

EVALUATING STRATEGIES FOR RESTORING PARROTFISH POPULATIONS IN
BELIZE

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ABSTRACT

Courtney Ellen Cox: Evaluating Strategies for Restoring Parrotfish Populations in Belize
(Under the direction of John F. Bruno)

Parrotfish populations have declined throughout the Caribbean due to overfishing. Functional loss of these key grazers has contributed to a shift in reef community structure from coral to algal dominance. Marine protected areas (MPAs) and a national ban on the harvesting of herbivorous fishes are two management strategies implemented in Belize to recover parrotfish populations. Restricting or eliminating fishing is thought to promote high biomass of herbivorous fish that suppress macroalgae facilitating coral recruitment and population recovery. The success of these strategies not only depends on reduced fishing pressure, but also on the connectivity between parrotfish populations.

My doctoral dissertation research examined the effectiveness of the MPA network in Belize and the ban on herbivorous fish harvesting in restoring fish communities and coral reef assemblages. From 2009 to 2013, I quantified the density and biomass of reef fishes, coral cover, and macroalgal cover at 16 reefs in Belize, including 8 protected sites and 8 unprotected sites. I then tested the effects of MPAs and the ban on herbivorous fish harvesting on coral reef community structure, projected parrotfish population recovery, and assessed connectivity between parrotfish populations in Belize and Honduras using nine nuclear microsatellite loci. Over the five year monitoring period, density or biomass increased for four parrotfish species. Population models indicate recovery is underway, and predict that a minimum of 9 years is needed to reach complete population recovery. Although

the ban has been beneficial for parrotfish populations, my results also suggest that Belize's current network of MPAs have not provided general and measurable ecological benefits for parrotfish biomass or the benthic community. I found only weak genetic population structure among populations within the Southern Mesoamerican Barrier Reef suggesting that these populations are connected via larval dispersal. My results highlight the importance of establishing a management approach that crosses international boundaries and suggest that improved enforcement of MPAs and additional restrictions on fishing effort may be necessary to restore parrotfish populations and coral reef health.

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CHAPTER 1: GENETIC TESTING REVEALS SOME MISLABELING BUT GENERAL COMPLIANCE WITH A BAN ON HERBIVOROUS FISH HARVESTING IN BELIZE

Introduction

In recent decades, coral reef ecosystems have experienced a substantial decline in coral health and fish abundances (Hughes 1994, Jackson et al. 2001, Gardner et al. 2003) and thus resource managers have implemented various measures to mitigate coral loss and restore fish populations. However, overexploitation of fish populations continues to occur despite conservation efforts in part because of illegal, unregulated, and unreported (IUU) fishing and fish mislabeling (Baker et al. 2007, Jacquet and Pauly 2008, Miller et al. 2011). The Belize Fisheries Department has developed a number of progressive marine management strategies including the establishment of no-take zones, protection of spawning aggregation sites, bans on bottom trawling and on the capture and possession of herbivorous fishes (Scaridae and Acanthuridae). Parrotfish comprised an average of 28% of the catch at Glover's Reef from 2005 to 2008 (Wildlife Conservation Society 2010). In 2009, resource managers implemented the national ban on herbivorous fish harvesting to mitigate high (~50%) macroalgal cover on much of the Belize Barrier Reef, which has largely been attributed to the loss of herbivorous fishes (Hughes et al. 2005). The new regulation was communicated to the public, specifically local fishermen, through public meetings in coastal fishing towns. Belize is the first country to implement a regional ban on herbivorous fish harvesting.

However, evaluation of compliance with the ban is needed to fully assess the value of the approach.

Fishing is economically, culturally, and socially important for many coastal communities in Belize with finfishes historically being an important local fishery and more recently an important export fishery (Belize Ministry of Agriculture and Fisheries 2008). Specifically, snapper and grouper are highly sought after by fishermen to meet demand from locals and tourists. Other important species in Belize include common snook, mackerels, kingfish, cobia, small tunas, bonito, pompano, permit, and hogfish (Belize Ministry of Agriculture and Fisheries 2008). In Belize, the Nassau grouper is protected from December 1 to March 31 and only individuals between 20 cm and 30 cm can be harvested year round. In addition, snapper and grouper aggregation sites require special permits from the fisheries department. However, snapper and grouper populations have been declining throughout the Caribbean including in Belize (Sala et al. 2001, Graham et al. 2008, RT et al. 2009, Paddock et al. 2009, Stallings 2009b, Mumby et al. 2012). Despite the declines in snapper and grouper populations, purported fillets of these species are still readily available in restaurants, fish markets, and supermarkets, which suggests that fish vendors may be selling less desirable species - including herbivorous species such as parrotfish and surgeonfish - as snapper and grouper. Mislabeling fillets of less desirable fish species as more popular and more expensive fish species has been well documented in the U.S. and other parts of the world (Jacquet and Pauly 2008, Miller and Mariani 2011). Marko et al. (2004) found that 77% of fish labeled as the overfished red snapper (*L. campechanus*) on the East Coast of the US were identified as less desirable species. Logan et al. (2008) found that 56% of fish labeled as Pacific red

snapper (genus *Sebastes*) in California and Washington were identified as overfished species of *Sebastes*. In the US, studies such as these have resulted in fines for seafood fraud (up to \$1 million) and states developing programs to use DNA testing to prevent mislabeling.

According to marine reserve managers, few arrests have been made for the possession of herbivorous fish (Annelise Hagan, person. comm, 2011). However, it is difficult to evaluate true compliance with this ban based on arrest records because of the lack of detailed record keeping by enforcement rangers. An alternative approach to detecting illegal fishing is to use genetic identification to determine if illegal species are being sold as fillet in markets. This study documents the prevalence of illegal, herbivorous fish and fish mislabeling in local markets from five major Belizean towns over a two year period.

Methods

Sample and data collection

We designed the sampling methodology to maximize spatial coverage within Belize, maximize the type of vendors sampled, and replicate the data collection over time. We purchased 111 fish fillets from open fish markets, supermarkets, restaurants, and/or fishing co-operatives in five major fishing and/or tourist towns along the Belize coast in May/June and October/November from 2009 to 2011 (Appendix A). We removed approximately 1 gram of muscle tissue from the fillets and stored in either 95% ethanol or 150 proof liquor in 2 ml screw cap tubes. The number of fish fillets purchased varied between towns and sampling periods due to availability from fishermen and number of fish vendors. A detailed account of sampling conducted in each town is included in Appendix A. We could not be

certain whether each fillet was cut from a different fish or if multiple fillets were cut from one large fish. Therefore, we analyzed the data under two assumptions; 1) each fillet was cut from a different fish and 2) fillets identified as the same species purchased from one vendor were cut from one large fish. For the purpose of proportion comparisons, we defined an individual sampling as data collected at one vendor in one town during one sampling period as summarized in Table S1.

DNA Extraction and PCR Amplification

We extracted genomic DNA from sample tissue with the Qiagen Puregene Mousetail kit (former Gentra cat. no. D-7010B) and stored at -20°C . A 658 base pair (bp) fragment of the mtDNA cytochrome oxidase I (COI) gene was amplified by PCR using a combination of LCO1490/HCO2198 (Folmer et al. 1994) or FishF1/FishR1 (Ward et al. 2005) oligonucleotide primers. A detailed description of the PCR reactions is included in Appendix A. We ran PCR products on a 1% agarose gel to confirm amplification of the correct fragment.

DNA Sequencing and Sequence Alignment

We purified PCR products using Zymo DNA Clean and Concentrator -25 (cat. no. D4033). All DNA was sequenced in one direction using PCR primers. Sequence identification was determined using both BOLD to search the Barcode of Life Data Systems and BLAST to search GenBank. We established confidence values for both BLAST (e-value $< 1\text{e-}100$) and BOLD (probability of placement $> 95\%$) to ensure that only high quality sequences were used to identify samples. Sequences obtained from unknown samples and

reference species were aligned with ClustalX.

Phylogenetic Analysis

We constructed phylograms using MEGA 5.1 using neighbor joining analysis and a Kimura two parameter (K2P) model to provide a graphic representation of the patterning of divergence between species (Tamura et al. 2011). The K2P model was selected by the MEGA 5.1 Best Fit DNA Model. Confidence in phylograms was assessed by the nonparametric bootstrap method with 1000 replications. Deep nodes within the phylogram could not be resolved using COI alone; therefore, BLAST and BOLD were used to verify our sample identification.

Statistical Analyses

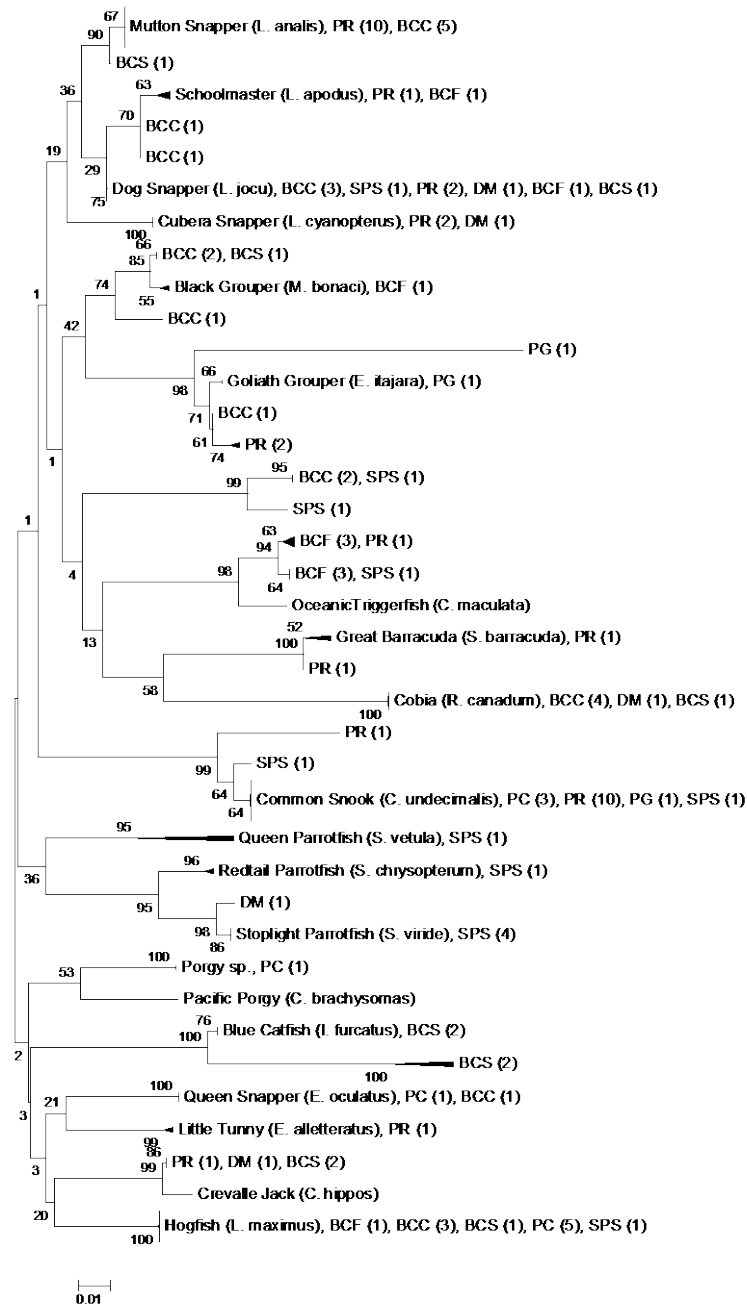
We utilized Fisher's Exact Tests to analyze differences in mislabeling proportions between sampling periods, vendors and towns. The Bonferroni correction was used to adjust the level of significance when conducting multiple significance tests.

Results

The purchased fish fillets were from fifteen fish genera (Fig. 1.1). Most samples were identified to species using BOLD and/or BLAST. We confirmed that a total of 69 out of the 111 fillets were cut from different individual fishes. It is possible that all fillets were cut from different fishes; however, 42 of the fillets were identified as the same species and sold from the same vendor.

Figure 1.1: Evolutionary relationships of samples

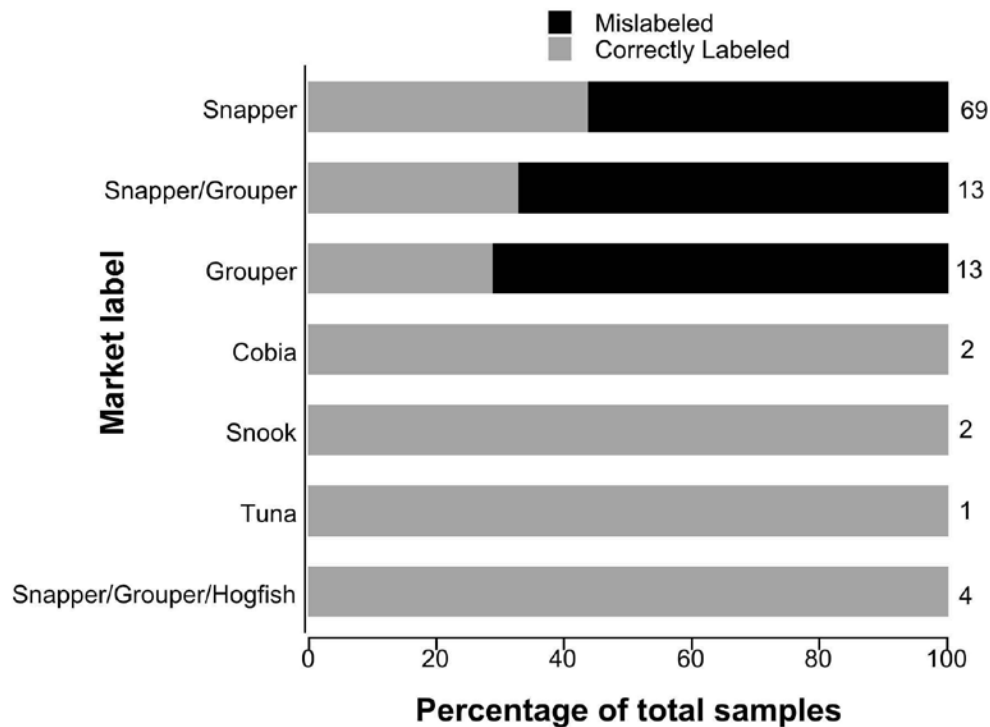
The evolutionary history was inferred using the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Tips are labeled by town and vendor (number of fillets) or reference species. PG=Punta Gorda, P=Placencia, D=Dangriga, SP=San Pedro, BC=Belize City, M=fish market, S=supermarket, R=restaurant, C=fishing co-operative.



Therefore, we assumed that these 42 fillets were cut from one of the 69 fishes when calculating minimum proportions of mislabeling and parrotfish. When we treated each fillet as an individual fish, the mean proportion, (*i.e.*, across towns, vendors and sampling periods) of mislabeled samples was $51 \pm 25\%$ (± 1 SE), $7 \pm 17\%$ of which were herbivorous fish. When we treated multiple fillets of the same species purchased from an individual vendor as one large fish, the mean proportion of mislabeled samples was $32 \pm 24\%$ (± 1 SE), $5 \pm 13\%$ of which were herbivorous fish. Fillets purchased were labeled as snapper, grouper, snapper/grouper, snapper/grouper/hogfish, cobia, tuna or snook. Only fillets labeled as snapper or grouper were mislabeled (Fig. 1.2).

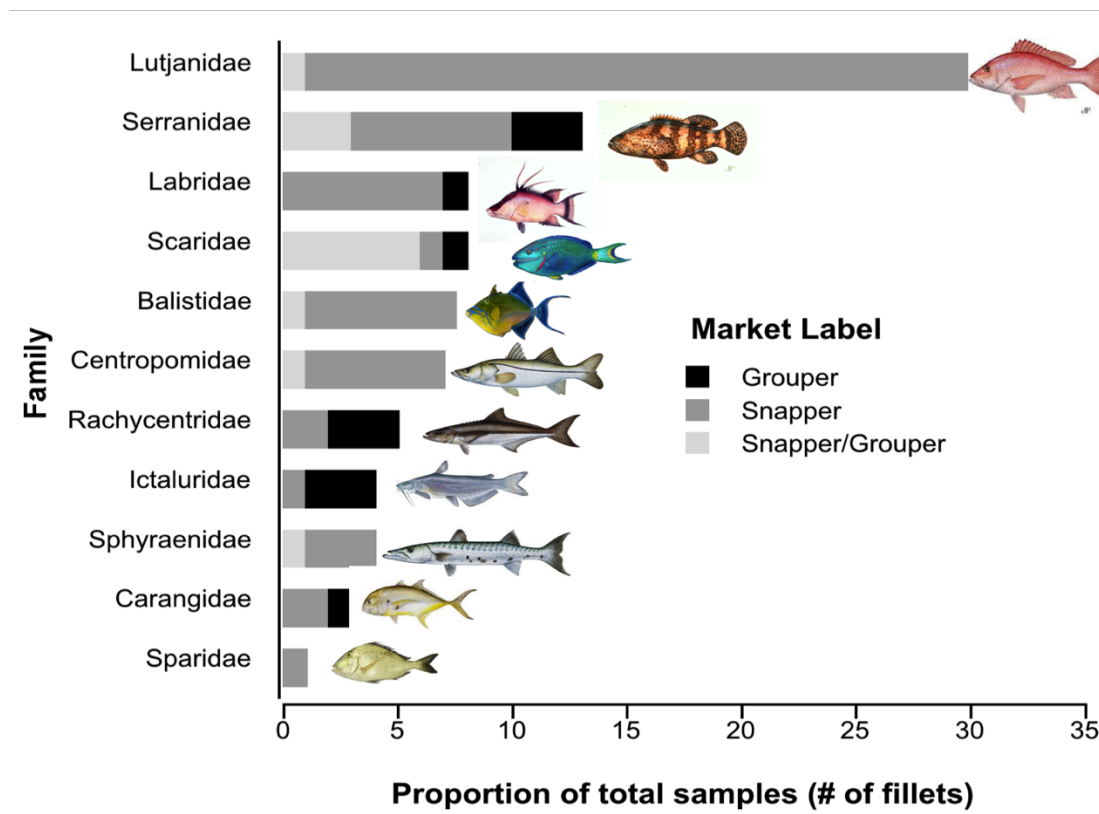
Figure 1.2: Proportion of Mislabeling by market label.

