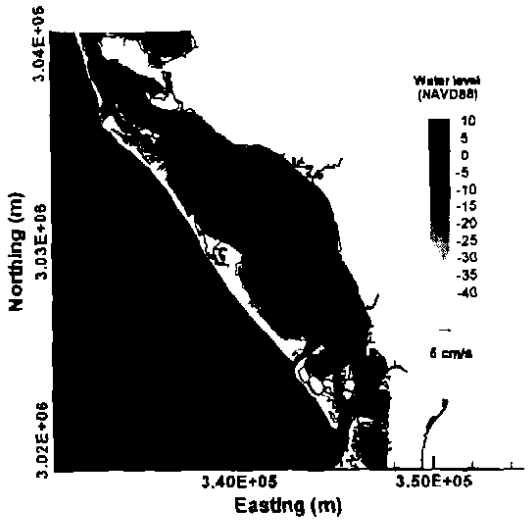


Figure 12 Sarasota Bay residual flow during May, 2001

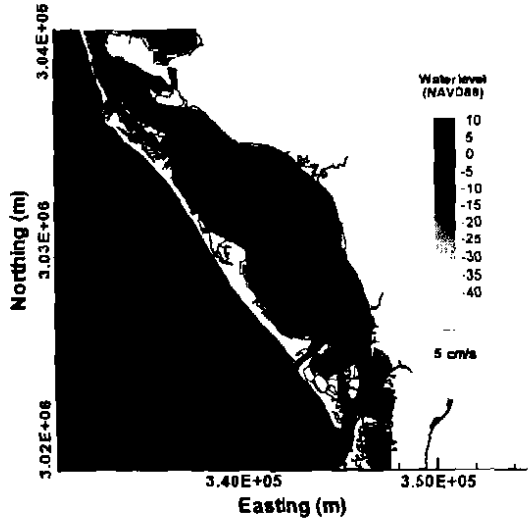


Figure 13 Sarasota Bay residual flow during June, 2001

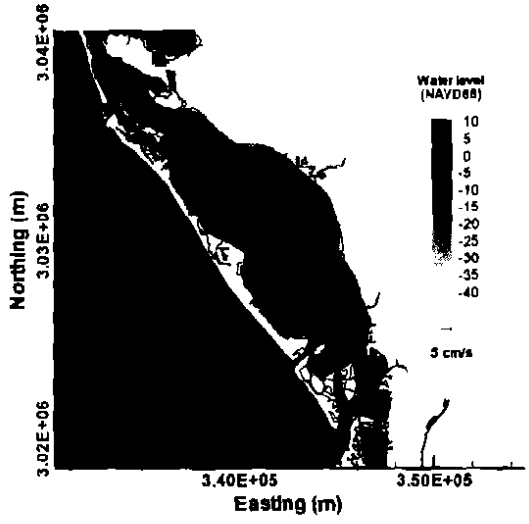


Figure 14 Sarasota Bay residual flow during July, 2001

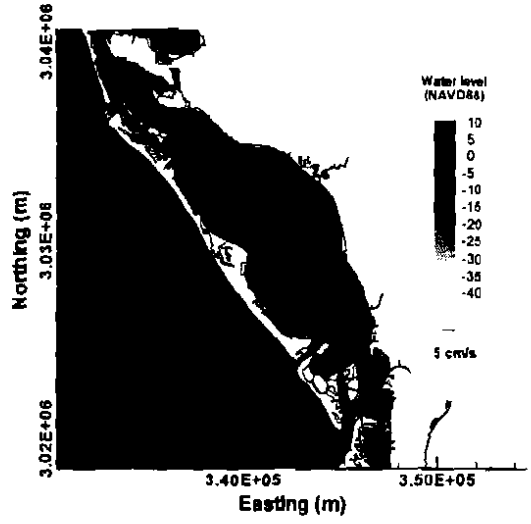


Figure 15 Sarasota Bay residual flow during August, 2001

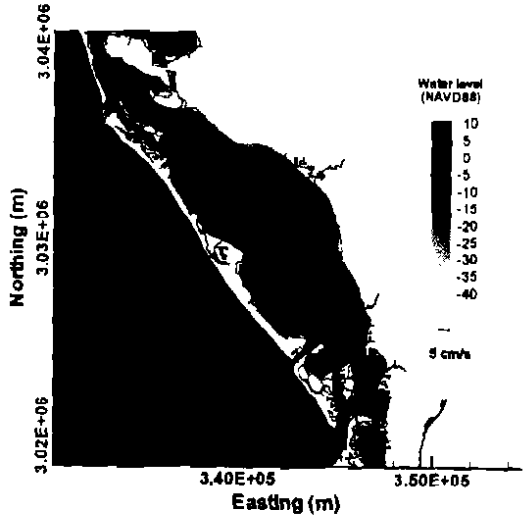


Figure 16 Sarasota Bay residual flow during September, 2001

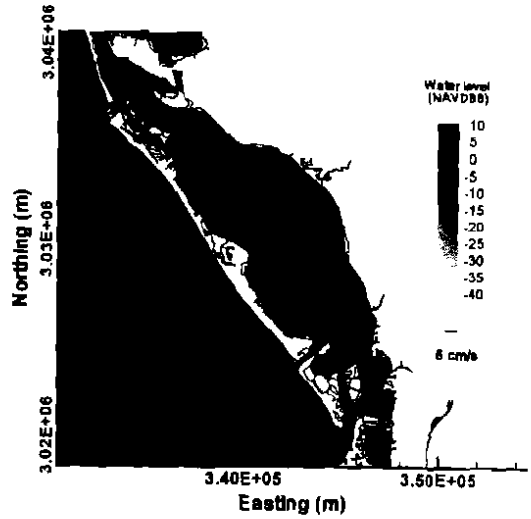


Figure 17 Sarasota Bay residual flow during October, 2001

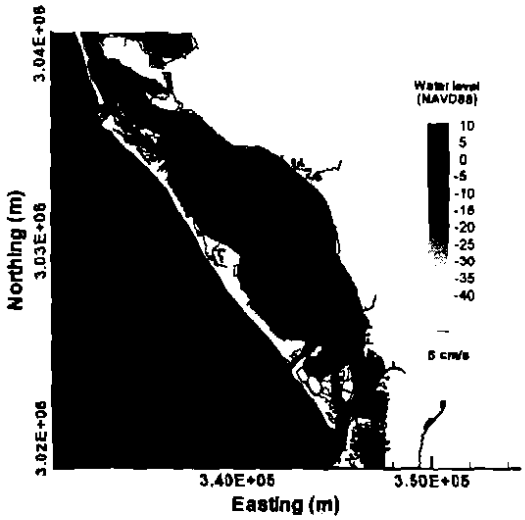


Figure 18 Sarasota Bay residual flow during November, 2001

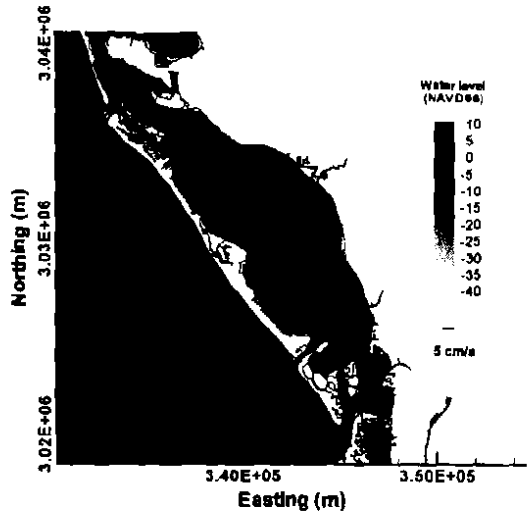


Figure 19 Sarasota Bay residual flow during December, 2001

Residual Salinity field

The monthly averaged salinity plots of Sarasota Bay for 2001 are shown in Figures 20 through 31. During the dry season (November to April), the salinity field remains approximately constant with some slight variation between 34 to 36 ppt. During the wet season, however, salinity drops more noticeably with increased precipitation and discharge from rivers and creeks.

