SPATIAL AND TEMPORAL TRENDS IN THE EPIFAUNAL COMMUNITY STRUCTURE ON TAMPA BAY ARTIFICIAL REEFS

Technical Report to the Florida Fish & Wildlife Conservation Commission in fulfillment of Grant Agreement # FWC-14018

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Abstract

The Environmental Protection Commission of Hillsborough County conducted a survey of the epifaunal communities on three artificial reefs in Tampa Bay in spring and fall 2016 in order to evaluate the current condition of these reefs and to compare results with a previous survey conducted in 2004. The three reefs selected represented different locations along the estuarine salinity gradient in Tampa Bay. These reefs were the Howard Frankland Reef in Old Tampa Bay; the Bahia Beach Reef in Middle Tampa Bay; and the Egmont Key Reef in Lower Tampa Bay. Each reef was sampled seasonally in the spring (April-May 2016 = dry season) and fall (August 2016 = wet season) to look at seasonal changes in the epifaunal community on the reef as well as differences between reefs within each season. Ten samples were collected on each reef during each seasonal sampling event by SCUBA divers using a 16 cm x 16 cm area epifaunal sampler. Epifaunal species were sorted and identified to the lowest practical taxonomic level and enumerated. Wet weight biomass was also measured for larger specimens and colonial organisms.

Results showed seasonal changes in the species composition, with the oyster *Ostrea equestris* dominating the community in the spring while barnacles were dominant in the fall. Spatially, species richness increased with the salinity gradient with highest number of taxa being present on the Egmont Key Reef. Comparison with the 2004 study found changes in the epifaunal community between years. Most notable was a large drop in the abundance and biomass of the invasive Asian Green Mussel, *Perna viridis*, which was dominant in 2004 but nearly absent in 2016.

Salinity and temperature appear to be the driving factors influencing the epifaunal communities on the artificial reefs. Salinity had a strong correlation with the epifaunal community structure between the three reefs within a given season and between 2004 and 2016. The combination of salinity and temperature were correlated with seasonal changes on the reefs.

Introduction

The Artificial Reef Program for Tampa Bay has been administered by the Environmental Protection Commission of Hillsborough County (EPC) since October 1986. EPC's Artificial Reef Program has a total of eight sites that span from as far north as the Courtney Campbell Causeway in Old Tampa Bay to as far south as Egmont Key at the mouth of the bay (Figure 1). The artificial reefs were deployed in Tampa Bay between 1987 and 2006 and each reef site accommodates various types and amounts of material at different stages of development. The artificial reefs in Tampa Bay are 10 feet or less in relief and deployed at depths ranging between 8 feet mean lower low water (MLLW) and 24 feet natural depth. A main objective of the Artificial Reef Program is to increase biological diversity and productivity in Tampa Bay by providing hard bottom substrates and communities which might not otherwise be available and to enhance recreational fishing opportunities. Since its inception, the EPC Artificial Reef Program has extended this concept to include artificial habitats as restoration and mitigation alternatives. The program has increased hard bottom habitat by placing over 36,000 metric tons of concrete substrate, covering an approximate area of 0.51 km2. Determining the success of the program is, in part, dependent on the benthic species diversity and benthic biomass found on the artificial reefs. Enhancing the body of knowledge of artificial reef dynamics in estuarine systems in the State of Florida will provide reef managers additional resources for artificial reef siting, monitoring, and maintaining healthy artificial reefs.

The EPC was awarded a grant in 2004 from the Florida Fish and Wildlife Conservation Commission (FWC) (Grant Agreement #FWCC-03045) to study the epibenthic macroinvertebrate communities on artificial reefs in Tampa Bay. This study focused on three artificial reefs managed by the EPC and was one of the first such surveys in an estuarine environment. The three reefs studied were located in Old Tampa Bay (Howard Frankland Reef; HFR), Middle Tampa Bay (Bahia Beach Reef; BBR) and Lower Tampa Bay (Egmont Key Reef; EKR). These three reefs were chosen as they represented an environmental gradient of salinity and water clarity from the upper to lower portions of the Tampa Bay estuary. Results from the 2004 study found a total of 124,116 organisms, representing 385 taxa, and 14 phyla present on the three reefs and evident variations in community structure between reefs in different bay segments as well as seasonal differences. Three phyla (Annelida, Arthropoda, and Mollusca) comprised 80% of the taxa and over 88% of the total abundance.

A major concern from this study was the prevalence of the invasive Asian Green Mussel, *Perna viridis*, which dominated the biomass at the HFR and was abundant at the BBR. From the original 2004 study, the BBR's species composition was similar to the EKR. *Perna viridis* had a strong foothold on the BBR with the potential to spread throughout the reef. If this happens, then BBR's species composition and biomass would be more similar to the HFR over time and there would potentially be an overall loss in species diversity, which could result in a reduced supply of different prey items for foragers (Dix et al. 2005).

The EPC was awarded a second grant from the FWC in 2015 (Grant Agreement #FWC-14018) to survey the epibenthic macroinvertebrate communities on the same three artificial reefs in order to document any changes in the community structure since 2004 and evaluate the current status of the reefs. This current study had the following objectives:

Objective **1:** Determine if the epibenthic communities have changed since the first survey in 2004.

Measured by: Species diversity. Changes in epibenthic diversity will be related to the observed water quality trends of increasing water clarity and lower chlorophyll a observed over the past decade.

Objective 2: Assess current health status of the reefs.

Measured by: Community composition and related location and environmental conditions. Changes in species abundance, diversity, and composition will determine if climax communities are now in decline or if they are stable. This information will be used to determine if the reefs are in ideal locations, are candidates for adding additional reef material, and if it is economically sensible to create new artificial reef habitat in Tampa Bay.

Objective 3: Determine if a ten-year monitoring frequency is adequate for detecting changes in the epibenthic invertebrate communities, or if these reefs need to be monitored at a greater frequency.

Measured by: Dramatic shifts in community structure. Dramatic events in community structure may not be detected within a 10-year sampling period. Changes in species diversity, biomass, and community structure due to environmental events like cold spells, freshwater inflows, pollution from stormwater runoff, severe storm events, and climate change may occur at intervals shorter than a decadal period. This may suggest more frequent sampling intervals, or additional sampling events are necessary.

Objective 4: Determine if the reefs need to be re-nourished with new material.

Measured by: Species diversity. Less diversity may indicate deterioration of the benthic community, which in turn may indicate the reef has lost its ability to function as viable recreational fishery habitat, and signal to reef managers that re-nourishment may be warranted.

Objective 5: Discover if artificial reef placement within the estuarine environment is important to their success.

Measured by: Community metrics such as species diversity and biomass. The locations of the reefs spatially in Tampa Bay have different environmental parameters which influence the species diversity and biomass at each location. Certain locations may not be ideal as Essential Fish Habitat for recruitment of recreationally managed species. Species diversity and biomass are indicators of ideal or adverse environmental parameters which may indicate the success of reef placement.

Figure 1. EPC artificial reef locations in Tampa Bay

Material & Methods

Study Sites

The same three artificial reefs from Old Tampa Bay, Middle Tampa Bay and Lower Tampa Bay were sampled as in the original 2004 study. The Howard Frankland Reef center (HFR) (Old Tampa Bay) is at 27° 54.70' N and 82° 33.25' W at a depth of 16 feet with a relief of 4-8 feet and the reef dimensions are 182.8 m by 365.8 m for a total area of 0.067 km² (Figure 2). HFR was originally deployed in December, 1991 and material was added through 1995, primarily concrete bridge material for a total of 2,340 tons.

The Bahia Beach Reef (BBR) center (Middle Tampa Bay) is at 27° 44.89' N and 82° 30.92'W, and its dimensions are 182.8 m by 365.8 m for a total area of 0.067 km² (Figure 3). The reef depth ranges from 18 – 24 feet and has a relief of 6-8 feet. BBR was originally deployed in September, 1987 and additional concrete bridge material (pilings, bridge decking) was added through 2005 for a total of 10,346 tons.

The Egmont Key Reef (EKR) center (Lower Tampa Bay) is at 27° 35.00' N and 82° 44.60' W, and its dimensions are 365.8 m by 365.8 m for a total area of 0.134 km² (Figure 4). The reef is at a depth of 18 feet and has a relief of 6-8 feet. EKR was originally deployed in June, 1999 and consists of concrete bridge material and 22 concrete pyramids. Additional material was deployed between 2001-2002 for a total of 5,829 tons.

