Short-term effects of salinity declines on juvenile hard clams, *Mercenaria mercenaria.*

Final report to Florida Sea Grant, for a Program Development Award

Project title: Short-term effects of rapid salinity declines on newly planted seed clams (*Mercenaria mercenaria*) during La Niña conditions in Florida.

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Summary

Sudden salinity drops in clam growing areas in Florida have been suggested as a cause of seed (juvenile) clam mortality. Laboratory trials were used to assess short-term impacts of rapid salinity drops on hatchery-produced juvenile hard clams (*Mercenaria mercenaria*) in two separate trials. Response parameters measured were mortality and condition index. In Trial I, clams were exposed to a salinity drop of 5 parts per thousand (ppt), 15 ppt, or 24 ppt, over a span of 24 hours, and the duration of this exposure was either 3 or 6 days. In Trial II, clams were exposed to an immediate salinity drop of either 10 ppt or 20 ppt. Clams were either immediately immersed or dry-stored for 24 hours prior to immersion, representing common treatment patterns by clam growers. The duration of exposure was 8 days. Both trials were conducted at ambient seasonal temperatures.

In Trial I, seed clams proved resistant to all salinity drops except for the largest drop (28.5 ppt to 4.5 ppt) for the longest duration (6 days). Condition index declined significantly under these conditions, and observed mortality at the end of 6 days was 17%. In all other treatments, condition index did not change from initial levels, and mortality was not different from controls (0-2%). In Trial II, seed clams experienced 100% mortality at the 20 ppt drop after 8 days of exposure, but mortality in the 10% drop treatment was low and did not differ from the control. Dry storage did not significantly affect mortality in any treatment, and no significant differences in condition index were observed in any treatments. The salinity drop that caused significant mortality in either Trial I or II was of longer duration than those observed in recent field data. Thus, if salinity drops do account for short-term commercial clam seed mortalities, effects may be compounded or mitigated by other factors, such as other environmental conditions or handling effects.

Introduction

Florida ranks third in the United States in aquaculture production values, and the culture of hard clams, *Mercenaria mercenaria*, represents the fastest growing segment of the state's aquaculture industry. Florida farm-raised clams are now a recognized commodity on the national market with Florida clam production accounting for more than 10% of the nation's total (USDA, 1998; Sturmer, 2000). The growth of the Florida hard clam industry is due in part to job retraining programs. Clam farming is providing an important source of income to former fishermen and has given a major economic boost to rural coastal communities (Colson and Sturmer, 2000). Clam farming has been successful on both the Gulf and Atlantic coasts, but there have been some setbacks. For example, in 1998, clam growers in Levy County sustained unexplained mortalities of up to 100% in a span of several days.

In 2000, clam growers were the first aquaculturists in the United States eligible to purchase crop insurance. With input from extension agents, growers and scientists, the US Department of Agriculture (USDA) Risk Management Agency has developed a pilot program under which hard clam growers are able to buy subsidized Cultivated Clam Crop Insurance for the 2000-2002 crop years. Areas included in the pilot project are Florida's Dixie, Levy, Brevard, and Indian River Counties, as well as counties in South Carolina, Massachusetts, and Virginia. The clam crop insurance program covers losses due to "unavoidable damage" such as storms, low oxygen, and salinity changes. If the pilot Cultivated Clam Crop Insurance is successful, it may be expanded to include other geographic areas and other aquaculture species (Rheault, 2000; USDA, 2000).

At this time, there are inadequate provisions for correlating water quality or weather events with crop loss. In 2000, however, we had the opportunity to deploy two water quality monitoring systems on temporary loan. In cooperation with Department of Agriculture and Consumer Services (DACS) personnel, monitoring sites have been established on the Gulf Jackson High Density Lease Area (HDLA) in Levy County and the Horseshoe HDLA in Dixie County. We now have several months of continuous (every 30 minutes) data on temperature, salinity, and dissolved oxygen from both sites.

A `La Niña' weather phenomenon began in 1999 and conditions continued through into 2001. The primary effects of La Niña years on the climate of Florida are deficits of precipitation, seasons that are warmer and dryer than normal, and changes in prevailing wind patterns (The Florida Consortium, 1999). During the first half of 2000, salinities in Dixie and Levy counties averaged 29.6 ppt. Annual salinity values for these areas typically average 22 ppt (L. Sturmer, Florida Aquaculture Extension, unpubl. data).

Our water monitoring data reveals important details of temporal variability, previously unresolved from monthly samples, apart from increased mean salinities. For example, we discovered regular tidal oscillations in salinity of up to 6 ppt. In addition, we noted a recurring pattern in salinity; salinity gradually increases over periods of four to seven days, followed by decreases in salinity over one to three days. It is unclear whether these longer-term salinity patterns are predictable results of tidal, wind, or river influences, or a combination thereof, or whether they occur only in La Niña years.

In spring 2000, several growers reported high mortalities of seed (juvenile clams) recently planted in Levy and Dixie counties. Upon examination of the concurrent water-quality data, we discovered that the seed had been planted just hours before decreases in salinity. In these particular cases, the salinity dropped approximately 11 ppt over the next

28 h; for example, from 30 ppt to 19 ppt. Adjacent adult clams and previously planted seed were unaffected, making crop insurance claims difficult to substantiate since the mortalities could have been the result of poor seed quality or mistreatment of seed during transport. However, the reporting growers are known to be reliable, and the previously planted seed - which did not experience the same mortality - came from the same source. This suggests that the mortality of newly planted seed was the result of the additional stress of the salinity drop occurring before the seed clams had a chance to adapt.

