Effects of heavy metals and pesticides on health and physiology of oysters in the Caloosahatchee Estuary: implications for management of water quality and restoration of oyster reefs.

Aswani K. Volety
Florida Gulf Coast University, 10501 FGCU Blvd, Fort Myers, FL 33965.

Gordon Romeis
Florida Department of Environmental Protection, 2295 Victoria Avenue, Ste. 364, Fort Myers, FL 33901.

&

Bruce Boler
US Environmental Protection Agency, 3745 Broadway Street, Suite 301, Fort Myers, FL 33901.

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I. Abstract/Executive Summary.

The Charlotte Harbor National Estuary Program is a partnership of citizens, elected officials, resource managers and commercial and recreational resource users working to improve the water quality and ecological integrity of the greater Charlotte Harbor watershed. A cooperative decision-making process is used within the program to address diverse resource management concerns in the 4,400 square mile study area. Many of these partners also financially support the Program, which, in turn, affords the Program opportunities to fund projects such as this. The entities that have financially supported the program include the following:

U.S. Environmental Protection Agency
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Cities of Sanibel, Cape Coral, Fort Myers, Punta Gorda, North Port, Venice and Fort Myers Beach
and the Southwest Florida Regional Planning Council.

Executive Summary

The Caloosahatchee Estuary has been exhibiting signs of impaired ecological health due to the extensive hydrological alteration, agricultural land use, and increasing development that have been occurring within the watershed. In addition to loss of benthic habitat due to channelizing and dredging in the Caloosahatchee River, significant water chemistry concerns have been identified within the Caloosahatchee Estuary and its tributaries that contribute to further degradation of fish and wildlife habitat and health. This project evaluated areas within the Caloosahatchee Estuary which are expected to have water quality concerns to verify whether these waters currently meet their designated use and assess the potential for a large scale restoration of oyster habitat. *In situ* biological response studies are needed to correlate pollutant concentrations with impairment of the health and physiological functions of estuarine organisms to aid in assessment of the overall ecological condition of the waterbody. This project used the American oyster, *Crassostrea virginica,* as the “valued ecosystem component” to assess the effects of contaminants and water quality on the ecological health of the Caloosahatchee Estuary. Candidate biomarkers investigated include juvenile oyster growth, reproductive physiology, disease susceptibility, and recruitment of oyster spat in five locations in the Caloosahatchee River. In addition, levels of heavy metals, pesticides, and PCB concentration in the water as well as oyster tissue were examined. Effects on oysters due to changes in salinity from freshwater discharges from Lake Okeechobee and variations in season were also examined.

Heavy metal and organochlorine pesticide concentrations in oysters varied significantly between sampling locations and sampling months. PCB concentrations in oyster tissues were below detection limits. Pesticide and PCB concentrations in water at the sampling locations were below detection limits as well. Both heavy metal and pesticide concentrations decreased with increasing distance downstream indicating upstream source of contaminants that decrease with tidal flushing and freshwater flows. Average heavy metal concentrations were below national average (National Status and Trends program). Condition index, spat recruitment, and gonadal index showed a seasonal trend varying with spawning activity and increased downstream during the sampling period. In addition, prevalence and infection intensity of the oyster pathogen, *Perkinsus marinus* increased downstream; decreases in salinity during summer months

associated with heavy rains and freshwater releases from Lake Okeechobee resulted in a sharp decline in *P. marinus* infections. Juvenile oysters deployed at upstream locations grew faster than those deployed at downstream locations.

It appears that the interactive effects of season and sampling location, combined with spatial variability mask any obvious trends of metal accumulation in oysters from the Caloosahatchee River. It should also be noted that despite high concentrations of certain heavy metals in oysters from some locations during various sampling times, overall concentrations are below national average. Oyster responses varied more with seasonal programming (salinity), rather than due to contaminant levels. No significant correlations were noted between oyster responses and metal and/or pesticide concentrations. Decreasing heavy metal concentrations with increasing distance downstream, and lack of correlation between heavy metal concentrations and oyster responses suggest that oyster health in the Caloosahatchee River is influenced more by freshwater inflow and resulting salinity fluctuations, rather than due to contaminant (heavy metals, pesticides, and PCBs). It should be cautioned that the current study did not examine polycyclic aromatic hydrocarbon concentrations in oysters. Future studies should investigate PAH concentrations in oysters and their potential role on oyster responses from select locations. In addition, this study evaluated the suitability of these locations for enhancing existing oyster bars or restoring historic oyster bars in order to provide the valuable ecologic functions of oysters mentioned above. Locations around Iona Cove and Shell Point appear to be conducive for the development of oyster reefs, but are currently lacking suitable substrate. Results from this study can be used as baseline values to evaluate heavy metal and organochlorine pesticides in the Caloosahatchee estuary as the watershed is further developed in the coming years.

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Introduction

Southwest Florida is among the fastest growing population centers in the country. As a consequence, watersheds are heavily managed to accommodate development, thereby compromising habitat and wildlife conservation efforts. Additionally, runoff from agricultural lands, golf courses, and housing subdivisions is directed into sensitive estuarine environments. These water flow alterations have resulted in the input of organic pollutants and altered salinity regimes, stressing the organisms inhabiting these ecosystems. For example, evidence of heavy metals accumulation in Estero Bay sediments was documented by Clark (1986), and later by Ceilley and Kibbey (1990). Specifically cadmium, chromium, lead, and zinc were at levels that indicated a high probability the sediments were contaminated. "Point and non-point sources including development, agricultural runoff, etc. contribute to such contamination" (Ceilley and Kibbey, 1990) and originate from freshwater inputs to the estuary. Elevated concentrations of these same heavy metals were documented in both the sediments and submerged aquatic vegetation (SAV) of 10-mile canal that drains 60± square miles of urban/suburban land and discharges into Estero Bay (Lee Co. Env. Lab unpublished data). The Caloosahatchee Estuary in SW Florida has been significantly altered both by hydrologic modifications and by increased development of adjacent lands for agriculture, residential use, and commercial use. These alterations have resulted in water quality degradation and loss of fish and wildlife habitat within the Estuary including oysters. In order to improve these conditions it is critical to understand the connections between land use and impacts on the ecosystem. Such information is clearly necessary, but currently lacking.

American oyster, *Crassostrea virginica* is prolific throughout temperate and subtropical latitudes of the western Atlantic and Gulf of Mexico, including SW Florida estuaries and occurs over a broad range of salinities. Oysters provide critical functions within the estuary ecosystem by filtering the water column, creating a reef structure for habitat and/or refuge, and providing a food source. Wells (1961) lists 303 species that depend, either directly or indirectly, on oyster reefs. In addition, the role of oysters in maintaining a healthy water column cannot be under-estimated. Oysters filter 4 to 40 liters of water per hour per oyster (Galtsoff 1964) and thus remove sediment, organic detritus, microbial pathogens, and contaminants from the water column (Bahr and Lanier 1981, Newell 1988), thereby “cleaning” the water and increasing light penetration. The filtered and deposited organic matter serves as a food source to other benthic organisms. This filtration role coupled with the secondary habitat provided by oyster reefs makes oysters a key species in benthic - pelagic coupling and maintaining a healthy ecosystem.

Historical accounts suggest that oyster growth and distribution has changed drastically since the 1960s, a time predating much of the extensive development of this coast. It is speculated that poor watershed management practices, diseases due to the protozoan parasite *Perkinsus marinus*, freshwater diversions or drought, and exacerbation of disease due to pollutants have contributed to the decline of oyster populations. Ultimately the impacts upon oyster health will help define watershed restoration conditions and guide restoration efforts.

The physiological and ecological effects of environmental stress are numerous. While the ultimate endpoint of stress is mortality, sub-lethal stress may interfere with the normal physiological activities of animals such as increased expenditure of energy reserves resulting in reduced growth, fecundity, and larval survival (Thompson et al. 1996, Capuzzo 1996); or an impaired defense system resulting in increased disease susceptibility (Anderson et al. 1996, Chu and Hale 1994). Consequently, an organism’s reproductive output, growth, and defense response are indirect measures of the health of an organism. Since a single response (for example, just a low growth rate) may be due to numerous factors, an integrated, multifaceted approach is preferred. Several studies have documented the physiological and ecological effects of environmental stress in oysters (Chu and La Peyre 1993a, Chu and La Peyre 1993b, Chu et al. 1993, Fisher 1988, Fisher and Newell 1986). The role of artificial reefs in the distribution and progression of diseases by *P. marinus* and *Haplosporidium nelsoni* on settlement and growth of oyster larvae were examined by several investigators (Bartol and Mann 1997, Bartol et al. 1999, Lenihan et al. 1999 and Volety et al. 2000) in Chesapeake Bay and mid-Atlantic regions. Most of these studies have focused on individual measures of various responses and none have used these measures for restoration purposes.

This project utilized the American oyster, *Crassostrea virginica*, as the “valued ecosystem component” to assess the effects of contaminants and water quality on the ecological health of the Caloosahatchee Estuary. We have investigated the potential effects of season and environmental contaminants on the physiological and ecological responses of oysters. Growth of juvenile oysters, condition index, reproductive state, spat recruitment and disease prevalence of *Perkinsus marinus* (Dermo) in oysters inhabiting potentially contaminated and reference sites were used as candidate biomarkers. By conducting *in situ* biological response studies on oysters as a valued ecosystem component in areas with known exposure to pollutants (such as at sites near marinas) compared to a reference site, organismal responses can be correlated with pollutant concentrations. If organisms in the pristine site lack effects while those in impacted sites are less healthy, then a cause and effect relationship between contaminants and health can be subsumed. Additionally this result will help determine what the desired water quality conditions should be for the estuary, thereby giving resource managers a target for restoration. This correlation will be valuable to better understand the cumulative impacts of land use management practices and to identify areas that need further attention to restore ecological function.

Project Objectives.

The objectives for the proposed study are:

1) Investigate various heavy metals, pesticides, and PCBs in the water and in the oyster tissues, and evaluate water quality parameters at four potentially impacted and one reference site.

2) Examine seasonally the condition index, disease prevalence *of P. marinus*, and reproductive potential using sentinel adult oysters.

3) Investigate growth and survival of caged juvenile oysters deployed at various sites.

4) Study oyster spat settlement on oyster shells deployed at various sites.

5) Examine the various macroinvertebrates and collect a baseline data of major taxa present in or near the reefs.

6) Contrast and correlate the measured health and ecological parameters with contaminant concentrations.

7) Involve students and the public in the project and increase their environmental awareness and their interest in and knowledge about environmental restoration.

Material and Methods.

Chemical analyses:

Oysters collected during the sampling scheme were immediately frozen at -70ºC upon arrival to the laboratory. Frozen samples were placed on dry ice and shipped to the Florida Department of Environmental Protection laboratory in Tallahassee for chemical analyses (trace metals, PCBs, and organochlorine pesticdes). Oyster tissue from 20 oysters from each sampling location was pooled for the analyses of metals and pesticides. Metals analyzed were Mercury, Antimony, Arsenic, Cadmium, Chromium, Copper, Lead, Manganese, Nickel, Selenium, Silver, Thallium, Tin, Zinc and Iron. Organochlorine analyses included Aldrin, Alpha-BHC, Beta-BHC, Delta-BHC, Gamma-BHC, Cis-Chlordane, Trans-Chlordane, DDD-p,p’, DDE-p,p’, DDT-p,p’, DDD-o-p’, DDE-o,p’, DDT-o,p’, Dieldrin, Endsulfan I, Endosulfan II, Endosulfan sulfate, Endrin, Heptachlor, Heptachlor Epoxide, Hexachlorobenzene, Methoxychlor, Mirex, Cis-Nonachlor, Trans-Nonachlor, PCB 8, PCB 18, PCB 28, PCB 52, PCB 44, PCB 66, PCB 101, PCB 105, PCB 118, PCB 128, PCB 138, PCB 153, PCB 170, PCB 180, PCB 187, PCB 195, and PCB 206. Mercury in tissue samples was analyzed using atomic fluorescence while metals were analyzed using trace-ICP emission spectroscopy (EPA 6010B) and pesticides analyzed using gas chromatograph with an electron capture detector (EPA8081). Metal (mg/kg (ppm)) and pesticide (ug/kg (ppb)) concentrations were determined on a wet weight basis. Results were normalized to dry weight of tissues by multiplying chemical analyte concentrations by 7.5 according to O’Connor (2002).

Water quality sampling:

Bi-monthly water quality sampling occurred concurrently with oyster sampling and survey of major taxa and habitat at one reference site and at four sites that are expected to contain heavy metal and organic pollutant concentrations. Florida Department of Environmental Protection (FDEP, 1998) staff conducted the water quality sampling in accordance with the FDEP Standard Operating Procedures and Quality Assurance Rule. Field parameters of specific conductance, salinity, pH, temperature, and dissolved oxygen were measured using a Hydrolab or YSI multiprobe sonde and data logger. PAR will be measured in the field with a LI-COR meter and data logger. Water samples were collected one foot above the bottom for determining TOC, BOD, TSS, TDS, Chl-a, color, turbidity, bacteria (total and fecal coliforms), and nutrients (nitrogen and phosphorus). All samples were collected and preserved in accordance with FDEP standard operating procedures. Samples were analyzed either at the Punta Gorda Branch Office Lab or the Department's Central Lab in Tallahassee.

Sampling locations:

Five sampling locations were selected in the Caloosahatchee Estuary in Lee County that are in the Tidal Caloosahatchee River Watershed region of the Charlotte Harbor, located just east of the Pine Island/Barrier Islands region of the CHNEP study area (Fig. 1). Sampling sites were (from upstream to downstream) at Iona Cove, Shell Point, Tarpon Point, Port Sanibel, and Greg's reef (reference site) located along the estuarine axis of the river. Iona Cove and Shell Point are located close to residential developments with boat docks; Tarpon Point and Port Sanibel are in close proximity to boat marinas, while Greg's reef is along the main channel in the lower San Carlos Bay.

Distribution and prevalence of disease:

For nearly fifty years, American oyster populations along the Atlantic and Gulf coasts of the United States have been ravaged by the highly pathogenic protozoan parasite *Perkinsus marinus*. Multiple stressors such as sediment loading, pollution, watershed alteration, salinity, temperature, and hypoxia enhance susceptibility to *P. marinus* infections. In order to determine effects of contaminants, *P. marinus* disease susceptibility / prevalence in oysters among impacted and reference sites were measured.

A total of fifty oysters (ten oysters per site, five sites) from various locations (see above) were collected bi-monthly. Oysters were assayed for the presence of *P. marinus* using Ray's fluid thioglycollate medium technique (Ray 1954, Volety et al. 2000). Samples of gill and digestive diverticulum were incubated in the medium for 4-5 days. *P. marinus* meronts enlarge in thioglycollate medium and stain blue-black with Lugol's iodine allowing for visual identification under a microscope. The intensity of infections were recorded using a modified Mackin scale (Mackin 1962) in which 0 = no infection, 1 = very light, 2 = light, 3 = light-moderate, 4 = moderate, 5 = moderate-heavy, and 6 = heavy.

***Oyster Condition Index*:**

The physiological condition of an oyster can be measured by its condition index, the ratio of meat weight to shell weight (Lucas and Beninger 1985). Since the metabolic energy remaining after reproduction and daily maintenance is converted to biomass, an oyster stressed either by its water quality or by disease has less energy for growth. Consequently, a comparison of oyster condition index among the oysters at impacted and reference sites should be indicative of oyster health and the influence of environmental and contaminant stress.

Condition index of oysters (n = 50, see above) were collected bi-monthly from various sites were analyzed according to Lucas and Beninger (1985). Oysters were shucked open, and meat separated and placed into pre-weighed aluminum boats. Both meat and shell were dried in an oven at 60°C for 24 - 48 hours. The dry meat weight and shell weight were determined and the condition index estimated as: dry meat weight/dry shell weight x 100.

***Oyster Growth*:**

Juvenile oysters grow at a faster rate than adults. For this reason, juvenile oysters were chosen to investigate growth of oysters deployed at various sites. Prior to the beginning of the study, 500 juvenile oysters (< 1 inch length) were collected. Five sets of 100 oysters were caged (mesh size 0.5 cm) and placed at five different sites. Growth of 50 randomly selected oysters was determined through bi-monthly measurements using a caliper. Oyster growth was estimated monthly during the study period and expressed as mm growth.

***Oyster Recruitment*:**

Water quality also influences larval recruitment. Oyster larvae respond to water flow, salinity, temperature, adult oysters, hard substrates, and old oyster shells colonized by bacteria. The net result is that oyster larvae typically settle more frequently in areas of low flushing, higher salinities, low contaminant levels and a dense accumulation of adults. In contrast, low salinities result in poor spat settlement and lower growth rates (Shumway 1996). Changes in water quality or poor oyster health may cause a shift in patterns of recruitment. New oyster recruits also provide opportunities to measure growth rates. If the time of settlement is known and the oyster's precise location can be recorded, an ontogenetic growth series can be determined. This information can be used to determine sites for reef restoration in the future.

