Summary Report to Gray's Reef National Marine Sanctuary:
Fish and Habitat Community Assessments, August 18-21, 2010

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Summary

Gray's Reef National Marine Sanctuary (GRNMS) is located 16 miles offshore of Sapelo Island, Georgia, in the South Atlantic Bight (SAB), where it protects 22 square miles of live-bottom habitat. NOAA is proposing to establish a research area in GRNMS to increase the opportunity to scientifically discriminate between natural and human-induced change to living sanctuary resources. One goal proposed for evaluation with the research area is to determine the effect of bottom fishing on benthic fish populations. To this end, in August 2010 we conducted benthic habitat and fish community surveys of six sites located within the proposed research area and six sites located outside the research area. At each site, we measured structural ledge characteristics (e.g., height, undercut height), determined benthic habitat biota with a series of stationary photoquadrats, and censused the conspicuous and cryptic fish communities with a combination of linear band transects and stationary quadrats. Overall, no significant differences in conspicuous fishes, cryptic/juvenile prey fishes, or habitat community structure were seen between management zones. However, robust comparisons between management zones were not possible nor expected given the lack of statistical power from the low samples sizes of this preliminary study. The dominant members of the fish community that we observed were consistent with previous studies from GRNMS spanning nearly 25 years, suggesting stability in the species composition of the top five to ten most abundant species on the live-bottom reefs. Our results provide a detailed census of live-bottom fish and benthic community structure that offer information as to the stability of the populations and also a template for additional comprehensive sampling scheduled for Spring/Summer 2011. We include a brief discussion and comparison of potential sampling methods for benthic habitat and fishes that may be useful when selecting protocols for future comprehensive sampling. Once accomplished, the planned increase in sample size of 20-48 medium to high relief sites per management zone will provide an ample pre-implementation baseline for live-bottom areas inside and outside the research area, anticipated for 2011.

Statement of Need/Introduction

Designated in 1981, Gray's Reef National Marine Sanctuary (GRNMS) is located 16 miles offshore of Sapelo Island, Georgia, in the South Atlantic Bight (SAB). The sanctuary protects 22 square miles of live-bottom habitat, one of the largest nearshore live-bottom reefs in the southeastern United States. The sanctuary focuses on the protection and conservation of all natural marine resources within its boundaries. NOAA is proposing to establish a research area in GRNMS consistent with the National Marine Sanctuaries Act (NMSA) (16 U.S.C. 1431 et. seq.). The purpose of a research area would be to increase the opportunity to scientifically discriminate between natural and human-induced change to living sanctuary resources. The proposed regulatory changes in the research area would prohibit all fishing and diving activities and vessel transit of the research area would be allowed only without interruption (see ONMS 2010 for further details).

In 2004, the Sanctuary Advisory Council (Advisory Council) convened a working group to determine the best management strategies for the proposed research area (hereafter, research area, or RA). A series of recommendations were made by this Research Area Working Group (RAWG) to the Advisory Council that would allow GRNMS to evaluate the research area concept. One goal established by the RAWG for evaluation with the research area is to determine the effect of bottom fishing on benthic fish populations. To address this goal, the RAWG recommended the establishment of a diver visual fishery independent survey whose objective would be to track populations and other characteristics of targeted and non-targeted fish species, both inside and outside the research area. The variables of interest would be the number and length of fish by species, as well as habitat characteristics (e.g. ledge height). The RAWG envisioned that this study would be conducted approximately three times per year and that the study would continue for greater than five years.

This report details the results of a pilot study undertaken in August 2010 at the request of GRNMS in response to RAWG recommendations. The results must be regarded as preliminary due to the low number of sites visited, but the report demonstrates the types of data returned from quantitative survey methods that are currently utilized by National Ocean Service (NOS) National Centers for Coastal Ocean Science (NCCOS) and National Marine Fisheries Service (NMFS) researchers, at the Beaufort, North Carolina laboratory. These results should contribute to the establishment of baseline estimates of population status for sanctuary resources and should allow GRNMS to evaluate the utility of conducting similar surveys in the future on a larger scale that are more statistically robust.

Methods

Site Selection & Survey Overview

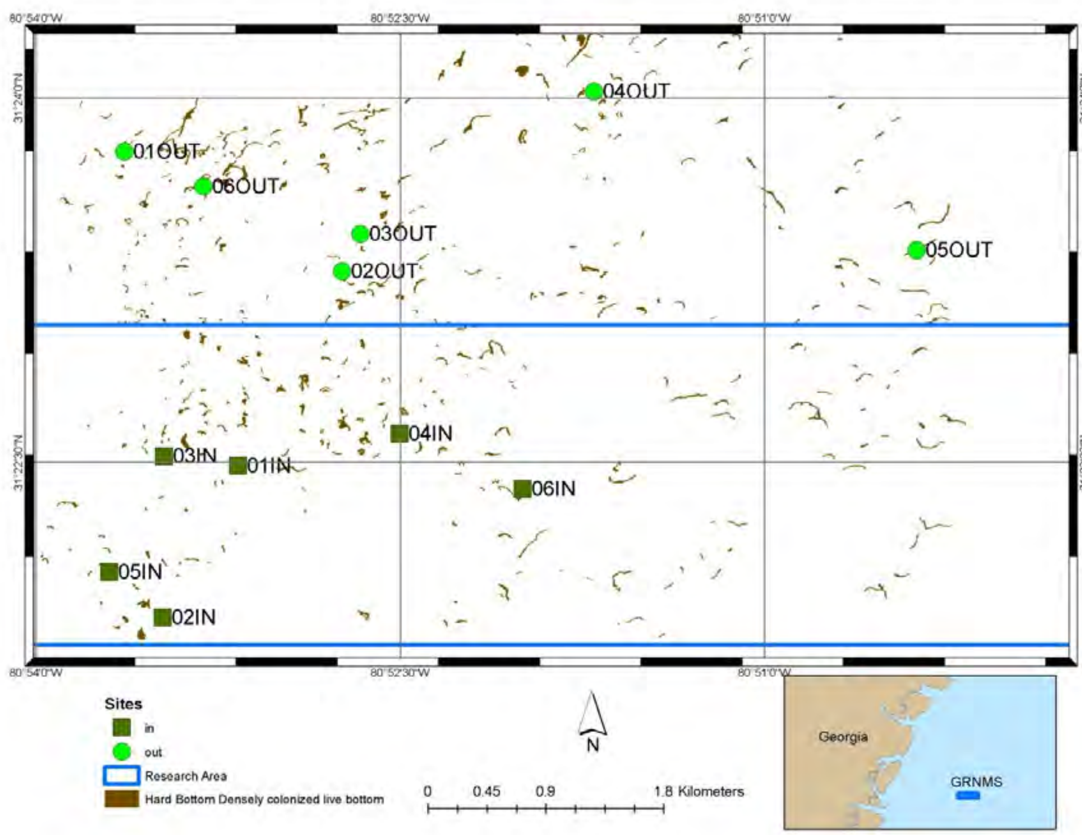
The preferred boundary for the research area (Fig 1) encompasses 8.27 square miles (21.43 km^2), roughly the southern third of the sanctuary. Ledges were selected that were medium to high relief, consisted of medium to large ledge area, and characterized as densely colonized hard bottom based on sites previously classified with multibeam data or by divers (Kendall et al. 2007). A potential universe of ledges that met the above criteria was created in ArcGIS and random points were then overlaid with a minimum separation distance of 50 meters. Twelve study site locations were selected from the random points; six sites were located within the research area and six sites were located outside the research area. At each site, we conducted surveys of the fish community, habitat, and algal sampling for both species identification as well as harmful dinoflagellate analysis. Each site was sampled in two dives (see below for details). The first dive team included a fish (conspicuous and cryptic/juvenile prey) transect diver, a habitat characterization diver, and a video diver. The second dive team included two stationary fish quadrat divers and one habitat characterization diver.

Habitat Community Assessments

Habitat Structure

Habitat surveys were conducted concurrently with fish assessments (see below). To better quantify reef structure, we also measured ledges and structural organisms (e.g., algae, sponges, tunicates) at each site. At fixed intervals along the fish survey transects (5, 15, 25, 35, and 45 m) and during stationary fish surveys (as time permitted), three ledge measurements were collected following methods described in Kendall et al. (2007). Total ledge height was the distance from the substrate to the top of the ledge, excluding all sessile organisms attached to the substrate. Undercut width quantified the amount of overhang of each ledge, and was measured from the leading edge of the ledge to the inner most portion of the ledge. Undercut height, or the height under the ledge, was measured from the substrate surface to the underside of the leading edge of the ledge. All measurements were collected using a measurement tape or when measurements exceeded 40cm, they were visually estimated using the transect tape as a guide. At each ledge measurement location, macroalgae and invertebrate height were recorded. Maximum height of an individual plant or invertebrate was recorded to the nearest centimeter.

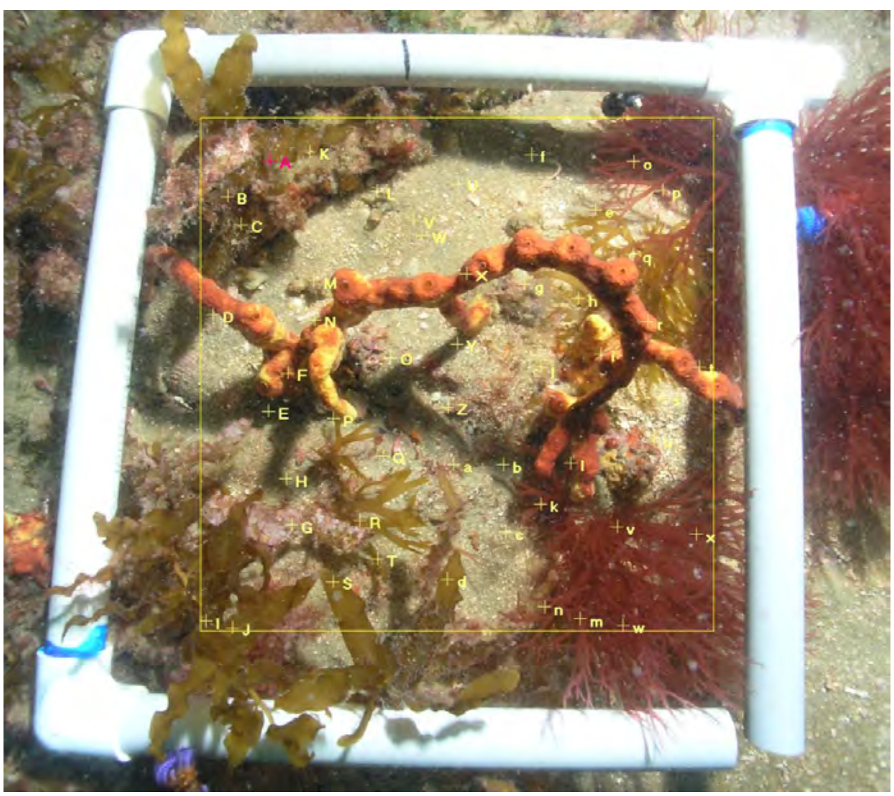
Figure 1.Location of research area off the Georgia coast (inset, outlined in blue), and locations of surveyed sites within (dark green squares) and outside (light green circles) the research area (in blue) at Grays Reef National Marine Sanctuary. Densely colonized hard bottom ledges, as defined by Kendall et al. (2007), are designated by brown polygons.



Habitat Biota

Using the fish transect as a guide, the habitat surveyor took photographs (standardized with $0.25m^2$ quadrats) every five meters, beginning at zero. All analyzed photographs were of the highest quality and resolution attainable (7.0 megapixels). Percent cover of habitat groups was determined using Coral Point Count (CPCe) software (Kohler & Gill 2006). Fifty stratified random points were overlaid on each photograph and the habitat below each random point was classified to the finest level possible (Fig 2). The number and spacing of points was chosen after rarefaction curves identified the sample size at which more points did not add additional information (Buckel et al. unpublished). These tests were previously conducted on images from North Carolina rocky reefs. In instances where species or genus could not be identified, a classification based on morphology was made. For example, where species was unknown but phyla were identifiable, a point could be classified as 'rod shaped soft coral' or 'strap-like red algae'. Where phyla were unidentifiable, generally due to shadowing, image quality, or obstruction, the point was labeled as unknown and removed prior to analysis (see Table 1 for sample sizes). Organism identification was informed by multiple resources including Schneider and Searles (1991), Devictor and Morton (2010), and the online species guide to Gray's Reef (Gleason et al. 2011; http://www.bio.georgiasouthern.edu/gr-inverts/index.html).

