Oyster monitoring in the northern estuaries on the southeast and southwest coasts of Florida

Final Report

2005 - 2018

Florida Fish and Wildlife Conservation Commission

Fish and Wildlife Research Institute

Molluscan Fisheries Research Group

100 Eighth Avenue SE

St. Petersburg, Florida 33701

March 13, 2020

Melanie L. Parker

Richard Radigan

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

EXECUTIVE SUMMARY

This report summarizes oyster population monitoring at study sites within the St. Lucie Estuary (SLE) and the Loxahatchee River Estuary (LRE) on the southeast coast of Florida from 2005 through 2018 and within the Caloosahatchee River Estuary (CRE) on the southwest coast of Florida from 2017 through 2018 as part of the monitoring and assessment component of the Comprehensive Everglades Restoration Plan. Study locations included the North Fork, South Fork and central estuary of the SLE (3 sites), the NW Fork and SW Fork of the LRE (2 sites), and an upstream and downstream region of the CRE (2 sites). Settled oyster density was monitored twice per year at all stations within each study site; surveys were conducted on a quarterly basis at one station in the SLE and at two stations in the CRE beginning in 2017. Disease (*Perkinsus marinus*) prevalence and intensity, reproductive development, juvenile recruitment, and juvenile growth and mortality were monitored on a monthly basis. Water quality monitoring, which included measurements of salinity, temperature, dissolved oxygen concentration, pH, depth and clarity, was conducted in conjunction with monthly sampling at all stations.

Estuarine salinity regimes, and the frequency and magnitude of the variations in those regimes, were the driving force behind patterns of oyster survival, abundance and health in the SLE, LRE and CRE. Salinity varied greatly among the study sites. The LRE SW Fork site (LX-S) and the CRE downstream site (CR-W) had the highest salinity regimes, with annual means that ranged from 21 to 29. Moderate salinity regimes, with annual means from 6 to 24, were found in the LRE NW Fork site (LX-N), the SLE central estuary site (SL-C) and the CRE upstream site (CR-E). The SLE North Fork (SL-N) and South Fork (SL-S) sites had low salinity regimes and exhibited annual means that ranged from 4 to 19. All three estuaries were impacted, to varying degrees, by active tropical storm seasons as well as local water management practices during the study. The SLE was most severely impacted by managed freshwater releases, which frequently caused salinities to fall below the optimal range and become stressful or damaging to oysters, leading to widespread oyster mortality events.

Although the impacts from low salinity events were more acute, long-term exposure to high salinities also negatively affected oysters in the estuaries. Salinities often exceeded the optimal range in the LRE and downstream CRE, and even in the SLE in 2011 and early 2012, and, during those periods, *P. marinus* prevalence in oysters was high. Increased predation and disease rates are typically associated with

higher salinities and temperatures, but the extent to which a higher salinity regime can affect an oyster population was most exemplified by the dramatic increase in *P. marinus* prevalence in oysters from the SLE during 2011 and 2012.

The timing of reproductive development in oysters varied among sites and years, but active reproductive development, including gametogenesis, spawning, and gonadal recycling, typically occurred from March to October with oysters entering the resting stage during the winter months. Analyses of gonadal tissues showed that during periods of low salinity related to storm activity and water releases, most oysters, as long as they survived, continued to develop gametes and spawn as expected; however, there were significantly fewer oysters developing gametes in those years. Recruitment patterns were also similar among estuaries, with recruits commonly present in arrays retrieved from April through December. Recruitment rates in the LRE and CRE approached or exceeded 2 spat/shell/month, whereas rates in the SLE were generally less than 1 spat/shell/month. Each estuary experienced periodic decreases in oyster density following declines in estuarine salinities, but, in most cases, juvenile recruits were detected shortly after conditions returned to tolerable levels. This suggests that even in sites where oysters almost disappeared completely, small relict populations, an exogenous larval source, or most likely, a combination of the two, contributed recruits for recovery.

Oyster abundance, health and population ecology within the three estuaries generally fell within expected ranges for south Florida oyster populations; however, the occurrence of heavy rainfall and subsequent freshwater releases frequently forced estuarine salinities outside tolerable ranges and, as a result, those oyster populations were negatively affected. Although oysters in the SLE repeatedly exhibited the capacity to recover when salinities stabilized following low-salinity events (and in the upstream CRE site in 2018), continued perturbations may so degrade oyster populations that appropriate substrate may no longer exist as a site of larval settlement, even if a supply of larvae persists. In the LRE and at the downstream CRE site, the higher salinity regime also negatively affected oyster populations by favoring higher disease and predation rates, but those effects were more slowly realized. The benefits of increased periods with optimal salinity conditions are often immediate; however, those benefits may extend into the future by building in a greater capacity for oysters to deal with the stressors that arise when environmental conditions decline.

INTRODUCTION

The eastern oyster, *Crassostrea virginica*, occupies estuarine and nearshore habitats along the Atlantic and Gulf of Mexico coasts of the United States. This species supported a subsistence fishery even before Europeans colonized what is now the United States (MacKenzie et al. 1997), and throughout recent history has provided an important economic and cultural resource to coastal residents. In addition to its direct economic benefits, the oyster provides essential habitat for many other estuarine inhabitants (Bahr and Lanier 1981). The eastern oyster is one of the most culturally, economically, and ecologically important species of U.S. coastal waters.

Oysters are frequently used as indicators of water quality in East Coast and Gulf estuaries. Oysters thrive under the variable salinity regime that is a natural component of a healthy estuary (La Peyre et al. 2009), such that too much stability can be detrimental; however, changes in freshwater inflow can have varied effects on the health and distribution of oyster populations and on economic factors such as commercial oyster landings (Turner 2006). Those impacts can be estuary specific, leading to increases or decreases in oyster productivity, depending on the magnitude and timescale of the change in freshwater supply (Wilber 1992). One of the most studied instances of altered freshwater flow concerns the Apalachicola River in northeast Florida. In that region, reduced river flow has led to higher estuarine salinities, which in turn have allowed for increased predation on oysters and decreased primary production (Livingston et al. 1997). Water management strategies such as diversion of freshwater out of a river before it reaches the estuary, as seen in the Apalachicola River, can disrupt natural cycles for oysters and other estuarine organisms. At the opposite extreme, management strategies such as the channelization of tributaries have led to increased delivery of nutrients and organic matter to many estuaries, such as Galveston Bay in Texas, as well as increased flows of freshwater that drastically alter the ecology and health of such estuaries (Klinck et al. 2002).

In Florida, oysters occur along both the Atlantic and Gulf coasts in almost all estuarine and nearshore waters. Along the both coasts, oysters are generally confined to estuaries, bays, and lagoons, such as the St. Lucie Estuary. Those waters, and other coastal waters on the southeast and southwest coasts of the state, have experienced altered patterns of freshwater delivery and quantity as a result of water management practices related to the St. Johns and Kissimmee river basins, Lake Okeechobee, and the Everglades. In particular, channelization and redirection of freshwater out of inland basins and into coastal estuarine waters has altered both the frequency and the rate of salinity variations in those coastal waters. The impacts of those alterations are often most detrimental during the wet season (May to October), when rainfall can be 400% higher than during the dry season (Carriker and Borisova 2011).

The Comprehensive Everglades Restoration Plan (CERP) was implemented as a means of restoring, to the greatest degree possible, natural freshwater flow to the Everglades wetland system and reducing detrimental flow to both coasts of South Florida (U.S. Army Corps of Engineers Jacksonville District and South Florida Water Management District 1999). The monitoring and assessment component of CERP was designed to provide a diverse approach to documenting and describing the impacts of changed freshwater flow to the flora and fauna of those inland landscapes and coastal waters. Because it is so widely distributed and has such essential habitat value, the eastern oyster was chosen as a target species for inclusion in the estuarine monitoring component of CERP.

The objective of this study was to implement a long-term monitoring program to document the response of oysters in south Florida estuaries to restoration efforts associated with CERP. Specifically, this study examined the distribution and abundance of settled oysters, prevalence and intensity of infection by the parasitic protozoan *Perkinsus marinus*, reproductive development, juvenile recruitment, and juvenile growth and mortality in the St. Lucie and Loxahatchee River Estuaries on the southeast coast, and in the Caloosahatchee River Estuary on the southwest coast. All three estuaries are located in regions that have been strongly affected by freshwater management activities and stand to benefit from CERP restoration activities. Results of this study will not only provide a baseline description of oysters in each estuary, but also will serve as a guide for determining whether CERP water management strategies (Barnes et al. 2007) are appropriate for achieving restoration goals. This report summarizes results from monitoring efforts conducted by the Fish and Wildlife Research Institute (FWRI) from 2005 though 2018 in SLE and LRE on the southeast coast of Florida and from 2017 through 2018 in the CRE on the southwest coast of Florida.

SOUTHEAST FLORIDA

Methods

Study Sites

Oyster sampling was conducted from January 2005 through December 2018 on oyster reefs within two southeast Florida estuaries: the St. Lucie Estuary (SLE) and the Loxahatchee River Estuary (LRE). Within the SLE, the north fork, the south fork, and the central estuary were considered to be separate sites each with three sampled oyster stations (i.e., oyster reefs), or three potential stations if no oysters were present. Similarly, in the LRE, the northwest fork and the southwest fork were considered to be separate sites, each with three sampled stations. This strategy resulted in a total of five separate study sites (St. Lucie-North, St. Lucie-South, St. Lucie-Central, Loxahatchee-North and Loxahatchee-South) each with three stations. Station coordinates are listed in Table 1 and locations are shown in Figure 1.

Table 1. Station coordinates for CERP oyster monitoring sites in southeast and southwest Florida.

Site	**Station**	**Latitude ºN**	**Longitude ºW**
St. Lucie North Fork	1	27 13.232	80 16.737
St. Lucie North Fork	2	27 12.686	80 15.846
St. Lucie North Fork	3	27 12.459	80 17.072
St. Lucie South Fork	1	27 11.691	80 15.636
St. Lucie South Fork	2	27 11.228	80 16.149
St. Lucie South Fork	3	27 09.949	80 15.673
St. Lucie Central Estuary	1	27 12.743	80 14.599
St. Lucie Central Estuary	2	27 12.087	80 14.493
St. Lucie Central Estuary	3	27 12.096	80 15.282
Loxahatchee NW Fork	1	26 58.164	80 07.688
Loxahatchee NW Fork	2	26 58.237	80 07.649
Loxahatchee NW Fork	3	26 58.370	80 07.686
Loxahatchee SW Fork	1	26 56.574	80 07.112
Loxahatchee SW Fork	2	26 56.630	80 07.280
Loxahatchee SW Fork	3	26 56.560	80 07.257

Water Quality

Monthly water quality sampling was conducted in conjunction with field sampling at all stations within each study site from 2005 through 2018. Recorded parameters included salinity, temperature, dissolved oxygen concentration, pH, depth and clarity. Depth was determined with a sounding line or incremented meter stick and clarity was obtained by using a standard Secchi disk. Water clarity 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 calibrated, multiparameter YSI. Additional salinity data from continuous data loggers deployed by the South Florida Water Management District (SFWMD), United States Geological Survey (USGS) and the Loxahatchee River District (LRD) were also analyzed. Graphical presentations in Appendix A show the monthly values measured by FWRI at each station within each site and the daily values measured by each data logger. Flow rates as recorded by the SFWMD and USGS were analyzed and included for comparisons.

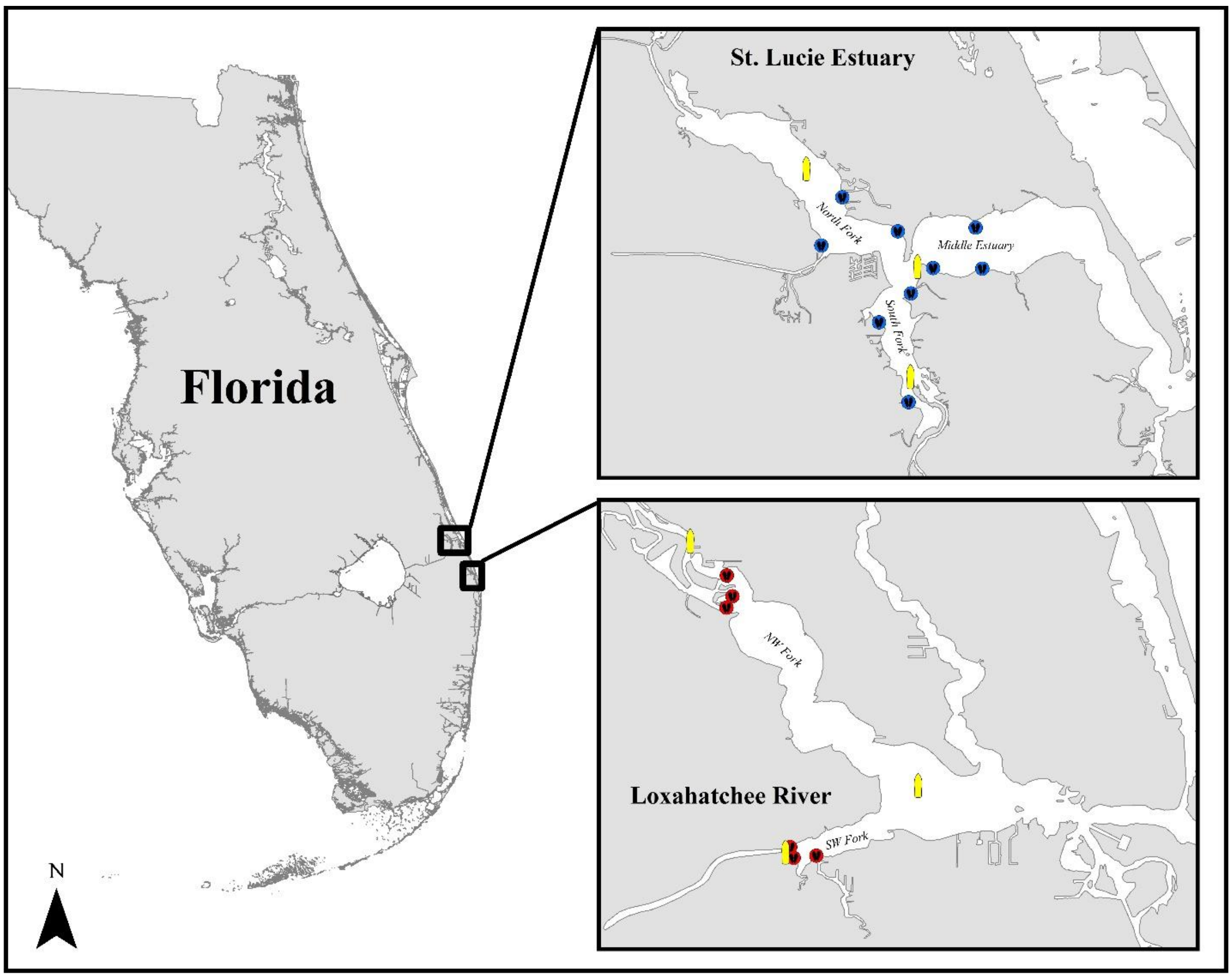

Figure 1. CERP oyster monitoring stations (blue and red symbols) and continuous water temperature and salinity data loggers (yellow symbols) in the St. Lucie and Loxahatchee River Estuaries on the southeast coast of Florida.

Settled Oyster Density

Oyster population surveys were conducted twice per year at all stations within each study site from 2005 through 2018. In 2005, sampling occurred during the winter and summer. After 2005, it was determined that surveys in the spring and fall would be more appropriate because those would occur near the beginning and end, respectively, of the oyster spawning season. The oyster survey methodology used in the present study was based on that of Lenihan and Peterson (1998) and Grizzle et al. (2005). From 2005 – 2007, 10 replicate 1-m^2 quadrats were haphazardly deployed on each sampled reef. After 2007, 15 replicate ¼-m^2 quadrats were haphazardly deployed on each sampled reef. Throughout the present study, all oysters within each quadrat were collected for determination of the total number of live oysters and of dead oysters with articulated shells, as well as the proportion of dead oysters to the total number of live oysters and dead oysters (dead ratio). In addition, shell height (SH; maximum linear distance from the umbo to the ventral shell margin) was measured for a maximum of 50 live oysters per 1-m^2 quadrat and 10 live oysters per ¼-m^2 quadrat. Beginning in 2017, additional quarterly surveys were conducted during the summer (June) and winter (December) at St. Lucie-Central Station 1. Mean live oyster density, ratio of dead oysters to total oysters, and mean live oyster SHs were calculated and plotted for each station within each site (Appendix B). The mean SH was calculated for each quadrat, resulting in 10 (2005 – 2007) or 15 (2008 – 2018) data points per station each survey, and used for all statistical analyses.

Disease and Reproductive Development

Live oysters were collected monthly from February 2005 through December 2018 for analysis of gonadal development stage. From March 2005 through December 2018, those live oysters were also processed for determination of the prevalence and intensity of the oyster disease *Perkinsus marinus* (dermo). Each month, a sample of five oysters from each of the stations within a study site (total N = five oysters * fifteen stations = maximum 75 per month) were transported, live and chilled, to the FWRI laboratory for processing. If no live oysters were available at one or two of the stations within a site, additional oysters were collected from another station within that site if possible. Each individual oyster was processed for reproductive stage and disease status according to the methods described below.

For *P. marinus* (dermo) disease analyses, prevalence and intensity were diagnosed with Ray's fluid thioglycollate media (RFTM) method (Ray 1966). Sections of mantle and gill tissue, each approximately 1 cm^2 in area, were clipped from each individual using sterile surgical scissors, placed in RFTM treated with antibiotics and antifungals, and incubated for 7 days in the dark at room temperature. After the incubation period, tissues were placed on glass slides, macerated with sterile razor blades, and stained with Lugol's solution. Mantle and gill tissues were then examined at ×40 magnification for the presence of *P. marinus* hypnospores. Parasite density (infection intensity) was ranked according to the Mackin scale (Table 2; Mackin 1962). Mean infection intensity for each oyster was calculated as the average infection intensity from mantle and gill tissues. Parasitic prevalence was calculated as the percentage of oysters infected, regardless of infection level. Mean infection intensity and the percentage of infected oysters were plotted for each station within each site (Appendix C).

Table 2. Mackin scale of *Perkinsus marinus* infection intensity stages (Mackin 1962)

Stage	Category	Number of cells
0	Uninfected	None detected
0.5	Very light	<10
1	Light	11–100 cells
2	Light to moderate	Local concentrations of 24–50 cells
3	Moderate	3 cells in all fields at 100×
4	Moderate heavy	High numbers in all tissues
5	Heavy	Enormous numbers

The tissues remaining after dermo analyses were preserved for histological determination of reproductive development stage. Tissues were fixed in a modified Davidson's fixative solution (Shaw and Battle 1957), the main difference being no glycerin was included, for a minimum of 2 days. Once fixed, cross-sections were taken approximately halfway between the adductor muscle and the anterior margin, to include the gonad. Cross-sections were placed in tissue-embedding cassettes, rinsed, and then transferred to a 70% solution of ethanol. Histological preparation consisted of dehydrating each oyster in 95% ethanol then embedding the tissue in paraffin. The sections were stained with hematoxylin and eosin and mounted on glass slides for analysis. Histological cross-sections were examined at ×200-400 magnification to ascertain sex and assigned one of four reproductive stages (Table 3) according to a classification scheme modified from the work of Fisher et al. (1996). Percentages of oysters developing gametes and percentages of oysters undergoing active gametogenesis (developing, ripe/spawning or spent/recycling stages) were plotted for each station within each site (Appendix D).

Table 3. Qualitative reproductive staging criteria for oysters collected from Florida waters (Fisher et al. 1996)

Value	Stage	Observations
1	Developing	Gametogenesis has begun
		immature gametes located on follicle walls
		mature gametes may be present
2	Ripe/Spawning	Follicles distended and full of ripe gametes
		ova compact/sperm with visible tails
		no immature gametes on follicle walls
		active spawning, but less than 2/3 depleted
3	Spent/Recycling	Most gametes evacuated from the follicles
		more than 2/3 depleted
4	Indifferent	Gonads devoid of gametes, cytolysis ongoing

Juvenile Recruitment

Juvenile oyster (spat) recruitment was monitored monthly from February 2005 through December 2018 at all stations within each study site. Spat monitoring arrays were constructed and processed, as adapted from Southworth and Mann (2004). Each array consisted of 12 axenic adult oyster shells (SH, 5-10 cm) strung onto two lengths of galvanized wire. The shells were oriented on the wire with their inner surfaces facing downward, then the shell strings were suspended from the arms of a T-shaped PVC frame and the PVC frame was pushed into the sediment until the bottommost shell was approximately 5 cm above the sediment surface. Upon retrieval, the shell strings were labeled and bagged, and new shell strings were placed immediately on the PVC frame. The retrieved shell strings were returned to the laboratory, where each shell was examined for oyster spat with the aid of a magnification lamp or dissecting microscope (maximum magnification, ×65).

Juvenile oyster recruitment was estimated by counting settled spat on the underside of the middle 4 shells on each shell string (Southworth and Mann 2004). Recruitment rates were obtained by dividing the number of spat per shell by the number of days the shell had been deployed, and then standardizing to a 28-day month. Those standardized values were then used to compute the mean spat per shell per month for each successfully retrieved replicate, or spat monitoring array, resulting in a maximum of 3 data points per station each month. Mean numbers of spat per shell per month were plotted for each station within each site (Appendix D). Recruitment rates are reported by retrieval date throughout.

Juvenile Growth and Predation

Several methods were used to estimate juvenile oyster growth and predation during the present study. From 2005 – 2007, broodstock were collected from the LRE and SLE and delivered to Harbor Branch Oceanographic Institution (HBOI) for conditioning and production of juvenile oysters. In 2005, after rearing cultchless juvenile oysters to a mean size of 10 to 20 mm SH, the juvenile oysters were transported from HBOI to their respective field sites for planting into wire mesh cages. Each cage included an open and closed compartment in order to estimate mortality attributable to macrofaunal predation. Monthly monitoring occurred for approximately one year and involved counting the total number of live oysters remaining in each compartment and measuring the SHs of 30 randomly selected oysters (or all remaining if < 30 live oysters) from each compartment. In 2006, the methodology was similar to that of 2005 with two major differences. First, instead of producing cultchless juvenile oysters, HBOI spawned the broodstock in tanks that contained axenic oyster shell as settlement substrate. Second, the shells with settled juvenile oysters were transported and planted into cages when the juveniles reached a mean SH of 1 to 5 mm. The 2007 methodology followed that of 2006 with one major exception. No successful spawns were completed with broodstock from SLE, so an alternative method was adapted. This method involved planting cages in the field with “blank” or clean axenic oyster shells that would serve as settlement substrate for wild juvenile oysters. Those wild juvenile oysters were then monitored monthly following standard protocols.

After 2007, hatchery production of juvenile oysters was terminated. Instead, axenic oyster shells were planted at the field sites for settlement of wild juvenile oysters, as with the SLE component of the 2007 study. From 2008 – 2010, the axenic shells were attached by fishing line to a 0.6-m L x 0.6 m W wire mesh growth array. Monitoring involved measuring the SHs of 30 randomly selected oysters (or all present if < 30 live oysters) monthly for one year. From 2011 – 2013, each study was initiated by planting large numbers of axenic shell in bags in each estuary. After enough wild juvenile oysters had settled on the shells in the bags, the shells with wild spat were sorted and counted. A shellfish tag was glued to each individual juvenile oyster and the SH of the animal was recorded. Tagged oysters were left attached to the large axenic oyster shells they initially settled on to create a more realistic, reef-like environment. After tagging, oysters were planted into one fully enclosed wire mesh cage and one open-top wire mesh cage at each station. The SH of all tagged oysters and the number of live tagged oysters was recorded during each monthly sampling trip. The 2011, 2012 and 2013 studies continued for 16, 18 and 13 months, respectively.

In January 2015, monthly mortality and shell height monitoring was initiated at one station in each of the southeast Florida estuaries (St. Lucie-Central Station 1 and Loxahatchee-North Station 2). At each station, 90 wild oysters with SHs of 10 mm or greater, were collected, measured (SH, mm) and planted into 3 open cages (n = 30 oysters per cage). Cages were constructed from 25.4-mm plastic-coasted wire mesh with dimensions of approximately 0.6-m L x 0.6-m W x 0.2-m H; the bottom and sides of the cages were lined with 6.35-mm plastic mesh to prevent loss of smaller oysters. One month later, all remaining oysters were counted, measured and released. A new set of 90 wild oysters were then immediately collected, measured and planted into the cages. This process was repeated monthly through December 2018. Mortality rates were calculated by first determining the survivorship rate (divide the number of remaining live oysters in the cage by the number of live oysters initially planted in the cage) and then subtracting that number from 1. Mean mortality, mean deployed SH and mean retrieved SH were plotted for each station within each site (Appendix E).

Statistical Analyses

Statistical analyses were performed with SAS Enterprise Guide version 7.1 (SAS Institute Inc., Cary, NC) and results were considered significant at $\alpha = 0.05$. All data were tested for normality by examining model residuals and then testing them for goodness of fit with the Shapiro-Wilk test (Shapiro and Wilk 1965). Because no data met normality assumptions, all statistical comparisons were performed using generalized linear mixed modeling with the GLIMMIX procedure (Littell et al. 2006). Statistical tests of all parameters included fixed factors of site and year.

Results

Water Quality

Salinity was highly variable at all study sites, often ranging from near 0 to more than 30 within a single year (Appendix A); however, the salinity regimes among sites varied greatly. Statistical comparison of salinities measured during monthly sampling trips revealed that each study site fell into one of three primary groups: a high salinity group, a moderate salinity group, and a low salinity group ($F_{4,2470}$=108.18, $P < 0.01$; Figure 2A). The Loxahatchee-South study site comprised the high salinity group, with an overall mean salinity of 24 for the 14-year study, and annual means that ranged from 21 to 29. The moderate salinity group included the Loxahatchee-North and St. Lucie-Central sites, where overall mean salinities were 15 and 16, respectively, and annual means ranged from 6 to 24. The St. Lucie-North and South sites, with overall mean salinities of 12 and 11, respectively, comprised the low salinity group, and exhibited annual means that ranged from 4 to 19. Salinity patterns also differed significantly among years within each of the sites ($F_{52,2470}$=3.00, $P < 0.01$; Figure 3). The lowest salinities occurred in 2005 and 2016 when annual means ranged from 3 to 7 in the SLE sites and from 11 to 21 in the two LRE sites. At the opposite extreme, salinities were significantly higher at the LRE sites in 2006 when annual means exceeded 29 in Loxahatchee-South and 21 in Loxahatchee-North. At the three SLE sites, the greatest salinities were measured in 2011 when annual means ranged from 19 to 24.

The mean daily salinities measured by data loggers deployed in the North Fork and at the US1 Roosevelt Bridge in the SLE and in the NW Fork, SW Fork and central LRE strongly paralleled the monthly measures by FWRI (Appendix A). Statistical comparisons of the daily salinity data also revealed significant differences among years at each logger station (Table 4; Figure 4). The lowest salinities measured by the two data loggers in the SLE occurred in 2005 and 2016, as identified by comparisons of the FWRI monthly measures. The highest mean salinities occurred in 2009, 2011, and 2013 in the North Fork and in 2009 and 2011 at the US1 Roosevelt Bridge. In the LRE, there were fewer parallels between the daily and monthly salinity measures. This is probably because the data loggers were not consistently deployed throughout the 14-y study. Data was only recorded in the NW Fork from 2007 to 2018, in the SW Fork from 2008 to 2011 and in 2018, and in central LRE from 2005 to 2011 and in 2018. The years with significantly greater salinities were 2009 and/or 2011. The lowest salinities were recorded in 2010 in the NW Fork, in 2018 in the SW Fork, and in 2005, 2008 and 2010 in the central estuary.

Table 4. Results from comparisons of mean daily salinities recorded by continuous data loggers deployed in the SLE and LRE.

Estuary	Location (Logger)	Factor	Num DF	Den DF	F Value	P Value
SLE	North Fork (HR1)	Year	13	3662	47.82	< 0.01
SLE	US1 Roosevelt Bridge	Year	13	4799	70.41	< 0.01
LRE	NW Fork (OY)	Year	11	4036	21.76	< 0.01
LRE	SW Fork (72)	Year	4	1160	51.13	< 0.01
LRE	Central (PP)	Year	7	2156	39.58	< 0.01

Flow rates from structures located upstream of the study sites were compiled to assess freshwater inflows and their effects on estuarine salinities (Appendix A). For the SLE, flow rates from four separate structures were summed to estimate the total freshwater inputs to the estuary. These structures included the C44S80 into the South Fork, and the C23S97, C24S49 and Gordy structures into the North Fork. Flows into the SLE were greatest in 2005 when the annual mean exceeded 2700 cfs ($F_{13,10198}$=33.19, P < 0.01; Figure 5). This corresponds with the significantly low salinities recorded at the three SLE sites (annual means from 4 to 6) and at the North Fork and US1 loggers (annual means of 6 and 7, respectively) in 2005. SLE flow rates were also significantly greater in 2013, 2016 and 2017 when annual means were 1300 cfs or greater. In 2005, 2013 and 2017, salinities decreased precipitously following increased flow rates and remained low for several consecutive months. In 2016, increased rainfall associated with the 2015/2016 El Nino event kept flow rates elevated from January through October. Subsequently, the prolonged freshwater releases kept salinities low during that same period. The lowest flow rates occurred in 2006, 2007, 2009 and 2011 when annual means were 400 cfs or less. Two of those years, 2009 and 2011, coincide with significantly high salinities measured by the US1 logger. Mean annual flow rates during the other years of the study were moderate and ranged from 585 to 921 cfs; however, two other events are worth mentioning. The first event occurred in August 2008 when Tropical Storm Fay made landfall in Florida. Heavy rains associated with the storm impacted the SLE by increasing the mean monthly flow rate from 881 cfs in July to over 3700 cfs in August; flow rates remained high through October. The second event occurred in 2010 and was similar in timing to the El Nino event previously described for 2016. In 2010, a prolonged freshwater release increased mean monthly flow rates to more than 1000 cfs for 5 consecutive months (March through July). During both of these events, salinities in the SLE decreased rapidly and remained low for the duration.

Additional analysis of flow and salinity data was conducted to further define environmental conditions in the SLE during the 14-yr study. Since flow from all four structures into the SLE likely converges near the middle estuary, salinity data from the logger deployed in the same general location (US1 Roosevelt Bridge) was selected for these comparisons. It is also worth noting that the most robust SLE oyster population is found just downstream of this location in the middle estuary. Mean salinity and flow as well as the percentage of measures that fell below, within and above the optimal salinity range (10 – 25) were calculated for each calendar year (Table 5). Each calendar year was also classified as wet, dry or moderate. The year was considered wet if 25% or more of the measures were below the optimal range (2005, 2010, 2013 and 2016). Similarly, the year was considered dry if 25% or more of the measures exceeded the optimal range (2007, 2009 and 2011). In 2017 and 2018, more than 25% of the measures were below optimal and an additional 25% of the measures were above optimal, exhibiting both extremes in each year. Moderate years were those years when 70% or more of the measures were within the optimal range.

