Changes in motile benthic faunal community structure following large-scale oyster reef restoration in a subtropical estuary

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Running head: Faunal shifts following oyster restoration

Abstract

Restored oyster reefs support diverse communities of motile benthic organisms (small fishes and mobile macroinvertebrates). These communities represent a critical component of estuarine food webs, and frequently include juveniles of ecologically and economically important species that utilize oyster reefs as nurseries. Oyster-related metrics are commonly used to quantify restoration success, yet it is also important to examine communities of oyster-associated organisms when attempting to identify ecological convergence between natural and restored oyster reef systems. Here, we compare the community composition of motile benthic organisms over time at one restored and three natural oyster (*Crassostrea virginica*) reefs in the Loxahatchee River estuary (Florida, USA) as one means of assessing restoration success. We also examine the effects of vertical relief on motile benthic organisms following restoration. The community of motile benthic organisms at a 1.93-hectare restoration reef gradually began to resemble natural communities in the months following reef construction. Within ~22 months, biomass and community composition were similar between natural and restored habitats. At that point, the mean biomass of motile benthic organisms at the restoration site had reached 83.6 g/m^2 (versus 89.8 g/m^2 at nearby natural reefs), and the restored reef supported > 1,600 kg of small, motile, oyster-associated organisms. Biomass values increased more rapidly in high-relief sections of the restored reef (30 cm vs. 15 cm reef height), particularly during the first year following restoration. High-relief areas were also characterized by increased oyster densities, greater oyster-generated rugosity, and decreased sedimentation. Our study suggests that small differences in reef design can have important implications for restoration success as well as food web structure and dynamics via shifts in community composition.

Keywords:

Oyster reef restoration; Food web; *Crassostrea virginica*; Habitat complexity; Nursery habitat; Ecosystem function

1. Introduction

With more than 60% of Earth's population living in the coastal realm, estuarine ecosystems have been extensively altered by human activities (Ray 2006). In many temperate and subtropical estuaries, oyster reefs represent a critical habitat type, providing numerous ecosystem services, including habitat provisioning, a food base for economically important fishery species, benthic-pelagic coupling, shoreline stabilization, improvements to water quality, and carbon sequestration (Coen et al., 2007; Grabowski et al., 2012; Kellogg et al., 2016; Officer et al., 1982). Oysters are a key foundation species (Bruno et al., 2003; Dayton 1972), and their presence can facilitate the colonization, survival, and growth of a myriad of other small organisms, including crabs, shrimp, mollusks, and fishes (Stunz et al., 2010; Tolley and Volety 2005). Such communities of small, motile, oyster reef-associated organisms represent a critical component of oyster reef food webs, serving as a food source for numerous ecologically, commercially, and recreationally important species (Abeels et al., 2012; Grabowski et al., 2005). By creating a forage-rich, structurally complex environment, oyster reefs provide critical nursery habitat for numerous higher trophic level marine and estuarine organisms (Beck et al., 2003; Coen et al., 2007; Gilby et al., 2018; Peterson et al., 2003).

Over the past century, oyster reefs throughout North America have experienced significant declines as a result of overharvesting, degraded water quality, altered salinity patterns, and disease (Beck et al., 2011; Jackson et al., 2001; Kirby 2004; Rothschild et al., 1994). As the ecological and economic importance of oyster reefs has become more widely recognized, habitat restoration is increasingly being used to slow or reverse these declines (Bersoza Hernández et al., 2018; Brumbaugh and Coen 2009; Schulte et al., 2009; Taylor and Bushek 2008). Although some oyster reef restoration projects are designed primarily to increase oyster production for commercial purposes, a more common goal of oyster reef restoration is to restore the suite of ecosystem services associated with an intact natural oyster reef community (Benayas et al., 2009; Coen and Luckenbach 2000; Grabowski and Peterson 2007; Luckenbach et al., 2005; Palmer et al., 2004). In addition to increasing oyster production, the construction of living oyster reefs has the potential to enhance populations of many other organisms that utilize these habitats during all or part of their life history, including commercially and recreationally valuable species (Peterson et al., 2003; Tolley and Volety 2005). For this reason, the success of an oyster reef restoration project should be measured not only by the recovery of a population of living oysters but also by the reestablishment of natural oyster reef community structure and species interactions (e.g., food web structure) (Coen and Luckenbach 2000).

Many studies focus on oyster-related metrics, including density, abundance, size, recruitment rates, survival, etc., as a means of assessing the success of restoration projects (Nestlerode et al., 2007; Schulte et al., 2009). Other responses, such as the community composition of motile benthic oyster reef-associated organisms, are less studied. These non-oyster variables are useful to more fully quantify potential similarities/differences (e.g., in species composition and organismal biomass) between natural and restored oyster systems (Humphries et al., 2011b; Rodney and Paynter 2006; Walters and Coen 2006). Long-term data for motile benthic faunal communities often are not available before the initiation of restoration efforts, so selecting ecologically appropriate restoration goals from a community ecology perspective and determining when those goals have been reached can present a challenge for managers (La Peyre et al., 2019).

Here, we utilize a long-term dataset to characterize the structure of motile benthic faunal communities (e.g., small crustaceans, motile mollusks, and demersal fishes) that utilize natural and restored oyster (*Crassostrea virginica*) reefs in the Loxahatchee River (Jupiter, Florida). The Loxahatchee River is near the southern extent of natural oyster reef development along the Atlantic coast of Florida, and oyster reef-associated communities have not been well studied in this subtropical system. We identified patterns in biomass and community composition of motile benthic organisms at several natural oyster reef "reference sites" in the system, creating baselines to facilitate comparisons between natural reefs and a large-scale, human-made restoration reef. We then used these baseline values to track the development of the motile benthic faunal community at the restored reef over time, reflecting shifts in food web structure, as a means of assessing the success of the restoration project. Additionally, since habitat complexity is known to affect motile benthic organisms that occupy oyster reefs (Karp et al., 2018; Margiotta et al., 2016), we tested the hypothesis that small increases in habitat complexity (i.e., greater vertical relief) within an oyster restoration reef would lead to an increase in biomass of motile benthic organisms. This study focuses on the southernmost large-scale oyster restoration reef along the Atlantic coast of the United States, and due to the geographic location of the system, represents an important addition to the existing oyster reef restoration literature. We hope that our findings will inform future restoration and management decisions in this region, and will be used as a basis for including more nuanced goals during restoration projects, including those focused on the overall structure of oyster reef food webs.

2. Materials and Methods

2.1. Study system

The Loxahatchee River (26°57’ N, 80°06’ W) is a 27-kilometer coastal river that flows into the Atlantic Ocean through Jupiter Inlet near Jupiter, Florida, USA (Fig. 1) (VanArman et al., 2005). Human actions over the last century have negatively impacted oyster reef habitats in the Loxahatchee River. Widening and stabilization of Jupiter Inlet beginning in the 1920s, as well as extensive dredging in the lower estuary during the 1940s and 1970s, allowed marine water to flow farther into the river. Dam construction and flood control practices have decreased freshwater flow into the system. These disturbances combined to increase overall salinity in the estuary, resulting in degraded oyster reef habitats and an upstream shift in the optimal salinity zone for oysters, i.e., 10–28 ppt (Loosanoff 1965). Oysters reefs presently occur ~4–7.5 km upriver from their historical location, at an area where a narrowing and shallowing of the river channel create a geomorphic barrier to marine water intrusion (SFWMD 2006; VanArman et al., 2005). The section of the river where salinities presently favor oyster growth is substrate limited. In this portion of the river, the benthos is composed largely of sand and silt, and lacks appropriate settlement habitats for larval oysters, e.g., remnants of historical oyster reefs. Present-day oyster reef development in this section of the Loxahatchee River is limited to patchy, subtidal, fringing reefs, often associated with mangrove shorelines (SFWMD 2006). Fallen mangrove branches and roots represent one of the only hard substrates available for oyster settlement, facilitating the formation of these fringing reefs (Aquino-Thomas and Proffitt 2014). Natural reefs in the system are structurally complex and are characterized by ridges, depressions, exposed sediment patches, and rapid drop-offs (Loxahatchee River

District, unpublished data). These reefs are generally 20–30 cm thick. Relic oyster shells can be found at historical reef locations closer to the ocean; however, benthic salinities in these areas are presently too high (consistently > 30 ppt) to support extensive reef development (SFWMD 2006).

