

ABSTRACT

HENSEL, ENIE. Power of Predators – The Role of Marine Fishes as Consumers and Nutrient Providers in a Changing Tropical Back-reef Ecosystem. (Under the direction of Dr. Robert R. Dunn).

Coastal habitats protect dynamic shorelines, sequester carbon, filter pollutants, and support whole fisheries. Coasts are vulnerable to both global climate change and local human impacts, e.g., nutrient loading, shoreline development, and overharvest, which threaten the provision of these ecosystem services through changes in community structure and functioning. This combination of global and local change affects species both at the top and bottom of food webs simultaneously. Therefore, realistic studies of multi-level interaction networks is the best way to predict the consequences of these changes on ecosystem services and functions. Here, I tested how human activities alter top-down and bottom-up processes within tropical marine food webs. I used a combination of surveys and experiments along Great Abaco Island, The Bahamas to ask the following questions: (1) how does shoreline development affect the relative abundance and distribution of marine megafauna, (2) what are the separate and interactive effects of overfishing and habitat degradation on reef fish community assemblage, (3) what is the relative importance between predation and nutrients shifts in determining seagrass invertebrate community composition, and (4) how does the introduction of artificial reefs alter the trophic ecology of a *Critically Endangered* predator?

(1) To determine how shoreline development affects the abundance and distribution of megafauna, I used a consumer-grade drone to survey sharks, rays, and turtles in developed and non-developed tidal creeks. Importantly, a detection success experiment determined that drones are an effective, non-invasive tool to monitor megafauna in shallow, clear waters. My surveys showed, on average, 35% more megafauna along non-developed shorelines than developed

shorelines. (2) To determine the relative importance of overfishing (top down) and habitat degradation (bottom up) on reef fish communities, I designed a fully factorial experiment manipulating predator presence and reef structural complexity on 16 artificial reefs. Over three months, I found the presence of a predator and increased reef habitat complexity had an additive effect on reef fish total abundance, with increased fish abundance by 250% and 300% compared to reefs without predators and decreased complexity, respectively. (3) To mechanistically test the role of fishes' consumptive and fish-derived nutrients provision effects on seagrass invertebrate communities, I conducted surveys and predator exclusion experiments within an ongoing, seven year factorial manipulative field experiment that manipulated local ambient nutrient dynamics. In low nutrient waters, I found that invertebrate biomass was strongly decreased by fish predation. However, when human-derived nutrients were present, additive effects of top-down (negative) and bottom-up (positive) control on invertebrate biomass and species density were present. (4) Lastly, I examined changes in the diet and movement behavior of juvenile Nassau grouper residing on natural and artificial reefs. Over a seven month study period, I found individuals located on natural reefs were larger on average than individuals on artificial reefs, grouper diet depended on individual fish size and home reef surrounding habitats, and for shifts in grouper movement, reef type altered how often individuals resided away from reef habitat. I found artificial reefs may be a simple management tool to enhance complex habitat within coastal systems, but because there were shifts in the trophic ecology of an important reef predator, wildlife managers should consider the potential cascading consequences for changes in inter- and intraspecific species interactions before artificial are introduced into a system.

Overall, my dissertation research has shown that both shifts in top-down and bottom-up processes to the coast create different food web composition, trophic position, and ultimately, ecosystem functioning for coastal animal communities. Determining relative importance of rapid anthropogenic changes depend on trophic group and strength of stressors but predicting overall effects of simultaneous change on whole ecosystems requires examination of these effects in tandem.

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Power of Predators – The Role of Marine Fishes as Consumers and Nutrient Providers in a
Changing Tropical Backreef Ecosystem

by
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DEDICATION

I dedicate my thesis to the people of The Abacos, The Bahamas. I hope my work aids in mitigating the effects of human-driven disturbances in Bahamian nearshore environments to help achieve ecosystem resiliency and resource sustainability.

*“There is no guarantee that people will care if they know,
but it is certain they cannot care if they do not know.”* – Dr. Sylvia Earle

“Unless someone like you cares a whole awful lot, nothing is going to get better. It's not.”
— Dr. Seuss, The Lorax

BIOGRAPHY

I grew up along the coastlines of Florida, and have experienced firsthand how dependent we are on coastal ecosystem services including protection from storms, food and job security, and recreation with incredible views. My background and professional experiences has led me to pursue a career that will help mitigate negative human impacts on coasts, promote nearshore ecosystem resiliency, and highlight the positive human impacts of restoration and sustainable efforts.

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**CHAPTER 1: Using a small, consumer-grade drone to identify and count marine
megafauna in shallow habitats**

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Research Article

Using a small, consumer-grade drone to identify and count marine megafauna in shallow habitats

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ABSTRACT. Large-bodied animals, megafauna, are disproportionately threatened and yet, remain relatively difficult to monitor, particularly true in the ocean. Consumer-grade drones have high definition imagery and offer a non-invasive way to monitor a subset of marine megafauna, especially those species that spend part of their life near the water's surface. However, a key question is the extent to which drone imagery data offer reliable abundance estimates due to potential detection restraints, and the ability to compare data from different locations. Here we tested the efficacy of a quadcopter drone to collect megafauna abundance data in multiple shallow-water habitats in the realistic background variation of shoreline development. On Great Abaco Island, The Bahamas we repeated drone surveys in nearshore habitats from June to August 2015 at three paired high and low human population sites. We tested the drone's detection probability using decoy organisms and found no effect of water quality or benthic characteristics on detectability. In short, the drones appear to work to monitor these species. We also noted patterns in the occupancy of the species on which we focused. We observed three shark, two ray, and two sea turtle species, finding higher abundances of all species in our low human population sites compared to high human population sites. Our results highlight the ability of consumer-grade drones to estimate the abundance and distribution of large-bodied elasmobranchs and sea turtles in shallow water habitats. Further, our study supports their capability to evaluate issues related to the conservation and management of nearshore ecosystems.

Keywords: Unmanned Aerial Vehicles (UAVs), non-invasive monitoring, human impacts.

INTRODUCTION

Large-bodied animals, megafauna, are some of the animals most vulnerable to and impacted by human activities (Lewison *et al.*, 2004; Dirzo *et al.*, 2014). Many megafauna species, particularly marine species, remain difficult to monitor due to their large home ranges and sensitivity to being captured and handled (Hueter & Manire, 1994). Aerial surveys are one methodology used to measure the size, density, and distribution of megafauna populations that spend part of their life near the ocean's surface (Loughlin *et al.*, 1992; Pollock *et al.*, 2006; Koski *et al.*, 2009). Aerial surveys allow researchers to monitor animals with low to no intrusion, thereby minimizing biases in observer presence and a detriment to animals (Jolly 1969; Hodgson *et al.*, 2013; Christie *et al.*, 2016). Traditionally conducted with observers on a small aircraft or a type of Unmanned Aerial Systems (UASs), such as

military Unmanned Aerial Vehicles (UAVs), aerial surveys were limited to studies that could obtain proper aircraft permits and meet budgetary requirements (Pollock *et al.*, 2006). In the last decade, small, consumer-grade UAVs, herein drones, have become readily available, increasing the use of aerial imagery to study a wide range of fauna and flora, likely due to these drones being more affordable and easier to use than more traditional UASs (Koh & Wich, 2012). Also, technological advancements in camera imagery have increased detection probability from traditional aerial monitoring methods (Grier *et al.*, 1981; Hodgson *et al.*, 2013). Because of these improvements, small, consumer-grade drones may be a promising tool to assist conservation and management agencies in assessing how human activities affect sensitive marine megafauna.

Aerial surveys conducted in marine environments, either by manned aircraft or drones, have focused mainly

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on monitoring megafauna species that breach the surface; e.g., mammals and sea turtles (Marsh & Sinclair, 1989; Loughlin *et al.*, 1992; Pollock *et al.*, 2006; Koski *et al.*, 2009). However, the high definition imagery produced by drones has led to a rise in investigations that use aerial surveys to study flora below the ocean's surface (Casella *et al.*, 2017; Chirayath & Earle, 2016; Kiszka *et al.*, 2016). For some fauna species, such surveys could also work well. Shark and ray species, for example, are large-bodied organisms that can be easily detected from an aerial image, especially in shallow habitats that are less than 3 m deep (Kessel *et al.*, 2013; Kiszka *et al.*, 2016). Despite this, little research has been conducted with drones to monitor the abundance and distribution of submersed megafauna in shallow water habitats (see Kiszka *et al.*, 2016).

In this study, we used a DJI Phantom Vision 2+® drone to test the accuracy of drones for identification and estimation of sharks, rays, and sea turtles abundance in shallow, clear water habitats along Great Abaco Island, The Bahamas. We selected our survey sites in the context of the realistic background variation of shoreline development to investigate how human activities alter the distribution of these populations. Our primary objectives for this study were to determine the efficacy of small, consumer-grade drones to collect reliable data in multiple shallow water habitats, provide baseline megafauna data for newly established protected parks, and test the capability of using such data for comparison studies.

MATERIALS AND METHODS

To assess the ability of drones to estimate the abundance and distribution of marine megafauna, we surveyed six tidal creek sites along the shoreline of Great Abaco Island, The Bahamas (26°25'N, 77°10'W) from June to August 2015 (Fig. 1).

These tidal creek habitats are shallow (<3 m deep), tidally-influenced estuaries that have narrow mouths, with creek width expanding inland (Fig. 2). Each site is bordered with red mangroves (*Rhizophora mangle*), and the benthic substrate is comprised of a mosaic of sand, dense and sparse seagrass meadows, macroalgae beds, hard bottom, and patch reefs. Island-wide, Abaco's tidal creek habitats are known foraging grounds for juvenile green sea turtles (*Chelonia mydas*) (Musick *et al.*, 1997) and have locally established shark and ray populations (Valdivia *et al.*, 2017). Any location on the island known or suspected to be a potential mating site for sharks, rays, and sea turtles was omitted from this study.

We also sought to determine if drone-based aerial surveys collect marine megafauna data appropriately to compare areas of conservation concern. To test this, we used sites selected in Stoner *et al.* (2011) which were assigned one of two categories: adjacent to high human impact (shoreline development) or adjacent to uninhabited, low human impact areas. The proxy for human impact level was the number of buildings within a 3 km radius from the midpoint of each site. We considered sites adjacent with a higher number of buildings to be more disturbed by nutrient loading, construction of artificial structures, and sedimentation (Stoner *et al.*, 2011 for details). We selected three high (84-1712 buildings; Treasure Cay, Cherokee, and Sandy Point) and three low human impact sites (0-10 buildings; Hills Creek, Snake Cay, and Cross Harbour; Figs. 1-2) and, using a paired design, we coupled a high and low human impact site within the same region of the island (Fig. 2). We selected site pairs within each region for similar creek mouth shape and average creek depth. Within each region, sites had a similar location on the island, benthic substrate, and distance to open ocean and currents, but we did not measure these variables for this study. Additionally, three of our sites are national parks (Snake Cay, Cherokee, and Cross Harbour), allowing us to provide baseline data of marine megafauna abundances to local and national management agencies.

To quantify the number of sharks, rays, and sea turtles at each site, we sampled the sites within each region on the same day and outgoing tide cycle, with a total of four sampling dates. For each site survey, we conducted two consecutive drone flights, in order to switch the drone's battery and maximize survey area. Each survey began closest to the tidal creek mouth and moved up the creek to avoid double counting; we assumed individuals were unlikely to swim against the tide within the time we replaced the drone's battery. We chose the initial starting position at each creek mouth from a stratified random sampling design (Marsh & Sinclair, 1989). Then we conducted parallel line transect surveys oriented perpendicular to the shoreline, covering the entire width of the creek. For paired sites, the order of site surveyed was chosen at random. We selected sampling time and conditions to ensure that water depth was between 1-1.5 m with good clarity. To maximize aerial-view detection, we completed all transect surveys in calm sea conditions (<1 Beaufort scale), allowing us to avoid potential view obstruction from surface waves.

Additionally, we conducted surveys during an outgoing diurnal tide to minimize individuals hidden within mangroves at high tide. The travel time between surveys of the high and low human impact sites within

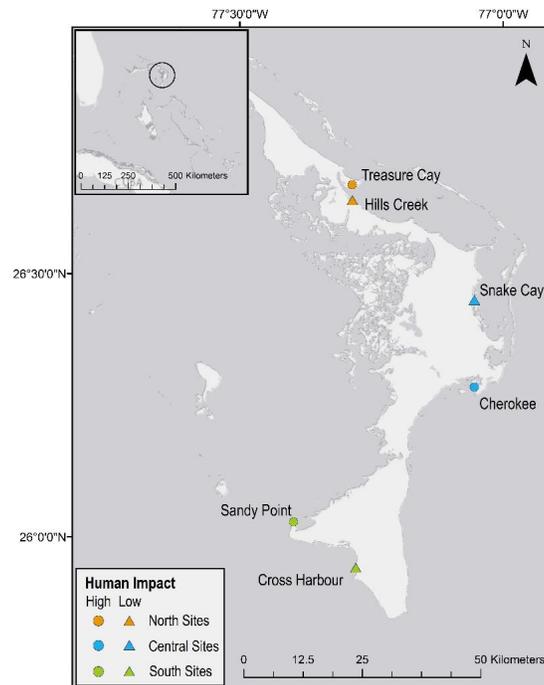


Figure 1. We conducted aerial surveys from June to August 2015 at six sites on Great Abaco Island, The Bahamas, quantifying the number of sightings of marine megafauna in nearshore habitats. Sites were classified as high or low human impact based on the number of buildings present within a 3-mile radius from the center of each human population center.

the central and north region was 20 min. For the south region, the time between site surveys was one hour, due to land access constraints.

For our aerial surveys, we used recorded video footage from a DJI Phantom Vision 2+[®] quadcopter drone (1.2 kg with camera, propellers, and battery) with a polarized lens attached to the camera to reduce glare. The camera had 14 megapixels with a resolution of 4384×3288 and high definition recording of 1080p30 & 720 p. Following the protocol of Kiszka *et al.* (2016), we flew the drone at a constant speed of 4.8 km h⁻¹ at an altitude of 7.6 m. We tilted the camera 10° from parallel to the water's surface to reduce sun glare, increasing the frame size of usable recorded footage; *i.e.*, total viable survey area covered. The total distance of each site's transect survey was determined by drone flight time, range limit of its ground controller, the shape of the tidal creek to mouth, and then modified to match its paired site optimally. Each Phantom 1-3 DJI[®] drone battery offers up to 25 min of flight time;

however, we found that the setup and total flight time for each battery gave us about 12 min of survey time. We manually conducted each drone flight path using premeasured visual landscape and seascape markings. Notably, we did not use any automated flight path software. There were compatibility issues with the DJI Phantom Vision 2+[®], and we did not have access to cellular data or wireless internet at remote locations.

To determine the detection probability of submersed individuals from the deployed drone, we set up an experiment using mock-shark individuals at our central region sites (Fig. 3). Our mock-sharks mimicked juvenile lemon sharks, (*Negaprion brevirostris*) because they were the hardest to detect in the post-video process, due to their light color and slender shape.

Two snorkelers placed 10 mock-sharks, made of grey foam weighted down with twine and weights, randomly within the transect survey area at varying depths. The drone pilot was blind to the mock-shark locations. We conducted three surveys at each central



Figure 2. North, central, and south region, paired sites were: Treasure Cay (TC) & Hill's Creek (HC), Cherokee (CH) & Snake Cay (SC), and Sandy Point (SP) & Cross-Harbour (CROSS), high and low human impact respectively. The yellow rectangles indicate where we conducted drone surveys at each site.

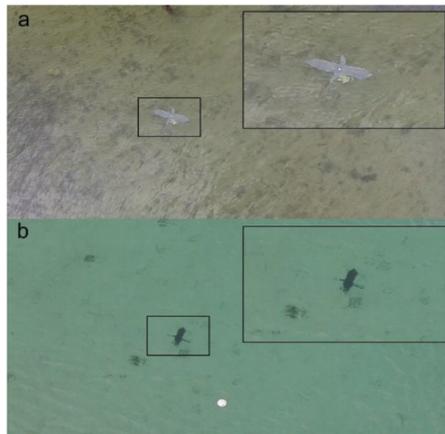


Figure 3. Images showing the mock-sharks used in our detection probability experiment at a) Cherokee (high human impact, and b) Snake Cay (low human impact). Both the drone pilot and the observer reviewing the video footage detected 100% of the mock-sharks in all six surveys.

region site, with the mock-sharks being relocated before each survey. The amount of individual mobility (e.g., resting or swimming), was not a detection variable of concern because our aerial footage was

recorded, allowing us to playback and review frames, and the movement of the drone eliminated the ability to detect whether an individual itself was moving. The central region's high human impact site had the most turbid water compared to all other sites and had the identical benthic substrate to the north region's high human impact site (i.e., sand and macroalgae beds). The central region's low human impact site contained all benthic substrates (i.e., dense and sparse seagrass meadows, sand, hard bottom, and macroalgae beds) and had same water clarity to the other low human impact sites, as well as the southern high human impact site. We then reviewed the recorded video footage in the laboratory by the drone pilot and an additional observer, blind to the number and location of mock sharks, and recorded the number of individuals detected.

To quantify the species' abundances for each site, we reviewed video recordings from both flights at each site, per sampling date, independently in the laboratory by two observers (Fig. 4). For all surveys, counts and species identification from observers were identical.

Since each drone flight recording had slightly different times (recordings were between 11-13 min), we determined the amount of reviewed footage by the region's site which had the shortest recording for each sampling date. We recorded abundance estimates for each survey as the average number of individuals per minute, over the course of both flights, per sample date.

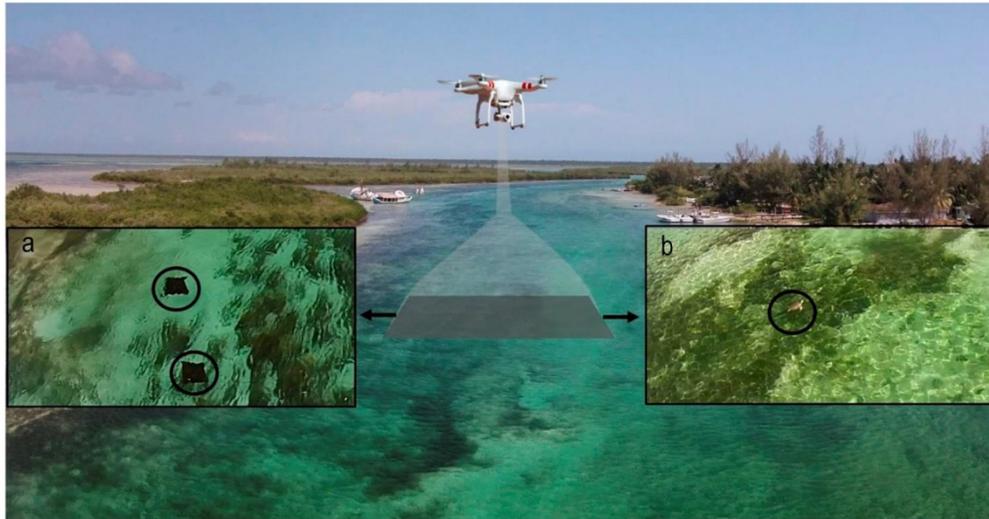


Figure 4. Example screenshots of recorded drone footage capturing a) Spotted eagle ray (*Aerobatus narinari*) and b) either a Green (*Chelonia mydas*) or Hawksbill (*Eretmochelys imbricata*) sea turtle.

Using a two-way Analysis of Variance (ANOVA), we tested the differences in average total marine megafauna, elasmobranch, and sea turtle abundance per minute between high and low human impact sites within each region. Data were log-transformed to meet model assumptions and verified using the Shapiro-Wilk test as well as diagnostic plots including Q-Q and residuals vs. fitted. We performed all statistical analyses in R v3.3.1 (R Core Team 2016).

RESULTS

Across all of our sites, we recorded five species of elasmobranchs, including lemon sharks (*Negaprion brevirostris*), nurse sharks (*Ginglymostoma cirratum*), bonnethead sharks (*Sphyrna tiburo*), southern stingrays (*Dasyatis americana*), and spotted eagle rays (*Aetobatus narinari*). We observed two species of turtles, hawksbill (*Eretmochelys imbricata*) or green turtles (*Chelonia mydas*), but we were unable to distinguish between these species in aerial imagery. “Sea turtles” (pooling the two species) were the most frequently observed taxon in our videos; we observed 145 total turtles across all sites and sample dates. Elasmobranchs were less abundant across Abaco’s creeks, observing 13 sharks and 28 rays total in all of our videos (Table 1).

For the detection probability experiment, all 10 mock-sharks were detected in all surveys ($n = 6$) in both central region sites. Our 100% detection mock-sharks gave us high confidence in the ability of our drone to detect live organisms in our survey sites. Abundances of live organisms were estimated as the average number of animals observed per minute from four replicate drone surveys per site. The south region sites had more elasmobranchs than did any other region ($F = 4.6$, $df = 2$, $P < 0.01$), estimating 0.1 ± 0.1 SE and 0.4 ± 0.1 SE elasmobranchs, for SP and CROSS respectively. Sites in our north region had the highest total abundance of megafauna, driven by a large number of sea turtles in these two sites ($F = 6.4$, $df = 2$, $P < 0.01$). For TC, we observed 0.3 ± 0.1 SE sea turtles and 0.8 ± 0.3 SE sea turtles at HC. For the central region sites, Cherokee and Snake Cay, we observed 0 and 0.3 ± 0.3 SE sea turtles and 0 and 0.1 elasmobranch, respectively.