Figure 2. Example of point intercept analysis of GRNMS habitat quadrats. Within a 25x25cm area (yellow square with yellow letters marking point locations), fifty points are overlaid in a stratified random (2 points per cell in a uniform 5 x 5 cell matrix) pattern; actual quadrat size is 30x30cm. Prominent biota in the picture below are *Halymenia trigona/Sciniaia complanata* (red macroalgae), *Sargassum sp.* and *Dictyota sp.* (brown macroalgae), and rope sponge.



Fish Community Assessments

All sampling protocols have inherent biases that can favor or exclude species based on factors such as behavior, habitat preference, or size (Allen et al. 1992, MacNeil et al. 2008). For example, traditional underwater visual census (UVC) transects geared towards conspicuous and highly mobile species may miss or underestimate smaller, benthic dwelling fishes. These smaller species are often cryptically colored and can either be the juvenile stage of conspicuous species or may remain cryptic and small throughout their life cycles, where they may function as important prey species within the fish community. For this reason, we combined three different sampling approaches in order to better capture the entire fish community within a given area. We conducted the following surveys:

1. 50 m UVC band transects with an estimated width of 5m on each side that targeted mobile conspicuous fishes. Area surveyed = 500 m^2.

2. 50 m UVC band transects with an estimated width of 1m on each side that only targeted the cryptic (or juvenile) prey species 10 cm and less in length. Area surveyed = 100 m^2.

3. Stationary UVC of 1 m^2 quadrats placed on the benthos for a predetermined (6 min) time period. Four quadrats were sampled per dive. This method favors the less mobile cryptic prey species less than 20 cm but does not exclude conspicuous species. Area surveyed = 4 m^2. Six photographs were also taken of each stationary fish quadrat: one photo on each corner, one photo of the entire quadrat, and one photo taken obliquely as a reference for upright biological growth associated with each quadrat. These data will be analyzed separately and are not presented here.

Densities were determined by dividing the number of fish observed by the area surveyed, and are reported in hectares. Fifty meter video surveys were also taken along the same transect as visual surveys for reference purposes, to confirm species identifications, and to document habitat types.

For the three surveys, the species identification and lengths of all fishes observed were recorded. Similar looking pairs or groups of species that proved difficult to identify to the species level included *Seriola dumerili* and *S. rivoliana, Pareques umbrosus* and *P. acuminatus*, *Decapterus macarellus* and *D. punctatus*, as well as certain porgies, gobies, blennies, and searobins. These species were identified to the genus level (Kendall et al. 2007).

We also estimated biomass for each fish species. Biomass was calculated from the length-weight relationship, $W = aL^b$, where W = weight in g and L = length in cm. The midpoint of the size categories was calculated for each 10cm category. For example if the size category was 20-30cm then the length was considered 25cm for the equation (Kendall et al. 2009). Values for the a and b parameters were obtained for each species from http://www.Fishbase.org (Froese & Pauly 2010). If the parameters for a particular species could not be found then the parameters for a morphologically similar species within the same genus were used. We were unable to obtain parameters for whitespotted soapfish (*Rypticus maculatus*) and blue goby (*Ptereleotris calliurus*) so these were not included in biomass analyses.

Kendall et al. (2009) identified two distinct groups of fishes from GRNMS associated with tall (mean 55 cm high) and short (mean 14 cm high) ledges, and determined that the split between fish communities appeared to occur at a ledge height of 25 cm. We therefore classified ledges at our study sites as tall (>25 cm high) or short (25 cm or less in height) and examined whether fish communities differed between these two ledge types.

Results

Habitat Community Assessments

Habitat Structure

All sites generally conformed to the description in Kendall et al. (2007), and the location of study sites is identified in Table 1 and Figure 1. High relief, densely colonized ledges were targeted for this survey; diver observations and structural measurements identified that nearly all sites met the criteria for this habitat (described by Kendall et al. 2007) except 02IN, which was predominantly sand with no defined ledge. For all sites and nearly all measurements, ledge measurements taken in 2010 were lower/smaller than those collected by Kendall et al (2007). Table 2 reports biological parameters measured at each site together with the corresponding Shannon (H′) estimates of conspicuous fish diversity. Multivariate analysis of structural habitats (Table 2) measured among sites indicated no significant differences between management zones (Analysis of Similarities, ANOSIM, $R = -0.004$, $P = 0.52$, Fig 3). Water samples collected for dinoflagellate analysis were found to contain no *Gambierdiscus* species. Other harmful algal bloom species were present but were not isolated for molecular identification and future analysis.

Table 1. Locations of sites surveyed in August 2010 at Gray's Reef National Marine Sanctuary, corresponding site name from Kendall et al. (2007), management zone designation, and number of analyzed habitat images and classifiable points.

Site Name	Kendall et al. (2007) Name	Research Area	Longitude W	Latitude N	N images (points)
01IN	LBM24_AUG_05	IN	-80.8881	31.37854	11 (538)
02IN[a]	LAM10AUG_05	IN	-80.8940	31.39630	11 (546)
03IN	LAM10MAY_05	IN	-80.8913	31.36437	11 (527)
04IN	LAM7AUG_05	IN	-80.8790	31.38810	11 (534)
05IN	LAM22AUG_05	IN	-80.8912	31.37541	11 (538)
06IN	LAM5AUG_05	IN	-80.8778	31.39065	11 (546)
01OUT	D7AUG_04	OUT	-80.8940	31.39630	11 (529)
02OUT	LAM11AUG_05	OUT	-80.8913	31.36437	11 (525)
03OUT	LBM12AUG_05	OUT	-80.8790	31.38810	11 (527)
04OUT	LAM14AUG_05	OUT	-80.8912	31.37541	11 (542)
05OUT	LAM17AUG_05	OUT	-80.8778	31.39065	11 (522)
06OUT	LAM4AUG_05	OUT	-80.8750	31.37695	11 (529)

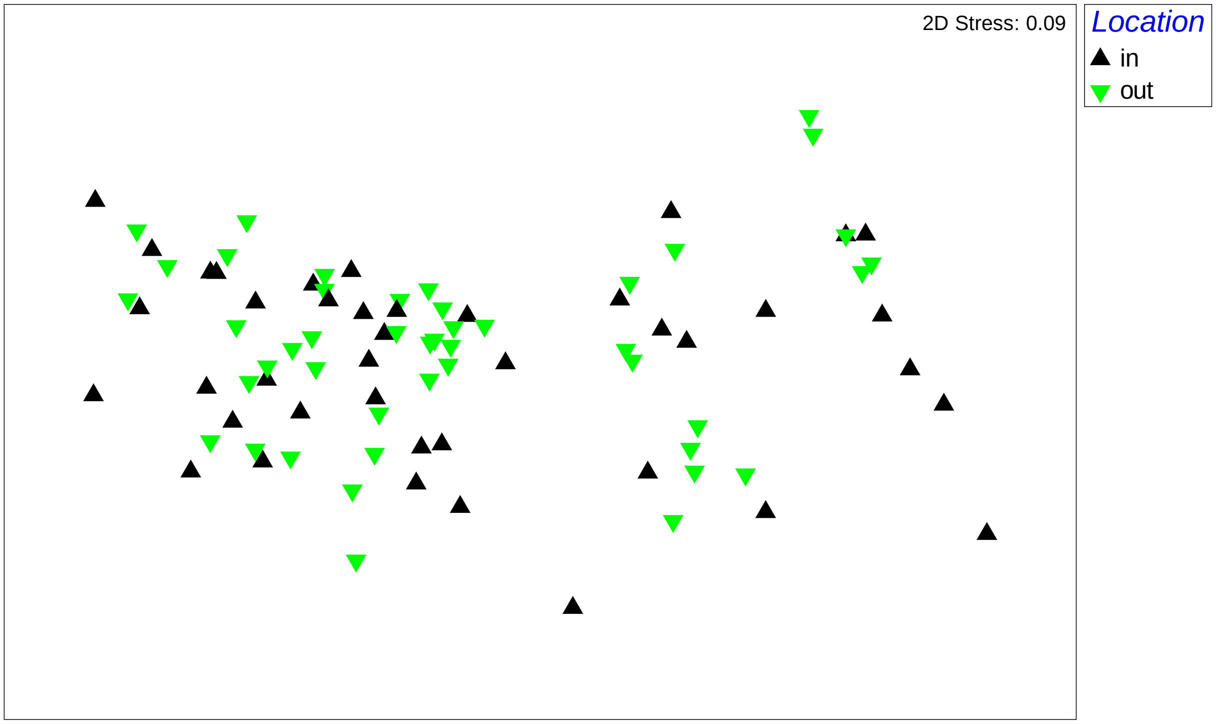
[a]Predominantly sand, very sparse reef patches, no defined ledge. This site was excluded from analyses comparing management zones.

Table 2. Description of invertebrate, macroalgae, and ledge characteristics in centimeters (mean ± SE, n = 5 – 10 per measurement), and fish diversity index (H′) at all sites surveyed in August 2010 at Gray's Reef National Marine Sanctuary.

Site	Invert. Height	Algae Height	Ledge Height	Undercut Height	Undercut Width	Fish Diversity H′
01IN	10 ± 1.9	5.9 ± 1.3	32.5 ± 6.8	21.6 ± 6.5	31.6 ± 12.3	0.89
02IN	15 ± 1.4	10 ± 2.1	0 ± 0	0 ± 0	0 ± 0	(1.62)[a]
03IN	9.8 ± 1.8	13.7 ± 3.1	33.6 ± 8.8	26.1 ± 8	42.5 ± 22.9	1.29
04IN	20.8 ± 3.4	12.4 ± 2.2	10.1 ± 3.2	4.4 ± 2.6	2.63 ± 1.0	1.55
05IN	10.4 ± 1.3	12±2.1	22.8 ± 11.6	17.4 ± 12.5	29.8 ± 22.8	0.77
06IN	22.8 ± 4.1	9.1 ±1.8	23.6 ± 5.0	16.25 ± 4.4	12.9 ± 4.6	1.79
Mean IN	**14.8 ± 1.3**	**10.4 ± 1.0**	**22.7 ± 3.2**	**15.85 ± 0.93**	**22.0 ± 6.4**	**1.26 ± 0.19**
01OUT	13.1 ± 2.7	12.8 ± 2.6	41.4 ± 9.6	20.3 ± 4.1	33.6 ± 12.9	1.90
02OUT	10.4 ± 2.9	10.4 ± 3.5	17.8 ± 7.1	3.6 ± 2.4	5.2 ± 3.3	1.82
03OUT	16 ± 2.5	10.5 ± 1.3	17.1 ± 5.1	6.0 ± 2.8	14.5 ± 8.1	1.71
04OUT	23.8 ± 2.8	18.9 ± 3.7	20.6 ± 6.8	12.0 ± 4.1	10.4 ± 5.8	1.30
05OUT	22.7 ± 3.4	13.5 ± 1.8	16.4 ± 2.5	7 ± 1.8	3.6 ± 1.1	1.75
06OUT	18 ± 2.8	11.7 ± 1.6	46.8 ± 8.5	27.6 ± 6.9	56.2 ± 21.7	0.36
Mean OUT	**17.8 ± 1.3**	**13.0 ± 1.0**	**27.8 ± 3.3**	**14.0 ± 2.2**	**22.8 ± 5.81**	**1.47 ± 0.24**

[a]Site 02IN not used in calculation of mean

Figure 3. MDS plot of structural habitat from sites inside and outside proposed research area at Gray's Reef[a].



[a]Figure does not include measurements from site 02IN.

Habitat Biota

Epibiota of GRNMS were found to be highly diverse and densely colonized (55% cover). Data presented here are preliminary; six inside RA and six outside RA sites are presented. Emerging patterns within the data are discussed, but no statistical analysis was completed on the biological habitat data due to its preliminary nature.

Macroalgae Community

More total algae cover was observed outside (31%) than inside (23%) the RA. Overall, fewer green algae than brown and red were found. Cover of red and brown algae within the RA was similar, however, comparison between strata identified that cover outside the RA was greater than inside (Fig 4A). There was greater between site variability for both red and brown algae, while chlorophyta cover was generally uniform among sites, excluding site 04IN which was densely covered with green turf-like algae (Fig 5A). Among all sites, predominant algae included turf (listed are mean percent cover, 5%), crustose coralline / *Peysonnelia sp.* (3%), and unknown red algae (4%). Unknown red algae may be comprised of multiple species, but could not be differentiated further due to image quality. A total of 22 different algae species or morphological groups have been identified to date (Table 3) and a greater number of rhodophyta were documented than other two algae divisions (Table 3; 11 red, 5 green, & 6 brown).

