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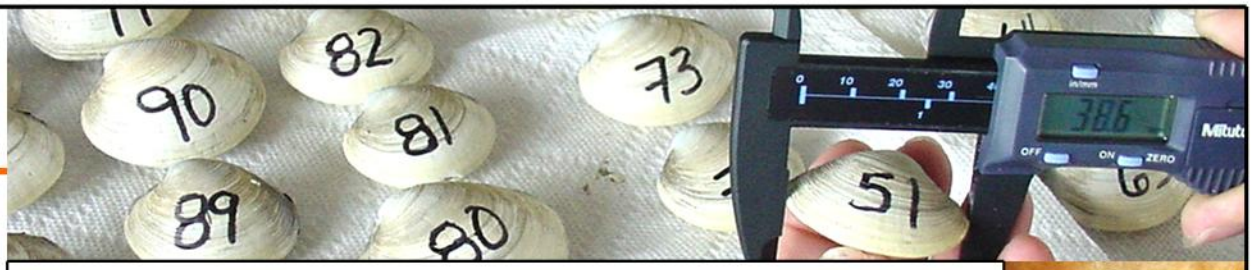


Sea Grant Florida

USDA

Clam Industry Workshop

Wednesday, September 24, 2008

Community Center, Cedar Key

1:30-3:00 PM

Welcome and Introductions
Karl Havens, Florida Sea Grant Director

Consistent hatchery and nursery yields for bivalves through health management principles and analysis of production systems
Ralph Elston, AquaTechnics, Sequim, Washington

Temperature monitoring and chlorophyll mapping of high-density lease areas in Cedar Key
Chuck Mulligan, Citrus County Academy of Marine Sciences

Monitoring of clam health during summer months
Denise Petty, UF/IFAS College of Veterinary Medicine

Effects of multiple stressors on clam survival in SW Florida
Vincent Encomio, Florida Oceanographic Society;
Aswani Volety, Florida Gulf Coast University

3:15-3:30 PM Break

3:30-4:45 PM

Initial assessment of soil landscapes in clam lease areas
Rex Ellis, Todd Osborne, and Mark Clark,
UF/IFAS Soil and Water Science Department

Enhancing stress resistance of cultured clams through triploidy
John Scarpa, Harbor Branch Oceanographic institute (HBOI);
Shirley Baker, UF/IFAS Fisheries and Aquatic Sciences

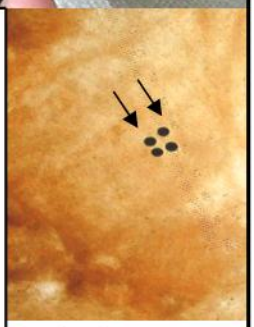
Evaluation of clam stock improvement through hybridization
Leslie Sturmer, UF/IFAS Shellfish Aquaculture Extension;
John Scarpa, HBOI at Florida Atlantic University

Evaluation of the sunray venus clam for species diversification
John Scarpa, HBOI at Florida Atlantic University;
Leslie Sturmer, UF/IFAS Shellfish Aquaculture Extension