For our comparison study testing, as a simple case example, the effects of shoreline development on marine megafauna abundance, development and region had a significant effect on the average total marine megafauna, elasmobranch, and sea turtle abundance per minute. For all response variables, there was no interaction effect between development and region: all fauna ($F = 0.9$, $df = 2$, $P = 0.88$), elasmobranchs ($F = 3.2$, $df = 2$, $P = 0.06$), and sea turtles ($F =$

Table 1. Average individuals per survey from four surveys at each site. Paired sites were surveyed on the same diurnal, outgoing tide cycle. A high (H) and low (L) human impact site is paired by island region: south, central, and north and are shaded green, blue, and orange, respectively.

Site	Average number of observed individuals per survey			
	Sea turtles	Sharks	Rays	All fauna
Sandy Point (H)	0.8 ± 0.5 SE	0.3 ± 0.3 SE	1.5 ± 0.9 SE	2.5 ± 1.3 SE
Cross Harbour (L)	3.3 ± 0.6 SE	0.8 ± 0.5 SE	3.8 ± 1.0 SE	8.0 ± 0.6 SE
Cherokee (H)	0.5 ± 0.5 SE	0.3 ± 0.3 SE	0	1.0 ± 0.6 SE
Snake Cay (L)	6.0 ± 1.2 SE	0	1.3 ± 0.6 SE	8.3 ± 2.3 SE
Treasure Cay (H)	5.2 ± 1.2 SE	0.4 ± 0.4 SE	0.6 ± 0.4 SE	6.2 ± 1.6 SE
Hills Creek (L)	13.0 ± 5.9 SE	1.0 ± 0.4 SE	0.2 ± 0.2 SE	14.8 ± 6.1 SE

0.1, $df = 2$, $P = 0.92$). For all marine megafauna (elasmobranchs and sea turtles combined), we observed twice as many animals in low human impact sites compared to high human impact sites (Fig. 5a; $F = 14.7$, $df = 1$, $P < 0.01$). Region had a slight effect on total marine megafauna ($F = 3.5$, $df = 2$, $P = 0.05$). The north region sites had more sea turtles whereas the south region sites had more elasmobranchs (Figs. 5b-5c, Table 1). Across all regions, we observed $2.5\times$ more elasmobranchs and $7.5\times$ more sea turtles in low human impact sites compared to high human impact sites ($F = 4.6$, $df = 1$, $P = 0.04$ and $F = 10.7$, $df = 1$, $P < 0.01$, elasmobranchs and sea turtles respectively; Figs. 5b-5c).

DISCUSSION

Our data suggest using small, consumer-grade drones may be an effective and non-invasive method for detecting and estimating the abundance of marine megafauna in shallow water habitats, particularly at relatively small spatial scales (*e.g.*, $<3\text{ km}^2$). Specifically, we provide evidence that these drones can detect elasmobranch species and sea turtles varying in size, shape, color, and mobility. With repeated surveys at six sites and directly testing our drone's detection probability with a mock-animal experiment, we show that drone's high definition imagery can detect elasmobranchs and sea turtles in shallow habitats within a range of water clarity residing over seagrass meadows, hard bottom, sand, and/or scattered patch reefs. Lastly, our study shows that consumer-grade drones are a useful tool to compare the abundance and distribution of marine megafauna in nearshore habitats exposed to varying intensity of human activities.

We consistently found fewer individuals of sharks, rays, and sea turtles in our high human impact sites compared to our low human impact sites (Fig. 5). However, we did not directly measure other environ-

mental variables (*e.g.*, water temperature and ocean current) that may have been the ultimate drivers for differences among our selected sites (Speed *et al.*, 2010; Schlaff *et al.*, 2014). There are a few reasons why our observed pattern could be related to various human-driven causes. There may be a higher frequency of boats (which was observed during surveys), increasing the likelihood of collisions with animals and generating substantial underwater noise (Slabbekoorn *et al.*, 2010). The removal of habitat-forming species associated with shoreline development is also likely to affect the abundance of fauna (Davenport & Davenport, 2006; Jennings *et al.*, 2008). The loss of mangroves along shorelines, for example, can decrease shelter availability for both elasmobranchs and their prey, as well as increase sedimentation affecting nearby seagrass beds or patch reefs (Rogers, 1990). In general, our findings of fewer elasmobranchs in nearshore habitats, although preliminary, are consistent with previous research conducted in Bimini, The Bahamas. Researchers found that shoreline development and the associated loss of habitat, introduction of toxic pollutants, and decrease in prey populations led to decreases in lemon shark abundance, increased mortality rates and reduced body condition of individuals. Lemon sharks, like many megafaunas, are known to provide cross-ecosystem linkages due to their extensive ranges and ontogenetic habitat shifts (Jennings *et al.*, 2008), which is essential to consider for the potential cascading or other indirect effects shoreline development can have on neighboring ecosystems (McCauley *et al.*, 2012).

The region of the island also influenced abundance trends, with the north region sites having the highest sea turtle abundance estimates and south region sites having the highest elasmobranch abundance estimates. Although The Abacos are known foraging grounds for juvenile green sea turtles, particular locations on the island may have higher sea turtle densities because of a wide range of drivers within both life history

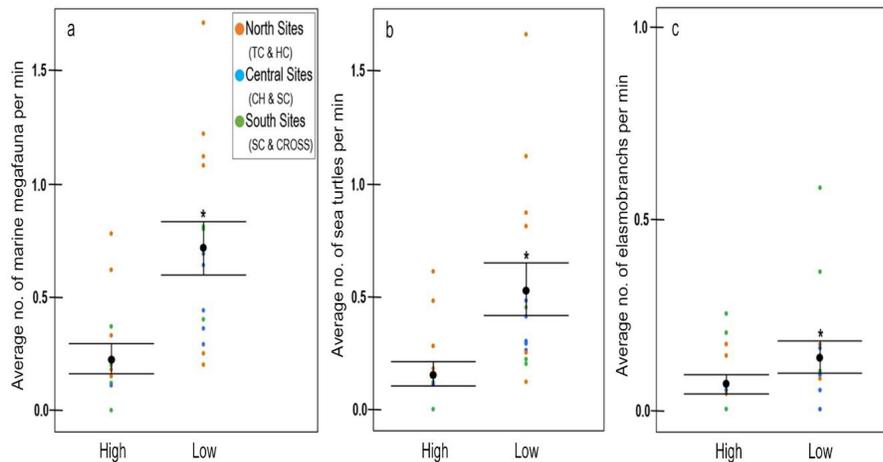


Figure 5. Sites with low shoreline development had, on the average, (a) higher abundance of total megafauna, (b) sea turtles, and (c) elasmobranchs, per minute of drone footage, than sites with high shoreline development. Between regions, the north sites had more overall sightings of sea turtles (C , $P < 0.01^*$) but there were no other differences found among regions. Asterisks indicate statistical differences ($P < 0.05$) between these site classifications.

characteristics, *e.g.*, proximity to hatching location, and local habitat characteristics, *e.g.*, benthic substrate composition and seagrass productivity (Bjorndal & Bolten, 1988; Heithaus *et al.*, 2002). We did not directly measure variables to predict why the south region sites had higher abundance estimates of elasmobranchs. However, from personal communications with local research stations and fishers, the shorelines of southern Great Abaco Island are historically well-known for their shark and ray densities. Potential environmental variables include, but not limited to, proximity to deep-waters and high densities of prey species (Speed *et al.*, 2010; Clavelle & Jylkka, 2013).

Previous studies using consumer-grade drones to estimate the abundance and distribution of fauna have typically been short-term (*i.e.*, <1 month) and/or conducted within a single survey site. Our study is one of the first to show that these drones can repeatedly survey multiple sites and collect consistent abundance and distribution data. Confidence in abundance estimates was strengthened by testing the detection probability of marine megafauna in all ranges of the benthic substrate and water clarity of our sampled sites. Similar to Kiszka *et al.* (2016), we also limited our survey sites to locations where aerial imagery could only clearly see to the benthic substrate, thereby limiting water depth and turbidity to maximize detection. Although we did not use any software to program automated drone flights,

newer drone models are compatible with most autopilot programs which can significantly expand survey area per drone flight and improve replicability of surveys. Noteworthy, for surveying remote locations, it is essential to review the calibration requirements as some drone models still require access to the wireless internet or cellular data before flying a pre-programmed flight.

We have shown that consumer-grade drones are a promising tool that is affordable, non-invasive, and easy to use for long-term monitoring of large-bodied species in subtropical and tropical nearshore habitats. Consumer-grade drones are becoming more technologically advanced, and new models typically have longer battery life and range extensions, potentially providing for increased survey length and time. Importantly, one should be aware of the potential limitations of drone models and be familiar with the local government regulations on the use of drones before using them. Monitoring how human activities affect marine megafauna is only one step in aiding in their protection and conservation-education, and public outreach is critical. The high definition footage recorded during monitoring can be used for engaging the public through social media, environmental presentations in schools, and the development of educational videos. There is still much to be considered regarding the use of drones in a conservation and management context, but our data suggest they can be a powerful tool.

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**CHAPTER 2: Effects of predator presence and habitat complexity on reef fish
communities in The Bahamas**

Prepared for publication

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Abstract

Reef ecosystems are highly diverse habitats that harbor many ecologically and economically significant species. Yet, globally they are under threat from multiple stressors including overexploitation of predatory fishes and habitat degradation. While these two human-driven activities often occur concomitantly, they are typically studied independently. Using a factorial design, we examined effects of predator presence, habitat complexity, and their interaction on patch reef fish communities in a nearshore ecosystem on Great Abaco Island, The Bahamas. We manipulated the presence of Nassau groupers (*Epinephelus striatus*), a reef predator that is critically endangered largely due to overharvest, and varied patch reef structure (cinder blocks with and without PVC) to reflect high or low complexity- four treatments in total. To assess changes in fish community composition we measured fish abundances, species richness, and evenness. We found that predator presence and high reef complexity had an additive, positive effect on total fish abundance: fish abundance increased by ~250% and 300%, compared to predator absence and low complexity reef treatments, respectively. Species richness increased with reef complexity. Variation in community structure was explained by the interaction between factors, largely driven by juvenile tomtate (*Haemulon aurolineatum*) and white grunt (*Haemulon plumieri*) abundances. Specifically, predator presence was positively and negatively associated with tomtate and white grunt abundance, respectively, suggesting predator presence altered interspecies competitive interactions. Our data suggest that both fisheries management of large-bodied piscivores and reef habitat restoration are critical to the management and conservation of reef ecosystem functions and services.

Key Words: Community structure, Nassau grouper, predator-prey, coral reef, artificial reefs, food webs, fishing, habitat degradation, trait-mediated indirect effects interactions

Introduction

Coral dominated reefs are highly productive ecosystems that can harbor large and diverse fish communities, but are threatened worldwide by myriad stressors (Hughes & Connell 1999, Hoegh-Guldberg et al. 2007). Two primary stressors are overfishing at higher trophic levels and habitat degradation (Dulvy et al. 2004, Lee 2006). These stressors can fundamentally change processes that act from both the top-down (e.g., trophic cascades) (Baum & Worm 2009, Allgeier et al. 2016, Valdivia et al. 2017) and bottom-up (e.g., nutrient cycling regimes and provision of refugia) (Beukers & Jones 1998, Lee 2006, Smith et al. 2006, Graham & Nash 2013) of interaction networks. Overexploitation of predators and the loss of habitat complexity are typically concomitant. Yet because these factors are most often studied independently, our understanding of how their simultaneous effects combine or interact to alter reef communities remains limited (but see Wilson et al. 2008).

Predators and habitat structural complexity play a central role in determining post-settlement coral reef fish communities (Hixon & Carr 1997). Predator impacts on fish communities can be direct (consumptive) and/or indirect (non-consumptive) (Almany 2004a, Stallings 2008, Chamberlain et al. 2014), both of which can be mediated by the structural complexity of reefs (Wilson et al. 2008). Specifically, habitat complexity can decrease predators' direct effects by reducing prey encounter rates (Swisher et al. 1998, Almany 2004b, Warfe & Barmuta 2004). In contrast, structural complexity can increase predators' indirect effects due to predators and their prey residing in close proximity to one another (Grabowski et al. 2008). To date, most studies examining predator effects in marine systems have tended to use simplified interaction webs within mesocosms and/or focused on small-bodied predator species. However, fishing typically targets large-bodied predators (e.g., sharks, tunas, and groupers) that are much

more difficult to study. Further complicating this scenario is the challenge of understanding how losses of predators may interact with concomitant changes in habitat complexity that is a result of major shifts in foundation species (e.g., coral to sponge and/or macroalgae) that can render reefs flat, reducing available refugia for fauna. This potential interaction has important implications for management and conservation efforts seeking to mitigate human impacts on coral reefs.

Here we ask: how do predator presence and reef complexity affect reef fish communities in terms of total abundance, species richness, evenness, and overall community composition? We conducted an in-situ experiment designed to examine effects of large predator presence, habitat complexity, and their interaction on artificial patch reef communities in a nearshore ecosystem on Great Abaco Island, The Bahamas. Nearshore, patch reef habitats are ideal for this experiment because they are isolated, complex, vertical habitats surrounded by hard or soft low-relief substrate and, being located in nearshore habitats, they are strongly influenced by local stressors (Stallings 2009). Artificial patch reefs are particularly ideal because they are relatively easy to manipulate and subsequently study whole community effects (Carr & Hixon 1997). In a 2 x 2 factorial design, we manipulated presence of a locally abundant reef predator, Nassau grouper (*Epinephelus striatus*), on patch reefs of low and high complexity. We used Nassau grouper as our predator species because they are an important fishery species that has experienced drastic population declines throughout the Caribbean from overexploitation (Dahlgren et al. 2016, Sherman et al. 2018). Overall, we found predator presence and high reef complexity to have an additive effect on total reef fish abundance, species richness was only affected by reef complexity, and these two factors interacted when examining overall community composition.

Methods

Our study occurred in the Sea of Abaco along the eastern shoreline of Marsh Harbour on Great Abaco Island, The Bahamas, from May - August 2014 (Fig. 1). Water visibility is typically ≥ 5 m and low tide depth ranges from 2-5 m. The nearshore seascape is a mosaic of hard-bottom, sand, algal beds, seagrass meadows dominated by turtle grass (*Thalassia testudinum*), and scattered coral and artificial patch reefs. Coral patch reefs are characterized by low relief (≤ 2 m height) and typically covered with some variation of encrusting or soft coral (e.g. *Orbicella* spp. or *Gorgonian* spp.), exposed limestone, sponges, and macroalgae (e.g. *Halimeda* spp. and *Sargassum* spp.). Artificial patch reefs are broadly defined as any human-introduced structure that is submerged on the benthos, usually introduced to mimic the function of patch reefs by providing structural complexity for biota to use as refugia and foraging grounds (Seaman 2000). Our experimental units were cinder block artificial reefs, which have been used widely over the past four decades to study reef fish assemblages (e.g. Hixon & Beets 1989, Carr & Hixon 1997). In April 2014, we constructed 16 artificial reefs (~ 1.4 m³), each using 35 cinder blocks (15 x 20 x 40 cm) on mixed sand/seagrass substrate. For all 16 artificial reefs (hereafter reefs), low tide depth was ~ 2.5 -3.0 m and each was located >200 m from other artificial or natural patch reefs; location of experimental reefs followed Yeager et al. (2014). We measured seagrass density (within a 2 m radius of the reef) at the beginning of the experiment as a potential covariate (Yeager et al. 2011).

To test how predator presence and structural complexity affect reef fish communities, we randomly assigned each of the 16 reefs to one of four treatments: predator presence x high complexity (PH), predator absence x high complexity (AH), predator presence x low complexity (PL), or predator absence x low complexity (AL; Fig. 1). For predator treatments we used

Nassau groupers which have a complex life cycle undergoing a series of ontogenetic shifts in both habitat and diet (Eggleston et al. 1998, Dahlgren et al. 2006). When they are ~3 months old, individuals begin to migrate from nearshore macroalgae beds to hard bottom or patch reef habitat. At this stage, individuals show strong site fidelity to their home activity area, i.e., often returning and reusing the same patch reef habitat (Eggleston et al. 1998). Due to their high site fidelity, grouper additions are difficult, and thus removals are the optimal method to manipulate their presence/absence (Stallings *personal communication*). Prior to the start of the experiment in May 2014, all 16 experimental reefs had at least two Nassau groupers present, ranging from 16-33 cm total length (TL). For predator absence treatments, we removed Nassau groupers with either trap or hand nets and relocated each individual to a reef habitat >3 km from our study site to reduce the chance of their return (Stallings *personal communication*). We tagged each individual before release, and we did not observe the return of any individuals during the duration of our experiment. We also removed any non-native, invasive lionfish (*Pterois volitans*) throughout the study period.

To establish high complexity reef treatments, we installed a PVC tree structure simulating historically important reef-building corals within our study system, i.e., *Acropora cervicornis*, *A. palmatta*, and *A. prolifera*. Low complexity reefs had no PVC structure, and the cinder block holes were filled with cement, leaving only three large gaps for potential refugia (Fig. 1). This low complexity reef architecture mimics a shift from branching, reef building corals to boulder-like reef heads dominated by encrusting corals and macroalgae.

To determine if fish community composition differed among treatments, we monitored all reefs weekly with Underwater Visual Census (UVC) surveys and also deployed GoPro[®] cameras three times at each reef during the study to monitor fish assemblages when we were not

present to verify predator treatments. E. Hensel conducted UVC surveys using a mask and snorkel. A UVC survey entailed monitoring each reef for 10 minutes and recording all fishes within 1 m of the reef at the species-level. Once all the active swimming fish were recorded, a flashlight was used to search every reef hole twice for less active or cryptic species. During the last two weeks of the experiment, visual estimates for the TL of each fish were recorded to the nearest centimeter. We used UVC surveys to quantify species richness and evenness, as measured by the reciprocal Simpson's $D = 1/\sum(n/N)^2$, where n is the abundance of that species per survey and N is the number of total species per survey (Simpson 1949).

For all analyses, we used UVC surveys from 60 days after reef construction. We did not include Nassau groupers in fish community response variables. We used two-way analysis of covariance (ANCOVA) to test for independent and interactive treatment effects on total fish abundance, species richness, and the reciprocal Simpson's Index of Diversity (Simpson 1949) at the species-level. For main factor effects, we used a one-way ANCOVA. We log transformed fish abundance prior to analysis to meet homoscedasticity assumptions, and included seagrass density in each model as a potential covariate (but removed this variable when not significant for reasons of parsimony).

To analyze fish community structure across treatments, i.e., relative abundance of the constituent species, we used non-metric multidimensional scaling (nMDS), permutational multivariate analysis of variance (PERMANOVA), and two-way crossed analysis of similarity (ANOSIM) on square-root transformed fish abundances at the species-level. We then conducted similarity percentage analysis (SIMPER) computations using the Bray-Curtis similarity coefficient, limited to the species contributing to the top 70% of dissimilarity between treatment's reef fish assemblage (Bray & Curtis 1957). The ANOSIM test statistic was computed under 999

permutations. Herein, we refer to the results of this nMDS analysis of reef fish assemblages as community structure. We conducted all analyses using program R (Team 2017).

Results

For UVC surveys at 60 days after reef construction, we documented a total of 2,461 fishes from 45 species and 19 families. Wrasses (Labridae), parrotfish (Scaridae), damselfish (Pomacentridae), and grunts (Haemulidae) were present on all 16 reefs.

Community abundance and biodiversity

Predator presence and reef complexity had significant positive effects on total fish abundance, but their interaction was not significant (Table 1). The highest fish abundance was found in predator presence x high complexity (PH) treatments, with an average of six times more fish than predator absence x low complexity (AL) treatments (Fig. 2a; Fig. 3). We also tested for the same effects on total fish biomass - trends followed those of total fish abundances (see supplemental material). Species richness was only significantly affected by reef complexity, with high complexity reefs averaging ~4 more fish species compared to low complexity reefs (Fig. 2d, e; Table 1). Inverse Simpson's index of diversity at the species-level was not affected by either factor or their interaction (ANOVA global model (1, 4) = 1.27, P = 0.33; Table 1).

Community composition

Fish community structure, i.e., relative abundance of the constituent species, of high and low complexity reefs differed (PERMANOVA R = 0.28, P <0.01; Table 1; Fig. 4). For high complexity treatments, reefs with and without predators differed from each other (PERMANOVA permutations = 999, R = 0.13, P = 0.03; Table 1; Fig. 4). Based on Bray-Curtis similarity indices, differences in fish communities among reef treatments were largely due to differences in Tomtate grunt (*Haemulon aurolineatum*) abundances (ANOSIM permutations =

999, $R = 0.30$, $P < 0.01$); treatment comparisons' similarities differed between 26.9-72.9% (SIMPER). PH reefs versus AL reefs had the largest disparity in Tomtate grunt abundances, with predator presence x high complexity reefs having an average of 15x more Tomtate grunts than predator absence x low complexity reefs (Fig. 5).

Discussion

Overharvest of fishes and habitat degradation are two main anthropogenic threats to coastal and nearshore ecosystems (Lotze et al. 2006). We manipulated Nassau grouper presence and artificial patch reef complexity to simulate how simultaneous overexploitation of large-bodied predator species and reef degradation alter coral reef fish communities. We found predator presence and high habitat complexity to have a positive, additive effect on total reef fish abundance (Fig. 2 and 3). Comparing our two most extreme treatments that simulated healthy versus degraded reefs, total reef fish abundance differed by 300% (Fig. 2 and 3). High reef complexity was positively associated with species richness as well as fish abundance for almost all 45 fish species recorded, except three species in which complexity had no effect (Fig. 2). The effect of predator presence on fish abundance was dependent on fish species identity and reef type (Fig. 5). Overall, our study suggests that both top-down and bottom-up up changes (i.e., predators and habitat architecture) to interaction networks can have far-reaching effects on entire fish communities and, by extension, coral ecosystems. Further, rather than isolating top-down or bottom-up impacts, it is valuable to consider these effects in tandem.