Invertebrate Community

The invertebrate community of Gray's Reef was the most speciose of the benthic community, spanning eight phyla, with 33 different species or groups (see Table 3 for spp list), 14 which were cnidarians. Hard coral, mainly *Oculina sp.,* was found at all sites except 04IN and percent cover was similar between strata (Fig 5B). Octocoral cover was similar between the two RA strata (1.8% inside, 1.2% outside; Fig 4B); however at some sites, octocoral cover was well above strata average (02IN 5%, 05OUT 4.8%; Fig 5B). Percent cover of other cnidarians, including hard corals, was similar inside and outside the research area (Fig 4B). Other cnidarians (Table 3) were largely comprised of hydroids, particularly at 06IN, and 06OUT where they made up 8% of the total site cover (compared to 2.6% inside and 3.2% outside, RA mean cover).

There was a greater combined cover by invertebrates other than cnidarians inside (27%) than outside (15.8%) the research area (Fig 4C). Primary differences were found between the two strata for sponge, tunicates, and other invertebrates (species listed in Table 3). Elevated sponge and other invertebrate cover inside the RA were largely driven by percent cover of other invertebrates (mainly bryozoans) and sponge (rope and ball shape sponges) at site 03IN (Fig 5C). Among surveyed sites, there was a range of cover differences but within each habitat group, they were generally similar between strata (Fig 5C). Dominant sponge types included ball sponge, rope sponge, and encrusting sponge. Predominant 'other invertebrates' consisted of bryozoans (including *Amathia sp., Schizoporella floridana*) and unidentifiable invertebrates which require further investigation for positive identification.

Abiotic Community

Overall, total exposed substrate cover was similar inside and outside of the RA (Fig 4D; 44% vs. 47%, respectively). Sediment/sand was the primary abiotic cover (mean 38% IN and 34% OUT), followed by rock (4% IN, 8% OUT), shell/shell hash, microalgae mat, and crack/crevice/hole combined to form the balance. Percent cover of exposed substrate (mainly sediment/sand cover) was greatest at site 02IN (67%; Fig 5D) which was not surprising given that divers did not find a discernible ledge (Table 2); measurements at this site were taken along sand-dominated, patchily distributed low relief hard bottom. Site 03IN had the lowest cover of exposed substrate, and interestingly, this site had one of the more complex ledges surveyed (Table 2) as well as highest cover of invertebrates (Fig 5C) and above-average rock cover (6.3%).

Figure 4. Mean percent cover (± SE), by major category, for all sites combined by inside and outside proposed research area (n=6 per research area). Note different scales on Y axis for each plot.

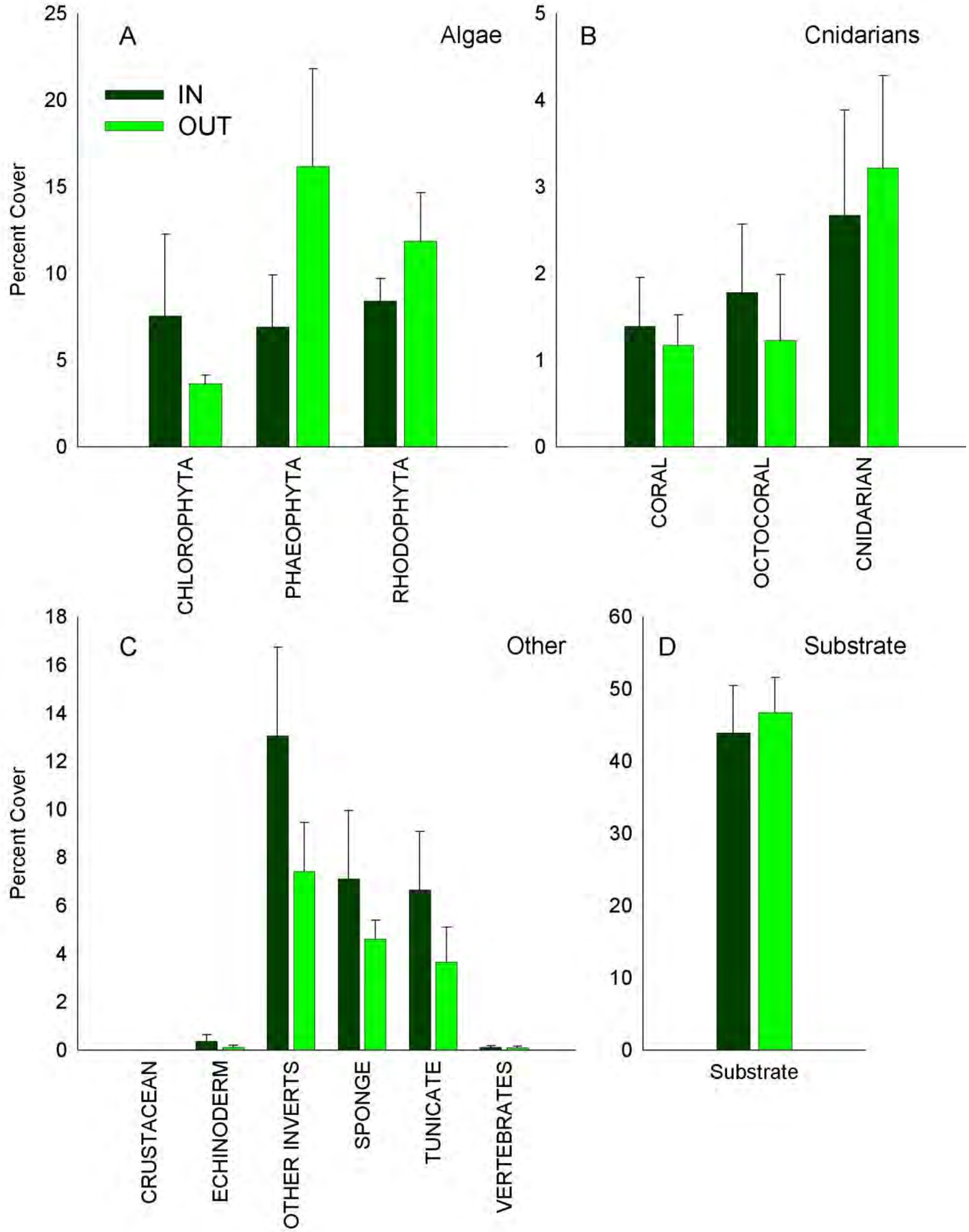
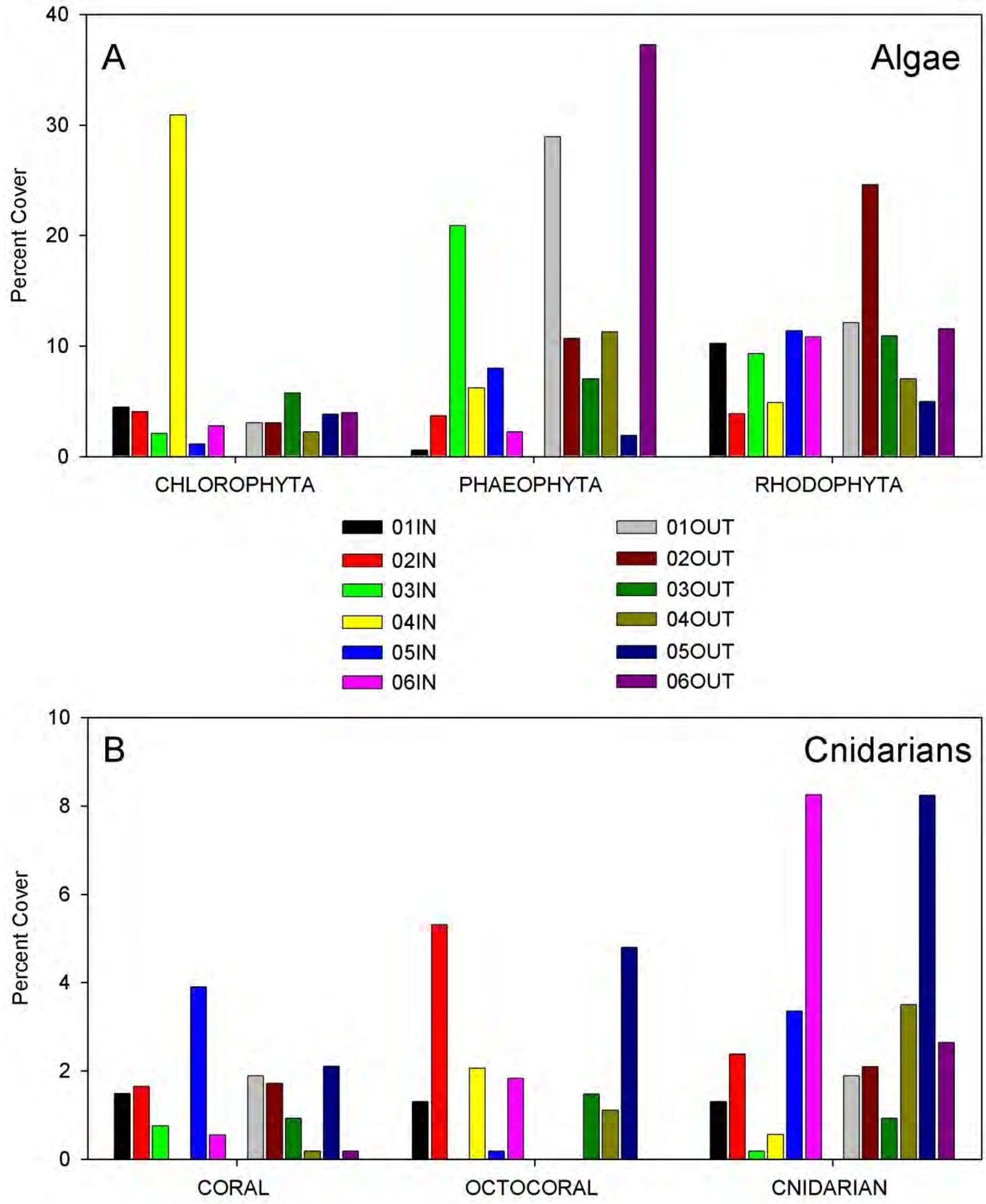


Figure 5. Percent cover by major habitat category for each site surveyed during August 2010 (n = 11 images, 525 – 546 classification points per site).



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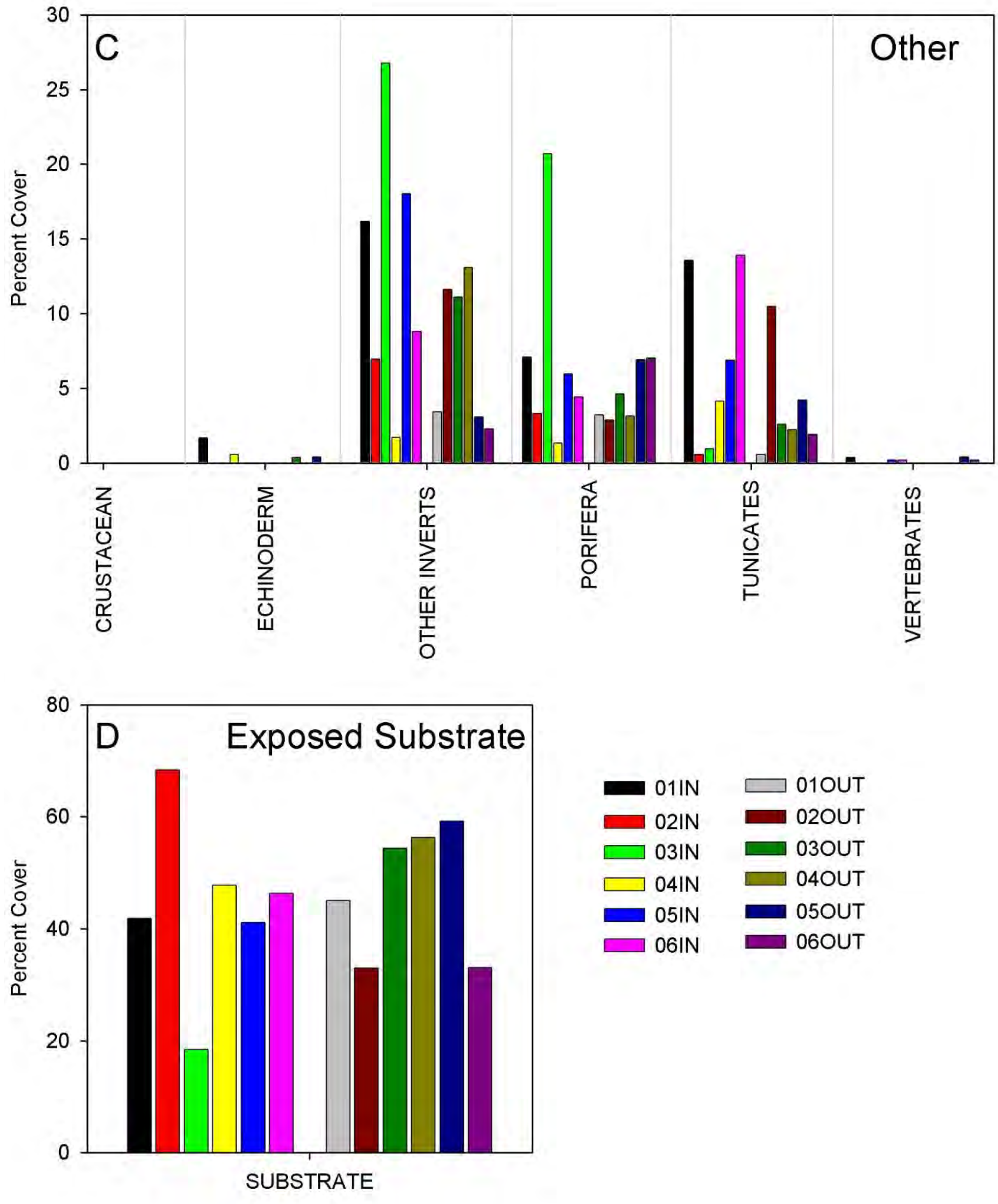