4:45-5:30 PM

Industry feedback session / Discussion groups

5:30-6:30 PM Social Hour



SFRC

University of Florida
Department of Fisheries & Aquatic Sciences
IFAS

FLORIDA DEPARTMENT OF AGRICULTURE AND CONSUMER SERVICES
DIVISION OF AQUACULTURE

CONSISTENT HATCHERY AND NURSERY YIELD FOR BIVALVE MOLLUSCS USING HEALTH MANAGEMNT AND ANALYSIS OF PRODUCTION SYSTEMS

Ralph Elston, *AquaTechnics, Sequim, Washington*

Bivalve shellfish hatchery and nursery management goals include predictable production, high survival before and after sale, high growth rate, high health and condition and minimization of waste. Achieving these goals will lead to efficient and profitable operation. Application of health management principles has been necessary to achieve consistent and efficient production in all forms of animal husbandry. Health management topics for bivalve shellfish intensive production include prevention and management of bacterial contamination, animal condition assessment and water quality monitoring and management. Contamination of hatcheries and nurseries is prevented by managing each component of the culture system that can serve as a source of contamination. These components include brood stock, algal feed stocks and the seawater source, in addition to the larval and juvenile cultures. High humidity in hatchery environments must be managed at critical steps to prevent maintenance of contamination. Water source suitability is site specific and water treatment and monitoring for both bacteriological contamination and water quality parameters need to be adapted to site specific needs. Microalgal food contamination by shellfish pathogenic bacteria can be a persistent and important source for introducing toxigenic and invasive bacteria into animal cultures. Approaches and methods for the management of larval and juvenile bivalve health, with emphasis on bacterial management, evaluation of animal condition and water quality evaluation will be presented.

VARIABILITY OF WATER TEMPERATURES DURING SUMMER MONTHS AT CLAM AQUACULTURE LEASE AREAS

Chuck Mulligan, *Citrus County Academy of Environmental Sciences*
Leslie Sturmer, *UF/IFAS Shellfish Aquaculture Extension*

Background: Water temperature plays an important role in biology and directly affects the bodily functions of aquatic organisms necessary for growth and survival. It also influences water quality parameters, such and dissolved oxygen and pH. Currently, five water quality monitoring stations (CLAMMRS sondes) are located at lease areas around the state to provide timely information on water temperature and other parameters so clam farmers can make more informed decisions about crop management. These stations measure continuously at a depth of 6 inches above the bottom. The limited number of stations does not provide for an in-depth understanding of water temperatures across the lease areas or the influence of temperature on clam production.

Objective: The intent of this project is to provide both detailed and broad coverage of water temperatures by deploying inexpensive data loggers at multiple leases to adequately describe variability possibly due to water depth, substrate characteristics, currents, and other parameters.

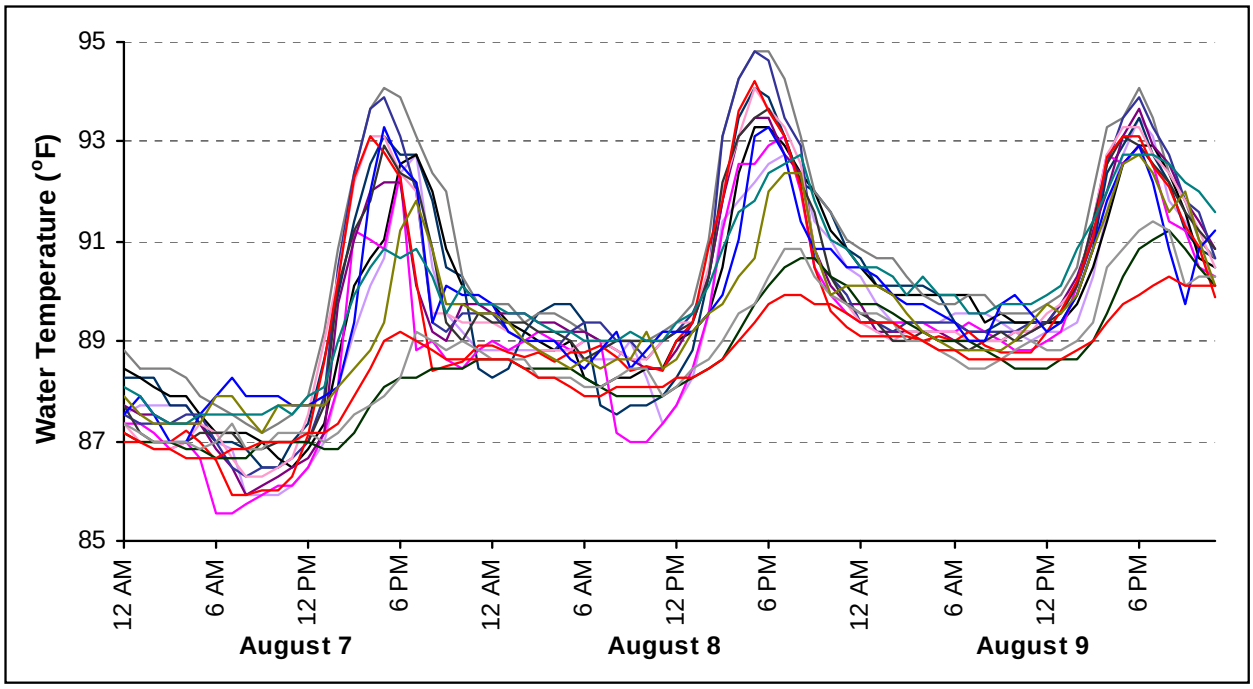
Methods: The waterproof data loggers used for this project (pictured at right) are small, about 2" long. Because of their size, they can be placed either inside of or attached to the outside of a clam bag on the lease site. This provides for temperature measurements at the same depth and location of the clams. From July through November of 2007, eight clam farmers placed data loggers on 17 leases in Cedar Key. Although this represents a small percentage of leases in the area, it is anticipated that the spread of leases used will provide useful information on temperature variability. In addition, temperature measurements were collected from the two CLAMMRS stations in the area.