Reefs on which predators, in this case Nassau groupers, were present tended to have a higher total abundance of fish (Fig. 2). This result may be counterintuitive because Nassau groupers at this life stage are known to consume fishes (Eggleston et al. 1998). Our result is also in conflict with studies focused on groupers in fringing or barrier reef habitat that have typically

found grouper presence to decrease prey abundance (Hixon & Carr 1997, Almany 2004c). There are a few possible explanations for this observation. First, Nassau groupers can initiate trophic cascades in which their presence has an indirect, positive effect on smaller organisms. For example, Stallings (2008) showed that the presence of Nassau groupers reduced the movement of two smaller-bodied grouper species, which indirectly increased reef fish recruitment. Such a behaviorally-mediated trophic cascade could be occurring in our study system because, even though we did not observe any small-bodied grouper species, we did observe other residential piscivores such as moray eels (Moridae) and transient predators such as jacks (Carangidae), albeit at low abundances. Another explanation might be due to unique life history characteristics of Nassau groupers. For instance, Nassau groupers are known to make nocturnal hunting migrations to nearby seagrass meadows. Therefore, the diurnal fish assemblages found on patch reefs may not be especially susceptible to predation from Nassau groupers residing on the same reef (Eggleston 1996, Sadovy & Eklund 1999). A third possibility is that Nassau groupers may select particular prey species and, in doing so, release those species' competitors; we expand on this idea below.

Our findings with respect to habitat complexity reinforce previous studies that reef structural complexity affects fish communities (Table 1 and Fig. 2; Hixon & Beets 1993, Almany 2004a, Graham & Nash 2012). Reef complexity and heterogeneity in the morphology of a reef's structure have been shown to be positively associated with fish abundance and species diversity (Hackradt et al. 2011, Kerry & Bellwood 2011), likely due to a combination of reducing competition for refugia and providing varied shapes and sizes of refugia to match fishes' morphologies. Throughout the study, we observed different fishes consistently using different parts of the reefs, which may to some degree reflect habitat niche-partitioning. For example,

grunt species were frequently observed aggregating within the high complex reefs' PVC structures that mimicked an architecture of *Acropora* spp. (Lirman 1999), while squirrelfish and soldierfish (Holocentridae) were often found within cinder block holes. Therefore, high complexity reef treatments likely reduced competition for refugia by providing space for many individuals and species to cohabitate. Our results also show that even in the presence of predators, high structural complexity seems to be an important factor mediating overall fish community composition (Fig. 4). A plausible explanation could be that our high complexity treatments decreased the consumption rate of Nassau groupers or other piscivores through the provision of smaller-sized refugia where individual piscivores could not enter (Hixon & Menge 1991, Almany 2004b).

We did not observe an interaction between predator presence and reef complexity for total fish abundance, but did observe a clear interaction when comparing fish community structure, i.e., relative abundance of the constituent species (Table 1). Our nMDS plot shows a separation between fish communities on high complexity reefs with and without predators present (Fig. 4). Results from ANOSIM and SIMPER analyses show that differences in Tomtate grunt abundances, the most abundant species on all experimental reefs, largely explain the treatment effect on reef fish community composition (Table 1). For high complexity reefs, Nassau grouper presence had a positive effect on Tomtate grunt abundance, while on low complexity reefs, the presence of Nassau groupers had a no effect on Tomtate grunt abundances (Fig. 5). A plausible explanation for this trend is that on complex reefs the presence of Nassau groupers may have altered the outcome of interspecific competition between Tomtate grunts and other fish species that utilize similar resources but that are inferior at finding refugia from groupers (Persson 1991). Examining high complexity reefs only, we compared average

abundances of similarly-sized (≤ 5 cm in TL) Tomtate and White grunts (*Haemulon plumieri*), the latter being the second most abundant species on all experimental reefs. Although statistically insignificant, Nassau grouper presence had a negative effect on White grunt abundance (one-way ANOVA $P = 0.18$; Fig. 6). Thus, Tomtate grunts may have been superior than White grunts accessing refugia when Nassau groupers were present. Lastly, we did not study the direct mechanism for predator effects on Tomtate grunt abundances and another potential explanation could be density-independent factors. For example, pelagic fish larvae settlement is known to be influenced by a reef's soundscape and both Nassau grouper and grunt species are well known to be vocal animals, and therefore, could have affected reef fish settlement patterns (Hazlett & Winn 1962, Freeman & Freeman 2016).

The high abundance of Tomtate grunts on complex reefs with groupers present could have effects on other facilitative species interactions (Meyer & Schultz 1985). For example, Huntington et al. (2017) suggested that large fish aggregations on high complexity reefs provide sufficient consumer-mediated nutrients to facilitate coral growth and survivorship, which in turn can increase reef complexity over time and feedback to support larger fish aggregations. In general, grunt species form dense, diurnal aggregations on reefs, and make nocturnal migrations to nearby seagrass and mangrove habitats to hunt (Meyer et al. 1983). Because they often makeup a large percentage of the biomass found on reefs and make nightly migrations, they are thought to be important transporters of critical nutrients for primary producers including corals (Allgeier et al. 2017). In light of our results that show grunt abundances are sensitive to predator presence, we argue that current impact assessments could be underestimating the ecological impact of intense fishing pressure on large-bodied reef predators like Nassau grouper.

Our study is one of few to examine how fisheries-targeted predator removal, declining reef habitat complexity, and their interaction affect fish community assemblage using in situ manipulations. Currently, most coral reef ecosystems throughout the world are faced with multiple stressors and, consequently, it is important to understand not only how species interactions may change, but also how these changes may scale up to alter community composition and ecosystem function. In the context of fisheries management and coral reef restoration and conservation, we have shown how the removal of large-bodied piscivores and the decrease in reef complexity can alter important interactions among reef fish communities. We suggest that in order to maintain biodiverse coral reef communities and preserve ecosystem processes, management should focus on both the conservation of large-bodied piscivores and the restoration of reef habitat complexity, through either reintroducing live coral or introducing artificial structure mimicking lost coral morphology.

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Compliance with Ethical Standards

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Conflict of Interest: E. Hensel declares that she has no conflict of interest. C. A. Layman declares that he has no conflict of interest. J. E. Allgeier declares that he has no conflict of interest.

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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Tables and Figures

Table 1 Results of two-way ANOVA and PERMANOVA for reef fish communities based on fish species' abundances at 60 days after reef construction

	Source	df	SS	MS	F	P
Total Fish Abundance						
ANOVA	Complexity	1	2.99	2.99	9.97	<0.01*
	Predator	1	2.44	2.44	8.12	0.02*
	Complexity*Predator	1	0.50	0.50	1.67	0.22
	Residuals	12	3.60	0.30		
<i>Main effects</i>	Complexity	1	2.99	2.99	9.48	<0.01*
	Predator	1	2.44	2.44	7.72	0.02*
	Residuals	13	4.10	0.32		
Tomtate Abundance						
ANOVA	Complexity	1	12.37	12.37	6.50	0.03*
	Predator	1	6.00	6.00	3.13	0.10
	Complexity*Predator	1	11.43	11.43	6.00	0.03*
	Residuals	12	22.85			
Species Richness						
ANOVA	Complexity	1	64.00	64.00	11.73	<0.01*
	Predator	1	2.25	2.25	0.41	0.53
	Complexity*Predator	1	4.00	4.00	0.73	0.41
	Residuals	12	65.50	5.46		
<i>Main effect</i>	Complexity	1	64.00	64.00	12.49	<0.01*
	Residuals	14	71.75	5.12		
Simpson's Index						
ANOVA	Complexity	1	0.97	0.10	0.82	0.38
	Predator	1	0.38	0.38	3.20	0.10
	Complexity*Predator	1	<0.01	<0.01	<0.01	0.10
	Residuals	12	1.41	0.11		
Community Structure						
PERMANOVA Permutations = 999	Complexity	1	0.06	0.06	0.28	<0.01*
	Predator	1	0.02	0.02	0.08	0.16
	Complexity*Predator	1	0.03	0.03	0.13	0.03*
	Residuals	12	0.11	0.01	0.52	
Community Structure						
ANOSIM Permutations = 999					R 0.30	<0.01*

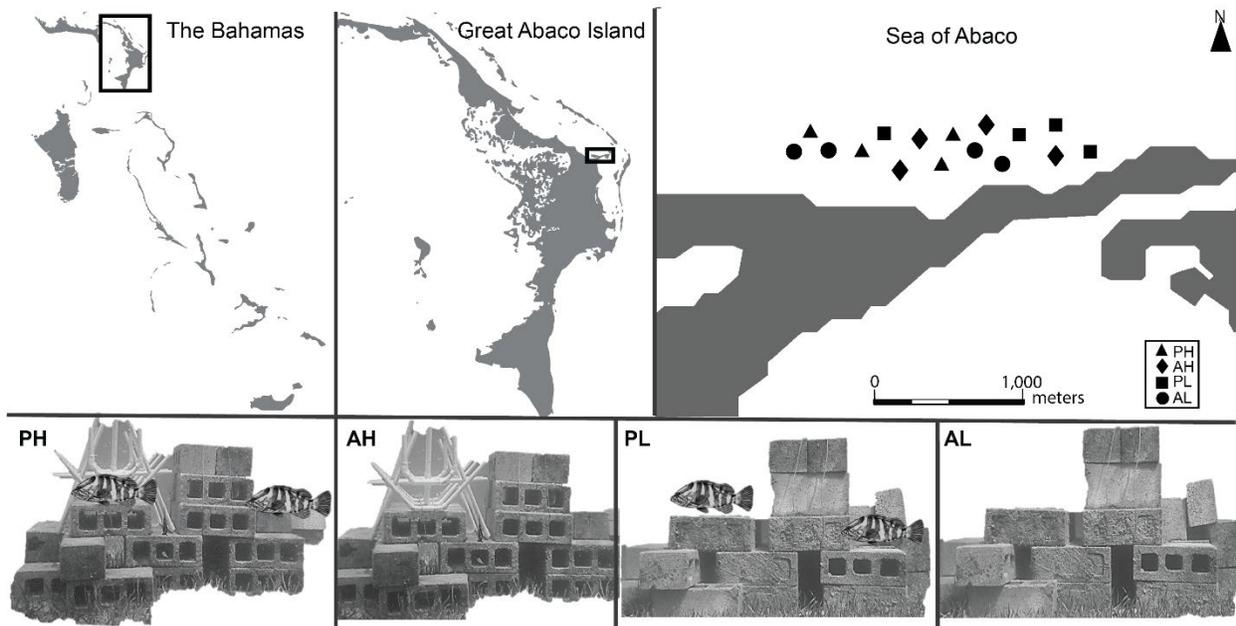


Fig. 1 Sixteen artificial reefs constructed in the Sea of Abaco, The Bahamas, in April 2014. They were constructed on mixed sand and seagrass benthic habitat >80m away from hard bottom substrate and >200m from natural or artificial patch reefs. At the bottom are representative images of the four treatments from left to right: predator presence x high reef complexity (PH), predator absence x high reef complexity (AH), predator presence x low reef complexity (PL), and low predator absence x low reef complexity (AL)

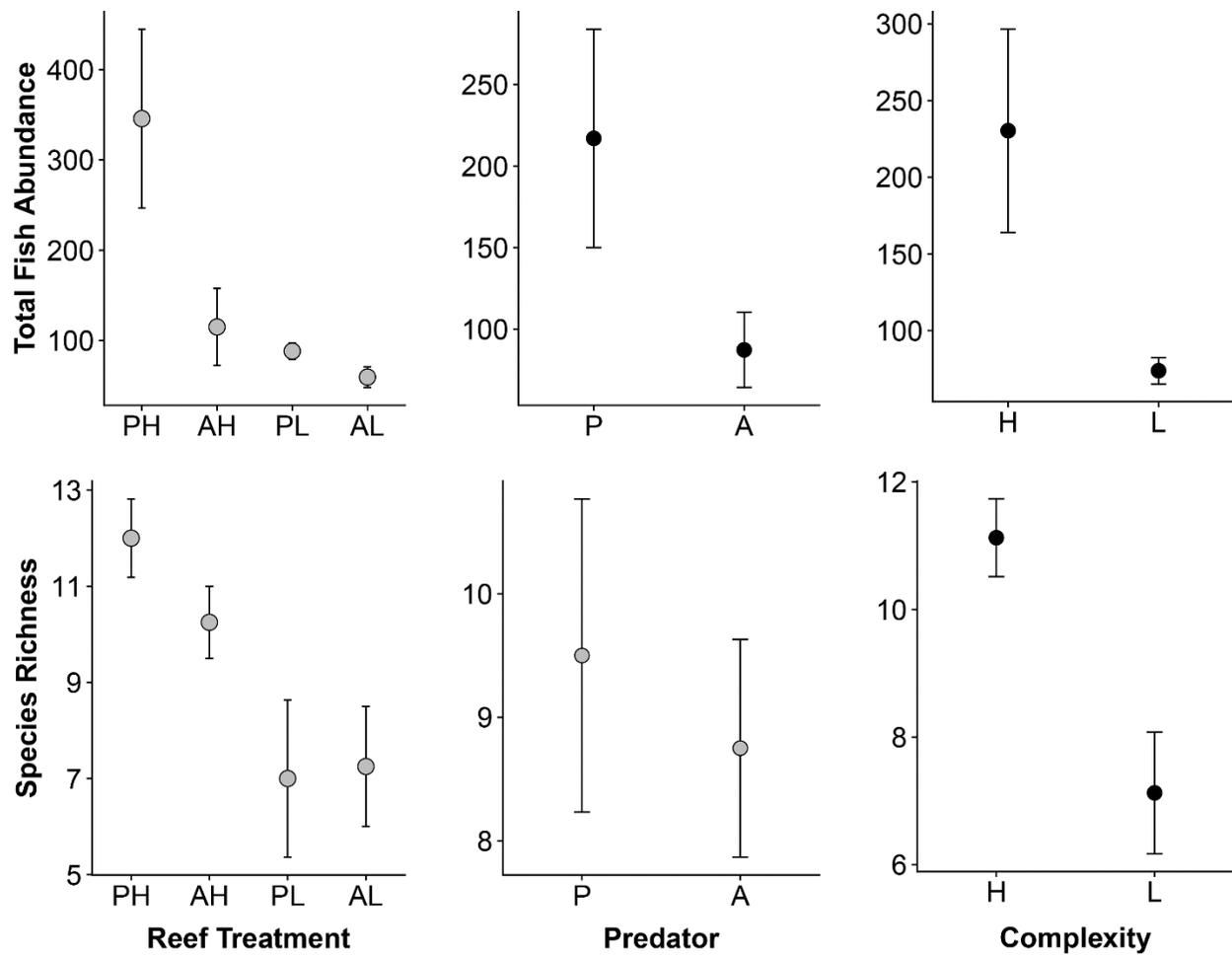


Fig. 2 Effects of Nassau grouper presence (present, P or absence, A) and reef structural complexity (high, H or low, L) for end-of-experiment (60 days) reef fish total abundance and species richness (n=16). Black data points indicate a statistically significant difference ($P < 0.05$) and grey data points indicate statistical insignificance for two-way ANOVAs or one-way ANOVAs investigating main effects. Error bars are standard error

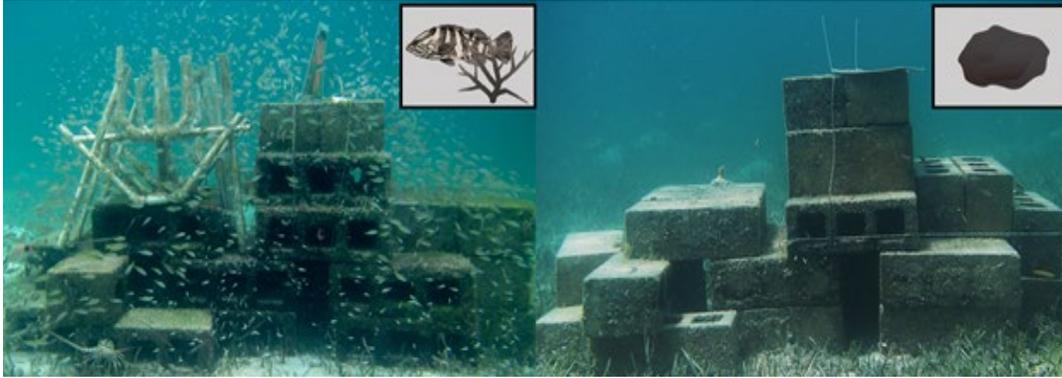


Fig. 3 Photographs demonstrate an example of the difference in total fish abundance between the two extreme reef treatments at 60 days after reef construction: predator presence x high complexity (left) and predator absence x low complexity (right)

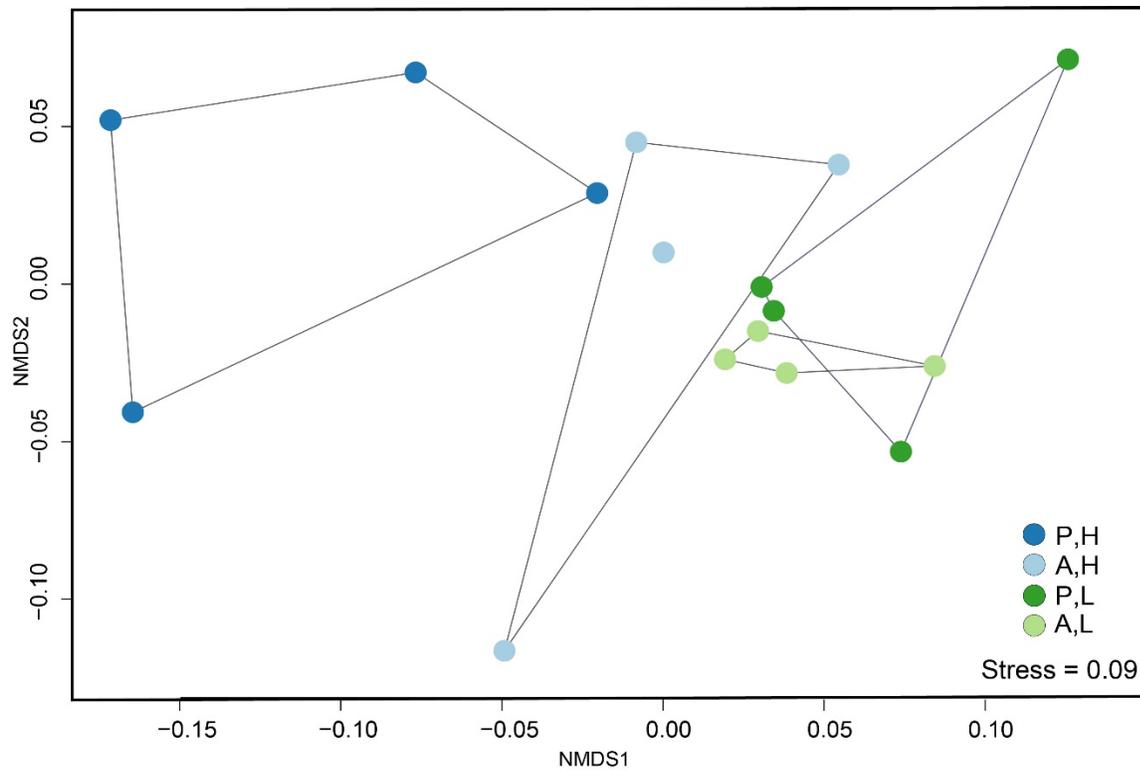


Fig. 4 Non-metric multidimensional scaling (nMDS) plot for fish community structure for four reef treatments (n=16). Reef fish communities were analyzed using reef fish abundances at day 60 of experimental treatments. “P” and “A” represent Nassau grouper presence or absence, and “H” and “L” represent high and low reef complexity treatments. Each colored point represents one reef. Reef treatment dissimilarities were calculated on square-root, transformed data (ANOSIM permutations = 999, A = 0.30, $p < 0.01$)

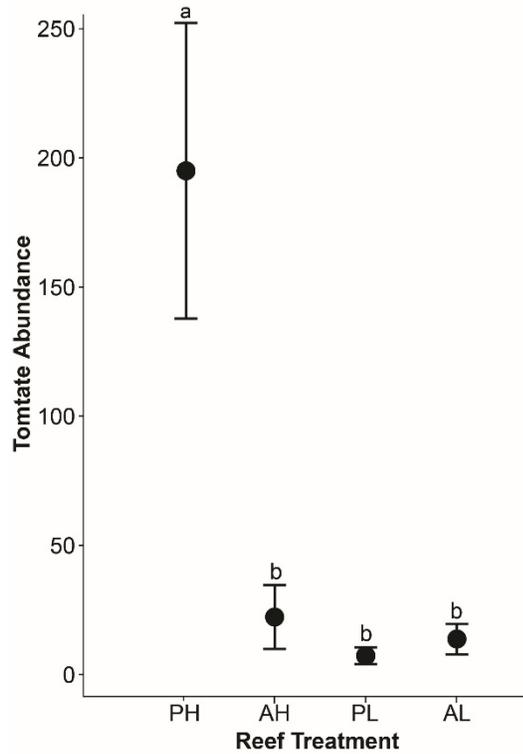


Fig. 5 Reef treatment effects on end-of-experiment (60 days) Tomtate grunt abundance (n=16, two-way ANOVA $p = 0.03$). Error bars are standard error. Letters above each point indicate statistical difference between treatments; treatments that do not share the same letter are significantly different from one another. Reef treatments included predator presence x high reef complexity (PH), predator absence x high reef complexity (AH), predator presence x low reef complexity (PL), and low predator absence x low reef complexity (AL)