2.2. Identifying natural oyster reef communities

Between May 2007 and May 2012, we sampled motile benthic organisms at three natural oyster reef reference sites (upstream, midstream, downstream) in the Loxahatchee River (Fig. 1). Natural reef sites were located 6.2 to 9.2 km upstream from the ocean, spanning the entire upstream-to-downstream range of present-day oyster reef development in the main branch of the river. We acknowledge that utilizing multiple sites within each river section would have been ideal, but the distribution of oysters in the river did not lend itself to such a design (e.g., see areal coverage estimates below). We chose sites that were representative of the respective river sections to characterize general temporal (wet season vs. dry season) and spatial (upstream vs. downstream) patterns in communities of motile benthic organisms at naturally occurring oyster reefs in the system. The three natural sites were fringing reefs with similar water depth, vertical relief, and oyster cover. The upstream natural reef reference site was ~250 m^2, the midstream site was ~3,000 m^2, and the downstream site was ~100 m^2. We sampled once every two months to balance the ability to identify seasonal patterns in community composition with logistical considerations.

To sample motile benthic macroinvertebrates and small demersal fishes, we deployed benthic sampling trays (n = 4/site) at ~2–10 m intervals at each of the three natural reef reference sites. Tray spacing was dictated by the size of each natural reef, as well as efforts to maximize inter-tray distance while minimizing depth/substrate variation among sub-sites. The benthic sampling trays were 64 × 52 × 10 cm plastic bakery trays lined with polyethylene mesh shade cloth (Plunket and La Peyre 2005; Rodney and Paynter 2006). Each tray was initially filled with 19 l of cleaned, dried oyster shell obtained from local restaurants. The design of these benthic trays allowed us to collect motile organisms that occupied interstitial spaces within the reef, a habitat that is difficult to sample using other methodologies.

At the time of deployment, each sampling tray was placed into a shallow depression that we excavated in the natural oyster reef substrate, such that the top surface of the shell in the tray was flush with the surrounding live oyster matrix. We placed all trays in subtidal areas, at depths ranging from ~0.6–0.8 m below mean low water. After a two-month soak time, a pair of divers using snorkeling gear sampled each tray by lifting it vertically out of the water and walking it to a nearby boat for processing. As water ran through the mesh on the tray bottom, motile benthic macroinvertebrates and small demersal fishes were trapped within the tray. By lifting the trays slowly, we found that demersal fishes would typically take shelter at the bottom of the tray, rather than swimming up and over the tray's edge, negating the need to utilize a cover during retrieval. All fishes, crabs, shrimp, and motile mollusks were collected by hand in the field, kept on ice, and returned to the laboratory for later processing. In the laboratory, we identified each organism to the lowest possible taxonomic level ("taxon" for the remainder of the paper), measured wet mass, and counted abundances. We did not include (1) fishes > 10 cm, (2) amphipods, copepods, and polychaetes, or (3) sessile invertebrates in our analyses, as our tray methodology was not designed to effectively quantify these organisms. After trays were processed, they were refilled with the same shell that was removed during sampling and returned to their original location in the oyster reef. By refilling trays with the same shell after each sampling, we minimized impacts on living oysters, sessile invertebrates, algae, bacterial biofilms, etc. that had colonized the substrate over time.

To characterize natural oyster reef-associated communities, we used a one-way analysis of variance (ANOVA) to compare overall mean biomass and density values for motile benthic fauna among the three natural reef sites across five years of sampling. Post-hoc comparisons were made using the Tukey HSD (honestly significant difference) test (SPSS v.16). We then used a series of nonparametric multivariate analyses to compare patterns of community composition (based on biomass) among sites and across sampling dates. Organism biomass is a key variable in assessing ecological patterns, as it reflects productivity and food-web dynamics, and thus is an important metric to represent community structure (Brown et al., 2004; Persson 1999; Saint-Germain et al., 2007). A Bray-Curtis similarity matrix was created using the mean biomass (g/m^2) of each taxon (Clarke 1993; Clarke et al., 2006), averaged at the site level for each sampling date. Biomass values were fourth-root transformed to down-weight abundant taxa and allow less-common taxa to influence similarity values (Clarke and Warwick 2001). A non-metric multidimensional scaling (NMDS) ordination was created to provide a visual representation of community similarity or dissimilarity among the three natural reference sites. Each data point in the NMDS ordination represents the community that was present during a single sampling date at a single site, utilizing the mean of four benthic sampling trays per data point. The relative proximity of two points to one another in the NMDS ordination reflects the relative similarity of the communities represented by those points. A 1-way analysis of similarities (ANOSIM) was used to test for significant differences in community composition among the three reference sites. Finally, we used similarity percentages (SIMPER) to identify which taxa were most responsible for differences in community structure among sites. Although SIMPER output may be disproportionately influenced by abundant species, it provides a basis for identifying those species accounting for overall differences in community structure. All community-level analyses were carried out using PRIMER v.6.1.6 software.

2.3. Restoration reef construction

In July 2010, the government of Martin County, FL, constructed 2.36 hectares of oyster restoration reef in the Loxahatchee River (Fig. 1) as part of a larger project funded by the National Oceanic and Atmospheric Administration (NOAA). Location, design, thickness, and substrate material for the restoration reef were determined by the contractor tasked with reef construction, within the constraints of the project’s permit. The overall goal of this restoration project was to create a self-sustaining living oyster reef with a similar structure and function to natural oyster reefs in the same system, thereby recovering critical ecosystem services provided by living oyster reefs. The project created suitable settlement habitat for larval oysters and other oyster reef-associated organisms through the construction of a carbonate-based reef in a substrate-limited section of the estuary. Since the Loxahatchee River does not support an oyster fishery, the production of oysters for commercial harvest was not a restoration goal.

Before reef construction, benthos at the restoration site was largely 2-dimensional, composed primarily of sand and coarse silt substrates. The reef was constructed by spreading a continuous and relatively homogeneous 15 cm layer of limestone/sandstone rocks and mollusk shells (~5–20 cm in diameter) across the river bottom, such that the entire restoration site was uniformly covered by a thin layer of calcareous material. The resulting reef was thinner and less structurally complex than natural reefs in the system. The limestone/sandstone rock and mollusk shell material that was used to build the reef was obtained as a byproduct of a nearby beach nourishment project. This material was mechanically sifted out of sand that was dredged from an offshore borrow site and pumped onto local beaches. Because of the large scale of the restoration project, heavy equipment was used to deploy the rock and shell aggregate. All areas of the completed reef remained submerged at low tide. Our sampling was conducted on a continuous 1.93-hectare section of the restoration reef ("restoration reef site" for the remainder of the paper), which was located ~6.75 km from the ocean, in the part of the river that currently supports natural oyster reef growth. The restoration reef site was ~2.5 km from the upstream reference site, ~100 m from the midstream reference site, and ~500 m from the downstream reference site used in our long-term natural oyster reef community structure study described in Section 2.2. This section of the restoration reef had a roughly rectangular footprint, measuring ~165 m × 120 m.

2.4. Comparing restored and natural oyster reef communities

To identify motile benthic organisms utilizing the restoration site before the construction of the reef, we began sampling this area six months before the reef was built. At that time (January 2010), we deployed four benthic sampling trays (see Section 2.2) within the future footprint of the restoration reef. Since our goal was to document community composition on the 2-dimensional soft-bottomed habitat before the addition of a 3-dimensional calcareous restoration reef, we filled each sampling tray with 19 l of unsifted ambient sand/coarse silt substrate excavated directly from the site (rather than oyster shell, as described in Section 2.2). Trays were then placed in the resulting holes, flush with the surrounding river bottom. We sampled these pre-restoration trays at two months (March 2010) and four months (May 2010) following deployment. At each sampling, trays were quickly lifted from the water, and the material contained within was sifted through a 1 mm mesh sieve to recover all motile benthic organisms. Trays were then refilled with unsifted ambient sand/coarse silt substrate and returned to their previous locations. Trays were temporarily removed from the river after the May 2010 sampling event in preparation for the construction phase of the restoration project.

Following reef construction in July 2010, we redeployed the four sampling trays at the restoration reef site. Each tray was filled with 19 l of restoration reef substrate. For the next 22 months, these trays were sampled bimonthly, using the same methodology outlined in Section 2.2. Trays were allowed to soak for two months between sampling events, and the substrate in each tray was removed and replaced during the sampling process.