Oyster recruitment experiments were conducted using old adult oyster shells strung together by a weighted galvanized wire and deployed at various sites with differing salinities. Shell strings consisting of 12 oyster shells, each 5.0-7.5 cm long, and each with a hole drilled in the center and oriented inner surface down, were suspended off the bottom at various sites (Haven and Fritz 1985). Oyster spat settlement was monitored monthly by counting the numbers of spat settled on the strung shells. Spat settlement is expressed as number of spat settled per oyster shell per month. By employing shellstrings to assess spat settlement, we investigated the recruitment potential of oysters to a given area, provided suitable habitat can be established in similar sites in the future. These studies serve as a baseline for future restoration activities and also examine the feasibility of reef development should water quality and substrate suitability improve (or can be improved).

***Reproductive potential and recruitment*:**

Unfavorable environmental conditions and *P. marinus* infections retard oyster growth (Paynter and Burreson 1991, Volety et. al. 2000), and thus reproduction and subsequent recruitment into the population. Histological procedures were used to examine gonadal state and reproductive potential of oysters from different sites during the study period. Ten oysters each from 5 sites were taken bi-monthly to analyze gonadal condition. Gametogenic stage was identified under a microscope and gonadal index expressed (Heffernan et al. 1989, Kennedy 1977, Kennedy and Krantz 1982).

Bio-assessments of major taxa:

The habitat value of oyster reefs will be qualitatively and semi-quantitatively evaluated through macroinvertebrate surveys conducted in conjunction with the collection of oyster tissue and water quality samples. Macroinvertebrate sampling was conducted by taking benthic grab samples and sieving them through successive mesh sizes, stained with rose Bengal and identified to the taxa level.

Statistical analyses:

The relationship between season, sampling location and oyster responses (condition index, *P. marinus* intensity, growth of juvenile oysters, and spat recruitment) was analyzed using a two-way ANOVA. Results were deemed significant at $P < 0.05$. When significant differences between means were detected, a multiple comparison of means (Dunnett's T-3) was used to detect the differences between two treatments assuming unequal variances. Correlation analysis was used to detect relationships between oyster responses and contaminant concentrations. All analyses were conducted using SPSS software (SPSS Inc., Chicago, IL). Due to differences in sediment size and composition, sampling for benthic invertebrate taxa was inconsistent, and hence no statistical analyses were conducted on that data.

Results and Interpretations.

Water Quality - Temperature and Salinity: As expected, temperature varied with season: temperature was highest in August (31ºC) and lowest during the month of February (19ºC) (Fig. 3). Differences in temperature between stations was < 2ºC in any given month. In contrast, salinity varied widely between sampling months and between sampling locations (Fig. 2). Salinity at the upstream locations, Iona Cove, Tarpon Point, and Shell Point was < 5 ppt in August 2001 and increased during the cooler, drier months (late fall - winter) (Fig. 2) to over 30 ppt. Other water quality parameters monitored during the sampling period are presented in Tables 1 and 2.

Chemical Contaminants: Pesticides were analyzed for all sites on several occasions. All pesticide results were below detection limits. As a result the pesticide data was not statistically analyzed. Iron was found in levels that exceed the standard in numerous water samples. Additional study would be required to determine if the iron is naturally occurring. There were some other metals that occasionally exceeded the standards at a few sites (Table 3).

Tissue analyses of oysters for heavy metals, and organochlorine pesticides and PCBs revealed that concentrations of these analytes varied between sampling locations and sampling months (Figs. 4 – 7). No appreciable levels of organochlorine pesticides and PCBs were detected in any of the sampled oysters (Tables 6 – 7). However, while metal concentrations at all sampling locations were at or below national average, few locations showed high concentrations of the metal analytes (Tables 4 - 5; Figs 8 - 14). However, no station showed consistently high concentrations of any chemical. Concentrations of metals were compared with national averages of heavy metals in oyster tissue from the NOAA's National Status and Trends Program (NS&T). Concentrations of metals from the current study that exceeded "High" concentrations in NS&T program are highlighted and italicized (Tables 4 - 5). Since metal concentrations in this study are computed on a wet weight basis, for comparison purposes with NS&T, values in the current study were multiplied by 7.5 to get tissue concentrations on a dry weight basis assuming 85% water content (O'Connor 2002). Preliminary studies confirmed that the water concentrations in oysters from the Caloosahatchee River is ~85% (results not reported). Since contaminant analyses were conducted on pooled oyster tissue from each location and sampling date, no replicate values were obtained for statistical purposes. However, values were transformed into a numeric ranking based on tissue concentrations at each location in comparison with the mean of all sampled locations (Fisher et al. 2000). Measured average value of total metals and /or pesticides for each station (data pooled from all sampling dates) were divided by the average value of total metals from all the stations. This results in unitless values, where a value of 1 is the average value of that metals and / or pesticides. Similar computations were made for each sampling month (data pooled from all locations). A value >1 suggests an above average value while a value of <1 suggests a below average value. These values were used to correlate total metal concentrations, or total pesticide concentrations with oyster responses from each sampling location averaged over the sampling period. Total metal concentrations decreased with increasing distance downstream (Table 4). While there are no obvious trends in metal concentrations in sampling months, metal concentrations tended to be higher during the August - October months (Table 5).

Condition Index: Condition index of oysters varied significantly between sampling stations and sampling months (Table 8; Figs 15 - 16). Condition index, a ratio of tissue weight: shell weight varies with reproductive cycle as oysters shed gametes during spawning season (May - October) (Volety et al., 2003). Overall, oysters in October of 2001 had significantly lower condition index compared to other sampling months (Fig. 15). Oysters from Tarpon Point and Shell Point had significantly lower than other sampling stations, Iona Cove, Port Sanibel, and Greg's reef (reference site) (Fig. 16). The reference site located downstream in the river had the highest condition index compared to other locations (Fig. 16).

P. marinus *intensity and Prevalence:* Prevalence (% infected oysters) of *Perkinsus marinus* infections varied between 13 - 62% during the sampling season (Fig. 17). Higher infection prevalences were observed during the late summer / early fall months and decreased during winter months and August, a month dominated by high freshwater flows into the river. Overall, oysters from downstream stations had higher infection rates compared to the two upstream stations (Fig. 18). Intensity (weighted prevalence) of *P. marinus* significantly varied both seasonally and spatially (Table 9). Intensity patterns mirrored those of prevalence, with higher infection intensities in oysters observed during late summer / early fall months (Fig. 19). In addition, oysters from the downstream locations (Shell Point, Port Sanibel, and Greg's reef) had significantly higher infection intensities compared to the two upstream locations, Iona Cove and Tarpon Point (Fig. 20).

Growth of juvenile oysters: Caged, juvenile oysters placed at various locations in the Caloosahatchee River showed significant differences due to sampling month and sampling locations (Table 10). Oysters at all sampling locations grew over the sampled months (Fig. 21). Overall, growth of juvenile oysters decreased with increasing distance downstream (Fig. 22). Oysters from Iona Cove and Shell Point showed the best growth.

Gonadal Index: Histological analyses of oysters suggested that oysters were actively spawning (gonadal index value 4-5) between May through October (Fig. 23). Oysters from upstream stations, Iona Cove and Shell Point, started spawning earlier compared to other locations. These results are supported by active spat recruitment at sampled locations (see below).

Spat recruitment: Results of spat recruitment support those of gonadal index. Spat recruitment significantly varied between sampling months and between sampling stations (Table 11). Oyster spat recruitment was observed at the sampled locations between April - through December suggesting that at least some oysters are spawning between March - November. Peak recruitment occurred between June - December (Fig. 24). Mean spat recruitment at all stations was highest during October 2002 reaching ~10 spat/shell while it was lowest from February to April 2002 (< 1 spat/shell). In general, spat recruitment increased with increasing distance downstream (Fig. 25).

Macroinvertebrate fauna: Macroinvertebrate fauna belonging to various taxa were observed in the shell hash around oyster reefs. These include: thread worms, mud worms, tube-worms, nematodes, arthropods - crustaceans, and shrimp, ribbon worms, and bivalves. Sediment texture and compositional differences (shell hash, mud, sand etc) made sampling difficult resulting in sampling differences. As a result, no statistical analyses were made on the macroinvertebrate data.

General Discussion and Recommendations.

Temperature and salinity values at various sampling stations in the Caloosahatchee River showed a trend that is typical for estuaries in SW Florida. Typically, salinity is lower in the Caloosahatchee River during the wet summer months and higher in the cooler, drier months. Results from this study validate the seasonal trends in the Caloosahatchee River observed in previous studies (Volety et al., 2003).

Seasonal sampling of oysters for various chemical analytes revealed that mercury, lead, arsenic, copper, zinc, chromium, selenium, and nickel were present in high concentrations (defined as higher than mean + 1 SD of the national average of the metal analyte). However, no spatial or temporal trends were discernable for individual metal analytes. Pesticide and PCB concentrations were below detection limits (Tables 6 - 7). However, total metal concentrations decreased with increasing distance downstream (Figs 4a & b, Tables 4 - 5). In addition, the total metal concentration was higher during the months of August - October. In SW Florida, this period coincides with freshwater releases from Lake Okeechobee and heavy rains, carrying water from the upstream agricultural areas and run-off from the City of Ft. Myers. The high concentrations of total metals during fall months combined with decreasing concentrations downstream suggest that the source of these metals is upstream and that as they reach downstream stations, combination of flushing and tidal mixing result in lower concentrations. Lack of detectable pesticide levels in oyster tissue suggests that pesticides in the water are short lived and pose no-threat to oysters in the lower Caloosahatchee estuary. It should be cautioned that while some metals showed high concentrations relative to the national average in the NS&T program, such levels are not known to cause harm to marine organisms or to man (O'Connor and Beliaeff, 1995).

When individual analytes were examined at various locations and sampling dates, several analytes showed high concentrations relative to the national average, no distinct trends were noticed. This implies that there are significant spatial and temporal variability. When metal concentrations were examined at various sampling stations (averaged for the year), or at various sampling months (averaged from all sampling locations), only arsenic exceeded the national average at the downstream most station (Table 4), and mercury, arsenic, selenium, and nickel exceeded national averages during December 2001 and August 2002 sampling months (Table 5). In many cases, the source of these contaminants is natural. For example, elevated arsenic levels in the southeast have been attributed to the natural occurrence of economically valuable phosphate deposits in the country (O'Connor 1992, 2002, Vallette-Silver et al. 1999). Overall, metal concentrations in oysters from Caloosahatchee River showed concentrations at a much lower level compared to national average.

Significant differences in condition index were observed both at the sampling stations as well as sampling months (Figs. 15 - 16). Condition index decreased from June to October 2001 suggesting that the decrease was related to spawning activity (see below). However, the decrease was less prominent in year 2. Previous studies showed decrease in condition index of oysters in relation to spawning stress (Volety et al., 2003). Similarly, with the exception of the upstream station, Iona Cove, condition index increased with increasing distance downstream (Fig. 16), again, a trend reported in previous studies. Tarpon Point had the lowest condition index of all the sampling locations.

P. marinus infection prevalence as well as the intensity varied with sampling location and sampling month (Figs. 17 - 20). Infection intensity and prevalence decreased with decreases in salinity resulting from freshwater releases and rainfall associated with summer months (July - August). The distribution and prevalence of *P. marinus* is influenced by temperature and salinity with higher values favoring the disease organism (Burreson and Ragone-Calvo 1996, Soniat 1996, Chu and Volety 1997). As expected, *P. marinus* infection prevalence and intensities increased with increasing distance downstream (Figs. 18 and 20). However, while the prevalence of infection was high (range 13 - 62%), the overall infection intensity was low (Fig. 20).

Juvenile oysters at upstream locations exhibited significantly higher growth compared to those that were deployed at the downstream locations (Fig. 22). Although oysters tolerate salinities between 0 and 42 ppt, growth is best achieved at salinities of 14-28 ppt; slower growth, poor spat production, and excessive valve closure occur at salinities below 14 ppt (see Shumway 1996). Mean salinities during the sampling period at the three upstream stations ranged from 20 - 23 ppt while those at the two downstream stations was 26 and 31 ppt respectively. The size of the oysters at all locations at the end of the sampling period was significantly higher compared to the initial deployment size (Fig. 22).

Recruitment of oyster spat onto shell strings during the months June - December (Fig. 24) combined with gonadal index (values 3-5) suggests that oysters in the Caloosahatchee estuary were reproductively active between May and October (Fig. 24). These results support previous studies on spat recruitment and reproductive responses of oysters in the Caloosahatchee River (Volety et al., 2003). Oysters at the upstream locations, Iona Cove and Shell Point were reproductively active 2 months earlier than at other locations (Fig. 23). However, numbers of spat recruited per shell increased with increasing distance downstream (Fig. 25). This may have been due to the higher numbers of oysters living at downstream stations in the Caloosahatchee River (Volety et al., unpublished results).

Summary.

It appears that the interactive effects of season and sampling location, combined with spatial variability mask any obvious trends of metal accumulation in oysters from the Caloosahatchee

River. It should also be noted that despite high concentrations of certain heavy metals in oysters from some locations during various sampling times, overall concentrations are below national average. Oyster responses varied more with seasonal programming (salinity), rather than due to contaminant levels. No significant correlations were noted between oyster responses and metal and/or pesticide concentrations. Decreasing heavy metal concentrations with increasing distance downstream, and lack of correlation between heavy metal concentrations and oyster responses suggest that oyster health in the Caloosahatchee River is influenced more by freshwater inflow and resulting salinity fluctuations, rather than due to contaminant (heavy metals, pesticides, and PCBs). The current study did not examine PAH concentrations in oysters. Future studies should investigate PAH concentrations in oysters and their potential role on oyster responses from select locations.

References cited:

Anderson, R. S., M. A. Unger, and E. M. Burreson. 1996. Enhancement of *Perkinsus marinus* disease progression in TBT exposed oysters *(Crassostrea virginica*). Marine Environmental Research. 42: 177-180.

Bahr, B. M., and W. P. Lanier. 1981. The ecology of intertidal oyster reefs of the south Atlantic: a community profile. US Fish and Wildlife Service, Office of Biological Services, Washington, D.C., FWS/OBS-81/15. pp. 105.

Bartol, I. K., and R. Mann. 1997. Small-scale settlement patterns of the *oyster Crassostrea virginica* on a constructed intertidal reef. Bulletin of Marine Science. 61:881-897.

Bartol, I. K., R. Mann, and M.W. Lukenbach 1999. Growth and mortality of oysters (*Crassostrea virginica*) on constructed intertidal reefs: effects of tidal height and substrate level. Journal of Experimental Marine Biology and Ecology. 237: 157-184.

Burreson, E. M. and L. M. Ragone-Calvo. 1996. Epizootiology of *Perkinsus marinus* disease of oysters in Chesapeake Bay with emphasis on data since 1985. Journal of Shellfish Research 15:17-34.

Capuzzo, J. M. 1996. The bioaccumulation and biological effects of lipophilic organic contaminants. In. *The Eastern Oyster Crassostrea virginica*. V. S. Kennedy, R. I. E. Newell, and A. F. Eble (eds). Maryland Sea Grant College Publication, College Park, MD. pp. 539-557.

Chu, F. L. E., and R. C. Hale. 1994. Relationship between pollution and susceptibility to infectious disease in eastern oyster, *Crassostrea virginica.* Marine Environmental Research. 38: 243-256.

Chu, F. L. E. and A. K. Volety. 1997. Disease processes of the *parasite Perkinsus marinus* in eastern oyster *Crassostrea virginica*: minimum dose for infection initiation, and interaction of temperature, salinity and infective cell dose. Diseases of Aquatic Organisms 28:61-68.

Ceilley, D.W. and K.A. Kibbey. 1990. South Estero Bay – Big Hickory Pass Report on water quality monitoring. Lee County Environmental Laboratory, Lee Co. Florida.

Chu, F. L. E., and J. F. La Peyre. 1993a. *Perkinsus marinus* susceptibility and defense-related activities in eastern oysters *Crassostrea virginica*: temperature effects. Diseases of Aquatic Organisms. 16: 223-234.

Chu, F. L. E., and J. F. La Peyre. 1993b. development of the disease caused by the parasite, *Perkinsus marinus* and defense-related hemolymph factors in three populations of oysters from Chesapeake Bay, USA. J. Shellfish Res. 12:21-27.

Chu, F. L. E., J. F. La Peyre, and C. S. Burreson. 1993. *Perkinsus marinus* infection and potential defense-related activities in eastern oysters, *Crassostrea virginica*: salinity effects. J. Invert. Pathol. 62:226-232.

Chu, F. L. E., and A. K. Volety. 1997. Disease processes of the parasite *Perkinsus Marinus* in eastern oyster *Crassostrea virginica*: minimum dose for infection initiation, and interaction of temperature, salinity and infective cell dose. Diseases of Aquatic Organisms 28: 61-68.

Clark, R.S. 1986. Water quality, circulation patterns and sediment analysis of the Estero Bay estuarine system. Department of Community Development, Lee County, Florida.

