A Comparison of Fish and Epibenthic Assemblages on Artificial Reefs with and without Copper-Based, Anti-Fouling Paint

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A Comparison of Fish and Epibenthic Assemblages on Artificial Reefs with and without Copper-Based, Anti-Fouling Paint

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Abstract.—Twenty artificial reefs were deployed early in October 2005 approximately 20 km south of Dauphin Island, Alabama (USA), in the Hugh Swingle General Permit Area. Each reef consisted of 12 concrete blocks (20 cm long × 20 cm wide × 41 cm high) arranged on a plywood base (1.5 m^2) and deployed on the bottom, 20 m deep. To quantify the epibenthic assemblage on the reefs, four removable bricks were attached to the reefs. Ten reefs were coated with copper-based, anti-fouling paint and 10 reefs were unpainted. Fish and epibenthic assemblages were compared between reef treatments (i.e., with and without copper-based paint). Reefs were surveyed 1 week after deployment in October 2005, then again in December 2005, May 2006, August 2006, and December 2006. During each survey, two scuba divers visually estimated the densities of all fish species and removed one of the removable bricks to identify and quantify the epibenthic organisms. The epibenthos (coverage area, biomass, diversity, species richness) and fish assemblages (total fish density, species diversity, species richness) were greater on unpainted reefs. Red Snapper *Lutjanus campechanus*, wrasses *Halichoeres* spp., Bank Sea Bass *Centropristis ocyurus*, and Atlantic Spadefish *Chaetodipterus faber* had higher densities on unpainted reefs. This study indicated that recruitment of fishes to artificial reefs was not just attraction to structure, but that growth of epibenthic assemblages had a significant influence on recruitment.

Introduction

Artificial structures have long been known to attract and aggregate fish species from surrounding waters. Artificial reefs provide shelter from predation, provide sites for orientation and spawning, and serve as substrate for epibenthic assemblages that may provide new and additional forage bases for fishes (Hueckel and Buckley 1987; DeMartini et al. 1994; Steele 1999; Szedlmayer and Lee 2004). Reef colonization typically is rapid, with many fish present before the in situ primary

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and secondary trophic levels have had sufficient time to develop (Hueckel and Stayton 1982; Shulman 1984; Bohnsack 1989). This suggests that artificial reefs initially provide shelter or structure from which fish orient. Many studies have reported a positive correlation between refuge availability and juvenile fish survival or abundance (Shulman 1984, 1985; Hixon and Beets 1989, 1993; Caley and St John 1996; Steele 1999; Lingo and Szedlmayer 2006; Piko and Szedlmayer 2007; Mudrak and Szedlmayer 2012). For example, Hixon and Beets (1993) reported higher reef fish abundance on artificial reefs with holes in the reef surface in comparison to fish associated with reefs lacking holes. In addition to their function as a refuge from predators, artificial reefs may provide food resources that are important for fish recruited to the reefs (Buckley and Hueckel 1985; Relini et al. 2002; Szedlmayer and Lee 2004; Redman and Szedlmayer 2009). The trophic structure of the entire assemblage develops over time as algae, invertebrates, and fish colonize artificial reefs. However, the development of epibenthic assemblages is a highly variable process that depends on substrate type and reef complexity, time since deployment, environmental variables, competition, and grazing pressure (Osman 1977; Hixon and Brostoff 1985). Pioneer trophic groups such as algae, barnacles, and serpulid worms are typically among the first colonizers on artificial reefs (Fager 1971; Ardizzone et al. 1989; Relini et al. 1994; Boaventura et al. 2006). Although initial colonization is rapid, slower colonizing taxa continue to settle, gradually replacing early colonizers (Ardizzone et al. 1989; Wendt et al. 1989). Epibenthic assemblages on artificial reefs may converge towards that of natural reefs with time, but more often, they appear to differ. Consequently, factors influencing the differences between natural and artificial reef assemblages remain unclear (Hixon and Brostoff 1985; Wendt et al. 1989; Carr and Hixon 1997; Perkol-Finkel and Benayahu 2007).

Many studies have shown that reef fishes can obtain substantial food resources from biota directly associated with artificial reefs (Hueckel and Stayton 1982; Hueckel and Buckley 1987; Vose 1990; Vose and Nelson 1994; Relini et al. 2002; Ouzts and Szedlmayer 2003; Szedlmayer and Lee 2004). For example, Szedlmayer and Lee (2004) reported that Red Snapper *Lutjanus campechanus* increased their dependency on reef-associated prey as they moved from open sand to artificial reef habitats. Gray Triggerfish *Balistes capriscus* also forage on artificial reefs, consuming a variety of epibenthic invertebrates including barnacles and bivalves (Vose 1990; Vose and Nelson 1994; Blitch 2000). Similarly, in the Mediterranean Sea, three of the four fish species examined fed on reef-associated prey, and 91% of the prey items consumed by Annular Seabream *Diplodus annularis* were exclusive to artificial reefs (Relini et al. 2002). Further, the contribution of reef-associated prey to the diets of reef fishes may depend on both fish species and their size. For example, medium and large Striped Seaperch *Embiotoca lateralis* and Quillback Rockfish *Sebastes maliger* fed chiefly on artificial reefs, whereas small fish foraged in the nearby sand (Hueckel and Stayton 1982).

In contrast, other studies have suggested fish depend little on food resources from artificial reefs (Randall 1963; Shulman 1984; Lindquist et al. 1994; Ibrahim et al. 1996; Nelson and Bortone 1996). Nelson and Bortone (1996) showed that many commercially important reef-associated fishes in the northern Gulf of Mexico fed on fishes, crabs, squids, polychaetes, and shrimps that were obtained from surrounding sand-bottom habitat.

Similarly, examination of stomach content showed that fishes associated with artificial habitat in St. John, Virgin Islands foraged in adjacent sea grass beds rather than on reef prey (Randall 1963).

It is clear that artificial reefs attract fish, but whether or not such reefs lead to increased fish production (e.g., $g \cdot m^{-2} \cdot d^{-1}$) remains unclear (Bohnsack and Sutherland 1985; Alevizon and Gorham 1989; Bohnsack 1989; Polovina 1989; Polovina and Sakai 1989; Bohnsack et al. 1994; DeMartini et al. 1994). Polovina (1989) suggested that artificial reefs may attract fish to locations where they are more easily harvested, potentially decreasing overall fish biomass through increased fishing mortality. Polovina and Sakai (1989) examined time series, catch, and effort data for flatfish catches near Shimamaki, Japan and reported that they were related to attraction to artificial reefs, not enhanced fish production. Bohnsack (1989) suggested that artificial habitats function as fish attractors when natural food and shelter resources are plentiful, and fish abundance is most likely limited by recruitment or exploitation. However, in areas lacking natural reef habitat, artificial reefs may increase fish carrying capacity by providing limiting factors such as food and shelter and thereby enhancing fish production.

Most studies on artificial reefs have focused on the fishery resources with limited attention to the development of epibenthic assemblages (Relini et al. 1994; Svane and Petersen 2001). Also, the few studies that have examined both fish and epibenthic assemblages involved variable reef complexity or low sample sizes (Buckley and Hueckel 1985; Hueckel and Buckley 1987; Relini et al. 2002). Usually, newly deployed artificial reefs provide new substrate for the settlement and recruitment of epibenthic species (Svane and Petersen 2001), especially those organisms with an affinity for hard substrate. Since epibenthos may be important in the diets of many reef fishes, the development of epibenthic assemblages on artificial reefs may affect fish recruitment and the retention of fish on the reef once recruited.

