MARTIN COUNTY OYSTER REEF RESTORATION PROJECT:

A COMPILATION AND SUMMARY OF FOUR SHORT-TERM BIOLOGICAL MONITORING STUDIES

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Oyster reefs are vital to the health of an estuary because they effectively stabilize shorelines, provide habitat and food for numerous species, and improve water quality by filtering organic matter and fine sediments. In Florida, oyster reefs are found in many estuarine and nearshore waters, including the St. Lucie and Loxahatchee River estuaries. Those waters, and other coastal waters in southeast Florida, have experienced altered patterns of water delivery and quality as a result of water management practices that are intrinsically tied to flood control to maintain water flow through Lake Okeechobee and the Everglades. In particular, the redirection of freshwater out of inland basins and into coastal waters has altered both the timing and range of salinity variation in many Florida estuaries. Large releases of freshwater laden with sediment and nutrients lead to muck accumulation and low salinities in the St. Lucie Estuary, while in the Loxahatchee, reduced freshwater inflows and the permanent opening of the inlet have allowed oceanic waters to encroach into the estuary, raising salinity. As a result, oyster populations have significantly declined in both estuaries over the past 50 years.

The Martin County Oyster Reef Restoration Project was implemented as a step towards restoring oyster populations to historic levels in both estuaries, a goal of the Comprehensive Everglades Restoration Plan (CERP). The project, managed by CSA International, Inc. (CSA) in Stuart, Florida, was completed in three phases, the first phase involved surveying each estuary to identify historic oyster reef locations and locate suitable substrate for oyster restoration sites via bathymetric and side scan sonar surveys. The Restoration Phase involved placement of over 40 million pounds of relict oyster shell, limestone rubble, and concrete at several sites in the St. Lucie Estuary and in the Northwest Fork of the Loxahatchee River.

Reef construction was conducted from August 2009 through July 2010. Barges navigated by GPS and heavy equipment were used to create 27.88 acres of oyster habitat in 110 working days. The Monitoring Phase consisted of post-construction surveys and several biological monitoring studies, which are discussed below. Post-construction surveys were conducted by CSA to document the location and areal extent of the constructed reefs. CSA also managed the biological studies conducted by scientists from the Florida Fish and Wildlife Research Institute (FWRI) of the Florida Fish and Wildlife Conservation Commission; Florida Oceanographic Society (FOS); Estuarine, Coastal and Ocean Science, Inc. (ECOS); Florida Atlantic University (FAU); and Florida International University (FIU).

Short-term Biological Monitoring Studies

Biological monitoring studies comprised three general concepts: assessment of (1) oysters and their biology, (2) the communities that rely on oyster reefs as habitat, and (3) the physical structure of the constructed reefs. Both FWRI and FAU had a component of their study devoted to monitoring the abundance of oysters on the restored reefs. FWRI incorporated experimental controls by monitoring abundance on natural reefs in the vicinity of restoration reefs in both the St. Lucie and Loxahatchee estuaries. The study by FWRI also measured reproductive development and recruitment of oysters to natural and restoration reefs and assessed the prevalence and intensity of the oyster disease *Perkinsus marinus* (dermo). FAU included an analysis of oyster densities relative to present and historic water quality data from the South Florida Water Management District database DBHYDRO. The community of organisms reliant on oyster reefs was monitored by FIU, FAU, and FOS/ECOS. FIU expanded an existing monitoring program in order to study the development of the faunal community in Loxahatchee restoration plots over time, and to compare those to communities at nearby natural reefs. Those studies were completed by enumerating organisms captured in benthic tray traps. FIU also incorporated a research component by comparing communities from high- and low-relief restoration reefs. FAU began community analyses by initiating a pilot study that determined the rate of colonization and enumerated

the macroinvertebrates, both motile and sessile, colonizing receptacles planted on bare sand. After the reefs were constructed, FAU sampled replicate patches on the restored reefs by inserting 30-cm diameter cores to a depth of 10 cm. FOS/ECOS used an entirely different methodology to study the reef community on both restored and natural reefs. They relied on acoustic signals produced by organisms in and around the reef to document changes in the abundance of biota on the developing restored reefs, allowing for comparison with nearby natural reefs. Finally, FAU determined the settlement, sedimentation, and accretion of new reef material by measuring changes in the height of the constructed reef over time.

Oyster Density and Biology

Results of the FWRI and FAU studies showed that live oysters settled and thrived at most restoration sites. FAU found that on some restoration reefs there was rapid initial colonization by oysters, which is indicative of high larval recruitment rates. Although there was not a linear progression in density at any one site, there was an overall increase in estuary-wide oyster abundance. Timing of reef deployment appeared to affect the rate of oyster recruitment. FWRI found that oysters settled quickly on sites that were constructed in early fall 2009 when larval oysters were abundant but did not appear until many months later at sites constructed in late fall and winter when larval oysters were scarce.

At three of the five restoration sites monitored by FWRI, two in the St. Lucie Estuary and one in the Loxahatchee River, oysters exhibited characteristics similar to those of oysters at nearby natural reefs: oysters were present at densities at or near natural reef densities, oysters were reproductively active, and disease and mortality rates were comparable to natural levels. For example, densities at the restoration site in the North Fork of the St. Lucie Estuary were stable and consistent throughout the study with approximately 100 organisms/m^2 measured during each bi-annual survey. Densities at the adjacent natural reef station were slightly less than 100/m^2 and exceeded those at the restoration station only in fall 2011 when densities increased to a mean of 158/m^2. At one middle estuary location, natural reef densities were more than double (1,573/m^2) those at the proximal restored reef (567/m^2), however, those densities also were some of the highest measured in the estuary in fall 2011. At the Loxahatchee restoration site, mean live density in fall 2011 was approximately 300/m^2, while that of the proximal natural reef was 483/m^2. At one of the less successful sites, oysters quickly colonized the reef once substrate was planted and grew at comparable rates, but experienced significant mortality during the winter of 2010/11 as a result of sedimentation and burial. The last site, located in the lower estuary in a narrow bottleneck with high current flow, had a low but relatively stable density of oysters (31/m^2 in fall 2011) that grew at normal rates to sizes of approximately 40-mm shell height, at which point most either stopped growing or died. Both shell and larger limestone rubble were planted as restoration substrate at this site, but most of the smaller pieces were either buried or washed away leaving only the upper surfaces of the larger rubble available for settlement and limiting success.

The varied success witnessed at each restoration site underlines the importance of the timing and placement of substrate for oyster reef construction in order to achieve maximum restoration goals. FAU's data analysis linking oyster densities at different sites to water quality via structural equation modeling showed that increasing variability in salinity and chlorophyll *a* had a moderate negative impact on oysters despite the drought and thus low canal flow conditions experienced during the sampling period. Other water quality parameters (e.g., turbidity, total suspended solids, pH, etc.) did not significantly affect oyster densities.

Oyster Reef Community Analyses

One of the most frequently cited benefits of oyster reefs is that they produce essential fisheries habitat and harbor abundant faunal communities. Those fauna serve as prey for higher trophic levels, including

important commercially and recreationally fished species. The reefs can also serve as refuges for early life stages of those same species. All three community assessment studies concluded that development of the community at the restoration reefs had not yet stabilized and that the community structure at restoration reefs may converge with the community structure at nearby natural reefs at some point in the future.

The FIU study found that abundance and biomass of oyster-associated fauna rapidly increased at the Loxahatchee restoration site after reef construction was completed and that after one year, biomass and abundance of taxa colonizing the restored reef approached or exceeded that of nearby natural reefs. Prior to reef construction, biomass at the site was only 8% to 15% of that found at the nearest natural reef site, but by May 2011, biomass was near the 4-year average from the nearest natural reef site at approximately 100 g/m^2. After reef construction was completed, abundance quickly reached and often exceeded values recorded at the natural reef site (approximately $500/m^2$ vs. $350/m^2$ in May 2011) most likely due to the large number of tiny recruiting organisms. A total of 11 fish taxa and 19 invertebrate taxa was collected by FIU from the restoration site. Nine of those taxa, which included the economically important stone crab, were found only at the restoration site. FIU found that community composition at the restored reef became more similar to natural reef communities over time, but still differed significantly by the end of the study. The most abundant organisms by weight on the natural reefs were depressed mud crabs, black-fingered mud crabs, and crested gobies. Initially, small xanthid crabs and depressed mud crabs were abundant on the restored reef, but by the end of the study black-fingered mud crabs, snapping shrimp, and swimming crabs were dominant.

Analyses by FAU indicated that restoration reef communities in the St. Lucie Estuary were initially dominated by barnacles, but by the conclusion of the study polychaetes, gastropods, and amphipods were all abundant. Total invertebrate densities exceeded $10,000/m^2$ in 2010 and reached more than $14,000/m^2$ in 2011. Invertebrate densities were positively influenced by not only the presence of cultch, but also by the presence of live oysters. Community composition in the St. Lucie Estuary differed among restoration sites and often within sites, suggesting both spatial and temporal patchiness in community composition. Although there were differences between FAU and FIU's sampling methods (e.g., mesh size of screening devices used to separate fine sediment from shell matrix and associated organisms), similar overall patterns were observed despite very different absolute abundances. FAU postulated that further community development and stability will likely be impacted in wetter years when high freshwater inputs to the system lead to low salinity disturbances.

Finally, the FOS/ECOS study used very different metrics based on passive acoustics to monitor development of the faunal community composition on restored reefs. They showed that the restored reefs had an initial increase in species richness above that of the neighboring natural reefs. The acoustic signal produced by the fauna at a mid-estuary restoration site was most similar to its neighboring natural reef. Upstream sites had lower signal strength (less diversity) and fewer snaps (lower abundance) at all times. Sites lower in the estuary had initial increases followed by subsequent reductions, resulting from periods of reduced salinity in the estuary.

The general conclusion was that the communities on restored reefs were approaching, but not yet identical, to natural reefs. Succession in the community as well as physical changes in the structure of the reef, which may result in maturation of niche breadth on the reef, likely both contributed.

Reef Structure

FAU used a portable leveling device called a sediment elevation table (SET) to provide a constant reference point for comparison of repeated measures of sediment elevation at the restoration sites in both estuaries. Results showed that there was a net loss of elevation (10.5 cm) in the St. Lucie Estuary during

the first 9 months of the study, which was due to a combination of subsidence and compaction of reef substrate. During the second year of the study, reef elevation continued to decline, but at a much slower rate. Finally, there was an elevation gain over the last 8 months of the study as a result of the colonization rate of reef-forming species such as oysters and mussels, exceeding the subsidence + sedimentation rate. The data indicated that the gain in vertical relief accumulated at a rate of approximately 3 mm/mo. A small net gain in elevation also was measured at the Loxahatchee restoration site.

FIU tested habitat structural complexity at the Loxahatchee restoration site by creating three parallel high-relief ridges within the reef matrix. Each ridge was 15 cm higher than the rest of the constructed reef. Comparisons of abundance and biomass indicated that both increased at much faster rates at the high-relief sites than at adjacent low-relief sites. In addition, the high-relief sites had greater biomass than anywhere else in the river (388 g/m^2 at the high relief site vs. a maximum of 175 g/m^2 at a natural reef site), only eight months after construction. FIU attributed the success of the high-relief sites to increased current flow and decreased sedimentation rates. The FIU and FAU studies both stressed the importance of incorporating structural complexity into the construction of restoration reefs and illustrate how reef structure influences community densities and compositions.

Summary

Salinity is one of the driving forces behind changes in oyster survival, abundance, and health in the St. Lucie and Loxahatchee estuaries. Although oysters in each estuary were subjected to large variations in salinity, the timing of the restoration project was auspicious in that it occurred during three relatively innocuous years. Just prior to the start of the restoration project, a combination of storm activity and water releases into the St. Lucie Estuary caused salinities to drop rapidly resulting in a widespread oyster die-off. Natural oyster beds were recovering in 2009 when construction of the restoration reefs began and by the fall, larval oysters were once again present and available for colonization of the newly constructed reefs. With the exception of a prolonged low-volume freshwater release into the estuary during the 2010 wet season, salinity levels were within tolerable limits, and often were near the optimal range for the duration of the study.

The constraints of the duration of funding provided by the American Recovery and Reinvestment Act of 2009 precluded long-term monitoring of the restored oyster reefs. Based on observations from preexisting long-term projects such as CERP-funded oyster monitoring, periods of reduced salinity in the St. Lucie Estuary and possibly in the Loxahatchee River can be expected. In each of the four studies described, there is evidence that while the status of oyster populations and communities on the restoration reefs is approaching those of the nearby natural reefs, the severity and duration of future low-salinity events will ultimately determine whether the restoration reefs can persist long term. Each study clearly demonstrates that in the short term, placement of settlement substrate is a viable option to increase the total acreage of oyster reef in targeted Florida estuaries, and should be retained as a tool for future restoration efforts.

The following appendices include the final reports submitted by scientists at FWRI, FOS/ECOS, FAU, and FIU detailing their short-term monitoring projects summarized in this Introduction.

APPENDICES

APPENDIX A

Martin County Oyster Reef Restoration Project Final Report
Florida Fish and Wildlife Research Institute

Martin County Oyster Reef Restoration Project

Florida Fish and Wildlife Research Institute

Final Report

30 November 2011

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EXECUTIVE SUMMARY

In June 2009, the National Oceanic and Atmospheric Administration (NOAA) awarded Martin County, Florida more than $4 million to construct 24.15 acres of reef, 20.7 acres in the St. Lucie Estuary (SLE) and 3.45 acres in the Loxahatchee River (LOX). The main objective of the Florida Fish and Wildlife Research Institute (FWRI) project was to monitor background oyster populations in concert with oysters populating restored reefs in each estuary. Scientists from the FWRI monitored settled oyster density, reproductive development, physiological condition, juvenile recruitment, and prevalence and intensity of the oyster disease *Perkinsus marinus* (dermo) at two background stations and four restoration stations in the SLE, and at one restoration station in the LOX. Water quality parameters were also monitored at each station. The same parameters were concurrently monitored at an additional nine natural reef stations (three in the North Fork, three in the South Fork, and three in the middle estuary) in the SLE and six natural reef stations (three in the Northwest Fork and three in the Southwest Fork) in the LOX as part of a separately funded component of the Comprehensive Everglades Restoration Plan (CERP). Those stations, along with the two natural reef stations funded by this project, served as reference populations against which to compare the success of the restored reefs.

Live oysters were present on each of the five restored reefs within 10 months of reef construction. Oysters at three of the five restoration stations (SL-R1, SL-R4, and LX-R1) exhibited characteristics similar to those of oysters at nearby natural reef stations and will likely persist. Each of those stations had oysters present at densities at or near background station densities, oysters that were reproductively active, and disease and mortality rates comparable to natural levels. At SL-R2, oysters colonized the reef quickly once substrate was planted and grew at comparable rates, but experienced significant mortality during the winter of 2010/11 and only a limited rebound during the 2011 recruitment season. The most likely factor driving the mortality at this site was sedimentation and burial. At SL-R3, there was a low density but relatively stable population of oysters that grew at normal rates to sizes of approximately 40 mm, at which point most either stopped growing or died. This station was located in a narrow bottleneck within the lower estuary that experienced very high current flow. Both shell and larger limestone rubble were planted at this station, but most of the smaller pieces were either buried and/or washed away leaving only the upper surfaces of the larger rubble available for settlement and limiting success.

The timing and placement of substrate for oyster reef construction are key factors to consider in achieving maximum restoration goals. Reef construction in the SLE and LOX was completed over a period when both estuaries had tolerable salinities and water quality as well as populations of existing oysters able to provide a sufficient supply of larvae for reef colonization. Most restoration patches were planted early enough in the year to coincide with recruitment season. However, some patches were planted in late fall or early winter when larval oysters were scarce. Many patches were planted in optimal locations, but some sites had less success due to sedimentation and dynamic local physical properties. Future efforts to optimize placement of restoration substrate will benefit from informed timing as well as from a better understanding of small-scale physical processes such as sedimentation, burial, wave energy, and localized variation in salinity at potential restoration and natural reef sites.

INTRODUCTION

Oyster reefs are vital to the health of an estuary because they effectively stabilize shorelines, provide habitat for numerous species, and improve water quality by filtering out nutrients and fine sediments. In Florida, oysters occur in nearly all estuarine and nearshore waters such as the St. Lucie and Loxahatchee River estuaries. Those waters, and other coastal waters in southeast Florida, have experienced altered patterns of water delivery and quality as a result of water management practices related to Lake Okeechobee and the Everglades. In particular, the redirection of freshwater out of inland basins and into the coastal waters mentioned above has altered both the timing and range of salinity variation in those waters. Large releases of freshwater laden with sediment and nutrients lead to muck accumulation and low salinities in the St. Lucie Estuary, while in the Loxahatchee, reduced freshwater inflows and the permanent opening of the inlet have allowed oceanic waters to encroach into the estuary. As a result, oyster populations in both estuaries have significantly declined over the past 50 years.

In June 2009, the National Oceanic and Atmospheric Administration (NOAA) awarded Martin County, Florida, more than $4 million for an oyster reef habitat restoration project funded by the American Recovery and Reinvestment Act (ARRA). That project, managed by CSA International, Inc. (CSA) of Stuart, Florida, used more than 40 million pounds of cultch to construct more than 27 acres of reef within the St. Lucie and Loxahatchee River estuaries providing not only social and economic benefit to the community, but also long-term ecological improvements. A barge navigated by a GPS supported placement of relict oyster shell and small limestone rock within the estuaries.

As one measure of success of the restoration effort, the Molluscan Fisheries research group at the Florida Fish and Wildlife Research Institute (FWRI) monitored several oyster biological parameters at two natural reef sites in the St. Lucie Estuary (SLE), at four restoration sites in the SLE, and at one restoration site in the Loxahatchee River (LOX). Those measures included monitoring changes in oyster distribution and abundance, reproductive development, physiological condition, juvenile recruitment, and prevalence and intensity of the oyster disease *Perkinsus marinus* (dermo). FWRI biologists also concurrently monitored the same parameters at an additional nine natural reef stations (three in the North Fork, three in the South Fork, and three in the middle estuary) in the SLE and six natural reef stations (three in the Northwest Fork and three in the Southwest Fork) in the LOX as part of a separately funded component of the Comprehensive Everglades Restoration Plan (CERP). Those stations, along with the two natural reef sites funded by this project, served as reference populations against which to compare the success of the restored reefs.

METHODS

Study Sites

In late August 2009, a scouting survey was conducted in the lower and central portions of the St. Lucie Estuary (SLE) to locate existing oyster reefs for background monitoring. One existing reef, located in the lower estuary within Willoughby Creek, was chosen as a monitoring station (SL-LE2). Although the project objectives called for two background monitoring stations in the lower estuary, another existing oyster reef not already being monitored by another organization could not be found in that section of the SLE. Instead, a reef on the south side of the middle estuary was chosen as a second background monitoring station (SL-LE1). Monitoring at those two stations was initiated in August 2009.

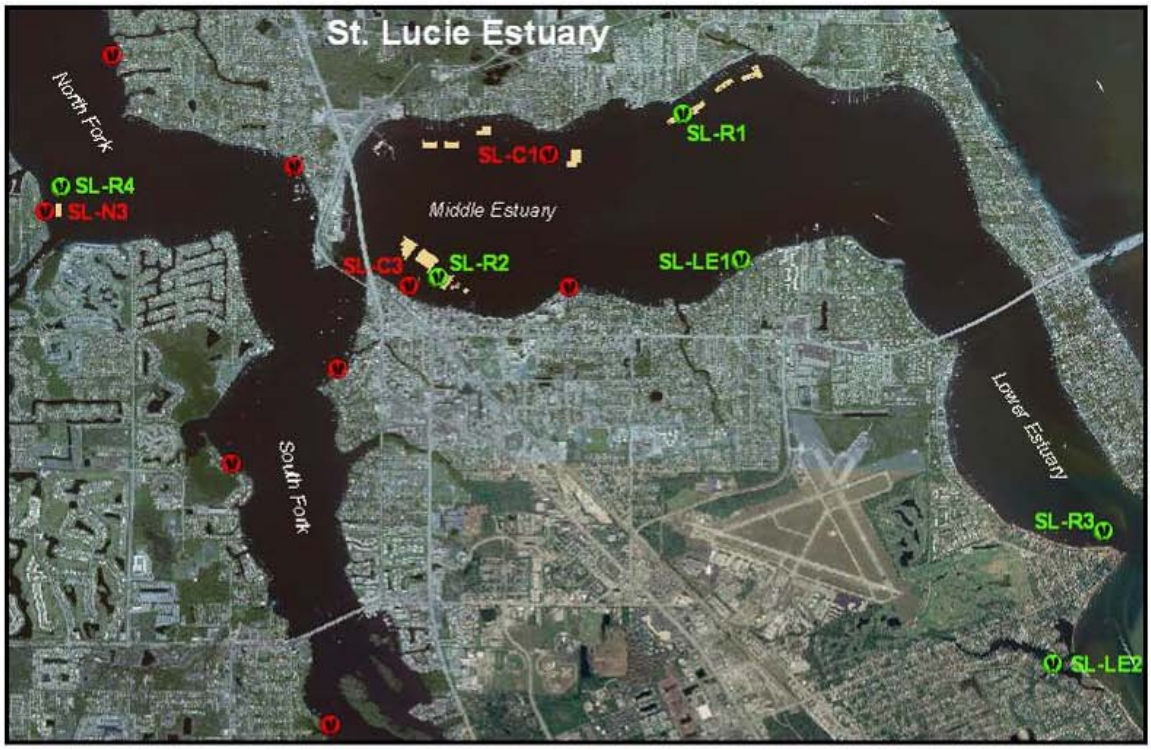
Monitoring at restoration stations was initiated as construction was completed at each site. In November 2009, restoration Stations 1 and 2 were deployed on the north and south side of the SLE central estuary, respectively. Restoration monitoring included only those two stations until February 2010 when SLE stations 3 and 4 were deployed. Station 3 was located at a restoration site in the lower estuary near Hell's Gate, while station 4 was located in the North Fork near the golf course. In July 2010, restoration construction was completed in the Loxahatchee River (LOX) and subsequently monitoring was initiated at the Loxahatchee restoration station in August 2010.

Within the SLE, the background monitoring stations and the restoration stations were identified as separate sites; St. Lucie-Lower Estuary and St. Lucie-Restoration. The restoration station in the LOX was identified as Loxahatchee-Restoration Station 1 (LX-R1). For some statistical analyses, comparisons were made between restoration stations and adjacent natural reef stations monitored as part of the Comprehensive Everglades Restoration Plan (CERP). All station locations are shown in **Figure A-1**, and coordinates for ARRA stations are provided in **Table A-1**.

Table A-1. Geographic coordinates of ARRA oyster monitoring stations in the St. Lucie Estuary and Loxahatchee River.

Site	Habitat Type	Station	Latitude (N)	Longitude (W)
St. Lucie Lower Estuary (SL-LE)	Natural Reef	1	27˚13.232'	80˚16.737'
St. Lucie Lower Estuary (SL-LE)	Natural Reef	2	27˚12.686'	80˚15.846'
St. Lucie Restoration (SL-R)	Restoration Reef	1	27˚12.743'	80˚14.599'
St. Lucie Restoration (SL-R)	Restoration Reef	2	27˚12.087'	80˚14.493'
St. Lucie Restoration (SL-R)	Restoration Reef	3	27˚12.096'	80˚15.282'
St. Lucie Restoration (SL-R)	Restoration Reef	4	27˚11.691'	80˚15.636'
Loxahatchee Restoration (LX-R)	Restoration Reef	1	26˚58.182'	80˚07.679'

a)



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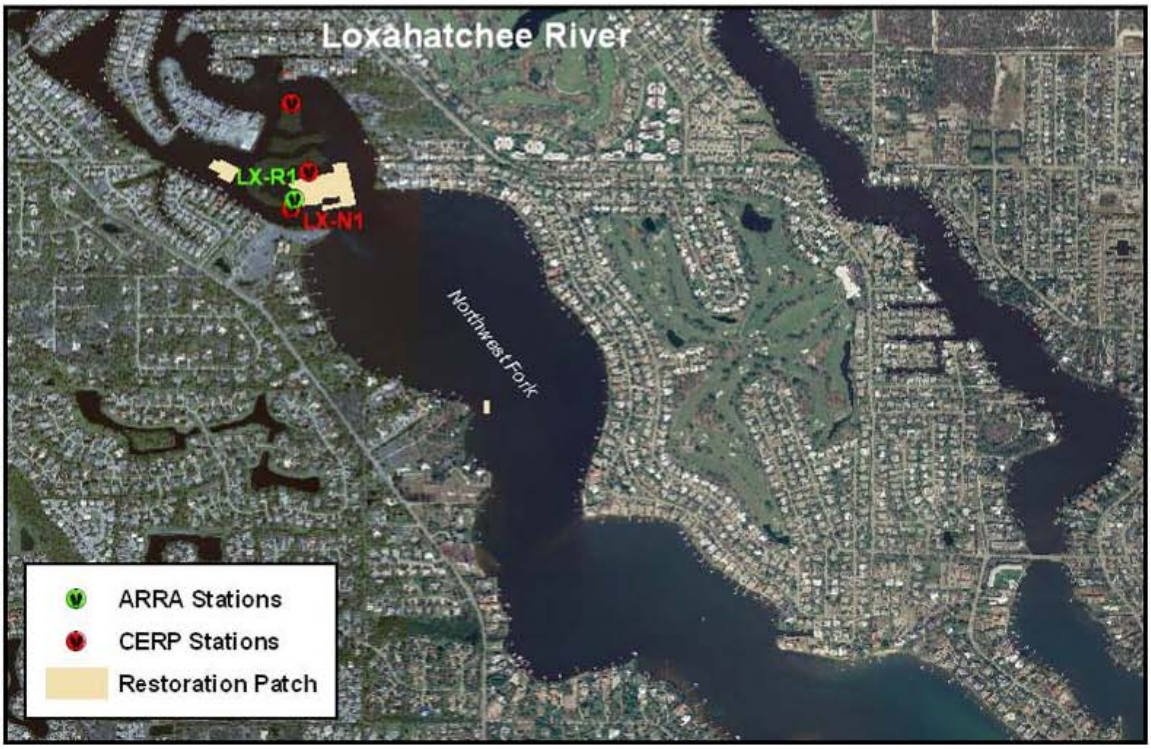

Figure A-1. Oyster monitoring stations in the St. Lucie Estuary a) and the Loxahatchee River b). Tan patches indicate reef construction areas in both estuaries. Green symbols indicate ARRA monitoring stations at two natural reef stations (SL-LE1 and SL-LE2) and five restoration stations (SL-R1 to -R4 and LX-R1). Red symbols indicate CERP-funded oyster monitoring stations.

Settled Oyster Abundance

Oyster density sampling was conducted biannually, in the spring and fall, at each station once monitoring was initiated. The first surveys were conducted in fall 2009 at the two SLE natural reef stations (SL-LE 1 and 2), in spring 2010 at the four restoration stations in the SLE, and in fall 2010 at the LOX restoration station. Fifteen replicate 0.25-m^2 quadrats were haphazardly deployed at each station. All oysters were then harvested from each quadrat to determine the number of live and dead oysters with articulated shells. A maximum of 10 live oyster shell heights (SH = maximum linear distance from the umbo to the ventral shell margin) were also measured from each quadrat. Mean densities of live and dead oysters as well as mean live oyster SHs were calculated and plotted for each station. Statistical comparisons between surveys and stations and between restoration and adjacent natural reef stations were performed using the GLIMMIX procedure for mixed models in the SAS 9.2 software package (Littell et al., 2006).

Condition, Disease, and Reproductive Monitoring

Oysters were collected for analysis of physiological condition, gonadal development state, and prevalence and intensity of the oyster disease *Perkinsus marinus* (dermo) on a monthly basis whenever present. Oyster samples from each station were transported, live and chilled, to the FWRI laboratory for processing. Each individual oyster was measured (SH [mm]), shucked, and the tissues processed for reproductive stage, disease status and physiological condition, when possible, according to the methods described below.

For condition analysis, oysters collected from each station were processed by thoroughly cleaning each individual, measuring the shell height, weighing the whole animal, and then shucking each oyster to obtain the tissue wet weight. The shells and tissues were dried for 48 hours and then the shell and tissue dry weights were recorded. At the two natural reef stations in the SLE, oysters were collected for condition index analyses each month from October 2009–October 2011. At the SLE restoration stations, oysters were not large enough to be sampled for condition index from Stations 1 and 2 until March 2010 and from Station 4 until May 2010. Oysters were not collected from SLE restoration station 3 for any analyses due to the scarcity of oysters and the difficulty in harvesting from that location. Oysters were large enough for condition index analyses at the LOX restoration station in November 2010. Condition index was calculated as the ratio of tissue dry weight to shell dry weight. Mean condition index and SH were calculated and plotted for each station. Statistical comparisons of condition index among stations and between restoration and adjacent natural reef stations were performed using the GLIMMIX procedure for mixed models in the SAS 9.2 software package (Littell et al., 2006).

For *Perkinsus marinus* (dermo) disease analysis, prevalence and intensity were diagnosed with Ray's fluid thioglycollate method (RFTM) as described by Bushek et al. (1994). Small (1 cm^2) pieces of gill and mantle tissue were incubated in RFTM media with antibiotics for 7 days in the dark at 25 ^oC. Tissue pieces were then placed onto glass microscope slides, macerated with razor blades, stained with Lugol's, and examined at 400× for the presence of hypnospores. Parasite density (infection intensity) was ranked using the Mackin scale, which ranges from 0 to 5 (**Table A-2**). At the two natural reef stations in SLE, oysters were collected for dermo analyses each month from October 2009–October 2011. At the restoration sites in SLE, oysters were initially collected for dermo analyses from station 1 in November 2009, Station 2 in December 2009, and Station 4 in February 2010. In LOX, dermo analyses began in October 2010 at the restoration station. Average parasite densities were calculated for each individual sample and from those values mean dermo infection intensity and prevalence were calculated for each station. Statistical comparisons of dermo infection intensity among stations and between restoration and adjacent natural reef stations were performed using the GLIMMIX procedure for mixed models in the SAS 9.2 software package (Littell et al., 2006).

Table A-2. Mackin scale showing different stages of *Perkinsus marinus* (dermo) infection intensity.

Stage	Category	Cell Number	Notes
0	Uninfected	No cells detected	--
0.5	Very light	<10 cells in entire preparation	--
1	Light	11-100 cells in entire preparation	Cells scattered or in localized clusters of 10-15 cells
2	Light-moderate	--	Cells distributed in local concentrations of 24-50 cells; or uniformly distributed so that 2-3 cells occur in each field at 100X
3	Moderate	3 cells in all fields at 100X	Masses of 50 cells may occur
4	Moderate heavy	Cells present in high numbers in all tissues	Less than half of tissue appears blue-black macroscopically
5	Heavy	Cells in enormous numbers	Most tissue appears blue-black macroscopically

For reproductive analysis, the tissues remaining from the dermo dissections were preserved in Dietrich's fixative solution (Barber, 1996). Following 20 hours of fixation, the oyster tissues were thoroughly rinsed in tap water and preserved in 70% ethanol for subsequent histological preparation. Histological preparation consisted of dehydrating each oyster in 95% ethanol for a minimum of three hours, then embedding the tissue in paraffin. Depending upon the size of the individual oyster, a minimum of one to a maximum of six 3.5-µm sections were cut from each embedded sample using a microtome mounted with a glass knife, maintaining a minimum separation of 60 µm (the approximate maximum diameter of an oocyte) between sections. The sections were stained with hematoxylin and eosin, and then mounted onto prelabeled glass slides for analysis. Resultant slides were examined at 200–400x on a compound microscope and each sample was assigned to a reproductive stage following the classification scheme (**Table A-3**) modified from the work of Fisher et al. (1996).

Table A-3. Qualitative reproductive staging criteria for oysters collected from Florida waters.

Value	Observations
0	Neuter or resting stage with no visible signs of gametes
1	Gametogenesis has begun with no mature gametes
2	First appearance of mature gametes to approximately one-third mature gametes in follicles
3	Follicles have approximately equal proportions of mature and developing gametes
4	Gametogenesis progressing, but follicles dominated by mature gametes
5	Follicles distended and filled with ripe gametes, limited gametogenesis, ova compacted into polygonal configurations, and sperm have visible tails
6	Active emission (spawning) occurring; general reduction in sperm density or morphological rounding of ova
7	Follicles one-half depleted of mature gametes
8	Gonadal area is reduced, follicles two-thirds depleted of mature gametes
9	Only residual gametes remain, some cytolysis evident
10	Gonads completely devoid of gametes, and cytolysis is ongoing

For graphical presentation, the 11 reproductive stages were simplified by combining them into four different categories. The indifferent category includes those oysters that have no visible gametes and are in the neuter or resting stage (stages 0 and 10). The developing category includes those oysters that are undergoing gametogenesis but show no evidence of recent spawning (stages 1–4). The ripe category includes those oysters that have follicles filled with ripe gametes and are nearly ready to spawn or have just begun to release gametes (stages 5 and 6). Finally, the spent/recycling category includes those oysters that have follicles containing both mature and immature gametes, and an apparent reduction in

gonadal area (stages 7–9). At the two natural reef stations in SLE, oysters were collected for reproductive analyses each month from October 2009–October 2011. At the restoration sites in SLE, oysters were initially collected for reproductive analyses from Stations 1 and 2 in March 2010 and Station 4 in May 2010. In LOX, reproductive analyses began in October 2010 at the restoration station.

Oysters from SL-R3 could not be collected for condition, disease, or reproductive analyses. Although there were live oysters present at that station, those oysters were attached and settled on large chunks of heavy substrate that were almost completely buried in the sand. That, coupled with the depth and fast moving currents at that station rendered our standard collection methods ineffective.

Spat Recruitment

Juvenile oyster recruitment was monitored by deploying and retrieving three replicate spat monitoring arrays at each station on a monthly schedule. Each array consisted of 12 axenic adult oyster shells (5-10 cm SH) strung onto two separate lengths of galvanized wire. The shells were oriented with their inner surface facing downward when suspended off the bottom. After a month long deployment, the shell strings were recovered and spat recruitment was estimated by discarding the top and bottom shells of each string, and counting the number of settled spat on the underside of the remaining strung shells. Spat monitoring was initiated at the SLE natural reef stations in late August 2009, at SLE restoration stations 1 and 2 in November 2009, Stations 3 and 4 in February 2010, and at the LOX restoration station in August 2010. Mean number of spat per shell per month were calculated and plotted for each station. Statistical comparisons of spatfall among stations and between restoration and adjacent natural reef stations were performed using the GLIMMIX procedure for mixed models in the SAS 9.2 software package (Littell et al., 2006).

Water Quality

Monthly water quality sampling was conducted in conjunction with field sampling at all stations. Recorded parameters included water depth, temperature, salinity, turbidity, pH, and dissolved oxygen concentration. Water depth was determined with a sounding line and turbidity was obtained using a standard Secchi disk. Turbidity is presented as a Secchi penetration value which is calculated as the percentage of the water column through which the Secchi disk could be seen. All other parameters were measured with a YSI instrument. Water quality monitoring was initiated at the SLE natural reef stations in late August 2009, at SLE restoration stations 1 and 2 in November 2009, stations 3 and 4 in February 2010, and at the LOX restoration station in August 2010. Graphical presentations show the values measured at each station each month.

RESULTS

Settled Oyster Abundance

An overall comparison of live oyster densities among stations revealed that abundances differed between natural and restoration stations as well as among the restoration stations. The highest live densities were measured at the two background stations (SL-LE1 and 2) and SL-R1 where overall mean live densities were near 300/m^2. Moderate live densities were recorded at SL-R2 and the LOX restoration station where overall means were 192 and 177/m^2, respectively. The two remaining SLE restoration stations had the lowest overall live densities with the mean for station 4 at 95/m^2 and station 3 at just 32/m^2. Dead densities were highest at the two background stations with approximately 50/m^2 at each (P<0.01). Dead densities at the five restoration stations were relatively low with overall means of less than 20/m^2.

Density patterns at each station varied among surveys (**Figure A-2**). Live densities at SLE background station 1 varied from survey to survey with the highest abundances, approximately 500/m^2, occurring in spring 2010 and fall 2011. In contrast, live densities at background station 2 were more consistent and ranged from 200 to 400/m^2 from fall 2009 through fall 2011 (P<0.05). Dead densities at both stations also varied by survey and ranged from approximately 15 to 85/m^2. Live densities at SL-R1 increased from spring to fall each year with spring abundances near 300/m^2 and fall abundances near 500/m^2 in 2010 and 2011. Live densities peaked at SLE restoration stations 2 and 3 in fall 2010 but decreased significantly in spring 2011 (P<0.01) falling approximately 79% at SL-R2 and 63% at SL-R3. Live densities at SL-R2 and 3 remained low as of the fall 2011 survey. At SL-R4, live densities reached a mean of approximately 100/m^2 in spring 2010 and remained consistent through fall 2011 (P>0.05). Mean live densities at the LOX restoration station increased significantly from 14/m^2 in fall 2010 to approximately 300/m^2 in both spring and fall 2011 (P<0.01). Dead oyster densities at the five restoration stations were generally low throughout the study with means of less than 25/m^2. Two exceptions occurred when dead densities increased significantly at SL-R3 in spring 2011 and at SL-R1 in fall 2011 (P<0.01).

Comparisons of live abundances at each restoration station with those at nearby natural reef sites show that although oysters are present at all of the restoration stations, they have not yet reached natural densities at most stations. The two most mature stations, SL-R1 and -R2, were compared with densities measured at two nearby CERP monitoring stations, SL-C1 and -C3. In an overall comparison, it was found that densities at the CERP stations were twice those at the adjacent restoration stations. However, densities at SL-C1 and SL-R1 are some of the highest in the estuary, with means of 1,573/m^2 and 567/m^2, respectively, in fall 2011. Because there were no monitored natural reefs nearby, comparisons for SL-R3 were made to the two background stations, SL-LE1 and -LE2, since one was located upstream and the other downstream of the restoration station. Densities at both background stations were generally an order of magnitude greater than densities recorded at the restoration station. For example, in fall 2011, mean density at SL-R3 was just 31/m^2 compared with densities of 541/m^2 at SL-LE1 and 272/m^2 at SL-LE2. Even in fall 2010 when mean live density at SL-R3 was slightly higher at 87/m^2, it still did not compare to natural densities. Densities at SL-R4 were also compared to densities measured at the nearby CERP monitoring station SL-N3. In contrast to the other restoration stations, densities at SL-R4 have reached and often exceeded those measured at the proximal natural reef station. Live densities remained stable and consistent at SL-R4, near 100/m^2, since the first survey was completed in spring 2010. Densities at nearby SL-N3 were slightly less than 100/m^2 and only exceeded those at SL-R4 in fall 2011 when densities increased to a mean of 158/m^2. In the LOX, densities at the restoration station were compared to the nearby CERP monitoring station LX-N1. In fall 2011, mean live density at LX-R1 was approximately 300/m^2 while that of the natural reef was 483/m^2.

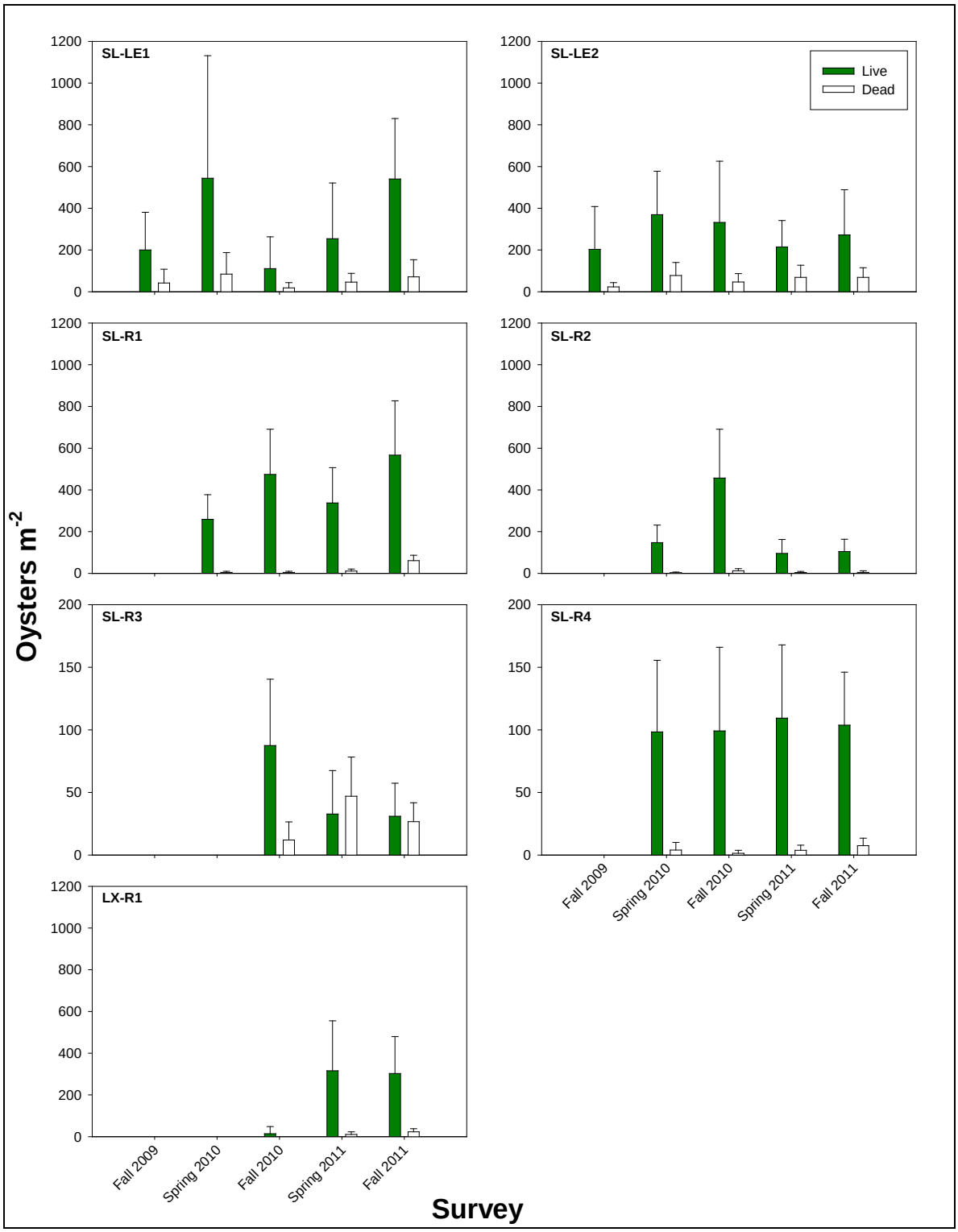

Figure A-2. Mean density (±SD) of live and dead oysters present at two natural reef stations (SL-LE1 and -LE2) and five restoration stations (SL-R1 to -R4 and LX-R1) during biannual surveys. SL-R stations 1–4 were not sampled until spring 2010; LX-R1 was not sampled until fall 2010. (Note differences in the y-axis range among stations.)

Dead oyster densities were substantially lower at the restoration stations than those at proximal natural reef stations. However, a comparison of live to dead ratios revealed that live ratios were very similar to ratios measured at most adjacent natural reef stations. Specifically, live percentages at SL-R1, SL-R2, and SL-R4 ranged from 90% to 99% while those at the corresponding natural reef stations ranged from 81% to 94% for the duration of the study. Live percentages were also high at LX-R1 with 93% live in fall 2011. However, live ratios at LX-N1 were consistently lower and decreased to 53% in fall 2011. The live ratio at SL-R3 (88%) was almost identical to those at the two background stations (86% and 88%) in fall 2010, but decreased to 41% in spring 2011 and remained low in fall 2011.

Oyster shell heights spanned a wide range at most natural reef and restoration stations (**Figure A-3**). Mean shell heights at the two SLE background stations fell between 40 and 65 mm with the exception of fall 2011, when shell heights at both stations significantly decreased to mean sizes of less than 40 mm ($P<0.01$). Shell heights at most of the restoration stations gradually increased as those stations, and the oysters settled there, became more mature. Shell heights at two restoration stations peaked in spring 2011 when mean sizes reached 60 mm at SL-R1 and 67 mm at SL-R4. Mean shell height at SL-R2 increased significantly from 33 mm in spring 2010 to 46 mm in fall 2010 and remained consistent throughout 2011 ($P<0.01$). Shell heights at SL-R3 were markedly smaller, with means that never exceeded 25 mm. Shell heights at the LOX restoration station increased significantly each subsequent survey reaching a mean size of 45 mm in fall 2011 ($P<0.01$), which was very similar to the mean shell height measured at the nearby natural reef station (LX-N1).

Disease Monitoring

Prior to September 2010, dermo infection was almost absent in oysters collected from most stations in the study (**Figure A-4**). Exceptions include detection in one oyster at SL-R2 in February 2010 and the frequent, but intermittent, presence of dermo at SL-LE2 since the study began in fall 2009. After becoming more prevalent in fall 2010, dermo infection was present in most collections at SL-LE1, SL-LE2, and SL-R1, but was present only sporadically at the remaining stations. Infection prevalence was high at both background stations in late 2011 with 100% of sampled oysters at SL-LE2 and 60% to 80% at SL-LE1 showing infection during the last 3 months of the study. Infection rates were more moderate at the SLE and LOX restoration sites, with 20% to 60% of the oysters infected. Despite the moderate to high prevalence of dermo, infection intensities were low with mean intensities only occasionally exceeding a Mackin score of 1, indicating that the sampled oysters were only lightly infected with the parasite (**Figure A-5**).

Physiological Condition and Reproductive Development

Analysis of gonadal tissues of oysters collected from each station indicated that in most months individual oysters were in various stages of reproductive development, including gametogenesis, active spawning, and gonadal recycling throughout the majority of each year (**Figure A-6**). However, developing oysters were most prevalent in the spring months, specifically March through June. Ripe and/or spawning oysters were most common in the summer months from May through July, and spent oysters were prevalent in September and October. The majority of the oysters collected during the winter months, specifically December through February, were in the resting or indifferent stage. There were no apparent differences in reproductive development between oysters collected from the background stations and those from the restoration stations.

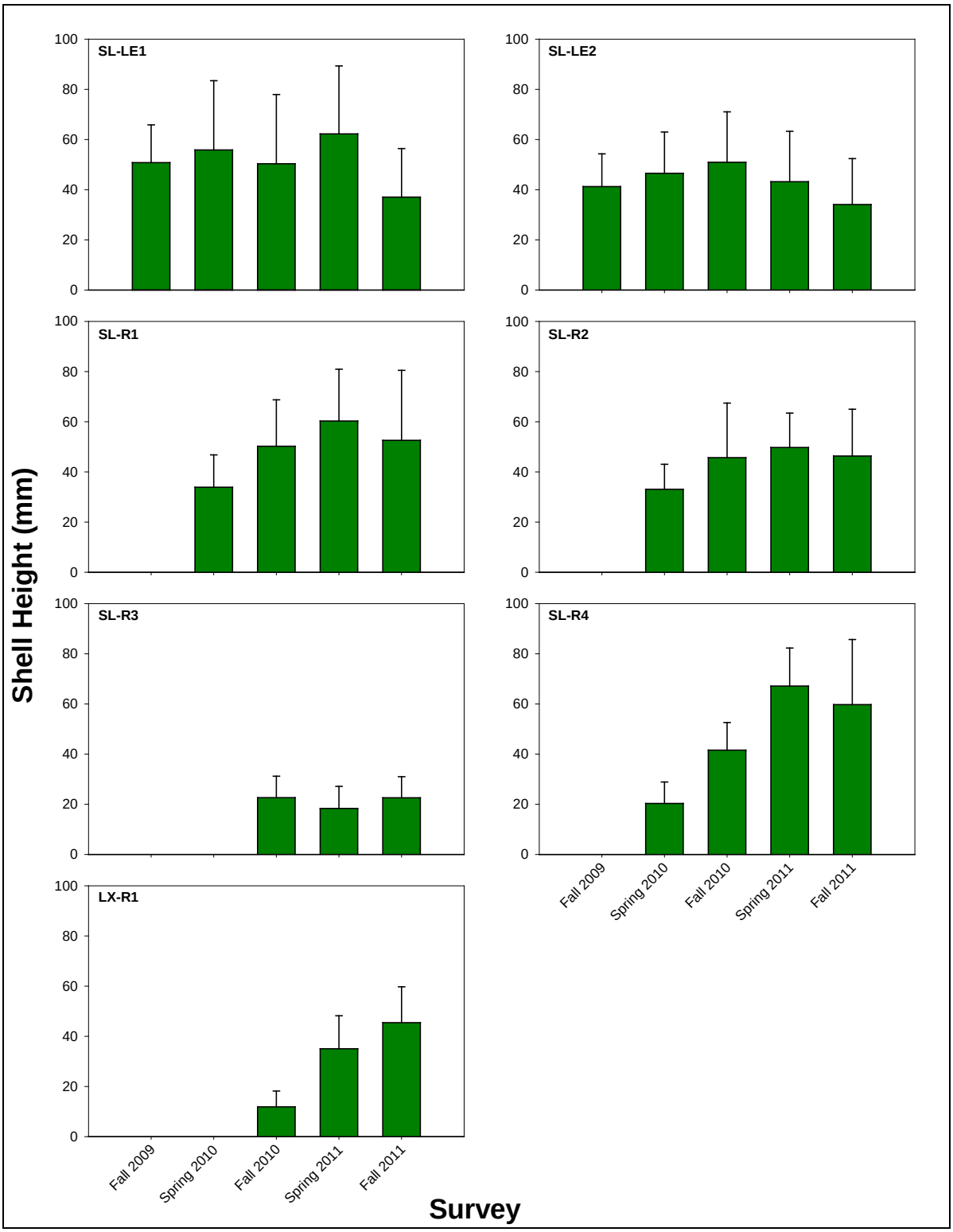

Figure A-3. Mean shell height (±SD) of live oysters present at two natural reef stations (SL-LE1 and SL-LE2) and five restoration stations (SL-R1 to -R4 and LX-R1) during biannual surveys. SL-R stations 1–4 were not sampled until spring 2010; LX-R1 was not sampled until fall 2010.