Since the spring 2000 mortality event, we have continued to download water quality data from data loggers installed at the Gulf Jackson and Horseshoe Beach High Density Lease Areas (HDLAs). Over twelve months of continuous data reveal that short-term water quality fluctuations are greater than originally suspected, and that this fluctuation is both temporal and spatial in nature. Both Gulf Jackson and Horseshoe Beach HDLAs exhibit strong daily salinity fluctuations. From 6/29/00 to 7/29/01, salinity values ranged from 5.4 to 37.2 ppt and 14.9 to 39.2 ppt at Gulf Jackson and Horseshoe Beach HDLAs, respectively (Figure 1). Mean salinity was 26.7 ppt (s.d.=4.8) at Gulf Jackson HDLA and 28.7 ppt (s.d.=4.8) at Horseshoe Beach HDLA. The magnitude of 24-hour salinity changes sometimes approached the overall magnitude for the entire study period. Absolute 24-hour salinity changes for Gulf Jackson and Horseshoe Beach are illustrated in Figures 2 and 3. From 6/29/00 to 7/29/01, mean 24-hour range in recorded salinity values at Gulf Jackson was 5.3 ppt (s.d.=3.2) with a maximum 24-hour change of 24.51 ppt. The range of 24-hour salinity values at Horseshoe Beach HDLA was less than at Gulf Jackson HDLA with a mean of 2.7 ppt and a maximum of 11.62 ppt.

A limited number of studies have explored the effects of salinity on the ability of seed clams to survive. For example, Chanley (1957) determined the minimum salinity at which hard clam seed from Milford Bay, CT, could survive following direct transfer to lower salinity. Roegner and Mann (1991) reviewed various data and predicted high mortality at salinities below 12 ppt, with no populations persisting below about 20 ppt. We are not aware, however, of any studies on the effects of drops in salinity on newly planted seed clams of the magnitude, or over the short time period, that appear to occur at the Gulf Coast aquaculture sites. In this study, we initially examined the short-term impacts of strong but gradual salinity declines of several different magnitudes, on the mortality and condition index of juvenile seed clams, at two duration lengths (Trial I). Subsequently, we examined the effect of immediate salinity declines, also of several magintudes, under two typical commercial handling regimes (Trial II).

Methods

Trial I. Salinity declines integrated over 24 hours

Seed clams (juvenile hard clams, *M. mercenaria*) were obtained from a commercial hatchery and held in flowing seawater at about 29 parts per thousand (ppt) at a private Cedar Key clam facility until use. Mean seed clam shell height was 5.98 mm (s.d. = 0.46 mm). Subsequent research was conducted at the Department of Fisheries and Aquatic Sciences, University of Florida, in Gainesville, Florida.

Salinity treatments were conducted in 10-gallon (38 liter) aquaria, each with a 3-cm layer of cleaned river sand in the bottom, and equipped with a power-head water

circulator. Clams were divided into lots of 150 each and placed inside scaled-down duplicates of commercial clam culture bags; this density approximated commercial planting densities. Bags were made of woven polyester mesh, about 25 x 25 cm. Bags were assigned randomly, two to each aquaria, in the experimental and control treatments.

Clams were fed with preserved algal paste (Coast Seafoods, Inc.). Paste was resuspended in well water and fed to the clams in aliquots throughout the trial; volumes of well water used were too small to significantly alter salinity in tanks. The clams were fed approximately 2% of their total wet weight per day, or about 0.058 g (dry weight) per tank (300 clams each). Temperatures were kept constant at 23^{o}C (s.d. = 1ºC) throughout the trials by use of aquaria heaters with thermostats, and temperatures were monitored regularly. Initial salinities in all aquaria were 28.5 ppt (s.d. = 0.6 ppt), or approximately equal to salinities at which seed clams were held prior to the treatments.

Treatments included salinity drops of two durations (extended salinity drop and short-term salinity drop) plus a control (no salinity drop). The extended salinity drop exposed seed clams to reduced salinities for six days, and the short-term drop, which started three days after the extended drop, ran for half that time. Three salinity drops were used: 5 ppt, or 28.5→23.5 ppt; 15 ppt, or 28.5→13.5 ppt; and 24 ppt, or 28.5→4.5 ppt. These salinity drops were selected based upon initial results indicating weak clam response at lower-magnitude salinity drops. Logistical constraints prevented all three drop trials from being run simultaneously, so trials ran in sequential weeks, with the smallest drop first and the largest drop last. We had four replicates of each treatment drop magnitude and duration.

In all treatments, salinities in the experimental treatments were lowered over a period of 24 hours (immediately in extended salinity drop treatments, and after three days in short-term salinity drop treatments), by incremental additions of reverse osmosis-purified water. Identical volumes of seawater were removed and replaced in the control treatments. Salinity was monitored regularly.

