RESEARCH ARTICLE

Oyster Reef Restoration in the Northern Gulf of Mexico: Effect of Artificial Substrate and Age on Nekton and Benthic Macroinvertebrate Assemblage Use

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Abstract

In the northern Gulf of Mexico (GOM), reefs built by eastern oysters, *Crassostrea virginica*, provide critical habitat within shallow estuaries, and recent efforts have focused on restoring reefs to benefit nekton and benthic macroinvertebrates. We compared nekton and benthic macroinvertebrate assemblages at historic, newly created (<5 years) and old (>6 years) shell and rock substrate reefs. Using crab traps, gill-nets, otter trawls, cast nets, and benthic macroinvertebrate collectors, 20 shallow reefs (<5 m) in the northern GOM were sampled throughout the summer of 2011. We compared nekton and benthic assemblage abundance, diversity and composition across reef types. Except for benthic macroinvertebrate abundance, which was significantly higher on old rock reefs as compared to historic reefs, all reefs were similar to historic reefs, suggesting created reefs provide similar support of nekton and benthic assemblages as historic reefs. To determine refuge value of oyster structure for benthic macroinvertebrates compared to bare bottom, we tested preferences of juvenile crabs across depth and refuge complexity in the presence and absence of adult blue crabs (*Callinectes sapidus*). Juveniles were more likely to use deep water with predators present only when provided oyster structure. Provision of structural material to support and sustain development of benthic and mobile reef communities may be the most important factor in determining reef value to these assemblages, with biophysical characteristics related to reef location influencing assemblage patterns in areas with structure; if so, appropriately locating created reefs is critical.

Key words: complexity, habitat, oyster, refuge, restoration, structure.

Introduction

Structure adds complexity to habitats and increases species richness and abundance (MacArthur & MacArthur 1961; Gratwicke & Speight 2005). Richness and abundance are thought to increase because of reduced predation (Grabowski et al. 2008), increased refuge value for smaller organisms (Dittel et al. 1996), greater larval retention (Tegner & Dayton 1981), as well as a greater number of foraging sites, thus reducing competition (MacArthur 1958). In marine systems, structure provided by submerged aquatic vegetation (SAV), coral reefs, and oyster reefs is also associated with greater species richness and abundance (Heck & Whetstone 1977; Harding & Mann 2001; Munday 2004).

Structural habitat loss due to anthropogenic factors currently threatens all marine ecosystems. For example, coral reefs face coral bleaching and increased sedimentation rates, and have decreased by over 80% in some areas (Gardner et al. 2003), while seagrass beds are negatively impacted by boat propellers and land-based pollution (Zieman 1976). Oyster reefs also have suffered an estimated 85% decrease in area from historical abundance due to overharvest, disease, and dredging (Beck et al. 2011).

Along the northern Gulf of Mexico (GOM), oyster reefs often provide the only natural hard substrate and three-dimensional structure in estuaries, and loss of this structure likely decreases biodiversity (Lotze et al. 2006; Airoldi et al. 2008). Numerous benthic macroinvertebrate species use structure provided by oyster reefs, whether the reef has live oysters or consists of dead shell (Tolley & Volety 2005). In addition, reefs may provide increased shoreline protection (Piazza et al. 2005; Scyphers et al. 2011), nutrient filtration, de-nitrification

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(Kellogg et al. 2013), and carbon sequestration (Grabowski & Peterson 2007) with their potential ecosystem services recently valued at over $5,500 per ha/year (Grabowski et al. 2012). Recognition of the recent decline of oyster reefs has resulted in extensive restoration efforts in many regions (Coen & Luckenbach 2000; Beck et al. 2011).

Restoration of oyster reefs has become a best management practice (BMP) for states along the northern GOM, as well as the eastern Atlantic coast. In the northern GOM alone, over 400 artificial oyster reefs have been created since 1990 (Furlong 2012). Reefs were initially created to restore oyster fisheries, but more recently have been created to restore multiple lost ecosystem services (Grabowski & Peterson 2007). Salinity, reef height, and dissolved oxygen all have significant effects on the survival of oyster reefs (Lenihan 1999), and may also significantly affect recruitment of oysters to created reefs (Grabowski et al. 2005; Luckenbach et al. 2005). Since oysters are gregarious, the recruitment of juvenile oysters further adds to the structural complexity and growth of the reef.