Table 5. Mean salinity and total number of salinity measurements in the SLE per calendar year, the number (n) and percentage of days when salinity was less than 10, ranged from 10 to 25, and exceeded 25, and mean flow. Blue bold and red bold font denotes significantly low or high values. Shading indicates wet years (blue), dry years (red), or years with both extremes (purple). Salinity data is from USGS and flow data (summed daily mean from the C44S80, C23S97, C24S49 and Gordy structures) is from SFWMD.

St. Lucie Estuary US1 Roosevelt Bridge			< 10		10 – 25		> 25		
Year	Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2005	**7.3**	349	228	65.3	121	34.7	0	0.0	**2724.2**
2006	17.3	270	14	5.2	217	80.4	39	14.4	**384.3**
2007	18.3	310	64	20.6	131	42.3	115	37.1	**397.0**
2008	15.9	363	62	17.1	270	74.4	31	8.5	784.0
2009	**20.2**	349	41	11.7	178	51.0	130	37.2	**401.9**
2010	14.7	344	105	30.5	199	57.8	40	11.6	704.6
2011	**21.3**	364	28	7.7	150	41.2	186	51.1	**397.5**
2012	18.2	366	60	16.4	280	76.5	26	7.1	606.6
2013	14.8	365	125	34.2	190	52.1	50	13.7	1378.3
2014	17.2	365	61	16.7	297	81.4	7	1.9	585.5
2015	16.5	364	54	14.8	306	84.1	4	1.1	636.7
2016	10.0	341	186	54.5	153	44.9	2	0.6	1841.6
2017	16.2	362	110	30.4	103	28.5	149	41.2	1738.9
2018	18.2	301	82	27.2	124	41.2	95	31.6	921.4
Mean	**16.1**			**25.2**		**56.4**		**18.4**	**964.5**

Similar metrics were calculated for salinities measured during the dry season months (January – April; November – December) and wet season months (May – October) of each calendar year. During the dry season months, those years when 50% or more of the salinity measures exceeded optimal were considered *extreme*-dry seasons; years with 25% or more of the salinity measures below optimal were classified as *wet*-dry seasons (Table 6). Specifically, the 2009, 2011 and 2017 dry seasons were considered *extreme*-dry seasons since 61.4 to 66.3% of the salinity measures during those years exceeded optimal. At the opposite extreme, more than 40% of the salinity measures during the 2005 and 2016 dry seasons were below optimal, thus they were considered *wet*-dry seasons. During the wet season months, years when 50% or more of the salinity measures were below optimal were considered *extreme*-wet seasons while those with 25% or more above optimal were considered *dry*-wet seasons (Table 7). The wet seasons of 2005, 2013, 2016 and 2018 were classified as *extreme*-wet while those of 2006, 2007 and 2011 were classified as *dry*-wet seasons. In most years, the mean dry season salinity was approximately 7 points higher than the mean wet season salinity. One exception occurred in 2006, when the mean wet season salinity exceeded the mean dry season salinity (19.5 vs. 14.7). Other exceptions occurred in 2013 and 2018 when differences between the dry and wet season means were much larger (22.0 vs. 7.6 in 2013; 23.7 vs. 10.4 in 2018) and in 2015 when the mean salinity was similar in both seasons (~16).

Nonlinear regression of monthly mean salinity at the US1 Roosevelt Bridge and flow into the SLE showed a significant negative correlation (Figure 6). By observing where the best fit curve extends beyond the optimal salinity band (10-25), it appears that that mean monthly flow rates exceeding 1200 cfs will decrease salinities below the optimal range. The mean minimum monthly flow rate necessary for keeping salinities from exceeding the optimal range was less discernable but likely falls between 50 to 100 cfs. A time series plot of the same flow and salinity data shows how flow rates that exceed the 1200 cfs threshold decrease salinities to below the optimal range (Figure 7). During most wet years, the mean monthly flow rate exceeded the maximum for 2 or more consecutive months.

Freshwater inflows to the LRE were less impactful than those recorded in the SLE (Appendix A). Because monitored oyster reefs in the LRE are found in the NW Fork and SW Fork, not in the central part of the estuary, flow rates measured at the Lainhart Dam and S46 structures were analyzed independently to estimate total freshwater inputs into the two forks of the estuary. In the NW Fork, flow rates were statistically similar (73 to 142

Table 6. Mean salinity and total number of salinity measurements in the SLE during the dry season months (January – April; November – December) each calendar year, and the number (n) and percentage of days when salinity was less than 10, ranged from 10 to 25, and exceeded 25, and mean flow. Blue-shading denotes *wet*-dry seasons and red-shading denotes *extreme*-dry seasons. Salinity data is from USGS and flow data (summed daily mean from the C44S80, C23S97, C24S49 and Gordy structures) is from SFWMD.

St. Lucie Estuary US1 Roosevelt Bridge			< 10		10 – 25		> 25		
Year	**Dry Season** Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2005	10.4	174	83	47.7	91	52.3	0	0.0	2257.2
2006	14.7	120	3	2.5	117	97.5	0	0.0	406.7
2007	21.5	148	5	3.4	74	50.0	69	46.6	124.9
2008	18.5	179	0	0.0	179	100.0	0	0.0	183.7
2009	24.4	166	0	0.0	64	38.6	102	61.4	147.4
2010	19.0	172	33	19.2	99	57.6	40	23.3	490.7
2011	22.5	181	6	3.3	55	30.4	120	66.3	154.4
2012	20.7	182	0	0.0	181	99.5	1	0.5	158.9
2013	22.0	181	0	0.0	134	74.0	47	26.0	128.7
2014	19.8	181	5	2.8	172	95.0	4	2.2	226.8
2015	16.1	181	28	15.5	153	84.5	0	0.0	577.8
2016	11.9	172	76	44.2	94	54.7	2	1.2	1810.9
2017	20.3	180	40	22.2	27	15.0	113	62.8	1059.8
2018	23.7	177	0	0.0	91	51.4	86	48.6	110.9
Mean	**19.0**			**11.5**		**64.3**		**24.2**	**559.9**

Table 7. Mean salinity and total number of salinity measurements in the SLE during the wet season months (May – October) each calendar year, and the number (n) and percentage of days when salinity was less than 10, ranged from 10 to 25, and exceeded 25, and mean flow. Blue-shading denotes *extreme*-wet seasons and red-shading denotes *dry*-wet seasons. Salinity data is from USGS and flow data (summed daily mean from the C44S80, C23S97, C24S49 and Gordy structures) is from SFWMD.

St. Lucie Estuary US1 Roosevelt Bridge			< 10		10 – 25		> 25		
Year	**Wet Season** Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2005	4.2	175	145	82.9	30	17.1	0	0.0	3183.6
2006	19.5	150	11	7.3	100	66.7	39	26.0	362.3
2007	15.3	162	59	36.4	57	35.2	46	28.4	664.6
2008	13.4	184	62	33.7	91	49.5	31	16.8	1377.8
2009	16.3	183	41	22.4	114	62.3	28	15.3	652.1
2010	10.5	172	72	41.9	100	58.1	0	0.0	915.0
2011	20.1	183	22	12.0	95	51.9	66	36.1	636.7
2012	15.7	184	60	32.6	99	53.8	25	13.6	1049.4
2013	7.6	184	125	67.9	56	30.4	3	1.6	2607.6
2014	14.7	184	56	30.4	125	67.9	3	1.6	938.4
2015	16.8	183	26	14.2	153	83.6	4	2.2	694.6
2016	8.0	169	110	65.1	59	34.9	0	0.0	1871.9
2017	12.3	182	70	38.5	76	41.8	36	19.8	2407.0
2018	10.4	124	82	66.1	33	26.6	9	7.3	1718.8
Mean	**13.2**			**39.4**		**48.6**		**12.0**	**1362.8**

cfs) in all years except 2006, when mean flow was significantly lower (56 cfs) than other years ($F_{26,15292}$=33.07, $P <$ 0.01; Figure 5). This corresponds with the higher mean salinity recorded during monthly sampling efforts that same year. In the SW Fork, annual mean flow rates fell into three statistically similar groups. The first group included 2008, 2010, 2014 and 2016 which all had annual means similar to the long-term 14-y mean flow rate (30 to 37 cfs). The second group included those years with lower annual means including 2009, which statistically had the lowest annual mean of 6 cfs, and 2006, 2011 and 2015 with means ranging from 13 to 17 cfs. The remaining years had annual means that were greater than the long-term average and ranged from 65 to 121 cfs.

As with the SLE, additional analysis of flow and salinity data was conducted to further define environmental conditions in the LRE during the 14-yr study. Flow from the two structures were analyzed independently with the corresponding logger and monthly salinity data. For the NW Fork, flow data from the LRE structure and salinity data from the LRD OY data logger were compiled to compare mean salinity and mean flow, as well as the percentage of measures that fell below, within and above the optimal salinity range (10 – 25) during each calendar year (Table 8). Since salinity data was only recorded by the LRD data logger from Feb 2007 to Dec 2018, salinity data collected by FWRI during monthly sampling trips was used for 2005 and 2006. Each year was classified as wet, dry or moderate using the same criteria as for the SLE. Two years, 2005 and 2010, were classified as wet years while 2006, 2009 and 2018 were considered dry years in the NW Fork. During the dry season months, the largest percentage of salinity measurements fell within the optimal range in most years (Table 9). The exception occurred in 2018, when 54.1% of the salinity measurements exceeded the optimal range, thus characterizing that year as having an *extreme*-dry season. During the wet season months, the majority of salinity measures were within optimal in all years except in 2006 when 50% of the measures exceeded optimal (Table 10). That year, as well as 2011, were considered *dry*-wet seasons since more than 25% of the salinity measures were above the optimal range. In most years, the mean dry season salinity was 4 to 5 points higher than the mean wet season salinity. Exceptions occurred in 2008 and 2015, when means were very similar during the wet and dry seasons (~19 in 2008; ~20 in 2015), and in 2013 and 2018, when differences in mean salinity between seasons were much larger (21.4 vs. 11.2 in 2013; 24.7 vs. 12.2 in 2018).

Table 8. Mean salinity and total number of salinity measurements in the LRE NW Fork per calendar year, the number (n) and percentage of days when salinity was less than 10, ranged from 10 to 25, and exceeded 25, and mean flow. Blue bold and red bold font denotes significantly low or high values. No logger data was available for 2005 and 2006, so statistics were calculated using *FWRI monthly salinity data. Shading indicates wet years (blue) or dry years (red). Daily salinity data is from the LRD (OY data logger) and flow data (Lainhart Dam structure) from the SFWMD.

Loxahatchee River Estuary NW Fork			< 10		10 – 25		> 25		
Year	Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2005*	12.6	10	3	30.0	6	60.0	1	10.0	112.2
2006*	**21.8**	12	1	8.3	6	50.0	5	41.7	**55.8**
2007	17.7	277	35	12.6	173	62.5	69	24.9	90.7
2008	19.0	243	16	6.6	193	79.4	34	14.0	98.0
2009	**22.4**	326	3	0.9	216	66.3	107	32.8	76.0
2010	**15.2**	328	98	29.9	191	58.2	39	11.9	117.9
2011	19.8	345	25	7.2	258	74.8	62	18.0	80.8
2012	17.9	361	51	14.1	277	76.7	33	9.1	101.7
2013	16.2	365	84	23.0	231	63.3	50	13.7	131.7
2014	18.0	360	45	12.5	282	78.3	33	9.2	142.0
2015	20.4	365	30	8.2	265	72.6	70	19.2	88.7
2016	17.8	366	29	7.9	303	82.8	34	9.3	114.2
2017	18.7	347	39	11.2	251	72.3	57	16.4	72.9
2018	18.4	365	72	19.7	179	49.0	114	31.2	104.4
Mean	**18.3**			**13.7**		**67.6**		**18.7**	**150.8**

Nonlinear regression of monthly mean salinity (LRD OY data logger) and flow (LRE structure) into the NW Fork of the LRE showed a significant negative correlation (Figure 8). On this plot, the best fit curve extended beyond the optimal salinity band where flow rates were below 25 cfs or where they exceeded approximately 235 cfs. The time series plot of the salinity and flow data show that, although there weren't many excursions outside the optimal salinity range, there was one occurrence in 2013 when flow rates exceeded the upper threshold and salinities were less than optimal (Figure 9). Other excursions occurred in 2007 and 2018 when flow rates were below the lower threshold resulting in salinities that exceeded optimal.

For the SW Fork, flow data from the S46 structure and salinity data from the LRD 72 logger were compiled to compare mean salinity and mean flow, as well as the percentage of measures that fell below, within and above the optimal salinity range (10 – 25) during each calendar year. Salinity data from the LRD data logger was only recorded from Apr 2008 to Apr 2011 and May 2018 to Dec 2018, so monthly data collected by FWRI was used to fill in the gaps. Unfortunately, that means there were substantially fewer salinity measures available for the

Table 9. Mean salinity and total number of salinity measurements in the LRE NW Fork during the dry season months (January – April; November – December) each calendar year, and the number (n) and percentage of days when salinity was less than 10, ranged from 10 to 25, and exceeded 25, and mean flow. No logger data was available for 2005 and 2006, so statistics were calculated using *FWRI monthly salinity data. Red-shading denotes *extreme*-dry seasons. Daily salinity data is from the LRD (OY data logger) and flow data (Lainhart Dam structure) from the SFWMD.

Loxahatchee River Estuary NW Fork			< 10		10 – 25		> 25		
Year	**Dry Season** Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2005*	14.7	5	1	20.0	3	60.0	1	20.0	75.9
2006*	23.7	6	0	0.0	4	66.7	2	33.3	35.7
2007	20.9	111	5	4.5	61	55.0	45	40.5	59.0
2008	18.8	169	10	5.9	143	84.6	16	9.5	90.9
2009	24.2	165	2	1.2	81	49.1	82	49.7	49.8
2010	19.3	152	29	19.1	84	55.3	39	25.7	83.5
2011	20.7	178	8	4.5	150	84.3	20	11.2	66.5
2012	20.3	179	1	0.6	156	87.2	22	12.3	74.2
2013	21.4	181	3	1.7	132	72.9	46	25.4	61.0
2014	20.8	176	9	5.1	136	77.3	31	17.6	107.6
2015	20.4	181	25	13.8	102	56.4	54	29.8	89.9
2016	18.8	182	18	9.9	132	72.5	32	17.6	107.5
2017	21.5	163	2	1.2	113	69.3	48	29.4	57.0
2018	24.7	181	0	0.0	83	45.9	98	54.1	42.3
Mean	**20.7**			**6.2**		**66.9**		**26.9**	**71.5**

Table 10. Mean salinity and total number of salinity measurements in the LRE NW Fork during the wet season months (May – October) each calendar year, and the number (n) and percentage of days when salinity was less than 10, ranged from 10 to 25, and exceeded 25, and mean flow. No logger data was available for 2005 and 2006, so statistics were calculated using *FWRI monthly salinity data. Red-shading denotes *dry*-wet seasons. Daily salinity data is from the LRD (OY data logger) and flow data (Lainhart Dam structure) from the SFWMD.

Loxahatchee River Estuary NW Fork			< 10		10 – 25		> 25		
Year	**Wet Season** Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2005*	10.5	5	2	40.0	3	60.0	0	0.0	147.8
2006*	19.9	6	1	16.7	2	33.3	3	50.0	75.7
2007	15.6	166	30	18.1	112	67.5	24	14.5	121.9
2008	19.6	74	6	8.1	50	67.6	18	24.3	105.0
2009	20.6	161	1	0.6	135	83.9	25	15.5	101.9
2010	11.7	176	69	39.2	107	60.8	0	0.0	151.8
2011	18.9	167	17	10.2	108	64.7	42	25.1	94.9
2012	15.5	182	50	27.5	121	66.5	11	6.0	128.8
2013	11.2	184	81	44.0	99	53.8	4	2.2	201.3
2014	15.3	184	36	19.6	146	79.3	2	1.1	175.9
2015	20.3	184	5	2.7	163	88.6	16	8.7	87.5
2016	16.9	184	11	6.0	171	92.9	2	1.1	120.9
2017	16.2	184	37	20.1	138	75.0	9	4.9	88.5
2018	12.2	184	72	39.1	96	52.2	16	8.7	165.5
Mean	**16.0**			**20.8**		**67.6**		**11.6**	**126.2**

analysis. Despite those shortcomings, the results are presented to serve as a starting point for gaining a better understanding of environmental conditions in the SW Fork. In all years except 2016, the largest percentage of salinity measurements exceeded the optimal range; however, 25% or more of the salinity measurements were also below the optimal range in 2005 and 2013 characterizing those years as having both extremes (Table 11). During the dry season months, the majority of salinity measurements exceeded the optimal range in all years except 2012 and 2016 (Table 12). In 2012, half of the salinity measures fell within and half exceeded the optimal range. In 2016, most measures were within the optimal range, making it a year with a more moderate dry season. All remaining years were characterized as having *extreme*-dry seasons. During the wet season months of most years, the majority of salinity measures exceeded the optimal range (Table 13). Exceptions occurred in 2007, 2016 and 2018 when the greatest percentage of measures were within the optimal range. Another exception occurred in 2017, when salinity measures were equally divided among the three categories. The final exception occurred in 2013

Table 11. Mean salinity and total number of salinity measurements in the LRE SW Fork per calendar year, the number (n) and percentage of days when salinity was less than 10, ranged from 10 to 25, and exceeded 25, and mean flow. Blue bold and red bold font denotes significantly low or high values. For those years when logger data was not available, statistics were calculated using *FWRI monthly salinity data. Shading indicates dry years (red) or years with both wet and dry extremes (purple). Daily salinity data is from the LRD (72 data logger) and flow data (S46 structure) from the SFWMD.

Loxahatchee River Estuary SW Fork			< 10		10 – 25		> 25		
Year	Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2005*	20.5	10	3	30.0	1	10.0	6	60.0	105.4
2006*	29.3	12	0	0.0	1	8.3	11	91.7	17.5
2007*	22.6	12	1	8.3	4	33.3	7	58.3	66.7
2008	23.9	200	17	8.5	60	30.0	123	61.5	36.7
2009	**29.4**	305	0	0.0	24	7.9	281	92.1	**5.9**
2010	26.2	336	8	2.4	91	27.1	237	70.5	32.1
2011	**31.8**	79	0	0.0	0	0.0	79	100.0	13.2
2012*	22.0	12	1	8.3	5	41.7	6	50.0	79.9
2013*	21.8	12	3	25.0	3	25.0	6	50.0	64.9
2014*	23.2	12	2	16.7	3	25.0	7	58.3	29.3
2015*	25.9	12	0	0.0	3	25.0	9	75.0	15.2
2016*	21.1	12	1	8.3	7	58.3	4	33.3	37.0
2017*	23.1	12	2	16.7	4	33.3	6	50.0	120.5
2018	**20.5**	245	57	23.3	74	30.2	114	46.5	101.2
Mean	**24.4**			**10.5**		**25.4**		**64.1**	**51.8**

Table 12. Mean salinity and total number of salinity measurements in the LRE SW Fork during the dry season months (January – April; November – December) each calendar year, and the number (n) and percentage of days when salinity was less than 10, ranged from 10 to 25, and exceeded 25, and mean flow. For those years when logger data was not available, statistics were calculated using *FWRI monthly salinity data. Red-shading denotes *extreme*-dry seasons. Daily salinity data is from the LRD (72 data logger) and flow data (S46 structure) from the SFWMD.

Loxahatchee River Estuary SW Fork			< 10		10 – 25		> 25		
Year	**Dry Season** Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2005*	22.8	5	1	20.0	1	20.0	3	60.0	24.0
2006*	30.5	6	0	0.0	0	0.0	6	100.0	0.1
2007*	26.7	6	0	0.0	1	16.7	5	83.3	11.6
2008	27.9	60	0	0.0	9	15.0	51	85.0	1.4
2009	30.6	138	0	0.0	1	0.7	137	99.3	1.3
2010	29.0	165	1	0.6	19	11.5	145	87.9	9.4
2011	31.8	79	0	0.0	0	0.0	79	100.0	9.8
2012*	24.6	6	0	0.0	3	50.0	3	50.0	0.2
2013*	29.4	6	0	0.0	1	16.7	5	83.3	0.0
2014*	27.3	6	0	0.0	2	33.3	4	66.7	6.1
2015*	25.9	6	0	0.0	1	16.7	5	83.3	23.8
2016*	19.4	6	1	16.7	3	50.0	2	33.3	45.4
2017*	28.3	6	0	0.0	2	33.3	4	66.7	58.6
2018	31.6	61	0	0.0	0	0.0	61	100.0	8.9
Mean	**27.5**			**2.7**		**18.9**		**78.5**	**14.3**

Table 13. Mean salinity and total number of salinity measurements in the LRE SW Fork during the wet season months (May – October) each calendar year, and the number (n) and percentage of days when salinity was less than 10, ranged from 10 to 25, and exceeded 25, and mean flow. For those years when logger data was not available, statistics were calculated using *FWRI monthly salinity data. Blue-shading denotes *extreme*-wet seasons and red-shading denotes *dry*-wet seasons. Daily salinity data is from the LRD (72 data logger) and flow data (S46 structure) from the SFWMD.

Loxahatchee River Estuary SW Fork			< 10		10 – 25		> 25		
Year	**Wet Season** Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2005*	18.2	5	2	40.0	0	0.0	3	60.0	185.6
2006*	28.1	6	0	0.0	1	16.7	5	83.3	34.6
2007*	18.4	6	1	16.7	3	50.0	2	33.3	120.8
2008	22.2	140	17	12.1	51	36.4	72	51.4	71.6
2009	28.4	167	0	0.0	23	13.8	144	86.2	10.4
2010	23.4	171	7	4.1	72	42.1	92	53.8	54.4
2011	25.9	5	0	0.0	2	40.0	3	60.0	16.5
2012*	19.3	6	1	16.7	2	33.3	3	50.0	158.8
2013*	14.3	6	3	50.0	2	33.3	1	16.7	128.6
2014*	19.1	6	2	33.3	1	16.7	3	50.0	52.1
2015*	25.9	6	0	0.0	2	33.3	4	66.7	6.8
2016*	22.9	6	0	0.0	4	66.7	2	33.3	28.6
2017*	18.0	6	2	33.3	2	33.3	2	33.3	181.5
2018	16.7	184	57	31.0	74	40.2	53	28.8	193.9
Mean	**21.5**			**16.9**		**32.6**		**50.5**	**88.9**

when more than 50% of the measures were below optimal, making 2013 an *extreme*-wet season. Because more than 25% of the salinity measures exceeded optimal, all other years were considered *dry*-wet seasons.

Nonlinear regression of monthly mean salinity (LRD 72 data logger) and flow (S46 structure) in the SW Fork of the LRE showed significant negative correlation (Figure 10). Although there wasn't much data available for this analysis, it appears that flow rates ranging from approximately 35 to 340 cfs will maintain salinities within the optimal range. The time series plot shows that flow into the SW Fork is intermittent and inconsistent thus salinities regularly exceed the optimal range (Figure 11). During 2008 and 2010, flow rates increased for a month or more, allowing salinities to decrease to optimal levels. In June 2018, flow exceeded the upper threshold which led to a mean salinity that was below the optimal range.

Water temperatures exhibited typical seasonal patterns throughout the 14-yr study but were slightly higher in the LRE than the SLE ($F_{4, 2459}$=11.55, $P < 0.01$; Figure 2B). The highest temperatures were recorded during July or August when means frequently exceeded 29° C in both estuaries (Appendix A). Temperature minima occurred in December, January, February or March, depending on the year and the estuary. The warmest year was 2015 when the mean temperature was 26.5° C; the coolest year was 2010 when the mean temperature was 24.6° C ($F_{13, 2459}$=1.75, $P = 0.46$). No significant differences were detected in water temperature patterns within sites during the 14-yr study ($F_{52, 2459}$=0.22, $P = 1.00$).

Dissolved oxygen concentrations were similar but slightly higher in Loxahatchee-South, St. Lucie-North and St. Lucie-South than in the remaining two sites ($F_{4,}$ 2187=5.41, $P < 0.01$; Figure 2C). At all sites, annual means ranged from 5.1 to 7.9 mg/L but did not significantly differ over the 14-yr study ($F_{48,}$ 2187=0.35, $P = 1.00$; Appendix A). As with dissolved oxygen concentrations, pH was similar among sites but slightly lower in the two LRE sites ($F_{4,}$ 2194=2.64, $P < 0.03$; Figure 2D). Annual means ranged from 7.7 to 8.2 but there were no significant differences among years within sites ($F_{48, 2194}$=0.79, $P = 1.00$). Dissolved oxygen concentrations and pH were not recorded in 2005.

Water clarity was significantly greater at the two LRE sites, where overall means were 98% or higher ($F_{4,2448}$=118.86, $P < 0.01$; Figure 2E). The lowest water clarity occurred in St. Lucie-South where the overall mean was 71%. Although water clarity differed significantly among years at each of the sites ($F_{52,2448}$=5.63, $P < 0.01$), those differences were most pronounced in the SLE sites where annual means were much lower in 2005 (32 to 70%) and 2016 (43 to 69%; Figure 12).

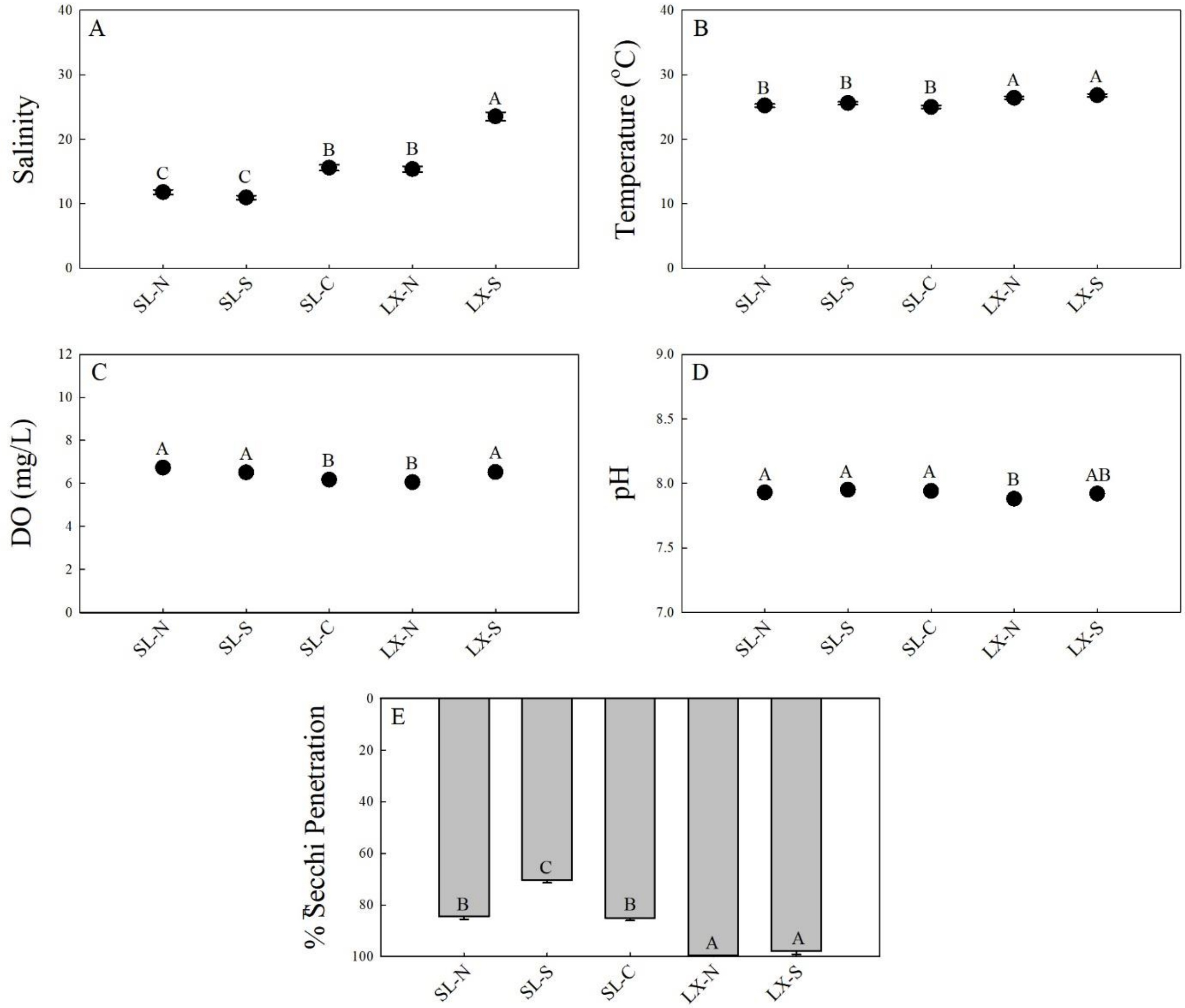

Figure 2. Estimated mean and standard error from overall comparisons of salinity (A), temperature (B), dissolved oxygen concentration (C), pH (D) and % Secchi penetration (E) at the five southeast Florida study sites from 2005 to 2018. Letter designations denote differences in least-squares means between sites for the 14-y study; those with the same letter do not differ significantly ($P > 0.05$).