Sample Selection

Samples were collected at each reef during two seasonal sampling eventsin 2016: April - May representing the dry season and in September representing the wet season. Ten samples were collected from each reef during each season for a total of 60 samples (Figure 5). Sampling locations were selected at each reef from random coordinates which were re-randomized between the two sampling events. The boat was anchored at each sample location and the coordinates, time, date, and conditions were recorded. Divers were deployed at the location and collected samples from the closest reef material to the designated sampling coordinates. Sample sites on the reef were randomly selected for one of the three different reef levels (top, middle or bottom of reef) and for one of three surface orientations (horizontal, vertical or inverted).

Hydrographic profiles

A water column profile was taken at each sample site with a Hydrolab Quanta multiparameter sonde. Measurements for temperature, salinity, depth, dissolved oxygen, and pH were taken at the surface (depth = 0.1 m), mid depth, and at the bottom. Secchi disk measurements were taken at each site to measure water clarity.

Field Collection and Sample Processing

Epifaunal samples were collected by SCUBA divers from each sample site. Prior to collecting the sample, a 16cm x 16cm metal frame was placed over the area to be sampled and a digital photograph was taken and the depth of the epifaunal growth was measured to the nearest centimeter.

A stainless steel epifaunal sampler (Figure 6) was used to remove attached organisms and to transport the sample to the surface. The sampler is rectangular with dimensions: 16 cm wide x 10.5 cm high x 20.5 cm deep (bottom) and 16 cm wide x 10.5 cm high x 14.7 cm deep (top), with one handle on top. The back of the sampler has an opening (6.9 cm wide x 4.6 cm high) with 0.5 mm metal screen mesh attached to allow water to escape. One diver scraped the 16 x 16 cm area until the artificial substrate is exposed, while a second diver placed a 0.5 mm dip net down current to catch any material that escaped from the sampler. The opening of the sampler was then sealed with a plastic cover and secured with a bungee cord for transporting the sample to the surface. The sampler and dip net were thoroughly rinsed with seawater into a 0.5 mm mesh sieve and sieved in a plastic dish pan of seawater. The retained material was transferred into prelabeled, plastic, screw-top one gallon jars, relaxed with a solution of seawater and Epsom salts and stored on ice for transport back to the laboratory. Upon return to the lab, the samples were fixed in 100% NOTOXhisto™ (Scientific Device Laboratory, Des Plaines, IL) for a minimum of 3 days and then transferred into 70% isopropyl alcohol until processing. The samples were later sorted under a dissecting microscope and the organisms were identified to the lowest practical taxonomic level.

Howard Frankland Reef Hillsborough County

Figure 2. Howard Frankland Reef (HFR) location and dimensions.

Bahia Beach Reef

Figure 3. Bahia Beach Reef (BBR) location and dimensions.

Egmont Key Reef

Figure 4. Egmont Key Reef (EKR) location and dimensions.

Figure 5. 2004 and 2016 artificial reef sampling locations.

Figure 6. Epifaunal sampler.

Data Analysis

Descriptive statistics, Analysis of Variance (ANOVA) and/or Kruskal-Wallis Nonparametric test (KW), relative percent, and graphs for hydrographic and biological data were generated using SigmaPlot 13.0 and SigmaStat 3.5 (SYSTAT Software, Inc. 2014). The biomass will be measured as wet weight (shell included) in grams. Ecological diversity metrics, multivariate analysis (cluster analysis, multi-dimensional scaling, Principal Coordinate Analysis, SIMPER, SIMPROF) and multivariate graphics were done using PRIMER 7.0 and PERMANOVA+ (Primer-E Ltd. 2016). Species abundance data was square root transformed and environmental data was log (n+1) transformed and normalized prior to analysis. Maps were generated using GIS ArcView ver. 10.

Results

Reef Characteristics

The Tampa Bay artificial reefs are constructed primarily from concrete bridge and road materials that have been donated from Florida Department of Transportation (FDOT) demolition projects. Table 1 shows the percent of samples collected on various reef materials in 2016 on each reef. Concrete bridge pilings were the predominate reef material on all three reefs and 44 of the 60 samples (73%) were collected on this substrate, followed by concrete bridge decking and concrete slabs. Concrete pyramids were sampled on the EKR. These structures were specifically constructed to serve as artificial reef enhancements and placed on the EKR in 1999 as mitigation for an EPCHC enforcement case.

Table 1. Percentage of 2016 samples by reef substrate type.

The percentage of samples collected at different relative heights on the reefs is shown in Table 2. A total of 47 of the 60 samples (78%) were collected near the bottom of the reef structure while only a single sample was collected near the top of the reef.

The reef surface orientation of each sample is presented in Table 3. The majority of the samples were collected on vertical surfaces (57%), while 38% were collected on horizontal surfaces. Only 5% of the samples were collected on inverted surfaces due to the placement of the reef material on the bottom sediments.

Table 3. Percentage of samples by surface orientation.

Hydrographic Profiles

The summary statistics for the measured field hydrographic parameters are shown in Tables 4-6 for each reef and season.

Depth

There were significant differences in the site depths between the reefs ($p<0.001$) but not between seasons (p = 0.895). The HFR had a mean depth of 5.0 meters during the spring and 4.7 meters during the fall sampling periods (Table 4) and was significantly shallower than the BBR and EKR in both seasons (Figure 7). There was no significant difference in the depths between the BBR and EKR in either season. The BBR sites had a mean depth of 5.8 meters in the spring and 5.9 meters in the fall (Table 5) and the EKR sites had a mean depth of 5.7 and 5.8 meters in the spring and fall respectively (Table 6).

The mean reef depths were significantly different between 2004 and 2016 at BBR and EKR within both the spring and fall seasons but there was no significant difference in the depth at HFR between years or seasons (Figure 8).

Table 4. 2016 Howard Frankland Reef bottom hydrographic summary statistics.

Table 5. 2016 Bahia Beach Reef bottom hydrographic summary statistics.

Table 6. 2016 Egmont Key Reef bottom hydrographic summary statistics.

Figure 7. 2016 mean site depth by reef and season.

Figure 8. 2016 vs. 2004 mean site depth by reef and season.

Temperature

The mean bottom temperatures were significantly different between seasons on all three reefs and among the reefs within a given season (Figure 9; p<0.001). Mean spring bottom temperature at the HFR was 23.06°C and increased to 28.48°C in the fall (Table 4). The bottom temperatures at the BBR were higher than at HFR with a mean of 24.73°C in the spring and 30.55°C in the fall (Table 5). EKR had the highest mean bottom temperature in the spring with a mean of 25.61°C while fall temperatures were higher than at HFR, but lower then BBR with a mean of 29.21°C (Table 6).

The mean spring bottom temperatures were significantly higher in 2016 than in 2004 while 2004 had significantly higher fall temperatures at all reefs (Figure 10; p<0.001).

Figure 9. 2016 mean bottom temperature by reef and season.

Figure 10. 2016 vs. 2004 mean bottom temperature by reef and season.

Salinity

Bottom salinity was significantly different between the three reefs within each season, with an increasing trend from the HFR to the EKR and was significantly lower in the fall at each reef (Figure 11; p<0.001). The HFR had the lowest salinity during both seasons with a mean of 24.63 psu in the spring and 18.58 psu in the fall (Table 4). The mean bottom salinities at the BBR were 27.44 in the spring and 22.26 in the fall (Table 5). Both HFR and BBR had salinities in the polyhaline range (18-30 psu) during both seasons (Figure 11), although the fall salinity at HFR was just above the upper limit of the high mesohaline range (10-18 psu). Salinity at the EKR were in the euhaline range (30-40 psu) during both seasons, with a mean value of 34.21 psu in the spring and 31.40 psu in the fall (Table 6; Figure 11).

The spring bottom salinity was significantly lower in 2004 at HRF and EKR. The fall bottom salinities were significantly higher in 2004 on all three reefs (Figure 12). In both sampling years, the HFR and BBR bottom salinity was in the polyhaline range and EKR was euhaline across seasons (Figure 12).

EPCHC Artificial Reef

Figure 11. 2016 mean bottom salinity by reef and season. Blue dashed line indicates euhaline conditions (>30 psu); green dashed line indicates polyhaline conditions (18-30 psu).