It is unclear how differences in restoration techniques and substrate materials contribute to reef structural differences, either immediately after reef creation or as reefs develop. If oyster reef restoration along the northern GOM is to enhance nekton assemblages, better understanding how nekton recruit to, and use these created reefs is critical. Understanding the effects of initial reef substrate on nekton use of the reef over the short-term (<5 years post-construction), and whether the effects of different initial substrates are minimized over the long-term (>6 years old) would provide valuable information. We compared nekton and benthic macroinvertebrate assemblages at new and old reefs created with oyster shell and rock, the two dominant materials used to construct reefs in this region (Furlong 2012), to those found on historical reefs. As an additional way to understand the role of reef structure, we conducted a laboratory experiment to examine the hypothesis that more complex reef habitat provides preferred refuge for an important reef resident (juvenile blue crabs, *Callinectes sapidus*).

Methods

Field Study: Nekton and Benthic Macroinvertebrate Artificial Reef Use

Study Area. To determine how artificial oyster reef construction materials affect nekton and benthic macroinvertebrate use over time, 20 reefs were chosen spanning the northern GOM, from Copano Bay, Texas, to Apalachicola Bay, Florida (Fig. 1). The northern GOM is micro-tidal (<0.5 m) and is dominated by shallow estuarine waters. The eastern oyster, *Crassostrea virginica*, is the dominant reef building organism in the region, and occurs both inter- and sub-tidally, although reefs have limited vertical structure because of the small tidal range.
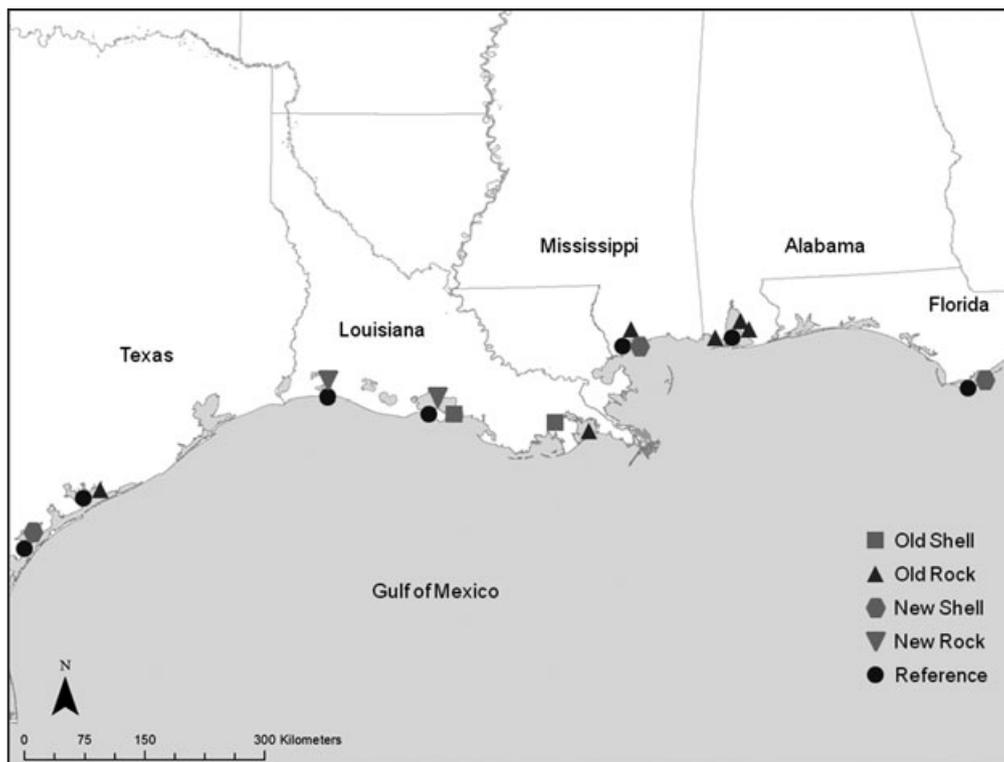

Figure 1. Locations of the 20 oyster reefs sampled along the northern Gulf of Mexico, with 6 old rock (OR) reefs, 2 new rock (NR) reefs, 2 old shell (OS) reefs, 3 new shell (NS) reefs, and 7 reference reefs sampled throughout the summer of 2011. Rock refers to reefs created with limestone or concrete bases; shell refers to reefs created with oyster shell. New refers to reefs created after 2006; old refers to reefs created before 2005.

Sampling Design. Thirteen created reefs were selected for sampling. On the basis of the base construction material, reefs were classified as rock (limestone or concrete), or shell (oyster shell), and as new (built after 2006) or old (built before 2006). Seven nearby historic (reference) reefs were also selected. Sample sizes were not distributed evenly among groups as reef availability was limited: old rock reefs (OR; $n = 6$), new rock reefs (NR; $n = 2$), old shell reefs (OS; $n = 2$), new shell reefs (NS; $n = 3$), and reference reefs (RF; $n = 7$). Reefs were subtidal, with an average depth of 2.1 ± 0.6 m, and a minimum distance of 100 m offshore. Reefs selected were created for conservation purposes only.

