Survey of Select Eastern Oyster (*Crassostrea virginica*) Populations in Lake Worth Lagoon, Palm Beach County, Florida

2010 Annual Report

Palm Beach County, Department of Environmental Resources Management

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Introduction

Oysters provide numerous ecological benefits including habitat diversity, erosion control and improvement of water quality. Restoration and enhancement of natural oyster reefs and the creation of additional oyster reefs are important components of Palm Beach County Department of Environmental Resources Management's (PBC-ERM) mission to restore and manage the Lake Worth Lagoon (LWL) ecosystem. To this end, monitoring projects are necessary to determine the health and productivity, not only of existing natural oyster beds, but to determine the success of artificially created oyster beds.

This report presents the findings of a two year (March 2008-February 2010) monitoring project by Harbor Branch Oceanographic Institute at Florida Atlantic University of two natural oyster reefs located in the northern and central Lake Worth Lagoon, and one artificially created oyster habitat in the central Lagoon. The sites chosen by PBC-ERM were a natural bed located at John D. MacArthur Beach State Recreation Park located in the estuary off the northern end of the Lake Worth Lagoon, a natural bed located at Ibis Isle in the central Lagoon, and a created bed centrally located at the Snook Islands Natural Area in the central Lagoon.

Objectives

The major objective of this study was to determine the health and productivity of each site and the potential for expanding reefs at or near these locations. This was accomplished by monitoring changes in: 1) environmental and water quality parameters, 2) size and density of adult oysters, 3) physiological condition and reproductive potential of adult oysters, 4) recruitment of larval oysters, 5) growth of newly set oysters, and 6) prevalence and intensity of oyster diseases, with emphasis placed on Dermo (*Perkinsus marinus*) and MSX (*Haplosporidum nelsoni*).

Methods

Sites were visited monthly, typically at low tide, where temperature, salinity, dissolved oxygen, pH and water clarity measurements were taken, and ten (MacArthur, Ibis Isles) to twelve (Snook Island) adult eastern oysters, *Crassostrea virginica*, were collected. All oysters were weighed and measured. The physiological condition of half of the sampled oysters at each site were analyzed by condition index (comparison of dry tissue to dry shell weight), while the remaining oysters were processed for reproductive and disease analysis using histological and other (Ray's fluid thioglycollate medium) methods for parasitic determination. Oyster spat recruitment was monitored by counting the number of settled spat that collected on strings of oyster shell deployed along the edge of the oyster beds that were removed and replaced each month. Juvenile growth was monitored by measuring the shell height of settled oysters on fixed oyster arrays likewise containing oyster shell that were deployed near the spat collectors. Twice a year, in spring (April) and fall (October), a survey of the adult oyster population was conducted to determine the density and size of live and dead oysters at each site.

Results

Water Quality Parameters

No variation was seen between the northern and central sites in regards to temperature or other environmental parameters measured, except for salinity. Salinity varied significantly between sites, with the two central sites experiencing wide variation in salinity. Differences were especially apparent in summer, when salinities at the central sites (Ibis Isle and Snook Island) were 10-15 ppt lower than at the northern MacArthur site. MacArthur had the highest average salinity and Ibis Isle the lowest salinity of the three sites (Fig. 1).

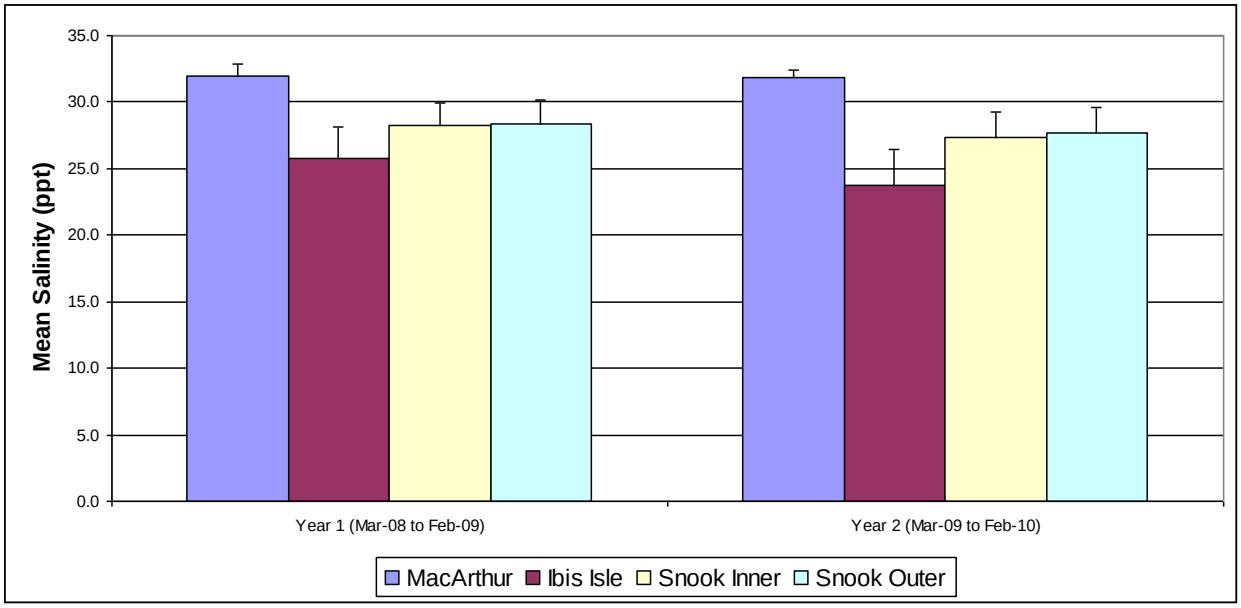

Figure 1. Annual average salinity (±SE) at oyster monitoring sites in Lake Worth Lagoon from March 2008-February 2010.

Adult Oyster Density

Live oyster density was significantly greater at Ibis Isle compared to MacArthur for both years of the study, and significantly greater compared to Snook Island in year one (Fig 2). Densities were higher in fall than in spring at all sites. Oyster density at Snook Island showed no vertical distribution differences between the base and middle of the riprap, but oysters were rarely found at the top of the Island.

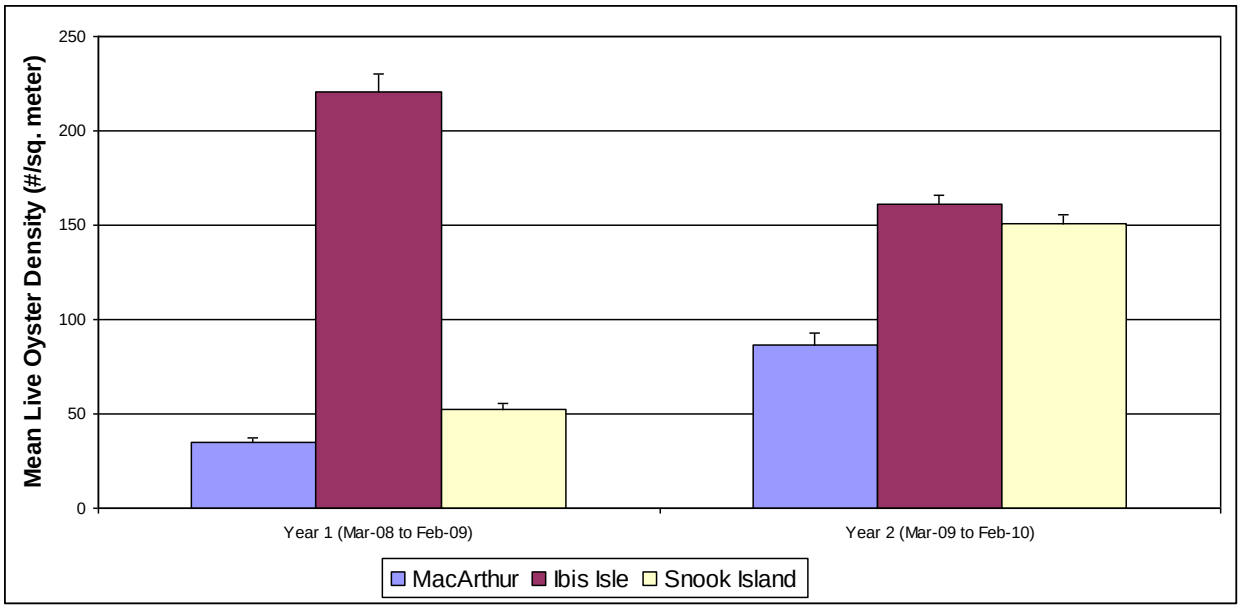

Figure 2. Average annual oyster density (±SE) of sampling sites in Lake Worth Lagoon from March 2008 to February 2010.

Physiological Condition

Oysters at Snook Island had the highest condition index (CI) in year one, while oysters at MacArthur had the highest CI in year two (Fig 3). CI was high at all sites during the winter. Low CI's were seen in the spring of year one at both MacArthur and Snook Island. Oysters at MacArthur had significantly higher CI's from August through December 2009 than the two central Lagoon sites.

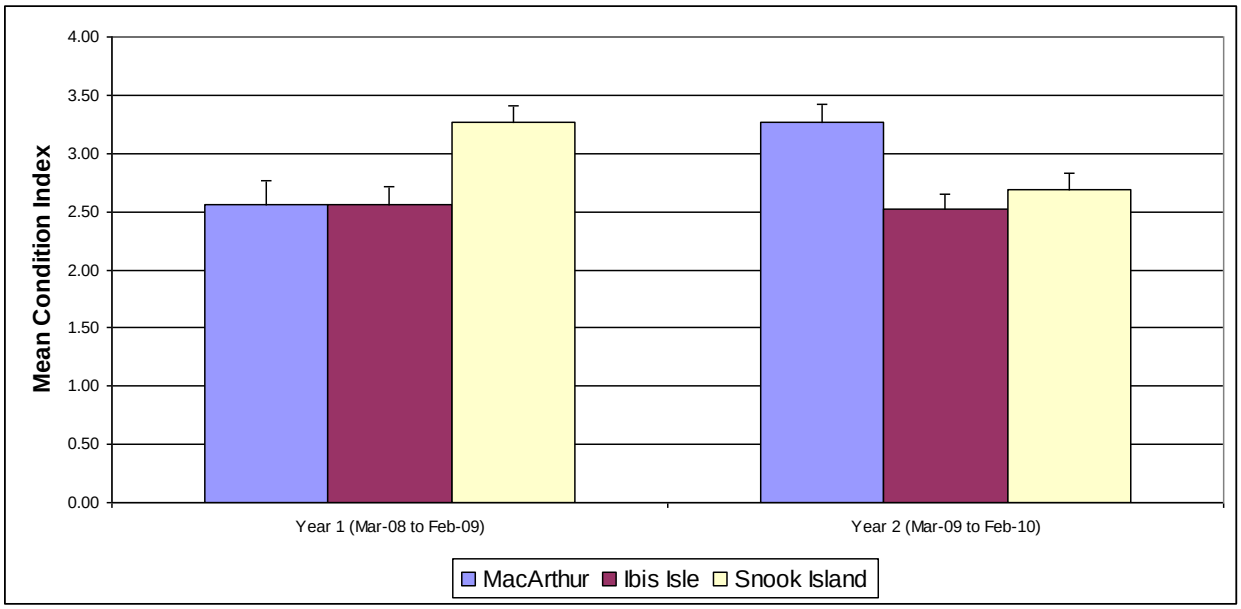

Figure 3. Average annual (±SE) condition index (CI) of oysters sampled from sites in Lake Worth Lagoon from March 2008 to February 2010.

Reproduction

Oysters were reproductively active at all sites, except during the winter. Oysters at the central Lagoon sites had a prolonged spawning period in year one compared to year two. Reproduction of oysters at MacArthur significantly lagged behind those at the two central Lagoon sites in year one but not in year two. Two reproductive peaks were seen at all three sites in year one in early summer and fall. In year two only oysters at Snook Island had two reproductive peaks, one in the spring and another in the fall. Reproductive peaks occurred only in the fall at both Ibis Isle and MacArthur in year two.

Disease

Dermo (*Perkinsus marinus*) was present year round at all three sites. Annual prevalence was similar, although levels were slightly lower at Snook Island (Fig. 4). Prevalence was lowest at all sites during the winter and low at the central sites concurrent with low salinity events. Intensity, as defined on a 0-5 Mackin scale, ranged from 0-4.5. Average annual intensities ranged from 0.85-1.2, with MacArthur having the highest annual intensities and Snook Island the lowest. No MSX (*Haplosporodium nelsoni*) was found at any of the sites. Other parasites of note included the gregarine protozoan *Nematopsis sp.*, turbellarians, the trematode *Bucephalus*, the cestode *Tylocephalum*, cnidarians of the genus *Eutima sp.* and the pea crab *Pinnotheres sp*.

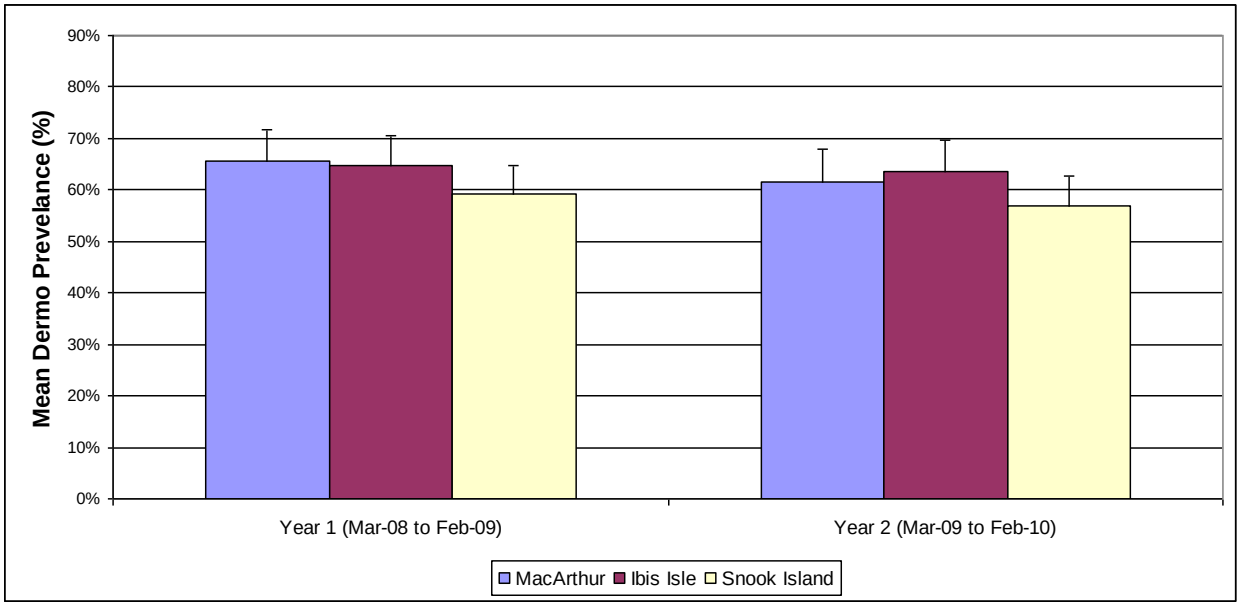

Figure 4. Annual average (±SE) Dermo prevalence at sampling sites in the Lake Worth Lagoon from March 2008 to February 2010.

Larval Recruitment

The highest larval recruitment occurred at Snook Island and the lowest at MacArthur (Fig. 5). Bi-annual recruitment peaks occurred only at Snook Island. Larval recruitment occurred from late spring/early summer through fall at all sites, and followed the same pattern as reproductive activity. Level of recruitment appeared to be related to adult oyster density.

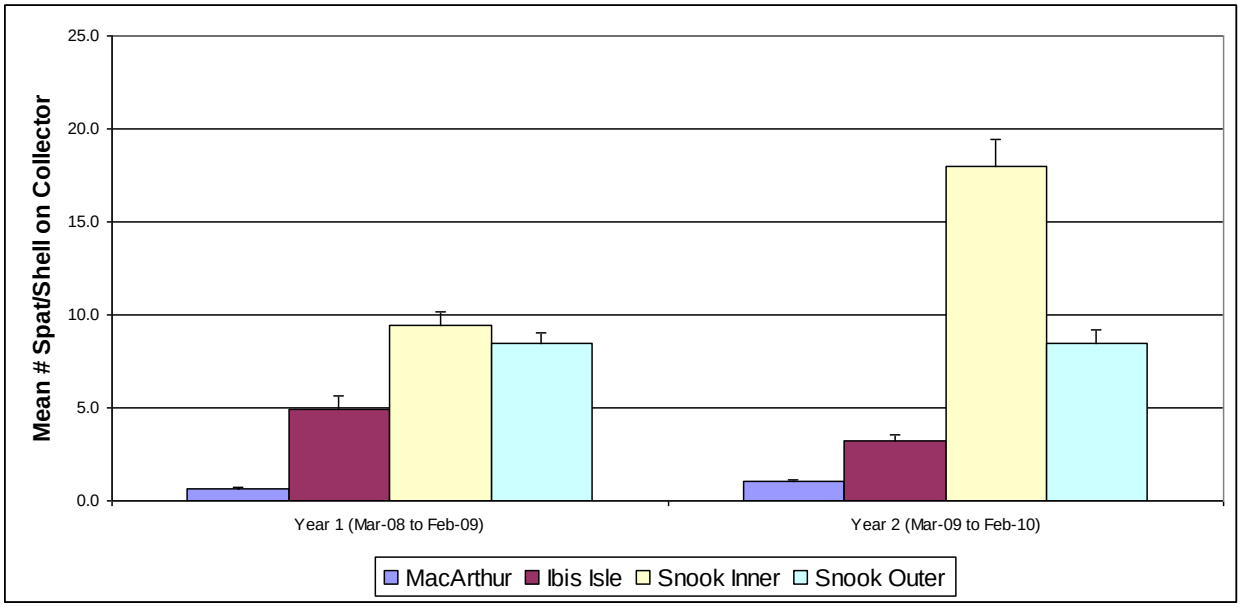

Figure 5. Average annual (±SE) number of spat collected at sampling sites in the Lake Worth Lagoon from March 2008 to February 2010.

Juvenile growth

Growth, as defined by shell height, was highest at Ibis Isle in year one and at MacArthur in year two (Fig. 6). Growth was lowest at Snook Island both years, and lower at Snook Island-Outer than Snook Island-Inner, although numerous problems plagued the collection of data at Snook Island-Outer.

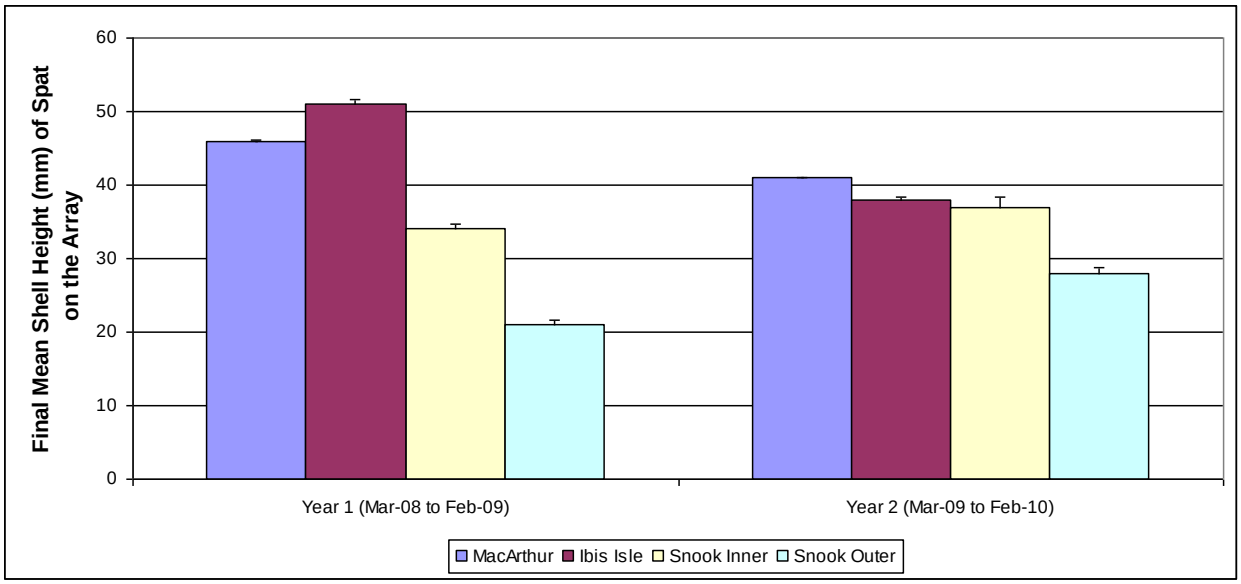

Figure 6. Final annual mean shell height (±SE) of juvenile oysters on shell arrays at sampling sites in Lake Worth Lagoon.