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Figure 12. 2016 vs. 2004 mean bottom salinity by reef and season. Blue dashed line indicates euhaline conditions (>30 psu); green dashed line indicates polyhaline conditions (18-30 psu).

Dissolved Oxygen

Bottom dissolved oxygen (DO) concentrations were significantly higher in the spring than in the fall at the BBR and EKR sites (p<0.001) but there was no seasonal difference at HFR (p=0.319). All dissolved oxygen measurements were above the 4mg/l state water quality criteria at all three reefs and during both seasons (Figure 13). EKR had significantly higher bottom dissolved oxygen than HFR (p=0.009) and BBR (p=0.027) during the spring (Figure 13). All reefs were significantly different from each other during the fall, with HFR having the highest and EKR the lowest bottom DO values (Figure 13).

The overall 2004 spring samples had higher bottom DO across all three reefs (p<0.001; Figure 14). The fall bottom DO were lower in 2004 at HFR and BBR and higher at EKR (p<0.001; Figure 14).

Figure 13. 2016 mean bottom dissolved oxygen by reef and season. Red dashed line indicates state water quality standard of 4 mg/l.

Figure 14. 2016 vs. 2004 mean bottom dissolved oxygen by reef and season. Red dashed line indicates state water quality standard of 4 mg/l.

Dissolved Oxygen Saturation

The bottom dissolved oxygen percent saturation was significantly different among the three reefs (p=0.002) and between seasons (p<0.001) but was above the state water quality criteria of 42% at all reefs (Figure 15). The HFR had the lowest percent saturation during the spring season with an increasing trend towards EKR, while having the highest saturation in the fall with a decreasing trend towards EKR (Tables 4-6; Figure 15). Seasonal differences were significant at all three reefs. The DO saturation increased in the fall at HFR ($p=0.002$) but was significantly lower in the fall at BBR and EKR ($p<0.001$).

The spring dissolved oxygen saturation was significantly higher in 2004 than in 2016 at HFR (p=0.002) and lower in 2004 at EKR (p<0.001). There was no significant difference at BBR between the spring 2004 and spring 2016 DO saturation (p=0.732; Figure 16). The fall DO saturation was significantly lower in 2004 at HFR and BBR, but significantly higher at EKR (all p<0.001; Figure 16).

Figure 15. 2016 mean bottom dissolved oxygen saturation by reef and season. Red dashed line indicates state water quality standard of 42% saturation.

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Figure 16. 2016 vs. 2004 mean bottom dissolved oxygen saturation by reef and season. Red dashed line indicates state water quality standard of 42% saturation.

Bottom pH

Bottom pH was significantly lower in fall than in the spring at all reefs (p<0.001; Figure 17). There were significant differences among all three reefs in the spring, with EKR having the highest mean pH (mean = 8.15: Table 6) and HFR having the lowest pH (mean = 8.09; Table 4). HFR had a significantly lower pH in the fall than BBR and EKR (p≤0.001), however there was no significant difference between the fall bottom pH values at BBR and EKR (p=0.206).

There was no statistically significant difference in the spring bottom pH between 2004 and 2016 on the HFR (p=0.315; Figure 18). Bottom pH was higher in spring 2016 than in spring 2004 at both BBR and EKR (p<0.001; Figure 18). The fall bottom pH was significantly lower in 2016 vs. 2004 on all three reefs (p<0.001; Figure 18).

Figure 17. 2016 mean bottom pH by reef and season.

Figure 18. 2016 vs. 2004 mean bottom pH by reef and season.

Water Clarity

Water clarity as measured by Secchi disk depth was significantly lower in the2016 fall season at HFR and BBR (p<0.001; Figure 19). This was most pronounced at HFR where the mean Secchi depth dropped from 3.5 meters in the spring to 1.6 meters in the fall (Table 4, Figure 19). There was no seasonal difference in the Secchi depth at EKR (p=0.198). The Secchi depth among the three reefs differed significantly during the spring with the shallowest Secchi depths measured at HFR and the deepest at BBR (mean = 5.1 meters; Table 5). HFR had significantly shallower Secchi depths in the fall relative to BBR and EKR (p<0.001; Figure 19) while BBR and EKR were not significantly different from each other (p=0.229; Figure 19).

Spring Secchi depths were significantly shallower in 2004 on all three reefs (p<0.001 for HFR and BBR; p-0.002 for EKR; Figure 20). Fall Secchi depths were also shallower in 2004 at BBR and EKR (p<0.001; Figure 20) but both years were similar at HFR (p=0.621).

Figure 19. 2016 mean Secchi depth by reef and season.

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Figure 20. 2016 vs. 2004 mean Secchi depth by reef and season.

Water Quality multivariate analysis

The principal coordinates analysis (PCO) on the 2016 bottom hydrographic parameters shows the individual sites group together by reef and season (Figure 21). The three reefs were more similar in their hydrographic characteristics during the spring as indicated by the closer distribution of the data points, while the fall data shows HFR was more dissimilar to the other reef x season groups. The reef x season data groups separate out by season along the first PCO axis (PCO1) which accounts for 44.3 % of the variation in the data and (Figure 21). PCO1 is correlated most strongly with pH (ρ=0.92), temperature (ρ=- 0.79), dissolved oxygen saturation and DO concentration (ρ=0.72 and 0.70 respectively; Table 7). The data groups separate out by reef generally along the second PCO axis (PCO2) which accounts for 36.8% of the variation in the data. PCO2 is most strongly correlated with depth $(p=0.82)$, dissolved oxygen concentration (ρ=-0.69), Secchi depth (ρ=0.68) and salinity (ρ=0.61) (Figure 21; Table 7).

SIMPER analysis on the Euclidian distance matrix of the 2016 hydrographic data was performed to evaluate the similarity within each reef x season group and the seasonal dissimilarity at each reef.

The average squared distance among the spring HFR samples was 0.53 with pH and depth contributing 41.80% and 39.55% respectively. The fall HFR samples had an average squared distance of 0.65 with DO saturation (20.41%) and DO (12.10%) accounting for the sample grouping. The average squared Euclidian distance between the spring and fall HFR samples was 10.58 with Secchi depth (31.21%), pH (20.50%), salinity (20.35%) and temperature (19.30%) contributing to the seasonal differences.

The spring BBR samples had an average squared distance of 1.73 with depth contributing 82.96%. The fall BBR samples had an average squared distance of 0.11 with pH (30.54%), Secchi depth (23.42%), and depth (20.46%) contributing to the sample group. The average squared distance between the spring and fall BBR samples was 9.21 with temperature (22.40%), pH (15.57%), and dissolved oxygen (15.47%) contributing to the difference between seasons.

The EKR spring samples had and average squared Euclidian distance of 0.21 with depth and pH contributing 53.36% and 25.44% to the sample group respectively. The fall EKR samples had an averaged squared distance of 0.58 with depth (36.53%), pH (28.41%) and DO saturation (16.86%) contributing to the sample group. The average squared distance between the spring and fall EKR samples was 14.74. The DO saturation (41.35%), DO (29.71%) and pH (19.30%) contributing to the seasonal difference.

The average squared Euclidian distance between HFR and BBR during the spring was 5.08 with depth and Secchi depth contributing 51.90% and 20.80% respectively. HFR and EKR had and average square distance of 7.82 due to salinity (38.09%) and pH (20.79%). The average squared distance between the spring BBR and EKR samples was 4.85 with depth and salinity contributing 28.95% and 27.83% respectively.

The HFR and BBR fall samples had and average squared Euclidian distance of 12.14 with Secchi depth (34.77%) and depth (29.59%) contributing to the difference between reefs. The fall HFR and EKR samples had an average squared distance of 22.16 with Salinity contributing 33.90% and Secchi depth 17.35%. BBR and EKR fall samples had an average squared distance of 4.95 with salinity contributing 65.81% to the difference between the reefs.

Figure 21. Principal coordinate analysis of 2016 bottom hydrographic parameters by reef and season.

Table 7. Eigenvalues and Pearson correlations for PCO axes and bottom hydrographic parameters (2016 data only).