Water Quality and Habitat Variables. Salinity, dissolved oxygen (mg/L), and temperature (°C) were measured monthly from May to August 2011 with a YSI 85 meter, and water clarity (Secchi depth, cm) was recorded during each site visit. SCUBA divers sampled oyster densities at each site once in October of 2011. Five 0.25 m^2 quadrats were haphazardly thrown at each site and divers collected samples by removing the top 10 cm of reef substrate in each quadrat. All materials were placed in labeled buckets, kept on ice, and returned to the laboratory. Materials were sorted into live oysters, shell (hash, dead oysters), other attached organisms (mussels, clams, barnacles), and rock (concrete, gravel). Number of live oysters and number of live mussels and clams were counted. Volume of live oysters, shell, other organisms, and rock were measured by water displacement (L).

Nekton Sampling. Nekton assemblages were sampled at each reef with a gill-net, cast net, otter trawl, and crab pots. Reefs were sampled once a month in May, June, July, and August by gill net, cast net, and otter trawl, and once in May and once in July with crab pots. For each sample event, two experimental gill-nets (10 m long $\times$ 1 m high; 7.6, 10.2, 12.7, and 15.2 mm mesh) were fished perpendicular to the dominant wind or current at each reef. After 1 hour, fishes were collected, identified to species, then weighed (g), measured (TL, cm), and released (4 sample dates $\times$ 2 gill nets $\times$ 20 reefs = 160). A cast net (radius of 2.44 m; 3 mm) was thrown seven times over the reef. All organisms captured were placed on ice and returned to the laboratory for identification, and length (TL, cm) and weight (g) measurements (4 sample dates $\times$ 7 casts $\times$ 20 reefs = 560). Samples from two 1-minute otter trawl (mouth opening of 2.44 m; 5.2 mm; 5 knots) pulls along the reef edge were placed on ice and returned to the laboratory for identification, and total length and weight measurements (4 sample dates $\times$ 2 trawls $\times$ 20 reefs = 160). For all gear types, species richness, catch-per-unit-effort (CPUE), and Shannon's Index of diversity were calculated. To determine adult blue crab abundances at each site, two baited commercial crab traps were soaked overnight, and CPUE recorded. Crab pots were set overnight in May and July 2011 (2 sample dates $\times$ 2 pots $\times$ 20 sites = 80).

Benthic Macroinvertebrate Assemblage Sampling. To sample benthic macroinvertebrate assemblages, 30 cm $\times$ 30 cm bags constructed from 2 cm Vexar mesh were filled with clean, unbleached oyster shell and attached to a cinderblock anchor. Two bags were deployed at each site in May and July (2 sample dates $\times$ 2 bags $\times$ 20 reefs = 80). After 1 month, bags were retrieved and placed in a plastic tub to minimize escape. Samples were kept on ice until returned to the laboratory where oyster shells were washed over a 1-mm mesh sieve, and all captured organisms placed in 80% ethanol and identified to the lowest practical taxon. For each site, species richness, total abundance, and Shannon's index of diversity were calculated.

Statistical Analyses

Water quality, total substrate volume (L), and live oyster density were analyzed using a one-factor ANOVA (factor: reef type), blocking on bay. The model used was a generalized linear mixed model in SAS 9.2 (Proc Glimmix, SAS, Cary, NC, U.S.A.) with a binomial, negative binomial, Poisson, or normal distribution determined by the lowest AIC score. Significant differences were examined using Tukey's a posteriori test.

Nekton and benthic macroinvertebrate abundance, richness, and diversity were analyzed separately by gear type with a one-factor ANCOVA (factors: reef type; covariate: salinity) using a generalized linear mixed model in SAS 9.2 (Proc Glimmix) with a binomial, negative binomial, Poisson, or normal distribution determined by the lowest AIC score. Factors of month, water depth, temperature, and bay were examined, but found not to be significant and not explored further in the models. Significant differences were examined using Tukey's a posteriori test. Results were considered statistically significant at $\alpha \leq 0.05$.

Non-metric multidimensional scaling (nMDS; metaMDS in the Vegan package of R 2.13.1) was used to examine patterns between nekton (castnet, trawl, and gill-net combined) and benthic macroinvertebrate abundances and habitat variables (dissolved oxygen, temperature, salinity, Secchi depth, total structure volume (L), and oyster density). The nMDS measured dissimilarity of samples using the Bray–Curtis distance measure, while ordination of habitat variables were fit using the ENVFIT function in the Vegan package.