Two response parameters in seed clams were measured at the end of each treatment: mortality and condition index. Mortality is an absolute but insensitive indicator of stress, while condition index is potentially more sensitive, but does not necessarily predict mortality. Condition index (CI) is the ratio of a sensitive numerator, tissue weight, to a relatively insensitive denominator, shell cavity volume (Rainer and Mann, 1992), and is a measure of the degree of "fattening" or nutritive status. CI is indicative of an organism's exposure to a variety of recent stressors and is also predictive of an organism's ability to withstand future stress (Mann, 1978). Condition index is sensitive to experimental error, particularly if the bivalves are small. To reduce this error in our experiments, CI was estimated for groups of 20 juvenile *Mercenaria* from a bag. These 20 clams comprised a single replicate, and resulted in a single datum. Shell cavity volume was estimated from the difference between the volume of water displaced by whole live animals, and the volume displaced by clean, separated valves (shells). Tissues were dried (100ºC) to constant weight. CI was calculated according to Rainer and Mann (1992), where CI = (dry meat weight x 100) / shell cavity volume.

Two-factor analysis of variance with interactions (Zar, 1996) was used to test null hypotheses of a) no effect of salinity drop treatment (extended, short-term, and control), and b) no effect of absolute salinity drop (5, 15, or 24 ppt) on response parameters. Response parameters were tested separately. Mortality data was transformed by a

modified Freeman and Tukey arcsine-square root transformation of actual numbers, as opposed to proportions (Zar, 1996), to normalize the data.

Trial II. Immediate salinity declines under two handling regimes

Clams were obtained from a commercial hatchery outlet in Cedar Key, Florida, and transported directly to the Fisheries and Aquatic Sciences Department facilities for trials. Mean seed clam shell height was 6.34 mm (s.d.=0.72), and salinity of the system they had been held in was 24 ppt at the time of collection.

Treatments were conducted in three separate but identical systems. Twenty-four aquaria were set up as for Trial I, but aquaria were also in water baths, with 8 aquaria each. Additionally, circulation in each aquariium was closed, with mechanical and biological filters. Treatments included a non-change control and two salinity drop levels of 10 and 20 ppt, resulting in test salinities of 14 ppt, 4 ppt, and 24 ppt, respectively. Actual mean salinity values, based on dairly measurements, were 24.15 ppt (s.d.=0.15), 14.11 ppt (s.d.=0.06), and 4.11 ppt (s.d.=0.03), respectively. Water temperature was maintained at ambient room temperature, which remained within 0.5°C of 26°C throughout the trial in all tanks.

As in Trial I, seed clams were placed inside scaled-down duplicates of commercial clam culture bags in the experimental system. About 150 seed clams were assigned to each bag. Half (24) of the bags were assigned randomly one each to the 24 aquaria in all three treatments, and immediately immersed (8/7/01). The remaining half of the bags was dry-stored in a cooler (16°C) utilizing commercial hatchery recommended storage and handling protocols (E. Quesenberry, Harbor Branch Oceangr. Inst., pers. comm.) Following 24 hours of dry storage (8/8/01), stored bags were assigned and immersed randomly, one to each aquaria. As a result, each aquaria had 2 bags each; one immediately immersed and one immersed following 24hr dry storage. This resulted in six total treatments: two handling treatments x three salinity treatments.

Beginning 24 hrs after immersion of the dry-storage bags and continuing for four days (8/9/01 through 8/12/01), 2 replicate bags from each treatment (immediate vs. delayed immersion) from each system (24, 14, or 4 ppt salinity) were randomly selected for analysis. Because of unresolved problems with condition index data for very small bivalves, the only response parameter we measured in Trial II was mortality, which was quantified as counts dead per replicate bag.

Chi-square analysis of contingency tables (Zar, 1996) was used to test the following null hypotheses: a) mortality is independent of salinity treatment (24, 14, or 4 ppt), and b) mortality is independent of time of initial immersion (immediate or delayed). Chi-square analysis was conducted on two- and three-dimensional contingency tables. Two-way contingency tables were subdivided for further chi-square analysis. In addition, mortality trends over the course of the trial in each of the six salinity-handling treatment combinations was compared using analysis of covariance with *a posteriori* multiple comparisons (SAS Institute, 1995). Mortality data was transformed by a modified Freeman and Tuckey arcsine-square root transformation of actual numbers, as opposed to proportions (Zar, 1996), to normalize the data.

Results

Trial I. Salinity declines integrated over 24 hours

Clams appeared to feed and produced feces and pseudofeces in all trials and in most treatments. None of the clams were able to burrow while within the clam bags in any of the trials, but the occasional clam which escaped from the bag always burrowed.

Mortality was slight (0-2%) and not significantly different from controls in all treatments except for a mean mortality of 17.0% (s.d. = 2.2%) following the extended salinity drop of 24 ppt (Figure 5). In the two-way ANOVA, this was accounted for by both treatment effect ($\mathbf{p} < 0.001$), where treatment = extended salinity drop, short-term salinity drop, or control; and by salinity drop trials effects ($\mathbf{p} < 0.001$), where trials = 5, 15, or 24 ppt drops. There were significant interaction effects, but all were accounted for by the high response at the 24 ppt extended salinity drop.

Condition index was highly variable relative to mortality, but the same pattern was detected, with the only significant decline following the extended salinity drop of 24 ppt (Figure 6). In the two-way ANOVA, this was accounted for by treatment effect ($\mathbf{p} = 0.036$). There were no significant interaction effects.