Results to Date: Water temperature trends in 2007 appeared to be similar across the lease sites from month to month. Regardless of lease site, there was a rise in temperature through the day followed by a cooling during the night. The average difference in temperatures over a 24-hour period did not change from month to month (4.3°F in August versus 4.1°F in November). What did change was the average temperature recorded from month to month (88.3°F in August versus 68.0°F in November). Based on the data collected, significant differences in temperatures across the leases were observed on a day-to-day basis. This suggests to a grower that when comparing temperatures at different leases, it is more important to look at hourly changes as opposed to monthly trends. One factor influencing water temperature is depth. We are still processing the data to understand how much of a role depth plays in temperature variability across these leases. It is anticipated that information obtained from this monitoring project may be useful in determining planting and harvesting strategies during the summer months.

Peak water temperatures were recorded during the month of August, with the hottest temperature (94.8°F) recorded at two of the leases on August 8th at 5:00 PM (see figure below). This peak occurred one hour before a predicted low tide (+0.2'). Temperatures ranged from 89.4°F to 94.1°F on the other 15 leases at that time, representing over a 5°F difference. The CLAMMRS sondes also recorded peak water temperatures at that time (94.2°F). This becomes important as clams cease growth processes around 88°F, and stop filtering at temperatures above 90°F. Looking at water temperatures during the month of August at all leases, there were 26 days on which temperatures reached 88°F, and of those days, temperatures exceeded 90°F on 17 occasions. Also of note is that regardless of lease site, peak temperatures during the day were consistently recorded around 6 PM. This strongly suggests to growers that in order to minimize stress and maximize clam survival and shelf life, farming activities should be conducted in the cooler morning hours when possible. The project provided a lot of useful information this year, and should provide more in the coming years.
In 2008, we have increased the number of leases in this project. In May, 39 data loggers were deployed

providing 10% coverage of the Gulf Jackson and Pelican Reef lease areas and 25% coverage of the Dog Island and Corrigans Reef lease areas. These will be recovered in October. In addition, a new publication entitled "The Role of Water Temperature in Hard Clam Aquaculture" (EDIS # FA151) is available online through the UF Electronic Data Information Source at the website, http://edis.ifas.ufl.edu.

Funded by USDA Hatch



Water Temperatures (°F) Recorded at 19 Leases in Cedar Key during August 7-9, 2007

MONITORING OF CLAM HEALTH DURING SUMMER MONTHS AT HIGH-DENSITY LEASE AREAS IN CEDAR KEY

[1,2]B. Denise Petty, [2,3]Leslie N. Sturmer

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The health assessment is one component of a project designed to augment existing research and education programs for Florida's shellfish aquaculture community. While a preliminary health assessment conducted in 2003 did not reveal any significant pathogens in Florida hard clams, some growers have reported crop losses during the summer months. In addition clam hatcheries have reported low larval survival which impacts production of seed.

To address these concerns, 6 harvest sized clams from each of 12 growers were collected from Cedar Key lease sites once a month in July, August, September, and October 2007, and repeated in May, June, July, and August 2008. Lengths, widths, and weights were obtained from these clams, which were then subjected to gross examination and gill biopsy. Finally, two sections of each clam were prepared for routine histologic examination.