The highest monthly salinity of 36 ppt occurs in June, in contrast to the lowest monthly salinity of 32 ppt in September. Within Sarasota Bay, the salinity concentration is approximately uniform.

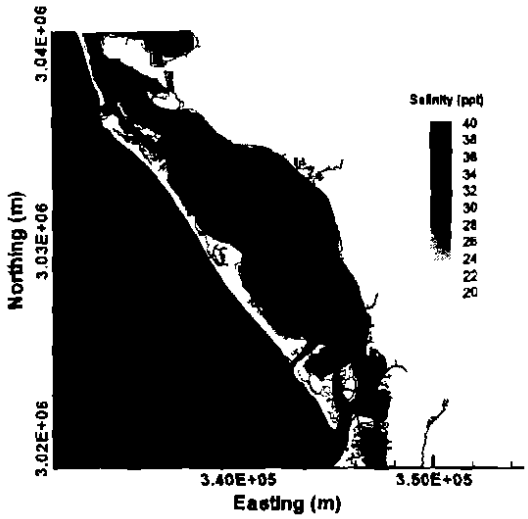


Figure 20 Sarasota Bay salinity field during January, 2001

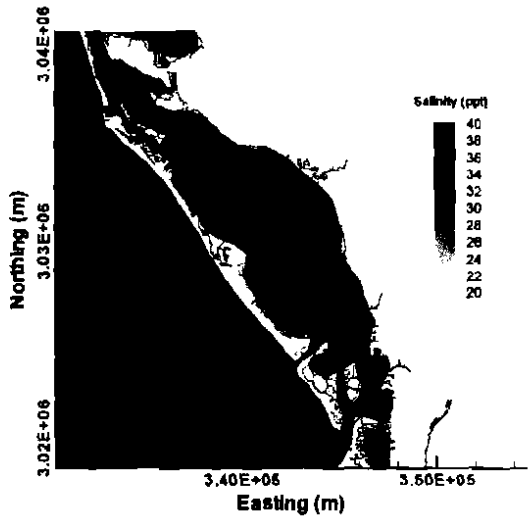


Figure 21 Sarasota Bay salinity field during February, 2001

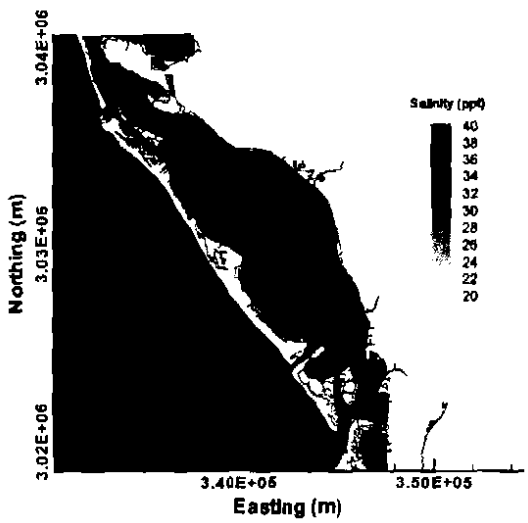


Figure 22 Sarasota Bay salinity field during March, 2001

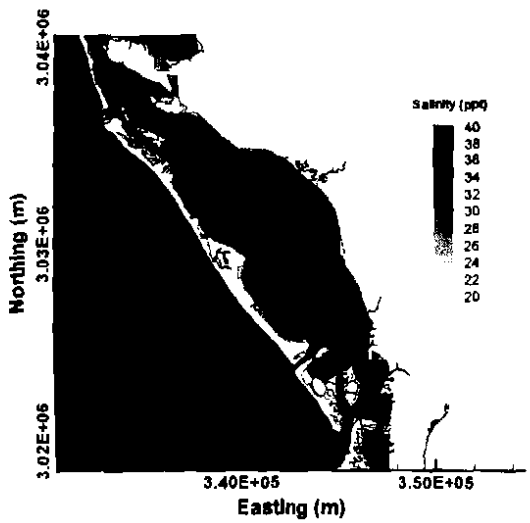


Figure 23 Sarasota Bay salinity field during April, 2001

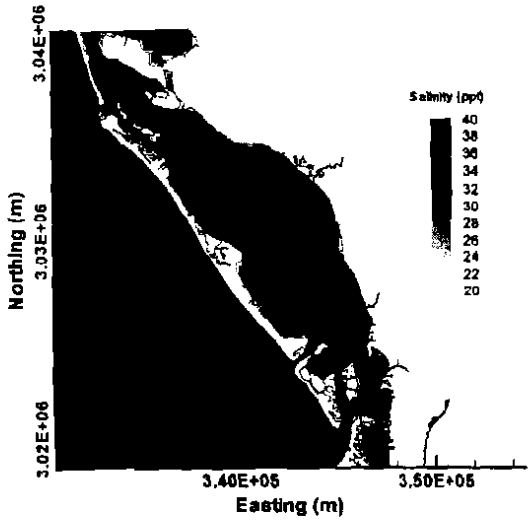


Figure 24 Sarasota Bay salinity field during May, 2001

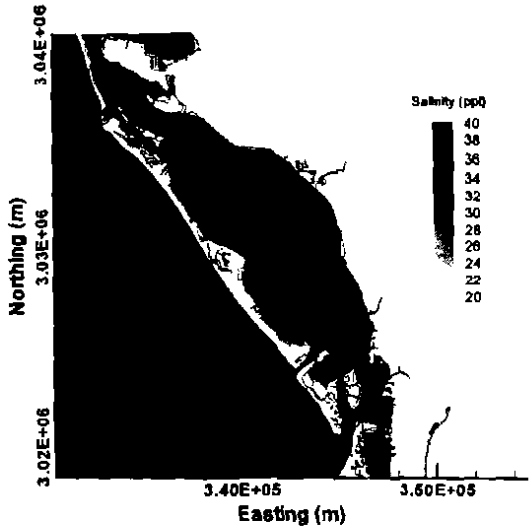


Figure 25 Sarasota Bay salinity field during June, 2001

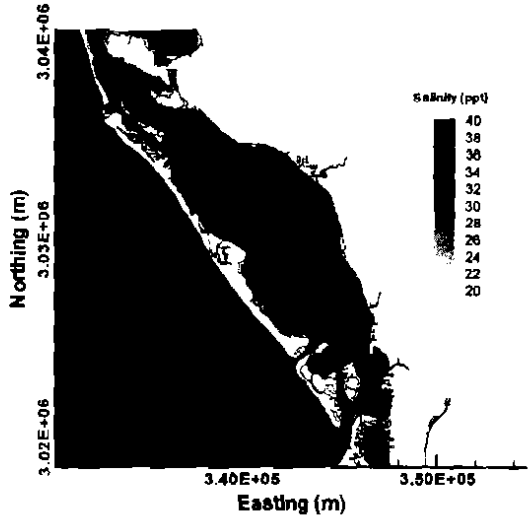


Figure 26 Sarasota Bay salinity field during July, 2001

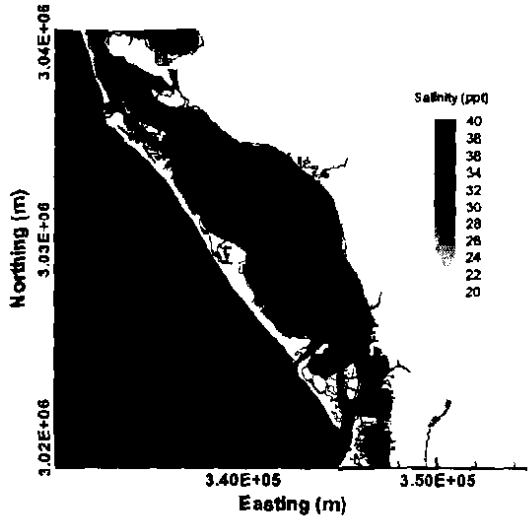


Figure 27 Sarasota Bay salinity field during August, 2001

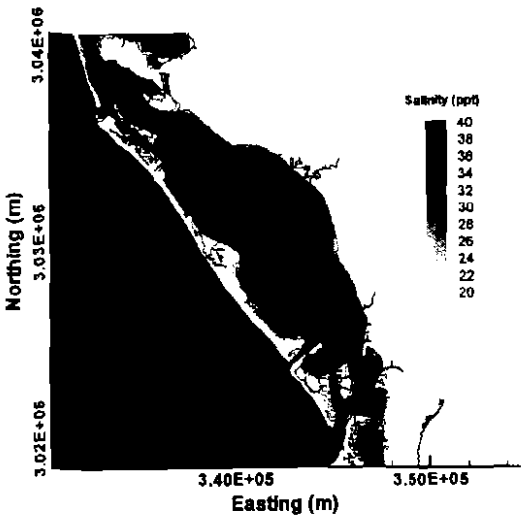


Figure 28 Sarasota Bay salinity field during September, 2001

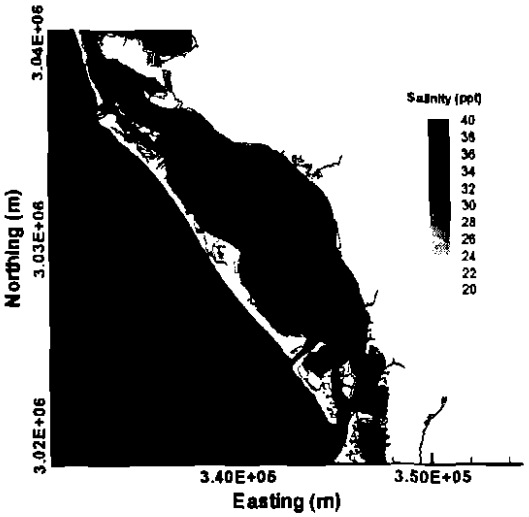


Figure 29 Sarasota Bay salinity field during October, 2001

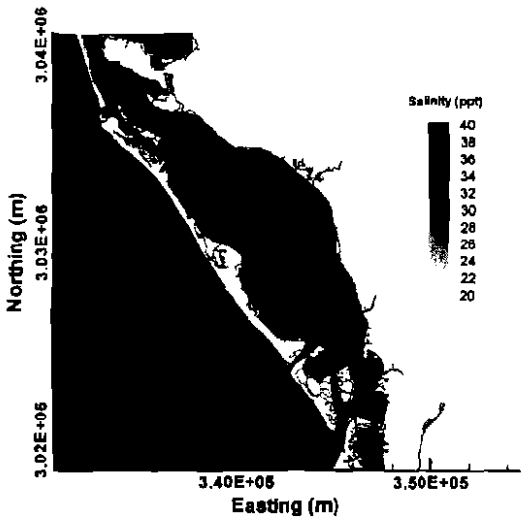


Figure 30 Sarasota Bay salinity field during November, 2001

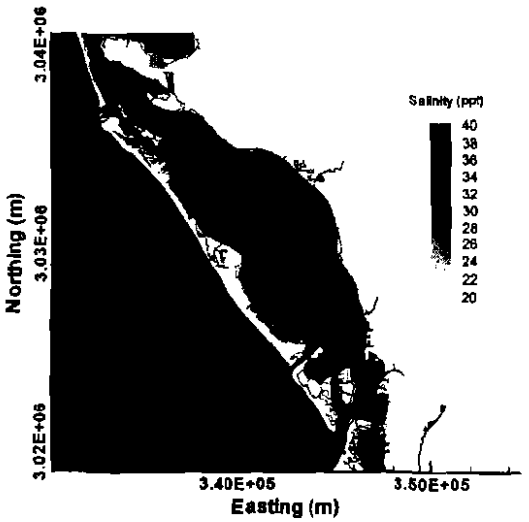


Figure 31 Sarasota Bay salinity field during December, 2001

Particle tracking simulation - which release site and what release time are the best

Using a 3-D particle tracking model, a 14-day particle tracking simulation was conducted and results are presented in this section. The primary purpose of this simulation is to compare the trajectories of particles released from three candidate release sites (see Figure 32). The simulation time covers from Nov 1st, 2004 to Nov 14th, 2004. First, a set of selected simulations