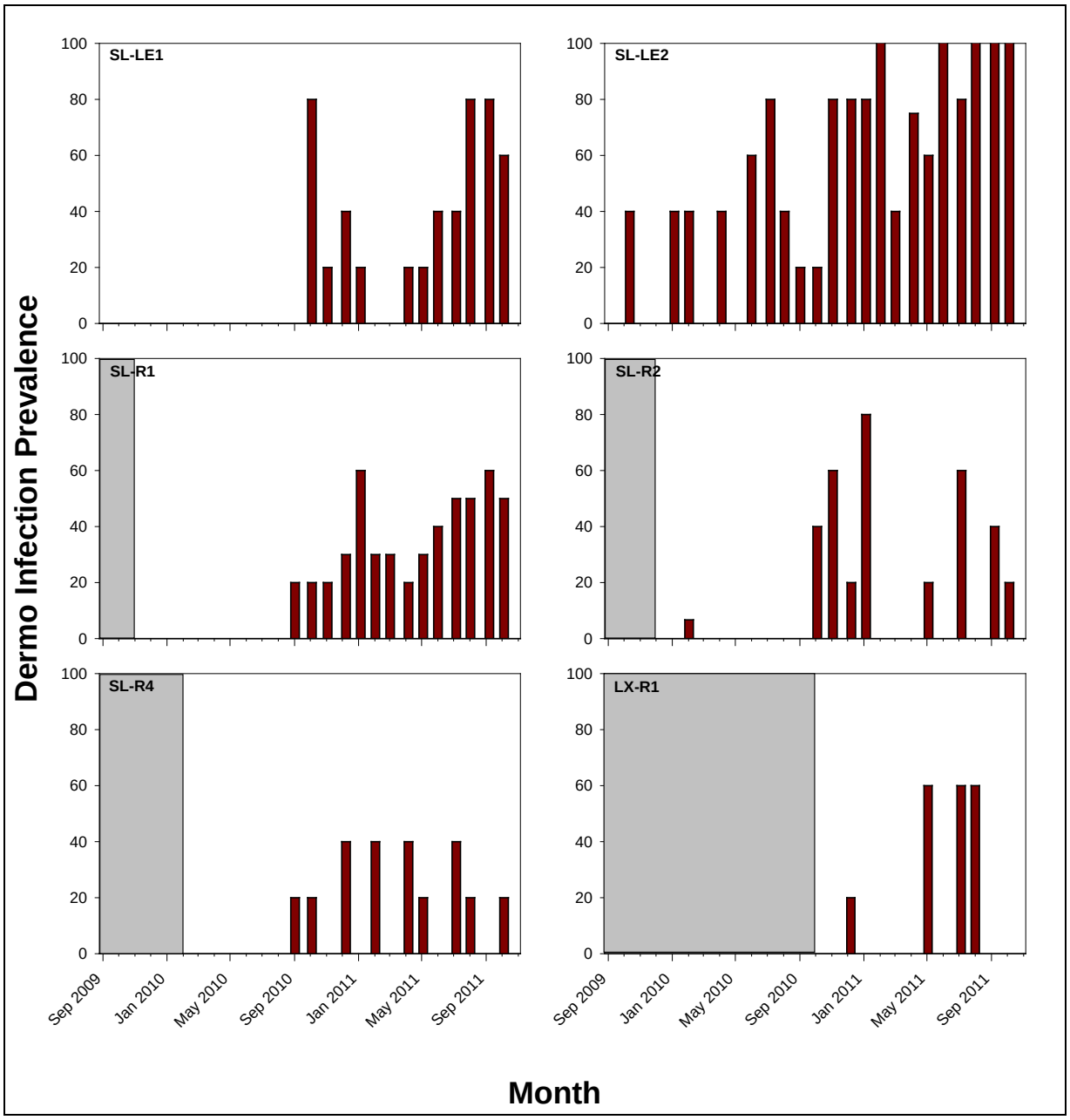

Figure A-4. Prevalence (%) of oysters infected with *Perkinsus marinus* at two natural reef stations (SL-LE1 and SL-LE2) and four restoration stations (SL-R1, -R2, -R4, and LX-R1) during monthly collections. Oysters were not collected for disease analyses from SL-R3 due to their scarcity and difficulty in harvesting at that location. Gray boxes indicate months when oysters were not collected for analysis.

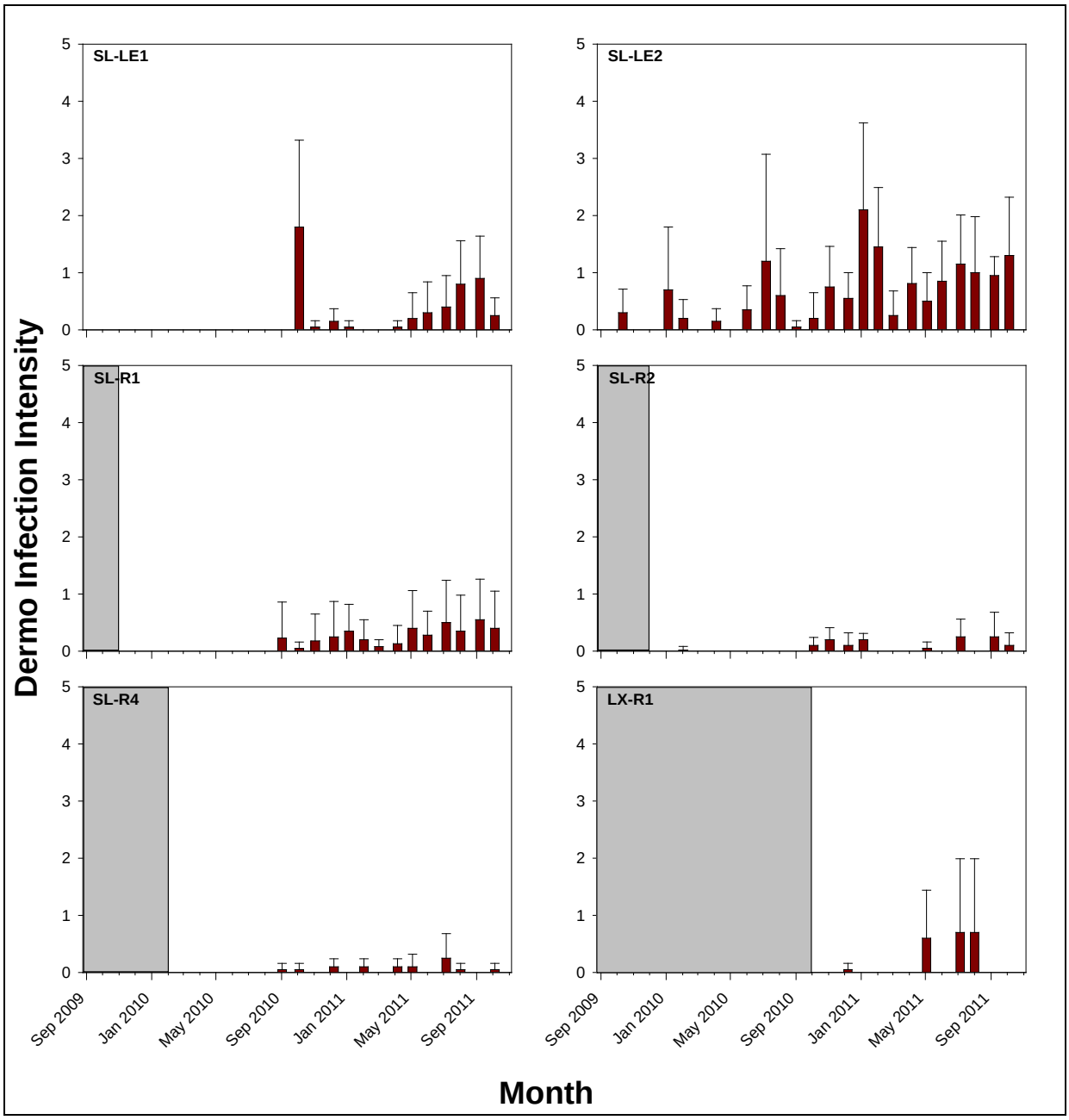

Figure A-5. Mean infection intensity (±SD) of oysters infected with *Perkinsus marinus* at two natural reef stations (SL-LE1 and SL-LE2) and four restoration stations (SL-R1, -R2, -R4, and LX-R1) during monthly collections. Oysters were not collected for disease analyses from SL-R3 due to their scarcity and difficulty in harvesting at that location. Gray boxes indicate months when oysters were not collected for analysis.

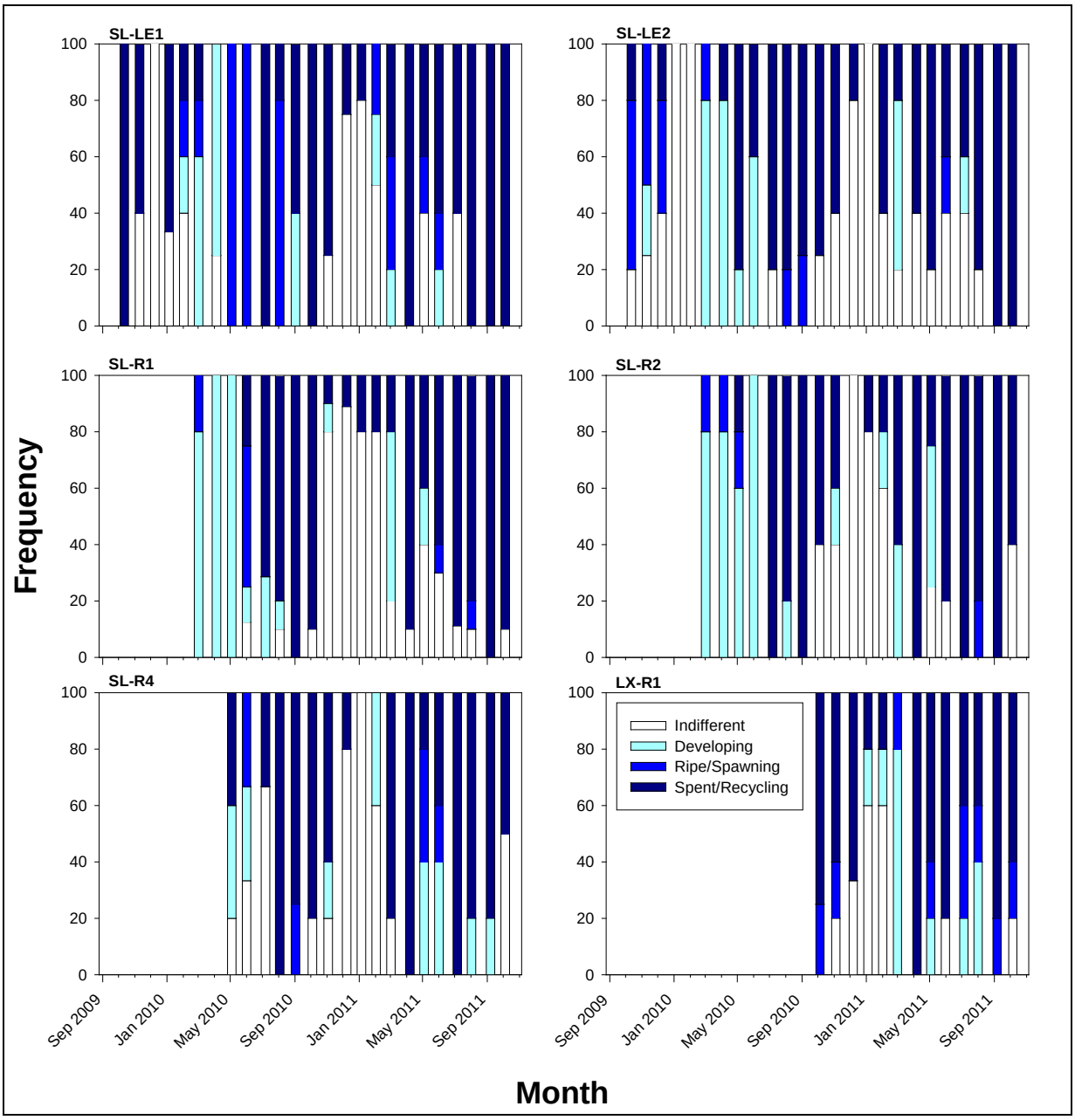

Figure A-6. Reproductive development of oysters collected monthly from two natural reef stations (SL-LE1 and SL-LE2) and four restoration stations (SL-R1, -R2, -R4, and LX-R1). Oysters were not collected for reproductive analyses from SL-R1 and -R2 until March 2010, from SL-R4 until May 2010, and from LX-R1 until October 2010. Oysters were not collected for reproductive analyses from SL-R3 due to their scarcity and difficulty in harvesting at that location.

Condition index values in 2010 peaked at most stations in March (**Figure A-7**), corresponding with peak gonadal development from reproductive analyses. Condition index values decreased steadily throughout the summer, with another smaller peak occurring at some point, until reaching the lowest values in early fall. Condition index patterns were similar in 2011, but the spring peak was not as pronounced. The missing data point in August 2010 at SL-R4 was due to a small oyster die-off. There were a few recently settled oysters at that station, but they were too small to process for condition index analyses.

Spat Recruitment

Recruitment patterns were similar among stations in the study with peak recruitment rates occurring in the spring and/or fall at most stations (**Figure A-8**). In late 2009, larval recruits were collected at both of the SL-LE background stations and a single spat was observed at SL-R1. The first recruits of the 2010 spawning season were observed on collector arrays retrieved in June from the two SLE background stations and in July from stations SL-R1 to –R3. Recruits were observed at LX-R1 after the first monitoring period at that restoration reef on arrays collected in September 2010. At SL-R4 in 2010, recruits were only detected in November. In the 2011 season, the first larval recruits were detected in March or April at all stations and were present continuously for the remainder of the study. The largest recruitment peaks in 2010 were detected at SL-R3 in November when the mean reached 18 spat shell^{-1} month^{-1}. Peak recruitment rates at the remaining SLE restoration stations were near 1 spat shell^{-1} month^{-1} in 2010 but increased to means ranging from 3 to 6 spat shell^{-1} month^{-1} in 2011. Mean peaks at the LOX restoration station were near 2 spat shell^{-1} month^{-1} in both years. At the background stations, peak recruitment rates reached approximately 3 spat shell^{-1} month^{-1} in both years, with the only exception being a peak near 6 spat shell^{-1} month^{-1} at SL-LE1 in May 2011. No recruits were detected at any of the stations in the month of February for the duration of the study.

Water Quality

Salinity was variable in both estuaries, ranging from lows near 0 ppt to highs near or exceeding 30 ppt during the study (**Figure A-9**). However, salinities were much more variable in 2010, reaching lows of less than 10 ppt at most stations in the early summer months before steadily increasing throughout the remainder of the year. Maximum salinities in 2010 only exceeded 30 ppt at SL-LE2 and SL-R3. In 2011, salinities were high and relatively stable until July when salinities began decreasing. In contrast to 2010, maximum salinities in 2011 exceeded 30 ppt at all stations with two exceptions occurring at SL-R4 and LX-R1 where maximums were slightly less than 30 ppt. Salinities were higher on average at SL-LE2 and SL-R3 with overall means in 2010 near 23 ppt and in 2011 near 31ppt. Salinity at those two stations never fell below 20 ppt in 2011. Average salinities at SL-LE1, and SL-R stations 1, 2 and 4 ranged from 11 to 17 ppt in 2010, but increased to 20 to 26 ppt in 2011. Mean, maximum, and minimum salinities at LX-R1 were almost identical in 2010 and 2011. In both years, means were 20 ppt, maximums were 28 ppt, and minimums were 3 to 7 ppt.

Temperatures at each station followed common seasonal patterns each year, with highs near 30 ^{o}C in the summer and lows in the teens during the cooler months (**Figure A-10**). Measurements of pH ranged from 7.0 to 8.1 and were relatively stable with the exception of a rapid decrease in September 2011, which was most likely due to freshwater runoff resulting from several late summer storms (**Figure A-11**). Dissolved oxygen concentrations ranged from 2 to 11 mg/L, with lows occurring during late summer and early fall months (**Figure A-12**). Turbidity was lowest at the two background stations and at the LOX restoration station where Secchi disk penetration was near 100% during most months of the study (**Figure A-13**). At the SLE restoration stations, turbidity was variable and higher in the spring and summer months.

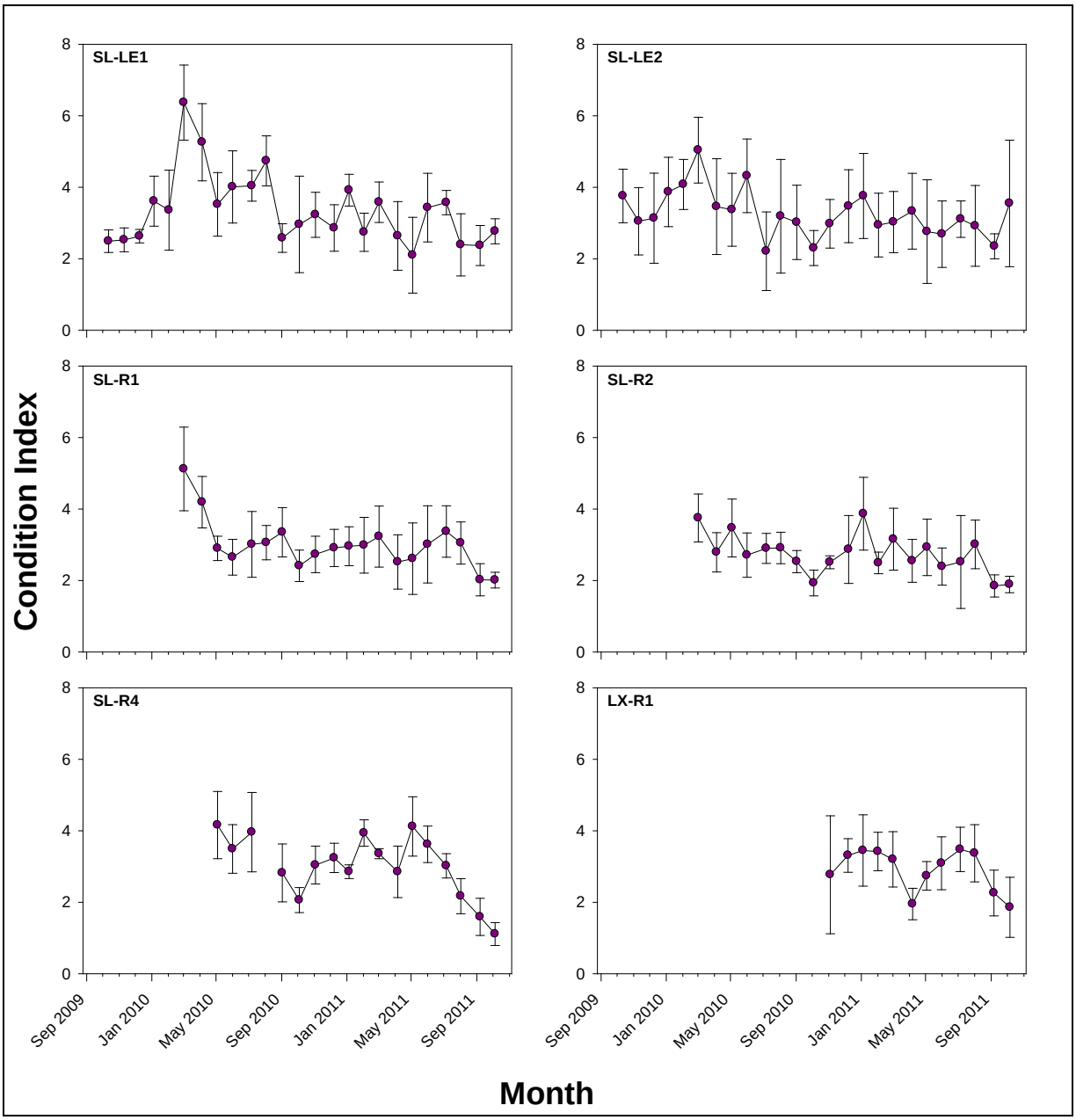

Figure A-7. Mean condition index (±SD) of oysters collected monthly from two natural reef stations (SL-LE1 and SL-LE2) and four restoration stations (SL-R1, -R2, -R4, and LX-R1). Oysters were not collected for condition analyses from SL-R1 and 2 until March 2010, from SL-R4 until May 2010, and from LX-R1until November 2010. Oysters were not collected for condition index analyses from SL-R3 due to their scarcity and difficulty in harvesting from that location.

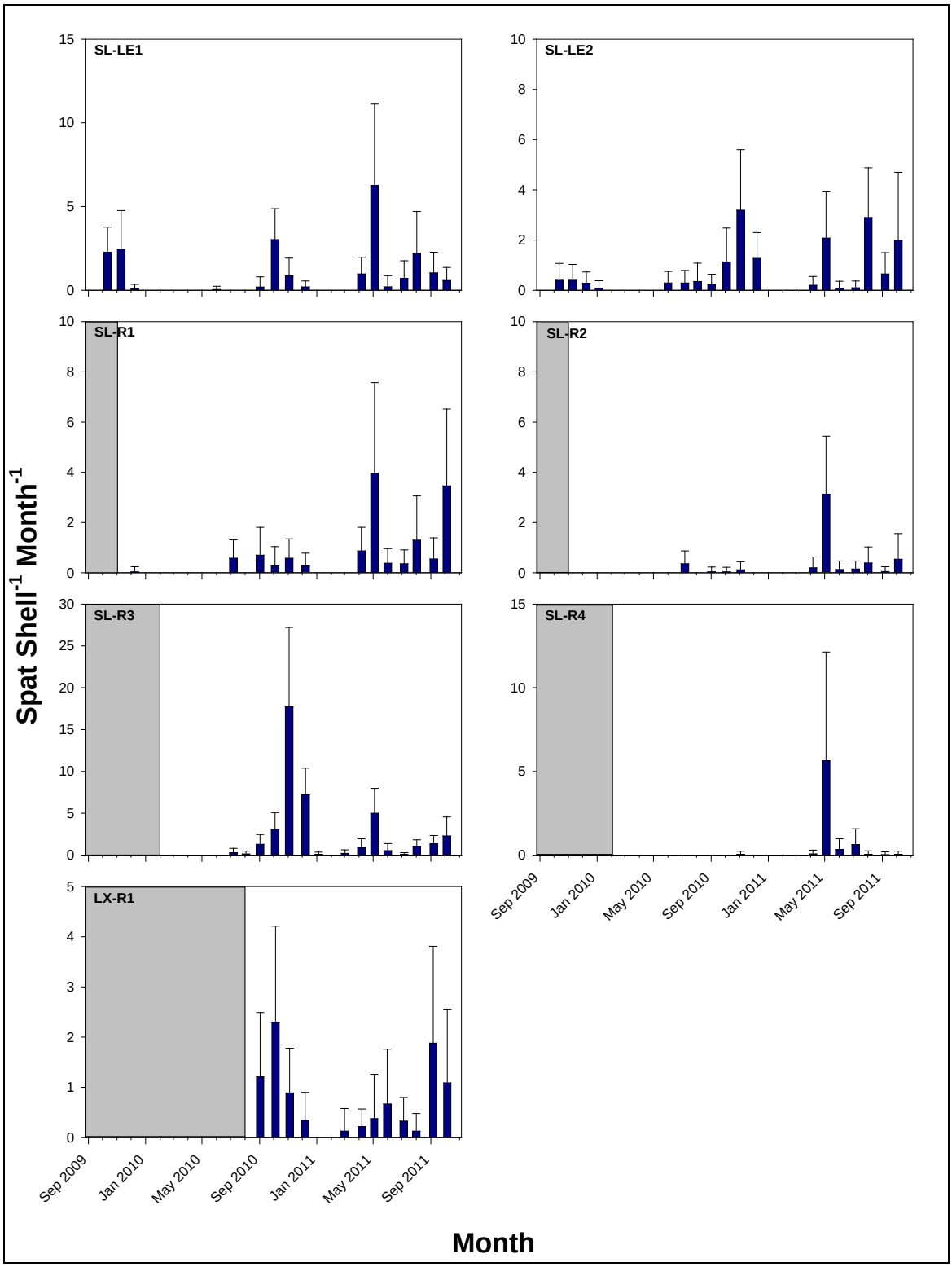

Figure A-8. Mean number (±SD) of oyster recruits collected per shell each month from two natural reef stations (SL-LE1 and SL-LE2) and five restoration stations (SL-R1 to -R4 and LX-R1). Gray boxes indicate months when spat arrays were not deployed for larval recruit collection. (Note differences in y-axis range among stations.)

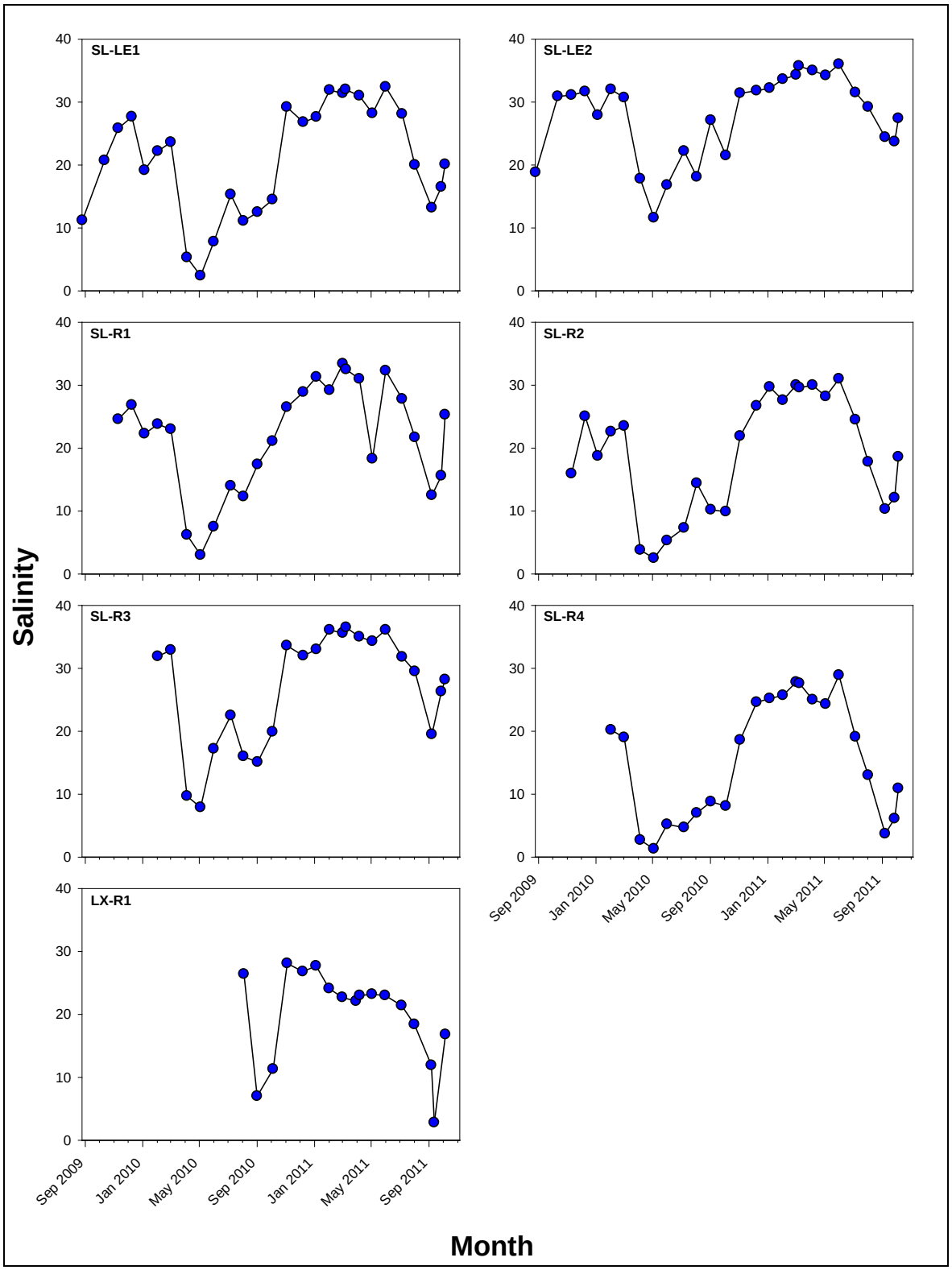

Figure A-9. Salinity recorded at two natural reef stations (SL-LE1 and SL-LE2) and five restoration stations (SL-R1 to -R4 and LX-R1) during monthly monitoring trips.

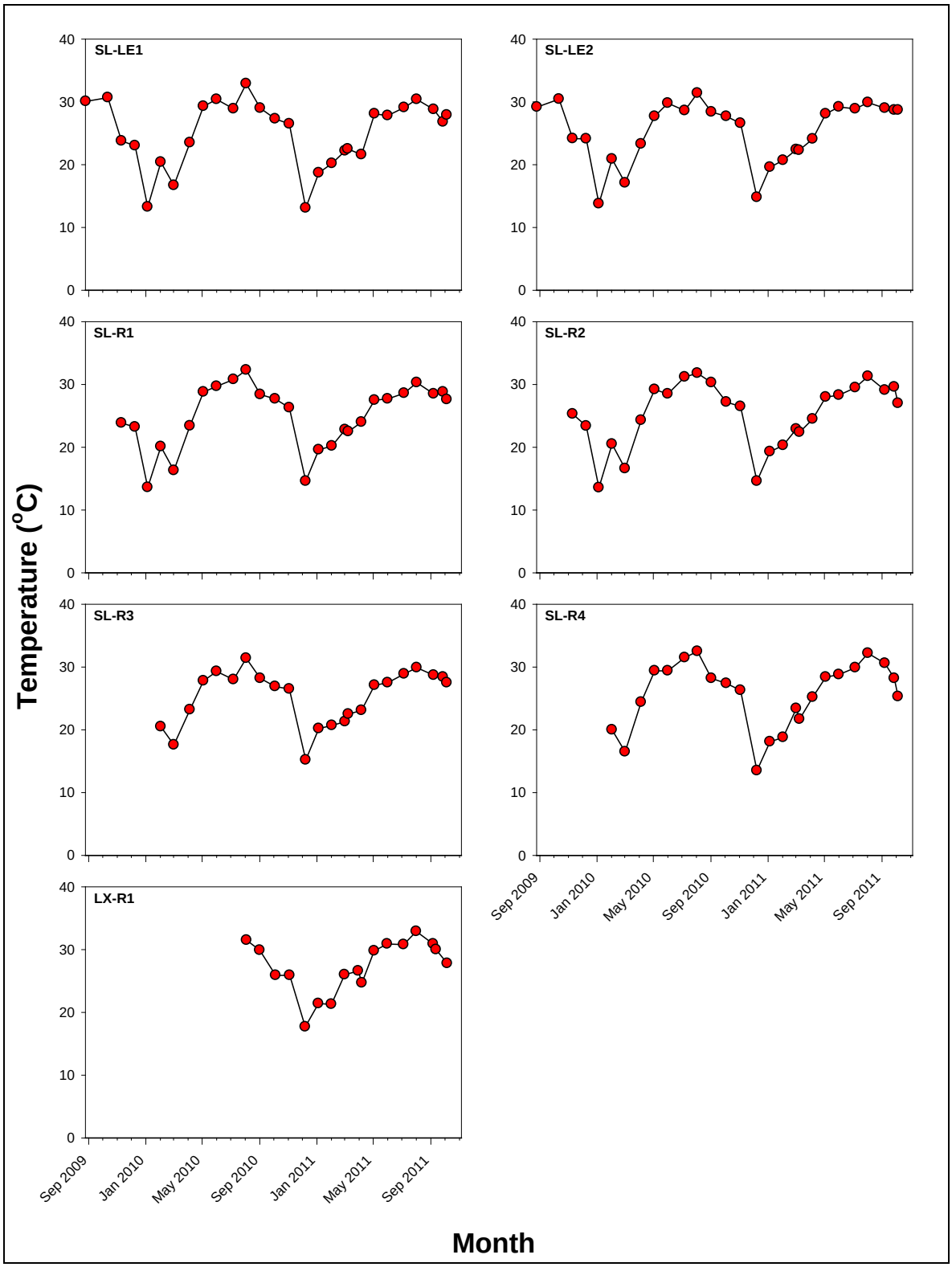

Figure A-10. Temperature recorded at two natural reef stations (SL-LE1 and SL-LE2) and five restoration stations (SL-R1 to -R4 and LX-R1) during monthly monitoring trips.

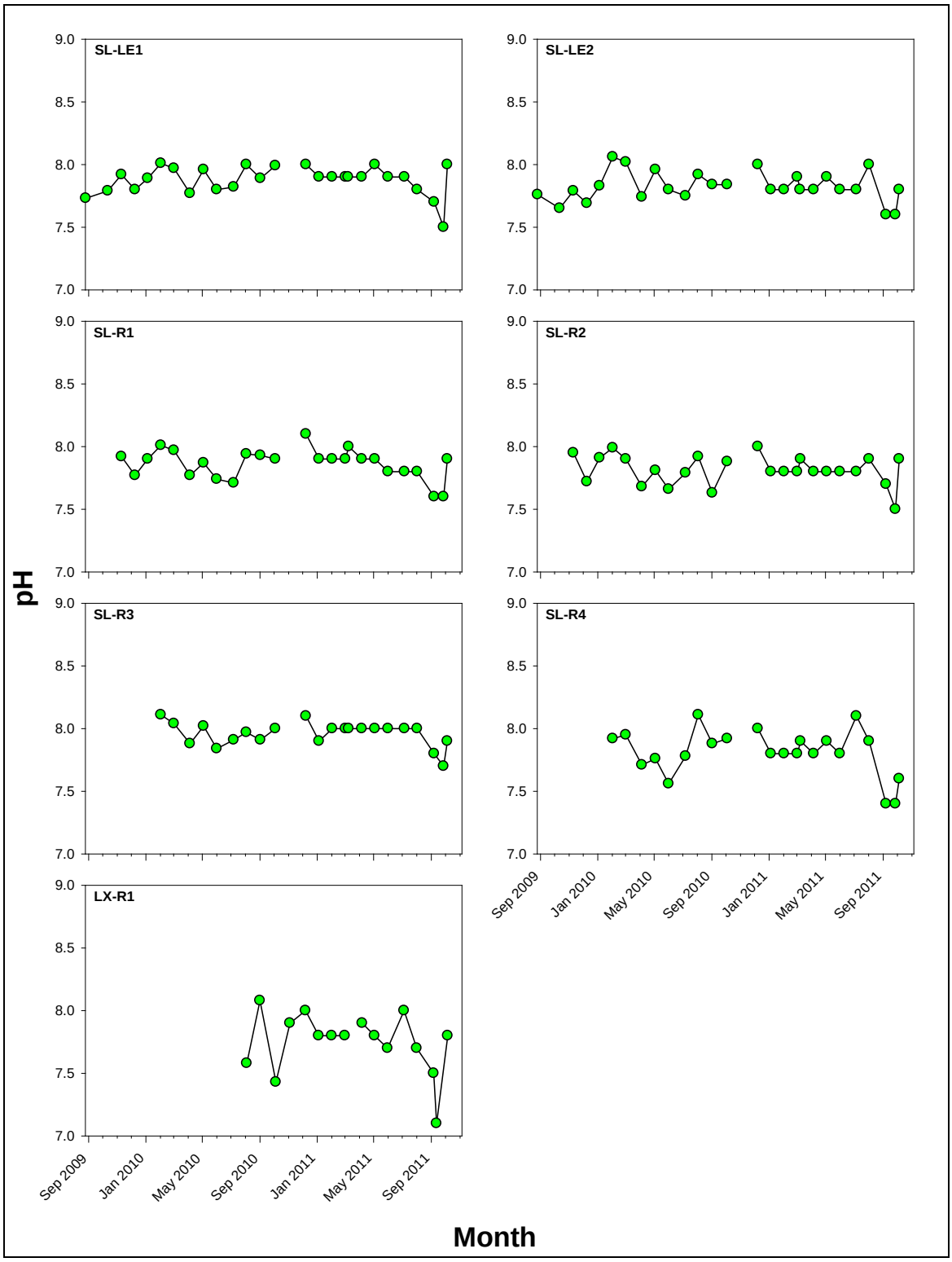

Figure A-11. pH recorded at two natural reef stations (SL-LE1 and SL-LE2) and five restoration stations (SL-R1 to -R4 and LX-R1) during monthly monitoring trips.

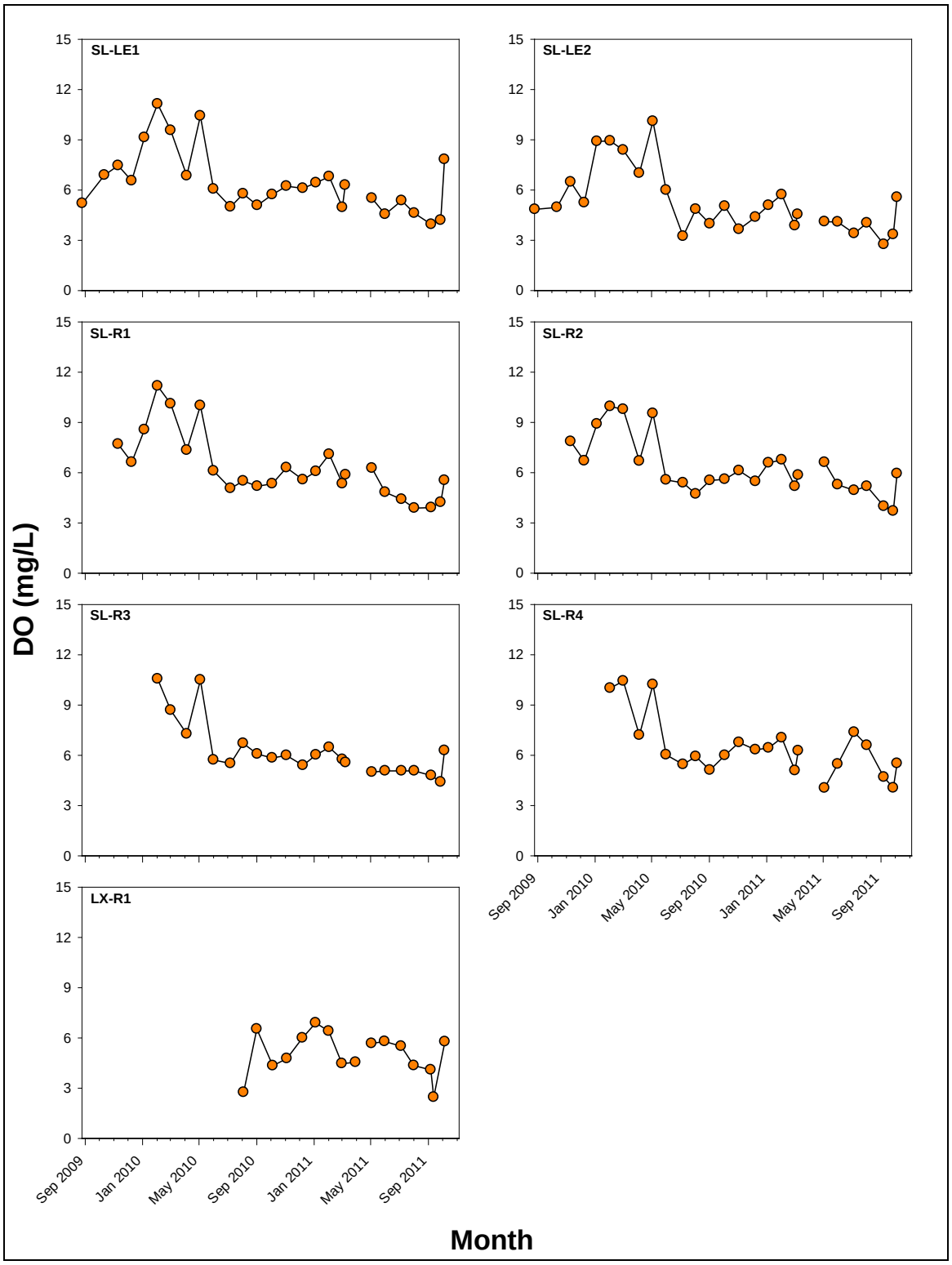

Figure A-12. Dissolved oxygen (DO) concentration (mg/L) recorded at two natural reef stations (SL-LE1 and SL-LE2) and five restoration stations (SL-R1 to -R4 and LX-R1) during monthly monitoring trips. (Data points missing in April 2011 due to malfunctioning DO probe.)

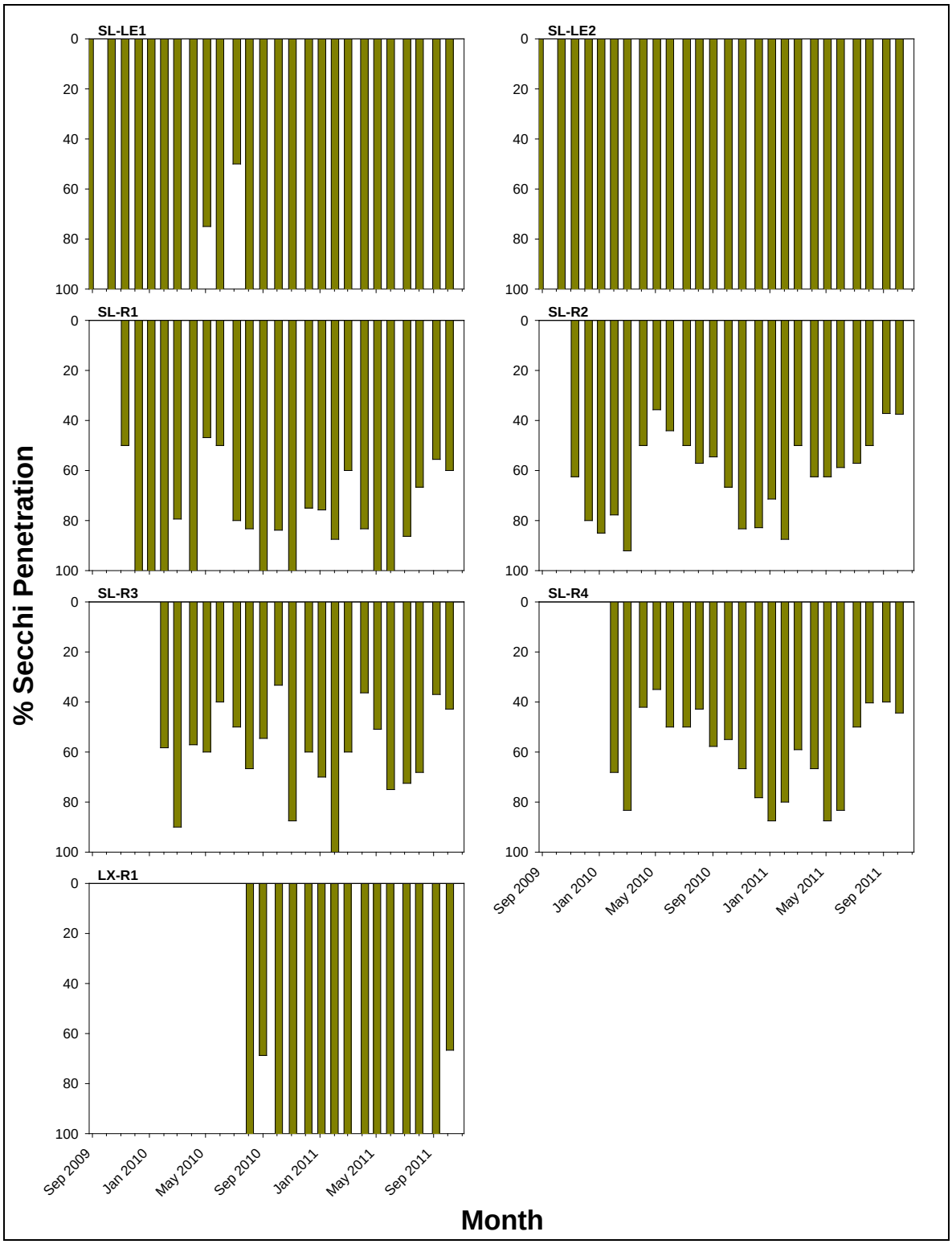

Figure A-13. Secchi penetration, reported as percent of water column penetrated, recorded at two natural reef stations (SL-LE1 and SL-LE2) and five restoration stations (SL-R1 to -R4 and LX-R1) during monthly monitoring trips.

DISCUSSION

Salinity is a driving force behind changes in oyster survival, abundance, and health in both the St. Lucie and Loxahatchee River estuaries. Although oysters in each estuary were subjected to large variations in salinity, the timing of the restoration project was auspicious in that it occurred during three relatively innocuous years. Prior to the start of the restoration project, storm activity coupled with water releases into the SLE in fall 2008 caused salinities to drop at all CERP-monitored stations resulting in a widespread oyster die-off in the estuary. Oysters were recovering in 2009 when construction of the restoration reefs began. No extreme storm events occurred for the remainder of the study, but there was a prolonged freshwater release in 2010.

The eastern oyster thrives in salinities from 10 to 30 ppt, but does poorly when salinity falls below 5 ppt for extended periods of time (Shumway, 1996). Salinities were consistently high in the SLE throughout the first several months of 2009, but decreased rapidly in June. Salinities in the estuary varied during the summer months with minima (<5 ppt) occurring in August, after which they began steadily increasing, reaching the optimal range (15–20 ppt) for oyster survival and recruitment success by October.

Reef construction was completed at three major sites, Sites 1 and 7 in the middle estuary and Site 11 in the North fork, by October 2009, coinciding with near optimal salinities and the final months of the recruitment season. In previous years, recruitment peaks at CERP-monitored SLE stations typically occurred bimodally, in summer and fall, with the strongest peaks occurring in the summer (Arnold et al., 2008, Parker and Geiger, 2009). However, because SLE oysters were recovering from a die-off in fall 2008, the majority of the 2009 spawning population comprised young, newly developing oysters that did not reach reproductive maturity until mid-summer. As a result, there was no summer recruitment peak but instead a higher magnitude fall peak ranging from 1 to 3 spat shell^{-1} month^{-1} at the middle estuary stations (Parker and Geiger, 2010). Recruitment continued through December 2009, and was even detected on a spat array deployed at SL-R1, indicating that spat were available to settle on substrate at the newly constructed restoration sites.

Construction at the SLE lower estuary restoration site (Site N6) was not completed until January 2010. By that time, the 2009 recruitment season had ended and recruits were not detected again at any SLE stations until June 2010. As a result, there were few, if any, larvae available to settle at this new site until many months after construction. The freshwater release event that impacted the SLE in 2010 may have inhibited settlement at that site and all other restoration sites in 2011. The freshwater release began in mid-March and caused salinities to drop from an estuarine average of 20 ppt to less than 3 ppt within a month. The release continued for the next 6 months during which time salinities remained below 10 ppt until July in the middle estuary and until October in the North Fork. Although there was no major oyster die-off related to the freshwater release event, the beginning of the event coincided with peak oyster condition index values and preceded the start of the 2010 spawning season. Recruitment rates in 2010 were substantially lower, 1 spat shell^{-1} month^{-1} or less, and there were no apparent peaks at any of the SLE restoration stations. Analyses of reproductive tissues and physiological condition of SLE oysters indicated that those oysters were developing and preparing to spawn as expected in early 2010. This suggests that adult oysters were spawning in 2010 but that the majority of newly spawned larvae were killed by low salinities and/or physically flushed out of the estuary as a result of the freshwater release event. The extraordinarily high recruitment rate, >15 spat shell^{-1} month^{-1}, measured at SL-R3 in November 2010 provides evidence for the physical flushing theory. That station is located downstream in the lower estuary at a narrows and it appears that while some larvae settled on the restoration substrate, many larvae passed through before moving into the Indian River Lagoon or oceanic waters.

Reef construction was completed at the Loxahatchee restoration site in late July 2010. Salinities in the LOX were near 5 ppt from April through June but increased to optimal levels by July. Recruitment is also bimodal in Loxahatchee, and in 2010 reached peaks at CERP-monitored stations of approximately 8 spat shell^{-1} month^{-1} in June and again in September (Parker and Geiger, 2011a). As in the SLE, recruitment continued throughout the rest of the year providing an abundance of larvae for settlement at the restoration site.

Salinities were considerably higher and more stable in early 2011 due to several months of low rainfall and the absence of any freshwater releases into the estuaries. Ideal salinity conditions and the presence of adult oysters at restoration stations likely contributed to the significantly higher recruitment rates recorded in 2011. In fact, at the CERP-monitored stations in the SLE, recruitment rates in May were the highest recorded since 2005, reaching means of 3 spat shell^{-1} month^{-1} in the middle estuary and more than 10 spat shell^{-1} month^{-1} in the North Fork (Parker and Geiger, 2011b). Unfortunately, increased rainfall in the summer led to substantial drops in salinity that appeared to reduce recruitment rates and peaks at most restoration stations in late 2011.

Higher salinities can lead to increased predation and disease (Shumway, 1996), which was a concern in summer 2011 after months of elevated salinities. While the prevalence of *Perkinsus marinus* (dermo) did increase in oysters collected from the SLE and LOX restoration stations, infection intensities remained light. Interestingly, dermo intensities, although still low, are typically higher at CERP-monitored stations in the LOX than those in the SLE (Parker and Geiger, 2010, 2011a, 2011b). The most straightforward explanation for the differences in dermo prevalence and intensity between estuaries is that the salinities experienced in the SLE are commonly too low for completion of the life history cycle of the parasite. It appears that, because salinity fluctuates so regularly in the SLE, the parasite is present at or near its physiological tolerance limits and cannot thrive, so the infection intensity remains low. Dermo infection patterns in the SLE may become more similar to those recorded in the LOX if salinities remain higher on average due to multiple dry years and/or there are changes in water management practices. There appears to be a source of infective agents sufficient enough for oysters throughout the estuary to be rapidly infected as soon as salinity and temperatures reach levels favorable to *Perkinsus marinus*.

Physical factors besides salinity may influence rates of biological properties of oysters, but are less likely to be acutely limiting. Hypoxia has been shown to delay metamorphosis, reduce larval settlement by roughly half (Baker and Mann, 1994a), and reduce larval and post-settlement oyster feeding rates (Baker and Mann, 1994b). Anoxia may result in near complete recruitment failure (Baker and Mann, 1992). Settled oysters are also affected by reduced oxygen concentrations, but the effects are more likely to be limiting (reduced available energy) than to cause mortality, especially in the short term. The impacts would be greatest when salinity is low and temperature is high (Shumway and Koehn, 1982). Deep hypoxia layers or severe stratification may not be present to the same extent in Florida's estuaries as they are in the Carolinas (Lenihan and Peterson, 2004) or Chesapeake Bay (Breitburg, 1990). The observed levels only rarely fell below 40% of saturation, well above those levels anticipated to be detrimental, but the once per month water quality sample may not have captured all hypoxia events. Many factors could influence water column dissolved oxygen levels, such as resuspension of bottom layers of organic matter, stratification during freshwater discharge or runoff, and intense phytoplankton blooms. Typically the most severe hypoxia occurs overnight when phytoplankton respiration exceeds photosynthesis and during summer, when biological respiration rates are high and water holds less oxygen. Thus, while we did not observe dissolved oxygen concentrations that inhibited growth of oysters, it appears the conditions stressful to oysters could occur in some years, in particular when early life stages are present in summer.