The present study examined the influence of epibenthic assemblages on the recruitment of reef fishes to artificial habitats by comparing fish assemblages on reefs with and without epibenthos. To make this comparison, copper-based paint was applied to inhibit the development of epibenthos on artificial reefs. Several studies have used copper paint to manipulate the development of epibenthic assemblages (Bosman and Hockey 1988; Farrell 1988), but few have used this approach to examine the associated effect of copper-based paint on reef fish recruitment (Redman and Szedlmayer 2009).

Methods

Laboratory Experiment

The potential for fish avoidance of painted reefs due to the presence of copper was examined in the laboratory. Two common species of reef fish, Red Snapper and Gray Triggerfish, were placed in 1,200-L circular tanks, which were part of a 14,080-L recirculating seawater system. Each experimental tank contained three copper-painted blocks (34% copper, 214-7070, Ameron International; 20 cm long × 20 cm wide × 41 cm high concrete construction blocks) on one side of the tank and three unpainted blocks on the opposite side. For each trial, six Red Snapper, six Gray Triggerfish, or a combination (three Red Snapper and three Gray Triggerfish) were placed in the circular tank (1,200 L) and their behaviors were video recorded for 1 h. Eleven trials with six fish per trial were completed (n = 66 total fish examined). Video recordings were reviewed with Image Pro-Plus 4.5 software and fish positions recorded in relation to the painted and unpainted blocks.

Field Study

Artificial reefs (n = 20) were deployed on October 10 and 12, 2005, 20 km south of Dauphin Island, Alabama (USA) in the Hugh Swingle General Permit Area (Figure 1). Each reef consisted of 12 concrete blocks (20 × 20 × 41 cm) arranged on a plywood base (1.5 m^2) and placed on the bottom at 20-m depths (Figure 2). Concrete blocks were secured to the plywood base using 1.2-m plastic cable ties. Additionally, four small, removable concrete bricks (9 × 6 × 20 cm) were secured to the larger blocks with 30-cm plastic cable ties to allow assessment of the associated epibenthic assemblage (Figure 2). Reefs were anchored in place with a 1.5-m nylon rope attached to a 1.2-m metal ground anchor embedded in the substrate. All reefs were labeled with numbered metal or plastic tags to facilitate reef identification. Concrete blocks in 10 reefs were painted with copper-based, anti-fouling paint (painted treatment, same copper paint as above), and concrete blocks in 10 additional (control) reefs were not painted (unpainted treatment). Reefs were

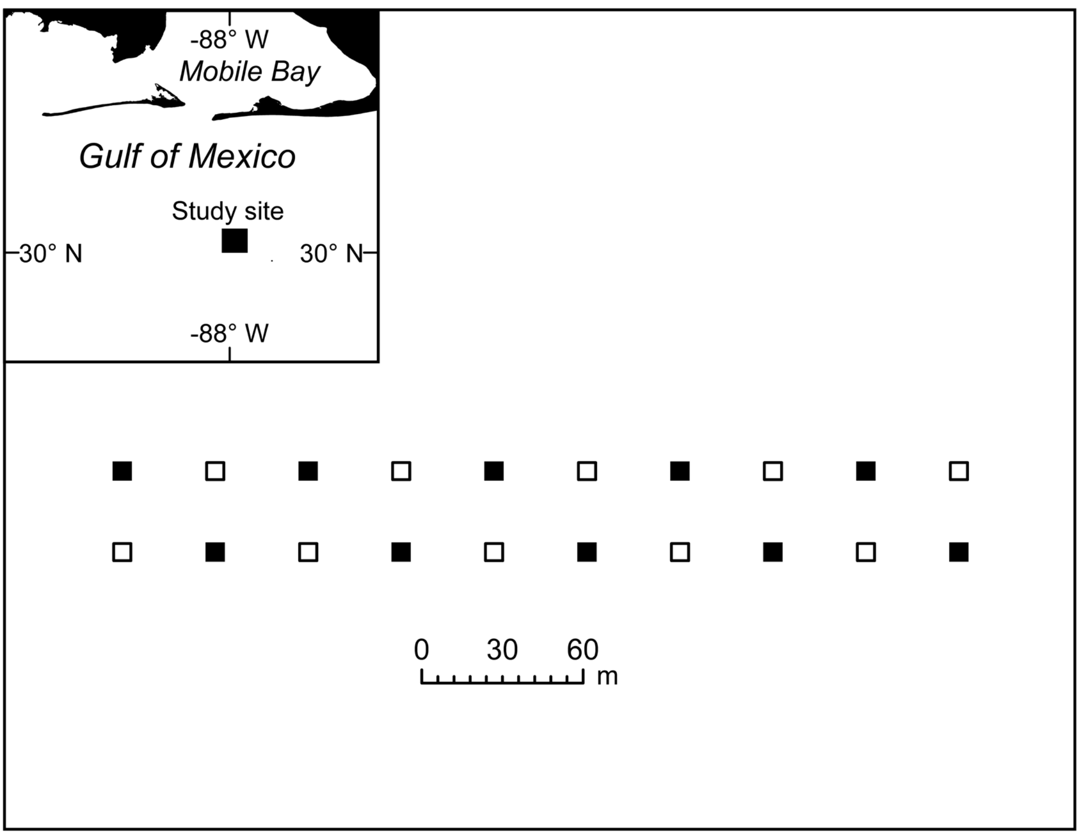

Figure 1. Location of study sites in the northern Gulf of Mexico and reef array design alternating between unpainted (open squares) and painted (black squares) reefs at 30-m intervals.