are made with particles released from three candidate release sites (#1, #2, #3 in Figure 32) and four particle release times - flood tide, high tide, ebb tide and low tide (see Figure 33). Model simulated percents of particles remaining within the Sarasota Bay are summarized in Table 2. Generally only small variations were found among the results for varying horizontal diffusion coefficients and number of particles used in the simulation (1,000 and 10,000 in the tests).

At Site #1, the result is very sensitive to the release time. With a release time at high tide, particles are quickly carried out into the Gulf of Mexico and there is no particle left within Sarasota Bay at the end of 14 days. For the other release times, approximately 70-80 percent of the total particles remain in the bay. Site#1 appears to be the worst release site.

This is quite different for Site #3, however, since it is far away from the inlet, hence the release time has little effect. On the average, the low tide release time appears to be the best since the highest percent of particles remain within the bay. In fact, low tide release time is the best for all release sites.

Site #2 appears to be an intermediate release site, since the model results show that particles remaining in the bay are somewhere in between those for Site #1 and Site #3.

With particles released from Site #3 at low tide (Nov 1st, 14:20, 2004), a final 14-day particle tracking simulation was made with hourly interval output. The Smagorinsky diffusion formula was applied in the simulation. Initially 1000 particles were released and transported by mean current and turbulent diffusion. Even though the particles' positions were saved every hour, only daily-updated locations were shown in Figures 34 through 47. At the end of 14 days, over 90% of the particles remain within the Sarasota Bay with distributed locations around central eastern part of the Bay. The other particles were flushed out of the Bay through the inlets. Figures 48 to 50 show three representative particle tracks with different final locations including north,

center and south of the particle cloud.