Fisher, W. S., L. M. Oliver, J. T. Winstead, and E. R. Long. 2000. A survey of oysters Crassostrea virginica from Tampa Bay, Florida: associations of internal defense measurements with contaminant burdens. Aquatic Toxicology 51: 115-138.

Fisher, W. S. 1988. Environmental influence on bivalve hemocyte function. In*. Disease Processes in marine bivalve molluscs*. W. S. Fisher (ed). Amer. Fish. Soc. Spec. Publ. 18., Bethesda, Maryland.

Fisher, W. S., and R. I. E. Newell. 1986. Salinity effects on the activity of granular hemocytes of American oyster, *Crassostrea virginica.* Bio. Bull. 170: 122-134.

Florida Department of Environmental Protection. 1998 Draft – Marine benthic habitat assessment data sheet and physical/chemical characterization field data sheet. FDEP Tallahassee, Florida.

Galtsoff, P. S. 1964. *The American Oyster: Crassostrea virginica Gmelin*. Fish. Bull. US. Fish and Wildlife. Service Publication 64. pp.480.

Haven, D. S. and L. W. Fritz. 1985. Setting of the American oyster *Crassostrea virginica* in the James River, Virginia, USA: temporal and spatial distribution. Marine Biology 86:271-282.

Heffernan, P. B., R. L. Walker and J. L. Carr. 1989. Gametogenic cycles of three marine bivalves in Wassaw Sound, Georgia II *Crassostrea virginica* (Gmelin, 1791). Journal of Shellfish Research 8:61-70.

Kennedy, V. S. 1977. Reproduction in *Mytilus edulis aoteanus* and *Aulaconmya maoriana* (Mollusca: bivalvia) from Taylor's Mistake, New Zealand. New Zealand Journal of Marine and Freshwater Research 11:253-267.

— and L. B. Krantz. 1982. Comparative gametogenic and spawning patterns of the oyster *Crassostrea virginica* (Gmelin), in central Chesapeake Bay. Journal of Shellfish Research 2:133-140.

Lenihan, H. S., F. Micheli., S. Shelton., and C. H. Peterson. 1999. The influence of multiple stressors on susceptibility to parasites: An experimental determination with oysters. Limnol. Oceanogr. 44: 910-924.

Lucas, A. and P. G. Beninger. 1985. The use of physiological condition index in marine bivalve aquaculture. Aquaculture 44:187-200. Ray, S. M. 1954. Biological studies of *Dermocystidium marinum*. The Rice Institute Pamphlet. Special Issue. November 1954, pp. 65-76.

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

Newell, R. I. E. 1988. Filtration capacities of oysters based on historic evidence. Proceedings of the Chesapeake Research Consortium 1988 meeting, Baltimore, MD. Chesapeake Research Consortium Publication. 129: 536-546.

O’Conner, T. P. 2002. National distribution of chemical concentrations in mussels and oysters in the USA. Marine Environmental Research 53: 117-143.

O’Connor, T. P. 1992. Mussel Watch: Recent Trands in Coastal Environmental Quality. NOAA Rockville, Maryland. 46 pp.

— and B. Beliaeff. 1995. Recent trends in coastal environmental quality: results from the mussel watch project. NOAA Silver Spring, Maryland. 25 pp.

Paynter, K. T. and E. M. Burreson. 1991. Effects of *Perkinsus marinus* infection in the eastern oyster *Crassostrea virginica* II. Disease development and impact on growth rate at different salinities. Journal of Shellfish Research 10:425-431.

Ray, S. M. 1954. Biological studies of *Dermocystidium marinum*. The Rice Institute Pamphlet. Special Issue. November 1954, pp. 65-76.

Shumway, S. E. 1996. Natural environmental factors. Pages 467-513 *in* V. S. Kennedy, R. I. E. Newell, and A. F. Eble (eds.), The Eastern Oyster *Crassostrea virginica*. Maryland Sea Grant College Publication, College Park, Maryland.

Soniat, T. M. 1996. Epizootiology of *Perkinsus marinus* disease of eastern oysters in the Gulf of Mexico. Journal of Shellfish Research 15:35-43.

Thompson, R. J., R. I. E. Newell, V. S. Kennedy, and R. Mann. 1996. Reproductive processes and early development. In. *The Eastern Oyster Crassostrea virginica*. V. S. Kennedy, R. I. E. Newell, and A. F. Eble (eds). Maryland Sea Grant College Publication, College Park, MD. pp.335-370.

Vallette-Silver, N.J., G.F. Riedel, E.A. Crecilius, H. Windom, R.G. Smith, and S.S. Dolvin. 1999. Evlevated arsenic concentrations in bivalves from the southeast coasts of the USA. Marine Environmental Research, *448*, 311-333.

Volety, A. K., F. O. Perkins, R. Mann and P. R. Hershberg. 2000. Progression of diseases caused by the oyster parasites, *Perkinsus marinus* and *Haplosporidium nelsoni*, in *Crassostrea virginica* on constructed artificial reefs. Journal of Shellfish Research. 19: 341-347.

Volety, A. K., S. G. Tolley, and J. T. Winstead. 2003. Effects of seasonal and water quality parameters on oysters (*Crassostrea virginica*) and associated fish populations in the Calooshatchee River. Final Report (C-12412-A1) to South Florida Water Management District.

Wells, H. W. 1961. The fauna of oyster beds with special reference to the salinity factor. Ecological Monographs. 31: 239-266.

Table 1. Bacteria, chlorophyll, TOC, TDS, TSS, and BOD levels of water collected at the time of oyster collection during sampling period.

6/6/2001	**Tarpon Pt**	**Iona Cove**	**Shell Pt**	**Port Sanibel**	**Greg's Reef**
Fecal Coli (/100mL)	2K				
Total Coli (/100mL)	8B				
Chl A (ug/L)					
Phaeo (ug/L)					
TOC (mg/L)	5				
TDS (mg/L)					
TSS (mg/L)					
BOD (mg/L)	1.7				

8/8/2001	**Tarpon Pt**	**Iona Cove**	**Shell Pt**	**Port Sanibel**	**Greg's Reef**
Fecal Coli (/100mL)	64B	12B	24B	24B	4K
Total Coli (/100mL)	260B	1540	280B	480	100B
Chl A (ug/L)		8.9	22.4	22.1	2.56I
Phaeo (ug/L)					
TOC (mg/L)					
TDS (mg/L)					
TSS (mg/L)					
BOD (mg/L)	1.2		1.3	2.9	1.9

10/17/2001	**Tarpon Pt**	**Iona Cove**	**Shell Pt**	**Port Sanibel**	**Greg's Reef**
Fecal Coli (/100mL)	4K	8B	60B	4B	NR
Total Coli (/100mL)	120	36B	100	32B	NR
Chl A (ug/L)		4.9J	NR	1.00U	3.92I
Phaeo (ug/L)					
TOC (mg/L)					
TDS (mg/L)					
TSS (mg/L)					
BOD (mg/L)	1.1				

12/17/2001	**Tarpon Pt**	**Iona Cove**	**Shell Pt**	**Port Sanibel**	**Greg's Reef**
Fecal Coli (/100mL)	24B	60	172	4K	16B
Total Coli (/100mL)	140B	NR	220B	60B	340B
Chl A (ug/L)		3.0I	2.5I	3.3I	2.2I
Phaeo (ug/L)					
TOC (mg/L)	0.03I				
TDS (mg/L)					
TSS (mg/L)					
BOD (mg/L)	1.4		1.7	1.3	1.3

2/18/2002	**Tarpon Pt**	**Iona Cove**	**Shell Pt**	**Port Sanibel**	**Greg's Reef**
Fecal Coli (/100mL)	4B	4B	4B	4B	4K
Total Coli (/100mL)	20B	20B	20B	20B	20K
Chl A (ug/L)		1.4I	2.1I	1.7I	1.3I
Phaeo (ug/L)					
TOC (mg/L)					
TDS (mg/L)					
TSS (mg/L)					
BOD (mg/L)	0.75I		1.7	0.9	1

4/29/2002	**Tarpon Pt**	**Iona Cove**	**Shell Pt**	**Port Sanibel**	**Greg's Reef**
Fecal Coli (/100mL)					
Total Coli (/100mL)					
Chl A (ug/L)		0.96U	0.85U	0.85U	0.96U
Phaeo (ug/L)		0.96U	0.85U	0.85U	0.96U
TOC (mg/L)	5.8I	6.4I	5.9I	4.6I	3.3I
TDS (mg/L)					
TSS (mg/L)					
BOD (mg/L)					

Table 1 Continued

6/5/2002	Tarpon Pt	Iona Cove	Shell Pt	Port Sanibel	Greg's Reef
Fecal Coli (/100mL)	2K		8B	2B	2B
Total Coli (/100mL)	20B		12B	8B	4K
Chl A (ug/L)					
Phaeo (ug/L)					
TOC (mg/L)					
TDS (mg/L)					
TSS (mg/L)					
BOD (mg/L)					

8/7/2002	Tarpon Pt	Iona Cove	Shell Pt	Port Sanibel	Greg's Reef
Fecal Coli (/100mL)	4K	2B	34B	6B	2K
Total Coli (/100mL)	4B	12B	24B	12B	4K
Chl A (ug/L)					
Phaeo (ug/L)					
TOC (mg/L)					
TDS (mg/L)					
TSS (mg/L)					
BOD (mg/L)					

Table 2. Water quality parameters at time of oyster collection during study period.

6/6/2001	Tarpon Pt	Iona Cove	Shell Pt	Port Sanibal	Greg's
Time (24 hr)	1018	1121	1145	1226	1300
Depth (ft)	0.75	0.75	0.75	1.5	0.75
SpC (uS/cm)	53420	53840	53770	55840	56220
Salinity (ppt)	35.15	35.4	35.42	36.96	37.21
pH (SU)	7.84	7.88	7.93	8	8.17
Temp (C)	29.11	29.78	29.48	29.5	29.91
Secchi (m)	0.46L	0.46L	0.46L	1L	0.46L
DO (mg/L)	4.81	6.16	5.8	5.71	6.5
Chl A (ug/L)					
Turb (NTU)	7				
Color (PCU)	10i				
NH3 (mg N/L)					
TKN (mg N/L)	0.66J				
NO2/NO3 (mg N/L)	0.007I				
TP (mg P/L)	0.087				
OP (mg P/L)		0.15			
BOD (mg/L)					

8/8/2001	Tarpon Pt	Iona Cove	Shell Pt	Port Sanibal	Greg's
Time (24 hr)	1000	1130	1100	1215	1240
Depth (ft)	0.5	0.5	0.5	0.5	0.5
SpC (uS/cm)	9495	6468	7279	NR	NR
Salinity (ppt)	28.63	3.49		NR	NR
pH (SU)	7.55	7.91	7.99	NR	NR
Temp (C)	28.63	30.22	28.78	32	29
Secchi (m)	NR	NR	NR	NR	NR
DO (mg/L)	4.56	7.8	7.93	NR	NR
Chl A (ug/L)	11.9				
Turb (NTU)	5.7I	6.2I	6.8	6.5	10.7
Color (PCU)	140	200	120	110	120
NH3 (mg N/L)					
TKN (mg N/L)	1.4	1.4	1.4	1.4	1.5
NO2/NO3 (mg N/L)	0.36	0.4	0.4	0.18	0.26
TP (mg P/L)	0.24	0.28	0.3	0.16	0.18
OP (mg P/L)	0.14	0.15	0.15	0.08	0.08
BOD (mg/L)		2.1			

10/17/2001	Tarpon Pt	Iona Cove	Shell Pt	Port Sanibel	Greg's
Time (24 hr)	1150	1015	1105	1207	1330
Depth (ft)	0.5	0.5	0.5	0.5	0.5
SpC (uS/cm)	36970	24760	25054	33921	43657
Salinity (ppt)	22.9	15.05	15.27	21.11	28
pH (SU)	7.76	7.84	7.68	7.82	7.9
Temp (C)	27.1	25.19	24.66	26.55	26.9
Secchi (m)	0.8L	NR	0.3	0.4L	0.4L
DO (mg/L)	5.5	7.2	6.83	6.62	6.46
Chl A (ug/L)	3.28I				
Turb (NTU)	2.7I	11.2	24	6.9	11.3
Color (PCU)	40	60	60	50	30
NH3 (mg N/L)					
TKN (mg N/L)	0.87	1.4	1.5	0.96	0.78
NO2/NO3 (mg N/L)	0.01U	0.07	0.07	0.01U	0.01U
TP (mg P/L)	0.1	0.14	0.19	0.11	0.1
OP (mg P/L)	0.08	0.05	.08I	0.05	0.03I
BOD (mg/L)		1.8			

12/17/2001	Tarpon Pt	Iona Cove	Shell Pt	Port Sanibel	Greg's
Time (24 hr)	1015	1045	1120	1155	1220
Depth (ft)	0.5	0.5	0.5	0.5	0.5
SpC (uS/cm)	46285	47072	46374	46183	48024
Salinity (ppt)	30.02	30.99		29.99	31.28
pH (SU)	7.71	7.75	7.74	7.82	7.88
Temp (C)	24.89	25.57	25.2	25.16	24.55
Secchi (m)	1.2	1.5	1.1L	0.9	2
DO (mg/L)	5.4	5.59	5.55	6.28	6.14
Chl A (ug/L)	2.0I				
Turb (NTU)	5.3I	5.0I	5.1I	10.3	3.8I
Color (PCU)	30	30	30	30	30
NH3 (mg N/L)					
TKN (mg N/L)	0.88	30	0.72	0.85	0.69
NO2/NO3 (mg N/L)	0.01U	0.01U	0.01U	0.01U	0.01U
TP (mg P/L)	0.09	0.09	0.08	0.09	0.07
OP (mg P/L)		0.041	0.04I	0.3I	0.03I
BOD (mg/L)		1.4			

Table 2 continued.

2/18/2002	Tarpon Pt	Iona Cove	Shell Pt	Port Sanibal	Greg's
Time (24 hr)	935	1020	1120	1220	1220
Depth (ft)	0.5	0.5	0.5	0.5	0.5
SpC (uS/cm)	42167	37877	38383	44715	44486
Salinity (ppt)	27.14	24.1	24.47	28.94	28.8
pH (SU)	7.83	7.86	7.93	7.91	7.87
Temp (C)	19.27	17.46	18.03	19.01	18.71
Secchi (m)	2.0L	0.5L	0.4L	1.2	1.9
DO (mg/L)	7.46	8.15	8.77	8.47	7.67
Chl A (ug/L)	1.4I				
Turb (NTU)	3.1I	7.3	13.4	16.8	16.8
Color (PCU)	20I	20I	30	20I	15I
NH3 (mg N/L)	0.014I	0.023J	0.013I	0.014I	0.011I
TKN (mg N/L)	0.62	0.77	0.83	0.71	0.6
NO2/NO3 (mg N/L)	0.013	0.006I	0.004U	0.008I	0.008I
TP (mg P/L)	0.062	0.078	0.097	0.075	0.073
OP (mg P/L)		0.039	0.035	0.03	0.032
BOD (mg/L)		2.7			

4/29/2002	Tarpon Pt	Iona Cove	Shell Pt	Port Sanibal	Greg's
Time (24 hr)	945	1010	1040	0	1155
Depth (ft)	0.5	0.5	0.5	0.5	0.5
SpC (uS/cm)	NR	NR	NR	NR	NR
Salinity (ppt)			NR	NR	NR
pH (SU)		7.82	7.74	7.8	7.99
Temp (C)	28.02	27.71	28.49	28.17	28.49
Secchi (m)	1.3	5.27	0.8L	1.3	1.9
DO (mg/L)	4.87	1.4	4.49	4.7	6.16
Chl A (ug/L)	0.96u				
Turb (NTU)					
Color (PCU)					
NH3 (mg N/L)	0.013i	0.015I	0.011I	0.012I	0.01U
TKN (mg N/L)	0.78	0.80A	0.83	0.69	0.64
NO2/NO3 (mg N/L)	0.005i	0.004U	0.004U	0.006I	0.004I
TP (mg P/L)	0.1	0.084A	0.09	0.07	0.066
OP (mg P/L)	0.23	0.04	0.044	0.03	0.021
BOD (mg/L)					

6/5/2002	Tarpon Pt	Iona Cove	Shell Pt	Port Sanibel	Greg's
Time (24 hr)	930	1010	1045	1130	1147
Depth (ft)	0.5	0.5	0.5	0.5	0.5
SpC (uS/cm)	45126	43213	45385	46949	54909
Salinity (ppt)	26.78	25.66	26.75	30.6	33.09
pH (SU)	7.32	8.02	7.96	7.93	8.09
Temp (C)	29.35	28.75	29.44	29.15	29.39
Secchi (m)	0.7L	0.65L	0.75L	1.05L	2
DO (mg/L)	5.75	5.5	4.72	4.39	6.78
Chl A (ug/L)					
Turb (NTU)					
Color (PCU)					
NH3 (mg N/L)	0.02U	0.02U	0.02U	0.02U	0.02U
TKN (mg N/L)	0.81 JA	0.91J	0.79J	0.75J	0.51J
NO2/NO3 (mg N/L)	0.006I	0.004U	0.005I	0.004U	0.004U
TP (mg P/L)	0.077	0.084	0.08	0.07	0.032A
OP (mg P/L)	0.047	0.051	0.051	0.032	0.016
BOD (mg/L)					

8/7/2002	Tarpon Pt	Iona Cove	Shell Pt	Port Sanibel	Greg's
Time (24 hr)	1010	1044	1117	1157	1226
Depth (ft)	0.5	0.5	0.5	0.5	0.5
SpC (uS/cm)	1700	7360	8480	23479	40062
Salinity (ppt)	10.17		5	14.09	25.62
pH (SU)	7.7	8.01	7.94	7.88	8.03
Temp (C)	30.86	29.68	30.11	30.64	31.78
Secchi (m)	0.7	0.6	0.6	0.78	1.43L
DO (mg/L)	4.67	6.73	6.37	5.59	5.6
Chl A (ug/L)					
Turb (NTU)					
Color (PCU)					
NH3 (mg N/L)	0.086	0.023	0.03	0.059	0.029
TKN (mg N/L)	1.2	1.5	1.5	1.3	0.75
NO2/NO3 (mg N/L)		0.13	0.13	0.089	0.026
TP (mg P/L)	0.17	0.19	0.18	0.15	0.093
OP (mg P/L)	0.12	0.11	0.11	0.077	0.032
BOD (mg/L)					

Table 3. Metal concentrations of water samples taken at time of oyster collection.