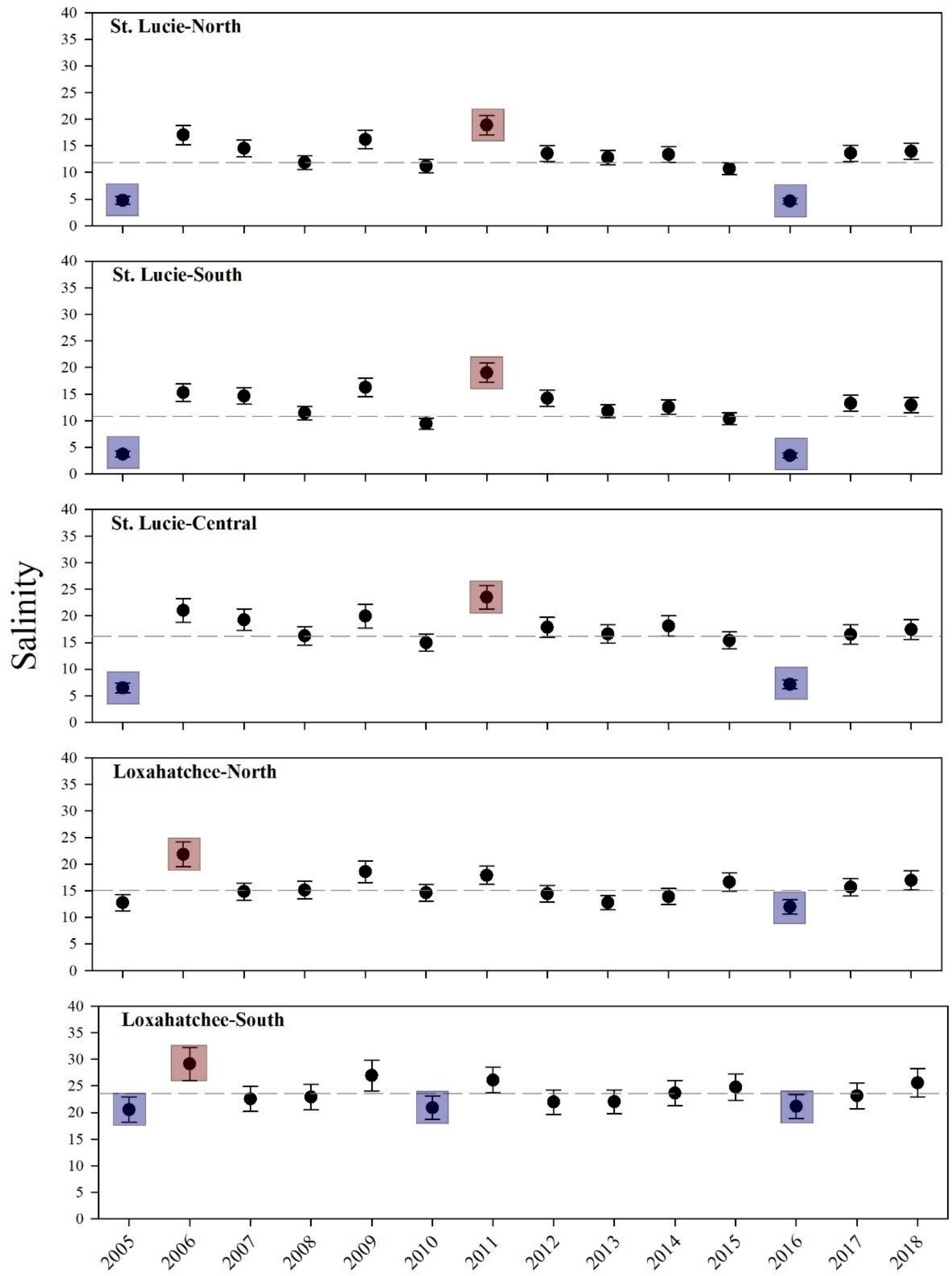

Figure 3. Estimated annual means and standard errors from comparisons of salinity within the five southeast Florida study sites from 2005 to 2018. Red-shaded data points indicate years with significantly greater salinities and blue-shaded data points indicate years with significantly lower salinities ($P < 0.05$). The dashed lines represent the overall estimated mean for each site during the 14-y study.

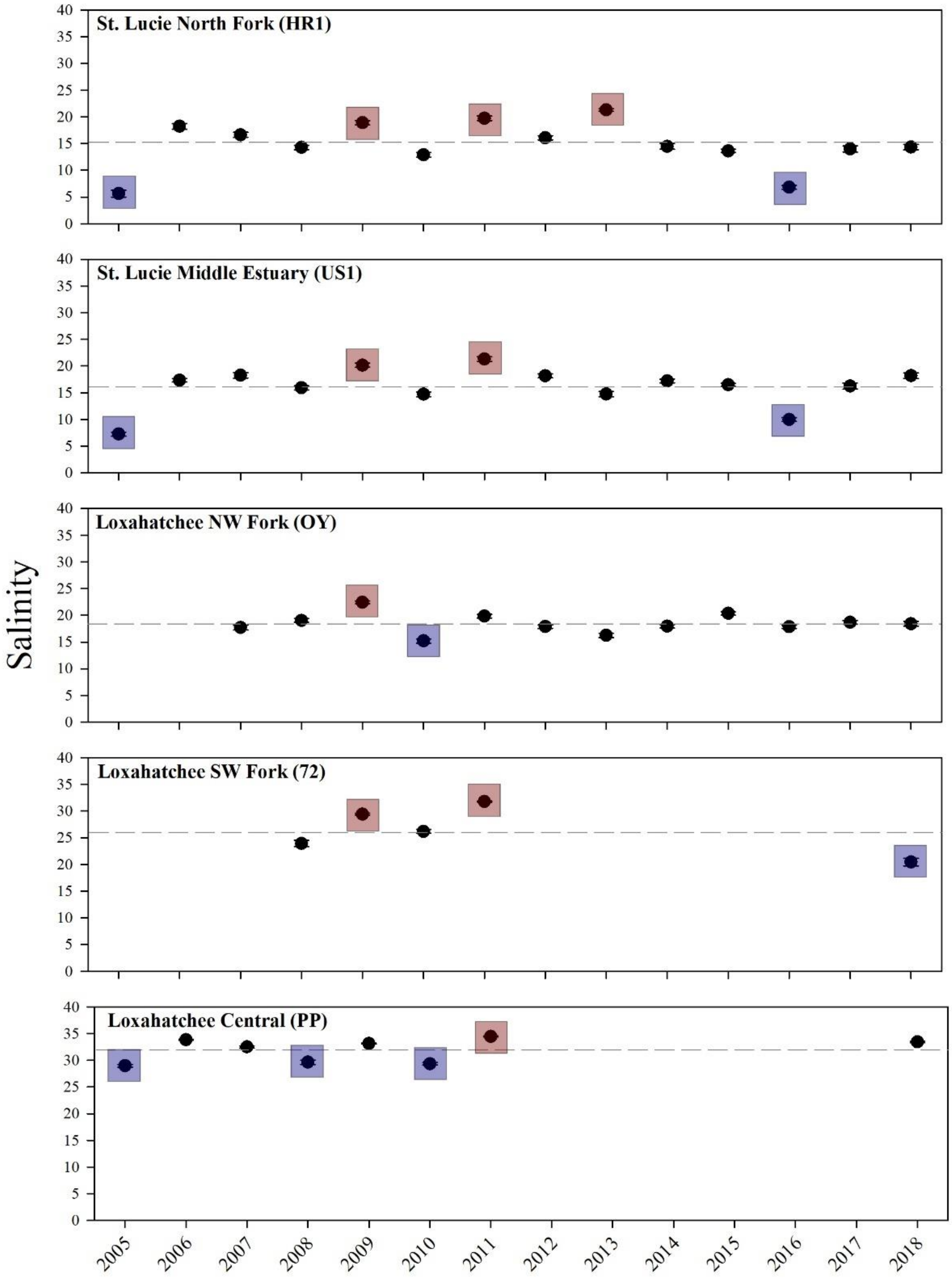

Figure 4. Estimated annual means and standard errors from comparisons of salinity measured by continuous data loggers deployed by the SFWMD, USGS and the LRD within the St. Lucie and Loxahatchee River Estuaries from 2005 to 2018. Red-shaded data points indicate years with significantly greater salinities and blue-shaded data points indicate years with significantly lower salinities ($P < 0.05$). The dashed lines represent the overall estimated mean for each site during the 14-y study.

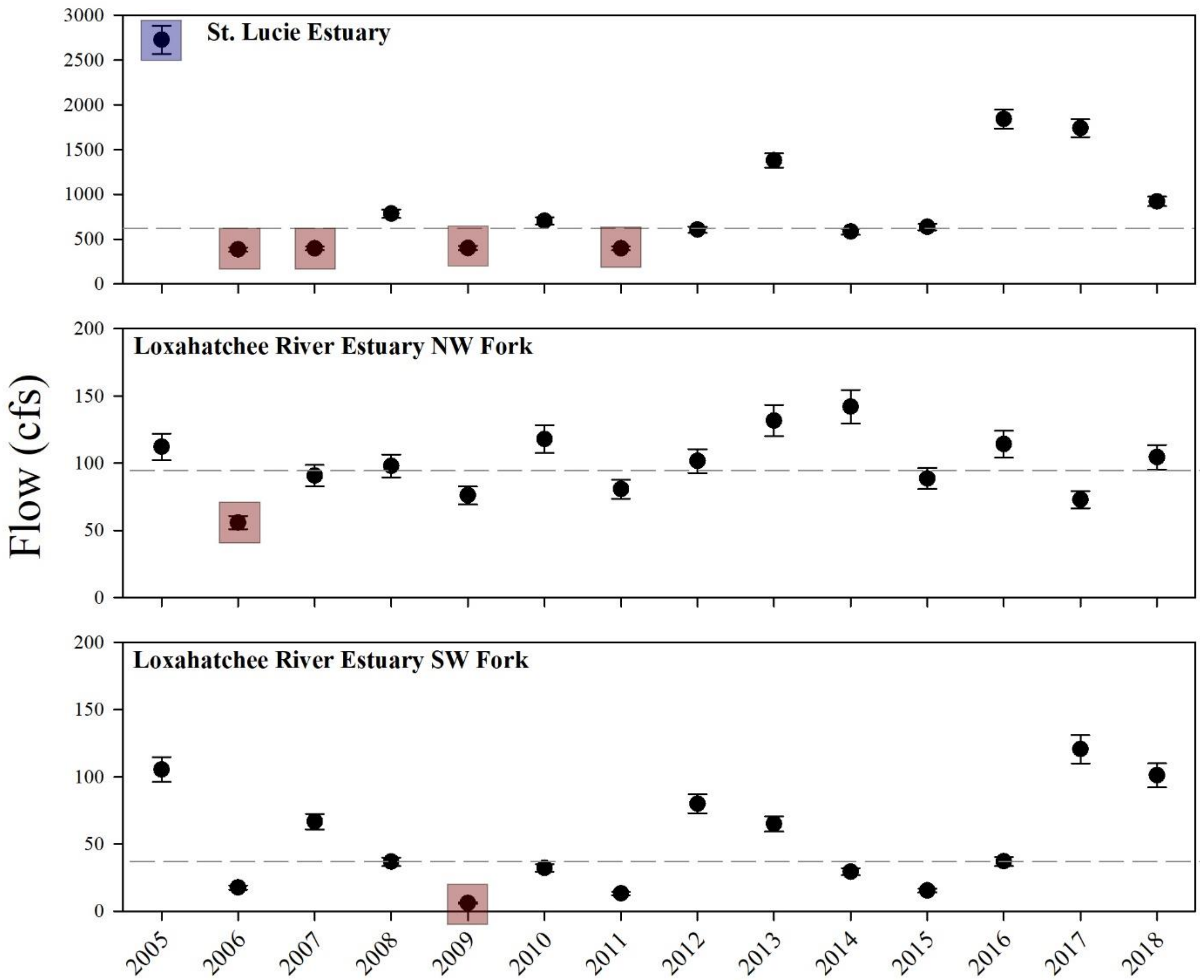

Figure 5. Estimated annual means and standard errors from comparisons of summed flows into the St. Lucie Estuary (S80, S97 and S49 structures) and into the NW Fork (Lainhart Dam structure) and SW Fork (S46 structure) of the Loxahatchee River Estuary from 2005 to 2018. The blue-shaded data point indicates year with significantly greater flow rates and red-shaded data points indicate years with significantly lower flow rates ($P < 0.05$). The dashed lines represent the overall estimated mean for each location during the 14-y study. Flow data from the SFWMD and USGS.

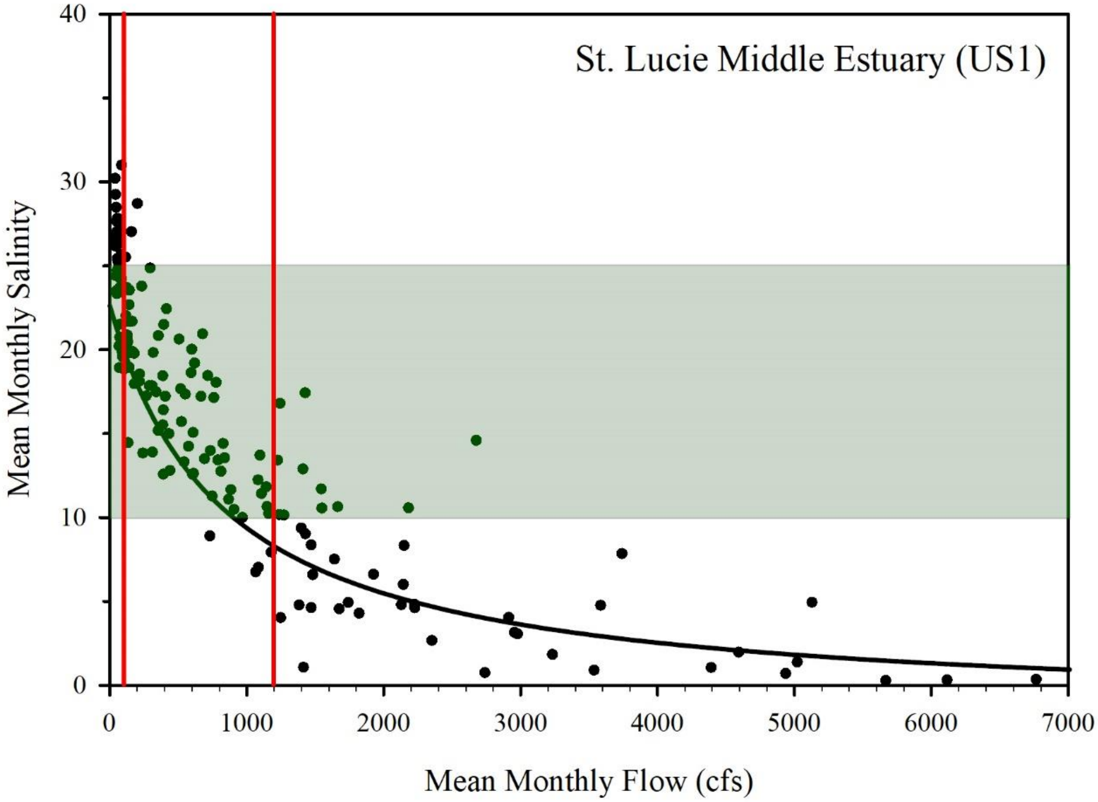

Figure 6. Nonlinear relationship between mean monthly salinity measured at the US1 Roosevelt Bridge and mean monthly flow in the SLE. The green band highlights the optimal salinity range (10 – 25) for oysters in the SLE. The red lines indicate the minimum (~50 cfs) and maximum mean monthly flow rates (~1200 cfs) allowable for maintaining salinities within the optimal range. Salinity and flow data from the USGS and SFWMD.

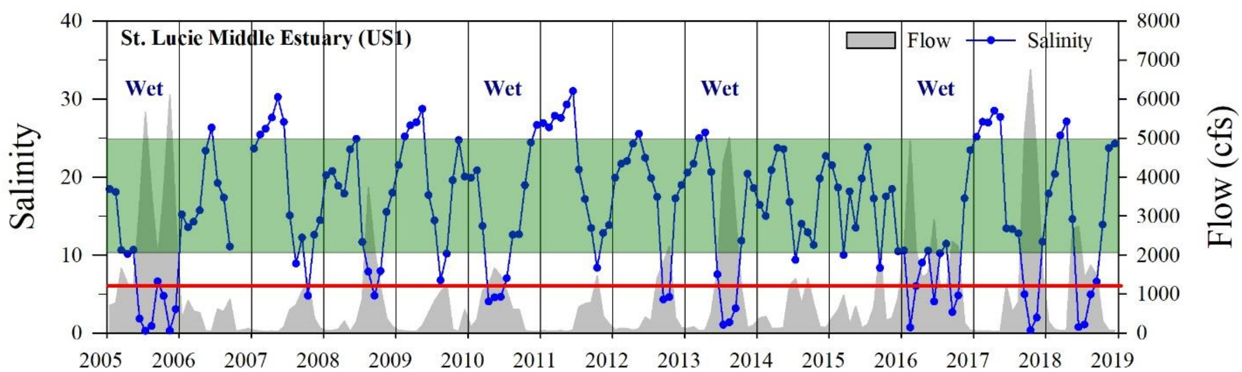

Figure 7. Mean monthly salinity measured at the US1 Roosevelt Bridge and mean monthly flow in the SLE. The green band highlights the optimal salinity range (10 – 25) and the red line shows the estimated maximum mean monthly flow rate (~1200 cfs) allowable for maintaining salinities above the lower boundary of the optimal range. Salinity and flow data from the USGS and SFWMD.

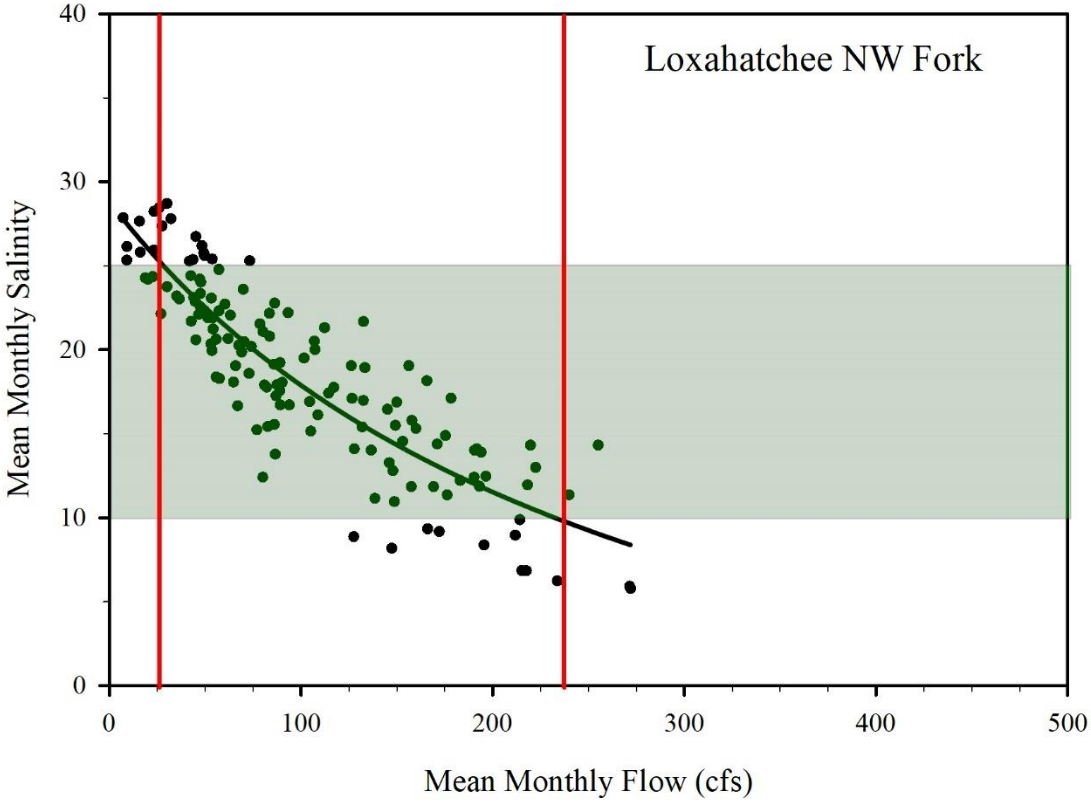

Figure 8. Nonlinear relationship between mean monthly salinity and mean monthly flow in the NW Fork of the LRE. The green band highlights the optimal salinity range (10 – 25) for oysters in the LRE. The red lines indicate the minimum (~25 cfs) and maximum mean monthly flow rates (~235 cfs) allowable for maintaining salinities within the optimal range. Salinity and flow data from the LRD and SFWMD.

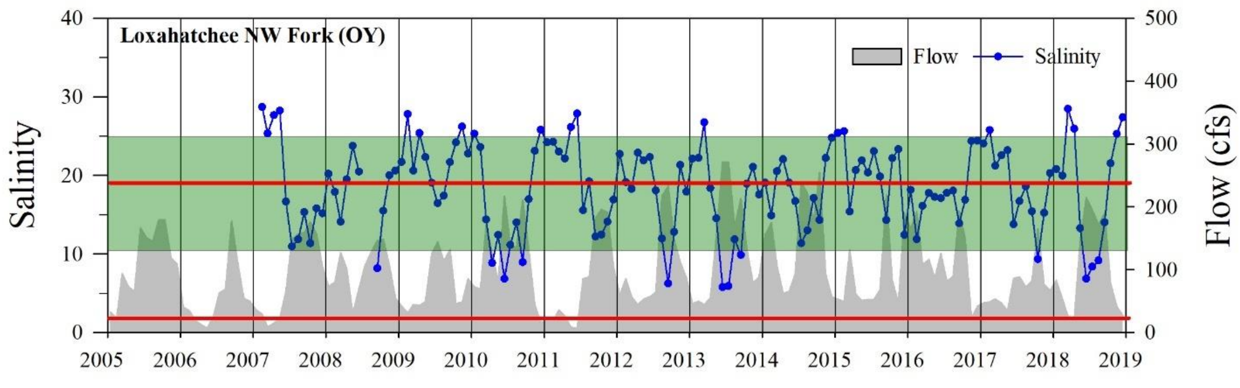

Figure 9. Mean monthly salinity and mean monthly flow in the NW Fork of the LRE. The green band highlights the optimal salinity range (10 – 25) and the red lines show the estimated minimum and maximum mean monthly flow rates (~25 and 235 cfs, respectively) allowable for maintaining salinities within the optimal range. Salinity and flow data from the LRD and SFWMD.

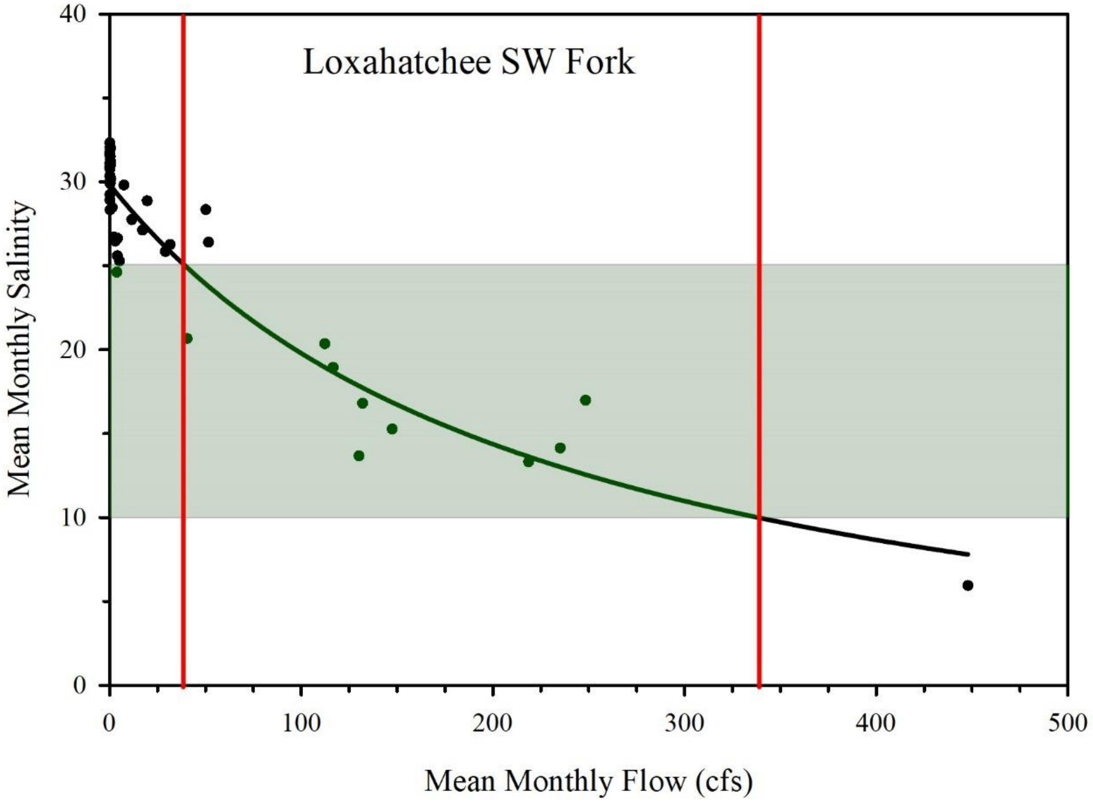

Figure 10. Nonlinear relationship between mean monthly salinity and mean monthly flow in the SW Fork of the LRE. The green band highlights the optimal salinity range (10 – 25) for oysters in the LRE. The red lines indicate the minimum (~35 cfs) and maximum mean monthly flow rates (~340 cfs) allowable for maintaining salinities within the optimal range. Salinity and flow data from the LRD and SFWMD.

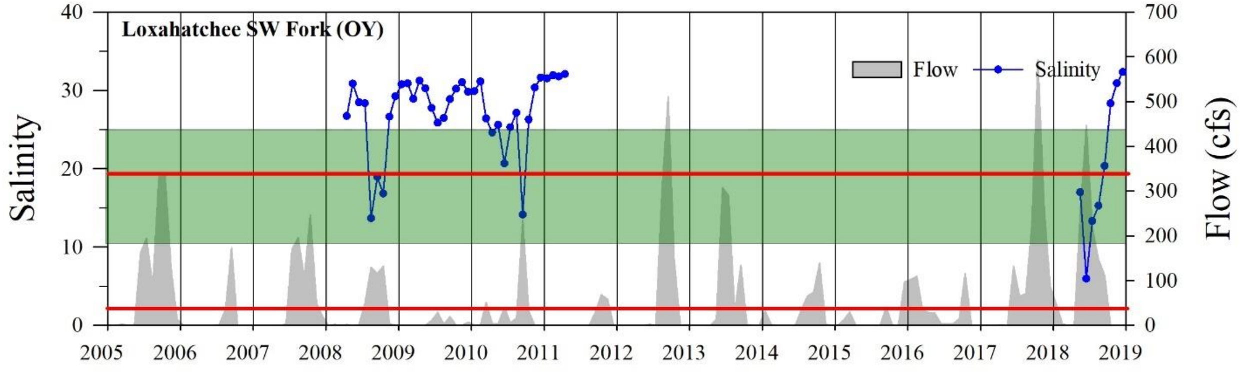

Figure 11. Mean monthly salinity and mean monthly flow in the SW Fork of the LRE. The green band highlights the optimal salinity range (10 – 25) and the red lines show the estimated minimum and maximum mean monthly flow rates (~35 and 340 cfs, respectively) allowable for maintaining salinities within the optimal range. Salinity and flow data from the LRD and SFWMD.

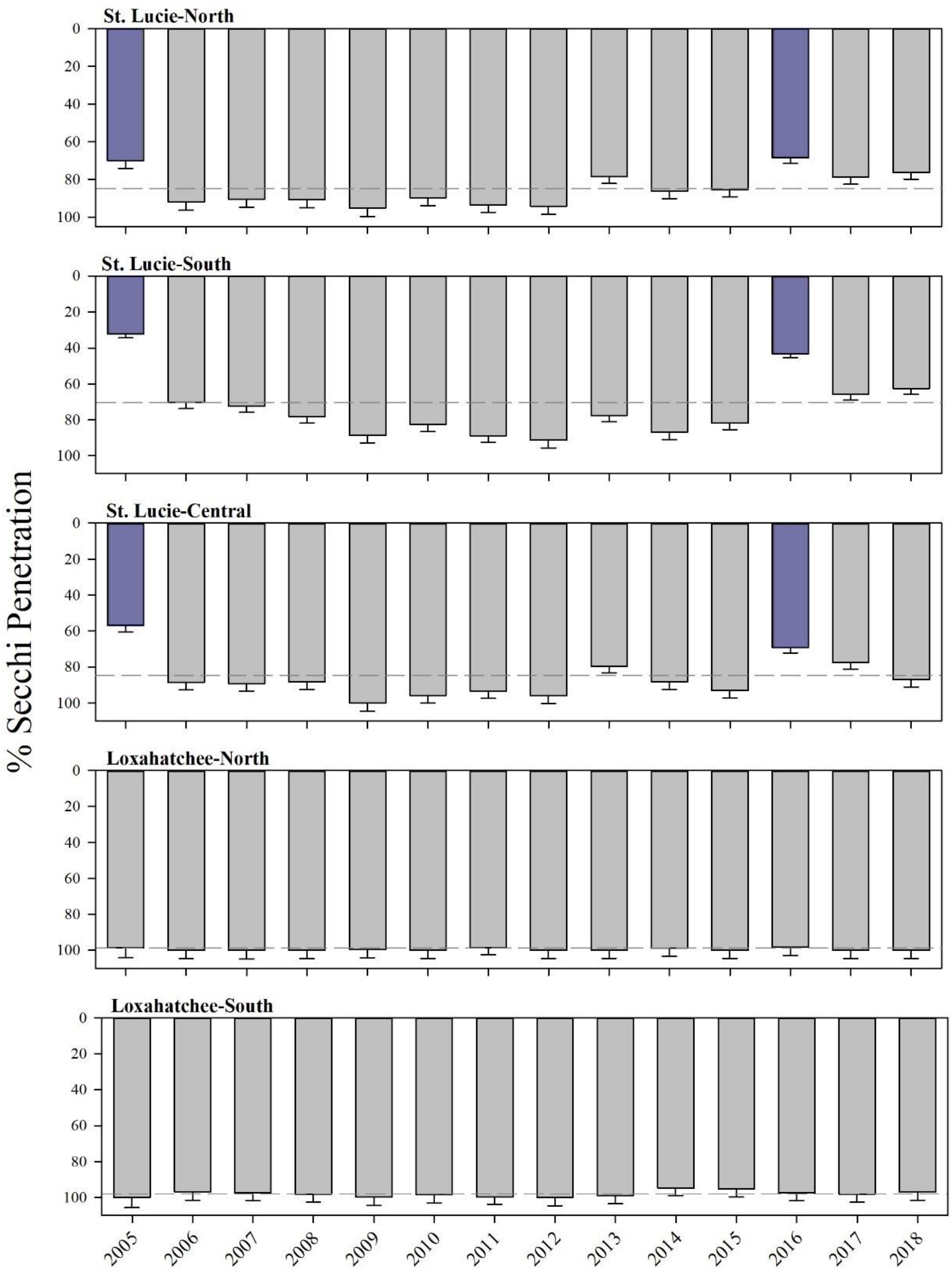

Figure 12. Estimated annual means and standard errors from comparisons of percent Secchi penetration within the five southeast Florida study sites from 2005 to 2018. Blue-shaded bars indicate years with significantly lower salinities ($P < 0.05$). The dashed lines represent the overall estimated mean for each site during the 14-y study.