Laboratory Experiment: Benthic Macroinvertebrate Refuge Choice Experiment

Juvenile and adult *Callinectes sapidus* and were collected from Grand Isle, Louisiana and brought back to the laboratory. All crabs were maintained in aquaria with undergravel filters at a salinity of 15 and temperature of 29°C throughout the experiment and fed every 3 days for 1 week to allow for acclimation.

To provide a choice of water depths, each tank had three water depths (7, 20, and 42 cm), as in Dittel et al. (1995). To vary oyster reef complexity, one of three treatments was constructed in the deep end of each tank: (1) no oyster shell, (2) low oyster shell = 10 cm relief, and (3) high oyster shell = 40 cm relief. Treatment 1 tested if juvenile crabs

Table 1. Water quality data including salinity, dissolved oxygen, temperature, and Secchi depth presented as a mean $\pm$ SE (range) for all treatments during sampling trips from May through October 2011.

Category	Salinity	DO (mg/L)	Temperature (°C)	Secchi Depth (cm)
Reference	17.7 ± 1.0 (0.6–31.2)	6.5 ± 0.1 (4.6–9.0)	28.9 ± 0.4	50 ± 3 (1–117)
Old rock	17.2 ± 2.7 (2.7–31.7)	6.9 ± 0.2 (4.5–8.8)	29.3 ± 0.4	63 ± 3 (36–109)
New rock	13.9 ± 2.3 (2.1–29.5)	7.4 ± 0.2 (5.4–8.9)	29.7 ± 0.9	36 ± 3 (12–61)
Old shell	8.2 ± 1.6 (0.2–16.0)	6.4 ± 0.3 (4.8–7.7)	30.7 ± 0.4	33 ± 4 (9–57)
New shell	18.6 ± 1.7 (9.5–29.7)	6.8 ± 0.2 (5.5–9.5)	28.5 ± 0.7	70 ± 5 (30–113)

prefer deep water or shallow water as a refuge. Treatment 2 mimicked a reduced oyster reef in nature (shallow water versus a low complexity deep refuge). Treatment 3 mimicked a high complexity, healthy oyster reef (shallow water vs. high complexity deep refuge). With only two identical tanks available (1.25 m × 0.5 m × 0.45 m), treatments were randomized and conducted in that random order during July 2011.

Experimental trials were conducted over 48 hour periods. At time 0, a juvenile *C. sapidus* (mean carapace width = 2.7 ± 0.3 cm) was placed in the medium depth, and allowed to acclimate for 24 hours. At 24 hours, the position of the crab was marked, and an adult *C. sapidus* (mean carapace width = 10.7 ± 0.8 cm) was placed in the deep end of the tank. After another 24 hours, juvenile and adult crab positions were noted. Each treatment was replicated four times with new crabs for each trial (n = 12; trials total). A chi-square test (SAS 9.2) tested if the juvenile *C. sapidus* moved from deeper water to a shallower depth with the addition of the predator.

Results

Field Study: Nekton and Benthic Macroinvertebrate Artificial Reef Use

Water Quality and Habitat Variables. Temperature was similar among all sites, while salinity (p = 0.02), Secchi depth (p = 0.0004), and dissolved oxygen (p = 0.04) all differed among sites (Table 1). Reference (RF) reefs had higher salinities than OS reefs, with other reef sites not differing significantly from one another or from RF or OS reefs. Total substrate volume (L) was significantly correlated with shell and other organisms so only total substrate volume and rock were analyzed by reef category. There was a significant difference in total volume of structure among reef categories (p = 0.0004) with OR reefs having higher volumes of structure than NS reefs and NR, RF, and OS not having significantly different volumes from one another, or from OR and NS reefs (Fig. 2a). Rock also differed significantly with NR and OR reefs having similar and significantly greater volumes of rock material as compared to OS (p < 0.0001) (Fig. 2b). No rock material was collected off of RF or NS reefs. Live oyster density and other shellfish density (clams, mussels), were significantly and positively correlated, so only live oyster density data are presented. There was a significant difference in live oyster density with RF, OR, and NR reefs having greater density as compared to NS and OS reefs (p < 0.0001; Fig. 2c).
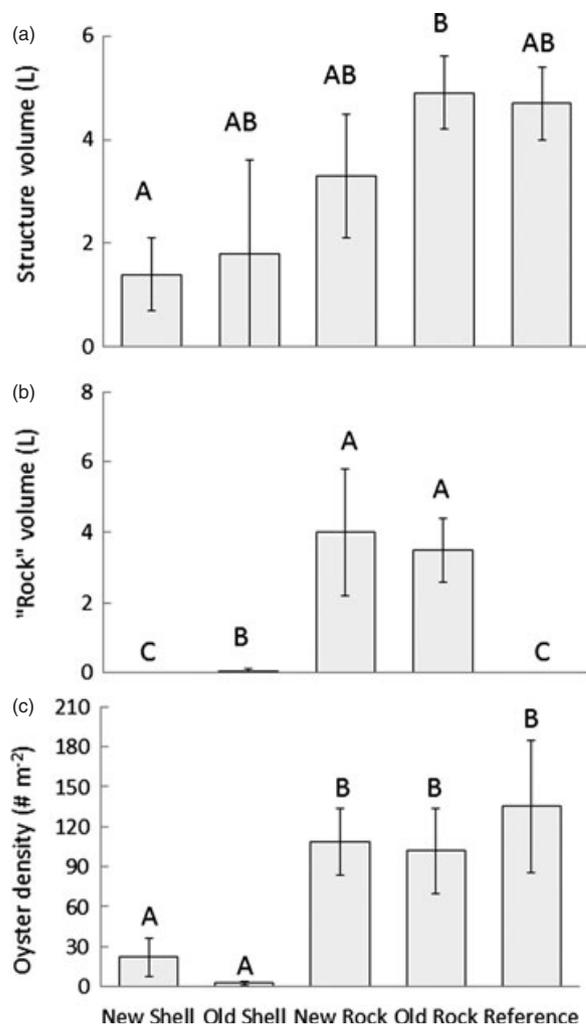