Temperature in the estuaries may serve to synchronize the timing of reproduction but is unlikely to be a source of mortality. Some reefs in SLE are occasionally exposed, so the maximum temperature those oysters are exposed to is around 30 ^{o}C for brief periods in the summer. A temperature of 30 ^{o}C may require heavy costs for routine metabolism, reducing available energy for reproduction and growth. The restored reefs, with lower elevation and planted in slightly deeper water, are unlikely to be exposed. In Loxahatchee, the relatively shallow nature of the site may allow the reef to function intertidally, raising the possibility of exposure to very high summer temperatures during daytime low tides. However, the upper thermal tolerance limits of Florida oysters remain poorly studied therefore the possible effects of such exposure are difficult to predict. Neither estuary should be affected by detrimental cold spells in the winter when minima temperatures range from the mid to upper teens, especially considering the minimal time a reef would be exposed at low tide. One negative consequence of mild winter temperatures is that dermo infection is possible at almost all times of the year because temperatures are only briefly low enough (<20 ^{o}C) to be limiting in the winter (Villalba et al., 2004).

Accompanying changes in salinity, brief periods of reduced pH and frequent changes in turbidity (measured by Secchi depth) were observed. Reductions in pH are known to affect shell formation processes (Watson et al., 2009) resulting in weaker shells, particularly in larvae (Miller et al., 2009). The magnitude of pH reductions, as low as 7.5, is much greater than pH changes studied in research associated with potential climate change, suggesting that either reduced shell thickness or increased energetic costs of shell formation may be occurring in southeast Florida estuaries. The turbidity measured by the Secchi disk cannot distinguish phytoplankton from suspended particulate matter, which is typically less valuable as a food source for oysters. The scale that is presented, percentage of the water column penetrated, allows a rough estimate of water clarity, but also carries a slight bias in that the restoration stations are generally deeper than the background stations. Nonetheless, the pattern of less light penetration farther upstream in the estuary and during periods of lower salinity reflects reduced water clarity nearer to the sources of freshwater. The freshwater inputs, whether natural or management-related releases, carry particulate matter capable of disrupting feeding patterns through increased filtration costs. Freshwater releases also cause siltation on the newly planted substrate, potentially reducing the area available for settlement of new oysters. An additional explanation for the differences in turbidity among stations in the SLE is that the large and very successful reefs on the northern side of the middle estuary filter water in those locations more completely.

Some of the differences in oyster densities among restoration stations can be attributed to the age, location, and substrate of the constructed reef. Although live oysters were found at each of the monitored restoration sites, abundances were greatest at SL-R1. While SL-R1 was one of the most mature restoration stations, that observation alone cannot account for the differences since SL-R2 was established within the same month but had densities that were significantly lower in 2011. Densities were initially similar between stations SL-R1 and SL-R2, but there was a considerable decline at SL-R2 in spring 2011 that may be attributed to substantial deposits of fine, silty muck noted during that survey. There was also a slight decline in density at SL-R1 from fall 2010 to spring 2011, but density at that reef rebounded by fall 2011. Live densities also decreased at SL-R3 in spring 2011, for similar reasons because that constructed reef was located in an area with sandy substrate and high currents. The restoration substrate used at that site was a combination of shell and large limestone rocks and it appeared that most of the shell was washed out of the reef area and/or buried almost immediately after deployment. The larger limestone rocks remained, but as the months progressed they became buried in the sand with only the upper portions exposed for oyster settlement. At SL-R4, oyster densities were very stable throughout the study and similar to those recorded at a nearby natural reef. It would seem that the densities achieved at this station were appropriate and that the lower abundances are more likely due to its upstream location in the North Fork where salinities are often lower and more variable than those in the middle estuary. Despite the fact that in October 2011 the LOX restoration site was only 14 months old, densities at that station were almost as high as those at SL-R1, which was nearly 2 years old. It is important to note that

both of those stations were located adjacent to some of the healthiest and most abundant natural oyster reefs in each of the estuaries, reflecting the importance of choosing appropriate restoration sites to achieve maximum success.

Fecundity has been shown to increase rapidly in oysters of greater than 400 mg dry weight (Thompson et al., 1996). This phase of life marks a stage where the oysters begin to devote an increasingly high percentage of available energy from somatic growth into reproductive efforts. In the SLE and LOX that would correspond to oysters of roughly 50-mm SH, based on data collected while measuring condition index. The shell heights of oysters at SL-R1 and -R2 had individuals approaching 100 mm by spring 2010 (at an age of approximately 6 months) and a few individuals exceeding 110 mm by fall 2010, at an age of approximately 1 year. There was very little change in the maximum size of observed oysters beyond that point, with typical maximum sizes during each survey between 110 and 120 mm. The oysters at the LOX restoration site achieved similar growth rates with the largest oysters approaching 100 mm at an age of approximately 1.5 years. If we assume that the largest oysters at each restoration station follow similar developmental processes as those described from the mid-Atlantic and Gulf Coast (Thompson et al, 1996), then at an age of approximately 6 months they should begin the life-history phase that allows most of their energy to be dedicated to reproduction rather than rapid somatic growth. Because oysters at three of the SLE stations (SL-R1, -R2, and -R4) and the LOX restoration station exceeded shell heights of 50 mm, it can be assumed that they transitioned to the reproductive phase of life and began to contribute to the larval pool within the estuary. Most oysters at SL-R3, however, never achieved this phase; the maximum SH measured during any survey was 48 mm. The total fecundity of an individual at this site would be expected to be very small. Contrary to some studies, we detected individuals of both genders at sizes as small as 25 mm that were reproductively active. The sex ratio is predominantly male until average shell height is approximately 35 mm, is roughly even until a mean size of around 60-mm SH, and then becomes female dominant at larger sizes, though some males were present in all size classes including two males larger than 100-mm SH. Results were similar at both background and restoration stations in the SLE and LOX. At the LOX restoration station, a single reproductively active female of 17.1-mm SH shows that oysters are capable of maturing very quickly (maximum age of 3 months) such that spat which settle in the spring would be capable of contributing larvae in the fall. Providing any larval supply exists, newly settled oysters should begin contributing reproductively to the population and become self-sustaining within their first year.

This monitoring project showed that reef restoration in the St. Lucie and Loxahatchee estuaries was successful. Powers et al. (2009) used three criteria to define oyster reef restoration success in North Carolina: presence of vertical structure, presence of live oysters, and evidence of recruitment. Specifically, they required a minimum of 20 cm vertical relief, densities of greater than $10/m^2$ with at least a few oysters per $0.25\text{-}m^2$ quadrat of sizes greater than 25-mm SH, and spat recruitment during at least one year of the study to meet minimum success criteria. All restoration stations in this study met the minimum requirements, with the possible exception of adequate vertical relief at SL-R3. Results showed that if adequate substrate is planted at an appropriate time, just prior to or during larval recruitment season, and if water quality is maintained within tolerable limits for oyster growth, there are sufficient numbers of naturally occurring oysters available to provide larval recruits for settlement on that substrate. Within 6 to 12 months, the newly settled oysters will grow and begin contributing to the estuary both ecologically and biologically once they become reproductively viable. When restoration substrate is planted at an inappropriate time, naturally settling oyster larvae may not be present and other species in the estuary might dominate and preclude future settlement by oysters. Alternatively, settling particulate matter may result in burial of the newly planted substrate before juvenile oysters have an opportunity to colonize.

Long-term databases, such as those in the CERP monitoring program, provide an ideal tool to predict optimal substrate planting times. If sufficient real-time data also existed, planting conditions for both estuaries could be optimized for the year planting was to occur. In estuaries where long-term monitoring programs have been discontinued, such planning exercises would have to be conducted with limited data and therefore would have lower reliability. As CERP progresses, and nears the time when more active restoration of the estuaries is considered, managers should consider reestablishing, or developing for the first time, monitoring programs for those estuaries where they do not exist. The incorporation of appropriate baselines and control sites allows for assessment metrics that are relevant to the individual project. This process could aid restoration and fisheries-based enhancement/rehabilitation efforts anywhere oysters are chosen as a target organism.

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ATTACHMENT: LABOR HOURS

The grant subcontract CSA International, Inc. Job No. 2245 to FWRI was used to fund, in part, eight individual employees during the life of the contract: Mark Gambordella, Anthony Vasilas, Michael Drexler, Janessa Cobb, Bethany Pierce, Octavia Poole, Richard Radigan, and Sarah Stephenson. The total number of hours of salary provided was 5,375.75, roughly 2.5 person-years of salary.

	2009	2010	2011	Total
Mark Gambordella	301.00	1,479.00	--	1,780.00
Anthony Vasilas	78.50	455.50	1,189.25	1,723.25
Michael Drexler	--	76.50	563.25	639.75
Janessa Cobb	--	--	603.75	603.75
Bethany Pierce	--	--	89.00	89.00
Octavia Poole	--	--	112.00	112.00
Richard Radigan	--	--	322.25	322.25
Sarah Stephenson	--	--	105.00	105.00
Total	379.50	2,011.00	2,985.25	5,375.75

APPENDIX B

Passive Acoustics as a Monitoring Tool for Evaluating Oyster Reef Restoration in the St. Lucie River, Florida
Florida Oceanographic Society

Passive Acoustics as a Monitoring Tool for Evaluating Oyster Reef Restoration in the St. Lucie River, Florida

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ABSTRACT

Historically, oysters in the St. Lucie Estuary and the Indian River Lagoon covered an estimated 567 acres. However, recent surveys report that their distribution in the St. Lucie Estuary has decreased by 84% in the last seven decades. The aim of this research was to use passive acoustic technology as a tool to measure the progress (reef use and colonization) of a large restoration project. Total power and number of snapping shrimp snaps were calculated for each acoustic recording. The present study focused on the sound production of snapping shrimp because they are one of the most abundant decapod crustacean species in oyster reefs, and they are well known for their sound production. Results indicated that total power and number of snaps can be useful in detecting differences between seasons, regions, habitats, and periods. In addition, number of snaps can be useful to estimate number of species present in an oyster reef. Spectral (frequency vs. amplitude) and time series were also examined to determine if these analyses provided acoustic signatures that could represent acoustic differences between sites as a result of salinity (spatial – upstream vs. downstream; temporal (dry season vs. wet season); and habitat type (restored vs. natural oyster reefs). Qualitative spectral analyses were able to distinguish salinity effects due to spatial and temporal differences between restored and natural reef sites. Additionally, acoustic signatures varied according to habitat type and corroborated results examining habitat differences due to total power, number of snaps, and associated species sampled from lift net surveys. However, considerable within-site variation in spectra also was observed, indicating that faunal assemblages or behavior of those species may change quite readily with environmental conditions and species interactions. These studies report the first use of passive acoustics to evaluate restored oyster reef habitats. There is considerable potential to use these techniques for rapid biological assessments of changes in water quality, with implications specifically in evaluating effects of freshwater discharges.

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INTRODUCTION

The Eastern Oyster (*Crassostrea virginica*)

Eastern oysters (*Crassostrea virginica*, Gmelin, 1791) range from the St. Lawrence River in Canada to the Atlantic Coast off Argentina (Kennedy et al., 1996). To reproduce, adult oysters release gametes into the water column; once the eggs are fertilized, the larvae take 2 to 3 weeks to develop (Cole and Knight-Jones, 1939). Oyster larvae settle onto hard substrates, often forming aggregations (Butler, 1954; Price, 1954; Coen and Luckenbach, 2000). Over time, generations of oysters settle on each other forming oyster reefs. Oyster reefs are primarily found in brackish waters. The distribution of the reefs is mainly limited by salinity and temperature of the body of water where they are located (Shumway, 1996). Eastern oysters have been found to tolerate full strength marine waters and salinities as low as 2 psu; however, they normally occur at salinities between 10 and 30 psu where they can grow, reproduce, and survive (Stanley and Sellers, 1986). Nonetheless, the effect of salinity on oysters is highly dependent on the ambient temperature. Adult eastern oysters have a wide temperature tolerance, and they can be found in areas where normal annual temperatures vary between -2 °C to 36 °C (Kennedy et al., 1996). Oyster reefs have important ecological roles. They help stabilize neighboring salt marshes (Bahr and Lanier, 1981; Meyer et al., 1996), provide habitat for various species (Coen et al., 1999; Coen and Luckenbach, 2000; Grabowski, 2002) and filter water (Bahr and Lanier, 1981; Newell, 1988). The importance of the habitat function provided by oyster reefs has been compared to that of seagrass beds and salt marshes (Coen et al., 1999; Coen and Luckenbach, 2000; Grabowski, 2002). Studies have shown that some fishes and invertebrates prefer to use oyster reef habitat over sand bottom or mudflat habitat (Lehnert and Allen, 2002; Grabowski et al., 2005).

This study was particularly concerned with the oyster reefs in the St. Lucie Estuary (SLE), southeast Florida. Historically, oysters in the SLE and the Indian River Lagoon covered an estimated 567 acres (Sime, 2005). However, recent surveys report that oyster reef distribution in the SLE has decreased by 84% in the last seven decades (Woodward-Clyde, 1998; IBIS, 2004; Sime, 2005; **Figure B-1**). Oyster reefs in the SLE are frequently affected by freshwater releases. During freshwater discharges from Lake Okeechobee, the salinity in the estuary can be lower than 5 psu for periods of 1 to 8 weeks (Wilson et al., 2005). At salinities below 2 psu, oysters are under stress and begin to die (Stanley and Sellers, 1986). Therefore, the loss of oyster reef habitat in the SLE has been attributed to discharges from the Lake Okeechobee (Graves et al. 2004; Sime 2005). Salinity in the SLE is also affected by changes in season. Southeast Florida is marked by a wet season during the summer and early fall months and a dry season during the fall and winter months. In the SLE, salinities vary significantly throughout the two seasons (Ji et al., 2007). Changes in salinity due to freshwater releases from Lake Okeechobee and changes in season have an important role in shaping the distribution of oyster reefs and their inhabitants. Sedimentation and oxygen levels also play an important role in shaping the abundance and distribution of the species that depend on oyster reefs (Wells, 1961; Crabtree and Dean, 1982; Shumway, 1996; Bartol et al., 1999; Tolley et al., 2005).

Some studies on eastern oyster reefs have shown that diversity was greater at higher salinities (Wells, 1961, Tolley and Volety, 2005, Tolley et al., 2005). However Bergquist et al. (2006) found that the abundance of two dominant species (mytilid bivalve *Ischadium recurvum* and xanthid crab *Eurypanopeus depressus*) was negatively correlated with salinity. In addition, Lenhert and Allen (2002) found that the catch of fish associated with subtidal oyster reefs in South Carolina was significantly higher during wet season (summer months) when salinity was lower. These contrasting results may be explained by the physical differences that exist among sites. The relationship between oyster density and salinity is stronger at lower intertidal reefs than at higher intertidal reefs (Berquist et al., 2006). Increased

water-born parasites and predators whose distribution is at least partially determined by reef height may explain these differences between results in the aforementioned studies.

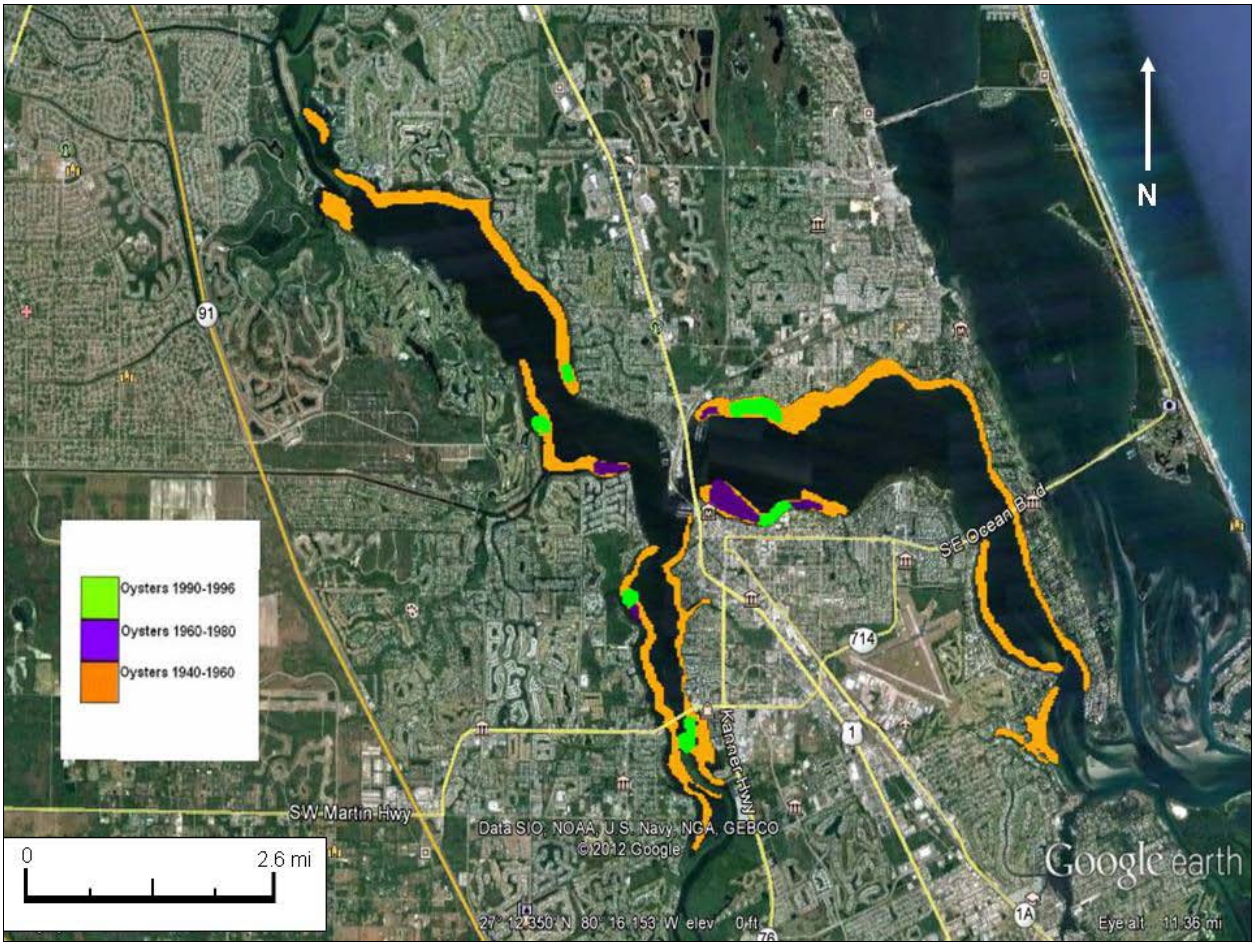

Figure B-1. Historic oyster reefs in the St. Lucie Estuary, Florida. Figure adapted from Woodward-Clyde (1998) and Steward et al. (2003).

Oyster Reef Restoration

The growing recognition of the ecological importance of oyster reefs has caused an increase in the efforts to restore oyster reefs over the last 20 years (Coen and Luckenback, 2000; Brumbaugh et al., 2006). In Martin County, the plummeting populations of oyster reefs are of special concern. Consequently, as part of the American Recovery and Reinvestment Act of 2009, the National Oceanic and Atmospheric Administration (NOAA) awarded Martin County federal funding for a large-scale oyster reef restoration project in the SLE and LOX. Over 22 acres of subtidal oyster reef habitat were created in the SLE between August 2009 and January 2010 (Oyster Reef Restoration, 2011).

Every oyster reef restoration project has a specific goal; therefore, restoration projects normally fall within one of the following categories: recruitment and growth of oyster reefs, provision of habitat for associated species, direct and indirect improvements of local water quality, and shoreline protection (Brumbaugh et al., 2006). Although all of these ecological services were desired benefits of the Martin County oyster reef restoration project, the goal of the studies described herein was to evaluate provision of habitat for both transient and resident oyster reef fauna (Oyster Reef Restoration, 2011).

Different methodologies exist to monitor the faunal assemblages of oyster reefs. Some of these methods are lift nets, core samples, drop nets, haul seine and gill nets, sampling trays, video surveys, and diver surveys/fish counts. In general, these methods may be time consuming and expensive because the numerous specimens collected require sorting and taxonomic identification. Brumbaugh et al. (2006) suggested that measuring presence/absence of organisms may be an efficient and statistically robust method to monitor reefs and measure biodiversity; however, this method still requires direct sampling of the oyster reefs.

Passive Acoustics as a Monitoring Tool

The present study introduces passive acoustics as a new method to monitor oyster reefs. Passive acoustics uses the naturally occurring sounds (bioacoustics) produced by marine organisms to study their behavior, biology, and location (Roundtree et al., 2006). Many marine organisms may produce sounds to communicate with each other during mating, aggression, or feeding. Also, organisms can produce accidental sounds associated with swimming, moving, and feeding (Roundtree et al., 2006; Mellinger et al., 2007). These intentional and non-intentional sounds can convey important information about the behavior, location, and abundance of organisms. The added value to this methodology is that it can be extrapolated to other marine ecosystems, such as coral reefs, kelp forests, and rocky reefs.

Most marine acoustical work performed in the past has focused on fish and cetaceans (Mellinger et al., 2007; Luczkovich et al., 2008) and little is known about marine invertebrate acoustics. Passive acoustics has been used to study sound in fishes for more than 60 years. Fisheries biologists have been able to use sound production to locate concentrations of fish species and to link sound production to spawning behaviors (Mok and Gilmore, 1983; Roundtree et al., 2006). In the last six decades, cetacean biologists have increasingly used passive acoustic monitoring techniques to determine range, seasonality, and abundance of cetaceans across the globe (Mellinger et al., 2007). Passive acoustics has a wide variety of uses such as assessing biodiveristy, monitoring water quality, and comparing differences in marine habitats (Watanabe et al., 2002; Radford et al., 2008; Sueur et al., 2008; Radford et al., 2010).

Various studies have used sound to study terrestrial and marine environments. Watanabe et al. (2002) used counts of snapping shrimp snaps to monitor changes in the water quality. They made 2-minute acoustic recordings of snapping shrimp and related the snap counts to the water temperature and dissolved oxygen levels. They found that under normal dissolved oxygen conditions (>3.0 mg/L) the number of snap counts increased exponentially with increasing temperature. However, at locations where dissolved oxygen was below 3.0 mg/L, snap counts did not increase exponentially with increasing temperature. Watanabe et al. (2002) suggest that snaps can be used as a rapid indicator of water quality. As water quality is a major factor affecting oysters in the SLE because of the Lake Okeechobee discharges (Graves et al., 2004), snapping shrimp sound production may be an important tool to monitor water quality in the SLE. Radford et al. (2010) used ambient sound signatures and snapping shrimp snap count as methods to detect differences among three types of habitats (macroalgal-dominated rocky reef, sea urchin-dominated rocky reef, and open sandy beach bottom). Radford et al. (2010) calculated the proportion of total sound intensity ($Prms^2$, %) for different frequency bands (100–800; 801–2,500; 2,501-20,000; and 20,001-24,000 Hz) for each habitat type. The results of this study showed that each site had a different dominant frequency band. Radford et al. (2010) concluded that different habitat types have characteristic sound signatures, and these signatures can convey information to compare habitat quality. Like other marine environments, sounds produced by movement, feeding, and callings made by different organisms in the oyster reefs create a unique acoustic signature. Oyster toadfish *(Opsanus tau)*, naked goby *(Gobiosoma bosc)*, mud crabs (*Panopeus* spp. and *Eurypanopeus depressus*), barnacles (ivory barnacle [*Balanus eburneus*], striped barnacle [*Balanus amphitrite*]), and snapping shrimps (*Alpheus floridanus*) are organisms that inhabit oyster reefs and are known for their sound production capabilities (Fish and Mowbray, 1970; Tavolga et al., 1981; Fine and Lenhardt, 1983). The present study

focused on the sound production of snapping shrimps because they are one of the most abundant decapod crustacean species in oyster reefs (Tolley et al., 2005; Boudreaux et al., 2006) and are well known for their sound production capabilities (Everest et al., 1948). In Florida oyster reefs, the most common snapping shrimp species is *Alpheus heterochaelis*, Say 1818 (Tolley et al., 2005; Boudreaux et al., 2006).

Snapping Shrimp (*Alpheus* spp.)

The snapping shrimp family Alpheidae is composed of approximately 600 species with 36 genera (Anker et al., 2006). Alpheids are exclusively benthic, and they frequently are the primary dominant decapod on marine hard bottom substrates (Everest et al., 1948; Knowlton and Moulton, 1963; Anker et al., 2006). They are more commonly found in shallow tropical and subtropical marine areas, but can be abundant in cooler deepwater areas as well (Knowlton and Moulton, 1963; Kropp, 1987). Therefore, it may be possible to use snapping to monitor marine environments across the world at tropical, subtropical, and temperate areas of the world. Snapping shrimp are characterized by their enlarged and powerful snapping claw (Schein, 1977). The snap is an important tool that is used for a number of tasks, including defense, prey capture, inter- and intraspecific signaling (Hazlett and Winn, 1962; Watanabe et al., 2002), rock boring, and excavation of soft sediment (Silliman et al., 2003).

Snapping shrimp sound production was first investigated by Everest et al. (1948). They examined underwater noise off the coast of Southern California because an unknown crackling sound interfered with submarine sonar systems. After some work, the unknown noise source was identified to be of biological origin. Later that year, the unknown noise was identified as the sound produced by snapping shrimp (Everest et al., 1948). In some habitats, the sound produced by snapping shrimp is so loud that on a semi-quiet day, the crackling can be heard above the water surface (Everest et al., 1948). Since then, snapping shrimp acoustics have been heavily studied (Everest et al., 1948; Hazlett and Winn, 1962; Knowlton and Moulton, 1963; Au and Banks, 1998; Versluis et al., 2000; Duffy and Morrison, 2002; Tóth and Duffy, 2005; Anker et al., 2006; Chitre et al., 2006). Most snapping shrimp studies have focused on studying the sound of snapping shrimp and the biological aspects of the snap (Everest et al., 1948; Schein, 1977; Au and Banks, 1998; Anker et al., 2006; Chitre et al., 2006). Early studies believed that the snap was produced by the quick and powerful closing of the claw (Hazlett and Winn, 1962; Knowlton and Moulton, 1963). However, a more recent study has shown that the snap is the result of the bursting of the cavitation bubble that is created when the shrimp closes its claw and it ejects water (Versluis et al., 2000). **Figure B-2** shows the hydrophone signal of a snap by *A. heterochaelis*. Initially, the snapper claw goes into its cocked position and 600 µsec later the claw is fully closed. At the moment that the claw is closed, a water jet escapes from the claw, and a cavitation bubble is produced. The collapse of this bubble is what causes the extremely loud and short snapping sound (Versluis et al., 2000). Studies suggest that the snapping sound is merely a side effect caused by the bubble collapsing, as no auditory organs have been detected in snapping shrimp (Schmitz and Herberholz, 1998).

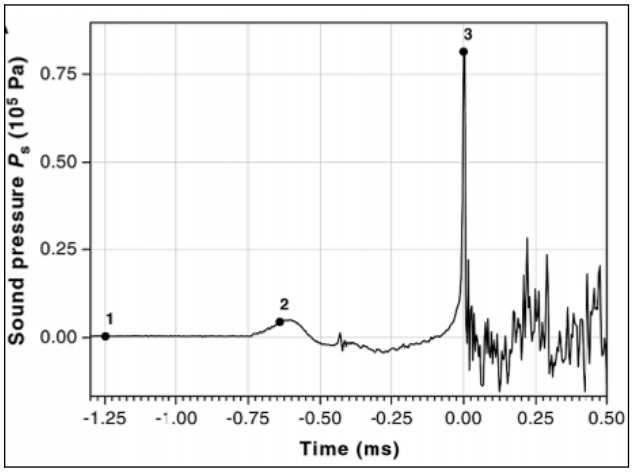

Figure B-2. Hydrophone signal of a snap by an *Alpheus heterochaelis* female measured at a distance of 4 cm. The number points correspond to respective claw movements: (1) claw is cocked; (2) claw is closed; (3) collapse of the cavitation bubble. Figure modified from Versluis et al. (2000).

Snapping shrimp use the water jet produced after the claw closure for inter- and intraspecific communication. They use mechanosensory hairs to detect hydrodynamic and tactile signals (Schmitz and Herberholz, 1998). According to Schmitz and Herberholz (1998), snapping is not used to damage conspecifics, but the snaps are viewed as threat displays that allow the opponents to evaluate their strength and fighting ability. Hazlett and Winn (1962) suggested that snapping shrimp snap is a territorial behavior, and that they rarely snap for food procurement. The territorial defense reaction is only exhibited when an intruding animal (inter- and intraspecific) approaches the living space of the shrimp. Silliman et al. (2003) explained that in saltmarshes, *A. heterochaelis* and black-clawed mud crab (*Panopeus herbstii*) co-defend their shared habitat. *A. heterochaelis* alerts *P. herbstii* of intruders using its snap, *P. herbstii* provides *A. heterochaelis* with lair maintenance, active protection from predators, and access to food left over from predation events. Snapping shrimp have also shown mutualistic interactions with goby fish. Gobies (*Nes longus* and *Ctenogobius saepepallens*) act as sentinels at the entrance of burrows while snapping shrimp (*Alpheus floridanus*) provide shelter by excavating burrows (Randall et al., 2005). Snapping shrimp have been shown to snap as a result of their interaction with conspecifics and other species; therefore, number of snaps could be used as a proxy to estimate inter- and intraspecific interaction between snapping shrimp and other oyster reef inhabitants. It would be expected that the more organisms there are in an oyster reef, the more snaps would be produced by snapping shrimp due to the increase in number of inter- and intraspecific interactions.

To a lesser extent there have been studies that have focused on other characteristics of snapping sound production, such as diel variations in snapping sounds (Lammers et al., 2006; Radford et al., 2008) and using snapping shrimp as a monitoring tool for shallow sea environments (Watanabe et al., 2002; Radford et al., 2008; Radford et al., 2010). Everest et al. (1948) conducted some of the earlier work on diel variations on snapping shrimp sound production off the coast of Hawaii in Kaneohe Bay. After conducting 11 nocturnal and diurnal recordings, they found that shrimp sound production peaked before sunrise and after sunset with a higher peak at sunset. More recent studies have found diel variations that corroborate these findings (Knowlton and Moulton, 1963; Radford et al., 2008), but the reason for these variations is unknown.

Because snapping shrimp sound production is known to change with water quality, habitat, and intra- and interspecific interactions (Hazlett and Winn, 1962; Watanabe et al., 2002; Randall et al., 2005; Radford et al., 2010), they can potentially be useful in detecting changes in the faunal communities and in environmental factors that affect oyster reefs.

Goals and Hypotheses

The aim of this research was to use passive acoustic technology as a tool to measure the progress (reef use and colonization) of the restoration project and to determine if there is a salinity influence (particularly in the context of freshwater releases) on biological sound production emanating from oyster reef formations in the St. Lucie River. We also sought to determine if restored oyster reef sites show a biological acoustic signature similar to that of natural oyster reef formations. In addition, this research focused on the snapping shrimp (*Alpheus* spp.) because their sound production has been shown to be an excellent tool to monitor habitat value and water quality changes in marine habitats (Watanabe et al., 2002; Radford et al., 2008; Radford et al., 2010).

The four main hypotheses for this study were: 1) Sound production will be different among the three different river regions. These regions represent different salinities; 2) Sound production will be different between wet and dry season; 3) Sound production and acoustic signatures will be different between natural and restored reefs; and 4) Sound production will correlate to faunal assemblages (decapod crustaceans and fishes).

MATERIALS AND METHODS

Study Area

All research was conducted in the St. Lucie Estuary (SLE) located on the southeastern coast of Florida. It is the largest tributary of the southern Indian River Lagoon (Graves et al. 2004). Historically, the Indian River Lagoon and the SLE have been influenced by ephemeral connections to the Atlantic Ocean (Brech, 2004). In 1892, when the St. Lucie Inlet was constructed, the SLE transitioned from a freshwater system into an estuary. In 1937, a drainage canal was built that connected Lake Okeechobee with the SLE (Woodward-Clyde, 1998; Wilson et al., 2005). In south Florida, seasons are determined by temperature and rain fall. The seasonal rains are common from mid-June through mid-October (Tolley et al., 2005). During intense rainfall events, large volumes of freshwater that carry muck, sediments and high levels of nutrients are released into the SLE. The discharges alter the salinity in the estuary and studies have shown that the salinity fluctuations affect fish communities (Gilmore, 1977; Gilmore et al., 1983), seagrass beds, oysters and other bivalves that inhabit the SLE (Sime, 2005).

The SLE is divided into three major regions: upstream, mid-estuary and downstream regions (Wilson et al., 2005). These three regions represent areas with different salinities. Salinity is highest in the downstream region, and progressively decreases upstream (Ji et al., 2007). The upstream region bifurcates into the north and south forks. The North Fork connects to the C-23 and C-24 canals, and the South Fork connects to Lake Okeechobee via the C-44 canal. The mid-estuary region is bordered by the Roosevelt Bridge to the west and the Evans Crary Bridge to the east. The downstream region is demarcated by the Evans Crary Bridge on the west, and the Indian River Lagoon on the east.

To accomplish the research goals, two separate studies were conducted: a spatial and seasonal study that was performed in the three river regions of the SLE and a lift net study that included only the mid-estuary river region of the SLE.

For the spatial and seasonal study, a restored and an adjacent natural reef were chosen for each of the three regions of the SLE (**Figure B-3**). In this study, a natural reef was defined as a reef that supported high densities of oysters and there was no obvious evidence of restoration (Rodney and Paynter, 2006). A restored reef was defined as a reef where cultch material such as fossil shell, rocks, and rubble was intentionally deployed for oyster spat to settle on it. Construction of restoration sites commenced in August 2009 and was completed July 2010 (Oyster Reef Restoration, 2011).

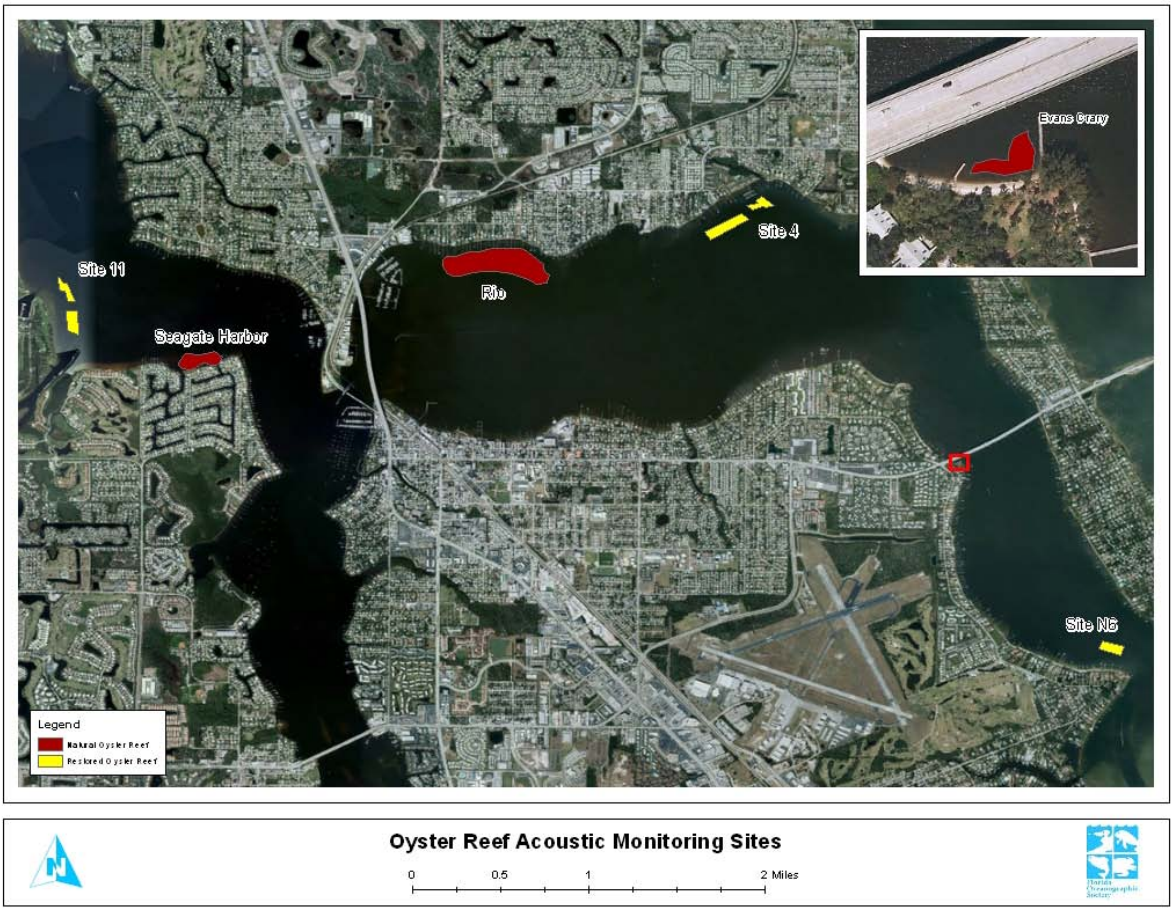

Figure B-3. Position of restored (yellow) and natural (red) oyster reefs monitored acoustically in the three regions of the St. Lucie Estuary (upstream, mid-estuary, and downstream). The St. Lucie Estuary is characterized by a salinity gradient where salinity is lowest upstream and highest downstream. The St. Lucie Estuary is located on the southeast of Florida.

The upstream region natural reef is located adjacent to the seawall for the Seagate Harbor neighborhood. The upstream region restored reef is Site 11. The mid-estuary natural reef is the Rio reef, which is located on the northern shores of the SLE. It is the most extensive natural reef in the SLE. The mid-estuary restored reef is Site 4, also located on the northern shores of the SLE 1 km east of the Rio reef. The downstream natural reef is located just south of the western part of the Evans Crary Bridge. The downstream restored reef is Site N6 located in the Hell's Gate region of the SLE (see **Table B-1** for GPS locations for each site).

Table B-1. Geographic coordinates of study site locations in the St. Lucie Estuary.

Reef Name	Region	Habitat Type	Latitude (N)	Longitude (W)
Site 11	Upstream	Restored oyster reef	27˚12.587'	80˚16.980'
Site 11	Upstream	Barren bottom	27˚12.598'	80˚16.946'
Seagate Harbor	Upstream	Natural oyster reef	27˚12.293'	80˚16.414'
Site 4	Mid-estuary	Restored oyster reef	27˚13.104'	80˚13.649'
Site 4	Mid-estuary	Barren bottom	27˚13.133'	80˚13.686'
Rio	Mid-estuary	Natural oyster reef	27˚12.742'	80˚16.586'
N6–Hell's Gate	Downstream	Restored oyster reef	27˚10.904'	80˚11.827'
Evans Crary	Downstream	Barren bottom	27˚11.812'	80˚12.543'
Evans Crary	Downstream	Natural oyster reef	27˚11.841'	80˚12.618'

For the lift net study, only one natural reef and one restored reef were chosen from the mid-estuary region. The mid-estuary region was selected for this study because the salinity at the mid-estuary region is intermediate between the upstream and downstream sites (Ji et al., 2007). The Rio reef, the most extensive natural reef in SLE, was chosen as the natural reef. The restored reef is Site 4 located on the northern shores of the SLE.

Experimental Design

Seasonal and Spatial Variation in Oyster Reef Bio-Acoustics

The study was divided into two sets of experiments: (1) spatial and seasonal study and (2) lift net study. For the spatial and seasonal study, acoustic signatures of natural and restored reefs were compared. Sampling was conducted during 4 days in the dry season (cool, dry season: 2 and and 16 February; warm, dry season: 7 and 16 June 2011) and during 2 days in the wet season (warm, wet season: 18 and 30 August 2011) at each site. Due to an exceptionally long dry season in south Florida, the dry season was sampled two more days than the wet season (4 days vs. 2 days). Acoustic recordings were 30 seconds in duration, with four replicate recordings made at each site. Dry season recordings were made late in the season because of extreme drought conditions in southern Florida that lasted until the beginning of June (South Florida Water Management District [SFWMD], 2011). Preliminary recordings also were made in 2010 (see interim report). These recordings, however, were performed using a digital recorder (Olympus DS-71) with automatic gain. A portable digital recorder without volume level enhancement, Microtrack II (M-Audio), was used for all recordings made during 2011. To maintain consistency and accuracy in acoustic measurements, only sounds recorded during 2011 were used for analyses in this report.

A High Tech Inc. HTI-94-SSQ hydrophone (2-30,000 Hz range; maximum -165 dB re: 1 V/µPa; spectral 54 dB re: 1 µPa/square root Hz at 10 Hz; maximum operating depth -6,096 m) and Microtrack II 2-channel mobile digital recorder (24-bit at 96 kHz) were used for acoustic sampling. The hydrophone was attached to a buoy at a fixed distance from the buoy's anchor (30 cm from bottom) and deployed off the boat (**Figure B-4**). The boat engine was turned off while the recordings took place. Replicate sites were randomly selected each time before sampling by grid and random number selection. During preliminary recordings, barren-bottom areas near the oyster reef were sampled. Distinct differences in sound intensities were observed between barren bottom and oyster reef habitats. Recordings from barren bottom, in general, were relatively quieter compared to oyster reefs, indicating that the hydrophone was not recording sounds beyond the sampled area. Among habitat types (restored reef, natural reef and barren bottom) recordings were performed at least 100 m away from each other. Furthermore, whenever possible, point recordings within habitat type were made at least 10 m away from each other, to avoid potential overlap between recordings.

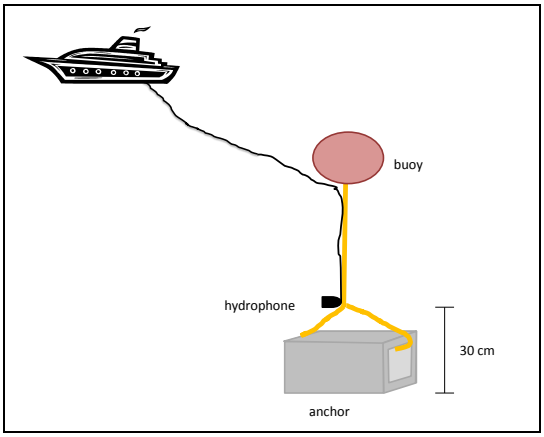

Figure B-4. Diagram (not to scale) of deployed buoy with attached High Tech, Inc. hydrophone.

Each site was acoustically sampled in the morning, at mid-day, and at dusk. Dusk is commonly defined as the period of time when illumination values are in the lower 50th percentile of each 24-hour period and rapidly decreasing (Erickson and Hightower, 2007). Recordings at the different sites were not done

simultaneously because of equipment limitations. It took approximately 2 hours to record all sites, therefore, temporal variation among the sites was probably not due to differences in the time of the recording. Temporal differences are noticeable over longer periods of time (>2 hr; Radford et al., 2008).

Sampling of Faunal Assemblages – Lift Net Study

The lift net study used four replicate nets (0.25 m^2) with a 3 mm-mesh size. A volume displacement of 4 L of oyster shell was placed in each net. The nets were then deployed in a natural and in a restored oyster reef at the mid-estuary region of the SLE. Shell for the lift nets was excavated from the reef where the lift net was placed. The shell was not cleaned or scraped off, therefore, the shell in the lift net was of the same material and age as the oyster reefs where it was placed. Lift nets (Crabtree and Dean, 1982) used for the collection of oyster reef inhabitants were constructed of 3.2-cm diameter PVC pipe, the netting was 3-mm mesh with a bag depth of 0.5 m. Lift nets were secured to the substrate with stakes. After approximately 30 days, the lift nets and substrate were pulled out along with the organisms that inhabited the shell.

Dry season nets were deployed on 2 May 2011 and collected on 10 June 2011 (33 days). The wet season nets were deployed on 12 July 2011 and collected on 10 August 2011 (30 days). A few days prior to collection, the eight nets were sampled acoustically at dawn, dusk, and noon. The lift net study used the same hydrophone, recorder, and methodology that were described for the spatial and seasonal study.

After the *in situ* sound surveys, the lift net contents (organisms and shells) were collected and brought back to the lab in aerated water collected on site. In the lab, all the decapod crustacean and fish species were identified to the lowest possible taxonomic level. The number of live and dead oysters was calculated for each net. Only articulated empty shells were considered as dead oysters. Average shell heights were calculated for each net from 10 randomly selected oysters. Afterwards, the contents of each lift net were placed in separate 3-gallon tanks. The lift net contents were acclimated for a period of 30 minutes before being placed in a flow through system at the Florida Oceanographic Society (FOS) Coastal Center in Stuart, Florida. The water from the flow through system at FOS was pumped directly from the ocean. All lift nets samples were exposed to the same water conditions.

Data Analysis

Sound Analysis

To facilitate understanding of this acoustical study, important terms are defined next. Sound is what we hear. In marine environments, waves of sound energy travel through water as vibrations of the fluid particles (i.e., water). These vibrations reach our ears and exert pressure on our eardrums enabling humans to hear. Human ears detect the relative loudness between two sounds in a logarithmic scale. Hence, the scale for sound intensity is logarithmic, and it is denoted in decibels (dB). Sound has different properties such as frequency, amplitude, and intensity. Frequency is the number of oscillations per second; it is the property of sound that determines its pitch. Acoustic intensity is a fundamental measure of the propagation of sound. The intensity, power, and energy of an acoustic wave are proportional to the average of the pressure squared (mean square pressure) (Richardson et al., 1998).

Sound can be represented in time series, spectrum, or spectrogram (**Figures B-5** to **B-7**). The time series displays raw digitized audio data with time on the x-axis and amplitude on the y-axis. The spectrum displays frequency on the x-axis and amplitude on the y-axis. The spectrogram displays time on the x-axis, frequency on the y-axis, and amplitude shows in color. In this study, the spectral analysis total power was calculated. Total power is defined as the total root mean square (rms) power level for the entire spectrum. Power is the measure of acoustic energy per unit of time (Richardson et al., 1998). In other words, intensity, power, and energy represent the loudness of a sound.

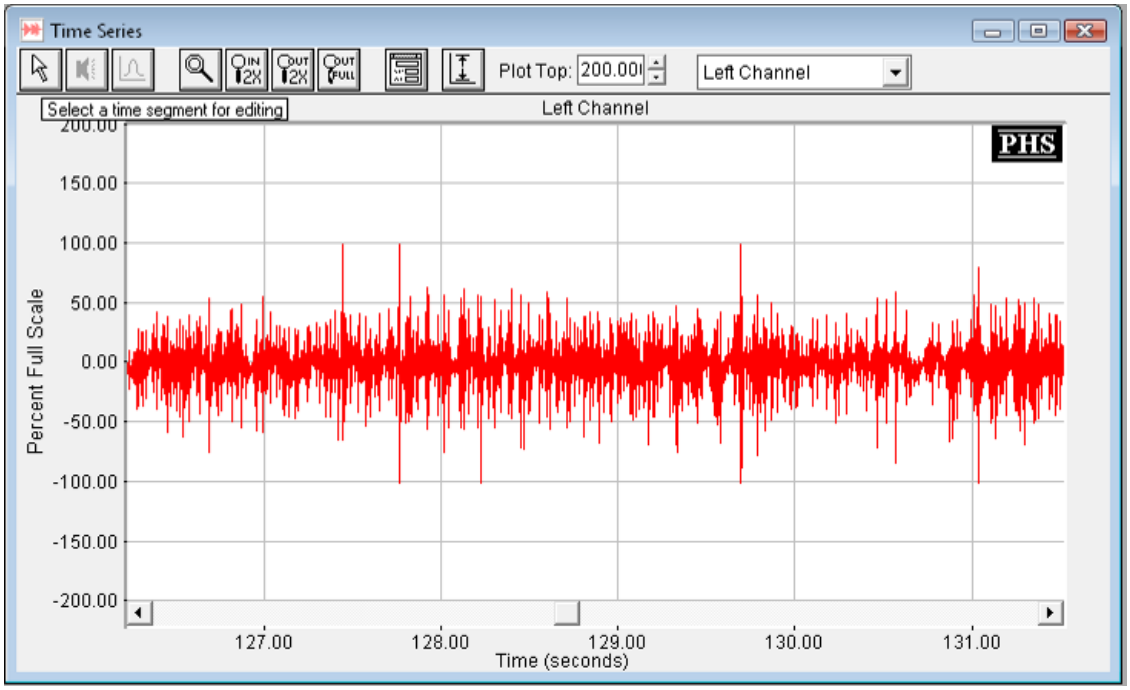

Figure B-5. Time series for a recording in the St. Lucie Estuary. Snapping shrimp and fish sounds are present in the recording. The x-axis shows time in seconds; the y-axis shows intensity level. (Frame captured from Spectra Plus.)

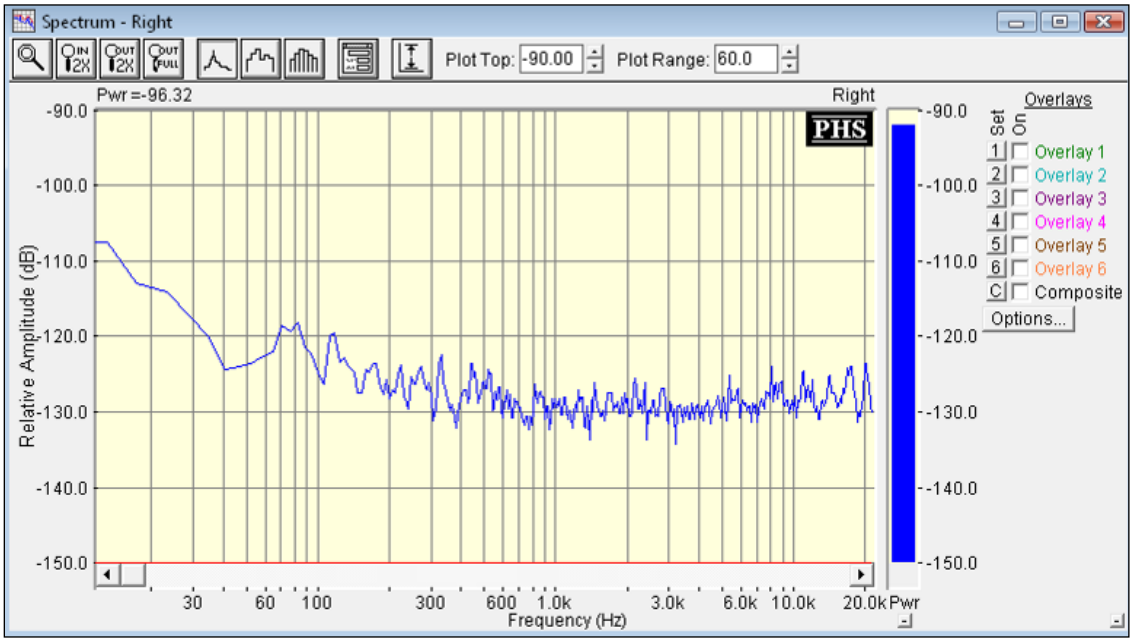

Figure B-6. Spectrum for a recording in the St. Lucie Estuary. Snapping shrimp and fish sounds are present in the recording. The x-axis shows frequency (Hz); the y-axis shows intensity level in decibels (dB). (Frame captured from Spectra Plus.)