Discussion

Both temperature and salinity played a role in determining reproduction, recruitment and disease prevalence and intensity. Expected seasonal temperature variation was seen, with the exception of an unprecedented cold spell in January of 2010. Although no immediate ramifications were apparent during the final February monitoring, effects may have occurred after the study's conclusion. No variation was seen between the northern and central sites in regards to the other environmental parameters measured, with the exception of salinity. Salinity varied significantly between sites, with the two central sites experiencing wide variation in salinity. MacArthur had the highest overall salinity and Ibis Isle had the lowest overall salinity.

Based on oyster density alone, Ibis Isle was the most productive of the three areas, followed by Snook Island and lastly MacArthur. The oyster bed at MacArthur had the lowest oyster density in spring and fall of both years, whereas the oyster bed at Ibis Isle had the highest density. Increases in density from spring to fall were seen at all sites with the exception of Snook Island in year one. This increase is expected based on recruitment that takes place in the Lake Worth Lagoon from spring to fall.

None of the sites exhibited a consistently higher condition index (CI) than another. CI was highest for Snook Island in year one and for MacArthur in year two. Salinity stress may have lowered the CI at the two central sites in year two due to fresh water releases. A prolonged period of low salinity occurred in the central Lagoon during the summer of year two compared to the previous summer. Restoration activities occurring at Ibis Isle during the summer likely added to oyster stress. However, this higher CI did not translate into increased reproductive activity or recruitment for MacArthur in year two.

Reproductive activity occurred continuously at all sites from spring to fall. Greater and longer reproductive activity was generally seen in year one in comparison to year two. Reproductive peaks occurred in either April or June and again in September. As CI decreased in the central Lagoon during the summer of year two so did reproductive activity. Recruitment followed the same pattern as reproductive activity in year two and was concentrated in the fall.

Dermo (*Perkinsus marinus*) was prevalent at all sites year round, though variability was seen between sites, seasons, and years. Intensities generally followed prevalence. Lowered prevalence and intensity occurred concurrent with lowered winter temperatures. As temperature was similar at all sites, salinity contributed to the variability seen between sites. Ibis Isle and Snook Island experienced a more variable salinity than MacArthur, and consequently a lower prevalence and intensity of Dermo. MSX (*Haplosporidum nelsoni*) was not noted in samples from any site. In addition to Dermo, parasites detected in this study included the gregarine protozoan *Nematopsis sp.*, turbellarians, the trematode *Bucephalus*, and the cestode *Tylocephalum*. Mollusks are intermediate hosts for most of these species and their presence in the oysters is indicative of a healthy ecosystem.

Recruitment occurred at all sites from spring through fall, but the level varied significantly depending on the site. Recruitment was constant and low at MacArthur, while major peaks occurred in the fall in the central Lagoon. Two periods of peak recruitment were seen at Snook Island; summer and fall (year one) and spring and fall (year two). A relationship appears to exist between adult density and recruitment as the lowest recruitment was seen at MacArthur, which also had the lowest adult density.

Growth of juvenile oysters varied between sites and years. Growth was greater at MacArthur and Ibis Isle than at Snook Island. Final shell height was greater in year one than in year two at MacArthur and Ibis Isle, but greater at Snook Island in year two. Growth was consistently lower at Snook Island-Outer than at the other sites. Several factors, including heavy predation by oyster drills in year one and continual overlay of new spat, may explain the lower growth at this site. Snook Island was the only site with two recruitment peaks. The overlay of new spat settling on arrays may have had the effect of decreasing the overall mean shell height.

Conclusions

Oyster densities on Florida's west coast (Caloosahatchee River) are much higher than all east coast sites, including the LWL. In all other parameters measured (i.e., reproductive activity, juvenile growth, and condition index) LWL oysters are comparable to those of other Florida east and west coast oysters within year to year variability. Based on these studies further oyster reef restoration projects within LWL should be successful whether projects considered are large, such as the Snook Island project, or small in scope, such as envisioned in the Living Shoreline projects.

All three sites monitored over the past two years have healthy oysters present. Although the natural oyster bed at MacArthur is subjected to less salinity pressure, it had the lowest density

and recruitment. Still, oysters at MacArthur had as high or higher CI and juvenile growth than oysters in the central Lagoon. The natural oyster beds at Ibis Isle had the highest density and single-month recruitment, but the site is substrate limited. The artificially constructed Snook Island environmental enhancement area had the highest annual recruitment (twice that of Ibis Isle and ten times that of MacArthur), but oyster density was only one-fifth to three-quarters that of Ibis Isle, though twice as high as at MacArthur, indicating this site may be substrate limited. Although no oysters grew on the top of the vertical structure, it is recommended that the height not be decreased due to the eventual wear and movement of rock at the base of the Island.

Lake Worth Lagoon is a productive system with patches of healthy oyster beds that provide the recruitment necessary to seed large (Snook Island) and small restoration projects, as long as other environmental factors (salinity, hydrology, food availability) and substrate type (hard bottom, mud) are considered. Based on this two year monitoring study, additional restoration projects should not only be successful, but would improve water quality, provide erosion control and increase habitat for associated species such as other invertebrates, fish, and birds.

INTRODUCTION

The Eastern (or American) oyster *Crassostrea virginica* is abundant throughout much of the Atlantic and Gulf of Mexico coasts of the U.S. (Carriker and Gafney 1996). This commercially valuable species is typically found in shallow, moderate salinity waters and attaches to both hard and soft substrata (Shumway 1996). The Eastern oyster has been the subject of farming and aquaculture since the 1800's and in the last two decades the focus of restoration (e.g., Coen et al. 1999) as oyster reefs or beds are considered an imperiled marine habitat (Beck et al. 2009).

In addition to their economic importance as a fishery, oyster reefs are important in providing a habitat for numerous other species, in improving water quality and in nutrient cycling. Species commonly associated with oysters include encrusting organisms such as bryozoans, barnacles, sponges, mussels and other bivalves (Wells 1961). Species that live within oyster reefs include various crustaceans (crabs, shrimp), worms, gastropods (snails) and fish (gobies, blennies, pipefish). Species of commercial value associated with oyster reefs include flounder, striped bass, cobia, Spanish mackerel, pinfish, blue crab, stone crab, penaeid shrimp, black drum, and several species of mullet.

Oysters are suspension feeders and remove particles from the water column. The filtration activity of *Crassostrea virginica* in Chesapeake Bay has been estimated to be 163 liters per gram (of oyster tissue) per day (Newell 1988). Many of the encrusting organisms that live on oysters (tunicates, barnacles) and other organisms that live within the oyster reefs are also suspension feeders and contribute to improving water quality.

In Florida, restoration of Eastern oyster populations is an important component of the Comprehensive Everglades Restoration Program (CERP) and is a matter of prime importance for local governments, environmental organizations, and the general public. The Eastern oyster has been selected as a target species for monitoring and restoration because of its wide distribution, historical context, and essential habitat value (e.g., Coen and Grizzel 2007, Volety et al. 2009). Restoration and management efforts have to be based upon historical and present knowledge of populations and their potential for expansion. Returning function to a habitat is the ultimate goal of a restoration effort. Monitoring is an essential part of all restoration projects and should be performed before, during, and after restoration to assess changes throughout the course of the project in order to evaluate, among other things, the habitat, the restoration technique, the stressors, the sites proposed for restoration, and to facilitate management. Environmental managers estimate there are over 185 acres of suitable area for oyster reef enhancement in the Lake Worth Lagoon. Oyster reefs are found in several locations within the Lagoon including Munyon Island, Ibis Isle, Snook Island, Everglades Island, Audubon Island, John's Island and John D. MacArthur Beach State Park. To that end, health and abundance of oysters were measured at three of those sites within the Lake Worth Lagoon (LWL) system of Palm Beach County. Growth, condition index, recruitment, reproduction, and disease status of Eastern oysters, *Crassostrea virginica*, at two natural (Ibis Isle, MacArthur) and one man-made (Snook Island) sites in the Lake Worth Lagoon system (Fig. 7) were measured from March 2008 through February 2010. Methodology generally followed that of similar oyster monitoring projects (Arnold et al. 2008; Parker and Geiger 2009; Volety et al. 2009) to assist in data comparison.

The data generated from this study will augment ongoing Eastern oyster research being conducted through CERP.

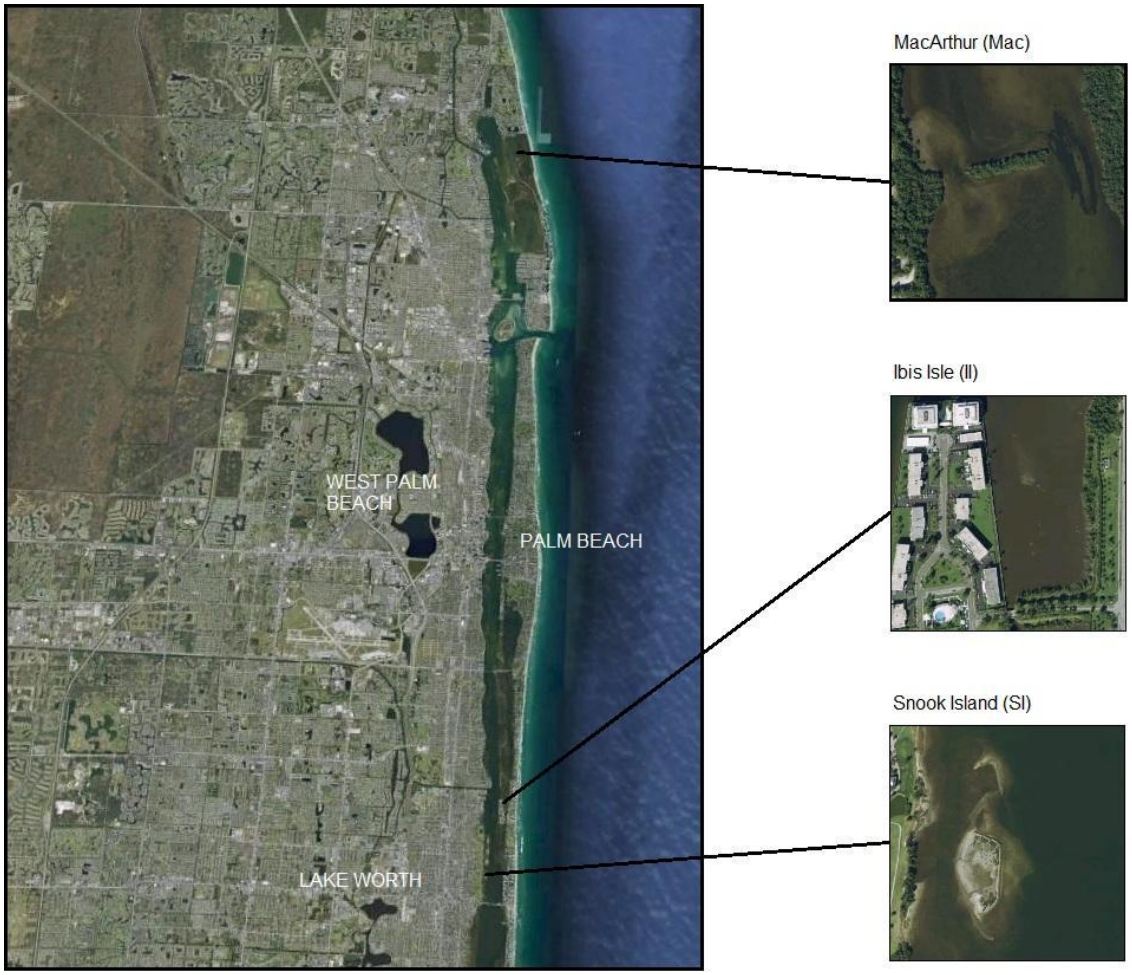

Figure 7: Satellite view of north and central portion of the Lake Worth Lagoon detailing the MacArthur, Ibis Isle and Snook Island sampling sites.

MATERIALS AND METHODS

Sampling Sites, Dates and Environmental Conditions:

The project was initiated on 21 March 2008. Oyster populations monitored in the Lake Worth Lagoon (LWL) of Palm Beach County, Florida were located at the northern portion of the LWL within John D. MacArthur Beach State Park (Fig. 1A), central LWL near Ibis Isle (Fig. 1B), and central LWL at Snook Islands (inner and outer side of one island) near the Lake Worth Municipal Golf Course (Fig. 1C). The Snook Islands are a series of man-made mangrove and rock islands that were constructed in 2005 of fill and limestone boulder riprap laid upon a sand base (Carson 2007).

Figure 8. Oyster monitoring sites in Lake Worth Lagoon: A) John D. MacArthur Beach State Park, B) Ibis Isle, and C) Snook Islands. Arrows indicate location of spat collectors and shell array.

Sites were examined near low tide. Sampling occurred in 2008 on 21 March, 16-18 April (survey month), 16 May, 16 June, 1-2 July, 1-2 August, 11 September, 13-15 October (survey month), 12 November, and 9 December. In 2009, sampling occurred on 9 January, 9 February, 9 March, 21-24 April (survey month), 21 May, 23-24 June, 21 July, 18 August, 15-16 September, 13-16 October (survey month), 17 November, and 14 December. In 2010, sampling occurred on 11 January and 10 February.

The following environmental measurements were taken during each sampling:
a) Dissolved oxygen (mg/L and % saturation) using a YSI-85 meter (Yellow Springs Instruments),
b) Salinity (ppt) using a YSI-85 meter,
c) Water temperature (^{o}C) using a YSI-85 meter,
d) Air temperature (^{o}C) using a standard thermometer,
e) Water clarity or light penetration (cm) using a 20 cm diameter secchi disk, and
f) pH using a handheld pH meter (pHep, Hanna Instruments).

Condition Index, Reproductive Stage, and Disease Monitoring:

Ten to twelve Eastern oysters, greater than 51 mm in height (hinge to lip), were collected monthly at each site. Oysters from each site were placed in a cooler with ice packs and transported live to Harbor Branch Oceanographic Institute in Fort Pierce, Florida for processing the same or next day. Oysters were processed for condition index, reproductive stage and disease status as follows. For condition index, five to six oysters from each site were thoroughly cleaned of external fouling organisms and oyster spat under fresh running water. Oyster height

(dorsal-ventral or hinge to lip) was measured using digital Vernier calipers (Fisher Scientific, model 06-664-16). Oyster whole-weight, meat weight and shell weight were measured using a digital balance (Denver Instruments, model P-230). Oysters were shucked by hand with a shucking knife and meat was separated from the shell halves. Meat and shells were placed in separate numbered pre-weighed aluminum pans, weighed, and then dried in an oven for 24-48 hrs at 80-100°C. Condition index was calculated as the proportion of tissue dry weight to shell dry weight, multiplied by 100 (Wilson et al. 2005).

The remaining five to six collected oysters were processed for reproductive and disease analyses. For reproductive analysis, a 5-10 mm thick section of tissue encompassing the gonad was cut transversely with a razor blade. The tissue was placed in a tissue holder and placed in Davidson's fixative for 48-72 hr before being transferred to 70% ethanol for subsequent histological preparation (Barber 1996, Wilson et al. 2005). Histological preparation consisted of dehydrating each gonad sample through a series of ethanol solutions (70-100%) for a minimum of one hour each, followed by clearing with toluene and then embedding the tissue sample in paraffin. Multiple 5-8 µm sections were cut from each embedded sample using a microtome, maintaining a minimum separation of 60 µm (the approximate maximum diameter of an oocyte) between sections. Thin sections were stained with hematoxylin and eosin and then mounted on pre-labeled glass slides for examination. Resultant slides were examined at 100-400x with the aid of a compound microscope and each sample assigned to a reproductive stage following a classification scheme (Table 1) modified from the work of Fisher et al. (1996).

Table 1. Qualitative reproductive stage criteria for Eastern oysters, *Crassostrea virginica*.

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

The tissue that remained following the extraction of the gonad sample was utilized for assessment of Dermo disease, a bivalve protozoan parasite (*Perkinsus sp.*). Dermo was quantitatively assessed using Ray's fluid thioglycollate medium (RFTM) as described by Bushek et al. (1994) as follows. Small pieces (approximately 1 cm^2) of gill and mantle tissue were incubated in RFTM with antibiotics/antimycotics for 5-7 days at room temperature (20-25°C).

Tissue pieces were then placed on glass microscope slides, macerated with a razor blade, stained with Lugol's iodine solution, and examined microscopically (400x) for the presence of hypnospores. Parasite density (i.e., infection intensity) was ranked using the Mackin scale, which ranges from 0 (no infection) to 5 (heavy infection). Average parasite density and prevalence (i.e., proportion of oysters with Dermo) were calculated monthly for each site.

Slides used for reproductive stage analysis were also used to determine the presence of MSX and other parasites. Finished slides were examined microscopically (400-1000x) for MSX and other parasites. Infections were classified as light, moderate, or heavy. Mean prevalence (i.e., proportion of oysters infected) of parasite infections was calculated monthly for each site.

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

Stage	Category	Cell Number	Notes
0	Uninfected	No cells	
0.5	Very light	< 10 cells in entire preparation	
1.0	Light	11-100 cells in entire preparation	Cells scattered or in clusters of 10-15 cells
2.0	Light-moderate		Localized infections of 25-50 cells or 2-3 parasites in each field at 100 X
3.0	Moderate	2-3 parasites in all fields at 100X	Majority are localized
4.0	Moderate-heavy	Parasites present in large numbers	Less than 50% of tissue appears blue black macroscopically
5.0	Heavy	Enormous numbers of parasites	Majority of tissue stains blue black when examined macroscopically

Spat Recruitment:
Oyster spat recruitment was monitored by using spat collectors (Arnold et al. 2008, Parker and Geiger 2009). At each site, three replicate spat collectors were deployed along the edge of the oyster bed facing open water (Figs. 2A-D). Each collector consisted of two sets of six axenic adult oyster shells (5.5-7.5 cm shell height) strung together on galvanized wire and hung from a 1.9 cm diameter PVC T-bar (approximately 79 cm tall and 33 cm across at the T); one set of shells on each side of the T. Shells were oriented with their inner surface facing down when suspended off the bottom; lowest shell was 5-10 cm off the bottom. Oyster recruitment was calculated by counting the number of settled spat on the underside of each strung shell and dividing by the number of total shells. Visualization of oyster spat was assisted by using a lighted magnifier (LumaPro, 5 Diopter Lens, Grainger Intl., IL). Spat collectors were deployed in March 2008 and recovered and replaced monthly.

Oyster Growth Monitoring:
Growth of oysters was monitored at each site by using shell arrays. A single array was deployed close to the spat collectors at each site (Fig. 2C & 2D). Each array consisted of twenty five axenic oyster shells (51-75 mm shell height with a 6.8 mm hole drilled centrally) anchored by

fishing line to a 60x60 cm piece of PVC-coated wire mesh (12.5 mm openings). The array was placed horizontally at the edge of each reef site and kept off the bottom by two PVC pipes (10 cm diam.). The array was anchored to the substrate by nylon rope secured to a trailer anchor, which was screwed into the sand/mud bottom, and marked with a small white foam buoy (Fig. 2C & 2D). The oyster growth monitoring arrays were examined monthly and the shell height of 30 random oyster recruits, or all oysters if less than 30 oysters, were measured. The arrays set out in April 2008, which coincides with the beginning of oyster spawning season, were removed in March 2009 and a new set of arrays were deployed. Shells in the new arrays were attached to the wire mesh using small plastic cable ties, which were easier to use compared to fishing line.