We pooled samples from each town/vendor to calculate the percentage of mislabeling. Sample size is listed at the end of each row.



We genetically identified fillets labeled as snapper or grouper to one of 11 families (Fig.1. 3). The proportion of fillets that were identified as parrotfish in San Pedro (43%) was significantly higher than Placencia (0%) and Belize City (2%) when each fillet was treated as an individual fish (Table 1.1). However, the proportion of parrotfish was not significantly different between towns when we treated multiple fillets of the same species purchased from an individual vendor as one large fish. The proportion of mislabeling was not significantly different between Belize City, Placencia, Dangriga, and San Pedro (Table 1.1).

Figure 1.3: Species composition of samples labeled as snapper, grouper, and snapper/grouper.



The proportion of mislabeled samples and parrotfish were zero in Punta Gorda; however there was not a significant difference between this town and other towns with much

higher proportions. The small sample size in Punta Gorda (N=3) likely accounts for the lack of significance between Punta Gorda and any of the other towns. We calculated total proportions by averaging (weighted average) the proportion of mislabeling or parrotfish estimated by each sampling in a particular town (Appendix A).

Table 1.1: Summary of Fish Mislabeling by Town.

The proportions of mislabeled samples were not significantly different between towns for total mislabeled samples ($p=0.09$). The proportion of parrotfish was significantly higher in San Pedro than in Placencia ($p<0.001$) and Belize City ($p<0.001$) when each fillet was treated as an individual fish. Proportions were not significantly different when fillets identified as the same species from an individual vendor were treated as one fish.

Town	Number of Samplings	Number of fillets (minimum number of individual fishes)	Fillets Identified as Parrotfish (Mean% \pm SE)	Total Mislabeled Fillets (Mean% \pm SE)
Punta Gorda	2	3 (3)	0 \pm 0	0 \pm 0
Belize City	11	46 (28)	2 \pm 10 - 4 \pm 10**	50 \pm 42 - 60 \pm 42**
Placencia	4	44/37* (24/23)	0 \pm 0	39 \pm 32** - 47 \pm 35
Dangriga	1	5 (5)	20	60
San Pedro	3	13 (10)	33 \pm 23** - 43 \pm 33	66 \pm 24** - 73 \pm 25

* 7 of these samples were confiscated from restaurants by the Belize Fisheries Department and the market label was not known. These samples were only used to calculate proportions of parrotfish.

** Mean calculation assumed that fillets identified as the same species at an individual vendor were cut from one fish.

The proportion of parrotfish sold was significantly higher in supermarkets than in restaurants or co-operatives and the proportion of mislabeling was significantly higher in open fish markets than in restaurants when fillets were treated as individual fishes, but was not significantly different when fillets of the same species were treated as one fish (Table 1.2). We calculated total proportion of mislabeling per vendor type by averaging (weighted average) the proportion of mislabeling from each individual vendor at each sampling period (Appendix A).

Table 1.2: Summary of Fish Mislabeling by Vendor.

The proportion of total mislabeled samples was significantly higher in the open fish markets than in restaurants ($p=0.004$) and the proportion of parrotfish sold was significantly higher in supermarkets than in restaurants ($p<0.001$) and in supermarkets when compared to that in co-operatives ($p<0.001$) when each fillet was treated as an individual fish. Proportions were not significantly different when fillets identified as the same species from an individual vendor were treated as one fish.

Vendor	Number of Samplings	Number of fillets (minimum number of individual fishes)	Fillets Identified as Parrotfish (Mean%±SE)	Total Mislabeled Fillets (Mean%±SE)
Restaurant	3	34/29* (23/22)	0±0	28±32** - 32±33
Fish Market	4	15 (11)	7±9 - 9±10**	53±20** - 80±20
Co-op	7	39 (20)	0±0	34±50** - 54±50
Supermarket	7	24 (19)	10±25** - 21±25	33±34** - 50±37

* 7 of these samples were confiscated from restaurants by the Belize Fisheries Department and the market label was not known. These samples were only used to calculate proportions of parrotfish.

** Mean calculation assumed that fillets identified as the same species at an individual vendor were cut from one fish.

The proportion of total mislabeled samples was significantly lower in June 2011 than in all other sampling periods; however, the proportion of fillets identified as parrotfish was not significantly different between sampling periods when fillets were treated as individual fishes and when fillets of the same species were treated as one fish (Table 1.3). Fish mislabeling and parrotfish sold in local markets increased from November 2009 to May 2010 and then decreased from May 2010 to June 2011.

Discussion

We found that 5% to 7% of fish fillets sold in local markets were illegal, parrotfish species and 32% to 51% were mislabeled. The proportion of mislabeling is similar to that in

many parts of the world (Baker et al. 2007, 2008, Wong and Hanner 2008, Ardura et al. 2010, Garcia-Vazquez et al. 2011, Marko et al. 2011).

Table 1.3: Summary of Fish Mislabeling by Sampling Period.

The proportion of total mislabeled samples was significantly lower in June 2011 when compared to proportions calculated for all other sampling periods ($p < 0.004$). The proportion of fillet identified as parrotfish was not significantly different between samplings periods.

Sampling Period	Number of Samplings	Number of fillets (minimum number of individual fishes)	Fillets Identified as Parrotfish (Mean%±SE)	Total Mislabeled Fillets (Mean%±SE)
November 2009	5	22 (15)	0±0	53±34** - 64±34
May 2010	5	22 (14)	14±19** - 23±29	71±23** - 82±17
October 2010*	6	39/32 (29/28)	5±10 - 8±17**	63±20** - 72±23
June 2011	4	28 (17)	0±0	4±3 - 6±4**

* 7 of these samples were confiscated from restaurants by the Belize Fisheries Department and the market label was not known. These samples were only used to calculate proportions of parrotfish.

** Mean calculation assumed that fillets identified as the same species at an individual vendor were cut from one fish

Low proportions of parrotfish were detected in Belize City (2%-4%), Placencia (0%), and Punta Gorda (0%) while proportions were higher in Dangriga (20%) and San Pedro (33%-43%). Proportions of mislabeling were relatively consistent among towns except for Punta Gorda where no mislabeling was detected. Local fishing culture, population size, and tourism activity varies between towns, which may provide insight into proportional differences in mislabeling and parrotfish sold in the markets (Table 1.4). The Belize Tourism Board reports the contribution of each region or town to the national hotel room revenue, which was used as a proxy for tourism activity (Belize Tourism Board 2008). Most of the fishermen are from rural and coastal communities and travel long distances (> 50 km) to

fishing grounds; therefore, it is difficult to determine where the fishermen in each town are harvesting.

San Pedro, is the main town on Ambergris Caye, and the most popular tourist destination in Belize. Ambergris Caye generates the highest national hotel revenue in Belize. Fishermen on Ambergris Caye mostly sell their catch directly to restaurants and hotels. Placencia is the fastest growing tourist destination and generates the second highest national hotel revenue. The number of fishermen in Placencia has decreased by approximately 95% (Noella Gray et al. 2010).

Table 1.4: Town Tourism and Population Statistics

District	Town	Population	% National Hotel Revenue	Number of fishermen	Fillets Identified as Parrotfish (Mean%)	Total Mislabeled Fillets (Mean%)
Toledo	Punta Gorda	5,205	1.2	≈107	0	0
	Toledo Rural	25,333				
Belize District	Belize City	53,532	12.1	>500	2 - 4**	50 - 60**
	Belize Rural	24,305				
	Ambergris Caye/ San Pedro	11,510	42.3	*	33** - 43	66** - 73
Stann Creek	Dangriga	9,096	7.3	≈30	20	60
	Stann Creek Rural	23,070				
	Placencia	750	12.4	≈25	0±0	39** - 47

* data not available

** Mean calculation assumed that fillets identified as the same species at an individual vendor were cut from one fish.

Belize City is the largest town in Belize and main port of entry, but only ranks third in national hotel revenue. Fishermen from all over Belize come to Belize City to sell their catch to the two major fishing co-operatives, local fish markets and restaurants. Dangriga is the cultural center of the Garifuna people and with the surrounding rural areas ranks fourth in national hotel revenue. Fishermen sell their catch in one fish market in Dangriga and directly to restaurants and hotels. Punta Gorda is a small fishing village and with the surrounding rural areas generates the lowest national hotel revenue. Approximately 107 fishermen are based out of Punta Gorda (Heyman and Graham 2000). Fishermen sell their catch in one fish market in Punta Gorda, one small co-operative and directly to restaurants and hotels.

Although proportional differences were not significant, our data show that high levels of mislabeling are associated with towns that have relatively high tourist activity (San Pedro, Placencia, and Belize City). In contrast, a high level of mislabeling was also found in Dangriga, which has relatively low tourist activity. Small sample size may account for the high level of mislabeling in Dangriga. Punta Gorda has relatively low tourist activity and a low level of mislabeling. Tourist activity may be increasing the demand for snapper and grouper fillet and thereby increasing mislabeling.

Sufficient data was not available to calculate a national average of harvested parrotfish prior to implementation of the ban; however, we compared our results to catch data collected at Glover's Reef Marine Reserve from 2005 to 2008 to determine the extent of the decrease in parrotfish harvesting (Wildlife Conservation Society 2010). Across Belize, 5% to 7% of fillets were parrotfish, which is significantly lower than the proportion of parrotfish

(28%) harvested from 2005 to 2008 at Glover's Reef Marine Reserve ($p < 0.001$). The proportion of parrotfish was much higher in San Pedro (43%) and Dangriga (20%). A small sample size may account for the relatively high percentage of parrotfish fillets sold in Dangriga. Parrotfish fillets were also found in Belize City at a much lower frequency (2%). Although overall compliance with the ban seems to be fairly high, spatial variation in the proportion of parrotfish fillets indicates a need for stronger enforcement in San Pedro and possibly Dangriga. Fisheries officers have a strong presence in Belize City and Placencia. These towns have low proportions of parrotfish in the markets suggesting that the presence of enforcement officers may be discouraging parrotfish marketing.

Snapper and grouper population declines have reduced the availability of snapper and grouper in Belize, forcing fishermen to supply the high demand for these target species with alternative fish species (Sala et al. 2001, Graham et al. 2008, RT et al. 2009, Paddock et al. 2009, Stallings 2009a, Mumby et al. 2011). We identified most of the mislabeled fillet samples as Labridae (hogfish), Scaridae (parrotfish), and Balistidae (triggerfish) (Fig. 3). Hogfish - a wrasse - is a popular fish in Belize and is often referred to as a hog snapper throughout the Greater Caribbean region. Culturally, labeling hogfish as a snapper would not be considered mislabeling. However, for the purposes of fisheries management, it is important to report fish correctly by taxonomic classification. We identified 9% of the mislabeled samples as triggerfish (Balistidae), which are not considered a desirable fish species in Belize. We identified 4% of the mislabeled samples as catfish (Ictaluridae). These are brackish species that are often seen in open canals in Belize City and rarely eaten by Belizeans. The remaining species that were sold as snapper or grouper are not necessarily

undesirable, but are less expensive than snapper and grouper.

The main incentives for fish mislabeling are meeting consumer demand and increasing profits. The supply chain in Belize is fairly short. Belizean fishermen sell their catch directly to locals in local fish markets, to co-operatives who then export catch or sell to local businesses, or directly to restaurants and hotels. It is unclear where along the supply chain most mislabeling is occurring and it is possible that vendors are unknowingly mislabeling fillets, but marketing and selling an undesirable fish as a popular and expensive fish can be highly profitable. For example, in Belize, snapper can be sold for twice the price of non-target species (Wendy's Restaurant (local restaurant), pers. comm. 2011). We found that snapper, grouper and hogfish fillets were sold at an average of US\$5.55 per pound, while cobia and snook fillets were sold at an average of US\$2.60 per pound (Table S2). Therefore, consumers may be unknowingly overpaying for desired fish, restaurant and hotel owners may be unknowingly deceiving customers, and honest fishermen may be losing profits to fraudulent competitors. Many fishermen have observed the decline in snapper and grouper abundances and support increased enforcement and fishing regulations (Heyman & Graham 2000). However, demand in restaurants remains high and supply seems to meet the demand potentially because of mislabeling. Therefore, many consumers are unaware of the fragile state of popular fish species. Fish mislabeling produces a false sense of availability, which reduces consumer power to control the market and causes even sustainable consumer choices to lead to overexploitation. For example, Marko et al. (2011) found Chilean sea bass with the Marine Stewardship Certification (MSC) labels, which indicate that the fish was harvested from the sustainable fishery, actually came from the unsustainable fishery.

Conclusions and Recommendations

In Belize, recovery of fragile coral reef ecosystems would support the local economy by directly benefiting tourism and fishing industries. Random fillet analysis would provide additional catch data that could be used to identify herbivorous fish sold in markets. However, funds for enforcement are already limited and providing resources for a project to analyze fillet may not be reasonable for Belize. An alternative approach would be to develop conservation campaigns that encourage local consumers and tourists to purchase more abundant species. Reducing the demand for snapper and grouper would reduce the prevalence of illegal fish in markets and the level of mislabeling. In addition, increasing demand for other species would benefit fishermen by increasing the cost of currently less desirable species. The results of this study suggest a decrease in parrotfish harvesting after implementation of the ban indicating that a regional harvesting ban has the potential to contribute to coral reef ecosystem recovery.

CHAPTER 2: NATIONAL FISHING BAN PROMOTES RECOVERY OF PARROTFISH POPULATIONS ON CARIBBEAN CORAL REEFS

Introduction

Species loss due to overfishing leads to potentially devastating shifts in species composition particularly in reef systems with limited functional diversity (Roberts 1995a). The region-wide mass mortality of the black sea urchin (*Diadema antillarum*) in 1983/84 resulted in the loss of a keystone grazer on Caribbean coral reefs leaving parrotfishes (*Scarus* sp. and *Sparisoma* sp.) as one of the few herbivores capable of controlling the abundance of fleshy macroalgae (Carpenter 1986, 1990, Liddell and Ohlhorst 1986, Mumby et al. 2006b). Consequently, the subsequent functional loss of these herbivorous fishes (i.e., grazing pressure) caused by overfishing (Hughes 1994, Pauly et al. 1998, Hughes et al. 2005, Paddock et al. 2009) has largely caused observed shifts in Caribbean reef communities from coral to algal dominance (Knowlton 1992, Done 1992, Hughes 1994, Hughes et al. 2003, McManus and Polsenberg 2004). Recent conservation management strategies designed to reverse algal dominance and improve coral reef health have focused on restoring parrotfish populations by reducing fishing pressure. However, no studies have assessed the response of these populations over time to reduced fishing pressure, which is the first step needed to test the effectiveness of this management approach. The number and body size of parrotfish on the Belize Barrier Reef (BBR) have declined considerably in the past decade, including reefs inside marine protected areas (MPAs) (Mumby et al. 2012).

Parrotfish biomass at Glover's Reef marine reserve declined by 41% from 2002 to 2008/09, with a major decline in the large and dominant fish herbivore, the Stoplight parrotfish (*Sparisoma viride*) (Mumby et al. 2012). Concurrently, macroalgal cover has increased across the BBR since the late 1990s from roughly 10% to 50% (McClanahan and Muthiga 1998, McClanahan et al. 1999). With *D. antillarum* functionally extinct in Belize and recovering at very slow rates and evidence that MPAs may not be effective at restoring herbivorous fishes (Huntington et al. 2011), resource managers implemented a national ban on herbivorous fish harvesting in April 2009. The new regulation prevents the harvesting of any species of parrotfish (Scarids) or surgeonfish (Acanthurids) nationwide (Statutory Instrument No. 49 of 2009). Parrotfish comprised an average of 28% of the catch at Glover's Reef in Belize from 2005 to 2008 (Wildlife Conservation Society 2010). Genetic testing revealed that only 7% of fish fillets collected in five major towns in Belize from 2009 to 2011 were parrotfish suggesting a decrease in parrotfish harvesting following the ban (Cox et al. 2013).

The response of fish populations to overfishing and subsequent reduced exploitation varies among species and is related to the life history traits of the species (Adams 1980, Hutchings 2001, Fernandes and Cook 2013). Fish populations with a long life span, late maturity, large body size and low rates of natural mortality and recruitment are expected to decline in abundance quickly due to overfishing and recover slowly when fishing stops, relative to populations with opposite life history characteristics (Adams 1980, Russ and Alcala 1998). Parrotfishes are relatively fast growing species that reach maturity at an early

age (approximately 1-2 years) and spawn year round (Robertson and Warner 1978). These life history traits may improve their potential to recover relatively quickly from overfishing (Adams 1980).

The rate of population recovery is also directly correlated with the extent of population decline, fishing mortality during recovery, intrinsic rate of increase, and exploitation history (Neubauer et al. 2013). Recovery can be predictable and achievable within a decade for fish populations with average exploitation histories and intrinsic rates of increase if fishing mortality is rapidly reduced prior to collapse (Neubauer et al. 2013). However, reducing fishing pressure may not always result in the recovery of depleted species potentially due to an Allee effect, a phenomenon characterized by reduced reproductive success resulting from very low population density (Kuparinen et al. 2014). For example, most Northwest Atlantic cod (*Gadus morhua*) populations collapsed in the early 1990s due to overexploitation (Hutchings and Myers 1994) and few populations have exhibited signs of recovery despite significant reductions in fishing pressure (Hutchings and Rangeley 2011). Results of model simulations predicting the recovery of Northwest Atlantic cod populations suggest that Allee effects not only slowed populations recovery but also increased the uncertainty of recovery time (Kuparinen et al. 2014).

If overharvesting was the prime cause of the decline of herbivores, the ban on herbivorous fish harvesting should lead to some extent of ecosystem recovery measured first by an increase in parrotfish density and biomass. The extent of ecosystem recovery (i.e., reduction of macroalgae cover) will depend on the top-down forces exhibited by herbivorous

fishes on algae communities in the absence of *D. antillarum*. In this paper, we first assess the effect of the ban and other factors potentially influencing parrotfish density after 4 years of ban establishment, and second, we predict the recovery response of *S. viride* over time using a size structured model and then compare model projections to observed post-ban changes in parrotfish demography (Crouse et al. 1987, White et al. 2010b, 2011). *S. viride* was chosen as a model species because they are one of the most abundant species on the BBR, one of the most targeted parrotfishes, and sufficient data required to conduct model simulations is available on life history traits such as growth and mortality rates.