2/18/2002	**Tarpon Pt**	**Iona Cove**	**Shell Pt**	**Pt Sanibel**	**Greg's**
Al (ug/L)	70U	100I	140I	140I	70U
As (ug/L)	7.0U	18U	18U	34U	22U
Ca (mg/L)	328	300	320	357	349
Cr (ug/L)	3.5I	4.6U	54.9	35.3	6
Cu (ug/L)	3.5U	3.5U	4.3I	3.5U	3.5U
Fe (ug/L)	32I	116	228	175	71
Mg (mg/L)	1040	921	967	1.13E+03 (1130)	1.11E+03 (1110)
Pb (ug/L)	50U	50U	50U	50U	50U
Zn (ug/L)	8.8U	8.8U	8.8U	8.8U	8.8U
Hg (ug/L)	0.10U	0.10U	0.10U	0.10U	0.10U
Cd (ug/L)	5.0U	5.0U	5.0U	5.0U	5.0U
Mn (ug/L)	4.8	6.1	6.4	4.4	4.7
Ni (ug/L)	2.0U	2.0U	9.3	7.3I	4.7I

4/29/2002	**Tarpon Pt**	**Iona Cove**	**Shell Pt**	**Pt Sanibel**	**Greg's**
Al (ug/L)	75	46	48	66	65
As (ug/L)	8.5I	7.0U	7.0U	11I	9.5I
Ca (mg/L)	361	327	357	392	391
Cr (ug/L)	5.0U	4.2U	5.8U	4.8U	4.0U
Cu (ug/L)	3.5U	3.5U	3.5U	3.5U	3.5U
Fe (ug/L)	58	48	52	62	57
Mg (mg/L)	1130	1010	1140	1260	1280
Pb (ug/L)	1.2U	1.2U	1.2U	1.2U	1.2U
Zn (ug/L)	20.0U	14.0U	4.0U	13	20U
Hg (ug/L)	0.10U	0.10U	0.10U	0.10U	0.10U
Cd (ug/L)	0.12U	0.12U	0.12U	0.12U	0.12U
Mn (ug/L)	7	8.6	10.3	5.6	5.2
Ni (ug/L)	2.0U	3.0I	2.2I	2.0U	2.0U

6/5/2002	**Tarpon Pt**	**Iona Cove**	**Shell Pt**	**Pt Sanibel**	**Greg's**
Al (ug/L)	50U	56I	58I	72I	50U
As (ug/L)	30U	30U	30U	30U	30U
Ca (mg/L)	351	338	353	386	422
Cr (ug/L)	20U	20U	20U	20U	20U
Cu (ug/L)	30U	30U	30U	30U	30U
Fe (ug/L)	56I	65I	57I	81I	50U
Mg (mg/L)	1140	1020	1060	1210	1340
Pb (ug/L)	50U	50U	50U	50U	50U
Zn (ug/L)	90U	74U	86U	96U	100U
Hg (ug/L)	0.1U	0.1U	0.1U	0.1U	0.10U
Cd (ug/L)					
Mn (ug/L)					
Ni (ug/L)					

8/7/2002	**Tarpon Pt**	**Iona Cove**	**Shell Pt**	**Pt Sanibel**	**Greg's**
Al (ug/L)	63	84	127	111	52
As (ug/L)	19U	7.0U	7.0U	19.0U	38.0U
Ca (mg/L)	150	92.5	101	198	352
Cr (ug/L)	2.0U	2.0U	8.7	8.2U	11.0U
Cu (ug/L)	3.0U	3.0U	3.0U	3.0U	3.0U
Fe (ug/L)	192	287	348	184	59
Mg (mg/L)	359	150	179	536	1060
Pb (ug/L)	34.0U	30.0U	26.0U	48.0U	58.0U
Zn (ug/L)	4.0U	4.0U	4.0U	4.0U	13.0U
Hg (ug/L)	0.10U	0.10U	0.10U	0.10U	0.10U
Cd (ug/L)					9.9
Mn (ug/L)	12.1	10.3	18.1	8.8	
Ni (ug/L)					

All Sites

Al	As	Ca	Cr	Cu	Fe	Mg	Pb	Zn	Hg	Cd	Mn	Ni
<=1.5mg	<=50ug		<=50ug	<=3.7ug	<=0.3mg		<=8.5	<=8.6ug	<=0.012ug	<=9.3ug		<=8.3ug

Table 4: Concentrations of heavy metals in oyster tissues (mg/kg; ppm dry weight) from the Caloosahatchee Estuary. Data presented is the average of six samplings from each site. Total metal concentration for each site was divided by the average total metal concentration for all the sites to get a unitless value that was ranked. A Value of 1 represents average value while > 1 and < 1 represent values above and below the mean concentrations for the estuary. Individual metal analytes that were in "high" concentrations are highlighted in yellow. "High" concentrations are defined as those that exceed mean + SD of national average from National Status and Trends Program.

Site	Mercury	Cadmium	Lead	Silver	Arsenic	Copper	Iron	Manganese	Zinc	Chromiun	Selenium	Tin	Nickel	Total	Rank
Iona Cove	0.15	0.71	0.85	1.58	4.66	375.63	355.50	21.98	5668.75	2.78	0.94	1.14	0.61	6435.26	1.62
Tarpon Point	0.16	1.43	1.34	1.55	7.51	298.13	627.50	24.99	3866.25	3.06	1.43	2.30	0.75	4836.39	1.22
Shell Point	0.16	0.66	1.04	1.36	5.51	198.75	349.88	24.30	3453.75	4.01	1.36	1.96	1.30	4044.05	1.02
Port Sanibel	0.15	0.70	0.93	1.21	11.39	128.13	318.63	18.93	2088.75	3.44	1.53	1.70	1.36	2576.83	0.65
Reference	0.12	1.20	0.73	1.23	21.25	97.56	349.50	22.49	1495.00	5.63	1.79	1.79	1.04	1999.31	0.50

Table 5: Concentrations of heavy metals in oyster tissues (mg/kg; ppm dry weight) from the Caloosahatchee Estuary. Data presented is the average of tissue analyses from 5 sites in the estuary. Total metal concentration for each sampling date was divided by the average total metal concentration for all the sampling dates to get a unitless value that was ranked. A Value of 1 represents average value while > 1 and < 1 represent values above and below the mean concentrations for the estuary. Individual metal analytes that were in "high" concentrations are highlighted in yellow. "High" concentrations are defined as those that exceed mean + SD of national average from National Status and Trends Program.

Date	Mercury	Cadmium	Lead	Silver	Arsenic	Copper	Iron	Manganese	Zinc	Chromiun	Selenium	Tin	Nickel	Total	Rank
6/6/01	0.14	0.76	1.25	0.74	11.40	136.50	268.50	35.25	1840.50	0.00	0.00	0.00	0.00	2295.04	0.58
8/7/01	0.14	1.00	1.58	1.25	9.90	194.48	479.40	28.05	3205.50	2.87	2.75	3.99	0.00	3930.89	0.99
10/17/01	0.15	1.29	1.38	1.97	9.66	348.60	574.95	24.00	4323.00	8.13	3.74	6.68	0.81	5304.35	1.33
12/17/01	0.12	1.28	0.90	1.46	22.80	195.75	337.65	15.45	2893.50	4.70	0.00	0.00	3.63	3477.23	0.87
8/21/02	0.24	0.48	0.00	1.77	0.00	339.60	453.75	18.30	5106.00	0.00	0.00	0.00	0.00	5920.14	1.49
10/21/02	0.11	0.82	0.77	1.14	6.63	102.90	286.95	14.16	2518.50	7.01	1.97	0.00	1.64	2942.58	0.74

Table 6: Concentrations of organichlorine pesticides in oyster tissues (ug/kg; ppb dry weight) from the Caloosahatchee Estuary. Data presented is the average of tissue analyses from 5 sites in the estuary. Total pesticide concentration for each sampling date was divided by the average total pesticide concentration for all the sampling dates to get a unitless value that was ranked. A Value of 1 represents average value while > 1 and < 1 represent values above and below the mean concentrations for the estuary. Concentrations of pesticides decreased with increasing distance downstream suggesting an upstream source of contaminants that get diluted with tidal influence.

Collection Site	**DDE-p,p'**	**DDD-p,p'**	**DDT-p,p'**	**DDT-o,p'**	**Trans-Clordane**	**Trans-Nonachlor**	**Cis-Chlordane**	**Total**	**Rank**
Iona Cove	11.98	0.33	0.00	0.00	1.36	1.56	0.81	16.04	1.55
Tarpon Point	7.65	0.00	0.61	0.18	1.95	1.74	1.94	14.06	1.36
Shell Point	7.69	0.00	0.00	0.00	0.63	0.90	1.01	10.23	0.99
Port Sanibal	5.93	0.00	0.00	0.00	0.13	0.50	0.49	7.04	0.68
Reference	2.29	0.00	0.84	0.00	0.33	0.44	0.48	4.36	0.42

Table 7: Concentrations of organichlorine pesticides in oyster tissues (ug/kg; ppb dry weight) from the Caloosahatchee Estuary. Data presented is the average of tissue analyses from 5 sites in the estuary. Total pesticide concentration for each sampling date was divided by the average total pesticide concentration for all the sampling dates to get a unitless value that was ranked. A Value of 1 represents average value while > 1 and < 1 represent values above and below the mean concentrations for the estuary.

Collection Date	**DDE-p,p'**	**DDD-p,p'**	**DDT-p,p'**	**DDT-o,p'**	**Trans-Clordane**	**Trans-Nonachlor**	**Cis-Chlordane**	**Total**	**Rank**
6/6/01	6.23	0.00	0.00	0.00	0.00	0.00	0.00	6.23	0.60
8/7/01	16.65	0.00	0.00	0.00	2.99	3.38	3.00	26.01	2.51
10/17/01	8.40	0.00	0.00	0.00	1.34	1.49	1.85	13.07	1.26
12/17/01	4.05	0.00	0.00	0.00	0.00	0.00	0.00	4.05	0.39
8/21/02	4.10	0.39	1.74	0.21	0.95	0.98	0.83	9.18	0.89
10/21/02	3.21	0.00	0.00	0.00	0.00	0.33	0.00	3.54	0.34

Table 8: Analysis of variance of condition index in oysters:

Source	Type III sum of squares	df	F	Significance
Month	75.41	9	8.38	0.000
Station	59.58	4	14.90	0.000
Station*Month	112.04	36	3.11	0.000

Table 9: Analysis of variance of *Perkinsus marinus* intensity in oysters:

Source	Type III sum of squares	df	F	Significance
Month	16.71	9	5.455	0.000
Station	17.91	4	5.682	0.010
Station*Month	21.70	36	0.797	0.795

Table 10: Analysis of variance of juvenile oyster growth:

Source	Type III sum of squares	df	F	Significance
Month	37766.31	6	51.57	0.000
Station	46399.25	4	95.04	0.000
Station*Month	8789.51	23	3.13	0.000

Table 11: Analysis of variance of spat recruitment on shell strings:

Source	Type III sum of squares	df	F	Significance
Month	19661.33	8	191.44	0.000
Station	4016.88	4	78.23	0.000
Station*Month	23528.24	31	59.12	0.000

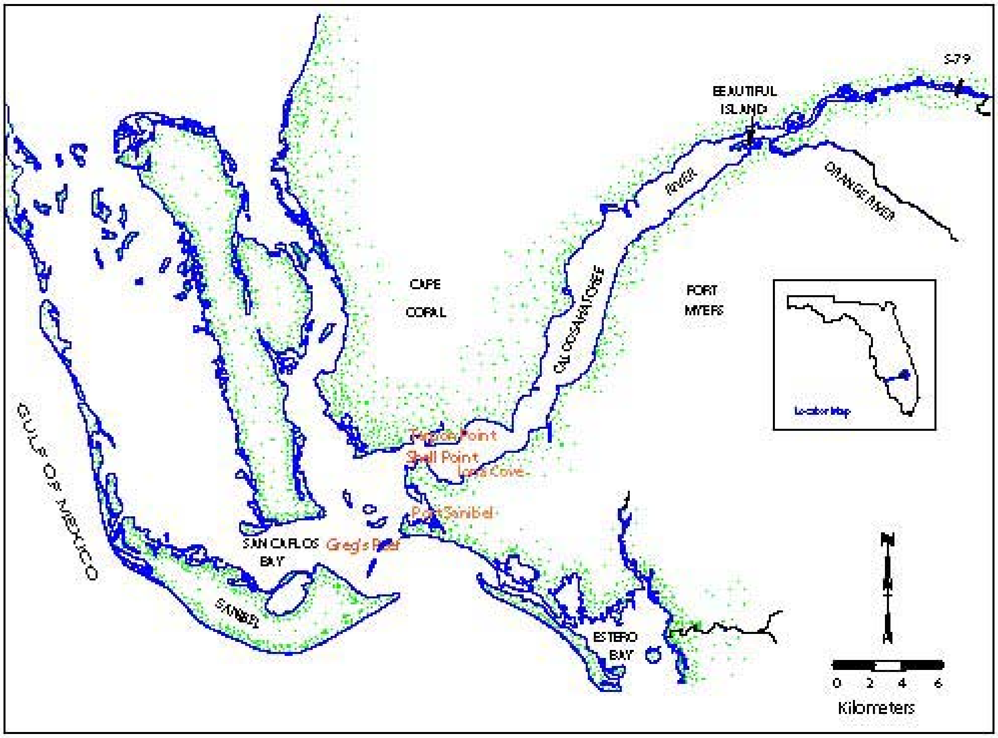

Figure 1. Map of the study area in the Caloosahatchee River and estuary with the sampling location, Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Greg's Reef (reference site), in red.

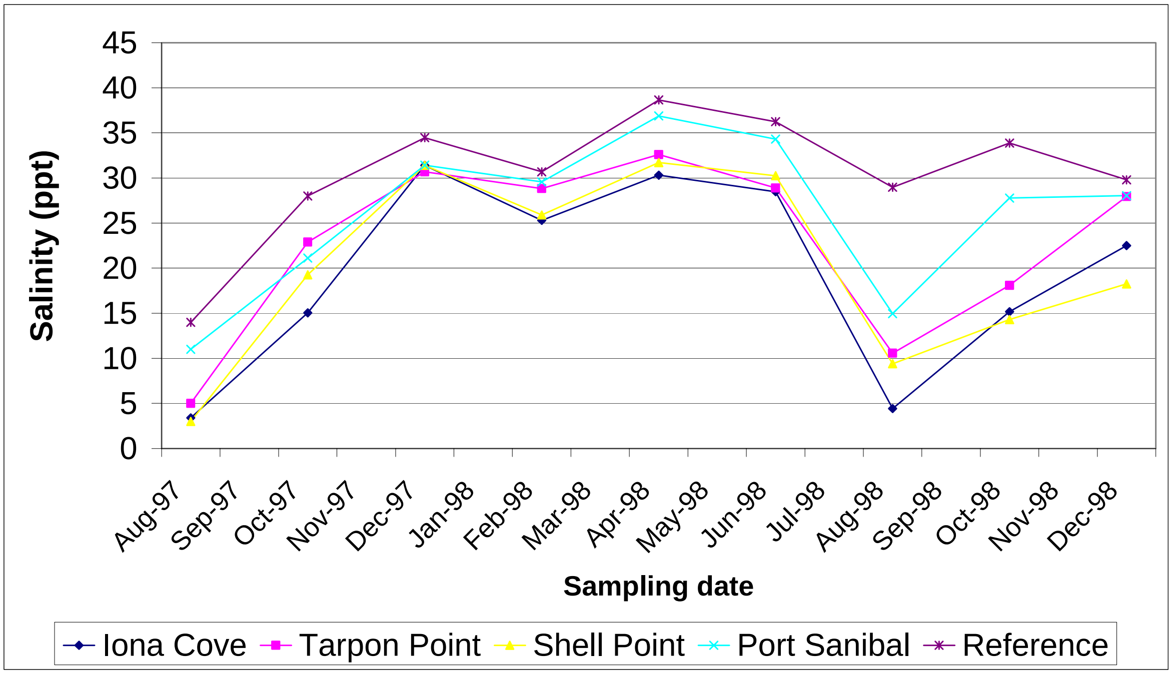

Figure 2. Salinity at sampling locations in Caloosahatchee River was measured bimonthly at the time of oyster collection.