To address hatchery concerns, clam hatcheries were visited in January and February 2008, which are the peak seed production months. Hatchery owners/managers were questioned about sanitation practices, recognized problems, and hatchery water source. Pediveligers, post-set clams, and broodstock were collected for histologic examination, and water and algae samples were collected for analysis.

Funded by USDA Hatch

THE EFFECTS OF MULTIPLE ENVIRONMENTAL STRESSORS ON HARD CLAM SURVIVAL AND PHYSIOLOGY IN SOUTHWEST FLORIDA

Encomio, Vincent; Goncalves, Madeleine; Abeels, Holly; Griffith, Andrew; Volety, Aswani K.
Coastal Watershed Institute - Florida Gulf Coast University Fort Myers, FL 33928

In Southwest Florida, hard clams (*Mercenaria mercenaria*) experience multiple stressors (freshets, high temperatures and red tide). Red tides, caused by the dinoflagellate, *Karenia brevis*, causes closure of harvesting when concentrations reach 5000 cells per liter. Closure of shellfish beds means clams can be exposed to prolonged environmental stress, particularly during the rainier summer months when low, stressful salinities will coincide with high temperatures. Red tides may also occur during the dry winter months. In 2005 a red tide lasted throughout the year in southwest Florida, spanning both dry and wet seasons. Little is known how hard clams respond to red tide and its interaction with natural environmental stressors such as low salinity and high temperature. Several lab experiments simulating these conditions were conducted.

In the first experiment hard clams were exposed to salinities at 10, 20 and 30 ppt for 2 weeks. Clams were then heat shocked at 100°F for one hour (lethal temperature), returned to ambient temperatures and monitored for survival for two weeks. There were significant differences in survival between treatments. Survival was significantly lower at 10 ppt. In a subsequent experiment, clams were exposed to *Karenia brevis* (500,000 cells per liter, 2 times per week) for 2 weeks and transferred to variable salinities (*K. brevis* → Δ salinity; 10, 20 and 30 ppt) for 2 weeks, simulating rainy season effects after red tide. Cellular response (phagocytosis – a measurement of clam immune function) and condition were measured after 2 weeks in variable salinities to characterize sublethal effects of red tide and lowered salinity. Results showed that cellular response was higher in clams due to a sub-lethal heat shock (96.8°F) and salinity, but not *K. brevis*.

A third experiment examining clams' responses to *K. brevis* after prior exposure to low salnity (Δ salinity → *K. brevis)* simulated red tide following rainy season. Clams were sampled prior to and after exposure to variable salinities (10, 20 and 30 ppt for 2 weeks). After salinity exposure, clams were returned to ambient salinities (30 ppt) and exposed to *K. brevis* (500,000 cells per liter, 2 times per week) for 2 weeks. In this experiment, cellular response was also elevated at 10 ppt, which may indicate a stressed condition. However, effects of red tide were not significant. The simulated bloom conditions may not have been severe enough to induce a significant response in clams. However, we maintained clams under aerated conditions throughout these experiments. In nature, depleted oxygen conditions may occur after bloom events. Future experiments must also consider hypoxia as an interactive stress. A significant finding was that effects of low salinity remained prolonged, even after return to optimal salinities. Closure of harvest due to red tide may lead to reductions of clam survival or condition if significant freshets, due to freshwater releases or storms, precede or even follow bloom events.

ENSURING SUSTAINABILITY OF THE FLORIDA HARD CLAM AQUACULTURE INDUSTRY THROUGH EVALUATION OF SOIL AND SEDIMENT QUALITY ON LEASES

Investigators:
Mark Clark, *University of Florida, Soil and Water Science Department*
L. Rex Ellis, *University of Florida, Soil and Water Science Department*
Todd Osborne, *University of Florida, Soil and Water Science Department*

Initial Assessment of Soil/Landscapes in Clamming Areas:

Objectives

The global objective of our soils-based research with respect to shellfish aquiculture is to conduct an initial land assessment of high-use and unused clam lease areas using a soils-based approach. Supporting this objective are several specific objectives outlining this research:

- Create a digital terrain model of the study area
- Sample soils to capture variability within and between leased and unleased areas
- Analyze samples for bulk soil characteristics: particle size distribution, bulk density, and organic matter content, to establish relationships between soil characteristics and clam productivity

Spatial Distribution of Soil Properties

Bathymetry and soil were sampled throughout the Dog Island clam lease area. Soils were analyzed for organic matter, particle size distribution, and bulk density. These variables were selected because they control the physical and chemical behavior of the soils in which the clams live. All resultant soil and bathymetry data were spatial referenced, thus allowing geostatistical analysis. The result are a variety of spatial maps of soil properties (Figure 1). Spatial relationships between soil properties such as organic matter and clay content are evident. These spatial patterns may help explain biogeochemical differences (e.g. enhanced H_2S) throughout the clam lease area.

Sulfate and Hydrogen Sulfide

Sulfate (SO_4-) is a ubiquitous ion in seawater that is transformed to hydrogen sulfide (H_2S) during anaerobic decomposition of organic matter in anoxic water columns and soils. This form of sulfur can be detrimental to biota, including aquaculture clams, when ambient concentrations of H_2S reach toxic levels. This often occurs in the summer months when water temperatures are high or organic matter concentrations are elevated. As we conducted the soils-based portion of this research, we created and additional objective: develop a rapid method for clam lease evaluation of H_2S concentrations whereby lease operators can bring samples to the co-op for analysis and determination of H_2S toxicity risk. This work is ongoing and two similar methods are being tested now. Further, we are also developing a relationship between water temperature and H_2S concentration for selected lease soils through laboratory experiments. This information, when the study is completed, may allow lease operators to determine H_2S toxicity risk during extreme temperature events based on current or projected water temperatures at the lease site. It is our goal that the information provided from this work be used by clam lease operators to minimize losses of clam stock to H_2S toxicity.