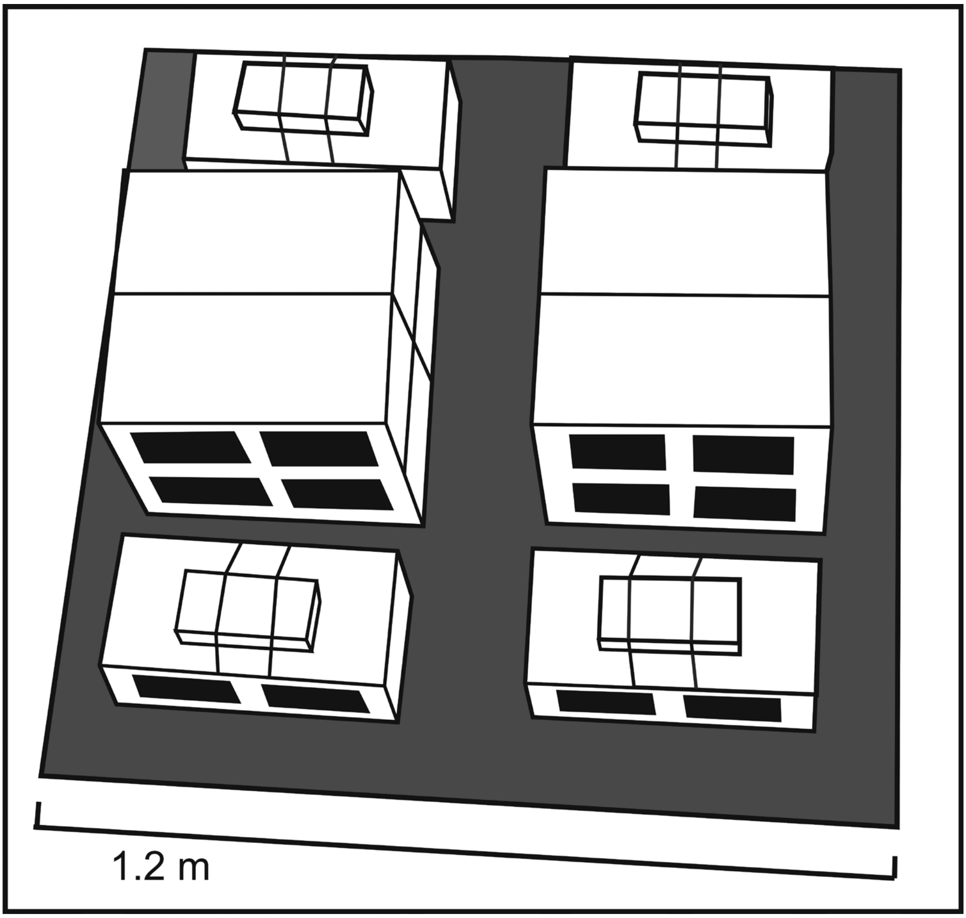

Figure 2. Artificial reef design indicating the placement of 12 concrete blocks on the plywood base and four small "break-away" bricks.

deployed along two transects, with 10 reefs per transect, alternating between painted and unpainted reefs at 30-m intervals (Figure 1).

Reef Surveys

All reefs were surveyed on October 20, 2005, December 1, 2005, May 29, 2006, August 2, 2006, and December 14, 2006. During each survey, two scuba divers used the discrete group census method (Greene and Alevizon 1989) to identify fish species densities and size-class (total length in 25-mm intervals). Since reefs were small (1.5 m^2), all fish were counted. Reefs and the surrounding area were also video recorded to later verify species identifications and record rare or unknown species. A small removable concrete brick was collected per reef during each survey. Bricks were placed in cloth sample bags (25 × 43 cm, #5250, Hubco Protexo) and preserved in alcohol-based preservative (NOTOXhisto fixative, Scientific Device Laboratory) for later analysis of epibenthic assemblages. Water samples were collected within 1 m of each reef to determine copper levels in the surrounding environment. Salinity (‰), temperature (°C) and dissolved oxygen (mg/L) were recorded during each survey with a YSI 6920 meter.

Epibenthos Analysis

All epibenthic organisms on the upper surface of removed bricks were counted, measured, and identified to the lowest taxa possible. The upper surface of each brick was photographed with a digital camera (Canon PowerShot A530). Images in the photographs were size-calibrated and analyzed with Image Pro-Plus 4.5 software to determine surface area (mm^2) of each individual organism or taxonomic group. The area covered by each taxon was recorded relative to the brick area measured as $cm^2/100\ cm^2$. Areas of both mobile and sessile epibenthic organisms were determined. In some cases, individuals overlapped spatially so that the total epibenthic area per brick may be greater than the total brick area (i.e., percent coverage per brick may be >100%). Areas of colonial or encrusting species (e.g., Sabellidae worms, sea mat bryozoan *Membranipora tenuis*, bugula bryozoan *Bugula neritina*, and Demospongiae sponges) were measured in clusters since it was difficult to distinguish individual organisms. The sample bags used to contain the bricks contained many mobile epibenthic organisms and additional sessile organisms that had become dislodged from the bricks. To count and measure these organisms, bag contents were rinsed through a 500-μm sieve to remove sand and mud and retrieve any organisms that had become unattached from the bricks. These organisms were counted, photographed, measured with Image Pro-Plus software, and identified to the lowest taxa possible.

Following measurement of areas, all epibenthos were removed from the top surface of each brick. These samples were then combined with the organisms from the corresponding sample bags and then dried in a drying oven at 60°C for 24 h. Samples were weighed to the nearest 0.01 g, and weight was reported as dry weight $g/100\ cm^2$.

Statistical Analyses

Reefs were surveyed multiple times; therefore, repeated measures generalized linear models (rmGLM) were used with negative binomial distributions to compare mean fish density per reef (number of fish/m^2), dominant fish species densities (i.e., >1% of the total density), epibenthos area ($cm^2/100\ cm^2$), epibenthos biomass (dry weight $g/100\ cm^2$), and dominant epibenthos taxa area (>1% of the total area, $cm^2/100\ cm^2$) among reef treatments and survey date or time after deployment (Seavy et al. 2005; Bolker et al. 2009).

Fish species densities and epibenthic taxa areas were square-root-transformed prior to diversity measure calculations. Fish and epibenthos assemblages were compared using the Shannon–Weiner diversity index (H'), species richness or the total number of species (S) present in the sample, and evenness ($J = H'/H'$max; Magurran 1988). These community variables (H', S, and J) for fish and epibenthos assemblages were compared among reef treatments and survey date with repeated measures analysis of variance (rmANOVA). If significant differences ($P \leq 0.05$) were detected with rmGLM or rmANOVA, specific differences were assessed with Tukey's post-hoc test (Zar 2010).

Fish and epibenthic assemblages were compared among reef treatments and survey dates using nonparametric multidimensional scaling (MDS; Szedlmayer and Able 1996; Lingo and Szedlmayer 2006). Bray–Curtis similarity coefficients were calculated as the similarity between all samples based on square-root-transformed fish densities and epibenthos areas for all species (Field et al. 1982). These similarities were calculated among individual reef samples and plotted as MDS ordinations labeled by reef treatment and survey date. We used two-way permutational multivariate analysis of variance

(PERMANOVA) to statistically compare fish assemblage similarities and separately epibenthic assemblage similarities among reef treatments, survey date, and interactions (Anderson et al. 2008).

Results

Laboratory Experiment

In the avoidance behavior experiment, fish had a significant preference for painted compared to unpainted block treatments. Thus, it was concluded that fish would not avoid the reefs owing to the presence of copper paint in field studies.

Field Unpainted versus Painted Artificial Reefs

During the reef surveys, water samples were collected from the vicinity of each reef and tested for the presence of copper. Copper was detected in seawater 0.5 m from painted reefs (mean + SE = 0.27 + 0.1 parts per million) during the first survey (October 2005), after which copper was not detected in any subsequent water samples. Water temperature (°C) was typically low in the winter while dissolved oxygen (mg/L) and salinity (‰) changed little during the surveys (Figure 3).