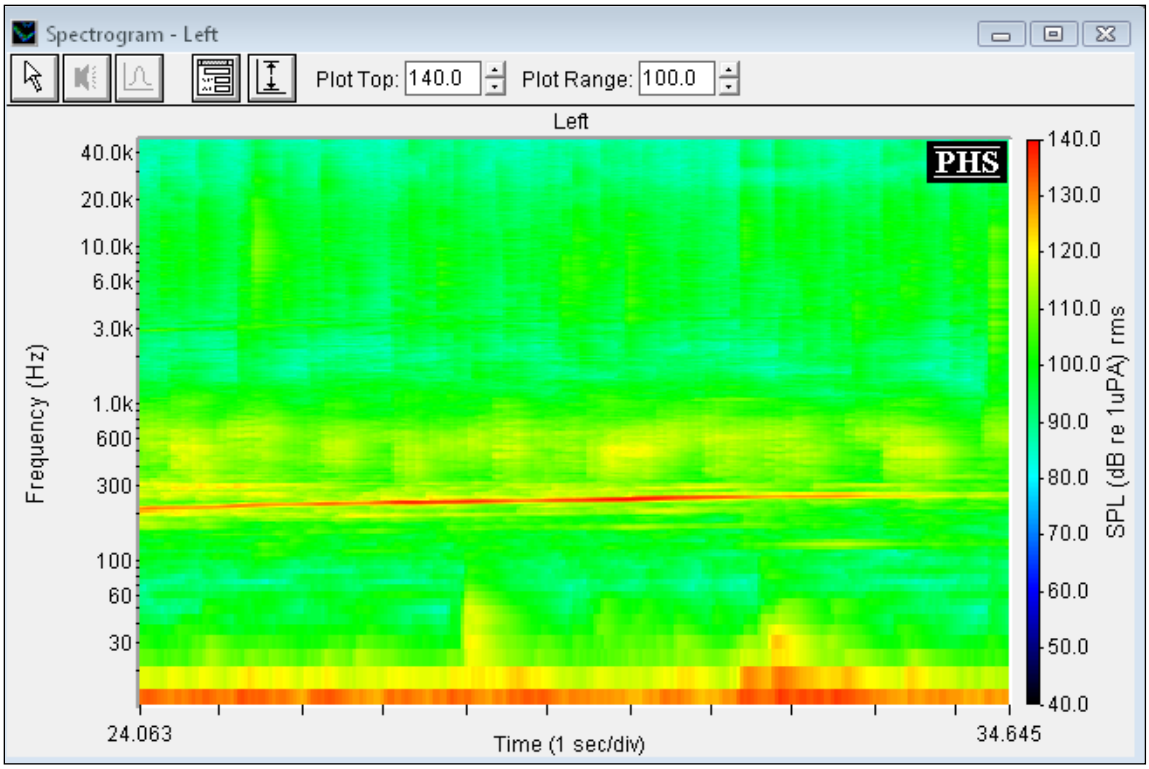

Figure B-7. Spectrogram for a recording in the St. Lucie Estuary. Snapping shrimp and fish sounds are present in the recording. The x-axis shows time (1 sec/div); the y-axis shows frequency (Hz). The color scale indicates intensity in sound pressure level (dB re: 1µPa). (Frame captured from Spectra Plus.)

The sound analysis was the same for both the spatial and seasonal study and the lift net study. The acoustic samples were filtered with a 2,500 to 20,000 Hz band-pass (dominant snapping shrimp frequency; Au and Banks, 1998) using GoldWave by GoldWave software (GoldWave Inc., St. John's, Newfoundland). Sound samples were analyzed with Matlab (MathWorks, Natick, Massachusetts) to count the number of snapping shrimp snap spikes above a predetermined pressure threshold. Radford et al. (2010) reported that snapping shrimp snaps have a specific pressure threshold (4 µPa). In a spectrogram, every point on the sound wave that peaked above this pressure threshold was counted as a snap. This method allowed a broad calculation of the number of snaps for each acoustic sample.

In addition, SpectraLab (Pioneer Hill Software, Poulsbo, Washington) and SpectraPro (Sound Technology, Inc.) software were used to generate spectra (frequency vs. amplitude) for each recording, total power for the 2,500 to 20,000 Hz frequency band was calculated for each recording. SpectraPro allows the spectrum for the entire sound replicate to produce a standard curve. Settings for spectral analyses were standardized at a sampling rate of 44,100 Hz, Fast Fourier Transform (FFT) of 1,024 overlap percentage of 0.0% (thus giving a frequency resolution of 43 Hz), decimation of 1, Hamming smoothing window, average size 2, logarithmic amplitude scaling , linear frequency scaling, 16 bit mono, with flat standard spectral scaling. Spectral range was set at 20,000 Hz with linear rather than log images. Linear spectral images were preferred because oyster reef invertebrate sounds, particularly alpheid shrimp sounds, are broad-band sounds often extending from less than 1,000 Hz to greater than 20,000 Hz. Replicate spectral analysis produces a maximum dB – frequency curve based on maximum dB levels sampled throughout the 10 to 20,000 Hz range of the replicate. This curve is analyzed for harmonic signatures and each set frequency interval can build a spreadsheet containing the

dB value for each frequency value, and allows other acoustic characters to be stored as spreadsheets for statistical analyses and as an overlay. Each spectral curve is stored as an overlay labeled to site, time and replicate number to allow a comparison between at least three other spectral curves from other analyses. In this study these various curves are color coded and labeled in all illustrations.

SpectraLab and SpectraPro automatically calculate the frequency of the maximum recorded decibel level (peak amplitude, dB × 10 re: 1µPa), total rms power level of the sound sample, and the signal to background sound ratio (SNR). All these values were used for analyses. However, it should be noted that the entire spectral sample is used for these values, thus including low-frequency ambient sounds that can be associated with water noise or other physical phenomena. It is uncertain whether these ambient sounds were included in the spectral sample as a detailed study of all sounds originating from an oyster reef have not been conducted. A long-term goal is to classify all invertebrate, fish, and physical sounds produced in these complex hard bottom communities. That is beyond the scope of this study.

Statistical Analysis

Seasonal and Spatial Study

Pearson correlation analysis was used to test for any correlation among response variables (number of snaps and power) to environmental variables (salinity, water temperature, and dissolved oxygen). A multivariate analysis of variance (MANOVA) was used to test the effect of season, region, habitat, and day period on these response variables because there was a significant correlation between number of snaps and power. To homogenize variances, the number of snaps and total power variables were log transformed (log+1). All data were analyzed using Statistical Analysis Software (SAS), Cary, North Carolina.

Lift Net Study

Pearson correlation analysis was used to test for correlations in response variables (number of snaps and total power) and in the environmental variables (salinity, water temperature, and dissolved oxygen). ANOVA was used to analyze the effect of season, day period, and habitat type on number of snaps. To homogenize variances, the number of snaps, total power, and number of species were log transformed (log+1). All data were analyzed using SAS.

Influence of Decreased Salinity on Snapping Shrimp Activity – Comparison of Dry vs. Wet Periods

The wet season in southeast Florida typically starts in the summer and ends in the early fall (Ji et al., 2007). However, the 2011 dry and wet seasons in southeast Florida were unusual. The dry season was extremely long, and the wet season was short and relatively dry. The onset of the wet season was dry and it was not until later in the season that the salinities in the SLE decreased. For this reason, we compared number of snapping shrimp snaps at the start and at the end of the wet season. For the beginning of the wet season, we sampled on 18 and 30 August, and for the end of the wet season, we sampled on 27 October 2011. ANOVA was used to compare the effects of season (beginning of wet season [dry/wet] and end of wet season) on snapping shrimp snaps.

Qualitative Acoustic (Spectra and Time Series) Analyses

Additional qualitative analyses of spectra (frequency vs. amplitude) and time series were also performed for the 2011 data. The data used for this study was taken over a period of two years at nine study sites five of which was used in the following analyses. Hydrophone sampling was performed as previously described (four 30-sec recordings of each habitat type). The following analyses were made using SpectraPro sound analysis software on 10 to 30 sec subsamples of four replicates taken randomly from each recording site and session.

Influence of Salinity on Acoustic Signatures (Spectra and Time Series)

In addition to examining potential salinity influences on snapping shrimp activity (number of snaps) spectra and time series were compared to determine the influence of salinity. Since there is a documented influence of water temperature on sound production rates in alpheid shrimp, the principal sound producer in oyster reefs, winter sound recordings were eliminated from the salinity analyses but included in spatial comparisons between recording sites. Warm dry (15 June and 5 July) and warm wet (18 and 30 August) season recordings were used for the comparative salinity studies. Recordings were made at each study site during the morning between 0500 and 1000 hr; afternoon between 1100 and 1500 hr; and dusk between 1600 and 2130 hr.

Time series, spectral, and spectrograph images were made of each replicate series to compare with times, stations, and replicates. Time series studies allowed replicates with extraneous sounds to be eliminated. Extraneous sounds were often produced by unknown yet rarely heard biological sound sources, wave action, boat sounds, anchor drags, and other mechanical non-biological sounds. Only replicates that revealed consistent sound levels, typically only alpheid shrimp snaps, were used for the analyses. When all four 30-sec replicates contained considerable extraneous sound they were subsampled to 10-sec sections comprising alpheid shrimp snap sounds for the comparative studies. This only occurred once at Site 6. All replicates (n = 76) for particular sites and time of day were examined for quality and extraneous sounds. Those burdened with large sections of extraneous sound were eliminated from these analyses.

Diel time series analyses of replicates from upstream and downstream stations (n = 120) indicated that extraneous sound production was lowest during morning recordings (0500 to 1000 hr). For this reason, dawn recordings were used for all of the analyses presented in this report. On occasion, all 30-sec files furnished for a location and time contained too much extraneous noise to use the entire sample and 10-sec subsamples were taken that were free of interference sounds. Detailed study of extraneous sounds (non-alpheid shrimp bio-acoustics, physical environmental sounds) and diel bioacoustic patterns associated with oyster reefs is possible from these data, but are not included due to time constraints and degree of relevancy to the primary goals of this study. A detailed classification and comparative analysis of extraneous sounds produced in a 30-sec replicate often takes several hours.

Three locations were used for salinity influence studies: Site 6, Seagate Harbor, and Site 11. Site 6 is a restored reef site located near the mouth of the St. Lucie River at Hells Gate. This site was always tidally influenced with salinities varying from 34.3 to 18.6 psu. Acoustic patterns from Site 6 were compared to those produced at two upstream sites, a natural oyster reef labeled as Seagate (Seagate Harbor) where salinities ranged from 10.0 to 29.5 psu and an adjacent restored oyster reef site (Site 11) where salinities ranged from 8.8 to 29.3 psu (**Figures B-23** to **B-28**; **Table B-7**).

The previously described stations allowed a comparison between adjacent oyster reefs upstream, restored oyster reef Site 11 and one natural oyster reef Seagate Harbor, that have nearly identical regional environmental parameters of salinity and water temperature. Both these sites are impacted by lower salinities than downstream sites and have a more variable low salinity regime. These latter sites are compared to a high salinity restored oyster reef site, Site 6, near the mouth of the St. Lucie River. Therefore, both temporal and spatial salinity effects are examined.

Acoustic Signature (Spectra and Time Series) Comparisons between Natural and Restored Oyster Reefs

Two adjacent locations that experienced similar salinity and temperature regimes were compared to determine acoustic signatures from restored oyster reefs, Site 4, and the Rio reef, a natural oyster reef. In this case, seasonal changes in temperature were not a factor, as all sites had similar seasonal water temperature regimes. Therefore, winter-dry season, 16 February, recordings were included in the study.

RESULTS

Salinity and Temperature Data

Salinity and water temperature data were obtained from DBHYDRO, an environmental database operated and maintained by the SFWMD. Salinity data were obtained at stations SE01, SE02, and HR1 located near the downstream, mid-estuary and upstream sites, respectively. Salinity and water temperature data were collected from 1 February to 30 August 2011. Water temperature data were collected from the mid-estuary station (SE02). Data are reported as averages with standard deviations. Mean salinities during acoustic sampling days at upstream, mid-estuary, and downstream were: 26.0 psu ± 4.1 SD, 29.8 psu ± 4.6 SD, and 32.5 psu ± 3.7 SD, respectively. Average salinity during the wet season at the three sites was 23.6 psu ± 4.44 SD whereas salinity during the dry season was 31.6 psu ± 3.4 SD. Salinity in the mid-estuary, during the wet season was 24.34 psu ± 4.42 SD and 32.20 psu ± 1.69 SD during the dry season. Water temperature in the mid-estuary during the wet season was 29.88 °C ± 0.63 SD and 24.01 °C ± 3.51 SD during the dry season.

Seasonal and Spatial Study

The number of snaps and power were correlated ($r=0.62$; $n=648$; $p=<0.0001$); therefore, the effects of season, region, habitat, and day period on the number of snaps and power were analyzed using MANOVA. Season, region, habitat, and day period had a significant effect on number of snaps and total power. **Table B-2** shows the Wilk's lambda values and significance; **Table B-3** shows the values for each protected ANOVA. Total power and number of snaps were significantly higher during the wet season compared to the dry season (**Figure B-8**). Both power and number of snaps were highest in the mid-estuary river region; however, total power was lowest at the upstream region, and number of snaps was lowest at the downstream region (**Figure B-9**). Total power was highest at the restored reef followed by the natural reef. Number of snaps was higher at the natural reef than at the restored reef. The barren-bottom habitats had the lowest total power and number of snaps (**Figure B-10**). Total power and number of snaps were highest during dusk (**Figure B-11**); however, there was a significant interaction between season and day period. Total number of snaps was highest during dusk and lowest during dawn in the wet season, but total power was highest at mid-day during the wet season (**Figure B-12**). The interaction between region and day period was significant (**Figure B-13**). Dusk had higher power and snaps than the other two-day periods. The mid-estuary region had highest power and number of snaps, but at dawn power was highest at the downstream site. The effects of season, region, and day period was the only significant three-way interaction (Wilk's=0.9301; n DF=8; sig<0.0001; **Figures B-14** and **B-15**). During dry season, the mid-estuary had higher power and number of snaps, but in the wet season number of snaps was highest downstream at mid-day.

Table B-2. MANOVA results for the effects of season (wet vs. dry), region (upstream, mid-estuary, and downstream), habitat (restored, natural, and barren bottom), and day period (dawn, mid-day, and dusk) on power and number of snaps.

Multivariate	S	R	S*R	H	S*H	R*H	S*R*H	D	S*D	R*D	S*R*D	H*D	S*H*D	R*H*D	S*R*H*D
Wilk's Lambda	0.987	0.9116	0.9714	0.8318	0.9916	0.8331	0.976	0.8946	0.9735	0.9357	0.9301	0.9851	0.9865	0.9714	0.972
N Degrees of freedom	2	4	4	4	4	8	8	4	4	8	8	8	8	16	16
D Degrees of freedom	593	1,186	1,186	1,186	1,186	1,186	1,186	1,186	1,186	1,186	1,186	1,186	1,186	1,186	1,186
F value	3.92	14.05	4.34	28.6	1.26	14.16	1.82	16.98	4	5.01	5.4	1.12	1.01	1.8	1.06
N	295.5	295.5	295.5	295.5	295.5	295.5	295.5	295.5	295.5	295.5	295.5	295.5	295.5	295.5	295.5
Significance	0.0203	<0.0001	0.0017	<0.0001	0.2856	<0.0001	0.0702	<0.0001	0.0031	<0.0001	<0.0001	0.3485	0.4279	0.3675	0.3908
Significance	**	***	***	***	NS	***	*	***	***	***	***	NS	NS	NS	NS

***p=0.005, **p=0.05 significance, *p<0.10.

NS = not significant (p≥0.05); S = season; R = region; H = habitat; D = day period. Response variables: Power log (power+1) and Snaps log (snaps+1).

Table B-3. Protected univariate ANOVA is presented for factors that were significant in the multivariate test (Wilk's lambda). For all univariate tests, the error DF=594. Response variables and covariate were log (x+1).

Power R^2=0.3416	S	R	S*R	H	S*H	R*H	S*R*H	D	S*D	R*D	S*R*D	H*D	S*H*D	R*H*D	S*R*H*D
Degrees of freedom	1	2	2	2	2	4	4	2	2	4	4	4	4	8	8
MS	0.0506	0.1442	0.0682	0.2373	0.006	0.0491	0.0065	0.2652	0.0522	0.0648	0.0814	0.0101	0.0064	0.0091	0.0059
F-value	6.04	18.41	8.14	28.33	0.72	5.87	0.77	32.01	6.23	7.73	9.71	1.2	0.76	1.09	0.71
Significance	0.014	0.0001	0.0003	0.0001	0.4885	0.0001	0.5445	0.0001	0.0021	0.0001	0.0001	0.3088	0.5521	0.3696	0.6872
Significance	**	***	***	***	NS	***	NS	***	***	***	***	NS	NS	NS	NS
Snaps R^2=0.3029	S	R	S*R	H	S*H	R*H	S*R*H	D	S*D	R*D	S*R*D6	H*D	S*H*D	R*H*D	S*R*H*D
Degrees of freedom	1	2	2	2	2	4	4	2	2	4	4	4	4	8	8
MS	27.933	16.5624	23.5094	209.5957	5.5993	52.7827	12.6974	26.3399	1.03	11.1498	7.4068	3.4383	1.6571	5.4183	4.333
F-value	6.63	3.93	5.58	49.71	1.33	12.52	3.01	6.25	0.24	2.64	1.76	0.82	0.39	1.29	1.03
Significance	0.0103	0.0202	0.004	0.0001	0.2658	0.0001	0.0178	0.0021	0.7833	0.0328	0.136	0.5155	0.8137	0.2483	0.4136
Significance	*	**	**	***	NS	***	**	**	NS	**	NS	NS	NS	NS	NS

***p=0.005, **p=0.05 significance, *p<0.10.
NS = not significant (p≥0.05); S = season; R = region; H = habitat; D =day period.

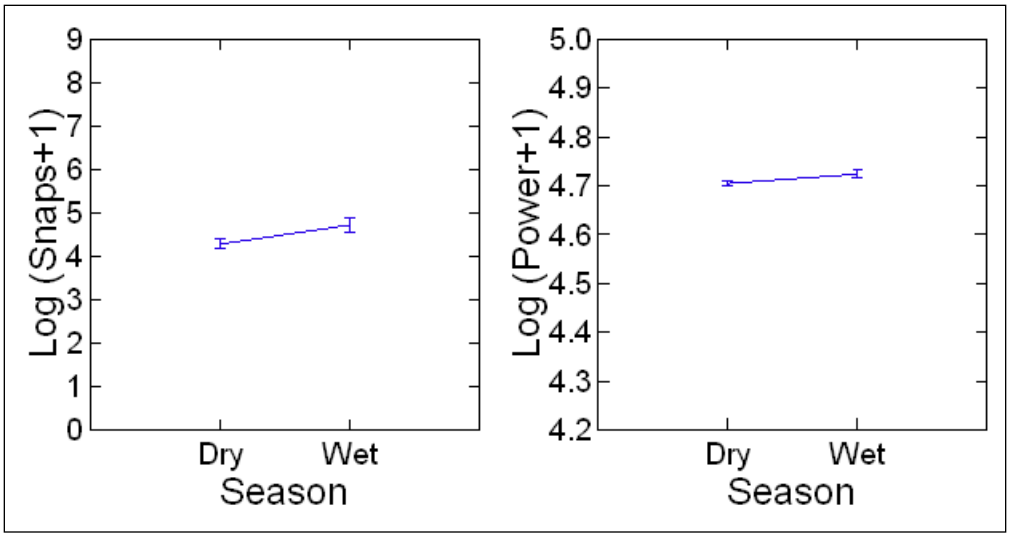

Figure B-8. The effect of season (wet and dry seasons) on number of snapping shrimp snaps and total power for the 2,500 to 20,000 Hz frequency band. Data were log transformed log (x+1) to homogenize variances. The unit for total power is dB re:1 μPa; the number of snaps was calculated for a 30-sec recording (number of snaps per 30 sec). Means and standard errors are shown.

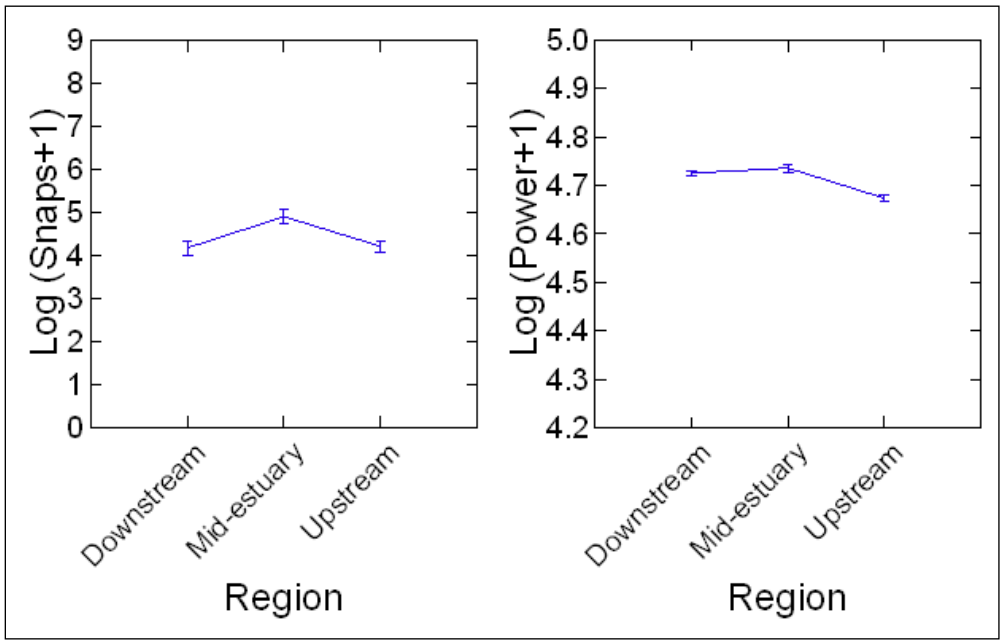

Figure B-9. The effect of region (downstream, mid-estuary, and upstream) on number of snapping shrimp snaps and total power for the 2,500 to 20,000 Hz frequency band. Data were log transformed log (x+1) to homogenize variances. The unit for power is dB re:1 μPa; the number of snaps was calculated for a 30-sec recording (number of snaps per 30 sec). Means and standard errors are shown.

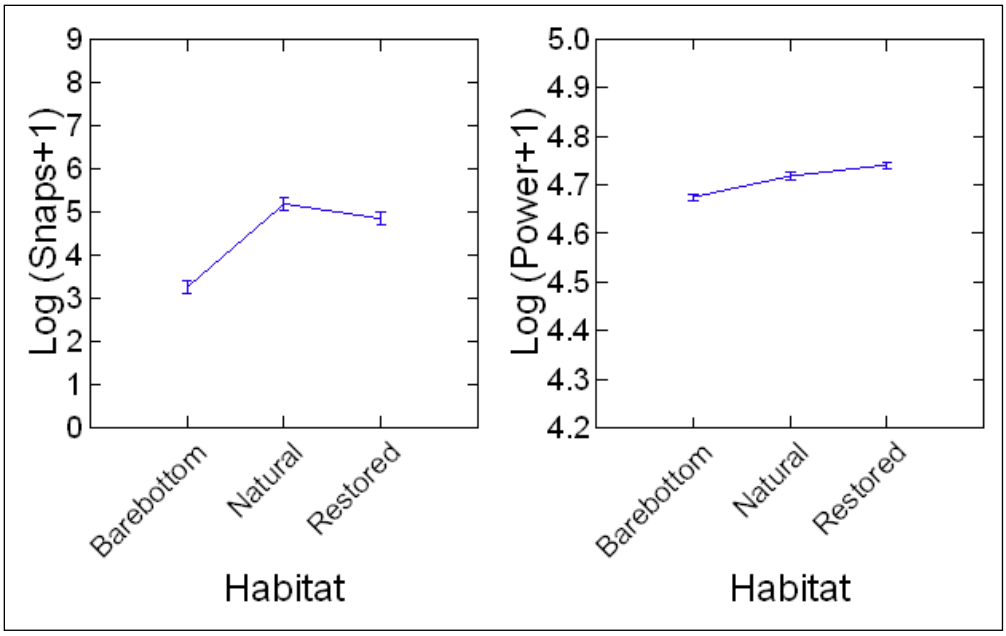

Figure B-10. The effect of habitat (barren bottom, natural, and restored) on number of snapping shrimp snaps and total power for the 2,500 to 20,000 Hz frequency band. Data were log transformed log (x+1) to homogenize variances. The unit for power is dB re:1 µPa; the number of snaps was calculated for a 30-sec recording (number of snaps per 30 sec). Means and standard errors are shown.

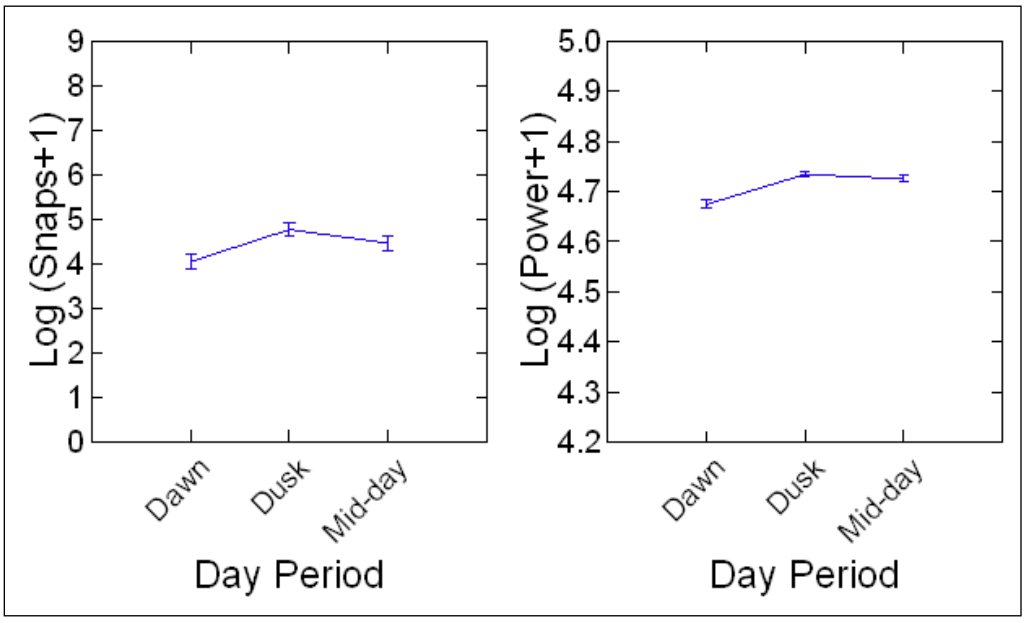

Figure B-11. The effect of day period (dawn, dusk and mid-day) on number of snapping shrimp snaps and total power for the 2,500 to 20,000 Hz frequency band. Data were log transformed log (x+1) to homogenize variances. The unit for power is dB re: 1µPa; the number of snaps was calculated for a 30-sec recording (number of snaps per 30 sec). Means and standard errors are shown.

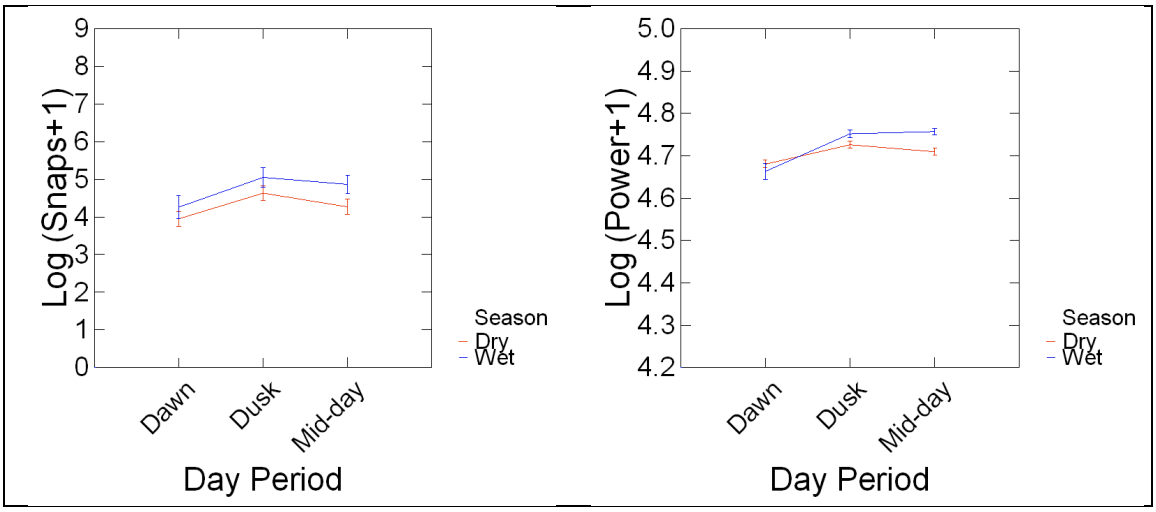

Figure B-12. The interaction effect of season (wet and dry) and day period (dawn, dusk, and mid-day) on number of snapping shrimp snaps and total power for the 2,500 to 20,000 Hz frequency band. Data were log transformed log (x+1) to homogenize variances. The unit for power is dB re:1 µPa; the number of snaps was calculated for a 30-sec recording (number of snaps per 30 sec). Means and standard errors are shown.

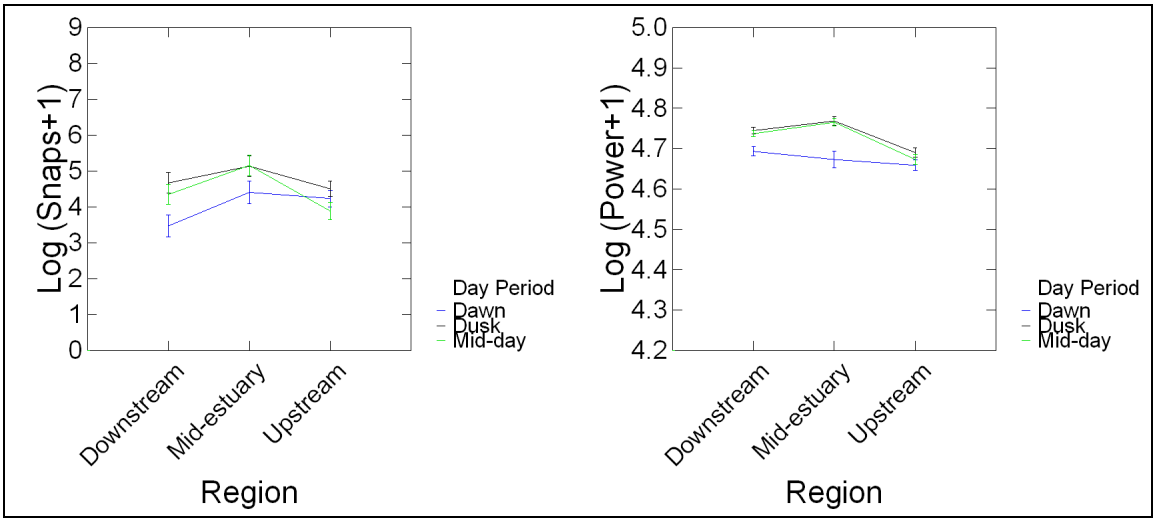

Figure B-13. The interaction effect of region (downstream, mid-estuary, and upstream) and day period (dawn, dusk, and dawn) on snapping shrimp snaps and total power for the 2,500 to 20,000 Hz frequency band. Data were log transformed log (x+1) to homogenize variances. The unit for power is dB re:1 µPa; the number snaps was calculated for a 30-sec recording (number of snaps per 30 sec). Means and standard errors are shown.

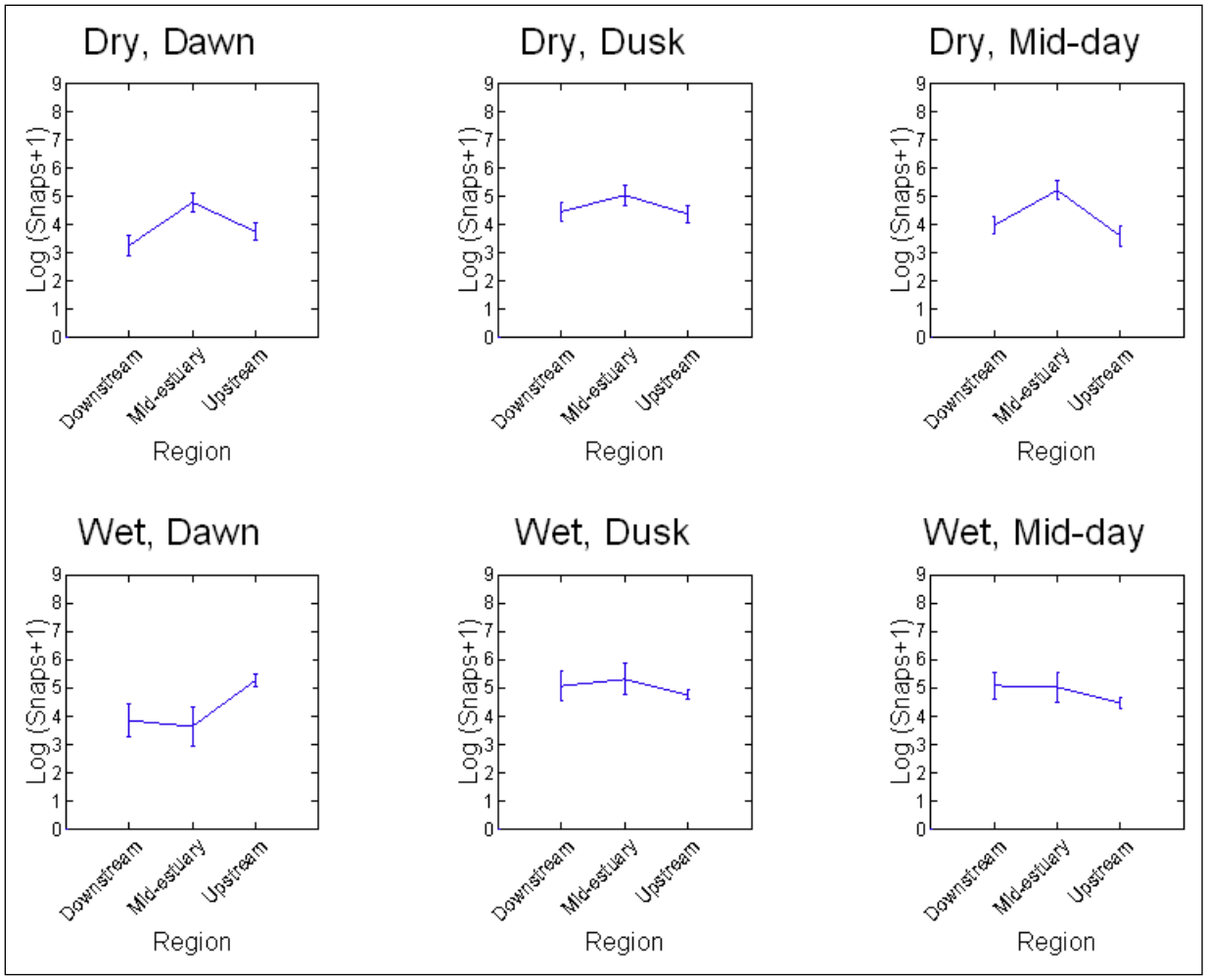

Figure B-14. The interaction effect of season (wet and dry), region (downstream, mid-estuary, and upstream) and day period (dawn, dusk, and dawn) on number of snapping shrimp snaps. Data were log transformed log (x+1) to homogenize variances. The number of snaps was calculated for a 30-sec recording (number of snaps per 30 sec). Means and standard errors are shown.

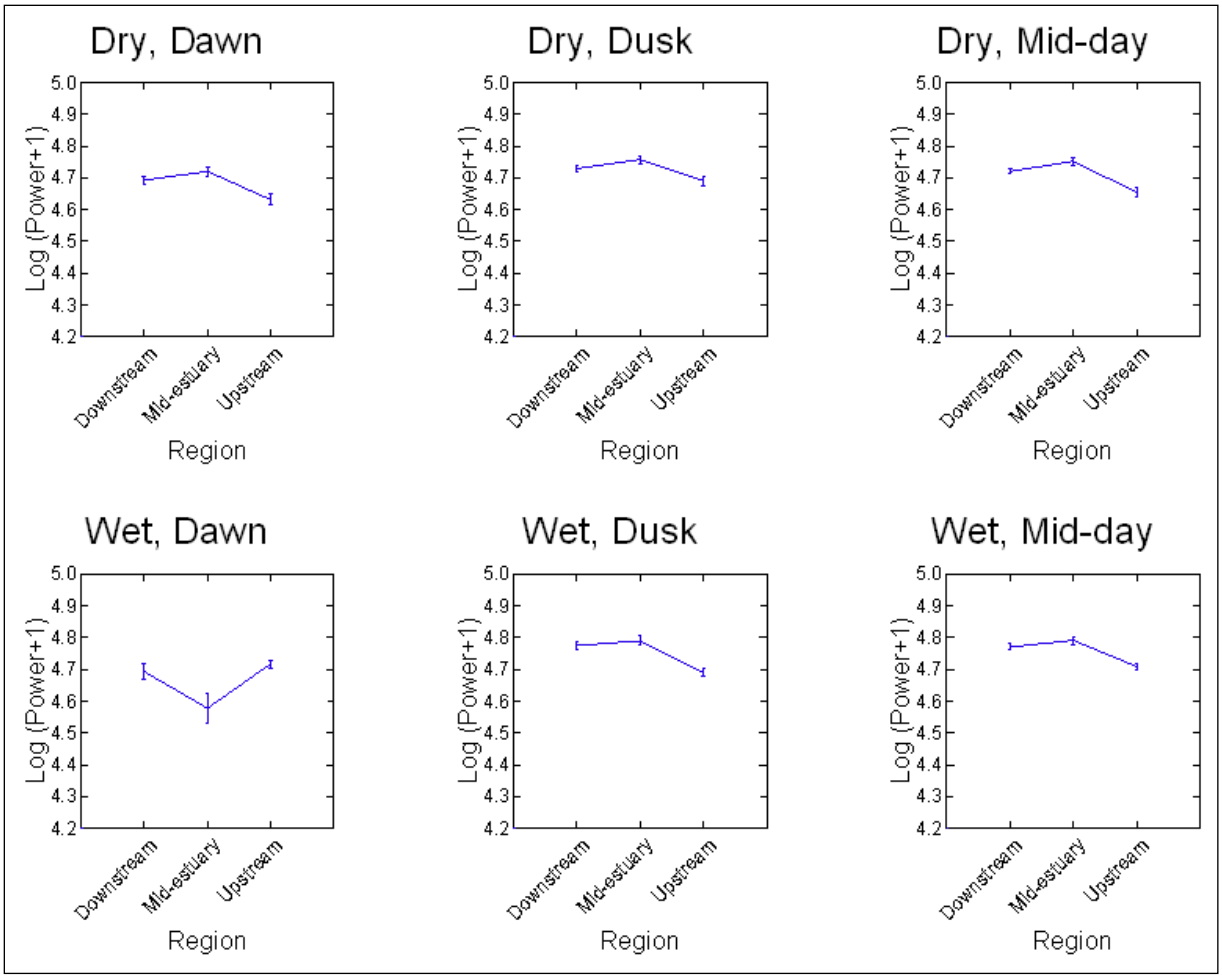

Figure B-15. The interaction effect of season (wet and dry), region (downstream, mid-estuary, and upstream) and day period (dawn, dusk, and dawn) on total power for the 2,500 to 20,000 Hz frequency band. Data were log transformed log (x+1) to homogenize variances. The unit for power is dB re: 1µPa. Means and standard errors are shown.

Lift Net Study

Oyster Survival

Four lift nets were sampled at each reef during wet and dry seasons. During the dry season oyster survival was very similar between the natural and restored reefs. The average number of live oysters found in the dry season at the natural reef was 81.75 ± 12.91 SD and 150.50 ± 74.93 SD at the restored reef. The average number of live oysters found in the wet season at the natural reef was 213.50 ± 68.40 SD and 117.00 ± 18.40 SD at the restored reef. Percent of live oysters was higher during the wet season than during the dry season. During the wet season, percent live oysters were 93% at the natural reef and 96% at the restored reef; whereas, it was 91% at the natural reef and 94% at the restored reef during dry season. The average shell height decreased during the wet season at the natural reefs. Average shell height in natural reefs declined from 77.93 mm ± 22.93 mm SD in the dry season to 51.30 mm ± 23.38 mm SD in the wet season. The average shell height at the restored reef also decreased during the wet season. The restored reef shell heights decreased from 73.83 mm ± 37.83 mm SD in the dry season to 69.08 mm ± 17.01 mm SD in the wet season.

Species Abundances

A total of 15 decapod crustaceans and 5 fish species were identified between the wet and dry season lift net samples over both sites (**Tables B-4** and **B-5**). Decapods were both more abundant and diverse than fish. The most abundant decapod species was the *Eurypanopeus depressus* (depressed mud crab). The second and third most abundant decapod species were *Petrolisthes armatus* (porcelain crab) and *Alpheus heterochaelis* (big claw snapping shrimp), respectively. The restored reef was more diverse than the natural reef during both dry and wet seasons. *Alpheus* spp. were more abundant at the restored site than the natural site. Number of shrimp remained similar during the wet and dry seasons. Although there was no significant difference (R=0.5037; DF=1; p=0.0909) between number of species and seasons, it is important to highlight that number of species at the restored reef decreased from 12 species during dry season to 6 species in the wet season. Number of species remained the same during both seasons at the natural reef (7 species).

Table B-4. Decapod crustaceans collected at the St. Lucie Estuary oyster reefs. A restored reef and a natural reef were sampled during one wet and one dry season. The total indicates the combined number of individuals found for the four replicate lift nets at each reef.

Taxa	Common Name	Dry		Wet		Total
		Natural (Rio)	Restored (Site 4)	Natural (Rio)	Restored (Site 4)	
Alpheus heterochaelis	Bigclaw snapping shrimp	7	67	15	59	148
Alpheus spp.	Snapping shrimp	0	0	1	0	1
Alpheus normanii	Green snapping shrimp	0	0	0	4	4
Clibanarius vittatus	Hermit crab	0	2	0	0	2
Eurypanopeus depressus	Depressed mud crab	246	188	191	73	698
Hexapanopeus angustifrons	Smooth mud crab	0	1	0	0	1
Hyppolyte zostericola	Zostera shrimp	0	2	0	0	2
Libinia dubia	Decorator crab	2	4	0	0	6
Menippe mercenaria	Stone crab	1	0	0	0	1
Mithrax forceps	Spider crab	0	1	0	0	1
Palaemoneks spp.	Grass shrimp	0	123	4	0	127
Panopeus herbstii	Blackfingered mud crab	0	1	1	25	27
Panopeus lacustris	Knotfingered mud crab	2	0	0	0	2
Panopeus spp.	Panopeus spp.	13	37	0	13	63
Petrolisthes armatus	Porcelain crab	3	163	1	41	208
Petrolisthes galathinus	Porcelain crab	0	1	0	0	1
Sesarma reticulata	Marsh crab	0	0	1	0	1

Table B-5. Fishes collected at the St. Lucie Estuary oyster reefs. A restored reef and a natural reef were sampled during one wet and one dry season. The total indicates the combined number of individuals found for the four replicate lift nets at each reef.

Taxa	Common Name	Dry		Wet		Total
		Natural (Rio)	Restored (Site 4)	Natural (Rio)	Restored (Site 4)	
Bathygobius soporator	Frillfin goby	2	0	0	0	2
Gobiosoma bosc	Naked goby	9	2	1	2	14
Hypleurochilus pseudoaequipinnis	Oyster blenny	0	1	0	0	1
Myrophis punctatus	Speckled worm-eel	1	0	0	0	1
Gobiesox strumosus	Skillet fish	0	1	0	0	1

Fishes were more diverse and abundant during the dry season than the wet season. During our sampling, 16 fishes were observed during the dry season and 3 fishes during the wet season. *Gobiosoma bosc* (naked goby) was the only fish that was found in both natural and restored reefs during wet and dry season; however, its abundance was greater during the dry season. Eleven *G. bosc were collected during the dry season* and three *G. bosc* were collected during the wet season.

Sound Production and Faunal Assemblages

The number of snaps and number of species were correlated (r=0.44067; n=48; p=0.0017; **Figure B-16**). Also, number of shrimp was strongly correlated to the number of snaps (r=0.63131; n=48; p<0.0001). A regression procedure showed that number of snaps can be used to estimate the number of species (R^2=0.2023; n=48; SD=0.02938; DF=1; p=0.0013) in oyster reefs. The later model explains 20% of the variability (R^2=0.2023). Power was not correlated either to number of snaps or number of shrimp. In the spatial and seasonal study, the number of shrimp was not estimated. Season, habitat, day period, and their interactions had a significant effect on number of snaps (**Table B-5**). Numbers of species (**Figure B-17**) and numbers of snapping shrimp snaps were higher at the restored reef (**Figure B-18**). Numbers of snaps were higher at dawn (**Figure B-19**). The diel differences intensified during the wet season (**Figure B-20**). The natural reefs exhibited a more marked diel difference than the restored reefs. In addition, natural reefs had higher sound production during dawn and restored reefs had higher sound production during mid-day (**Figure B-21**).

Influence of Decreased Salinity on Snapping Shrimp Activity – Comparison of Dry vs. Wet Periods

The ANOVA results show that the number of snapping shrimp snaps was significantly higher during the start of the wet season (August 2011) than the end of the wet season (October 2011; F-value=14.65; DF=1; P-value=0.0002; **Figure B-22**). In addition, salinity at station SE 02 averaged 21.17 psu ± 5.58 SD from August to September, whereas salinity in October averaged 5.82 psu ± 5.47 SD at the same station.

Spectra and Time Series Analyses

Salinity Effects

The following series of spectral and time series illustrations for each station and wet/dry season should not be interpreted as quantitative data. **Table B-7** presents the quantitative analyses of the spectra depicted in the figures. These tabular values are most valuable in determining the relative change in biological acoustic energy measured from an oyster reef at the various ambient water salinity conditions.

Examination of all replicates from each station for all dates revealed that there was considerable variation in the variety of sound types recorded at Site 6 located proximate to the river mouth of the St. Lucie where there was greater tidal influence and therefore higher salinities than at other sites (**Figures B-23** to **B-28**). Tidal influence on organism activity and higher species richness and biodiversity at Site 6 was likely responsible for the greater acoustic diversity. Examination of variation in power, max dB and SNR for each period also shows great variation with the only trend being minimum values in max dB and power when salinities were most depressed 18.6 ppt at this location on 30 August.

The most consistent acoustic salinity response with all spectral values, max dB, power, and SNR occurred at the natural oyster reef site, Seagate (**Table B-7**). Max dB, power, and SNR all declined to minimum values as salinities declined in the wet season. The highest acoustic spectral values were observed at the highest salinity (39.5 psu) on 16 June 2011.

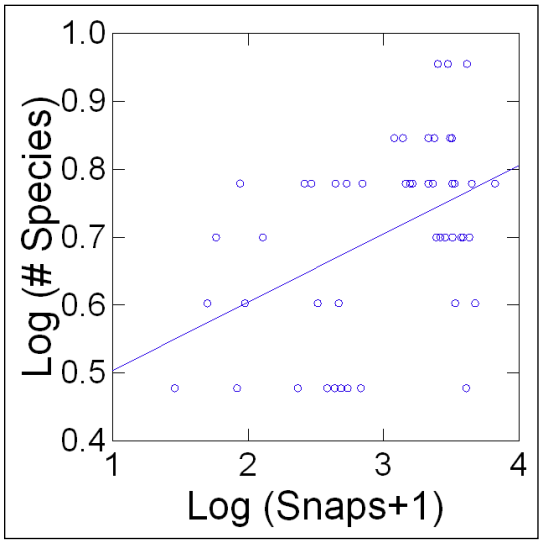

Figure B-16. Scatter plot for number of species and number of snaps at a restored reef and a natural reef in the mid-estuary region of the St. Lucie Estuary, southeast Florida. Number of species and number of snaps data were log transformed log (x+1) to homogenize variances. The number of snaps was calculated for a 30-sec recording (number of snaps per 30 sec). r=0.44067; n=48; p=0.0017.

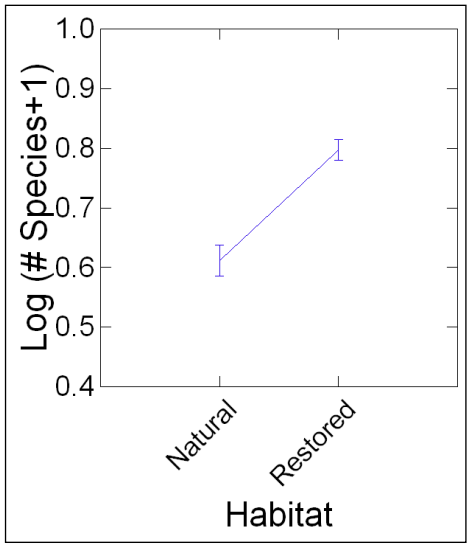

Figure B-17. Number of species at natural and restored reefs in the St. Lucie Estuary, southeast Florida. Number of species data were log transformed log (x+1) to homogenize variances. Means and standard errors are shown.

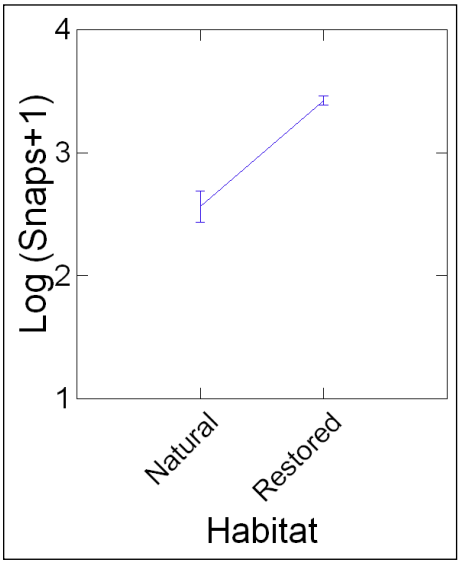

Figure B-18. Effect of habitat (natural and restored) on number of snapping shrimp snaps. Number of snaps data were log transformed log (x+1) to homogenize variances. The number of snaps was calculated for a 30-sec recording (number of snaps per 30 sec). Means and standard errors are shown.