To assess convergence between motile benthic communities on natural and restored oyster reefs, we compared biomass and organismal density between the three natural reef reference sites and the restoration site. We then used nonparametric multivariate analyses to compare community structure at the restoration reef site to the three natural reef sites over time. Organismal biomass data from each restoration reef sampling date were incorporated into the natural reef NMDS ordination (see Section 2.2) to visualize changes in community composition following reef construction. To avoid redundancy, a single ordination plot is shown, containing natural and restoration reef data. Each restoration reef data point in the NMDS ordination represents the community composition found at the restoration site on a single sampling date (mean of four trays per data point). Hierarchical agglomerative cluster analysis was used to identify groupings of similar restored and natural communities. We conducted a 1-way ANOSIM to test for differences among natural reef communities (i.e., the reference sites), pre-restoration communities, and post-restoration communities. For this analysis, we divided the post-restoration period into four- to six-month time blocks (two to three sampling dates) to look for community convergence over time. We then used SIMPER to identify primary taxa that contributed to the dissimilarity between natural reefs and the restored reef during each time block in the 22 months following reef construction. Community-level analyses were carried out using PRIMER v.6.1.6 software.

2.5. Habitat complexity in a restored oyster reef

To test the effects of habitat complexity on motile benthic community structure at the restored oyster reef, we created three parallel 10 m × 7 m experimental blocks within the continuous restoration reef matrix, each containing two levels of bottom relief. The three experimental blocks were located near the center of the restoration reef and were spaced at ~25 m intervals. We created a 1 m border around each block by clearing away the rock and shell aggregate down to the natural sand/silt substrate. Within each of the three experimental blocks, we built a high-relief plot and a paired low-relief plot, where high relief refers to rapid (i.e., sub-meter scale) changes in reef height relative to the surrounding benthos. The three high-relief plots, which were constructed using an excavator and hand tools, measured 10 m long × 2 m wide × 30 cm tall. The height of the high-relief plots corresponded to the greatest elevation allowed by the construction permit, the approximate height of most natural fringing reefs in the system (Loxahatchee River District, unpublished data), and a threshold height identified by Colden et al. (2017) above which oyster density and survival may be maximized. Each paired low-relief plot measured 10 m long × 4 m wide × 15 cm tall. The height of the low-relief plots was equal to the height of the surrounding restoration reef matrix. Low-relief plots were twice as wide but half as tall as high-relief plots to ensure that both treatments contained the same volume of limestone/sandstone rock and mollusk shell substrate (6 m^3). The paired high- and low-relief plots within each experimental block were adjacent to each other. We created a 1 m wide strip of exposed sand substrate between each high- and low-relief plot. Based on pre-restoration bathymetric surveys (conducted by Continental Shelf Associates, Inc., Stuart, FL, USA), all three blocks were placed at the same initial base elevation within the larger restoration reef footprint. Since each pair of high- and low-relief treatments within an experimental block were parallel and only separated by a 1 m border, they were subject to similar environmental and physical conditions (e.g., current velocity and direction, distance to mangroves, salinity, etc.).

In August 2010, one week after reef construction was completed, we deployed 42 benthic sampling trays across the three experimental blocks (14 paired trays per block). Within each experimental block, we created two parallel rows of sampling trays, with seven trays running down the long axis of the high-relief plot, paired with seven trays running down the long axis of the low-relief plot. Trays were spaced ~1 m apart within rows. Each tray was filled with 19 l of rock and shell restoration substrate that was excavated directly from the reef surface. Trays were then placed into the resulting depressions, such that the surface of the material in the tray was even with the surrounding substrate. The initial 19 l of material that was collected from the reef and placed into each tray was treated as the day 0 sample. At this time, all motile benthic organisms were removed from the substrate and retained before the initial filling of each tray to characterize the community that was present at the start of the study. Rather than sampling this set of trays at a fixed bimonthly time interval, we chose *a priori* to sample at approximately day 0 (date of deployment), 14, 28, 60, 120, 240, 365, and 480. On each sampling date, one randomly selected pair of trays (high/low) was removed from each experimental block and processed (six trays per sampling date). Unlike the sampling protocol described in sections 2.2 and 2.4, these trays were left undisturbed from the time of deployment to the time of sampling, at which point they were permanently removed from the river. By utilizing a range of different soak times, rather than re-sampling every two months, we were able to more accurately identify cumulative changes in community structure that occurred in the 16 months following reef construction. This method allowed motile faunal communities to develop over time without being disturbed every two months, as occurred with our long-term monitoring protocol.

To compare biomass between high- and low-relief treatments over time, we ran a General Linear Model using relief level and days since construction as fixed factors (SPSS v.16). We initially included the location of each experimental block within the reef as a random factor, but location was not a significant predictor of biomass, so we removed it from the model. Data were fourth-root transformed to meet assumptions of homogeneity of variance. To visualize changes in community structure between the two vertical relief treatments over time, we created an NMDS ordination from a Bray-Curtis similarity matrix using fourth-root transformed biomass values (g/m^2) from each tray (Primer v.6.1.6). We then used a 2-way crossed ANOSIM to test for differences in community composition between relief treatments and across sampling dates.

Although this study focused primarily on motile benthic organisms, we observed changes in oyster density and surface rugosity in high- and low-relief sampling trays that had been allowed to soak undisturbed for extended periods of time. At the time of our final sampling (day 485), we quantified the number of live oysters in the three remaining pairs of high- and low-relief sampling trays. Additionally, we measured surface rugosity in these trays by pressing a piece of copper wire into the contours, recesses, and surface irregularities along lines running across the center of each tray's long and short axes, generating two bent-wire measurements per tray. The bent piece of wire was then straightened and measured. Rugosity measurements were reported as the ratio of bent-wire distance to straight-line distance. A paired t-test was used to compare final rugosity between treatments (SPSS v.16).

3. Results

3.1. Temporal and spatial variability in natural oyster reef communities

Between May 2007 and May 2012, we collected and identified nearly 27,000 individual organisms representing 11 fish and 19 invertebrate taxa from natural oyster reefs in the Loxahatchee River (Table 1, 2). We were able to identify many taxa at the species level. In cases where we were not able to make positive species-level identifications (typically due to difficulties in differentiating juveniles of closely related species), we grouped organisms at the lowest possible taxonomic level for subsequent analyses (e.g., mud crabs < 9 mm carapace width were combined as Panopeidae spp.). Dominant motile organisms (by biomass) in these natural oyster reef communities were black-fingered mud crabs (*Panopeus herbstii*), followed by depressed mud crabs (*Eurypanopeus depressus*), crested gobies (*Lophogobius cyprinoides*), unidentified mud crabs < 9 mm (Panopeidae spp.), snapping shrimp (*Alpheus* spp.), green porcelain crabs (*Petrolisthes armatus*), and frillfin gobies (*Bathygobius soporator*) (Table 1). Each of the remaining 24 taxa accounted for ≤ 2% of total natural reef biomass. Unidentified Panopeidae spp. < 9 mm were the most numerically abundant benthic organisms at natural oyster reefs, followed by *Alpheus* spp., *P. armatus*, *E. depressus*, *L. cyprinoides*, and *P. herbstii*, with each of the remaining taxa representing ≤ 2% of the total sample (Table 2).

We observed a distinct seasonality in the biomass of motile reef-associated organisms. Although there was year-to-year and site-to-site variability, we found that biomass at natural oyster reefs in the Loxahatchee River was typically greatest during May or July. The timing of annual biomass minima was less consistent among years but usually occurred between November and March. This region typically experiences a June through October wet season and a November through May dry season. At all three natural reef sites, mean annual maximum biomass values (spring/summer) were approximately two times greater than mean annual minimum biomass values (fall/winter) (Table 3). When averaged across all natural reef reference sites and months, the mean biomass of motile oyster reef-associated organisms at natural reefs was 93.8 ± 34.6 g/m^2 (mean ± SD), and mean organismal density was 266.6 ± 158.4 individuals/m^2.