Fish Assemblages

There were 33 fish taxa identified (lowest taxa: 26 species, five genera, two families) among 1,119 individuals on unpainted reefs and 766 individuals on painted reefs during the first four survey periods (80 reef surveys). Survey data recorded during the last survey, December 2006—14 months after deployment, were not included in the analyses because the reefs were broken apart and

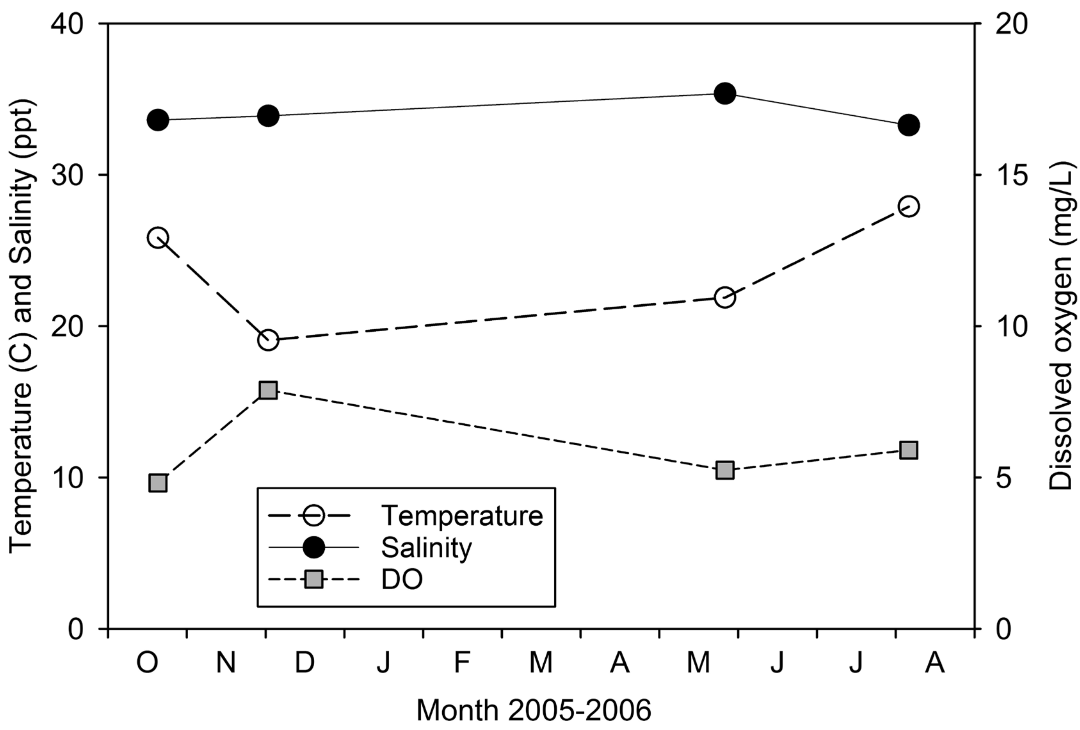

Figure 3. Temperature (°C), salinity (‰) and dissolved oxygen (mg/L) by survey date in 2005–2006.

more than 50% of the reef materials were buried in the substrate. Mean fish densities per reef were higher on unpainted compared to painted reefs ($F_{1, 18}$ = 5.9, P = 0.026) and increased with survey date ($F_{3, 54}$ = 93.7, P < 0.001; Table 1). Fish densities were not associated with reef treatment and survey date interactions ($F_{3, 54}$ = 1.2, P < 0.307; Table 1). Dominant species on the reefs included Red Snapper, wrasses *Halichoeres* spp., Tomtate *Haemulon aurolineatum*, Gray Triggerfish, Lane Snapper *Lutjanus synagris*, sand perch *Diplectrum* spp., Cocoa Damselfish *Stegastes variabilis*, Pigfish *Orthopristis chrysoptera*, Bank Sea Bass *Centropristis ocyurus*, Blenniidae, Rock Sea Bass *Centropristis philadelphica*, Greater Amberjack *Seriola dumerili*, and Atlantic Spadefish *Chaetodipterus faber*. Each of these 13 taxa were more than 1% of the total density and together accounted for 95.3% of all the fish observed during the study (Table 1).

Densities of Red Snapper, wrasses, Bank Sea Bass, and Atlantic Spadefish were higher on unpainted reefs compared to painted reefs (Table 1). Densities of Red Snapper, Tomtate, Gray Triggerfish, Gray Snapper, Pigfish, Bank Sea Bass, Blenniidae, and Atlantic Spadefish increased with time after deployment (i.e., survey date; Table 1). Also, fish densities for wrasses, Pigfish, Bank Sea Bass, and Atlantic Spadefish were significantly associated with an interaction between reef treatment and survey date (Figure 4). No significant differences in fish densities were detected for the other common fish species relative to reef treatment or survey date (Table 1).

All fish observed were either juveniles or small-sized adults (<300 mm total length [TL]; Table 2). Few species had different sizes (TL mm) between reef treatments, but wrasses and Greater Amberjack were larger on unpainted reefs and Atlantic Spadefish were larger on painted reefs. Red Snapper were associated with an interaction of larger fish (TL mm) on unpainted reefs during the December 2005 survey ($F_{3, 612}$ = 4.2, P < 0.006). There was a general trend of increasing size (TL mm) with survey date for several species that included Red Snapper, Lane Snapper, sand perch, and Gray Triggerfish (Table 2).

Higher fish diversity (H') and species richness (S) were observed among fish associated with unpainted reefs. Species diversity and richness increased with survey date, but these assemblage characteristics were not associated with interactions between reef treatment and survey date (Table 3). Examination of the MDS plot and Bray–Curtis similarities for fish assemblages allowed visual recognition of assemblages and PERMANOVA analyses detected significance differences by reef treatment for survey dates in December 2005 (pseudo-$F_{1, 18}$ = 2.9, P = 0.034) and August 2006 (pseudo-$F_{1, 18}$ = 1.8, P = 0.041; Figure 5).

Epibenthos

Epibenthos samples (n = 57) were collected in December 2005, May and August 2006, and 33 epibenthic taxa were identified (lowest taxa: 12 species, six genera, six families, and nine higher taxonomic groups). Mean coverage area was greater on unpainted reefs, increased with survey date (Table 3), and was associated with an interaction between reef treatment and survey date (Figure 6). Epibenthos dry weight was greater on unpainted reefs, and this increased with survey date (Figure 7).

Barnacles (*Balanus* spp.) covered the greatest area, followed by Sabellidae worms, purse-oysters *Isognomon* spp., mud crabs *Panopeus* spp., Demospongiae sponges, brown bryozoan, and Florida dovesnail *Costoana-*

Table 1. Mean and percent densities (number/m^2) for all fish and dominant fish taxa (≥1% of the total density) for all surveys and mean ± SE densities for all fish and dominant fish taxa by reef treatment and survey date. Different letters indicate significant differences ($P \leq 0.05$) between reef treatments (UP = unpainted, P = painted) and among survey dates.