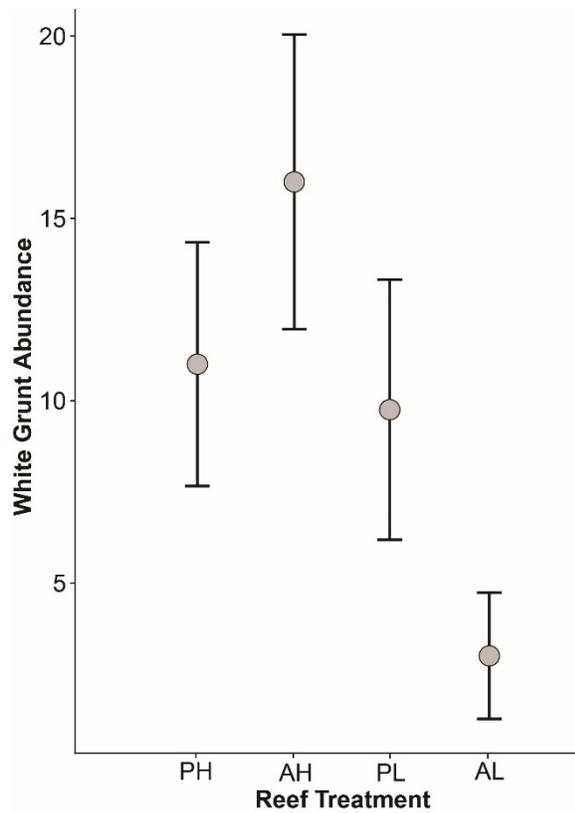


Fig. 6 Average White grunt abundances for reef treatments at end-of-experiment (60 days) with standard error bars. White grunt abundance was comprised from White grunt individuals that were estimated ≤ 5 cm in total length during UVC surveys to compare with similarly-sized Tomtate grunts. One-way ANOVA was conducted to compare white grunt abundances on predator presence x high complexity reefs (PH) versus predator absence x high complexity reefs (AH; $n=8$, $P = 0.18$)

**CHAPTER 3: The relative roles of top-down and bottom-up control of reef fishes within a
tropical seagrass community**

Authors: Enie Hensel, Marc J. S. Hensel, and Jacob E. Allgeier

Abstract

Humans have altered coastal ecosystems through changes in top-down and bottom-up processes via a myriad of stressors including overfishing and eutrophication. High fishing pressures can hinder food web stability through shifts in predator-prey interactions, as well as have strong effects on ambient nutrient dynamics by removing fish-derived nutrients which are known to aid in primary production. Human nutrient loading alters primary producer communities through increasing nutrient availability and changing its stoichiometry (nitrogen to phosphorous). Even though there is evidence that primary producers can also be strongly influenced by fish- and human- derived nutrients, most research still focuses on effects of human nutrient loading. This creates a potential gap in our understanding of the complete and cascading effects of human-driven stressors. Here we tested the relative role between reef fishes direct, top-down (predation) and indirect, bottom-up (altering seagrass vegetation composition and density) control on a *Thalassia testudinum* seagrass epifauna community in the context of different ambient nutrients. To mechanistically separate fishes' top-down versus bottom-up effects, we conducted surveys and embedded a predator exclusion experiment within a long-term (>7.5 yr), full factorial field experiment that manipulates local, ambient nutrient availability (fish- and human-derived nutrients). Our results show that in low nutrient waters, seagrass epifauna invertebrate communities are only affected by predation. Total invertebrate biomass increased by 166% when predators were excluded. We did not observe any indirect, cascading effects on invertebrate communities from the direct effects nutrient shifts have on seagrass primary producer communities. Overall, our study offers a next step forwards to understanding how multiple anthropogenic stressors mechanistically alter important top-down and bottom-up process through multi-level interaction webs.

Introduction

Humans have altered both top-down and bottom-up processes within natural ecosystems (Hulot et al. 2000, Hautier et al. 2015). Historically, human impacts on top-down processes are examined by experimental removal of a high-trophic level predator species, which can exert strong top-down control on their surroundings and help maintain food web stability (Estes et al. 2001, Doughty et al. 2016). Human impacts on bottom-up processes are primarily observed by changes in nutrient dynamics of natural ecosystems through nutrient enrichment (Smith et al. 1999, Isbell et al. 2013). In aquatic environments, where consumers are the major sources of nutrients through excretion and egestion, human-driven loss of large-bodied consumer species can also affect nutrient dynamics by removing natural sources of vital nutrients (Schmitz et al. 2010, Allgeier et al. 2014). Primary producers are strongly influenced by consumer-mediated nutrients in oligotrophic environments (Meyer and Schultz 1985, Holbrook et al. 2008, Allgeier et al. 2015), but the structural and functional consequences of predator loss from a nutrient dynamics perspective remains relatively unexplored (Hughes et al. 2007, Mumby et al. 2007, Burkpile and Hay 2010). In this paper, we focus on how the decline of consumers and human-derived nutrient enrichment shift the relative strengths in top-down and bottom-up control in a tropical coastal ecosystem.

Fishes can exert both top-down and bottom-up control in coastal and other marine ecosystems. For example, herbivorous fish graze algae and seagrasses, thereby regulating primary productivity (Valentine and Heck Jr 1999, Campbell et al. 2006, Burkpile and Hay 2008), while carnivorous fish directly and indirectly affect prey densities and their distribution via predation (Stallings 2008, Hixon 2011, Valdivia et al. 2017). In tropical and subtropical nearshore benthic ecosystems, excretion from fishes are often the main sources of nutrients for

primary producers in extremely oligotrophic environments (Meyer et al. 1983, Holbrook et al. 2008, Layman et al. 2011, Allgeier et al. 2014, Huntington et al. 2017). Tropical and subtropical fish communities aggregate on reefs creating biogeochemical hotspots, which increases primary production that fuels some of the most diverse and productive ecosystems in the ocean, e.g., coral reefs and seagrasses (Shantz et al. 2015). In the last half century, reef fishes have suffered population declines, predominantly due to overharvest and habitat degradation, which can affect ecosystem structure and function (Pauly et al. 1998, Dulvy et al. 2004, Airoidi et al. 2008). Importantly, overharvest and human-driven habitat degradation typically occur simultaneously in coastal ecosystems and yet, these impacts are often studied independently (but see Wilson et al. 2008). Our understanding of how human activities may alter the ecological role of marine fishes remains incomplete without examining the main and interactive effects of multiple human-derived stressors on their top-down and bottom-up control within their surrounding communities.

Coastal and nearshore habitats are important ecosystems because they provide services such as buffering storm surge from severe weather events and provide structural refuge for important fishery species, yet, they are susceptible to multiple anthropogenic stressors because they face impacts from both marine and terrestrial sources (Smith et al. 2006). For example, overharvest of marine predator species and shoreline dredging that removes nearshore habitats, respectively. Because these impacts reach species at the top and bottom of food webs, we need to expand empirical research to multi-level interaction webs to better evaluate how these simultaneous stressors alter community structure and ecosystem functions (Duffy 2002). Current research has shown how coastal primary producers are altered from multiple human-derived stressors. For example, Burkepile et al. (2013) found that human nutrient enrichment on coral reefs systems can reverse the relationship between fish aggregations and reef-building coral

species. Specifically, fish-derived nutrients, in combination with human nutrient enrichment, altered the amount and stoichiometry (nitrogen to phosphorous) of ambient nutrients which enhanced macro-algal growth and suppressed coral growth (Burkepile et al. 2013). Similarly, in *Thalassia testudinum* dominated seagrass meadows, shifts in the amount and source of nutrients were found to alter primary producer community composition in *T. testudinum* dominated seagrass meadows. Such that fish- and human-derived nutrient additions increased seagrass shoot density and productivity where fish-derived nutrients were primarily responsible for changes in species density of seagrasses (Allgeier et al. 2018). Because reef fishes have been shown to affect whole seagrass communities through top-down and bottom-up processes through an accumulation of studies, we suggest they are an ideal model system to mechanistically tease apart how prevalent, simultaneous anthropogenic stressors can alter ecosystems through a multi-level interaction web.

Here we conducted surveys and a predator exclusion experiment within an ongoing, long-term (>7.5 yr), factorial manipulative field experiment to examine how changes in reef fish communities, both fish density and species composition, and anthropogenic nutrient enrichment, affect seagrass-associated invertebrate communities in a *T. testudinum* seagrass meadow in The Bahamas. Specifically, we examine the potential indirect changes in invertebrate biomass and community assemblage due to the direct changes in primary producer communities including species density, seagrass shoot density, and seagrass growth rate from altered nutrient dynamics (see Allgeier et al. 2018 for primary producer community details). To examine potential changes in predator-prey interactions, i.e., direct consumption effects, we also embedded predator exclusion cages into our experimental design to mechanistically examine the relative role of

fishes' bottom-up and top-down control in a seagrass community. This research was guided by three questions:

1. Does reef-fish predation alter seagrass invertebrate biomass and community composition (herein, *Q1 fish predation on invertebrates*)?
2. Does reef fish community size (density) affect the relative role of fishes' top-down control (direct or indirect consumption effect) and bottom-up (consumer derived nutrients) on seagrass invertebrate communities (herein, *Q2 fish top-down vs. bottom-up role*)?
3. How do two different sources of nutrient enrichment, i.e., fish-derived and human-derived nutrients, separately and interactively affect nearby seagrass invertebrate biomass and community composition (herein, *Q3 shifts in fish- and human-derived nutrient enrichment*)?

Methods

Study Area

Our study was conducted June-November 2017 within a long-term (>7.5 yrs), ongoing manipulative field experiment in The Bight of Old Robinson, a semi-enclosed bay along the central coastline of Great Abaco Island, The Bahamas (Figure 1). The experimental units are located in a mosaic of seagrass meadows and sand flats. Water visibility is normally > 5 m and depth is approximately 3m at low tide. The seagrass meadows are dominated by turtle grass, *T. testudinum*, with manatee grass, *Syringodium filiforme*, and macroalgae, e.g., *Udotea* spp. and *Penicillis* spp., also present. Ambient background nutrients in this system are extremely low (~3.5 lg/L Soluble Reactive Phosphorus, ~3 lg/L NO₃, and ~7 lg/L NH₄⁺; Allgeier et al. 2010, Stoner et al. 2011).

In December 2010, we constructed 16 artificial reefs in a blocked 2 x 2 factorial design (four reefs per block) to manipulate two types of nutrient supply to nearby seagrass communities: (1) altered fish-derived nutrients through manipulation of artificial reefs: low fish community density (– Fish), high fish community density (+ Fish), and (2) nutrient enrichment by the addition of fertilizer to simulate eutrophication (– Fert, + Fert; Figure 1). (1) Artificial reefs, herein reefs, are a useful experimental unit which have been used widely over the past four decades to study reef fish assemblages and manipulate the biomass of reef-associated fishes as well as the trophic levels present (Hixon and Beets 1989, Carr and Hixon 1997, Eggleston et al. 1997). We created a pyramid reef shape (~100 cm x 80 cm at base, 90 cm height) with 30 cinder blocks (40 cm x 20 cm x 20 cm). We constructed two reef types: (i) reefs made from cinderblocks, which provide fishes with varied sized-refugia holes to mimic healthy reef habitat and low fishing pressure, i.e., high and low trophic level species present and high fish-derived nutrient supply (+ Fish), and (ii) reefs with the cinder block holes filled in with cement, creating a smooth-sided reef (reducing refugia availability) to mimic human-driven habitat degradation and high fishing pressure that reduces large-bodied predator presence (high trophic level species) and fish-derived nutrient supply (– Fish).

To confirm fish-derived nutrient availability and trophic level species presence manipulations, we conducted underwater visual surveys at least four times per year since reef construction. Because fish community structure per reef treatment was similar to past surveys, we used estimated fish nutrient supply rates and ratios for N and P from Allgeier et al. (2018) whom applied models generated by Allgeier et al. (2014, 2015). For this particular experiment, we conducted three additional fish surveys between June-November 2017 to confirm fish

community structure did not have temporal differences from previous surveys (Supplemental material). To manipulate human-derived nutrient enrichment, we suspended seven PVC diffusers in the water column ~ 0.5m above the substrate, each filled with ~ 500g of slow release fertilizer (Florkan 18-6-8 NPK, type 270) on a glass fritted pole around the reef, diffusers were replaced every three months to maintain consistent nutrient addition and release rate. Effects of nutrient manipulations were previously confirmed prior to this particular experiment by assessing changes in nutrient stoichiometry in nearby seagrass tissue as a proxy, and determined that nutrient manipulations alter seagrass communities 0 to ~4 m from the base of each reef (for details see Allgeier et al. 2017, 2018). For treatments without nutrient enrichment, we placed empty PVC diffusers to control for PVC structure. Lastly, each reef was located >150 m from any other reef so as to reduce among-reef movement of transient reef fish species (Carr and Hixon 1997). For human-derived nutrient manipulations, cross-reef enrichment among reefs due to fertilizer was not a concern due to the highly oligotrophic nature of the system and high rates of nutrient uptake by producers (Allgeier et al. 2018).

Predation exclusion experiment | Q1 fish predation on invertebrates and Q2 fish top-down vs bottom-up role

To by tease apart the relative role of top-down (predation) and bottom up (fish excretion) control of reef fishes on seagrass invertebrate communities (Q1 fish predation on invertebrates and Q2 fish top-down vs bottom-up role), we conducted a predator exclusion experiment from September to November 2017 alongside two of the four reef treatments (Figure 1; n = 8 reefs total): ‘– Fish, – Fert’ and ‘+ Fish, –Fert’. Exclusion cages (100cm length x 100cm width x 50cm height) were made out of a plastic mesh material with 3cm mesh-size holes. For each reef, we placed a 1m² cage and an open control plot at 0.5 m and 30 m from each reef’s base (n= 64

plots total). Noteworthy, we did have fishes present that were small enough to fit through our mesh cages (e.g., Labridae and juvenile Scaridae species), however we did not observe fishes within cages during our weekly surveys. We had two distances (0.5 m and 30 m from base of reef) to help distinguish between bottom-up effects from fish-derived nutrients (close proximity to reef). We randomly placed the 0.5m distance plots near the base of each reef, with at least 1m between each plot. The 30 m distance plots were also at least 1m apart from one another and located in the same orientation to the base the reef (e.g., leeward side). We randomly selected reef base orientation for each reef (Figure 2). Before constructing predator exclusion treatments, we collected seagrass characteristic data for each plot (described below). We checked treatments weekly to remove any external cage debris accumulation and to verify cage integrity. In November 2017, we collected final seagrass and invertebrate data in the same manner described below.

Seagrass and Invertebrate sampling

For all areas where we collected invertebrates, we also measured several seagrass bed characteristics including primary producer community composition and seagrass density and canopy height. For seagrass bed community composition we use 1m² modified Braun-Blaquet surveys to identify primary producers present and their relative percent canopy cover within each quadrat (Fourqurean et al. 2001). Braun-Blaquet surveys are a rapid, visual assessment technique that uses a standardized shoot density score to record primary producer relative abundances. In addition within these plots, we measured *T. testudinum* seagrass bed canopy height at 10 random points and shoot density at five random points within a 15cm² quadrat. Allgeier et al. (2018) showed shifts in nutrient sources altered primary producer community composition and seagrass shoot height. Therefore, we used average primary producer species density and seagrass canopy

height as a continuous model factor as potential measure for indirect fish-derived effects on invertebrate communities.

We conducted invertebrate sampling for *Q1 fish predation on invertebrates* and *Q2 fish top-down vs. bottom-up role* in November 2017. For *Q3 shifts in fish- and human-derived nutrient enrichment*, we sampled subplots between June-July 2017. All invertebrate sampling occurred during dusk (18:00-19:30 US EST) when many invertebrate species are known to be more active (Eggleston et al. 1998, Lyon et al. 2019). For each sampling date, we randomly chose two reefs because sampling all 16 reefs at once was not possible. For *Q3 shifts in fish- and human-derived nutrient enrichment*, at each reef, we collected invertebrates at three distances (0.5 m, 2 m, 30 m) from the reef's base along three transects 120° from one another (Figure 2). Thus we had three subplots per distance ($n = 16 \text{ reefs} \times 9 \text{ subplots per reef} = 144 \text{ subplots total}$). We oriented our first transect on the leeward side of each reef (Figure 2), and recorded transect identity to test for reef orientation affecting invertebrate community responses. A 2m² quadrat was placed at each sampling subplot and a SCUBA diver (E. Hensel or M.J.S. Hensel) used a mesh dip net (0.5 mm mesh size) to collect epi-benthic fauna (Hammerschlag-Peyer et al. 2013, Stoner et al. 2014). The net was able to sweep ~3 cm below the sediment, and we thoroughly swept each subplot three times or until there was no loose debris or sediment left. We immediately transferred dip-net contents into a fine mesh bag underwater and, once surfaced, moved them to a sealed container placed on ice in the boat to reduce invertebrate movement and/or predation. We processed invertebrate samples within 24 hours upon collection. To process each sample, we spread all contents on a large lipped tray submerged in a thin layer of warm saltwater and examined plant material for invertebrates and before removing. We identified invertebrates to the lowest taxonomic level possible and measured their total length to the nearest

millimeter. Once all the plant material was removed, we sieved the remainder of the sample through a 20 (800 μ) mesh screen to maximize detection of any remaining invertebrates. The invertebrates sampled from the predator exclusion plots were stored in a 10% formalin saltwater solution to be further processed for biomass measurements in a laboratory at NC State University (Supplemental material).

For invertebrates collected in November 2017, we measured total length as well as wet and dry biomass (mg) with and without the shell (e.g., if a hermit crab or mollusc) for each individual. For wet biomass, we placed each individual on a paper towel and removed excess liquid. For dry biomass, we placed individuals in a drying oven at 60°C for 48 hours and then weighed. We were not able to collect biomass measurements for invertebrate samples from our 2m² subplots addressing *Q3 shifts in fish- and human-derived nutrient enrichment*. Therefore, we used the biomass measurements from our predator exclusion experiment to estimate invertebrate biomass at the species-level based on our total length measurements (Supplemental material). Using the ‘Simple Fisheries Stock Assessment Methods’ R package (FSA version 0.8.24), we estimated invertebrate biomass using an allometric length-weight conversion ($W=a(TL)^b$) where weight (W) is in milligrams, total length (TL) is in millimeters, and parameter constant a and b were obtained from species-level logged regressions (Ogle 2016).

Statistical Analyses

To measure the effects of reef habitat complexity (reef with and without holes) on fish abundances we used two-way analysis of variance (ANOVA). We log transformed fish abundance prior to analysis to meet assumptions of homoscedasticity. To analyze changes in fish community composition between reef treatments we used non-metric multidimensional scaling (nMDS), permutational multivariate analysis of variance (PERMANOVA), two-way crossed

analysis of similarity (ANOSIM, 999 permutations) on square-root transformed fish abundances at the species-level, and similarity percentage analysis (SIMPER) computations using the Bray-Curtis similarity coefficient, limited to the species contributing to the top 70% of dissimilarity between treatment's reef fish assemblage (Bray and Curtis 1957). For each reef, we averaged total fish abundance and community composition over three underwater visual surveys conducted in 2017.

Q1 fish predation on invertebrates & Q2 fish top-down vs. bottom-up role | We used a three-way analysis of covariance (ANCOVA) to examine the separate and interactive effects of fish treatment, predator exclusion, and distance from reef treatment on invertebrate biomass, species density, and evenness. We defined invertebrate biomass as the sum of individuals collected and specifically, used wet biomass with shell if present. For evenness, we measured both the reciprocal Simpson's index $D = 1 - \sum(n - N)^2$, where n is the abundance of that species per survey and N is the number of total species per survey (Simpson 1949) as well as Shannon's index $H = - \sum_{i=1}^S p_i * \ln p_i$ (Hill 1973) where p_i is the proportion of individuals belonging to the i th species present. To examine invertebrate community compositional changes, i.e., changes in the biomass of constituent species, we used nonparametric analyses described above for changes in fish communities, i.e., nMDS, PERMANOVA, ANOSIM, and SIMPER.

Since changes in fish-derived nutrients directly alters seagrass primary producers communities (Allgeier et al. 2018), and may indirectly affect seagrass invertebrate communities, before we solely used fish treatment as a categorical factor (- FISH, + FISH) within the three-way ANOVA, we tested the main effects of primary producer species density and seagrass canopy on the invertebrate response variables to verify if they needed to be incorporated within our analyses. We did not observe any significant effects for both primary producer species

density and seagrass canopy for any respond variable (ANOVA global $P > 0.05$). Therefore, for our study we treated fish treatment as a categorical factor (– FISH, + FISH).

Q3 shifts in fish- and human-derived nutrient enrichment | We used a three-way ANCOVA (fish x fertilizer x distance) for all response variables, i.e., total invertebrate biomass, species density, species evenness, and community composition. For each plot, we took the average of our three subplots per distance for each reef then log transformed each variable. We also conducted nMDS, PERMANOVA, ANOSIM, and SIMPER analyses to measure community composition changes. We conducted all analyses using program R version 3.6.0 (Team 2017).