Table 2 Percent of particles left within Sarasota Bay

	Location 1				Location 2				Location 3			
	a	b	c	d	a	b	c	d	a	b	c	d
Time 1	76.2	78.9	65.1	76.4	86.6	88.4	86.2	86.3	90.1	94.4	88.0	90.0
Time 2	0.0	0.0	0.0	0.0	5.2	11.0	52.4	5.2	90.5	84.8	94.6	90.8
Time 3	72.8	58.6	79.8	70.7	75.9	77.8	88.0	80.0	89.0	86.1	85.5	89.3
Time 4	89.1	87.9	81.2	89.0	92.5	89.1	89.4	92.7	92.7	91.1	92.0	93.0

a: Ah = 1,000 cm^2/s; 1,000 particles
b: Ah = Variable (Smagorinsky formula) ; 1,000 particles
c: Ah = 1,00 cm^2/s; 1,000 particles
d: Ah = 1,000 cm^2/s; 10,000 particles

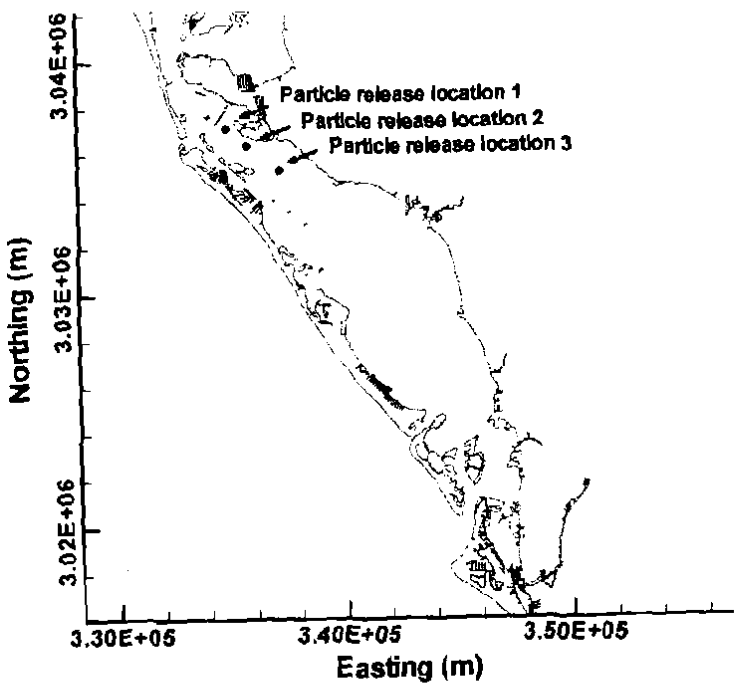


Figure 32. Locations of the three release sites (#1, #2, and #3).

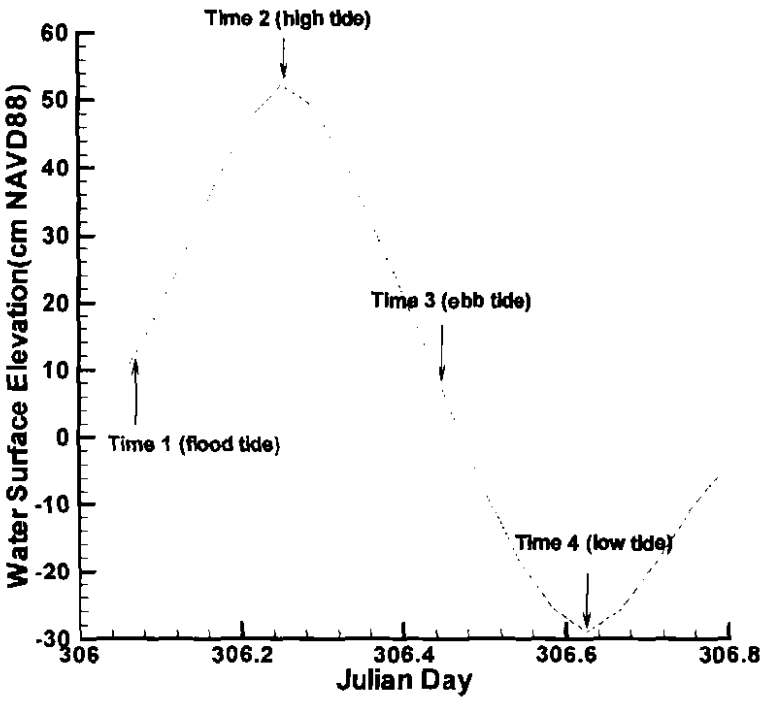


Figure 33 Four different release times for particle tracking.