Fish Community Assessments

A total of 44 species of fish were observed at GRNMS (Table 4). As expected, the three different sampling methods did show differences in the species of fishes observed and the richness of species sampled. The 50 m transects for conspicuous fishes portrayed a richer component of the live-bottom community (35 species) than either the 50 m cryptic/juvenile prey (hereafter prey) transects (20 spp) or the stationary prey quadrats (22 spp). Multivariate analyses of the densities of conspicuous and prey fish communities observed among sites indicated no significant differences between management zones (ANOSIM, $R_{conspicuous} = -0.091$, $p = 0.70$, Figs 6A & 7A; $R_{cryptic/prey} = -0.005$, $p = 0.38$, Figs 6B & 7B).

In terms of density of conspicuous fishes overall, three species composed 90% of the community: tomtate, scad, and slippery dick (Fig 8A). For the prey fish community overall, the pattern was similar, with tomtate, scad, slippery dick, and belted sandfish forming 91% of the community observed (Fig 8B).

The pattern of biomass among sites for conspicuous and prey fishes did not match that of densities. For conspicuous fishes, schooling species (blue runner, great barracuda, *Seriola* amberjack) or large bodied species (gag, red snapper) accounted for the majority of the biomass observed (Fig 9). For the prey fish community, species that occurred in the greatest densities (tomtate, scad, slippery dick, belted sandfish, black sea bass) were also those species that formed the majority of small bodied fish biomass observed (Fig 10).

Figure 6. Community composition (based on densities) of conspicuous fishes (A) and prey fishes (B) from sites inside and outside the proposed research area at Gray's Reef.

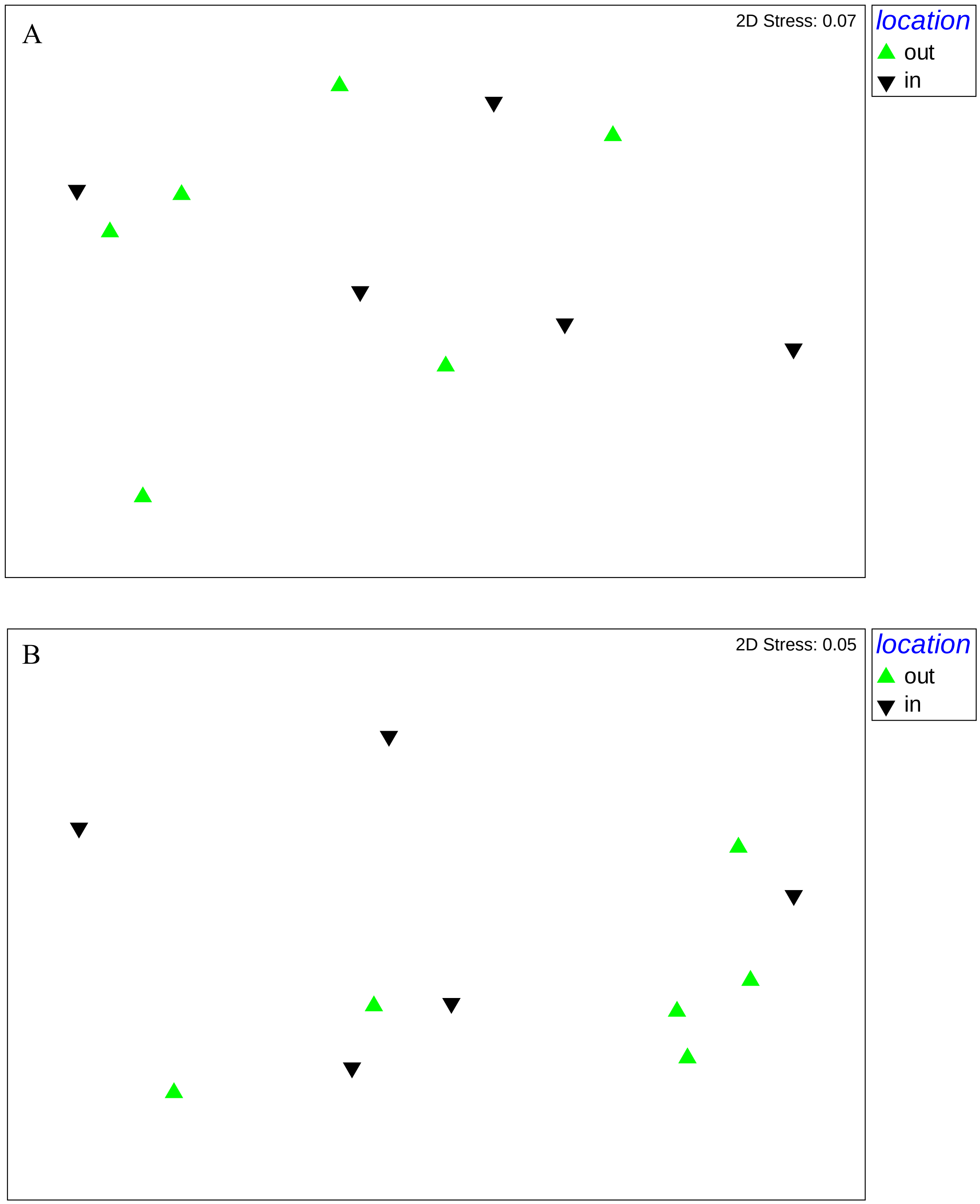
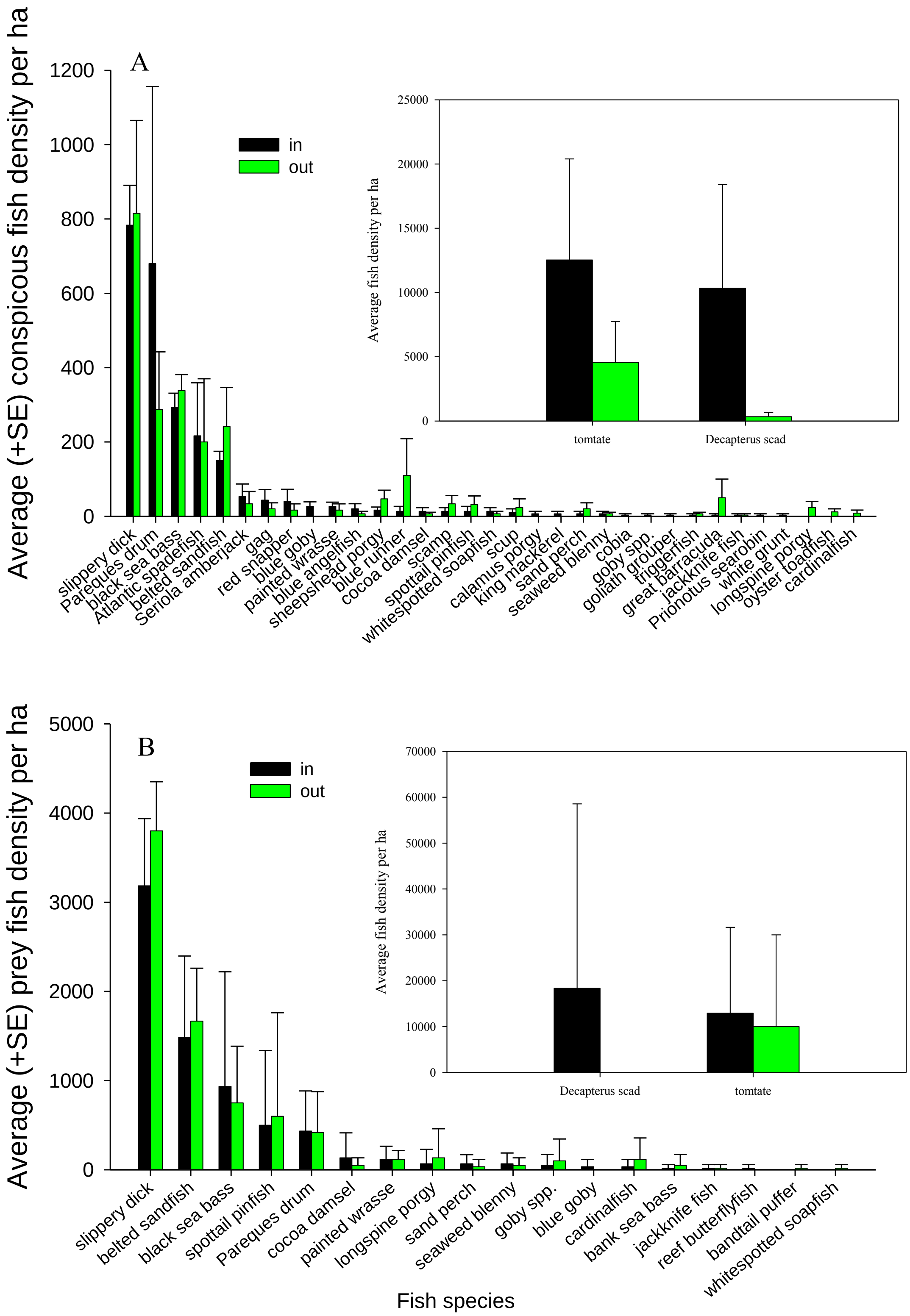


Figure 7. Average conspicuous fish density (A) and prey fish density (B) from sites inside and outside the proposed research area.



During August 2010, the size distributions of select economically important species did not show differences between management zones (Figs 11 & 12). Although there was a broader size range of gag in the research area (including 40 – 90 cm fish, Fig 11A) compared to outside (70 – 90 cm fish, Fig 11B), the pattern was reversed for scamp (60 – 80 cm fish inside, Fig 11C, versus 20 – 90 cm fish outside, Fig 11D). Red snapper showed a similar pattern to gag regarding size range and management zone (Figs 11E & F) while black sea bass showed similar size ranges between management zones (Figs 11G & H). When examined together, however, these differences were not significant (ANOSIM, $R = -0.016$, $p = 0.47$). The size ranges of representative prey fishes also did not show differences between management zones and largely overlapped (Fig 12, ANOSIM, $R = -0.035$, $p = 0.54$).

When classifying ledges at our study sites as either tall (>25 cm high) or short (25 cm or less in height) according to work by Kendall et al. (2009) based at GRNMS, we also found a distinct community structure of prey fishes associated with each type of ledge (Fig 13A, ANOSIM, $R = 0.336$, $p = 0.048$). The observed dissimilarity between ledge types was primarily driven by differences in the densities of tomtate, scad, spottail pinfish, and black sea bass, which together contributed nearly 65% to the observed dissimilarity (similarity percentages [SIMPER] analysis on square root transformed densities, $\text{tomtate}_{\text{tall ledge}} = 130.90$ vs. $\text{tomtate}_{\text{short ledge}} = 44.32$; $\text{scad}_{\text{tall}} = 0.0$ vs. $\text{scad}_{\text{short}} = 59.46$; spottail $\text{pinfish}_{\text{tall}} = 27.98$ vs. spottail $\text{pinfish}_{\text{short}} = 6.39$; black sea $\text{bass}_{\text{tall}} = 14.11$ vs. black sea $\text{bass}_{\text{short}} = 32.41$). Although a similar pattern is apparent for conspicuous fishes (compare the distribution of points in Fig 13B to the mélange associated with lack of structure in Fig 6), the observed differences were not significant (ANOSIM, $R = 0.135$, $p = 0.18$).