Results

Reef fish community manipulations

The mean abundance and species composition of reef fish varied significantly according to reef manipulations (two-way ANOVA $P < 0.01$, $F = 36.56$, $df = 1$; Table 1 and Figure 3). Mean fish abundance was an average 46.29 ± 3.88 SE for – FISH and 104.29 ± 13.68 SE for + Fish (Figure 3). Fish community structure, i.e., relative abundance of the constituent species, was also altered (SIMPER, PERMANOVA and ANOSIM permutations = 999, $P < 0.01$ and $R = 0.45$, respectively; Table 1 and Figure 3). Seventy percent of differences among communities were explained by four fish species: *Haemulon plumieri* (35.7%), *Halichoeres bivittatus* (18.1%), *Thalassoma bifasciatum* (12.7%), and *Ancanthurus coeruleus* (3.61%) (Figure 3). As would be expected given our study design we also found reduced abundances of large-bodied and/or predator species (those species likely to nest in holes in cinderblocks). Large-bodied and/or predator species were represented by these families: Carangidae, Holocentridae, Lutjanidae, Muraenidae, Scorpaenidae, and Serranidae (ANOVA $P < 0.001$, $F = 10.83$, $df = 1$). Our fish

abundances and community changes between treatments paralleled those from Allgeier et al. (2018). Therefore, in our study we did not explicitly measure fish biomass or the amount of nitrogen and phosphorous supply from the fish communities found on our experimental reefs because we were confident Allgeier et al. (2018) measurements for these variables were still relevant for our study.

Q1 fish predation on invertebrates & Q2 fish top-down vs. bottom-up role

The distance from reef treatments and exclusion of predators via our caged plots, regardless of mean total fish abundance (– Fish or + Fish reef treatments), increased total invertebrate biomass by an average of ~ 4,600 mg and ~2,800 mg, respectively (three-way ANOVA $P < 0.01$; Table 1: Figure 4). Only predator exclusion treatments altered species density (three-way ANOVA, main effect $P < 0.01$, $F = 16.33$, $df = 1$; Table 1), with an average of 8.18 ± 0.74 SE species in caged plots compared to 5.56 ± 0.49 SE observed in control plots. These results confirmed reef-associated fishes prey on nearby seagrass invertebrates within our study site. Species evenness, measured by both inverse Simpson's and Shannon's indices did not significantly change (three-way ANCOVA global $P = 0.27$ and global $P = 0.31$, respectively) in response to predator exclusion. Mean total fish abundance (– Fish or + Fish reef treatments), did not influence the effect of exclusions on invertebrate biomass or community composition (ANOSIM $R = 0.22$).

Q3 shifts in fish- and human-derived nutrient enrichment

To test for the effect of nutrient enrichment shifts on invertebrate biomass and community composition shifts we conducted a three-way ANOVA (fish x fertilizer x distance) as well as nonparametric analyses. Only distance from reef treatment (0.5m, 2m, and 30m) altered total invertebrate biomass (three-way ANOVA $P < 0.01$, $F = 6.64$, $df = 2$; Table 1; Figure 5). We

found no effect of either type of nutrient enrichment on invertebrate species density (global $P = 0.07$), Shannon's diversity index (global $P = 0.37$), or inverse Simpson's diversity index (global $P = 0.15$). For community structure, measured by potential changes in the biomass of constituent species amongst treatments, we did not observe any significant differences (ANOSIM $R = 0.18$).

Discussion

We demonstrated that coral reef fish communities have a strong top-down control on reef-adjacent seagrass invertebrate communities. Experimental shifts in the amount of both consumer-mediated and human-derived nutrients available, i.e., bottom-up control, did not alter invertebrate biomass. Overall our study suggests that tropical seagrass invertebrate communities are likely structured through top-down control processes such as predation. In the context of overfishing and eutrophication within our study system, i.e., decreases in predation pressure and increases in available nutrient, our findings suggest that fishing intensity may cause bigger changes in seagrass invertebrate communities than altering nutrient sources and availability.

Q1 fish predation on invertebrates

Invertebrate biomass increased by 166% when predators were excluded over a two-month period using our predator exclusion cages (Figure 4). Predation pressure was also detectable at 30m away from each reef, however, regardless of exclusions, on average total invert biomass was 230% greater 30 m away from the base of each reef. Combined, these results show how reef adjacent seagrass beds' epifauna communities are influenced by predation. Despite observable differences in the density (~50 more fishes) and species composition (more large-bodied, higher trophic level species) of reef fish between our – FISH and + FISH reef treatments, we found no differences in predation pressure as both sets of uncaged plots had similar densities of invertebrates, further indicating the strength of predation in our system

(Figure 3 & 4). Our results contradict others studies that show fishing (or simulation of the effects of fishing through complete fish removal) alters reef fish predation pressure on seagrass epifauna. One explanation for the difference between our results and those from other studies (Jones et al. 1991, Lewis and Anderson 2012) may be how we mimicked fishing with our fish aggregation density manipulations compared to other experiments. Specifically we did not completely remove or exclude all fish to simulate overfishing. Instead, through artificial reef manipulations, we mimicked the realistic outcome of fishing pressure on targeted species, i.e., large-bodied piscivores, and reef habitat degradation, i.e., less fish refugia availability, for the location of our study.

Q2 fish top-down vs. bottom-up role

Allgeier et al. (2018) showed that fish-derived nutrient enrichment altered plant/algal community structure. Using the same field sites we tested whether these effects can cascade to secondary trophic level organisms. Although we measured identical fish-derived nutrient trends on primary producer community as Allgeier et al. (2018), i.e., a negative relationship between fish excretion and primary producer species density, we did not detect any indirect, cascading effects for secondary trophic level organisms (Table 1; Figure 5). These results suggest that any cascading effects of fish-derived nutrient enrichment on invertebrates are likely to be modest or idiosyncratic. Noteworthy, we can't exclude the possibility that indirect, nutrient enrichment effects from reef fish aggregations on seagrass invertebrate communities may be measureable at lower fish abundance thresholds, e.g., if fish excretion has an effect on invertebrates only when seagrass and invertebrate biomasses are low. Still, our suspicion is that *T. testudinum* invertebrate communities are predominantly altered by fishes' top-down control then their potential bottom-up processes.

Q3 shifts in fish- and human-derived nutrient enrichment

In our invertebrate sampling plots that did not separate fish predation from consumer-mediated nutrient effects on seagrass communities, regardless of reef treatment, only distance from reef altered invertebrate biomass (Figure 5). Here we observed double the biomass of invertebrates at our furthest distance compared to directly adjacent to reefs. This relationship further supports that reef fish predation strongly influences reef-adjacent seagrass epifauna communities.

Here we mechanistically tested whether researchers may be underestimating community-level and ecosystem-level responses to fishing pressure when only fishes' top-down effects are measured. First, we built on previous studies that accumulatively have examined the effects of reef fishes' role on their primary producer communities by investigating how fishes' top-down and bottom-up control may affect one of their primary food sources, seagrass epifauna. By measuring their top-down and bottom-up control in tandem, we found fishes predation pressure is likely stronger than any potential indirect effects their provision of nutrients may have on seagrass epifauna. Secondly, our study examined how multiple stressors, overfishing, habitat degradation, and eutrophication, may shift interspecific species interactions leading to community-level changes which can alter ecosystem functions. Lastly, our study offers a next step forwards to understanding how multiple anthropogenic stressors mechanistically alter important top-down and bottom-up process through multi-level interaction webs. Further research, examining the separate and concomitant effects of fishing pressure and human nutrient loading along a continuous gradient will be necessary for wildlife and resource managers to identify potential thresholds where the interplay between these stressors shift the relative strengths in top-down and bottom-up processes.

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Tables and Figures

Table 1. ANOVA, ANCOVA, PERMANOVA, and ANOSIM outputs for all analysis conducted examining how artificial reef manipulation alters reef fish abundance and community structure as well as how shifts in nutrient amounts and source alter seagrass epifauna communities. Fish abundances were averaged over two surveys from 16 reef fish communities (4 per treatment) with an average total of 1203.8 fish observed per survey. Total invert biomass (mg) for Q1 and Q2 were averaged from 32, 1m² plots and for Q3 were averaged from 48, 2m² plots.

	Source	df	SS	F	P
Total Fish Abundance					
two-way ANOVA	Fish	1	2.45	20.46	<0.01*
	Fertilizer	1	0.11	0.91	0.36
	Block	3	0.13		
	Fish*Fertilizer	1	0.02	0.17	0.69
	Residuals	9	1.08		
Fish Community Structure					
PERMANOVA permutations = 999	Fish	1	0.05	<u>F (R²)</u> 4.21 (0.20)	<0.01*
	Fertilizer	1	0.04	2.95 (0.14)	<0.01*
	Fish*Fertilizer	1	0.02	1.44 (0.07)	0.015
	Residuals	12	0.15	0.58	
ANOSIM permutations = 999				<u>R</u> 0.45	<0.01*
Q1 & Q2 Total Invert Biomass					
three-way ANOVA	Predator Exclusion	1	3.69	13.89	<0.01*
	Distance	1	7.74	29.13	<0.01*
	Fish	1	0.14	0.53	0.47
	Block	1	2.89		
	Predator Exclusion*Distance	1	0.43	1.62	0.22
	Predator Exclusion*Fish	1	0.08	0.31	0.58
	Distance*Fish	1	0.02	0.07	0.80
	Fish*Predator Exclusion*Distance	1	0.44	1.65	0.21
	Residuals	23	6.11		
Q1 & Q2 Species Density					
ANOVA	Predator Exclusion	1	1.27	15.70	<0.01*
	Distance	1	0.32	4.00	0.06
	Fish	1	0.21	0.21	0.65
	Block	3	1.72		
	Predator Exclusion*Distance	1	0.01	0.14	0.71
	Predator Exclusion*Fish	1	0.18	2.18	0.15
	Distance * Fish	1	0.34	4.21	0.05
	Predator Exclusion*Distance*Fish	1	0.00	0.02	0.89
	Residuals	21	1.71		

Table 1 (continued).

Q3 Total Invert Biomass					
three-way ANOVA	Fish	1	0.07	0.15	0.70
	Fertilizer	1	1.27	2.78	0.10
	Distance	2	6.06	6.64	<0.01*
	Block	3	0.24		
	Fish*Fertilizer	1	0.44	0.96	0.34
	Fish*Distance	2	0.15	0.16	0.85
	Fertilizer*Distance	2	0.39	0.43	0.65
	Fish*Fertilizer*Distance	2	1.73	1.89	0.17
	Residuals	33	15.06		

Table 2. Seagrass epifauna species found within each invert sample (subplot and cage/control plots) collected between June – November 2017 for each reef treatment

Omnipresent Organisms	
Family	<i>Genus species</i>
Diogenidae	<i>Clibanarius tricolor</i>
Neritidae	<i>Smaragdia viridis</i>
Phasianellidae	<i>Euluthidium</i> spp.
Cerithiidae	<i>Cerithium</i> spp.

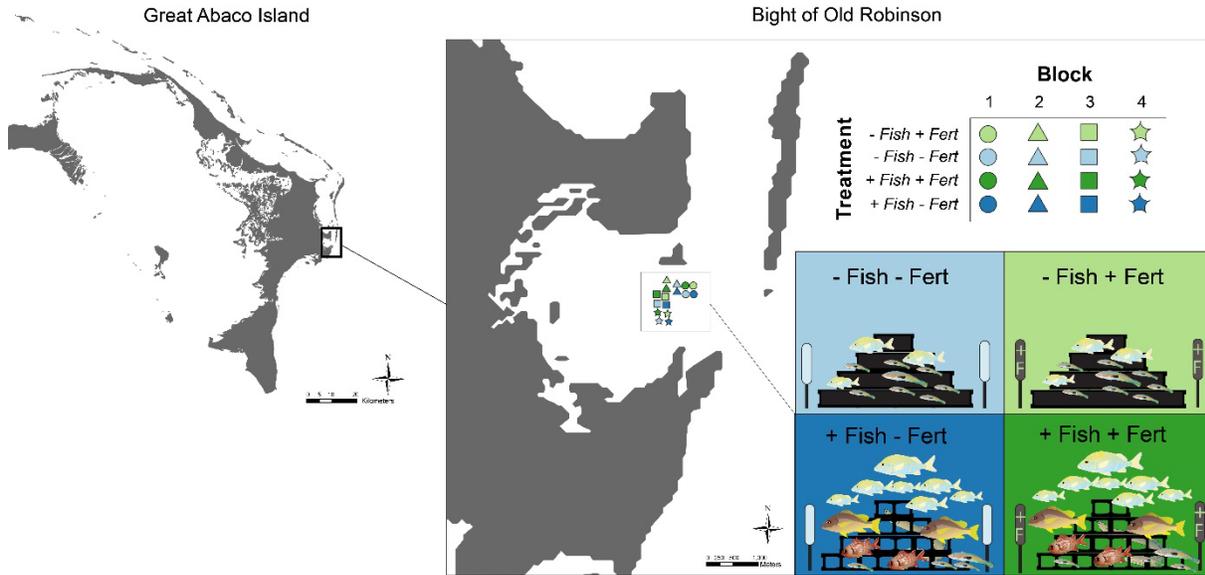


Figure 1. Sixteen artificial reefs constructed in December 2010 in a 2 x 2 factorial block design manipulating fish- and human-derived nutrients through reef structure complexity, and PVC fertilizer diffusers, respectively. Here we sampled reef adjacent seagrass epifauna communities between June-November 2017.

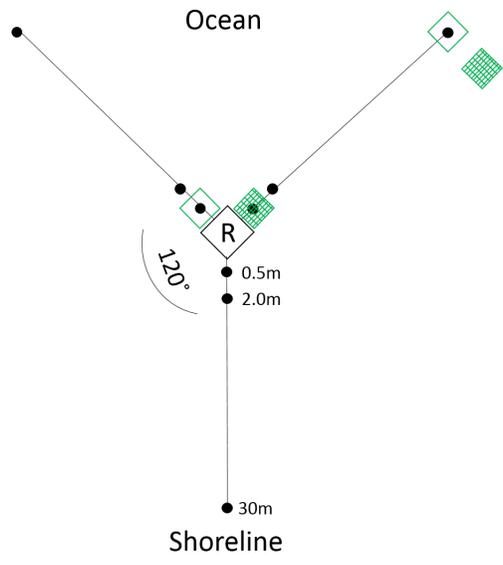


Figure 2. Diagram displaying transect orientation and distance from reef for each 2m² subplot (represented by a black dot). Green squares are two examples of 1m² cage or control plots for the predator exclusion experiment.

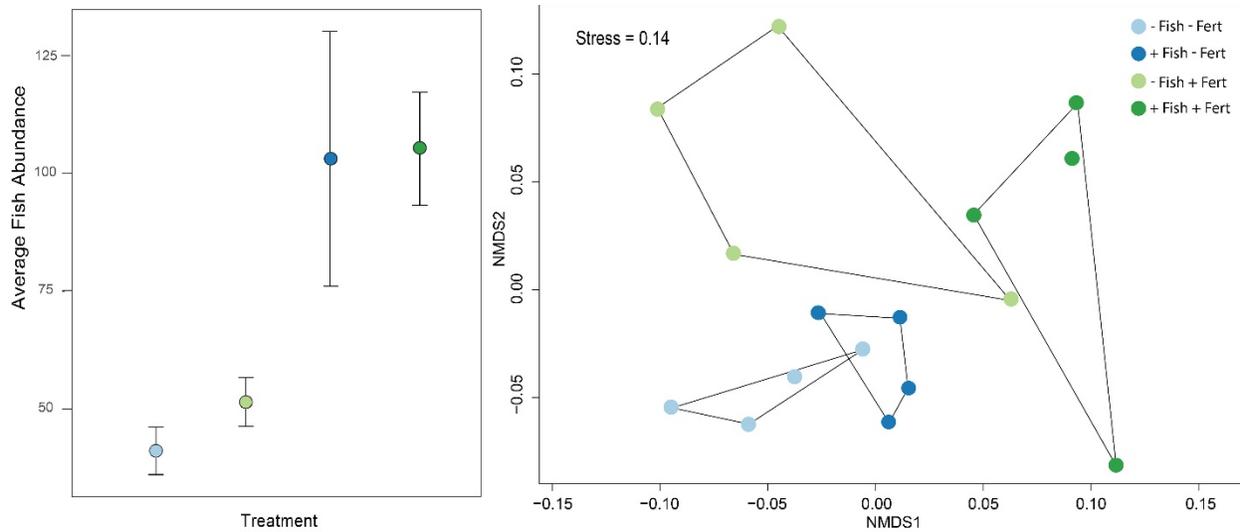


Figure 3. For changes in fish abundances and community composition per reef treatment, total average abundance and species present was comprised from three fish surveys conducted between June-November 2017. Reef fish community density manipulations were tested using a two-way ANOVA (\pm FISH main factor: $P < 0.01$, $F = 20.47$, $df = 1$). Non-metric multidimensional scaling (nMDS) plot for the composition of the number of individuals per species present within our fish aggregations per reef treatment.

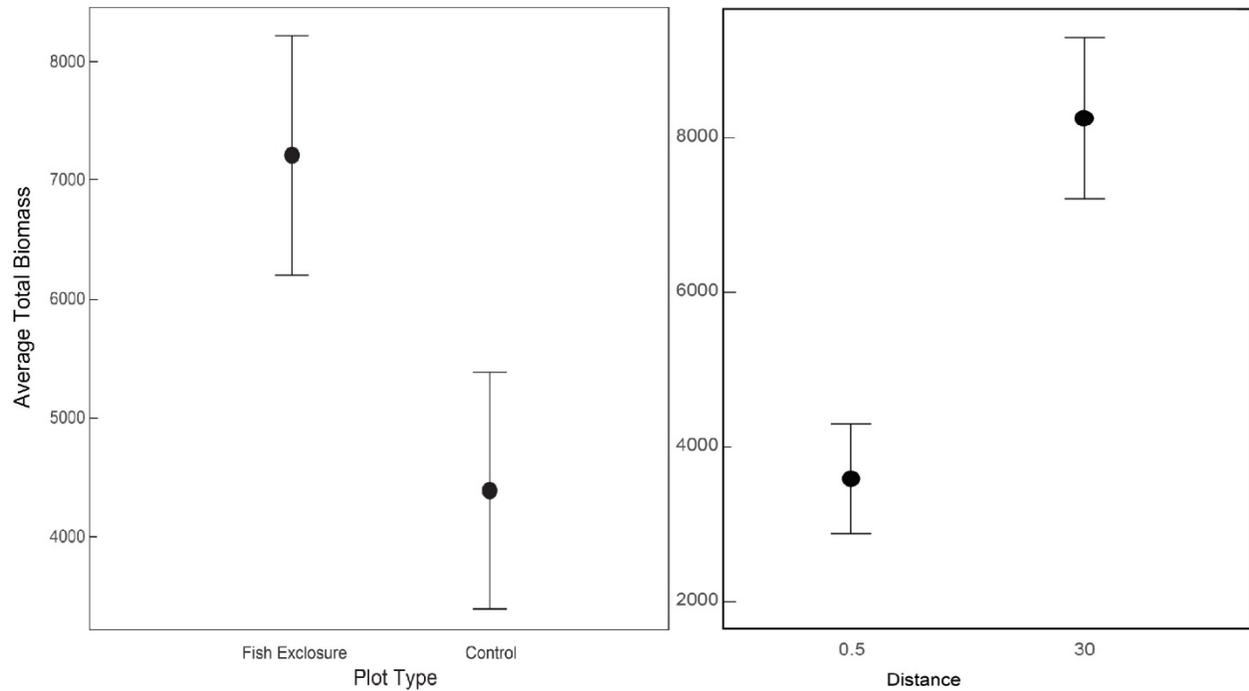


Figure 4. Average total invertebrate biomass (mg) is based on samples collected within 1m² predator exclusion caged or open control plots in November 2017. We had a total of eight replicated for caged and control plots, with four embedded within ‘-Fish x -Fert’ and ‘+Fish x -Fert’ reef treatments. Total invertebrate biomass was affected by fish predation and distance from reef treatments, i.e., 0.5m or 30m from reef base, (three-way ANOVA, predator exclusion $P < 0.01$, $F = 13.89$, $df = 1$, and distance $P < 0.01$, $F = 29.13$, $df = 1$).