			Reef treatment		Survey date			
Fish taxa	Mean	%	UP	P	Oct 2005	Dec 2005	May 2006	Aug 2006
All fish			28.0±4.1 z	19.2±2.6 y	4.0±1.0 w	12.7±1.3 x	23.1±1.5 y	54.5±4.6 z
Red Snapper	7.8	32.9	8.9±1.2 z	6.6±0.9 y	0.4±0.1 x	7.1±0.9 y	10.7±1.1 z	12.9±1.8 z
Halichoeres spp.	3.6	15.1	5.3±1.9 z	1.8±0.7 y				14.3±3.1
Tomtate	3.5	14.9	3.7±0.9	3.3±1.1	0.1±0.1 x	0.1±0.1 x	3.7±0.7 y	10.2±2.0 z
Lane Snapper	1.5	6.2	1.5±0.2	1.4±0.2	0.8±0.2 yx	0.7±0.1 x	1.6±0.3 zy	2.8±0.5 z
Diplectrum spp.	1.1	4.5	1.1±0.2	1.0±0.2	1.3±0.3	1.0±0.2	1.0±0.2	1.1±0.5
Gray Triggerfish	1.0	4.4	1.0±0.2	1.0±0.2	0.1±0.1 w	0.7±0.2 x	1.6±0.3 yx	1.8±0.3 y
Cocoa Damselfish	0.9	3.6	1.0±0.4	0.8±0.2				3.4±0.6 z
Pigfish	0.7	2.8	0.8±0.2	0.5±0.1	0.1±0.1 y	1.1±0.3 z	1.2±0.2 z	0.3±0.1 y
Blenniidae	0.6	2.4	0.9±0.3	0.3±0.1			0.7±0.5	1.7±0.5
Bank Sea Bass	0.6	2.4	0.7±0.1 z	0.5±0.1 y		0.2±0.1 y	1.0±0.2 z	1.2±0.2
Greater Amberjack	0.5	1.9	0.6±0.6	0.3±0.2				1.8±1.3
Mycteroperca sp.	0.4	1.6	0.3±0.3	0.5±0.5	1.0±1.0		0.5±0.5	
Rock Sea Bass	0.4	1.5	0.3±0.1	0.4±0.1			0.8±0.3	0.7±0.2
Atlantic Spadefish	0.3	1.1	0.4±0.2 z	0.1±0.1 y		0.9±0.3z	0.2±0.1 y	

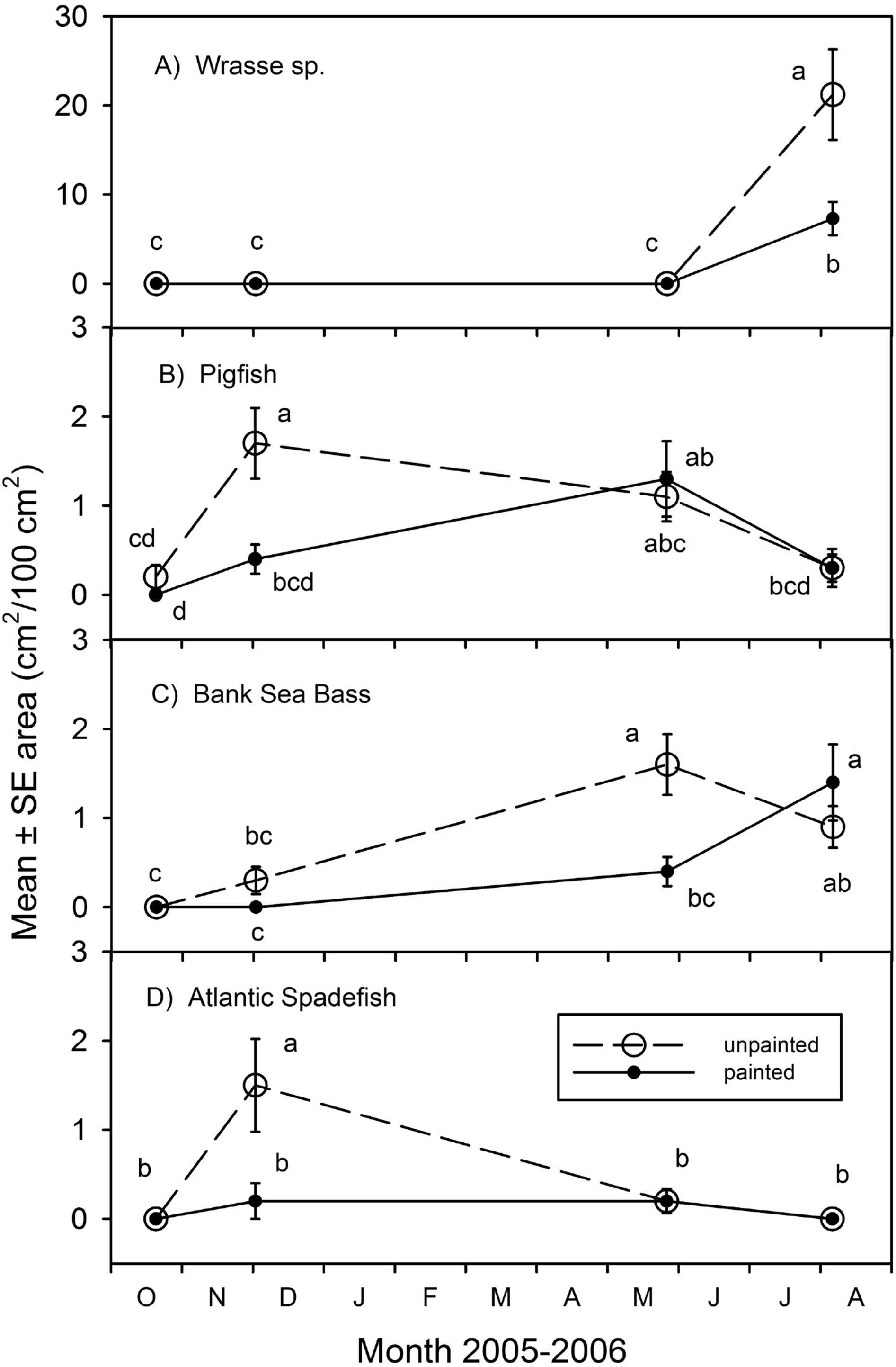

Figure 4. Mean ± SE densities (number/m^2) of **(A)** wrasses, **(B)** Pigfish, **(C)** Bank Sea Bass, and **(D)** Atlantic Spadefish by reef treatment and survey date. Different lowercase letters indicate significant differences ($P \leq 0.05$) between reef treatments and among survey dates.

Table 2. Mean size (TL mm) by reef treatment and survey date, for fish taxa ≥1% of the total density. Different letters indicate significant differences ($P \leq 0.05$) between reef treatments (UP = unpainted, P = painted) and among survey dates.

	Reef treatment		Survey date			
Fish taxa	UP	P	Oct 2005	Dec 2005	May 2006	Aug 2006
Red Snapper	129±2.0	123±2.1	100±13.5 y	102±3.2 y	118±2.2 y	148±1.5 z
Halichoeres spp.	39±0.4 z	37±0.7 y				39±6.2
Tomtate	142±2.4	144±1.8			138±3.1	145±1.8
Lane Snapper	132±6.2	136±6.2	102±9.4 y	77±5.2 y	138±9.2 z	155±4.6 z
Diplectrum spp.	92±8.5	87±7.8	65±4.6 y	86±4.3 y	156±13.9 z	61±8.6 y
Gray Triggerfish	253±8.9	227±11.7	166±25.5 zy	211±17.9 y	230±11.4 zy	265±10.8 z
Cocoa Damselfish	32±1.1	32±1.2				32±0.8
Pigfish	168±6.3	155±9.4	153±12.5	165±8.2	170±8.4	136±12.1
Blenniidae	53±3.3	42±6.1			25±0.0	61±2.4
Bank Sea Bass	122±12.7	125±14.2		106±8.7	102±15.7	143±11.8
Greater Amberjack	292±0.0 z	212±8.9 y				267±6.8
Mycteroperca sp.	216±0.0	89±0.0	89±0.0		216±0.0	
Rock Sea Bass	152±15.7	143±13.1			135±9.6	162±18.0
Atlantic Spadefish	112±5.3 y	153±24.1 z		112±4.8 y	153±26.4 z	

chis sertulariarum (Table 4). Areas for each of these taxa were more than 1% of the total area, and these seven taxa together accounted for 93.1% of the total area on the removable bricks. The remaining 26 taxa comprised 6.9% of the total area.