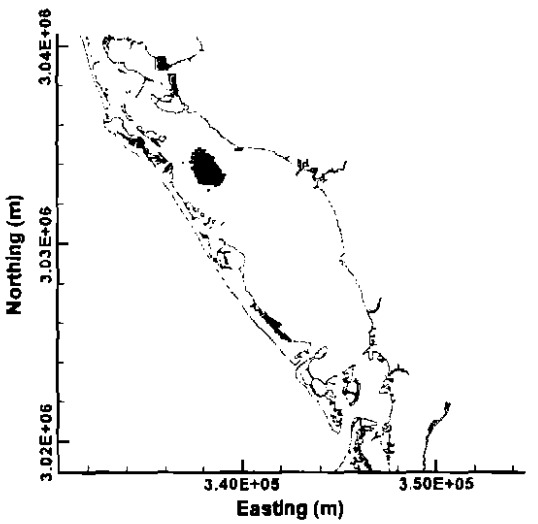


Figure 34 The particle locations after one day simulation

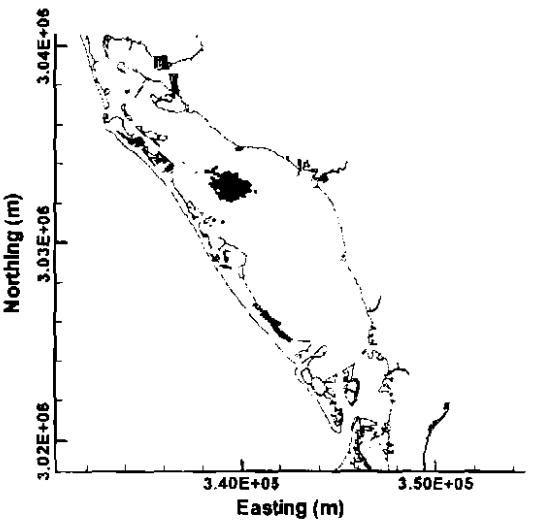


Figure 35 The particle locations after two day simulation

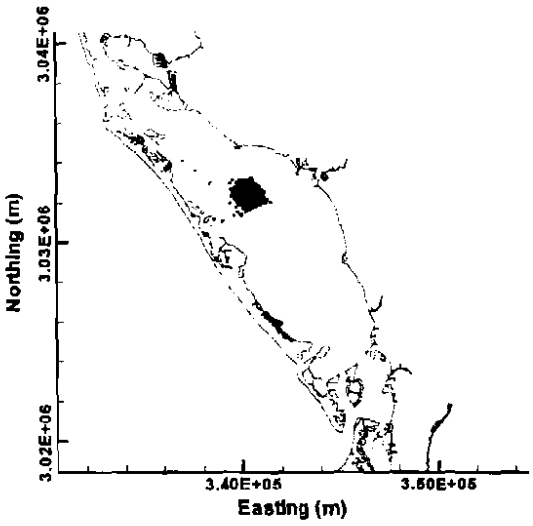


Figure 36 The particle locations after three day simulation

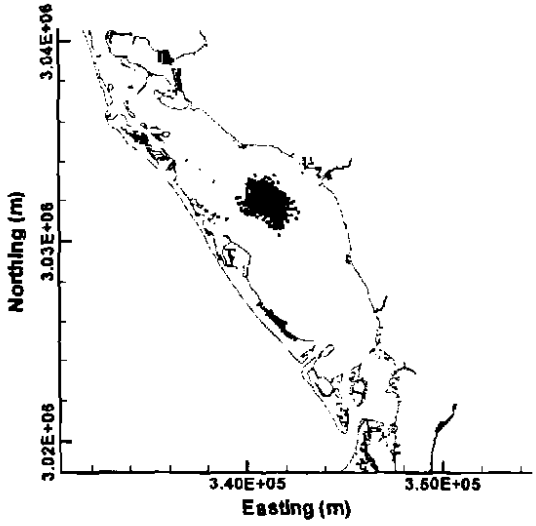


Figure 37 The particle locations after four day simulation

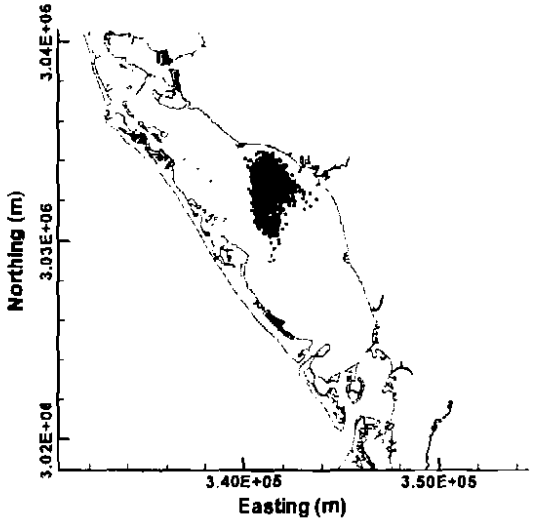


Figure 38 The particle locations after five day simulation

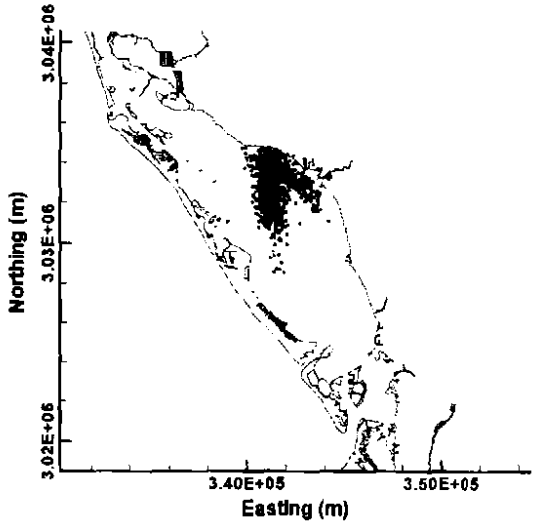


Figure 39 The particle locations after six day simulation

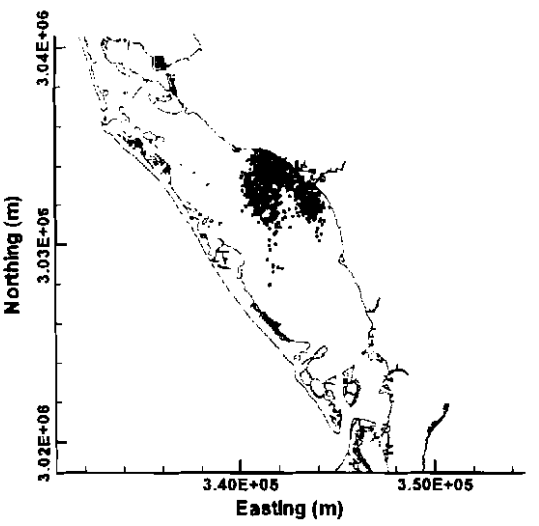


Figure 40 The particle locations after seven day simulation

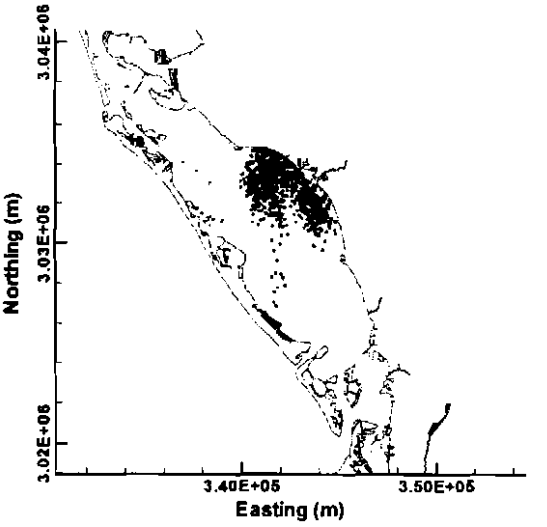


Figure 41 The particle locations after eight day simulation

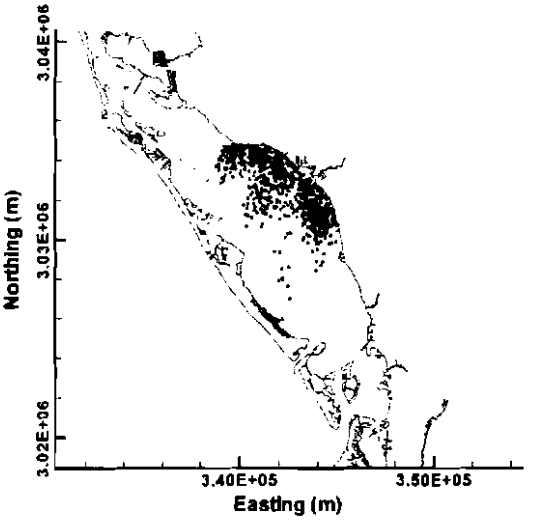


Figure 42 The particle locations after nine day simulation

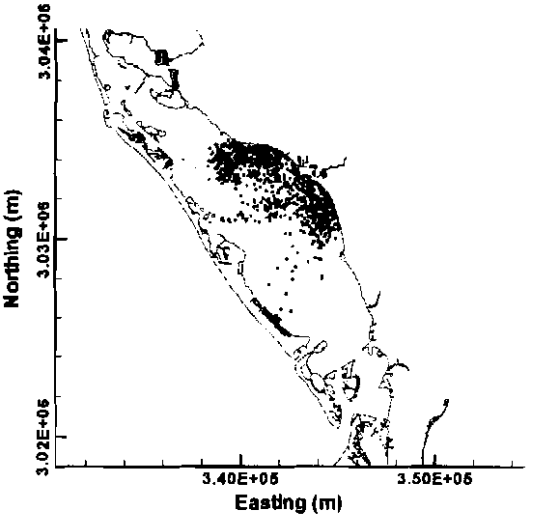


Figure 43 The particle locations after ten day simulation

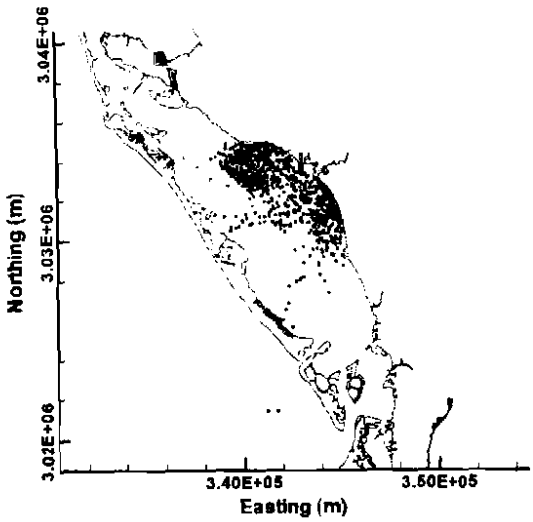


Figure 44 The particle locations after eleven day simulation

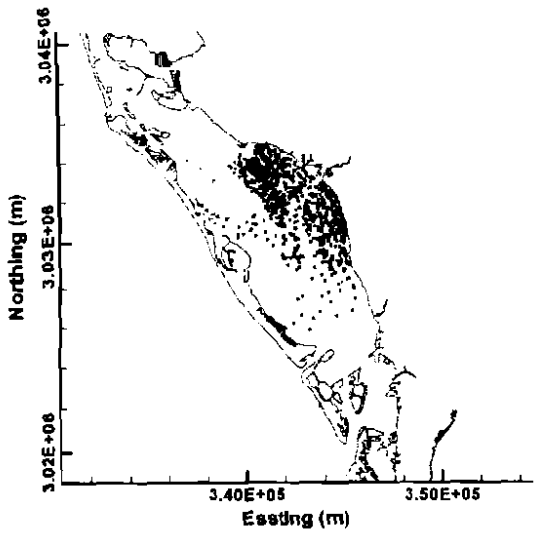


Figure 45 The particle locations after twelve day simulation

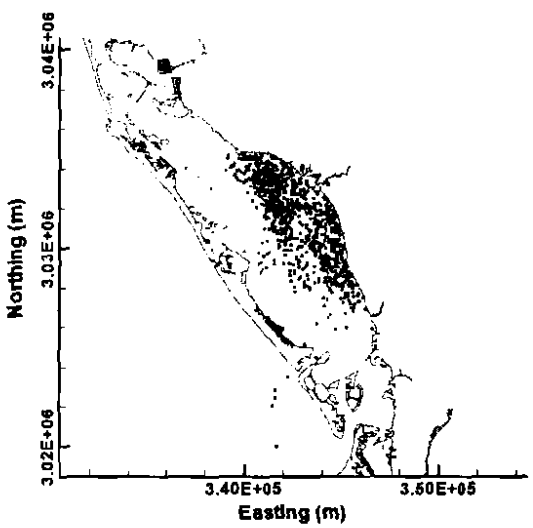


Figure 46 The particle locations after thirteen day simulation

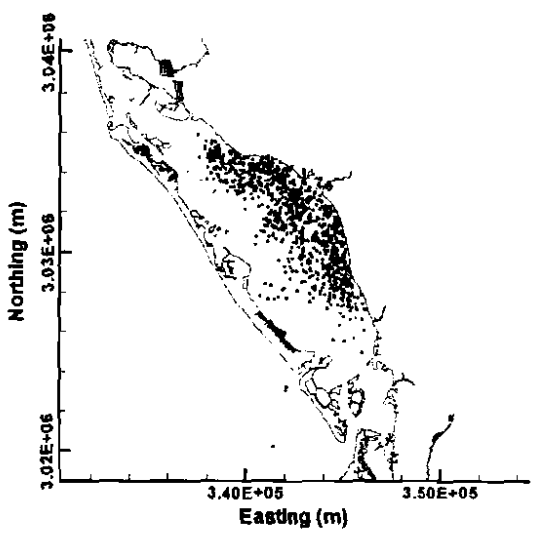


Figure 47 The particle locations after fourteen day simulation