Figure 9. Oyster spat collectors (PVC T-bars with strung shells) at each monitoring site in the Lake Worth Lagoon; A) MacArthur Beach State Park, B) Ibis Isle, C) Snook Island – Inner, and D) Snook Island – Outer. Oyster growth monitoring arrays with white marker buoy can be seen in C and D. Survey quadrat can be seen in B.

Semi-Annual Adult Oyster Survey:
A survey of oysters at each site was conducted semi-annually in spring (April) and fall (October). At MacArthur and Ibis Isle, up to 15 ¼-m^2 quadrats were randomly deployed on the reef (Fig. 2B), whereas at Snook Island transects were made over the rock riprap to accommodate vertical height and included both the inner and outer sections. All oysters within each quadrat were moved, except at Snook Island as they were firmly attached to the rock, for determination of the number of live and dead oysters with articulated shells. Shell height was measured with a flexible plastic ruler for a maximum of 30 randomly selected live oysters for each quadrat (max n=450 oyster measured/site). Quadrat location on Snook Island was noted as bottom, middle or top, as the oysters were attached to the limestone riprap at different heights.

Statistical Analysis:
Statistical analyses on monthly data sets were performed using SAS or Systat software. Measurements for each parameter were compared between sites using ANOVA or Robust ANOVA (to improve normality or homogeneity of ranked data) and means separated using Tukey's significant difference post-hoc test. All comparisons were considered significant if $P \leq 0.05$.

RESULTS

Environmental Conditions
Temperature was similar at all sites, and followed the typical seasonal pattern in year one. Temperature ranges were more extreme in year two, with winter temperatures being atypically low for Florida. Water temperature ranged from 16.8-32.0^{o}C in year one, and from 8.7-35.1^{o}C in year two (Fig. 10). Air temperature generally followed water temperature.

Salinity varied greatly between sites, but was generally higher in winter and spring and lower in summer and fall at all sites (Fig. 11). Salinity was consistently higher at MacArthur and ranged from 25-36 ppt (Fig. 11). Salinity was extremely variable in the central LWL (7-35 ppt) and although both sites exhibited a similar pattern, salinities at Ibis Isle were lower than at Snook Island from summer to fall (Fig. 11).

Dissolved oxygen (D.O.) concentration was similar at all sites and ranged from 3.8-9.9 mg/L, although a range of 5-8 mg/L was typically seen (Fig. 12). Lower levels occurred in summer and fall corresponding with higher summer temperatures. The pH ranged from 7.8-8.5, but generally fell between 7.8 and 8.1 and did not vary between sites or seasons (Fig. 13). Water clarity, measured by Secchi disk, was extremely variable (Fig. 14). As sites were typically visited near low tide, Secchi disk depth reached total water depth at all sites, with the exception of Snook Island-Outer. Because most Secchi disk depth measurements were limited by the depth of the site, measurements were more an indication of water depth than clarity, with the exception of Snook Island-Outer where measurements were taken farther east of the island in deeper water and provided a more complete representation of water clarity at the site.

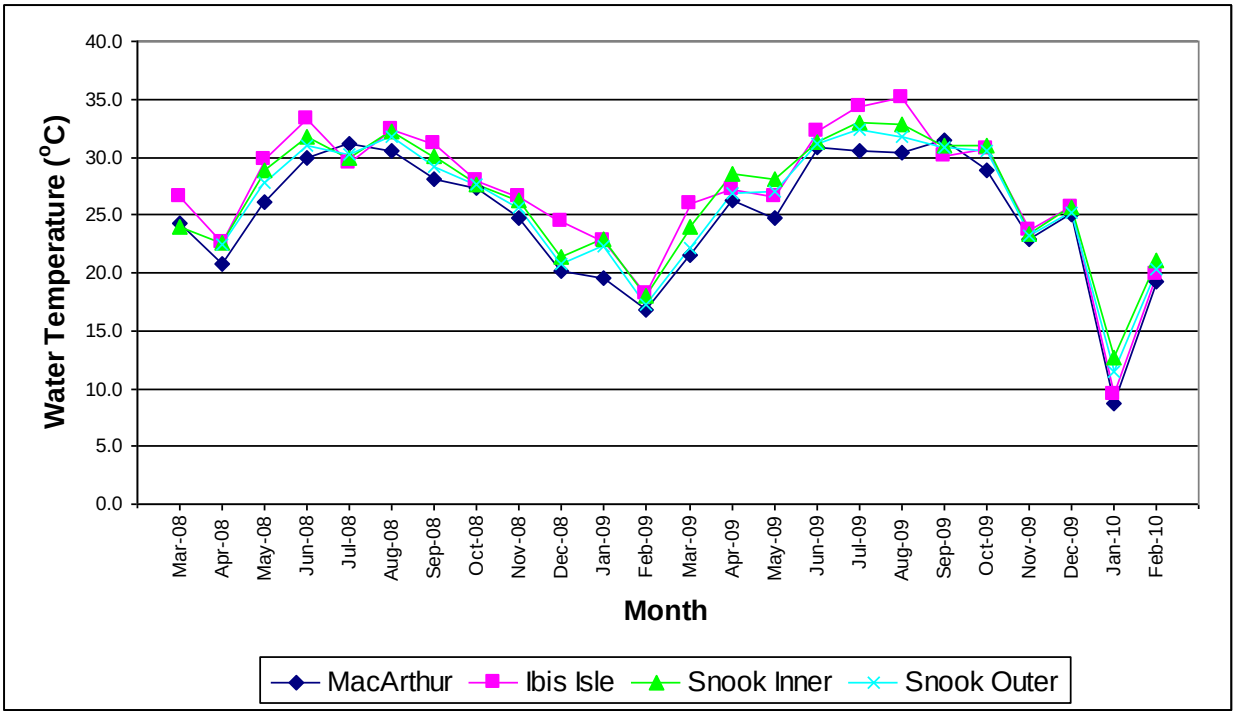

Figure 10. Water temperature at oyster monitoring sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.:* Only one water temperature was recorded at Snook Island in March 2008.

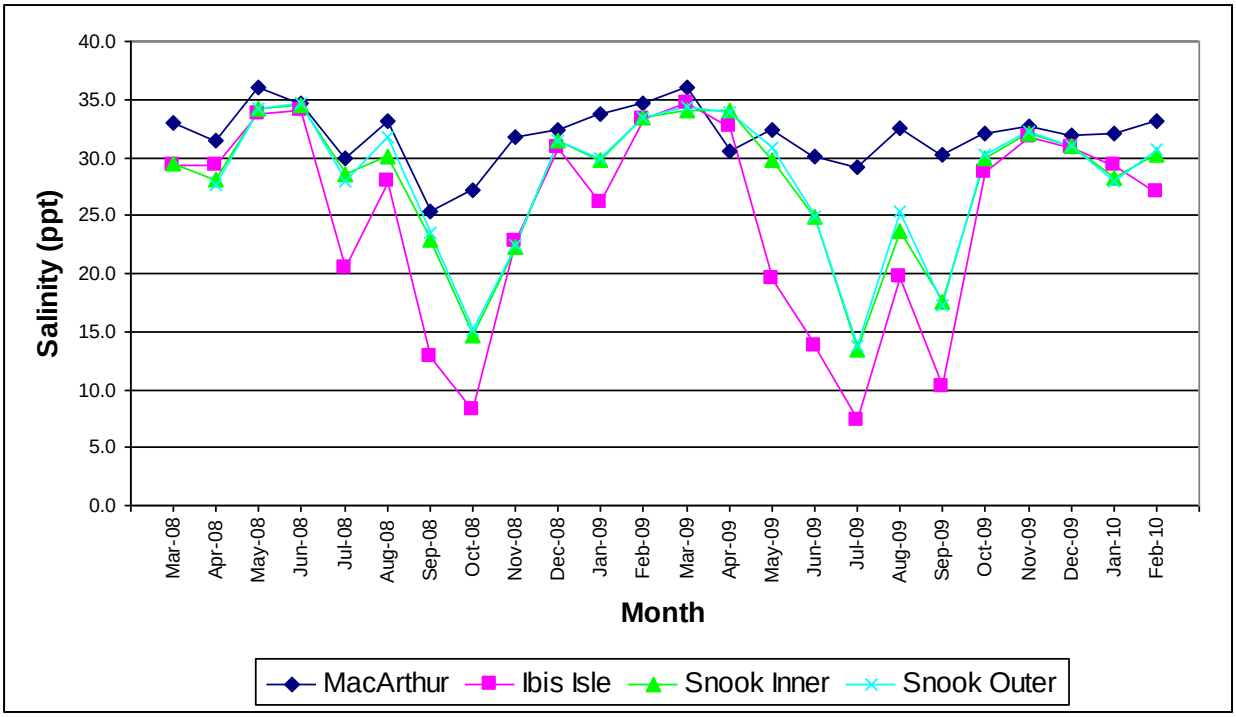

Figure 11. Salinity at oyster monitoring sites in Lake Worth Lagoon from March 2008-February 2010. *nb:* Only one salinity was recorded at Snook Island in March 2008.

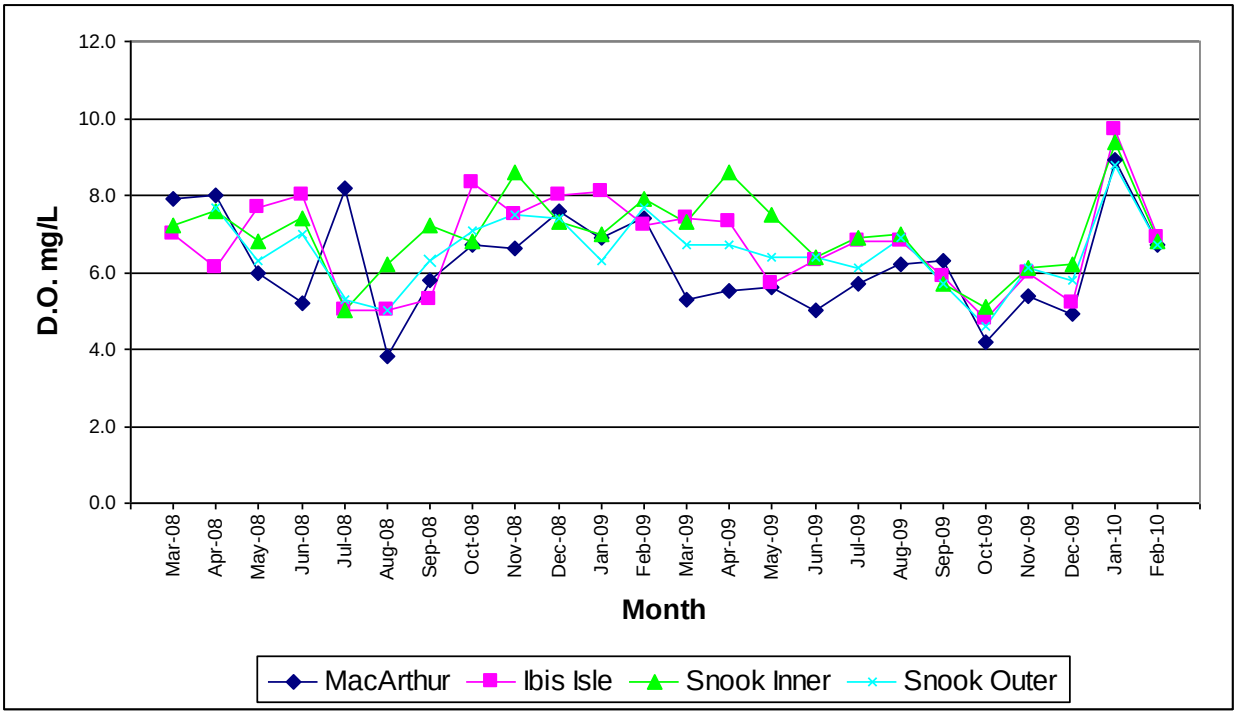

Figure 12. Dissolved oxygen (mg/L) at oyster monitoring sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.:* Only one dissolved oxygen reading was recorded at Snook Island in March 2008.

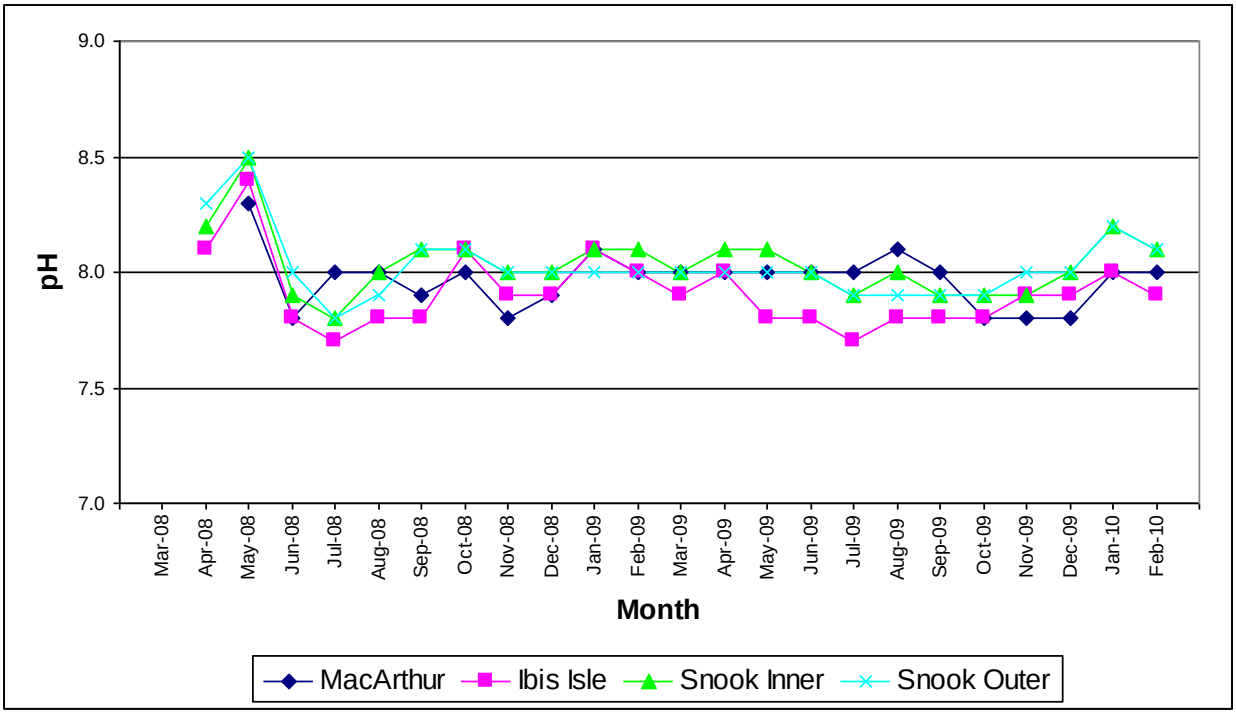

Figure 13. pH at oyster monitoring sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.:* No pH data was taken in March 2008. No pH data was taken at MacArthur in April 2008.

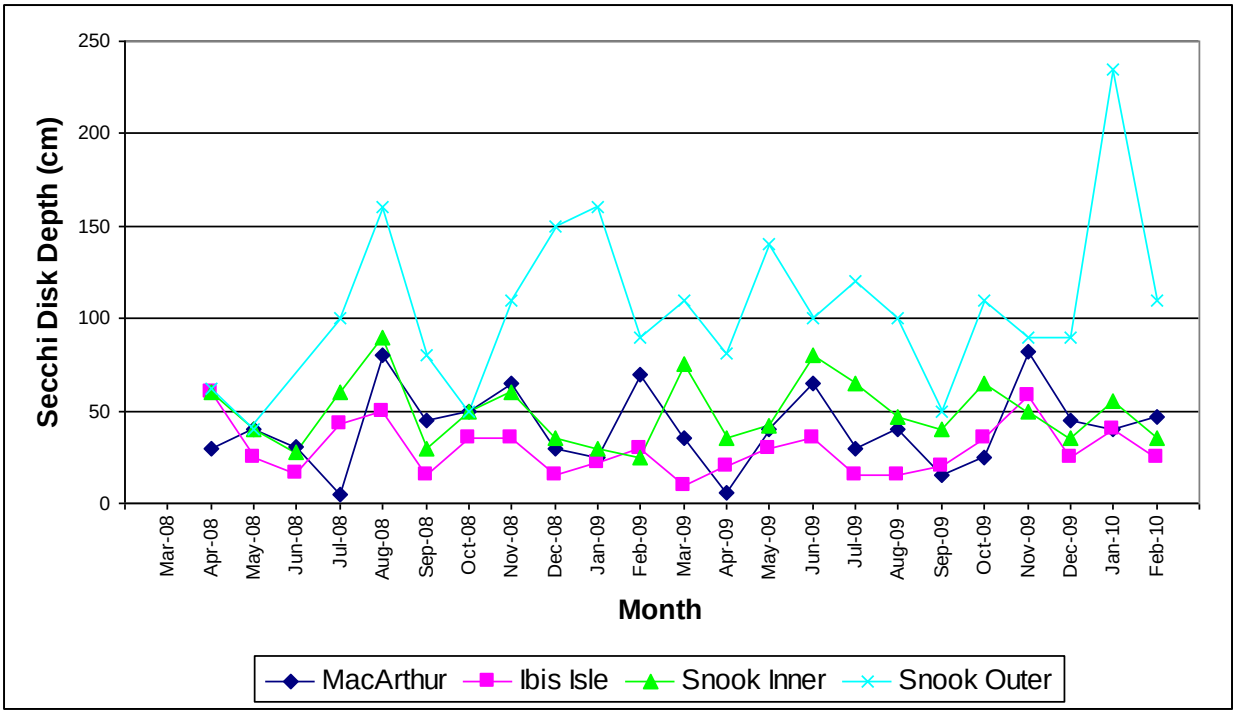

Figure 14. **Water clarity by Secchi disk depth at oyster monitoring sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.*: No reading was taken in March 2008 and most readings were at maximum depth.**

Oyster Survey

Surveys of adult oysters were conducted during the spring (April) and fall (October) of year one and year two. Every quadrat sampled at Ibis Isle contained oysters, while not all quadrats sampled at MacArthur or Snook Island contained oysters. Quadrat samples with no oysters (live or dead, herein referred to as "zeros") were not included in the statistical determination of average oyster density. Live oyster density was significantly greater at Ibis Isle in both years and in both seasons compared to MacArthur. Live oyster density was significantly greater at Ibis Isle compared to Snook Island in both seasons in year one (Fig. 15). There were no significant differences in oyster density between MacArthur and Snook Island.

There was an increase in average live oyster density from spring to fall in both years. The average live oyster density increase from spring to fall in year one of 62%, 94% and 4% at MacArthur, Ibis Isle and Snook Island, respectively, was not statistically significant (P=0.066). The average live oyster density increase from spring to fall in year two of 676%, 61% and 92% at MacArthur, Ibis Isle and Snook Island, respectively, was statistically significant (P=0.0002). The average density of dead oysters was greater than live oysters at MacArthur in year one and in the spring of year two (Fig. 15).

Average shell height of live oysters in both seasons of year one were significantly greater at MacArthur compared to both Ibis Isle and Snook Island (Fig. 16). Overall, average shell height of live oysters was greater in spring than fall in both years, although this difference was less apparent in year two than in year one.

Snook Island is the only site with any vertical height as it is a man-made rock barrier island that extends above the water surface even at high tide (Fig. 8C). With the exception of the fall of 2008, no oysters were present on the top most rock (Fig. 17). On the inner side of the island, oysters were found approximately 18cm from the top of the ridge, whereas on the outer side oysters were found approximately 10 cm below the ridge. At the base (48 cm below crest) of the riprap on the inner side of the island there were a considerable number of oysters (Fig. 9C), but on the outer side (71 cm below crest) fewer oysters were found. During surveys the location of the quadrat samples was noted (i.e., top = top of crest, middle = along incline, bottom = base at water's edge). Average live oyster density in the middle was twice as high as the bottom in the spring of year one, but not in the fall of year one or spring of year two. Density increased 3-4 times at both levels in the fall of 2009 (Fig. 17).