Axis	Eigenvalue	%	Depth (meters)	Temperature $(^{\circ}C)$	Dissolved O ₂ (mg/l)	O ₂ Saturation $(\%)$	Salinity (psu)	pH	Secchi Depth (meters)
PCO ₁	183.00	44.31	0.15	-0.79	0.70	0.72	0.60	0.92	0.54
PCO ₂	152.18	36.85	0.82	0.29	-0.69	-0.55	0.61	0.21	0.68
PCO ₃	36.24	8.77	0.41	0.47	0.14	0.41	-0.02	0.08	-0.14
PCO ₄	26.61	6.44	0.31	-0.18	0.13	-0.06	-0.47	-0.21	0.35
PCO ₅	10.47	2.54	-0.19	0.19	-0.04	0.01	-0.18	0.16	0.25
PCO ₆	4.48	1.09	0.06	-0.04	-0.02	-0.09	-0.13	0.18	-0.15
PCO7	0.01	0	0.01	0.00	0.02	0.00	0.00	0.00	0.02

The PCO on the combined 2004 and 2016 bottom hydrographic data grouped the data by reef and season within each year. The data groupings were distributed by season generally along the PCO1 axis and the temperature and DO vectors (Figure 22). PCO1 accounted for 40.4% of the variation in the data and was correlated with dissolved oxygen (ρ=0.93), DO saturation (ρ=0.91) and temperature (ρ=-0.84) (Table 8).

The data points separated out by reefs generally along PCO2 which accounted for 28.7% of the variation in the data (Figure 22). PCO2 was correlated with salinity (ρ=-0.79), depth (ρ=-0.75) and Secchi depth (ρ=-0.62) (Table 8).

Figure 22. Principal coordinate analysis of 2016 and 2004 bottom hydrographic parameters by year, reef and season.

Axis	Eigenvalue	%	Depth (meters)	Temperature $(^{\circ}C)$	Dissolved O21 (mg/l)	O ₂ Saturation $(\%)$	Salinity (psu)	pH	Secchi Depth (meters)
PCO ₁	336.57	40.41	-0.08	-0.84	0.93	0.91	0.45	0.07	0.44
PCO ₂	239.19	28.71	-0.75	-0.19	0.34	0.17	-0.79	-0.47	-0.62
PCO ₃	130.22	15.63	-0.45	0.31	0.01	0.22	0.06	0.85	-0.11
PCO ₄	67.96	8.16	0.34	-0.22	0.09	0.00	0.13	0.16	-0.60
PCO ₅	38.89	4.67	-0.33	-0.25	-0.11	-0.25	0.26	-0.02	-0.05
PCO ₆	20.12	2.42	0.07	-0.21	0.02	-0.16	-0.29	0.18	0.16
PCO ₇	0.04	0.00	0.00	-0.01	0.02	-0.02	-0.02	0.00	-0.01

Table 8. Eigenvalues and Pearson correlations for PCO axes and bottom hydrographic parameters (2016 and 2004 data).

SIMPER analysis of the Euclidian distance matrix for the combined 2004 and 2016 hydrographic dataset was performed to discern which parameters contributed to observed differences between the two years within reef x season groups. The average squared distance between the 2004 and 2016 spring HFR samples was 3.88 with Secchi depth contributing 54.85%. The 2004 and 2016 spring BBR samples had and average squared distance of 8.49 with pH contributing 41.01%. The average squared distance between the 2004 and 2016 spring EKR samples was 6.51 with pH and temperature contributing 43.87%and 37.20% respectively.

The 2004 and 2016 fall HFR samples had and average squared distance of 12.21 with pH contributing 36.89%. The fall BBR 2004 and 2016 samples had an average squared distance of 11.02 due mainly to Secchi depth which contributed 47.99% to the difference between years. The 2004 and 2016 fall EKR samples had an average squared distance of 6.75 with pH contributing 63.06% to the dissimilarity between years.

Epifaunal Community

Epifaunal Growth

The epifaunal growth height was variable on different substrate types (Figure 23) but was not significantly different statistically (p=0.084; Kruskal-Wallis ANOVA on ranks). Growth height was greater on horizontal surfaces relative to vertical surfaces on the reefs (Figure 24; p=0.003) but no significant difference was shown between inverted and horizontal or vertical surfaces (p=0.107 and 0.666 respectively) possibly due to the small sample size of inverted surfaces collected (n=3).

Figure 23. 2016 Epifaunal growth height by substrate type.

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Figure 24. 2016 Epifaunal growth height by surface orientation.
The epifaunal growth height of the 2016 artificial reef samples was not significantly different among the three reefs or spring and fall seasons (Figure 25; p=0.474).

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Figure 25. 2016 mean epifaunal growth height by reef and season.

The epifaunal growth height was significantly higher in 2004 on HFR during both seasons (Figure 26; p=0.003) but not on BBR or EKR.

Figure 26. 2016 vs. 2004 mean epifaunal growth height by reef and season.

Community Structure

The summary statistics for the 2016 artificial reef epifaunal community measures of Species Richness (S), Abundance (N), the Shannon Diversity Index (H') and Evenness (J') are shown in Tables $9 - 11$ for each reef and season and are presented in the following sections.

Table 9. 2016 Howard Frankland Reef epifaunal community summary statistics.

Highlighted = statistically significant higher seasonal values (p <0.05)

Table 10. 2016 Bahia Beach Reef epifaunal community summary statistics.

Highlighted = statistically significant higher seasonal values (p <0.05)

Table 11. 2016 Egmont Key Reef epifaunal community summary statistics.

2016 Egmont Key Reef	Species Richness $S = #$ of taxa		Abundance $N = ind/m2$		Diversity $H' = -\sum p_i \ln p_i$		Evenness $J' = H'/\ln S$	
	Spring	Fall	Spring	Fall	Spring	Fall	Spring	Fall
Mean	96	95	63,927	157,281	3.12	2.54	0.69	0.56
Median	97	93	59,388	95,736	3.22	2.55	0.73	0.57
Min	59	74	17,002	38,690	2.12	1.92	0.46	0.42
Max	128	140	160,523	693,379	3.74	2.94	0.83	0.67
SD	23	18	42,265	194,424	0.55	0.33	0.11	0.08

Highlighted = statistically significant higher seasonal values (p <0.05)

Species Richness

Mean species richness was highest on concrete culvert and concrete slab substrates (Figure 27), however there was no statistically significant difference shown among the different substrate types ($p = 0.061$). Species richness was significantly lower on inverted surfaces relative to horizontal and vertical surfaces (Figure 28; p=0.003).

Species richness was higher during the spring than in the fall, particularly on the HFR and BBR (Tables 9 & 10; Figure 29) while the EKR had a similar number of species present in both seasons (Table 11; Figure 29). Species richness overall was significantly higher in the spring than in the fall (p=0.023) and significantly lower on the HFR than on EKR (p<0.001) and BBR (P=0.002) within seasons. There was no significant difference between BBR and EKR (p=0.388) although the mean number of taxa was higher at BBR in the spring and higher at EKR in the fall (Tables 10 & 11; Figure 29).

Figure 27. 2016 mean epifaunal species richness by substrate type.

Figure 28. 2016 mean epifaunal species richness by surface orientation.

Figure 29. 2016 mean epifaunal species richness by substrate type by reef and season.

The species richness was significantly higher in 2016 than 2004 across all reefs and seasons (Figure 30; p<0.001). In 2016 there was a mean of 83 taxa per sample found compared to 70 taxa per sample in 2004. Both years exhibited similar trends with higher species richness in the spring and increasing from HFR in the upper bay to EKR in the lower bay within each season (Figure 30).

Figure 30. 2016 vs.2004 mean epifaunal species richness by reef and season.

Abundance

There was no significant difference in the abundance of epifaunal taxa among the different reef substrates (Figure 31; p=0.501 Kruskal –Wallis ANOVA on ranks). Epifaunal abundance was not significantly different among the different surface orientations (Figure 32; p=0.584, Kruskal-Wallis ANOVA on ranks).

The overall epifaunal abundance in 2016 was significantly different among the three reefs (Figure 33; p=0.009). This was most notably in the fall samples where HFR had a lower abundance of epifaunal taxa compared to EKR (p=0.001) and BBR (p=0.045) while there was no significant difference among the reefs in the spring. There was no significant difference between seasons at HFR (Table 9; p=0.372) or BBR (Table 10; p=0.955). Abundance was significantly higher at EKR in the fall (Table 11; p=0.01).

There was no significant difference in overall epifaunal abundance between 2016 and 2004 (Figure 34; p=0.189) however seasonally the abundances were higher in 2016 fall relative to 2004 fall (p=0.001). In 2004 the epifaunal abundance was significantly higher in the spring than in the fall across all three reefs (Figure 34; p<0.001) and the spring 2004 reefs generally had higher abundances than the 2016 spring reefs.