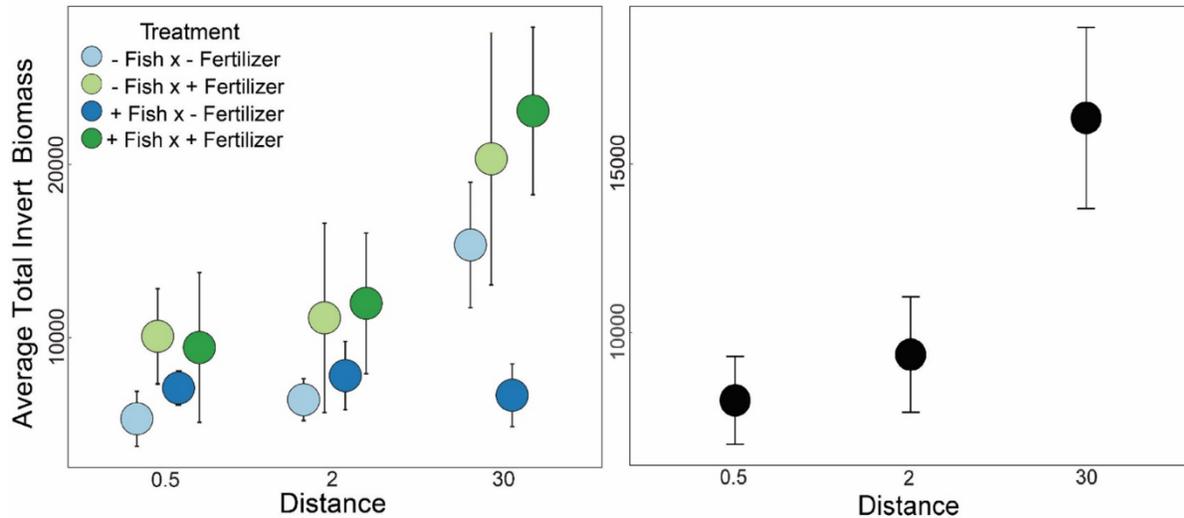


Figure 5. Average total invertebrate biomass (mg) per treatment is based on samples collected within 2m² subplots in June – September 2017. Three subplots were averaged for each distance at each reef for total biomass per reef (n = 4 reefs per treatment); therefore total subplots n = 144 and plot n = 48. Distance from reef increased invertebrate biomass per plot (three-way ANOVA, distance main effect P<0.01, F = 6.64, df = 2).

**CHAPTER 4: Do Artificial Reefs alter the Ecology of juvenile
Nassau Grouper (*Epinephelus striatus*) – a *Critically Endangered* species?**

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Abstract

Marine fish populations have suffered severe declines worldwide due to a myriad of stressors including overfishing and habitat degradation. One way to mitigate the loss of critical habitat is to introduce complex, artificial structure. For this management strategy to be effective it's key to first understand any potential direct or cascading consequences of introducing a non-native structure to an area of interest. Here, we investigate how artificial reefs within a back-reef system adjacent to shoreline development may alter the ecology of the *Critically Endangered* Nassau grouper (*Epinephelus striatus*). Using stomach contents and stable isotope analyses combined with acoustic telemetry, we examined changes in the diet and movement behavior of juvenile Nassau grouper (20-42 cm total length) residing on natural and artificial reefs. Over a seven month study period, we found individuals located on natural reefs were larger on average than individuals observed on artificial reefs. Our examination of stomach content showed that grouper diet depended on individual fish size (length). Through light stable isotope analysis ($\delta^{13}\text{C}$ & $\delta^{15}\text{N}$), we found reef type altered the habitat in which groupers hunted for prey. Specifically, natural grouper reefs preyed on nearby fauna within reef habitat while artificial reef groupers tended to consume prey from seagrass beds. Lastly, for shifts in grouper movement, reef typed altered how often individuals resided away from reef habitat. In the context of conservation and management, our findings demonstrate artificial reefs may be a simple tool for wildlife managers to apply in tropical nursery habitats that have experienced declines in complex habitat. However, due to changes in foraging habitat and movement behavior of a large-bodied predator species, we also suggest that managers should consider the potential cascading consequences for changes in inter- and intraspecific species interactions before artificial are introduced into a system, regardless of introduction purpose, e.g., conservation and ecotourism.

Introduction

Marine fish populations have been drastically declining over the last 50 years due to a myriad of stressors (Deegan 1993, Pauly et al. 1998, Jackson 2001). Population declines have major ecological and economic implications because fishes can have strong influences on their surrounding systems through both direct and indirect processes (Mumby et al. 2006, Layman et al. 2011), and many fishes have significant recreational and commercial value (Holmlund and Hammer 1999, Worm et al. 2006). Two main stressors marine fish populations face are overexploitation and habitat degradation (Jackson 2001, Hoegh-Guldberg and Bruno 2010). Many prized, large-bodied fishery species are particularly susceptible to these stressors because they tend to have complex life history traits including late maturity and/or dependency on multiple habitat types throughout their life stages (Olden et al. 2007).

Many coral reef fishes exhibit varying habitat needs throughout their ontogeny. Reef fish often depend on nearshore back-reef systems as nursery habitats early in life before they migrate to barrier reefs as adults (Nagelkerken et al. 2000, Parsons et al. 2015); back-reef systems are defined as nearshore environments between the coral barrier reef and shore in the tropics that are typically comprised of an interconnected mosaic of diverse habitat types (Adams et al. 2006). Then, as adults, they may still frequent nearshore ecosystems for food (Meyer and Schultz 1985, Deegan 1993). This dependency leaves many fishes vulnerable to shoreline development and its associated effects on nearshore ecosystems. For instance, nutrient loading from run-off pollution may degrade nearshore environments, and naturally complex habitats may be further degraded. Some nearshore habitats can be lost entirely with the introduction of hardened shorelines like seawalls and docks. One way to mitigate the loss of critical habitat is to introduce complex, artificial structure (Mercader et al. 2017). However, a consideration for this type of management

practice is to first understand the potential direct or cascading consequences of introducing a structure that is not native to the area of interest. In this study, we investigate the introduction of artificial reefs in a tropical, human-developed back-reef system and how they may alter the ecology of an ecological and economically important species, Nassau grouper (*Epinephelus striatus*).

Use of artificial reefs for conservation and management

Artificial reefs are broadly defined as any human introduced structure that is submerged on the benthos, usually introduced to mimic the function of patch reefs (small, isolated coral heads) by providing structural complexity for fauna to use as refugia and foraging grounds (Seaman 2000). Artificial reefs have been used globally for theoretical ecology research (Carr and Hixon 1997), as conservation and management tools, e.g., enhance coral restoration efforts, (Bohnsack and Sutherland 1985, Sherman 2002), and for promoting recreational ecotourism. Throughout the Caribbean, for example, many artificial reefs are destination SCUBA and snorkel locations and lack ecological monitoring and/or assessment. Importantly, the regulations for creating artificial reefs vary widely, typically depending on local government laws and enforcement regarding human introduced habitat enhancement objects, leaving many questions as to the potential contributions or consequences introduced habitat may have on community interaction webs and ecosystem functions. Although artificial reefs have been widely used for ecological research, few studies have compared the ecology of natural and artificial reefs (but see Carr and Hixon 1997).

Complex life history characteristics generates vulnerability to human activities

Nassau groupers are a top resident predator on coral reefs and have been observed on artificial reefs throughout their native range within the Caribbean (Beets and Hixon 1994,

Sadovy and Eklund 1999, Stallings 2009). Nassau groupers have a complex life cycle that has ultimately made them vulnerable to human stressors. For instance, they exhibit spawning aggregation behavior that has left adults vulnerable to major fishing exploitation (Sadovy 1997, Sherman et al. 2018). Consequently, the species is currently listed as *Critically Endangered* by the International Union for Conservation of Nature (IUCN) Red List (Sadovy 2018). During development, juvenile Nassau groupers undergo a series of ontogenetic shifts in both habitat and diet (Eggleston 1996, Eggleston et al. 1998, Dahlgren et al. 2006). Over their first ~six years they reside in nearshore, back-reef habitats including macroalgae beds, rock rubble, rock ledges, and patch or fringing coral reefs, with a shift from macroalgae beds to hard bottom of patch reef habitat beginning at ~three months old (Eggleston 1995). At this stage, individuals show strong site fidelity to their home activity area, often returning to and reusing the same patch reef coral head as refugia (Eggleston et al. 1998). Although variable, Nassau groupers can depend on their patch reef habitat as their main refugia and preying grounds for years until they mature and migrate to barrier reefs. Due to human population growth and sprawl, many critical Nassau grouper nursery habitats have experienced extreme changes including the loss of nearshore complex habitat. Therefore, in addition to high fishing pressure of adults, juveniles may be threatened due to loss of critical nursery habitat.

Study Objectives

Artificial reefs may be a simple management tool to enhance complex habitat within Nassau grouper nursery habitat. Here we examine how artificial reefs may alter the ecology of juvenile Nassau groupers within a back-reef system in The Bahamas. We examine potential changes in their diet and movement as well as potential patterns in individual size and weight by reef type. Our study was conducted along the eastern shoreline of Marsh Harbour, The Bahamas

– where urban development is present and there is evidence in abiotic and biotic changes within nearshore grouper habitats, i.e., nutrient enrichment, increased fishing pressure, and habitat flattening or removal (Stoner et al. 2011). In addition, within our study system, artificial reefs have been introduced over the last two decades for ecological research, conservation, and recreation purposes.

Methods

Study Site

Our study occurred in a back-reef ecosystem in the Sea of Abaco along the eastern shoreline of Marsh Harbour on Great Abaco Island, The Bahamas, from May – December 2016 (Figure 1). Our back-reef system consisted of hard-bottom, sand, algal beds, *Thalassia testudinum* dominated seagrass meadows, and scattered rock outcrops as well as coral and artificial patch reefs. Coral patch reefs ranged from $\sim 0.25 - 500\text{m}^3$ and artificial patch reefs, i.e., reef balls, submerged car frames, and piled cinderblocks, ranged from $\sim 0.25 - 6\text{ m}^3$ (Figure 2). Artificial reefs used in this study were at least 200m away from any other patch reef, however, distance between distinctive coral patch reefs varied from $\sim 15 - 200\text{m}$. We sampled a total of 20 natural and 22 artificial reefs.

Morphometric and diet data collection

Between May – August 2016, we captured 44 Nassau groupers, herein groupers, using two non-lethal methods: fish traps and hand net capture. Sampling effort was conducted as often as possible when weather permitted. For each sample date, we randomly selected one natural and one artificial reef and then soaked three baited traps at each reef the previous evening during dusk (19:00 – 20:00). Groupers are known to make nocturnal migrations to hunt, and return at dawn or early morning (5:30-10:30) to their home reef. (Eggleston 1996, Eggleston et al. 1998).

We checked our fish traps between 7:00 – 9:00 on each sampling date. We standardized our bait inside each trap, using frozen Queen Conch (*Lobatus gigas*) scrap in a fine mesh bag to prevent groupers from consuming it and thus, altering stomach content data. For artificial reefs only, we also used a hand net to capture any other groupers observed. Once we located where the grouper resided in the artificial reef, a SCUBA diver would cover that reef hole with the hand net and another diver would gently persuade the grouper out of the reef from the other side, orienting the grouper into the net. Once groupers were brought to the surface, we placed them into a holding tank that was filled with aerated saltwater and 25ml of API[®] Stress Coat Aquarium Water Conditioner. We recorded reef identity and type, GPS location, time caught, total length (TL), and weight (kg) for each grouper. Before groupers were released, we also tagged each grouper using an external identification Floy Tag & Mfg. Inc., inserted with a tag gun on the left lateral-side near the dorsal fin, approximately halfway between the fin origin and caudal peduncle.

To identify trophic level and diet base, we also collected a small fin tissue clip (~5 mm x 5 mm) from the tip of the anal fin for light stable isotope analysis. Fin clips were immediately deposited into a vial to avoid contamination and placed on ice. Frozen fin clips were shipped to NC State University and prepared for stable isotope analysis; samples were dried for 48 hours at 60°C, crushed into powder form using a Precellys[®] Homogenizer, and ~1-2 mg of each sample was stored in an isotope tin until shipment to the UC Davis Stable Isotope Facility for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. For basal resources stable isotope data, we used a compiled dataset from multiple projects from C. A. Layman that have occurred on Great Abaco Island over the last decade (S. T. Giery et al. *unpublished data*).

Lastly, if a grouper individual was not showing signs of stress, i.e., discoloration or swimming in a disoriented fashion, we performed a gastric-lavage to expel any potential stomach

contents. We used a clear, flexible tube ~1cm in diameter with a one-directional hand pump to direct clean, saltwater into the grouper's stomach cavity. Once the stomach was emptied, we measured the total weight of stomach contents (g), and identified contents to the lowest taxonomic level possible.

Acoustic telemetry array

To study individual grouper movement from May 1 – December 19 2016, we used an acoustic telemetry array, Vemco™ VR2W Positioning System (VPS) (Figure 1). The array was created with 10 synced, overlapping Vemco™ VR2W receivers that provided GPS points with ~35cm of accuracy for each fish. We placed an additional eight non-synced receivers 200 m from the array as a border starting July 29th, 2016 to record if any groupers left our study site. In our Vemco™ array (Figure 1), we tagged 18 grouper 20-29 cm TL on six natural and six artificial reefs. We used coral patch reefs as our natural reefs, which were a continuous gradient of reef size ranging from ~1-300 m³ and for artificial reefs we used cinderblock reefs ~2m³ (Figure 1). For movement analysis (described below) we only used groupers present from July 29– December 19, i.e., date when boarder receivers were implemented, resulting in a total of 13 individuals, five located on cinderblock reefs and eight individuals located on coral patch reefs.

Acoustic transmitter surgical procedure

We followed surgical protocols from Stump et al. (2017). All transmitters and surgical tools were properly disinfected using Benz-All followed with a distilled water rinse before every surgery. We placed each captured grouper into an aerated holding tank, as described above, and assessed overall health to verify if we could proceed with the surgery, e.g., stress coloration, disease presence, and potential predator wounds. We then transferred each grouper in a buffered tricaine methanesulfonate (MS-222, 75ppm) tank for anesthesia. While in the MS-222 tank, we

inserted a unique identification Floy Tag & Mfg., Inc., and recorded TL (cm) and weight (kg). Once fully anesthetized, we placed each grouper into an aerated surgical tank where it was held in a sling, ventral side up, and gills submerged. We made a small incision (~1.5cm) along the centerline posterior to the pelvic fins and inserted a Vemco™ V9 transmitter into the body cavity, lightly pinching the skin upward during incision to avoid puncturing the stomach. We closed the incision using 2-3 interrupted sutures. These sutures were tightened to fully close the incision; unlike mammals, fish wounds do not swell and it is important to ensure muscle contact. Each surgical procedure, from anesthetization to sutures, took ~ 10 minutes. After surgery, each grouper was placed into the holding tank to recover. Once each grouper showed signs of normal swimming behaviors (typically after 15-35 minutes), a diver returned the individual to its original capture location in a mesh bag and observed the fish to verify normal behavior.

Analysis

To assess whether grouper morphometric and stomach diet content varied by reef type (artificial or natural), we analyzed data using a series of one-way analyses of variance (ANOVAs). In two ANOVAs evaluating morphometric data, we used grouper TL and weight as the response variables and reef type as the predictor. We also used reef type as grouper TL and weight predictor variables for stomach diet content for an ANOVA. We additionally fit ANOVAs predicting stomach content total weight and content composition – contents were categorized as either crustacean, fish, mixed, or nothing (empty stomach). Light stable isotopes signatures were analyzed using two one-way ANOVAs to compare $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of groupers found on natural versus artificial reefs. For all data, we log transformed variables when necessary prior to analysis to meet homoscedasticity assumptions.

For grouper movement analysis, initial receiver data were processed by VemcoTM. For all statistical analysis, we used the number of detections and their locations for individual groupers at three minute intervals during the study period. Before statistical analysis, there were a couple modifications we had to adjust for our detections. First, we identified two different types of ‘zero’ detections for our grouper individuals due to receiver limitations. Receivers do not have the ability to detect an individual if they are within a reef. Therefore, if a grouper was last detected near a reef and the next detection was near that reef again, we identified the time passed between detections as within a reef hole. If they were not re-detected near a reef but instead detected elsewhere within the array, then the time passed between detections was identified as time spent out of array. Second, in order to create two unique identify the zero detections, we had to create a unified time series across all groupers, making all groupers have the same number of detections; this was possible through identifying a zero detection as either in a reef or outside the array.

We categorized a detection location for each grouper as one the following: on a reef, near a reef, within the array but not near a reef, or not located in the array. A grouper was identified as ‘on a reef’ if it was within 3 m of a reef’s boarder. For ‘near a reef’ a grouper was within 15 m along the border of a reef, and if a grouper was detected anywhere else within the array, we categorized its location as within the array, i.e., >15 m from any reef. Furthermore, we examined each grouper’s movement in relation to its home reef i.e., the reef of capture and compared how grouper movement was affected by grouper TL, weight, and home reef type using one-way ANOVAs. We conducted all analyses using program R (version 3.6.0; R Core Team 2017).

Results

Morphometric and diet data collection

We collected data from 21 groupers from natural reefs and 23 from artificial reefs. Grouper size ranged from 20.1 – 42.4 cm TL on both reef types, but on average, groupers found on artificial reefs were smaller than those on natural reefs (ANOVA $P < 0.01$, $F = 7.67$, $df = 1$; Figure 3). Specifically, 56.5% of the individuals located on artificial reefs were 20-29 cm TL and for natural reefs, 76.2% of individuals were 30-39 cm TL. One potential reason for this difference in size might be from reef type altering the growth rate of Nassau grouper. We did not measure growth rate for this study. However, if groupers on natural patch reefs are growing faster than those on artificial reefs, one assumption is that they would consume more prey than artificial reef groupers, i.e., more full stomachs and/or heavier stomach content. Our ANOVA results showed that reef type did not predict stomach content weight ($P = 0.85$, $F = 0.03$, $df = 1$), but we found larger groupers, measured by both grouper TL and body weight, had heavier stomach content (TL: $P = 0.01$, $F = 6.66$, $df = 1$; weight: $P = 0.02$, $F = 5.57$, $df = 1$; Figure 3). In addition, if reef type alters grouper growth rate, we might see a different relationship between grouper TL and body weight for artificial and natural reef grouper comparison, but again, we did not find any differences between reef types ($P = 0.54$, $F = 1.20$, $df = 1$; Figure 3). Stomach content type (i.e., crustacean, fish, mixed, or empty) was also affected by fish TL ($P < 0.01$, $F = 4.37$, $df = 3$). We only observed fish (i.e., the “fish” or “mixed” content categories) within gut content of groupers ≥ 34.0 cm TL (Figure 4). For light stable isotope comparisons between groupers on artificial and natural reefs, we did not find any difference in $\delta^{15}\text{N}$ signatures (ANOVA $P = 0.36$, $F = 0.85$, $df = 1$; Figure 5), but did find a significant difference in $\delta^{13}\text{C}$ signatures (ANOVA $P < 0.01$, $F = 16.18$, $df = 1$; Figure 5). Natural reef groupers had an average of $\delta^{13}\text{C} -11.07 \pm 0.15$ and

artificial reef groupers had an average $\delta^{13}\text{C}$ -10.43 ± 0.14 . Along the island of our study site, previous studies have shown that *Thalassia testudinum* seagrass as an average $-9.42 \delta^{13}\text{C} \pm 1.44$ SD (n = 24), where dominant macroalgae species within our study site range from $-17.5 - -18.9$ $\delta^{13}\text{C}$, i.e., *Sargassum* spp. ($-18.9 \delta^{13}\text{C} \pm 0.71$ SD, n = 4), *Laurencia* spp. ($-17.51 \delta^{13}\text{C} \pm 1.01$ SD, n = 18), *Batophora* spp. ($-18.7 \delta^{13}\text{C} \pm 3.39$ SD, n = 18), and *Dictyota* spp. ($-17.7 \delta^{13}\text{C}$, n = 1), (S. T. Giery unpublished data).

Grouper Movement

Grouper movement comparisons for eight individuals on natural reefs and five individuals on artificial reefs (TL ranged from 20.2 – 35.5 cm) suggested a difference in behavior between reef types. Natural reef groupers were detected over 2400 times more, on average, at least 15m away a reef than were artificial reef groupers ($P < 0.01$, $F = 17.05$, $df = 1$; Figure 6). In contrast, artificial reef groupers stayed within the boundaries of their home reef more often than did groupers on natural reefs.

Discussion

We found that juvenile Nassau groupers reside on natural and artificial reefs within a human-influenced back-reef system. We compared the ecology of groupers residing on natural and artificial reefs, with a focus on potential differences in their body size, diet, and movement patterns concerning their proximity to their home reef. We found groupers located on artificial reefs were on average, smaller in length than individuals observed on natural reefs (Figure 3), indicating a potential reef type preference related to grouper size (and assumed age). Our results show that grouper diet depends on individual size (TL and weight), but not reef type. Through comparing movement and light stable isotope data (inferred through variation in $\delta^{13}\text{C}$ signatures), however, we found that reef type predicted the habitat in which groupers hunted for

prey. Lastly, our movement results showed that groupers occupying different reef types exhibited different movement behaviors; groupers from natural reefs spent more time away from reef habitat.

We observed smaller (TL and weight) groupers on artificial reefs compared to natural reefs (Figure 3). The relationship between body size and reef type could have several explanations. First, there are morphological and architectural differences between the artificial and natural reefs within our study system. Specifically, there are differences in the size and number of available reef holes that juvenile Nassau grouper use for refugia. Many reef fauna, including Nassau groupers, prefer refugia holes that are close to the size of their own bodies (Eggleston et al. 1997). Most of the artificial reefs for our study were composed of cinderblocks with many small holes (~12 cm in diameter) close to the body size of groupers ranging from 20-32 cm TL. The natural patch reefs within our study site are characterized by larger holes (> 20-30 cm in diameter) or no refugia holes (Figure 2), including the small, isolated coral heads that are similar in size to our cinderblock reefs. The natural reefs within our study site may therefore not represent ideal habitat for < 30 cm TL juvenile groupers. A second explanation for the relationship between body size and reef type is that due to a combination of the loss of complex structure on extant natural reefs within our study site and the competition for available refugia on these patch reefs is likely higher than on artificial reefs (such that smaller individuals might be excluded). A final explanation is that residential predation pressure from larger predators, e.g., adult groupers, on natural patch reefs within our study system may be greater than on artificial reefs. Further research on grouper individual size/age and growth rate is necessary to elucidate whether reef type alters the biology of groupers or whether smaller groupers display reef type preference.