Figure 2. Mean structure (± SE liters) of material (live oysters, shell, rock) (a), mean rock volume (± SE liters) (b) and mean oyster density (number per m²) (c) sampled on reference and artificial (i.e. new and old shell and rock) reefs in the northern Gulf of Mexico. Letters above histograms indicate significant differences in volume of structure (p < 0.05).

Nekton Sampling. A total of 2,081 nekton (42 species) were collected in all samples. Gill-net catches included 217 individuals of 24 species. Trawl catches included 815 individuals of 27 species. Cast net catches included 1,055 individuals of 19 species (Table 2). There was no significant difference in CPUE, richness or diversity by reef type within gillnet or castnet

Table 2. Nekton species list with Latin names, type of gear caught in (O, otter trawl; G, gill net; C, cast net) and presence or absence on a reef category.

Latin Name	Gear Type	Reference	Old Rock	New Rock	Old Shell	New Shell
Achirus lineatus	O			X		
Anchoa mitchilli	OC	X	X	X	X	X
Arius felis	OGC	X	X	X	X	X
Bagre marinus	OGC	X	X	X	X	X
Bairdiella chrysoura	OC	X	X			
Bothus robinsi	O	X				
Brevoortia patronus	OGC	X	X	X	X	X
Callinectes sapidus	OG	X	X	X		
Carcharhinus leucas	G	X	X	X	X	
Chaetodipterus faber	OG	X		X		
Chloroscombrus chrysurus	OC	X				
Citharichthys spilopterus	O	X				
Cynoscion arenarius	OG	X	X	X	X	
Cynoscion nebulosus	OG	X	X	X	X	X
Dasyatis americana	G	X				
Dorosoma cepedianum	G	X				X
Elops saurus	G		X			X
Etropus crossotus	O	X				
Farfantepenaeus aztecus	OGC	X	X	X	X	X
Hexapanopeus paulensis	C	X	X	X	X	X
Lagodon rhomboides	OC	X		X		
Leiostomus xanthurus	OC				X	
Lironeca ovalis	OC			X		
Litopenaeus setiferus	OC	X		X	X	
Menidia beryllina	OC		X	X		X
Micropogonias undulatus	OGC	X	X	X	X	
Mugil cephalus	OGC		X	X	X	X
Palaemonetes pugio	OC	X	X	X		
Panopeus herbstii	O	X				
Paralichthys lethostigma	OG	X	X	X		
Peprilus alepidotus	G		X			
Pogonias cromis	GC	X	X			
Pomatomus saltatrix	G	X				
Rhinoptera bonasus	G	X				
Rhizoprionodon terraenovae	G	X	X			
Scomberomorus regalis	G		X			
Selene setapinnis	G					X
Sphyrna tiburo	G	X				
Symphurus plagiusa	OC	X		X		
Trachinotus carolinus	G		X			

X indicates occurrence within a particular reef type.

samples, even when corrected for salinity. Trawl catches revealed a significant reef effect on diversity ($p = 0.002$) and richness ($p = 0.002$), with significantly higher diversity and richness on NR reefs as compared to OR and NS. OR and NS had similar diversity and OS and RF had similar diversity and richness to all reef types (Fig. 3). Total abundance of trawl catches, however, did not vary among reef categories. There were no differences in *Callinectes sapidus* CPUE among reef categories. The nMDS of nekton was not significant (stress $= 0.22$), and reef categories did not separate based on nekton species.