Figure 31. 2016 mean epifaunal abundance by substrate type.

EPCHC Artificial Reef
2016 Epifauna Survey

Figure 32. 2016 mean epifaunal abundance by surface orientation.

Figure 33. 2016 mean epifaunal abundance by reef and season.

EPCHC Artificial Reef 2016 Epifauna Survey

Figure 34. 2016 vs. 2004 mean epifaunal abundance by reef and season.

Diversity and Evenness

The Shannon Diversity Index (*H'*) was not significantly different among the various reef materials sampled although mean values were apparently higher on concrete culvert and concrete slab substrates (Figure 35; p = 0.171, Kruskal –Wallis ANOVA on ranks). There was a significant difference in *H'* among the different surface orientations (Figure 36; p=0.005 KW). Diversity was higher on horizontal surfaces than on inverted surfaces (p=0.007) but there was no significant difference between horizontal and vertical surfaces (p=0.183) or between vertical and inverted surfaces (p=0.07) although mean *H'* values were higher on vertical reef surfaces (Figure 36).

The 2016 samples had significantly higher *H'* in the spring than in the fall across all three reefs (Figure 37; p<0.001) however, diversity within seasons was not different among the three reefs (Figure 37; p=0.690).

The overall H' was not significantly different between 2016 and 2004 (p=0.928) nor among the reefs within a sampling year or season (Figure 38; p=0.226). Spring diversity was higher in 2016 than in 2004 (p=0.013) while fall diversity was higher in 2004 compared to 2016 (p=0.019). In 2004 there was no significant difference in H' between seasons (p=0.395).

EPCHC Artificial Reef

Figure 36. 2016 mean epifaunal Shannon diversity by surface orientation.

EPCHC Artificial Reef
2016 Epifauna Survey

Figure 37. 2016 mean epifaunal Shannon diversity by reef and season.

Figure 38. 2016 vs. 2004 mean epifaunal Shannon diversity by reef and season.

The Shannon Evenness Index (*J'*) was not significantly different among the different substrate materials sampled in 2016 (Figure 39; p=0.398, KW), but was significantly higher on horizontal surfaces than on inverted surfaces (Figure 40; p=0.045).

Evenness was not significantly different among the three reefs in 2016 within seasons (Figure 41; p=0.739) but was significantly higher in the spring than in the fall (Figure 41; p=0.002).

There was no difference in *J'* overall between 2016 and 2004 (Figure 42; p=0.180). Fall 2004 J' values were significantly higher than fall 2016 across all reefs (Figure 42; p<0.001).

Figure 39. 2016 mean epifaunal evenness by substrate type.

Figure 40. 2016 mean epifaunal evenness by surface orientation.

EPCHC Artificial Reef 2016 Epifauna Survey

Figure 41. 2016 mean epifaunal evenness by reef and season.

Figure 42. 2016 vs. 2004 mean epifaunal evenness by reef and season.

Species Composition and Similarity

A total of 492 taxa were identified in 2016 across all three reefs and both seasons (Appendix A). Juvenile barnacles (Balanidae) were the most dominant taxon overall accounting for 22.7% of the abundance and occurring in 93.3% of the samples collected. Seven taxa (1.42% of the total taxa identified) accounted for 50% of the cumulative abundance. In addition to the Balinidae, these included juvenile anemones (Actinaria), the caprellid amphipod *Deutella incerta*, the oyster *Ostrea equestris*, the spionid polychaete *Polydora websteri*, the tanaid crustacean *Leptochelia/Hargeria* species complex, and the gammarid amphipod *Ericthonius brasiliensis* (Table 12)*.* Two taxa occurred in 100% of the samples collected: *Polydora websteri* and mud crabs (Panopeidae) while 83 taxa were found in only one sample.

Table 12. 2016 Artificial Reef dominant taxa.

Annelids were the most speciose phylum with 163 taxa (33% of the total) and making up 20.4% of the total abundance. Mollusks were represented by 136 taxa (27.6% of the total) which included 85 gastropods and 51 bivalves but accounted for only 9.87% of the overall abundance. Arthropods had 96 taxa (19.5%) and were the most abundant phylum with 59.3% of the total abundance. Amphipods had 39 taxa and Decapods had 31 taxa. In addition to juvenile barnacles (Balanidae), six barnacle taxa were recorded in 2016: *Balanus trigonus*, *Amphibalanus cf. amphitrite*, *A. eburneus*, *A. improvises*, *A. reticulatus* and *A. venustus.*

A total of 59 taxa (12%) were colonial species. These included 12 Bryozoan taxa, 12 of 16 Chordate (tunicates), 20 Cnidarians (3 Anthozoans, 17 Hydrozoans) and 18 Poriferans.

There was a seasonal shift in the dominant taxa in 2016. The oyster *Ostrea equestris* was the most abundant species in the spring accounting for 10.3% of the abundance and was present in 100% of the samples (Table 13). The fall samples were strongly dominated by juvenile barnacles which represented 34.5% of the total abundance and were found in 100% of the samples (Table 13). Additionally, the barnacle *Balanus trigonus* was also among the fall dominant taxa (Table 13). Actinaria and *Polydora websteri* were also recorded in all of the fall samples (Table 13).

Table 13. 2016 Overall dominant taxa by season.

A total of 201 taxa were found on the HFR during the spring 2016 sampling. The spring HFR epifaunal community was dominated by *Ostrea equestris* along with the amphipod *Stenothoe cf. georgiana*, Actinaria, juvenile barnacles and the amphipod *Paracaprella pusilla* (Table 14). The fall 2016 HFR epifaunal community had 163 taxa and was dominated by Actinaria which accounted for 25.35% of the abundance on the reef (Table 14). Additionally, the barnacles *Amphibalanus reticulatus* and juvenile Balanidae were among the most abundant taxa found and together made up over 25% (13.64% and 11.6% respectively) of the fall HFR abundance (Table 14). *Polydora websteri* and the amphipod *Monocorophium acherusicum* were also among the dominate fall taxa (Table 14).

Table 14 2016 Howard Frankland Reef dominant taxa by season.

A total of 260 epifaunal taxa were recorded on the BBR during the 2016 spring sampling. *Ostrea equestris* was the dominant species in the spring accounting for 15.49% of the abundance on the reef (Table 15). Other spring dominant taxa included tanaid crustaceans (*Leptochelia/Hargeria* species complex), juvenile anemones (Actinaria), juvenile barnacles (Balanidae) and the syllid polychaete *Syllis gracilis* (Table 15). A total of 222 taxa were recorded in the fall BBR samples. The fall epifaunal community was strongly dominated by juvenile barnacles which made up over 48% of the relative abundance on the reef and were present in 100% of the samples collected (Table 15). Other dominant taxa in the fall included Actinaria, the barnacle *Amphibalanus reticulatus*, *Syllis gracilis* and the caprellid amphipod *Deutella incerta* (Table 15).

The EKR had 300 epifaunal taxa recorded during the 2016 spring sampling. The caprellid amphipod *Caprella penantis* was the most abundant species in the spring EKR samples. Other dominant taxa included Actinaria, juvenile barnacles, tanaids in the *Leptochelia/Hargeria* species complex and the gammaridean amphipod *Elasmopus cf. rapax* (Table 16). A total of 228 taxa were identified in the fall EKR samples. The fall epifaunal community was dominated by juvenile barnacles which accounted for 32.68% of the abundance (Table 16). Other dominant taxa included the caprellid amphipod *Deutella incerta*, the barnacle *Balanus trigonus*, the decapod shrimp *Lucifer faxoni* and the gammaridean amphipod *Ericthonius brasiliensis* (Table 16).

	Spring	Fall			
%	%	%	%		
Abundance	Occurrence	Abundance	Occurrence		
	Caprella penantis	Balanidae			
14.18%	50.00%	32.68%	100.00%		
Actiniaria		Deutella incerta			
11.09%	90.00%	14.77%	30.00%		
	Balanidae	Balanus trigonus			
5.65%	70.00%	6.14%	100.00%		
	Leptochelia/Hargeria spp.	Lucifer faxoni			
4.05%	100.00%	5.43%	100.00%		
Elasmopus cf. rapax		Ericthonius brasiliensis			
3.96%	100.00%	4.72%	100.00%		

Table 16. 2016 Egmont Key Reef dominant taxa by season.