Trial II. Immediate salinity declines with two handling regimes

Clams appeared to feed and produce feces and pseudofeces in all treatment groups, although less so at the 4 ppt salinity level. Mortality was slight (0 – 4%) at the 24 and 14 ppt salinity levels, regardless of handling regime (immediate or delayed immersion), throughout the entire trial (Figure 7). Beginning on the second day (8/10/01) of the trial, mortality was significantly greater at the 4 ppt salinity level for both handling treatments, and reached 100% by 8/14/01.

Chi-square analysis of contingency tables (Appendix A) failed to reject the null hypothesis that clam mortality was independent of salinity at the 24 and 14 ppt levels. Mortality was not independent of salinity at the 4 ppt level beginning on 8/10/01 and throughout the remainder of the trial. Mortality was independent of time of immersion (immediate or 24-hour delay) for all treatment levels on all days except at the 4 ppt salinity level on 8/11/01. Analysis of covariance of mortality trends in the six handling-salinity treatment confirmed this; mortality was significantly higher in the 4 ppt treatments, regardless of handling treatment ($p < 0.005$), but the 14 ppt salinity treatment did not differ significantly from the 24 ppt salinity control, nor did handling treatments significantly affect mortality within salinity treatments. Percent mortality of clams for each of the six salinity-treatment combinations is presented in Table 1.

Discussion

Juvenile hard clams, *M. mercenaria*, are surprisingly robust to short term salinity drops, given predictions by Roegner and Mann (1991). Nonetheless, salinity declines of the magnitude sometimes observed at high-density lease areas (HDLAs) resulted in significant mortality in both Trial I and Trial II. In the first trial, with a step-wise salinity reduction, mortality was delayed for about six days, compared to only two days in the second trial. Furthermore, use of a common handling technique – dry storage for 24 hours – did not significantly affect mortality. Short-term salinity drops of these magnitudes appear to occur on an annual basis in the Dixie and Levy County high-

density clam lease areas, but we are unable to determine how often extended salinity drops occur, based on existing data.

Differences between Trial I and II in the onset of clam mortality may be due to the difference in how rapidly salinity was dropped (over 24 hours in Trial I versus immediate in Trial II), but other differences between the two trials may also at least partially account for the differences. Ambient water temperature was approximately 3°C cooler in Trial I, with potentially reduced metabolic rate. Both temperatures were well within normal growth and reproduction ranges for this species (Roegner and Mann, 1991). Existing information on juvenile hard clams is unsufficient to determine whether this temperature difference is physiologically significant. There may also be genetic differences across hatchery stocks used in this study; this is the topic of separate research by these authors.

Long-term effects of salinity drops remain to be tested. Salinity drops may have sublethal effects, measured in reduced growth rate (which affects profitability to growers), but this response parameter requires a longer-term study than could be conducted here. Alternately or additionally, salinity drops may have a chronic impact on clam survival, causing increased mortality over a period longer than examined in this study, or by reducing clam resistance to other environmental stressors.

Our study suggests that rapid salinity drops of a 10-15 ppt have little acute effect on seed clams. Rapid salinity drops in the Dixie-Levy County clam growing areas, however, is generally associated with freshwater runoff from land, and this water is likely to bring with it changes in addition to salinity. Additional potential stressors to clam health include sudden temperature shifts (Diaz, 1973), hypoxic events (Baker and Mann, 1992), and pathogens (Krauter et al., 1998). Thus, reduced salinity may signal a variety of stressors, so that even if the salinity drop itself is not severe, the stress in combination with temperature or oxygen changes may result in mortality. We are also unable to rule out variability in seed clam handling by growers, at this time.

This study also allowed us to examine the statistical behavior of two response parameters: condition index and absolute mortality. Condition index (CI) clearly has the potential to be more sensitive than absolute mortality as a response indicator of seed clam stress; CI is a continuous variable within each clam, while mortality is dichotomous for each clam (dead or alive). In these trials, however, CI proved not to be as sensitive as mortality across samples. In Trial I, the power of the tests, based on **p** values, was higher for mortality than for CI, even though the same overall patterns of response were observed. The probable reason for the apparent lack of sensitivity of CI is the high variability in CI measurements, which is undoubtedly compounded by the difficulty in measuring CI in such small organisms. We still feel that CI may prove to be a more sensitive response indicator, but because the variability differs from that of mortality, experimental protocols need refinement.

Mortality data present their own statistical difficulties. Most values in this data set were near zero, with a few in the 15-25% range in Trial I and a few in the 100% range in Trial II, resulting in a non-normal data distribution. Data transformations only partially corrected non-normality problems. Fortunately, both a balanced ANOVA and ANCOVA design are robust to modest violations of the assumption of normality, and the statistical tests bore out what is clearly apparent from a graphical presentation of the data (Figures 5 and 7). Non-parametric tests are an imperfect solution; while robust to

violations of the assumption of normality, they are also less powerful (Zar, 1996). If future studies on this problem present mortality data with a similarly non-normal distribution but without the non-overlapping variances seen between the single high-mortality treatment and all other treatments, the researcher must make the unpleasant choice between parametric tests and possibly spurious null hypothesis rejection, and nonparameteric tests and loss of statistical power. In this scenario, condition index data (above), with its normal distribution, becomes additionally attractive.