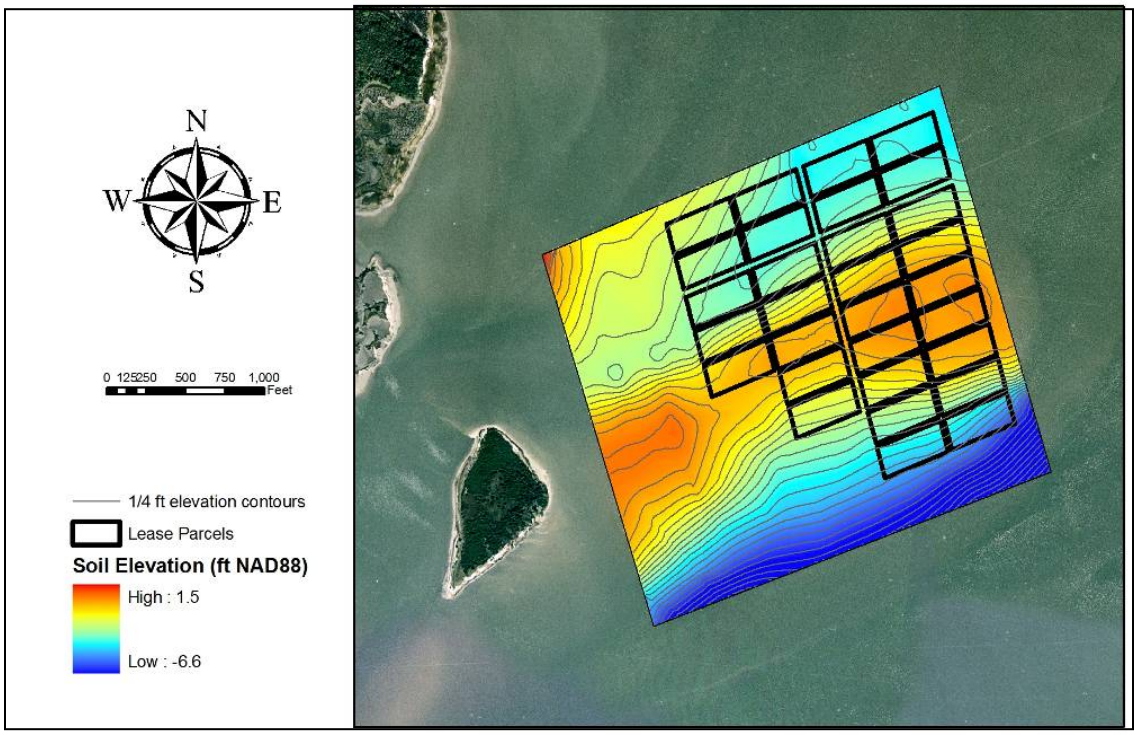


Figure 1. Bathymetric map of Dog Island lease area. Soil properties are similarly mapped for the lease area.

Funded by USDA CSREES Special Research Grants Program

ENHANCING STRESS RESISTANCE OF CULTURED CLAMS THROUGH TRIPLOIDY: FINAL REPORT ON LABORATORY CHALLENGES & FIELD TRIALS

Investigators:
John Scarpa, *Harbor Branch Oceanographic Institute at Florida Atlantic University*
Shirley Baker, *University of Florida, Fisheries and Aquatic Sciences Program*
Leslie Sturmer, *University of Florida, Cooperative Extension Service*
Chuck Adams, *University of Florida, Department of Food and Resource Economics*

Project Summary:
Stressors, such as high temperature, low dissolved oxygen, salinity extremes, and low body mass after spawning, may contribute to high summer mortality and unreliable production in the southeast United States; particularly Florida. Triploid induction is a basic breeding technique. Triploid organisms have three sets of chromosomes instead of the usual two, which results in reproductive sterility. Sterile clams may be beneficial as energy normally used for reproduction is diverted to growth and potentially stress resistance. Therefore, induced triploidy was investigated for improvement of hard clam survival and stress resistance in Florida waters. Specific objectives in this project were to: 1) create replicate diploid and triploid families, 2) compare growth and survival of diploid and triploid clams during growout, 3) compare physiological responses of diploid and triploid clams to stress, and 4) compare the economics of triploid production to diploid production.

Triploidy was successfully induced in hard clams, however, success varied (0-100%). Typical mass spawning techniques were problematic because eggs were pre-fertilized leading to incorrect timing of application of the chemical to induce triploidy, which resulted in low triploid induction or high mortality of clam embryos. If triploid clams are found to be advantageous for culture in Florida, a reliable technique for producing triploids must be developed.

Triploidy may increase survival, but only under certain conditions, such as hypoxia. Laboratory experiments compared the combined effects of temperature, salinity and low oxygen on clam survival. Clams were challenged at 90°F at salinities of 10ppt, 25ppt, and 40ppt, and normal (normoxia) or low (hypoxia) oxygen levels. At salinity extremes (10 and 40 ppt) triploid clams had no advantage over diploid clams. However, at 25ppt and hypoxia, diploid clams began to die 3 days sooner than triploids. After 22 days of exposure, about 4 times as many diploid clams had died as compared to triploid clams.

Triploidy may confer a metabolic advantage, but only under certain conditions, such as low salinity. Laboratory experiments were conducted to measure oxygen uptake rates (metabolism) of clams at various water temperatures and salinities. There was no difference in metabolism between triploid and diploid clams at water temperatures of 68, 77, 81, 86, and 90°F at 25 ppt. However, at 15 ppt and 81°F, triploid clams had lower metabolism than they did at 25 ppt, while diploid clams did not. Lower metabolism may allow triploid clams to survive longer than diploids under these stressful conditions.

Triploidy did not increase thermo-tolerance of clams. Laboratory experiments were conducted to asses if thermo-tolerance, as measured by survival and heat-shock protein (Hsp) concentrations in tissue, was increased by triploidy. Heat-shock proteins were found in the

tissues of hard clams under both normal and heat–shock conditions. Survival in hard clams was higher when initial Hsp concentrations were higher at the time of heat-shock. Therefore, selection for high Hsp clams may increase thermo-tolerance.