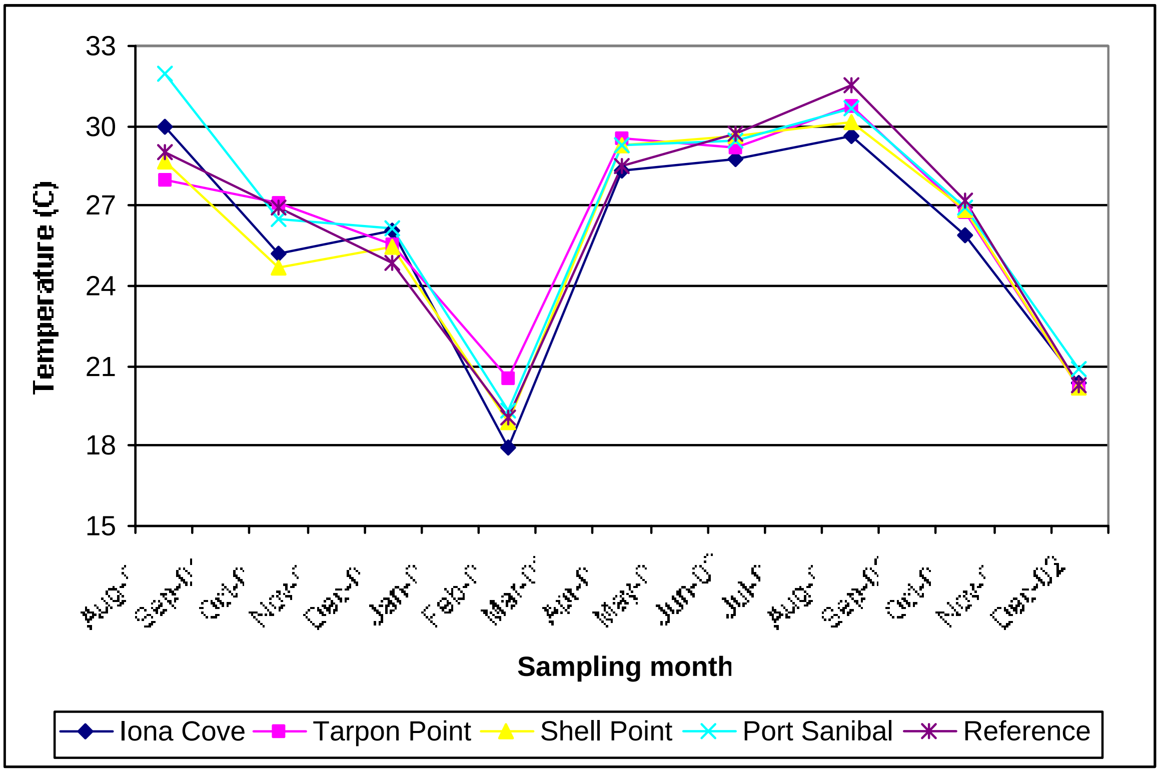

Figure 3. Temperature at sampling locations in Caloosahatchee River was measured during bimonthly collection of oysters.

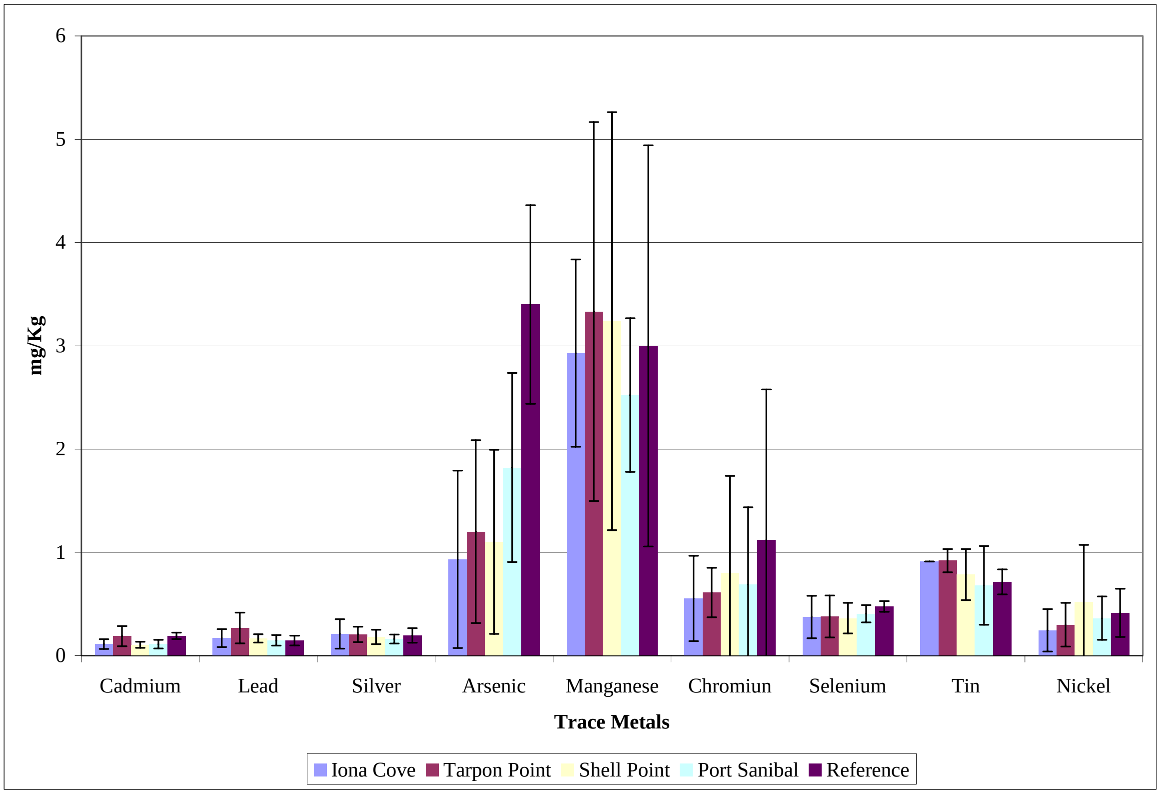

Figure 4a. Trace metal concentrations in oyster tissue averaged by sampling locations, Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Greg’s reef.

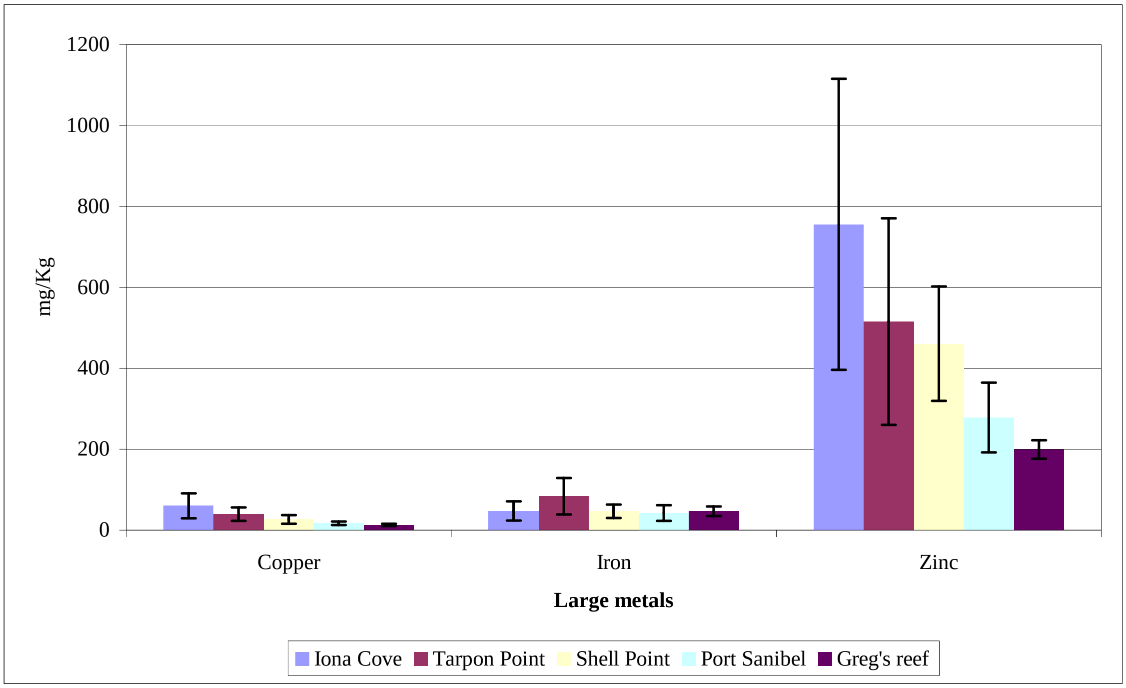

Figure 4b. Heavy metal concentrations in oyster tissue averaged by sampling locations, Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Greg’s reef

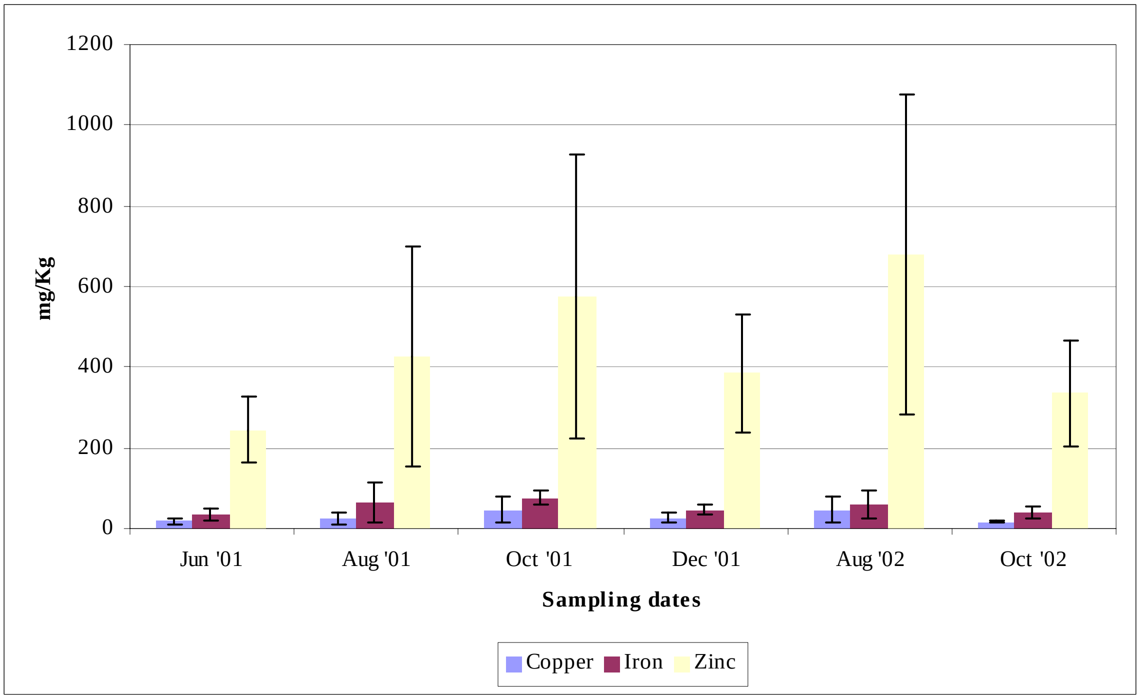

Figure 5a. Heavy metal concentrations in oyster tissue averaged by sampling dates.

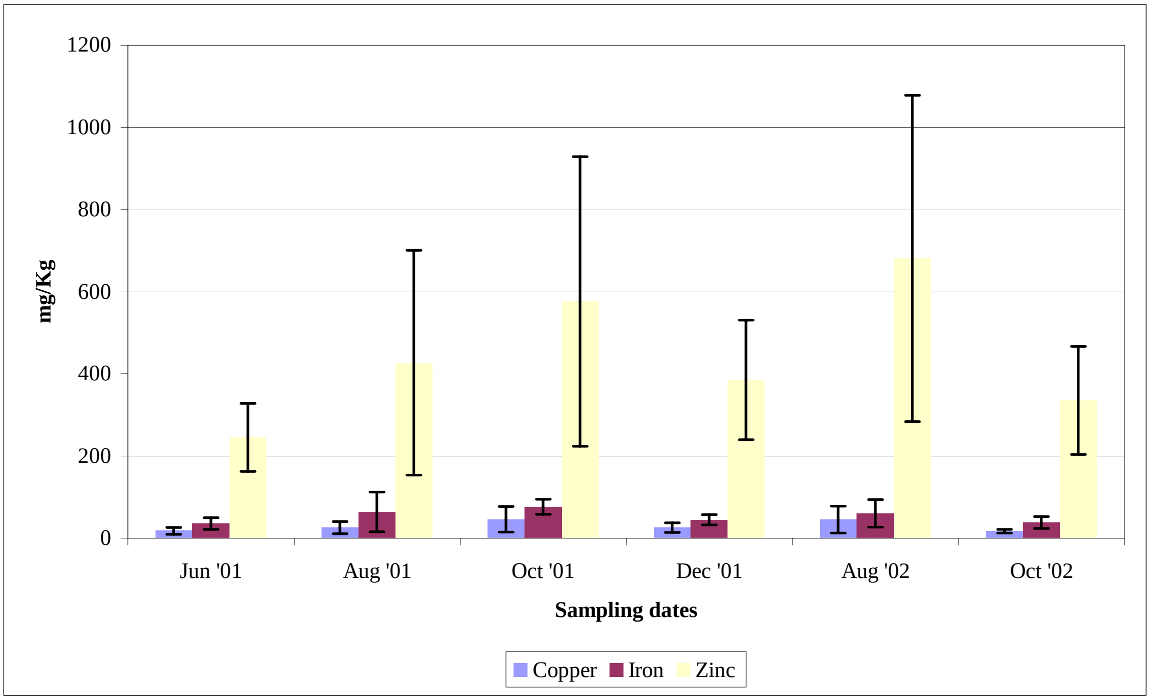

Figure 5b. Heavy metal concentrations in oyster tissue averaged by sampling dates.

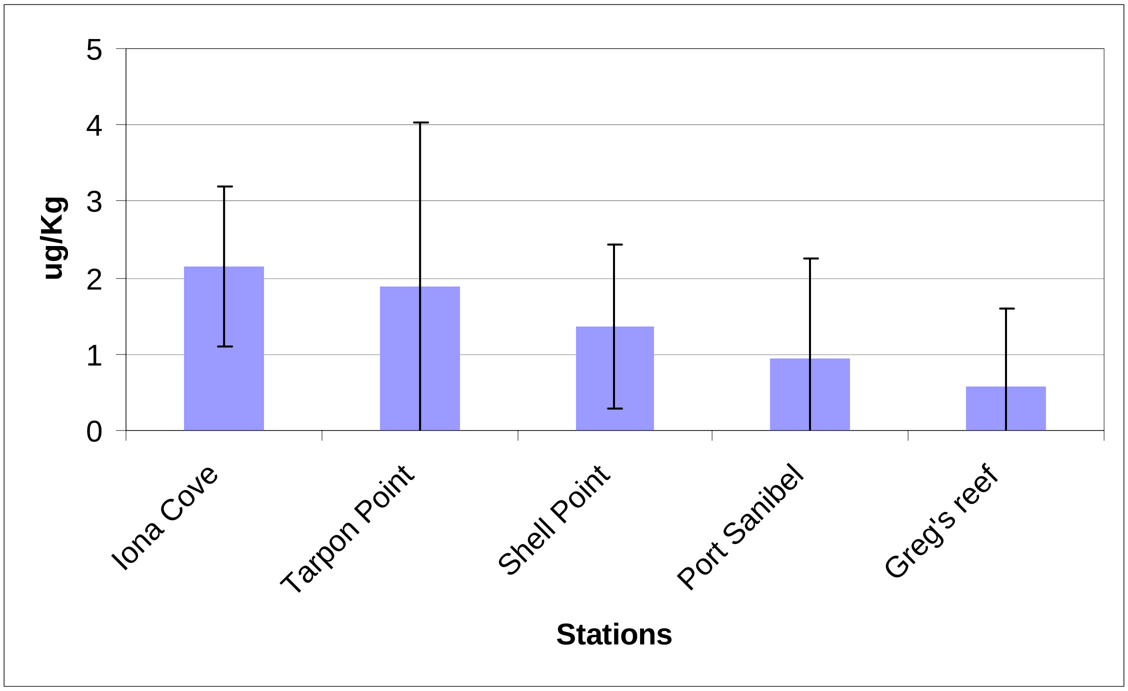

Figure 6. Pesticide concentrations in oyster tissue averaged per sampling location, Iona Cove, Tarpon Point, Shell Point, Port Sanibel; and Greg’s reef.