Most (88%) of the dominant epibenthos taxa covered more area on unpainted reef surfaces, and all taxa increased in area with survey date (Table 4). Changes in epibenthos surface area coverage were associated with interactions of reef treatment and survey date for several taxa, with higher areas for barnacles in December 2005, May and August 2006 ($F_{2, 33}$ = 36.8, P < 0.001), Sabellidae worms in December 2005 and August

Table 3. Mean fish and epibenthos assemblage diversity (H'), species richness (S), and evenness (J), by reef treatment and survey date. Different letters indicate significant differences ($P \leq 0.05$) between reef treatments (UP = unpainted, P = painted) and among survey dates.

	Type		Survey			
Fish	UP	P	Oct 2005	Dec 2005	May 2006	Aug 2006
H'	1.6±0.1 z	1.5±1 y	0.8±0.1 z	1.3±0.1 y	1.7±0.1 x	2.0±0.1 w
S	6.0±0.4 z	5.0±0.5 y	2.1±0.2 z	4.3±0.3 y	6.5±0.3 x	9.0±0.5 w
J	0.93±0.01	0.93±0.02	0.89±0.04	0.94±0.01	0.94±0.01	0.94±0.01
Epibenthos						
H'	2.0±0.1 z	1.5±0.1 y		1.3±0.1 x	1.8±0.1 y	2.0±0.1 z
S	12.5±0.9 z	5.8±0.7 y		3.3±0.7 x	10.4±1.0 y	13.0±0.9 z
J	0.82±0.01	0.76±0.01		0.81±0.04	0.81±0.01	0.77±0.02

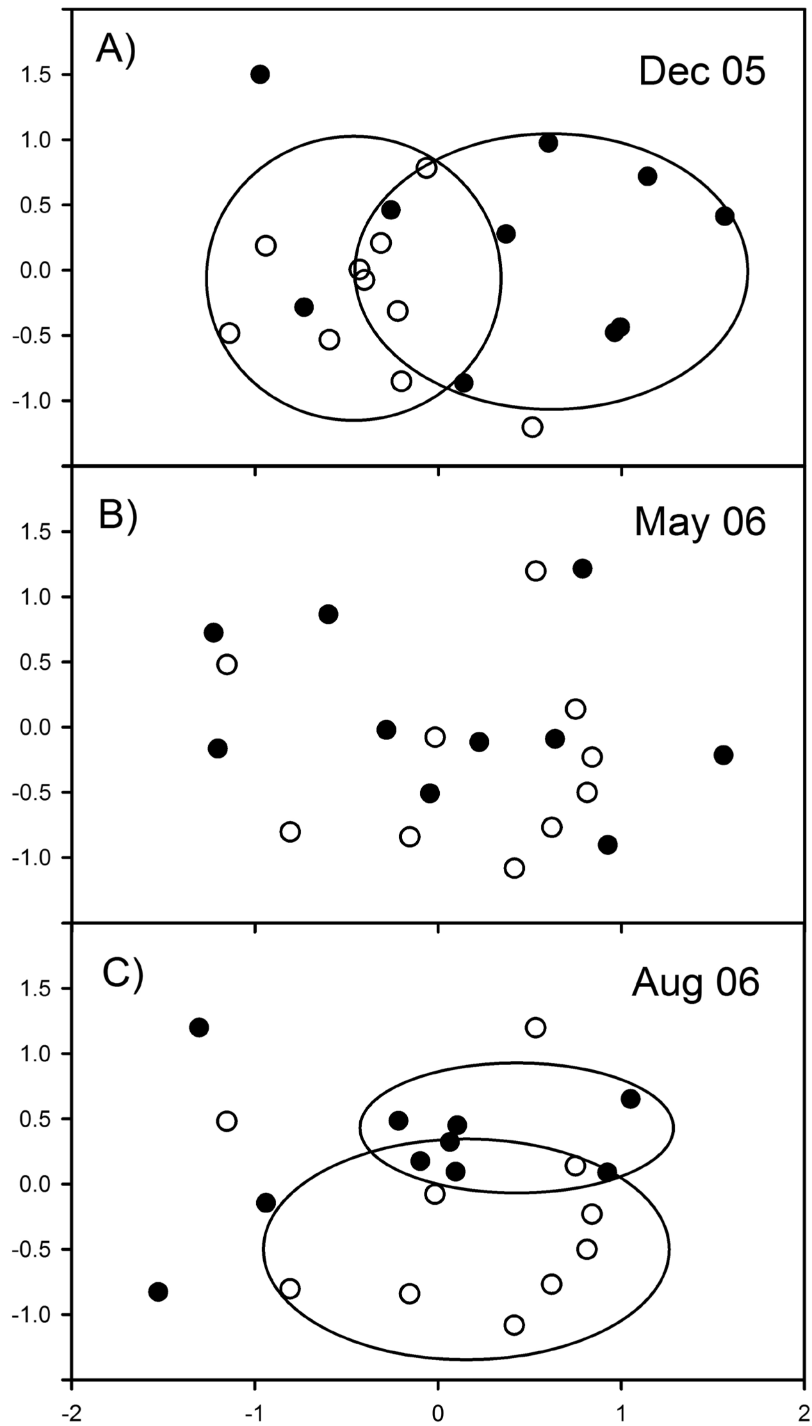

Figure 5. Nonmetric multidimensional scaling plots of fish assemblages based on Bray–Curtis similarities by reef treatment and survey date. Unpainted reefs = open circles and filled circles = painted reefs.

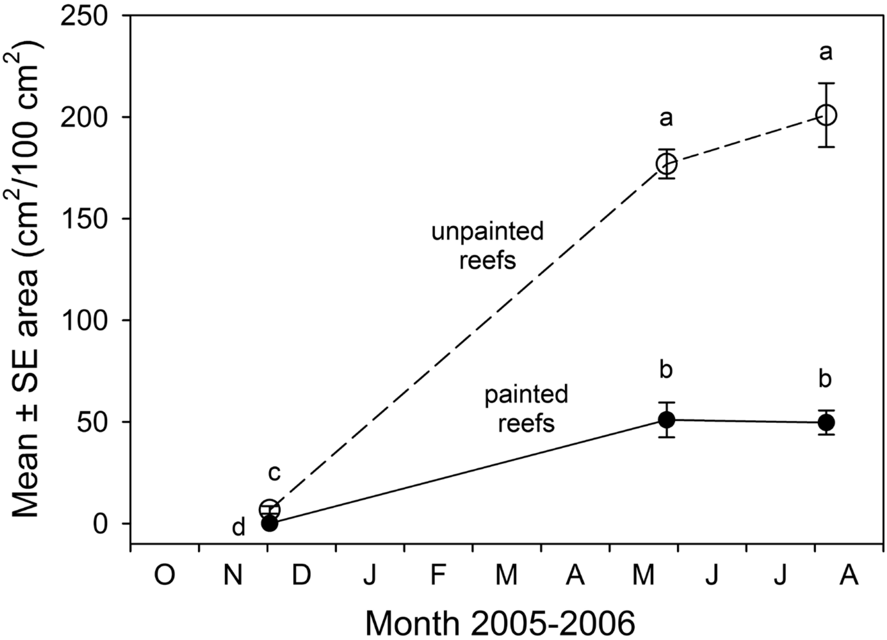

Figure 6. Mean ± SE epibenthos area ($cm^2/100\ cm^2$) by reef treatment and survey date. Different lowercase letters indicate significant differences ($P \leq 0.05$) between reef treatments and among survey dates.