Other response parameters are available for future consideration. Scope for Growth (SFG), the balance between processes of energy acquisition and energy expenditure, provides greater insight than CI into the underlying physiological changes associated with a response (Dame 1996). SFG is more difficult to measure than CI, however, and subject to the same level of variability, since it involves some of the same measurements. Actual growth, measured by changes in shell size (the parameter also used to grade clams in the industry) is more simple to measure, but unpublished data (these authors) indicate that variability is at least as high as for condition index, so much longer-duration trials are required to obtain a significant response.

In the future, we intend to modify the previous trials to **a)** enhance the statistical power of the tests to more finely resolve condition index changes, and **b)** to examine slightly longer-term (two weeks instead of one) impacts of salinity on condition index and mortality. We feel further laboratory resolution of the role of salinity drops in juvenile hard clam condition index and survival is warranted.

Laboratory trials should be used to guide and supplement field trials, but not to replace them. Laboratory experimentation is irreplaceable for hypothesis testing, but once we have clearly demonstrated the impact of an environmental parameter in the laboratory, field studies will be required to quantify their role under culture conditions. Such field trials will benefit from other research efforts ongoing in clam lease areas to measure water quality parameters.

Acknowledgements

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Table 1. Percent mortality of seed clams following either immediate or delayed (24-hour) immersion at three salinity levels (24, 14, and 4 ppt). Note: 8/9/01 represents two days post immediate immersion and one day post delayed immersion.

Treatment		Date				
Salinity (ppt)	Immersion	8/9/01	8/10/01	8/11/01	8/12/01	8/14/01
24	Immediate	0.57	1.02	0.82	0.87	1.23
24	Delayed	1.57	1.80	1.67	1.51	1.94
14	Immediate	0.59	1.02	1.72	2.67	2.39
14	Delayed	0.26	3.25	3.89	3.77	3.60
04	Immediate	1.12	3.02*	9.54*	35.74*	100.00*
04	Delayed	1.76	4.79*	16.73*+	35.63*	100.00*

* = Statistically significant ($\alpha = 0.05$), H_A (mortality is not independent of salinity), based on chi-square analysis of 2-way contingency table data.
+ = Statistically significant ($\alpha = 0.05$), H_A (mortality is not independent of immersion), based on chi-square analysis of 2-way contingency table data.

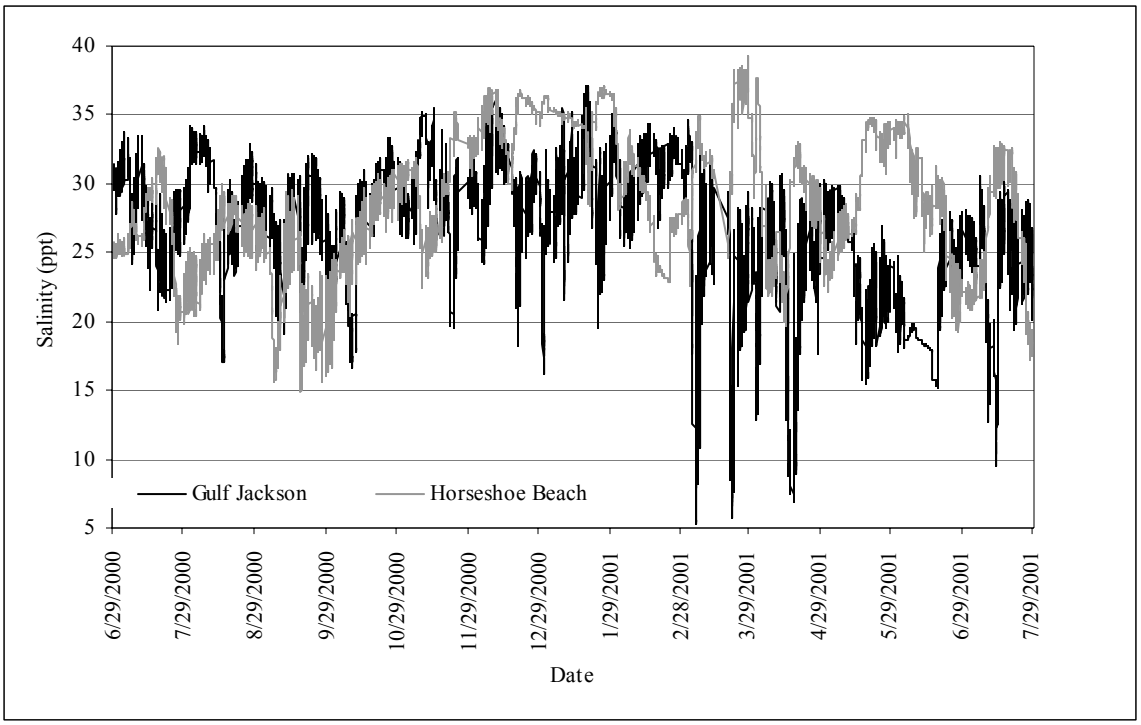

Figure 1. Salinity (ppt) values for Gulf Jackson and Horseshoe Beach high density lease areas, 6/29/00 through 7/29/01.