Triploidy did not consistently confer a growth or survival advantage in laboratory or field trials. Laboratory and field experiments were conducted to determine if triploid clams had better growth and survival. Two three-week laboratory growth experiments were performed on nursery-seed (~85 and 12 mg). Triploids grew significantly less by the end of both experiments (Exp I: Triploid 250-269% vs Diploid-341%; Exp II: Triploid-422% vs Diploid-549%). Field growth studies at different sites on the west coast of Florida were interrupted during the severe 2004 hurricane season, with loss of most groups. In samples taken from one site just after the hurricanes, triploids and diploids were similar in shell length and whole weight. In samples taken from just one family two months after the hurricanes (~12 months in age), it was found that triploids were smaller in shell length (19.7 vs 23.4 mm) and whole-weight (1.97 vs 3.33 g). However, triploids had a higher condition index (i.e., ratio of dry meat weight to shell weight, 6.6 vs 5.6). Histological examination of gonads indicated that 50% of the diploid clams had completed spawning and 40% had no gonad. In contrast, 100% of the verified triploids had no gonad. This data indicated that gonad development was inhibited in triploid hard clams as has been seen in other bivalve species. A new set of triploid clams was produced in late 2005. At the end of field culture, differences were found between triploids and diploids and between sites. At Cedar Key, triploids were similar in length (46mm) and weight (30g), but had a higher condition index (5.3 vs 4.8) and lower survival (69 vs 80%) compared to diploids. At Charlotte Harbor, triploids were similar in length (48-50mm) and condition index (4.6), but lower in weight (34 vs 44 g) and survival (43 vs 49%) compared to diploids.

Triploid induction methods modestly increased the cost of producing seed clams. An economic analysis was performed on the cost of producing triploid nursery-seed (1mm) clams. The additional cost associated with triploid production in the hatchery was calculated to be $0.14-0.27/1000 seed (~5-10% increase), depending on survival.

Recommendation: Triploid clams did not consistently produce higher growth or survival as compared to normal diploid clams in laboratory or field experiments. Therefore, triploid clams are not recommended for all Florida clam farmers.

Funded by USDA ARS and Florida Sea Grant

EVALUATION OF CLAM STOCK IMPROVEMENT THROUGH USE OF HYBRIDIZATION

John Scarpa, *Harbor Branch Oceanographic Institution at Florida Atlantic University*
Leslie N. Sturmer, *University of Florida, Cooperative Extension Service*
Shirley Baker, *University of Florida, Department of Fisheries and Aquatic Sciences*

Hard clam aquaculture in Florida has expanded primarily through increased acreage rather than increased productivity. Over the years, clam production in Florida has seen various mortality events resulting from hurricanes, low salinities, and, potentially, high water temperatures. The local southern hard clam (*Mercenaria. campechiensis*) may offer improved production characteristics and hybridizes readily with the northern hard clam (*M. mercenaria*). Therefore, an examination of production characteristics under commercial conditions has been undertaken of the parental species and their reciprocal crosses.

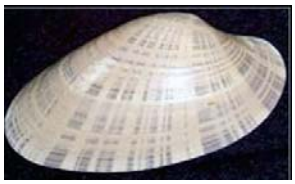
Cultured *M. mecenaria notata* and wild *M. campechiensis* were used as broodstock. Spawning was induced by thermal shock and single parent crosses performed. The resulting larvae from each group within a spawn (n=5 spawns performed) were cultured separately in 400-L vessels utilizing standard hatchery practices of daily water change and batch feeding of microalgae (T-Iso). Larvae were transferred to downwelling systems for metamorphosis after 7 days culture. Differences in fertilization were noted between some hybrid crosses, potentially being related to the female. Nuclear DNA amount, as measured by flow-cytometry using DAPI stain, differed slightly (six fluorescent units) between the parental species. Some hybrids groups exhibited values of the maternal parent, whereas as others exhibited values of the paternal parent, but not a value in between of the parents. Therefore, protein (aka, allozyme) gel electrophoresis of known markers for each species was conducted on parental tissue samples. Allozyme analysis revealed that natural hybrids were utilized in two trials, thereby leaving three groups of clams with true parents and hybrids for grow-out comparison. This genetic analysis reflects the difficulty of using visual characteristics to differentiate between species that naturally hybridize.