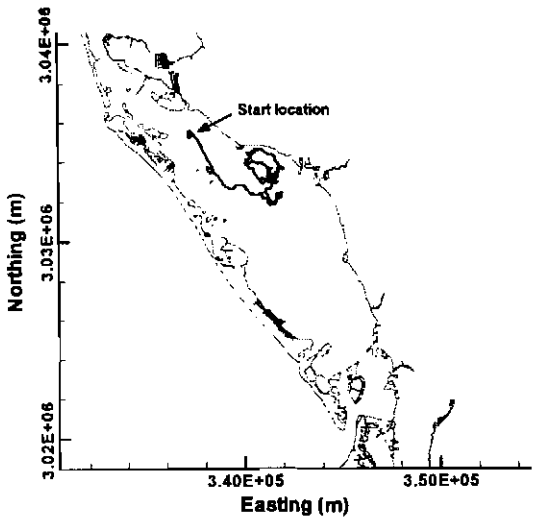


Figure 48 The representative track one of the 14-day particle tracking

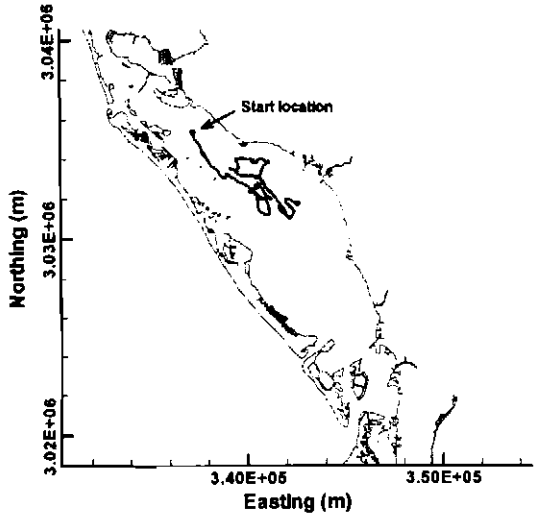


Figure 49 The representative track two of the 14-day particle tracking

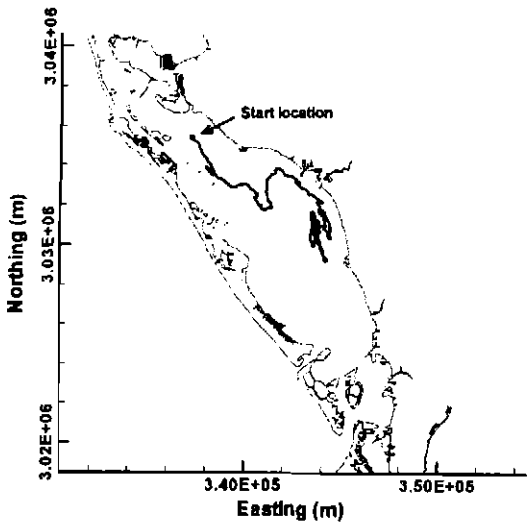


Figure 50 The representative track three of the 14-day particle tracking

Appendix 1 Measured data for Sarasota Bay simulation

Discharge

The daily discharge data at Alpha River, Little Manatee River and Hillsborough River were obtained from USGS. The daily discharge of Manatee River at Manatee dam was provided by Manatee County. Their time series discharges are shown in Figure A.1. As there is no measured discharge data during 2000 and 2001 period for South Creek, Catfish Creek, Walker Creek, Whitake Creek and Phillippee Creek, the historic monthly discharge for each Creek was obtained from USGS and was applied in the model (Figure A.2).

Precipitation and evaporation

The precipitation data was obtained from South West Florida Water Management District (SWFWMD) online database. The locations of the seven stations (station 114, 115, 117, 120, 285, 415 and 93) were shown in Figure 1. As to evaporation, there is only one set of data available during the simulation period at station BCBNAPLES from South Florida Water Management District (SFWMD) DBHydro database. The time series of precipitation and evaporation were shown in Figure A.2.