Benthic Macroinvertebrate Assemblage Sampling. A total of 15,205 individuals of 39 species were collected using shell bags. The highest proportion of organisms collected were of the Xanthidae family ($n = 3,338$) (Table 3). Benthic macroinvertebrate diversity differed significantly by reef category ($p = 0.01$), with NR and RF reefs having greater diversity as compared to OS, and with NS and OR reefs not differing significantly from any other category (Fig. 4a). Total abundance also differed significantly by reef category ($p < 0.001$) with OR reefs having significantly higher abundances as compared to all reefs except for NS, which did not differ significantly from reference reefs (Fig. 4b). OS and NR reefs supported lowest total abundance of all reef categories, but were not significantly different than reference reefs. Reef category had no effect on species richness, although salinity was significant as a covariate ($p < 0.0001$). The nMDS analysis indicated differences in assemblages (stress $= 0.17$) with salinity, Secchi depth, and temperature being significant environmental drivers ($p = 0.0001$, $p = 0.02$, and $p = 0.02$, respectively).
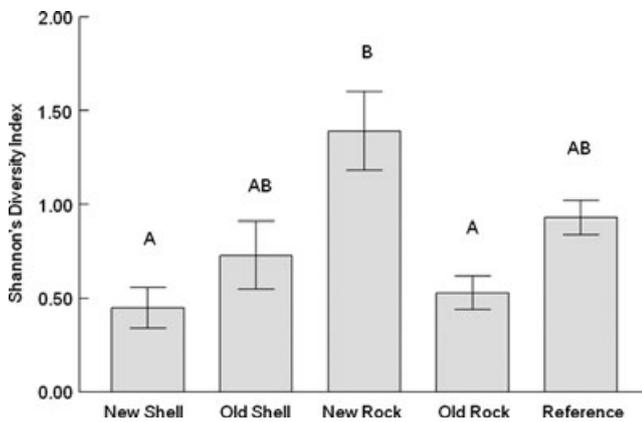
Figure 3. Mean Shannon's diversity index values (± SE) for nekton collected with trawl nets on artificial (i.e. new and old shell and rock) and reference reefs. Letters above histograms indicate significant differences in diversity ($p < 0.05$).

Laboratory Experiment: Benthic Macroinvertebrate Refuge Choice Experiment

There was a significant effect of structure on water depth selected by juvenile *C. sapidus* in the presence of predators ($p = 0.01$). In high structure reef treatments, juveniles remained in deeper water when predators were added. In low structure reefs, juveniles moved to shallower water in half of the trials. In trials where there was no structure, juveniles always moved to shallower water with addition of the predator (Fig. 5). For all trials, adult crabs were always found in deep water.

Discussion

Created reefs provided similar support for nekton and benthic macroinvertebrate assemblages as historic (reference) reefs regardless of reef construction materials or age. There were, however, subtle differences in nekton and benthic macroinvertebrate assemblages among several of the created reef categories but these differences were not related to structural volume or live oyster density. Provision of structural material to support and sustain development of reef communities may be the most important factor in determining reef value to nekton and benthic macroinvertebrate assemblages. Biophysical characteristics related to reef location or unmeasured complexity variables may, however, influence long-term sustainability of the reef, and ultimately assemblage patterns; if so, appropriately locating created reefs is of utmost priority.

Numerous studies indicate that structure increases species abundance and diversity in marine systems (Orth & Heck 1980; Diehl 1988; Wyda et al. 2002) but determining accurate measures of structure, and structural complexity has proven difficult (Bartholomew et al. 2000; Humphries et al. 2011). In this study, all the created reefs supported nekton and benthic macroinvertebrate assemblages similar to reference reefs, with one exception of old rock reefs which supported a greater abundance of benthic macroinvertebrate species. It is possible

Table 3. Benthic macrofaunal species list with Latin names and presence or absence on a reef category.