The Bray-Curtis (BC) similarity analysis of the 2016 and 2016 vs. 2004 samples indicates that the epifaunal community on the HFR was distinct from the other two reefs in both studies (Figures 43 & 44). The Bray-Curtis similarity among the 2016 spring HFR samples was 52.25 with *Ostrea equestris*, Actinaria, the syllid polychaete *Brania nitidula,* the gastropod *Astyris lunata* and juvenile barnacles contributing to the similarity among the samples. The fall 2016 HFR samples had an average similarity of 54.04 with relatively high abundances of Actinaria, *Amphibalanus reticulatus* and juvenile barnacles among the samples.

The average dissimilarity between the 2016 spring and the fall HFR samples as 58.39. The spring epifaunal community was characterized by higher abundances of *Ostrea equestris* and the gammarid amphipod *Stenothoe cf. georgiana* while the fall community had higher abundances of Actinaria and barnacles (unidentified juveniles + *Amphibalanus reticulatus*)

The spring 2016 BBR samples had an average BC similarity of 51.93 and were characterized by high abundances of tanaid crustaceans (*Leptochelia*/*Hargeria* spp.), *Ostrea equestris* and the gammarid amphipod *Leucothoe cf. spinicarpa* spp. complex. The average BC similarity among the 2016 fall samples was 46.05 with high abundances of juvenile barnacles, the syllid polychaete *Syllis gracilis*, and Actinaria contributing to the community structure. The spring 2016 epifaunal community on the BBR overall was more similar to the fall 2016 BBR and EKR communities but less similar to the spring 2016 EKR community, while the fall communities on the BBR and EKR reefs were more similar to each other due to the high seasonal abundance of juvenile barnacles (Figure 43).

The average dissimilarity between the 2016 spring and fall BBR samples was 59.86. The spring epifaunal community had higher abundances of *Leptochelia*/*Hargeria* spp., *Ostrea equestris*, and the gammarid amphipods *Podocerus brasiliensis* and *Elasmopus cf. rapax* while the fall community was characterized by juvenile barnacles and adult *Amphibalenus reticulus.*

The spring 2016 EKR samples had an average Bray-Curtis similarity of 40.88 with *Leptochelia*/*Hargeria* spp., Actinaria, *Ostrea equestris*, the gammarid amphipod *Elasmopus cf. rapax* and spionid polychaete *Polydora websteri* contributing to the similarity among samples. The fall EKR samples had an average BC similarity of 57.58 with unidentified juvenile barnacles and mature *Balanus trigonus* contributing to the similarity among the fall samples along with *Polydora websteri*.

The average dissimilarity between the 2016 spring and fall EKR samples was 60.23. The spring EKR epifaunal community had higher abundances of the caprellid amphipod *Caprella penantis* and the gammarid amphipod *Stenothoe cf. georgiana* while the fall EKR community had higher abundances of barnacles (juveniles + *Balanus trigonus*), the decapod crustacean *Lucifer faxoni*, the caprellid amphipod *Deutella incerta* and the spionid polychaete *Polydora websteri.*

Figure 43. 2016 Bray-Curtis similarity of epifaunal communities averaged by reef and season

Within the 2016 spring season the average dissimilarity between the HFR and BBR samples was 62.84. The spring HFR community had higher abundances of *Stenothoe cf. georgiana* while *Leptochelia*/*Hargeria* spp., and *Ostrea equestris* were more abundant at BBR. The 2016 spring HFR and EKR samples had an average dissimilarity of 71.65. The HFR community had higher abundances of juvenile barnacles, the syllid polychaete *Brania nitidula*, of *Stenothoe cf. georgiana* and *Ostrea equestris* and the EKR community had higher abundances of Actinaria and *Caprella penantis*. The average dissimilarity between the 2016 spring BBR and EKR samples was 63.47. The spring BBR community had higher abundances of *Leptochelia*/*Hargeria* spp., *Ostrea equestris,* juvenile barnacles, the ophiuroid *Ophiactis savignyi* gammarid amphipod *Leucothoe cf. spinicarpa* spp. complex while the spring EKR community had higher abundances of the caprellid amphipod *Caprella penantis* and the gammarid amphipod *Photis cf. longicaudata.*

Within the 2016 fall season the average dissimilarity between the HFR and BBR samples was 62.54. The fall HFR epifaunal community had higher abundances of Actinaria, the barnacle *Amphibalanus reticulatus* and the gammarid amphipod *Monocorophium acherusicum* and the fall BBR community had higher abundances of juvenile barnacles, the spionid polychaete *Polydora colonia*, *Leptochelia*/*Hargeria* spp., and *Ostrea equestris.* The average dissimilarity between the fall HFR and EKR samples was 70.36 with higher abundances of *Amphibalanus reticulatus*, Actinaria and *Monocorophium acherusicum* at HFR while EKR had higher abundances of barnacles (juveniles + *Balanus trigonus*), *Lucifer faxoni,* and the gammarid amphipods *Ericthonius brasiliensis* and *Photis cf. longicaudata.* The 2016 fall BBR and EKR samples had a relatively low average dissimilarity of 57.07 with the two epifaunal communities being characterized by a higher abundance of the barnacle *Amphibalanus reticulatus* at BBR and higher abundance of *Balanus trigonus* and unidentified juvenile barnacles at EKR.

The similarity analysis between the 2016 and 2004 samples shows that the epifaunal communities on the Howard Frankland Reef were more similar across seasons and study years and more dissimilar than the communities on the other two reefs (Figure 44). The spring 2004 and Spring 2016 HFR communities had an average dissimilarity of 62.20. The 2004 spring HFR epifaunal community had higher abundances of the gammarid amphipods *Ericthonius brasiliensis* and *Stenothoe cf. georgiana,* the gastropod *Crepidula depressa*, Actinaria, and *Polydora websteri* as well as the oyster *Crassostrea virginica* and Asian Green Mussel *Perna viridis*. The 2016 spring HFR epifaunal community was characterized by higher abundances of *Ostrea equestris*, juvenile barnacles, and the polychaetes *Syllis gracilis* and *Spiocheatopterus costarum* the caprellid amphipod *Deutella incerta* and the decapod Panopeidae (mud crabs). The fall 2004 and 2016 HFR communities had an average dissimilarity of 68.55. The 2004 fall community had higher abundances of *Ostrea equestris*, *Anthopleura* sp., the gastropods *Crepidula depressa* and *Boonea impressa*, and the oyster *Crassostrea virginica*. The 2016 fall community had higher abundances of Actinaria, the barnacle *Amphibalanus reticulatus*, the spionid polychaete *Polydora websteri* and mud crabs (Panopeidae).

The 2004 and 2016 spring BBR epifaunal communities were similar and grouped together in the cluster analysis as did the 2016 fall BBR and EKR epifaunal communities, while the 2004 fall BBR community was more dissimilar than the other BBR and EKR communities (Figure 44). The 2004 spring and fall EKR communities were similar and clustered with the 2016 spring EKR community (Figure 44).

Figure 44. 2016 vs. 2004 Bray-Curtis similarity of epifaunal abundance averaged by year, reef and season.

Biomass

The 2016 total wet weight biomassfor each reef and season is presented in Table 17 and mean wet weight biomass by reef and season is presented in Figure 45. Epifaunal biomass was significantly higher in the fall on all three reefs (Figure 45; p=0.001) but was not significantly different between reefs within a given season (Figure 45; p=0.820).

Biomass (grams wet wt.)	2016						
	HFR		BBR		EKR		
	Spring	Fall	Spring	Fall	Spring	Fall	
Total $(n=10)$	715.17	1416.28	835.69	1295.53	878.15	1480.05	
Mean	71.52	141.63	83.57	129.55	87.82	148.00	
Median	70.63	105.96	84.15	120.24	65.56	132.80	
Min	27.35	41.30	23.71	40.85	18.21	59.25	
Max	111.48	280.61	162.08	247.08	170.28	286.79	
Standard Dev.	27.73	95.89	39.42	66.91	61.74	77.69	

Table 17. 2016 Artificial Reef wet weight biomass by reef x season.

Figure 45. 2016 Mean reef epifaunal wet weight biomass by reef and season.