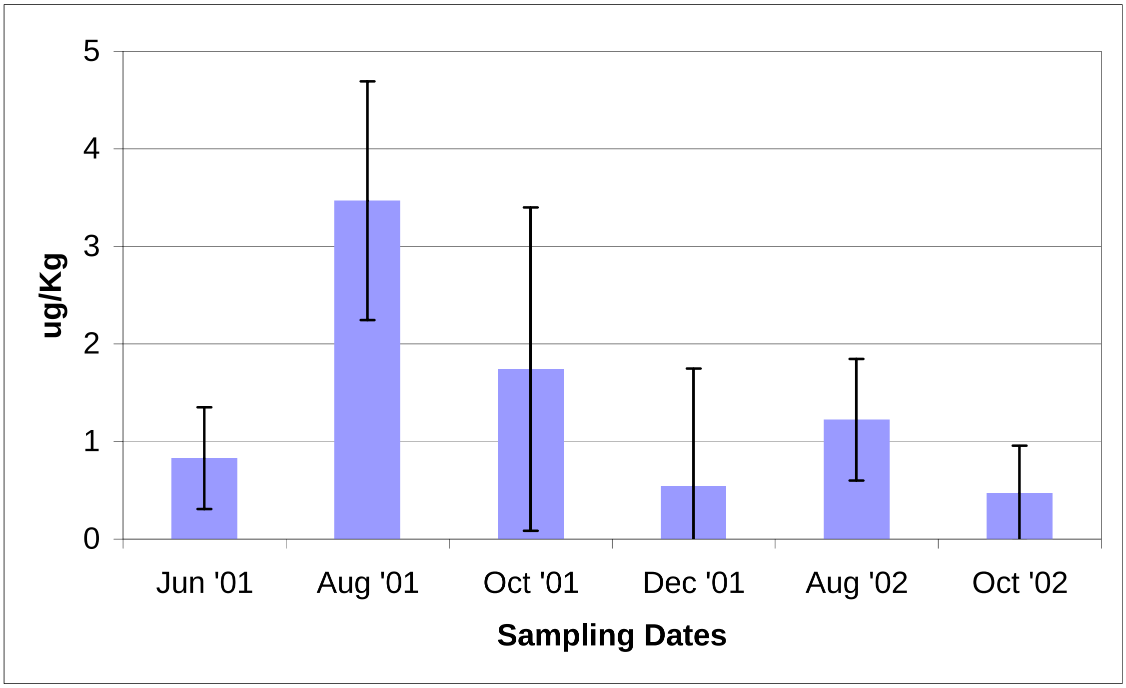

Figure 7. Pesticide concentration in oyster tissue averaged by sampling date.

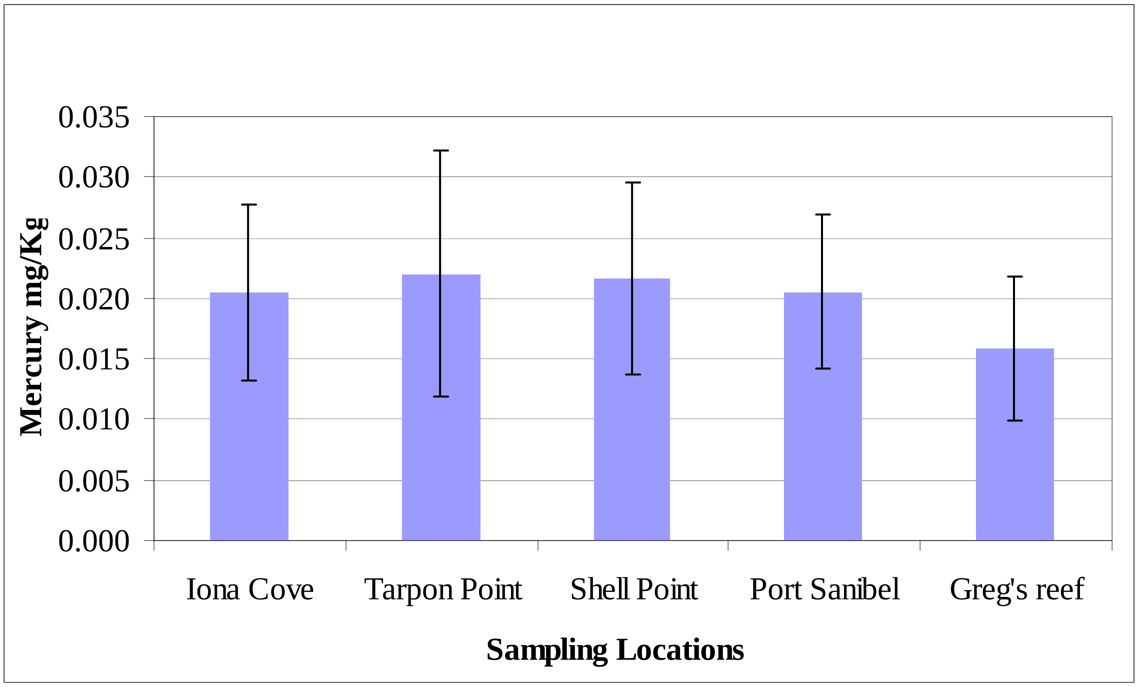

Figure 8. Mercury concentration in oyster tissue collected in the Caloosahatchee River averaged by sampling locations, Iona Cove, Tarpon Point, Shell Point, Port Sabinel, and Greg’s reef.

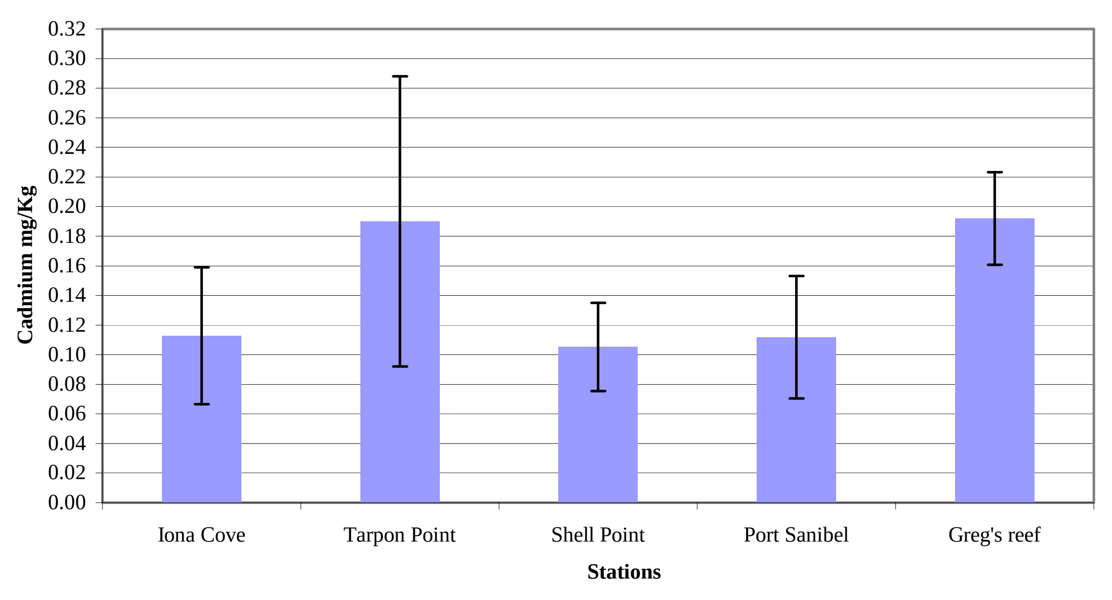

Figure 9. Cadmium concentration in oyster tissue collected in Caloosahatchee River averaged by sampling locations, Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Greg’s reef.

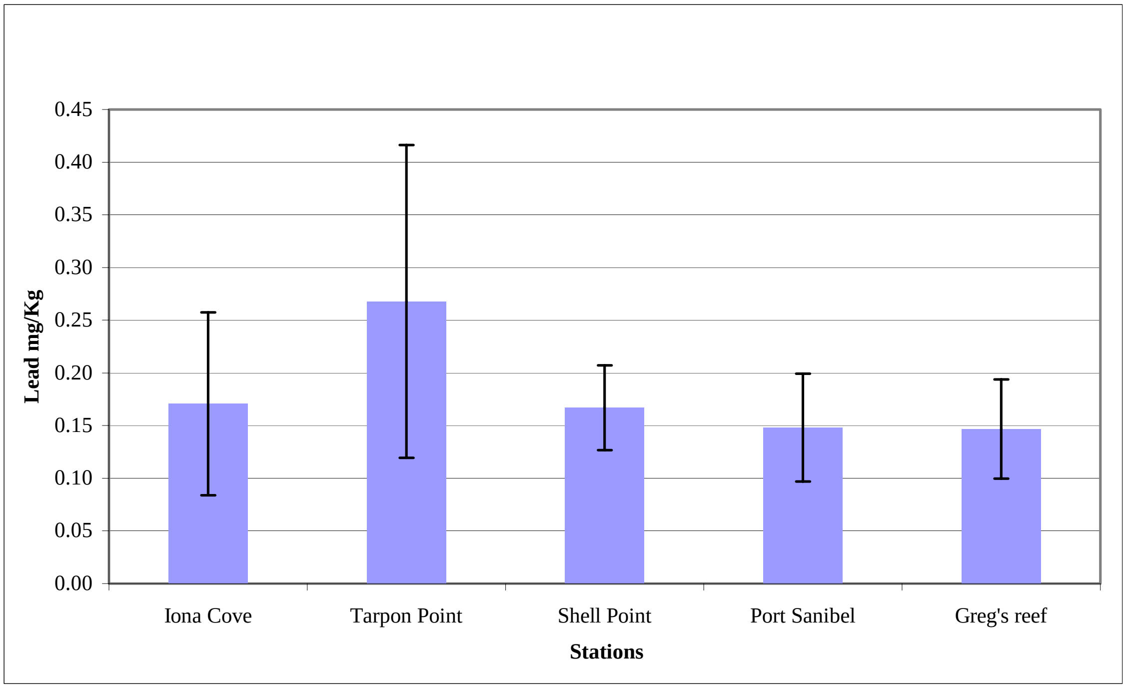

Figure 10. Lead concentration in oyster tissue collected in Caloosahatchee River averaged by sampling locations, Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Greg’s reef.

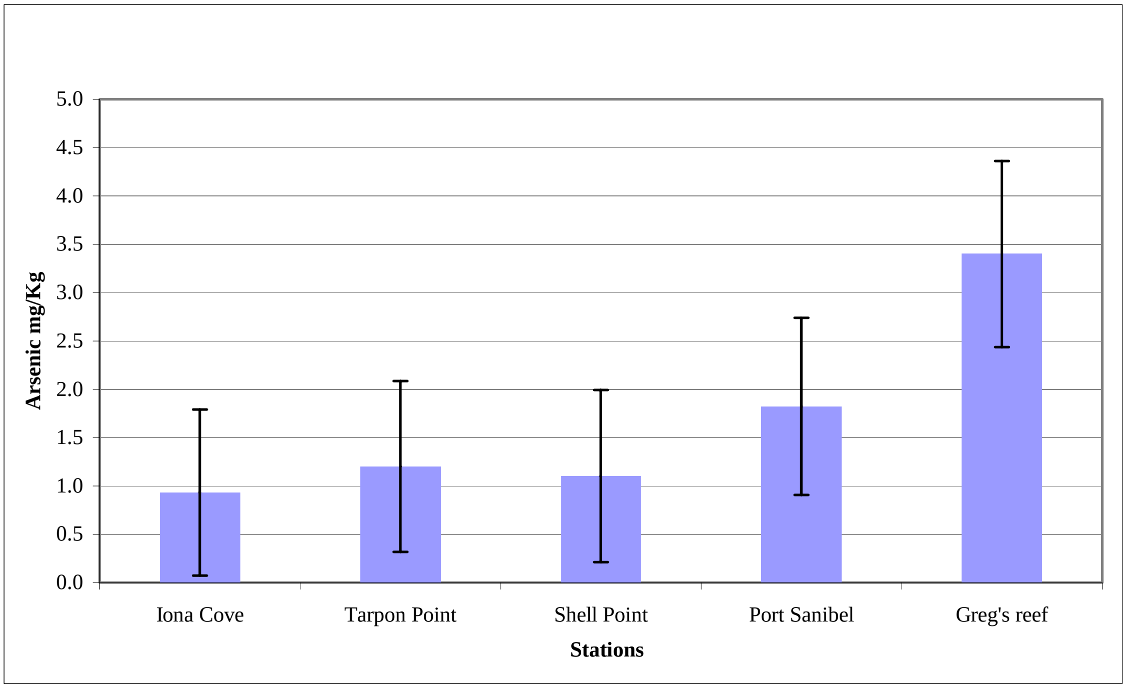

Figure 11. Arsenic concentration in oyster tissue collected in Caloosahatchee River averaged by sampling locations, Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Greg’s reef.

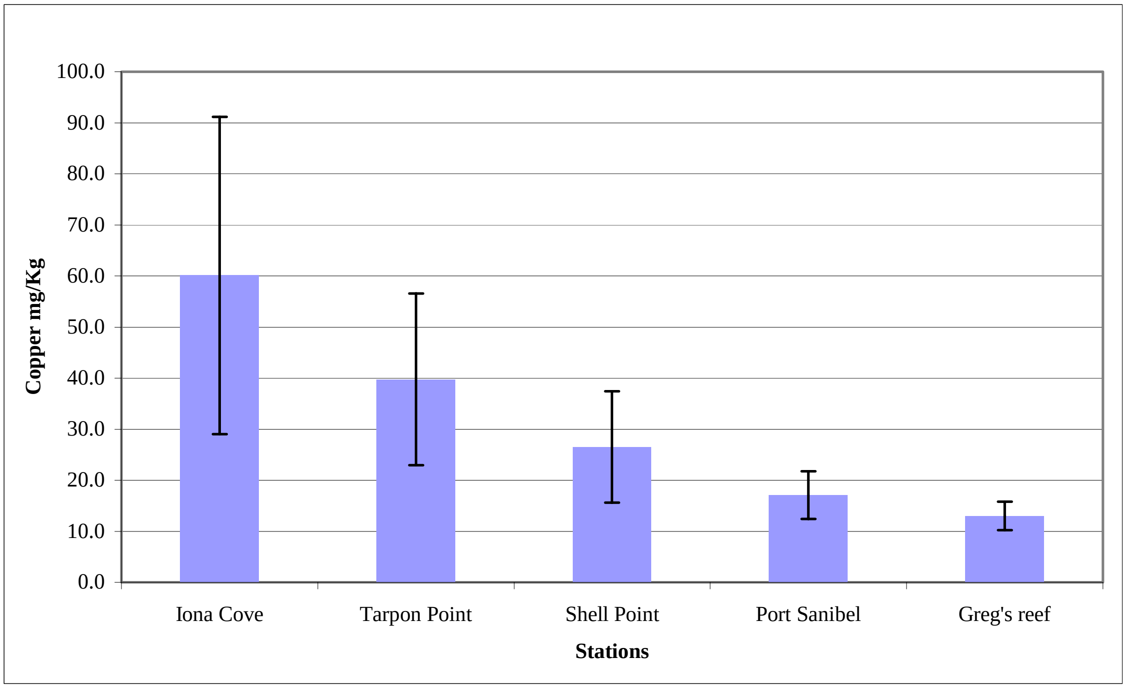

Figure 12. Copper concentration in oyster tissue collected in Caloosahatchee River averaged by sampling locations, Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Greg’s reef.

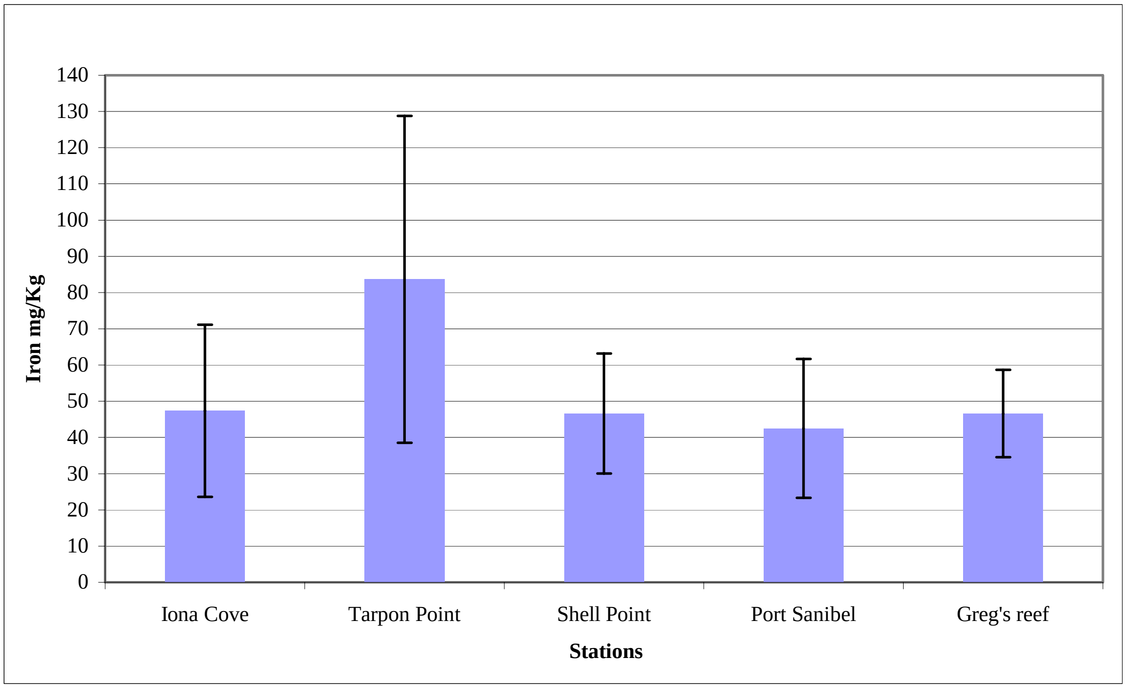

Figure 13. Iron concentration in oyster tissue collected in Caloosahatchee River averaged by sampling locations, Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Greg's reef.