Settled Oyster Density

When sampling began in early 2005, no live oysters were observed at any of the stations in the St. Lucie-North or St. Lucie-South study sites (Appendix B). Settled oysters were not detected in quadrats deployed in either site until fall 2006. Although live oysters were present in the St. Lucie-Central site in 2005 and spring 2006, densities remained very low (< 2/m^2 at most stations) until fall 2006.

The greatest densities of oysters were found in the Loxahatchee-North site where the overall mean for the 14-year study was 489 oysters/m^2 ($F_{4,5645}$=62.69, P < 0.01; Figure 13A) and annual means ranged from 122 to 912 oysters/m^2. The St. Lucie-Central and Loxahatchee-South sites exhibited moderate live densities with overall means of 272 and 221 oysters/m^2, respectively. Although oysters were almost absent from St. Lucie-Central during the surveys in 2005, spring 2006, fall 2008, fall 2013, and spring 2018, live densities in the remaining surveys were some of the greatest recorded among all sites, especially in fall 2014 when means were greater than 2000 oysters/m^2 at Station 1. The lowest densities occurred in the St. Lucie-North and South sites, where the overall means were less than 10 oysters/m^2.

Oyster density also differed significantly among years at each of the sites ($F_{52,5645}$=25.71, P < 0.01). Although there was no consistent pattern for when each site exhibited the greatest oyster densities, the lowest densities often corresponded with periods of low salinity and high flow rates. At all sites, the lowest densities were recorded in 2005 when values ranged from 122/m^2 in Loxahatchee-North to less than 30/m^2 at the remaining sites (Figure 14). This corresponds with the significantly high flow rates and low salinities recorded in 2005. Oyster densities were also significantly low in the St. Lucie-South site in 2013 (approximately 3/m^2) and in both the St. Lucie-North and South sites in 2018 (<1 and 0 oysters/m^2, respectively). Although densities at the three SLE sites were not significantly lower in 2008 than most years, it is noteworthy that mean overall density decreased by two orders of magnitude from 229 oysters/m^2 in March to less than 3 oysters/m^2 in September. Oyster densities were relatively stable in the two LRE sites, ranging from approximately 500 to 900 oysters/m^2 in the NW Fork and 200 to 500 oysters/m^2 in the SW Fork during most years.

In order to assess the relative health of a particular oyster reef, the ratio of dead oysters to the total number of live oysters and dead oysters was determined (Appendix B). Statistical comparison of dead ratios revealed differences among sites ($F_{4,5339}$=17.29, P < 0.01; Figure 13B). The greatest ratios were found in St. Lucie-North and South where overall means were 0.59 and 0.80, respectively. Ratios fluctuated most at these two sites as well, with annual values ranging from 0.02 to 1.00 during the 14-y study. Ratios of dead oysters at the remaining three sites were similar (0.20) but varied the least at Loxahatchee-South where annual means ranged from 0.11 to 0.54. Ratios were similar at the remaining two sites, where annual means varied from 0.05 to 0.89.

Differences in ratios of dead oyster among years within sites were also detected ($F_{52,5339}$=1722.08, $P < 0.01$). At all sites, the greatest dead ratios occurred in 2005, when the means were 1.00 in St. Lucie-North and South, 0.89 in St. Lucie-Central, 0.75 in Loxahatchee-South and 0.54 in Loxahatchee-North (Figure 15). Ratios were also significantly high in the St. Lucie-South site in 2013 (0.94) and in the St. Lucie-North and South sites in 2018 (0.98 and 1.00, respectively) corresponding with large freshwater releases and low salinities that occurred during or just prior to those periods. Ratios of dead oysters were significantly low ($\leq$ 0.06) in the three SLE sites in 2006 and/or 2007 following the die-off that occurred in 2005. Other significantly low dead ratios were recorded in Loxahatchee-North in 2008 (0.11), St. Lucie-South in 2011 (0.06) and St. Lucie-Central and Loxahatchee-South in 2017 (0.05 and 0.10, respectively).

Oyster shell heights (SH) of live oysters were greatest and varied the least in the Loxahatchee-South site where the overall mean was approximately 42 mm ($F_{4,4640}$=39.02, $P < 0.01$; Appendix B and Figure 13C). Loxahatchee-North oysters exhibited the smallest SHs with an overall mean of 38 mm. Mean overall SH in St. Lucie-Central fell in the middle at 42 mm and varied more from year to year. Because there were large amounts of missing data from the St. Lucie-North and South sites due to frequent oyster die-offs, estimated means were not generated from the statistical model; however, overall mean SHs calculated from raw data from years when live oysters were present were 46 mm for the North Fork and 48 mm for the South Fork.

Mean SHs varied among years at all sites throughout the study ($F_{49,4640}$=26.44, $P < 0.01$), but were relatively stable in Loxahatchee-South, where annual means ranged from 35 to 48 mm, and in Loxahatchee-North, where annual means ranged from 26 to 44 mm (Figure 16). At both LRE sites, the lowest annual values were recorded in 2018 when there was a localized oyster die-off during the summer months that likely removed most of the larger oysters from the population. Significantly smaller SHs were also measured in 2018 at the St. Lucie-North and Central sites (not in St. Lucie-South because there were no live oysters). SHs were also smaller in 2006 and 2014 in St. Lucie-South, again, following oyster die-offs in previous years.

Results from analyses of salinity, live oyster density, dead oyster ratios and live oyster shell height were compiled for comparison. In the SLE middle estuary, live oyster density and the ratio of dead oysters were lowest in 2005 corresponding with the significantly low mean annual salinity (Table 14). That year was considered a wet year with both the dry season and wet season months also characterized as wetter than expected. There was a similar occurrence in 2016 when all three classifications were wet; however, despite those characterizations, live oyster density was among the highest recorded in the study. Those differences in oyster population responses were related to the duration of the low salinity events. In 2016, salinities fell below optimal in early February and remained sub-optimal most days through mid-October (186 days < 10). In 2005, salinities fell below optimal in March and remained sub-optimal through December (228 days < 10). The lowest ratios of dead oysters were measured in years following these low salinity events (2006 and 2017) and reflect recovery in the form of large numbers of spat settling to

Table 14. Wet, dry or moderate classifications for each calendar year, the dry season months (January – April; November – December), the wet season months (May – October) and the mean salinity, live oyster density, ratio of dead oysters and live oyster shell heights in the SLE middle estuary (St. Lucie-Central study site). Shading indicates wet (blue), dry (red), or both extremes (purple). Blue bold and red bold font denotes significantly low or high salinity values. Red bold or green bold font denotes significantly low or high biological values. Salinity data is from USGS.

St. Lucie Estuary US1 Roosevelt Bridge							
Year	Classification	Dry Season Classification	Wet Season Classification	Salinity	Live Density	Dead Ratio	SH
2005	Wet	Wet	Extreme	**7.3**	**9.52**	**0.89**	44.97
2006	Moderate		Dry	17.3	101.55	**0.05**	30.30
2007	Dry		Dry	18.3	**336.15**	0.08	47.61
2008	Moderate			15.9	**254.67**	0.45	37.21
2009	Dry	Extreme		**20.2**	**318.49**	0.23	47.09
2010	Wet			14.7	**532.09**	0.11	**55.95**
2011	Dry	Extreme	Dry	**21.3**	**536.44**	0.11	49.70
2012	Moderate			18.2	**426.13**	0.32	40.85
2013	Wet		Extreme	14.8	**309.60**	0.37	34.25
2014	Moderate			17.2	**633.64**	0.14	32.27
2015	Moderate			16.5	**705.42**	0.07	42.55
2016	Wet	Wet	Extreme	**10.0**	**471.42**	0.10	39.42
2017	Both	Extreme		16.2	**599.29**	**0.05**	45.76
2018	Both		Extreme	18.2	96.84	0.65	**17.30**

repopulate the reefs, thus outnumbering and minimizing the number of recently dead oysters. The smallest mean oyster size was measured in 2018 following a widespread oyster die-off associated with Hurricane Irma in September 2017. (The mean live density for 2017 does not reflect this die-off since the September survey was not conducted due to poor water quality conditions in the SLE following the storm; statistical results and Figure 14 only reflect the spring 2017 survey data.) As with dead ratios in 2006 and 2017, the small mean shell heights measured in 2018 are due to the recovering oyster population being primarily composed of recently settled spat. The largest oysters were measured in 2010, which was classified as a wet year – but one that did not negatively impact live oyster densities. Instead, juvenile recruitment was inhibited by the low salinities so that no spat were settling and most oysters present on the reefs were older and larger.

In the LRE NW and SW Forks, the lowest live oyster densities and greatest ratios of dead oysters were also recorded in 2005 (Tables 15 and 16). That year was classified as wet year in the NW Fork and as a year exhibiting both extremes in the SW Fork; monthly measured salinities were below optimal in June, September and November (no sampling was conducted in October). In the SW Fork, all remaining months except April had salinities that exceeded the optimal range. The largest oysters in the NW Fork were measured in 2005 and 2011. The greater mean shell height in 2005 was likely due to suppression of new recruits by the lower salinities, leading to a population comprised primarily of older and larger oysters. In 2011, the larger mean shell heights may have been the result of a moderate year with mostly optimal salinities (25 days < 10; 62 days > 25) and no extended periods with excursions outside the optimal range. The largest oysters in the SW Fork were found in 2006 and 2007. Both years were classified as dry with both the dry season and wet season months also characterized as drier than expected. Although measured salinities exceeded optimal in most months of both years, the relatively stable salinity regime was likely favorable for greater oyster survival and growth. As in the SLE, the smallest oysters were measured in 2018. In both forks, 2018 was characterized as dry, and the dry season months were drier than expected, but this appeared to have little impact on live oyster densities. However, there was a moderate oyster die-off in the NW Fork due to a period of sub-optimal salinities most days from mid-May through early August. As a result, live oyster densities decreased from 975 oysters/m^2 in March to 188 oysters/m^2 in September. Thus, the small mean shell height measured in the NW Fork in 2018 reflects a population

Table 15. Wet, dry or moderate classifications for each calendar year, the dry season months (January – April; November – December), the wet season months (May – October) and the mean salinity, live oyster density, ratio of dead oysters and live oyster shell heights in the LRE NW Fork. Shading indicates wet (blue) or dry (red). Blue bold and red bold font denotes significantly low or high salinity values. Red bold or green bold font denotes significantly low or high biological values. No logger data was available for 2005 and 2006, so statistics were calculated using *FWRI monthly salinity data. Daily salinity data is from the LRD (OY data logger).

Loxahatchee River Estuary NW Fork							
Year	Classification	Dry Season Classification	Wet Season Classification	Salinity	Live Density	Dead Ratio	SH
2005*	Wet			12.6	**122.10**	**0.54**	**43.68**
2006*	Dry		Dry	21.8	263.12	0.15	37.40
2007	Moderate			17.7	252.48	0.38	39.81
2008	Moderate			19.0	**518.04**	**0.11**	38.31
2009	Dry			**22.4**	**678.44**	0.19	38.56
2010	Wet			**15.2**	**697.47**	0.16	40.75
2011	Moderate		Dry	19.8	**606.40**	0.22	**43.02**
2012	Moderate			17.9	321.87	0.27	39.48
2013	Moderate			16.2	**498.04**	0.14	38.60
2014	Moderate			18.0	**582.09**	0.18	35.48
2015	Moderate			20.4	**903.51**	0.13	37.97
2016	Moderate			17.8	**830.89**	0.12	38.92
2017	Moderate			18.7	**912.04**	0.15	36.92
2018	Dry	Extreme		18.4	**581.82**	0.36	**26.09**

Table 16. Wet, dry or moderate classifications for each calendar year, the dry season months (January – April; November – December), the wet season months (May – October) and the mean salinity, live oyster density, ratio of dead oysters and live oyster shell heights in the LRE SW Fork. Shading indicates wet (blue), dry (red), or both extremes (purple). Blue bold and red bold font denotes significantly low or high salinity values. Red bold or green bold font denotes significantly low or high biological values. For those years when logger data was not available, statistics were calculated using *FWRI monthly salinity data. Daily salinity data is from the LRD (72 data logger).

Loxahatchee River Estuary SW Fork							
Year	Classification	Dry Season Classification	Wet Season Classification	Salinity	Live Density	Dead Ratio	SH
2005*	Both	Extreme	Dry	20.5	**29.82**	**0.75**	39.91
2006*	Dry	Extreme	Dry	29.3	**176.58**	0.12	**47.99**
2007*	Dry	Extreme	Dry	22.6	89.23	0.21	**48.03**
2008	Dry	Extreme	Dry	23.9	**224.31**	0.14	38.80
2009	Dry	Extreme	Dry	**29.4**	**212.62**	0.14	38.28
2010	Dry	Extreme	Dry	26.2	**266.22**	0.16	41.82
2011	Dry	Extreme	Dry	**31.8**	**449.56**	0.14	45.88
2012*	Dry	Extreme	Dry	22.0	**207.47**	0.52	42.05
2013*	Both	Extreme	Extreme	21.8	**532.67**	0.12	41.33
2014*	Dry	Extreme	Dry	23.2	**433.51**	0.14	43.41
2015*	Dry	Extreme	Dry	25.9	**271.33**	0.21	43.00
2016*	Dry		Dry	21.1	**267.20**	0.18	42.88
2017*	Dry	Extreme	Dry	23.1	**312.49**	**0.10**	38.98
2018	Dry	Extreme	Dry	**20.5**	**221.64**	0.17	**35.13**

comprised primarily of new recruits that settled after the period of low salinities. In the SW Fork, no oyster die-off occurred after the wet summer months, but there was a large recruitment event that decreased the overall mean for 2018.

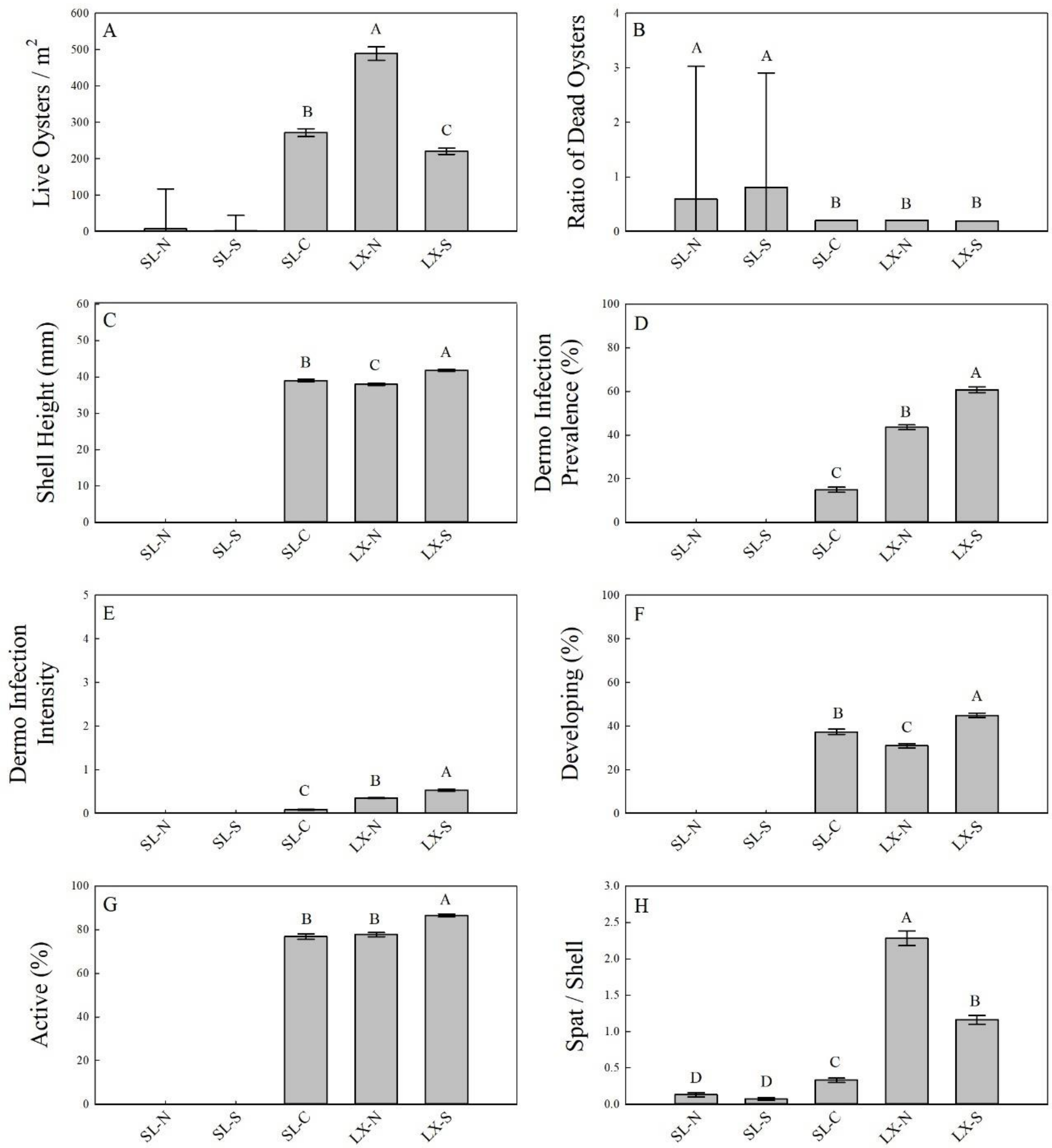

Figure 13. Estimated mean and standard error from overall comparisons of live oyster density (A), the ratio of dead oysters (B), shell height of live oysters (C), number of spat recruits per shell (D), the percentage of oysters infected with *Perkinsus marinus* (E), *Perkinsus marinus* infection intensity in oysters (F), percentage of oysters developing gametes (G) and percentage of oysters undergoing active gametogenesis (H) within the five southeast Florida study sites from 2005 to 2018. Letter designations denote differences in least-squares means between sites for the 14-y study; those with the same letter do not differ significantly ($P > 0.05$).

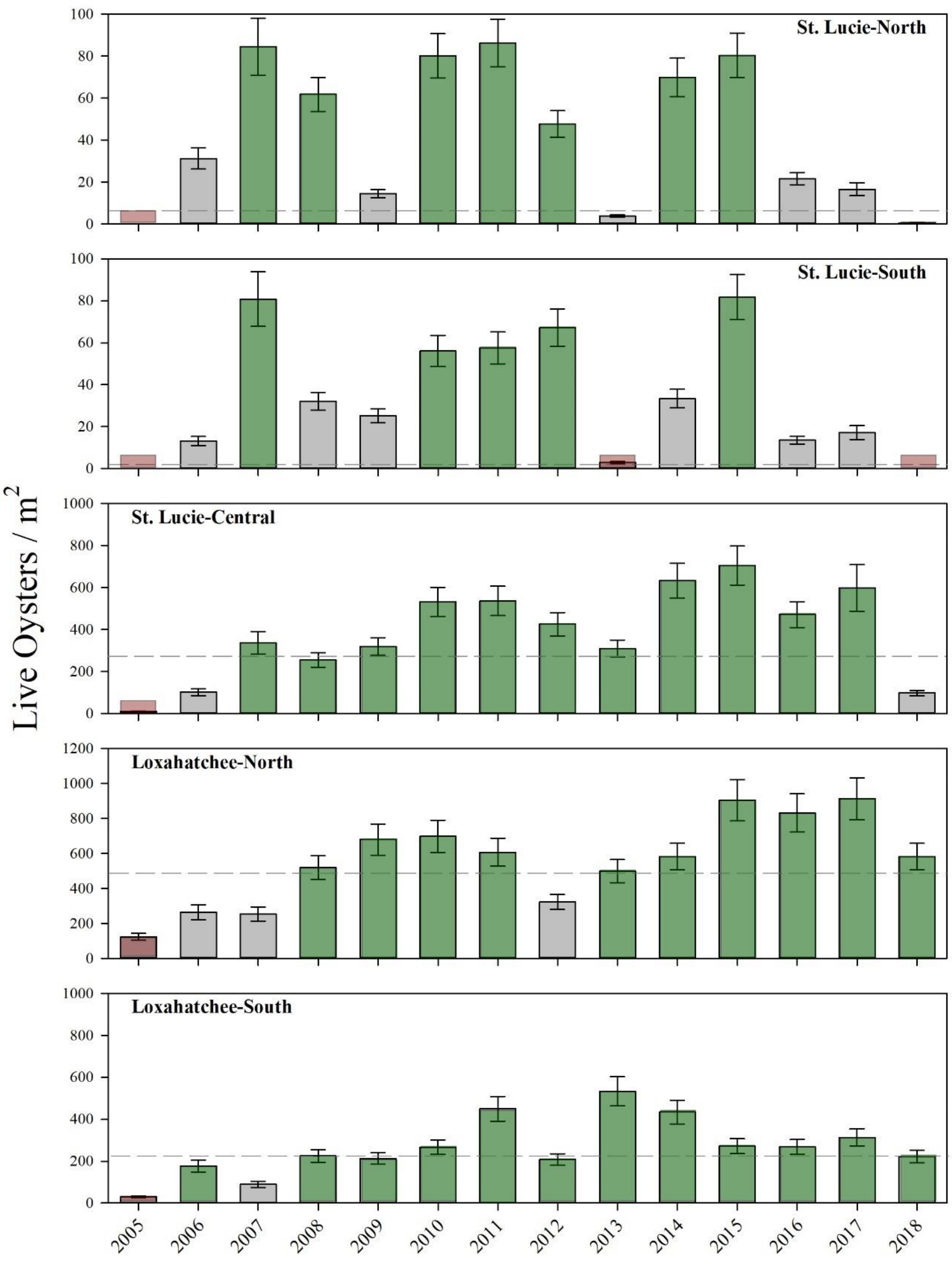

Figure 14. Estimated annual means and standard errors from comparisons of live oyster density within the five southeast Florida study sites from 2005 to 2018. Red-shaded bars indicate years with significantly lower densities and green-shaded bars indicate years with significantly greater densities ($P < 0.05$). The dashed lines represent the overall estimated mean for each site during the 14-y study.

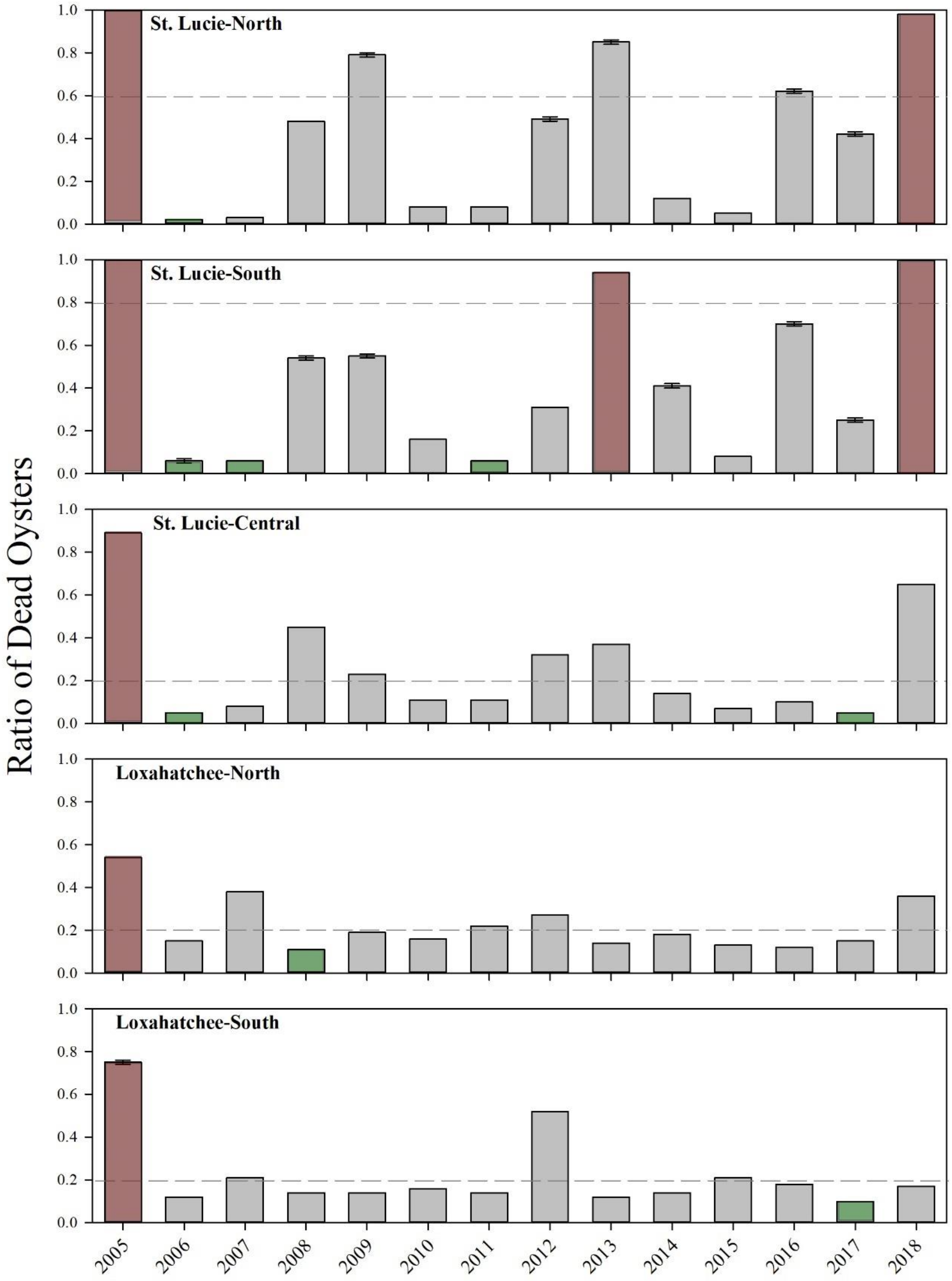

Figure 15. Estimated annual means and standard errors from comparisons of the ratio of dead oysters within the five southeast Florida study sites from 2005 to 2018. Red-shaded bars indicate years with significantly greater ratios and green-shaded bars indicate years with significantly lower ratios ($P < 0.05$). The dashed lines represent the overall estimated mean for each site during the 14-y study.

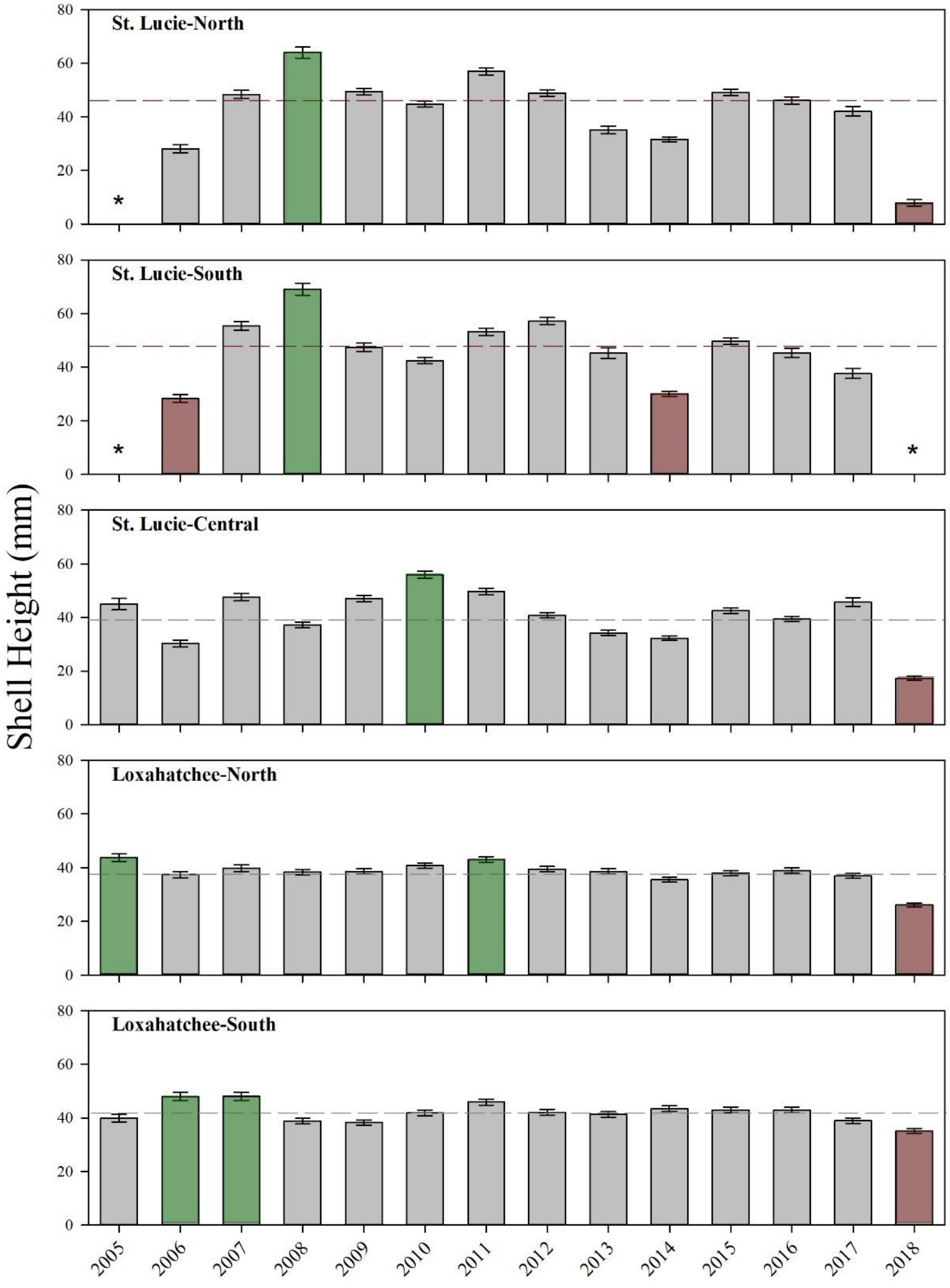

Figure 16. Estimated annual means and standard errors from comparisons of shell height of live oysters within the five southeast Florida study sites from 2005 to 2018. Red-shaded bars indicate years with significantly smaller shell heights and green-shaded bars indicate years with significantly greater shell heights ($P < 0.05$). The gray dashed lines represent the overall estimated mean for each site during the 14-y study; red dashed lines are estimated from raw data. Asterisks indicate years when no live oysters were present for measurement.

Disease

Sampling for *Perkinsus marinus* disease analyses began in March 2005. Due to the frequent occurrence of low salinity events in the SLE, there were several periods when live oysters were not collected and processed for disease analyses (Appendix C; Table 17). In addition, oysters were present but not collected from the LRE sites in October 2005 due to the sampling trip being postponed in order to avoid the effects of Hurricane Wilma.

Table 17. Months following mortality events when live oysters were either not available or too small (< 15 mm shell height) to collect from the SLE and process for disease analyses.