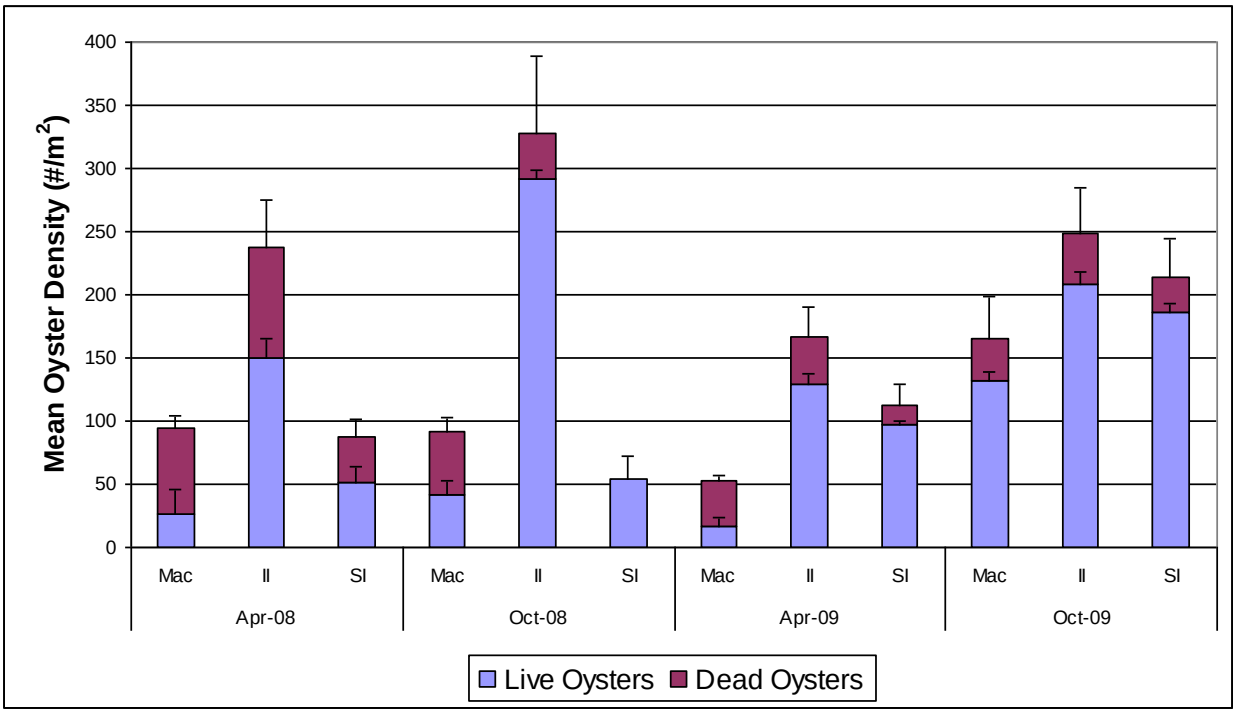

Figure 15. Mean (±SE) oyster density from sampling sites in Lake Worth Lagoon from the 2008 and 2009 spring and fall surveys. *nb.*: Mac=MacArthur, II=Ibis Isle, SI= Snook Island. Mean densities were determined without the inclusion of zero quadrats.

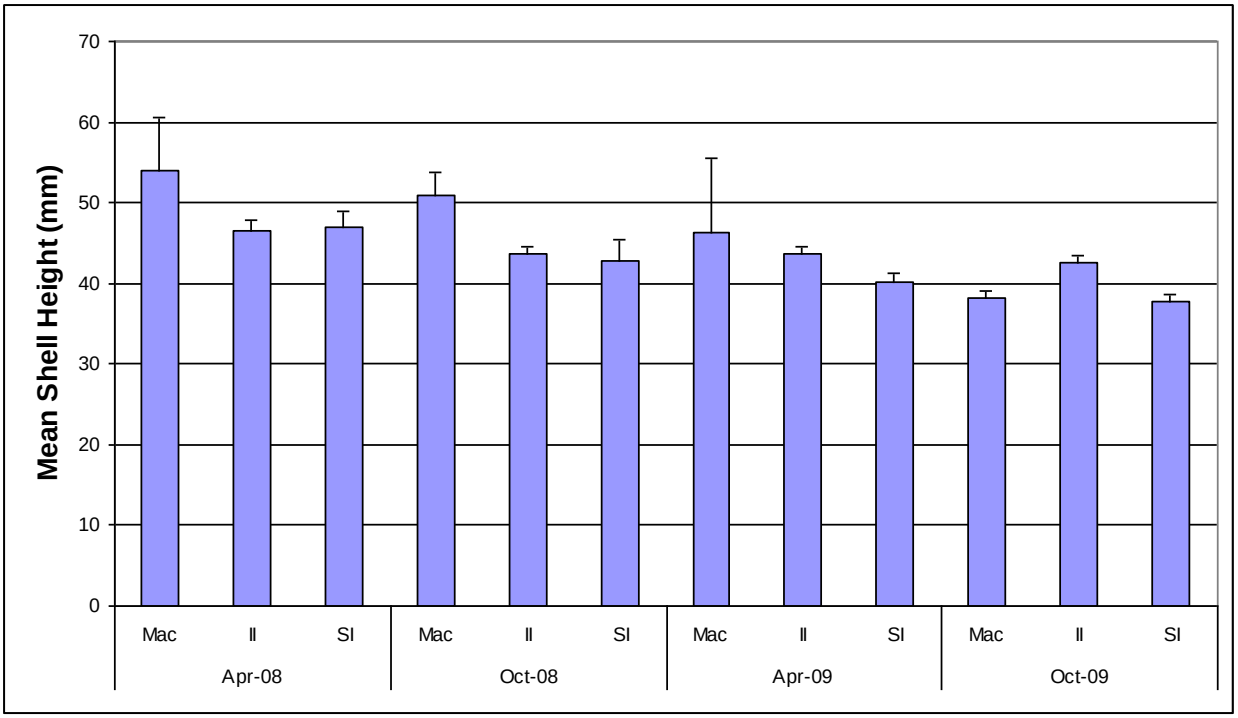

Figure 16. Mean (±SE) oyster shell height from sampling sites in Lake Worth Lagoon from the 2008 and 2009 spring and fall surveys. *nb*.: Mac=MacArthur, II=Ibis Isle, SI= Snook Island.

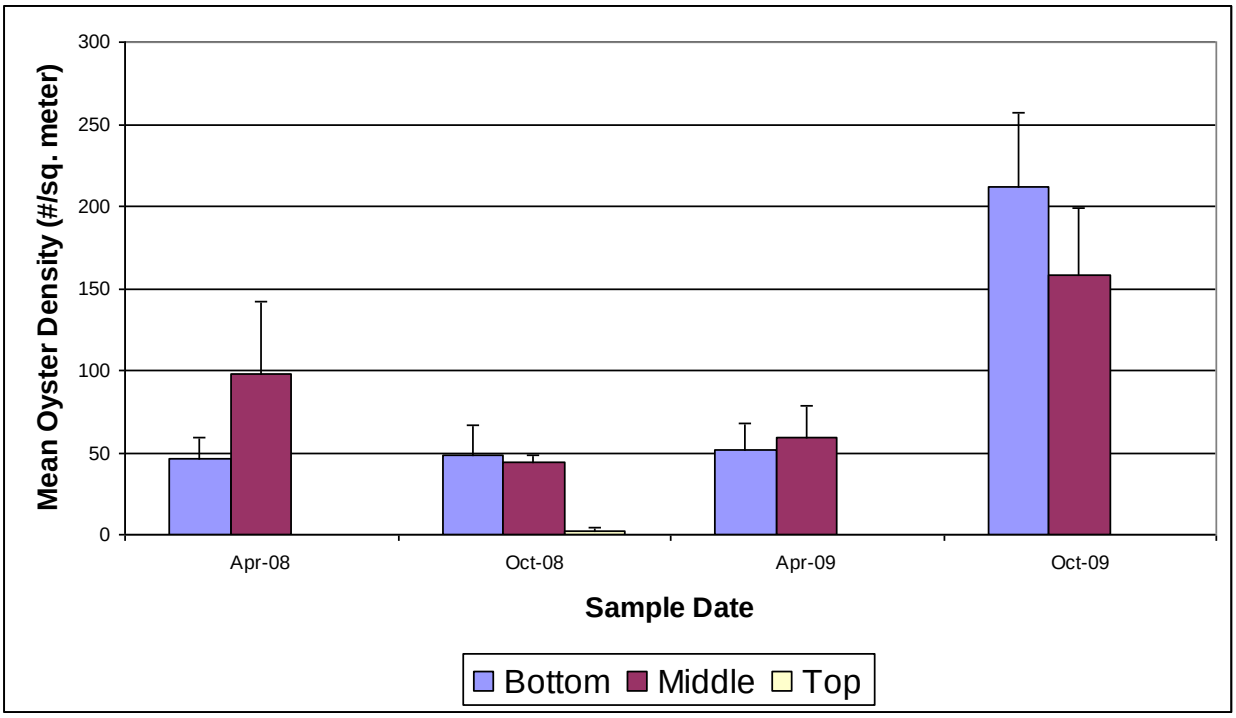

Figure 17. Mean (±SE) density of oysters at different heights on Snook Island riprap. Bottom is the base of the riprap, closest to water at low tide. Middle is between the bottom and top. Top is the crest, which is not covered by water, but crevices contain oysters.

Adult Oyster Samples

Condition Index

Mean shell height ranged from approximately 50 to 80 mm (Fig. 18), while whole weight ranged from approximately 30 to 50g (Fig. 19). Whole weight differences occurred less than height differences. Statistically significant differences in shell height were seen in 10 out of 24 months, with oysters from Ibis Isle generally exhibiting higher shell height. Statistically significant differences in whole weight occurred in 5 out of 24 months, with oysters from MacArthur generally having greater weight.

Condition index (CI) differed between sites both seasonally and annually. Oysters at Snook Island had the highest CI in year one, while oysters at MacArthur had the highest CI in year two (Fig. 20). CI was high at all sites during the winter in both years. Low CI's were seen in the spring of year one at both MacArthur and Snook Island. In contrast to year one, oysters at MacArthur had significantly higher CI's from August through December 2009 than the two central Lagoon sites.

Reproductive Stage

Reproduction varied between sites both seasonally and annually. In year one oysters at all sites exhibited a similar pattern of reproductive stage, with the exception of spring in which the mean reproductive stage of oysters at MacArthur significantly lagged behind those at the two central Lagoon sites (Figs. 21, 22, & 23). This same lag was not apparent in year two. Oysters in year one appeared to have a prolonged spawning period compared to year two as indicated by the proportion of oysters in the mature stage. Bi-modal reproduction was evident at all sites in year one with reproductive peaks occurring in both early summer and fall. Bi-modal peaks in year two were only evident at Snook Island with peaks occurring in both spring and fall. Reproductive activity deceased at all sites from December through February in both years as indicated by the lack of oysters in mature stage (Figs. 21, 22, & 23).

The sex of each sampled oyster was noted during reproductive staging (Fig 24). If sex could not be determined (stage 0-1), the oyster was noted as undifferentiated. The proportion of undifferentiated oysters (Fig. 25) followed the pattern of reproductive stage at each site (Figs. 21, 22, & 23). A greater preponderance of female (2-3x) compared to male oysters was found at all sites. As the number of oysters sampled for reproductive assessment at each site was small (n=5-6), the ratio of males to females on a monthly basis may not be indicative of the population. A greater number of oysters were undifferentiated at MacArthur compared to the other two sites, which corresponds to the fewer number of months that oysters at this site were reproductive (Figs. 21, 22, & 23).

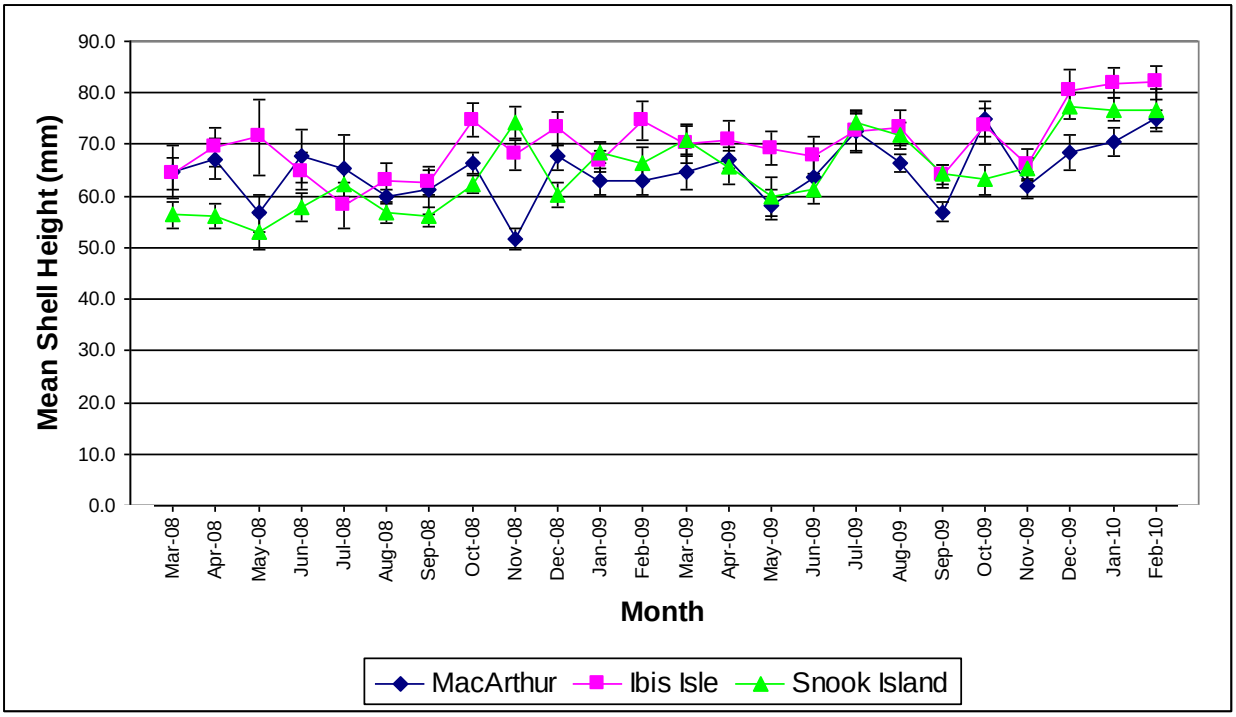

Figure 18. Mean (±SE) shell height of oysters sampled from sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.* Snook Island is combined inner/outer.

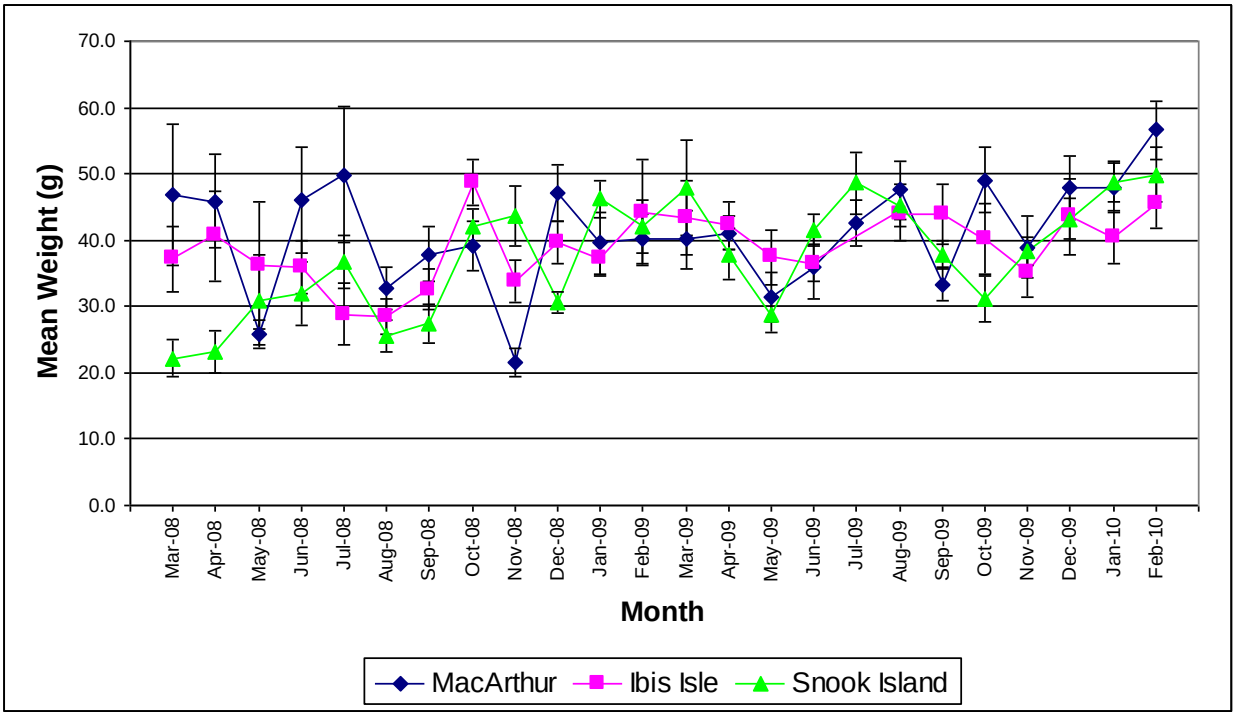

Figure 19. Mean (±SE) whole weight of oysters sampled from sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.* Snook Island is combined inner/outer.

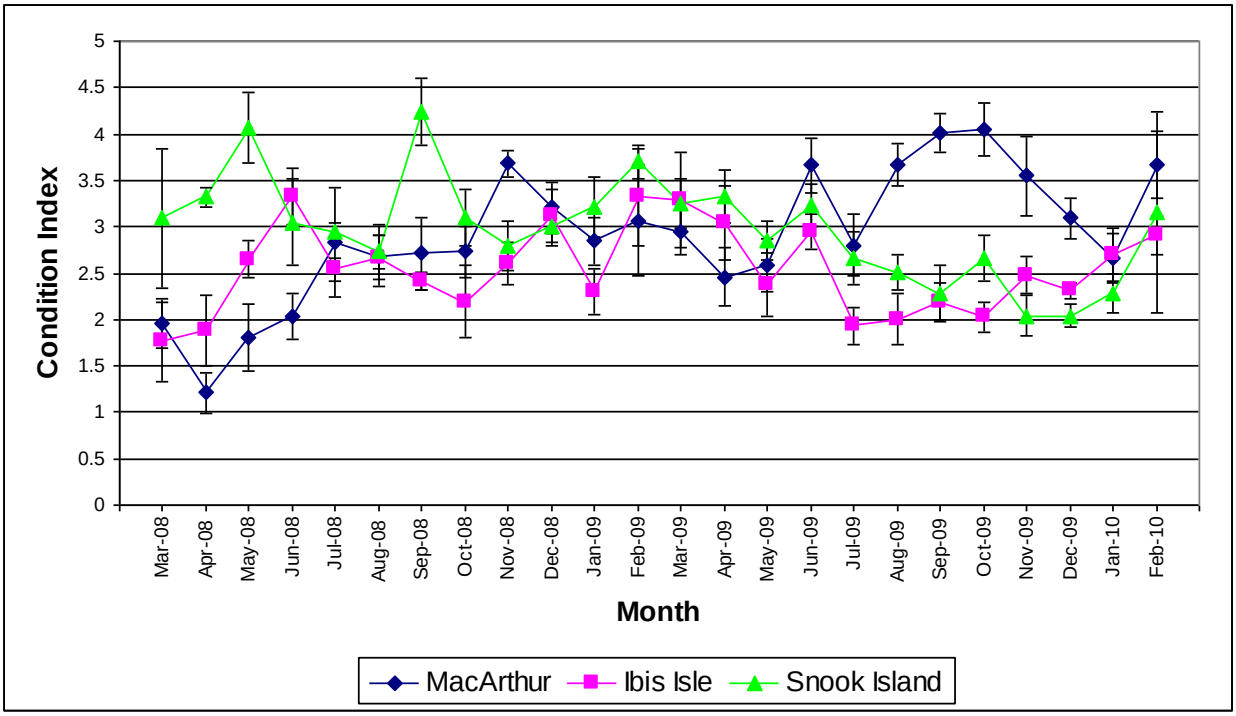

Figure 20. Mean (±SE) condition index of oysters sampled from sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.* Snook Island is combined inner/outer.