Methods

Fish Censuses

We monitored reef fish communities at 16 sites (15-18 m) along the BBR during May and June in 2009, 2010, 2012 and 2013 (Fig. S1, Table S1). Sites were selected to maximize spatial coverage along the fore reef track, include a range of protection zones, and to coincide with sites monitored in previous years by local NGOs. Monitoring sites included (a) fully-protected reserves in which all fishing is banned (Conservation Zone 1), (b) reserves where some fishing and extractive activities are allowed (Conservation Zone 2) and (c) unprotected reefs on which fishing is permitted (None). To minimize habitat variability of survey sites, we only surveyed spur-and-grove reef formations at each site and focus on *Orbicella* (former *Monstastrea*) dominated habitat. Refer to Appendix S1 for additional details regarding the visual fish censuses.

Statistical Analysis

We used linear mixed effects models to test the effect of the ban on total parrotfish biomass and density annually over the five-year monitoring period. We also used generalized linear mixed effects models to test the effect of the ban on individual parrotfish species and to test the effect of the number of mature individuals on juvenile density in the following year. Total parrotfish density (log-transformed) and parrotfish biomass were modeled with a Gaussian distribution and identity link through a linear mixed effect model. The density of each individual parrotfish species was modeled with an inverse Gaussian distribution through a generalized linear mixed effects model. The number of years following ban implementation (i.e., post 2009) and 10 covariates that could influence parrotfish biomass and density were coded as fixed effects and sites were coded as random effects. Covariates included protection status (C1, C2 or none), human population density within 50 km radius of each site, reef structural complexity, reef area and mangrove perimeter within 10 km of each site, minimum average sea surface temperature (2002-2011), wave exposure, predator biomass, macroalgal cover, and average oceanic net primary productivity (2002-2012). Refer to Appendix S2 for details regarding covariate data collection. To evaluate collinearity among all explanatory variables, we calculated the variance inflation factors (VIF). We sequentially removed each covariate for which the VIF value was above 2 (Graham 2003). Wave exposure and protection status were sufficiently correlated to compromise interpretation when modeled together (*Spearman rank correlation* $r_s = 0.50$): therefore, we modeled these covariates separately (Graham 2003). Model A included wave exposure and a covariate and Model B included protection status as a covariate. We generated sets of models with combinations of the terms in each global model. To select the best model we averaged the subset of models

with a delta Akaike's Information Criterion corrected (AICc) for small samples of less than 2. Homogeneous and normal distribution errors of final models were confirmed in the plot of residuals against predicted values and quantile-quantile plots, respectively (Zuur et al. 2009). Spline spatial correlograms were plotted to corroborate that the final model residuals were not spatially autocorrelated (Zuur et al. 2009). All analyses were performed in R v.2.15.2 (R Core Team 2013) using the package *nlme* v.3.1-113 for the linear mixed-effect models, *lme4* v.0.99-2 for generalized linear mixed-effect models and *MuMin* v. 1.9.13 for the model averaging.

Stage-Structured Model

To predict parrotfish population recovery we developed a closed population stage-structured model based on recruitment rate, growth rate, and natural survival rate of individuals in four stage classes. We assumed a closed population because adequate recruitment data is not available to build a reliable open population model.

Density data was categorized into four stage classes: (1) juveniles 0-10 cm, (2) sub-adults 11-20 cm, (3) adults 21-30 cm, and (4) large adults 31-50 cm. For each stage class, we estimated the recruitment (R), the probability of surviving and growing into the next stage size class (G) and the probability of surviving and remaining in the same stage (P). We used the best available vital parameters taken from the literature or calculated parameters based on our observed density data (Table 1). The resulting stage-based projection model is as follows:

$$A = \begin{bmatrix} P1 & R2 & R3 & R4 \\ G1 & P2 & 0 & 0 \\ 0 & G2 & P3 & 0 \\ 0 & 0 & G4 & P4 \end{bmatrix}$$

The transition probabilities G, and P, were estimated using Equation 1 and Equation 2 (Crouse et al. 1987).

$$P_i = \left(\frac{1-p_i^{d_i-1}}{1-p_i^{d_i}} \right) p_i \quad (1)$$

$$G_i = \frac{(p_i^{d_i})(1-p_i)}{1-p_i^{d_i}} \quad (2)$$

where p_i is the stage-specific survival probability and d_i is the stage duration (in years). We calculated the stage duration (d_i) using the von Bertalanffy equation:

$$L_t L_t = L_\infty (1 - e^{-k(di)} e^{-k(tn-to)}), \quad (3)$$

where L_∞ is the asymptotic maximum size, k is the growth rate, and L_t is the change in length over d_i (Table 2.1).

Table 2.1: Life History Traits for Stoplight Parrotfish, *Sparisoma viride*
S=survival rate, R=recruitment rate

Size Class	S (%/yr) ^a	R (#/yr)	Growth Rate (#/yr) ^b	Asymptotic maximum size (cm) ^c	Duration in size class (yr)	c ^e	b ^{d,e}
0-5 cm	44	0	0.45	35.7	0.28	0.0004	2.93
6-10 cm	44	0	0.45	35.7	0.39	0.0004	2.93
11-20 cm	54	0.034	0.45	35.7	1.10	0.0004	2.93
21-30 cm	81	0.499	0.45	35.7	2.95	0.0004	2.93
31-50 cm	86	1.337	0.45	35.7	5.28	0.0004	2.93

a. van Rooij and Videler1997

b. Choat et al 2003

c. Chaot et al 2003

d. Bohnsack and Harper 1988

e. Parameters in equation $R = cTL^b$

Fishing presumably ceased in 2009 following implementation of the ban on herbivorous fish harvesting. Therefore, the model assumed zero fishing pressure and survival rates in the 11-50 cm size classes were based only on natural mortality rates estimated by van Rooij and Videler. (1997). The projected population densities are based on size-specific natural survival

rates estimated from *S. viride* populations in Bonaire, Netherlands Antiles in 1997 (van Rooij and Videler 1997) because natural survival rates have not been estimated for *S. viride* populations in Belize. Natural survival rates are dependent on numerous factors including predation pressure, resource limitation, and competition (Shulman and Ogden 1987, Hixon 1991, Carr and Hixon 1995, Stewart and Jones 2001). We acknowledge that natural mortality rates for *S. viride* in Belize may differ from those estimated in Bonaire due to variation in ecological or environmental conditions; however, estimating parrotfish mortality at each size class in Belize was out of the scope of this study. Survival rate for the 0-10 cm size class was estimated by calculating the proportion of individuals recorded in the 0-10 cm class size that were again recorded in the 11-20 cm size in the following year. *S. viride* were estimated to spend approximately 8 months in the 0-10 cm size class and 1 year in the 11-20 cm size class; therefore, the juveniles that survived would have grown into the next size class by the following year.

Population Projection

We conducted model simulations to project population density over 50 years by multiplying successively higher powers of the projection matrix and by an initial population vector consisting of densities observed in 2010. The fishing ban in Belize was implemented in April 2009 and our monitoring surveys were conducted in May 2009. Parrotfish harvesting most likely continued for a period of time after implementation as the new regulation was disseminated through the fishing community. Therefore, we concluded that the assumption of zero fishing pressure used in the population projection model was most closely met after

2010 and used 2010 size class densities as the initial population vector to predict long-term population dynamics.

This type of model has asymptotic (i.e., long-term) behavior described by the dominant eigenvalue (λ) of A : geometric growth ($\lambda > 0$) or decline ($\lambda < 0$). We examined the resulting stable size distribution (SSD), and the elasticity of λ to parameter changes. In the long term, the matrix will converge to a stable size distribution, i.e., a consistent proportion of individuals in each age class, given by the dominant right eigenvector of A . The model predicted the number of years to reach SSD, which was used as an estimate for the time required for *S. viride* populations to recover. We identified the matrix parameters that are most important contributors to λ through an elasticity analysis, which measures the change in λ in response to a proportional change in the parameters.

This model does not predict the stable nonzero equilibrium density because it lacks density dependence. However, this is adequate for describing the initial transient response of a population that is at low density because of harvesting (White et al. 2013). Population modeling was performed in R v.2.15.2 (R Core Team 2013) using the package *popbio* v.2.4

Results

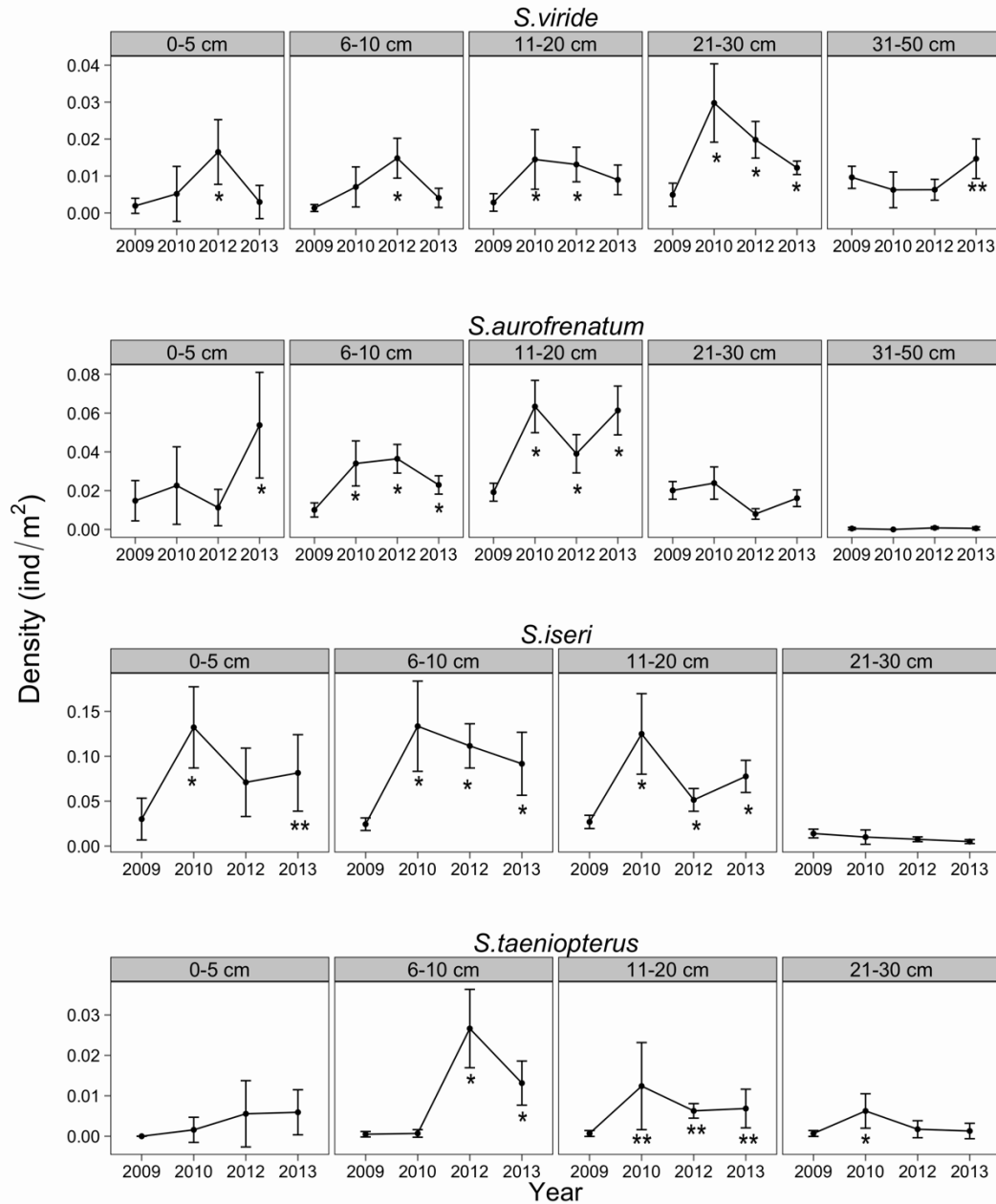
We observed 10 parrotfish species during the monitoring surveys (Table S2). Of the 10 species, only four exhibited an average annual density over 2 individuals/100m² and a significant change in density over time. The four most abundant species were the striped

parrotfish (*Scarus iseri*), princess parrotfish (*Scarus taeniopterus*), stoplight parrotfish (*S. viride*), and redband parrotfish (*Sparisoma aurofrenatum*).

Overall parrotfish density increased by 188% from 2009 (0.17 (± 0.01) individuals/m²) to 2013 (0.50 (± 0.05) individuals/m²). The most substantial increase of 269% occurred between 2009 and 2010 and the average annual increase over the 5-year monitoring period was 84%. Density changes in the five size classes varied by species (Fig. 1). *S. iseri* density was greater in each year following 2009 for the 6-20 cm size classes ($p < 0.01$ for each year) and greater in 2010 and 2013 for the 0-5 cm size class ($p < 0.01$ and $p < 0.05$, respectively). *S. taeniopterus* density was greater in 2012 and 2013 for the 6-10 cm size class ($p < 0.01$ for each year) and the 11-20 cm size class ($p < 0.05$ for each year). *S. taeniopterus* density was greater in 2010 for the 21-30 cm size class ($p < 0.01$) and subsequently decreased in 2012, while *S. viride* density increased in the 0-10 cm size classes in 2012 ($p < 0.01$) and then decreased in 2013. The 11-20 cm and 21-30 cm size classes densities show similar trends with density increasing in 2010 and 2012 ($p < 0.01$) and decreasing in 2013 for *S. viride*. *S. viride* showed an increase in the largest size class (31-50 cm) in 2013 ($p < 0.05$) relative to all other previous years. *S. aurofrenatum* increased in the 0-5 cm size class in 2013 relative to densities observed in 2009 ($p < 0.01$). *S. aurofrenatum* increased in the 6-10 cm and 11-20 cm size classes in 2010 and remained greater than 2009 densities in 2012 and 2013 ($p < 0.01$) (Fig. 2.1). Average total length of terminal phase *S. taeniopterus*, *S. aurofrenatum* and *S. iseri* individuals falls within the 21-30 cm size class (Böhlke and Chaplin 1993). Therefore, we did not expect to observe individuals in the 31-50 cm size.

Figure 2.1: Mean density by class size for four species of parrotfish across 16 reefs from 2009 to 2013.

Error bars represent the 95% confidence interval. Significant increases from densities observed in 2009 are identified by * or ** (* indicates a p-value of <0.01 and ** indicates a p-value of <0.05).

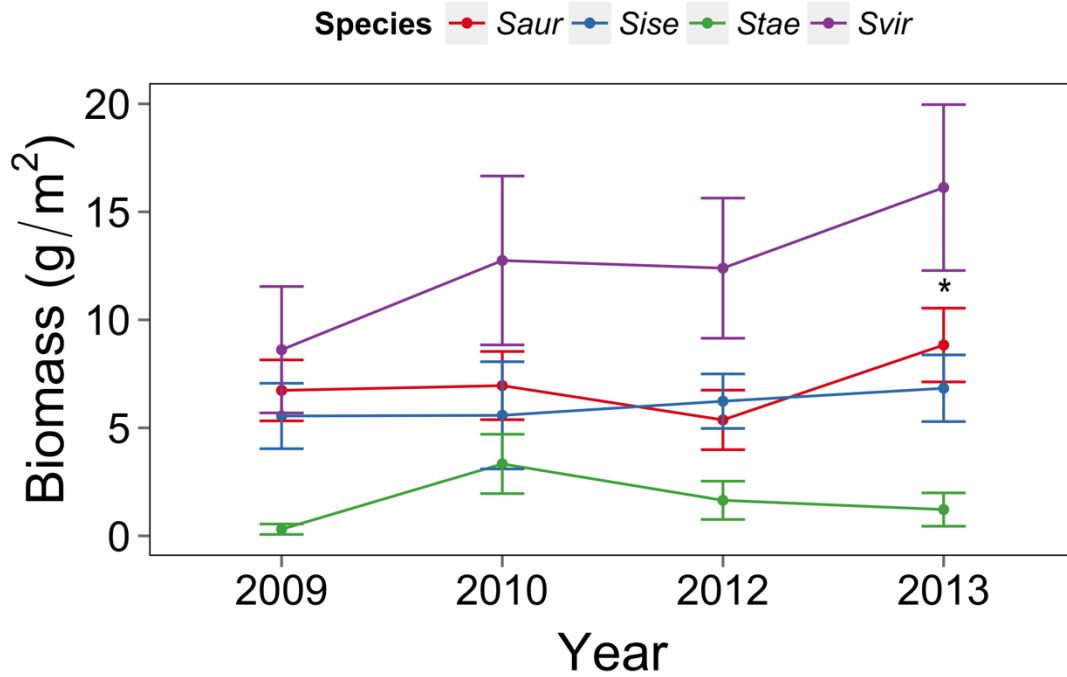


Parrotfish biomass increased by 54% from 2009 (25.14 (± 1.74) g/m²) to 2013 (38.79 (± 3.58) g/m²) with an average annual increase of 17%. *S. viride*, which is the largest of the four species, contributed an average of 46% to the total parrotfish biomass over the five-year

monitoring period. *S. taeniopterus*, *S. aurofrenatum* and *S. iseri* contributed 6%, 26%, and 23% to the total parrotfish biomass, respectively. We found a significant increase in *S. viride* biomass from 2009 (8.62 ± 1.49) to 2013 (16.12 ± 1.96) ($p < 0.01$), but did not find increases in biomass for the other three species (Fig 2.2.).

Figure 2.2: Mean Biomass (\pm 95% confidence interval) by class size for four species of parrotfish across 16 reefs from 2009 to 2013.