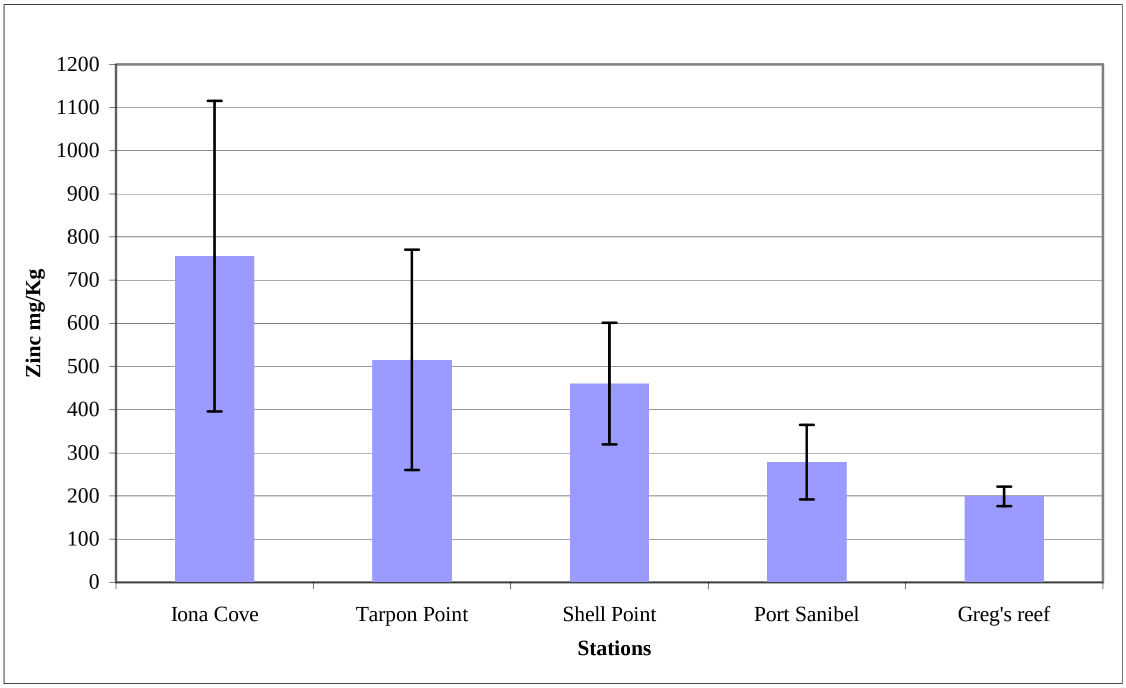

Figure 14. Zinc concentration in oyster tissue collected in Caloosahatchee River averaged by sampling locations, Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Greg's reef.

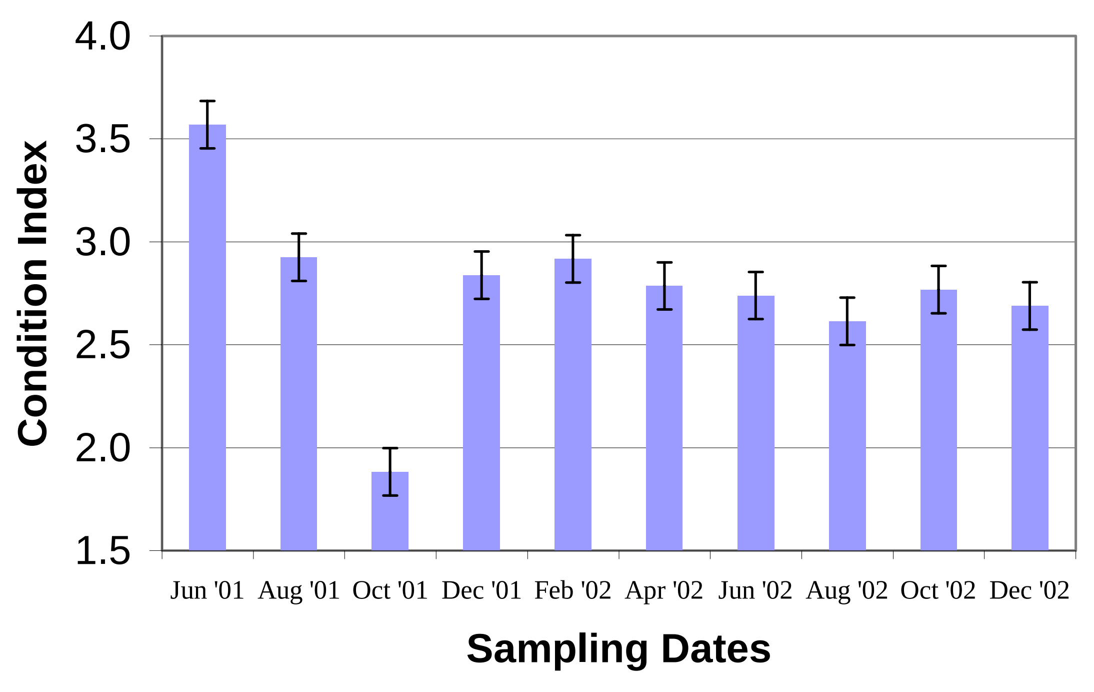

Figure 15. Mean condition index of oysters from all the sampling locations during the study period.

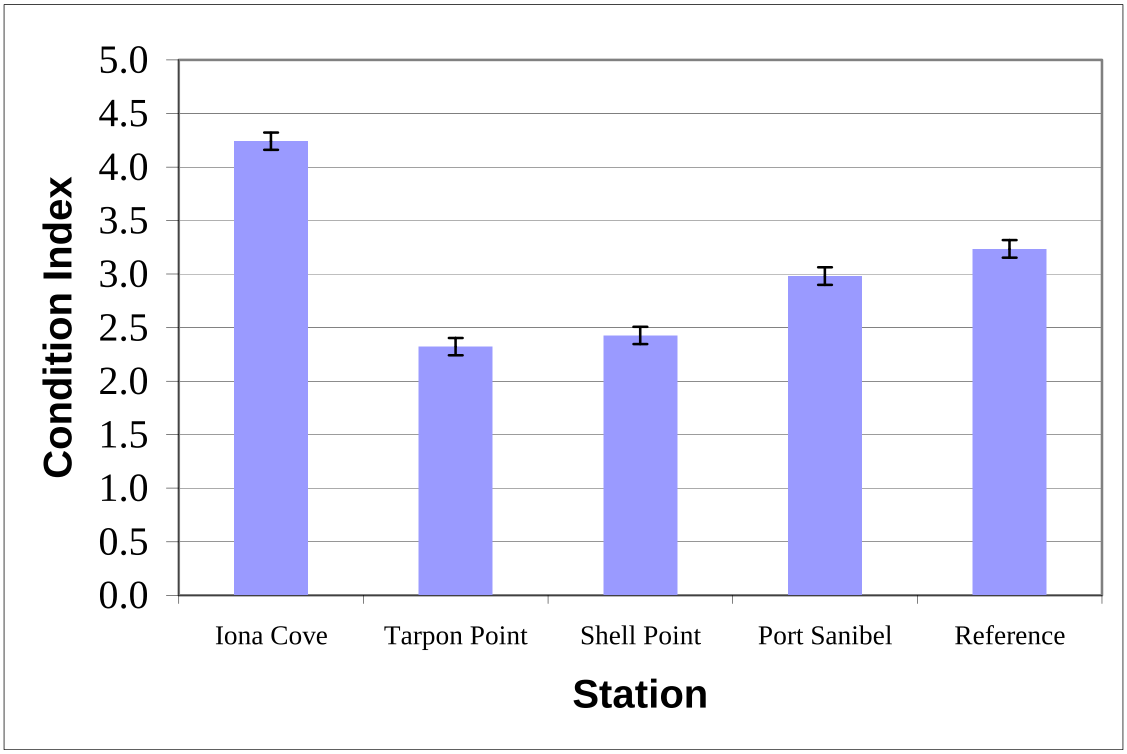

Figure 16. Mean condition index of oysters at the sampling locations Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Reference during the study period.

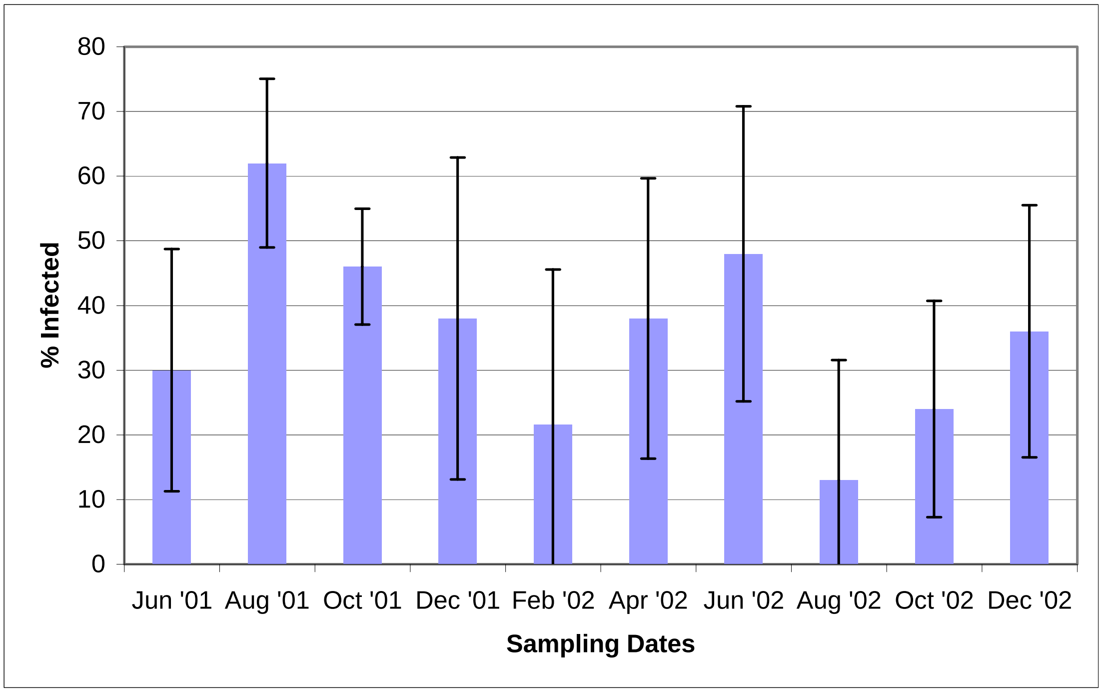

Figure 17. Mean *P. marinus* prevalence in oysters collected during study period.

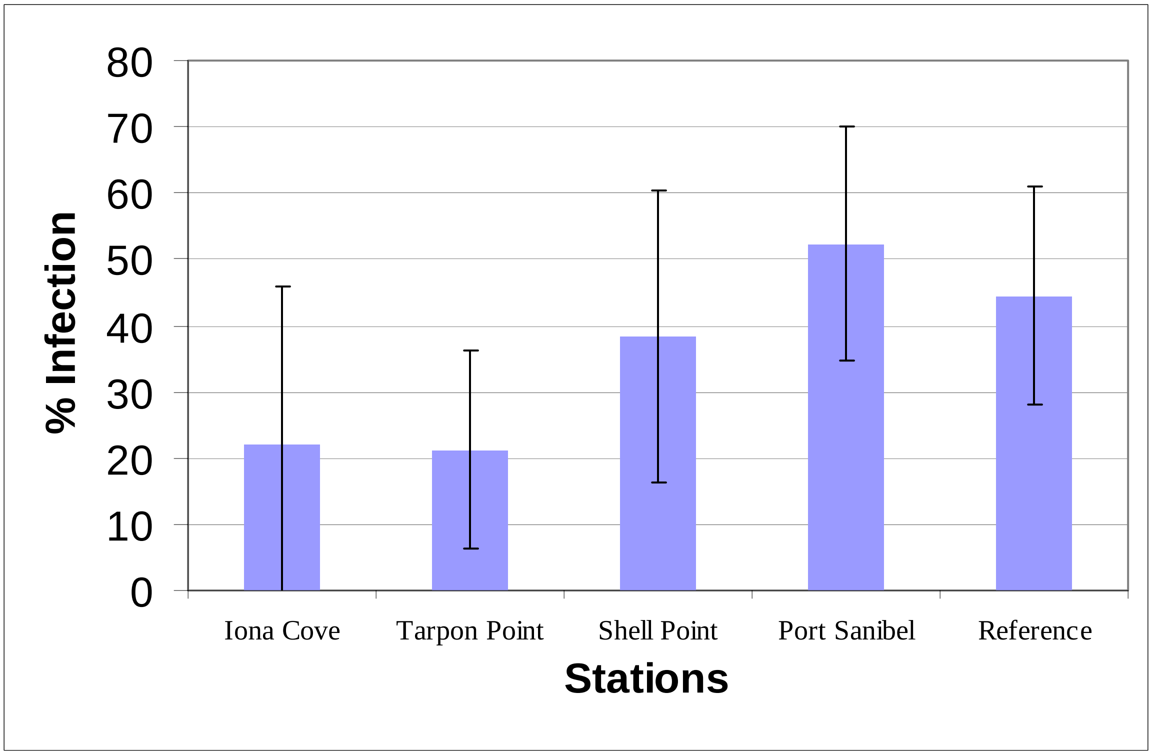

Figure 18. Mean *P. marinus* prevalence in oyster at sampling locations Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Reference.

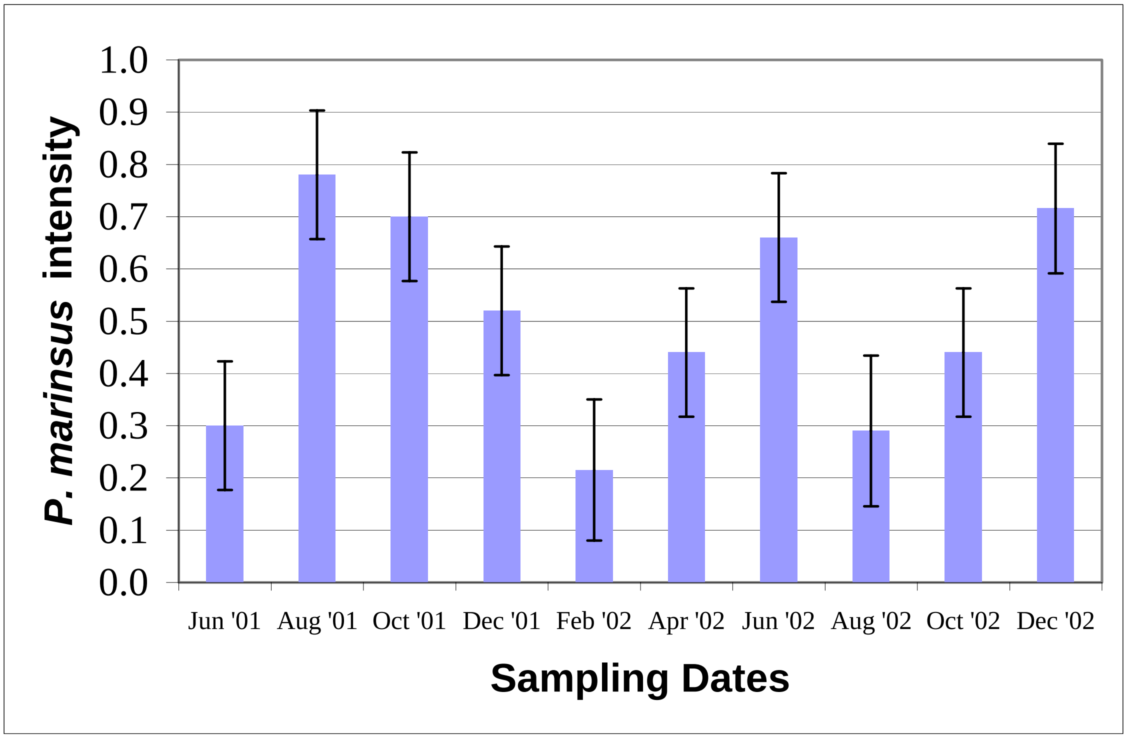

Figure 19. Mean infection intensity of *P. marinus* in oysters during the study period.