Figure 8. Average density of conspicuous (A) and prey fishes (B) overall from all sites.

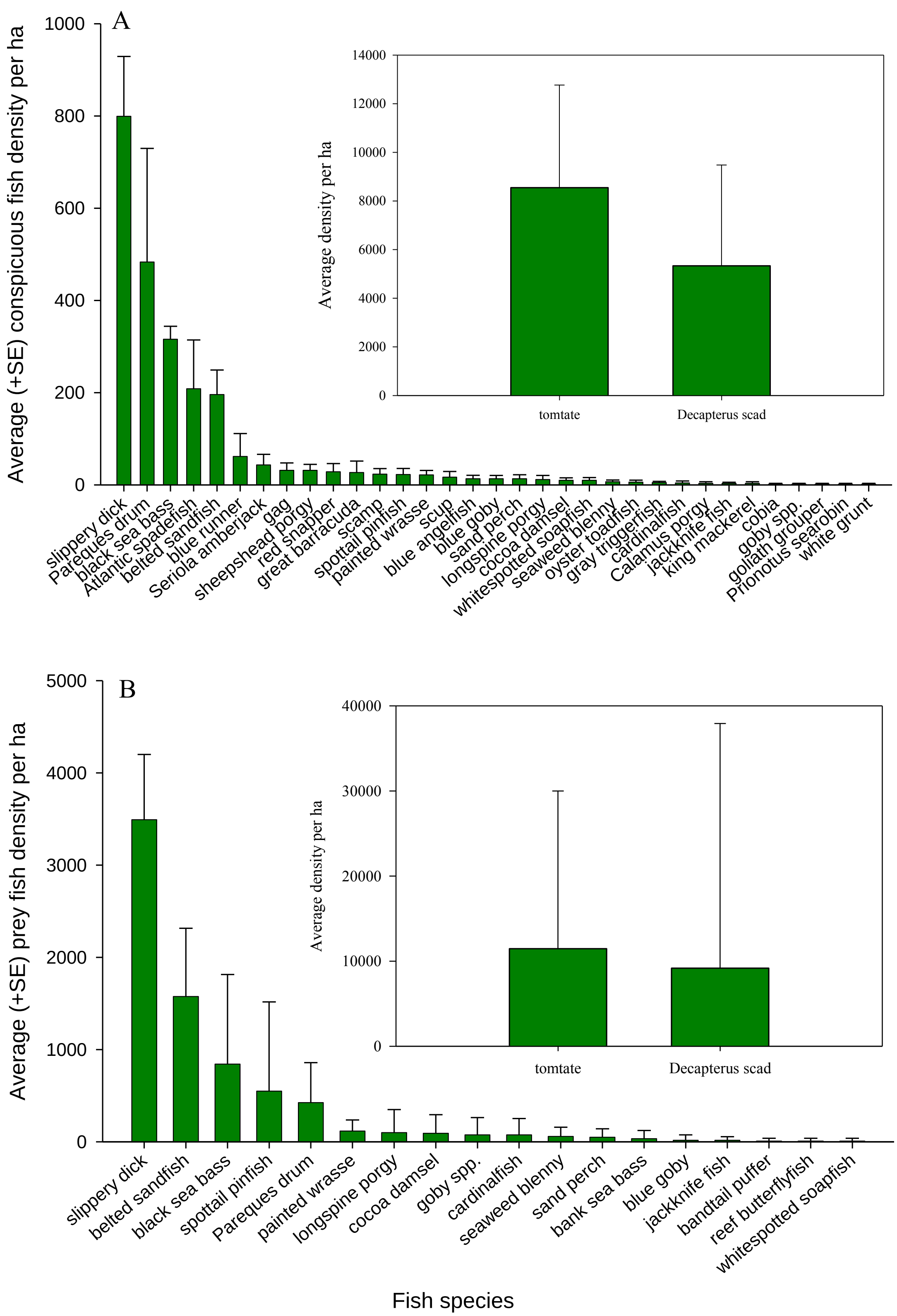


Figure 9. Average total biomass of conspicuous fishes overall from all sites (A) and by management zone (B).

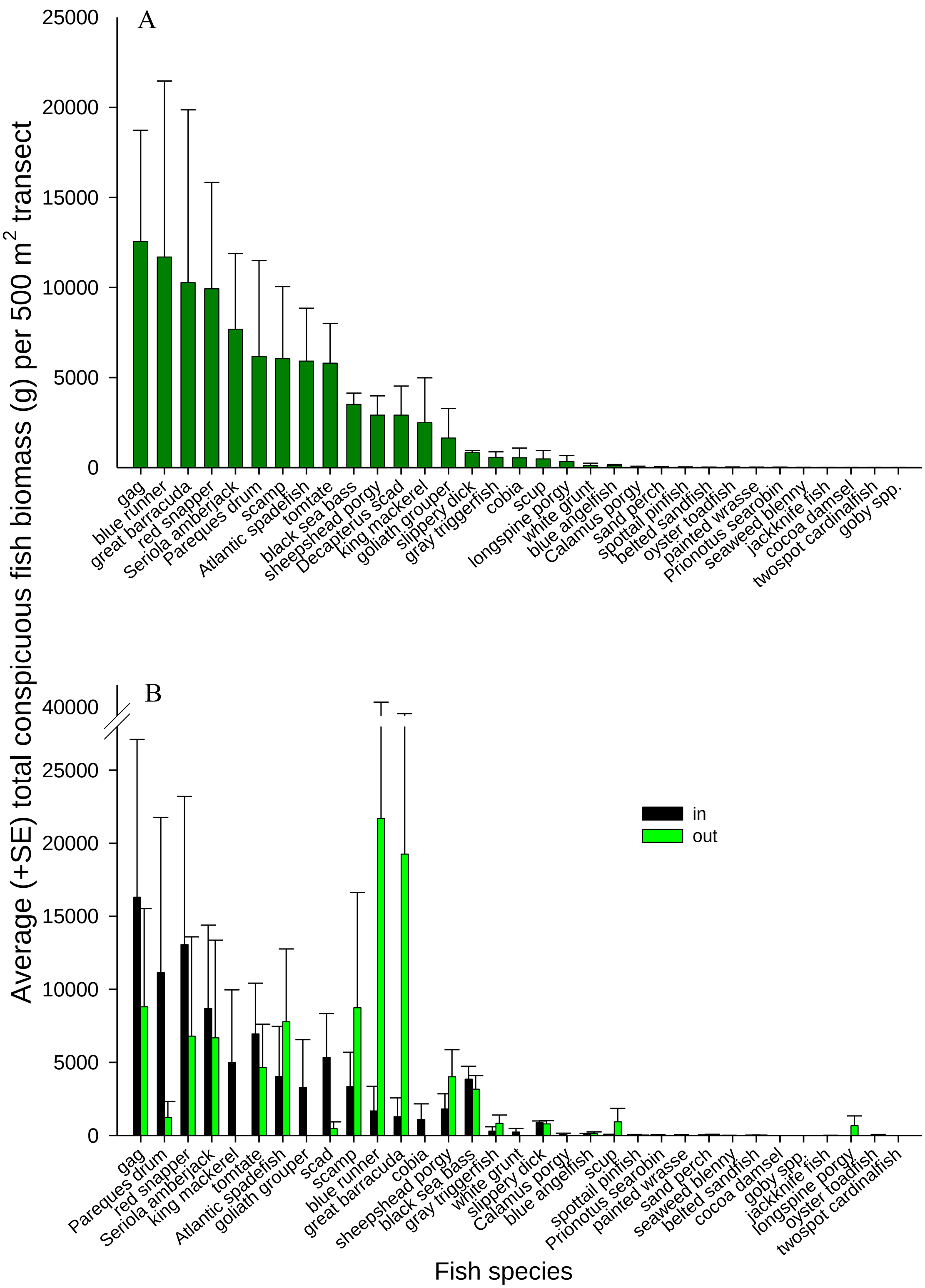


Figure 10. Average total biomass of prey fishes overall from all sites (A) and by management zone (B).

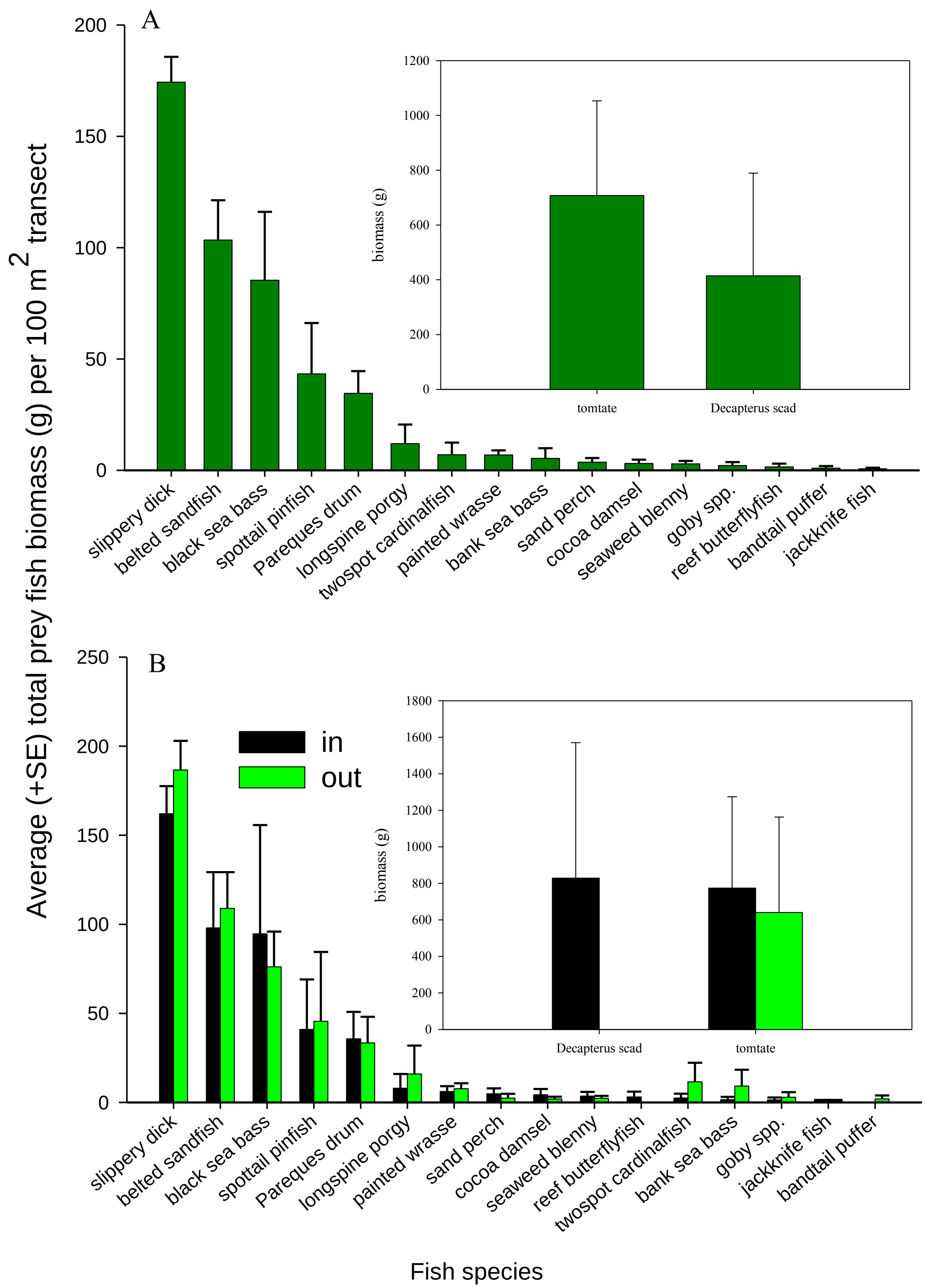
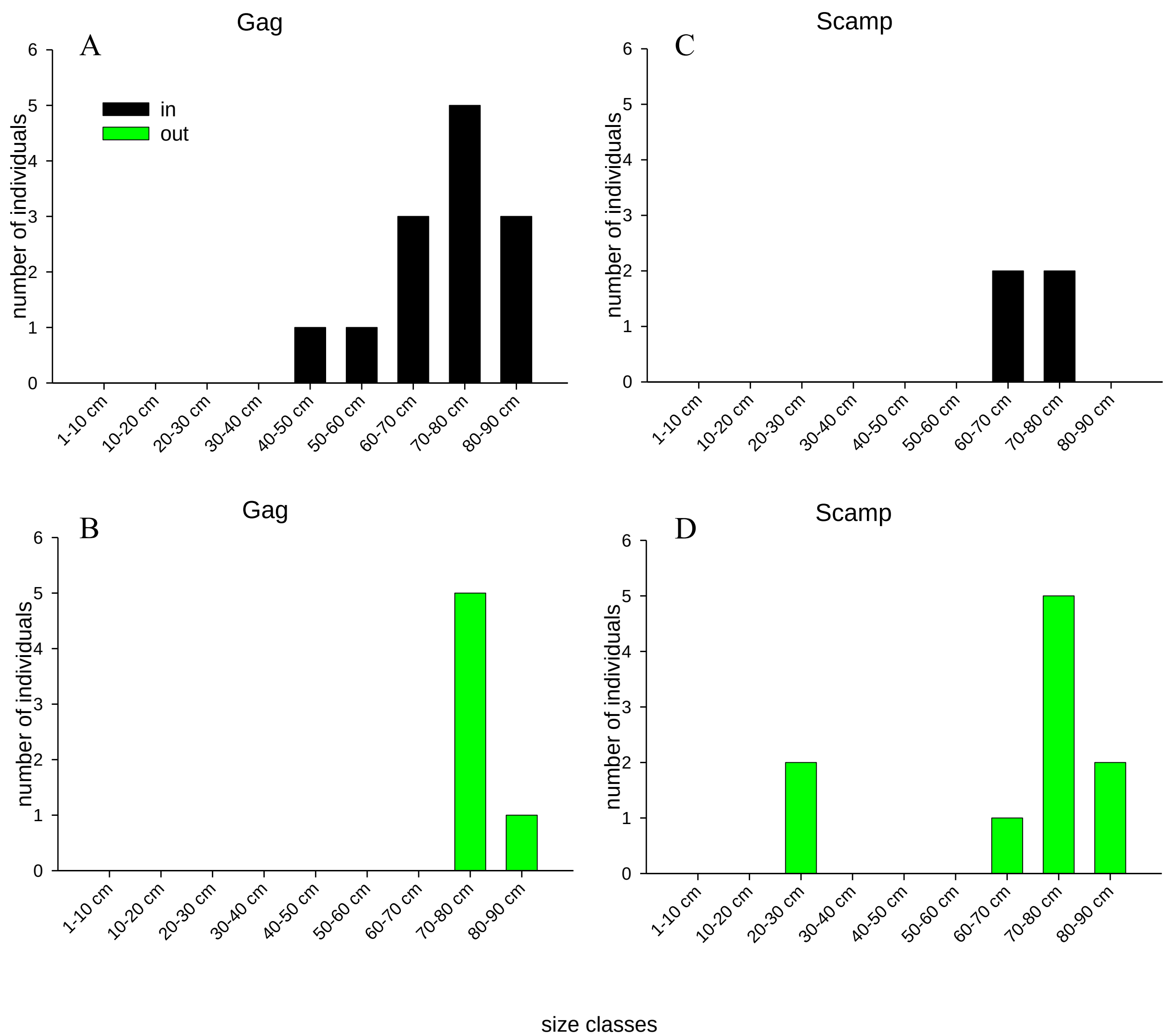