S. viride biomass increased from 2009 to 2013 ($p < 0.05$) indicated by * in the figure.



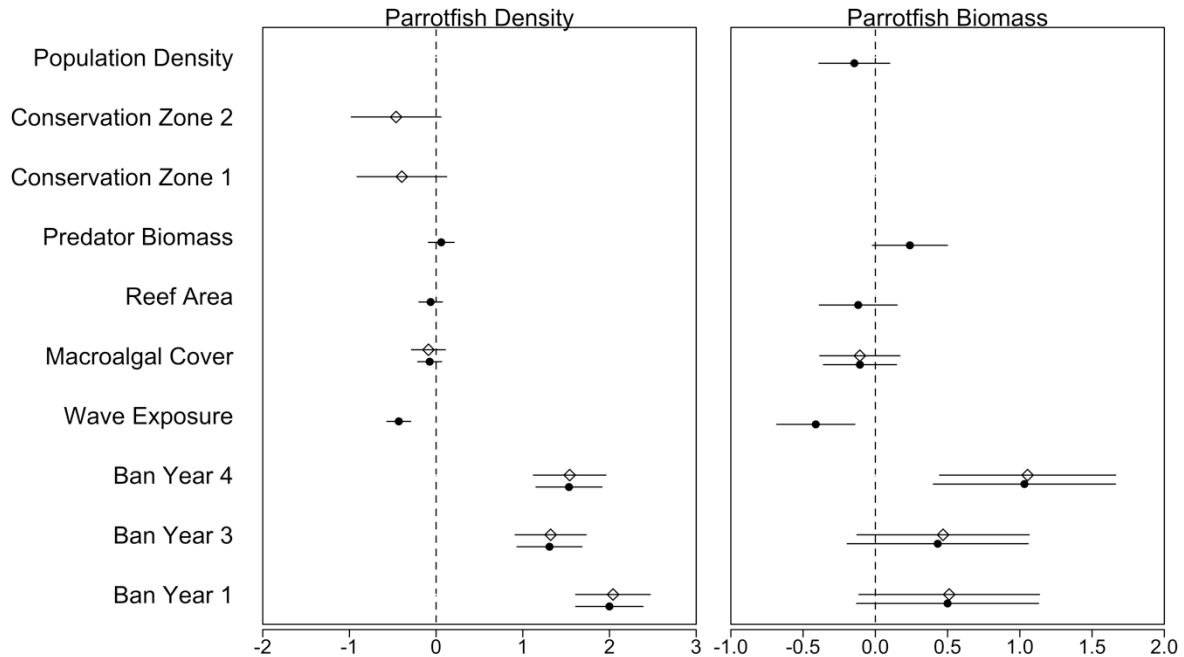
Model averaging for total parrotfish density resulted in final linear mixed effects models that included wave exposure, reef area, years since implementation of the fishing ban (ban year), and human population density (Density Model A) and predator biomass, ban year, and SST minimum (Density Model B) as the best predictor variables. The final models for parrotfish biomass included wave exposure, predator biomass, macroalgal cover, and

population density (Density Model A) and predator biomass, population density, mangrove area, and macroalgal cover (Density Model B) as predictor variables. These models revealed that each year following implementation of the harvesting ban had a positive effect on total parrotfish density ($p < 0.02$ for each year) and a positive effect on parrotfish biomass in 2013 (Fig. 2.3). Wave exposure had a negative effect on both parrotfish density and biomass, human population density and macroalgal cover had a weak negative effect on parrotfish biomass, and predator biomass had a weak positive effect on parrotfish biomass (Fig. 2.3, $p < 0.001$, $p = 0.04$, $p = 0.04$, and $p = 0.02$, respectively). Parrotfish density increased at 12 out of 16 sites, but protection status had no effect on parrotfish density or biomass.

For *S. viride*, the SSD was projected as 39.7% (0-10 cm), 17.6% (11-20 cm), 18.7% (21-30 cm), and 23.9% (31-50 cm). The elasticity analysis of the matrix parameters showed that *S. viride* populations were more sensitive to adult and large adult survival (21-50 cm size class) than to changes in juvenile survival or recruitment rates. The closed population projection predicts that SSD will be reached by year 2019 if fishing pressure ceased in 2010 and remains constant. In 2019, the total *S. viride* density at SSD is estimated at 0.06 ± 0.01 individuals/m² (greater than 21 cm in length), which is equal to 29.7 ± 3.9 g/m². We did not find a difference between projected and observed *S. viride* density in 2012 ($p = 0.84$) or 2013 ($p = 0.15$) nor did not find a difference between projected and observed *S. viride* biomass in 2012 ($p = 0.07$) or 2013 ($p = 0.35$) (Fig 2.4).

Figure 2.3: Coefficient estimates (mean \pm 95% confidence interval) resulting from linear mixed effects models testing the effect of the harvesting ban and 10 covariates on parrotfish density and biomass over time.

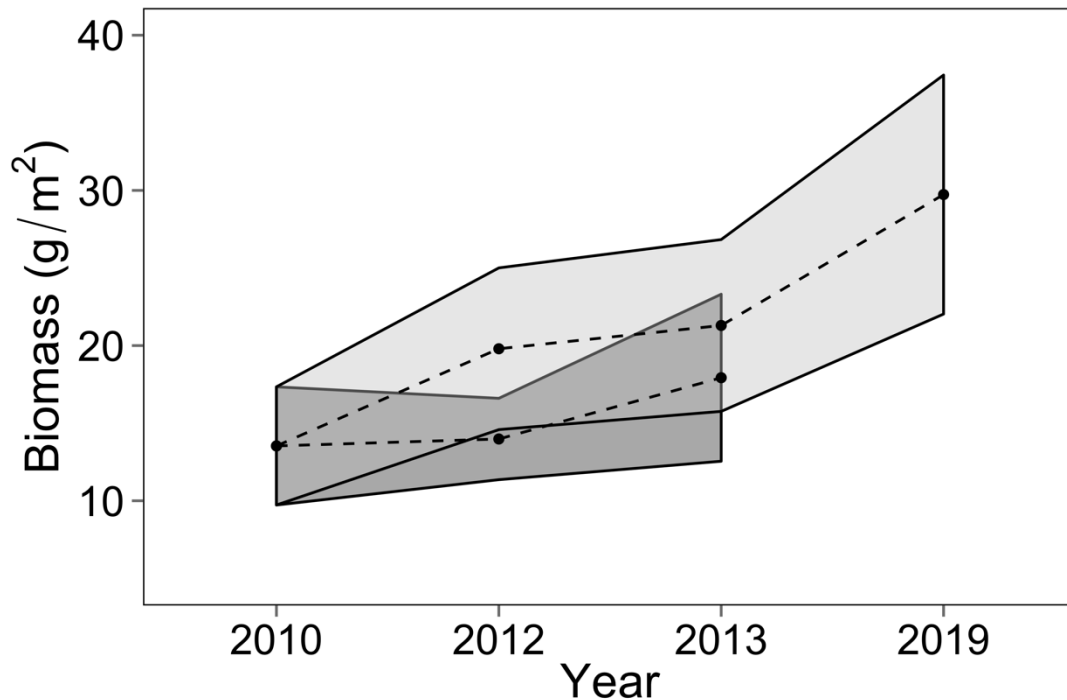
Covariates identified here are the variables included in the final averaged models. Solid circles represent coefficient values from the model that included wind exposure as a fixed effect and the open diamonds represent coefficient values from the model that included protection status as a fixed effect. These variables were highly correlated (e.g., $r_s=0.50$) and could not be run in the same model.



Discussion

Restoring parrotfish populations is the primary management strategy for reducing macroalgal cover and reestablishing coral dominance on Caribbean coral reefs. Our results suggest reduced fishing pressure achieved through the harvesting bans Belize has implemented could be effective in a relatively short time frame for fast growing species such as parrotfish. However, reduced fishing does not guarantee population recovery even if this regulation is enforced with full compliance (Kuparinen et al. 2014). Therefore, monitoring these populations over time is needed to track the progression of recovery and determine whether further measures are needed to reduce macroalgal cover.

Figure 2.4: Comparison of stage-structured population model predictions (light grey) and data collected during monitoring surveys (dark grey) for *S. viride* biomass (mean \pm 95% confidence interval).



We found that density increases for four parrotfish species and an increase in biomass for one species over the five-year monitoring period following the ban on parrotfish harvesting. We accounted for other covariates that can affect fish population dynamics over time and our statistical models suggest the observed initial recovery could be due to the ban.

Prior to the ban on parrotfish harvesting, fishermen typically used spear guns to target parrotfish greater than 20 cm. Being the largest of the four abundant parrotfish species currently observed on the BBR, *S. viride* was the most fished species (James Azueta pers. comm. 2012). We predicted to see an increase in the 21-50 cm size classes over time as a result of reduced spear fishing, specifically in *S. viride*. *S. viride* densities increased by an average of 89% in these larger size classes over the 5 year monitoring period suggesting

decreased fishing pressure for *S. viride* and preliminary evidence that the ban has been effective for this species (Fig 2.1).

We observed increases in density for the 0-20 cm size classes for *S. taeniopterus*, *S. aurofrenatum* and *S. iseri* (Fig. 2.1). These sizes and species are not typically targeted by spear fishermen. However, parrotfish greater than 5 cm, particularly those between 15 and 34 cm, are vulnerable to fish traps (Recksiek et al. 1991, Rakitin and Kramer 1996, Mumby et al. 2006a). Observed increases in these species could suggest a decrease in individuals being caught in fish traps or an increase in juvenile survival rate.

The average length at maturity for most parrotfish species is between 15 and 17 cm (Reeson 1983). Therefore, an increase in the 11-20 cm size class would increase the population reproductive potential possibly explaining increases in the 0-10 cm size classes in the following year. However, we did not find a relationship between the number of mature individuals and the number of juveniles (0-10 cm) in the following years on a regional scale ($p > 0.14$ for each species). Juvenile density is not only influenced by the number of mature fishes, but also by variability in natural mortality due to environmental factors and predation pressure (Hixon and Carr 1997) which most likely explains the temporal variation we observed in juveniles.

S. viride, population projection models predicted that a minimum of 9 years is required for the population to reach a stable stage distribution (i.e., population recovery) if fishing pressure ceased in 2010 and remains at zero. This projection provides resource

managers with a goal for population recovery. If parrotfish harvesting ceased in 2010 (with minimal poaching), managers should expect to see 0.06 ± 0.01 individuals/m² or 29.7 ± 3.9 g/m² of large (>21 cm) of *S. viride* by 2019. Projected biomass values can be used by managers to assess population recovery by comparing the observed biomass of large individual *S. viride* to the projected biomass. For instance, the population model predicted *S. viride* biomass to be 21.3 ± 2.8 g/m² in 2013. The *S. viride* biomass observed in 2013 (17.9 ± 2.7 g/m²) was not significantly different from that predicted (Welch t-test, $t = -0.959$, $p = 0.347$). Based on this comparison, *S. viride* biomass is consistent with the population projection models suggesting that these populations are beginning to recover (Fig. 2.4).

Our results suggest that the ban on parrotfish harvesting has had a positive effect on parrotfish density and biomass over a five-year period. This indicates that fishing pressure has been reduced as a result of the harvesting ban. For the ban to fully accomplish its goals parrotfish population recovery must result in decreased macroalgal cover and coral recruitment must increase in response to reduced macroalgae. Because a minimum of 9 years is projected for populations to recover, further monitoring is needed to track increases in large individuals that exert strong grazing pressure in the next 5 years and the effect of increased parrotfish biomass on benthic structure. Overall, our findings provide promising evidence that the ban on parrotfish harvesting is an effective conservation strategy for restoring these key herbivores and would be beneficial to other nations in the Caribbean.

CHAPTER 3: ESTABLISHMENT OF MARINE PROTECTED AREAS ALONE DOES NOT RESTORE CORAL REEF COMMUNITIES

Introduction

Caribbean coral reef ecosystems have experienced more than three decades of coral mortality and habitat degradation (Hughes 1994, Jones et al. 2004, Bellwood et al. 2004), with coral cover declining from ~50% in the 1970s to ~15% at present (Gardner et al. 2003, Schutte et al. 2010). The proximate causes of coral loss in the greater Caribbean include disease outbreaks, hurricanes, altered land-use practices that lead to increased sedimentation and nutrient pollution, and coral bleaching events related to anthropogenic climate change causing mass mortalities (Woodley et al. 1981, Hughes 1994, Eakin et al. 2010). Declines in coral cover and subsequent loss of structural complexity (Alvarez-Filip et al 2011) can negatively affect reef fish abundance and biodiversity, as many species rely on the presence of living coral assemblages for habitat (Bell and Galzin 1984, Jones et al. 2004). Across the Caribbean, reef fish density has declined at rates of 2.7% to 6.0% per year for more than a decade (Paddack et al. 2009) associated with the loss of available habitat and overfishing. These ecosystem-level changes continue to have far-reaching effects on coastal communities that depend on coral reefs for fisheries, tourism, and other ecosystem services.

This decline in coral cover was followed by a drastic increase in benthic macroalgae resulting from a combination of interrelated factors including: 1) increased availability of habitable substrate (Aronson and Precht 2001), 2) a regional decrease in grazing pressure caused by a decline of the keystone sea urchin *Diadema antillarum* (Hughes 1994, Woodley 1999), and 3) overfishing of herbivorous/detritivorous including parrotfish (scarids) and surgeonfish (acanthurids) (Bellwood et al. 2004). Large amounts of macroalgae on a reef can suppress coral growth and recruitment through several mechanisms including shading, abrasion, allelochemicals, limiting suitable settlement substrate (e.g., by covering encrusting coralline algae that enhance coral settlement) and potentially via the enhancement of microbes and disease (River and Edmunds 2001, Kuffner et al. 2006, Smith et al. 2006, Box and Mumby 2007, Rasher and Hay 2010). The ecological role of grazers, including urchins and herbivorous reef fishes, and their importance in controlling macroalgal growth and enhancing coral recruitment has been well documented since the mid-1980's (Hay 1984, Carpenter 1986, Lewis 1986, Williams and Polunin 2001, Carpenter and Edmunds 2006, Mumby 2006, Mumby et al. 2007).

The establishment of marine protected areas (MPAs) is the principal strategy for restoring fish populations (including commercially important species and herbivorous fishes) and facilitating recovery of coral reef benthic communities (Polunin and Roberts 1993, Halpern and Warner 2002, Mumby et al. 2006a). Specifically, restoring the ecological role of grazers including urchins and herbivorous reef fish to control macroalgal growth and enhance coral recruitment has become a key goal of coral reef management (Mumby and Steneck 2008, Jackson et al. 2014b). Potential immediate and long-term benefits of MPAs include

reductions in fishing pressure and destructive fishing practices, increases in organismal biomass and diversity, and increases in fish stocks in adjacent fisheries due to larval export and adult migration (Lester et al. 2009, Harrison et al. 2012). Target fish populations are capable of responding quickly to reductions in fishing pressure resulting in increased density and biomass (Polunin and Roberts 1993, Mosquera et al. 2000, Côté et al. 2001, Halpern 2003). However, these benefits are not realized for some species because many MPA networks do not link larval supply and settlement areas or lack adequate enforcement (Roberts 1995b, Mora et al. 2006, Huntington et al. 2011).

Although reduced fishing pressure within MPAs has led to increased density and biomass of some fish species (Roberts 1995b, Aburto-Oropeza et al. 2011), there is little conclusive evidence that protection from fishing promotes positive effects on coral community structure under all environmental conditions (Mumby and Steneck 2008). For example, within the Exuma Cays Land and Sea Park, Bahamas, increases in coral cover over a 2.5 year period were higher at protected sites than fished sites. However, macroalgal cover was extremely low at these sites ($3.1 \pm 1\%$) throughout the duration of the study (Mumby and Harborne 2010). In contrast, in regions where macroalgal cover is higher such as Belize and Florida, MPAs have not influenced coral or macroalgal cover (McClanahan and Muthiga 1998, McClanahan et al. 1999, Huntington et al. 2011, Toth et al. 2014). For instance, no-take areas within the Florida Keys National Marine Sanctuary have not promoted an increase in coral cover or a decline in macroalgal cover despite having a higher abundance of adult herbivorous fishes than fished reefs (Kramer and Heck 2007, Toth et al. 2014). Similarly, after 10 years of reserve designation, Glover's Reef Marine Reserve in Belize has had no

effect on herbivorous fish abundance, macroalgal cover, or coral cover (Huntington et al. 2011). This failure of MPAs alone to promote herbivory and coral assemblage recovery suggests that new fisheries policies may be required to restore coral reef ecosystems (Mumby and Steneck 2008).

The Belize Barrier Reef has one of the most extensive MPA networks in the Caribbean consisting of 18 MPAs that cover approximately 2,525 km² of territorial waters. Two of these MPAs (Glover's Reef Marine Reserve and Hol Chan Marine Reserve) have been the focus of most reserve effect studies in Belize but have not consistently promoted positive effects on reef communities (Polunin and Roberts 1993, Roberts and Polunin 1994, McClanahan et al. 2001, Huntington et al. 2011). In 2009, the Belize Fisheries Department implemented a national ban on herbivorous fish harvesting as an additional conservation strategy to restore herbivorous fish populations. Here, we test the effectiveness of Belize's national MPA network in protecting and restoring reef fishes, as well as promoting the recovery of benthic communities at a regional scale. The uniqueness of our study is that we accounted for several abiotic and biotic variables that could affect coral reef community structure and potentially MPA success that have not been considered in previous studies allowing us to identify other key characteristics contributing to reef health and the performance of Belize's MPA network. We then compare the effects of the MPA network and the ban on herbivorous fish harvesting.

Methods

Study locations

We monitored reef fish communities at 16 fore reef sites (15-18 m) along the Belize Barrier Reef during the summer months of May and June in 2009, 2010, 2012 and 2013 (Appendix C, Table 3.1). Sites were selected to maximize spatial coverage along the fore reef, include a range of protection zones, and to coincide with sites monitored in previous years by local NGOs. To minimize habitat variability of survey sites, we only surveyed spur-and-grove reef formations at each site and focus on *Orbicella* (former *Monstastrea*) dominated habitat. Survey sites included (a) fully-protected MPAs where only non-extractive sport fishing was permitted (Conservation Zone 1); (b) partially protected MPAs where special restrictions were in place that include limited fishing licenses and banned use of traps, nets, and long-lines (Conservation Zone 2); and (c) unprotected reefs where fishing was unrestricted except for herbivorous fishes and Nassau grouper (see Belize National Statutory Instrument No. 49 of 2009) (control) (Appendix C, Table 3.1). Information regarding zoning of protected areas was provided by the Belize Fisheries Department (www.fisheries.gove.bz).