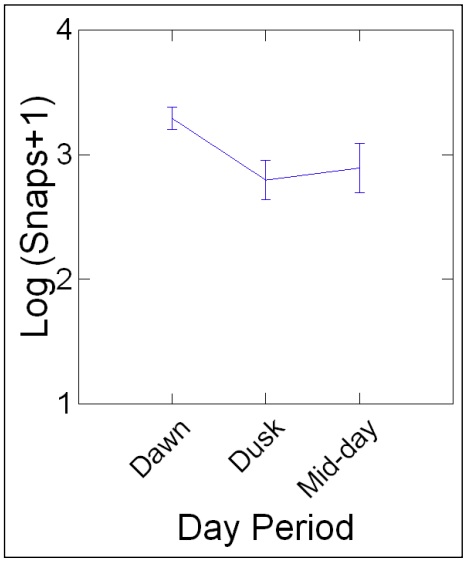

Figure B-19. Effect of day period (dawn, mid-day, and dusk) on number of snapping shrimp snaps. Number of snaps data were log transformed log (x+1) to homogenize variances. The number of snaps was calculated for a 30-sec recording (number of snaps per 30 sec). Means and standard errors are shown.

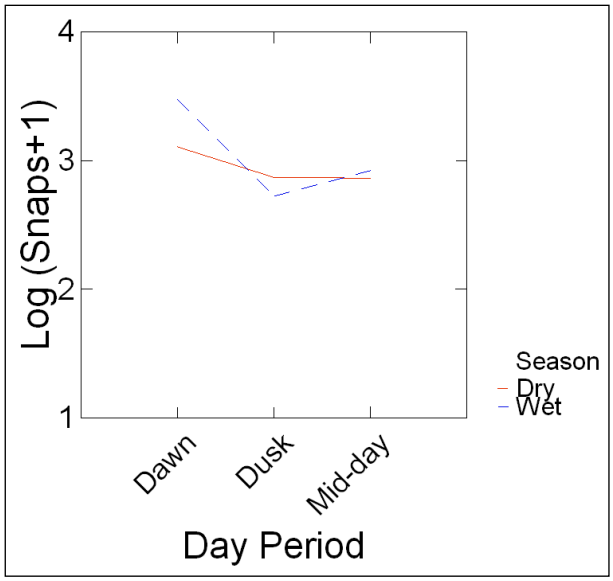

Figure B-20. Effect of the interaction of season (wet and dry) and day period (dawn, mid-day and dusk) on number of snapping shrimp snaps. Number of snaps data were log transformed log (x+1) to homogenize variances. The number of snaps was calculated for a 30-sec recording (number of snaps per 30 sec). Means and standard errors are shown.

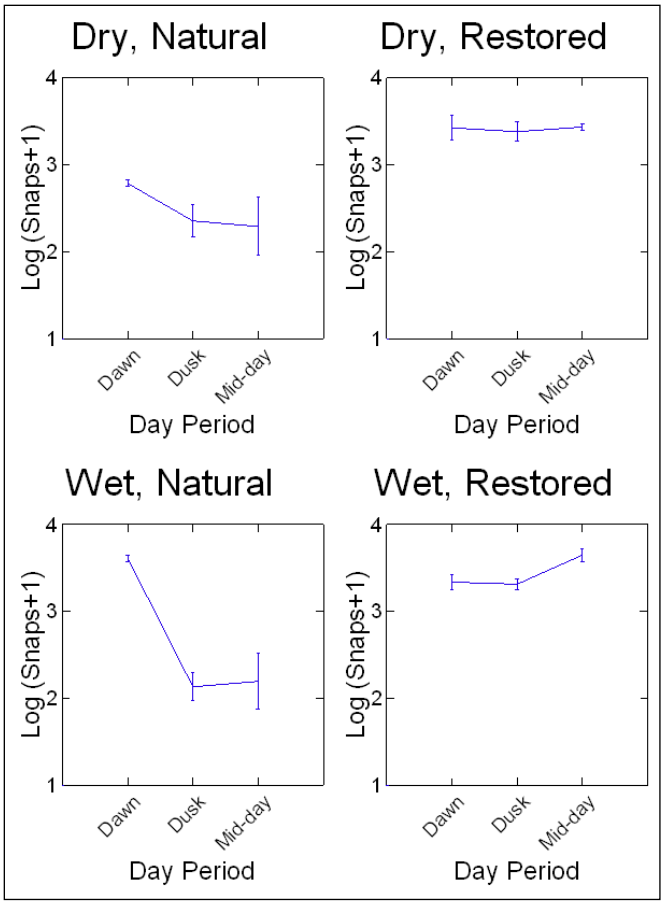

Figure B-21. Effect of season (wet and dry), habitat (natural and restored) and day period (dawn, mid-day, and dusk) on number of snapping shrimp snaps. Number of snaps data were log transformed log (x+1) to homogenize variances. The number of snaps was calculated for a 30-sec recording (number of snaps per 30 sec). Means and standard errors are shown.

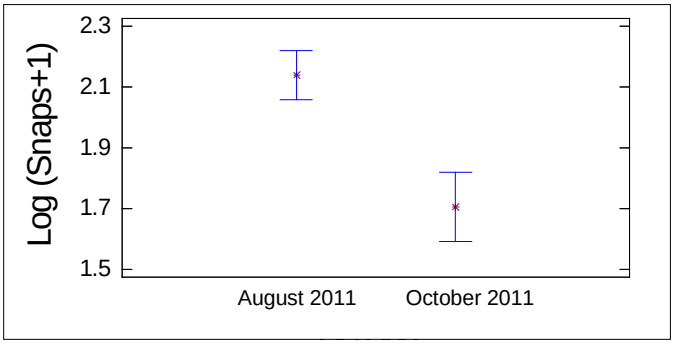

Figure B-22. Effect of wet season (August and October 2011) on number of snapping shrimp snaps measured in the mid-estuary region of the St. Lucie River. The early part of the wet season (August 2011) was characterized by still, dry conditions with regards to salinity (21.2 psu ± 5.6 SD) as a result of lingering drought conditions. Subsequent rainfall throughout the remainder of the dry season resulted in much lower mid-estuary salinities by the end of the dry season (October 2011; 5.8 psu ± 5.5 SD). Number of snaps data were log transformed log (x+1) to homogenize variances. The number of snaps was calculated for a 30-sec recording (number of snaps per 30 sec). Means and standard errors are shown.

Table B-6. ANOVA results for the effects of season (wet vs. dry), habitat (restored and natural), and day period (dawn, mid-day, and dusk) on number of snapping shrimp snaps. Number of snaps was log (x+1) transformed to homogenize variances.

Variable	DF	F-Value	P-Value
Season	1	1.37	0.2503
Habitat	1	116.37	<0.0001
Season*Habitat	1	0.88	0.3540
Day Period	2	14.38	<0.0001
Season*Day Period	2	3.51	0.0403
Habitat*Day Period	2	18.76	<0.0001
Season*Habitat*Day Period	2	5.78	0.0067

Table B-7. Tabular values of spectral analyses of upstream restored reef (Site 11), upstream natural reefs (Seagate), and downstream restored reef (Site 6). Salinity (ppt), maximum dB, power, and signal to noise ratio (SNR) for spectral curves sampled on 16 June, 5 July, 18 August, and 30 August 2011. Data analyzed were collected from dawn recordings on the indicated dates. Blue cells indicate maximum values; red cells indicate minimum values.

Date	Salinity (ppt)	Max dB	Power	Signal to Noise Ratio
Site 11				
16 June	29.3	-53.58	-28.28	-8.588
5 July	19.8	-13.79	-6.18	1.880
18 August	8.8	-40.91	-29.39	-5.700
30 August	10.6	-28.08	-20.90	-8.817
Seagate				
16 June	29.5	-6.41	-0.22	29.500
5 July	20.6	-28.14	-18.05	-7.430
18 August	10.0	-27.66	-17.00	-1.450
30 August	10.3	-49.91	-26.05	-8.546
Site 6				
16 June	34.3	-34.81	-23.88	-8.433
5 July	32.3	-12.50	-7.60	1.690
18 August	24.7	-13.60	-7.35	13.040
30 August	18.6	-48.01	-25.13	-7.792

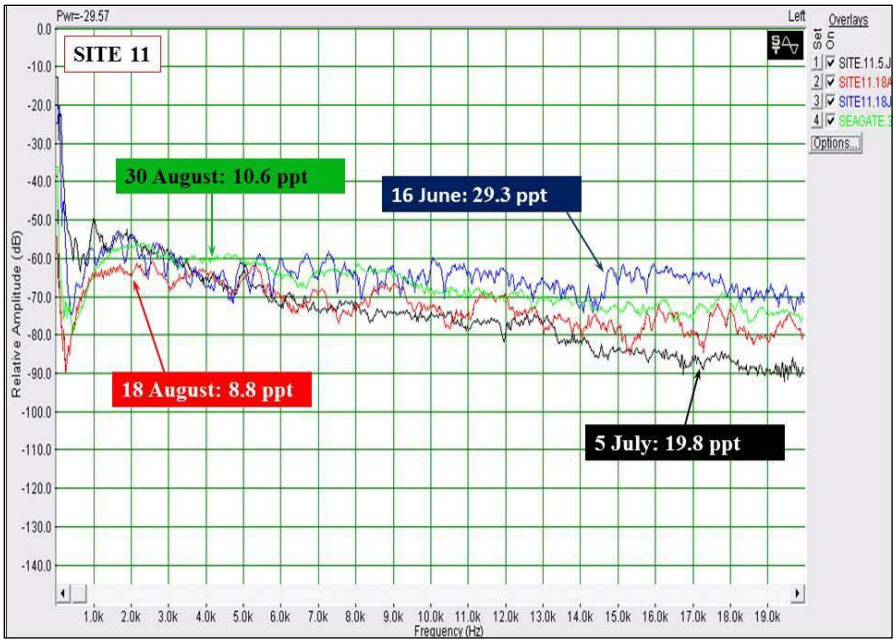

Figure B-23. Wet season, dry season acoustic spectral profiles for samples taken at Site 11, a restored oyster reef site in the upstream region of the St. Lucie River. All data shown are from dawn recordings. Specific sampling dates are indicated on the graph. Frequency (Hz) is shown on the x-axis; relative amplitude (dB) is shown on the y-axis.

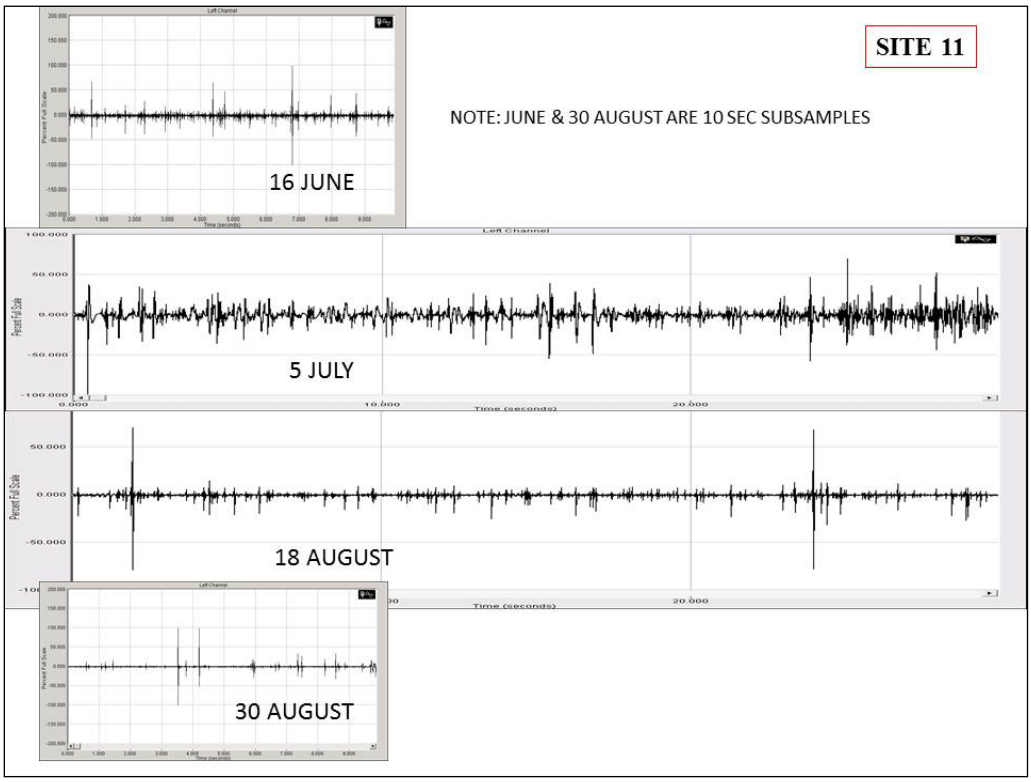

Figure B-24. Time series for wet and dry season acoustic samples at Site 11. All data shown are from dawn recordings. Time (seconds) is shown on the x-axis; relative intensity is shown on the y-axis.

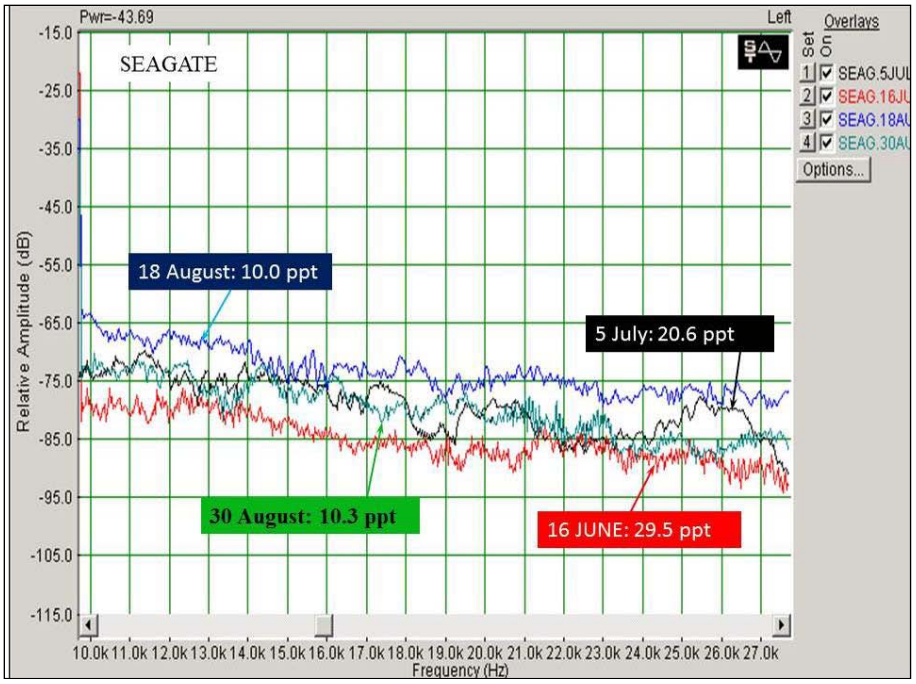

Figure B-25. Wet season, dry season acoustic spectral profiles for samples taken at Seagate, a natural oyster reef in the upstream region of the St. Lucie River. All data shown are from dawn recordings. Frequency (Hz) is shown on the x-axis; relative amplitude (dB) is shown on the y-axis.

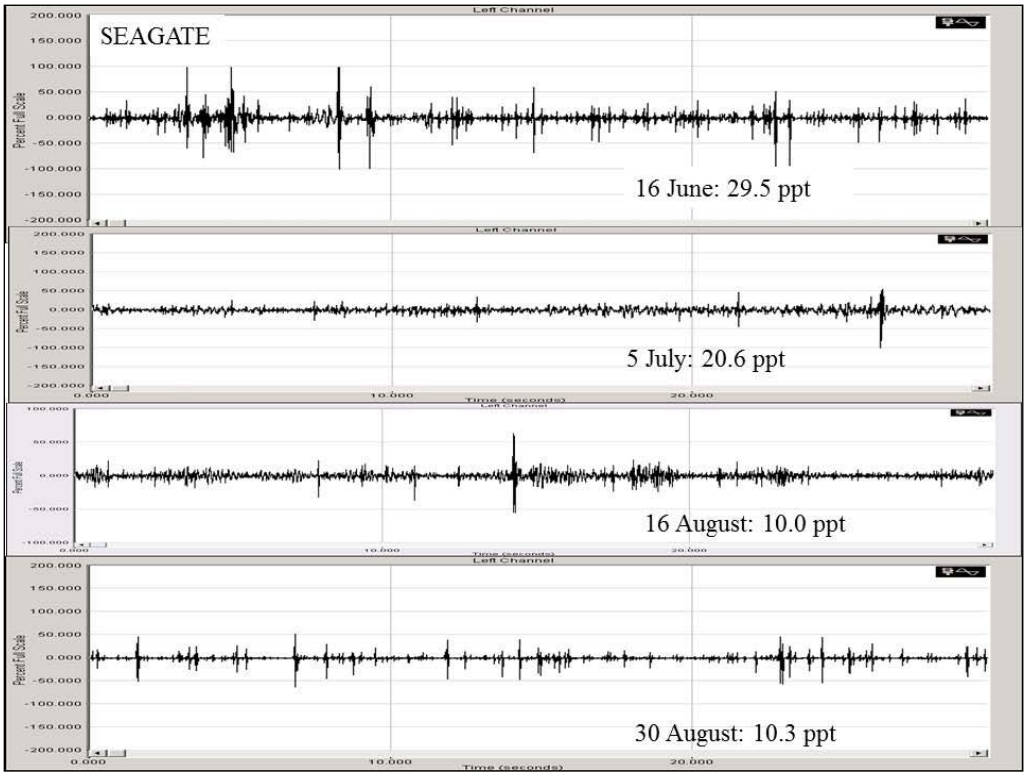

Figure B-26. Time series for wet and dry season acoustic samples at Seagate. All data shown are from dawn recordings. Time (seconds) is shown on the x-axis; relative intensity is shown on the y-axis.

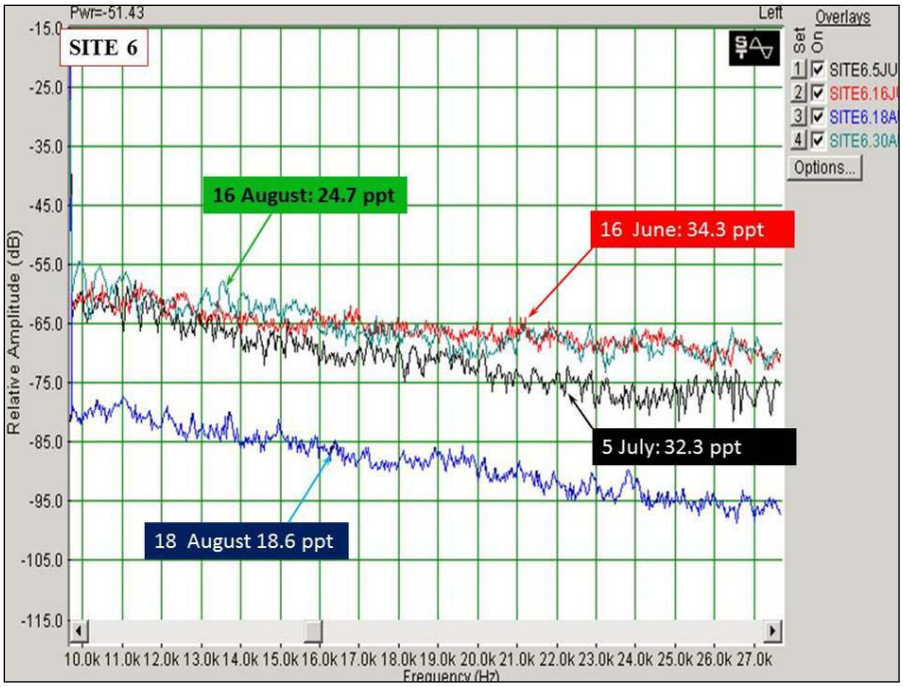

Figure B-27. Wet season, dry season acoustic spectral profiles for samples taken at Site 6, a restored oyster reef site located in the downstream region of the St. Lucie River. All data shown are from dawn recordings. Frequency (Hz) is shown on the x-axis and relative amplitude (dB) is shown on the y-axis.

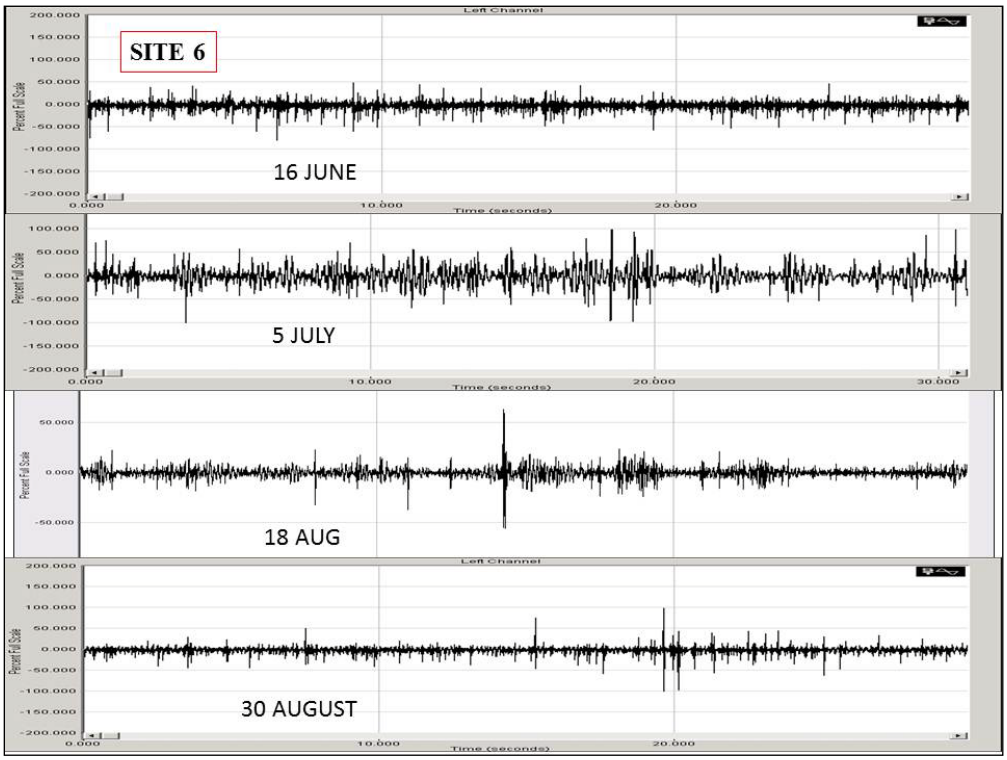

Figure B-28. Time series for wet and dry season acoustic samples at Site 6. All data shown are from dawn recordings. Time (seconds) is shown on the x-axis and relative intensity is shown on the y-axis.

The restored oyster reef, Site 11, did not show any clear acoustic spectral pattern associated with salinity. The lowest max dB was observed at the highest salinity (29.5 psu) on 16 June whereas the minimum spectral power was observed on 18 August at 8.8 psu, and minimum SNR on 30 August at 10.6 psu. This could be due to the fact that this was a restored reef and the alpheid shrimp and other potential sound producing biological assemblages are still recruiting to the new oyster habitat. Therefore, a relatively stable species complement and/or number of individuals resembling adjacent natural reef formations have not been reached. Further study of Site 11 after additional annual cycles may reveal a clear pattern as observed at Seagate, the adjacent natural oyster reef.

Seven of nine (78%) minimum values of max dB, power, and SNR were recorded during the wet season when salinities were most reduced. Seven of nine (78%) maximum values of max dB, power, and SNR were recorded during the dry season (**Table B-1**). All maximum values were recorded at salinities above 24.7 psu. These data indicate that there is a negative effect of lowered salinity on biological acoustic energy (max dB, power, SNR) originating in oyster habitats, particularly natural oyster communities and those near the mouth of the river where higher salinities are more typical.

In conclusion, natural oyster reef biological sounds reveal a clear influence of salinity on sound production. Seagate revealed synchrony in the three measurement parameters, maximum dB, power, and SNR. Restored oyster communities were more ambiguous, showing greater variation in sound production based on these three sound energy measurements. Sound energy was similar at the restored oyster reef site near the river mouth, Site 6, and the natural oyster reef sites at Seagate when salinities were lowest on 30 August.

In the St. Lucie Estuary two basic salinity patterns were examined to determine the influence of salinity on oyster reef sound production: (1) temporal patterns from dry (June–July) to wet season (August) and (2) spatial patterns from upstream (Site 11, Seagate) to downstream sites (Site 6). Temporal analyses examine single site changes in sound with changes in water salinity and reveal a clear negative impact on oyster reef bioacoustic levels with reduced salinities on mid-estuary natural oyster reefs. Spatial influence on salinity regime examines upstream and downstream sites across both high and low salinity periods. Upstream restored oyster reef, Site 11, acoustic data was ambiguous relative to salinity variation, while downstream restored sites showed a definitive reduction in bioacoustic energy at salinities below 24.7 psu at Hell's Gate. Animal communities producing sound differ between these locations and between wet and dry seasons but show most consistency in acoustic energy reduction with lower salinities at the natural oyster reef site, Seagate.

Natural vs. Restored Reefs

Two adjacent locations, Rio and Site 4, experiencing similar salinity and temperature regimes were compared to determine acoustic signatures from restored oyster reefs (Site 4) and natural oyster reef (Rio). In this case, seasonal changes in temperature were not a factor because all sites had similar seasonal water temperature regimes. Therefore, winter-dry season recordings on 16 February were included in the study.

When dry season and wet season spectral replicate analyses are placed in the same spectral image great temporal variation across months can be observed for both natural and restored oyster reefs in the mid-estuary region (**Figures B-29** and **B-30**). The natural reef at Rio (**Figure B-29**) reveals overlap between the months with the highest water salinity, 16 June, 32.3 psu, and that with the lowest salinity, 30 August, 16.4 psu. But an examination of max dB, power and SNR for each date show great separation with lower overall energy levels in June (**Table B-8**). The natural reef site shows an evident salinity effect with a consistent reduction in bioacoustic energy with declining salinities, similar to the pattern observed at the other natural oyster reef site examined at Seagate.

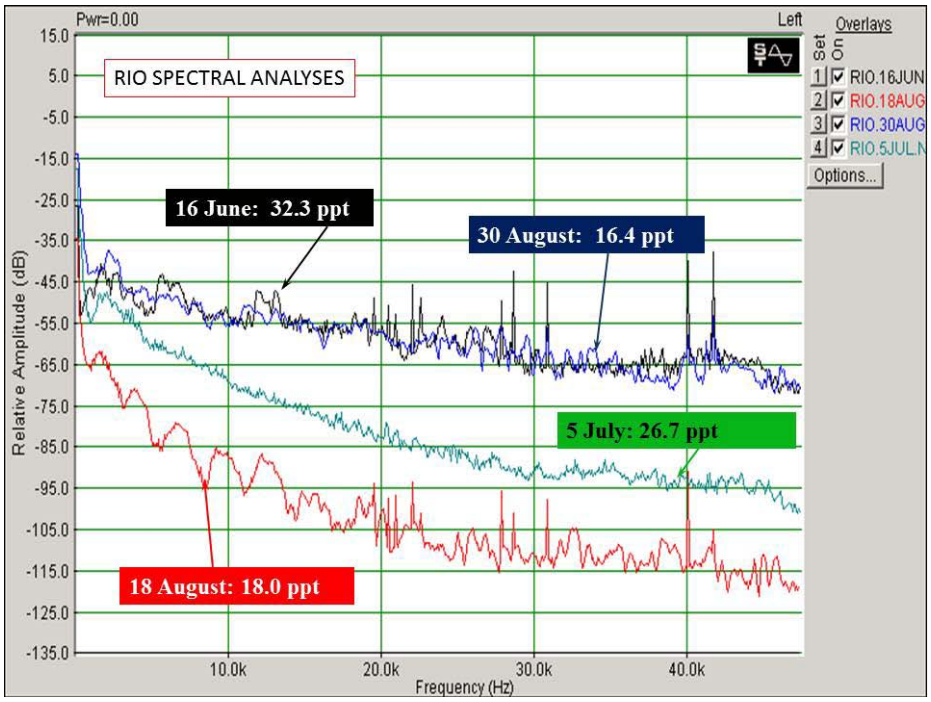

Figure B-29. Spectral curves for all dry-wet season recordings from Rio, a natural oyster reef site in the mid-estuary region of the St. Lucie River. All data shown are from dawn recordings. Frequency (Hz) is shown on the x-axis; relative amplitude (dB) is shown on the y-axis.

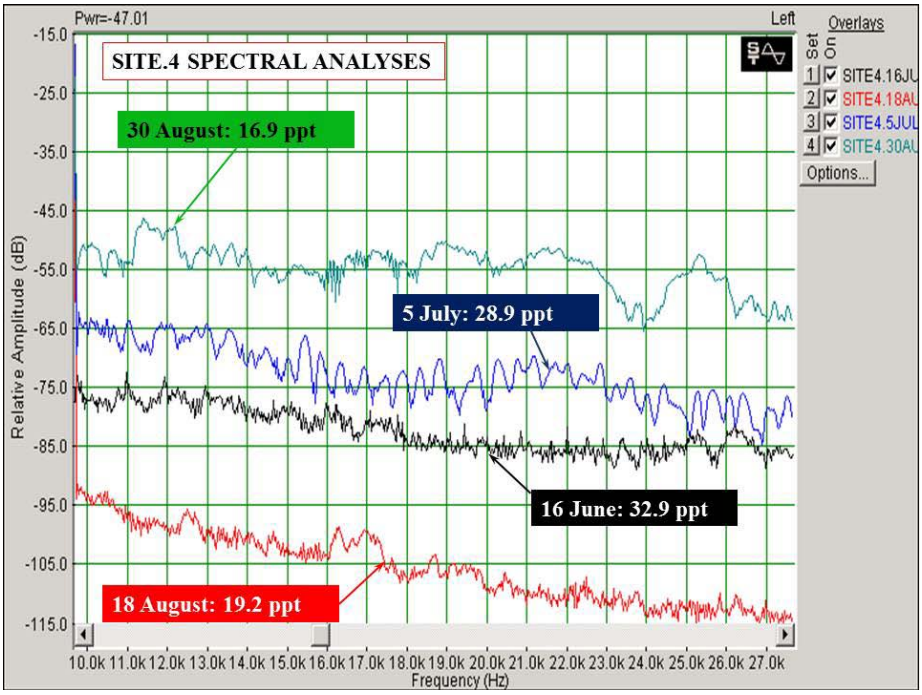

Figure B-30. Spectral curves for all dry (June, July 2011) to wet (August 2011) season recordings from the restored reef Site 4, located in the mid-estuary of the St. Lucie River. Although June and July are traditionally considered wet season months, drought conditions prevailed throughout 2011 and salinities during June and July (indicated on the graph) were reflective of these conditions. All data shown are from dawn recordings. Frequency (Hz) is shown on the x-axis; relative amplitude (dB) is shown on the y-axis.

Table B-8. Comparison of bioacoustic spectral energy (max dB) between Rio (natural reef) and Site 4 (restored reef). Both reefs are located in the mid-estuary region of the St. Lucie River and within dates sampled, experienced similar temperatures and salinities. Data analyzed were collected from dawn recordings on the indicated dates. Blue cells indicate maximum values; red cells indicate minimum values.

Date	Site 4	Rio
	Max dB	
16 February	-33.98	-40.81
16 June	-13.65	-27.33
5 July	-13.09	-15.18
18 August	-17.31	-35.01
30 August	-19.45	-5.61
	Power	Power
16 February	-23.60	-33.24
16 June	-12.12	-15.82
5 July	-8.32	-6.91
18 August	-9.70	-27.54
30 August	-12.76	-0.98
	Signal to Noise Ratio	
16 February	-3.955	-9.119
16 June	21.990	-5.704
5 July	-0.17	6.35
18 August	1.18	17.27
30 August	12.381	15.940

The mid-estuary restored reef site, Site 4, reveals an even broader separation between replicate spectral analyses based on temporal comparisons with no apparent salinity pattern (**Figure B-30**). The absence of a definitive bioacoustic salinity response at the mid-estuary and upstream restored oyster reef sites, Sites 4 and 11, indicates that the bioacoustic sources at these sites differ from those at natural reefs in their response to salinity and temporal change.

Figure B-31 reveals the dry season bioacoustic spectra for both the restored (Site 4) and natural reef (Rio) sites. The natural reef shows a much more energetic spectrum for the high salinity June recording than that for the restored site, but all three other spectra follow one another closely. Dry season recordings overlapped for all recordings except the Rio 16 June recording. The overlap between these two sites was so obvious that it appears that these two adjacent reefs have very similar bioacoustic assemblages, thus the restored reef largely resembled, acoustically, the natural reef.

The natural reef (Rio) and the restored reef (Site 4) showed comparable acoustic spectra during the wet season. Both the 18 August and 30 August spectra overlapped for both sites when taken on the same day (**Figure B-32**).

The spectra produced for each recording period for either Site 4 or Rio revealed great temporal variation. However, the spatial spectral comparisons within each month overlapped considerably, indicating that those two sites resembled one another based on spectral analysis.

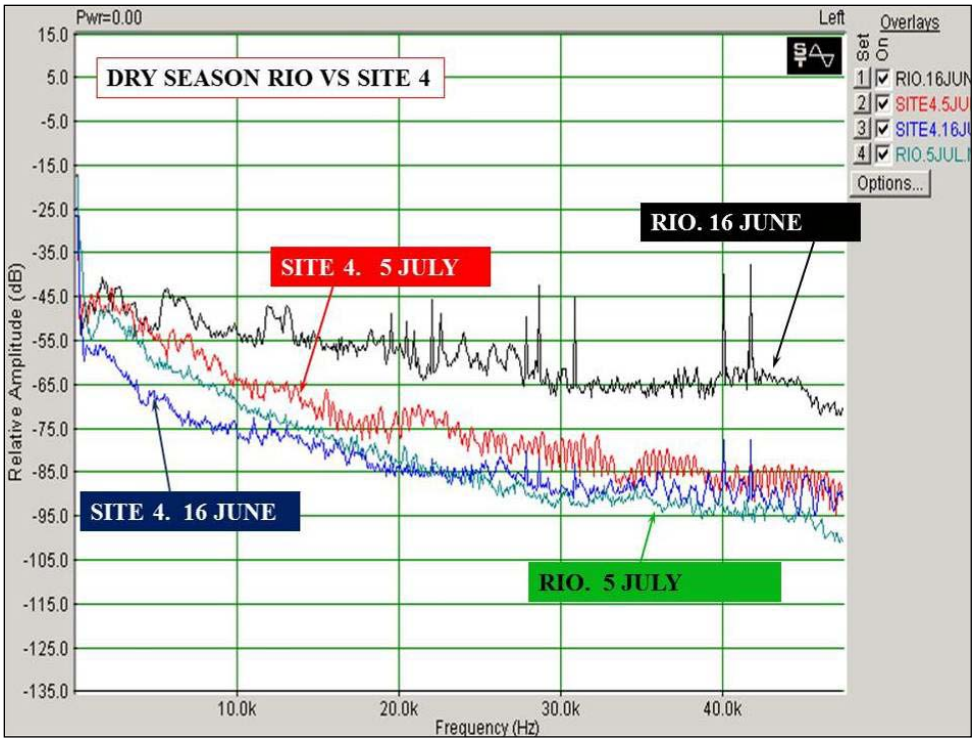

Figure B-31. Comparison of spectral curves from Rio and Site 4 during the warm period of the dry season (June, July 2011). All data shown are from dawn recordings. Frequency (Hz) is shown on the x-axis; relative amplitude (dB) is shown on the y-axis.

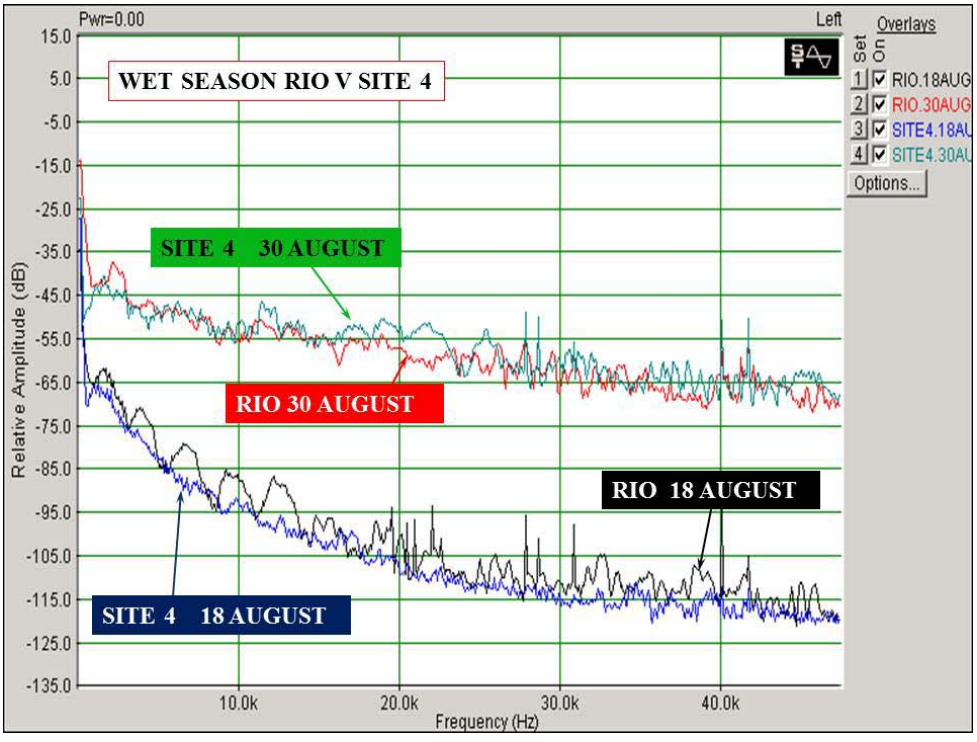

Figure B-32. Comparison of spectral curves from Rio and Site 4 during the warm period of the wet season (August 2011). All data shown are from dawn recordings. Frequency (Hz) is shown on the x-axis; relative amplitude (dB) is shown on the y-axis.

Table B-8 data indicate that there are no obvious similarities in bioacoustic energy values presented between sites other than minimum bioacoustic energy occurred at both sites in February at the lowest water temperatures recorded in 2011. Site 4 showed its highest acoustic energy during the dry season for all three parameters (**Table B-2**), while Rio showed its highest values for all three parameters during the wet season. There is a very clear and consistent pattern at Rio with highest bioacoustic energy values for max dB, power, and SNR in August and lowest in February. Data from Site 4 are more ambiguous.

In order for these energetic values to be further compared in detail, data in **Table B-2** need to be expanded to include all replicate recordings made, four per date per site. This would allow a detailed statistical study after each replicate is examined and carefully filtered for extraneous sounds. Taking 10-sec increments from the 30-sec replicates also would increase the number of replicates for energetic analyses, thus a more viable statistical comparison could be made. In addition, creating spreadsheets of hundreds of frequency and decibel distributions per spectrum would greatly increase these data and therefore strength of a comparison of restored vs. natural reef bio-acoustics using diagnostic spectral data. Until a more robust statistical comparison can be made, the strength of spectral curve overlap indicates that the bioacoustic energy in adjacent mid-estuary restored and natural oyster reefs resemble one another (see **Figures B-9** and **B-10**).

DISCUSSION

The distribution of oyster reefs is affected by substrate type, predation, and salinity; however, it is not completely clear what the major factors are that affect the distribution of the organisms that inhabit the oyster reefs (Wells, 1961; Harding, 2001; Lehnert and Allen, 2002; Tolley et al., 2005; Wilson et al., 2005). In oyster reef restoration, it is crucial to understand the faunal changes that restored reefs undergo over the course of time. Direct sampling can be time-consuming, lengthy and costly; passive acoustic sampling, therefore, offers a rapid method to monitor the distribution and changes in faunal assemblages in oyster reefs. One of the objectives of the present study was to describe the spatial and seasonal acoustic changes in oyster reefs of the SLE. Also, it was important to provide a baseline study for the use of snapping shrimp as a tool to monitor the changes in marine habitats. Analysis of the data showed that sound had significant differences between habitats, river regions, seasons, and time periods.

For the seasonal and spatial study, a correlation between total power and number of snapping shrimp snaps was found; however, for the lift net study there was no correlation between the two. Although, snapping shrimp are ubiquitous, and they are likely to be responsible for the majority of the sound produced at higher frequency bands (i.e., 2,500 to 20,000 Hz); other unknown sound producers are likely to produce sound within the same frequency band as snapping shrimp. It is possible that these unknown sound producers were more abundant at the mid-estuary site and for this reason, number of snaps and total power were not correlated there. Further studies are necessary to identify unknown invertebrate sound production.

Southeast Florida is marked by wet and dry seasons. During the wet season, temperatures are generally higher and rainfall increases. This study showed that season had a significant effect on sound production (number of snaps and total power). In addition, sound production varied among regions. These sound production variations could be explained by salinity differences. In 2011, the salinities during the wet season were lower than during the dry season. In addition, the SLE is characterized by a salinity gradient, where salinities are highest downstream and decrease upstream (Ji et al., 2007). Sound-producing organisms (i.e., snapping shrimp) may be responding to changes to salinity, temperature, or both. A study by Ferraris et al. (1994) commented that *Alpheus viridari* maintained a hyposmotic (lower osmotic concentration than the surrounding water) gradient regardless of the salinity range that it was exposed to (10–46 psu). Ferraris et al. (1994) concluded that *A. viridari* is capable of reacting rapidly and effectively to changes in temperature and salinity. In addition, results from an observational experiement on *A. heterochaelis* carried out at Florida Oceanographic Coastal Center indicated that shrimp exposed to salinities below 5 psu died within the first 48 hours of the experiment whereas most snapping shrimp exposed to salinities between 10 and 20 psu survived several weeks after the onset of the experiment.

Results of many studies indicate that salinity is a major environmental factor affecting faunal assemblages (Wells, 1961; Harding, 2001; Lehnert and Allen, 2002; Wilson et al., 2005; Tolley et al., 2005). Mean salinities during the 2011 wet season were lower than during the dry season. Oyster reef optimal salinity ranges from 10 to 30 psu (Stanley and Sellers, 1986). In the SLE, salinities during the dry season were near the maximum optimal salinity range for oysters. Previous studies have concluded that oyster reefs and their inhabitants thrive at intermediate salinities, 10 to 20 psu (Shumway, 1996; Tolley et al., 2005). Our results indicated that sound production was highest during the wet season. It is possible that sound production was highest during the wet season and at the mid-estuary region as a reflection of the thriving oyster reef community at intermediate salinities. Furthermore, predation is known to increase as salinity increases (Tolley et al., 2006); therefore, it is possible that at intermediate salinities, more organisms are present in oyster reefs due to a decrease in predation pressure.

In addition to sound production changes, shell height decreased from the dry season to the wet season. *Perkinsus marinus* prevalence was not studied in this research project; however, *P. marinus* has been documented to increase during the summer months when temperatures are highest in the SLE (Wilson et al., 2005). Although salinities also decrease in the wet season, in this study, salinity was still sufficiently high to support proliferation of *P. marinus*, the causative agent of dermo disease in oysters. It is possible that an increase in *P. marinus* during the summer months killed a percentage of the adult oyster population, resulting in a decline of shell height during the wet season.

Oyster reef restoration projects usually have one or more of the following objectives: recruitment and growth of oyster reefs, provision of habitat for associated species, direct and indirect improvements of local water quality, and shoreline protection (Brumbaugh et al., 2006). The study addressed the provision of habitat for associated species. The sound production was compared in natural reefs and restored reefs to evaluate the restoration progress. It was interesting to find that restored reefs had higher total power than the natural reefs and the barren-bottom habitats. The number of snaps was higher in the natural reef than in the restored reef and barren-bottom habitats in the spatial and seasonal study. However, the number of snaps was higher in the restored reef compared with the natural reef in the lift net study. The difference in sound production between natural and restored reef could be attributed to community succession. Oyster reef restoration is an example of a disturbance, and the sequential changes in a community after the disturbance is called succession (Ehrlich and Roughgarden, 1987). When cultch material was deployed in the SLE during the 2009 winter, newly exposed substrate became available to various species. When the new material became available shrimp and other species occupied the new material; however, the starting species composition may experience changes throughout time until it resembles that of a natural reef.

Snapping shrimp, a primary sound producer, is known to be territorial, and the snap is mostly used in aggressive interactions that occur during the defense or acquisition of a shelter (Rahma et al., 2002). One hypothesis that could be studied in the future is when new material becomes available, large numbers of shrimp and other species rapidly occupy the new material. Territories may not be clearly established, causing shrimp and other sound-producing species to produce more sound to defend their new territories.

A study by Manley et al. (2010) showed that in restored reefs, oysters, barnacles, and mussels were the first colonizers three months after the new cultch material was deployed. The accumulation of these organisms creates a more complex habitat that overtime can provide greater habitat surface. More habitat surface provides organisms more protection from predation. Manley et al. (2010) mentioned that species colonization in oyster reefs occurs rapidly. After six months the majority of the species had colonized the new material. It would be expected that as time progresses, the restored reef species composition will resemble that of a natural reef (convergence). Walters and Coen (2006) failed to identify similar faunal communities between natural and restored oyster reefs after sampling them for seven consecutive years. Nonetheless, assessing differences in faunal assemblages between natural and restored oyster reefs is an important metric to evaluate oyster restoration progress (Walters and Coen, 2006).

In this study, both total power and snaps were lowest at the barren-bottom sites where it is likely to have a lower diversity and density of marine organisms (Tolley et al., 2005; Wells, 1961). Previous studies that have found a difference in species composition between natural and restored reefs suggest that the cultch material used for restoration affected the species composition of the reef. Fossilized shell, coral, and rock rubble were used for the restored reef. On the other hand, the natural reef is formed by the settlement of generation upon generation of oyster clusters. A study by Nestlerode et al. (2007) showed that the material used for oyster reef restoration in the Chesapeake Bay had an effect on the reef complexity (the amount of cover in relation to the habitat area) and survival of oysters. Nestlerode et al. (2007) presented two different hypotheses that may explain this difference: (1) oyster larvae make material selection at the time of settlement, and they preferred one material over another and (2) the difference between reefs

occurred after oyster settlement; the different interstitial spaces host different predators, which in turn cause a difference in oyster survival and growth. Results of average shell height show similarity between the natural and the restored reefs. However, interstitial space was not measured in this study. Interstitial space may be important in shaping the complexity of a reef. Previous studies have shown that reef complexity is a factor that affects diversity and abundance of species.

Rodney and Paynter (2006) compared faunal diversity in restored reefs and non-restored reefs. They found that the mean number of macrofauna species was greater in restored plots than in non-restored plots. They hypothesized that the restored reef provided a more complex habitat; therefore, the restored reef provides more ecological services (e.g., foraging grounds, increase transfer of energy, habitat, and increase in filtering rates) than the non-restored reef. Similar results were found by Meyer and Townsend (2000); they found that created reefs had higher number of species than the adjacent natural reefs. The restored reefs provide good quality food and habitat; therefore, organisms succeed in restored reefs. It is likely that in the present study, like in the aforementioned studies, reef material and reef complexity are factors that contribute to the difference in number of species and number of snaps between natural and restored reefs.

The results from our research indicate that sound production varies throughout the day; the number of snaps and total power were highest at dusk and lowest at dawn for the spatial and seasonal study. These results are consistent with the results found by Radford et al. (2008) who studied the ambient noise off a fringing coral reef in New Zealand. They found that snapping shrimp had a significant diel periodicity and reported that snapping shrimps produced more snaps at dusk throughout the year, except during spring. Diel pattern is common in marine organisms. “Evening chorus” is a term that has been used in the literature to describe the ambient noise that a larger number of nocturnal animals produce at dusk and early night (Radford et al., 2008). Graves et al. (2004) studied the diel variation in seagrass dwelling shrimp, they showed that shrimp have a strong diel variation. Abundance of shrimp in seagrass beds increased shortly after sunset and declined throughout the night (Graves et al., 2004). Various decapod species and some *Alpheus* sp. shrimp were found buried during the day to avoid predation in seagrass beds (Greening and Livingston, 1982). Future studies can focus on testing the hypothesis that in oyster reefs, snapping shrimp hide themselves in crevices during the day to avoid predation on oyster reefs. This could potentially explain the decrease in number of snaps during the day. However, the lift net study has a different diel trend in sound production. There were a greater number of snaps at dawn than at dusk. These results are contrary to what was found in the spatial and seasonal study. The spatial and seasonal study included the downstream, mid-estuary, and upstream regions of the SLE and the lift net study, focused only on the mid-estuary. These opposing results in acoustic diel variation and region could be explained by the interaction that exists between day period and region. The MANOVA results from the spatial and seasonal study indicated that in the mid-estuary, both total power and snaps were highest during the mid-day and dusk. Abiotic factors such as current, sedimentation, and bathymetry that can vary across locations may cause differences in peak diel sound production.

There was a significant season and day period interaction in both studies. During the wet and dry season number of snaps and power increased at dusk; however, this difference strengthens during the wet season. The wet season coincides with the summer and fall when days are longer and temperature is highest, which may indicate that there was a temperature effect that influenced sound production. It is possible that day length may have an effect on shrimp behavior. Laboratory experiments comparing different day lengths could provide an insight to the behavioral changes in snapping shrimp. However, it may be difficult for researchers to acclimate reef inhabitants to tank conditions. In the experimental design, scientists planned to collect acoustic samples in the laboratory of the animals collected with lift nets. However, animals remained quiet once brought to the lab and recordings did not show any sound production. Future studies should focus on developing a methodology that will allow animals to behave as they do in the natural environment and produce sound.

Another possible explanation is that snapping shrimp increase their snaps during the wet season due to increased water temperatures. Snapping shrimp are poikilotherms, and the wet season in Florida is associated with increased water temperatures. Watanabe et al. (2002) concluded in their study that under normal dissolved oxygen conditions (>3.0 mg/L) the number of snaps increased with increasing temperature. However, when comparing *Alpheus* snaps within the 2011 wet season (August 2011 vs. October 2011 samplings), there was a significant decrease in the number of snaps in October, the end of the wet season and a period of much lower salinities (5.8 psu ± 5.5 SD in October vs. 21.2 psu ± 5.6 SD in August). Temperature effects on shrimp physiology and behavior may have predominated during drought conditions, masking any "wet" seasonal effects. Significant rainfall during this study did not occur until well into the wet season of 2011. The resulting salinity conditions allowed for a post-hoc comparison of snap counts between the "drier" portion of the wet season (August 2011) and at the culmination of the wet season (October 2011) when salinities were much lower. Water temperatures did not change significantly during this period and we believe that the decreased snap counts were due to chronically lower salinities as a result of increased rainfall and subsequent runoff. Canal discharges from C-23 and C-24 also increased during this period (see **Appendix C**, Martin County Oyster Reef Restoration: Reef Development and Community Structure).