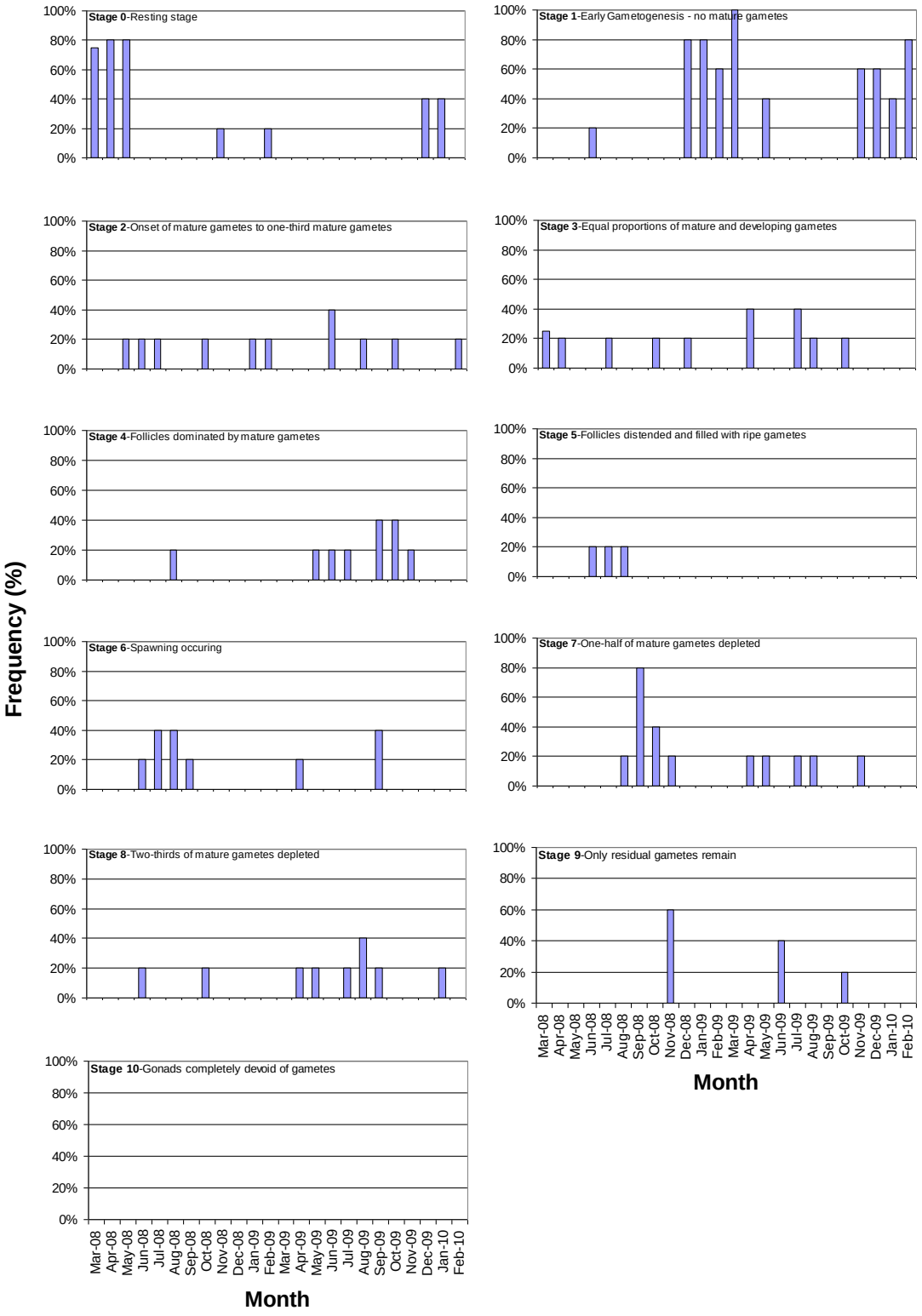

Figure 21. **Reproductive stage frequency of oysters sampled from MacArthur site in Lake Worth Lagoon from March 2008-February 2010.**

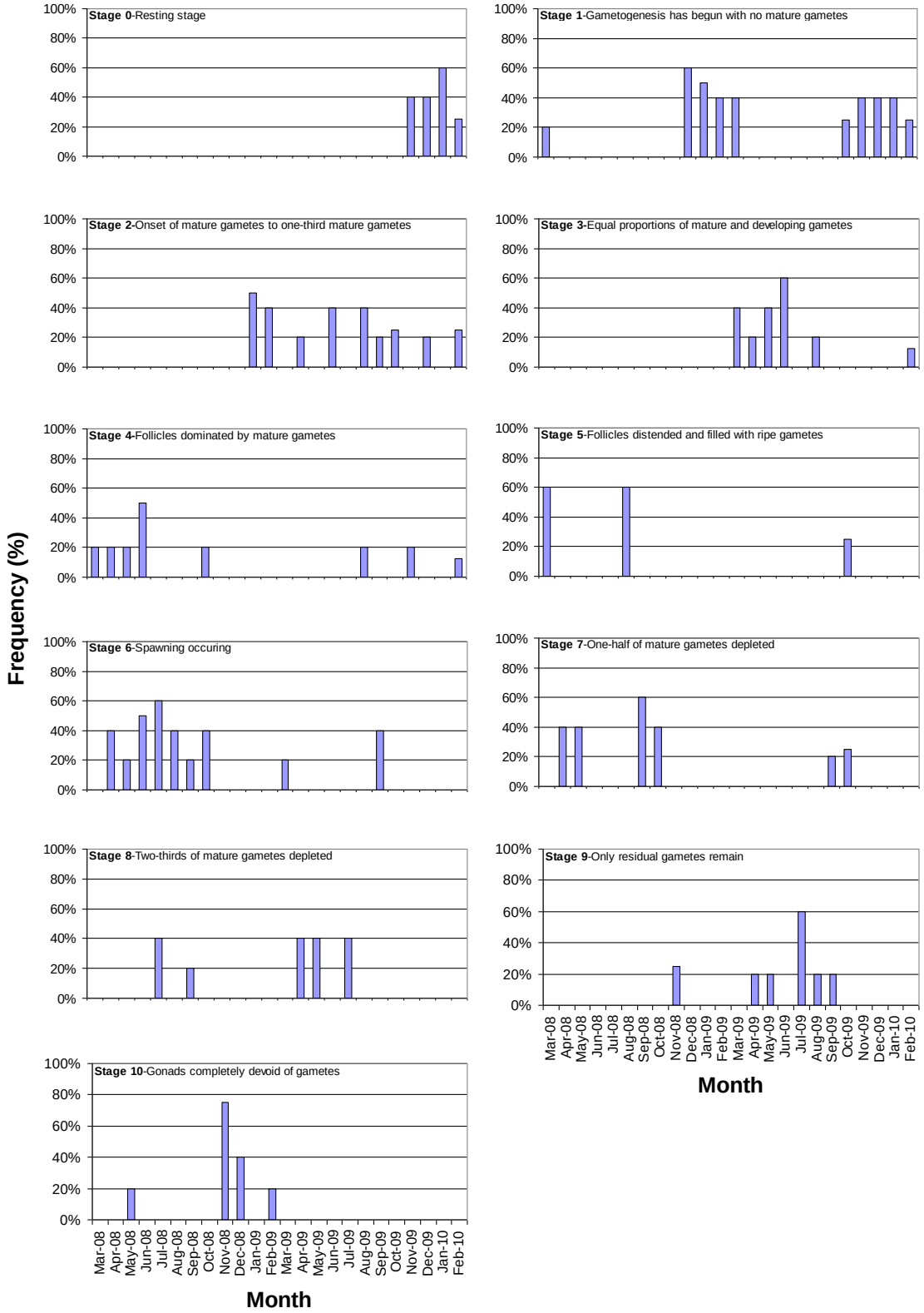

Figure 22. **Reproductive stage frequency of oysters sampled from Ibis Isle site in Lake Worth Lagoon from March 2008-February 2010.**

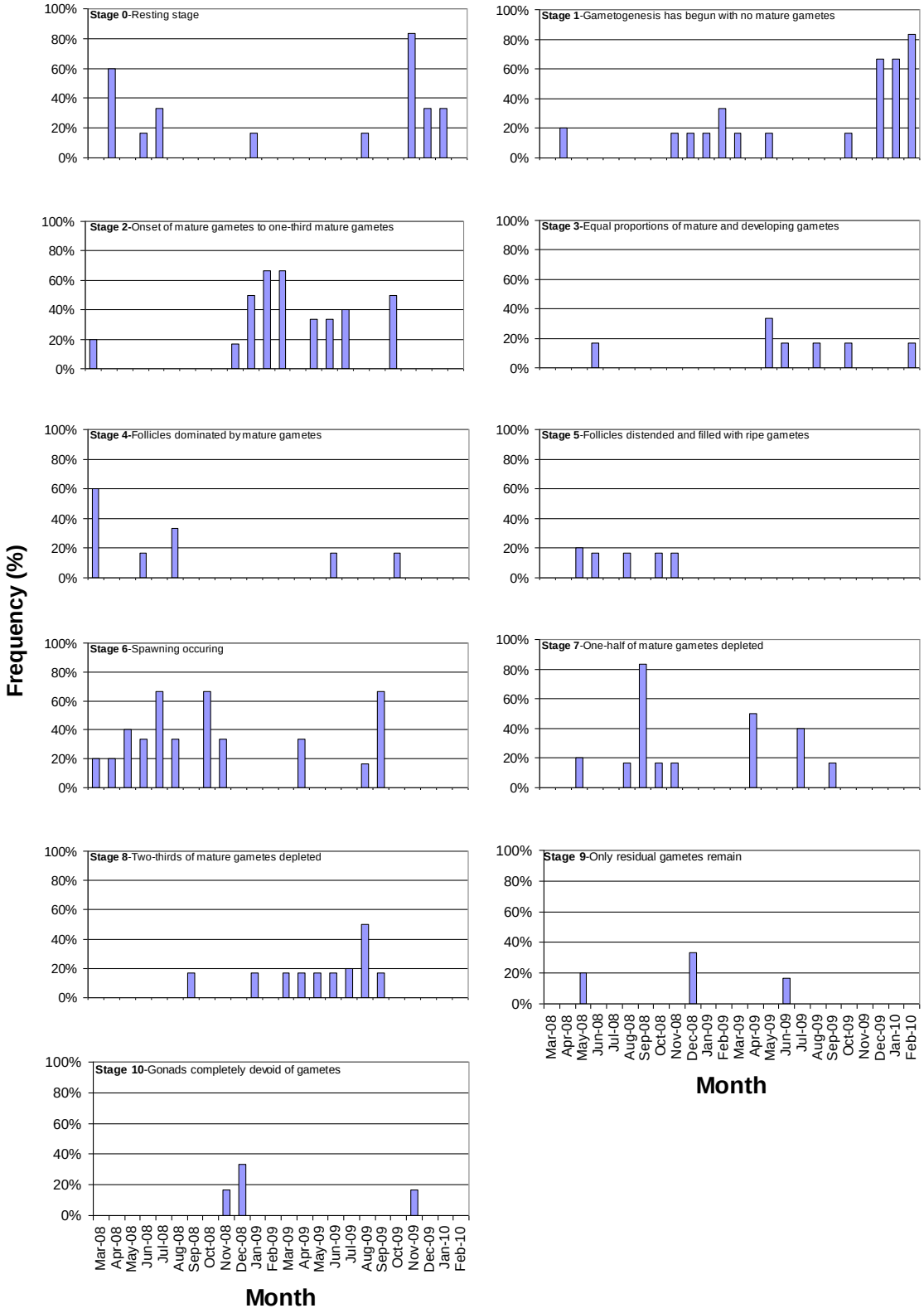

Figure 23. **Reproductive stage frequency of oysters sampled from Snook Island site in Lake Worth Lagoon from March 2008-February 2010.**

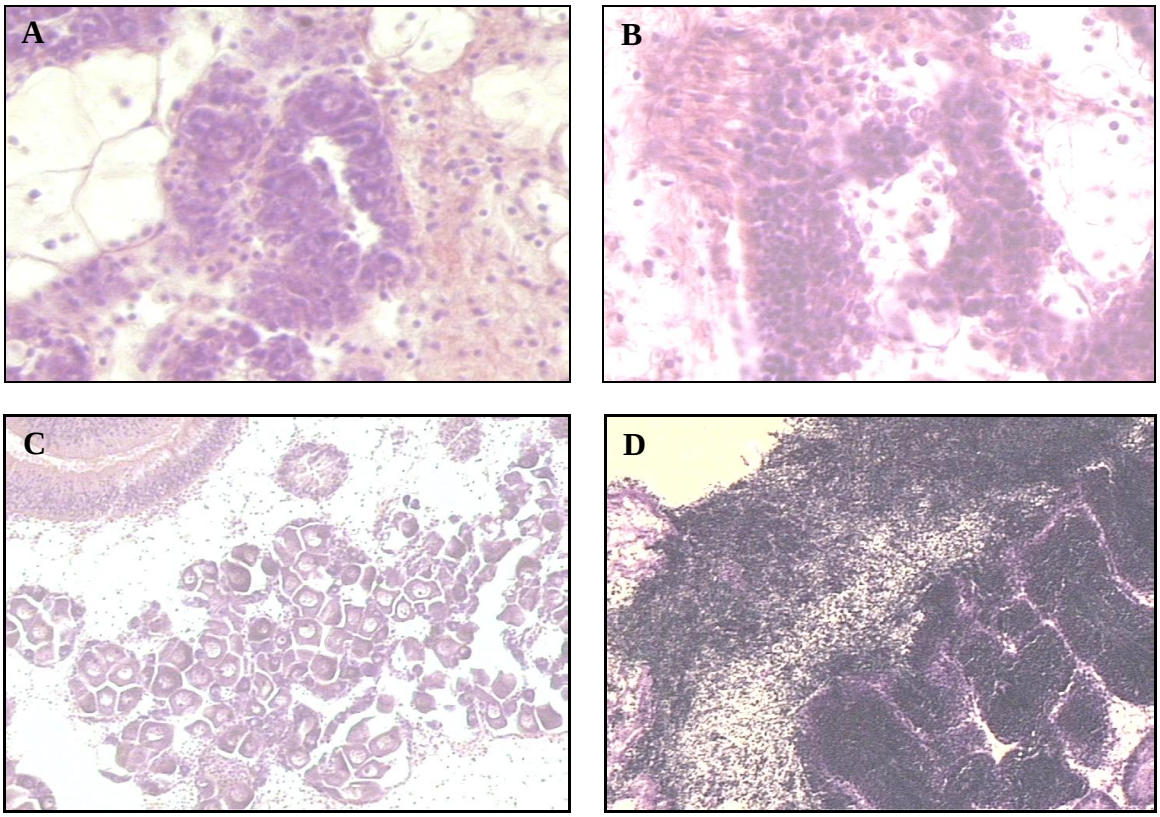

Figure 24. Photomicrographs (40x) of stage 1-2 (early gametogenesis), 6-7(spawning) gonads from female (A,C) and male (B,D) oysters sampled from Lake Worth Lagoon.

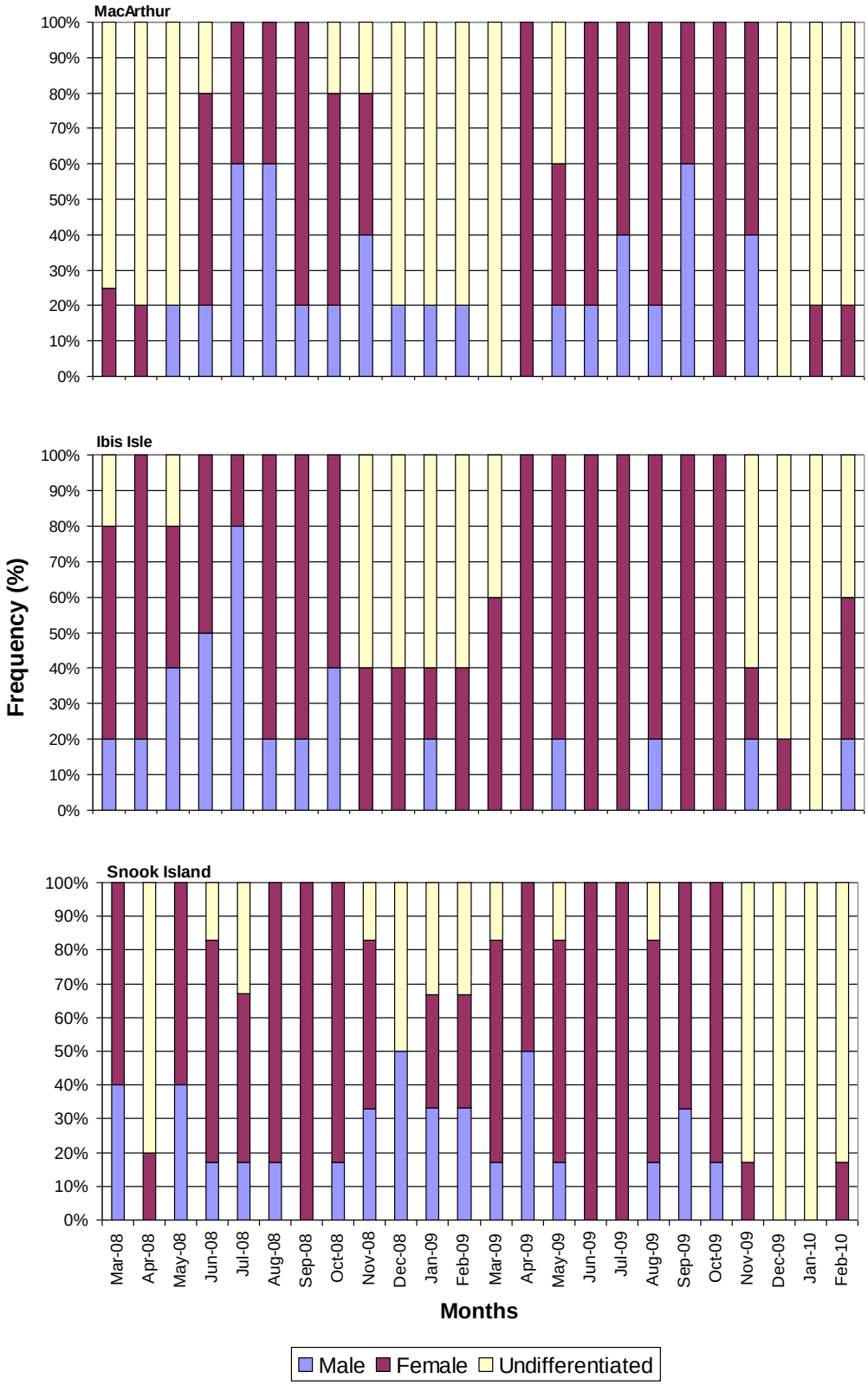

Figure 25. Gender frequency of oysters sampled from MacArthur, Ibis Isle and Snook Island sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.* Snook Island is combined inner/outer.

Oyster Disease

Five to six oysters were sampled monthly at each site to determine the prevalence (i.e., proportion of oysters with the pathogen, irrespective of intensity) and intensity (i.e., amount of the pathogen in an oyster) of Dermo, MSX, and other parasites (e.g., helminthes and protozoans). Mean Dermo intensity ranged from 0-4.4 (Mackin scale) and was lowest in winter at all sites (Fig. 26). Intensity was high (>1.5) at all sites during the summer of year one, but only at MacArthur Park during the summer of year two. MacArthur Park oysters had a significantly higher intensity than oysters at the two central sites from July to September 2009. Annual average intensity was similar at MacArthur Park and Snook Island both years, but lower at Ibis Isle in year two.