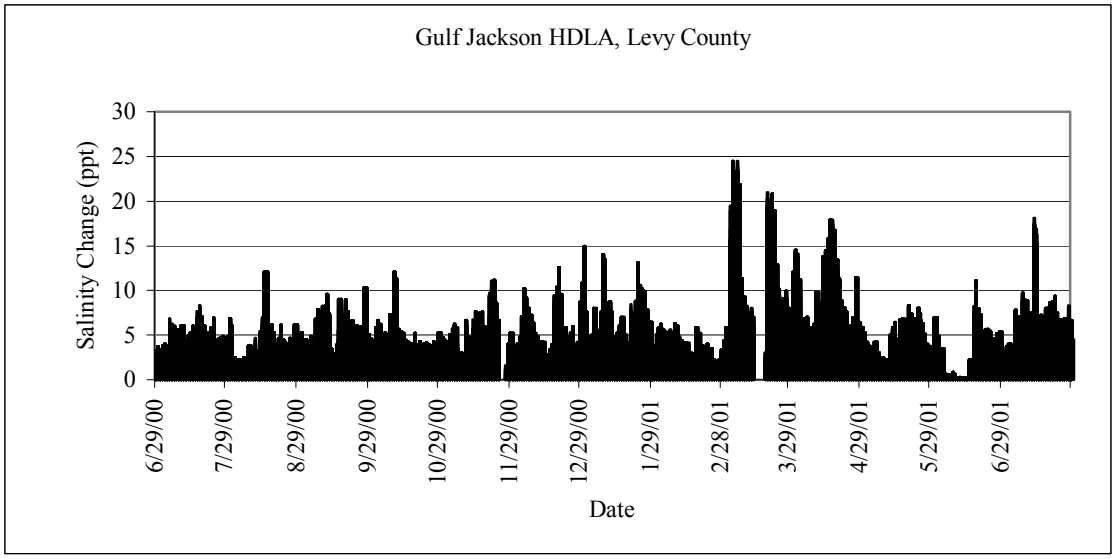

Figure 2. Maximum 24-hour absolute change in salinity (ppt) measured every 30 minutes at Gulf Jackson high-density lease area, Levy County from 6/29/00 through 7/28/01.

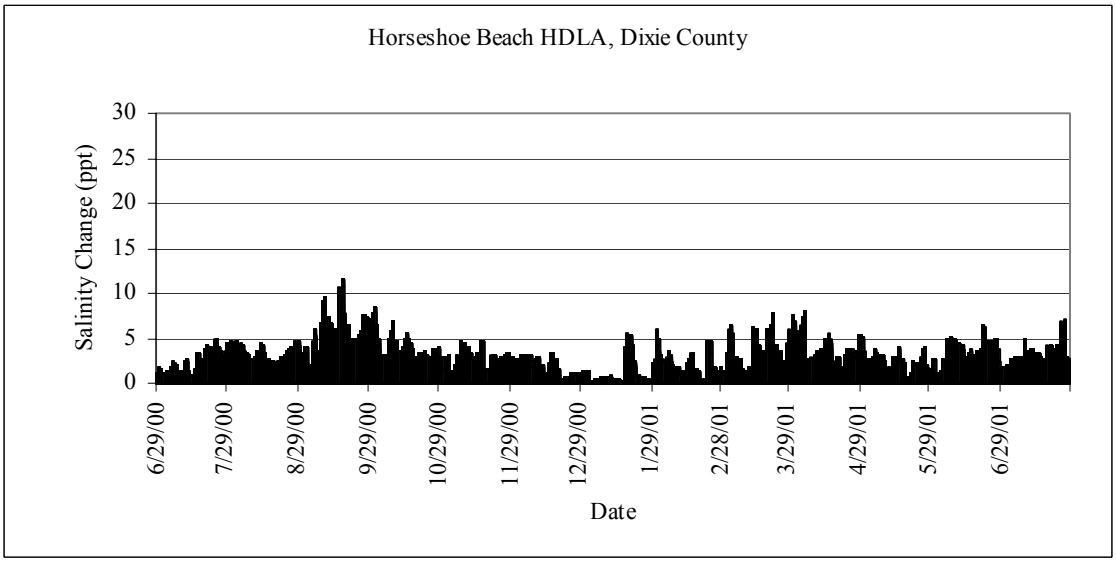

Figure 3. Maximum 24-hour absolute change in salinity (ppt) measured every 30 minutes at Horseshoe Beach high-density lease area, Dixie County from 6/29/00 through 7/28/01.