Results indicated that there was significant interaction between season, region, and day period in the spatial and seasonal study. It is important to mention that during dry season, at dawn the mid-estuary had higher power and number of snaps, but during wet season the upstream region had higher power and number of snaps than the mid-estuary. Also, during dry season at mid-day, number of snaps was highest at mid-estuary, but during wet season, number of snaps was highest downstream. These results indicate that sound production is likely to be affected by multiple factors, and not by one single factor. Organisms in oyster reefs are likely to be sensitive to abiotic changes such as temperature, salinity, reef complexity, and sedimentation, among others. Therefore, abiotic changes that affect organisms in oyster reefs may be reflected in their sound production.

The aim of this study was to conduct field experiments to assess the relationship between sound production and faunal assemblages and to determine if passive acoustics is a viable methodology to monitor oyster reef restoration. The results indicated that number of snaps is a good metric to estimate number of snapping shrimp and number of species in an oyster reef.

Species composition between the natural and restored reef was different. There was a greater number of species (16) in restored reefs than in the natural reefs (14). The higher levels of sound observed on the restored reefs vs. the natural reefs may be representative of a higher number of aggressive interactions among snapping shrimps and between snapping shrimps and other species as a result of increased available territories afforded by the introduction of new cultch.

The results showed that number of snaps and number of species varied at the natural and restored reefs. The restored reef had a greater number of snaps and number of species than the natural reef. Previous studies have shown similar findings where species composition differed between natural and restored reefs (Meyer and Townsend, 2000; Rodney and Paynter, 2006). It is difficult to compare the results in this study with other published studies due to differences in location, sampling methods, restoration methods, and other factors. However, the authors of these studies suggest reef complexity could explain the differences in species composition between natural and restored reefs.

Results show that *Eurypanopeus depressus* (depressed mud crab), *Petrolisthes armatus* (porcelain crab), and *Alpheus heterochaelis* (big claw snapping shrimp) were the most abundant species in the lift net samples. The results in this study are consistent with other lift net studies in the southeastern United States. Boudreaux et al. (2006) found that *A. heterochaelis* and *E. depressus* were the most abundant motile fauna in oyster reefs in the Mosquito Lagoon, Florida. Also, Tolley et al. (2005) found

that *E. depressus, Panopeous* spp., *P. armatus,* and *A. heterochaelis* comprised 84% to 94% of the total organisms sampled in their lift nets. These studies show that the species composition of oyster reefs in the St. Lucie Estuary is similar to other oyster reefs in the southeastern United States. Future research should focus on identifying species-specific sound production in species such as, *E. depressus, Panopeous* spp., and *P. armatus* because they comprise a significant number of the organisms that are found in oyster reefs. If species-specific sound production is identified for these species, it is likely that passive acoustics can be used to determine their abundance. Estimating abundances of the aforementioned species, snapping shrimp, and the naked goby could expedite evaluation of the oyster reef restoration with regards to one of the ecosystem benefits that oyster reefs provide – enhanced biodiversity. It is likely that number of snapping shrimp snaps was useful to estimate number of species present in a reef because of the interactions between the shrimp and other reef inhabitants. Snapping shrimp are known to share burrows with mud crabs and gobies and snapping is as a territorial defense mechanism. It could be expected that the more organisms that are present in an oyster reef, the more snaps would be produced by shrimp snapping because of an increase in the number of interactions among shrimp and other species in the reef. Because snapping shrimp are common in various ecosystems, this methodology could be extrapolated to monitor number of snapping shrimp and number of species in other ecosystems, including coral reefs, rocky reefs, kelp forests, and marshes.

Results from this study indicate that total power and number of snaps can be a method used to detect differences between seasons, regions, habitats, and time of day. Therefore, passive acoustics is a powerful tool that can be used to monitor changes in marine environment. The fact that number of snaps can be used to estimate number of species present in a reef is promising, and passive acoustics has tremendous potential to be used as a tool to monitor marine environments. Future studies should focus on using passive acoustics to determine abundance of organisms. These studies should identify other sound producers and estimate their abundance based on their sound production.

As snapping shrimp sounds are ubiquitous in marine environment, it is an excellent monitoring tool; however, there are other marine organisms that are potentially sound producers. Although snapping shrimp are the primary sound producers in oyster reefs and their acoustic frequency band is between 2,500 to 20,000 Hz, it is crucial to determine what other sound producers fall within this frequency band. In addition, the lower frequency bands should also be studied. Fish are the most common sound producers at lower frequencies (<570 Hz; Simpson et al., 2008). Naked gobies are sound producers, and Lederhouse (2009) reported that they can be use as indicator of oyster reef biodiversity. However, Lederhouse (2009) had to collect the naked gobies from the reef. Passive acoustics does not require direct sampling of the oyster reef, making its use less destructive and more time efficient than other methods.

Spectral and time series bioacoustic data from oyster communities examined during this study showed great variation depending on the phyletic source and sound producing method, impact of environmental conditions (salinity, temperature, tide, solar – lunar incidence, sediment type, hydrodynamics, and oceanography), and finally, status of community evolution (restored vs. natural). Sound producing organisms associated with oyster reefs include not only alpheid shrimp, but a variety of other crustaceans that potentially produce sound, sipunculid and polycheate worms, boring mollusks (radulae sounds), fish sounds and potentially filtering sounds by the oysters themselves. Local gobiid sound production, *Gobiosoma bosc*, in oyster communities has already been documented (Mok and Gilmore, 1983).

All these factors must be included in a more detailed long-term study of bioacoustic data collected during this study of oyster reefs within the St. Lucie River estuary. However, considerable information already analyzed in this study reveals the value of bioacoustic information in determining the impact of salinity on bioacoustic activity from oyster reefs and a comparison between natural and restored oyster reef bio-acoustic assemblages.

Lower salinities reduced sound production from a natural oyster reef formation (Seagate) and at a restored oyster reef in a high salinity tidal environment (Site 6). This could be the result of reduction in organism activity that produces the dominate sound or emigration from the oyster reef to more favorable higher salinity sites downstream. If the latter occurred, we would expect there to be potential increases in bioacoustic sound originating from oyster reefs downstream in the more favorable environs.

Biological sounds originating in restored oyster reefs were more ambiguous, indicating that they were changing, or evolving, likely having not yet reached the number of bioacoustic species or number of sound producing individuals as natural reefs. The Rio and Site 4 sites in mid-estuary showed greater resemblance to each other than the upper estuary sites, Seagate and Site 11.

In some cases, the sound originating in the restored reef was more energetic than in the adjacent natural reef, indicating that more sound producers were present or their behavior was different. It is possible that the new oyster reefs are experiencing a series of organism invasions and extinctions following island biogeographic theory. Therefore, restored oyster reefs may be experiencing immediate increases in species richness, more sound producers, before territories and resource partitioning reaches a level indigenous to the location and other environmental parameters influence the community. From our lift net studies we report increased species richness in a restored oyster reef (Site 4) compared to an established natural reef (Rio) in the same river region (mid-estuary). Further comparisons of natural and restored reefs within similar salinity regimes should be continued to determine if and when convergence between restored and natural habitats might occur. As indicated in acoustic and lift net surveys, the restorations performed in this project have resulted in initial increases in species richness, a desired benefit of this project.

Though a more detailed study is warranted, this work has demonstrated the value of bioacoustic information in examining oyster reef community condition based on a critical environmental parameter, water salinity. Since two pairs of artificial and natural oyster reef formations showed similar bioacoustic spectral comparisons, there appears to be some value in using bioacoustic surveys to determine the status of artificial oyster reef development relative to natural oyster reefs.

We were very fortunate that we were able to make these comparisons during this survey at a location that is as volatile as the St. Lucie River, a location that has experienced significant deleterious human impacts from freshwater diversions.

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APPENDIX C

Martin County Oyster Reef Restoration:
Reef Development and Community Structure Final Report
Florida Atlantic University

Martin County Oyster Reef Restoration:
Reef Development and Community Structure

Final Report

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EXECUTIVE SUMMARY

Oyster colonization and development of the invertebrate community in the St. Lucie River estuary (SLR) proceeded from 2009 – 2011 and was influenced by the variability in salinity but not the mean salinity under the low-runoff conditions that prevailed during this drought period. Colonization also proceeded well in the Loxahatchee reefs. Using sediment elevation table and marker horizon technologies developed from wetland science research, it was shown that subsidence, in this case reef sinkage into soft sediments and reworking and compaction of the fossil shell varied at different sites. In the SLR, 9.5 cm of subsidence plus 1 cm of sedimentation caused 10.5 cm of loss of elevation in the first 9 months. Over the next year however, losses were much slower, and then by the end of the study there was a net elevation gain. This gain coincided with continued colonization by reef-forming species, primarily oysters and mussels. A small net gain in elevation also was seen in the Loxahatchee site. These data indicate that vertical relief is accumulating through biogenic activity at a rate of about 3 mm/mo.

Community analysis of completed samples indicated dominance by polychaetes and amphipods with total invertebrate densities reaching >14,000/m^2. Invertebrates were influenced not only by the presence of fossil shell cultch, but also by the presence of live oysters, showing the importance of living reef formers, not just dead or inert shell, to the community as a whole. Community taxa similarities were different among sites, often within sites indicating patchiness, and over time. These results suggest that development of the community is still on-going and has not yet reached a stable point. Any stability, if reached at all, will be disrupted in wetter years when higher freshwater flows provide a pulse of low salinity disturbance to the system.

Data analysis by structural equation modeling (SEM) allows posing and testing true multivariate hypotheses with complex interrelations among the variables. The entire model can be tested for "fit" to the observed pattern of covariances in the data, and if the pattern of covariance implied by the hypothesized model is not significantly different from the true pattern of covariances in the dataset, then this is good evidence for cause-and-effect even though the data are observational. We linked the oyster and invertebrate community data to water quality data and flow data from DBHYDRO (SFWMD, 2011) to assess some of the relationships discussed above. We had hypothesized that salinity as a dominant stressor would be important, as would chlorophyll *a*, because it might represent food for filter feeders directly and indirectly to the food web.

Moreover, we undertook a supportive analysis by using SEM and rainfall, canal flow, and water quality data from DBHYDRO over the decade just prior to the oyster project (January 2000–March 2011). That analysis helped solidify our confidence in linking water quality parameters to flow because it encompassed wet as well as dry periods.

SEM of the decade of water quality, flow, and rainfall data showed that rainfall drove flows in the C23 and C24 canals to a lesser extent than the C44 because its flow is so tightly controlled by the U.S. Army Corps of Engineers. Distance from inlet (a proxy for tidal inputs of salt water) and canal flows (especially C44 and C24) explained 75% of the variability in salinity from 2000 – 2011. Salinity, total Kjeldahl nitrogen, color, and a 1 month time lag in chlorohpyll explained 37% of chlorophyll *a* values over the decade.

Over the 2-year oyster study timeframe, means of salinity and chlorophyll varied much less than in high runoff years. However, SEM showed that increasing variability in these two water quality parameters (measured as their coefficient of variation) had negative impacts on oyster abundances. This SEM analysis shows that even in low-flow years, there can be adverse effects of water quality on oysters. Oyster reefs however, continued to develop at all sites, just at different rates.

INTRODUCTION

Eastern oyster, *Crassostrea virginica* (Gmelin, 1791), populations occur along the West Atlantic and Gulf of Mexico coasts from Canada to the Yucatan Peninsula and have undergone significant declines due to freshwater disturbances, pollution and disease, and over-harvesting and habitat loss (Beck et al., 2011; NOAA, 2007; Rothschild et al., 1994; Woodward-Clyde, International Americas, 1998; Lenihan and Peterson, 1998). Oysters are sensitive to abrupt fluctuations and prolonged decreases in salinity, loss of suitable habitat, and increased levels of suspended particulate matter caused by unnatural freshwater disturbances (Rothschild et al., 1994; Barnes et al., 2005; Wilson et al., 2005). Changes in salinity may impact oyster reproductive efforts, larval recruitment, and settlement success as well as influence levels of predation and parasitism (Coen and Luckenbach, 2000; Barnes, 2005; Wilson et al., 2005,). Although oysters have the capability to filter large amounts of water per day, levels of suspended particulate matter exceeding the consumer's assimilation capacity may result in the accumulation of sediments and the mortality of oysters and other suspension feeders and contribute to the loss of suitable habitat for potential recruitment (Peterson and Black, 1988; Barnes, 2005; Sime, 2005; Thomsen & McGlathery, 2006; Taylor and Bushek, 2008; Volety et al., 2009). As a result of the declining oyster populations and habitats, several restoration efforts have been conducted along the Atlantic and Gulf of Mexico coasts of the U.S. (Rothschild et al., 1994; Peterson et al., 2000; Coen and Luckenbach, 2000; Luckenbach et al., 2005; Powers et al., 2009).

The St. Lucie River Estuary (SLE) in southeast Florida is a major human-controlled distributary of freshwater from Lake Okeechobee, and as such is one of the northern estuaries of the Comprehensive Everglades Restoration Plan (CERP). Oysters are one of the target species for restoration of this estuarine system. A technical report by Woodward-Clyde International Americas (1998) states that only anecdotal evidence has been reported on the abundance of oysters in the SLE prior to the 1940s. This evidence suggested that greater than 500 hectares (1,236 acres) of oyster reef occurred in the SLE. The current amount of oyster habitat within the estuary has been estimated at 300 to 1,000 m^2, a 99.9% reduction in the overall oyster population since the 1940s if the anecdotal accounts are accurate (South Florida Water Management District [SFWMD], 2009; Parker and Geiger, 2009). As a result of the decline of the oyster population in the SLE monitoring began in 2005 as part of CERP, the Comprehensive Everglades Restoration Plan (SFWMD, 2009; Parker and Geiger, 2009).

The largest oyster reef restoration effort in Southeast Florida, the Oyster Reef Restoration Project (ORRP), was undertaken by Martin County in August 2009, as a component of CERP. The project resulted in the creation of over 27.8 acres of mainly fossil shell material available for oyster and other reef-forming species to use as a basis for habitat formation. Previous restoration pilot studies conducted in the SLE by CSA International, Inc. as a subcontractor to Ecological Associates Inc. and Martin County in 2005 and 2006 incorporated the use of similar shell material and were successful in recruiting oysters.

The main objective of our study was to determine the development of reef structure and the associated invertebrate community of the restored oyster reefs in the SLE and the LOX. We then linked our faunal data to variation in water quality in statistical analyses via structural equation modeling (SEM), to assess the degree of change that could be associated with these environmental variables. Additionally, we determined the sedimentation and reef subsidence (including compaction by shell reworking and reef sinking) rates using sediment elevation table (SET) devices installed in selected restored reefs. SET methodology has been used extensively in wetlands to assess elevation change, but this is the first attempt to use it in oyster reefs.

METHODS

Pilot Study

In early August 2009, prior to the main restoration efforts, we initiated a pilot study to determine the rate of colonization and to establish a baseline of resident macroinvertebrates (motile and sessile species) colonizing the oyster reefs in the SLE. We placed six receptacles containing the same fossil shell material used for the reefs in the ORRP in shallow subtidal waters along the south shore of the middle estuary to evaluate invertebrate colonization throughout the duration of the project (white star on southwest side of Middle Estuary in **Figure C-1**). These receptacles were sampled at regular intervals throughout the duration of the project.

Figure C-1. Oyster reef study site locations in the St. Lucie Estuary. White star is the location of the receptacle site for the preliminary study; orange triangles are South Florida Water Management District water quality monitoring stations; yellow numbers are the oyster reef sites constructed during the Oyster Reef Restoration Project.

The six receptacles, three baskets (10-mm mesh) and three milk crates (20 mm mesh), collectively containing approximately 7.4 m^3 of harvested fossilized cultch were deployed on August 9, 2009 between dilapidated pier pilings on the southern side of the Middle Estuary. Receptacles were fully submerged at a shallow subtidal depth of approximately 0.5 m on bare sand. They were arranged in two columns with three rows from the shoreline edge out towards the channel. No oysters were noted living on the pier pilings immediately adjacent to the reciptacles, however, two small (3 m by 0.5 m) intertidal oyster reefs occurred nearby; one approximately 40 m southwest of receptacle location and the other approximately 60 m southeast.

Receptacles were sampled by quickly removing the basket or crate from its position and immediately placing it into a large plastic bucket to avoid loss of shell material or organisms. On shore, the samples were sorted into three plastic bins halfway filled with seawater, each designated for levels of sessile organism colonization; none, colonization without oysters, and colonization with oysters. The total number of fossilized shells was counted for each container and returned to the original receptacle with the

exception of 50 randomly selected shells with settled oysters. The water remaining in each bin and the large retrieval container was sieved through a 1-mm mesh sieve for motile organisms prior to replacing the original receptacle. All sieved samples were placed into plastic jars and filled with a 10% buffered formalin solution (seawater and buffer formalin stained with Rose Bengal).

Fifty collected shells were assayed in the field to quantify abundance of sessile species and to measure oyster colonization and size. These fossilized shells were generally identified by type (i.e., oyster, clam, whelk) and measured individually along the long and short axes, and an approximate surface area was estimated as an ellipse from these measures. The side of each shell was labeled: "A" for the interior of the shell and "B" for the exterior of the shell. All sessile organisms that had colonized were identified (oyster, barnacle, mussel, serpulid, tunicate) and quantified on both sides of the shell. The fossil shells were returned to the original receptacle which was then replaced in the water.

Samples were collected at 2- to 3-month intervals to provide comparative data on the invertebrates colonizing the area throughout the project as well as during fluctuations in water quality and data on oyster recruitment and growth.

Oyster and Community Studies

Between August 2009 and January 2010, multiple subtidal oyster reef sites, approximately 1.5 to 3 m deep, were created in the SLE as part of the ORRP (Sites 1, 2, 4, 7, 11, N1, N4, and N6). Sites were defined by the ORRP as permitted areas within the estuary for patch construction. Patches are composed of harvested fossil shell material (oyster, clam, whelk) and are approximately 30.5 m long × 7.6 m wide × 0.3 m thick with approximately 9 m between patches at a site.

Three replicate patches per site were randomly selected prior to sampling, located by a geographic positioning system (GPS), and sampled by divers collecting three cores per patch. Initial sampling of each site occurred 2 to 8 weeks post-patch construction from 11 September through 13 December 2009, except Site N6 (the most downstream site constructed in January 2010) that was first sampled in June 2010. The samples were collected by a benthic core 0.30 m in diameter; weighted with two 1.8-kg weights equidistant from each other and fitted with a 0.30-m long, 1-mm mesh net on one end and aluminum sheet metal to provide a 3.18 cm cutting end at the other end. Divers inserted the core to a depth of approximately 10 cm into the shell material and excavated the core using a wide-blade shovel. The core containing the sample was brought to the surface on the shovel (**Figure C-2A**). The sample was sieved through a 1-mm mesh sieve in the field, placed in jars, fixed with 10% buffered formalin, and stained with Rose-Bengal. Sampling occurred approximately every 6 months through June 2011.

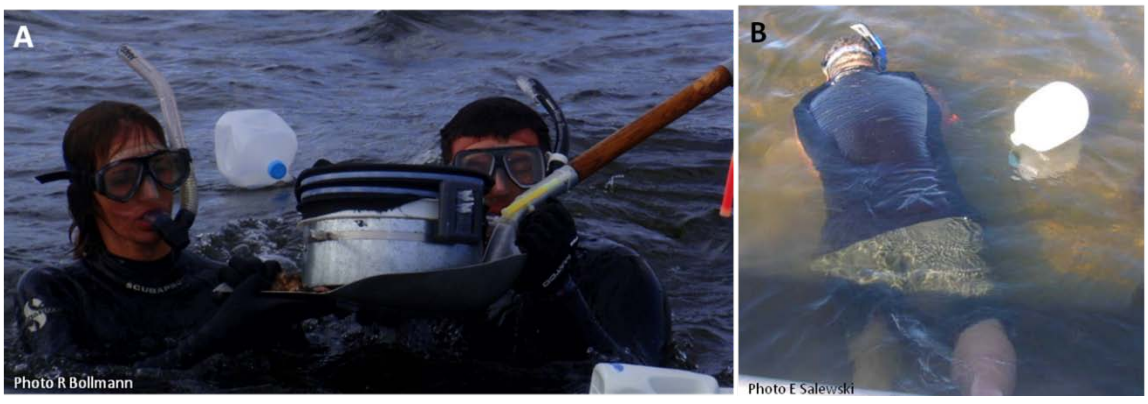


Figure C-2. (A) Invertebrate sample with core brought back to boat for collection in the SLE and (B) setting the benthic core in the Loxahatchee River.

In the lab, organisms were sorted into major taxonomic categories (e.g., crustaceans, polychaetes) and stored in 70% isopropyl alcohol until further identification. Organisms were identified in the laboratory by means of microscopy and dissection techniques. To provide an initial reference collection, a subset of samples was identified to the lowest practicable taxon (usually species) by Ecological Associates, Inc. Invertebrates in most samples, however, were identified by the research group at FAU.

Influence of Water Quality

Structural equation modeling was used in the following assessments:

1. Water quality, canal flow, and rainfall data from 2000–2011 mined from DBHYDRO stations in the St. Lucie River and the inlet used to assess the web of relationships among flows and suspected environmental drivers (salinity, turbidity, chlorophyll *a*, color, ammonia, nitrate, and phosphate); and
2. Oyster data from 2009–2011 linked in a separate SEM analysis to a subset of these environmental variables, including both the mean and monthly variations (coefficient of variation), as possible drivers of oyster reef development.

We posed *a priori* structural equation models and tested various complex and simple models. **Figures C-3A** and **-3B** show two simple conceptual SEMs posed as hypotheses. The first concerns the oyster populations per se colonizing the different sites. In the first SEM, oyster densities are related to age of patch, salinity, distance from inlet (as it affects salinity and relates to a source of larvae), and freshwater flow through three main canals (**Figure C-3A**). In the second SEM (**Figure C-3B**), the totality of all invertebrates colonizing sites is related to the same factors, but also to the abundance of live oysters as reef-forming species. This last point is important to assess because for restoration it is valuable to know if other invertebrate species react only to the physical structure of the cultch, or if they also respond to living reef-formers.

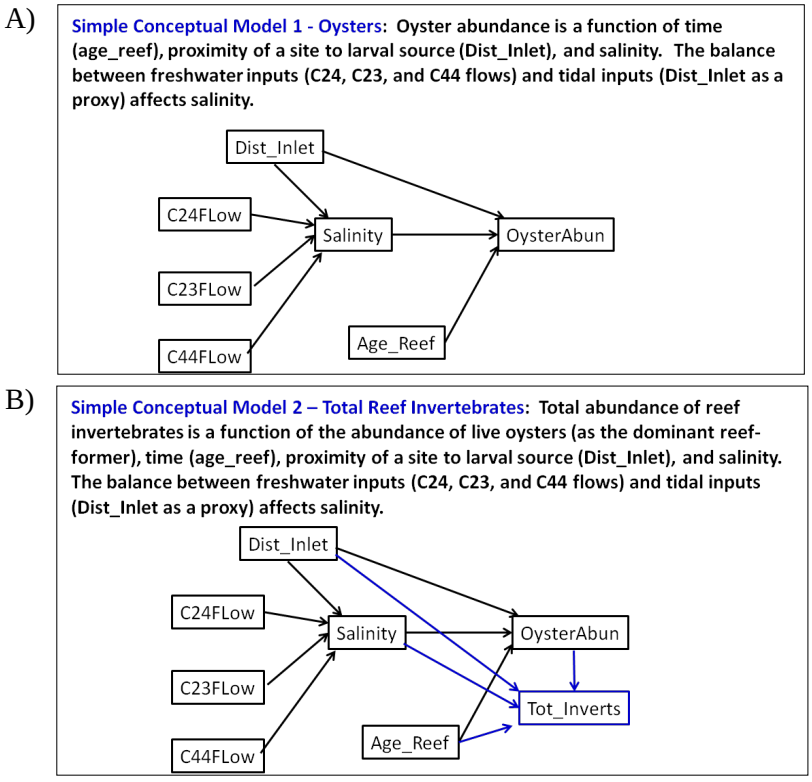


Figure C-3. Two structural equation models proposed as simple multivariate hypotheses: (A) oysters alone and (B) all invertebrates colonizing the constructed reefs.

Sediment Elevation Table (SET)

A sediment-erosion or sediment elevation table (SET) has been described as a portable leveling device that provides a constant reference from which sediment surface can be measured (Boumans and Day, 1993; Cahoon et al., 2003; 2004). Repeated measures of elevation or subsidence can be obtained with precision because the table remains fixed for each sampling, the only component that changes is the surface sediment or structure (Cahoon et al., 2002.) We are the first research group to install and use the SET device on oyster reefs to determine reef subsidence or accumulation.

Sediment elevation tables were installed with 1-m long steel rods driven into the constructed reef structure at Sites 1 (February 2010) and 2 in the SLE (September 2010), the LOX SET was installed in January 2011. Once the steel rods contacted bedrock/limestone (nine rods in Site 1 and eight in Site 2), a receiver was attached to the exposed rod and left at the site. Receivers extended no more than 15 cm above reef shell surface. To measure the reef surface, a removable arm rod was attached to the receiver. The arm rod is then fitted with the SET arm, which was positioned north (0°) and secured with two clamps. Nine 9-m fiberglass pins were inserted into the arm and allowed to touch the top surface of the reef, then secured with clips. The top of each pin was measured for that direction. The arm was unclamped and moved 90° in the next direction and the pin placement and measuring was repeated for east, south, and west directions (**Figure C-4A**, **B**, **C**). (Refer to United States Geological Survey's SET website for further SET information http://www.pwrc.usgu.gov/set/.)

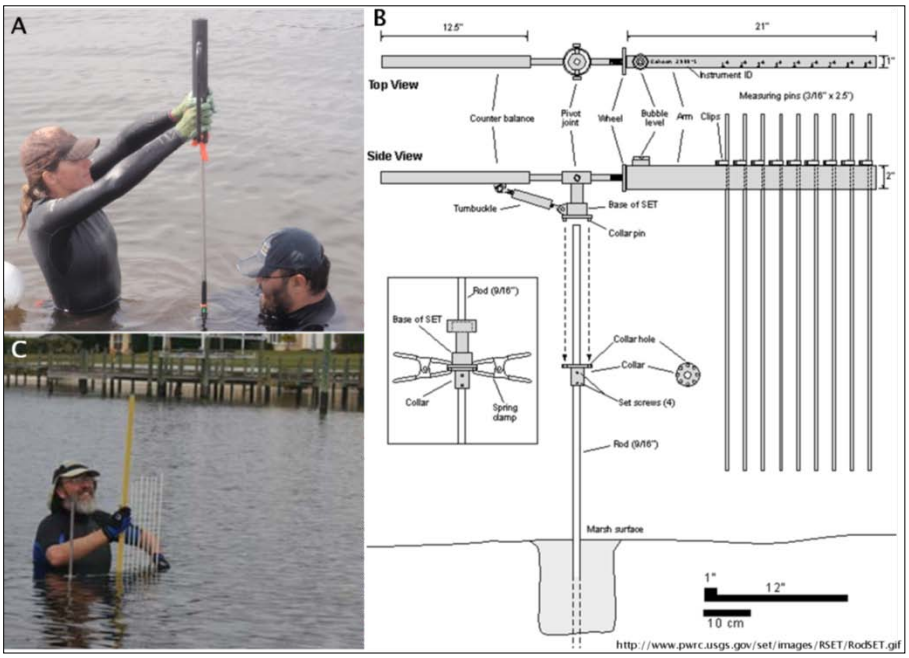


Figure C-4. Sediment elevation table (SET) benchmark installation (A); SET schematic (U.S. Geological Survey) (B); and measuring SET pins (C).

In addition to installing the SET, marker horizons (MH) (one each at Sites 1 and 2) were installed, which enabled us to determine the rate of sedimentation. Marker horizons were established flush with the reef surface by digging a 40-cm diameter hole into the restored reef, approximately 35 cm deep. One half of a 5-gallon bucket was used as the MH, which was placed inside the hole, anchored with shell material around the inside and outside base, and then filled with clean, white beach sand. The white beach sand was used because it provides a stark visual contrast to the sediment in the SLE. Using a clear, 4-cm benthic core, a sample was taken from the center of the MH and the amount of sedimentation on the reef measured as the depth of darker sediment overlying the beach sand.

RESULTS

Oysters

Reef development proceeded at various rates, depending on the site (**Figure C-5**). Densities and sizes were greatest at Site 4; densities of larger animals were 2× and smaller recruits were 54× those of Site 1 (**Table C-1**). The pilot studies (data not shown) indicated extremely rapid colonization of fossil shell cultch by amphipods and certain other species in the very shallow subtidal area of the mid estuary. This report focuses mainly on results from the SLR.

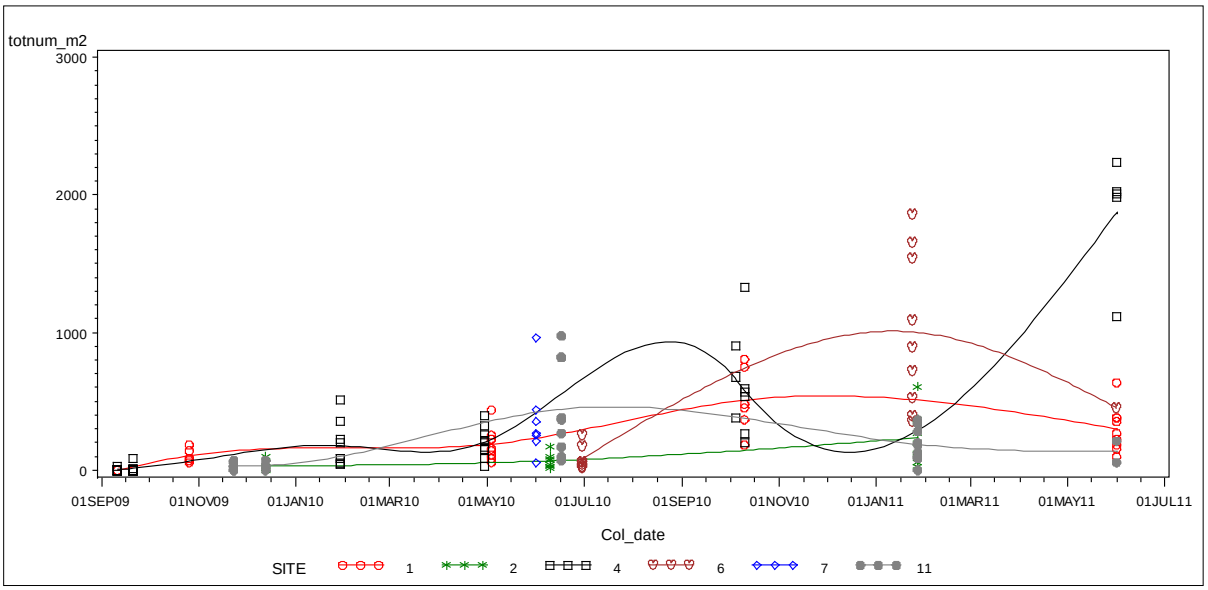


Figure C-5. Total oyster abundance/m^2 of all size classes by site. SAS graph spline curves indicate trends.

Table C-1. Densities/m^2 and shell lengths of oysters at two St. Lucie River sites. Values are means and 1 SE.

June 2011 (final Sampling)	SITE 1			SITE 4		
	MEAN	SE	n	MEAN	SE	n
DENSITIES / m2						
Oysters Greater than 20 mm	274.5	60	9	519.7	63.1	5
Oysters Shorter than 20 mm	25.2	8.1	9	1360.4	188.7	5
SIZES (length in mm)						
Mean Length	43.3	1.3	9	24.3	2.8	5
Max (largest individual) length	74.5	5.8	9	103.4	2.9	5

The largest size class was abundant at Site 4, but not at the other sites (**Figure C-6**). Other sites had far fewer large individuals, suggesting elevated mortality or slow growth or both. Especially interesting was the extremely low densities of large individuals at the most downstream site (Site 6). Note the difference in scales on the Y axes in **Figures C-5** to **C-7**. The largest individual living oysters per site follow somewhat similar trends (**Figure C-8**). Largest animals occurred at Site 4 and smallest at Site 6. The maximum size either leveled off or declined, depending on the site beginning about September 2010, about 1 year into the project. June 2011 data were not available for all sites by report time.

However, new recruits, indicated by the proxy variable of abundance of individuals <20 mm, revealed extensive settlement at the more downstream sites (Sites 4 and 6) in June and January 2011, respectively (**Figure C-7**). Interestingly, Site 4 (mid-eastern SLR) had considerable juveniles and small individuals in June 2011 but the more downstream Site 6 did not. This suggests further work on the recruitment dynamics (e.g., from outside the SLR or from within) and growth (varying at different sites) is warranted.

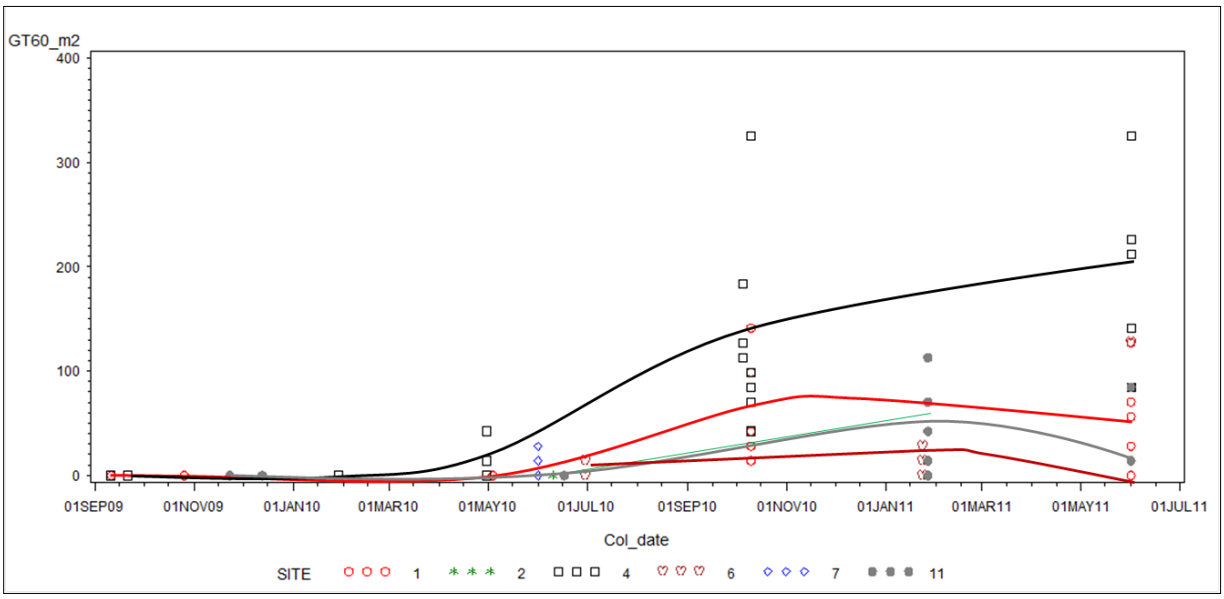
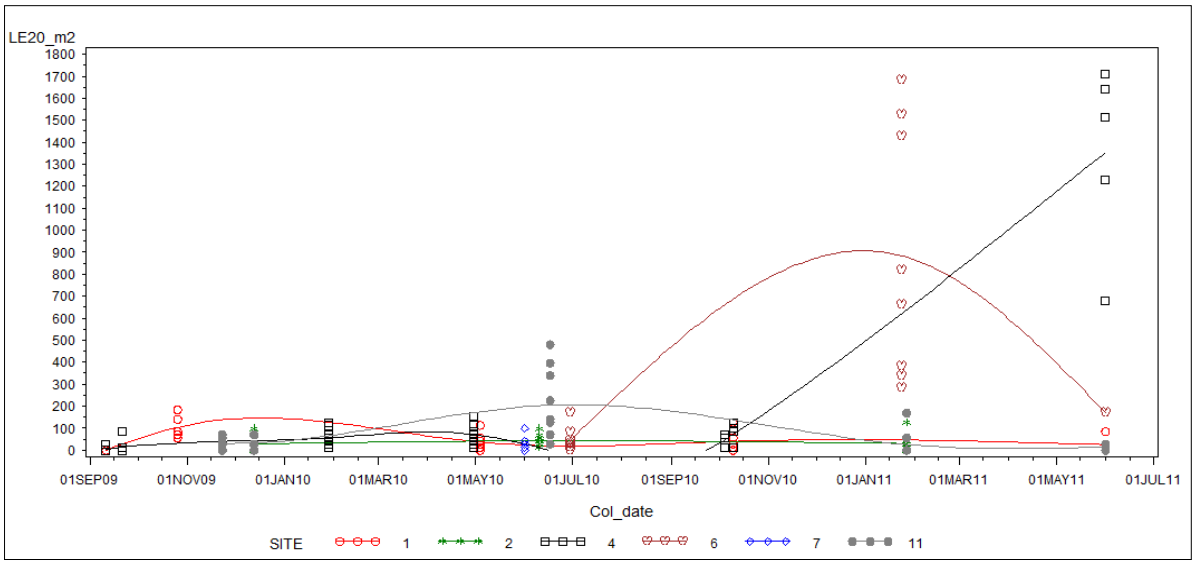


Figure C-6. Number of oysters/m^2 in largest size class category (≥60-mm shell length). Trend lines are hand-drawn.

A)



B)

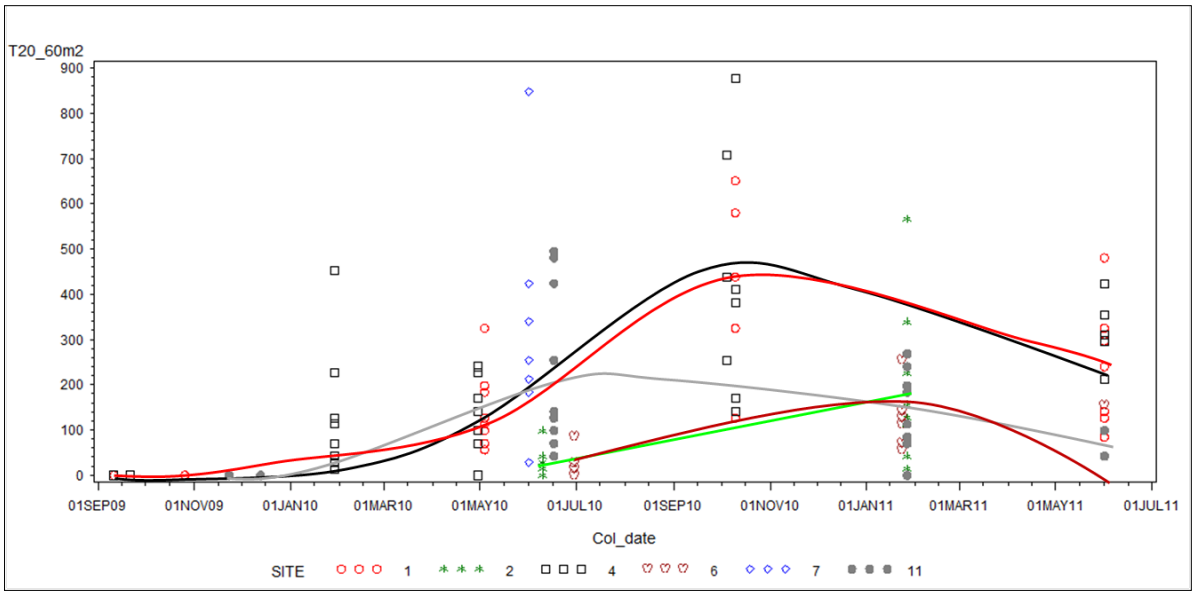


Figure C-7. Number of oysters/m^2 in the smallest size class category (≤20-mm shell length) (A) and in the mid-size class category (>20–60 mm) (B). Spline curves drawn by SAS graph (A) and by hand (B).

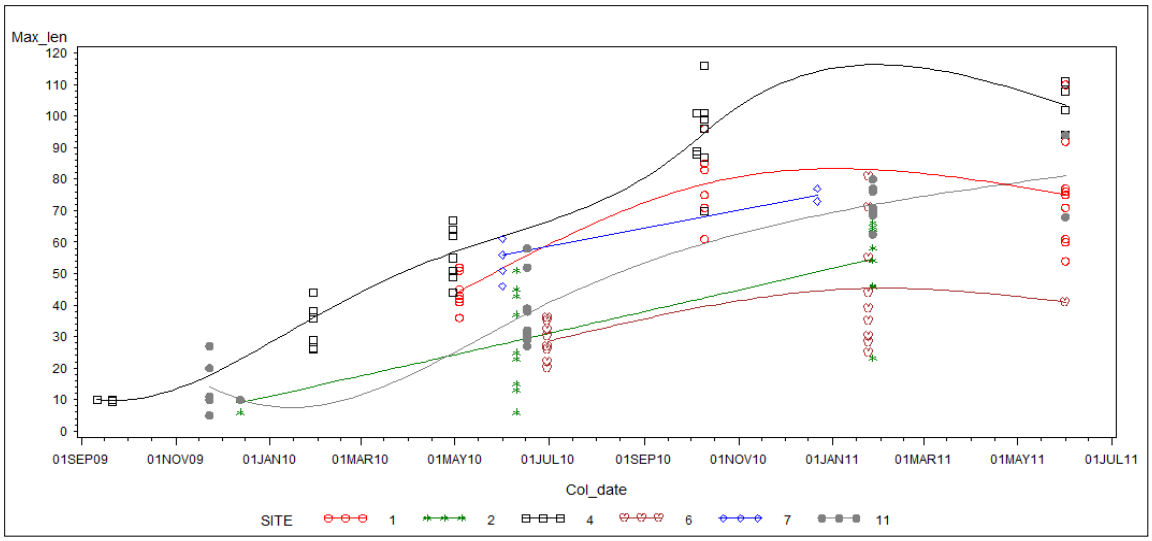


Figure C-8. The largest live oysters in collections by sample, station, and time.

Structural Equation Modeling Analyses of Water Quality (2000-2011)

Some water quality variables (potential "environmental drivers" of oyster reef development) were linked to canal flow while others were not over the decade 2000 – early 2011 (**Figure C-9**). Chlorophyll *a* was postulated to be a food source for oysters. Nutrients (various forms of nitrogen [N] and phosphorus [P]), light penetration (color, secchi, total suspended solids [TSS]), and salinity (as the diluting effects of tidal inputs) were hypothesized to affect chlorphyll *a* and one another. Freshwater flow from the three main gauged canals was hypothesized to be an important driver of all of these water quality variables, directly or indirectly and precipitation (mainly rainfall) was expected to be the 'driver' of flow. Certain environmental variables (e.g., turbidity, TSS, dissolved oxygen [DO], pH, Secchi) were evaluated and discarded if not significantly linked to water flow or positively correlated with another variable already in the model (e.g., color and phosphate). The covariance structure implied by the model posed *a priori* was not significantly different from the actual covariance structure of the data (**Figure C-10**, RMSE test), which is presumed evidence of possible cause-and-effect relationships among variables. In the SEM, single-headed arrows indicate cause and effect (regression) and double-headed arrows show correlation without postulated causality. Coefficients are standardized path coefficients, or partial regression and correlation coefficients. Width of arrows in **Figure C-11** is proportional to the magnitude of these coefficients. Each endogenous variable (those receiving arrow heads and thus response variables) also has R-square values that indicate the proportion of variance explained for that endogenous variable.

The SEM indicates that 75% of the variation in salinity over that decade is explained by distance of the collector from the inlet and the flows of the C44 and C24 canals. The C23 canal had smaller effects on salinity by itself, but was highly correlated with the C24 canal flow (r=+0.89). Rainfall was a strong driver for C23 and C24 flow, but a weak driver (R-square = 5% of variation explained) of C44 flow probably because discharge of this canal is highly regulated.

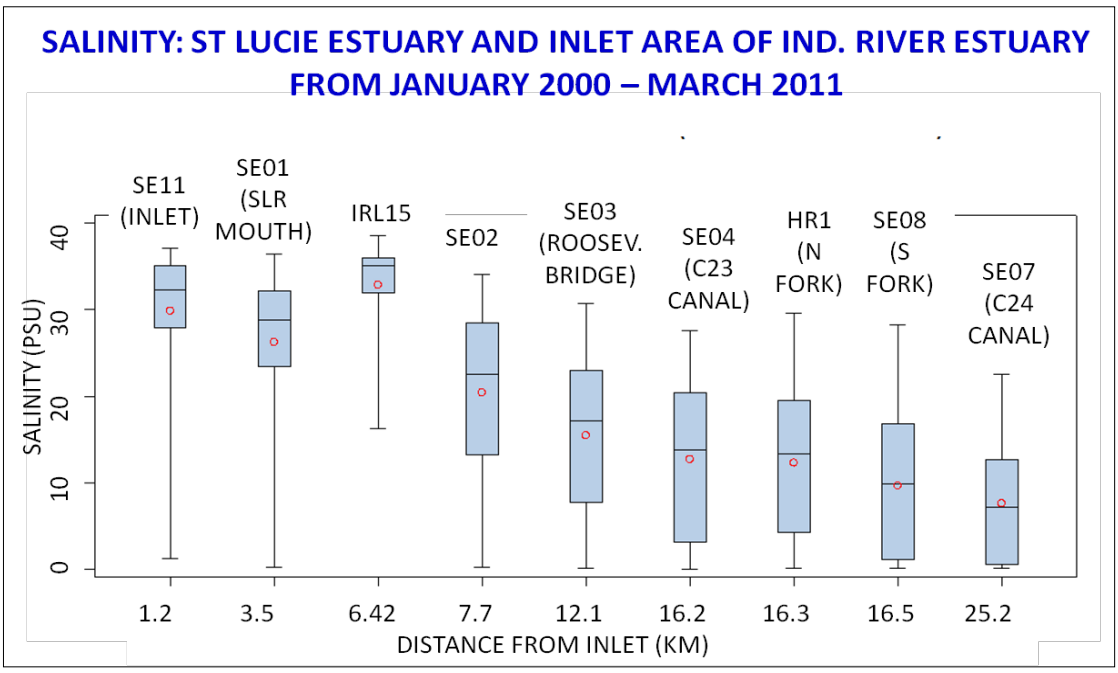
Color was not influenced by C44 flow, but was by C23 and C24 flow and the distance from inlet and salinity (a proxy for volume of tidal water input). Total nitrogen was a function of C24 inputs, and was diluted by oligotrophic marine waters (salinity variable, -.53 path coefficient).

Chlorophyll *a* (postulated as a food source for oysters) was positively affected by color, salinity, and nitrogen, and a 1-month lag (i.e., the previous month's chlorophyll *a*).

Structural Equation Modeling of Oyster Colonization (2009-2011)

Oysters are known to be sensitive to extremely low salinities, which sometimes exist in the SLR. Both the mean and variability of salinity changed with distance from inlet (**Figure C-9**). As shown in **Figure C-10**, freshwater flow from the C44, C24, and C23 canals and distance from the inlet accounted for 75% of the variation in salinity over the decade 2000-2010. The SEM analysis presented next uses only a small fraction of those data from the times of sampling for invertebrates. Consequently, it was necessary to combine the individual canal flows into a total flow variable.

A)



B)

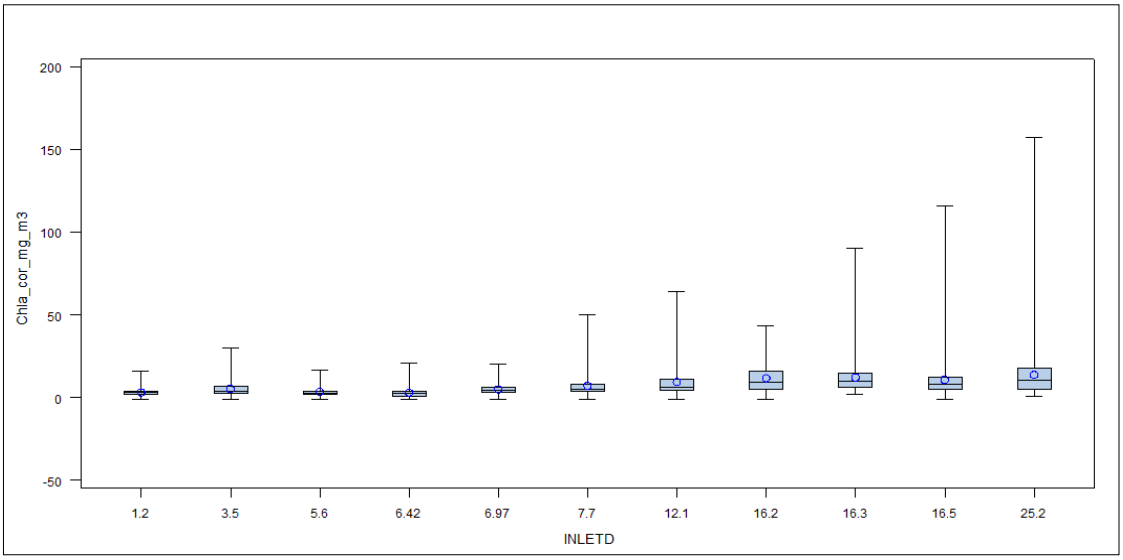


Figure C-9. Boxplots of salinities (A) and chlorophyll *a* (B) at selected South Florida Water Management District sites from fall 2000 to spring 2011.