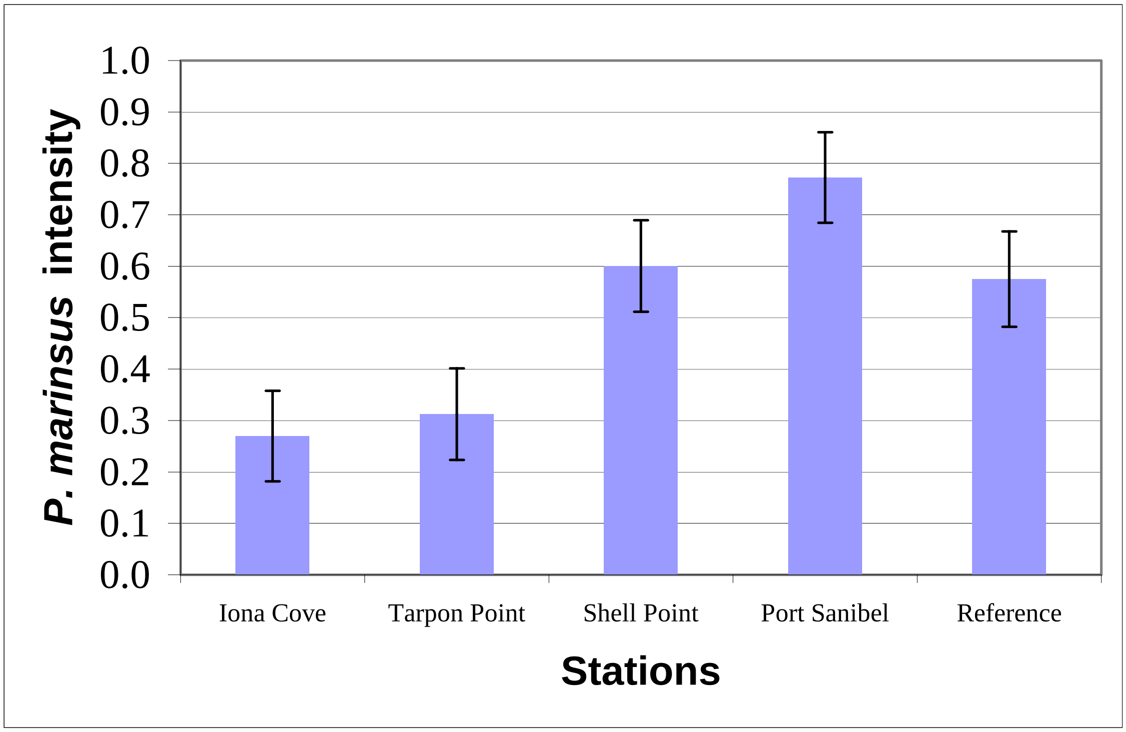

Figure 20. Mean infection intensity of P. marinus in oyster at sampling locations Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Greg's Reef (reference).

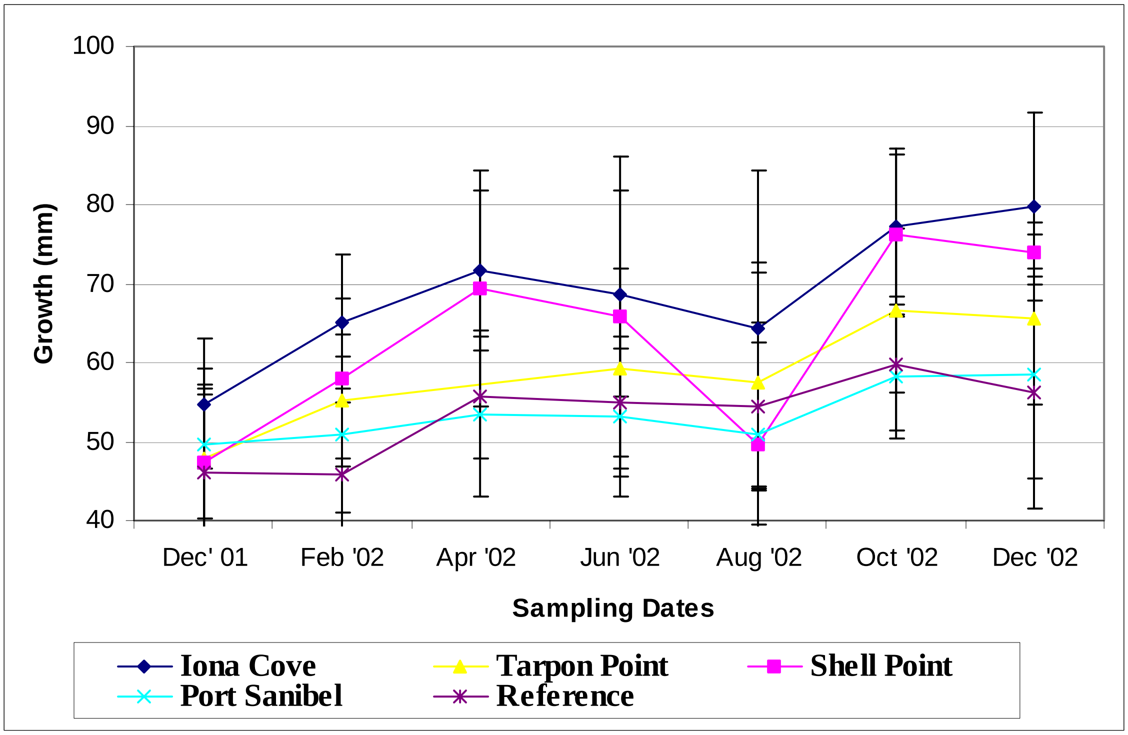

Figure 21. Growth of caged oysters placed at the sampling locations during the study period.

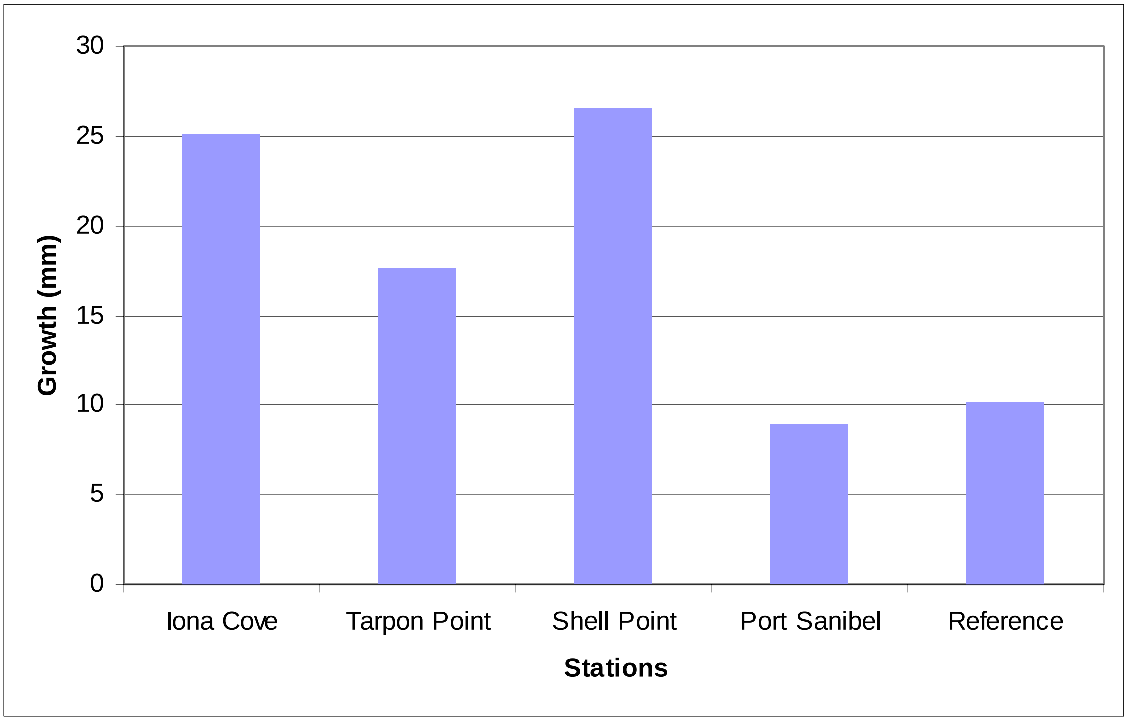

Figure 22. Overall growth of caged oysters placed at the sampling locations Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Reference.

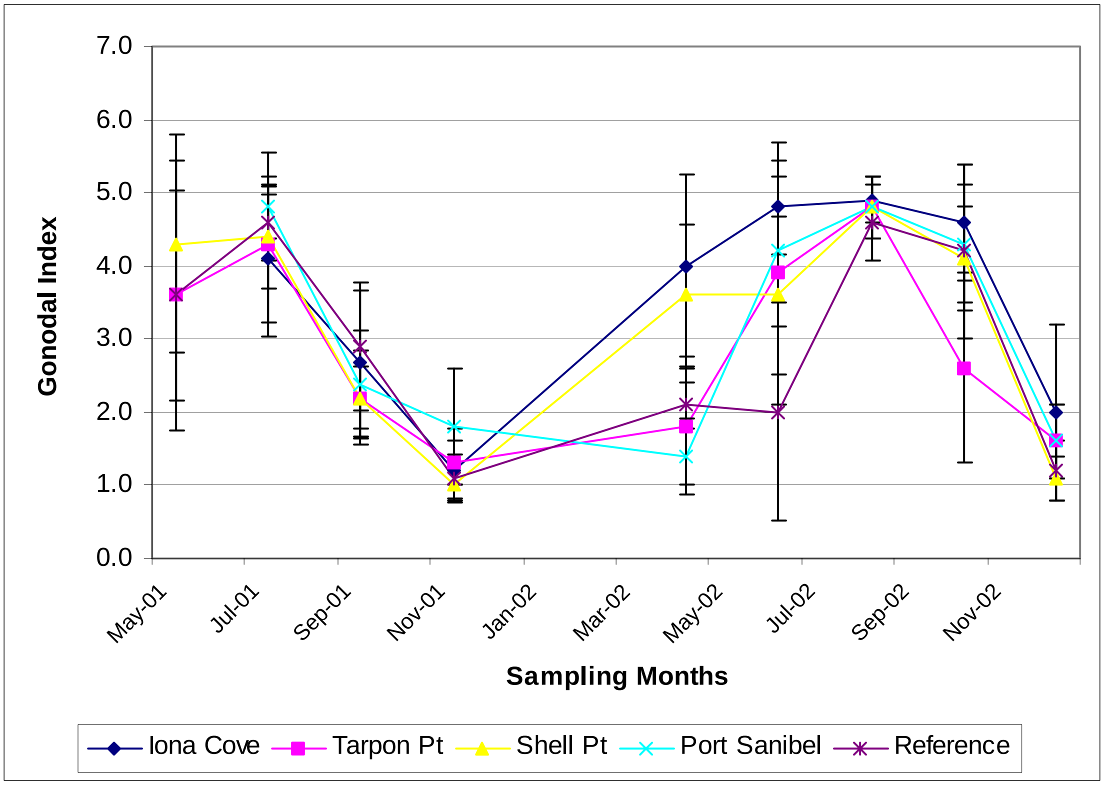

Figure 23. Gonadal index of oysters from sampling locations during the study period.

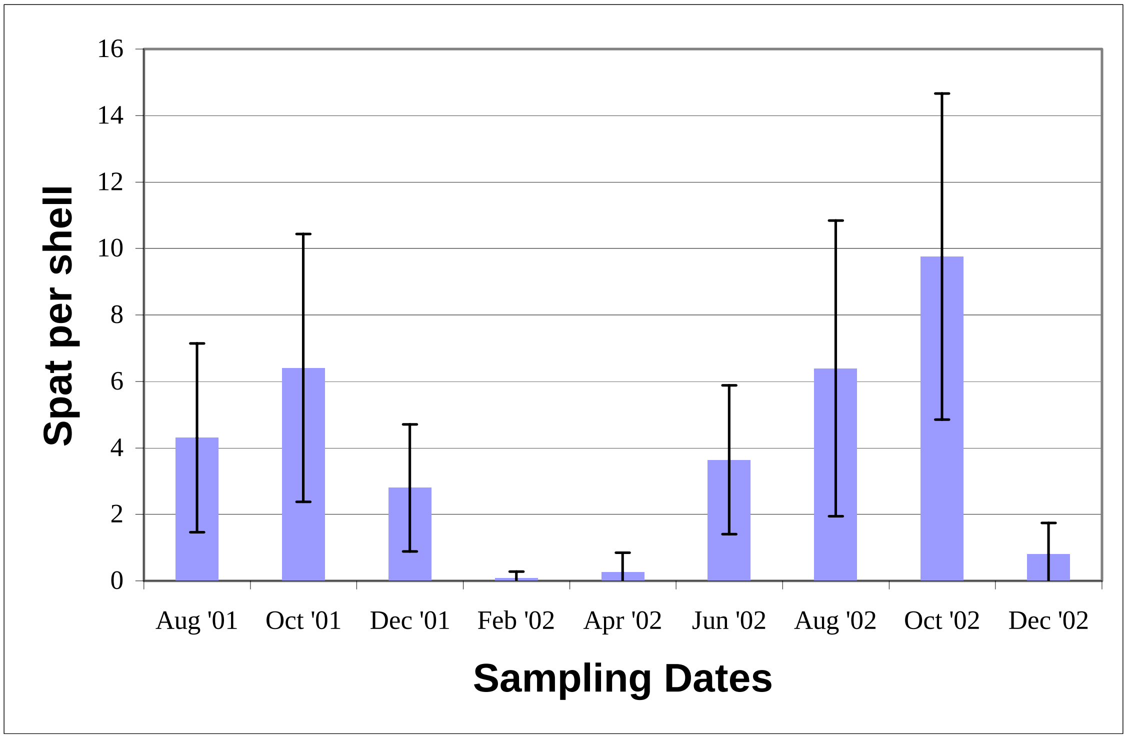

Figure 24. Mean recruitment of spat for all sampling locations during study period. Data are reported as an average number of spat/shell.

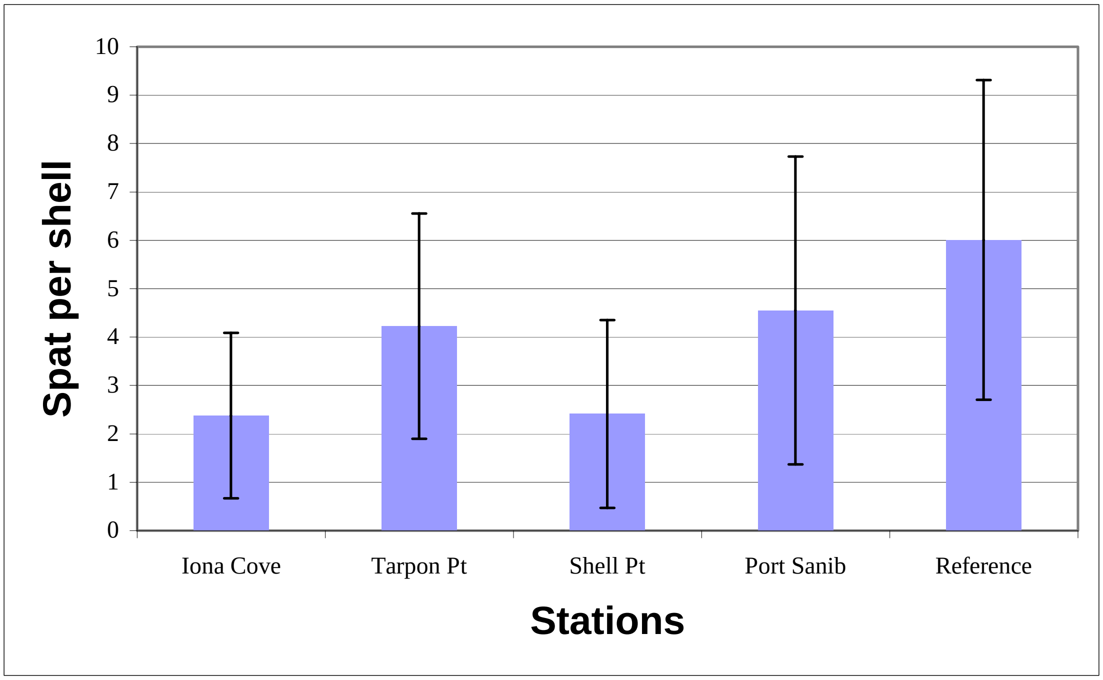

Figure 25. Mean recruitment of spat at the sampling locations Iona Cove, Tarpon Point, Shell Point, Port Sanibel, and Reference. Data are reported as an average number of spat/shell.