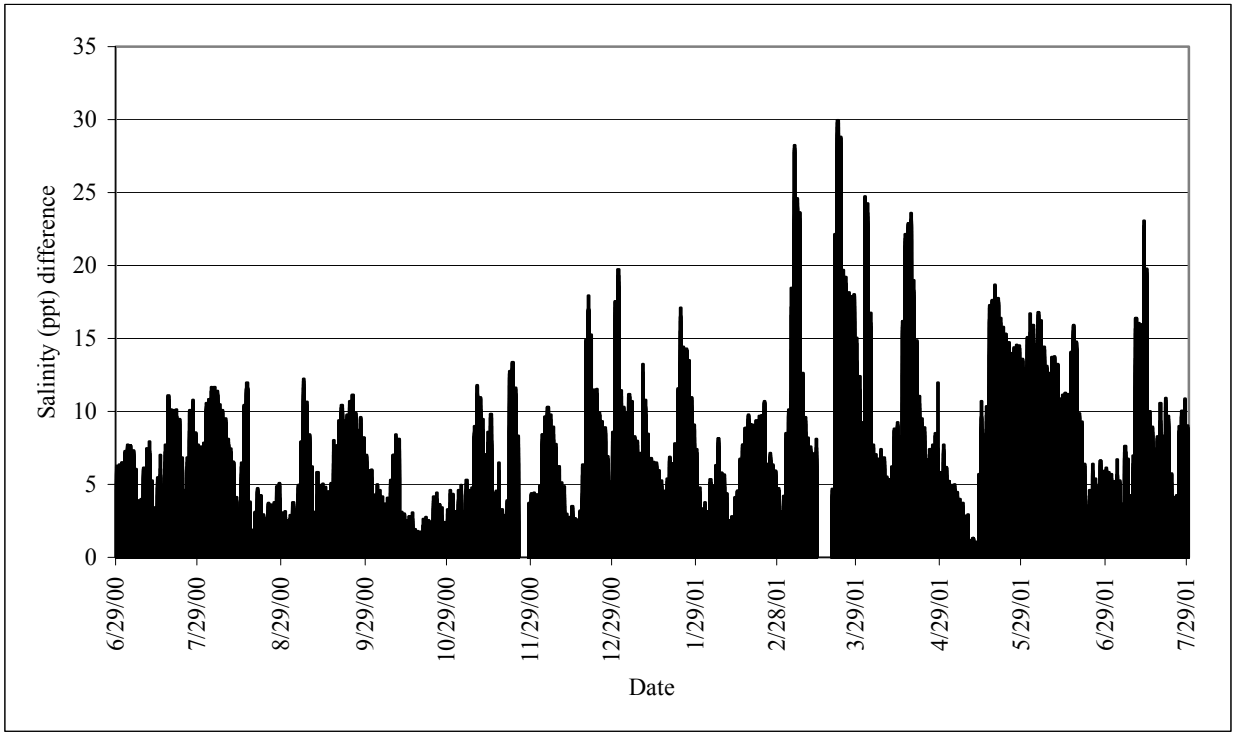

Figure 4. Salinity (ppt) difference between Gulf Jackson and Horseshoe Beach high density lease areas, 6/29/00 through 7/29/01.

Need figures 5 and 6 (Figures 1 and 2 from interim report.

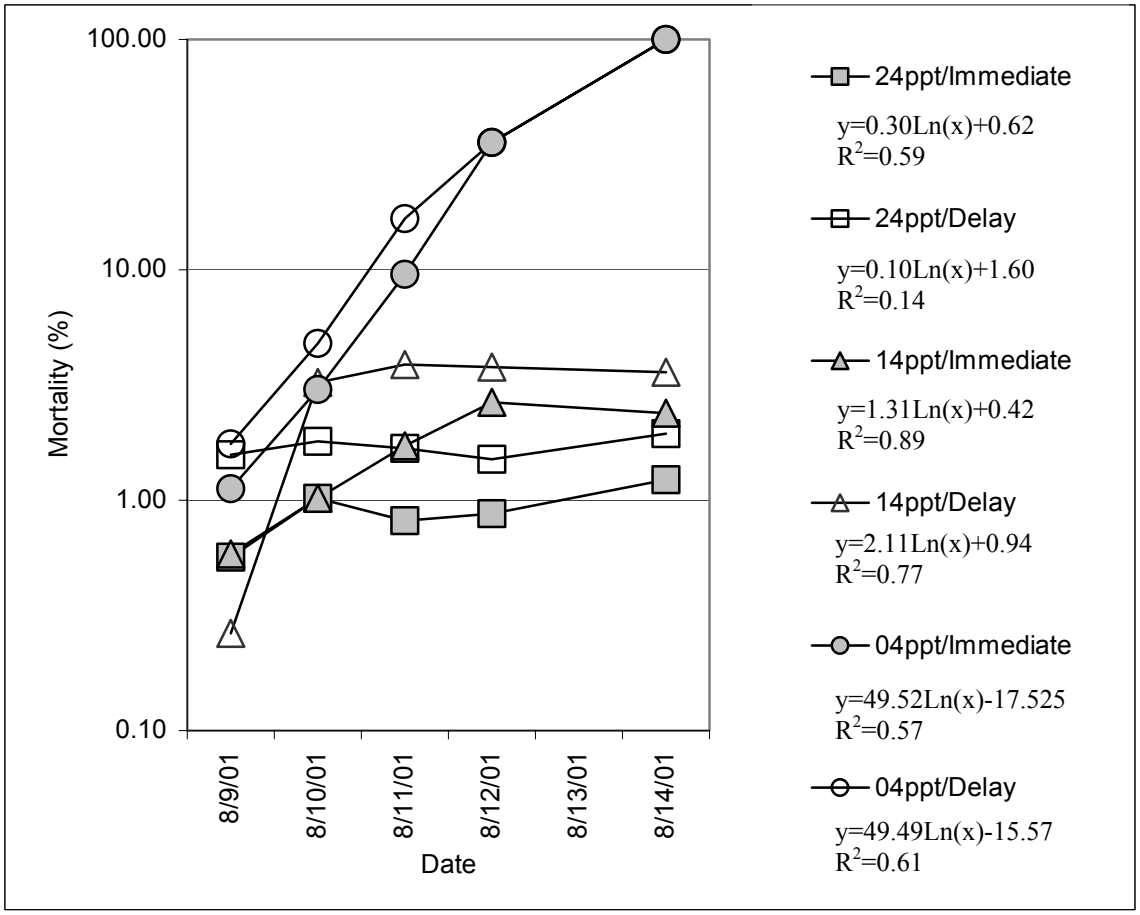

Figure 7. Mortality (%) of seed clams, *Mercenaria mercenaria*, over time following declines in salinity of 0 ppt (24 ppt), 10 ppt (14ppt), and 20 ppt (4ppt).