Figure 11. Length frequency comparison between management zones for select conspicuous fishes. Size classes are total length (TL).



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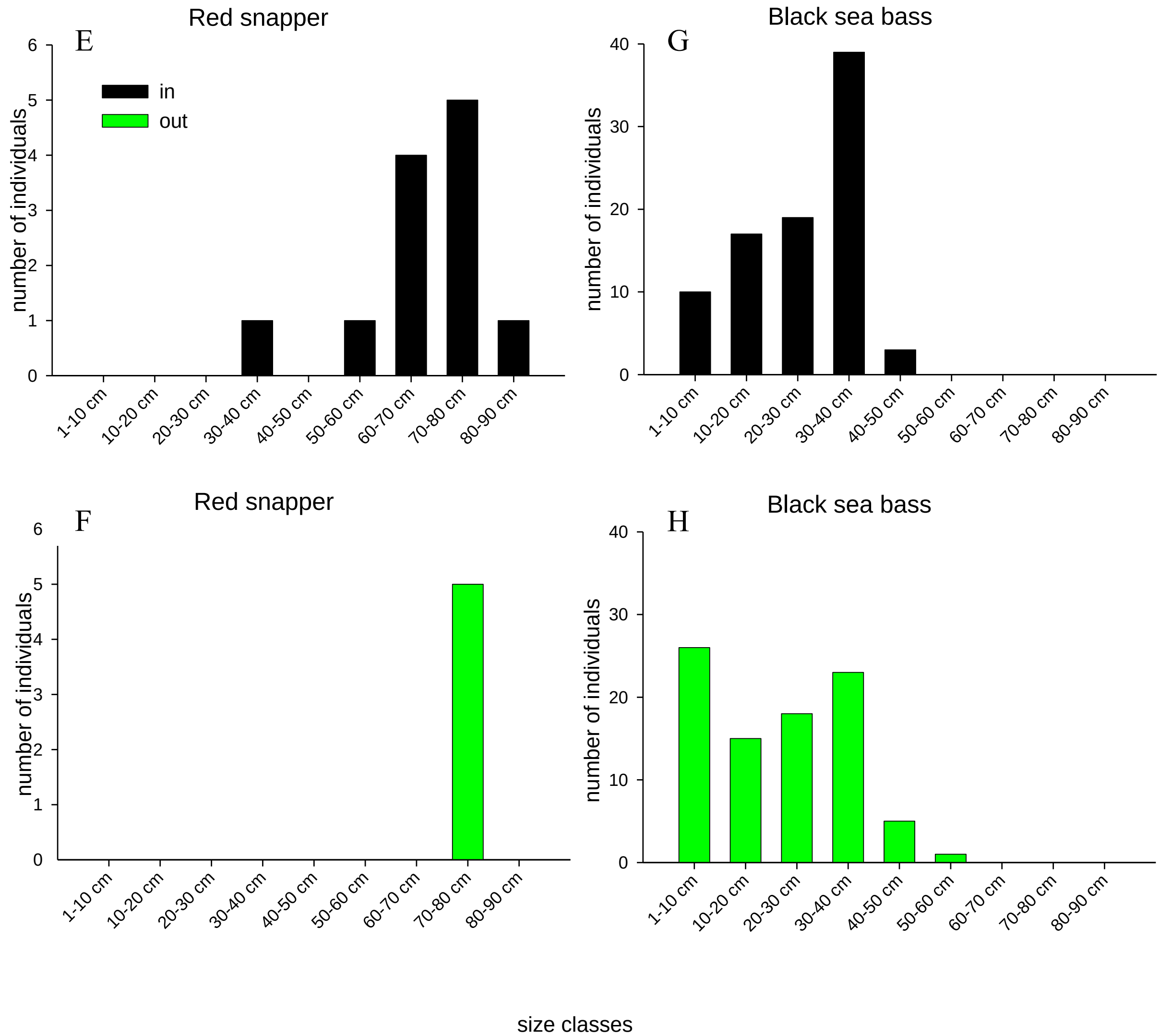


Figure 12. Length frequency comparison between management zones for select prey fishes. Size classes are total length (TL).