Latin Name	Reference	Old Rock	New Rock	Old Shell	New Shell
Acteocina canaliculata	X	X			X
Alpheus heterochaelis	X	X	X		X
Amphipoda	X	X	X	X	X
Anadara ovalis	X	X			X
Callinectes sapidus	X	X	X	X	X
Chasmodes bosquianus	X	X	X		X
Eurypanopeus depressus	X	X	X	X	X
Farfantepenaeus aztecus	X	X	X	X	X
Geukensia demissa	X	X	X	X	X
Gobiesox strumosus	X	X	X		X
Gobiosoma	X	X			X
Harpacticoida					X
Hydrobiidae		X			
Ilynassa obsoleta	X	X	X		X
Libinia dubia	X	X			
Litopenaeus setiferus	X	X	X	X	
Macoma mitchelli	X	X	X		
Menippe adina	X	X	X		X
Mulinia pontchartrainensis	X	X	X	X	X
Nassarius acutus	X	X			X
Nassarius vibex	X	X	X	X	X
Oliva sayana		X			
Ophichthus puncticeps		X			
Opsanus beta		X			X
Palaemonetes pugio	X	X			X
Panopeus obesus	X	X	X		X
Panopeus simpsoni	X	X		X	X
Petrolisthes armatus	X	X	X		X
Polychaeta	X	X	X	X	X
Probythinella louisianae	X	X	X		
Rhithropanopeus harrisii	X	X	X	X	X
Stramonita haemastoma	X	X			
Styela plicata	X		X	X	X
Tagelus plebeius		X		X	X
Texadina barrette	X			X	

X indicates occurrence in a particular reef type.

that these old rock reefs differed in the size and number of interstitial spaces as compared to other treatments, to the extent that these reefs provided more suitable refuge space for small organisms. While differences could not be attributed to structural volume or construction material, per se, differences in size and number of voids could be critical; rock reefs could be constructed of gravel, or large concrete blocks thus affecting interstitial space. Determining accurate measures of structure, and structural complexity, which address issues of interstitial space size and number, has proven challenging (Bartholomew et al. 2000; Warfe et al. 2008; Humphries et al. 2011).

Past studies have found that structure may be most important in defining nekton species assemblages and abundance (Diehl 1992; Humphries et al. 2011), and results from this project would support this contention. The minimal differences in nekton assemblages between reef types found in this study are not uncommon considering the mobile nature of nekton (Shervette & Gelwick 2008; Gregalis et al. 2009). In this
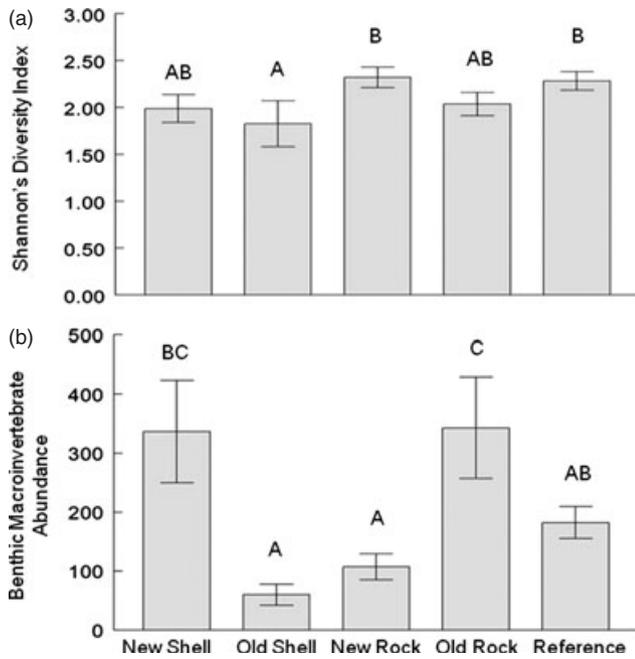
Figure 4. Mean Shannon's diversity index values (a) and mean abundances (b) ($\pm$ SE) for benthic macroinvertebrate species collected on artificial (i.e. new and old shell and rock) and reference reefs. Letters above histograms indicate significant differences in Shannon's diversity index ($p < 0.05$).

Figure 5. Mean ($\pm$ SE) juvenile *Callinectes sapidus* position (shallow water = 0, intermediate water = 1, deep water = 2) with or without an adult *C. sapidus* present. Experimental trials consisted of four replicates of each of three structure treatments: no structure (none), low complexity of structure (low), and high complexity of structure (high).

study, difference were only found in trawl data, and suggested decreased diversity in old rock and new shell compared only to new rock reefs. Given that across the northern GOM fish are not thought to be forage or habitat limited, nekton may use similar sub-tidal habitat structures (i.e. oyster reefs, SAV) equally with differences attributable to factors such as wind speed, salinity, wave energy, or adjacent habitats, and subtle habitat or substrate differences (Shervette & Gelwick 2008; Gregalis et al. 2009).