Although intensity was generally low, prevalence was generally high (60-100%) except during the winter and early spring (Fig. 27). Snook Island exhibited the most variability and prevalence was slightly lower at this site both years.

MSX was not noted in oyster samples from any site. Numerous parasites were detected histologically including the gregarine protozoan *Nematopsis sp.*, turbellarians, the trematode *Bucephalus*, and the cestode *Tylocephalum*. Symbiotic parasites, such as the cnidarian *Eutima* and the pea crab *Pinnotheres*, were also present. Symbiotic pea crabs were infrequently found during preparation of oysters for condition index and histological analysis. In addition, one oyster sampled at MacArthur in April 2008 was infected with an ovacystis-like virus. In August 2009, 60% of the oysters at Ibis Isle and 20% of the oysters at Snook Island were noted to have a gut ciliate (*Ancistrocoma* sp.).

Nematopsis sp. oocysts were found in both oyster gill and mantle connective tissue in oysters examined at all sites with a prevalence range of 0-100% (Fig. 28). Prevalence was higher at all sites in year one (40-100%) than in year two (0-100%). Prevalence was similar at all sites in year one, but decreased at both MacArthur and Ibis Isles in year two. Intensity was usually low, though moderate and heavy intensities were seen in oysters at all sites: MacArthur in November 2008, May, April and June 2009, at Ibis Isle in July, August and November 2008, and March, April, May and June of 2009 and at Snook Island in June, July, September 2008 and February, April, May, June, November of 2009 and February of 2010. No immune response was evident, nor was there any other indication that the parasite negatively affected the infected oysters.

Sporocytes of the trematode *Bucephalus sp.* were intermittently found in gonadal tissue at low prevalence (0-20%) at all sites in year one; none were found in year two (Fig. 29). Intensities were typically low, although moderate infections were noted at Ibis Isles in November 2008 and Snook Island in January 2009.

Metacestodes of the genus *Tylocephalum* sp. were found localized to connective tissue near the digestive diverticulum with a prevalence range of 0-67% (Fig. 30). The parasites were absent at all sites in the fall of year one and in February, May and July of 2009. Intensities were low (1-3

worms/oyster), except for April and December 2009, when moderate intensities (7 worms/oyster) were seen at Snook Island.

Turbellarian cestodes were found sporadically throughout both years, generally at low prevalence and at low intensities (1-3 worms/oyster) (Fig. 31). The parasite was absent at all sites from summer to fall in year one and in the fall of year two.

Hydrozoan polyps of *Eutima sp.* were found in histological sections of oyster gills with a prevalence range of 0-80% (Fig. 32). Hydrozoans were absent in oysters collected from the central Lagoon sites in the fall and winter of year one and the summer and fall of year two. Hydrozoans were less prevalent in year two than in year one at all sites.

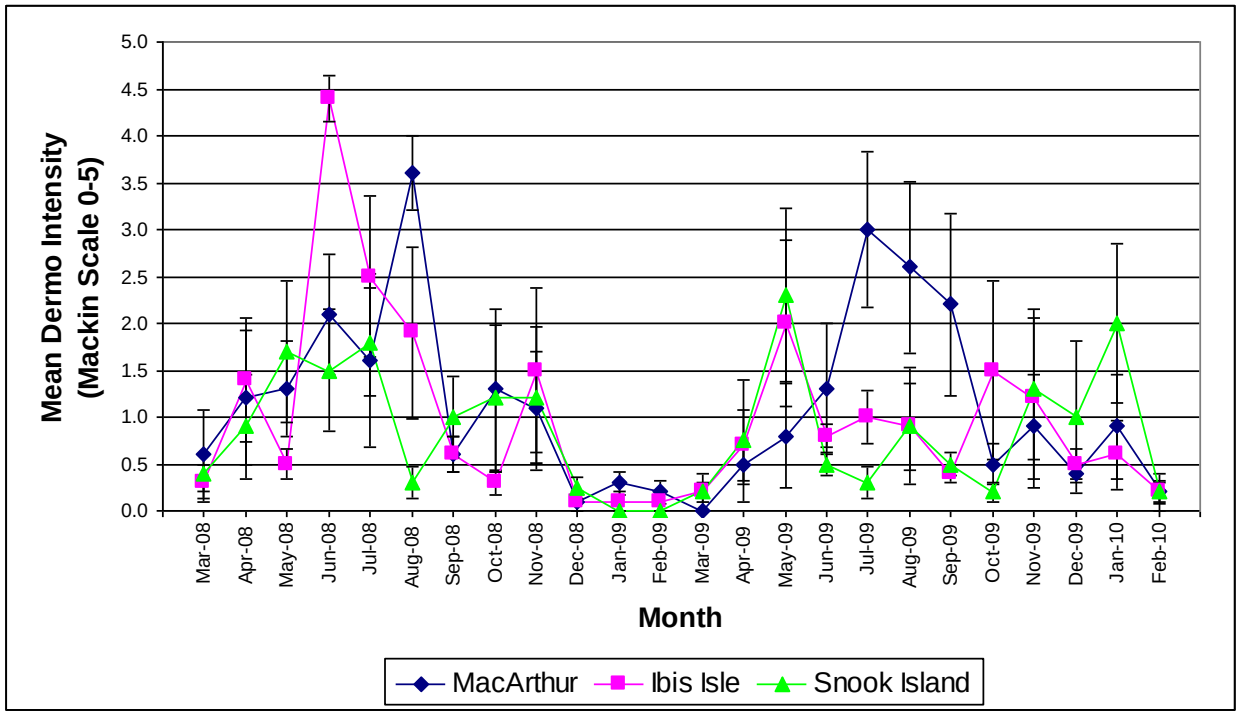

Figure 26. Mean (±SE) Dermo (*Perkinsus* sp.) intensity in oysters sampled from sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.* Snook Island is combined inner/outer.

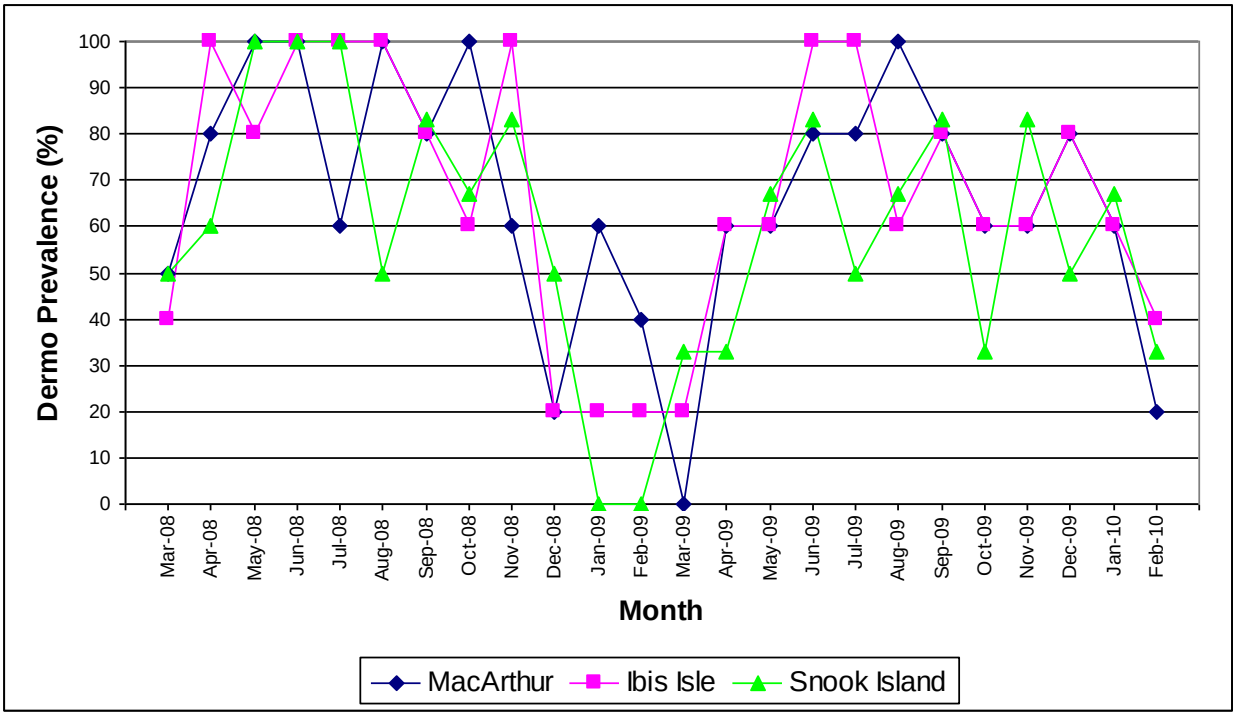

Figure 27. Dermo prevalence of oysters sampled from sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.* Snook Island is combined inner/outer.

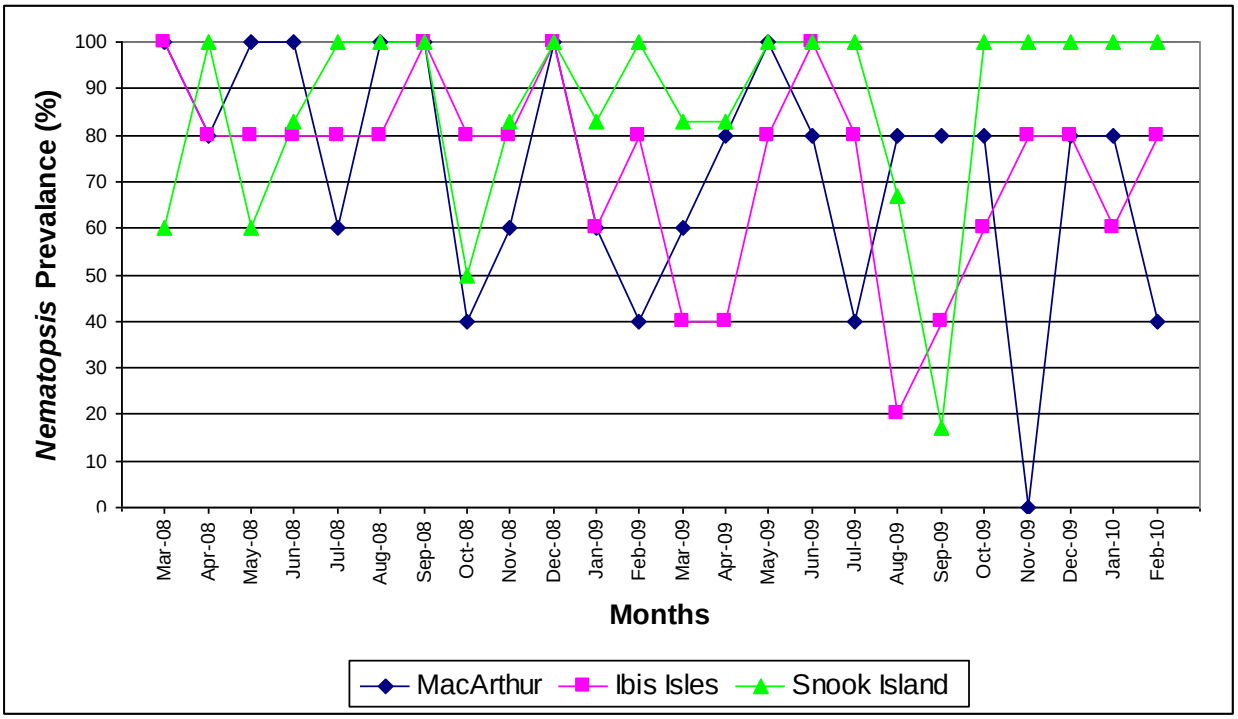

Figure 28. *Nematopsis* sp. prevalence of oysters sampled from sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.* Snook Island is combined inner/outer.

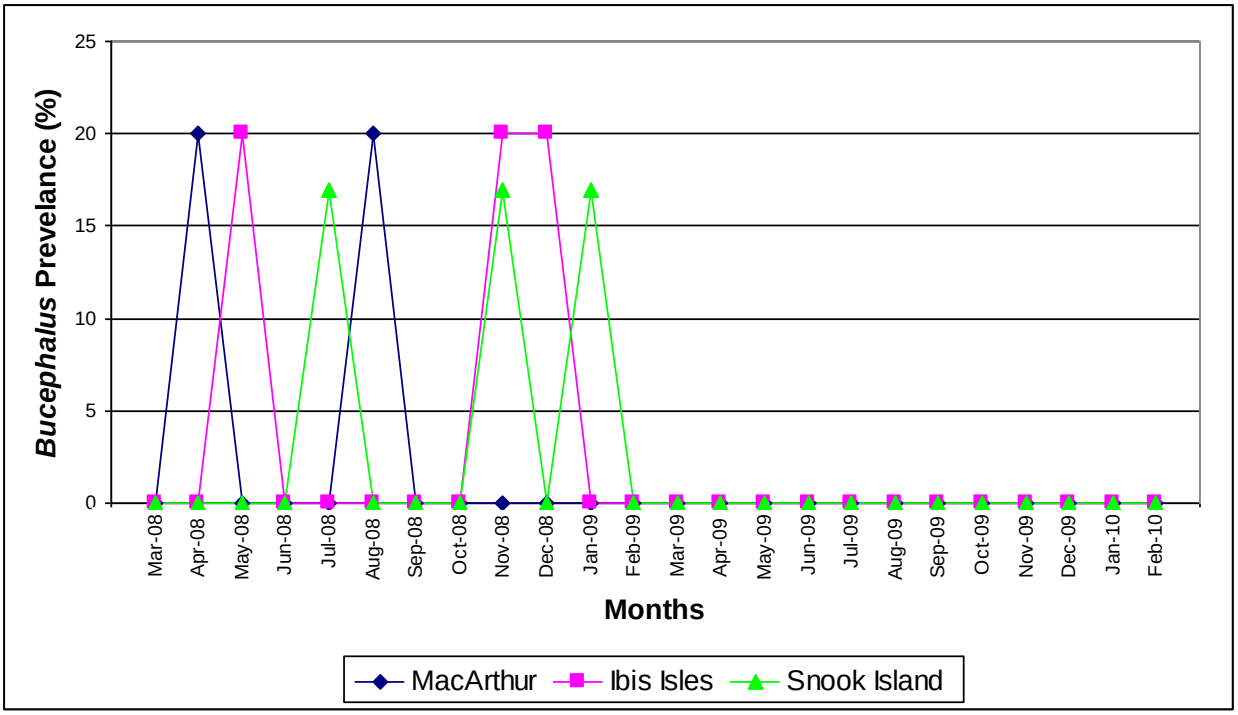

Figure 29. ***Bucephalus*** **sp. prevalence of oysters sampled from sites in Lake Worth Lagoon from March 2008-February 2010.** ***n.b.*** **Snook Island is combined inner/outer.**

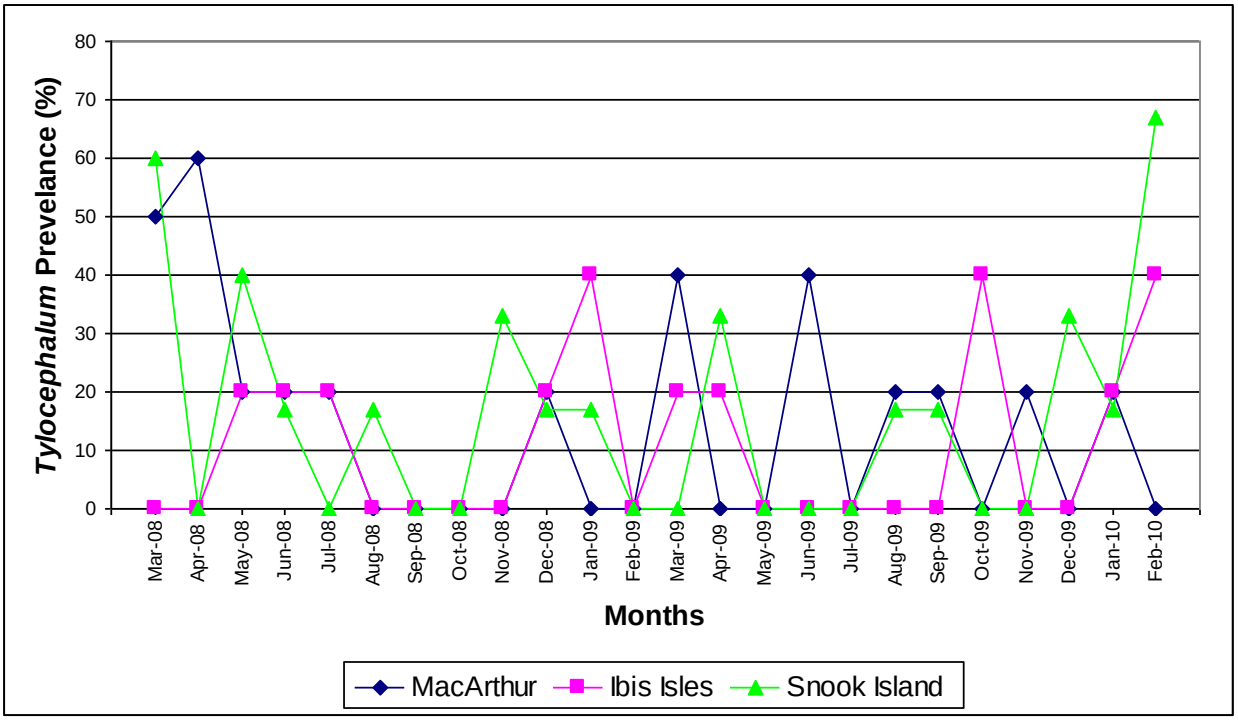

Figure 30. ***Tylocephalum*** **sp. prevalence of oysters sampled from sites in Lake Worth Lagoon from March 2008-February 2010.** ***n.b.*** **Snook Island is combined inner/outer.**

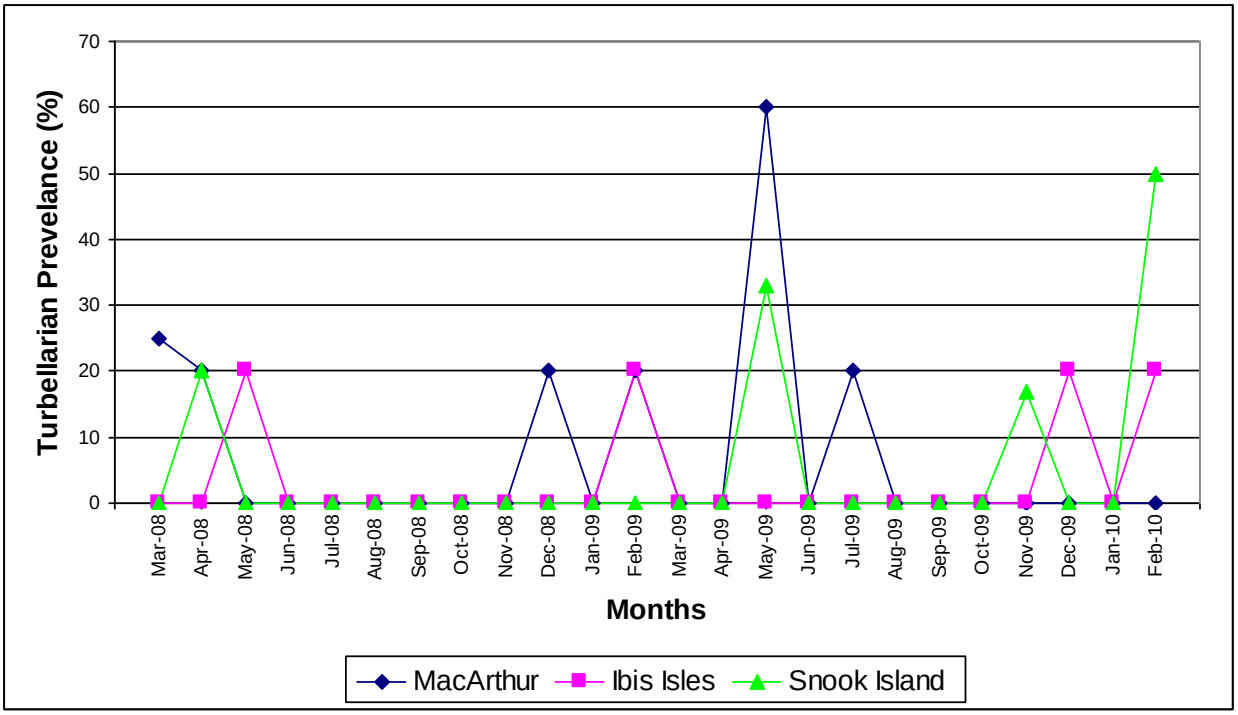

Figure 31. Turbellarian prevalence of oysters sampled from sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.* Snook Island is combined inner/outer.