Triplicate families of parental stocks and their reciprocal hybrids were land-based and field nursed following standard protocols used in hard clam culture. During September 2008, seed (12-20 mm shell length) of each family is being planted to evaluate performance under commercial conditions. Production characteristics (growth, survival, condition index) will be compared between these families at several stocking densities (960-1360/bag, or 60-85/ft^2) and site locations using both the bag and bottom plant methods. At harvest in September 2009, shelf life of these stocks in refrigerated storage will be documented as well as market acceptance. Controlled laboratory experimental challenges will examine the combined effects of temperature, salinity, and oxygen levels on survivorship of these stocks (both growout-sized seed and littleneck-sized clams) using natural ranges found in Florida. The physiological mechanism by which hybridization may improve field survival will be determined.

Funded by USDA CSREES Special Research Grant Program

EVALUATION OF ALTERNATIVE BIVALVE SPECIES FOR DIVERSIFICATION: SUNRAY VENUS CLAMS

Principal Investigators:
John Scarpa, *Harbor Branch Oceanographic Institute at Florida Atlantic University*
Leslie N. Sturmer, *University of Florida (UF), Cooperative Extension*
Chuck Adams, *UF Food and Resource Economics Department*
LeRoy Creswell, *Florida Sea Grant*



Project Summary:
The sunray venus, *Macrocallista nimbosa*, was commercially fished in Florida during the 1970s. Although natural growth rates were estimated to be high, its patchy distribution limited commercial exploitation. The sunray venus clam is now being evaluated as a potential new aquaculture species to diversify the hard clam culture industry in Florida.

Adult sunray venus clams were collected near St. Teresa Beach and Cedar Key, Florida and shipped to HBOI by overnight courier. Clams were then placed in temperature-controlled tanks (20-25^{o}C, 30-32 ppt), fed microalgae daily, and observed for mortality. Broodstock clams did not exhibit excessive mortality from overnight shipping (i.e., <11% after one week). Histological analysis of gonad tissue indicated an approximate 1:1 female to male ratio.

Induced spawning was attempted using thermal-cycling (10^{o}C increase) and dissected-sperm addition, which resulted in the first ever successful induced spawning of both male and female sunray venus clams. Approximately one month later a second induced spawning was accomplished using thermal-cycling and serotonin injection. Both spawns yielded viable gametes that resulted in D-stage larvae the following day. Larvae were fed microalgae (*Isochrysis* sp., clone T-ISO) daily and water (78-82^{o}F, 30-31 ppt) was changed daily. Larval development was similar to hard clams; pediveligers (~220 μm) were noted at day seven at which time they were placed in downwellers without or with substrate (sand, aragonite). Metamorphosis occurred over several days in all treatments. Settled clams were fed mixtures of T-ISO and a diatom (*Thallasiosira* sp.) and water changed approximately every other day. Three months after settlement, clams from both spawns were enumerated by volumetric sampling. The first spawn exhibited a 63% return and the second spawn exhibited a 46% return. Nursery seed was distributed to project partners for further nursery culture.

A feed density trial was conducted with juveniles to determine maximum cell density for growth. Triplicate 4-L beakers each containing 24 clams were fed 0, 50, 100, or 200 K cells/mL of *Isochrysis* sp. twice/day over a four week period. Growth (absolute and % weight change) did not increase above the 100K cells/mL treatment.

Approximately 118,000 sunray venus nursery clam seed (9-18 mm shell length) were field-planted in soft bags or hard cages. After two-four months, survival ranged from 32-94% with 3.4-6.5 mm/month shell length growth. Sunray venus clams are being further cultured in soft bags, soft bags with internal PVC pipe frames, cages, and bottom plants at densities of 38-70/ft^{2}; a portion of which will be used for market perception tests. Sunray venus clam culture methods to date are exhibiting little difference from hard clam methods for spawning, larvae culture, nursery culture, and early growout culture.

Funded by Florida Sea Grant

CLAM WORKSHOP PRESENTERS AND CONTACT INFORMATION

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