In addition to seasonal variability, long-term mean biomass of motile oyster-reef associated organisms at natural reef reference sites showed considerable spatial variability. There were significant differences in average biomass among sites ($F_{2,84} = 8.79$, $p < 0.001$), with values increasing upstream to downstream (Table 3). Post-hoc testing revealed that the long-term mean biomass at the downstream natural reef site was 24% greater than at the midstream natural site (114 ± 42 g/m^2 vs. 92 ± 27 g/m^2) and 44% greater than at the upstream natural site (114 ± 42 g/m^2 vs. 79 ± 26 g/m^2) (Table 3). Differences in biomass between the midstream and upstream sites were not significant. We observed similar spatial differences in mean organismal density (organisms/m^2) among sites ($F_{2,84} = 9.42$, $p < 0.001$), with densities at the downstream site (372.4 ± 181.5 individuals/m^2) significantly greater than the midstream site (218.2 ± 94.9 individuals/m^2) and the upstream site (229.5± 153.1 individuals/m^2). Densities at the upstream and midstream sites were not significantly different.

Community composition of motile benthic organisms differed among the three natural reef reference sites across 31 sampling dates (Fig. 2; ANOSIM Global $R = 0.54$, $p = 0.001$). Pairwise comparisons suggested that the upstream and downstream sites had the most dissimilar communities ($R = 0.80$, $p = 0.001$). *Petrolisthes armatus*, *P. herbstii*, *E. depressus*, *L. cyprinoides*, *Nassarius vibex*, and *Lupinoblennius nicholsi* were the primary taxa driving community-level differences between the upstream site and the downstream site (based on biomass, Table 1). *Petrolisthes armatus*, *P. herbstii*, and *N. vibex* made up a greater percentage of the downstream community, whereas *E. depressus*, *L. cyprinoides*, and *L. nicholsi* made up a greater percentage of the upstream community (Table 1). In most cases, biomass values for these taxa at the midstream reference site were intermediates of upstream and downstream values. Overall species richness was greater at the downstream reference site (25 species) than at the midstream or upstream sites (20 species each).

3.2. Convergence between natural and restored reef communities

In 26 months of bimonthly sampling at the restoration site (March 2010 to May 2012), we collected ~4,000 motile benthic organisms representing 20 invertebrate taxa and 10 fish taxa (Table 1, 2). Ten of these taxa, including the economically important Florida stone crab (*Menippe mercenaria*), were not found at natural reef sites during the study. During the four months before restoration, biomass values measured from the sandy and silty substrate at the future restoration site were substantially lower than values from natural reefs, representing ~10% of the mean biomass present at the three natural oyster reef reference sites (Fig. 3). Motile benthic organisms began to colonize the restoration reef site shortly after construction ended. At the time of our first post-restoration sampling, two months after reef construction, biomass values at the restored reef site were just 22% of the mean biomass of the three natural reef reference sites; however, abundance values had already reached 72% of the mean organismal density on natural reference reefs. Biomass increased slowly during the first 6 months following the completion of the restoration project. Between months 6 and 8, the mean biomass at the restoration site doubled (Fig. 3). From month 8 to 18, biomass values at the restored reef began to exhibit seasonal fluctuations that were like those observed at nearby natural reference reefs. By the final two sampling dates of the study (March 2012, 20 months post-restoration, and May 2012, 22 months post-restoration), biomass values at the restored reef were similar to mean biomass values at the natural reference reefs (Fig. 3). The simultaneous increase in biomass from month 20 (March 2012) to 22 (May 2012) at both natural and restored sites is indicative of the seasonal variation we detected in our long-term dataset.

When viewed across the duration of the study, there were significant differences among natural, pre-restoration, and post-restoration communities (Fig. 2; ANOSIM Global $R = 0.70$, $p = 0.001$). Pre-restoration communities, which were characterized by low taxonomic richness (15 taxa) and low biomass (8.8 ± 4.2 g/m^2), differed from natural reef communities ($R = 1.00$, $p = 0.001$). Of the 15 taxa identified during pre-restoration sampling, all except for *Eucinostomus* sp. also occurred at natural reef sites. At the time of our first post-restoration sampling, two months after reef construction, several taxa that were common at natural reference reefs were already present at the restoration site (e.g., *E. depressus*, *Alpheus* spp., Panopeidae spp. < 9 mm, *P. armatus*, *Gobiosoma bosc*), primarily as small, newly recruited, juveniles. Several larger benthic species (e.g., *P. herbstii*, *L. cyprinoides*, *B. soporator*, *L. nicholsi*) that were abundant at nearby natural reference reefs were initially absent from the restoration reef community.

In the 22 months following the construction of the restoration reef, motile benthic communities at the restoration site slowly became more like natural reference reef communities (Fig. 2; through time, restoration reef data points get closer to the cluster of natural reef data points in ordination space). This convergence was gradual, with post-restoration communities differing from natural reef communities during the first six months after restoration ($R = 0.96$, $p = 0.001$), the second six months after restoration ($R = 0.85$, $p = 0.001$), and the third six months after restoration ($R = 0.42$, $p = 0.008$). In the first six months following restoration reef construction, *L. cyprinoides*, *P. herbstii*, and *B. soporator* were the primary taxa affecting community differences between natural and restored reefs. Juvenile *P. herbstii* were first found at the restoration reef six months after construction was completed; however, it took eight months for biomass and abundance values to approach those found at natural reference reefs. Following the appearance of *P. herbstii* in month six, differences between restored and natural reef communities during the second six-month period following restoration were driven primarily by *L. cyprinoides*, *Portunus* spp. (swimming crabs), and *B. soporator*. Although *G. bosc* recruits were present within two months of reef construction, colonization of the restoration reef by other demersal fish species occurred more slowly. *Lophogobius cyprinoides* was not found at the restored reef until month 14. During this time (the third six-month period following restoration), community differences between natural and restored reefs were largely affected by *B. soporator*, *P. armatus*, and *Portunus* spp.

Communities measured toward the end of the study (months 16, 20, and 22) were more similar to natural reference reef communities (at a 60% similarity level) than they were to earlier post-restoration communities. In the last four-month period of the study (the final two sampling dates, 20 and 22 months post-construction), motile benthic community composition at the restoration reef closely resembled that found at natural reference reefs ($R = 0.17$, $p = 0.22$), particularly the downstream reference site (Fig. 2). Note that the data points representing three of the final four sampling dates lie within the cluster of natural reef data points in the ordination space. With the appearance of *B. soporator* 20 months post-restoration, community differences during months 18 to 22 of the study were primarily driven by *Stramonita haemastoma* (Florida rock shell), *P. armatus*, and *Palaemonetes* spp. (grass shrimp).

3.3. Effects of habitat complexity at a restored oyster reef

To assess the effects of vertical relief on post-restoration oyster reef communities, we sampled paired high-relief and low-relief experimental plots within the restoration site 8 times during the 16 months immediately following reef construction. During this period, we collected > 3,000 motile benthic organisms from the experimental treatments. Mean biomass at high-relief plots was significantly greater than at low-relief plots ($F_{1, 26} = 68.1$, $p < 0.001$), and there was a significant effect of time since construction on biomass values for both levels of vertical relief, with a general trend of increasing biomass over time ($F_{1, 26} = 24.7$, $p < 0.001$; Fig. 4). We observed a significant interaction between the effects of relief and time since construction on the biomass of benthic organisms ($F_{6, 26} = 3.20$, $p = 0.017$). For the first eight months of the study, biomass increased at both high- and low-relief plots; however, the overall rate of increase at high-relief plots during this period was 10 times greater than at adjacent low-relief plots.

After peaking in month eight (April), biomass values at high-relief plots slowly began to decrease. The timing of this decrease corresponded to seasonal biomass declines that were simultaneously occurring at nearby natural reference reefs. Low-relief plots experienced a similar decline in biomass, but the decrease began three months later (July). When high-relief biomass peaked on day 240, we recorded a single-tray biomass of 388 g/m^2, higher than any natural reef biomass value measured during the study. At this point, mean high-relief biomass was > 900% greater than mean low-relief biomass. Community composition at high- and low-relief treatments changed over time, but for any single sampling date, communities for both treatment levels exhibited overlap. We observed significant differences in community structure between the two treatment levels across all sampling dates (*R* = 0.47, *p* = 0.001), as well as among dates for both treatments levels (*R* = 0.60, *p* = 0.001).