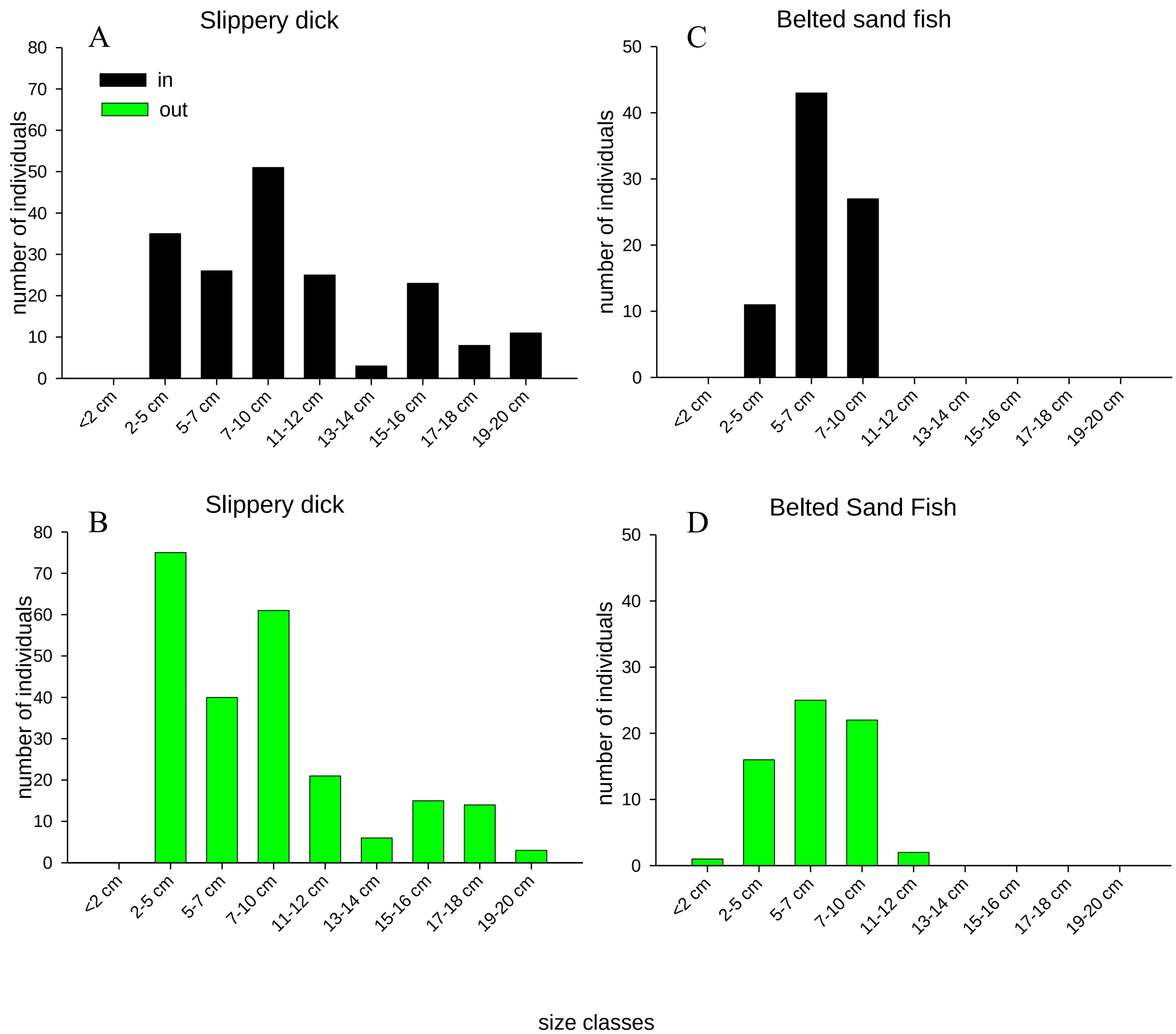


Figure 12 continued

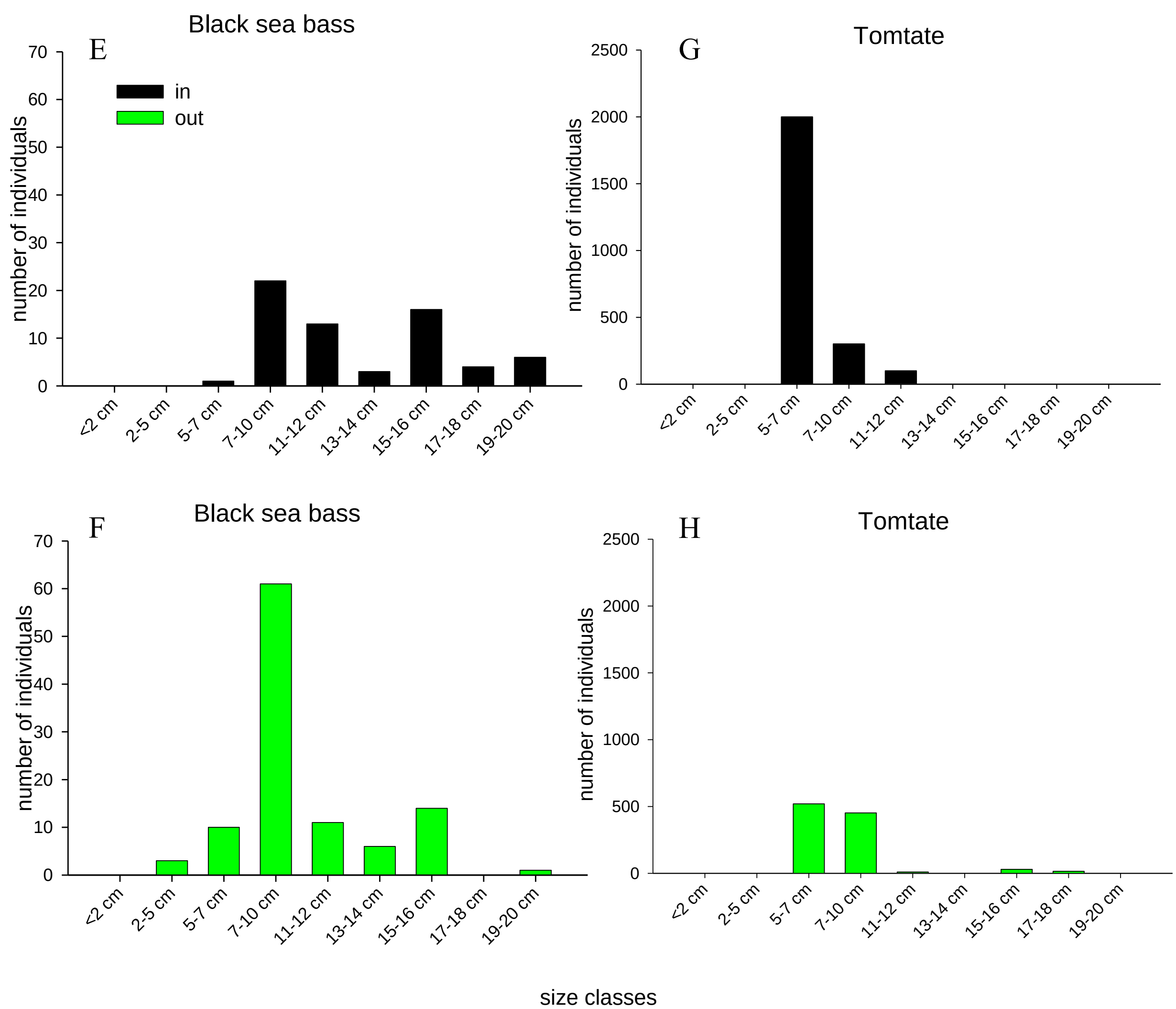
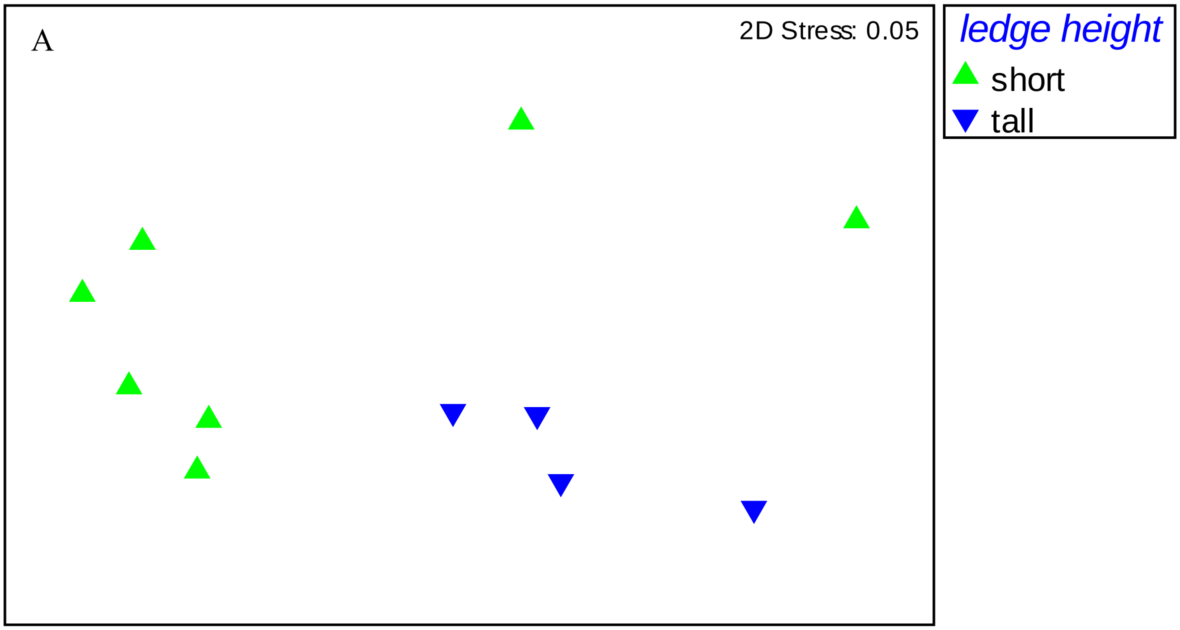


Figure 13. Community composition (based on densities) of prey fishes (A) and conspicuous fishes (B) associated with two different ledge types at Gray's Reef.



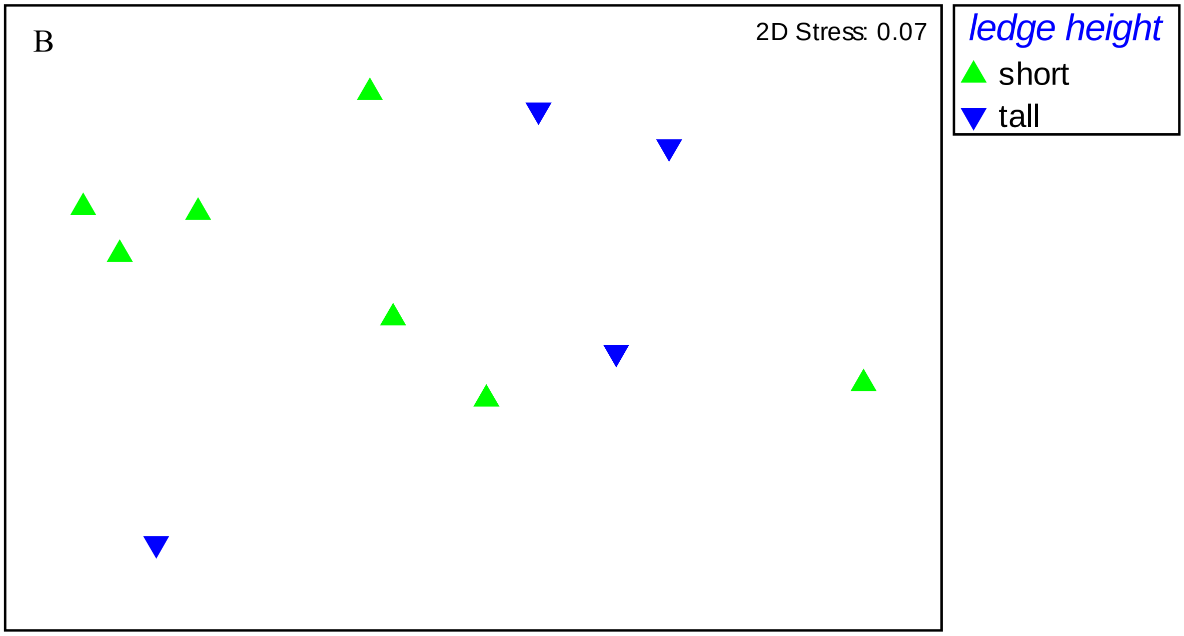


Table 3. Habitat species and groups listed by major category found at all sites in August 2010 at Gray's Reef National Marine Sanctuary.

Major Category	Species Group	Species or Description of species group
Algae	Chlorophyta	
		Codium species
		Caulaerpa mexicana
		Cladophora prolifera
		Unknown Green
		Fuzzy Green / Turf
	Phaeophyta	
		Dictyopteris species
		Dictyota species
		Sargassum filipendula
		Turf / filamentous
		Fuzzy brown / *Hinksia species*
		Unknown Brown
	Rhodophyta	
		Botryocladia occidentalis
		Crustose coralline / *Peysonnelia species*
		Eucheuma isiformis
		Rhodymenia pseudopalmata / Gracilaria mammalaris
		Halymenia trigona / Scinaia complanata
		Halymenia sp. (wide blade)
		Solieria filiformis
		Tubular red
		Strap-like red
		Filamentous red
		Unknown red
Cnidarian	Coral / Scleractinia	
		Oculina sp.
		Cup coral (Phyllangia americana, Paracyathus pulchellus)
	Octocoral	
		Leptogorgia hebes
		Leptogorgia virgulata
		Telesto sp.
		Titanideum frauenfeldii
		Sea fan

	Sea rod Soft Coral, unknown spp.
Other Cnidarians	Hydroid Anemone Zoanthids Other / Unidentifiable
Porifera	*Aplysina fulva* *Clathria prolifera* *Spirastrella sp.* *Ciocalypta gibbsi / Axinyssa ambrosia / Raspailia sp.* Ball Sponge, unknown spp. Encrusting sponge, unknown spp. Rope sponge, unknown spp. Other sponge, unknown spp.
Tunicates	*Euhermania gigantean* *Styela – Molgula sp.*(sea squirts) *Symplegma sp.* Tunicate, unknown spp.
Echinoderms	*Arbacia punctulata*
Other Invertebrates	*Amathia sp.* *Schizoporella cornuta* *Schizoporella floridana* Bryozoans Worms Unknown / Unidentifiable Invertebrate *Chondrosia – Didemnum spp.*
Vertebrates	Fish (unspecified species)
Bottom Types	Microalgae mat Film (organic / inorganic deposit) Sediment / sand Shell or shellhash Crack / crevice / hole Rock

Table 4. Species list from fish surveys in August 2010 at 12 stations inside and outside the proposed research area at Gray's Reef National Marine Sanctuary.

Family	Common Name	Genus	species	Sampling methodology[a] C, P, Q
Acanthuridae				
	doctorfish	*Acanthurus*	*chirurgus*	Q
Apogonidae				
	twospot cardinalfish	*Apogon*	*pseudomaculatus*	C, P, Q
Balistidae				
	gray triggerfish	*Balistes*	*capriscus*	C
Batrachoididae				
	oyster toad	*Opsanus*	*tau*	C
Blenniidae				
	crested blenny	*Hypleurochilus*	*geminatus*	Q
	seaweed blenny	*Parablennius*	*marmoreus*	C, P, Q
	blenny species			Q
Carangidae				
	Atlantic bumper	*Chloroscombrus*	*chrysurus*	Q
	blue runner	*Caranx*	*crysos*	C
	scad species	*Decapterus*		C, P, Q
	amberjack species	*Seriola*		C
Chaetodontidae				
	reef butterflyfish	*Chaetodon*	*sedentaris*	P
Ephippidae				
	atlantic spadefish	*Chaetodipterus*	*faber*	C
Gobiidae				
	blue goby	*Ptereleotris*	*calliurus*	C, P
	goby species	.	.	C, P
Haemulidae				
	tomtate	*Haemulon*	*aurolineatum*	C, P, Q
	white grunt	*Haemulon*	*plumieri*	C

Labridae				
	painted wrasse	*Halichoeres*	*caudalis*	C, P, Q
	slippery dick	*Halichoeres*	*bivittatus*	C, P, Q
Lutjanidae				
	red snapper	*Lutjanus*	*campechanus*	C
Paralichthyidae				
	gulf flounder	*Paralichthys*	*albigutta*	Q
Pomacanthidae				
	blue angelfish	*Holacanthus*	*bermudensis*	C
Pomacentridae				
	cocoa damselfish	*Stegastes*	*variabilis*	C, P, Q
Rachycentridae				
	cobia	*Rachycentron*	*canadum*	C
Sciaenidae				
	drum species	*Pareques*		C, P, Q
	jackknife fish	*Equetus*	*lanceolatus*	C, P
Scombridae				
	king mackerel	*Scomberomorus*	*cavalla*	C
Serranidae				
	goliath grouper[b]	*Epinephelus*	*itajara*	C
	belted sandfish	*Serranus*	*subligarius*	C, P, Q
	black sea bass	*Centropristis*	*striata*	C, P, Q
	bank sea bass	*Centropristis*	*ocyurus*	P, Q
	gag	*Mycteroperca*	*microlepis*	C
	sand perch	*Diplectrum*	*formosum*	C, P, Q
	scamp	*Mycteroperca*	*phenax*	C
	whitespotted soapfish	*Rypticus*	*maculatus*	C, P, Q
Sparidae				
	longspine porgy	*Stenotomus*	*caprinus*	C, P
	scup	*Stenotomus*	*chrysops*	C, Q
	sheepshead	*Archosargus*	*probatocephalus*	C
	spottail pinfish	*Diplodus*	*holbrookii*	C, P, Q
	pinfish	*Lagodon*	*rhomboides*	Q
	porgy species	*Calamus*		C

Sphyraenidae				
	great barracuda	*Sphyraena*	*barracuda*	C
Tetraodontidae				
	bandtail puffer	*Sphoeroides*	*spengleri*	P
Triglidae				
	searobin species	*Prionotus*		C, Q

[a]C = observed with transects of conspicuous fishes, P = observed with transects of cryptic/prey fishes, Q = observed with stationary quadrats of cryptic/prey fishes; [b] = not recorded from previous studies of fishes at Gray's Reef by Kendall et al. (2007, 2009), Gilligan (1989), or Parker et al. (1994).