We classified level of enforcement at each MPA site according to qualitative estimations published in the Healthy Reefs 2014 EcoAudit for Belize (McField 2014). Managers at each reserve were asked to score the overall level of enforcement as good, moderate, or inadequate. Sites with good enforcement were those that had regular patrols and overall satisfactory compliance. Sites with moderate enforcement were those with regular patrols, but limited poaching and insufficient legal outcomes. Sites with inadequate

enforcement were those with irregular patrols, poaching, insufficient legal outcomes, and a high level of concern from the local community.

Fish surveys

We performed visual fish censuses to estimate reef fish species composition and density using a modification of the standard Atlantic and Gulf Rapid Reef Assessment (AGRRA) v5.4 techniques (Lang et al. 2010). Fish species were identified, counted, and sizes were estimated in 10 cm intervals. Total lengths were recorded for species with rounded or truncated caudal fins, while fork lengths were recorded for all other species. At each site, we recorded fishes within 2 x 30 m transects for individuals < 40 cm in length and within 10 x 50 m transects for individuals > 40 cm in length. We also counted and identified all smaller fish (< 5 cm) within 15x1 m transects. We deployed six to eight belt transects per site at least 10 meters apart and conducted surveys during daylight from 0800 to 1600hr. Experienced and trained divers performed all fish surveys. Divers were trained by estimating fish sizes in the water against artificial fish models of known size and comparing these sizes to those estimated by a diver experienced in fish surveys. Fish biomass was calculated through the allometric weight-length relationship, $W = aTL^b$, where W is the weight of each individual (in grams), TL is the length of each fish (in cm) estimated from visual surveys, and the parameters a and b are species specifics (Froese and Pauly 2011). When these variables were not available, we used the values of congeneric species of similar size and morphology. We used the mid-point of the 10 cm interval to calculate biomass.

Table 3.1: Monitoring Site Details

Site	Latitude	Longitude	Reserve ID	Year Established	Size (km ²)	Protection Status	Enforcement Level
Calabash	17.261470	-87.819700	No Status	N/A	N/A	None	None
Half moon	17.205600	-87.546790	Half Moon Caye National Monument	1982	39.2	C1	Moderate
Middle Caye	16.737030	-87.805360	Glover's Reef Marine Reserve	1993	350.7	C2	Moderate
South of Middle Caye	16.728750	-87.828670	Glover's Reef Marine Reserve	1993	350.7	C2	Moderate
Tobacco Caye	16.919110	-88.047570	No Status	N/A	N/A	None	None
South Water Caye	16.813460	-88.077560	South Water Caye Marine Reserve	1996	476.7	C2	Inadequate
Alligator Caye	17.196600	-88.051150	No Status	N/A	N/A	None	None
Tackle Box	17.910560	-87.950830	No Status	N/A	N/A	None	None
Hol Chan	17.863430	-87.972380	Hol Chan Marine Reserve	1987	4.2	C1	Good
Mexico Rocks	17.987820	-87.903820	No Status	N/A	N/A	None	None
Bacalar Chico	18.162820	-87.822220	Bacalar Chico Marine Reserve	1996	9.2	C1	Moderate
Gallows	17.495920	-88.042550	No Status	N/A	N/A	None	None
Pampion Caye	16.373100	-88.089130	No Status	N/A	N/A	None	None
Ranguana Caye	16.285010	-88.150310	No Status	N/A	N/A	None	None
Nicholas Caye	16.112300	-88.255860	Sapadilla Cayes Marine Reserve	1996	4.9	C1	Inadequate
Southwest Caye	16.71087	-87.8461	Glover's Reef Marine Reserve	1993	350.7	C2	Moderate

Benthic surveys

Benthic cover was estimated using point-intercept methods according Lang *et al.* (Lang et al. 2010). At each site, six 10 m lead-core transect lines were laid on the substrate along the spur and groove formation (45-50 ft deep) spaced approximately 10 m apart. Benthic groups were identified at every 10 cm intervals along the 10 m transect line. We broadly categorized the benthos in hard corals, macroalgae, crustose coralline algae, turf algae, zoantids, sponges, gorgonians, rubble, sand, pavement, and other live categories that included bryozoans, anemones, and corallimorpharians. Hard corals and macroalgae were identified up to species and genus, respectively. Benthic transects occurred along the first 10 m of the fish transects.

Covariates

To examine the effectiveness of the Belize Barrier Reef MPA network, we examined four reef community indicators of reef performance and health (predator and parrotfish biomass, coral and macroalgae cover). Additionally, we accounted for 8 additional variables that could influence coral reef community structure and potentially compromise management efforts including sea surface temperature anomalies, average oceanic net primary productivity (2002-2012), wave exposure, reef structural complexity, mangrove perimeter within 5 km, reef area within 5 km, distance to deep water, and year (Table 1). For detailed descriptions, measurements, and justifications for each covariate refer to Appendix C.

Data Analysis

We used linear mixed effects models to test the effect of protection status and enforcement level of Belize's MPAs on predatory fish and parrotfish biomass, and coral and macroalgal cover. We generated a global model with protection status, enforcement level and a subset of 12 covariates that could influence fish biomass or benthic community structure coded as fixed effects and sites coded as random effects. To evaluate collinearity among all explanatory variables and generate models without correlated variables, we calculated the variance inflation factors (VIF) and sequentially removed each covariate for which the VIF value was above 2 (Graham 2003). Among the covariates, wave exposure, protection status, and enforcement level were sufficiently correlated to compromise interpretation when modeled together (*Spearman rank correlation* $r_s > 0.50$), therefore, we modeled these covariates separately (Graham 2003). Predatory fish biomass was $x^{(1/6)}$ transformed and coral cover was square root transformed to improve homogeneity of variance and model fit. Numerical covariates were standardized and centered (mean of zero and standard deviation of one) to aid in model comparisons. Meaningful interactions and quadratic terms were included in exploratory models.

Based on the global model we ran all possible combinations of co-variables fitted by maximum likelihood to identify the top models that best explain the response indicators. Final models (those with a $\Delta AIC_c < 2$) were then run and averaged fitted by restricted maximum likelihood (Burnham and Anderson 2002). For each final model, a marginal and conditional R squared was calculated, which gives an estimation of model fit (Nakagawa and Schielzeth 2013).

Homogeneous and normal distribution errors of final top models were confirmed in the plot of residuals against predicted values and by using the normal scores of standardized residuals deviance, respectively (Zuur et al. 2009). Spline spatial correlograms were plotted to corroborate that the final model residuals were not spatially autocorrelated (Zuur et al. 2009). All analyses were performed in R v.2.15.2 (R Core Team 2013) using the package *nlme* v.3.1-113 for the linear mixed-effect models and *MuMin* v. 1.9.13 for the model averaging.

Results

We found no difference between coral cover or macroalgal cover at protected or well-enforced sites and control sites between 2009 and 2013 (Table 3.2, Fig 3.3 and Fig 3.4). While parrotfish biomass and predator biomass increased over time ($p < 0.001$, Fig 3.2), there was no difference between protected or well-enforced sites and control sites (Table 3.2, Fig 3.3 and Fig 3.4).

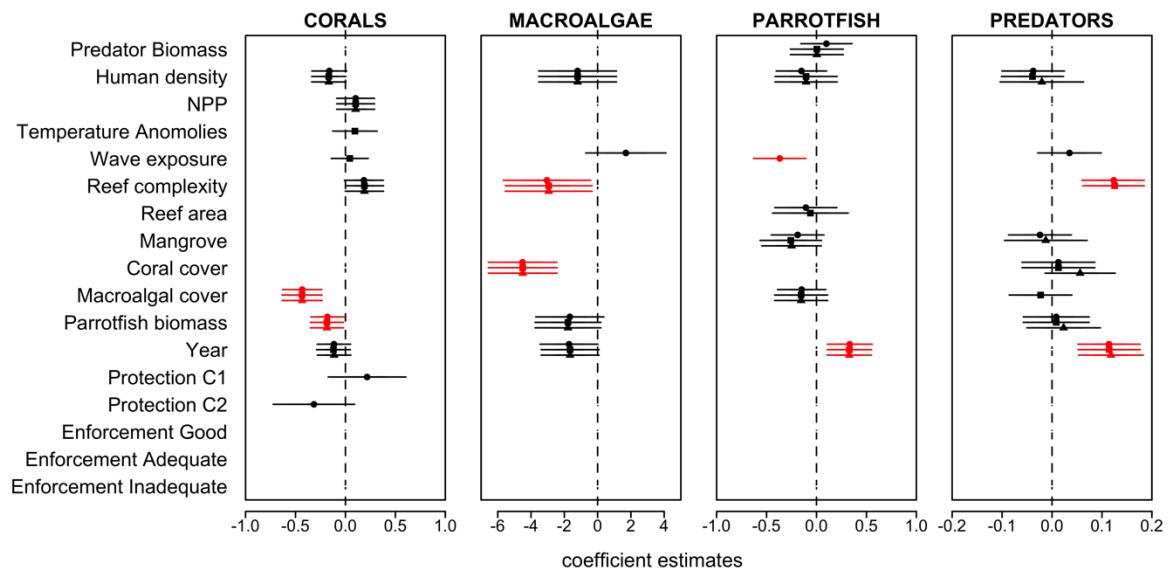
Final linear mixed effects models showed that reef complexity, wave exposure, coral cover, macroalgal cover, and parrotfish biomass had a significant effect on at least one of the four response variables (Fig. 3.1 and Fig. 3.2, Table 3.3). Reef complexity had a positive effect on predator biomass ($p < 0.001$) while wave exposure had a negative effect on parrotfish biomass ($p = 0.01$). Macroalgal cover and parrotfish biomass had a negative effect on coral cover ($p < 0.001$ and $p = 0.03$, respectively). Reef complexity and coral cover had a negative effect on macroalgal cover ($p = 0.03$ and $p < 0.001$, respectively).

Table 3.2: Mean value and standard error(SE) of each response variable by protection level and enforcement level. p-value represents results of linear mixed effects models.

Response Variable		Mean	SE	df	p-value
Coral Cover (%)					
<i>Protection Status</i>					
	C1	22.4	1.9	10	0.69
	C2	14.5	0.9	10	0.35
	None	20.6	1.3	--	--
<i>Enforcement Level</i>					
	Good	23.9	5.1	9	0.83
	Moderate	14.5	1.5	9	0.79
	Inadequate	19.0	1.5	9	0.50
	None	20.6	1.3	--	--
Macroalgal Cover (%)					
<i>Protection Status</i>					
	C1	48.1	3.1	10	0.50
	C2	55.1	2.0	10	0.41
	None	47.1	1.5	--	--
<i>Enforcement Level</i>					
	Good	48.2	4.6	9	0.57
	Moderate	58.0	1.7	9	0.60
	Inadequate	49.5	2.8	9	0.04
	None	47.1	1.5	--	--
Predator Biomass (g/m²)					
<i>Protection Status</i>					
	C1	29.0	12.3	10	0.42
	C2	9.4	1.5	10	0.17
	None	19.2	4.0	--	--
<i>Enforcement Level</i>					
	Good	17.2	8.0	9	0.30
	Moderate	9.0	2.3	9	0.59
	Inadequate	24.2	10.3	9	0.12
	None	19.2	4.0	--	--
Parrotfish Biomass (g/m²)					
<i>Protection Status</i>					
	C1	31.1	3.9	10	0.35
	C2	31.9	2.8	10	0.50
	None	32.7	2.4	--	--
<i>Enforcement Level</i>					
	Good	17.4	4.7	9	0.08
	Moderate	34.4	5.9	9	0.93
	Inadequate	33.3	2.4	9	0.93
	None	32.7	2.4	--	--

Figure 3.1: Coefficient estimates (mean \pm 95% confidence interval) resulting from linear mixed effects models testing the effect of the protection status and enforcement level and 15 covariates on predatory reef fish biomass, parrotfish biomass, macroalgal cover, and coral cover.

Solid circles represent coefficient values from the model that included protection status as a fixed effect, solid squares represent coefficient values from the model that included wave exposure as a fixed effect and solid triangles represent coefficient values from the model that included enforcement level as a fixed effect. These variables were highly correlated (e.g., $r_s > 0.50$) and could not be run in the same model.



Discussion

By quantifying fish biomass and benthic community composition at 16 reef sites inside and outside of five MPAs, we found that on average sites within MPAs or those well enforced did not have higher fish biomass, lower macroalgal cover, or higher coral cover when compared to unprotected or poorly enforced sites. Many MPA efficacy studies have found positive effects of MPA establishment on fish communities. For instance, protected areas within the Florida Keys show increased abundances of large predatory and adult

herbivorous fishes after more than two decades of reserve establishment (Kramer and Heck 2007).

Similarly, within the Exuma Cays Land and Sea Park (ECLSP) reserve, parrotfish biomass was two times higher than in unprotected areas along the same reef tract (Mumby et al. 2006a). In Belize, fish biomass was higher in Hol Chan Marine Reserve than outside the reserve during a 1993 study (Polunin and Roberts 1993). However, not all studies report a positive effect of protection on fish communities, highlighting the important role of poaching or lack of enforcement that can compromise the performance of MPAs. For example, a recent study at Glover's Reef Marine Reserve found that the biomass of herbivorous fishes was similar within reserve sites when compared to adjacent control sites potentially due to a lack of sufficient enforcement or increased predatory reef fishes within reserves (Huntington et al. 2011). We found no positive effect of protection or enforcement on either predatory reef fishes or parrotfishes. These results broaden the scale of the findings of Huntington et al. 2011 demonstrating that current (2009-2013) mean fish biomass within Belize's national MPA network is not higher than that in fished areas. The large spatial extent of our monitoring sites highlights that the poor performance of Belize's MPAs is evident at a larger scale.

Table 3.3: Top linear mixed-effects models testing the effects of 15 covariates on four response variables

Among the covariates, wave exposure, protection status, and enforcement level were sufficiently correlated to compromise interpretation when modeled together (*Spearman rank correlation* $r_s > 0.50$), therefore, we modeled these covariates separately (Model A: Wave Exposure, Model B: Protection Status, Model C: Enforcement Level). The top models considered are those with $\Delta AICc < 2$. Relative variable importance (RI) is the sum of the weights of all models that contain that particular variable. Covariate abbreviations are as follows: PdB: Predator Biomass, HD: Human Density, NPP: Net primary productivity, W: Wave exposure, RC: Reef complexity, RA: Reef area, MP: Mangrove perimeter, C: Coral cover, M: Macroalgal cover, PB: Parrotfish Biomass, Y: Year, C1: Conservation zone 1, C2: Conservation zone 2. Enforcement level, distance to deep water and sea surface temperature anomalies were not retained in any of the top models.

Response Variable	PdB	HD	NPP	W	RC	RA	MP	C	M	PB	Y	C1	C2	df	logLik	AICc	$\Delta AICc$	wt
Coral Cover																		
<i>Model A</i>																		
1		X			X				X	X				7	-50.15	116.46	0.00	0.42
2		X			X				X	X	X			8	-49.49	117.80	1.34	0.21
3		X	X		X				X	X				8	-49.49	117.81	1.35	0.21
4					X				X	X				6	-52.41	118.41	1.95	0.16
RI		0.84	0.21		1.00				1.00	1.00	0.21							
<i>Model B</i>																		
1		X			X				X	X				7	-50.15	116.46	0.00	0.35
2		X			X				X	X	X			8	-49.49	117.80	1.34	0.18
3		X	X		X				X	X				8	-49.49	117.81	1.35	0.18
4									X	X		X	X	7	-51.02	118.19	1.73	0.15
5		X							X	X				6	-52.41	118.41	1.95	0.13
RI		0.72	0.18		0.85				1.00	1.00	0.18	0.15	0.15					
<i>Model C</i>																		
1		X			X				X	X				7	-50.15	116.46	0.00	0.42
2		X			X				X	X	X			8	-49.49	117.80	1.34	0.21
3		X	X		X				X	X				8	-49.49	117.81	1.35	0.21

Response Variable	PdB	HD	NPP	W	RC	RA	MP	C	M	PB	Y	C1	C2	df	logLik	AICc	Δ AICc	wt
	4				X				X	X				6	-52.41	118.41	1.95	0.16
	RI	0.84	0.21		1.00				1.00	1.00	0.21							
Macroalgal Cover																		
<i>Model A</i>																		
	1				X			X			X			6	-198.19	409.96	0.00	0.19
	2			X	X			X			X			7	-196.94	410.04	0.08	0.18
	3				X			X		X	X			7	-197.04	410.23	0.27	0.16
	4				X			X		X				6	-198.45	410.49	0.53	0.14
	5			X	X			X		X	X			8	-196.32	411.46	1.50	0.09
	6	X			X			X		X				7	-197.74	411.64	1.68	0.08
	7	X			X			X		X	X			8	-196.42	411.67	1.71	0.08
	8	X			X			X			X			7	-197.78	411.72	1.76	0.08
	RI	0.21		0.27	1.00			1.00		0.56	0.78							
<i>Model B</i>																		
	1				X			X			X			6	-198.19	409.96	0.00	0.26
	2				X			X		X	X			7	-197.04	410.23	0.27	0.22
	3				X			X		X				6	-198.45	410.49	0.53	0.20
	4	X			X			X		X				7	-197.74	411.64	1.68	0.11
	5	X			X			X		X	X			8	-196.42	411.67	1.71	0.11
	6	X			X			X			X			7	-197.78	411.72	1.76	0.11
	RI	0.32			1.00			1.00		0.64	0.69							
<i>Model C</i>																		
	1							X			X			5	-200.65	412.41	0.00	0.19
	2							X		X	X			6	-199.61	412.80	0.38	0.16
	3	X	X					X		X				7	-198.52	413.19	0.78	0.13
	4							X		X				5	-201.18	413.48	1.06	0.11
	5	X	X					X		X	X			8	-197.37	413.56	1.14	0.11