At the culmination of the habitat complexity experiment (day 485), high- and low-relief treatments exhibited differences in live oyster densities and surface rugosity (Fig. 5). On average, high-relief treatments had more than twice as many live oysters per m^2 as low-relief treatments (420 ± 100 vs. 206 ± 114 oysters per m^2; mean ± SD). As a result, surface rugosity was significantly greater for the high-relief treatments than for the low-relief treatments (1.64 ± 0.15 vs. 1.20 ± 0.13; $t_5 = 4.66$, $p = 0.006$). We observed that the interstitial spaces in two of the three low-relief trays that were sampled on the final day of the experiment were densely packed with sediment. Sediment accumulations were minimal in high-relief trays, similar to our observations from long-term sampling trays at natural reef sites. By the end of the habitat complexity experiment (December 2011), at which time trays had been left undisturbed for 485 days, the biomass of motile benthic organisms in high-relief trays (147 g/m^2) was ~700% greater than in low-relief trays (18 g/m^2).

4. Discussion

Oyster reefs in the Loxahatchee River provide critical habitat for a variety of ecologically and economically important motile benthic organisms. The most abundant taxa on these oyster reefs—small mud crabs (Panopeidae), porcelain crabs (Porcellanidae), snapping shrimp (Alpheidae), and gobies (Gobiidae)—represent key nodes in the estuarine food web (Yeager and Layman 2011). These detritivores, primary consumers, and mesopredators are an important food source for larger estuarine predators, linking estuarine primary production to higher trophic levels (Abeels et al., 2012). Additionally, a number of economically important species utilize natural oyster reefs in the estuary as nursery habitat. Although benthic sampling trays are not designed to efficiently capture larger, more motile organisms, our long-term sampling of natural reefs revealed juvenile snapper, grouper, blue crabs, and commercial shrimp sheltering in the reef matrix, further illustrating the value of these habitats as nurseries. Our findings underscore the complexity of oyster reef food webs and highlight the need to assess restoration projects based on unique species interactions as inferred from community composition (Abeels et al., 2012; Rezek et al., 2017) in addition to oyster-specific metrics. We acknowledge that the spatial/geographic scale of our study was limited, as we focused on one restored oyster reef in a single estuary; however, our findings are applicable to other subtropical estuaries and complement previous findings from diverse geographical areas.

In the Loxahatchee River, the timing of biomass maxima and minima for motile benthic communities appears to be related to seasonal patterns of precipitation and freshwater inflow. The annual peaks in biomass that we observed in late spring and early summer corresponded to the end of the dry season (November to May) or early stages of the wet season (June to October). Annual minimum biomass values occurred in late fall and winter, at the start of the dry season. Although the timing of biomass peaks was relatively similar from year-to-year, the timing of annual minima was more variable. At the three natural reef reference sites, long-term mean biomass values approximately doubled between the end of the wet season and the end of the dry season. A similar temporal pattern was observed in the Caloosahatchee Estuary in southwest Florida, where oyster reef communities exhibited greater biomass during the dry season than during the wet season (Tolley et al., 2005). Intra-annual fluctuations in biomass may represent a direct response to water conditions, such as changes in salinity related to precipitation or changes in water temperature (Lehnert and Allen 2002; Shervette and Gelwick 2008), or may be a result of ingrained behavioral responses associated with seasonality (e.g., change in day length).

The spatial variability in biomass of motile benthic organisms that we observed may also be attributed to salinity differences within the estuary. The upstream natural site, which had the lowest mean biomass, was closest to the freshwater source of the river and experienced more rapid fluctuations in salinity, as well as longer periods of reduced salinity (Loxahatchee River District, unpublished data). The downstream reference site, where biomass values were typically highest, may have experienced smaller fluctuations in environmental parameters (e.g., salinity, temperature) due to its proximity to the ocean. A similar change in oyster reef community structure along an upstream-to-downstream salinity gradient has been observed in other systems (Quan et al., 2012; Shervette and Gelwick 2008; Tolley et al., 2005). These findings underscore the importance of utilizing system-specific natural reef data to determine the optimal location within an estuary to target oyster restoration efforts. This is particularly true for upstream locations within estuaries, where periodic episodes of reduced salinity may harm the benthic organisms that occupy oyster reefs (Marshall et al., 2019).

Patterns of motile benthic community composition that we identified at natural oyster reef reference sites in the Loxahatchee River allowed us to quantify the amount of time required for motile restored reef communities to begin to resemble motile natural reef communities in this subtropical estuary. In this case, the restored reef motile benthic community was similar to natural reef communities (in terms of biomass and species composition) after ~20 to 22 months. This was comparable to the convergence times identified by Meyer and Townsend (2000) in North Carolina estuaries. Rezek et al. (2017) reported an even more rapid convergence time in a Texas estuary, with restored reef community structure resembling natural reef community structure within 12 to 15 months following restoration. Rezek et al. (2017) also found that food web structure converged with a natural oyster reef food web within a 12- to 15-month time frame, suggesting that restored oyster reef food webs can function similarly to those found on naturally occurring reefs. We speculate that the relatively rapid convergence in community structure observed at restored oyster reefs may be a result of the low species richness found at healthy oyster reefs, combined with certain life-history traits exhibited by many reef-associated species (e.g., pelagic larvae, early maturation). Although community convergence can occur rapidly, some studies have documented changes in oyster reef communities continuing over longer time frames, up to 3 to 7+ years following restoration (Quan et al., 2009; Quan et al., 2012; Walters and Coen 2006). Further community-level convergence may occur at the Loxahatchee River restoration reef if certain less-common taxa (e.g., *Lupinoblennius nicholsi, Archosargus probatocephalus*, *Erotelis smaragdus*) appear over time. Additional convergence may occur if rare species that were only found at the restoration site (e.g., *Menippe mercenaria, Stramonita haemastoma, Mithrax* spp.) disappear from that community. Our study did not reveal the timeframe required for complete community-level convergence (i.e., including all rare taxa) to occur. Rare species may be important if species richness is used as the primary measure of restoration success; however, less-common species are unlikely to have major impacts from the perspective of restoring ecosystem function.

At the end of the study (22 months after restoration), the restoration reef community most closely resembled the communities found at the downstream natural reef reference site. Although the downstream reference site was not closest to the restoration reef spatially, both were located in the same shallow, open embayment. This is in contrast to the other two reference sites, which were located in narrower, mangrove-lined channels. This similarity in landscape context between the restoration site and the downstream reference site may account for the resemblance in community composition.

The gradual development of the motile benthic community at the restoration reef was likely driven by a complex interaction between habitat quality, specific settlement cues, and the presence of previous plant and animal colonists. Initial colonists may have been generalist species that possessed broader habitat or dietary requirements than later arrivals (Rezek et al., 2017). It is also possible that some of the later colonizers (e.g., certain blenny and goby species) were more reliant on living oysters or articulated oyster shells as habitat, and, as such, may have required a certain level of live oyster growth before utilizing the new reef. The continued accumulation of live oyster biomass at the restoration reef will be particularly important over time since positive interactions between living oysters and other oyster reef-associated species have been shown to help to shape post-restoration communities (Halpern et al., 2007; Meyer and Townsend 2000; Reeves et al., 2020). Further convergence between motile benthic communities at natural and restored reefs may be facilitated by the continued presence of living oysters at the restoration site into the future.

Motile benthic organisms that colonized the restoration reef likely represented new secondary production in the system, reflecting population-level (e.g., settlement) and community-level (e.g., trophic interactions) mechanisms following the creation of new habitat. The low biomass and high abundance values we observed shortly after the reef was constructed imply that the restoration reef was initially colonized by large numbers of tiny organisms. Most taxa first appeared at the restoration reef as small juveniles, suggesting that they had recently recruited from the plankton. Although biomass of motile benthic fauna steadily increased at the restoration reef site for the first 10 months following reef construction, we did not observe a simultaneous reduction in biomass at nearby natural reefs that would have been indicative of a redistribution of existing production to the new reef. Since habitat was likely limited for benthic oyster reef-dependent species in the Loxahatchee River, the addition of new structurally complex restoration reef habitat provided more places for larval organisms to settle (Bohnsack 1989; Pickering and Whitmarsh 1997). Based on our final biomass estimate from the restored reef site (83.6 g/m^2), the 1.93-hectare section of the restoration reef supported > 1,600 kg of new biomass of motile benthic organisms in May 2012, 22 months after the reef was constructed. Since restored oyster reefs are utilized by a variety of larger transient fish species (Harding and Mann 2001; Layman et al., 2014), this new benthic production at the base of the restored oyster reef food web may also serve to increase production at higher trophic levels, potentially linking oyster reef production to other habitats in the estuary. This also suggests that secondary production quantifications could have provided additional insight into the relative success of this restoration project (Layman and Rypel 2020).