For benthic macroinvertebrate assemblages, structure provides a valuable refuge and added complexity; for example, oysters may create a nearly impenetrable refuge from decapod predators (Glancy et al. 2003). Live oyster density, or differences in shell and rock substrate were not associated with any differences in nekton or benthic macroinvertebrate assemblage indicators. Even more noteworthy is that structurally all created reefs were statistically similar to reference reefs, suggesting that all provided similar support of nekton and benthic macroinvertebrate assemblages as historic reefs. Again, the measurement of structure used here does not account for variation in interstitial space size or incorporate landscape scale measures of structural complexity, such as reef size, patchiness, or surrounding habitat types which may be critical in determining assemblage characteristics (Grabowski et al. 2005; Geraldi et al. 2009; Gregalis et al. 2009).

The laboratory experiment allowed for more manipulation of structural volume and explicitly tested the refuge value of increased vertical complexity for juvenile *Callinectes sapidus*. Juvenile *C. sapidus* took refuge in more complex refugia, in
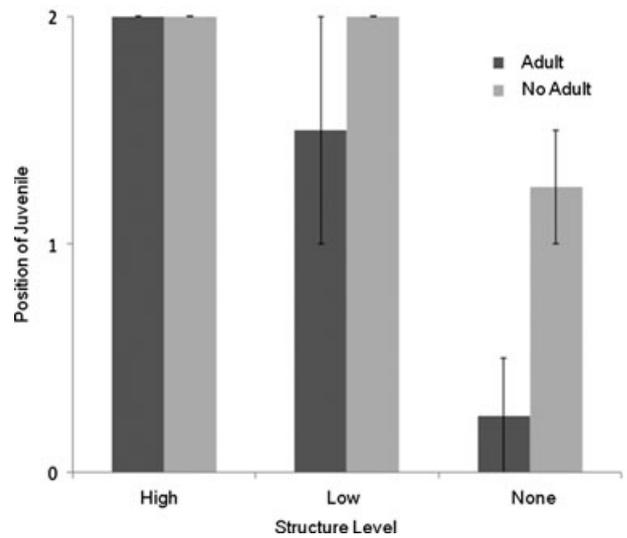
the presence of a predator. Specifically, juvenile *C. sapidus* in the presence of a predator were less likely to use reduced structure (oyster shell relief and volume) as refuge than when shells had high vertical relief. When greater vertical complexity was provided, juvenile *C. sapidus* took refuge in oyster shell 100% of the time. The importance of greater vertical complexity for oyster reefs is corroborated by other studies of reefs as refugia (Grabowski 2004; Soniat et al. 2004; Tolley & Volety 2005). Refuge is important as it can also enhance or inhibit the cascading indirect effects of predators (Pace et al. 1999).

If the goal of reef creation is to provide hard structure, sustainability of the reef is critical; reference, new rock, and old rock reefs all had similar and significantly higher numbers of live oysters as compared to new and old shell reefs. This may be a function of created reef location with rock and reference reefs being placed either by design or by chance in locations more suitable for good oyster recruitment, growth and survival, or reflect potential differences in construction, or material longevity (Choong-Ki et al. 2013). In Delaware Bay, half of the oyster shell added to reefs was lost over a period of 2–10 years (Powell et al. 2006). Oyster shell is less dense than rock and easily dispersed (Lenihan 1999), or is spread too thinly and sinks in mud. Shell also degrades over time and depending on the history and environmental conditions of the site (Waldbusser et al. 2011), the shell may possibly degrade before oyster spat recruit to that area (Mann & Powell 2007).

Clearly, the habitat value of both shell and rock reefs is dependent on the provision of hard structure. While there were some subtle differences in nekton and benthic macroinvertebrate assemblages, none could be related directly to structural volume, or the living reef community, and with one two exceptions (benthic macroinvertebrate abundance and Shannon's Diversity Index), all reefs provided similar support to

nekton and benthic macroinvertebrate assemblages as adjacent reference reefs. This suggests that some unmeasured complexity variable is critical, or, that in the presence of any structure, currents, water quality, and adjacent habitat types may determine community characteristics. With regard to the benthic macroinvertebrate assemblage, structure is a vital driver of recruitment success: reefs that provide continued structure over time are more likely to support diverse and abundant benthic macroinvertebrate assemblages. As restoration efforts increase due to a loss of structural habitat within the estuarine ecosystem, finding out exactly how structure affects ecosystem services, and long-term sustainability becomes even more critical.

Implications for Practice

- At early and late stages, both created rock and shell reefs support nekton and benthic macroinvertebrate assemblages similar to those found on historic reefs.
- Structure, regardless of material type, is critical in creating viable reefs; location is critical in determining nekton and benthic macroinvertebrate assemblages.
- Hard structure that is less likely to diminish over time provides more sustained opportunities for benthic macroinvertebrate and oyster recruitment at restored oyster reefs.

Acknowledgments

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