Mortality Event	Site	Timing	Duration
2005	St. Lucie-North	Mar 2005 - Nov 2006	21 months
	St. Lucie-South	Mar 2005 - Nov 2006	21 months
	St. Lucie-Central	Jul 2005 - Apr 2006	10 months
2008	St. Lucie-North	Sep 2008 - Mar 2009	7 months
	St. Lucie-South	Sep 2008 - Mar 2009	7 months
	St. Lucie-Central	Sep 2008 - Jan 2009	5 months
2013	St. Lucie-North	Aug 2013 - Dec 2013	5 months
	St. Lucie-South	Aug 2013 - Dec 2013	5 months
	St. Lucie-Central	Sep 2013 - Dec 2013	4 months
2016	St. Lucie-South	Jul 2016	1 month
2017	St. Lucie-North	Sep 2017 - Nov 2018	15 months
	St. Lucie-South	Sep 2017 - Dec 2018	16 months
	St. Lucie-Central	Sep 2017 - May 2018	9 months
2018	St. Lucie-Central	Jul 2018	1 month

Prevalence of infection by *Perkinsus marinus* (dermo) varied significantly among sites ($F_{4, 1854}$=165.55, $P < 0.01$; Figure 13D). The highest infection rates occurred in oysters from the Loxahatchee-South site where the overall mean was 61%. The overall infection rate was moderate in Loxahatchee-North (44%) and low in St. Lucie-Central (15%). Because there were large amounts of missing data from the St. Lucie-North and South sites due to frequent oyster die-offs, estimated means for those sites were not generated from the statistical model. Mean dermo prevalence calculated from raw data was 19 and 23% in St. Lucie-North and South, respectively.

Dermo prevalence also differed significantly among years at each of the sites ($F_{49, 1854}$=7.44, $P <$ 0.01; Figure 17). This was most pronounced in Loxahatchee-South where annual mean infection rates ranged from 5% in 2005 to approximately 89% in 2014 and 2015. Infection rates varied less in the

Loxahatchee-North site where the lowest prevalence (14%) occurred in 2005 and all remaining years were statistically similar (26 to 74%). In St. Lucie-Central, dermo prevalence was relatively low in most years, ranging from just 2 to 20%; however, prevalence increased significantly in 2011, 2012 and 2013 when rates were 49 to 64%. Annual dermo prevalence was similar among years in both the St. Lucie-North and South sites where rates ranged from approximately 1 to 40%.

Intensity of infection by *P. marinus* followed a pattern similar to that seen with dermo prevalence (Appendix C). The greatest mean overall infection intensities were found in oysters from Loxahatchee-South (0.53; Figure 13E). Loxahatchee-North oysters had slightly lower overall intensities (0.35) and oysters from St. Lucie-Central had intensities that were an order of magnitude lower (0.08). As with prevalence, missing data prevented the statistical model from estimating means for St. Lucie-North and South; however, means calculated from raw data were 0.08 and 0.10 for the north and south forks, respectively.

Intensities varied the most in Loxahatchee-South where annual means ranged from 0.03 in 2005 to approximately 1.25 in 2014 and 2015 (Figure 18). In Loxahatchee-North, the lowest intensity was also measured in 2005 (0.02); the highest levels occurred in 2011, 2012, 2015 and 2016 when intensity ranged from 0.59 to 0.80. Infection intensity levels were similar in most years in oysters from St. Lucie-Central, ranging from 0.01 to 0.22. The highest intensity level in St. Lucie-Central oysters occurred in 2012 (0.81). Infection intensities were similar among years in both the St. Lucie-North and South sites where values ranged from 0 to 0.24. Despite the differences detected among sites and years, most mean infection intensity values were less than 1, indicating that sampled oysters were only lightly infected with the parasite (Table 2).

Results from analyses of salinity, dermo prevalence and dermo infection intensity were compiled for comparison. In the SLE central estuary, dermo prevalence was greatest in 2011, 2012 and 2013. In 2011, mean annual salinity was also among the highest recorded (Table 18). That year was considered a dry year with both the dry season and wet season months also characterized as drier than expected. Higher salinity regimes bring higher predation and disease rates, as was demonstrated by the significant increase in infection prevalence from 2010 to 2011. Salinities were not as high in 2012 but prevalence rates remained high due to the moderate salinity regime that allowed the dermo parasite to persist and, because oysters

Table 18. Wet, dry or moderate classifications for each calendar year, the dry season months (January – April; November – December), the wet season months (May – October) and the mean salinity, *Perkinsus marinus* (dermo) infection prevalence and dermo infection intensity in the SLE middle estuary (St. Lucie-Central study site). Shading indicates wet (blue), dry (red), or both extremes (purple). Blue bold and red bold font denotes significantly low or high salinity values. Red bold or green bold font denotes significantly low or high biological values. Salinity data is from USGS.

St. Lucie Estuary
US1 Roosevelt Bridge

Year	Classification	Dry Season Classification	Wet Season Classification	Salinity	Dermo Prevalence	Dermo Intensity
2005	Wet	Wet	Extreme	**7.3**	**3.33**	**0.02**
2006	Moderate		Dry	17.3	**9.17**	**0.06**
2007	Dry		Dry	18.3	**2.78**	**0.01**
2008	Moderate			15.9	**17.50**	**0.14**
2009	Dry	Extreme		**20.2**	**2.00**	**0.04**
2010	Wet			14.7	**5.00**	**0.02**
2011	Dry	Extreme	Dry	**21.3**	**49.44**	0.47
2012	Moderate			18.2	**63.89**	**0.81**
2013	Wet		Extreme	14.8	**54.17**	0.57
2014	Moderate			17.2	**17.78**	**0.06**
2015	Moderate			16.5	32.22	**0.22**
2016	Wet	Wet	Extreme	**10.0**	**20.00**	**0.10**
2017	Both	Extreme		16.2	**16.67**	**0.08**
2018	Both		Extreme	18.2	**10.00**	**0.03**

were surviving longer, a larger percentage likely acquired the parasite. Although 2013 was considered a wet year, dermo prevalence was still high that year. The wet year classification for 2013 was due to heavy rainfall from June through October and the resultant sub-optimal salinities. However, prior to June, salinities were within or exceeded the optimal range and as a result, dermo prevalence remained high. Dermo prevalence was moderate in 2015, following two years of moderate salinities, indicating that even in optimal salinity conditions, the dermo parasite can be quite prolific.

In the LRE, the lowest dermo prevalence occurred in 2005 in both the NW and SW Forks (Tables 19 and 20). This coincides with a year exhibiting one of the lowest annual salinities and that was classified as wet in the NW Fork and as having both extremes in the SW Fork. The remainder of the years in the NW Fork were similar and exhibited moderate prevalence rates. Most of those years had salinities that were considered moderate which further illustrates the potency of the parasite. In the SW Fork, dermo prevalence was significantly greater in 2014 and 2015, years which were considered dry and were drier than expected during both the wet and dry seasons; however, that characterization of the salinity regime

Table 19. Wet, dry or moderate classifications for each calendar year, the dry season months (January – April; November – December), the wet season months (May – October) and the mean salinity, *Perkinsus marinus* (dermo) infection prevalence and dermo infection intensity in the LRE NW Fork. Shading indicates wet (blue) or dry (red). Blue bold and red bold font denotes significantly low or high salinity values. Red bold or green bold font denotes significantly low or high biological values. No logger data was available for 2005 and 2006, so statistics were calculated using *FWRI monthly salinity data. Daily salinity data is from the LRD (OY data logger).

Loxahatchee River Estuary NW Fork						
Year	Classification	Dry Season Classification	Wet Season Classification	Salinity	Dermo Prevalence	Dermo Intensity
2005*	Wet			12.6	**14.18**	**0.06**
2006*	Dry		Dry	21.8	**26.26**	0.20
2007	Moderate			17.7	**28.33**	0.25
2008	Moderate			19.0	**40.56**	0.28
2009	Dry			**22.4**	**43.89**	0.38
2010	Wet			**15.2**	**28.33**	0.31
2011	Moderate		Dry	19.8	**68.33**	**0.73**
2012	Moderate			17.9	**74.44**	**0.80**
2013	Moderate			16.2	**50.56**	0.38
2014	Moderate			18.0	**47.22**	0.41
2015	Moderate			20.4	**53.89**	**0.59**
2016	Moderate			17.8	**59.44**	**0.62**
2017	Moderate			18.7	**49.44**	0.40
2018	Dry	Extreme		18.4	**35.56**	0.31

Table 20. Wet, dry or moderate classifications for each calendar year, the dry season months (January – April; November – December), the wet season months (May – October) and the mean salinity, *Perkinsus marinus* (dermo) infection prevalence and dermo infection intensity in the LRE SW Fork. Shading indicates wet (blue), dry (red), or both extremes (purple). Blue bold and red bold font denotes significantly low or high salinity values. Red bold or green bold font denotes significantly low or high biological values. For those years when logger data was not available, statistics were calculated using *FWRI monthly salinity data. Daily salinity data is from the LRD (72 data logger).

Loxahatchee River Estuary SW Fork						
Year	Classification	Dry Season Classification	Wet Season Classification	Salinity	Dermo Prevalence	Dermo Intensity
2005*	Both	Extreme	Dry	20.5	**4.62**	**0.03**
2006*	Dry	Extreme	Dry	29.3	22.86	0.22
2007*	Dry	Extreme	Dry	22.6	46.37	0.38
2008	Dry	Extreme	Dry	23.9	55.56	0.50
2009	Dry	Extreme	Dry	**29.4**	61.67	0.68
2010	Dry	Extreme	Dry	26.2	67.22	0.67
2011	Dry	Extreme	Dry	**31.8**	80.56	0.92
2012*	Dry	Extreme	Dry	22.0	77.22	0.94
2013*	Both	Extreme	Extreme	21.8	49.44	0.49
2014*	Dry	Extreme	Dry	23.2	**89.44**	**1.25**
2015*	Dry	Extreme	Dry	25.9	**88.33**	**1.20**
2016*	Dry		Dry	21.1	78.89	0.94
2017*	Dry	Extreme	Dry	23.1	66.67	0.71
2018	Dry	Extreme	Dry	**20.5**	57.78	0.64

was not unique and was, in fact, applicable to most years in the SW Fork. Unfortunately, there was limited salinity data available for analysis since a continuous data logger was only deployed for a few years of the study. It is likely that more detailed salinity data would reveal a cause for the higher disease incidence during those years.

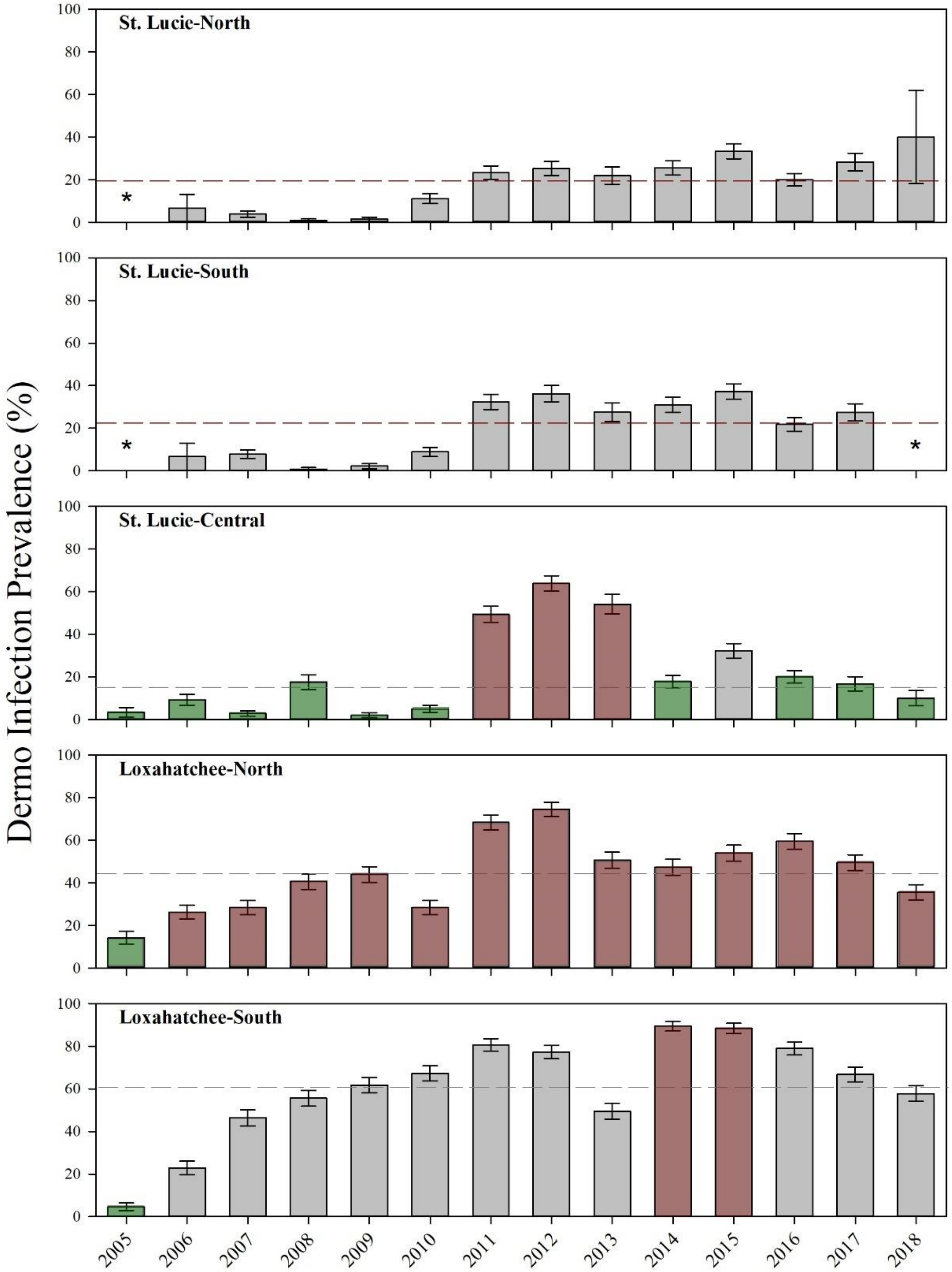

Figure 17. Estimated annual means and standard errors from comparisons of the percentage of oysters infected with *Perkinsus marinus* within the five southeast Florida study sites from 2005 to 2018. Red-shaded bars indicate years with significantly higher intensities and green-shaded bars indicate years with significantly lower intensities ($P < 0.05$). The gray dashed lines represent the overall estimated mean for each site during the 14-y study; red dashed lines are estimated from raw data. The asterisks indicate years when means could not be estimated by the model.

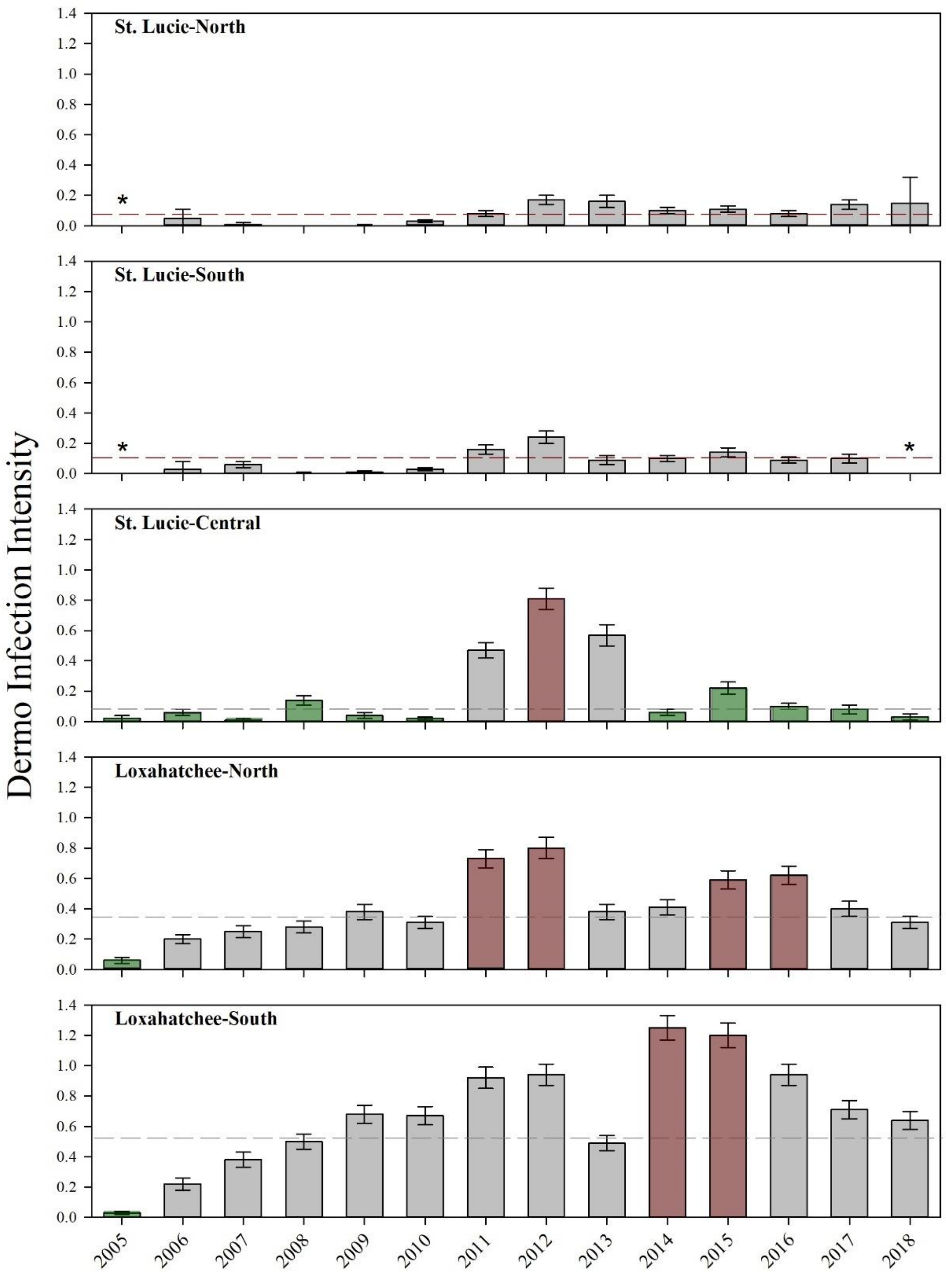

Figure 18. Estimated annual means and standard errors from comparisons of *Perkinsus marinus* infection intensity in oysters within the five southeast Florida study sites from 2005 to 2018. Red-shaded bars indicate years with significantly higher intensities and green-shaded bars indicate years with significantly lower intensities ($P < 0.05$). The gray dashed lines represent the overall estimated mean for each site during the 14-y study; red dashed lines are estimated from raw data. The asterisks indicate years when means could not be estimated by the model.

Reproductive Development and Juvenile Recruitment

Sampling for reproductive analyses began in February 2005. Due to the frequent occurrence of low salinity events in the SLE, there were several periods when live oysters were not collected and processed for analysis (Table 21). In addition, oysters were present but not collected from the LRE sites in October 2005 due to the sampling trip being postponed in order to avoid the effects of Hurricane Wilma. Oysters were collected from Loxahatchee-South in September 2006 and from St. Lucie-Central in October 2006, but the samples were destroyed during histological preparation.

Table 21. Months following mortality events when live oysters were either not available or too small (< 15 mm shell height) to collect from the SLE and process for reproductive analysis.

Mortality Event	Site	Timing	Duration
2005	St. Lucie-North	Feb 2005 - Nov 2006	22 months
	St. Lucie-South	Feb 2005 - Nov 2006	22 months
	St. Lucie-Central	Jul 2005 - Apr 2006	10 months
2008	St. Lucie-North	Sep 2008 - Mar 2009	7 months
	St. Lucie-South	Sep 2008 - Mar 2009	7 months
	St. Lucie-Central	Sep 2008 - Jan 2009	5 months
2013	St. Lucie-North	Aug 2013 - Dec 2013	5 months
	St. Lucie-South	Aug 2013 - Feb 2014	7 months
	St. Lucie-Central	Sep 2013 - Dec 2013	4 months
2016	St. Lucie-South	Jul 2016	1 month
2017	St. Lucie-North	Sep 2017 - Nov 2018	15 months
	St. Lucie-South	Sep 2017 - Dec 2018	16 months
	St. Lucie-Central	Sep 2017 - May 2018	9 months
2018	St. Lucie-Central	Jul 2018	1 month

Although the patterns and timing of reproductive development varied among sites and years, gametogenesis and spawning typically occurred between March and October (Appendix D). In most years, the majority of oysters entered the resting, or indifferent, stage in November or December and remained inactive through February. A comparison of percentages of oysters in the gonadal development stage found differences among sites ($F_{4, 633}$=23.53, P < 0.01; Figure 13F). The greatest number of oysters developing gametes was found in Loxahatchee-South where the overall mean was 45%. The overall percentage was intermediate in St. Lucie-Central (37%) and Loxahatchee-North (31%). Because there were large amounts of missing data from the St. Lucie-North and South sites due to frequent oyster die-offs, estimated means for those sites were not generated from the statistical model. Mean percentages of oysters in the developing stage were calculated from raw data and were approximately 40% for both sites.

The percentage of oysters developing gametes also differed significantly among years at some of the sites ($F_{49, 633}$=3.87, $P < 0.01$; Figure 19). Percentages varied most widely in oysters from St. Lucie-North; however, most years had means that were statistically similar and ranged from 0 to 50%. Two years had statistically greater percentages, 2013 and 2017, when means were 74 and 70%, respectively. The pattern was similar in St. Lucie-South with greatest percentages (76 and 80%) occurring in 2013 and 2017 and the lowest percentages (7 to 47%) occurring during the remainder of years. In Loxahatchee-North, the lowest number of oysters developing gametes was measured in 2007 and 2011 when percentages were 10% and 17%, respectively; the greatest percentages were recorded in 2013 (48%) and 2018 (57%). No statistical differences among years were detected for oysters from the Loxahatchee-South (31 to 62%) and St. Lucie-Central (16 to 64%) sites.

Additional comparison of the percentages of oysters in any of the three active reproductive stages (developing, ripe/spawning and spent/recycling) revealed that the greatest percentage occurred in oysters from Loxahatchee-South where the mean was 86% ($F_{4, 633}$=18.22, $P < 0.01$; Figure 13G). The remaining sites had similar percentages, ranging from 77 to 79%; however, percentages for St. Lucie-North and South were not generated by the model and were instead calculated from raw data. Percentages of reproductively active oysters differed among years in the St. Lucie-North and South sites, but were similar at the remaining sites ($F_{49, 633}$=2.58, $P < 0.01$; Figure 20). In St. Lucie-North and South, significantly greater numbers of oysters (97 to 100%) were reproductively active in 2013 and 2017. The lowest numbers of reproductively active oysters in St. Lucie-North were recorded in 2006, 2007, 2011 and 2016 (0 to 67%). In St. Lucie-South, the lowest numbers occurred in 2006 (13%).

Juvenile recruitment exhibited seasonal patterns similar to those seen with reproductively development (Appendix D). Oyster spat were typically detected on arrays retrieved in the spring, summer and fall. Spring recruits were most commonly first detected in April at the LRE sites and in May at the SLE sites. After initiation of recruitment in the spring, juvenile recruits were often present on arrays collected every month through December. In the three SLE sites, recruitment was often sporadic and inconsistent, and often was not detected until much later in the season, especially in years following an oyster die-off. The LRE sites often exhibited a bimodal recruitment pattern, with peaks occurring in the spring (April, May, June or July) and fall (September, October or November). Occasionally, in each of the sites, low rates of recruitment continued through December and into January or February of the following year.

Recruitment rates were greatest in Loxahatchee-North, where the mean for the duration of the study was 2.28 spat/shell/month ($F_{4, 7075}$=168.64, P < 0.01; Figure 13H). The lowest recruitment rates, which were an order of magnitude lower than LRE, were recorded in the three SLE sites ($\leq$ 0.33 spat/shell/month). Mean recruitment rates in Loxahatchee-South were moderate (1.16 spat/shell/month). The maximum rate for a single sample was 58.94 spat/shell/month, retrieved from Loxahatchee-North Station 1 in April 2012. Recruitment rates were similar among years at most sites but did differ significantly in St. Lucie-North and Loxahatchee-South ($F_{4, 7075}$=9.13, P < 0.01; Figure 21). In St. Lucie-North, recruitment was greatest in 2011 when the mean was 1.50 spat/shell/month. Rates ranged from 0 to 0.43 spat/shell/month and did not differ significantly during the remaining years. In Loxahatchee-South, recruitment in 2007 was lower (0.19 spat/shell/month) than recorded in all other years (0.43 to 3.81 spat/shell/month).

Results from analyses of salinity, reproductive development and juvenile recruitment were compiled for comparison. No statistical differences in the percent of oysters developing gametes, the percent of oysters actively undergoing gametogenesis, or the number of spat/shell/month were detected in samples from the SLE middle estuary (Table 22). It is interesting to note that some of the highest annual means for percent developing and percent active occurred in years classified as wet or exhibiting both extremes (2005, 2013, 2018). One possible explanation for this is that the lower salinities were inhibiting spat settlement therefore most oysters collected for analysis were older and therefore more reproductively active. One of the highest recruitment rates in the middle estuary was recorded in 2016 during a year classified as wet with wetter than expected dry and wet seasons. This result is counter to expected in that lower salinities often kill oyster larvae or the high flow rates simply flush the larvae downstream. However, that high annual mean is due to a large recruitment event captured by arrays deployed in May and retrieved in June, which coincided with a brief reduction in flow and a subsequent increase in salinity

Table 22. Wet, dry or moderate classifications for each calendar year, the dry season months (January – April; November – December), the wet season months (May – October) and the mean salinity, percent of oysters developing gametes, percent of oysters undergoing active gametogenesis and number of spat recruits per shell in the SLE middle estuary (St. Lucie-Central study site). Shading indicates wet (blue), dry (red), or both extremes (purple). Blue bold and red bold font denotes significantly low or high salinity values. Red bold or green bold font denotes significantly low or high biological values. Salinity data is from USGS.

St. Lucie Estuary
US1 Roosevelt Bridge

Year	Classification	Dry Season Classification	Wet Season Classification	Salinity	% Developing	% Active	Spat / Shell
2005	Wet	Wet	Extreme	**7.3**	61.64	93.15	0.02
2006	Moderate		Dry	17.3	58.24	80.22	0.64
2007	Dry		Dry	18.3	25.29	58.24	0.49
2008	Moderate			15.9	15.65	67.83	0.11
2009	Dry	Extreme		**20.2**	43.12	79.38	0.20
2010	Wet			14.7	23.56	59.77	0.09
2011	Dry	Extreme	Dry	**21.3**	28.00	65.71	0.47
2012	Moderate			18.2	19.77	73.45	0.81
2013	Wet		Extreme	14.8	47.86	89.74	0.29
2014	Moderate			17.2	33.52	76.70	0.77
2015	Moderate			16.5	29.41	73.53	1.01
2016	Wet	Wet	Extreme	**10.0**	32.76	63.22	1.20
2017	Both	Extreme		16.2	57.26	82.91	0.16
2018	Both		Extreme	18.2	63.77	85.51	0.99

(Appendix D). The mean recruitment rate recorded that month ranged from 9 to 14 spat/shell at the three St. Lucie-Central stations; in all other months of that year, rates ranged from 0 to 1 spat/shell.

In the LRE NW Fork, there were statistical differences in the number of oysters developing gametes (Table 23). The greatest percentages occurred in 2013, which was a moderate year, and in 2018 which was a dry year with an extreme dry season. In 2013, salinity conditions were optimal so it's not surprising a larger percentage of oysters were successfully developing gametes. In 2018, conditions were less optimal, so the high number may instead reflect high productivity by the large recruitment class that settled in 2017 (Appendix D). The lowest numbers of developing oysters were recorded in 2007 and 2011, both of which were considered moderate years. In 2007, predation rates may have been elevated following 2006, which was a dry year with a dry wet season, thus causing a decline in the number of developing oysters. In 2011, very few sampled oysters were classified as developing gametes; most were collected in February and March with only one other collected in August. In the SW Fork, the only statistical difference occurred in 2007 when the number of spat/shell/month was significantly lower than other years

(Table 24). Recruitment rates were depressed at all three SW Fork stations that year but there is no apparent cause for the decline.

Table 23. Wet, dry or moderate classifications for each calendar year, the dry season months (January – April; November – December), the wet season months (May – October) and the mean salinity, percent of oysters developing gametes, percent of oysters undergoing active gametogenesis and number of spat recruits per shell in the LRE NW Fork. Shading indicates wet (blue) or dry (red). Blue bold and red bold font denotes significantly low or high salinity values. Red bold or green bold font denotes significantly low or high biological values. No logger data was available for 2005 and 2006, so statistics were calculated using *FWRI monthly salinity data. Daily salinity data is from the LRD (OY data logger).

Loxahatchee River Estuary NW Fork							
Year	Classification	Dry Season Classification	Wet Season Classification	Salinity	% Developing	% Active	Spat / Shell
2005*	Wet			12.6	34.46	90.54	1.35
2006*	Dry		Dry	21.8	27.27	81.17	2.97
2007	Moderate			17.7	**10.11**	68.54	0.84
2008	Moderate			19.0	28.49	75.98	1.99
2009	Dry			**22.4**	33.90	73.45	1.13
2010	Wet			**15.2**	31.82	68.75	1.29
2011	Moderate		Dry	19.8	**17.22**	70.00	1.37
2012	Moderate			17.9	32.39	80.11	3.42
2013	Moderate			16.2	**48.31**	88.20	2.13
2014	Moderate			18.0	35.59	78.53	2.76
2015	Moderate			20.4	27.27	78.98	4.52
2016	Moderate			17.8	32.58	64.61	2.58
2017	Moderate			18.7	33.14	72.57	6.64
2018	Dry	Extreme		18.4	**56.74**	82.58	4.76

Table 24. Wet, dry or moderate classifications for each calendar year, the dry season months (January – April; November – December), the wet season months (May – October) and the mean salinity, percent of oysters developing gametes, percent of oysters undergoing active gametogenesis and number of spat recruits per shell in the LRE SW Fork. Shading indicates wet (blue), dry (red), or both extremes (purple). Blue bold and red bold font denotes significantly low or high salinity values. Red bold or green bold font denotes significantly low or high biological values. For those years when logger data was not available, statistics were calculated using *FWRI monthly salinity data. Daily salinity data is from the LRD (72 data logger).