Habitat complexity plays an important role in the outcome of oyster reef restoration. We have shown that even small differences (i.e., 15 cm) in vertical relief can have large effects on restored oyster reef communities, particularly during the first year after restoration. In our study system, several factors may have led to the increased organismal biomass we documented in high-relief plots within the restoration reef. Similar to Schulte et al. (2009), we observed greater live oyster densities in treatments with slightly higher vertical relief. High-relief reefs have been found to experience increased current flow velocities, decreased sedimentation rates, and reduced occurrence of hypoxia (Lenihan 1999; Lenihan and Peterson 1998), all of which favor the survival and growth of oysters (Schulte et al., 2009). This increased oyster growth can gradually lead to greater surface rugosity, another form of habitat complexity, which was apparent in the high-relief treatment at the end of our study. Increased rugosity, in turn, leads to hydrological conditions that favor larval oyster settlement (Soniat et al., 2004; Whitman and Reidenbach 2012), creating positive feedback that results in increased oyster recruitment on high-relief reefs (Gregalis et al., 2008). Reduced sedimentation and compaction rates can also lead to greater rugosity by maintaining open interstitial space in high-relief reefs, creating a refuge for reef-dwelling organisms. This is an important factor in restoration reef success, as interstitial space has been shown to affect community structure in restored reefs (Callaway 2018). Additionally, habitat complexity can affect food web structure on oyster restoration reefs as a result of altered predator-prey interactions (Grabowski 2004; Grabowski et al., 2008; Grabowski and Powers 2004; Hughes and Grabowski 2006; Humphries et al., 2011a). Our findings thus extend previous observations of the importance of habitat complexity in oyster restoration projects, in this case for a subtropical estuary. These results may guide future restoration efforts in South Florida, a region that has not been the focus of extensive oyster restoration research.

Although many possible mechanisms could explain the differences in biomass we detected between high- and low-relief sites, our observations suggest that increased sedimentation in low-relief areas, and its related impact on live oyster growth and rugosity, may be the primary driver in the Loxahatchee River. Initial surface rugosity did not differ between treatments, since both were constructed from the same substrate. Through time, low-relief areas appeared to lose surface rugosity due to sedimentation and compaction, while rugosity at high-relief areas remained constant or increased due to oyster growth. Early in the post-restoration phase, before live oysters grew, sedimentation in the low-relief treatments likely reduced the amount of interstitial space available for organismal colonization. This is apparent in our data, as high-relief biomass was more than five times greater than low-relief biomass within the first month following reef construction, despite just a 15 cm difference in vertical relief. Over time, as some oysters grew in low-relief areas, the negative impacts of sedimentation appeared to decrease, resulting in the gradual convergence in biomass values that we observed.

The results of this study emphasize the importance of incorporating even small increases in vertical relief into the design of future oyster restoration projects. Although flat, 2-dimensional restored reefs have been shown to increase the abundance of macroinvertebrates and small fishes when compared to unstructured (i.e., non-reef) habitats (Plunket and La Peyre 2005), studies like ours that directly compare high- and low-relief habitats typically show an increased response with greater vertical relief (Gratwicke and Speight 2005; Harding and Mann 2001). Colden et al. (2017) found that reefs with an elevation of 30 cm or greater, the height of our high-relief treatment, had oyster densities that were 3.5 times greater than reefs below that height threshold. Whereas high-relief restoration reefs may become permanent, low-relief reefs are less likely to persist over time due to burial by sediments and insufficient oyster accretion rates (Colden et al., 2017; Schulte et al., 2009; Taylor and Bushek 2008).

Overall, our findings illustrate a relatively rapid convergence in motile benthic community structure between restored and natural oyster reefs. From the perspective of motile oyster-associated organisms, this restoration project appears to have successfully achieved the pre-construction goal of creating a self-sustaining oyster reef with a similar structure and function to a natural reef through the addition of carbonate-based material to a substrate-limited section of the estuary. Although healthy motile benthic communities only represent one component of the ecological success of a large-scale oyster restoration project, these findings are of broad importance, as they illustrate how quickly food web components and habitat provisioning can be restored through restoration efforts. The recovery of ecosystem services represents a rapid ecological and economic return on the initial investment made to create the restoration reef.

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Table 1 Relative gravimetric abundance of motile benthic organisms collected in sampling trays at natural (upstream, midstream, downstream) and restored oyster reefs in the Loxahatchee River (Florida, USA). Taxa are arranged by total overall gravimetric abundance (natural and restored sites combined). The Restored Reef column includes all organisms collected during bimonthly sampling following reef construction, as well as the high/low-relief time series. Asterisks (*) indicate taxa that were identified only at the restored oyster reef. NP = not present.

Taxon	**Common Name**	**Natural Reef (Total) % by biomass**	**Natural Reef (Up) % by biomass**	**Natural Reef (Mid) % by biomass**	**Natural Reef (Down) % by biomass**	**Restored Reef (Total) % by biomass**
Panopeus herbstii	black-fingered mud crab	24.49	8.53	23.97	39.94	20.26
Eurypanopeus depressus	depressed mud crab	16.42	25.32	19.27	5.15	18.65
Lophogobius cyprinoides	crested goby	15.86	24.54	18.43	5.08	2.76
Panopeidae spp.	mud crab (< 9 mm)	13.24	15.34	14.46	10.11	11.22
Alpheus spp.	snapping shrimp	8.91	8.36	6.15	12.30	14.86
Petrolisthes armatus	green porcelain crab	7.53	1.21	6.28	14.73	8.63
Bathygobius soporator	frillfin goby	5.40	6.29	7.01	2.88	1.58
Nassarius vibex	bruised nassa snail	2.35	NP	NP	6.99	0.71
Lupinoblennius nicholsi	highfin blenny	1.38	3.55	0.57	0.19	NP
Portunus spp.	swimming crab	0.24	0.52	0.01	0.22	6.44
Gobiosoma bosc	naked goby	0.59	0.60	0.32	0.88	3.85
Menippe mercenaria	Florida stone crab	NP	NP	NP	NP	*6.79
Lutjanus griseus	gray snapper	0.77	1.44	0.43	0.51	0.39
Neritina clenchi	Clench's nerite snail	0.64	2.00	0.01	0.04	0.06
Palaemonetes spp.	grass shrimp	0.16	0.01	0.06	0.40	0.97
Pachygrapsus transversus	mottled shore crab	0.24	0.40	0.32	NP	0.18
Archosargus probatocephalus	sheepshead	0.20	0.63	NP	NP	NP
Erotelis smaragdus	emerald sleeper	0.17	NP	0.48	0.01	NP
Hypleurochilus aequipinnis	oyster blenny	0.01	NP	0.03	0.02	0.71
Stramonita haemastoma	Florida rock shell	NP	NP	NP	NP	*0.68
Epinephelus itajara	goliath grouper	0.10	NP	0.29	NP	NP
Mithrax spp.	clinging crab	NP	NP	NP	NP	*0.45
Farfantepenaeus aztecus	brown shrimp	0.04	0.04	0.06	0.03	0.09
Libinia spp.	spider crab	0.02	NP	0.05	0.01	0.12
Lutjanus synagris	lane snapper	NP	NP	NP	NP	*0.23
Upogebia spp.	mud shrimp	0.02	NP	NP	0.05	0.04
Haemulon spp.	grunt	0.02	NP	NP	0.06	NP
Mercenaria spp.	hard clam	0.02	0.06	NP	NP	NP
Callinectes sapidus	blue crab	0.01	0.01	0.01	NP	0.01
Tagelus spp.	razor clam	0.01	0.01	NP	0.01	NP
Clibanarius vittatus	striped hermit crab	0.01	NP	NP	0.01	0.01
Lysmata wurdemanni	peppermint shrimp	0.01	0.01	NP	0.01	NP
Gobiesox strumosus	skilletfish	0.01	NP	NP	0.01	NP
Eucinostomus sp.	mojarra	NP	NP	NP	NP	*0.01
Malacoctenus macropus	rosy blenny	NP	NP	NP	NP	*0.02
Alpheus formosus	striped snapping shrimp	NP	NP	NP	NP	*0.01
Hypsoblennius ionthas	freckled blenny	NP	NP	NP	NP	*0.01
Syngnathus spp.	pipefish	NP	NP	NP	NP	*0.01
Isopoda spp.	isopod	0.01	NP	NP	0.01	NP
Pinnixa spp.	pea crab	NP	NP	NP	NP	*0.01

Table 2 Relative numerical abundance of motile benthic organisms collected in sampling trays at natural (upstream, midstream, downstream) and restored oyster reefs in the Loxahatchee River (Florida, USA). Taxa are arranged by total overall numerical abundance (natural and restored sites combined). The Restored Reef column includes all organisms collected during bimonthly sampling following reef construction, as well as the high/low-relief time series. Asterisks (*) indicate taxa that were identified only at the restored oyster reef. NP = not present.