Appendix A.
Chi-square Analysis of Contingency Tables
Summary of Contingency Table Subdivision

Analysis of three-dimensional contingency tables:
r = immersion (immediate and delayed), c = salinity (24, 14, and 14 ppt), t = overall mortality
Test for mutual independence
H_O = Mortality, salinity, and time of immersion are mutually independent in the population tested.
H_A = Mortality, salinity, and time of immersion are not all mutually independent in the population tested.

Date	$\Sigma\chi^2$	$\chi^2_{0.05,8}$	Result
8/9/01	7.31	15.507	H_O
8/10/01	19.95	15.507	H_A
8/11/01	126.36	15.507	H_A
8/12/01	454.69	15.507	H_A

Overall mortality is independent of salinity and time of immersion only on 8/9/01.

Analysis of subdivided two-dimensional contingency tables:

Mortality vs. Salinity
H_O = Mortality, and salinity are independent.
H_A = Mortality and salinity are not independent.

For date = 8/10/01
r = salinity (24, 14, and 4 ppt), c = mortality
$\Sigma\chi^2 = 9.631$, $\chi^2_{0.05,2} = 5.991$, H_A
r = salinity (24, and 14 ppt), c = mortality
$\Sigma\chi^2 = 1.16$, $\chi^2_{0.05,1} = 3.841$, H_O
Mortality is not independent of salinity at the 24, 14 and 4 ppt level, combined.
Mortality is independent of salinity at the 24 and 14 ppt levels, combined.
∴Mortality is not independent of salinity at the 4 ppt level.

For date = 8/11/01
r = salinity (24, 14, and 4 ppt), c = mortality
$\Sigma\chi^2 = 109.3$, $\chi^2_{0.05,2} = 5.991$, H_A
r = salinity (24, and 14 ppt), c = mortality
$\Sigma\chi^2 = 2.179$ (with Yates correction), $\chi^2_{0.05,1} = 3.841$, H_O
Mortality is not independent of salinity at the 24, 14 and 4 ppt level, combined.
Mortality is independent of salinity at the 24 and 14 ppt levels, combined.
∴Mortality is not independent of salinity at the 4 ppt level.

For date = 8/12/01
r = salinity (24, 14, and 4 ppt), c = mortality
$\Sigma\chi^2 = 112.2$, $\chi^2_{0.05,2} = 5.991$, H_A
r = salinity (24, and 14 ppt), c = mortality
$\Sigma\chi^2 = 0.12$, $\chi^2_{0.05,1} = 3.841$, H_O
Mortality is not independent of salinity at the 24, 14 and 4 ppt level, combined.
Mortality is independent of salinity at the 24 and 14 ppt levels, combined.
∴Mortality is not independent of salinity at the 4 ppt level.

For date = 8/14/01
r = salinity (24, 14, and 4 ppt), c = mortality
$\Sigma\chi^2 = 674.8$, $\chi^2_{0.05,2} = 5.991$, H_A
r = salinity (24, and 14 ppt), c = mortality

$\Sigma\chi^2 = 1.842$, $\chi^2_{0.05,1} = 3.841$, H_O

Mortality is not independent of salinity at the 24, 14 and 4 ppt level, combined.
Mortality is independent of salinity at the 24 and 14 ppt levels, combined.
∴Mortality is not independent of salinity at the 4 ppt level.

Mortality vs. Immersion
H_O = Mortality and time of immersion are independent.
H_A = Mortality and time of immersion are not independent.

For date = 8/10/01
r = immersion (immediate and delayed), c = mortality
$\Sigma\chi^2 = 2.794$, $\chi^2_{0.05,1} = 3.841$, H_O
*Mortality is independent of time of immersion***.**

For date = 8/11/01
r = immersion (immediate and delayed), c = mortality
$\Sigma\chi^2 = 11.07$, $\chi^2_{0.05,1} = 3.841$, H_A
r = immersion (immediate and delayed) for 24 and 14 ppt salinity levels only , c = mortality for 24 and 14 ppt salinity levels only.
$\Sigma\chi^2 = 3.838$, $\chi^2_{0.05,1} = 3.841$, H_O
Mortality is not independent of time of immersion for the 24, 14 and 4 ppt salinity levels, combined.
Mortality is independent of time of immersion for the 24 and 14 ppt salinity levels, combined.
∴Mortality is not independent of immersion at the 4 ppt salinity level.

For date = 8/12/01
r = immersion (immediate and delayed), c = mortality
$\Sigma\chi^2 = 2.794$, $\chi^2_{0.05,1} = 3.841$, H_O
Mortality is independent of time of immersion.

For date = 8/14/01
r = immersion (immediate and delayed), c = mortality
$\Sigma\chi^2 = 2.794$, $\chi^2_{0.05,1} = 3.841$, H_O
Mortality is independent of time of immersion.