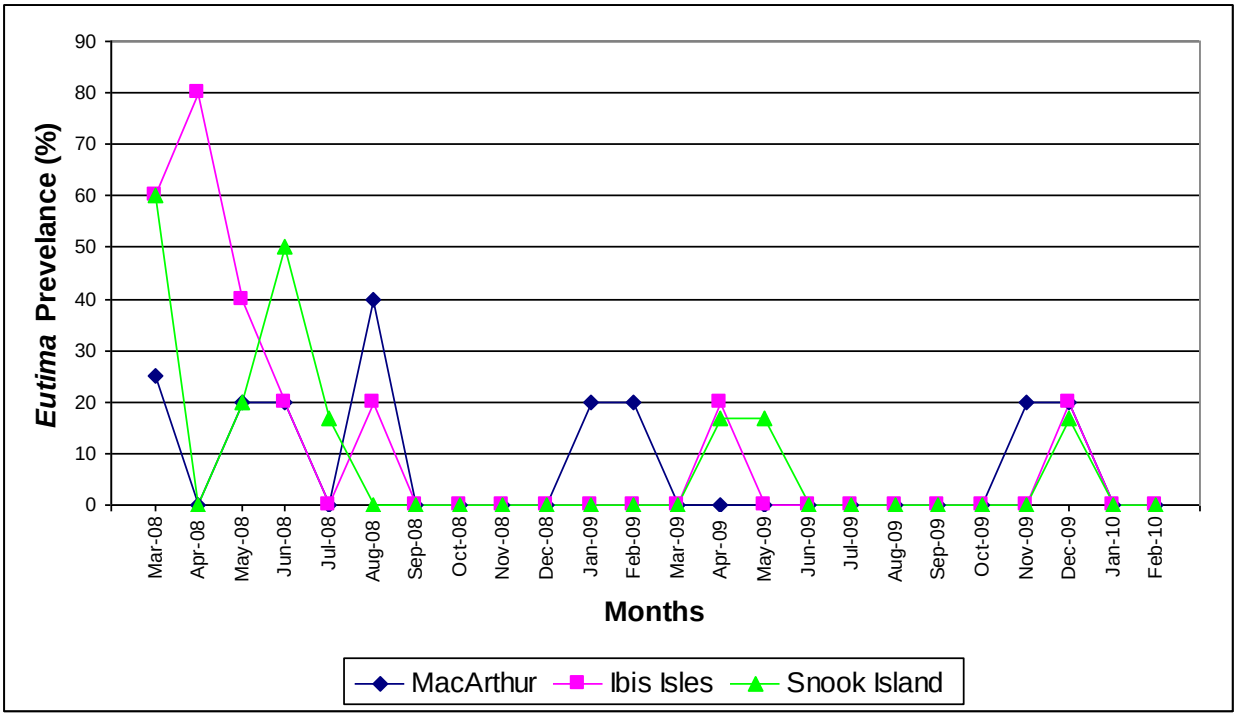

Figure 32. *Eutima* sp. prevalence of oysters sampled from sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.* Snook Island is combined inner/outer.

Oyster Spat Recruitment

Spat collectors (n=36 shells total/site, Fig. 9) were placed at sites in March 2008 and thereafter retrieved and changed monthly. All sites had light to heavy barnacle recruitment on the shells (Fig. 33).

Oyster spat recruitment differed significantly at each site (Fig. 34). Recruitment at all sites was greatest from late spring/early summer through fall in both years and absent in winter. Recruitment was lowest at MacArthur. Total recruitment for year one at MacArthur averaged 0.7 spat/shell and for year two 1.1 spat/shell. Peak recruitment in year one occurred in September and in year two in June.

Oyster spat recruitment at Ibis Isle was also low, although higher than at MacArthur. Total recruitment for year one averaged 4.9 spat/shell and decreased slightly in year two to 3.2 spat/shell. Peak recruitment occurred in the fall in both years.

Oyster recruitment at Snook Island-Inner and Snook Island-Outer were similar in pattern in 2008, but differed in total number with Snook Island-Inner typically having the higher recruitment. Recruitment at Snook Island appeared to be bi-modal at both inner and outer sites in year one, but only for the inner site in year two. Total average recruitment at Snook Island-Inner was lower in year one (10 spat/shell) than in year two (18.1 spat/shell). Peak recruitment occurred in June and again in August remaining high through October. Peak recruitments in year two occurred in May and again in October.

Total oyster recruitment at Snook Island-Outer was similar in year one (avg. 7.0 spat/shell) and year two (avg. 8.5 spat/shell). Peak recruitment in year one occurred in June 2008, and again in September. Peak recruitment in year two occurred in October 2009, which coincided with the second peak recruitment event at Snook Island-Inner.

Figure 33. Oyster spat (arrow, left shell) and barnacles (right shell) on spat collector shells from Ibis Isle site of Lake Worth Lagoon in June 2008.

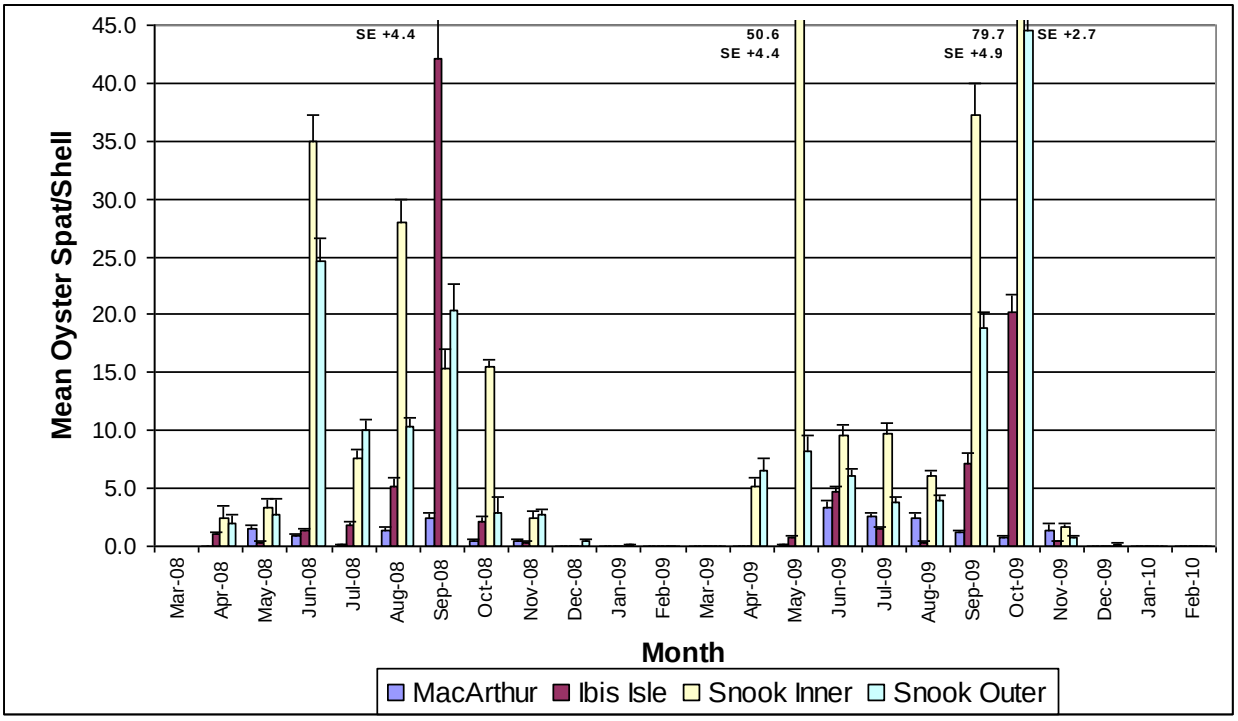

Figure 34. Mean (±SE) number of oyster spat/shell from sites in Lake Worth Lagoon from March 2008-February 2010. *n.b.*: In March 2008 there was no data as spat collectors were initially placed in the water that month. The number of spat/shell was >45 in May and October of 2009 and is indicated by written notation (including SE) adjacent to the bar.

Juvenile Oyster Growth

Shell arrays (25 oyster shells attached to a piece of wire mesh and raised about 10 cm off bottom) were anchored in place at each site in year one in April 2008 and in year two in March 2009 (Fig. 9). Shell arrays were examined monthly and the shell height of 30 random oyster recruits (or all oysters if less than 30) were measured (Fig. 35).

Significant differences were observed between sites in all months in year one, two months after arrays were deployed, and one month after arrays were deployed in year two (Fig. 36). Average growth, as measured by shell height varied more in year one than in year two, and a decrease in growth from year one to year two was seen at MacArthur and Ibis Isle. Newly recruited oysters showed the greatest growth in year one at Ibis Isle and slowest growth at Snook Island-Outer. Juvenile oysters at MacArthur and Ibis Isle were significantly larger than at Snook Island-Inner from August 2008 until February 2009 and oysters at Ibis Isle were significantly larger than those at MacArthur from December 2008 to January 2009. The array at Snook Island-Outer was removed and replaced in September 2008 which led to the decision not to include Snook Island-Outer data in statistical analysis. Continual problems occurred with the Snook Island-Outer array through-out year one. No data was taken in June 2008 due to an approaching lightening storm. Encrusting algae covered the array in September 2008 killing all the oysters. In December of 2008 the array was found 10 m from its normal location, but still upright. No live oysters were found in February 2009.

Overgrowth and predation were problems at both MacArthur and Snook Island. In October 2008 at MacArthur the shell array was covered with the green algae *Caulerpa sertularioides* and in November with the orange tunicate *Ecteinascidia turbinata*, as well as barnacles. At Snook Island-Inner in October 2008, a heavy set of oyster spat was noted on the underside of the array. In November 2008, the Snook Island-Inner array was found overturned and buried in the mud. In December 2008 a heavy infestation of oyster drills (Fig. 37) was noted on the Snook Island-Inner array oysters, along with many dead oysters showing a single drill hole.

In March 2009, the array at each site was removed and replaced with a new array. The shells on the arrays had noticeable new oyster spat at Snook Island in April, at Ibis Isle in May, and at MacArthur in June 2008. Although oysters at MacArthur set and grew slower than those at the two central Lagoon sites, the average annual growth at all sites in year two was remarkably similar, except for Snook Island-Outer in year two. Oysters at Snook Island-Outer were significantly smaller compared to the other sites from November 2008 through February 2010.

Figure 35. Oyster spat (arrow) being measured on shell array at Ibis Isle.

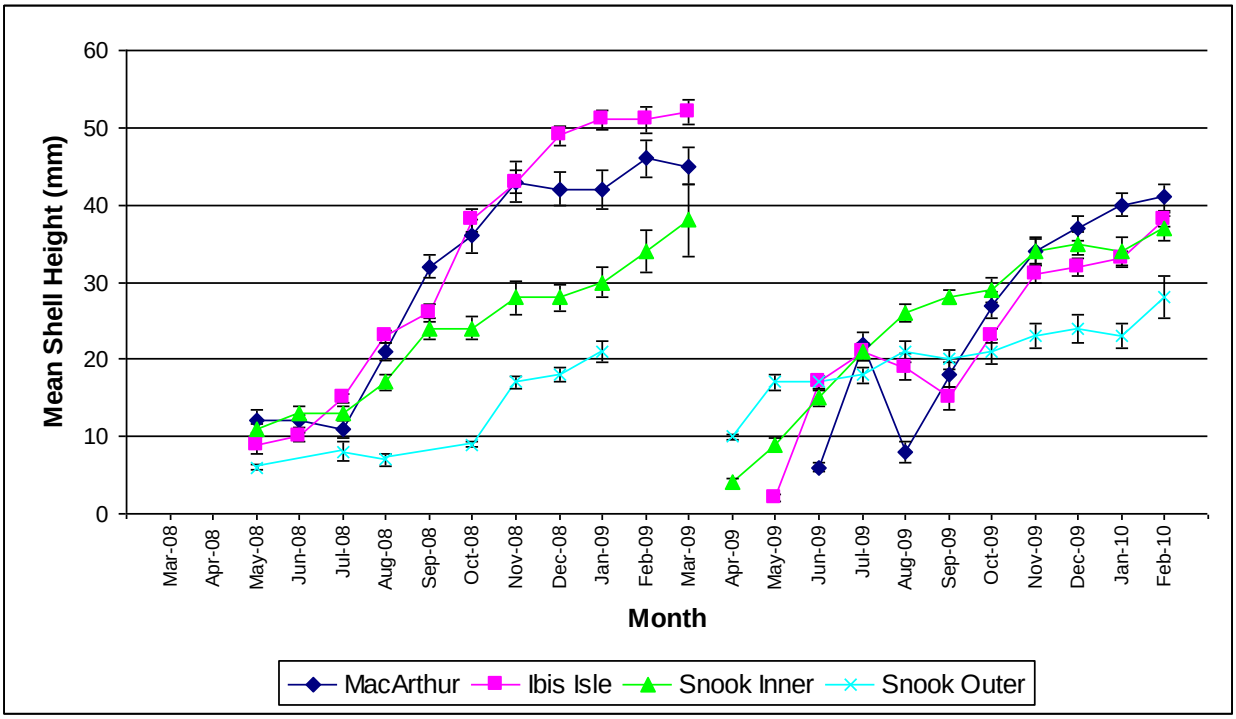

Figure 36. Mean (±SE) shell height of juvenile oysters on shell arrays from all sites in Lake Worth Lagoon from March 2008- February 2010.

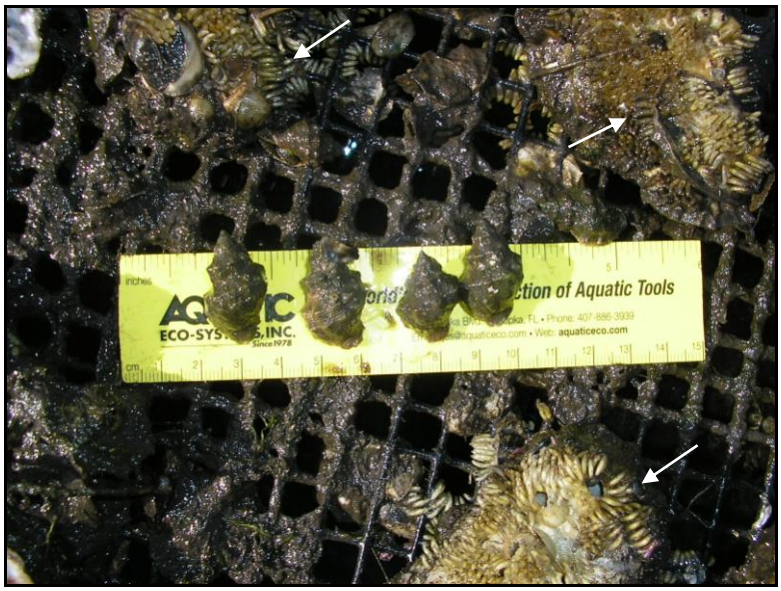

Figure 37. Oyster drills *Urosalpinx* sp. or *Eupleura* sp. (on ruler) and their eggs cases (white arrows) on oyster shell array at Snook Island-Inner in December 2008.

DISCUSSION

This study monitored Eastern oysters (*Crassostrea virginica)* at three sites within the Lake Worth Lagoon (LWL) estuary over a two-year period (March 2008-February 2010). Located in Palm Beach County, Florida the LWL is an estuarine habitat approximately 34 km in length (1165 sq km watershed) influenced by human activities and canal drainage. Sites monitored in this study were located at both the northern (John D. MacArthur State Recreation Area) and central (Ibis Isle, Snook Islands) portions of the Lagoon and included both natural oyster beds (MacArthur and Ibis Isle) and an environmental enhancement area (Snook Islands). The primary objectives of this study were to determine recruitment, survival, growth, reproduction, condition index, and health status of established oysters at these locations. The present study complements studies performed between 2005-2009 at natural oyster beds within the LWL estuary (Arnold et al. 2008, Parker and Geiger 2009, Volety 2010)

Environmental Conditions

Environmental parameters were generally similar between sites for the entire monitoring period and fell within ranges reported to be tolerant by Eastern oysters (Shumway 1996). As parameters were measured on a monthly basis this sampling scheme provides only a seasonal picture, and does not reflect daily variations that occur. With the exception of an extreme and prolonged period of lower than normal temperatures that occurred throughout South Florida in January of 2010 (9ºC) water temperatures in this study (17-35ºC) were comparable to ranges reported in previous studies (Arnold et al. 2008, Geiger and Parker 2009, Volety 2009). Air temperatures followed a similar pattern to water temperature. Although Eastern oysters may survive extreme temperatures, the rate of temperature change directly affects survival (Shumway 1996).

The optimum salinity range for the Eastern oyster is 14-28 ppt (Shumway 1996). Oysters within the two central sites were subjected to large variations in salinity (7-35 ppt) due to freshwater releases from the C-51 canal, while those within the northern LWL were higher and less variable (25-35 ppt). Tropical Storm Fay and freshwater releases combined to depress salinities in the central LWL during the fall of year one, while rainfall and freshwater releases depressed salinities in the area throughout the entire summer of year two. Although oysters can survive a salinity of 5 ppt for extended periods (Shumway 1996), combinations of high temperature (>25 ^{o}C) and very low salinities (<5 ppt), which can occur in Florida estuaries during the tropical storm season, are known to affect overall health and survival of oysters (Heilmayer et al. 2008).

Dissolved oxygen (D.O.) levels were within acceptable ranges (4-10), typically lower in the summer and higher in the winter. The pH of the water was usually near or slightly lower (7.8-8.0) than that of seawater (8.2), and was generally lower during the summer, particularly at Ibis Isle, which coincides with lowered salinity. The large difference between the May 2008 sample (pH of 8.3-8.4) and the remaining sampling periods (pH of 7.8-8.1) is likely the result of an incorrect pH meter calibration. Secchi disk measurements for this study did not adequately represent water clarity as the sites were visited near low tide and measurements were usually limited by water depth. Ibis Isle has an extremely silty bottom that is easily resuspended during tidal changes. The Snook Island-Inner site is similarly shallow and affected by silt resuspension. Snook Island-Outer was the only site not limited by water depth. Establishing water clarity by Secchi disk may be of limited value and is more a comment on the condition of bottom sediments than on nutrient availability.

Adult Surveys

The oyster bed at MacArthur had the lowest oyster density in spring and fall of both years, whereas the oyster bed at Ibis Isle had the highest density. Increases in density from spring to fall were seen at all sites with the exception of Snook Island in year one. This increase is expected based on recruitment that takes place in the LWL from spring to fall. Similar densities and seasonal patterns have been reported previously for LWL sites (Arnold et al. 2008, Parker and Geiger 2009, Volety 2010). In contrast to oysters on the east coast, Volety (2010) reported high densities for sites located within the Caloosahatchee (100-1500/m^2) with peak densities occurring in fall rather than spring.