Taxon	**Common Name**	**Natural Reef (Total) % abundance**	**Natural Reef (Up) % abundance**	**Natural Reef (Mid) % abundance**	**Natural Reef (Down) % abundance**	**Restored Reef (Total) % abundance**
Panopeidae spp.	mud crab (< 9 mm)	41.21	50.78	44.96	30.03	36.12
Alpheus spp.	snapping shrimp	14.32	8.31	12.09	21.23	24.13
Petrolisthes armatus	green porcelain crab	13.58	3.88	10.41	24.41	7.37
Eurypanopeus depressus	depressed mud crab	11.09	15.84	15.35	3.65	9.68
Lophogobius cyprinoides	crested goby	6.71	11.58	6.75	2.49	0.62
Panopeus herbstii	black-fingered mud crab	3.53	1.81	4.35	4.35	4.00
Gobiosoma bosc	naked goby	1.53	1.30	0.65	2.43	7.90
Palaemonetes spp.	grass shrimp	1.68	0.02	0.69	3.88	7.07
Nassarius vibex	bruised nassa snail	2.06	NP	NP	5.44	0.37
Bathygobius soporator	frillfin goby	1.44	1.41	1.82	1.16	0.40
Lupinoblennius nicholsi	highfin blenny	0.94	2.44	0.27	0.18	NP
Neritina clenchi	Clench's nerite snail	0.41	1.18	0.01	0.07	0.04
Pachygrapsus transversus	mottled shore crab	0.29	0.42	0.51	NP	0.09
Portunus spp.	swimming crab	0.04	0.06	0.01	0.04	0.74
Farfantepenaeus aztecus	brown shrimp	0.10	0.02	0.17	0.11	0.04
Mithrax spp.	clinging crab	NP	NP	NP	NP	*0.40
Menippe mercenaria	Florida stone crab	NP	NP	NP	NP	*0.36
Libinia spp.	spider crab	0.04	NP	0.05	0.08	0.13
Lutjanus griseus	gray snapper	0.07	0.05	0.06	0.09	0.04
Hypleurochilus aequipinnis	oyster blenny	0.01	NP	0.02	0.02	0.21
Upogebia spp.	mud shrimp	0.03	NP	NP	0.07	0.07
Erotelis smaragdus	emerald sleeper	0.04	NP	0.11	0.01	NP
Mercenaria spp.	hard clam	0.02	0.06	NP	NP	NP
Tagelus spp.	razor clam	0.01	0.02	NP	0.02	NP
Callinectes sapidus	blue crab	0.01	0.01	0.02	NP	0.01
Lysmata wurdemanni	peppermint shrimp	0.01	0.01	NP	0.02	NP
Isopoda spp.	isopod	0.01	NP	NP	0.03	NP
Stramonita haemastoma	Florida rock shell	NP	NP	NP	NP	*0.04
Archosargus probatocephalus	sheepshead	0.01	0.02	NP	NP	NP
Alpheus formosus	striped snapping shrimp	NP	NP	NP	NP	*0.03
Pinnixa spp.	pea crab	NP	NP	NP	NP	*0.03
Clibanarius vittatus	striped hermit crab	0.01	NP	NP	0.01	0.01
Epinephelus itajara	goliath grouper	0.01	NP	0.01	NP	NP
Gobiesox strumosus	skilletfish	0.01	NP	NP	0.01	NP
Haemulon spp.	grunt	0.01	NP	NP	0.01	NP
Eucinostomus sp.	mojarra	NP	NP	NP	NP	*0.01
Hypsoblennius ionthas	freckled blenny	NP	NP	NP	NP	*0.01
Lutjanus synagris	lane snapper	NP	NP	NP	NP	*0.01
Malacoctenus macropus	rosy blenny	NP	NP	NP	NP	*0.01
Syngnathus spp.	pipefish	NP	NP	NP	NP	*0.01

Table 3 Spatial variation in mean biomass of motile benthic oyster reef-associated fauna at three natural reef sites along an upstream-to-downstream gradient (mean ± standard deviation). Overall mean biomass includes all sampling dates. Annual maximum biomass is the mean of each year's maximum biomass value, which typically occurred at the end of the dry season or the beginning of the wet season. Annual minimum biomass is the mean of each year's minimum biomass value, which usually occurred near the beginning of the dry season. Capital letters in parenthesis represent the results of Tukey HSD post-hoc tests comparing overall mean biomass at the three sites, where different letters indicate significantly different overall mean biomass values at $p < 0.05$.

Site	**Overall Mean Biomass (g/m^2)**	**Annual Maximum Biomass (g/m^2)**	**Annual Minimum Biomass (g/m^2)**
Upstream	79 ± 26 $^{(A)}$	108 ± 22	50 ± 20
Midstream	92 ± 27 $^{(A)}$	129 ± 31	62 ± 14
Downstream	114 ± 42 $^{(B)}$	171 ± 75	82 ± 11

Figure Captions

Fig. 1. Map of the Loxahatchee River estuary (Jupiter, Florida, USA), showing the location of the upstream (Up), midstream (Mid), and downstream (Down) natural reef reference sites, as well as the oyster restoration reef (Rest).

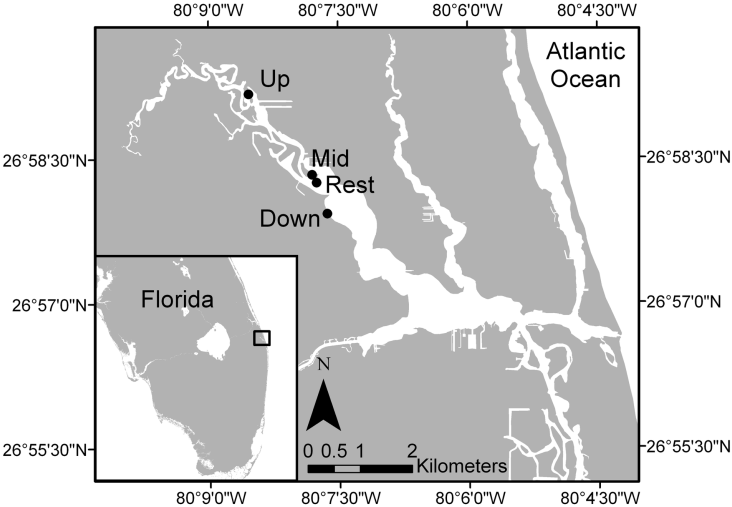
Fig. 2. Non-metric multidimensional scaling (NMDS) ordination showing relative similarity/dissimilarity between natural (Up = upstream reference site, Mid = midstream reference site, and Down = downstream reference site) and restored (Pre = pre-restoration, Rest = post-restoration) motile oyster reef communities. Each data point represents a single sampling date at a single site (mean of four trays). The relative proximity of two points to one another in the NMDS ordination reflects the relative similarity of the communities represented by those points (i.e., closer points indicate more similar communities). Natural reference reef data were collected from May 2007 to May 2012. Pre-restoration data were collected in March and May 2010, and post-restoration data were collected from September 2010 to May 2012.

Fig. 3. Changes in biomass of motile oyster reef-associated organisms following oyster reef restoration. Dashed black lines represent biomass at the restoration reef site, before reef construction (first two data points, March and May 2010), and after reef construction (all points after July 2010). Biomass at three natural reef reference sites is represented by black (upstream site), dark gray (midstream site), and light gray

(downstream site) solid lines. The asterisk (*) is the date of restoration reef construction. Error bars have been omitted for simplicity.