The total epifaunal biomass was significantly higher in 2004 than in 2016 on the HFR (p<0.001) and on the BBR (p=0.017) but not on the EKR (Figure 46). This was most prevalent at HFR where the mean spring biomass in 2004 was 807 grams vs. 71.5 grams in spring 2016 (Figure 46). Overall spring biomass was higher than in the fall during 2004 (p=0.002) but there was no significant difference between seasons in 2016 (p=0.360). In 2004, all three reefs were significantly different with HFR having the highest and EKR having the lowest biomass while in 2016 there was no significant difference in biomass among reefs with a given season.

Figure 46. 2016 vs. 2004 mean reef epifaunal wet weight biomass by reef and season.

The 2016 HFR biomass was dominated by barnacles in both the spring and the fall seasons. Barnacles accounted for 69.48% of the total spring biomass and over 90% of the fall biomass (Table 18). Other taxa contributing to the 2016 HFR biomass included the ascidian *Styela plicata* (5.00%), the sponge Cliona sp. A (3.14%), *Ostrea equestris* (2.57%), and the bryozoan *Aeverilla armata* (2.12%) (Table 18). In contrast, the 2004 HFR spring biomass was dominated by the Asian Green Mussel *Perna viridis*, which comprised over 78% of the biomass and the Eastern Oyster *Crassostrea virginica* which accounted for 13.8% of the biomass (Dix et al. 2005). Both species were present in 2016 but *P. viridis* only contributed to 0.01% of the spring biomass and *C. virginica* only 0.42%.

Other taxa contributing to the 2016 fall HFR biomass in addition to barnacles included the sponges *Lissodendoryx cf. carolinesis* (1.84%) and *Mycale* sp. (1.81%), the ascidian *Diplosoma cf. listerianum* (0.91%) and the sponge *Haliclona* sp. (0.82%) (Table 18). The 2004 fall HFR biomass was also dominated by *Perna viridis* and *Crassostrea virginica* which comprised 65.46% and 10.28% of the biomass and barnacles contributed 7.31% of the fall biomass that year (Dix et al. 2005). In 2016 *P. viridis* only accounted for 0.16% of the fall HFR biomass and *C. virginica* was not present in the fall samples.

Barnacles accounted for 42.33% of the 2016 spring biomass on the BBR (Table 19). Other dominant taxa contributing to the biomass included the sponges *Heteroscleromorpha* sp. A of EPC (23.63%) and *Mycale* sp. (6.28%), *Ostrea equestris* (3.52%), and the anthozoan *Carijoa riisei* (3.39%) (Table 19). *Perna viridis* dominated the 2004 spring BBR biomass accounting for 73.47% while barnacles contributed 8.68% of the biomass that year. *P. viridis* was present in the 2016 spring BBR samples but only accounted for 0.12% of the total biomass.

Barnacles dominated the 2016 fall BBR biomass contributing 73.69% (Table 19). Other dominant taxa included the sponges *Heteroscleromorpha* sp. A of EPC and *Lissodendoryx cf. carolinesis* which comprised 10.84% and 2.73% of the total biomass respectively (Table 19). The 2004 fall BBR biomass was dominated by *Perna viridis* (58.93%) which only accounted for 0.39% of the 2016 fall biomass and barnacles contributed 27.69% of the 2004 biomass.

Barnacles made up 20% of the 2016 spring EKR biomass (Table 20). Other top contributing species included the anthozoan *Cladocora arbuscular* (17.17%), the ascidian *Aplidium cf. stellatum* 16.52%), the sponge *Ircinia cf. campana* (12.28%) and the hydrozoan *Eudendrium carneum* (5.07%). Barnacles and *Ostrea equestris* dominated the 2004 spring EKR biomass contributing 57.24 and 8% respectively. *O. equestris* accounted for 4.92% of the 2016 spring EKR biomass by comparison.

The 2016 fall EKR biomass was also dominated by barnacles which comprised 43.22% of the total. Other top contributing species included the sponge *Ircinia cf. felix* (21.86%), the ascidian *Aplidium cf. stellatum* (7.47%), *Ostrea equestris* (5.27%) and the hydrozoan *Eudendrium carneum* (4.18%). In 2004 the fall EKR biomass was dominated by several of the same taxa including barnacles (36.54%), *Aplidium cf. stellatum* (30.58%) and *Ostrea equestris* (6.95%). *Perna viridis* was present in both seasons of 2016 and 2004 but only comprised a small fraction of the total biomass in any year or season. In 2016, *P. viridis* accounted for only 0.02% of the spring biomass and 0.08% of the fall biomass. In 2004 it contributed 0.46% in the spring and 0.88% in the fall.

Barnacles made up the largest proportion of the biomass on all three reefs during both seasons (Tables 18-20), but were significantly higher in the fall on all reefs (Figure 47; p<0.001) and barnacle biomass was significantly higher on the HFR than on the EKR within seasons and particularly in the fall (Figure 47; p=0.043). There was no significant difference in the barnacle biomass between HFR and BBR (p=0.226) or between BBR and EKR (p=0.361) within seasons.

The oyster *Ostrea equestris* biomass was not significantly different between seasons on any of the reefs (p=0.967) however the overall mean biomass was lower in the fall at HFR and BBR and higher in the fall on EKR (Figure 48). There was no significant difference among the three reefs during the spring while EKR had a significantly higher biomass of *O. equestris* in the fall than at HFR and BBR (Figure 48; p=0.007).

Table 19. 2016 Bahia Beach Reef spring and fall total and percent wet weight biomass by taxa.

Table 20. 2016 Egmont Key Reef spring and fall total and percent wet weight biomass by taxa.

Figure 48. 2016 wet weight biomass of Ostrea equestris by reef and season.

The Bray-Curtis similarity analysis and SIMPROF test on the 2016 epifaunal biomass indicates that the communities on the HFR and BBR were similar across both seasons while the spring and fall EKR community was distinct from the other two reefs (Figure 49). The HFR and BBR epifaunal communities had an average similarity of 65.17 and were characterized by high biomass of juvenile barnacles and other crustaceans and the sponge *Lissodendoryx cf. carolinesis*. The spring and fall EKR communities had an average similarity of 62.59 and also had a high biomass of barnacles and the ascidians *Aplidium cf. stellatum* and *Aplidium constellatum*, the hydrozoan *Eudendrum carneum* and *Ostrea equestris*.

Figure 49. 2016 Bray-Curtis similarity of epifaunal biomass averaged by year, reef and season.

The Bray-Curtis similarity analysis and SIMPROF test on the 2016 and 2004 biomass clustered the 2004 spring and fall HFR and BBR together and separate from the 2004 EKR and all 2016 reefs (Figure 50). The SIMPER analysis indicated that the 2004 HFR + 2004 BBR communities had an average similarity of 62.39 due to the high biomass of *Perna viridis* on these reefs. Within this grouping, the 2004 HFR spring and fall communities and the 2004 BBR spring and fall communities each formed a significant subgroup as indicated by the SIMPROF test. The 2004 HFR spring and 2004 HFR fall subgroup had an average similarity of 67.23 and had a high biomass of *Crassostrea virginica*. The 2004 BBR spring + 2004 BBR fall subgroup had an average similarity of 73.77 and had higher biomass of the sponge *Heteroscleromorpha* sp. A of EPC.

The remaining samples grouped together with and average similarity of 55.61 with higher biomasses of barnacles and *Ostrea equestris* contributing to the similarity among them (Figure 50). Within this group, the 2016 HFR and BBR spring and fall communities clustered together with an average similarity of 65.17. This group was characterized by a high biomass of barnacles and other crustaceans and the sponge *Lissodendoryx cf. carolinesis*. The 2004 + 2016 EKR spring and fall communities clustered together with an average similarity of 60.32 with barnacles, *Aplidium cf. stellatum*, *Ostrea equestris* and the ascidian *Distaplia cf. bermudensis* contributing to the similarity among samples (Figure 50).

Figure 50. 2016 vs. 2004 Bray-Curtis similarity of epifaunal biomass averaged by year, reef and season.

Epifaunal Community and Hydrographic Parameters

BIOENV analysis was done in PRIMER ver 7 in order to correlate the reef physical parameters with the epifaunal community structure based on the Bray-Curtis similarity for both the epifaunal abundance and biomass results for 2016 and the 2016 vs. 2004 datasets.

The results for the 2016 epifaunal abundance data found the combination of bottom temperature and salinity had the highest correlation with the epifaunal community structure (ρ = 0.538) with salinity having the strongest correlation of any single parameter (ρ = 0.531).