Response Variable	PdB	HD	NPP	W	RC	RA	MP	C	M	PB	Y	C1	C2	df	logLik	AICc	ΔAICc	wt
	6	X						X		X	X			7	-198.75	413.66	1.25	0.10
	7	X						X			X			6	-200.05	413.68	1.26	0.10
	8	X						X		X				6	-200.20	413.98	1.57	0.09
	RI	0.53	0.24					1.00		0.70	0.67							
Parrotfish Biomass																		
<i>Model A</i>																		
	1			X							X			5	-230.06	471.23	0.00	0.23
	2			X			X				X			6	-229.17	471.93	0.69	0.16
	3			X					X		X			6	-229.44	472.46	1.23	0.12
	4	X		X							X			6	-229.50	472.59	1.36	0.12
	5			X			X		X		X			7	-228.35	472.86	1.62	0.1
	6	X		X			X				X			7	-228.41	472.97	1.74	0.1
	7	X		X							X			6	-229.78	473.15	1.91	0.09
	8			X		X	X				X			7	-228.52	473.20	1.97	0.09
	RI	0.09	0.21	1.00		0.09	0.44		0.22		1.00							
<i>Model B</i>																		
	1						X				X			5	-232.21	475.54	0.00	0.27
	2										X			4	-233.51	475.75	0.22	0.24
	3						X		X		X			6	-231.47	476.52	0.98	0.16
	4								X		X			5	-232.96	477.02	1.49	0.13
	5					X	X				X			6	-231.93	477.45	1.91	0.10
	6	X					X				X			6	-231.95	477.48	1.94	0.10
	RI		0.10			0.10	0.63		0.29		1.00							
<i>Model C</i>																		
	1						X				X							
	2										X			5	-232.21	475.54	0.00	0.30
	3						X		X		X			4	-233.51	475.75	0.22	0.27

Response Variable	PdB	HD	NPP	W	RC	RA	MP	C	M	PB	Y	C1	C2	df	logLik	AICc	ΔAICc	wt
	4								X		X			6	-231.47	476.52	0.98	0.18
	5	X					X				X			5	-232.96	477.02	1.49	0.14
	RI	0.11					0.59		0.32		1.00							
Predator Biomass														6	-231.95	477.48	1.94	0.11
Model A																		
	1				X						X			5	5.19	0.74	0.00	0.46
	2	X			X						X			6	5.98	1.62	0.88	0.30
	3			X	X						X			6	5.80	1.98	1.24	0.25
	RI	0.30		0.25	1.00						1.00							
Model B																		
	1				X						X			5	5.19	0.74	0.00	0.61
	2	X			X						X			6	5.98	1.62	0.88	0.39
	RI	0.39			1.00						1.00							
Model C																		
	1										X			4	-0.46	9.65	0.00	0.73
	2							X			X			5	-0.25	11.61	1.96	0.27
	RI						0.27				1.00							

Our results are also in agreement with Huntington et al. (2011) and Kramer and Heck (2007) who found no significant differences in coral cover inside and outside of Caribbean reserves. Without higher parrotfish biomass, it is not surprising that we did not observe lower macroalgal cover or higher coral cover with MPAs. However, mean macroalgal cover across protection levels, ranging from 47.1% to 55.1%, was higher than the average for the Caribbean (approximately 23%) (Bruno et al. 2009, Jackson et al. 2014b).

Figure 3.2: Significant correlations between response variables and explanatory variables. Points are means per site.

Black line is the mean (\pm 95% confidence interval) of the predicted response variable as a function of a given covariate.

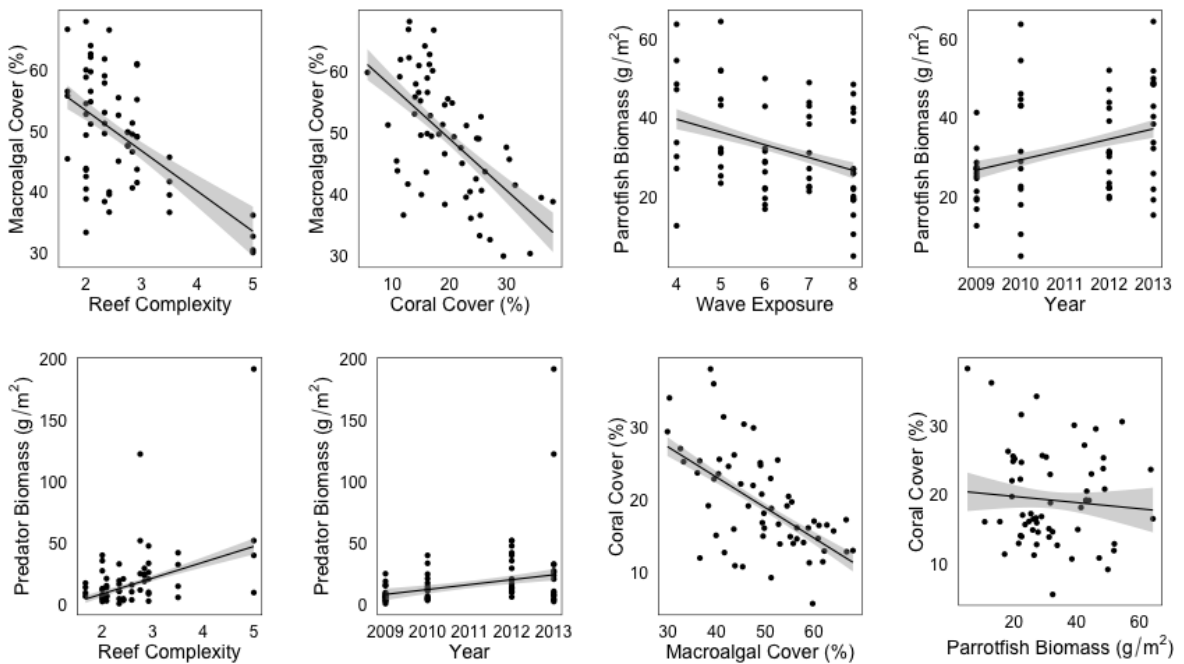


Figure 3.3: Comparison of parrotfish biomass, predatory reef fish biomass, macroalgal cover, and coral cover within protected areas (C1 and C2) and unprotected areas.
Boxplots represent means per site year.

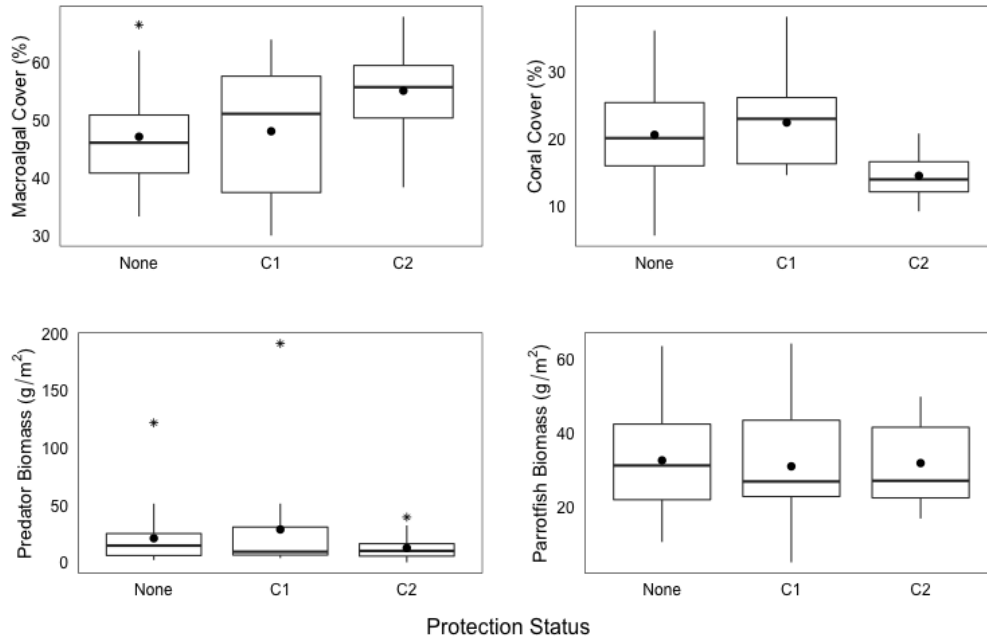
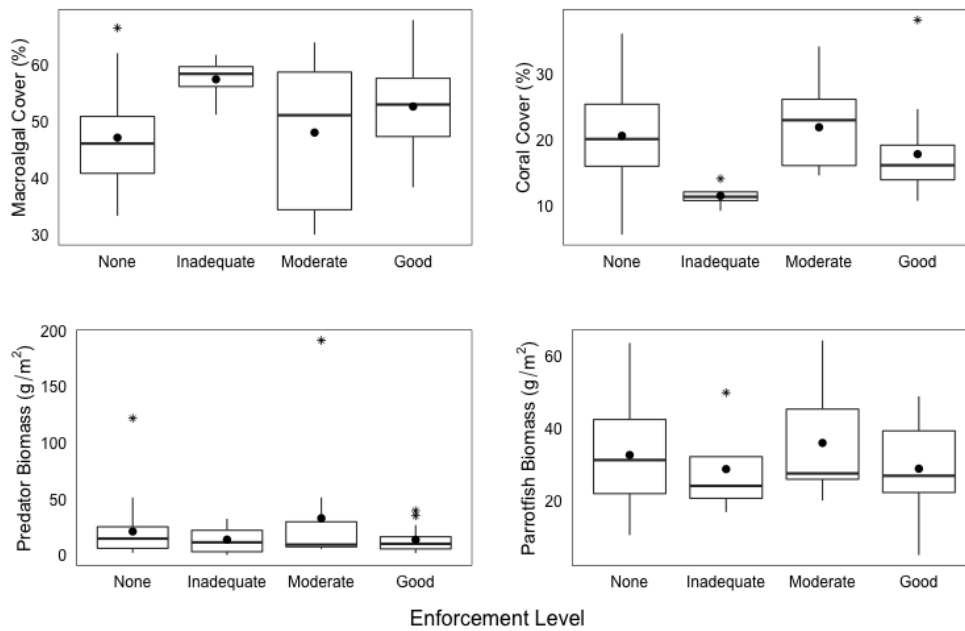


Figure 3.4: Comparison of parrotfish biomass, predatory reef fish biomass, macroalgal cover, and coral cover by enforcement level.
Boxplots represent means per site year.



Macroalgal cover in our study was also higher on average than Mumby and Harborne (2010) reported in the Bahamas ($3.1 \pm 1\%$) where increases in coral cover were significantly higher at reserve sites than those in non-reserve sites as a result of higher parrotfish grazing within the reserve. Regional declines in coral cover and recruitment coupled with above average macroalgal cover in Belize suggests that a more severe coral to macroalgal phase-shift has occurred in this area than has occurred elsewhere in the Caribbean, which may preclude a potential cascading effect of herbivores on macroalgae abundance.

While we did not observe positive effects of MPAs, there was an increase in predatory reef fish and parrotfish biomass over the five-year monitoring period (Fig 3.2). Parrotfish biomass increased by 54% from 2009 ($25.1 \pm 1.7 \text{ g/m}^2$, mean \pm standard error) to 2013 ($38.8 \pm 3.6 \text{ g/m}^2$) most likely due to a ban on herbivorous fish harvesting implemented in 2009 (Cox et al. in press), suggesting that effective restrictions on fishing effort are an important tool for coral reef management in addition to MPAs. We also observed an increase of approximately 300% in predatory reef fish biomass from $8.4 \pm 1.8 \text{ g/m}^2$ in 2009 to $34.2 \pm 13.5 \text{ g/m}^2$ in 2013. Two sites (Tackle Box (fished) and Half Moon (C1) exhibited substantially higher predator biomass in 2012 and 2013 compared to other sites, driving the overall increase in mean predator biomass. Further analysis is necessary to understand site-specific community dynamics and environmental factors resulting in higher predator biomass.

Enforcement is a key issue in the performance of Belize's national MPA network. While 19.6% of Belize's territorial waters are within MPAs, only 2.7% are within areas fully

protected from fishing. Of the 18 MPAs in Belize, 10 (56%) are considered to be relatively well-enforced (“good” or “moderate” enforcement ranking) (McField 2014). Our study included one fully protected, well-enforced MPA (Hol Chan Marine Reserve), two fully protected, moderately enforced MPAs (Bacalar Chico Marine Reserve and Half Moon Caye National Monument), and one partially protected, moderately enforced MPA (Glover’s Reef Marine Reserve). The other two MPA sites are partially protected and inadequately enforced (South Water Caye Marine Reserve and Sapadillo Caye Marine Reserve). Despite being designated as protected areas or being considered by local managers as well enforced or moderately enforced, these coral reef communities do not appear to be benefiting from local management suggesting that poaching may still be occurring in these areas. Our findings support a recent global analysis that found no-take, old (>10 years), well enforced, large (>100 km²) and isolated marine reserves have the most benefits for fish communities (Edgar et al. 2014). The MPAs evaluated in this study range from approximately 5 to 477 km² size and were established 18 to 32 years ago (Table 3.1). Interestingly, two of the five MPAs included in this study are above the 100km² size threshold and the 10 year threshold identified by Edgar et al (2014), but without effective enforcement even large and old marine reserves do not restore fish biomass (Table 3.1 and Fig. 3.4).

It is important to consider sites that support coral reef ecosystems that are healthier than the average across the MPA network. For example, of the 16 reef sites, Half Moon Caye (where fishing is prohibited and enforcement is moderate) exhibits relatively high average annual predator biomass (72 ± 4 g/m²), relatively high coral cover (30 ± 2 %), and low macroalgal cover (37 ± 2 %) (Appendix C). Parrotfish biomass (41 ± 5 g/m²) at this reserve was

only higher than 4 other sites and was similar to the average annual parrotfish biomass across sites (Figure S2). As our models predicted, high reef complexity at Half Moon Caye (index of 5) supported high predator biomass and low macroalgal cover. Higher structural complexity is a strong correlate of fish biomass (Wilson et al 2007) including herbivorous fishes (Alvarez-Filip et al 2011). Our results indicated that structural complexity is of vital importance to the health of this reef leading to both relatively high fish biomass and low algal cover.

While our results suggest that the reef management in Belize at the time of this study appeared to be limited in promoting fish and coral recovery, further temporal and geographical analyses will be necessary to identify subtler or delayed benefits of Belize's protection programs. Benefits of protection and effective enforcement may take years or decades to be realized through the dynamic responses of the ecosystem (Babcock et al. 2010). Continued monitoring at these sites would strengthen our ability to detect patterns that may arise over longer time scales such as reductions in macroalgal cover as herbivore biomass continues to increase.

As a key conservation management strategy, MPAs have the potential to promote coral reef recovery by restoring reef fish populations and potentially reducing macroalgal cover. However, establishing MPA boundaries is not enough. Enforcement is imperative to MPA success particularly as reef systems increasingly face a multitude of natural and anthropogenic stressors that transcend MPA boundaries such as ocean warming and acidification, poor water quality, and introductions of invasive species. A lack of funding in

Belize and other Caribbean countries often limits enforcement efforts due to a wide range of challenges to be addressed such as watershed and invasive species management (McField 2014). If faced with limited funds for reef conservation, our results suggest that local managers focus their enforcement efforts on those reefs with higher structural complexity, which support high predatory fish biomass and lower macroalgal cover. Other management efforts are also important such as the recent ban on herbivorous fish harvesting in all national waters that provides additional protection that may promote parrotfish population recovery independent of MPA designation or enforcement. Strengthening enforcement, limiting poaching within MPA boundaries, and implementing additional fisheries policies such as Belize's national ban on herbivorous fish harvesting, promote faster recovery of fish communities that would contribute to reversing the phase shift back toward coral dominance.

CHAPTER 4: GENETIC POPULATION STRUCTURE REVEALS CONNECTIVITY OF PARROTFISH POPULATIONS IN THE SOUTHERN MESOAMERICAN BARRIER REEF

Introduction

A primary focus of Caribbean coral reef management is restoring herbivorous fish populations to reduce macroalgae that inhibits coral assemblage recovery (Jackson et al. 2014b). Overfishing of herbivorous fishes such as parrotfishes results in the functional loss of one of the few herbivores left on Caribbean coral reefs. This loss has largely contributed to observed shifts in Caribbean reef communities from coral to algal dominance (Knowlton 1992, Done 1992, McManus and Polsenberg 2004). Marine protected areas and regional scale fishing regulations are management strategies that have been implemented to reduce fishing pressure on herbivorous fish populations. However, the success of these strategies not only depends on reduced fishing pressure, but also on the connectivity of these fish populations across national boundaries as population recovery may be dependent on larval input from other sources.

Reef fish connectivity is influenced by both ocean surface currents that transport pelagic larvae between both near and distant populations (up to 100 km from source population) (Sponaugle et al. 2002, Ezer et al. 2005, Cowen et al. 2006) and by the behavior of adult fishes and pelagic larvae, which can restrict or direct dispersal contributing to local retention (Cowen et al. 2000, 2003, Jones et al. 2005). Major surface currents in the Mesoamerican Barrier Reef that potentially link fish populations via passive larval dispersal include the Caribbean Current, a northwestward offshore flow in the deep water off the

continental shelves of Honduras and Belize; an equatorial coastal current that flows along the coasts of Belize, Guatemala and Honduras, and a cyclonic circulation in the Gulf of Honduras (Craig 1966, Ezer et al. 2005, Chérubin et al. 2008). Connectivity patterns also reflect adult fecundity and time and location of spawning events relative to currents, gyres, and tides (Shulman and Bermingham 1995, Sponaugle et al. 2002), vertical and horizontal swimming behavior and sensitivity to environmental cues of pelagic larvae (Cowen et al. 2000, Paris et al. 2007); pelagic larval duration (PLD) (Jones et al. 2009); and larval mortality (Sponaugle et al. 2002, Paris et al. 2007). Late-stage pelagic larvae are competent swimmers that can control their trajectories including vertical migrations or directed horizontal swimming which may reduce passive dispersal and enhance self-recruitment (Leis and Carson-Ewart 1997, Stobutzki and Bellwood 1997, Sponaugle et al. 2002). Fish larvae are also capable of orienting themselves toward natal sites or other suitable habitat by sensing environmental cues such as variation in water chemistry, sound and vibrations, hydrography, magnetism, visibility and electrical fields (Kingsford et al. 2002, Lecchini et al. 2005, Gerlach et al. 2007). High connectivity between populations has been linked to a long pelagic larval duration resulting in long dispersal distances in some species, but this is not a universal trend among all fishes and even fish with long PLDs may recruit back to source populations (Shulman and Bermingham 1995, Swearer et al. 1999, Purcell et al. 2006, 2009, Jones et al. 2009).