Loxahatchee River Estuary SW Fork							
Year	Classification	Dry Season Classification	Wet Season Classification	Salinity	% Developing	% Active	Spat / Shell
2005*	Both	Extreme	Dry	20.5	47.30	93.92	0.43
2006*	Dry	Extreme	Dry	29.3	41.48	88.15	1.08
2007*	Dry	Extreme	Dry	22.6	31.28	84.36	**0.19**
2008	Dry	Extreme	Dry	23.9	38.37	80.81	1.48
2009	Dry	Extreme	Dry	**29.4**	48.86	87.50	0.89
2010	Dry	Extreme	Dry	26.2	44.00	81.71	1.14
2011	Dry	Extreme	Dry	**31.8**	36.72	80.79	0.55
2012*	Dry	Extreme	Dry	22.0	36.52	85.96	2.46
2013*	Both	Extreme	Extreme	21.8	55.06	89.89	1.70
2014*	Dry	Extreme	Dry	23.2	46.67	86.11	1.26
2015*	Dry	Extreme	Dry	25.9	40.22	87.15	2.59
2016*	Dry		Dry	21.1	43.82	80.34	0.92
2017*	Dry	Extreme	Dry	23.1	57.39	90.91	2.35
2018	Dry	Extreme	Dry	**20.5**	61.93	85.80	3.81

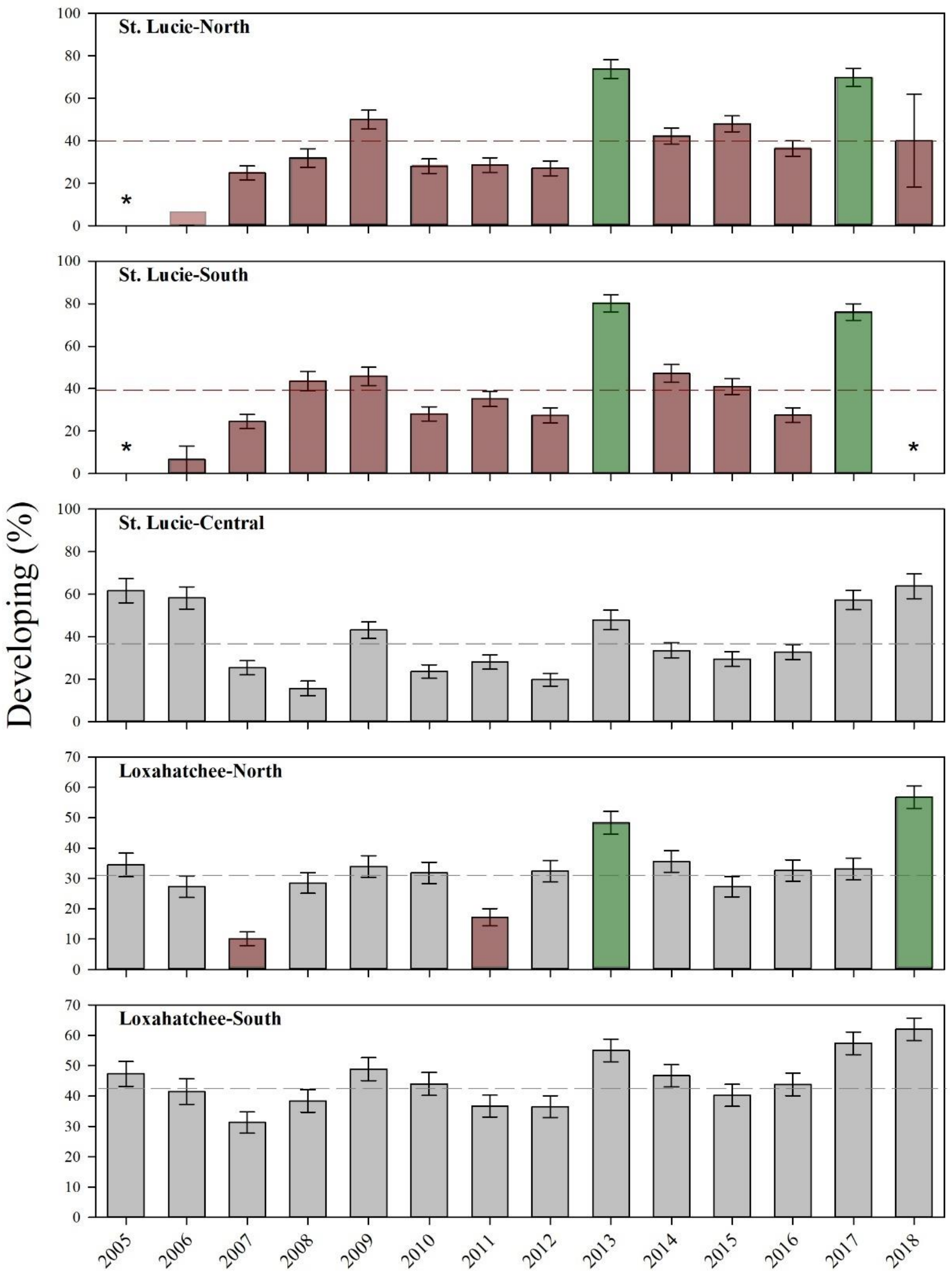

Figure 19. Estimated annual means and standard errors from comparisons of the percent of oysters developing gametes within the five southeast Florida study sites from 2005 to 2018. Red-shaded bars indicate years with significantly fewer developing oysters and green-shaded bars indicate years with significantly more developing oysters ($P < 0.05$). The gray dashed lines represent the overall estimated mean for each site during the 14-y study; red dashed lines are means estimated from raw data. The asterisks indicate years when means could not be estimated by the model.

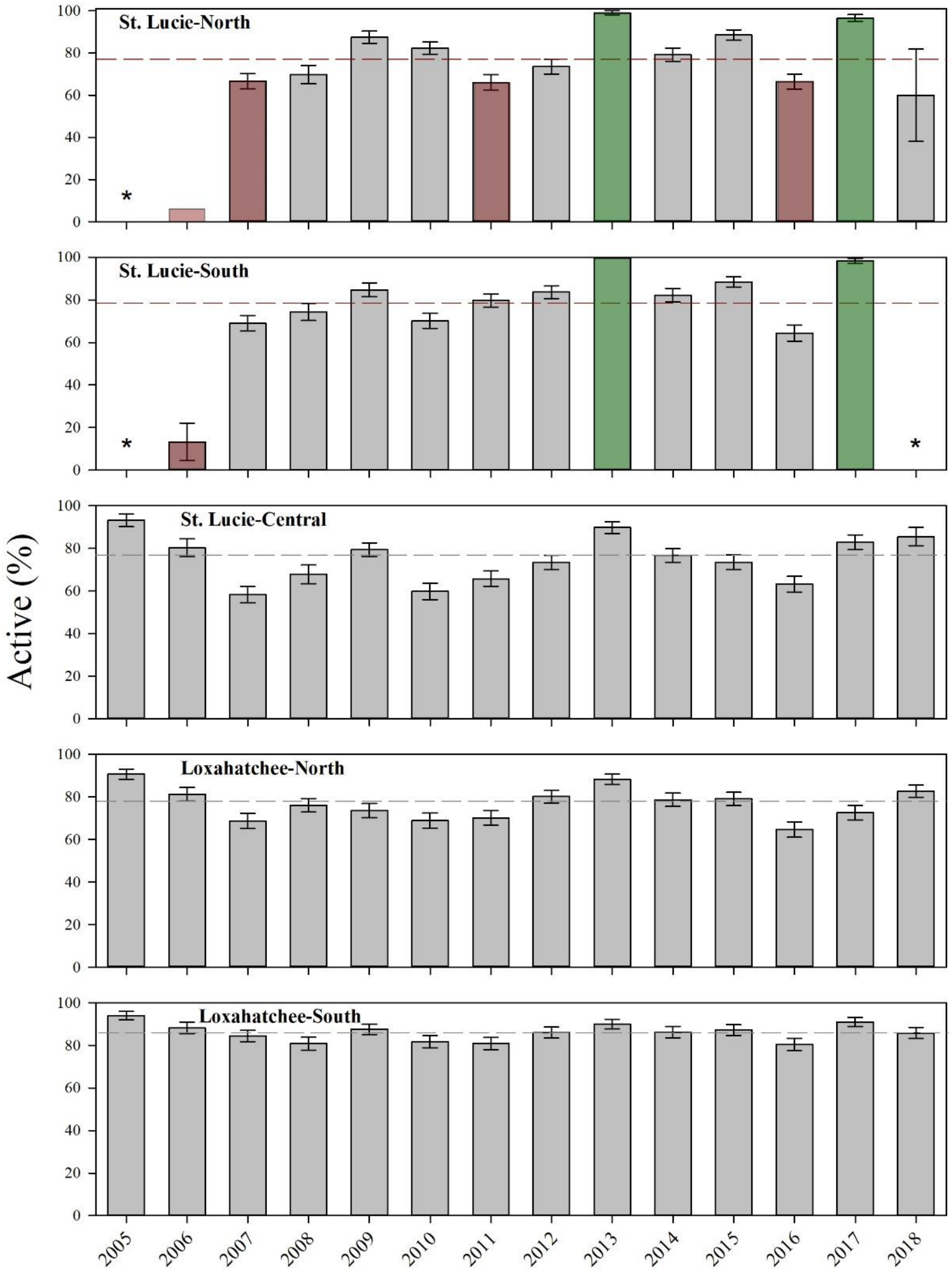

Figure 20. Estimated annual means and standard errors from comparisons of the percent of oysters undergoing active gametogenesis within the five southeast Florida study sites from 2005 to 2018. Red-shaded bars indicate years with significantly fewer active oysters and green-shaded bars indicate years with significantly more active oysters ($P < 0.05$). The gray dashed lines represent the overall estimated mean for each site during the 14-y study; red dashed lines are means estimated from raw data. The asterisks indicate years when means could not be estimated by the model.

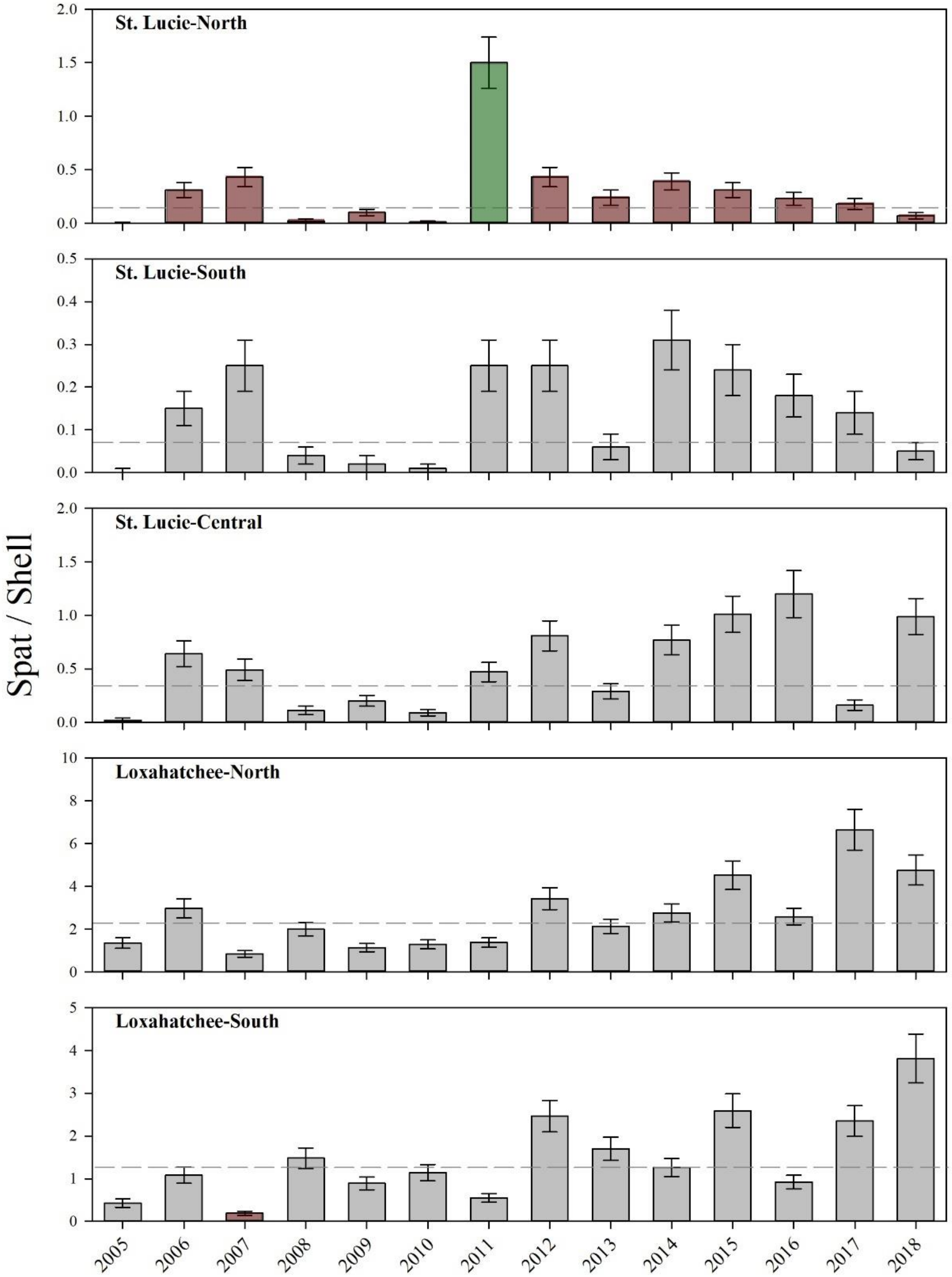

Figure 21. Estimated annual means and standard errors from comparisons of the number of spat recruits per shell within the five southeast Florida study sites from 2005 to 2018. Red-shaded bars indicate years with significantly lower rates and green-shaded bars indicate years with significantly higher rates ($P < 0.05$). The gray dashed lines represent the overall estimated mean for each site during the 14-y study.

Juvenile Growth and Predation

2005 – 2007

The 2005 juvenile growth study began in September 2005 when the single, cultchless oysters produced by Harbor Branch Oceanographic Institute (HBOI) were transported and planted into open and closed cages in the LRE; juveniles were planted into open and closed cages in the SLE in November 2005. Mean shell height (SH) at planting was approximately 20 mm in all sites. Juveniles planted into cages in the SLE sites were all dead when sampled in December, most likely due to persistent low salinities in the estuary. Juveniles planted into open cages in the LRE were also all dead by December 2005, possibly due to heavy predation and/or being washed out of the cages by water currents. Remaining juveniles in the LRE sites were monitored for 10 months and reached mean SHs of 50 and 72 mm, respectively (Appendix E). Mean overall growth rates differed among sites, with 2.99 mm/month in Loxahatchee-North and 5.11 mm/month in Loxahatchee-South.

The 2006 juvenile growth study began in May 2006 when spat settled on axenic shell were transported from HBOI and planted into open and closed cages in the LRE sites; spat were planted into open and closed cages in St. Lucie-Central and into closed cages in St. Lucie-North and South in July 2006. Mean SH at planting was approximately 3.5 mm in the LRE sites and 2 mm in the SLE sites. Spat planted into open cages in the two LRE sites were all dead by the first sampling date in July 2006. Juveniles in the LRE site were monitored for 12 months and reached mean sizes ranging from 36 to 46 mm. Juveniles in St. Lucie-Central were monitored for 11 months with mean SHs reaching 43 mm in the closed cages and 58 mm in the open cages. Juveniles in St. Lucie-North and South were monitored for 10 and 11 months, respectively; mean SH reached 46 mm in St. Lucie-North and 92 mm in St. Lucie-South. At all sites, mean SH decreased at some point during the experiment, suggesting that either larger oysters were dying or new, smaller recruits were settling on the shell substrate. Mean overall growth rates differed substantially among sites, ranging from approximate 2.99 mm/month in Loxahatchee-North to 8.17 mm/month in St. Lucie-South. Growth rates were similar between closed and open cages in St. Lucie-Central, with means of 3.71 and 5.05 mm/month, respectively.

The 2007 juvenile growth study began in July 2007 when spat settled on axenic shells were transported from HBOI and planted into open and closed cages in the LRE and axenic shells for wild spat settlement were planted into open and closed cages in the SLE sites. Mean SH at planting was approximately 13.5 mm in the LRE sites. Juveniles in the LRE were monitored up to 11 months and reached larger mean SHs in closed cages (33 to 57 mm) than in open cages (26 to 42 mm). Juvenile recruits first appeared on shell substrate planted in the SLE sites in July 2007. SLE juveniles were monitored for 5 to 7 months and reached a mean SH of approximately 40 mm in both open and closed cages in the St. Lucie-North and South sites. Juveniles in open and closed cages in St. Lucie-Central were substantially smaller, reaching just 24 mm. As with the 2006 experiment, mean SH decreased at some point at most sites as a result of larger oysters dying or smaller recruits settling on the shell substrate. Growth rates differed among sites, with the highest occurring in Loxahatchee-South, St. Lucie-North and St. Lucie-South where the means ranged from 3.18 to 7.47 mm/month. At the remaining sites, mean rates ranged from 1.42 to 2.14 mm/month. Growth rates were similar between open and closed cages within a site.

<u>2008 – 2010</u>

The 2008 juvenile growth study began in March 2008 when axenic shells for wild spat settlement were planted into open growth arrays at each site. Juvenile spat were first detected on shells in Loxahatchee-South in April, in Loxahatchee-North and St. Lucie-Central in May, in St. Lucie-South in June, and in St. Lucie-North in July. However, survival of juveniles in the SLE sites was short-lived, as most were dead by September following Tropical Storm Fay and the resultant low estuarine salinities. Salinities increased quickly after the storm and juvenile recruits from the last pulse of the spawning season settled onto the SLE growth arrays. Juveniles at all sites were monitored through February 2009. Final SHs in the Loxahatchee-North and South sites reached means of 31 and 42 mm, respectively (Appendix E). Final mean SHs in the SLE sites reach 28 to 32 mm in February, after only 4 or 5 months of growth. Growth rates were moderate in the two LRE sites (~3 mm/month) and greatest in the SLE sites (~5.5 mm/month).

The 2009 juvenile growth study began in March 2009 when axenic shells for wild spat settlement were planted into open growth arrays in each site. Juvenile spat were first detected on shells in Loxahatchee-South in April, in Loxahatchee-North in May and in the three SLE sites in June. Mean SH increased steadily at all sites but decreased substantially after the summer months, especially in the SLE sites, due to a recruitment pulse that brought numerous small recruits. Juveniles at all sites were monitored through March 2010. Final SHs ranged from 27 mm in Loxahatchee-North to 33 mm in Loxahatchee-South. Mean overall growth rates were similar among sites, ranging from 1.38 to 2.51 mm/month.

The 2010 juvenile growth study began in March 2010 when axenic shell for wild spat settlement were planted into open growth arrays in each site. Juvenile spat were first detected on shells in the two LRE sites in May, in St. Lucie-North and Central in July and in St. Lucie-South in August. Juveniles at all sites were monitored through March 2011. Final SHs in Loxahatchee-North and St. Lucie-Central were smaller (31 to 32 mm) than those at the other sites where means ranged from 42 to 49 mm. Despite the differences in final SH, overall growth rates were similar among sites, ranging from 0.35 to 1.38 mm/month.

<u>2011 – 2013</u>

The 2011 juvenile growth study began in February 2011 when wild oyster spat were tagged, measured and planted into open and closed cages in each site. Mean SH at planting was approximately 29 mm in the LRE sites and 43 mm in the SLE sites. Tagged juveniles were monitored through May or June 2012. Final mean SHs were smallest in the St. Lucie-Central open cages (41 mm). The largest final mean SHs were measured in St. Lucie-North (63 to 65 mm), St. Lucie-South (61 to 66 mm) and Loxahatchee-South (56 to 66 mm). Mean overall growth rates were relatively low among sites and cages, ranging from < 1 mm/month in St. Lucie-Central to approximately 2 mm/month at the remaining sites. Rates of survivorship differed among sites (Appendix E). Survivorship was greatest in closed cages where 10 to 18% remained alive in June 2012. Less than 5% of the tagged oysters remained alive by June 2012 in the open cages, with the only exception occurring in St. Lucie-North where 14% remained alive.

The 2012 juvenile growth study began in July 2012 when wild oyster spat were tagged, measured and planted into open and closed cages in each site. Mean SH at planting was approximately 18 mm in the two LRE sites and 15 mm in the SLE sites. Most of the tagged oysters were dead by July 2013, but a few persisted in the Loxahatchee-North cages through December 2013. Final mean SHs were similar among most sites and cages, ranging from 46 to 61 mm. Exceptions occurred in the Loxahatchee-North open cage, where one oyster reached 70 mm, and in the Loxahatchee-South closed cages, where two oysters reached a mean SH of 101 mm. Mean overall growth rates were similar among open cages (~3 mm/month), except in the Loxahatchee-South cages where the mean rate was 7 mm/month. Mean overall growth rates were also near 3 mm/month in the open cages, except in St. Lucie-North and South where the mean rates were near 5 mm/month. Survivorship decreased rapidly in the Loxahatchee-South, St. Lucie-North and St. Lucie-South cages, falling to less than 8% by October 2012. Survivorship was greater in the St. Lucie-Central closed and open cages where 13 and 2%, respectively, remained alive before the cages were lost in August 2013. A few oysters remained alive in the Loxahatchee-North cages through December 2013.

The 2013 juvenile growth study began in December 2013 when wild oyster spat were tagged, measured and planted into open and closed cages in each site. Mean SH at planting was approximately 18 mm in the LRE sites and 22 mm in the SLE sites. The tagged oysters planted in three cages were lost within the first months of the study; two of those cages (St. Lucie-Central Station 1 and Loxahatchee-South Station 2 open cages) were flipped upside down between sampling trips thus spilling all tagged oysters and one cage (St. Lucie-Central Station 1 closed cage) was completely missing upon arrival in February 2014. Final mean SHs were smallest in the Loxahatchee-North (33 mm) and St. Lucie-South (46 mm) open cages. At the remaining sites, the final mean SHs ranged from 54 to 70 mm. Mean overall growth rates were similar among the LRE sites and cages, ranging from 2.21 to 3.00 mm/month. In the St. Lucie-North and South sites, growth rates were lower in the closed cages (~3.75 mm/month) than in the open cages (~5.75 mm/month). Survivorship in the Loxahatchee-North, St. Lucie-North and St. Lucie-South open cages was lowest, with all tagged oysters at those sites dead by July 2014. By the end of the study in December 2014, survivorship at the remaining sites ranged from < 1 to 10%.

<u>2015 – 2018</u>

Measures of mortality and shell height were initiated in January 2015 when wild oysters were collected, measured and planted in open cages at St. Lucie-Central Station 1 and Loxahatchee-North Station 2 (Appendix E). The experiment was repeated monthly through December 2018; however, no live oysters were available from September 2017 through May 2018 at the SLE station. Additionally, no mortality or shell height measures were recorded from the SLE cages in July 2016 due to poor water quality conditions.

Mortality rates were similar between sites with overall means of 47% for SLE and 49% for LRE ($F_{1, 244}$=1.97, P = 0.16); however, there were significant differences in mortality among years within each site ($F_{3, 244}$=129.02, P < 0.01; Figure 22). In SLE, the highest mortality rate was recorded in 2015 when the annual mean was 74%. The lowest mortality rate in the SLE was 26% in 2017 when mortality rates from January through August ranged from approximately 3 to 43%; no live oysters were present for the experiment for the remainder of the year. In the LRE, the highest mortality rate (59%) was recorded in 2015 and the lowest rates were measured in 2015 and 2017 (40 to 44%).

Mean SHs of oysters deployed in cages were larger in the LRE than in the SLE (48 vs. 42 mm; $F_{1, 76}$=13.28, P < 0.01). In the LRE, there were no significant differences in deployed SHs among years; annual means ranged from 45 to 53 mm. In the SLE, deployed SHs were smallest in 2018 when the mean was 24 mm ($F_{3, 76}$=20.23, P = 0.01; Figure 22). Deployed SHs in the SLE ranged from 49 to 53 mm in other years. Mean SHs of retrieved oysters were also larger in the LRE than the SLE (50 vs. 45 mm; $F_{1, 76}$=7.74, P = 0.01). As with deployed SHs, there were no differences in retrieved SHs among years in the LRE but retrieved SHs in the SLE were smaller in 2018 ($F_{3, 76}$=9.89, P = 0.01; Figure 22). Retrieved SHs from the SLE in 2018 were 26 mm but ranged from 50 to 56 mm the other years.

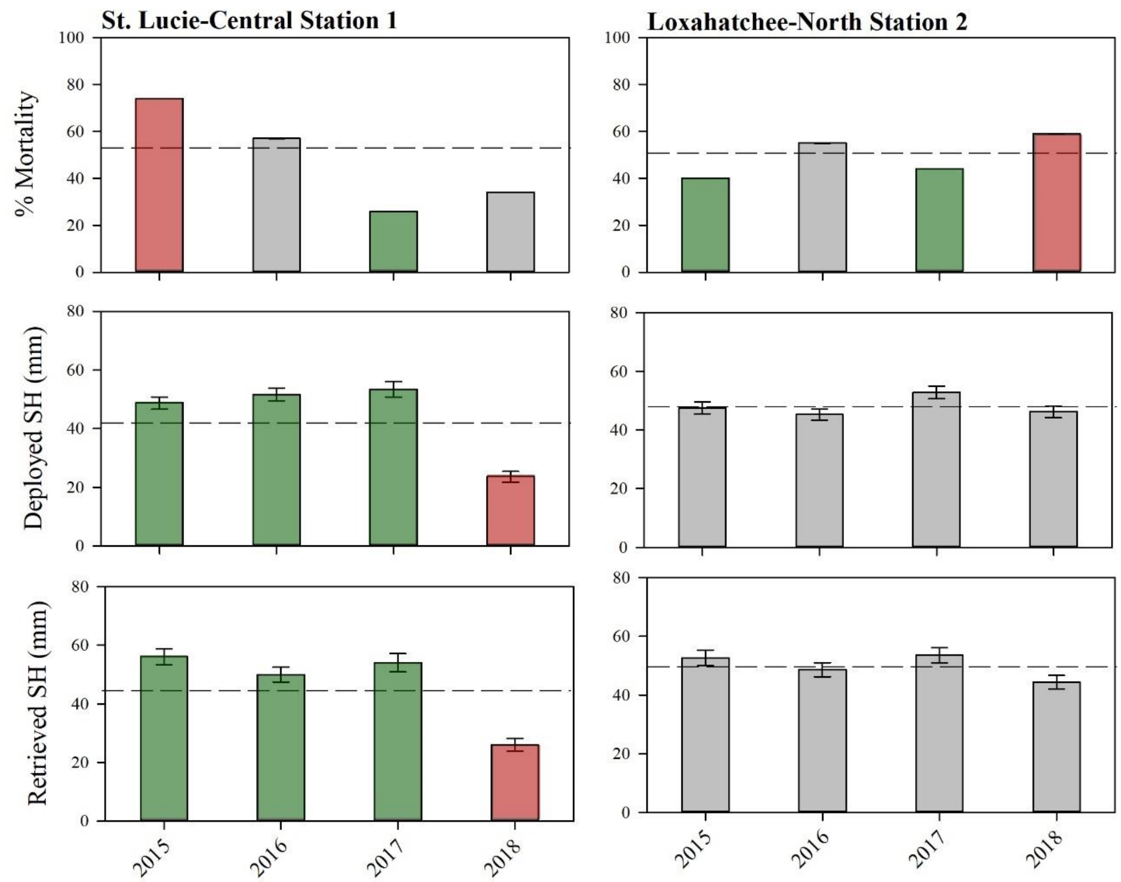

Figure 22. Estimated annual means and standard errors from comparisons of percent mortality, deployed shell height and retrieved shell height of oysters planted into open cages at Station 1 in St. Lucie-Central and Station 2 in Loxahatchee-North from 2015 – 2018. Red-shaded bars indicate years with significantly greater mortality rates/smaller shell heights and green-shaded bars indicate years with significantly lower mortality rates/larger shell heights ($P < 0.05$). The gray dashed lines represent the overall estimated mean for each site during the 4-y study.

SOUTHWEST FLORIDA

Methods

Study Sites

Oyster sampling was conducted by FWRI from February 2017 through December 2018 on oyster reefs in the Caloosahatchee River Estuary (CRE). Within the CRE, oysters were sampled from an upstream site with stations located at Peppertree Pointe (PPT) and Iona Cove (IC) and from a downstream site with stations located at Bird Island (BI) and Kitchel Key (KK). This strategy resulted in a total of two separate study sites each with two stations. Station coordinates are listed in Table 25 and locations are shown in Figure 23.

Table 25. Station coordinates for CERP oyster monitoring sites in southwest Florida.

Site	Station	Station Name	Latitude ^oN	Longitude ^oW
Caloosahatchee River East	1	Peppertree Pointe	26 31.629	81 57.366
Caloosahatchee River East	2	Iona Cove	26 30.804	81 58.754
Caloosahatchee River West	3	Bird Island	26 30.829	82 01.964
Caloosahatchee River West	4	Kitchel Key	26 29.777	82 00.336

Water Quality

Monthly water quality sampling was conducted in conjunction with field sampling at all stations within each study site from February 2017 through December 2018. Recorded parameters included salinity, temperature, dissolved oxygen concentration, pH, depth and clarity. Depth was determined with a sounding line or incremented meter stick and clarity was obtained by using a standard Secchi disk. Water clarity 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 calibrated YSI. Additional salinity data from continuous data loggers deployed by the South Florida Water Management District (SFWMD) was also analyzed. Graphical presentations in Appendix F show the monthly values measured by FWRI at each station within each site and the daily values measured by each data logger. Flow rates as recorded by the SFWMD, the United States Geological Survey (USGS) and Army Corps of Engineers (ACOE) were analyzed and included for comparisons.