Fig. 4. Biomass of oyster reef-associated organisms at high- and low-relief experimental plots during the first 16 months following restoration. Mean biomass at high-relief plots was significantly greater than at low-relief plots, and there was a significant effect of time since construction on biomass values for both levels of vertical relief. We observed a significant interaction between the effects of relief and time since construction on the biomass of benthic organisms. Error bars = standard deviation.

Fig. 5. Representative examples of benthic sampling trays from low-relief (left) and high-relief (right) plots at the end of the habitat complexity experiment, 485 days after initial deployment. At this time, high-relief treatments had more than twice as many live oysters per m^2 as low-relief treatments (420 ± 100 vs. 206 ± 114 oysters per m^2; mean ± SD). Surface rugosity was significantly greater for the high-relief treatments than for the low-relief treatments (1.64 ± 0.15 vs. 1.20 ± 0.13; $t_5 = 4.66$, $p = 0.006$).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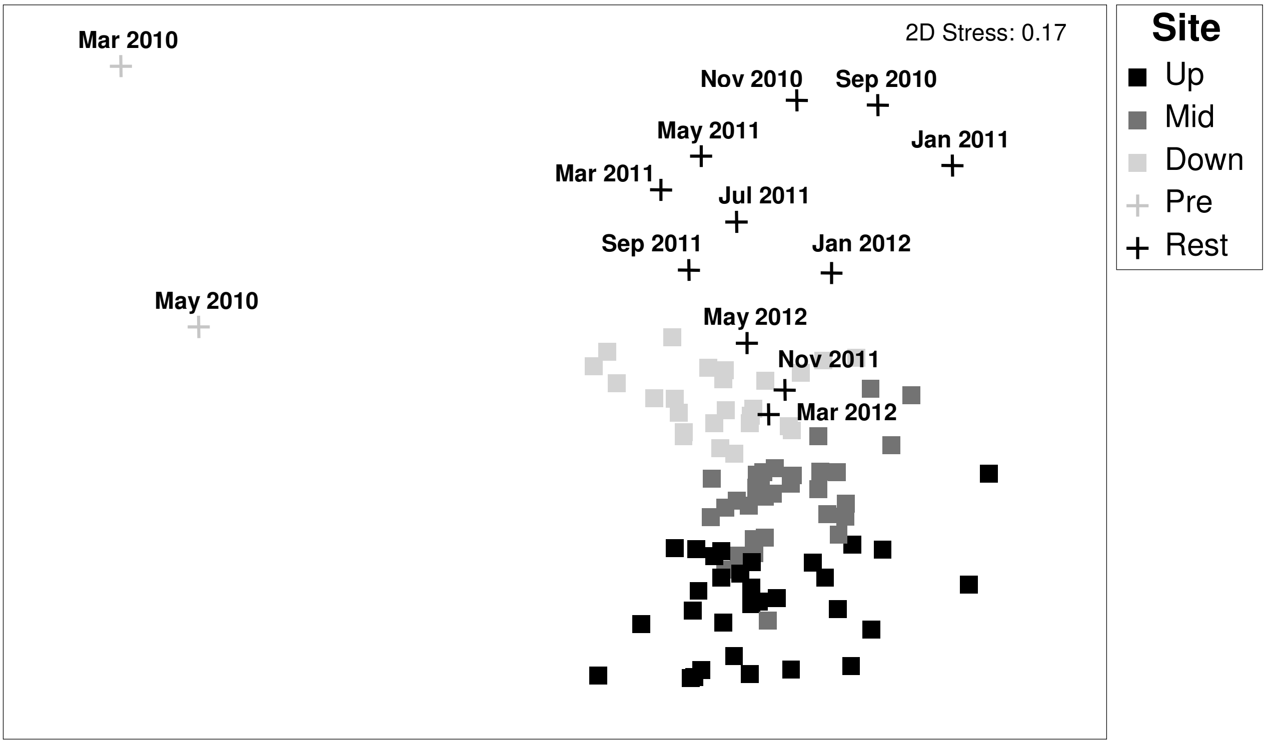
2D Stress: 0.17
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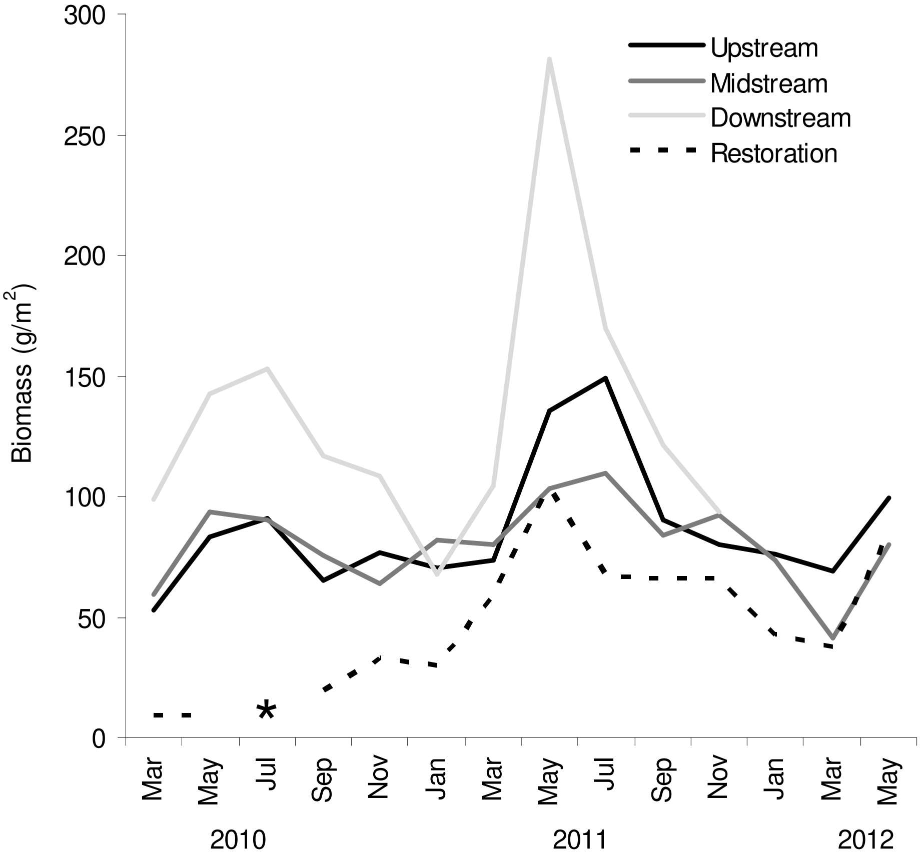
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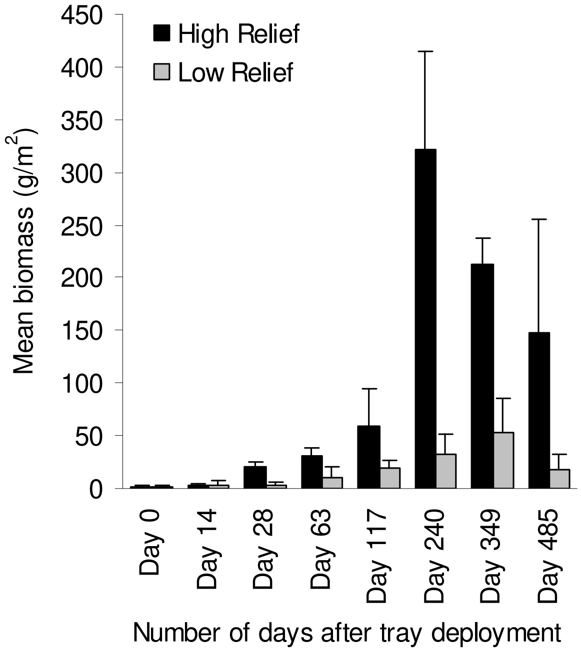

High Relief
Low Relief
Mean biomass (g/m²)
0
50
100
150
200
250
300
350
400
450
Day 0
Day 14
Day 28
Day 63
Day 117
Day 240
Day 349
Day 485
Number of days after tray deployment


Low-relief treatment
High-relief treatment