Molecular markers have been widely used to detect genetic population structure and make inferences about larval dispersal in marine organisms (Hellberg et al. 2002, Jones et al. 2009). Within the Greater Caribbean Region, barriers to larval dispersal identified through

genetic analysis of fish, invertebrates and corals separate the eastern and western Caribbean, the Mesoamerican Barrier Reef and the eastern Caribbean, and coastal and offshore sites in Belize (Purcell et al. 2009, Hogan et al. 2012, Foster et al. 2012, Iacchei et al. 2013, Jackson et al. 2014a). The separation between the Gulf of Honduras and the northern Mesoamerican Barrier Reef System was also detected by dispersal modeling and an assessment of larval fish assemblages across the Mesoamerican Barrier Reef (Cowen et al. 2006, Paris et al. 2007, Muhling et al. 2013). Muhling et al. (2013) suggest that the Gulf of Honduras is a potentially important retention area for pelagic larvae, whereas conditions further north favor dispersion. However, the degree of potential connectivity between the southern and northern MBRS varies seasonally and by species (Purcell et al. 2006, Hogan et al. 2010, Villegas-Sanchez et al. 2010, Kool et al. 2010, Jackson et al. 2014a).

Parrotfishes are territorial fish species that forage and occasionally spawn in shallow reef areas and travel up to several hundred meters to deeper areas to spawn and find suitable sleeping habitat (Ogden and Buckman 1973, Lobel and Ogden 1981, Dubin and Baker 1982). They are relatively fast growing species that reach maturity at an early age (approximately 1-2 years or 17 cm to 27 cm) and spawn year round releasing gametes into the water column (Munro et al. 1973, Robertson and Warner 1978, Koltes 1993). The mean pelagic larval duration for parrotfishes ranges from 28 to 53 days (Schultz and Cowen 1994, Jones et al. 2006). These life history traits may improve larval dispersal potential, but little is known about parrotfish larval behavior that may enhance self-recruitment. One previous study assessing connectivity of parrotfish in the Greater Caribbean, found high gene flow between Stoplight Parrotfish (*Sparisoma viride*) populations in five eastern Caribbean islands

(Geertjes et al. 2004). However, no studies have assessed potential larval dispersal and connectivity among western Caribbean populations including the Mesoamerican Barrier Reef.

In 2009, the Belize Fisheries Department implemented a national ban on herbivorous fish harvesting including parrotfish to restore populations and reduce macroalgal cover. Currently, parrotfish are not legally protected in Honduras outside of no-fishing zones, which constitute only 2% of territorial seas, and none of these zones are well-enforced (McField 2014). We expect the surface gyre between Belize and Honduras to facilitate larval dispersal and connect populations between these regions (Purcell et al. 2009). If larval dispersal effectively links these populations, high fishing pressure in Honduras may greatly impede population recovery (Gobert et al. 2005). Here, we assess the genetic population structure of *S. viride* populations within Belize and Honduras to detect connectivity or larval retention in these regions.

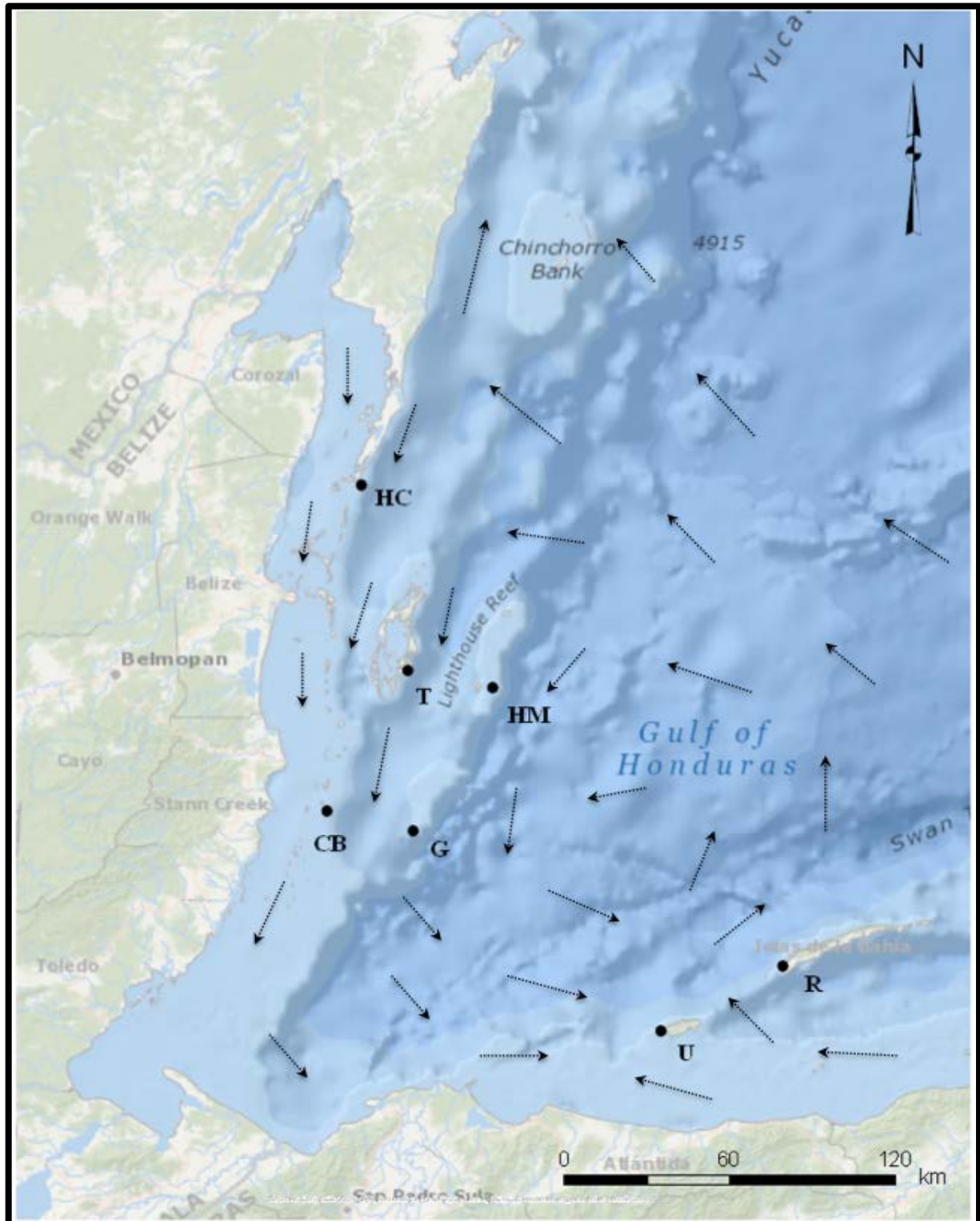
Methods

Sample Collection and Genomic DNA Isolation

We collected fin clippings from 20-50 individual *S. viride* at 10 sites within Belize and Honduras (Fig. 4.1). We chose *S. viride* as a model species because it is a well-studied parrotfish species and is common throughout the Mesoamerican Barrier Reef.

Figure 4.1: Topographic map of mean current direction and sampling locations.

Arrows represent the direction of mean ocean surface currents based on Ezer et al 2005 and Craig 1966. Points represent sampling locations. T=Turneffe, CB=Carrie Bow, HC=Hol Chan, HM=Half Moon, R=Roatan, and U=Utila.



Using either SCUBA or snorkel, we captured adult *S. viride* in a hand net between the hours of 9:00 pm and 12:00 am and removed a small portion (approximately 5 mm²) of the upper lobe of the caudal fin with scissors in situ. This was a non-lethal method of collecting genetic material and fish were released within 2 minutes. We stored the fin sample in 2 ml tubes filled with 95% ethanol. We isolated genomic DNA from sample tissue following the manufacturer's protocol for the Qiagen Puregene Mousetail kit (former Gentra cat. no. D-7010B) and stored at -20°C.

Amplification, Genotyping and Data Analysis

We amplified microsatellite loci (Table 4.1) in 5 multiplex PCR reactions with a Qiagen Type-it Microsatellite PCR kit. Primers were optimized under following conditions: DNA polymerase was activated in an initial activation step (95°C for 5 min), followed by 45 thermocycles of denaturation (95°C for 30 s), annealing (60°C for 90 s), and extension (72°C for 30 s), and a final extension (30 min at 60°C). Florescent- labeled PCR products were size-separated and analyzed in a CEQ 8000 Genetic Analysis System (Beckman Coulter).

We scored all microsatellites loci using GeneMapper version 3.7 (Applied Biosystems) and binned allele sizes with the MstatAllele package v1.05 (Alberto 2009) for R v3.0.2 (R Development Core Team 2013). An individual tissue sample was included in the genetic analyses if we could genotype at least seven of the ten microsatellite loci.

Table 4.1: Summary of 9 polymorphic nuclear microsatellite loci in *Sparisoma viride*.

Number of alleles (Na), size range of amplicons (size of the cloned allele in parentheses), observed (Ho) and expected (He) heterozygosities.

Locus ID	Primer Sequences (5' to 3')	Repeats	Na	H _o	H _e
PFish7	F: TGAAAGCTTCACTGATGTCGC R: CAGAGTGACAAATGTTTGAGGC	ATCT(144) ATCT(104)	17	0.930	0.945
Pfish 14	F: CCATGAAAAGTGTCCCAGCC R: CAAACTGAGAAAGAAAAGTTGCTAGG	ATCT(96) ATCT(124)	20	0.885	0.908
Pfish19	F: GGTGGAACCCAAAGAAAGACC R: CATTTTCAGGTTGCTATTCCCC	ATCT(104) ATCT(104)	20	0.949	0.918
PFish48	F: TGTGGTCTTCACAAGTCAGAGG R: CCCCTGGAGATAGACTGGC	ATCT(68) ATCT(108)	22	0.693	0.908
PFish51	F: TTTCCTTTTGAGCAGTTACTTTAGC R: AATGACATTGATTTTGAGTTTGG	ATCT(72) ATCT(104)	35	0.831	0.820
Pfish69	F: AGAGGACAAATGGTCTCCCG R: ACCTTCCTGTTTGTGAGGTGC	ATCT(88) ATCT(72)	22	0.825	0.880
Pfish158	F: TGCCAATATAATTGTGTTCCATACC R: TTTCCTTTTCGCTTTGTTTCG	ATATC(60) ATATC(60)	33	0.808	0.838
Pfish164	F: CTTTGGCTTACTGAGCGGG R: AAGGATTCAGATAGACATCCAGCC	ATCT(32) ATCT(84)	46	0.928	0.915
Pfish187	F: ATTCAGAGGCAGTAGCGCCC R: CAGTGCAAAGTTTGATTGAGATCC	ATCT(56) ATCT(56)	26	0.768	0.806

We calculated number of alleles, expected heterozygosity (H_e), and observed heterozygosity (H_o) using the adegenet package v1.4-2 (Jombart 2008) for R v3.0.2 (R Development Core Team 2013) (Table 2). Exact tests for goodness of fit to Hardy–Weinberg equilibrium (HWE) using the Markov chain method (10,000 permutations) for each locus within each population were performed in GenoDive v2.0b27 (Table 4.2) (Meirmans and Van Tienderen 2004). Statistical significance of HWE values was assessed after a Bonferroni correction ($p < 0.0008$). Departures from HWE can be caused by biological processes such as inbreeding or population substructure (i.e. the Wahlund effect) or by technical issues such as null alleles. We tested for the presence of null-alleles using MicroChecker v2.2.3 (Van Oosterhout et al. 2004) and for linkage disequilibrium using Genepop on the web (Morgan 2000, Rousset 2008). We calculated population and individual inbreeding coefficients (F_{IS}) using the inbreeding function available from the adegenet package v1.4-2 for R v3.0.2. We then examined the frequency distribution of inbreeding coefficients for all individuals.

We estimated the number of genetically differentiated clusters using discriminant analysis of principal components (DAPC) (Jombart et al. 2010) available in the adegenet package v1.4-2 for R 3.0.2. DAPC has been shown to accurately characterize population subdivision and resolve the underlying structuring in more complex population genetics models. DAPC uses principal component analysis (PCA) to transform as a prior step to discriminant analysis (DA) and does not rely on assumptions about Hardy-Weinberg equilibrium or linkage disequilibrium (Jombart et al. 2010). We ran successive K -means clustering and identified the optimal number of clusters using the Bayesian Information Criterion (BIC). In all analyses, 68 principal components of principal component analysis

were retained in the data transformation step, and six axes were retained in the discriminant analysis step. The first two principal components of the DACP were plotted to evaluate relationships among clusters. We conducted an analysis of molecular variance (AMOVA) to test for population structure among populations using GenoDive 2.0b27. Both global and pairwise F_{ST} were tested for significance with 10,000 permutations.

As a comparison to the DAPC analysis, we used the Bayesian clustering algorithm in STRUCTURE 2.3.4 (Pritchard et al. 2000). We ran STRUCTURE with an admixture model and assumed correlation among population allele frequencies. Default values for alpha and prior F_{ST} were used. Log-likelihood values were computed for each K (1-7) by running STRUCTURE 10 times with 100,000 repetitions each (burn-in: 100,000 iterations).

We tested for IBD by implementing Mantel tests (10,000 permutations) based on the degree of genetic similarity calculated using Nei's distance (Nei 1978) and Euclidean distance (km) between all sampling locations using the ade4 package v1.6-2 (Chessel et al. 2004) and the adegenet package v1.4-2 for R v3.0.2. We then plotted patterns of IBD among all sampling locations to determine if geographical distance was driving genetic differentiation.

Results

We scored 214 individuals over ten nuclear microsatellite loci with 17 to 46 alleles per locus. Microsatellite sequences were deposited to Genbank (*PFish48*, *PFish55*, *PFish51*, *PFish187*, *PFish69*, *PFish14*, *PFish7*, *PFish158*, *PFish164*, *PFish19*). We removed 8

individuals that could not be genotyped at seven of the ten loci from the analysis. Of the ten microsatellite loci developed for this study (Cox et al, in prep), one (*PFish55*) showed significant deviations from Hardy–Weinberg equilibrium at all sampling localities and showed evidence of substantial null alleles. Consequently, this locus was excluded from all analyses (although its inclusion did not alter the results substantially—data not shown). For the remaining nine loci, there were significant deviations from HWE in 2 of 56 (~4%) comparisons after correcting for multiple comparisons Table 4.2. We found no evidence of scoring errors due to large allele dropout or stutter; however, four of nine markers (*PFish7*, *PFish48*, *PFish69*, *PFish14*) showed patterns consistent with null alleles, which are the likely cause of the deviations from HWE. There were no consistent, across-samples effects of null alleles, and given that null alleles only have minor effects on F_{ST} estimates and the accuracy of assignment testing, these loci were included in the all analyses (Carlsson 2008).

For the nine loci, mean allelic richness per population ranged from 8.13 (site U) to 9.21 (site CB) and there appeared to be no geographic trend in values. Observed heterozygosities ranged from 0.87 to 0.90 and expected heterozygosities ranged from 0.85 to 0.88 (Table 4.3). Population level F_{IS} ranged from 0.14 to 0.19 with a mean of 0.17 (Table 4.3). The majority of individuals within each population had a low inbreeding coefficient with only four individuals (among four populations) having a probability of greater than 0.40 to inherit two identical alleles from a single ancestor (Fig 4.2).

Table 4.2: Results of Hardy-Wienburg Equilibrium analysis.

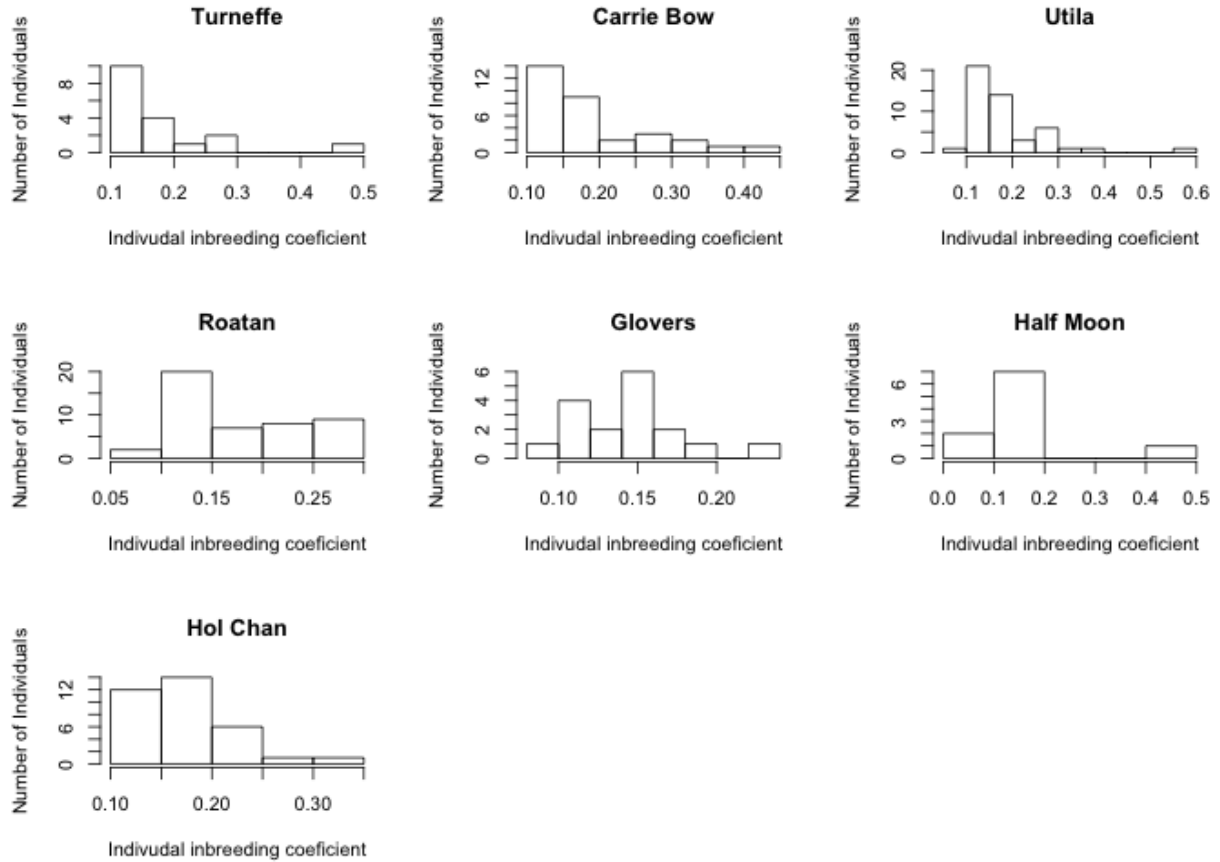
Values in bold represent significant p -values after Bonferonni correction ($p < 0.0008$).