The results for the 2016 vs. 2004 epifaunal abundance data found the combination of reef depth, bottom dissolved oxygen and bottom salinity had the strongest correlation with the epifaunal community structure between years (ρ = 0.581) with salinity having the strongest correlation of any single parameter (ρ = 0.523).

The results for the 2016 epifaunal biomass data found the highest correlation between the physical parameters and epifaunal community structure was a combination of bottom dissolved oxygen saturation and salinity (ρ = 0.243) with salinity having the strongest single parameter correlation (ρ = 0.218).

The results for the 2016 vs. 2004 epifaunal biomass data found the combination of depth and salinity had the highest correlation with the community structure between years (ρ = 0.297) with salinity having the strongest correlation of any single parameter ($\rho = 0.274$).

Discussion

The three reefs in this study did show differences in their epifaunal communities both spatially within Tampa Bay and temporally between seasons which correlated with the hydrographic conditions at the reef locations.

The HFR located in Old Tampa Bay and the furthest up the estuary from the Gulf of Mexico was characterized by lower salinity and generally lower water clarity than the other two reefs. This was most evident during the wet season (fall sampling period) due to higher rainfall and runoff entering the bay. The dissolved oxygen and percent saturation were also higher at HFR in the fall relative to the other two reefs. This was likely due seasonal booms of the dinoflagellate *Pyrodinium bahamense* that frequently occur during the summer in Old Tampa Bay (Karlen and Campbell, 2012).

The BBR located in Middle Tampa Bay is mid-way between the HFR and EKR and its salinity and other hydrographic parameters are intermediate between the other two reefs.

The EKR in Lower Tampa Bay is near the mouth of the bay and has greater tidal exchange with the Gulf of Mexico. This results in higher salinities than the other reefs and generally more stable hydrographic conditions overall.

Species richness was higher in the spring on HFR and BBR and correlated with higher seasonal salinities and generally better water quality conditions overall. EKR had high species richness during both seasons which reflects the more seasonally stable water quality conditions on that reef. Epifaunal abundance was higher in the fall on BBR and EKR and corresponded with the seasonal recruitment of barnacles, while abundance was lower in the fall at HFR along with a drop in the number of taxa. Higher Shannon diversity and evenness index values during the spring on all three reefs again correlate with the higher seasonal salinity and better water quality conditions during the dry season.

Overall, species richness and abundance on the artificial reefs was higher than has been found in Tampa Bay soft sediment habitats. Karlen et al. (2015) reported a historical median bay-wide species richness of 35 taxa and abundance of 5,813 ind/ m^2 for sediment samples collected from 1993-2012 as part of the Tampa Bay Benthic Monitoring Program. By comparison, the lowest median seasonal species richness on the reefs was 51 taxa (HFR fall) and the lowest seasonal mean abundance was 41,337 ind/m².

There was an observed shift in the dominant taxa between seasons, with the oyster *Ostrea equestris* dominating in the spring, which may reflect seasonal larval recruitment that time of year. Although *O. equestris* was present at all reefs during the spring, it was mainly dominant at HFR and BBR. Barnacles dominated in the fall which suggests a pulse of larval recruitment in the late summer months. Most of the barnacles were small, recently settled juveniles which could not be identified to species, however six distinct adult barnacle species were identified in this study: *Balanus trigonus*, *Amphibalanus cf. amphitrite*, *A. eburneus*, *A. improvises*, *A. reticulatus* and *A. venustus* (Appendix A)*.* Of these, *Amphibalanus reticulatus* was more dominant on HFR and BBR while *Balanus trigonus* was the dominant species on EKR.

The spring and fall epifaunal species composition on HFR was more similar to each other than with the other reefs indicating a relatively stable epifaunal community across seasons despite changes in hydrographic conditions. The spring epifaunal communities at BBR and EKR were dissimilar to the other reefs, while the fall BBR and EKR communities were more similar due largely to the high abundance of barnacles on both reefs.

The epifaunal biomass was much higher in the fall on all three reefs in 2016 which again is attributed to the greater abundance of barnacles observed during that season. Within seasons however the biomass on the three reefs was not different. The biomass composition on HFR and BBR was similar across both seasons due the contribution of barnacles and the sponge *Lissodendoryx cf. carolinesis* while the biomass composition on EKR across seasons was more similar to each other and dominated by barnacles and several species of ascidians and hydrozoans

Compared with the 2004 artificial reef study, the species richness was higher in 2016 while epifaunal abundance did not change. This suggests an overall healthier epifaunal community. Shannon diversity and evenness were also similar between years despite the increase in species richness in 2016.

One notable change in the epifaunal community between 2004 and 2016 was the decrease in the Asian Green Mussel, *Perna viridis* which was very abundant and dominated the biomass in 2004 particularly on HFR and BBR. This species was of concern in the original study because it is a non-native invasive species that had recently been introduced into Tampa Bay presumably through ship ballast water (Baker et al., 2007). Although still present in 2016, its contribution to the epifaunal community abundance and biomass was negligible. This drop in the *Perna viridis* population in Tampa Bay has been attributed to its low tolerance for cold temperatures (McFarland et al. 2015) and several winter mass die-offs had been recorded in Tampa Bay in the intervening years between studies including events in the winter of 2007/2008, 2009 and 2010 (Firth et al, 2011).

The abundance of the Eastern oyster*, Crassostrea virginica*, was also lower in 2016 than in 2004 on HFR while the Crested oyster, *Ostrea equestris*, was among the dominant species in 2016 particularly in the spring. This may be due to the observed difference in the spring salinity which was lower in 2004 than in 2016. *Ostrea equestris* has been reported to prefer higher salinities while *Crassostrea virginica* is more tolerant of lower salinity habitats (Galtsoff and Merrill, 1962).

The epifaunal community on Tampa Bay artificial reefs is influenced by several hydrographic factors but primarily by salinity and to a lesser extent by temperature. The salinity gradient from Old Tampa Bay to Lower Tampa Bay affects the epifaunal community structure spatially between the three reefs while salinity and temperature influence the seasonal changes in the epifaunal community. All three reefs have been in place for over 18 years with no new material being deployed more recently than 2005. Thanner et al. (2006) found that after 5 years, benthic assemblages (primarily sponges and corals) on artificial reefs were relatively stable and similar to adjacent natural reef communities. Given the age of the Tampa Bay reefs the existing epibenthic communities are in a stable state and most of the observed differences among the three reefs surveyed are attributed to the spatial and seasonal differences in the hydrographic conditions and seasonal recruitment patterns of the epifaunal species.

Conclusions

The purpose of this current survey of the epifaunal communities on Tampa Bay artificial reefs was to test five stated objectives:

Objective 1: Determine if the epibenthic communities have changed since the first survey in 2004.

The epibenthic communities have changed since 2004 most notably on the HFR and BBR while the EKR community was more stable over time. The most notable change was the near absence of the Asian Green Mussel, *Perna viridis,* in 2016.

Objective 2: Assess current health status of the reefs.

The increase in overall species richness in 2016 and the near absence of *Perna viridis* indicates that the current status of the reefs is healthy and has improved since the original survey in 2004.

Objective 3: Determine if a ten-year monitoring frequency is adequate for detecting changes in the epibenthic invertebrate communities, or if these reefs need to be monitored at a greater frequency.

The frequency between the 2004 and 2016 studies did detect community changes. While monitoring the reefs at a greater frequency may be preferable for detecting changes at a finer scale, the cost and benefits of more frequent monitoring need to be better evaluated. A five-year monitoring period may be a feasible option for future studies which would allow for a shorter period between surveys to detect changes in the reef community while distributing the cost and work load over several years. This would also contribute to a long-term data base over time that would be important for understanding future successional changes in the artificial reef epibenthic community structure (Nicoletti et al. 2007).

Objective 4: Determine if the reefs need to be re-nourished with new material.

The results of this study suggest that the reefs are still relatively diverse and recruiting new epifauna seasonally. Adding new material however would still increase available habitat and ensure the continued viability of the reefs. The addition of new reef material would further increase the reef biodiversity by providing a mosaic of substrates of different ages supporting multiple successional communities on the reef.

Objective 5: Discover if artificial reef placement within the estuarine environment is important to their success.

Reef placement did influence the species composition of the epifaunal communities based largely on the estuarine salinity gradient in Tampa Bay with lower diversity and species richness corresponding to lower salinities. However, all three reefs still appeared to support healthy, functioning epifaunal communities.

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Appendix A: Tampa Bay Artificial Reef Taxa List