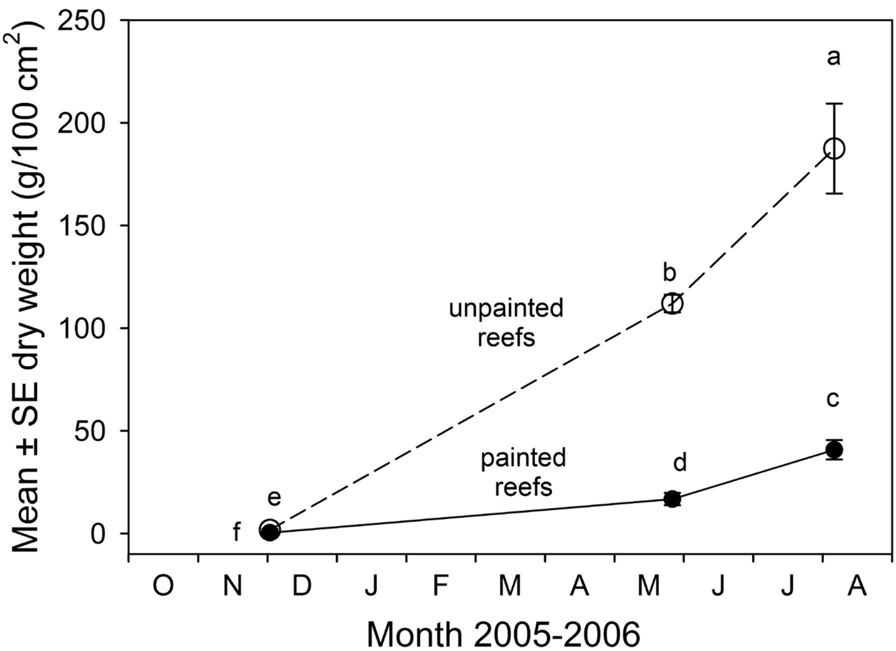

Figure 7. Mean ± SE epibenthos dry weights ($g/100\ cm^2$) by reef treatment and survey date. Different loiwercase letters indicate significant differences ($P \leq 0.05$) between reef treatments and among survey dates.

Table 4. Mean and percent area for all epibenthos and dominant epibenthos taxa (≥1% of the total density) for all surveys and mean ± SE areas for all epibenthos and dominant taxa by reef treatment and survey date. Different letters indicate significant differences ($P \leq 0.05$) between reef treatments (UP = unpainted, P = painted) and among survey dates.

			Reef type		Survey		
Epibenthos taxa	Mean	%	UP	P	Dec 2005	May 2006	Aug 2006
All epibenthos			132.4±17.5 z	35.9±5.7 y	3.5±1.3 y	114.0±15.4 z	125.3±19.2 z
Balanus spp.	56.5	66.5	87.7±11.1 z	24.2±4.0 y	2.8±1.2 y	72.9±11.7 z	85.8±10.9 z
Sabellidae	8.8	10.4	8.8±2.0	8.9±2.8	0.1±0.05 x	22.7±3.0 z	2.4±0.5 y
Isognomon spp.	4.9	5.7	9.4±1.6 z	0.1±0.05 y	0.1±0.05 x	5.1±1.6 y	8.7±2.1 z
Mud crabs	3.7	4.3	6.4±0.9 z	0.9±0.2 y	0.01±0.003 x	4.5±0.9 y	6.0±1.1 z
Demospongiae	2.7	3.2	5.3±2.3 z	0.03±0.04 y			7.8±3.2
Bugula bryozoan	1.6	1.9	3.0±1.2 z	0.1±0.1 y			4.5±1.7
Florida dovesnail	0.9	1.1	1.1±0.2 z	0.7±0.1 y	0.01±003 y	1.3±0.1z	1.4±0.2 z

2006 ($F_{2,33}$ = 11.5, P < 0.001), purse oysters in December 2005, May and August 2006 ($F_{2,33}$ = 58.1, P < 0.001), mud crabs in May and August 2006 ($F_{2,33}$ = 11.4, P < 0.001), Demospongiae in August 2006 ($F_{2,51}$ = 18.9, P < 0.001), and brown bryozoans in August 2006 ($F_{2,51}$ = 12.7, P < 0.001).

Species diversity (H') was higher for the epibenthos on unpainted reefs and H' increased with survey date. Similarly, there were more species (S) on unpainted reefs and S increased with survey date. There were no interaction effects detected between reef treatment and survey date for epibenthos H' or S (Table 3).

Visual inspection of the epibenthos MDS plot indicates almost complete separation by reef treatment and survey date. These differences were statistically significant based on the PERMANOVA analyses of Bray–Curtis similarities for epibenthos areas by reef treatment (pseudo-$F_{1,51}$ = 31.1, P = 0.001), survey date (pseudo-$F_{2,51}$ = 46.0, P = 0.001), and reef treatment and survey date interaction (pseudo-$F_{2,51}$ = 26.2, P = 0.001; Figure 8).

Discussion

Epibenthos and Fish Assemblages

The function of artificial reefs has long been debated (Bohnsack 1989; Shipp and Bortone 2009; Gallaway et al. 2009). It is obvious that artificial reefs attract many fish species, but whether or not any new production can be attributed to artificial reefs is the center of controversy. If production (biomass pro-