Our result showing that grouper diets varies with body size parallel the findings of Eggleston et al. (1998). Eggleston et al. (1998) found that when juvenile Nassau groupers reach 20–30 cm TL they experience ontogenetic diet shifts from crustaceans to fish, where groupers from > 30 cm TL consume mainly fish (Figure 4). These consistent findings support our hypothesis that differences in diet between grouper on natural and artificial reefs are likely due to grouper size (and assumed age), not reef type itself. Based on our light stable isotope results, we did observe that reef type shifts diet base, i.e., basal or primary resources (Figure 5). For our study site in particular, this likely indicates that home reef type and its immediate surroundings (0-15 m from the center of the reef) determined what habitat groupers' hunt in, where natural reef groupers prey on individuals from or within both macroalgae and seagrass based habitats (macroalgae beds or natural patch reefs) and artificial reef groupers prey on individuals only from or within seagrass beds. We could not identify the direct causal mechanism for difference in grouper diet base, but offer a plausible explanation. Regardless of body size, juvenile Nassau groupers have strong site fidelity, are generalist predators (Eggleston et al. 1998, Sadovy and Eklund 1999), and therefore, individuals' diet may be subject to their home reef's surroundings. For our natural patch reefs, the benthic substrate was a gradient of hard bottom between clumped coral heads, hard rock bottom with stands of macroalgae (*Sargassum* spp.), to soft sediment and seagrass beds. Note, the ecotone range between natural coral patch reefs to seagrass beds was different for each natural patch reef in our study. For our artificial reefs, there was no habitat gradient change as these reefs were placed within seagrass beds at least 200m from any other benthic habitats. This finding is important for habitat management decisions regarding how the placement of artificial reefs may influence interaction networks. In order to both replenish back-reefs systems with complex habitat and minimize human influence on its food web interactions,

our results suggest that artificial reefs should be placed within similar benthic substrate of natural patch reefs.

Using telemetry, we found that natural reef groupers moved away from reef habitat more often than groupers residing on artificial reefs. 31.38% of detections of natural reef groupers were at locations > 15 m away from reef habitat. In contrast, artificial reef groupers were only detected more than 15 m away from their reef habitat just 5.22% of their time. These findings may have important bioenergetic implications which further support our suggestion that the ecological differences between natural and artificial reef groupers may be explained by predation and competition pressure being higher on natural reefs than artificial reefs. Specifically, artificial reef groupers decreased range of movement indicates less energy towards either searching for food or predation-risk behavior and more towards growth (Dill 1987, Post and Parkinson 2001, Jørgensen et al. 2016). During early development (< 20 cm TL), changes in juvenile Nassau groupers' habitat use has been shown to be consistent with the “minimize μ/g hypothesis”, where ontogenetic habitat shifts minimize predation risk (mortality μ) and maximize growth rate (g) (Dahlgren and Eggleston 2000). Here, our findings suggest that movement patterns of larger (>20 cm TL), and assumed older, Nassau groupers still exhibit behaviors that are predictable and consistent with the minimize μ/g hypothesis. We observed natural reef groupers allocating more time and moving further distances away from reef habitat thereby spending more time in open areas, e.g., hard bottom, sand, or seagrass substrate, oppose to artificial reef groupers spending most of their time within or near their reef habitat. This supports that extant natural patch reefs in our study system probably have inadequate refugia from predators and/or prey availability for these juvenile groupers. Therefore, since Nassau groupers readily use artificial reefs, the introduction of artificial complex structure into reef depauperate back-reef systems may decrease

juvenile Nassau groupers' energy spent on predation risk behavior as well as lower competition for prey availability.

Additionally, movement pattern differences may indicate that reef type alters top-down control of juvenile groupers. Juvenile Nassau groupers themselves are large in body size compared to many resident patch reef fishes and are known to exude behaviorally-mediated trophic cascades by directly decreasing smaller-bodied grouper movement and indirectly increasing fish recruitment densities (Stallings 2008). Therefore, by artificial reef groupers having a smaller range of movement, their top-down control on their surroundings might be truncated due to decreases in the probability of interacting with smaller-bodied piscivores. In addition to differences among reef types in predation risk and competition, the location and placement of artificial reefs may also contribute to artificial reef groupers have a smaller range of movement from their home reef. Like our artificial reefs within our study site, most artificial reefs vary widely in size, materials, etc., but are typically a single entity and are spatially isolated from natural reefs for various reasons, e.g., introduce a new ecotourism location or protect extant reefs from artificial structures during severe storms. Because the artificial reefs within our study system were isolated from natural reefs and placed within seagrass beds, artificial reef groupers were faced with an obvious visual contrast between reef and seagrass habitat or refugia to open habitat. The natural patch reefs within our study system all had some form of a gradient from reef to seagrass habitat, and therefore range expansion from their home reef to open habitat may have visually not been determined as a risk.

We have shown that artificial reefs are readily used as home reefs by juvenile Nassau grouper, an iconic fishery and *Critically Endangered* species. In addition, due to fishes readily using artificial reefs, we have shown that human impacted nearshore ecosystems are likely

limited in complex habitats, and express both monitoring fishing regulations and protecting or restoring critical habitat is important for reversing human impacts and potentially maintaining sustainable fisheries. Therefore, artificial may be a simple tool for managers to apply in nursery habitats that have experienced a decline or loss in complex habitat. However, given the differences in foraging habitat and movement behavior by reef type that we observed from this piscivore species, managers should consider the potential cascading consequences for inter- and intraspecific interactions before artificial reefs are introduced into a system, regardless of introduction purpose (e.g., conservation or ecotourism).

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Tables and Figures

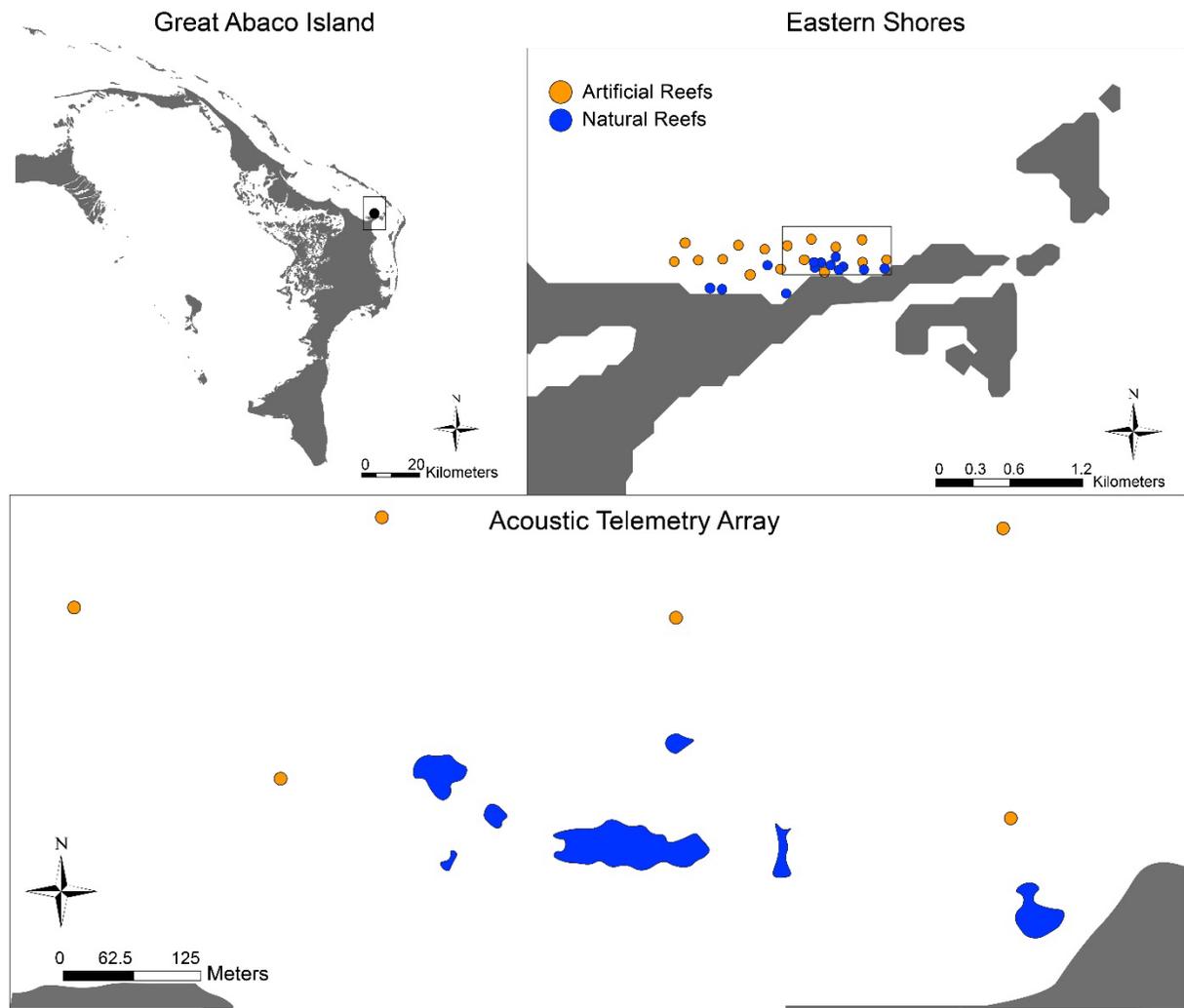


Figure 1. We studied juvenile Nassau grouper diet (n=44) and movement (n=13) along the eastern shoreline of Marsh Harbour, Abacos, The Bahamas from May – December 2016 on natural and artificial reefs (top right). From May – August, we collected fin-clips and stomach contents to document grouper trophic – level and diet base. Bottom image shows reef shapes and relative sizes to one another within VEMCO[®], VR2W Positioning System, i.e., a triangulated positioning array

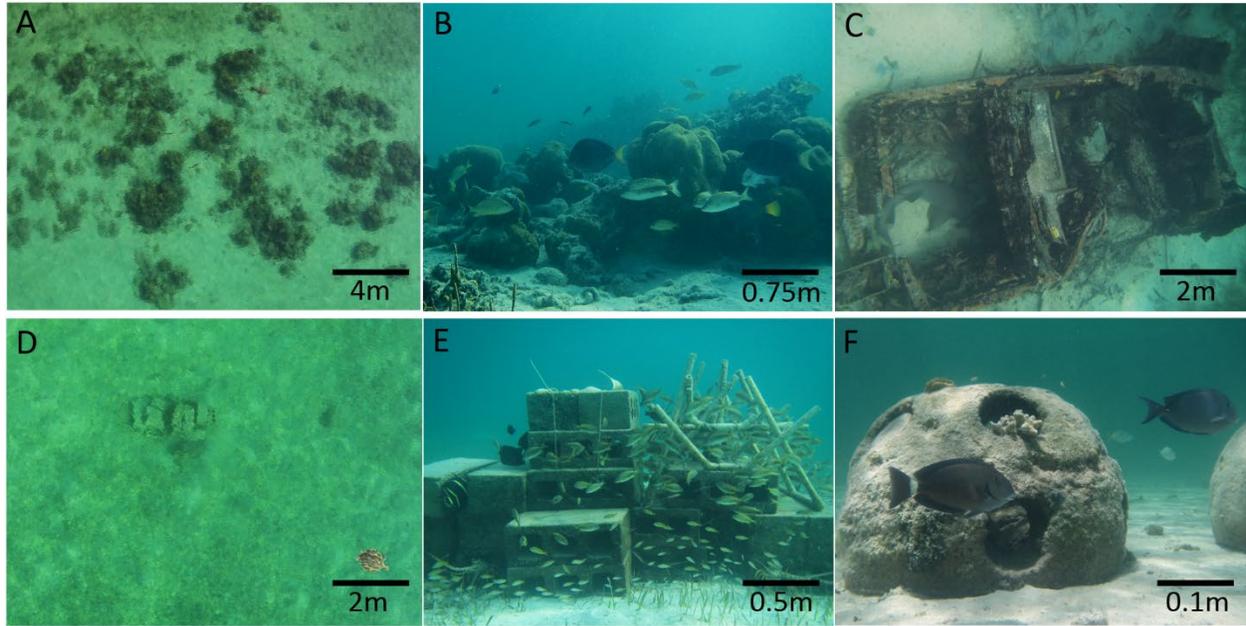


Figure 2. Different natural and artificial patch reef types found along the eastern shoreline of Marsh Harbour, Abacos, The Bahamas, are represented including: (A) aerial view of coral, (B) coral, (C) car frame, (D) aerial view of cinderblock, (E) cinderblock, and (F) cement reef ball.

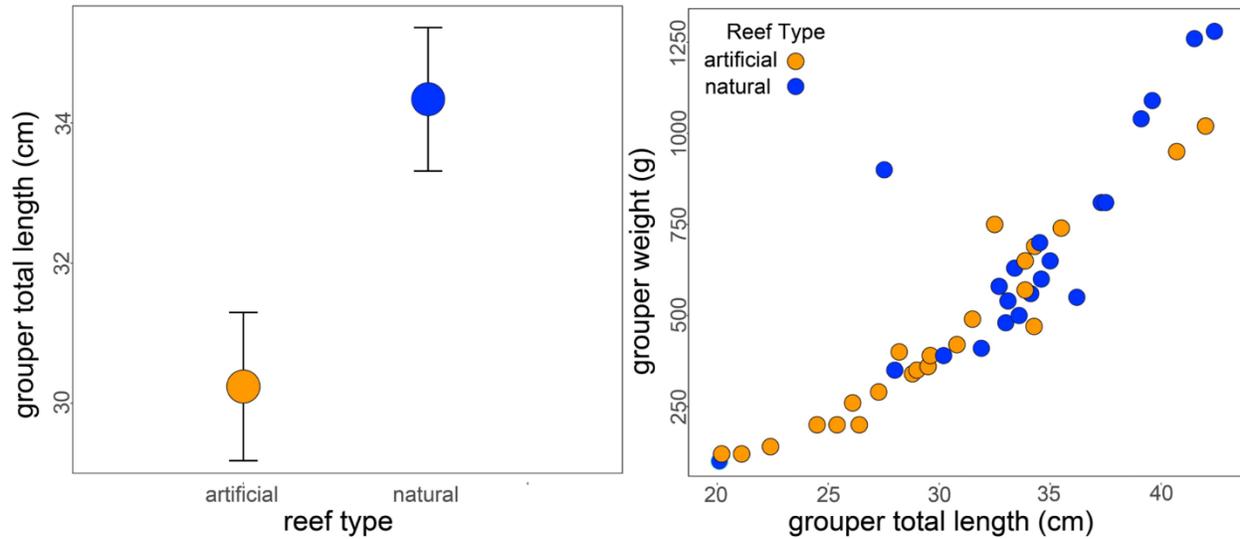


Figure 3. Shown on the left, mean Nassau grouper length (TL) was greater on natural compared to artificial reefs (ANOVA $P < 0.01$, $F = 7.67$, $df = 1$). A scatterplot of grouper body weight versus TL for all fish sampled ($n = 21$ natural reefs, $n = 23$ on artificial reefs) suggests a consistent relationship (slope) among reef types. Groupers were sampled along the eastern shoreline of Marsh Harbour, Abaco, The Bahamas May-August 2016.

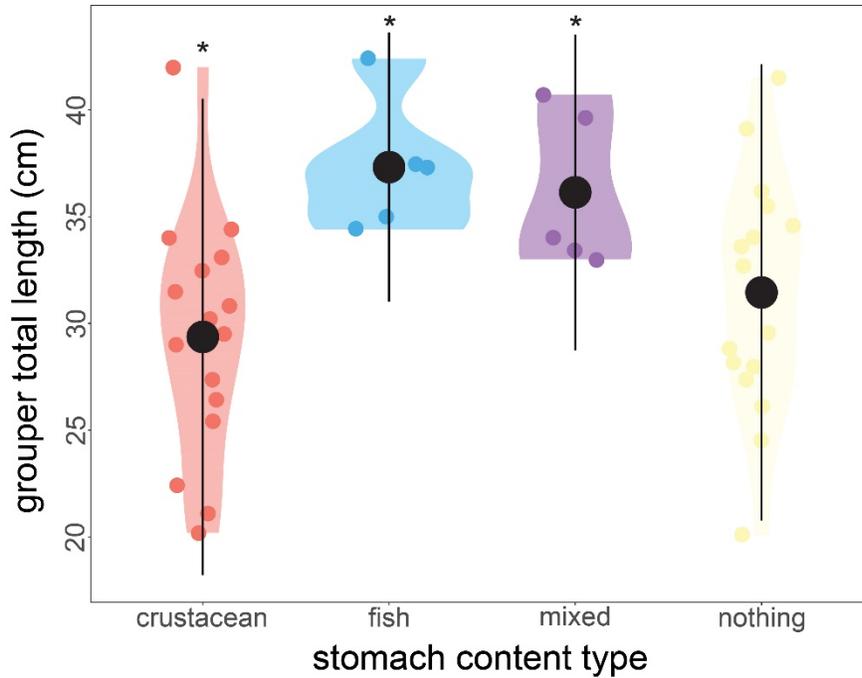


Figure 4. Diet type differed with juvenile Nassau grouper TL (ANOVA $P < 0.01$ $F = 4.37$ $df = 40$). A relationship between grouper TL and diet type is denoted with an asterisk. Diets were categorized from stomach contents sampled with gastric-lavage along the eastern shoreline of Marsh Harbour, Abaco, The Bahamas May-August 2016.

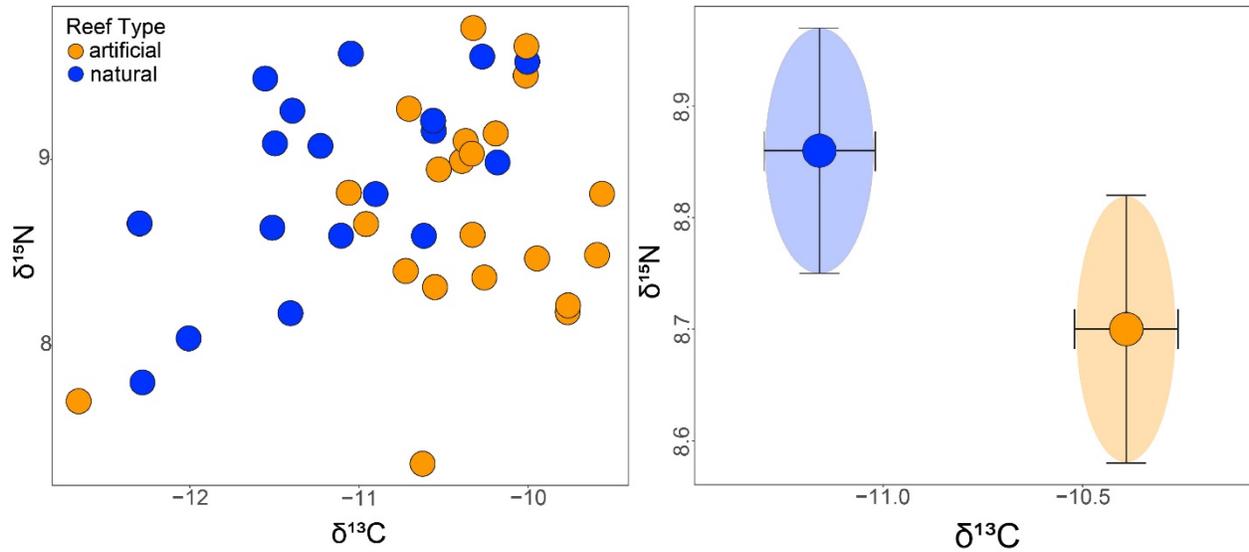


Figure 5. Light stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) from 44 unique fin clips collected from juvenile Nassau grouper along the eastern shoreline of Marsh Harbour, Abacos, The Bahamas from May-August 2016. Carbon signatures indicated a difference in basal resources between groupers located on natural versus artificial reefs (one-way ANOVA $P < 0.01$, $F = 16.18$, $df = 1$). Nitrogen signatures (indicating trophic-level) did not differ among groupers within our study (one-way ANOVA $P = 0.36$, $F = 0.85$, $df = 1$).