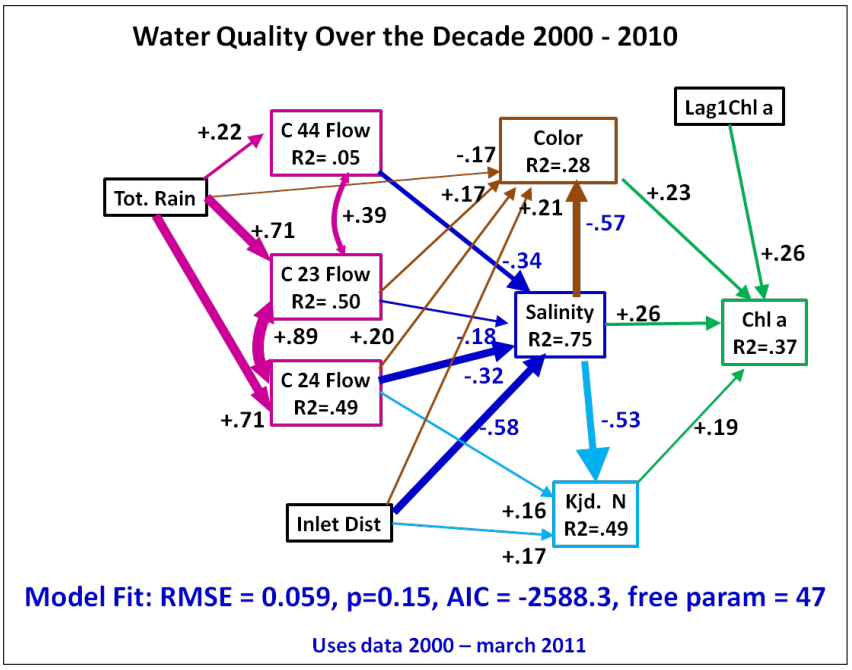
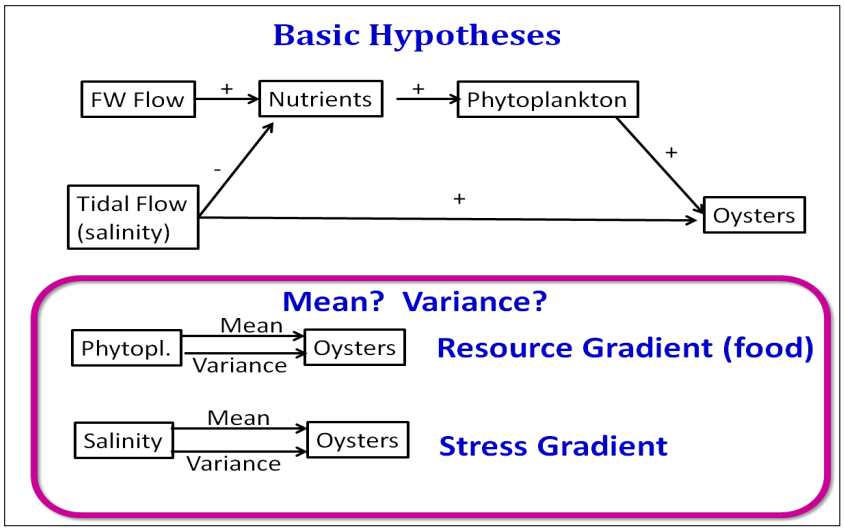


Figure C-10. The network of relationships among rainfall; flow of the C44, C23, and C24 canals; salinity, total Kjeldahl nitrogen, and color (environmental drivers); and chlorophyll *a* as a proxy measure of phytoplankton abundance.

Our basic hypotheses discussed and tested by SEM analyses were that salinity (as a primary environmental variable and occasionally a stressor) and chlorophyll *a* (as a potential food source) were important to the development of the oyster population (**Figure C-11A**). Further, we hypothesized that it was the variability in space and time of these variables, either in addition to or instead of the means that was an important driver of oyster viability. Here, we present and discuss this primary conceptual model. We also posed a number of other models using variables such as turbidity, total suspended solids, pH, and DO but the covariance structure of none of these fit when combined with the oyster data (nor by extension later the associated invertebrate assemblage data). Further, we employed nutrients in the model eventually tested assuming that they would aid in explaining variation in chlorophyll *a*, but these models did not fit the data either and were discarded.

Oyster abundance in the created patches increased over time, Age_reef variable, as indicated in the patch age variable in **Figure C-11B**. Overall, 62% of the variation in oyster densities was explained by the model. Mean salinity and chlorophyll *a* (removed from the final model) had no effect, but increasing variation in both these variables across the estuary and over time had moderate direct negative effects on oyster abundance. The variability of both salinity and chlorophyll *a* were driven by increasing distance from the inlet (i.e., reduced tidal influence) and freshwater inflows from the canals.

A)



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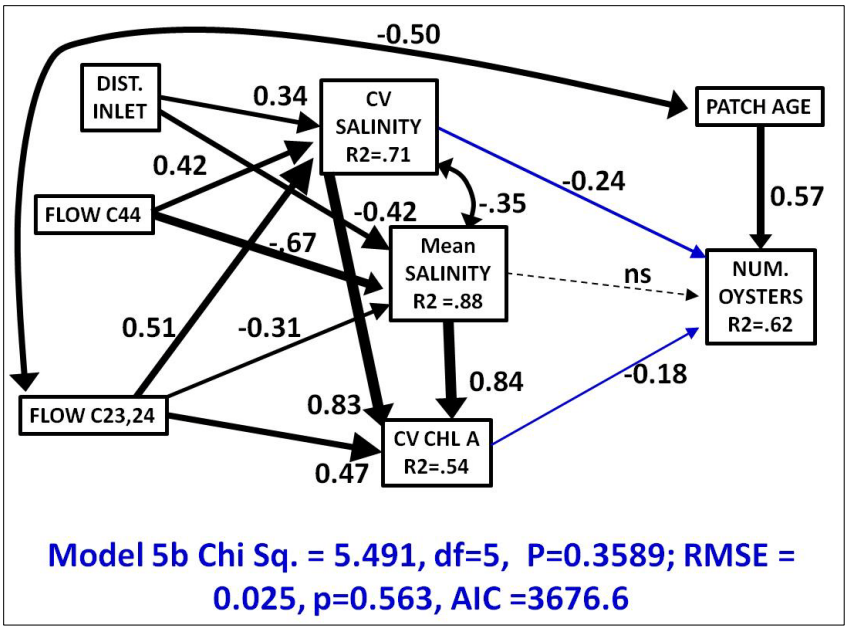

Figure C-11. Conceptual model posed as basic hypotheses (A); and oyster abundance as a function of patch age, salinity, chlorophyll *a*, distance from inlet, and canal flow (B). Flows from C23 and C24 canals were summed because they were highly correlated (r>0.9) and so may function as one variable. Values are standardized path coefficients (partial regression coefficients) to facilitate comparisons of the magnitudes of effects among predictor variables. Both mean and the coefficient of variation (CVs on variable names) for salinity and chlorophyll *a* were used as predictor variables.

The lack of effect of mean salinity does not imply that this variable is always unimportant. We expect that in wet years, when there is considerably more freshwater discharge, salinities would become low for extended periods and produce severe stress or mortality in the oyster population.

Our SEM did not separate the effects of the three main canals because C23 and C24 were summed. A separate multiple regression of salinity on distance of a site from inlet and mean monthly canal flows for all months shows that the C44 and C24 canals had significant, and similar magnitude, effects on salinity, but the C23 canal did not: LSAL = 1.9436 - .2903 (LC44) – 0.2398(LC24) - 0.5120(LINLETD); $R^2 = 0.74$). All values in the previous SEM and multiple regression analyses were $\log_{10}(x+1)$ transformed.

Sediment Elevation Table Analysis of Reef Elevation

At the SET in SLR Site 1, there was considerable net loss of elevation over the first 9 months of the project (**Figure C-12**). This resulted from a combination of elevation loss due to reefs sinking and compacting, and about 10 mm of sedimentation. Over approximately the next year, elevation loss continued but at a much slower rate. Then, during the last 8 months of the project, there was a gain in elevation probably resulting from a rate of oyster (and mussel) colonization and growth that exceeded the subsidence+sedimentation rate. This accretion in topographic relief occurred at an average rate of 3 mm/mo. If we assume that the monthly sedimentation rate can be estimated from the first 9 months of data (1.11 mm/mo), then the reef accretion rate is exceeding at least the sedimentation rate that occurs during drought flow conditions. This illustrates the production of reef structure and vertical relief off the bottom. Both of these are important for colonization by other invertebrates (Salewski and Proffitt unpublished field experiment data) and some vertebrates.

The SETs at SLR Site 2 and the Loxahatchee site were not installed as early as the SETat Site 1. If there were similar elevation losses they were not captured in the "net change" data (**Figure C-12**). However, elevations at all SET sites ended up in the positive range, indicating accretion due to reef development.

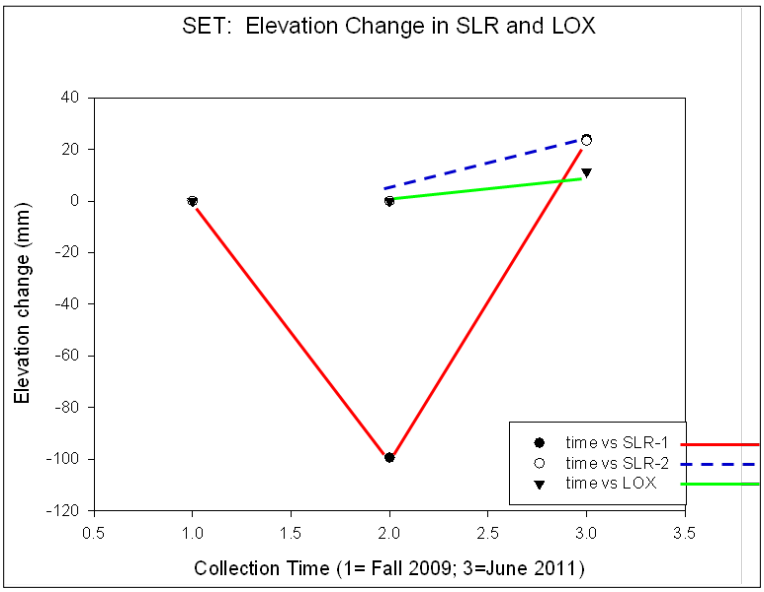

Figure C-12. Sediment elevation table (SET) results for Sites 1 and 2 in the St. Lucie River and the Loxahatchee site. All are normalized to 0 elevation at time of installation of the SET. Subsequent negative changes are elevation losses; positive changes are elevation gains.

Oyster Reef Community Analysis

Reef invertebrates include those that aid in reef-building (primarily oysters and mussels in this system) and those species, motile or sessile, that mainly use the oyster reefs as habitat. Averaged across all SLR sites, reef-building taxa increased over the time-course of the study (**Figure C-13**). When separated by site and time (**Figure C-14**), the major total reef-building taxa (oysters+mussels) showed increases followed by decreases that varied by SLR site. Thus, development of reef varied with location in the SLR and mostly tracked the oyster population development analyzed in the previous section.

Total invertebrate abundance averaged over all SLR sites increased rapidly over the first year, and more slowly thereafter (**Figure C-15**). Total invertebrates when separated by site, show patterns of increases in abundance during the first year followed by slight, but site specific, declines (**Figure C-16**). Further, total invertebrate abundances increased as a quadratic function of live reef-forming species, although both increased over time as well (**Figure C-17**).

For comparison, we sampled reefs created in 2005 and 2006 at the first of the study (fall 2009). At those sites, reef- building taxa (oysters+mussels mainly) were 47.2 +/- 48.7 SD live individuals/m^2. Maximum abundance in a single 2005-2006 reef sample was 1,373 animals/m^2. Total nonoyster fauna was 339.6 +/- 529 SD individuals/m^2 and was 51% amphipods and 32% polychaetes. Reefs created in 2009 surpassed the 2005-2006 reef starting mean density within a few months, and mean densities at July 2011 (**Figure C-15**) were comparable to the single sample maximum value seen in the 2005-2006 reefs.

The most abundant, or dominant, taxa had very different patterns of colonization in the SLR reefs (**Figure C-18**). Polychaetes and gastropods both increased over time, although the latter was much slower to recruit. Amphipods increased over the first year, and then leveled off. However, barnacles increased to great numbers in the first year, and then declined precipitously in the second year.

Individual major taxa showed interesting trends among sites, over time and with reef development. Polychaetes, for example, increased over time, but had very different patterns at different sites (**Figure C-19**). However, it is of interest to know if the polychaetes (as an example taxa) are colonizing at various rates because the substrate is new and population development simply takes time, or if they respond positively to the presence of live reef-formers like oysters. Graphing polychaetes against oyster abundance suggests a relationship with live reef formers that is different for different parts of the SLR estuary (**Figure C-20**). Polychaetes colonized at much lower rates per live oyster at the upper (Site 11) and lower (Site 6) estuary sites.

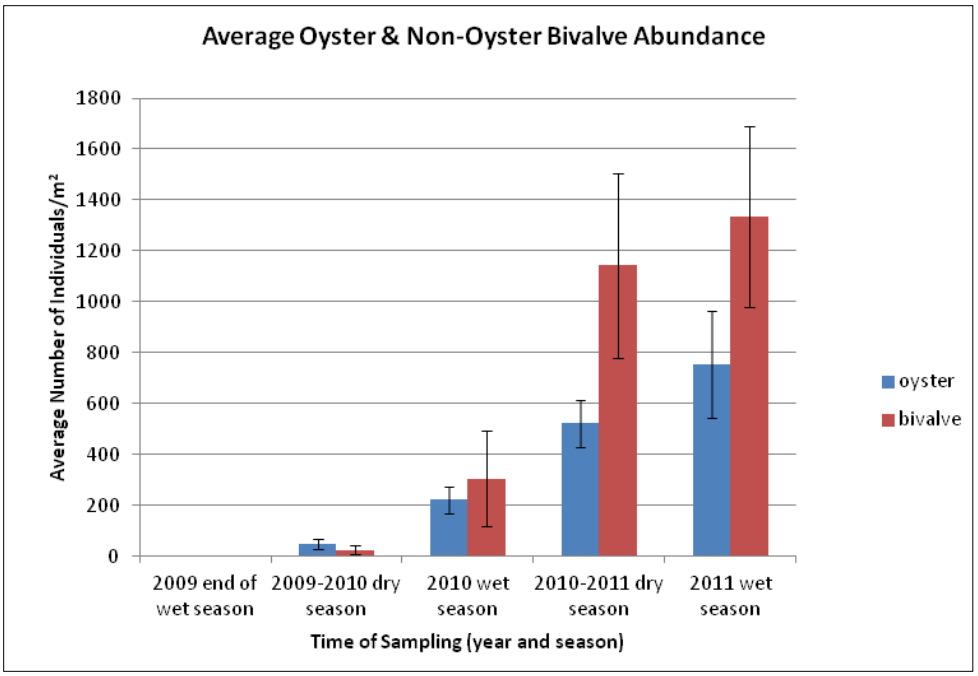

Figure C-13. Means ± 1 SD of oyster and other bivalve (primarily several species of mussels) shown by year and season.

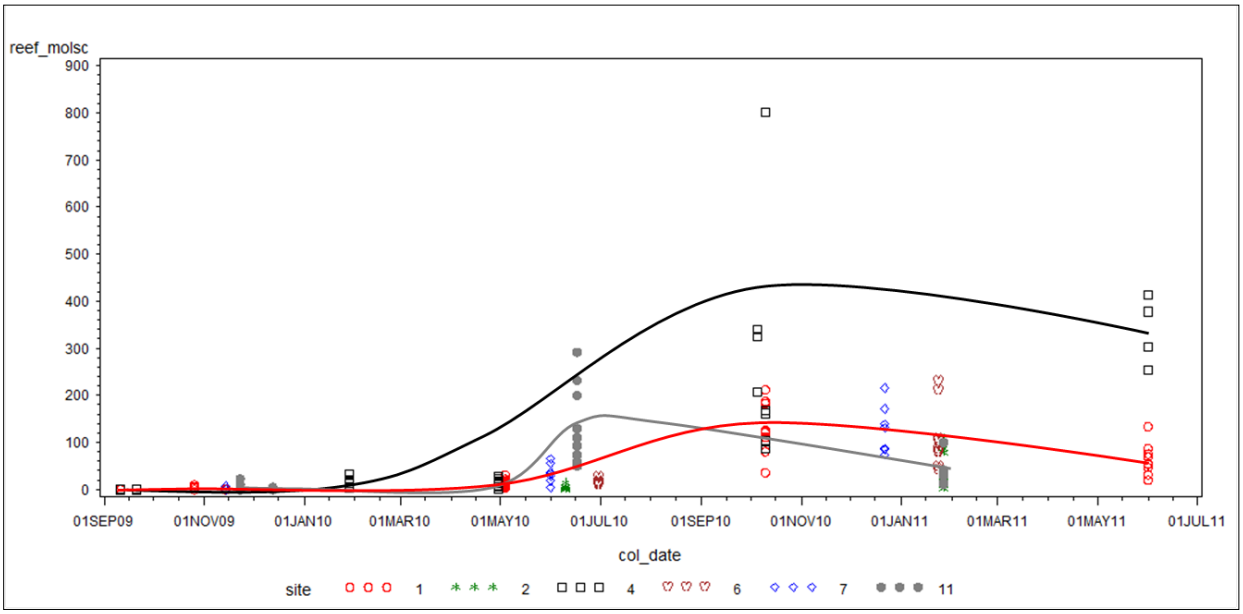

Figure C-14. Total reef-forming mollusks (oysters+mussels) by collection date and site in the St. Lucie River. Trend lines (hand-drawn for several sites) suggest increases followed by decreases in magnitude that differ by site.

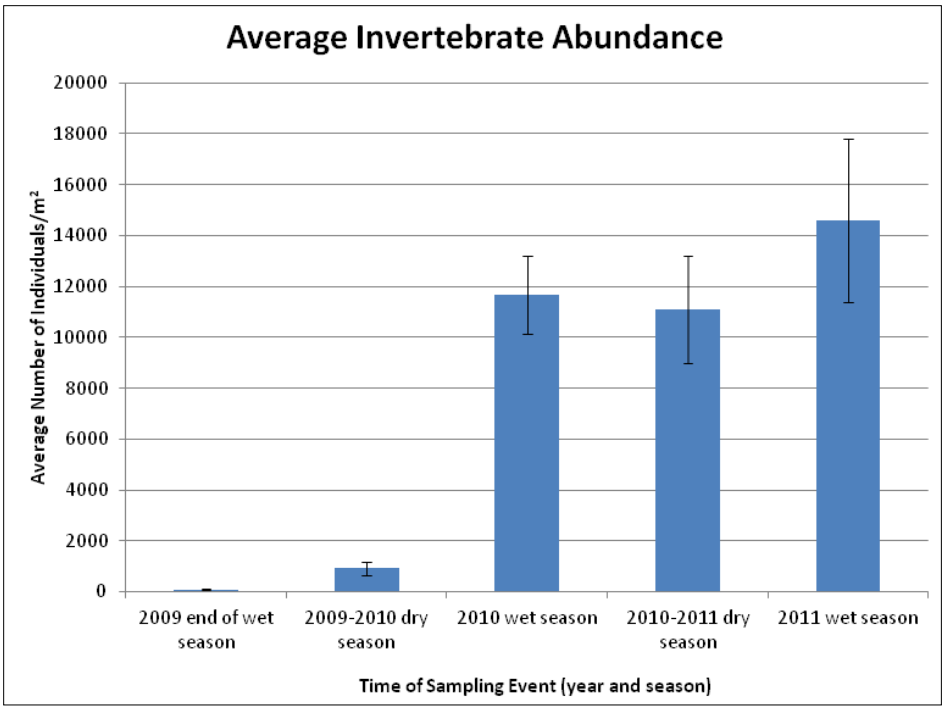

Figure C-15. Total invertebrates of all taxa on reefs in the St. Lucie River over the course of the study averaged across sites. Values are means ±1 SD.

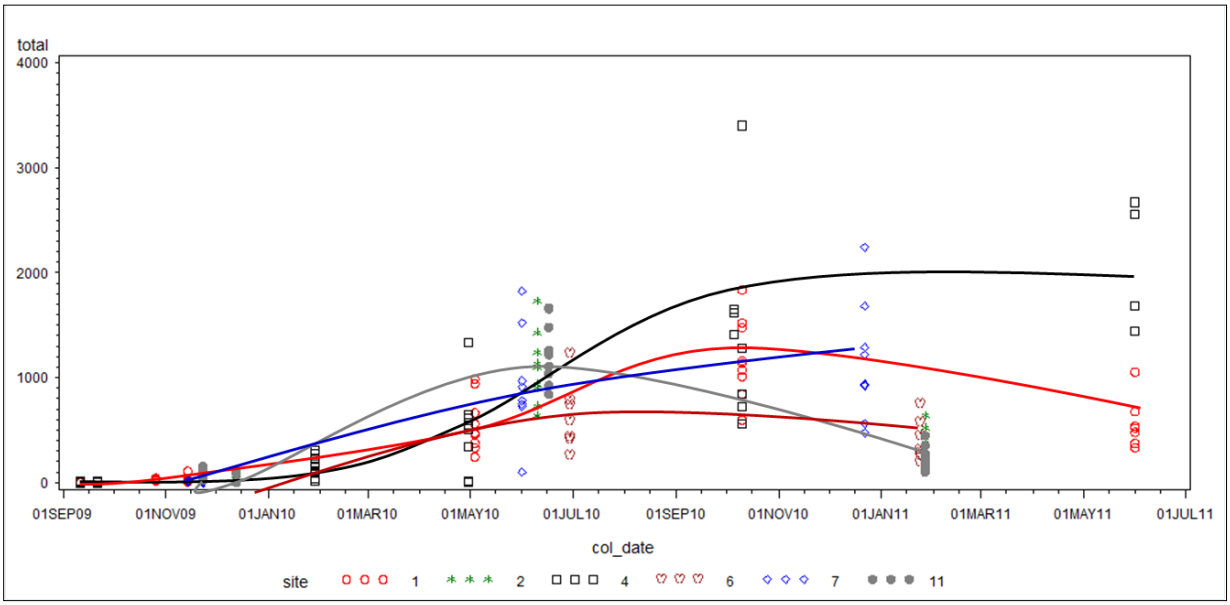

Figure C-16. Total invertebrate abundance by St. Lucie River site over time.

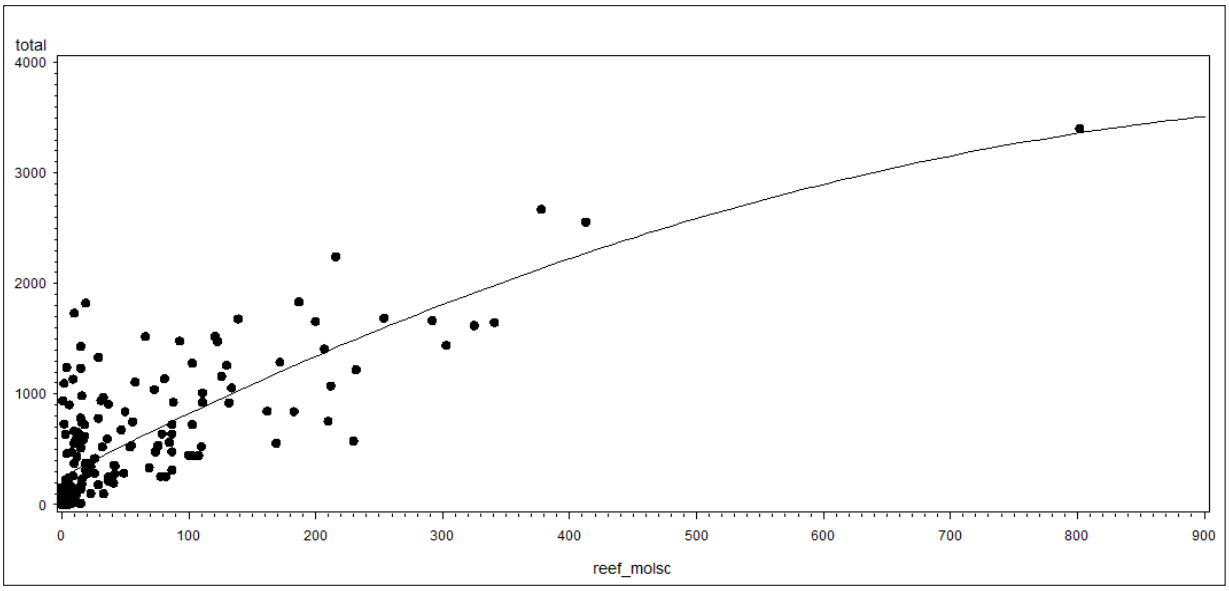

Figure C-17. Total invertebrates vs. live reef-forming taxa, all times and sites combined.

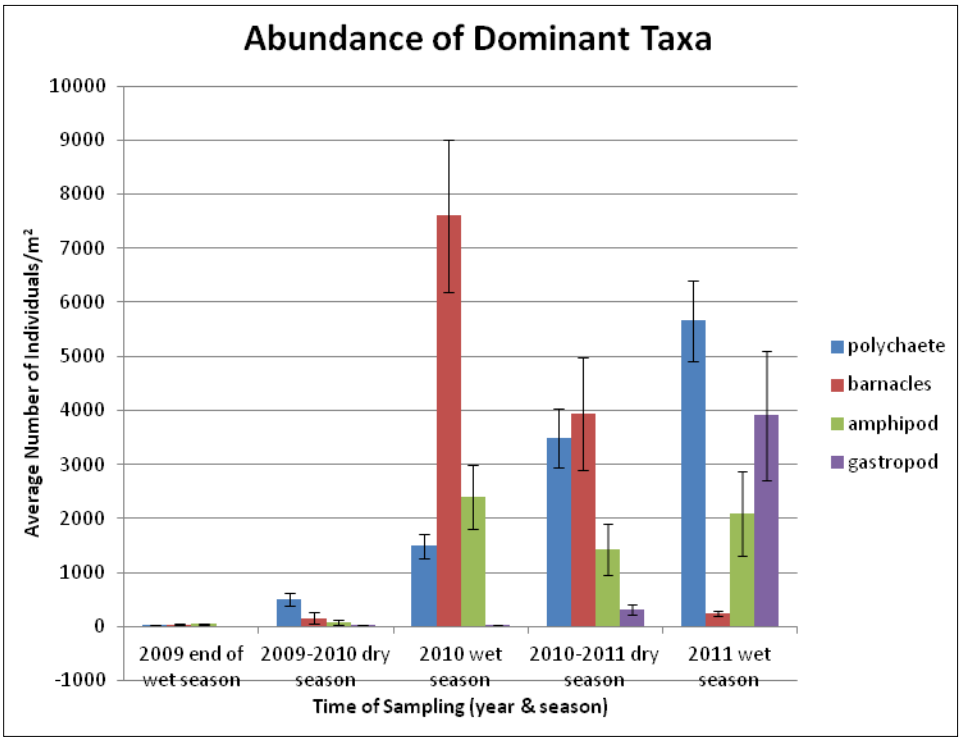

Figure C-18. Mean (±1 SD) abundances of the four most abundant taxonomic groups by year and season averaged over all St. Lucie River sites.

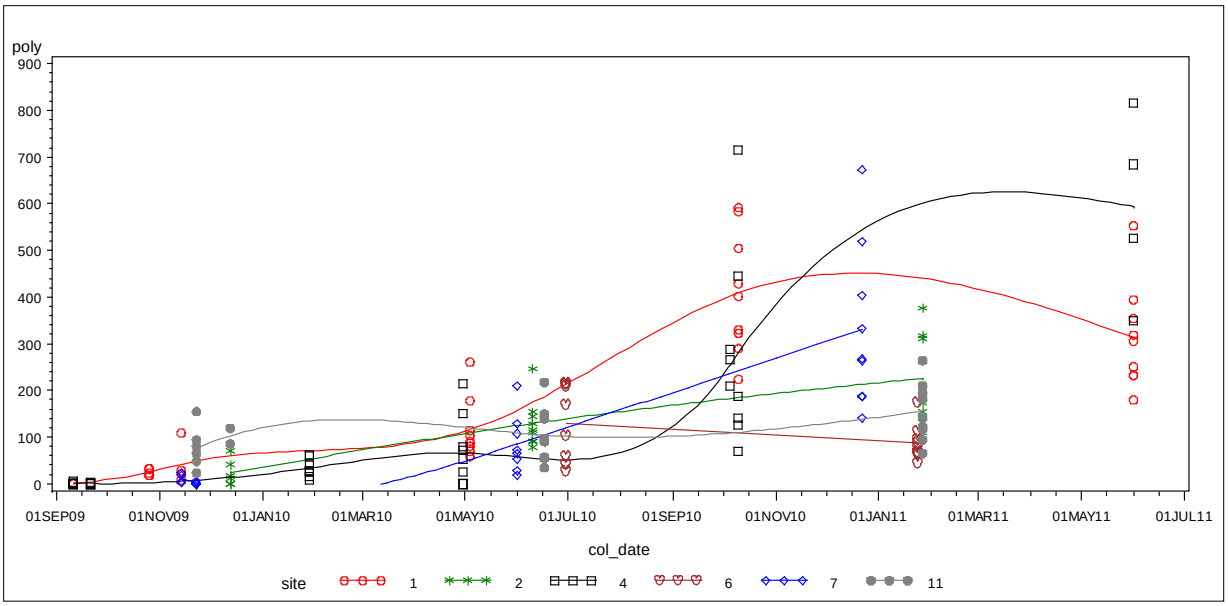

Figure C-19. Polychaete abundances at St. Lucie River reefs over time.

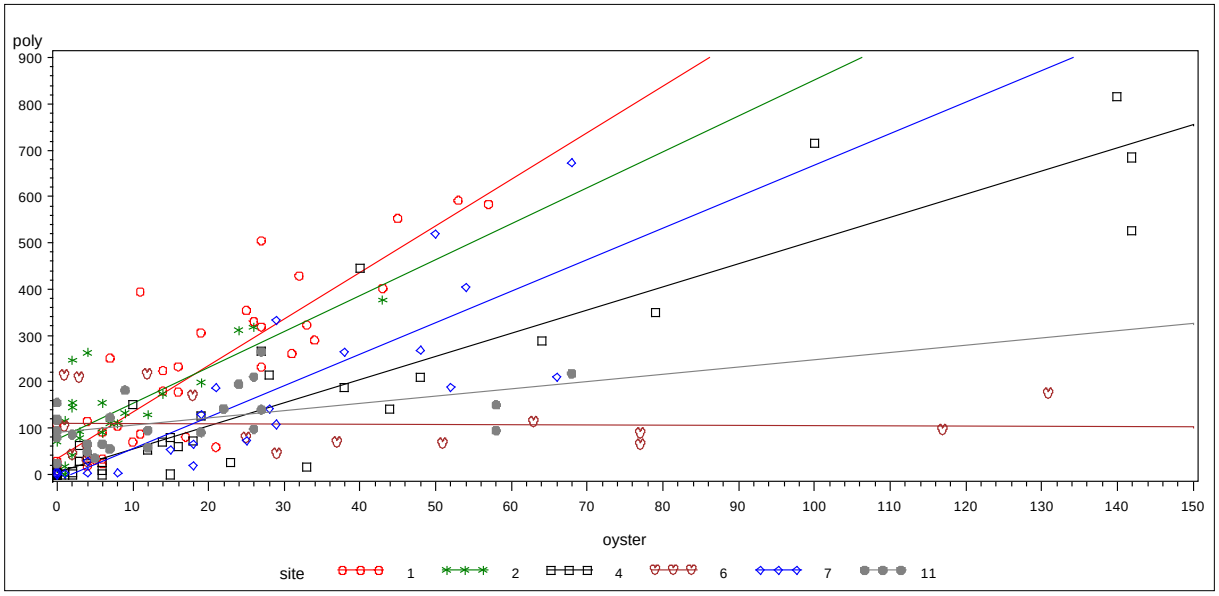

Figure C-20. Total polychaete abundances at different St. Lucie River sites plotted against abundance of live oysters. Trend lines are least squares regressions.

Stuctural Equation Modeling Analysis of Reef Community

Unlike oysters, plotting the total invertebrate fauna against salinity produced a hump-backed curve (**Figure C-21**) that necessitated the use of a quadratic (salinity-squared) term in addition to the salinity term. It is possible that this relationship reflects the differences in individual physiological tolerances to salinity of different taxa. Further, total invertebrate fauna increased in response to increasing numbers of total reef-building mollusks (**Figure C-22**) as well as to oysters alone.

The SEM explained 77% of the variation in total invertebrate abundance (**Figure C-23**). Salinity (path coefficient = +.38) and salinity-squared (-.57), and age of reef (.76) had large direct effects on total invertebrates. However, with those physical variables accounted for, live oyster abundance accounted for a significant amount of additional variability in the total invertebrates.

Flow had both direct (-.15) and indirect effects through two paths (Flow→Sal→inverts and Flow→oysters→inverts). Total effects were +.07 when these direct and indirect paths are summed, thus, although the direct effect of flow on the abundance of total invertebrates was negative, the total influence was a weak positive. Again, as mentioned in the oyster section, the study period had fairly low flows and thus moderate to high salinities. A wet year will likely produce stress that probably cannot be adequately modeled by extrapolating from the existing dataset.

Fewer samples have been completely identified to species, thus further community analyses in this analysis uses only a subset of samples across the first year and a half of the project. These samples were subjected to ANOSIM in Primer to assess species similarities among sites and across times. Community composition differed at large (site) and small (patch within site) spatial scales (Site global R=0.052, $p<0.03$; Patch global R=.106, $p<0.01$). Temporal differences in community composition occurred at both year (global R=0.418, $p<0.01$) and season (global R=.069, $p<0.01$) scales.

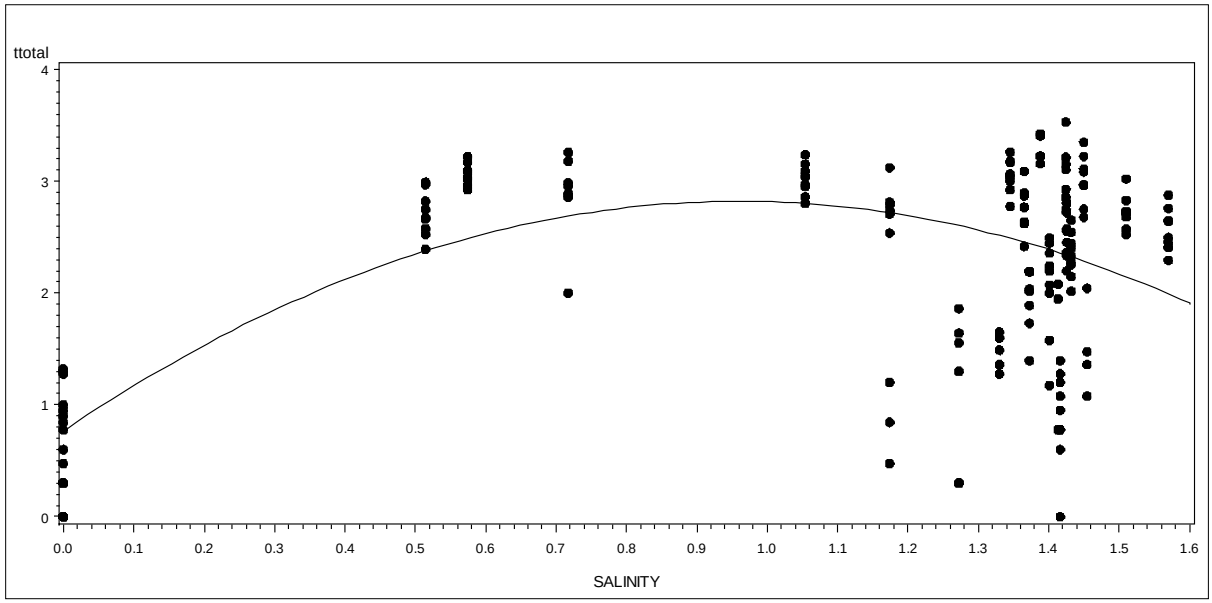

Figure C-21. The hump-backed relationship between log (salinity+1) on the x-axis and log (total invertebrate abundance+1) on the y-axis.

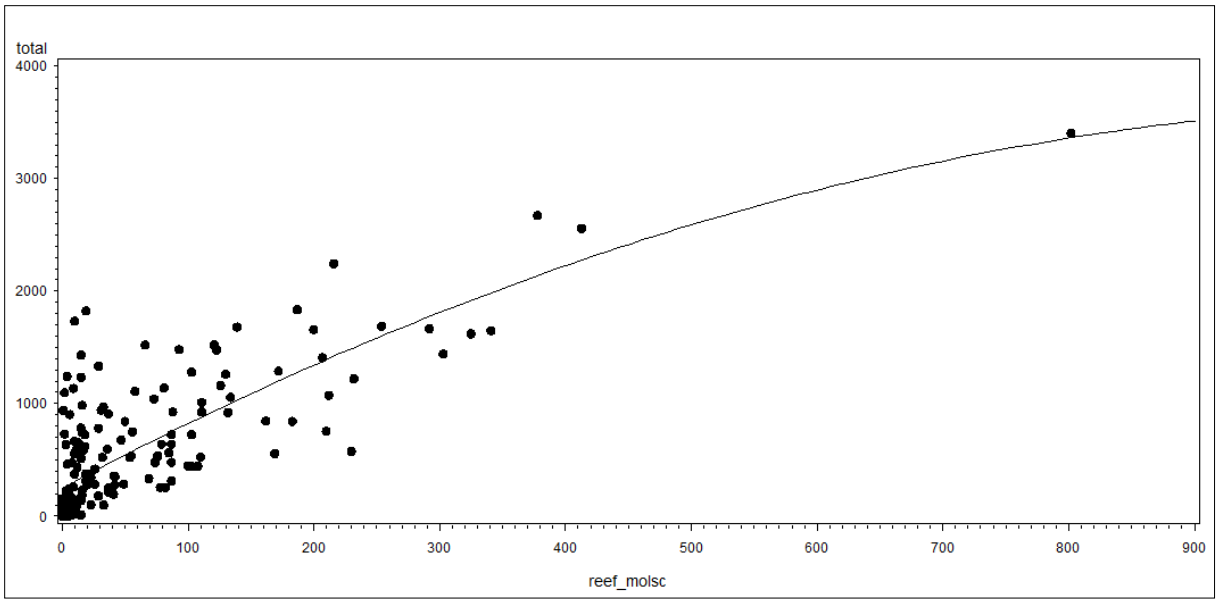

Figure C-22. Graph illustrating the increase in total reef invertebrate abundance with increasing live reef-forming mollusks.

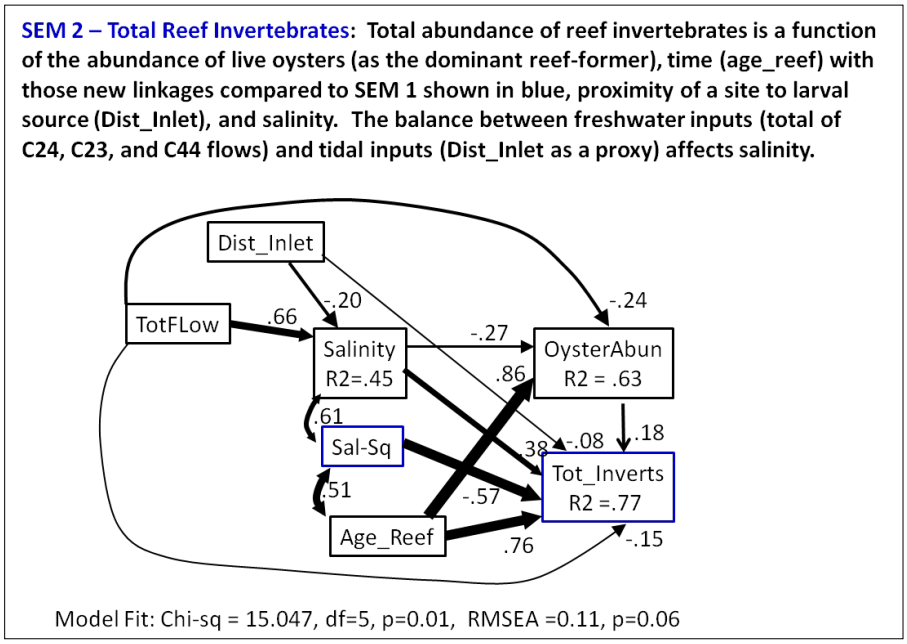

Figure C-23. The structural equation modeling (SEM) for total invertebrate abundance/m^2 indicates that presence of live oysters increases invertebrate densities, as does proximity to inlet. The quadratic response to salinity is indicated by the + effect of salinity and the – effect of salinity-squared. Blue indicates variables not in the oyster model. Chlorphyll *a* was not significant in this model, and was omitted.

DISCUSSION

Restoration of oyster communities in the SLR and LOX has been largely successful, although the extent of oyster and community development has varied substantially by site. From the SET data, it appears that reef accretion from oyster and mussel settlement and growth occur at an average of 3 mm/mo, and that this is 2 to 2.5 times the rate of sedimentation, at least during low flow times. Further work with SETs at all sites could determine this, as could studies on the development of reef structure and complexity.

The reef community developed via colonization and primary succession over the course of the 2-year study. Oysters and other invertebrate taxa colonize at rates that vary by position of the reef in the estuary, season, year, and with salinity. The presence of live oysters, not just cultch material, enhances the rate of development of the invertebrate assemblage. Part of this effect may be by oysters contributing to the overall food web, and part may be the development of additional structure and topographic relief. Reef development will probably continue to proceed toward a more complex oyster ecosystem resembling a natural reef until interrupted by disturbance of the kind that can be produced by canal runoff during wet years. Extreme disturbance will reset reef abundances to much lower levels and the process of colonization and succession will begin again. However, the next community that develops will have the benefit of considerable structure and vertical relief provided by the assemblage of animals existing before the disturbance. Oyster reef development in the SLR could become this iterative process of successive periods of colonization and growth interrupted by occasional massive disturbances. If so, reef development will depend on: (1) the rates of colonization and reproduction of reef species, (2) incidence and frequency of disturbance from high canal flow; (3) the rates of more frequent, smaller perturbations (e.g., sedimentation rates, algal blooms); and (4) the rate of elevation change from reef sinking or erosion of reef structure. These are avenues for future research.

The abundance of invertebrate fauna on reefs retained by a 1-mm mesh was striking, with values reaching 14,000 individuals/m^2. This represents populations of many species having ecological functions (e.g., detritivore, planktovore, predator, reef builder, tube dweller) and a diverse food web. Many of these species have short generation times, thus the total productivity far exceeds the numbers captured on any given sampling day. Therefore, this suite of species makes a vast contribution by supporting higher trophic levels such as secondary consumer fish species, and indirectly to top predators such as dolphins, sharks, and birds (see list in **Attachment)**. Some of the species listed are more typical of soft substratum systems suggesting an added degree of complexity as patches of soft sediment fill in at the base of shells.

Assessing colonization and recruitment are instructive. From 2009-2010 there were relatively low rates of recruitment of small individuals (<20 mm) at all sites. Because there were several months between sampling events, it was not possible to detect those colonizers that could have entered the reef communityand been lost between sampling events. However, assessment of the small, medium, and larger size classes each time suggests that recruitment data were adequate.

There was much greater colonization and recruitment in 2011, although the bulk was at more seaward Sites 4 and 6 which suggests either a large input of larvae entering the system at the mouth of the SLR or differential survival and recruitment or both. SEM models with “distance from natural reef” within the SLR did not fit the data although this is probably a source of larvae. The difference between spring and early summer seasons in 2010 and 2011 could reflect simple between-year variance or it might be reflective of greater production of larvae in the SLR as a whole because of the large number of resident oysters on the created reefs.

The maximum growth rate of oysters was estimated by the size of the largest live oyster at sites over time. Oysters grew fastest at Site 4 and slowest at Site 6 where the maximum size was only less than half that at Site 4. Site 6 was younger but only by a few months, so it is unlikely that this is an explanation for such a difference in size. More likely oysters in the middle size class are suffering extensive mortality, or, possibly growth is less because of stress factors. Differences in water velocity among sites were estimated, but it was not a significant factor in SEM models. Site 6 was a location with higher, less variable salinities, which might encourage predators. Our data on predators such as the gastropod *Boonea impressa* and the flatworm *Stylochus* sp., however, did not show differences among sites (abundance data not shown).

Structural Equation Modeling Using Monitoring Data

Carefully controlled and performed experiments are acknowledged to be the gold standard in establishing cause and effect relationships. However, it is often impossible to conduct experiments in the field over spatial scales that are relevant to populations and communities and, in this case, to the scale of the restoration. Structural equation modeling provides a mechanism for testing hypotheses of cause-and-effect using observational data (Shipley, 2000; Grace, 2006). In SEM, true multivariate hypotheses can be posed with multiple linkages producing direct and indirect hypothesized causal effects, and other linkages hypothesized to be correlative without causation, all in one statistical model. This arrangement of variables and proposed causal and noncausal relationships mathematically dictates an "implied covariance structure" among the variables in the model. One then gathers real data on these variables and tests the "observed covariance structure" in the dataset. If the observed covariance structure in the real data are not significantly different from the covariance structure implied by the model proposed, then this is a strong presumptive test for causality in the multivariate framework of the model postulated. Practitioners of SEM would take this as a presumptive test of causality, and would attempt to gather additional data for an independent test of the model.

Most usually, a scientist would gather data on all variables at the same time at all sites. In this case, we wanted to see if water quality and flow data from the South Florida Water Management District's DBHYDRO database could be coupled with biological data in a way that could explain some proportion of the variance of the biological data. This turned out to be the case, which suggests a strong role for monitoring datasets in testing ecological hypotheses such as posed here. Good predictive power in terms of R^2 values were found for both oyster and total invertebrate abundances despite the fact that water quality stations were sometimes a distance away from the biological sampling locations.

In order to link biological variables to a more complex web of physical variables, a considerable amount of additional biological data would be necessary. Thus, we tested relatively simple models herein. However, future research should focus on gathering biological data at many more locations and times in the SLR.

In conclusion, colonization and development of the oyster reefs and associated community progressed over the course of the 2-year study. Water discharges and certain water quality parameters affected the rate and trajectory of development at different reef locations. The presence of living oysters, not just cultch, positively influenced various taxonomic groups of invertebrates and ascidians colonizing the reefs. Reef development should continue until the present drought ends and heavy rains and canal flows begin and produce extended periods (i.e., 3+ months) of low salinities. It is then likely that wide-spread mortality will occur on the reefs and succession will be reset.

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ATTACHMENT: LIST OF TAXA

Grouping	Family	Species
Mollusca	Anomiidae	*Anomia simplex*
Mollusca	Arcidae	*Anadara transversa*
Mollusca	Assimineidae	*Assiminea* sp.
Mollusca	Bivalvia	*Bivalvia* sp.
Mollusca	Carditidae	*Glans dominguensis*
Mollusca	Calyptraeidae	*Crepidula depressa*
Mollusca	Calyptraeidae	*Crepidula fornicata*
Mollusca	Calyptraeidae	*Crepidula* sp.
Mollusca	Calyptraeidae	*Crepidula* spp.
Mollusca	Cerithiopsidae	*Cerithiopsis carpenteri*
Mollusca	Columbellidae	*Astyris lunata*
Mollusca	Corbulidae	*Corbula contracta*
Mollusca	Dressenidae	*Mytilopsis leucophaeata*
Mollusca	Mactridae	*Mulinia lateralis*
Mollusca	Mangeliidae	*Pyrgocythara plicosa*
Mollusca	Myidae	*Sphenia antillensis*
Mollusca	Mytilidae	*Amygdalum papyrium*
Mollusca	Mytilidae	*Brachidontes extusus*
Mollusca	Mytilidae	*Brachidontes* sp.
Mollusca	Mytilidae	*Geukensia demissa*
Mollusca	Mytilidae	*Ischadium recurvum*
Mollusca	Mytilidae	*Mytilidae* sp.
Mollusca	Mytilidae	*Musculus laterlis*
Mollusca	Neritidae	*Neritina reclivata*
Mollusca	Neritidae	*Neritina virginea*
Mollusca	Noetiidae	*Noetia ponderosa*
Mollusca	Ostreidae	*Crassostrea viriginica*
Mollusca	Pteriidae	*Isognom alatus*
Mollusca	Pyramidellidae	*Boonea impressa*
Mollusca	Semelidae	*Abra aequlais*
Mollusca	Semelidae	*Semelidae* sp.
Mollusca	Solecurtidae	*Tagelus divisus*
Mollusca	Tellinidae	*Macoma tenta*
Mollusca	Tellinidae	*Tellina* sp.
Mollusca	Triphoridae	*Marshallora nigrocincta*
Mollusca	Ungulinidae	*Diplondonta punctata*
Mollusca	Veneridae	*Dosinia discus*
Polychaeta	Ampharetidae	*Hobsonia florida*
Polychaeta	Capitellidae	*Capitella capitata*
Polychaeta	Capitellidae	*Heteromastus filiformis*
Polychaeta	Capitellidae	*Mediomastus californiensis*
Polychaeta	Capitellidae	*Mediomastus* sp.
Polychaeta	Capitellidae	*Capitellidae*
Polychaeta	Chaetopteridae	*Spiochaetopterus costarum*

Grouping	Family	Species
Polychaeta	Chaetopteridae	*Chaetopteridae*
Polychaeta	Chrysopetalidae	*Bhawania* sp. A
Polychaeta	Dorvilleidae	*Schistomeringos rudolphi*
Polychaeta	Eunicidae	*Lysidice ninetta*
Polychaeta	Eunicidae	*Marphysa sanguinea*
Polychaeta	Eunicidae	*Nematonereis hebes*
Polychaeta	Glyceridae	*Glycera abranchiata*
Polychaeta	Glyceridae	*Glycera* sp.
Polychaeta	Goniadidae	*Glycinde nordmanni*
Polychaeta	Goniadidae	*Goniadidae*
Polychaeta	Hesionidae	*Parahesione luteola*
Polychaeta	Hesionidae	*Podarkeopsis levifuscina*
Polychaeta	Hesionidae	*Podarke obscura*
Polychaeta	Hesionidae	*Hesionidae*
Polychaeta	Nereidae	*Laeonereis culveri*
Polychaeta	Nereidae	*Neanthes succinea*
Polychaeta	Nereidae	*Nereis falsa*
Polychaeta	Nereidae	*Nereis lamella*
Polychaeta	Nereidae	*Nereis riisei*
Polychaeta	Nereidae	*Nereis* sp.
Polychaeta	Nereidae	*Steninonereis martini*
Polychaeta	Nereidae	*Steninonereis tecolutlensis*
Polychaeta	Nereidae	*Nereidae* sp. A
Polychaeta	Nereidae	*Nereidae*
Polychaeta	Onuphidae	*Diopatra cuprea*
Polychaeta	Opheliidae	*Armandia maculata*
Polychaeta	Opheliidae	*Ophelina acuminata*
Polychaeta	Opheliidae	*Opheliidae*
Polychaeta	Orbiniididae	*Naineris grubei*
Polychaeta	Orbiniididae	*Naineris* sp.
Polychaeta	Orbiniididae	*Orbiniididae*
Polychaeta	Orbiniididae	*Proscolopos* sp. A
Polychaeta	Paraonidae	*Paraonidae*
Polychaeta	Pectinariidae	*Pectinaria gouldi*
Polychaeta	Polynoidae	*Harmothoe cf aculeata*
Polychaeta	Polynoidae	*Harmothoe* sp. A
Polychaeta	Polynoidae	*Harmothoe* sp.
Polychaeta	Polynoidae	*Lepidasthenia commensalis*
Polychaeta	Polynoidae	*Subadyte pellucida*
Polychaeta	Phyllodocidae	*Anaitides mucosa*
Polychaeta	Phyllodocidae	*Eulalia bilineata*
Polychaeta	Phyllodocidae	*Eulalia viridis*
Polychaeta	Phyllodocidae	*Nereiphylla castanea*
Polychaeta	Phyllodocidae	*Nereiphylla fragilis*
Polychaeta	Phyllodocidae	*Phyllodocidae*
Polychaeta	Sabellariidae	*Saellaria vulgaris vulgaris*
Polychaeta	Sabellidae	*Bispira melanostigma*

Grouping	Family	Species
Polychaeta	Sabellidae	*Branchiomma nigromaculata*
Polychaeta	Sabellidae	*Demonax microphthalmus*
Polychaeta	Sabellidae	*Fabricinuda* sp.
Polychaeta	Sabellidae	*Laonome* sp. A
Polychaeta	Sabellidae	*Notaulax cf midoculi*
Polychaeta	Sabellidae	*Parasabella lacunosa*
Polychaeta	Sabellidae	*Parasabella microphthalma*
Polychaeta	Sabellidae	*Parasabella* sp.
Polychaeta	Sabellidae	*Potamilla* sp.A
Polychaeta	Sabellidae	*Sabellastarte* sp. A
Polychaeta	Sabellidae	*Sabellidae*
Polychaeta	Serpulidae	*Ficopomatus enigmaticus*
Polychaeta	Serpulidae	*Ficopomatus miamiensis*
Polychaeta	Serpulidae	*Ficopomatus* sp.A
Polychaeta	Serpulidae	*Ficopomatus* spp
Polychaeta	Serpulidae	*Hydroides dianthus*
Polychaeta	Serpulidae	*Hydroides* sp. A
Polychaeta	Serpulidae	*Serpulidae*
Polychaeta	Spionidae	*Boccardiella hamata*
Polychaeta	Spionidae	*Dipolydora* sp.
Polychaeta	Spionidae	*Dipolydora socialis*
Polychaeta	Spionidae	*Paraprionospio pinnata*
Polychaeta	Spionidae	*Polydora barbilla*
Polychaeta	Spionidae	*Polydora cornuta*
Polychaeta	Spionidae	*Polydora* spp.
Polychaeta	Spionidae	*Polydora websteri*
Polychaeta	Spionidae	*Prionospio (Prionospio) cristata*
Polychaeta	Spionidae	*Pseudopolydora* sp. A
Polychaeta	Spionidae	*Spio pettiboneae*
Polychaeta	Spionidae	*Steblospio benedicti*
Polychaeta	Spionidae	*Spionidae*
Polychaeta	Syllidae	*Exogone lourei*
Polychaeta	Syllidae	*Odontosyllis enopla*
Polychaeta	Syllidae	*Syllis (Typosyllis)* sp. A
Polychaeta	Syllidae	*Syllidae*
Polychaeta	Terebellidae	*Loimia meduse*
Polychaeta	Terebellidae	*Pista palmata*
Polychaeta	Terebellidae	*Pista* sp. A
Polychaeta	Terebellidae	*Thelepus setosus*
Polychaeta	Terebellidae	*Terebella* sp. A
Polychaeta	Terebellidae	*Terebella* sp. B
Polychaeta	Terebellidae	*Terebellidae*
Oligochaeta	Tubificidae	Tubificidae
Oligochaeta	Oligochaeta	Oligochaeta
Crustacea	Alpheidae	*Alpheus cf. heterochaelis*
Crustacea	Alpheidae	*Alpheus* spp.
Crustacea	Ampeliscidae	*Ampelisca abdita*

Grouping	Family	Species
Crustacea	Amphilochidae	*Hourstonius laguna*
Crustacea	Amphilochidae	*Hourstonius* spp.
Crustacea	Aoridae	*Grandidierella bonnieroides*
Crustacea	Balanidae	*Balanus amphitrite*
Crustacea	Balanidae	*Balanus eburneus*
Crustacea	Balanidae	*Balanus improvisus*
Crustacea	Balanidae	*Balanus* spp.
Crustacea	Corophiidae	*Monocorophium* spp.
Crustacea	Corophiidae	Corophiidae sp.
Crustacea	Cytheridedae	*Peratocytheridea* sp.
Crustacea	Isaeidae	*Photis* sp.
Crustacea	Ischyroceridae	*Cerapus* sp.
Crustacea	Leptocheliidae	*Hargeria rapax*
Crustacea	Leptocheliidae	*Leptochelia/Hargeria rapax complex*
Crustacea	Melitidae	*Meilta nitida complex*
Crustacea	Melitidae	*Melita nitida*
Crustacea	Menippidae	*Menippe mercenaria*
Crustacea	Mysidae	Mysidae sp.
Crustacea	Paguridae	*Pagurus* spp.
Crustacea	Palaemonidae	*Periclimenes americanus*
Crustacea	Palaemonidae	Palaemonidae sp.
Crustacea	Porcellanidae	Porcellanidae sp.
Crustacea	Panopeidae	*Eurypanopeus depressus*
Crustacea	Panopeidae	*Rhithropanopeus harrisii*
Crustacea	Xanthidae	Xanthidae sp.
Crustacea	Portunidae	Portunus sp.
Crustacea	Tanaidae	*Sinelobus stanfordi*
Crustacea	Tanaidae	Tanaidae sp.
Tunicata	Ascidiacea	Ascidiacea sp.
Tunicata	Molgulidae	*Molgula occidentalis*
Tunicata	Styelidae	*Styela plicata*
Actiniaria		Actiniaria sp.
Nemata		Nemata sp.
Nematoda		Nematoda sp.
Nemertea		Nemertea sp.
Ophiuroidea		Ophiuroidea sp.
Platyhelmintes	Stylochidae	*Stylochus* sp.
Pycnogonidae		Pycnogonidae sp.