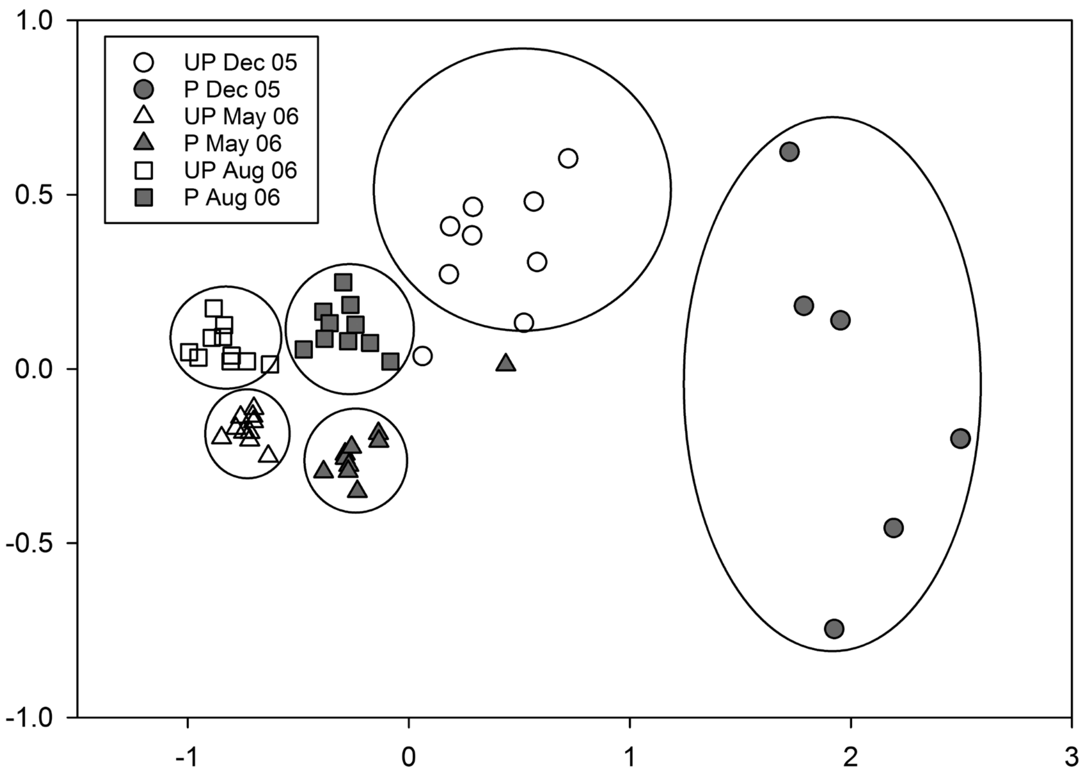

Figure 8. Nonmetric multidimensional scaling plots of epibenthos areas based on Bray–Curtis similarities by reef treatment and survey date. Unpainted reefs = UP open symbols and painted reefs = P closed symbols.

duced per unit time) is occurring, it must result from two features: increased shelter from predation (decrease in natural mortality) or increased food resources from the presence of benthic organisms associated with the addition of new hard structure that would not exist in shifting sand and mud substrates. The connection between protection from predation and artificial reefs has been reported (Topolski and Szedlmayer 2004; Mudrak and Szedlmayer 2012), but demonstration of increased fish abundance due to increased prey base has been more difficult to document (Hueckel and Buckley 1987; Redman and Szedlmayer 2009). The present study attempted to evaluate the food resource aspect of artificial reefs by limiting the growth of epibenthos on artificial reefs. This attempt was successful as the unpainted reefs clearly had higher epibenthos areas, biomass, diversity, and species compared to copper-treated or painted artificial reefs. Epibenthos areas and biomass increased with survey date for both reef treatments, but there were persistent substantial differences between these treatments over the 10-month survey period. These results were consistent with two previous studies that reported lower epibenthos coverage on copper-painted surfaces (Lee and Trott 1973; Redman and Szedlmayer 2009).

In the present study, clear differences in fish assemblages were detected between unpainted and painted reefs. The densities of all fish and of Red Snapper, wrasses, Bank Sea Bass, and Atlantic Spadefish were higher on unpainted reefs. Possible criticisms of the present results are that some other factor (or factors) could have caused the observed differences in fish assemblages between treatments. Possible factors may be differences in temperature, salinity, dissolved oxygen, substrate, or proximity to other reefs. However, we can discount such possibilities due to the study design, as reefs were alternated between unpainted and painted reefs over relatively short distances (30 m); thus, any changes in such variables would be equally distributed among both reef treatments.

An important consideration is the connection between the increased amounts of epibenthos to the diets of fishes. Prey for Red Snapper and Gray Triggerfish were part of the epibenthos in the present study and were consistent with the present study findings. These prey items included Ascidiacea and Polychaeta for Red Snapper (Ouzts and Szedlmayer 2003; Szedlmayer and Lee 2004) and barnacles for Gray Triggerfish (Vose 1990; Vose and Nelson 1994; Blitch 2000). However, many smaller epibenthos that can serve as potential prey items may have been missed in the present study, particularly the more motile Decapoda that would tend to escape as the removable bricks were retrieved from the reefs. For example, conspicuously absent from epibenthos samples were caprellid amphipods (Caine 1991; Woods 2009). These amphipods typically attach to scuba divers by the hundreds and remain attached after divers surface yet were absent in the breakaway samples (S. T. Szedlmayer, personal observation). In future epibenthos studies on artificial reefs, it is suggested that some type of net capture method should be employed to assure a more complete assessment.

Other studies have also reported connections between the epibenthos and fish assemblages on artificial reefs. Diet analyses indicate that increases in Copper Rockfish *Sebastes caurinus* and Quillback Rockfish *S. maliger* were correlated to successional epibenthos development on artificial reefs (Buckley and Hueckel 1985). Further, many fish species became more abundant on artificial reefs that have a more developed epibenthos

(Hueckel and Buckley 1987). Perhaps the most appropriate previous study for comparison to the present study was a survey of fish assemblages also on unpainted versus copper-painted concrete block reefs in the same study area of the northern Gulf of Mexico (Redman and Szedlmayer 2009). In this previous study, similar relations were reported for Red Snapper and Gray Triggerfish and their association with epibenthos. An important difference was that the Redman and Szedlmayer (2009) study did not collect epibenthos or attempt to identify the epibenthic taxa, but simply relied on underwater photographs to document the epibenthos on the concrete block surfaces. Thus, an important aspect in the present study was the identification and quantification of epibenthos on artificial reefs.

Increased shelter provided by the reef can also be linked to increases in the epibenthos. For example, the density of four species of Blenniidae on artificial structures in the north-central Gulf of Mexico were positively related to densities of barnacles as these fishes used empty barnacle shells as shelter (Topolski and Szedlmayer 2004). In the present study, blenniid densities may also have been related to barnacle areas on artificial reefs. Although blenniids did not differ significantly between reef types, their densities increased with the increase in barnacle area over survey date.

Copper Paint Treatment

A potential issue in the present study was that copper toxicity may inhibit fish recruitment. However, following submersion painted surfaces soon become covered with a thin biofilm that slows the release of copper to low levels (8–22 $\mu g \cdot cm^{-2} \cdot d^{-1}$) while still preventing epibenthos development (Dempsey 1981; Valkirs et al. 2003). Also, the constant water flow in the open Gulf of Mexico prevents accumulation of copper in the water immediately surrounding the painted reef surfaces. Water samples collected from the seawater surrounding the reefs indicated that copper was present at low concentrations (<1 parts per million) near the painted reefs 1 week after deployment, but copper was not detected on any reefs on subsequent surveys. Also, fish in the laboratory experiment showed no avoidance of copper-painted blocks. Therefore, any differences in fish density between reef treatments were not related to the presence of copper paint on the reef. More likely, the differences in epibenthos assemblages were responsible for the differences in associated fish assemblage parameters.

Conclusions

Copper-paint treatment of artificial reefs was associated with greater epibenthos areas, biomass, species diversity, and species richness on unpainted versus painted reefs. The increased epibenthos likely provided an increased forage base for fish that resulted in increased total fish density and increased densities of four fish species on the unpainted reefs compared to painted reefs. In addition, increased epibenthos may have increased the shelter aspect of artificial reefs, particularly for small cryptic species such as Blenniidae, thus leading to lower natural mortality. To further confirm such patterns, that an increase in the artificial reef associated fish assemblage was the result of an increase in the epibenthic assemblage, future studies should attempt to quantify fish diets simultaneously with unpainted and painted reef treatments. In addition, obtaining fish biomass estimates for both painted and unpainted reefs may provide greater insights on the value of artificial reefs.

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