Discussion

Habitat at GRNMS

All ledge measurements collected during this study were smaller than those measured by Kendall et al. (2007) but seasonal sand transport events that alternately cover and uncover rock ledges are a characteristic of live-bottom reefs of the Southeast U.S. (Renaud et al. 1997). Such an influx of sand between the two survey periods may explain the differences in measurements. Furthermore, although the present study sites utilized coordinates from Kendall et al. (2007), inherent variation in transect position once on the bottom as well as differences in sample sizes of ledge measurements (n=5 per transect by Kendall et al. [2007] vs. 5-10 in this study) may have contributed to the dissimilarity in ledge heights. Nonetheless, the ledges we sampled supported a high diversity and percent of biological benthic cover as well as fish species. No apparent differences in the density and diversity of habitat cover were found between the inside and outside research area (RA) strata. Combining all sites, mean biological cover was 55%, which is slightly higher than comparable ledge habitats in Kendall et al. (42%). Excluding macroalgae, cover of all other benthic organisms (coral, octocoral, sponge, and other benthic organisms) determined here was similar to that of Kendall et al (2007). Macroalgae cover measured here (31% outside RA, 23% inside) was greater than that reported by Kendall et al. (2007; 18%), but some of the variability between these two studies may be due to temporal (seasonal) differences.

Previous surveys of offshore North Carolina live-bottom reefs identified large seasonal variation in macroalgae species composition and cover (Peckol & Searles 1984, Freshwater et al. unpublished). Based on trends observed in North Carolina, we anticipate peak algal cover at GRNMS to occur in September – October, depending on the seasonal water temperature, although anecdotal observations indicate that the peak may occur earlier (Gleason D, personal communication). Minimum algal biomass and diversity typically are found during a period of minimum bottom water temperature (January – March). Kendall et al's (2007) surveys of GRNMS occurred in August (as the current study) but also in May. In addition, algal percent cover reported by Kendall et al. (2007) is combined between the two time periods, precluding a direct comparison between studies.

Methodological differences between Kendall et al. (2007) and the current study may have also contributed to the differing results in benthic community composition. A technique comparison study conducted in the Florida Keys National Marine Sanctuary (Edwards unpublished) examined data from stationary quadrats quantified *in situ* (method of Kendall et al 2007) and still photographs (photoquadrats) analyzed in the laboratory (method of the current study). In this methodological comparison Edwards (unpubl.) found that the biotic cover for the two techniques was not different, while abiotic cover was greater using *in situ* quadrats. Additionally, the benthic community composition from photoquadrats was identified to a finer taxonomic resolution than with *in situ* quadrats.

The additional species-level detail documented in the current study may be preferred when examining fine-scale (changes in species abundance/representation together with percent cover) community changes which may occur with changing climate, or via trophic cascades resulting from fish community shifts following changes in fishing pressure. Although total dive time at each site was similar, sample size and data processing time requirements of *in situ* vs. photoquadrat techniques were different. Habitat quantification of *in situ* quadrats was completed on site (n=5 samples per transect) but the method lacks a visual permanent record, such as photographs or video that can be reviewed or reanalyzed at a later date. In contrast, photoquadrats (n=11 images and 550 points per transect) provide a permanent historical record of each site and are classified in the laboratory, requiring approximately 1.5 hrs per transect. Although the post-collection processing time requirement is greater for photoquadrats, the larger sample size, species-level detail, and historical reference photos are improvements over *in situ* quadrat methods. In a similar study, Preskitt et al. (2004) also found estimates of percent cover of abundant species to be more precise using photoquadrat methods compared with *in situ* point intercept methods. If photoquadrat sampling is selected for habitat characterization as part of a larger scale survey at GRNMS, plans must include the higher level of commitment (e.g., habitat technician/outside collaboration) necessary for post-collection processing time requirements.

Fishes at GRNMS

The number of species of fish (44) observed in this study was lower than previous studies by Gilligan (1989, 91 spp), Parker et al. (1994, 60 spp), and Kendall et al. (2007, 2009, 72 spp) but these authors visited a larger number of sites or made more dives than the 12 sites of the current study (e.g., > 80 dives, 22 – 92 sites). These differences are expected given that sampling intensity affects species richness, which tends to increase with survey effort (Magurran 1988). Whereas Kendall et al. (2007) suggested a potential shift in community composition in comparison to the fish community sampled at GRNMS by Parker et al. (1994), we observed only one species (goliath grouper, *E. itajara*) that had not been previously observed by other investigators. There were differences among current survey methods (e.g., 50 m conspicuous fish transect yielded 16 unique species, 50 m prey transect yielded 2 unique species, stationary prey quadrat yielded 6 unique species) in the species richness generated and particular species sampled. Note that 16 unique species for the conspicuous fish transect represents species that may have been observed by the prey transects or quadrats but that would not have been recorded if over 10 or 20 cm, respectively. Conspicuous transects surveyed the greatest area (500 m^2) and also returned a greater species richness overall than prey transects (100 m^2) or prey quadrats (4 m^2). Stationary surveys are known to survey a greater diversity and density of fishes than transects (Colvocoresses & Acosta 2007, Kulbicki et al. 2010), yet surveys that encompass a larger area (such as transects) are necessary to adequately sample larger, more mobile species (Holzwarth et al. 2006, Kulbicki et al. 2010, Ward-Paige et al. 2010, Richards et al. 2011). At GRNMS, linear features such as ledge habitats may be best sampled by a linear survey method such as belt/strip transects (Kendall et al. 2007). If a goal of future surveys is to aim for a complete characterization of the fish community then a combination of transects and stationary quadrats may be desired, although this combination of methods as currently configured may reduce the number of stations that can be sampled. However, since the current management goal is to obtain a sufficient number of samples (thereby increasing statistical power) to allow the detection of any differences in fish communities between management zones, a reevaluation of current methods is justified. For example, cryptic/juvenile prey fishes might be sampled exclusively with the transect method by a single diver who would also survey conspicuous fishes. At the same time, another diver would characterize structural features of habitat such as ledge height and undercut height. Two additional divers would characterize biotic benthic cover with *in situ* quadrats for species identification and estimates of percent cover, combined with photoquadrats to provide a permanent visual record. Rather than sampling each site in two dives, substituting or combining all tasks into a single dive would allow a doubling of effort.

If only considering the densities of cryptic fishes, our overall estimates (2.8 fish/m^2) are similar to estimates from a comparable temperate reef fish community off California (~3 fish/m^2) that employed nearly identical methods to our own (Allen et al. 1992). Overall cryptic fish density was 1.75 times greater than conspicuous fish density, emphasizing the importance of including sampling methods to adequately characterize this often ignored fish community (Allen et al. 1992, Smith-Vaniz et al. 2006). Our estimates of overall (conspicuous and cryptic prey) fish densities (4.4 fish/m^2) are lower than previous estimates from GRNMS by Kendall et al. (2007) and Parker et al. (1994) (21 vs. 8-20 fish/m^2, respectively). Parker et al. (1994) employed diver video transects and this differing methodology may explain their higher density estimates. Kendall et al. (2007) utilized nearly identical methods to this study except the area surveyed in their shorter transects was five times smaller, which may explain their higher density estimates (Colvocoresses & Acosta 2007). Given the differences in methodology or area surveyed among studies, we compared the rank order of the five most abundant species that we observed with those from the earlier studies. All three studies ranked tomtate and scad as the top two most abundant species in GRNMS, and were in general agreement on the importance of slippery dick, *Pareques* drum, black sea bass, and longspine porgy in terms of abundance. Both Parker et al. (1994) and this study found slippery dick to be the third most abundant species, with either drum (this study) or black sea bass (Parker et al. 1994) occupying the fourth and fifth most abundant place. Similarly, Kendall et al. (2007) placed black sea bass and longspine porgy as the fourth and fifth most abundant species, and observed slippery dick as the 8^{th} most abundant. The similarities among studies in the top five to ten most abundant species suggests that the community structure of dominant species at GRNMS has remained relatively stable over a period of nearly 25 years, though a shift in the composition of less abundant species may have occurred since the mid eighties(Kendall et al. 2007). Less commonly observed, larger bodied species were ranked more abundant in the current study (gag, great barracuda, red snapper, *Seriola* amberjack, and scamp ranked 9-14) compared with Kendall et al.'s (2007) prior survey (although scamp ranked similar [14] between studies, gag tied with other species at rank 16-27, and the same remaining species ranked lower than 27).

Overall total biomass of prey fishes in this study (16.1 g/m^2) is higher than the temperate reef in California (5.2 – 5.7 g/m^2), and may be related to the large difference in body size between the two most abundant species at GRNMS (tomtate and scad, 25 and 34 cm maximum total length [TL], respectively) compared with California (island kelpfish, *Alloclinus holderi*, and spotted kelpfish, *Gibbonsia elegans*, 10 and 16 cm max TL, respectively). Regarding conspicuous fishes at GRNMS, 7 species were common between Kendall (2007) and this study when examining the rank order of the top ten species for overall biomass, although the species-specific rank order differed between studies. Both studies ranked blue runner in the top two in terms of biomass, but

Kendall et al. (2007) found relatively smaller bodied species accounted for the majority of fish biomass (*Pareques* drum, Atlantic bumper, black sea bass, sheepshead, Atlantic spadefish, as well as scamp). In contrast, this study ranked gag before blue runner for biomass, followed by the relatively large bodied great barracuda, red snapper, *Seriola* amberjack, and scamp, as well as *Pareques* drum.

Although not significantly different between management zones, the size ranges of select economically important species in this study show similar patterns to those from Kendall et al. (2007) who compared size ranges with two levels of boat density (a proxy for fishing effort). We found a broader size range for gag and red snapper in the research area compared to outside, similar to low versus high boat densities, although this pattern was reversed for scamp. Presumably, scamp should show a similar pattern to fishing effects and the pattern we observed may reflect the low sample size. Both studies showed similar size ranges between management zones (or boat densities) for black sea bass; the select prey fishes whose size ranges we examined also showed this pattern.

We found a distinct community of prey fishes associated with short or tall ledges, which was primarily differentiated by the higher abundances of tomtate and spottail pinfish at tall ledges. These results agree with previous studies (Kendall et al. 2007, 2009, Schobernd & Sedberry 2009). However, other species such as scad and black sea bass have also been found to be associated with tall ledges, though in the current study cryptic/juvenile size individuals of these two species showed the reverse pattern. These differences may reflect the patchy distribution of scad combined with our small sample size or reflect that our findings apply to cryptic/juvenile size individuals that may show different patterns from adults. Indeed, Kendall et al. (2007) showed that black sea bass (all size classes) abundance was negatively correlated with the presence of gag or scamp, and these groupers were typically associated with tall ledges(Kendall et al. 2009). The community of conspicuous fishes was not sufficiently distinct to allow statistical differentiation between short and tall ledges, although a trend was apparent in the analyses which might have been strengthened with a larger sample size.

No significant differences in conspicuous fishes, cryptic/juvenile prey fishes, or habitat community structure or cover were seen between management zones. However, robust comparisons between management zones were not possible nor expected given the limited time frame and low sample size of this study. We identified structural features (ledge characteristics) of sites at GRNMS, characterized the biological component of benthic habitats, and identified conspicuous and cryptic fish communities. The dominant members of the fish community that we observed were consistent with previous studies from GRNMS spanning nearly 25 years, suggesting stability in the species composition of the top five to ten most abundant species on the live-bottom reefs. Furthermore, our sampling was of sufficient resolution to allow detection of previously identified relationships between fishes and structural habitat for the cryptic fish community.

The results provide a detailed census of live-bottom fish and benthic community structure that can provide a template for additional comprehensive sampling scheduled for Spring/Summer 2011. The discussion and comparison of potential sampling methods for benthic habitat and fishes can aid in selection of protocols for future sampling. Once accomplished, the planned increase in sample size of 20-48 medium to high relief sites per management zone will provide an ample pre-implementation baseline for live-bottom areas inside and outside the research area, anticipated for 2011.

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