Wind

There were three wind stations with data used in the simulation. Two of them were from NOAA stations: St. Petersburg (8726520) and Port Manatee (8726384) and one from University of South Florida Coastal Ocean Monitoring and Prediction System (COMPS) station EGK. The time series of wind vector plots at these three stations were in Figure A4.

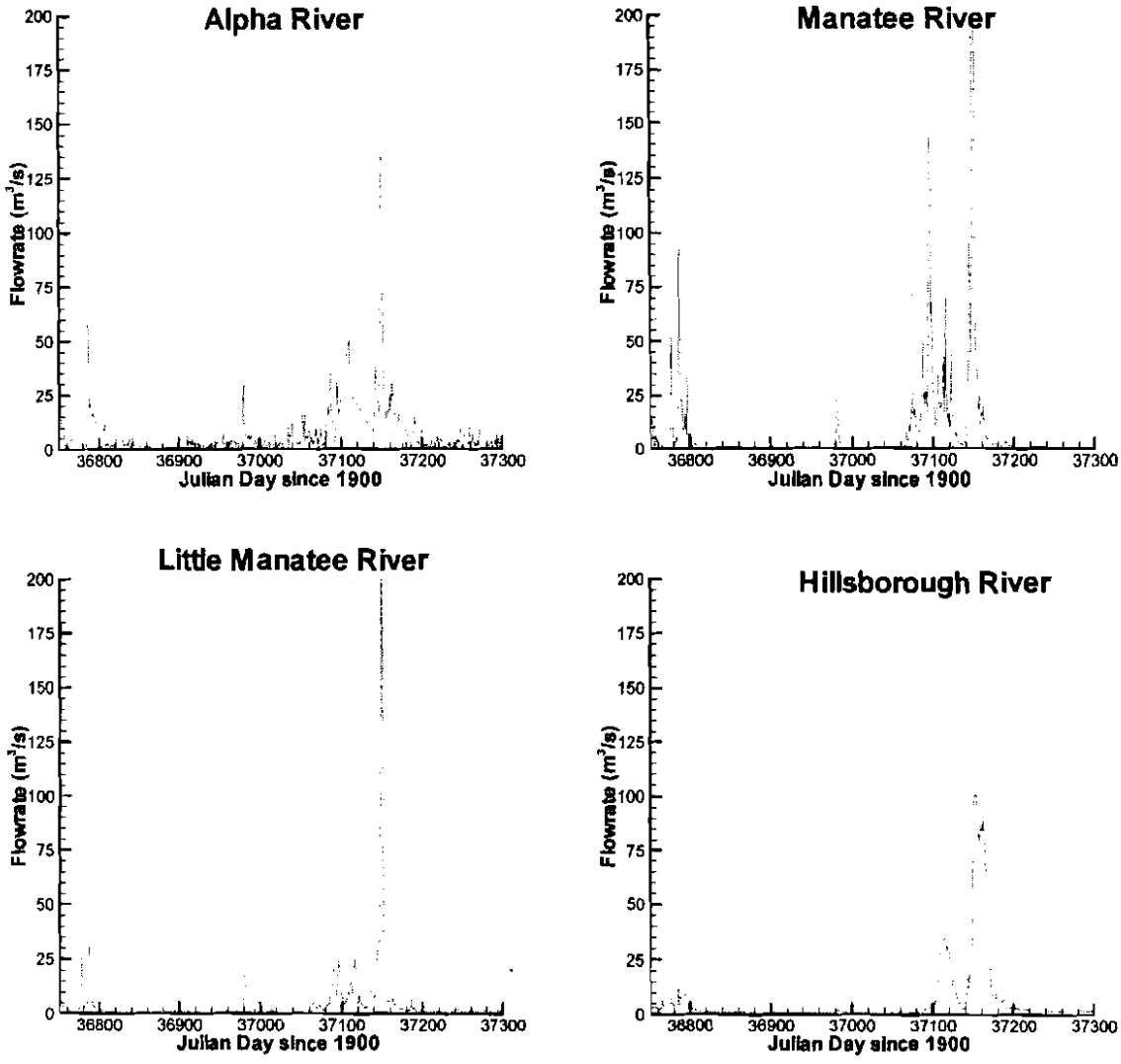


Figure A1 Measured discharge for Sarasota Bay simulation (part 1)

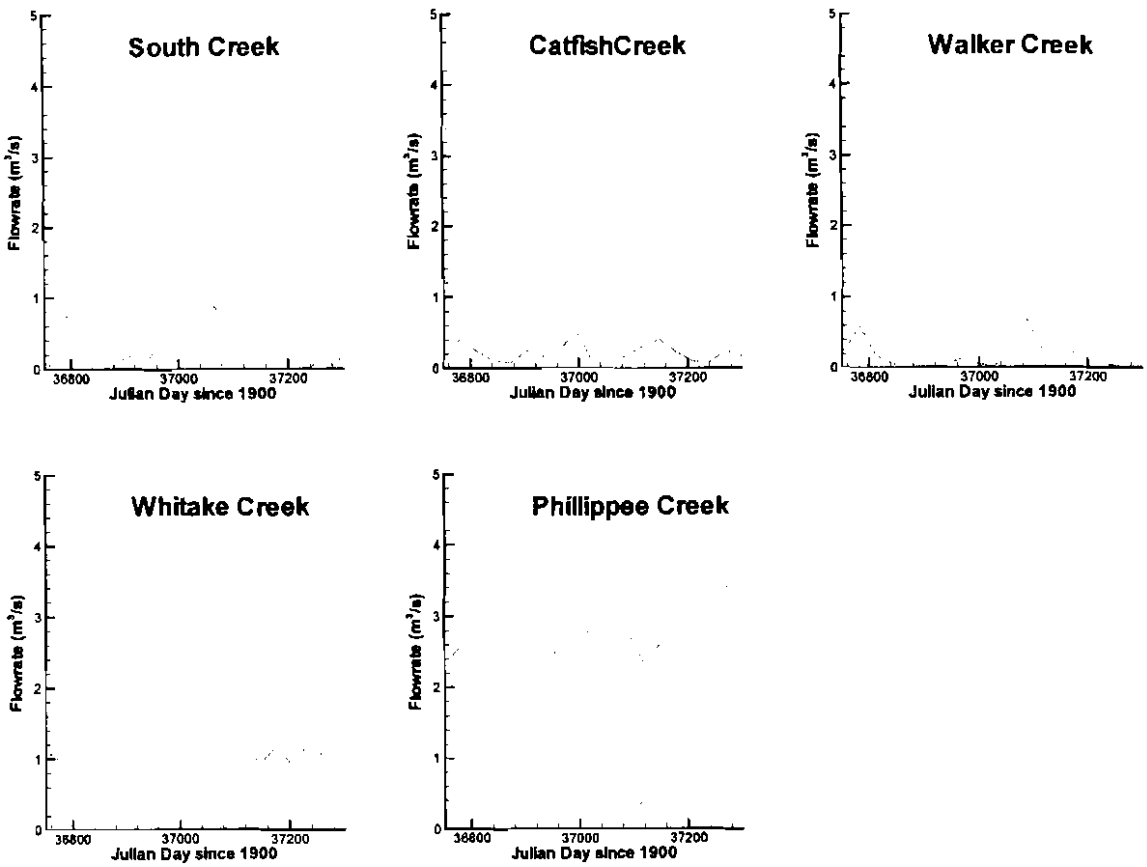


Figure A2 Measured discharge for Sarasota Bay simulation (part 2)