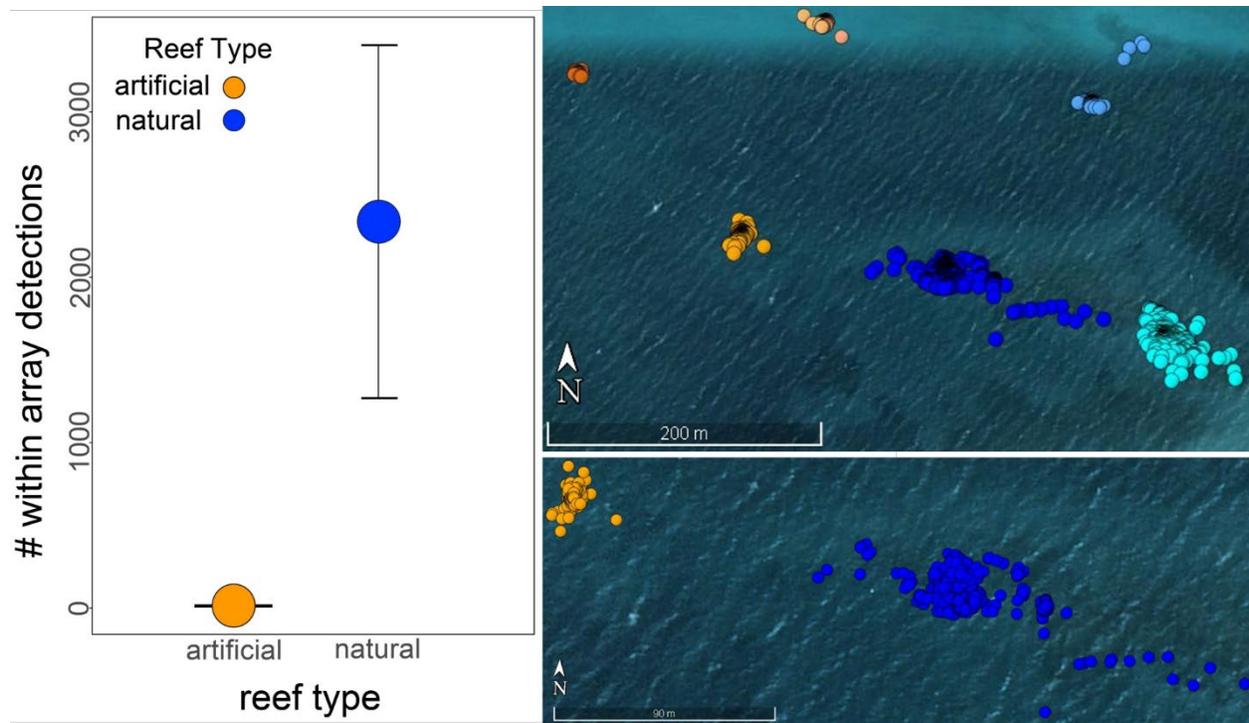


Figure 6. From July 29th – December 19th 2016, we used an acoustic telemetry array, VemcoTM VR2W Positioning System (VPS) and compared the average number of movement detections juvenile Nassau grouper were found away from reef habitat (>15m) for eight individuals using natural reefs and five individuals residing on artificial reefs as their home reef (one-way ANOVA $P < 0.01$, $F = 17.05$, $df = 1$). Images on the right show exemplary snapshots of individual grouper detections recorded for one day (here September 15th, 2016). Orange and blue shaded dots represent groupers using artificial and natural reefs as their home reef, respectively.

APPENDICES

Appendix A

TITLE

Effects of predator presence and habitat complexity on reef fish communities in The Bahamas.
Supplementary Material.

RUNNING PAGE HEAD

Predators, reef complexity, and fish community structure

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Supplemental Material – Total Fish Biomass

METHODS

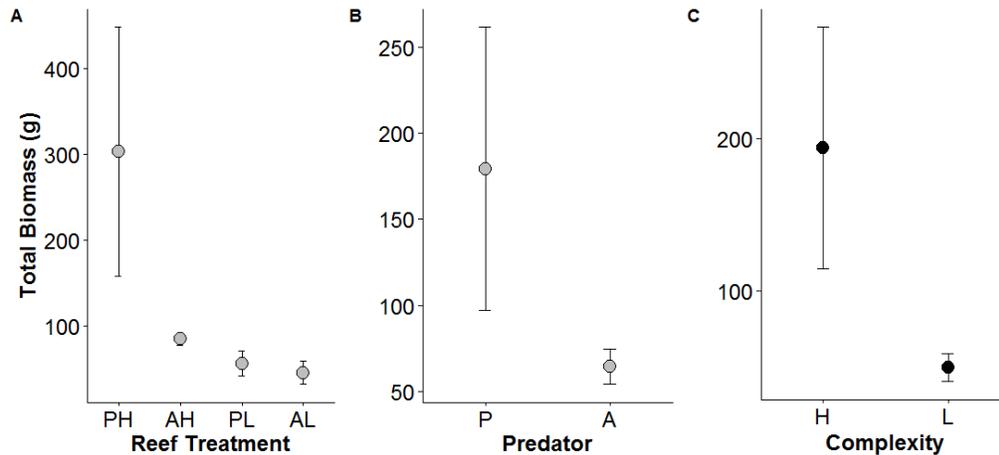
During the last two weeks of the experiment, the total length (TL) of each fish was visually estimated to the nearest centimeter. These data were used to estimate fish biomass with an allometric length-weight conversion ($W=a(TL)^b$) where weight (W) is in grams, TL is in centimeters, and parameter constant a and b were obtained from the literature at the species level (Froese 2005). We used two-way analysis of covariance (ANCOVA) to test for independent and interactive treatment effects on total fish biomass. For main factor effects, we used a one-way ANCOVA. We log transformed fish biomass prior to analysis to meet homoscedasticity assumptions and included seagrass density in each model as a potential covariate, but removed this variable when not significant for reasons of parsimony. We conducted all analyses using R software (Team 2017).

RESULTS

Predator presence and structural complexity had an additive effect on total fish biomass ($F_{1,1} = 8.91, p=0.01$ and $F_{1,1} = 16.02, p < 0.01$, respectively) and seagrass was a significant covariate ($F_{1,1} = 9.71, p < 0.01$, supplemental Table 1).

Supp. Table 1 Results of ANCOVA for total reef fish biomass based on fish species' abundances and total lengths at 60 days after reef construction

Total Fish Biomass	Source			df	SS		
	MS	F	P- value				
	Predator		1	1.35	1.35	4.66	0.05
	Complexity		1	5.03	5.03	17.33	<0.01*
	Predator * Complexity		1	<0.01	<0.01	<0.01	0.98
	Seagrass Density		1	2.66	2.66	9.15	0.01*
	Residuals		11	3.19	0.29		



Supp. Fig. 1 Separate and interactive effects of Nassau grouper presence (present, P or absent, A) and reef structure complexity (high, H or low, L) for average of end-of-experiment (60 days) reef fish total biomass (n=4). Black data points indicate statistical significance (p < 0.05) and grey data points indicate statistical insignificance from two-way ANOVAs

Appendix B

TITLE

The relative roles of top-down and bottom-up control of reef fishes within a tropical seagrass community. Supplementary Material.

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Supplemental Material

Estimating Invertebrate Biomass per Species

We were not able to record biomass measurements for invertebrate samples from our 2m² subplots addressing *Q3 shifts in fish- and human-derived nutrient enrichment*. Therefore, we used the biomass measurements from our predator exclusion experiment to estimate invertebrate biomass at the species-level based on our total length measurements. Using the ‘Simple Fisheries Stock Assessment Methods’ R package (FSA version 0.8.24), we estimated invertebrate biomass using an allometric length-weight conversion ($W=a(TL)^b$) where weight (W) is in milligrams, total length (TL) is in millimeters, and parameter constant a and b were obtained from species-level logged regressions (Ogle 2016). We only implemented invertebrate species when linear regressions were $R^2 \geq 0.30$. Therefore, we only used estimated biomass from 27 unique organisms out of a total of 99 unique organisms we observed within all of our 2m² subplots (Supp. Table 3).

SUPPLEMENTARY TABLES

Supp. Table 1. Table of mean fish abundance per species within the different fish communities found in each reef treatment type. Spiny lobster (*Panulirus argus*) were also included as they are a known predator for seagrass-associated invertebrates as well as organisms that are easily detected during surveys

Fish Species	Reef Treatment			
	- FISH, - FERT	- FISH, + FERT	+ FISH, - FERT	+ FISH, + FERT
<i>Abudefduf saxatilis</i>	0.00	0.00	0.25	0.00
<i>Acanthurus chirurgus</i>	1.38	1.38	0.75	0.75
<i>Acanthurus coeruleus</i>	0.25	1.75	1.50	0.75
<i>Apogon townsendi</i>	0.25	0.00	0.00	1.25
<i>Aulostomus maculatus</i>	0.25	0.00	0.25	0.00
<i>Balistes vetula</i>	1.00	0.00	0.50	0.25
<i>Canthidermis sufflamen</i>	0.00	0.00	0.25	0.00
<i>Canthigaster rostrata</i>	0.00	1.00	0.00	0.00
<i>Carangoides ruber</i>	0.00	0.00	1.25	2.25
<i>Chaetodon sedentarius</i>	0.25	0.00	0.00	0.00
<i>Epinephelus striatus</i>	0.25	0.00	0.75	1.00
<i>Gymnothorax funebris</i>	0.25	0.00	0.00	0.00
<i>Gymnothorax moringa</i>	0.25	0.25	17.75	0.25
<i>Gymnothorax vicinus</i>	1.25	0.50	0.00	0.25
<i>Haemulon aurolineatum</i>	0.00	0.00	0.25	14.50
<i>Haemulon melanurum</i>	0.00	0.25	0.00	1.25
<i>Haemulon parra</i>	0.00	0.00	0.00	5.00
<i>Haemulon plumieri</i>	6.13	23.50	22.88	47.13
<i>Halichoeres bivittatus</i>	12.13	6.08	7.38	6.13
<i>Halichoeres garnoti</i>	0.00	0.00	0.75	0.50
<i>Halichoeres poeyi</i>	0.75	2.00	1.25	1.63
<i>Halichoeres radiatus</i>	0.00	0.00	0.00	0.50
<i>Holacanthus ciliaris</i>	0.75	0.25	0.75	0.50
<i>Holocentrus adscensionis</i>	0.88	1.00	13.63	7.13
<i>Lutjanus apodus</i>	0.00	0.00	0.25	0.00
<i>Myripristis jacobus</i>	0.00	0.00	2.50	1.75
<i>Ocyurus chrysurus</i>	0.75	0.00	0.75	0.50
<i>Panulirus argus</i>	0.00	0.25	2.38	0.00
<i>Pareques acuminatus</i>	1.25	1.00	0.75	0.88
<i>Pomacanthus paru</i>	0.50	0.50	0.00	0.00
<i>Pterois volitans</i>	0.00	0.00	0.00	0.63
<i>Sargocentron coruscum</i>	0.25	0.00	0.00	0.00
<i>Scarus iserti</i>	2.00	0.88	2.50	2.63
<i>Scyllarides aequinoctialis</i>	0.00	0.00	0.25	0.00
<i>Sparisoma aurofrenatum</i>	0.00	1.00	0.38	0.50
<i>Sparisoma radians</i>	0.00	2.38	1.50	1.00

Supp. Table 1 (continued)

Fish Species	Reef Treatment			
	- FISH, - FERT	- FISH, + FERT	+ FISH, - FERT	+ FISH, + FERT
<i>Stegastes leucostictus</i>	0.88	0.25	0.88	1.25
<i>Stegastes partitus</i>	0.00	0.00	0.25	0.00
<i>Thlassoma bifasciatum</i>	9.50	7.25	20.13	4.88

Supp. Table 2. Table of seagrass-associated invertebrates sampled in predator exclusion plots (1m²) and survey subplots (2m²); listed to the lowest taxonomic level possible. An Asterisk indicated organisms that were used for estimated biomass for *Q3 shifts in fish- and human-derived nutrient enrichment*. There was a total of 7 phylums, 17 classes, at least 49 families, and 99 unique organisms observed. A total of 3033 organisms were sampled

Phylum	Class	Family	Unique Identity (or Genus species)
Annelida	Echuiria		Echuiria*
Annelida	Polychaeta	Amphinomidae	Amphinomidae
Annelida	Polychaeta	Eunicidae	Eunicidae_1*
Annelida	Polychaeta	Eunicidae	Eunicidae_2*
Annelida	Polychaeta	Eunicidae	Eunicidae_3*
Annelida	Polychaeta	Eunicidae	Eunicidae_4*
Annelida	Polychaeta	Opheliidae	<i>Armandia maculata</i>
Annelida	Polychaeta	Pectinariidae	<i>Lagis</i> spp.
Annelida	Polychaeta	Sabellidae	Sabellidae
Annelida	Polychaeta	Terebellidae	Terebellidae
Annelida	Polychaeta		polychaeta_1*
Annelida	Polychaeta		polychaeta_2*
Annelida	Polychaeta		polychaeta_3*
Arthropoda	Malacostraca	Calappidae	Calappidae
Arthropoda	Malacostraca	Carpiliidae	Carpiliidae
Arthropoda	Malacostraca		Tanaidacea
Arthropoda	Pycnogonida		Pantopoda
Arthropoda	Hexanauplia		Hexanauplia
Arthropoda	Malacostraca	Alpheidae	Alpheidae
Arthropoda	Malacostraca	Diogenidae	<i>Calcinus</i> spp.
Arthropoda	Malacostraca	Diogenidae	<i>Cilbanariu tricolor</i> *
Arthropoda	Malacostraca	Diogenidae	<i>Dardanus venosus</i> *
Arthropoda	Malacostraca	Diogenidae	<i>Paguristes puncticeps</i>
Arthropoda	Malacostraca	Epialtidae	Epialtidae_1
Arthropoda	Malacostraca	Epialtidae	Epialtidae_2
Arthropoda	Malacostraca	Mithracidae	Mithracidae_1*
Arthropoda	Malacostraca	Mithracidae	<i>Pitho aculeata</i> *
Arthropoda	Malacostraca	Mysidae	Mysidae
Arthropoda	Malacostraca	Pinnotheridae	Pinnotheridae
Arthropoda	Malacostraca	Portunidae	Portunidae_1

Supp. Table 2 (continued)

Phylum	Class	Family	Unique Identity (or Genus species)
Arthropoda	Malacostraca	Portunidae	Portunidae_2
Arthropoda	Malacostraca	Portunidae	Portunidae_3
Arthropoda	Malacostraca	Pseudosquillidae	Stomatopoda_1*
Arthropoda	Malacostraca	Pseudosquillidae	Stomatopoda_2*
Arthropoda	Malacostraca		Amphipoda_1
Arthropoda	Malacostraca		Amphipoda_2
Arthropoda	Malacostraca		Amphipoda_2
Arthropoda	Malacostraca		Decapoda_1
Arthropoda	Malacostraca		Diogenidae_1
Arthropoda	Malacostraca		Isopoda
Arthropoda	Malacostraca		Shrimp_1*
Arthropoda	Malacostraca		Shrimp_2*
Arthropoda	Malacostraca		Shrimp_3*
Arthropoda	Ostracoda		Ostracoda*
Chordata	Ascidiacea		Ascidiacea_1
Chordata	Ascidiacea		Ascidiacea_2
Chordata	Ascidiacea		Ascidiacea_3
Chordata	Ascidiacea		Ascidiacea_4
Echinodermata	Asteroidea	Oreasteridae	<i>Oreaster reticulatus</i>
Echinodermata	Echnoidea	Toxopneustidae	<i>Lytechinus variegatus</i>
Echinodermata	Echnoidea		Clypeasteroidea
Echinodermata	Echnoidea		Echnoidea*
Echinodermata	Holothuroidea		Holothuroidea
Echinodermata	Ophiuroidea	Ophiidermatidae	<i>Ophioderma appressum</i>
Echinodermata	Ophiuroidea	Ophiidermatida	Ophiidermatidae_1*
Echinodermata	Ophiuroidea	Ophiidermatida	Ophiidermatidae_2*
Mollusca	Bivalvia	Arcidae	<i>Arca zebra</i>
Mollusca	Bivalvia	Cardiidae	<i>Americardia</i> spp_1
Mollusca	Bivalvia	Cardiidae	<i>Americardia</i> spp_2
Mollusca	Bivalvia	Glycymerididae	<i>Glycymeris pectinata</i>
Mollusca	Bivalvia	Limidae	<i>Lima caribaea</i>
Mollusca	Bivalvia	Pectinidae	Pectinidae
Mollusca	Bivalvia	Pinnidae	Pinnidae
Mollusca	Bivalvia	Pteriidae	<i>Pteria colymbus</i>
Mollusca	Bivalvia	Solecurtidae	<i>Solecurtus</i> spp.
Mollusca	Bivalvia	Tellinidae	<i>Tellina</i> spp.
Mollusca	Bivalvia	Veneroidae	<i>Chione elevate</i>
Mollusca	Bivalvia	Veneroidae	<i>Pitar</i> spp_1
Mollusca	Bivalvia	Veneroidae	<i>Pitar</i> spp_2
Mollusca	Bivalvia	Veneroidae	<i>Pitar</i> spp_3
Mollusca	Bivalvia	Veneroidae	<i>Transennella</i> spp.
Mollusca	Bivalvia	Veneroidae	Veneroidae_1*
Mollusca	Gastropoda	Aplysiidae	Aplysiidae_1
Mollusca	Gastropoda	Aplysiidae	Aplysiidae_2
Mollusca	Gastropoda	Aplysiidae	Aplysiidae_3
Mollusca	Gastropoda	Bullidae	<i>Bulla occidentalis</i>
Mollusca	Gastropoda	Calliostomatidae	<i>Calliostoma</i> spp.
Mollusca	Gastropoda	Cerithiidae	<i>Cerithium</i> spp._1*

Supp. Table 2 (continued)

Phylum	Class	Family	Unique Identity (or Genus species)
Mollusca	Gastropoda	Cerithiidae	<i>Cerithium</i> spp._2*
Mollusca	Gastropoda	Columbellidae	<i>Suturoglypta albella</i>
Mollusca	Gastropoda	Conidae	<i>Conus</i> spp.
Mollusca	Gastropoda	Cymatiidae	<i>Cymatium</i> spp.
Mollusca	Gastropoda	Fissurellidae	<i>Diodora</i> spp.
Mollusca	Gastropoda	Lottidae	<i>Patelloida pustulata</i> *
Mollusca	Gastropoda	Modulidae	<i>Modulus modulus</i>
Mollusca	Gastropoda	Modulidae	<i>Modulus</i> spp.
Mollusca	Gastropoda	Modulidae	<i>Modulus</i> spp.
Mollusca	Gastropoda	Nassaridae	<i>Nassarius</i> spp.
Mollusca	Gastropoda	Neritidae	<i>Smaragdia viridis</i> *
Mollusca	Gastropoda	Olividae	Olividae
Mollusca	Gastropoda	Phasianellidae	<i>Euluthidium</i> spp.*
Mollusca	Gastropoda	Pseudomelatomidae	<i>Crassispira</i> spp.
Mollusca	Gastropoda	Tegulidae	<i>Tegula fasciata</i> *
Mollusca	Gastropoda	Turbinidae	<i>Astralium phoebium</i>
Mollusca	Gastropoda	Turbinidae	<i>Turbo</i> spp.*
Mollusca	Gastropoda		gastropoda_1
Mollusca	Polyplacophora		Polyplacophora
Mollusca	Scaphopoda	Dentaliidae	<i>Antalis</i> spp.
Porifera			Porifera

Supp. Table 3. Total length (mm) and total body wet biomass (mg) data from predator exclusion plots used to estimate biomass per unique organisms for total invertebrate biomass per 2m² plot for *Q3 shifts in fish- and human-derived nutrient enrichment*. Linear regressions were analyzed using ‘Simple Fisheries Stock Assessment Methods’ R package (FSA version 0.8.24)

Unique Identity	n	Biomass Range	Biomass average	Length range	Length average	Linear regression	R ²
Ophiidermatidae	56	5.28 - 473.93	194.28 ± 14.52	10.0 - 76.0	37.68 ± 2.02	y = 1.28x + 0.21	0.40
Cerithiidae	295	8.92-783.7	328.2 ± 10.17	4.0-22.0	13.98 ± 0.21	y = 2.16x -0.00	0.81
<i>Cilbanariu tricolor</i>	146	3.5-758.58	251.20 ± 16.17	3.0-20.0	11.5± 0.36	y = 2.12 + 0.04	0.73
<i>Dardanus venosus</i>	7	7.35 - 704.94	133.34 ± 95.76	5.0 -24	11.85 ± 2.47	y = 2.08x -0.46	0.53
Echuiria	21	3.43-32.97	17.12 ± 3.74	7.0-34.0	14.43 ± 1.34	y = 1.55x -0.70	0.36
<i>Euluthidium</i> spp.	30	13.53-55.86	34.65 ± 2.22	3.0-7.0	5.0 ± 0.18	y = 1.08x + 0.77	0.33
Eunicidae	34	3.91 - 73.09	18.88 ± 3.29	8.0 - 75.0	22.38 ± 2.6	y = 1.05x -0.24	0.37
Ostracoda	5	3.16-14.9	8.7 ± 2.60	3.0-7.0	4.8 ± 0.8	y = 1.88x -0.38	0.95
<i>Patelloida pustulata</i>	9	16.75 - 103.74	64.64 ± 10.22	6.0 - 11.0	9.33 ± 0.55	y = 2.82 -0.97	0.84
<i>Pitho aculeata</i>	16	15.72-416.53	150.7 ± 27.56	3.0-13	8.60 ± 0.72	y = 1.66x + 0.53	0.50
Polychaeta	5	6.44-73.09	33.59 ± 14.72	11.0-75.0	42.6 ± 12.67	y = 1.05x -0.24	0.37
Echnoidea	6	14.06-158.79	147.19 ± 55.23	73.0-12.0	7.67 ± 1.28	y = 2.30x + 0.02	0.89
Shrimp_1_2_3	23	2.21-279.44	5.98 ± 14.717	4.0-35.0	13.87 ± 1.54	y = 2.09x -0.77	0.71
<i>Smaragdia viridis</i>	28	7.4-78.41	40.27 ± 3.14	3.0-7.0	5.20 ± 0.18	y = 2.46x -0.18	0.85
<i>Tegula fasciata</i>	47	155.11-2953.95	1012.48 ± 80.92	6.0-18.0	12.06 ± 0.33	y = 2.25x + 0.52	0.54
<i>Turbo</i> spp.	9	86.49 - 2268.59	986.88 ± 331.633	5.0 - 20.0	11.0 ± 1.73	y = 2.57x + 0.17	0.96
Veneroidae_1	55	49.2-832.12	208.95 ± 24	3.0-26.0	8.54 ± 0.51	y = 2.14x + 0.25	0.68