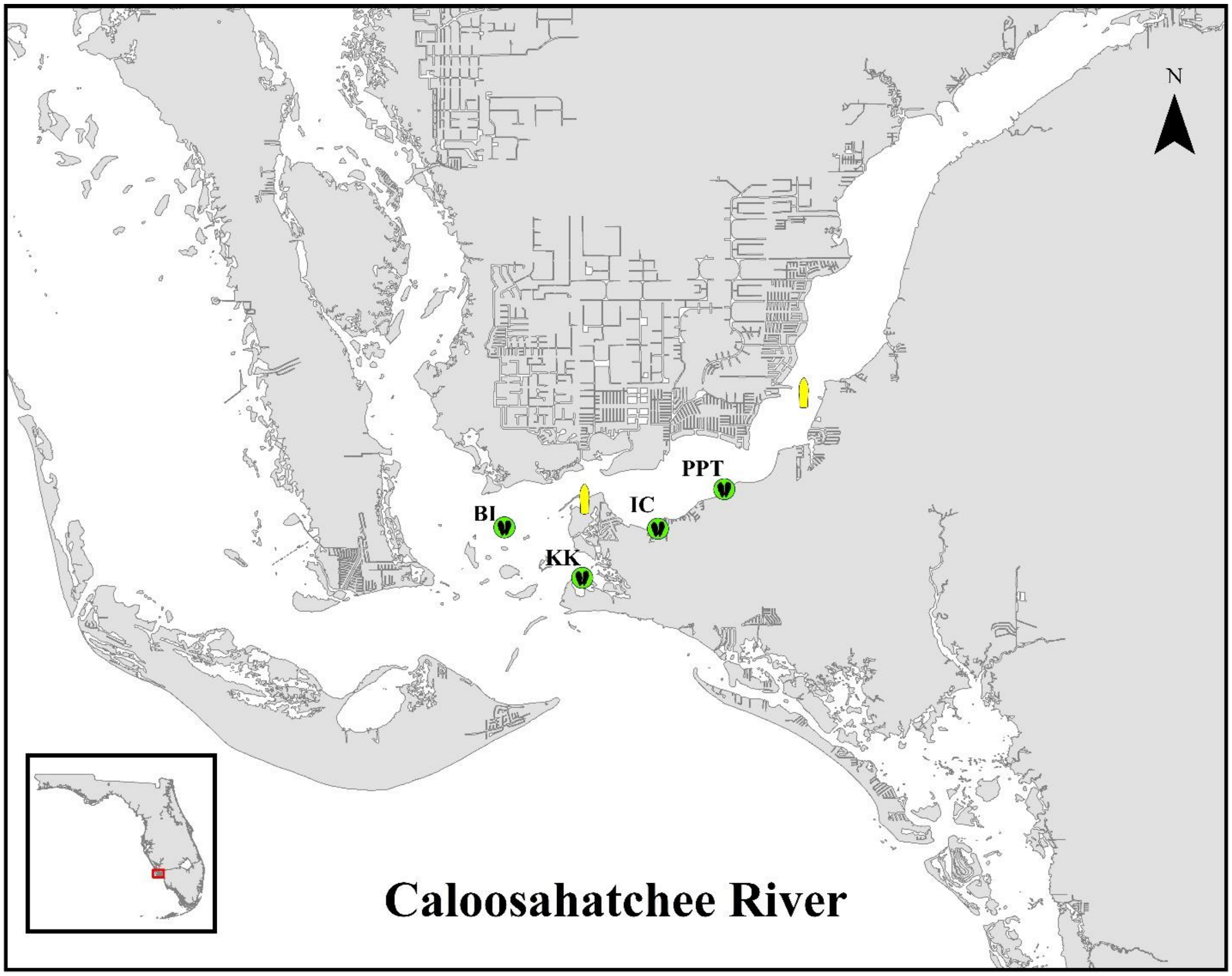

Figure 23. CERP oyster monitoring stations (green symbols) and continuous water temperature and salinity data loggers (yellow symbols) in the Caloosahatchee River Estuary on the southwest coast of Florida. PPT = Peppertree Pointe, IC = Iona Cove, BI = Bird Island, KK = Kitchel Key

Settled Oyster Density

Oyster population surveys were conducted twice per year at all sites and stations from 2017 through 2018. Additional quarterly surveys were conducted during the summer (June) and winter (December) at the Bird Island and Iona Cove stations. The oyster survey methodology used in the present study was based on that of Lenihan and Peterson (1998) and Grizzle et al. (2005). At each station, four replicate ¼-m^2 quadrats were haphazardly deployed and all oysters within each quadrat were collected for determination of the total number of live oysters and dead oysters with articulated shells, as well as the proportion of dead oysters to the total number of live oysters and dead oysters (dead ratio). In addition, shell height (SH; maximum linear distance from the umbo to the ventral shell margin) was measured for a maximum of 50 live oysters per quadrat. Mean live oyster density, ratio of dead oysters to total oysters, and mean live oyster SHs were calculated and plotted for each station within each site (Appendix G). The mean SH was calculated for each quadrat, resulting in 4 data points per station each survey, and used for all statistical analyses.

Disease and Reproductive Development

Live oysters were collected monthly from February 2017 through December 2018 for analysis of gonadal development stage and for the prevalence and intensity of the oyster disease *Perkinsus marinus* (dermo). Each month, a sample of five oysters from each of the stations within a study site (total N = five oysters * four stations = maximum 20 per month) were transported, live and chilled, to the FWRI laboratory for processing. If no live oysters were available at one or two of the stations within a site, additional oysters were collected from another station within that site if possible. Each individual oyster was processed for reproductive stage and disease status according to the methods described below.

For *P. marinus* (dermo) disease analyses, prevalence and intensity were diagnosed with Ray's fluid thioglycollate media (RFTM) method (Ray 1966). Sections of mantle and gill tissue, each approximately 1 cm^2 in area, were clipped from each individual using sterile surgical scissors, placed in RFTM treated with antibiotics and antifungals, and incubated for 7 days in the dark at room temperature. After the incubation period, tissues were placed on glass slides, macerated with sterile razor blades, and stained with Lugol's solution. Mantle and gill tissues were then examined at ×40 magnification for the presence of *P. marinus* hypnospores. Parasite density (infection intensity) was ranked according to the Mackin scale (Table 26; Mackin 1962). Mean infection intensity for each oyster was calculated as the average infection intensity from mantle and gill tissues. Parasitic prevalence was calculated as the percentage of oysters infected, regardless of infection level. Mean infection intensity and the percentage of infected oysters were plotted for each station within each site (Appendix G).

The tissues remaining after dermo analyses were preserved for histological determination of reproductive development stage. Tissues were fixed in a modified Davidson's fixative solution (Shaw and Battle 1957), the main difference being no glycerin was included, for a minimum of 2 days. Once fixed,

Table 26. Mackin scale of *Perkinsus marinus* infection intensity stages

Stage	Category	Number of cells
0	Uninfected	None detected
0.5	Very light	<10
1	Light	11–100 cells
2	Light to moderate	Local concentrations of 24–50 cells
3	Moderate	3 cells in all fields at 100×
4	Moderate heavy	High numbers in all tissues
5	Heavy	Enormous numbers

cross-sections were taken approximately halfway between the adductor muscle and the anterior margin, to include the gonad. Cross-sections were placed in tissue-embedding cassettes, rinsed, and then transferred to a 70% solution of ethanol. Histological preparation consisted of dehydrating each oyster in 95% ethanol then embedding the tissue in paraffin. The sections were stained with hematoxylin and eosin and mounted on glass slides for analysis. Histological cross-sections were examined at ×200-400 magnification to ascertain sex and assigned one of four reproductive stages (Table 27) according to a classification scheme modified from the work of Fisher et al. (1996).

Table 27. Qualitative reproductive staging criteria for oysters collected from Florida waters (Fisher et al. 1996)

Value	Stage	Observations
1	Developing	Gametogenesis has begun immature gametes located on follicle walls mature gametes may be present
2	Ripe/Spawning	Follicles distended and full of ripe gametes ova compact/sperm with visible tails no immature gametes on follicle walls active spawning, but less than 2/3 depleted
3	Spent/Recycling	Most gametes evacuated from the follicles more than 2/3 depleted
4	Indifferent	Gonads devoid of gametes, cytolysis ongoing

Juvenile Recruitment

Juvenile oyster (spat) recruitment was monitored monthly from February 2017 through December 2018 at all stations in the CRE. Spat monitoring arrays were constructed and processed, as adapted from Southworth and Mann (2004). Each array consisted of 12 axenic adult oyster shells (SH, 5-10 cm) strung onto one length of galvanized wire. The shells were oriented on the wire with their inner surfaces facing downward, then the shell strings were suspended from the arms of a T-shaped PVC frame and the PVC frame was pushed into the sediment until the bottommost shell was approximately 5 cm above the sediment surface. Upon retrieval, the shell strings were labeled and bagged, and new shell strings were placed immediately on the PVC frame. The retrieved shell strings were returned to the laboratory, where each shell was examined for oyster spat with the aid of a magnification lamp or dissecting microscope (maximum magnification, ×65).

Juvenile oyster recruitment was estimated by counting settled spat on the underside of the middle 10 shells on each shell string (Southworth and Mann 2004). Recruitment rates were obtained by dividing the raw number of spat per shell by the number of days the shell had been deployed, and then standardizing to a 28-day month. Those standardized values were then used to compute the mean spat per shell per month for each successfully retrieved replicate, or spat monitoring array, resulting in a maximum of 3 data points per station each month. Mean numbers of spat per shell per month were plotted for each station within each site (Appendix G). Recruitment rates are reported by retrieval date throughout.

Juvenile Growth and Predation

In November 2017, monthly mortality and shell height monitoring was initiated at the Bird Island and Iona Cove stations; however, no live oysters were available at the Iona Cove station through December 2018. At the Bird Island station, 90 wild oysters with SHs of 10 mm or greater, were collected, measured (SH, mm) and planted into 3 open cages (n = 30 oysters per cage). Cages were constructed from 25.4-mm plastic-coasted wire mesh with dimensions of approximately 0.6-m L x 0.6-m W x 0.2-m H; the bottom and sides of the cages were lined with 6.35-mm plastic mesh to prevent loss of smaller oysters. One month later, all remaining oysters were counted, measured and released. A new set of 90 wild oysters were then immediately collected, measured and planted into the cages. This process was repeated monthly through December 2018. Mortality rates were calculated by first determining the survivorship rate (divide the number of remaining live oysters in the cage by the number of live oysters initially planted in the cage) and then subtracting that number from 1. Mean mortality, mean deployed SH and mean retrieved SH were plotted for the Bird Island station (Appendix G).

Statistical Analyses

Statistical analyses were performed with SAS Enterprise Guide version 7.1 (SAS Institute Inc., Cary, NC) and results were considered significant at $\alpha = 0.05$. All data were tested for normality by examining model residuals and then testing them for goodness of fit with the Shapiro-Wilk test (Shapiro and Wilk 1965). Because no data met normality assumptions, all statistical comparisons were performed using generalized linear mixed modeling with the GLIMMIX procedure (Littell et al. 2006). Statistical tests of all parameters included fixed factors of site and year.

Results

Water Quality

Salinity was variable at both study sites, ranging from 0 to 30 in the Caloosahatchee River-East (CR-E) site and from 14 to 35 in the Caloosahatchee River-West (CR-W) site (Appendix F). Statistical comparison of salinities measured during monthly sampling trips revealed that they were greatest at the downstream CR-W site where the overall mean was 24 ($F_{1,84}$=16.63, $P < 0.01$); the overall mean at the upstream CR-E site was 15. Salinity patterns did not differ significantly among years within the two sites ($F_{1,84}$=0.10, $P = 0.77$). The mean daily salinities measured by the data loggers deployed at the upstream CCORAL station and the downstream MARKH station paralleled the monthly measures (Appendix F); no statistical differences among years were detected.

Flow rates from the S79 structure located upstream of the study sites were compiled to assess freshwater inflows and their effects on estuarine salinities (Appendix F). Flow rates were greatest in 2017 when the annual mean reached 3405 cfs ($F_{2,15228}$=4689.24, $P < 0.01$). The annual mean flow rate in 2018 was 2010 cfs.

Additional analysis of flow and salinity data was conducted to further define environmental conditions in the CRE during the 2-yr study. Since flow from the S79 structure is upstream of both continuous data loggers, salinity measures from both were included for these comparisons. It is also worth noting that the CCORAL data logger is upstream of the monitored oyster population while the MARKH data logger is located between the CR-E and CR-W sites. Mean salinity and flow as well as the percentage of measures that fell below, within and above the optimal salinity range (10 – 25) were calculated for each calendar year (Table 28). Each calendar year was also classified as wet, dry or moderate. The year was considered wet if 25% or more of the measures were below the optimal range (CCORAL: 2017 and 2018). Similarly, the year was considered dry if 25% or more of the measures exceeded the optimal range (MARKH: 2017 and 2018).

Table 28. Mean salinity and total number of salinity measurements in the CRE each calendar year, the number (n) and percentage of days when salinity was less than 10, ranged from 10 to 25, and exceeded 25, and mean flow. Blue bold font denotes significantly low flow value. Shading indicates wet years (blue) or dry years (red). Salinity and flow data (S79 structure) is from SFWMD.

Caloosahatchee River Estuary

CCORAL			< 10		10 – 25		> 25		
Year	Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2017	11.3	363	203	55.9	149	41.0	11	3.0	**3405.2**
2018	12.5	351	136	38.7	215	61.3	0	0.0	2009.6
Mean	**11.9**			**47.3**		**51.2**		**1.5**	**2707.4**

MARKH			< 10		10 – 25		> 25		
Year	Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2017	20.6	365	56	15.3	152	41.6	157	43.0	**3405.2**
2018	20.6	349	43	12.3	157	45.0	149	42.7	2009.6
Mean	**20.6**			**13.8**		**43.3**		**42.9**	**2707.4**

Similar metrics were calculated for salinities measured during the dry season months (January – April; November – December) and wet season months (May – October) of each calendar year. During the dry season months, those years when 50% or more of the salinity measures exceeded optimal were considered *extreme*-dry seasons; years with 25% or more of the salinity measures below optimal were classified as *wet*-dry seasons (Table 29). The dry seasons of 2017 and 2018 at the MARKH station were both considered *extreme*-dry seasons since 65.2 to 68.5% of the salinity measures each year exceeded optimal. At the opposite extreme, 33.5% of the salinity measures at CCORAL were below optimal in 2017 making that a *wet*-dry season. During the wet season months, years when 50% or more of the salinity measures were below optimal were considered *extreme*-wet seasons while those with 25% or more above optimal were considered *dry*-wet seasons (Table 30). The wet seasons of both 2017 and 2018 at the CCORAL station were considered *extreme*-wet since more than 75% of those salinity measures were below optimal. At both logger stations, dry season salinity was approximately 7 higher than the mean wet season salinity in 2017; the difference was greater in 2018, when mean dry season salinity exceeded the wet season salinity by approximately 12.

Table 29. Mean salinity and total number of salinity measurements in the CRE during the dry season months (January – April; November – December) each calendar year, and the number (n) and percentage of days when salinity was less than 10, ranged from 10 to 25, and exceeded 25, and mean flow. Blue-shading denotes *wet*-dry seasons and red-shading denotes *extreme*-dry seasons. Salinity and flow data (S79 structure) is from SFWMD.

Caloosahatchee River Estuary

CCORAL			< 10		10 – 25		> 25		
Year	**Dry Season** Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2017	14.7	179	60	33.5	119	66.5	0	0.0	2358.2
2018	18.4	181	7	3.9	174	96.1	0	0.0	820.8
Mean	**16.6**			**18.7**		**81.3**		**0.0**	**1589.5**

MARKH			< 10		10 – 25		> 25		
Year	**Dry Season** Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2017	24.2	181	8	4.4	55	30.4	118	65.2	2358.2
2018	26.3	181	1	0.6	56	30.9	124	68.5	820.8
Mean	**25.2**			**2.5**		**30.7**		**66.9**	**1589.5**

Table 30. Mean salinity and total number of salinity measurements in the CRE during the wet season months (May – October) each calendar year, and the number (n) and percentage of days when salinity was less than 10, ranged from 10 to 25, and exceeded 25, and mean flow. Blue-shading denotes *extreme*-wet seasons and red-shading denotes *dry*-wet seasons. Salinity and flow data (S79 structure) is from SFWMD.

Caloosahatchee River Estuary

CCORAL			< 10		10 – 25		> 25		
Year	**Wet Season** Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2017	8.0	184	143	77.7	30	16.3	11	6.0	4733.8
2018	6.2	170	129	75.9	41	24.1	0	0.0	3531.3
Mean	**7.1**			**76.8**		**20.2**		**3.0**	**4132.6**

MARKH			< 10		10 – 25		> 25		
Year	**Wet Season** Mean Salinity	Total n	n	% of Total	n	% of Total	n	% of Total	Mean Flow (cfs)
2017	17.1	184	48	26.1	97	52.7	39	21.2	4733.8
2018	14.5	168	42	25.0	101	60.1	25	14.9	3531.3
Mean	**15.8**			**25.5**		**56.4**		**18.0**	**4132.6**

Water temperatures exhibited typical seasonal patterns, were similar among sites ($F_{1,84}$=0.74, P = 0.39) and ranged from 16 to 34° C during the 2-yr study. The highest temperatures were recorded in August or September when means exceeded 31° C (Appendix F). Temperature minima occurred in December 2017 and January 2018 when lows reached 16 at the CR-E site and 17 at the CR-W site. Annual means for both years were approximately 26° C. No significant differences were detected in water temperature patterns between years within the two sites during the study ($F_{1, 84}$=0.01, P = 0.90).

Dissolved oxygen concentrations were similar among sites ($F_{1,84}$=0.08, P = 0.78; Appendix F). Annual means ranged from 7.0 to 7.5 but did not differ significantly between years ($F_{1,84}$=0.08, P = 0.78). As with dissolved oxygen concentrations, pH was similar among sites but slightly lower in CR-E ($F_{1,84}$=4.79, P = 0.03; Appendix F). Annual means ranged from 8.0 to 8.3 but there were no significant differences between years within each site ($F_{1,84}$=0.01, P = 0.91).

Water clarity was high and similar between sites, with overall means of 92% in CR-E and 100% in CR-W ($F_{1,84}$=0.00, P = 0.98; Appendix F). The lowest water clarity occurred in CR-E in October and November 2017 and again in June and July 2018; all other measures at CR-E and CR-W were 100%. No significant differences were detected in water clarity within the two sites between years ($F_{1, 84}$=0.00, P = 1.00).

Settled Oyster Density

Oyster surveys measuring live oyster density, number of dead oysters with articulated shells and live oyster shell heights were conducted at all stations in spring 2017, spring 2018 and fall 2018. No surveys were conducted in fall 2017 due to impacts from Hurricane Irma.

Overall densities of oysters differed between the two study sites ($F_{1,42}$=115.40, P < 0.01). The greatest densities of oysters were found in the CR-W site where the overall mean was 817 oysters/m^2 (Appendix G). Oyster density also differed among surveys within each of the sites ($F_{2,42}$=61.06, P < 0.01). In the CR-E site, the greatest live oyster densities were recorded in spring 2017 and fall 2018 when mean values ranged from 535 to 587 oysters/m^2. The lowest density was measured in spring 2018 following a period of low salinity and high flow rates associated with Hurricane Irma in late 2017 (1 oyster/m^2). In

CR-W, the greatest densities of live oysters were found in Fall 2018 (1677 oysters/m^2) while the lowest densities were recorded in spring 2017 and spring 2018 (509 and 640 oysters/m^2, respectively).

In order to assess the relative health of a particular oyster reef, the ratio of dead oysters to the total number of live and dead oysters was determined (Appendix G). Statistical comparison of dead ratios revealed differences among sites ($F_{1,42}$=107.58, $P < 0.01$). The greatest ratios were found in CR-E where the overall mean was 0.41; the overall mean in CR-W was 0.16. Differences among surveys within sites were also detected ($F_{2,42}$=375.96, $P < 0.01$). In the CR-E site, the greatest ratio of dead oysters was recorded in spring 2018 (0.99), when almost all oysters were dead following the low salinity event associated with Hurricane Irma in late 2017. In CR-W, the greatest dead ratio occurred in spring 2017 (0.51). The lowest dead ratios were measured at the CR-E and CR-W sites in fall 2018 when the means were 0.03 and 0.04, respectively.

Live oyster shell heights (SH) were significantly larger in CR-E than in CR-W ($F_{1,36}$=10.01, $P <$ 0.01; Appendix G). The overall mean SH for oysters in CR-E was 27 mm while those in CR-W were 22 mm. Mean SH also varied among surveys within each site ($F_{2,36}$=0.93, $P < 0.01$). At both sites, the largest oysters were measured during the spring surveys in 2017 and 2018; however, mean sizes were significantly larger in CR-E where means ranged from 43 to 45 mm. In CR-W, spring means ranged from only 23 to 26 mm. At both sites again, the smallest SHs were measured in fall 2018 when the mean SH was 11 mm in CR-E and 17 mm in CR-W.

Results from analyses of salinity, live oyster density, dead oyster ratios and live oyster shell height were compiled for comparison (Table 31). Biological data from the CR-E site was paired with salinity data from the CCORAL station since it is located upstream of those monitoring stations. Likewise, biological data from the CR-W site was paired with salinity data from the MARKH station. In 2017, the fall survey was not conducted in either the CR-E or CR-W sites due to impacts from Hurricane Irma. As a result, the annual means for 2017 only represent the density of oysters recorded during the spring surveys at stations in both the sites. In the CR-E, 2017 was considered a wet year with both the dry season and wet season months also characterized as wetter than expected. It is likely that if the fall survey was conducted at the CR-E stations, very few live oysters would have remained, thus decreasing the annual mean. In the CR-W, both years were classified as dry and the dry season was also characterized as drier than expected. Higher

Table 31. Wet or dry classifications for each calendar year, the dry season months (January – April; November – December), the wet season months (May – October) and the mean salinity, live oyster density, ratio of dead oysters and live oyster shell heights in the Caloosahatchee River-East and West study sites. Shading indicates wet (blue) or dry (red). Salinity data is from SFWMD.

Caloosahatchee River Estuary

Year	Classification	Dry Season Classification	Wet Season Classification	Salinity	Live Density	Dead Ratio	SH
CCORAL (CR-E)							
2017	Wet	Wet	Extreme	11.3	534.50	0.11	45.13
2018	Wet		Extreme	12.5	294.00	0.51	26.49
MARKH (CR-W)							
2017	Dry	Extreme		20.6	508.50	0.51	25.98
2018	Dry	Extreme		20.6	1158.25	0.09	20.20

salinity conditions, as are typical in the CR-W site, increase reproductive potential and spat settlement rates, as evidenced by the high oyster densities recorded in 2017 and especially in 2018. However, those high salinities also increase disease and predation rates and the impact of this can be seen when examining the ratio of dead oysters. In 2017, when data was only collected in the spring before the Hurricane, dead ratios were significantly higher in CR-W than in CR-E. In 2018, that pattern was reversed. In the CR-E, the higher dead ratios reflected the die-off of oysters after the hurricane caused salinities to decrease to below optimal. In the CR-W, the lower dead ratio reflected a decrease in predation and disease rates resulting from the lower storm-related salinities, as well as a population dominated by young, newly settled spat that far outnumbered the dead oysters. The largest mean oyster size was measured in 2017 in the CR-E, indicating an oyster population comprised of multiple spawning classes of oysters. In the CR-W in 2017, the mean size was smaller, suggesting that there were fewer larger oysters as a result of the high mortality rates associated with the higher salinity environment. In 2018, mean SHs were smaller in CR-E, indicating recovery after the hurricane in the form of large numbers of spat settling to repopulate the reefs. In the CR-W, the small SHs were also due to large numbers of spat settling on reef, especially in the fall of that year.

Disease

Sampling for *Perkinsus marinus* disease analysis began in February 2017. Due to the occurrence of low salinity events, there were several periods when live oysters were not available for collection and processing for disease analyses (Appendix G; Table 32). The sampling trip scheduled for September 2017 was cancelled due to Hurricane Irma, so no samples were collected from either site that month.

Table 32. Months following mortality events when live oysters were either not available or too small (< 15 mm shell height) to collect from the CRE and process for disease and reproductive analyses.

Mortality Event	Site	Timing	Duration
2017	Caloosahatchee River-East	Oct 2017 - Apr 2018	14 months
2018	Caloosahatchee River-East	Jun 2018	1 month

Prevalence of infection by *Perkinsus marinus* (dermo) did not vary significantly among sites ($F_{1, 63}$=2.05, P = 0.16). Overall infection rates were 25% in CR-E and 34% in CR-W. However, dermo prevalence did differ among years within the CR-E site ($F_{1, 63}$=6.58, P = 0.01). The mean annual rate of 43% in 2017 was significantly higher than the rate of 13% measured in 2018. In CR-W, annual means were 35% in 2017 and 32% in 2018. Intensity of infection by *P. marinus* did differ significantly among sites ($F_{1, 436}$=8.77, P < 0.01); overall intensity was greater in CR-W (0.31) than in CR-E (0.09). As with dermo prevalence, dermo infection intensity differed among years in the CR-E ($F_{1, 436}$=3.21, P = 0.07) with values of 0.20 in 2017 and 0.04 in 2018. In CR-W, annual means were 0.33 and 0.29 in 2017 and 2018, respectively. Despite these statistical differences in dermo infection intensity, there is little biological relevance as these values represent very light infections in both sites during both years.

Results from analyses of salinity, dermo prevalence and dermo infection intensity were compiled for comparison (Table 33). In the CR-E site, dermo prevalence and intensity were greatest in 2017. Although 2017 was considered a wet year, no live oysters were present for collection after the hurricane, so infection rates primarily represent oysters collected prior to the low salinity event. As those oysters were exposed to salinities within the optimal range, and occasionally exceeding the optimal range, dermo prevalence was moderate. In CR-W, salinities exceeded optimal during the dry months of each year and were most frequently within the optimal zone during the wet seasons. As a result, dermo prevalence in the CR-W site was moderate during both years.

Table 33. Wet or dry classifications for each calendar year, the dry season months (January – April; November – December), the wet season months (May – October) and the mean salinity, *Perkinsus marinus* (dermo) infection prevalence and dermo infection intensity in the Caloosahatchee River-East and West study sites. Shading indicates wet (blue) or dry (red). Green bold font denotes significantly greater biological values. Salinity data is from SFWMD.

Caloosahatchee River Estuary						
CCORAL (CR-E)						
Year	Classification	Dry Season Classification	Wet Season Classification	Salinity	Dermo Prevalence	Dermo Intensity
2017	Wet	Wet	Extreme	11.3	**42.86**	**0.20**
2018	Wet		Extreme	12.5	13.33	0.04
MARKH (CR-W)						
2017	Dry	Extreme		20.6	35.38	0.33
2018	Dry	Extreme		20.6	31.79	0.29

Reproductive Development and Juvenile Recruitment

Sampling for reproductive analyses began in February 2017. Due to the occurrence of low salinity events, there were several periods when live oysters were not available for collection and processing for disease analyses (Appendix G; Table 32). The sampling trip scheduled for September 2017 was cancelled due to Hurricane Irma, so no samples were collected from either site that month.

Although patterns and timing of reproductive development differed between years, gametogenesis and spawning typically occurred between March and October (Appendix G). The majority of oysters entered the resting, or indifferent, stage in November or December and remained inactive through February. A comparison of the percentage of oysters in the gonadal development stage found differences between sites ($F_{1, 33}$=16.55, $P < 0.01$). The greatest number of oysters developing gametes was found in CR-E where the overall mean was 51%. The overall percentage in CR-W was only 29%. The percentage of oysters developing gametes differed significantly between years in the CR-W site but was similar in CR-E ($F_{1, 33}$=3.00, $P < 0.01$). In CR-W, the percentage was slightly higher in 2018 (36%) than in 2017 (23%). In CR-E, percentages were 54 and 49% in 2017 and 2018, respectively.

Comparisons of oysters staged in any of the three active reproductive stages (developing, ripe/spawning, and spent/recycling) were also conducted. Results revealed that the greatest percentage occurred in CR-E where the overall mean was 86% ($F_{1, 33}$=24.97, $P < 0.01$). Only 59% of staged oysters were in active reproductive stages in CR-W. Percentages or reproductively active oysters were similar between years in both sites with 80 to 91% in CR-E and 57 to 62% in CR-W ($F_{1, 33}$=24.97, $P < 0.01$).

Juvenile recruitment exhibited patterns similar to those seen with reproductive development (Appendix G). Oyster spat were detected on arrays retrieved every month from March 2017 through December 2018 in CR-W and in all months except November 2017 through February 2018 in CR-E; arrays were not retrieved from either site in September 2017. In 2017, recruitment was somewhat sporadic and inconsistent at both sites in the early months but stopped completely after Hurricane Irma in September. In 2018, there was a clear fall recruitment peak in September at both sites. Those peaks reached a mean of 90 spat/shell in CR-W and 25 spat/shell in CR-E.

Recruitment rates were greatest in CR-W where the mean for the duration of the study was 11.7 spat/shell/month ($F_{1, 243}$=63.68, $P < 0.01$).   The overall recruitment rate was 1.39 spat/shell/month in CR-E. Recruitment rates were similar between years at the CR-W site (8.45 spat/shell/month in 2017 and 16.18 spat/shell/month in 2018) but were significantly greater in 2018 in CR-E (0.45 vs. 4.30 spat/shell/month; $F_{1, 243}$=9.07, $P < 0.01$).

Results from analyses of salinity, reproductive development and juvenile recruitment were compiled for comparison (Table 34). In CR-W, the percentage of oysters developing gametes was relatively low compared to oysters in CR-E. It is likely that the higher salinity environment and the resultant high disease prevalence may have caused physiological stress to the oysters thus rendering them less productive. Another possibility is that most of the oysters are newly settled immature spat. A similar pattern was detected in oysters that were classified as undergoing active gametogenesis. Recruitment rates in CR-E in 2017 were significantly lower than in 2018. This is primarily due to the loss of the fall recruitment peak in 2017 following Hurricane Irma and the associated low salinity event.

Juvenile Growth and Predation

Measures of mortality and shell height were initiated in November 2017 when wild oysters were collected, measured and planted in open cages at the Bird Island station in CR-W (Appendix G). The experiment was repeated monthly through December 2018. Mortality and shell height measures were also to be conducted on oysters in cages planted at the Iona Cove station in CR-E, but no live oysters were available during the 2-yr study period. Mortality rates were high in most months and ranged from 91 to

Table 34. Wet or dry classifications for each calendar year, the dry season months (January – April; November – December), the wet season months (May – October) and the mean salinity, percent of oysters developing gametes, percent of oysters undergoing gametogenesis and number of spat recruits per shell in the Caloosahatchee River-East and West study sites. Shading indicates wet (blue) or dry (red). Green bold font denotes significantly greater biological values. Salinity data is from SFWMD.

Caloosahatchee River Estuary

Year	Classification	Dry Season Classification	Wet Season Classification	Salinity	Developing	Active	Spat / Shell
CCORAL (CR-E)							
2017	Wet	Wet	Extreme	11.3	53.85	91.03	0.45
2018	Wet		Extreme	12.5	48.89	80.00	**4.30**
MARKH (CR-W)							
2017	Dry	Extreme		20.6	23.48	56.52	8.45
2018	Dry	Extreme		20.6	**35.83**	61.50	16.18

100%; exceptions occurred in February and March 2018 when mortality rates were 18 and 30%, respectively. Mean SHs of oysters deployed in cages were similar and ranged from 32 to 42 mm throughout the study. Upon retrieval, mean SH was more varied and ranged from 29 mm in July 2018 to 55 mm in September 2018. No live oysters remained upon retrieval of the cages in January 2018. In all months except May and July 2018, mean SH increased. In May 2018, mean SH of deployed and retrieved oysters was the same (33 mm). In July 2018, mean SH of retrieved oysters was almost 7 mm smaller than the mean SH of deployed oysters (35 mm vs. 28.5 mm). This was likely due to a greater loss of larger individuals over the course of the month-long deployment, resulting in an overall lower mean SH.