Differences in density among the sites sampled in the study may be due to a number of factors including temperature, salinity, disease, predation and recruitment. The lack of increase in density from spring to fall in year one at Snook Island was likely due to predation as recruitment was similar in both years. A large number of oyster drills were noted on Snook Island arrays in the fall of 2008. The greatest increase in density at Snook Island occurred at the base of the riprap. Temperature may have been a factor affecting vertical distribution as oyster density remained the same from spring to fall at the base of the riprap but actually decreased in the middle. Decreased Dermo pressure in the fall due to low summer salinities in the central LWL may also have played a role in increased fall densities at those sites. On the other hand, a prolonged decrease in salinity as seen in year two at Ibis Isle may have negatively impacted density by impacting recruitment. Ibis Isle may also have encountered changes in hydrology,

water quality or nutrient availability in year two due to restoration efforts which may have had a temporary negative impact on that site in terms of recruitment and density. The dramatic increase seen at MacArthur in year two did not appear to be the result of increased recruitment or measured environmental changes, but could be the result of increased nutrient availability or increased survival. This increased survival is reflected in the decrease in average shell height of oysters at all sites in year two compared to the previous year. The occurrence of overset (i.e., oyster spat settling upon earlier settled oysters) will reduce observed growth as average shell height does not change. A seasonal decrease in shell height concurrent with an increase in density from spring to fall in the LWL was likewise reported by Arnold et al. (2008) and Parker and Geiger (2009).

Adult Monitoring

Condition Index

None of the sites exhibited a consistently higher condition index (CI) than any other. The annual condition index was highest for Snook Island in year one and highest for MacArthur in year two. The CI at Snook Island was significantly higher in both the spring and fall of year one and at MacArthur from August through December of year two. Oyster morphology varies greatly depending on substrate and habitat condition. Mean shell height and weight varied from month to month at all three sites. Shell height was significantly different between sites in 10 of 24 months and oysters at Ibis Isle were more likely to have higher shell height. Weight was significantly different in 5 of 24 months and Snook Island oysters were most often heavier. This is representative of the substrate differences that exist at the three sites. Oysters that grow on muddy substrates, such as found at Ibis Isle, tend to be long and narrow while oysters adhering to hard substrate like rock or hard bottom, such as found at Snook Island, tend to be rounded.

Condition index is typically described as being high in the spring and declining later in the season as energy reserves are transferred into gamete production. Although this holds true of oyster reefs in the St. Lucie estuary and the Loxahatchee on Florida's east coast (Arnold et al. 2008, Parker and Geiger 2009, Volety 2010) as well as the Caloosahatchee on Florida's west coast (Volety 2010), oyster reefs in the LWL are atypical in having a relatively high condition index from spring through fall. In this study, variability was seen between both sites and years. Oysters at MacArthur had CI peaks in the summer and fall of both years. Ibis Isle had CI peaks in summer and fall in year one, but only in the spring of year two. Snook Island had CI peaks in the spring and fall in year one, and followed the same pattern as Ibis Isle in year two. Arnold (et al. 2008) reported two condition index peaks in the LWL, one in April and another in June. Parker and Geiger (2009) reported variability in condition index through-out the year in the LWL. Volety (2010) reported highest condition indexes in LWL during the summer. These results suggest that stress was generally low enough and/or nutrients generally high enough to support both growth and reproduction throughout the year. The CI at MacArthur was significantly higher in year two compared to the central sites from August through December. Both central sites were subjected to salinity stress in the summer of year two, which may have negatively depressed condition index at these sites. Restoration at Ibis Isle was occurring concurrently and may have further added to stress at that site during that time.

Reproduction

Reproductive activity occurred continuously at all sites from spring to fall, although reproductive peaks varied depending on location. Spawning (stages 5-8) was spread out over a longer period in year one and peaks were not as evident as in year two, although peaks generally occurred in either April or June and again in September. Prolonged reproductive activity (April to September) and year to year variability are common in the LWL (Arnold et al. 2008, Parker and Geiger 2009 and Volety 2010). Although oysters at MacArthur were in significant better physiological condition than those in the central Lagoon sites in year two, this did not translate to greater reproductive output. As CI decreased in the central Lagoon during the summer of year two so did reproductive activity. Recruitment followed the same pattern as reproductive activity in year two and was concentrated in the fall. A greater proportion of sampled adult oysters were female rather than male, especially those sampled during spawning season and more so at the central sites than at MacArthur. Oysters are protrandric, which results in larger oysters tending to be female (Thompson et al. 1996). This skewed male to female ratio is likely the effect of sampling adult oysters larger than 51 cm rather than a reflection on the gender ratio in the environment and was also reported by Parker and Geiger (2009). Two hermaphrodites have been found at Ibis Isle (June 2008, October 2009) among all sampled oysters (n=330, 0.61%), which falls within ranges reported (Thompson et al. 1996).

Disease

The most prevalent oyster disease in Florida is Dermo, a haplosporidian endoparasite (*Perkinsus marinus*), associated with warm and salty environments that results in death of infected oysters two years later (Ford and Tripp 1996). Dermo disease was prevalent at all sites year round, though variability was evident between sites, seasons, and years. Intensities generally followed prevalence. Lowered prevalence and intensity occurred concurrent with lowered winter temperatures. As temperature was similar at all sites, salinity contributed to the variability seen between sites. Ibis Isle and Snook Island experienced a more variable salinity than MacArthur and consequently a lower prevalence and intensity of Dermo, especially following prolonged periods of lowered salinity occurred during the summer of year two. Annual intensity and prevalence were highest at MacArthur and lowest at Snook Island. Although monthly variations were was seen, neither site experienced annual variation. Annual prevalence and intensity was lower at Ibis Isle in year two due to the extended period of low salinity that occurred that summer. Similar monthly and yearly variations related to temperature and salinity have been reported previously in the LWL (Arnold et al. 2008, Parker and Geiger 2009, Volety 2010). Mean Dermo disease prevalence and intensity are higher on the west coast of Florida than the east coast due to higher average annual salinities (Volety 2010). Higher levels of intensity were reported in this study than in previous studies. The disparity between data reported in this study and that reported by Arnold et al. (2008) and Volety (2010) may be explained by their averaging of sites and years, however Parker and Geiger (2009) did not report densities as high as were seen in the present study, and their data was not averaged across sites or years.

MSX (*Haplosporidum nelsoni*) was not noted in samples from any site. The range of MSX has been reported to extend from Maine to Florida (Hillman et al. 1988, Burreson and Ford 2004,

Ulrich et al. 2007). Although Ulrich et al. (2007) reported MSX in samples from the Gulf Coast of Florida, the report was based on polymerase chain reaction (PCR) assays and no MSX organisms were verified in histological sections. There are no other reports of MSX positive samples from Florida (Arnold et al. 2008, Parker and Geiger 2009, Volety 2010).

Neither Arnold et al. (2008) nor Parker and Geiger (2009) examined oysters for any parasites except Dermo and MSX and therefore no baseline data exists for other oyster parasites in the LWL. However, Winstead et al (2004) reported various parasites from natural oyster populations in the Gulf of Mexico. In addition to Dermo, parasites detected in this study included the gregarine protozoan *Nematopsis sp.*, turbellarians, the trematode *Bucephalus*, and the cestode *Tylocephalum*. Mollusks are intermediate hosts for most of these species. No one site exhibited a greater prevalence or intensity of these parasites than another.

Crustaceans are the definitive host for *Nematopsis sp*. and are common in oysters in the southeastern U.S. and Gulf of Mexico (Lauckner 1983, Kim et al. 1998). Crabs were typically encountered at all sites sampled. Annual prevalence was similar to the higher values reported by Winstead et al. (2004) in the Gulf of Mexico and higher than that reported by Scarpa et al. (2009) for a one-time examination in oysters in land-based tanks receiving water from the Indian River Lagoon. A lower prevalence of *Nematopsis sp*. was seen at Ibis Isle and Snook Island concurrent with periods of lowered salinity in accordance with results reported by Tuntiwaranuruk et al. (2008). Intensity, categorized as low, moderate or high was usually low and did not correspond to salinity values. As reported by Winstead et al. (2004) there was no immune response to indicate that the parasite negatively affected the infected oysters.

Fish are the definitive host for trematodes such as *Bucephalus* which affects oysters by destroying the gonads and eventually causing mortality (Lauckner 1983). Sporocytes of the trematode *Bucephalus sp.* were intermittently found, generally at low prevalence, in gonadal tissue at all three sites. Winstead et al. (2004) and Scarpa et al. (2009) reported similar low prevalence in the Gulf of Mexico and the Indian River Lagoon. There was no evidence of a correlation between low salinity and increased *Bucephalus* infection as reported by Winstead et al. (2004). The parasite was absent in year two, in which the central sites in particular experienced a prolonged period of lowered salinity. Another explanation for the absence of this parasite in year two might be the absence of the definitive (true gar, *Lepisosteus*) and second intermediate hosts (mullet, *Mugil cephalus*).

Elasmobranches are the final hosts for the metacestode *Tylocephalum* (Lauckner 1983). *Tylocephalum* sp. was found at all sites with an average annual prevalence similar to that reported by Winstead (et al. 2004) in the Gulf of Mexico. Intensities were generally low although two instances of moderate intensities were seen at Snook Island. Infection typically caused a severe host response as has been reported by other researchers (Lauckner et al. 1983, Winstead et al. 2004).

Invertebrates are typically the final hosts for the flatworm turbellarians (Shinn 1985). Turbellarians were found sporadically at all sites in both years. Prevalence was consistent with that reported by Winstead et al. (2004) in the Gulf of Mexico.

Symbiotic parasites, such as the cnidarian *Eutima* and the pea crab *Pinnotheres*, were also present. Symbiotic pea crabs (*Pinnotheres sp*.) were infrequently found during preparation of oysters for condition index and histological analysis. Hydrozoan polyps of *Eutima sp*. were found in histological sections of oyster gills. Warm water hydrozoan polyps of the genus *Eutima* have been previously reported in the southern Atlantic and Gulf coasts of Florida (Mulholland and Fredl 1996). Prevalence appeared to follow a seasonal pattern and was greatest from spring through summer, disappearing at all sides in the fall and at all sites but MacArthur in the winter. A decreased prevalence was seen in year two, although the seasonal pattern was the same. Similar seasonal prevalence was reported by Winstead et al. (2004) in the Gulf of Mexico and Scarpa et al. (2009) in the Indian River Lagoon. Intensities were typically low.

One oyster sampled at MacArthur in April 2008 was infected with an ovacystis-like virus. This virus has previously been reported in Florida in the Gulf of Mexico at low (<1%) prevalence (Winstead and Courtney 2003, Winstead et al. 2004). In August of 2009, a high prevalence and intensity of a gill ciliate (*Ancistrocoma* sp.) was detected in the digestive tract of oysters at Ibis Isle, while a lower prevalence and intensity was detected in oysters at Snook Island. Hemocytic response was evident in the more heavily infected oysters. Considered an opportunistic pathogen, this gill ciliate can invade the host tissue during periods of abnormal physiological stress (Lauckner 1983). It is postulated that lowered salinities coupled with the movement of sediment during the restoration project may have temporarily added additional stress to oysters in the central Lagoon, causing host tissue to be invaded during that time. Oysters quickly recovered and the parasite disappeared once salinities increased, temperatures decreased, and the capping of muck was completed.

Multiple parasites were found at all sites and many oysters in the study had multiple parasitic infections. Multiple parasitic infections in oysters are indicative of a healthy ecosystem that supports a diverse assortment of other species as oysters are typically the intermediate hosts for parasites with final hosts being crustaceans, fish or birds.

Larval Recruitment

Recruitment occurred at all sites spring through fall, but the level varied significantly depending on the site. Recruitment was constant and low at MacArthur, while major peaks occurred in the fall in the central Lagoon. Two periods of peak recruitment were seen at Snook Island; summer and fall in year one and spring and fall in year two. Recruitment from summer through fall, with peaks in the fall, appears to be the normal pattern in the central Lagoon (Arnold et al. 2008, Parker and Geiger 2009). A similar two-peak pattern, as was seen at Snook Island, has been reported for the Loxahatchee area (Parker and Geiger 2009). In contrast, recruitment in the St. Lucie Estuary occurs earlier than in the LWL (Parker and Geiger 2009). Oyster reefs on the west coast report a similar spring through fall recruitment with peaks in summer or fall (Volety 2010). A relationship appears to exist between adult density and recruitment. Lowest recruitment was seen at MacArthur, which also had the lowest adult density. Whether this has to do with environmental conditions or nutrient availability in the northern LWL is unclear. It is not certain that recruits necessarily remain in the same area in which they are spawned. Oyster

recruits have little control over their own fate and dispersal is strongly dependant on currents and flushing rates of the estuary (Andrews 1983). Oyster larvae have been documented to travel at least 30 miles (Bahr and Lanier 1981). Ibis Isle had a greater adult density than Snook Island but had fewer recruits and exhibited peak recruitment only in the fall. As these two central sites are subject to similar environmental conditions, other factors beyond the scope of this monitoring study, such as hydrodynamics, may play a crucial role in recruitment. Additional loss of early spat may be caused by predators (e.g., oyster drills and crabs).

Juvenile Growth

Growth of newly settled oysters varied between locations and over time. Shell height was greater in year one than in year two at MacArthur and Ibis Isle, but not at Snook Island. Growth was consistently lower at Snook Island-Outer than at the other sites. Several factors, including heavy predation by oyster drills in year one and excessive recruitment, may explain lower growth at this site. Snook Island was the only site with two recruitment peaks. The overlay of new spat settling on arrays may have had the effect of decreasing the overall mean shell height. More fouling was typically seen on the array at Snook Island-Outer and the array was often moved about due to the boat wave action at that location. No ready explanation exists to why juvenile oysters had less growth in year two than in year one at MacArthur and Ibis Isle as northern and central sites were subjected to different environmental pressures (i.e. salinity) and presumably to different nutrient inputs, although the low January temperatures could have been a factor. Still growth rates reported in this study are comparable to previous monitoring efforts in LWL (Arnold et al. 2008, Parker and Geiger 2009, Volety 2010). Consistent with the present study, Parker and Geiger (2009) reported variation in both recruitment and shell height between three central LWL sites. Arnold et al. (2008) reported average means from all three LWL sites therefore no variation can be seen between sites, however variation was seen from year to year. All researchers reported less growth in LWL than in the St. Lucie Estuary or in the Loxahatchee area (Arnold et al. 2008, Parker and Geiger 2009, Volety 2010). Parker and Geiger (2009) postulated that the greater recruitment at LWL may have decreased the mean shell height. Oysters exhibit greater growth on the west coast of Florida than on the east coast (Volety 2010). At sites along the Caloosahatchee, greater growth occurred in oysters deployed after low salinity events which Volety (2010) attributed to decreased disease and predator pressure. Salinity did not appear to be a factor in juvenile growth in this study, as the mean shell height at MacArthur was greater than or equal to that of oysters at the less saline, central LWL sites.

CONCLUSIONS

This report summarizes two years of monitoring efforts at three distinct oyster reefs in Lake Worth Lagoon: two natural and one artificial, in two distinct locations, north and central, and subject to different salinity pressures. Productivity of monitored oyster beds in this study is consistent with data from previous monitoring studies in the LWL (Arnold et al. 2008, Parker and Geiger 2009, Volety 2010). The LWL is as productive (Loxahatchee area, Mosquito Lagoon, Sebastian River) or more productive (St. Lucie Estuary, Biscayne Bay) than other oyster reefs on Florida's east coast (Arnold et al. 2008, Parker and Geiger 2009). Oyster densities on Florida's west coast (Caloosahatchee River) are much higher than all east coast sites, including

the LWL (Volety 2010). Oyster reefs in the Caloosahatchee River had about five times greater recruitment and twice the intensity of Dermo disease compared to east coast sites (Volety 2010). In all other parameters measured (i.e., reproductive activity, juvenile growth, and condition index) LWL oysters are comparable to those of other Florida east and west coast oysters within year-to-year variability. Based on these studies further oyster reef restoration projects within LWL should be successful whether projects considered are large, such as the Snook Island project, or small in scope, such as envisioned in Living Shoreline projects.

All three sites monitored over the past two years have healthy oysters present. Although the natural oyster bed at MacArthur is subjected to less salinity pressure it had the lowest density and recruitment. Still, oysters at MacArthur had as high or higher CI and juvenile growth than oysters in the central Lagoon. Low density at this site may be due to increased disease pressure (Dermo), lack of substrate, poor nutrient availability, predation (crabs and oyster drills were often seen at this area) or poaching (an ongoing problem cited by park rangers). This lowered density likely impacted recruitment, which could additionally be impacted by lack of substrate, poor nutrient availability, or hydrology. Currents run on either side of this island and may carry recruits and nutrients away from the monitored site.

The natural oyster beds at Ibis Isle had the highest density and single-month recruitment, but the site is substrate limited. The edge of the Ibis Isle oyster beds transition to soft mud and muck in which the shells sink, decreasing the availability of old shell as substrate for new recruits. Ibis Isle may likewise be impacted by hydrology caused by the narrow channel under the bridge, south of the beds, which may limit water flow. Capping of muck, such as was done at Snook Island and restoring flow under the bridge may enhance the area.

The artificially constructed Snook Island environmental enhancement area had the highest annual recruitment (twice that of Ibis Isle and ten times that of MacArthur), but oyster density was only one-fifth to three-quarters that of Ibis Isle though twice as high as at MacArthur. The rock substrate may be at its maximum density or other factors (e.g., wave action, competition, or predation) might be limiting settlement and growth of oysters at this site. Wave action from boat traffic over the two-year monitoring period appeared to have worn the rock substrate down, particularly on the outer side. As islands with more vegetation were not monitored it is unclear whether vegetation may have protected those sites from wave action to some extent. Although no oysters grew on the top of the vertical structure, it is recommended that the height not be decreased due to the eventual wear and movement of rock at the base of the Island

While fresh water input may have helped to decrease disease (Dermo) pressure, it also adversely affected the condition index in both central sites and perhaps recruitment at Ibis Isle, which is closer to the source of the release. The amount of man-made fresh water input needs to be considered when determining placement of restoration projects.

Lake Worth Lagoon is a productive system with patches of healthy oyster beds that provide the recruitment necessary to seed large (Snook Island) and small restoration projects, as long as other environmental factors (salinity, hydrology, food availability) and substrate type (hard bottom versus mud) are considered.

SUMMARY CONCLUSIONS

- Oyster density was greatest at Ibis Isle and lowest at MacArthur
- Condition index was highest at Snook Island in year one and at MacArthur in year two
- Reproductive activity was higher at both Ibis Isle and Snook Island than at MacArthur
- Dermo disease prevalence and intensity was highest at MacArthur and lowest at Snook Island
- Larval recruitment was greatest at Snook Island and lowest at MacArthur
- Juvenile growth was greater at MacArthur and Ibis Isle in year one than at Snook Island-Inner; all sites were similar in year two
- Juveniles grew slower at Snook-Island Outer than at the other sites in both years.
- Oyster reefs in Lake Worth Lagoon are as productive or more productive than oyster reefs in other east Florida locales
- Annual variation in oyster productivity may be the result of natural variations in environmental parameters (temperature, salinity, nutrient availability), man-made (fresh-water releases) or a combination of the two.
- Central Lagoon sites appear to be more productive than northern Lagoon sites despite the additional fresh-water input
- Created oyster reefs as exemplified by Snook Island are as productive as natural reefs
- Productivity may be enhanced by carefully considering placement of created reefs in relation to natural oyster beds

RECOMMENDATIONS

- Additional oyster restoration projects are recommended for the central LWL
- The construction of additional artificial reefs, similar to that created at Snook Island are recommended
- Improving, maintaining and increasing successful natural reefs, similar to the Ibis Isle restoration project, are recommended
- Due to year-to-year fluctuations seen, multi-year monitoring is recommended at potential sites
- Additional monitoring of sites after restoration projects have been completed in the area (such as at Ibis Isle) are recommended to see the impact of those restoration projects
- Encouraging student and community volunteers to participate in restoration and monitoring projects under the direction of a biologist
- Implementation of smaller volunteer projects such as Living Shorelines and monitoring of those projects in conjunction with homeowners, student and community volunteers under the direction of a biologist
- Monitoring multiple sites within sites
    - Monitoring other islands in the Snook Island Restoration Area to see if similar results would have been obtained at sites with similar vertical distribution, but with more vegetation or northern, middle and southern sampling sites
    - Monitoring additional northern sites, for example one natural, such as MacArthur and one artificial
- Increased sample size – 20 per site (10 for CI, 10 for reproduction/disease) increases variability seen
- More frequent surveys – four times per year rather than two

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