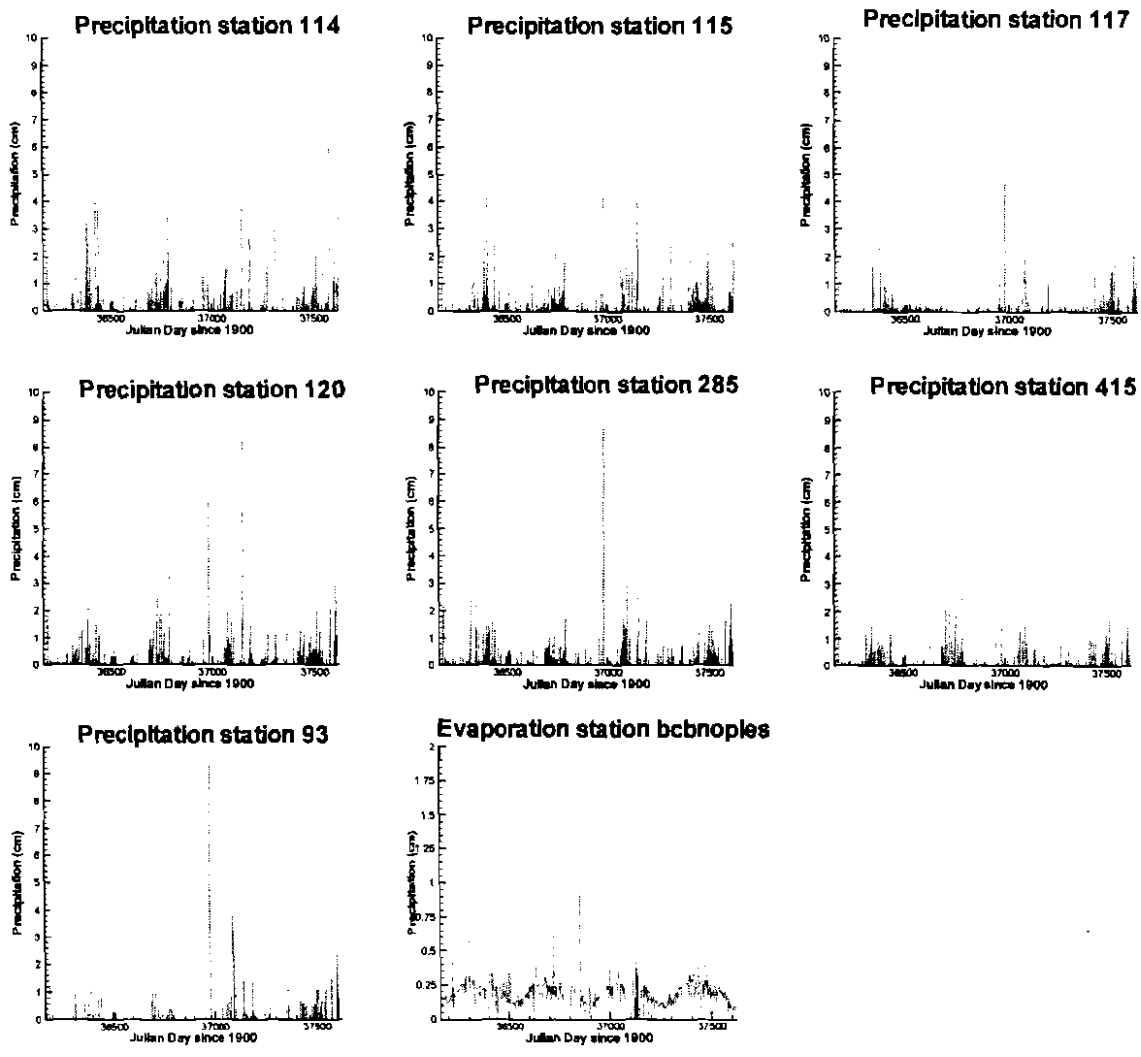


Figure A3 Measured precipitation and evaporation for Sarasota Bay simulation

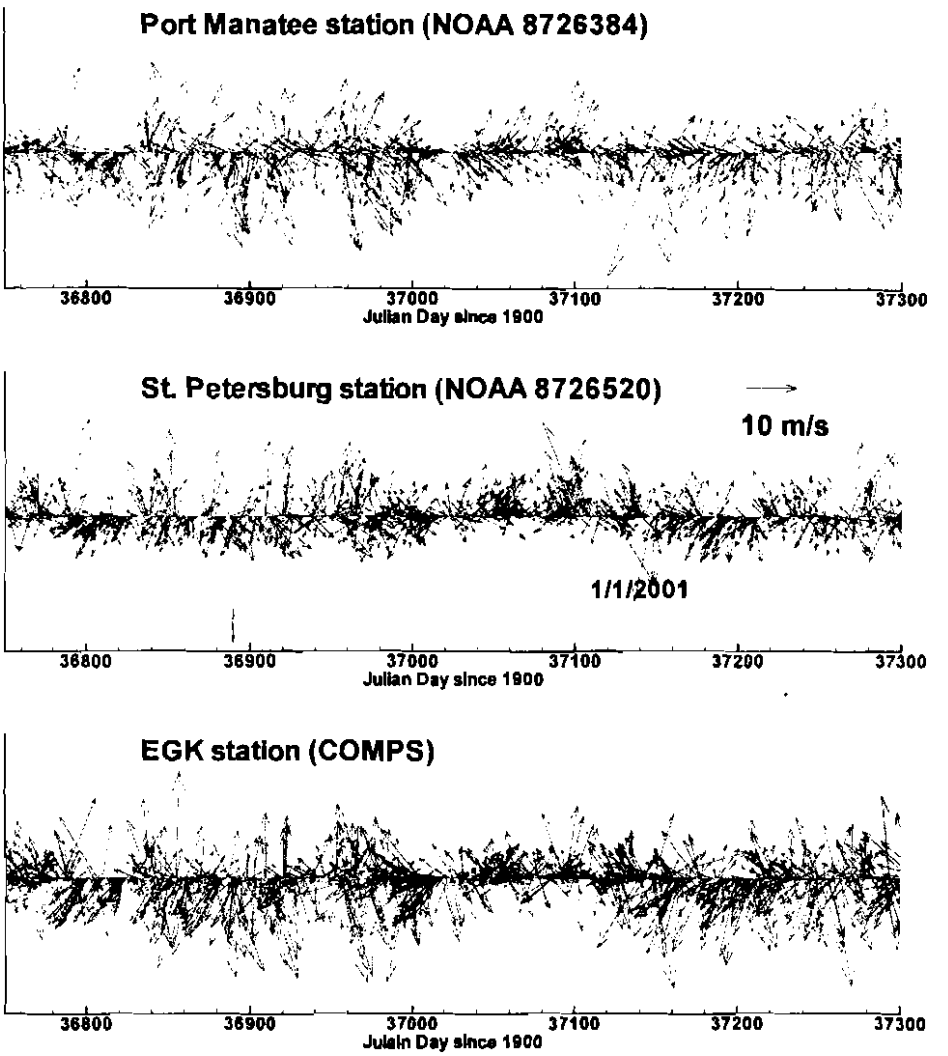


Figure A4 Measured wind data for Sarasota Bay simulation