DISCUSSION

This report summarizes oyster population monitoring results from five study sites located in the SLE and LRE on the southeast coast of Florida and from two study sites located in the CRE on the southwest coast of Florida. Monitoring in the southeast sites was conducted by FWRI from 2005 through 2018. Monitoring in the CRE was conducted by Dr. Aswani Volety and his team at FGCU from 2000 through 2016 (Volety et al. 2016). FWRI continued those monitoring efforts in the CRE beginning in early 2017; results from February 2017 through December 2018 are included in this report.

Oyster (*Crassostrea virginica*) monitoring in the SLE and LRE was initiated in 2005 after an extremely active 2004 hurricane season. The sequence of storms that occurred in 2004 resulted in considerable rainfall in Central and South Florida, significantly reducing salinity in many coastal waters via natural and anthropogenic contributions (Paperno et al. 2006, Steward et al. 2006). Those reductions in salinity were exacerbated by another active hurricane season in 2005. The effects of the 2004/2005 storms were best demonstrated in the SLE, where both the dry and wet seasons of 2005 were wetter than expected and the mean annual salinity was 7.3 (daily measures were < 10 for 228 days). Those low salinity values were not solely attributable to direct inputs of freshwater via rainfall and runoff, but also include inflow of freshwater from flood control releases implemented by local water management practices. The SLE watershed was tripled in size during the 20th century as a result of not only urban and agricultural development (Haunert et al. 1994), but also due to the addition early in the century of three flood control canals that flow into the estuary, the largest of which links it directly to Lake Okeechobee (Sime 2005).

Water quality has a large influence on oyster health and survivorship; rapid changes in salinity, high temperatures, low dissolved oxygen concentrations and siltation can all be stressors to oysters in Florida waters. Temperature and salinity are two of the most influential physical parameters affecting oyster populations (Shumway 1996), but during this study the magnitude, timing and frequency of salinity fluctuations were the major driving force behind changes in oyster ecology. Eastern oysters can tolerate exposure to a wide range of salinities, 1.2 to 36.6 in Apalachicola Bay, FL (Menzel et al. 1966), but the optimal salinity range is much narrower and may differ among populations. For the CERP RECOVER program, the current effort to update the Northern Estuaries Salinity Performance Measure has compiled study results, monitoring data and reviewed scientific literature to define an optimum salinity envelope for oysters of 10 – 25. Salinities that fall outside that range are considered stressful (5-9; > 25) or damaging (< 5) to oysters. The interaction of salinity and temperature also affects the survivorship of oysters (Shumway 1996), e.g., an abrupt salinity decrease in waters at high temperature will be much more stressful to oysters than a similar decrease in cooler waters. Florida oysters are commonly exposed to temperatures near their upper physiological tolerance limits; some years, water temperatures can exceed 30° C for four or more consecutive months, and oysters can experience even higher temperatures if they are exposed during afternoon low tides. When animals are subjected to environmental conditions that meet or exceed tolerance limits, their energetic capacity to deal with additional stresses, such as low salinity or disease, are diminished or lost.

Settled oyster density measurements from the SLE in 2005 reflected earlier conditions during which salinities fell below tolerance limits. Although early 2005 salinities were within the optimal range for oysters, the 2004 storms probably decreased salinities to intolerable levels that year, resulting in oyster mortalities in the north and south forks of the SLE. This conclusion is based on the high ratio of dead oysters present at monitored stations in early 2005, suggesting recent mortality of those oysters. Empty oyster shells remain intact 1 to 2 years before disarticulating, depending on size and environmental conditions (Christmas et al. 1997, Ford et al. 2006), so it's most likely those oysters died as a result of events that occurred in 2004. Although the validity of assessing the density of dead oysters from survey counts has been disputed, those counts are helpful in showing recent mortality events or when used as an indicator of relative changes in the proportion of dead oysters within a population (Mackin 1959). Regardless of the cause, few live oysters were observed at any of the sampled SLE stations during the 2005 surveys. Because live oysters were present in moderate densities in the nearby LRE during the 2005 surveys, the factors that contributed to the dearth of live oysters in the SLE appear to have been specific to that estuary.

In June 2005, salinities in the SLE fell below 10 and remained sub-optimal most days for the rest of the year; salinity was < 5 (damaging) for 171 of those days. Fortunately, salinities increased in early 2006 and generally remained within the optimal range for most of the year. The SLE oyster population continued to recover in 2007 despite a dry period from February to June when salinities exceeded optimal. In 2008, salinities were relatively moderate until August when Tropical Storm Fay impacted the estuary. Although this resulted in near complete mortality of oysters in the SLE, this event was less severe than the mortalities observed in 2005. Salinities recovered quickly after the storm, reaching optimal levels by late October, thus allowing for settlement and growth of new spat recruits before the end of the 2008 spawning season. Those recruits successfully overwintered, allowing for a more rapid recovery in 2009. This success is reflected in the 2009 survey results, when mean estuarine densities increased from near 0 oysters/m^2 in fall 2008 to just over 100 oysters/m^2 in the spring.

The reprieve for the SLE oyster population was short-lived as salinities decreased again in 2010 during a prolonged freshwater release event that lasted from March through July. Although there were not widespread oyster mortalities associated with this event, oysters at upstream stations in the north and south forks were exhibiting poor health and dying, especially during the hot, summer months. The timing of this event also coincided with the months of peak reproductive development and spawning. Analyses of oyster gonadal tissues revealed that oysters were undergoing gametogenesis and spawning as expected in 2010; however, recruitment rates in 2010 were among the lowest recorded during the entire 14-yr study. This suggests that the vast majority of newly spawned larvae were either physically flushed out of the estuary or killed by the low salinities.

SLE salinities were high in 2011, exceeding the optimal range for 186 consecutive days from January through early July, and more moderate in 2012 until the fall when rainfall and the subsequent freshwater releases associated with Hurricane Isaac in August negatively impacted oysters at the most upstream stations. Salinities rebounded quickly in late October and remained at or above the upper boundary of the optimal range through May 2013. Salinities then decreased rapidly to sub-optimal levels in June after heavy rainfall and high magnitude freshwater releases began affecting the estuary. This time the inundation of freshwater caused widespread oyster mortalities in all three SLE study sites. This freshwater event also led to high levels of enteric bacteria and a cyanobacterial bloom which prompted the Martin County Department of Health to issue a health advisory that recommended avoiding contact with SLE waters. When water quality finally improved in December, the fall 2013 survey was conducted and, of the sparse number of live oysters present, all were juvenile spat that had settled in the past few weeks. This was reflected in the significantly smaller SHs measured during that survey. To reiterate, no live, adult oysters were found at any sampled station in December 2013, suggesting that there was a complete loss of settled oysters.

Although there were frequent fluctuations in salinity, 2014 and 2015 were relatively innocuous in the SLE as there were no extreme rainfall events or prolonged freshwater releases. Salinity remained within the optimal range most days, with the only acute decreases occurring in July and September 2014 and March and September 2015, coincident with increased freshwater inflows. As a result, the oyster population stabilized and settled oyster densities in 2015 were among the highest recorded for the entirety of the study. Estuarine conditions declined again in early 2016 when increased rainfall associated with the 2015/2016 El Nino event caused salinities to decrease abruptly in February and remain sub-optimal for the next 9 months. Settled oysters in the middle estuary and the North Fork weathered this low salinity event, but oysters at the most upstream stations in the South Fork were dead by July and spat recruitment rates throughout the estuary were low and inconsistent.

In 2017, salinities in the SLE and at the downstream CRE site exceeded optimal most days from January through May. Salinities at the upstream CRE site were within the optimal range from January through May but then fell below optimal in early June and remained sub-optimal for the remainder of the year (204 days). In the SLE and the downstream CRE site, salinities also decreased abruptly, but not until September, after the powerful and intense Hurricane Irma. Excessive rainfall and runoff associated with the storm increased Lake Okeechobee water levels and subsequently large volumes of water were released into the CRE and the SLE. Flow rates into the CRE during the days immediately following the storm reached or exceeded 20,000 cfs and averaged more than 10,000 cfs for the months of September and October. In the SLE, flow rates exceeded 10,000 cfs for the first two days following the storm and averaged more than 5,700 cfs from September through November. Those flow rates into the SLE were among the highest recorded during the 14-yr study period and were only exceeded by those in 2005.

Prior to the storm, live oysters were present at all sampled stations in the SLE and at relatively high densities in the middle estuary. Less than a month after the storm, no live oysters were found at any station in the three SLE study sites. It's evident that the magnitude of the freshwater inputs into the SLE were large enough to cause widespread oyster mortalities, but the timing and duration of the event likely exacerbated the effects and prolonged the recovery period by suppressing larval recruitment. No larval recruits were detected in the SLE from September through December, so the next opportunity for recovery was pushed to spring 2018 when the new spawning season began. In the CRE, the effects of the low salinity event differed among sites. At the upstream site, salinities had already been sub-optimal since June but decreased to < 5 (damaging) in late August and remained so for more than 80 days. As in the SLE, no live oysters were found at either upstream station the month after the storm. Salinities at the downstream site decreased following the storm but remained well within the optimal range. As a result, there was no detectable die-off at those stations. In fact, recruitment at those stations peaked in August and remained high through November, suggesting that there were minimal negative effects at those locations following that storm. In contrast, no recruits were detected at the upstream stations from September through December.

Salinities in the SLE were within or above the optimal range in the early months of 2018 and, as anticipated, new spat recruits were detected at most stations by May. Unfortunately, salinities plummeted again in June when flow rates increased to more 2000 cfs following heavy rainfall in late May and all newly settled oyster recruits were killed. Salinities remained sub-optimal through early October further delaying oyster recovery until late fall 2018. In the CRE, salinities at the upstream site recovered quickly in early 2018 and remained within the optimal range until late May when they decreased to < 10 and remained so until October (129 days). Despite the low salinities, recruits were present at both upstream stations by May and live oysters were found at the Bird Island station during the June survey. Recruitment rates increased in August and remained high through October and, as a result, live oysters were also present at the Peppertree Pointe station by September. At the downstream CRE site, salinities exceeded optimal from February through May before decreasing to optimal levels for most days during the rest of the year. Since there was no oyster die-off at the downstream stations following Hurricane Irma, live oyster densities were relatively high at those stations in spring 2018. Recruitment rates were also high at this site from August through October, and, as a result, live densities during the fall survey were the highest recorded during 2-yr CRE study.

The recovery period for oysters following a die-off in the SLE was strongly influenced by the timing and duration of the low salinity events. The die-offs that occurred in 2008 and 2013 were less catastrophic and followed by a more rapid recovery than those that occurred in 2005 and 2017. In 2008, the low salinity event occurred in September, as with Hurricane Irma in 2017, but this event was much shorter in duration, thus allowing a small number of larvae to settle in the estuary before the end of the spawning season. In 2013, the low salinity event began in the summer but also concluded by October, again allowing successful larval settlement to occur in late fall before the end of the season. The oyster die-off associated with Hurricane Irma was similar to the event that occurred in 2005. During the 2005 event, salinities remained sub-optimal through the end of the year and recovery was delayed until initiation of the spawning season in spring 2006. Recovery following the 2017 die-off was delayed even longer due to the active 2018 wet season and the resultant small-scale mortality event that killed all the recently settled spat in June and July.

Although the impacts from low salinity events were more acute, long-term exposure to high salinities also had negative effects on the oyster populations. Salinities often exceeded optimal in the LRE and at the downstream CRE site, even in the SLE in 2011, and, during those periods, dermo prevalence in oysters was high. Increased predation and disease rates are typically associated with higher salinities and temperatures, but the extent to which a higher salinity regime can affect an oyster population is most exemplified by the dramatic increase in dermo prevalence measured in oysters from the SLE in 2011, 2012 and 2013. Prior to 2011, mean annual dermo rates ranged from 1 to 18% of sampled oysters. By 2012, infection rates had increased to approximately 30% in the north and south fork sites and to over 60% in the middle estuary. In June 2013, at those SLE stations were live oysters were present, 50 to 100% of sampled oysters were infected; however, infection intensity remained low with most oysters exhibiting only light to moderate infections. Oysters that are physiologically acclimated to high salinities may have reduced ability to cope with sudden decreases in salinity. In other words, the tolerance limits of a high-salinity oyster are unlikely to extend as low as those adapted to optimal or low salinity. It follows that a high-salinity oyster that is weakened by a parasitic infection, i.e., dermo, would be even less able to withstand extreme changes, and more rapidly succumb to death with salinities decrease abruptly.

The LRE was also affected by salinity fluctuations as a result of active wet seasons and water management practices, but there were no extensive oyster mortality events. Except during periodic storm-related inundations of freshwater, the LRE received minimal freshwater inflows and frequently experienced tidal encroachment of oceanic waters. This is a result of physical alterations that included a rerouting of upland waters from the NW Fork to the SW Fork via a flood control canal, and the stabilization of the natural opening of the Jupiter inlet (VanArman et al. 2005). Live oyster densities in the LRE were lower in 2005 than in subsequent years, indicating that the 2004/2005 storms did have some impact, but a rapid and substantial recovery was realized under the higher salinity regime of 2006.

Despite the fact that the LRE was rarely impacted by low salinity events, local salinity regimes did influence oyster density patterns both directly and indirectly. Oftentimes, densities increased between seasons in the NW Fork but remained the same or decreased slightly in the SW Fork. The probable explanation for those differences was the more moderate salinity regime in the NW Fork. While salinities in both forks varied considerably, the NW Fork typically experienced salinities that fell well within the optimal range. In contrast, salinities in the SW Fork were much higher and often exceeded the upper boundary of the optimal range. As a consequence, oyster density in the SW Fork was likely kept in check by the accompanying increase in disease and predation rates. In fact, this suggests that there are benefits to brief declines in salinity, especially if they decrease salinity to optimal levels, as evidenced by the high oyster densities and successful recruitment season recorded in the CRE in 2018.

Both the prevalence and intensity of dermo infections were markedly higher in the LRE than in the CRE or SLE, with the exception of 2011-2013 in the SLE. The most straightforward explanation for differences in parasite incidence is that the salinities in much of the CRE and SLE were too low for completion of life cycle of the *Perkinsus marinus* (dermo) parasite. It is well established that low salinity and low temperature are correlated with reduced levels of infection (Craig et al. 1989), thus oysters in the CRE and SLE exhibited reduced prevalence and intensity of the disease relative to oysters in the LRE. It appears that, because salinity fluctuated so consistently in the SLE prior to 2011 and after early 2013, dermo never gained a foothold and although the parasite was present, the intensity of the infection remained low. The frequent low salinity events in the SLE may have further reduced the success of the parasite by killing off its host organism. Because the LRE has a more stable and higher salinity regime, the oysters that occupy that estuary experience an elevated infection rate. Nonetheless, infection intensity remained low in all three estuaries with levels rarely exceeding a light infection.

Although the timing of reproductive development in oysters varied among sites and years, active reproduction (gametogenesis, spawning, and gonadal recycling) typically occurred from March to October; most oysters entered the resting, or indifferent, stage in November and remained resting through the winter months. Analyses of gonadal tissues showed that during periods with low salinities resulting from storm activity and water releases, most oysters, as long as they survived, continued to develop gametes and spawn as expected; however, there were significantly fewer oysters developing gametes in those years. Oftentimes in those circumstances, spawned larvae did not successfully recruit to the reefs. Whether the larvae were killed outright by the low salinities or simply flushed downstream and out of the estuary is unknown.

General recruitment patterns were similar among estuaries, with recruits commonly present on arrays retrieved from April through December; however, recruitment was often sporadic and inconsistent in the SLE and at the upstream CRE site. Not surprisingly, recruitment rates differed in the SLE, LRE and the CRE. Mean recruitment rates exceeded 11 spat/shell/month in the CRE downstream site, approached or exceeded 2 spat/shell/month in the CRE upstream site and in the LRE, whereas rates in the SLE were generally less than 1 spat/shell/month. Each estuary experienced periodic decreases in oyster densities related to changes in salinity, but. in most cases, recruits were detected shortly after conditions had returned to tolerable. This suggests that even in sites where oysters almost disappeared completely, small relict populations, an exogenous larval source, or most likely a combination of the two, contributed larvae for settlement.

Juvenile growth and predation were studied with a variety of methods, but each methodology yielded similar results. For experiments conducted from 2005 – 2014, growth rates differed among estuaries and were generally higher in the LRE SW Fork, SLE North Fork and the SLE South Fork, where means often reached or exceeded 5 mm SH/month. Those higher growth rates were typical of oysters in southeastern United States, particularly in the Gulf of Mexico, but were more rapid than those reported for more northern populations (Shumway 1996). Consistent with other bivalves, the more rapid growth rate in southern latitudes may be attributed to the longer growing season rather than to an inherently more rapid growth rate (Jones et al. 1990). This distinction is important since factors other than temperature also influence growth. In particular, oysters do not grow well at salinities below 10 (Loosanoff 1953). The ramifications of growth variations can be significant, as faster growing oysters will more quickly escape the ravages of size-limited predators and would also be expected to reallocate energy from growth to reproduction at an earlier age. Growth rates were lower in the LRE NW Fork and the SLE middle estuary, where means were typically between 2 and 3 mm SH/month. Growth rates also differed among years. Growth was slower in 2009 and 2011, but the lowest growth rates were measured in 2010 when means were less than 1 mm SH/month at most sites. This was likely due to the low estuarine salinities, especially in the SLE, caused by the prolonged freshwater release that occurred that year.

Survivorship of tagged juvenile oysters was measured in conjunction with the 2011, 2012 and 2013 growth studies in order to estimate the impacts of macrofaunal predators. The 2011 study (February

2011 – June 2012) was relatively successful as oysters in both the closed and open cages survived for the duration of the experiment at most sites. Predation rates were moderate at most sites with approximately 20% more oysters remaining in the closed cages vs. the open cages. In the 2012 study (July 2012 – December 2013), efforts to quantify predator impacts on oysters planted in the SLE were complicated by the freshwater induced oyster mortalities that occurred in late 2012 and again in 2013. Specifically, most oysters planted in the SLE north and south forks died in September following the sharp salinity decline associated with Hurricane Isaac in August 2012. At the remaining sites, overall survivorship was low but 10 to 15% higher in closed vs. open cages. Similar rates (5 to 15% higher in closed vs. open cages) were measured at most sites in the 2013 study (December 2013 – December 2014); however, survivorship in the SLE north fork was actually higher in the open cages than the closed cages, until the open cage was lost in August 2014. In summary, the mortality rates and the differential survivorship recorded in closed and open cages during these three studies suggest that in addition to death by natural causes, macrofaunal predators were present and actively killing oysters planted in open cages within both estuaries.

In January 2015, a new method of measuring macrofaunal predation rates was initiated in both estuaries. This method involved collecting wild oysters and placing them into open cages in the SLE middle estuary, the LRE NW Fork and at the Bird Island station in CRE downstream site. After a 1-month deployment, the cages were retrieved and all remaining oysters were counted to determine the mortality rate. The method was adopted so that measures of mortality would be more comparable to other monthly measures such as recruitment and disease incidence. Overall mortality rates were similar between LRE and SLE sites, but there were differences between estuaries each year. In the SLE, the highest mortality rates occurred in 2015 when 74% of the experimental oysters were killed. As previously discussed, in 2015 the SLE had moderate salinities and there were no extreme rainfall events or large freshwater releases. The high mortality rate was directly attributable to predation as the optimal salinity regime likely allowed for maximal predation rates. Mortality rates in the SLE were moderate in 2016 and probably tempered by the prolonged freshwater influx that year. SLE mortality rates were low in early 2017, prior to the die-off associated with Hurricane Irma, and low in late 2018 when live oysters were once again available. In the LRE, mortality rates were moderate during all four years. Mortality rates were substantially higher in the CRE than those recorded in the SLE and LRE, as almost 100% of the oysters were lost in most months.

One potential explanation for the high mortality rates in the CRE could be due to the presence of blue crabs. In many months, one or two crabs were found inside the cages when they were retrieved. Its much less clear as to why CRE mortality rates were so much lower in February and March 2018. It doesn't appear to be related to water quality, so one theory is that it could be related to the commercial blue crab fishery. If many crab traps with bait were deployed nearby, that may have kept the predators away from the oyster cages.

Conclusion

This section of the report summarizes oyster population monitoring at five study sites within the SLE and the LRE in southeast Florida from 2005 through 2018 and within two study sites in the CRE estuary in southwest Florida from 2017 through 2018. Oyster abundance, health and population ecology within the three estuaries generally fell within expected ranges for south Florida oyster populations; however, the occurrence of heavy rainfall and subsequent freshwater releases frequently forced estuarine salinities outside tolerable ranges and, as a result, those oyster populations were negatively affected. Although oysters in the SLE repeatedly exhibited the capacity to recover following low-salinity events (and the upstream CRE site in 2018), continued perturbations may so degrade oyster populations that larval supply or availability of appropriate settlement substrate may become limiting factors for recovery. Evidence from past mapping efforts in the SLE suggest that natural reef coverage has declined relative to the historic extent (URS Greiner Woodward Clyde 1999), likely as a result of shell disarticulation and burial by sedimentation. The loss of essential larval settlement substrate provided by an extant reef may require decades to rebuild without intervention (Mann and Powell 2007). In the LRE and at the downstream CRE site, the higher salinity regime also negatively affected oyster populations by favoring higher disease and predation rates, but those effects were more slowly realized. The immediate benefits of increased periods with optimal salinity conditions have been documented in this and previous reports; however, those benefits may extend into the future by allowing oysters a greater capacity to deal with the environmental stressors that arise when estuarine conditions decline.

ACKNOWLEDGMENTS

This research was funded by the South Florida Water Management District as part of the monitoring and assessment component of the Comprehensive Everglades Restoration Plan under the district's agreement numbers CP40614, 4600001084, 4600002548 and 4600003152. The authors would like to thank Barbara Welch and Patricia Gorman for their guidance and support. The authors would also like to thank numerous past and present FWRI Molluscan Fisheries staff, including Bill Arnold, Steve Geiger, Sarah Stephenson, Janessa Cobb, Brett Pittinger, Carla Beals, Mark Gambordella, Michael Hofmann, Brandy Brown, Jennifer Davenport, Anthony Vasilas, Michael Drexler, Lindsey O'Hern, Bethany Pierce, Octavia Williams-Poole, Ashley Hurley, Christopher Kirby, Michael Poniatowski, Benjamin Gutzler, Matthew Davis, Britt Burke, Nicole Martin, Nick Tolopka, Erica Levine, Heidi Waters, Nicole Maloney, Matthew LaGanke, Takoda Edlund, Austin Heil, Tomena Scholze and Michael Mosser for their invaluable assistance in the field and laboratory.

REFERENCES

Bahr, L. M. and W. P. Lanier. 1981. The ecology of intertidal oyster reefs of the South Atlantic coast: A community profile. U.S. Fish and Wildlife Service, Office of Biological Services, Washington, D.C. FWS/OBS-81/15, 105 pp.

Barnes, T. K., A. K. Volety, K. Chartier, F. J. Mazzotti and L. Pearlstine. 2007. A habitat suitability index model for the eastern oyster (*Crassostrea virginica*): a tool for restoration of the Caloosahatchee Estuary, Florida. *Journal of Shellfish Research* 26: 949-959.

Carriker, R. R. and T. Borisova. 2011. Florida's water resources. Florida Cooperative Extension Service technical series FE757. Gainesville, FL: University of Florida. 7 pp.

Christmas, J. F., M. R. McGinty, D. A. Randle, G. F. Smith and S. J. Jordan. 1997. Oyster shell disarticulation in three Chesapeake Bay tributaries. *Journal of Shellfish Research* 16: 115-123.

Craig, A., E. N. Powell, R. R. Fay, and J. M. Brooks. 1989. Distribution of *Perkinsus marinus* in Gulf coast oyster populations. *Estuaries* 12: 82-91.

Fisher, W. S., J. T. Winstead, L. M. Oliver, H. L. Edmiston and G. O. Bailey. 1996. Physiological variability of eastern oysters from Apalachicola Bay, Florida. *Journal of Shellfish Research* 15: 543-555.

Ford, S. E., M. J. Cummings and E. N. Powell. 2006. Estimating mortality in natural assemblages of oysters. *Estuaries and Coasts* 29: 361-374.

Grizzle, R. E., L. G. Ward, J. R. Adams, S. J. Dijkstra, and B. Smith. 2005. Mapping and characterizing subtidal oyster reefs using acoustic techniques, underwater videography, and quadrat counts. *American Fisheries Society Symposium* 41: 153-159.

Haunert, D. E., F. Lund, J. Steward, and R. Virnstein (eds.). 1994. Surface water improvement and management (SWIM) plan for the Indian River Lagoon. South Florida Water Management District, West Palm Beach, FL, USA.

Jones, D. S., I. R. Quitmyer, W. S. Arnold, D. C. Marelli. 1990. Annual shell banding, age, and growth rate of hard clams (*Mercenaria* spp.) from Florida. *Journal of Shellfish Research* 9: 215-225.

Klinck, J. M., E. E. Hofmann, E. N. Powell, and M. M. Dekshenieks. 2002. Impact of channelization of oyster production: a hydrodynamic oyster population model for Galveston Bay, Texas. *Environmental Monitoring and Assessment* 7: 273-289.

La Peyre, M. K., B. Grossman, and J. F. La Peyre. 2009. Defining optimal freshwater flow for oyster production: effects of freshet rate and magnitude of change and duration on eastern oysters and *Perkinsus marinus* infection. *Estuaries and Coasts* 32: 522.534.

Lenihan, H. S., and C. H. Peterson. 1998. How habitat degradation through fishery disturbance enhances impacts of hypoxia on oyster reefs. *Ecological Applications* 8: 128-140.

Littell, R. C., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schaenbenburger. 2006. SAS for mixed models, 2nd edition. Cary, NC: SAS Institute Inc. 814 pp.

Livingston, R. J., X. Niu, F. G. Lewis, III and G. C. Woodsum. 1997. Freshwater input to a gulf estuary: long term control of trophic organization. *Ecological Applications* 7: 277-299.

Loosanoff, V. L. 1953. Behavior of oysters in water of low salinities. Proceedings of the National Shellfisheries Association 43: 135-151.

MacKenzie, C. L., Jr., V. G. Burrell, Jr., A. Rosenfield and W. L. Hobart (eds.). 1997. The history, present condition, and future of the molluscan fisheries of North and Central America and Europe, Volume 1, Atlantic and Gulf Coasts. U.S. Department of Commerce, NOAA Technical Report 127, 234 pp.

Mackin, J. G. 1959. A method of estimation of mortality in oysters. *Proceedings of the National Shellfisheries Association* 50: 41-51.

Mackin, J. G. 1962. Oyster diseases caused by *Dermocystidium marinum* and other microorganisms in Louisiana. *Publication of the Institute of Marine Sciences, University of Texas* 7: 132-229.

Mann, R. and E. N. Powell. 2007. Why oyster restoration goals cannot be achieved. *Journal of Shellfish Research* 26: 905-917.

Menzel, R. W., N. C Hulings and R. R. Hathaway. 1966. Oyster abundance in Apalachicola Bay, Florida in relation to biotic associations influences by salinity and other factors. *Gulf Research Reports* 2: 73-96.

Paperno, R., D. M. Tremain, D. H. Adams, A. P. Sebastian, J. T. Sauer, and J. Dutka-Gianelli. 2006. The disruption and recovery of fish communities in the Indian River Lagoon, Florida, following two hurricanes in 2004. *Estuaries and Coasts* 29: 1004-1010.

Ray, S. M. 1966. A review of the culture method for detecting *Dermocystidium marinum*, with suggested modifications and precautions. *Proceedings of the National Shellfisheries Association* 54: 55-69.

Shapiro, S. S., and M. B. Wilk. 1965. An analysis of variance test for normality (complete samples). *Biometrika* 52: 591-611.

Shaw, B. L., and H. I. Battle. 1957. The gross and microscopic anatomy of the digestive tract of the oyster *Crassostrea virginica* (Gmelin). *Canadian Journal of Zoology* 35: 325-347.

Shumway, S. E. 1996. Natural environmental factors. In: V.S. Kennedy, R.I.E. Newell & A.F. Eble, editors. The Eastern Oyster *Crassostrea virginica*. College Park, Maryland: Maryland Sea Grant College, University of Maryland System, pp. 467-513.

Sime, P. 2005. St. Lucie Estuary and Indian River Lagoon Conceptual Ecological Model. *Wetlands* 25: 898-907.

Southworth, M., and R. Mann. 2004. Decadal scale changes in seasonal patterns of oyster recruitment in the Virginia sub estuaries of the Chesapeake Bay. *Journal of Shellfish Research* 23: 391-402.

Steward, J. S., R. W. Virnstein, M. A. Lasi, L. J. Morris, J. D. Miller, L. M. Hall, and W. A. Tweedale. 2006. The impacts of the 2004 hurricanes on hydrology, water quality, and seagrass in the central Indian River Lagoon, Florida. *Estuaries and Coasts* 29: 954-965.

Turner R. E. 2006. Will lowering estuarine salinity increase Gulf of Mexico oyster landings? *Estuaries and Coasts* 29: 345-352.

URS Greiner Woodward Clyde. 1999. Distribution of oysters and submerged aquatic vegetation in the St. Lucie estuary: final report. West Palm Beach, FL: South Florida Water Management District. 80 pp.

U.S. Army Corps of Engineers Jacksonville District and South Florida Water Management District. 1999. Central and southern Florida project comprehensive review study: final integrated feasibility report and programmatic environmental impact statement. Jacksonville, FL: U.S. Army Corps of Engineers. 4034 pp.

VanArman, J., G. A. Graves and D. Fike. 2005. Loxahatchee watershed conceptual ecological model. *Wetlands* 25: 926-942.

Volety, A., K. McFarland, E. Darrow, D. Rumbold, S. G. Tolley and A. N. Loh. 2016. Oyster monitoring network for the Caloosahatchee Estuary 2000-2015. Final Report submitted to the South Florida Water Management District, 73 pp.

Wilber, D. H. 1992. Associations between freshwater inflows and oyster productivity in Apalachicola Bay, Florida. *Estuaries and Coastal Shelf Science* 35: 179-190.