APPENDIX D

Loxahatchee River Oyster Reef Restoration Monitoring Report:
Using Baselines Derived from Long-term Monitoring of Benthic Community Structure on Natural Oyster Reefs to Assess the Outcome of Large-scale Oyster Reef Restoration
Florida International University

Loxahatchee River Oyster Reef Restoration Monitoring Report:

Using Baselines Derived from Long-term Monitoring of Benthic Community Structure on Natural Oyster Reefs to Assess the Outcome of Large-scale Oyster Reef Restoration

December 2011

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BACKGROUND

More than 60% of Earth's population lives in the coastal realm, rendering estuaries one of the most altered ecosystem types worldwide (Ray, 2006). In many temperate and subtropical estuaries, oyster reefs represent a critical habitat type, providing numerous ecosystem services to humans (Coen et al., 2007). As filter feeders, oysters remove plankton and organic particles from the water column. Individual oysters are capable of filtering up to 190 L (50 gal) of water per day, and thus oyster reefs can significantly improve water quality and clarity (Jonas, 1997; Officer et al., 1982; Ulanowicz and Tuttle, 1992). Oyster reefs play another role by supporting diverse communities of small benthic organisms (e.g., bottom-dwelling crabs, shrimp, mollusks, and fishes). Oysters are considered a foundation species, and their presence can facilitate the colonization, survival, and growth of myriad other organisms (Bruno et al., 2003). This community of small oyster reef-associated organisms often serves as a food source for numerous ecologically, commercially and recreationally important species. In addition, oyster reefs function as nurseries for juveniles of economically important species, like gray snapper and stone crabs.

In recent years, oyster reefs throughout North America have experienced significant declines. These declines have been linked to a variety of factors, including disease, over harvest, degraded water quality, and altered salinity patterns. As the ecological and economic importance of oyster reefs has become more widely acknowledged, increased efforts have been made to monitor oyster reef health and characterize the biotic and abiotic factors that are intrinsic to reef function. Additionally, the creation of new oyster reef habitat through restoration efforts has become an increasingly important tool to counteract the loss of natural reefs. While some oyster restorations may be constructed specifically to increase oyster production for commercial purposes, the goal in most cases is to restore multiple ecosystem services associated with natural oyster reefs. As a result, oyster reef restoration has the potential to enhance populations of many species, including commercially and recreationally valuable fishes (Peterson et al., 2003).

Since long-term datasets are often lacking for oyster reef communities, it can be difficult to assess the impacts of restoration efforts, as well as natural or anthropogenic disturbance events. Establishing baselines for what constitutes a healthy oyster reef, accounting for both spatial and temporal variability, is an important component of future efforts to conserve or restore oyster reef habitats. A major concern of oyster reef restoration is understanding how human-made reefs compare to natural reefs over time. The success of an oyster reef restoration should not only be measured by the recovery of living oyster populations, but also by the reestablishment of ecosystem function and an eventual convergence with pristine oyster reef community structure (Coen and Luckenbach, 2000).

In the Loxahatchee River, oyster reefs have been significantly degraded, largely as a result of anthropogenic alteration of freshwater inflow and associated salinity changes. Freshwater flow into the estuary has decreased over time due to flood control measures, while marine contributions increased following the widening and stabilization of Jupiter Inlet in the 1940s, resulting in a shift in the optimal salinity zone for oysters from its historical location. This spatial shift is critical to the survival of oyster populations, larval oysters require the presence of a hard, carbonate-based substrate (typically provided by preexisting oysters) in order to settle and survive. In the Loxahatchee River, present-day optimal salinity levels are found several kilometers upriver from optimal larval settlement habitats (i.e., remnants of historical oyster reefs) in an area that is substrate limited. Construction of a restoration reef (composed of mollusk shell and limestone rock) in this part of the river would immediately create a structurally complex habitat, while simultaneously providing carbonate substrate for settlement and growth of living oysters. In addition to oyster recruitment and growth, transformation of a restoration reef into something functionally analogous to a natural oyster reef requires recruitment of many other benthic organisms.

Positive interactions between living oysters and other oyster reef fauna may facilitate the eventual formation of a natural oyster reef community at the site of oyster reef restoration (Halpern et al., 2007).

The goal of this study was to utilize a long-term oyster reef monitoring dataset to characterize the structure of oyster reef faunal communities (e.g., small benthic crustaceans, mollusks, and demersal fishes) in the Loxahatchee River. Specifically, we identified spatial (i.e., upstream-to-downstream) and temporal (i.e., wet season vs. dry season) patterns in biomass, abundance, and community composition of infaunal organisms from natural oyster reefs, creating powerful baselines to allow for comparison between natural and human-made reefs. We then used these baseline values to assess and track the development of benthic infaunal communities at the restoration reef established during the Martin County Oyster Reef Restoration Project over time. Additionally, we designed a series of high relief ridges and low-relief plots within the restoration reef to experimentally test the effects of vertical relief on community composition and biomass of benthic organisms in a restoration setting. A condensed summary of our findings is provided at the end of this report.

PROJECT DESCRIPTION

Since May 2007, we have conducted bimonthly sampling of benthic organisms at three different natural oyster reef sites in the Loxahatchee River (**Figure D-1).** These sites were located along an upstream-to-downstream gradient, between river mile 4 and 6. Boy Scout Camp (BS) was our most upstream sampling site, Oyster Island (OI) was located in the middle of the sampling area, and Seventh Dock (SD) was near the downstream limit of oyster reef development in the Northwest Fork of the river. At each site, we deployed four replicate benthic sampling tray traps at approximately 2- to 10-m intervals (based on reef size). Benthic tray traps are a common approach for sampling demersal fishes and invertebrates that utilize oyster reefs as habitat. These sampling units consist of plastic bakery trays (64 × 52 × 10 cm) with fiberglass screening attached securely to the tray bottom (**Figure D-2**). Prior to deployment, oyster shells were collected and dried in ambient air conditions. A total of 19 L of shell was placed into each tray so that the entire bottom of the tray was covered. At each field site, an area equal to the dimensions of the tray trap was excavated and the trap placed into the excavated hole such that organisms could move laterally across the benthos and into the trap (**Figure D-2**). To collect organisms, the traps were lifted vertically, allowing water to run through the fiberglass screening on the tray bottom, trapping benthic organisms and small demersal fishes within the tray. All fishes and invertebrates were collected by hand, kept on ice in the field, and returned to the laboratory for processing (identification to the lowest possible taxonomic level, counting, and weighing). After the trays were sampled, they were refilled with shell and returned to their original location in the oyster reef. The organisms collected in the traps were used to characterize seasonal and spatial (upstream vs. downstream) patterns in oyster reef-associated communities, providing baseline values for natural oyster reef communities in the river.

Figure D-1. The three long-term natural oyster reef monitoring sites where samples have been collected bimonthly since March 2007. BS = Boy Scout Camp, OI = Oyster Island, and SD = Seventh Dock. REST is the location of Site 14 of the Martin County Oyster Reef Restoration Project.

Figure D-2. A benthic sampling tray filled with oyster shell (left) and a deployed tray (visible at low tide) located at one of the river's natural oyster reefs (right).

In January 2010, we added four new benthic sampling trays to Site 14 of the Martin County Oyster Reef Restoration Project (**Figure D-1**). Initially, these trays were filled with 19 L of sand and sediment from the river bottom (instead of oyster shell, as was used in the other long-term monitoring trays). By initiating our sampling 6 months prior to reef construction, we hoped to establish a pre-construction baseline for benthic community structure at the site. Following reef construction in July 2010, the four trays were redeployed, each containing 19 L of the loose limestone rock and mollusk shell aggregate that was used to build the reef. For the remainder of the study, these trays were sampled at the same bimonthly frequency (using the same methodology) as the natural reef monitoring trays.

To test effects of habitat complexity on oyster reef colonization, we created two levels of bottom relief within the Loxahatchee River oyster restoration reef. During the construction of the reef at Site 14, we worked with heavy equipment contractors to create three parallel ridges within the restoration reef matrix. These ridges were 10 m × 2 m × 30 cm thick (the greatest height allowed by the state permit). For each high-relief ridge, we created a paired low-relief plot (10 m × 4 m × 15 cm thick) in the adjacent reef matrix, using the same volume of rock and shell (**Figure D-3**). Since the restoration reef at Site 14 was constructed as a homogeneous 15-cm thick layer of limestone rock and shell material, the low-relief experimental plots served as controls for the remainder of the reef. Each experimental ridge/plot had an approximately 1 m wide perimeter of sand separating it from the rest of the reef matrix. Within each high/low experimental unit (block), 14 benthic tray traps were filled with 19 L of rock and shell and placed in rows approximately 1 m apart (seven trays per high-relief ridge, and 7 trays per low-relief plot). A total of 42 benthic tray traps were deployed across the three experimental blocks in August 2010.

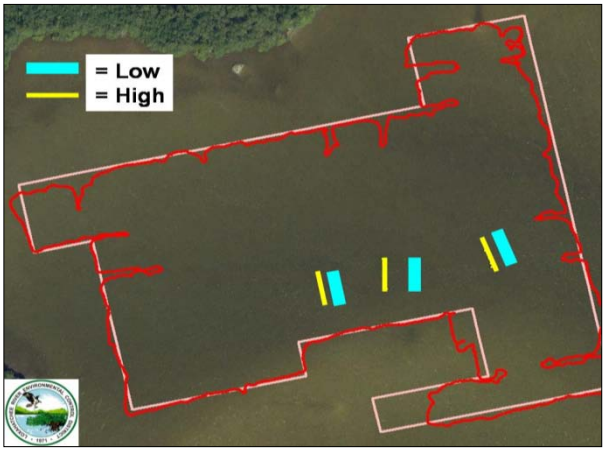

Figure D-3. Map of Loxahatchee River oyster reef restoration site No.14, showing the location of each paired high relief/low relief experimental plot. Each row contained seven benthic sampling trays. The red line indicates the actual boundary of the reef.

Rather than sampling these trays at a fixed bimonthly time interval, we chose *a priori* to sample at approximately day 0, 14, 30, 60, 120, 240, and 365. On each sampling date, one randomly selected pair of trays (high/low) was removed from each experimental block and processed (six trays per sampling date). Unlike the bimonthly monitoring trays, these were left undisturbed from the time of deployment to the time of sampling, at which point they were removed from the river.

To provide an initial estimate of community composition among the four sampling sites and between the high relief/low relief treatments, we calculated the relative abundance (number of individuals/m^2) and biomass (g/m^2) of each taxonomic group found during our sampling. We then used nonparametric multivariate analyses to compare patterns of community composition among sites and across sampling dates. This method allowed us to simultaneously examine all members of each community to see how composition varied spatially and temporally. We used the mean biomass (g/m^2) of each taxonomic group (averaged at the site level for each sampling date) as the dependent variable in these analyses. Values were fourth root transformed in order to down-weight abundant prey categories and allow less common categories to influence similarity values (Clarke and Warwick, 2001). Non-metric multidimensional scaling (MDS) ordinations were created to provide a visual representation of similarity or dissimilarity among the four sites. The relative proximity of two points to one another on the MDS ordination represents the relative similarity of the communities found at those sites. Points that are close to one another on the ordination plot represent communities that are similar, while points that are far apart represent communities that are relatively different. A one-way analysis of similarities (ANOSIM) was then used to test for significant differences in community composition among the four sites. All community-level analyses were carried out using PRIMER v6.1.9 software.

MONITORING FINDINGS

Between May 2007 and September 2011, we sorted, identified, and weighed >28,000 individual organisms captured in benthic tray traps at natural oyster reef sites in the Loxahatchee River, representing 13 fish taxa and 20 invertebrate taxa (**Table D-1**). In terms of abundance, 10 taxonomic groups accounted for >96% of the organisms we collected: small xanthid crabs, snapping shrimp (*Alpheid* spp.), green porcelain crabs (*Petrolisthes armatus*), depressed mud crabs (*Eurypanopeus depressus*), crested gobies (*Lophogobius cyprinoides*), grass shrimp (*Palaemonetes* spp.), black-fingered mud crabs (*Panopeus herbstii*), nassa snails (*Nassarius* sp.), naked gobies (*Gobiosoma bosc*), and frillfin gobies (*Bathygobius fuscus*) (**Table D-1**). We found a number of differences in community composition across the three study sites, based on both abundance (number of organisms) and biomass (weight of organisms). Community-level measures based on abundance are greatly affected by small but common species, while measures based on biomass are often influenced by less abundant but larger organisms. In terms of abundance, green porcelain crabs and nassa snails were more common at the downstream site (Seventh Dock) than at either of the other sites (**Figure D-4**). Depressed mud crabs were less abundant at this site. Crested gobies were most abundant at the upstream site, Boy Scout Camp. In terms of biomass, black-fingered mud crabs represented a larger percentage of the overall community at Seventh Dock than at Boy Scout Camp, with Oyster Island representing an intermediate value (**Figure D-5**). Depressed mud crab and crested goby biomasses were lowest at the Seventh Dock site.

Table D-1. Oyster reef-associated fauna (invertebrates and small benthic fishes) captured in benthic tray traps at natural oyster reef sites in the Loxahatchee River, June 2007–September 2011.

Species	Common Name	Quantity (Number of Individuals)
Invertebrate		
Eurypanopeus spp.	Small mud crab (<10 mm)	11,455
Alpheus spp.	Snapping shrimp	4,296
Petrolisthes armatus	Green porcelain crab	4,084
Eurypanopeus depressus	Depressed mud crab	2,607
Panopeus herbstii	Black-fingered mud crab	901
Palaemonetes spp.	Grass shrimp	859
Nassarius sp.	Nassa snail	558
Nerita spp.	Nerite snail	80
Pachygrapsus transverses	Mottled shore crab	76
Penaeus spp.	Penaeid shrimp	52
Tagelus spp.	Razor clam	49
Neopanope sayi	Say's mud crab	25
Portunus spp.	Swimming crab	22
Upogebia sp.	Mud shrimp	20
Libinia spp.	Spider crab	20
Ophionereis sp.	Brittle star	15
Callinectes sapidus	Blue crab	6
Synalpheus brevicarpus	Short-clawed sponge shrimp	5
Lysmata sp.	Peppermint shrimp	4
Clibanarius vittatus	Striped hermit crab	2
Fish Species		
Lophogobius cyprinoids	Crested goby	1,652
Gobiosoma bosc	Naked goby	519
Bathygobius soporator	Frillfin goby	404
Lupinoblennius nicholsi	Highfin blenny	193
Lutjanus griseus	Gray snapper	29
Erotelis smaragdus	Emerald sleeper	24
Haemulon sp.	Grunt	20
Hypleurochilus aequipinnis	Oyster blenny	8

Table D-1. (Continued).

Species	Common Name	Quantity (Number of Individuals)
Apogon binotatus	Barred cardinalfish	3
Astrapogon alutus	Bronze cardinalfish	2
Parablennius marmoreus	Seaweed blenny	2
Archosargus probatocephalus	Sheepshead	2
Epinephelus itajara	Goliath grouper	1

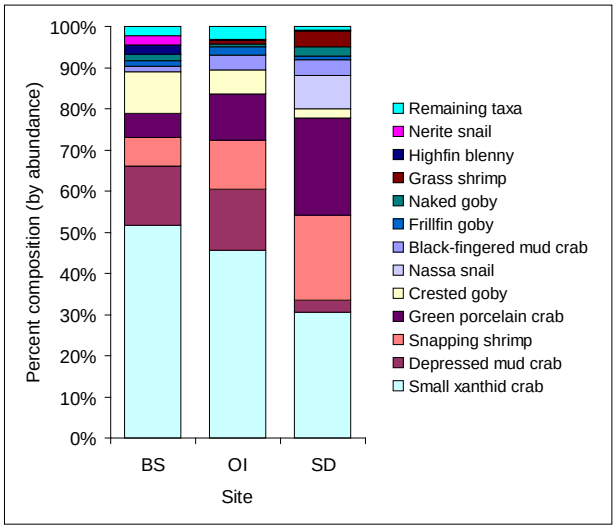

Figure D-4. Percent composition of oyster reef-associated communities based on abundance (number of individuals/m^2), averaged across all sampling dates, for the three bimonthly sampling sites; Boy Scout Camp (BS), Oyster Island (OI), and Seventh Dock (SD).

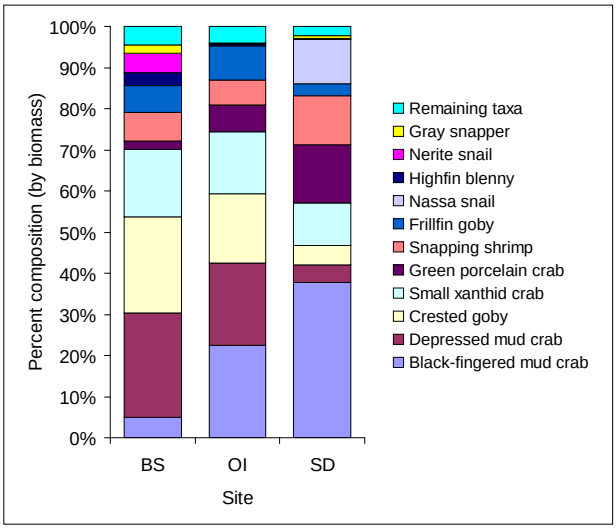

Figure D-5. Percent composition of oyster reef-associated communities based on biomass (g/m^2), averaged across all sampling dates, for the three bimonthly sampling sites; Boy Scout Camp (BS), Oyster Island (OI), and Seventh Dock (SD).

By creating a non-metric multidimensional scaling (MDS) ordination, we were able to compare community composition at the three natural oyster reef sites across all 25 sampling dates. MDS creates a two-dimensional ordination that facilitates visual comparisons of complex communities by representing relative similarity (or dissimilarity) by the relative distance between data points. The closer two data points are to each other, the more similar the overall community structure is between those points. An MDS ordination of our sampling data clearly shows that the community structure of oyster reef-associated organisms varies among the three study sites, but is similar within each site (**Figure D-6**). A one-way analysis of similarities (ANOSIM) revealed significant differences in community structure between Boy Scout Camp and Oyster Island (R=0.39, *P*=0.001), Boy Scout Camp and Seventh Dock (R=0.82, *P*=0.001), and Oyster Island and Seventh Dock (R=0.47, *P*=0.001). The greatest level of dissimilarity was between the two sites that were situated farthest apart, Boy Scout Camp and Seventh Dock. The taxa that were most responsible for driving the differences in community structure between sites were green porcelain crab, black-fingered mud crab, depressed mud crab, and highfin blenny. ANOSIM failed to detect differences in community composition among seasons (R=0.03, *P*=0.15).

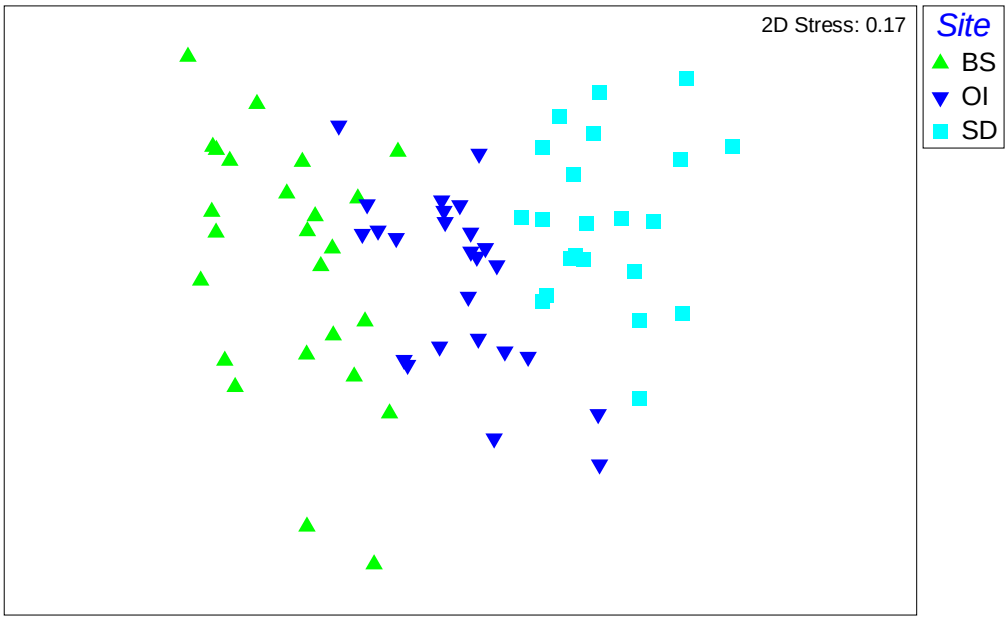

Figure D-6. Non-metric multidimensional scaling ordination of community biomass values, showing clear differentiation among oyster reef-associated communities at the three study sites, Boy Scout Camp (BS), Oyster Island (OI), and Seventh Dock (SD). Each point represents the community at a single site on a single date.

One of the most obvious ecological patterns that emerged after several years of sampling is that total biomass of oyster reef-associated organisms typically peaks at the end of the dry season. When mean biomass values from all sites (across all 4 years of the study) were averaged together by month, we found that biomass peaked in May, and was lowest in November (**Figure D-7**). Although there was some variability in this pattern from year to year, biomass was typically greatest in early summer (end of dry season) and lowest in early to mid winter (end of wet season). Recognizing this seasonal pattern is important when comparing natural and restored reef communities. Seasonal fluctuations in biomass varied slightly across the three natural oyster reef sites. While general patterns were similar at all three sites, the exact timing of maximum biomass varied. When averaged over the course of the study, biomass at Boy Scout Camp and Seventh Dock peaked in May, while biomass at Oyster Island typically peaked one sampling period later, in July. On average, biomass was lowest at Boy Scout Camp and Oyster Island in November and at Seventh Dock in January.

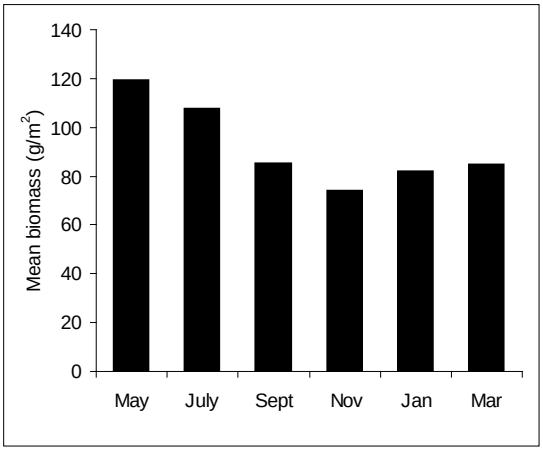

Figure D-7. Monthly biomass, averaged across all natural reef sites and sampling dates.

Biomass declined at all sites during the wet season, but this pattern was most apparent at the upstream site (Boy Scout Camp), where there was a roughly 50% reduction in biomass during the wet season. This site experiences greater seasonal fluctuations in salinity, since it is closest to the upstream source of freshwater inflow. The other two sites are exposed to greater saltwater influence from the ocean, even during the wet season. When viewed across all four years of the study, Boy Scout Camp consistently

experienced the greatest seasonal fluctuations in biomass, as well as the lowest overall biomass. Seventh Dock, the most downstream site, showed less seasonal variability and greater overall biomass than Boy Scout Camp. For most sampling dates, biomass increased along an upstream-to-downstream gradient.

The seasonal shifts in biomass that we observed in oyster reef-associated organisms were likely driven by fluctuations in salinity between the wet season and dry season. Although ANOSIM did not reveal seasonal differences in overall community structure, it appears that seasonal salinity patterns affect the density and/or size structure of certain organisms that occupy the oyster reefs. Biomass values for two species in particular (green porcelain crab and frillfin goby) seem to peak at the end of the dry season, and then rapidly decline at the start of the wet season. Other species exhibit clear seasonal shifts in average body size. For black-fingered mud crabs, the smallest size class is most dominant in July and September, suggesting that reproduction peaks towards the end of the dry season. Based on changes in body size, crested goby reproduction appears to peak earlier in the dry season. Additionally, size structure for many species varied from year to year, likely due to large interannual variation in the timing of recruitment, particularly for broadcast spawning species.

RESTORATION OUTCOME

As we began monitoring the Martin County Oyster Reef Restoration Project, we were able to use the above data to determine how the restored reef compared to natural reefs through time. Between March 2010 and September 2011, we processed approximately 5,300 individual organisms from the restoration site, representing 11 fish taxa and 19 invertebrate taxa (**Table D-2**). Nine of these taxa, including the economically important stone crab, were found only at the restoration site. Prior to constructing the restoration reef, biomass at the site was only 8 to 15% of that found at the nearest natural oyster reef monitoring site, Oyster Island (**Figure D-8**). Total biomass began to increase immediately following reef construction. By May 2011, the restoration reef reached a seasonal biomass peak (i.e., the end of the dry season) as predicted by our long-term monitoring dataset. At this peak, biomass at the restoration reef was close to the 4-year average biomass value at the nearest natural reef site; however, that was still approximately 11% less than the current year's value at that site. Following the seasonal peak associated with the end of the dry season, biomass values decreased at a more rapid rate at the restoration reef than at the nearby natural reef. During the post-restoration time frame, organismal abundance at the restoration reef quickly exceeded abundance values recorded at the nearby natural reef (**Figure D-9**). This was likely due to the large number of very small post-recruitment organisms that we identified at the restored reef site in the months following reef construction.

Table D-2. Oyster reef-associated fauna (invertebrates and small benthic fishes) captured in benthic tray traps at restoration Site No.14 in the Loxahatchee River, March 2010–September 2011. Asterisks indicate species that have only been identified at the restoration site.

Species	Common Name	Quantity (Number of Individuals)
Invertebrate		
Eurypanopeus spp.	Small mud crab (<10 mm)	1,948
Alpheus spp.	Snapping shrimp	1,136
Eurypanopeus depressus	Depressed mud crab	498
Petrolisthes armatus	Green porcelain crab	394
Palaemonetes spp.	Grass shrimp	332
Panopeus herbstii	Black-fingered mud crab	147
Bivalvia spp.	Juvenile clam	54*
Portunus spp.	Swimming crab	50
Tagelus spp	Razor clam	38
Nassarius sp.	Nassa snail	30
Mithrax sp.	Red Mithrax crab	27*
Menippe mercenaria	Stone crab	22*
Upogebia sp	Mud shrimp	11
Penaeus spp	Penaeid shrimp	9
Pachygrapsus transverses	Mottled shore crab	6
Nerita spp.	Nerite snail	6
Libinia spp.	Spider crab	3
Pinnotheres sp.	Pea crab	2*
Stramonita haemastoma fl.	Florida rock shell	1*
Fish		
Gobiosoma bosc	Naked goby	509
Hypleurochilus aequipinnis	Oyster blenny	13
Archosargus probatocephalus	Sheepshead	11
Lophogobius cyprinoids	Crested goby	9
Bathygobius soporator	Frillfin goby	9
Lutjanus griseus	Gray snapper	3
Lupinoblennius nicholsi	Highfin blenny	2
Lutjanus synagris	Lane snapper	1*
Syngnathus sp.	Pipefish	1*
Eucinostomus sp.	Mojarra	1*
Malacoctenus macropus	Rosy blenny	1*

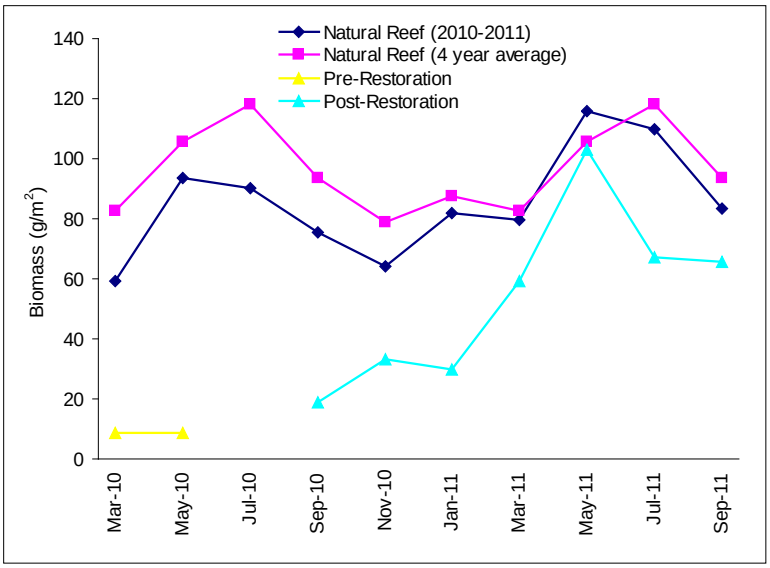

Figure D-8. Plot of mean organismal biomass (g/m^2) at natural and restored oyster reef sites in the Loxahatchee River. The long-term natural oyster reef monitoring site (Oyster Island) was located approximately 100 m from the restoration reef and was used as a control to compare community structure between natural and restored reefs. The dark blue line represents actual biomass measurements at the natural reef site taken between March 2010 and September 2011. The purple line represents biomass values at this site, averaged across the 4-year monitoring dataset. The gap in the restoration reef data at July 2010 represents the reef construction period.

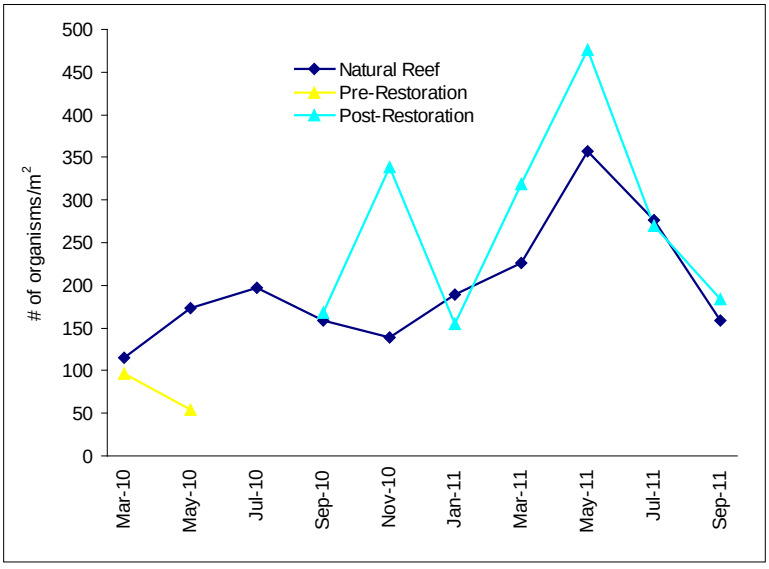

Figure D-9. Plot of mean number of individual organisms/m^2 at natural and restored oyster reef sites in the Loxahatchee River. The long-term natural oyster reef monitoring site (Oyster Island) was located approximately 100 m from the restoration reef and was used as a control to compare community structure between natural and restored reefs. The gap in the restoration reef data at July 2010 represents the reef construction period.

While patterns of total biomass (g/m^2) give an indication of productivity on the restored reef, overall community composition provides a stronger indication of how closely the restoration reef resembles a natural reef. By adding data collected from the bimonthly sampling trays at the new reef to the existing MDS ordination of natural reef community composition (**Figure D-10**), we were able to track community structure at the restoration site as it converged on the values that we would expect to encounter at a natural reef. In this ordination, the cluster of green and blue points represents natural oyster reef communities. The closer a point is to the green and blue cluster, the more closely that community resembles a natural reef community. Pre-restoration community structure (red) did not resemble a natural oyster reef community. The most abundant taxa in these samples were mollusks, penaeid shrimp, and swimming crabs (**Figure D-11**), organisms that are not common in natural reef habitats (**Figure D-12**). By the 14-month mark following restoration, some abundant natural oyster reef taxa (e.g., black-fingered mud crab) were beginning to become abundant at the restored reef (**Figures D-11** and **D-12**). However, some taxa that were uncommon at the natural reef site (e.g., snapping shrimp, swimming crab) were still highly abundant at the restored reef. A one-way analysis of similarities (ANOSIM) revealed significant differences in community structure between the pre-restoration points and the three natural oyster reef sites. Following restoration, community structure appeared to get progressively more similar to the values observed on natural reefs. Each post-restoration sampling date (pink) is closer to the natural reef cluster than the previous date. The point representing the final sampling date, September 2011, is closest to the cluster of natural reef points. Despite this apparent convergence in ordination space, post-restoration communities were still significantly different than communities at any of the three natural reef sites (ANOSIM). This suggests that 14 months is not a sufficient amount of time for community structure at the Loxahatchee River restoration reefs to fully converge with the community composition of natural oyster reefs in the system. Further monitoring is necessary to determine when and if this convergence will occur.

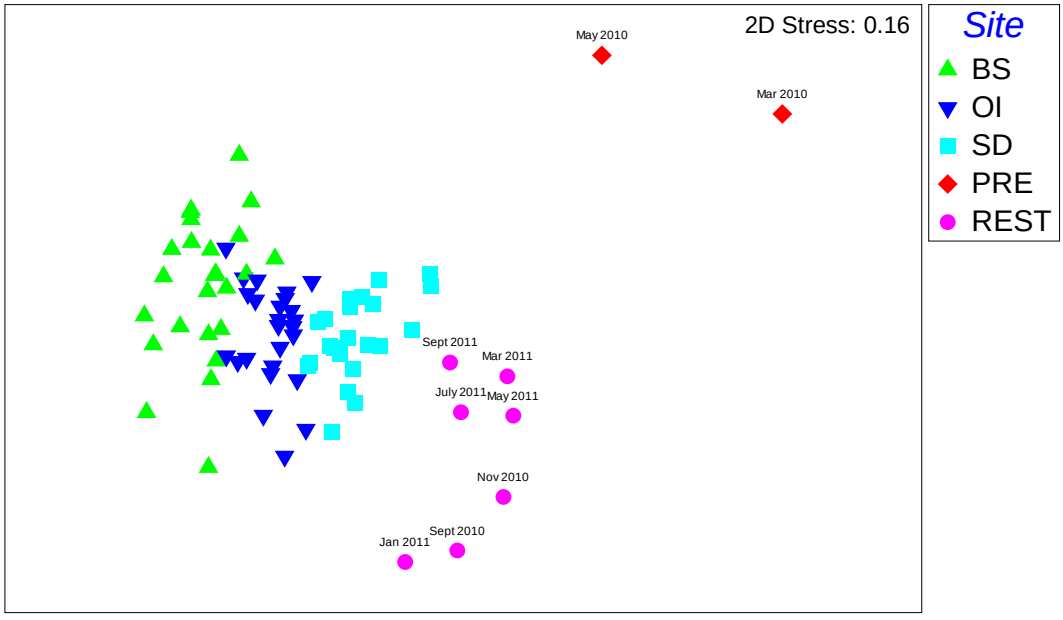

Figure D-10. Pre- and post-restoration data added to the baseline non-metric multidimensional scaling ordination of community biomass values at three natural oyster reef sites, Boy Scout Camp (BS), Oyster Island (OI), and Seventh Dock (SD). The two pre-restoration sampling dates (red) are clearly differentiated from the natural reef communities. Following restoration (pink), community composition becomes progressively more similar to natural reef communities over time. Each point represents a single site on a single date.

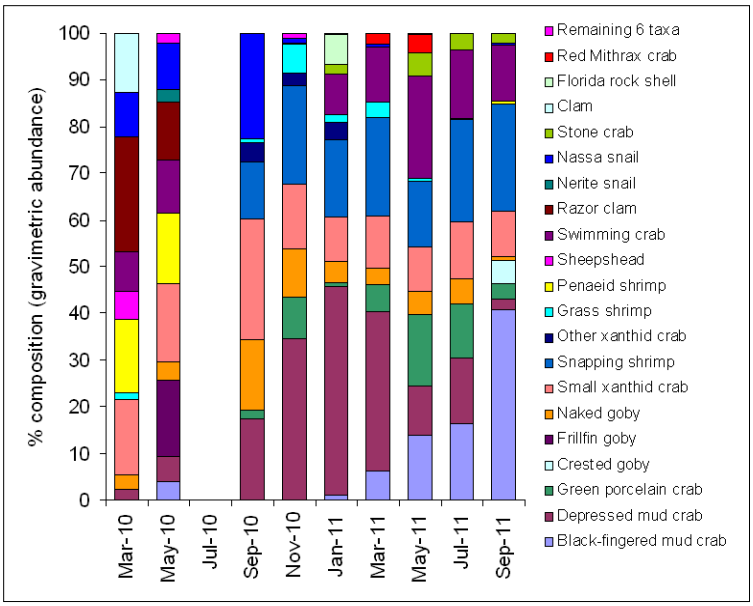

Figure D-11. Percent composition (based on gravimetric abundance) of benthic organisms collected in tray traps at the restoration reef. The first two bars represent samples collected before the reef was constructed. Prior to restoration, mollusks, penaeid shrimp, and swimming crabs were the most abundant members of the benthic community (by mass). Shortly after restoration, small xanthid crabs and depressed mud crabs were most abundant. Over time, black-fingered mud crabs, snapping shrimp, and swimming crabs became the most abundant members of the community.

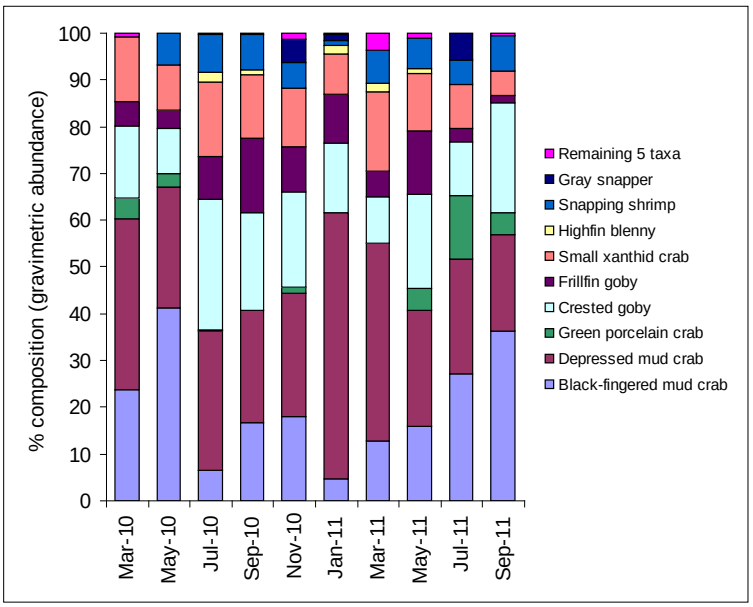

Figure D-12. Percent composition (based on gravimetric abundance) of benthic organisms collected in tray traps at a natural oyster reef site (Oyster Island) immediately adjacent to the restoration reef. In most months, depressed mud crabs, black-fingered mud crabs, or crested gobies were the most abundant organisms (by mass) at the natural oyster reef site.

While biomass and abundance measures at the restored reef site approached or exceeded natural reef values after 1 year, our findings at the experimental high relief ridges were even more pronounced. Biomass (**Figure D-13**) and abundance (**Figure D-14**) at the high-relief experimental ridges increased at a much faster rate than at the adjacent low-relief plots. Although the difference in vertical relief between the two treatments was small (only 15 cm), the effect of this slight variation in relief was very large. By the 8-month mark following restoration, the high-relief experimental ridges had higher biomass values than anywhere else in the river. A biomass value of 388 g/m^2 was recorded from a single high-relief tray on 3 April 2011. This was the highest oyster reef biomass value we have ever recorded anywhere in the river. This biomass was more than double the previous maximum of 175 g/m^2 and almost five times greater than the overall mean natural oyster reef biomass (81 g/m^2) obtained from 4 years of bimonthly sampling. Additionally, the size and density of live oysters at the high-relief ridges appeared to be greater than at the low-relief plots.

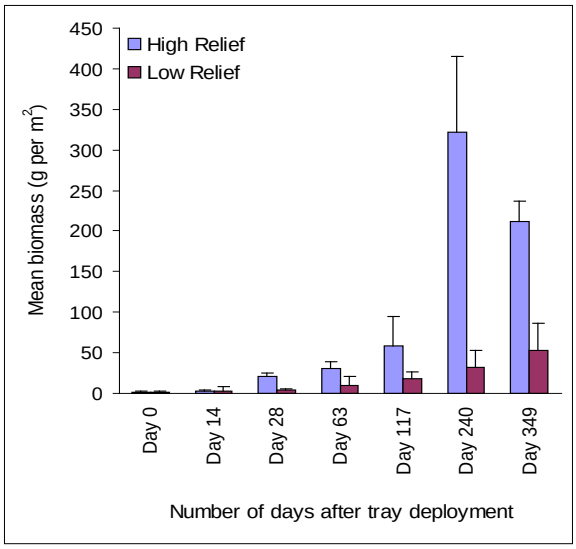

Figure D-13. Changes in organismal biomass over time at high- and low-relief sections of the restoration reef. On all sampling dates, abundance was greater at high-relief experimental ridges than at adjacent low-relief plots. Biomass at the high-relief ridges peaked on day 240 (3 April 2011), near the end of the dry season. This matches the temporal pattern we have observed in our long-term monitoring dataset. Biomass continued to increase at the low relief plots through day 349 (21 July 2011).

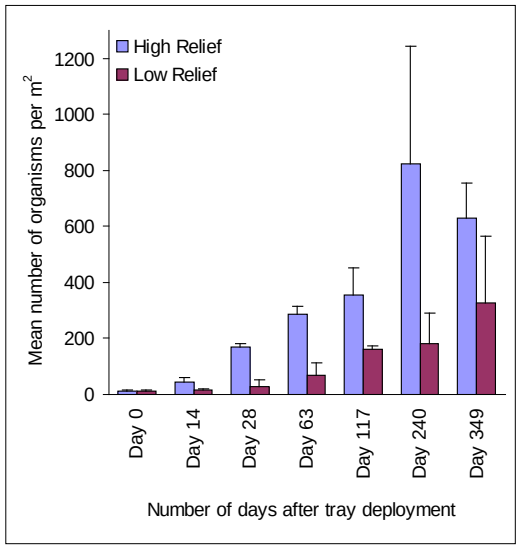

Figure D-14. Changes in organismal abundance over time at high- and low-relief sections of the restoration reef. On all sampling dates, abundance was greater at high-relief experimental ridges than at adjacent low-relief plots. Abundance at the high-relief ridges peaked on day 240 (3 April 2011), near the end of the dry season. This matches the pattern we have observed in our long-term monitoring dataset. Abundance continued to increase at the low-relief plots through day 349 (21 July 2011).

Structurally complex high-relief oyster reefs are often exposed to tidal currents and wave action, particularly in intertidal or immediately subtidal settings. These reefs have been found to experience increased current flow velocities and decreased sedimentation rates compared to low-relief reefs (Lenihan, 1999), both of which favor survival and growth of oysters (Schulte et al., 2009). Reduced sedimentation and compaction rates also can lead to greater rugosity and increased interstitial space in high-relief reefs, creating refuge for numerous reef dwelling organisms. Low-relief restoration reefs often experience hypoxic conditions (Lenihan, 1999) that could potentially harm oysters and associated benthic

communities. Additionally, habitat complexity can affect community composition on oyster restoration reefs as a result of altered predator-prey interactions (Grabowski et al., 2008; Grabowski and Powers, 2004; Hughes and Grabowski, 2006).

While any of the above possibilities may explain the large differences in biomass and abundance we detected between high- and low-relief sites, our observations suggest that sedimentation and compaction may play a major role in structuring benthic communities at the restoration reef. Although we did not directly measure sedimentation rates, we frequently observed large quantities of densely packed fine sediment in the low relief sampling trays. This was never observed in the high relief trays, despite just a 15 cm difference in vertical relief. Further research is needed to identify the mechanisms that are driving the relief-based patterns we observed.

CONCLUSIONS

This study evolved from a long-term monitoring program that has been carried out on the Loxahatchee River since 2007. Although our original goal was to identify spatial and temporal patterns of biomass and species richness among oyster reef-associated organisms purely from a monitoring perspective, the data collected provided an important means to measure the success of the oyster restoration project in the river. By establishing community-level baselines (e.g., MDS ordinations, ANOSIM analyses, relative abundance and biomass plots for all species, total biomass estimates) for healthy oyster reefs in the Loxahatchee River, we will now be able to follow the development of the Martin County Oyster Reef Restoration Project well into the future. The baseline values provided by our long-term monitoring study will enable us to track the progress of community assembly at future oyster restoration projects in the system. Furthermore, our findings of greatly increased biomass and abundance at high-relief sites within the reef emphasize the importance of incorporating vertical relief into future oyster reef restoration efforts in the Loxahatchee River.

SUMMARY

- We collected and processed approximately 5,300 individual organisms from the Loxahatchee River oyster reef restoration site between March 2010 and September 2011, representing 11 fish taxa and 19 invertebrate taxa. Nine of these taxa, including the economically important stone crab, were found only at the restoration site.
- An additional 28,000+ organisms were collected from natural oyster reefs in the river between May 2007 and September 2011. This intensive long-term sampling allowed us to assess community composition at natural oyster reefs and identify baseline values that could then be used to facilitate comparisons between natural and restored reefs.
- Biomass rapidly increased at the restoration site following reef construction.
- After one year, biomass and abundance values at the restoration reef were similar to those at natural reefs. However, community composition still differed between the restored reef and nearby natural reefs. Restored reef communities slowly became more similar to natural reef communities over time. Our baseline MDS plot will allow us to track community structure at the restoration reef into the future.
- Experimental high-relief ridges within the restoration reef had significantly greater biomass than low-relief areas of the reef, despite the fact that vertical relief at the ridges was only 15 cm greater than elsewhere in the reef. A biomass value of 388 g/m^2 was recorded from a single high-relief tray on 3 April 2011. This was the highest oyster reef biomass value we have ever recorded anywhere in the river, and was more than double the previous maximum of 175 g/m^2 and almost five times greater than the overall mean natural oyster reef biomass (81 g/m^2) obtained from 4 years of bimonthly sampling. Small differences in vertical relief greatly enhanced the productivity of the oyster restoration reef.

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