Population	<i>PFish48</i>	<i>PFish158</i>	<i>PFish51</i>	<i>Pfish164</i>	<i>Pfish14</i>	<i>Pfish187</i>	<i>Pfish19</i>	<i>Pfish7</i>	<i>PFish69</i>
Turneffe	0.002	0.550	0.272	0.212	0.021	0.461	0.633	0.520	0.454
Carrie Bow	0.000	0.411	0.422	0.424	0.294	0.243	0.317	0.021	0.002
Glovers	0.113	0.227	0.037	0.500	0.547	0.570	0.205	0.450	0.269
Hol Chan	0.004	0.192	0.233	0.201	0.330	0.470	0.544	0.512	0.068
Half Moon	0.540	0.308	0.637	0.460	0.087	0.604	0.459	0.512	0.598
Roatan	0.001	0.179	0.221	0.409	0.382	0.139	0.537	0.197	0.234
Utila	0.000	0.193	0.269	0.388	0.178	0.188	0.303	0.509	0.514

Table 4.3: Summary Statistics. Sample size (N); average allelic richness (N_a); expected and observed heterozygosity (H_E , H_O); and inbreeding coefficient (F_{IS}) for the 7 sampling locations included in this study.

Population	N	N_a	H_E	H_O	F_{IS}
Turneffe	18	8.72	0.85	0.87	0.17
Carrie Bow	32	9.21	0.88	0.89	0.19
Glovers	17	8.31	0.85	0.87	0.14
Hol Chan	34	8.45	0.86	0.87	0.17
Half Moon	10	8.78	0.86	0.9	0.16
Roatan	46	8.71	0.88	0.89	0.18
Utila	48	8.13	0.87	0.87	0.18

Figure 4.2: Frequency distribution of individual inbreeding coefficients by population.



Tests for linkage disequilibrium (LD) were significant in 4 of 196 comparisons (~2%) after correcting for multiple tests, and there were no locus-specific patterns. The four significant tests were detected from samples collected from Hol Chan Marine Reserve, Belize.

The among-population global F_{ST} value was low (0.001) and not significant ($p = 0.48$). Pair-wise (among sampling sites) F_{ST} values ranged between -0.001 and 0.011 (Table 4.4). Significant population differentiation was found in pairwise comparisons between

Turneffe and Glover's, Utila, and Roatan (Table 4.4). The DAPC analysis identified two clusters within the data. The two main components of the DAPC analysis based on the number of sampling locations ($k = 7$) supported the weak differentiation of Turneffe (Fig. 4.3).

Table 4.4: Pairwise F_{st} values (below diagonal) and corresponding p-values (above diagonal) after 10,000 permuntations.

Significant p-values are shown in bold ($p < 0.05$).

	Turneffe	Carrie Bow	Glovers	Hol Chan	Half Moon	Roatan	Utila
Turneffe	--	0.143	0.018	0.192	0.227	0.012	0.040
Carrie Bow	0.004	--	0.747	0.536	0.938	0.861	0.917
Glovers	0.012	-0.003	--	0.251	0.342	0.766	0.521
Hol Chan	0.003	0.000	0.003	--	0.646	0.108	0.324
Half Moon	0.005	-0.009	0.003	-0.003	--	0.936	0.671
Roatan	0.009	-0.003	-0.003	0.003	-0.008	--	0.731
Utila	0.007	-0.003	-0.001	0.001	-0.003	-0.001	--

STRUCTURE analysis did not detect the weak differentiation between Turneffe and the other six islands (Fig 4.4). However, because STRUCTURE clusters individuals by minimizing Hardy–Weinberg and gametic disequilibrium, complex spatial structure is often not identified (Pritchard et al. 2000, Evanno et al. 2005, Jombart et al. 2010). The multivariate analysis used in DAPC does not make any assumption on the population genetic models and may be more efficient at identifying genetic clines and hierarchical structure (Jombart et al. 2010).

Mantel tests of IBD over all populations did not show a positive relationship between geographical and linearized genetic distances. Instead, we detected a negative relationship between geographical and linearized genetic distances ($R^2 = -0.476$, $p = 0.06$) (Fig 4.5).

Figure 4.3: Discriminant analysis of principal components (DAPC) of *Sparisoma viride* allelic data.

The scatterplot shows the first two principal components of the DAPC, using sampling locations as prior clusters. Populations are shown by different colors and inertia ellipses and points represent individuals. T=Turneffe, CB=Carrie Bow, HC=Hol Chan, HM=Half Moon, R=Roatan, and U=Utila.

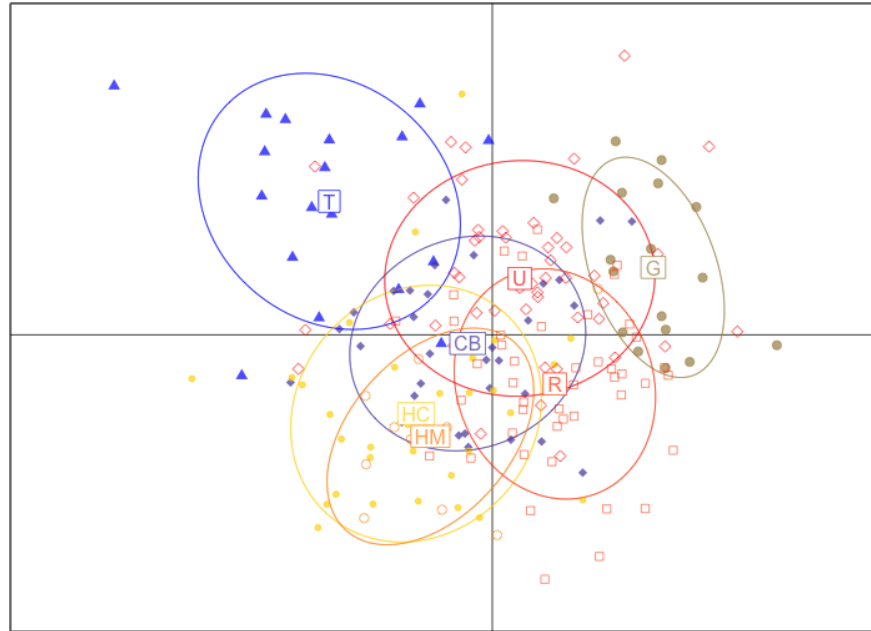


Figure 4.4: Triangular plot of STRUCTURE results.

No genetic differentiation was detected by this analysis.

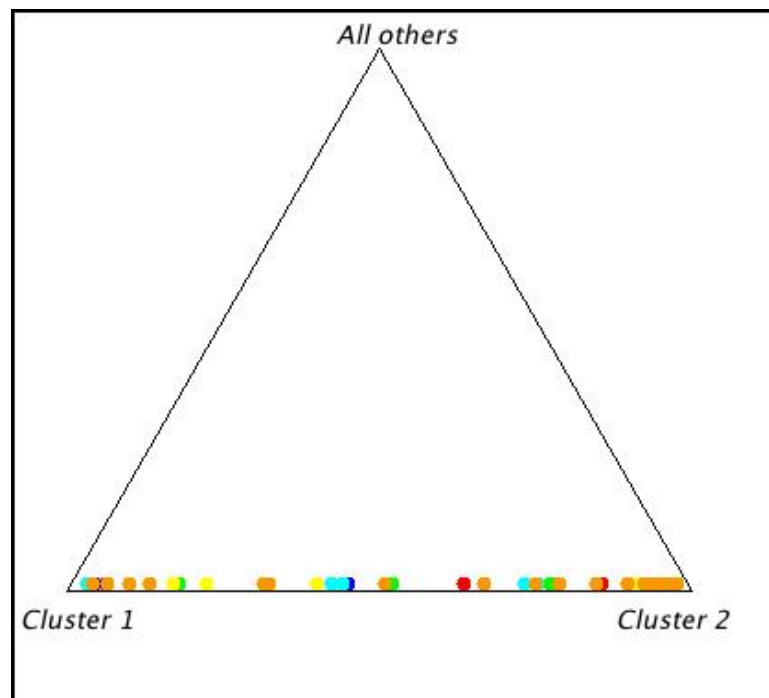
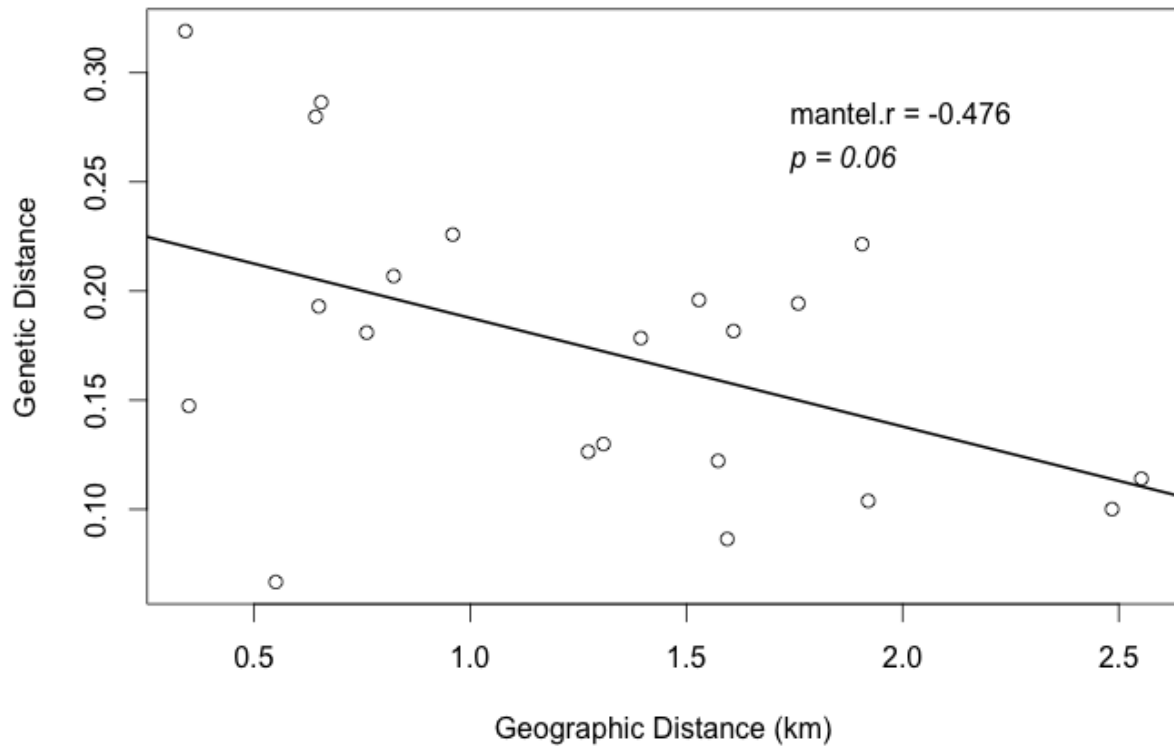


Figure 4.5: Correlation between genetic distance (Nei's Distance) and geographic distance (km) to evaluate isolation by distance (IBD).



Discussion

Effective management to foster grazer recovery requires a thorough understanding of ecological processes that control population gene flow including larval dispersal and population connectivity. Using nine microsatellite markers, we did not detect genetic differentiation between four *S. viride* populations in Belize and two populations in Honduras indicating that these populations are connected through larval dispersal. The population at Turneffe Atoll was weakly differentiated from one population in Belize (Glover's) and two populations in Honduras (Utlia and Roatan). Our findings of larval connectivity between populations in Belize and Honduras are consistent with Purcell et al. 2009, who found no

genetic differentiation between *S. partitus* populations from Glover's Reef, Belize and Cayos Cohcinos, Honduras

Connectivity of fish populations through larval dispersal is influenced by oceanographic circulation and larval behavior (Cowen et al. 2000, White et al. 2010a). Genetic differentiation between the eastern and western Caribbean was detected for populations of bicolor damselfish (*Stegastes partitus*) and french grunts (*Haemulon flavolineatum*), but not for bluehead wrasse (*Thalassoma bifasciatum*) suggesting that the former species have more restricted larval dispersal (Purcell et al. 2006, 2009). These genetic differences were attributed to differences in PLD. *S. partitus* and *H. flavolineatum* have PLDs of ranging from 14 to 30 days, while *T. bifasciatum* have a PLD of 45 days or more (McFarland et al. 1985, Wellington and Robertson 2001). There was a positive relationship between genetic and geographical distance for *S. partitus* and *H. flavolineatum* in the eastern Caribbean, but no spatial pattern in the western Caribbean, which highlights the importance of oceanographic currents in larval dispersal. However, local retention was identified for the California spiny lobster (*Panulirus interruptus*) despite a PLD of 240 to 330 days and high potential for connectivity through ocean surface currents, which underscores the importance of larval behavior in limiting dispersal (Iacchei et al. 2013). Thus, genetic population structure cannot be extrapolated from one species to another (Díaz-Ferguson et al. 2011) and predicting larval dispersal for species of particular importance such as parrotfish should be assessed independently and incorporated into fisheries management approaches (Reiss et al. 2009, Ovenden et al. 2011). However, in many cases, genetic population structure is not sufficiently considered in management practices resulting in potential loss of genetic

diversity and ineffective fish stock recovery or sustainability (Laikre et al. 2005, Reiss et al. 2009).

Extensive connectivity and gene flow between management areas should enhance genetic diversity and has the potential to promote population recovery (Berry et al. 2012). However, population recovery may be compromised if source populations located outside jurisdictional boundaries are overfished. In other words, depleted fish populations would not be replaced by immigrants or recruits from other populations. Local conservation organizations are monitoring the biomass of herbivores throughout the Mesoamerican Barrier Reef and encouraging local governments and communities to conserve this important functional group, but Belize is currently the only country with a regulation in place that nationally protects parrotfishes. Our results provide evidence of parrotfish population connectivity between Belize and Honduras highlighting the importance of establishing a management approach that crosses international boundaries to restore parrotfish populations and coral reef health.

APPENDIX A

Sample and data collection

Belize City is the largest coastal settlement in Belize and had three types of vendors selling fish fillets. There are two fishing co-operatives that sell seafood in the city; however only one sold fillets during the sampling periods. In addition, in Belize City we purchased fillets from two major supermarkets and two open fish markets. The fishing co-operative is the only establishment that sells fillets in Placencia. We purchased fish fillets from the Placencia co-operative in November 2009 and May 2010, but none were available during the October 2010 or June 2011 sampling periods. In October 2010 only whole fish was available and in June 2011 only lobster was available. A fisheries officer accompanied us to obtain samples from local restaurants in Placencia in October 2010 and June 2011. This was the only town in which a fisheries officer was available and willing to assist with restaurant surveys. Seven of the samples collected in Placencia were confiscated from restaurants by the Belize Fisheries Department and the market label was not known. These samples were only used to calculate proportions of parrotfish. Punta Gorda has one open fish market and one fishing co-operative office. The open fish market only sells whole fish and other seafood. The co-operative was selling fillets in June 2011 only. Fish fillets are only sold at one supermarket in San Pedro. We purchased fillets at this supermarket in November 2009, May 2010 and October 2010. The supermarket did not sell fillets in June 2011. One open fish market is located in Dangriga and fillets were only sold at this market in October 2010. We visited open fish markets when they were most active, which was in the early morning in Dangriga and Punta Gorda and midafternoon in Belize City. We visited co-operatives and

supermarkets in the morning to increase the chances that fillets would be available. In Placencia, we visited restaurants when fisheries officers were available to assist.

Due to differences in the availability of fish fillets between towns and vendors, our sampling design was unbalanced and replication was uneven. For instance, whole fish are sold more frequently than fillets in Punta Gorda and Dangriga resulting in low sample sizes. Only one supermarket in San Pedro sold fillets, which also resulted in a low sample size. The low sample sizes associated with these towns resulted in an uncertainty of the accuracy of mislabeling proportions. In contrast, we purchased or collected fillets from three vendor types (fish market, supermarket, and co-operative) in Belize City and two vendor types (restaurants and co-operative) in Placencia resulting in larger sample sizes.

PCR Amplification

Each PCR reaction mixture consisted of 1.0 µl 10x PCR buffer for Hotstart Taq (Apex), 0.5 µl of 50 mM MgCl₂, 0.5 µl of 10 mM dNTP mix, 0.3 µl each of 10 mM primer, 0.1 µl Hotstart taq (Apex), and 0.5-2.0 µl of template DNA. Reactions using LCO/HCO primers were amplified with a thermal program consisting of an initial step of 15 min at 95°C followed by 35 cycles of 1 min at 95°C, 45 s at 42°C, and 1 min at 72°C, followed by a final extension step at 72°C for 7 minutes. Reactions using FishF1/FishR1 primers were amplified with a thermal program consisting of an initial step of 15 min at 95°C followed by 35 cycles of 35 cycles at the following parameters: 1 min at 95°C, 45 s at 52°C, and 1 min at 72°C, followed by a final extension step at 72°C for 5 minutes

Table A1: Summary of Data Collection. Proportion of mislabeling (Number of fillet analyzed). M = open fish market, S = supermarket, C = co-operative, R = restaurant, NA = no fillet sold

Sampling Period	Belize City			Placencia		Punta Gorda		Dangriga	San Pedro	Mean (Total N)
	M	S	C	C	R	C	R	M	S	
November 2009	1.0 (6)	1.0 (2)	0.0 (5)	0.80(5)	NA	NA	NA	NA	0.40(4)	0.64 (22)
May 2010	1.0 (2)	1.0 (3)	0.57 (7)	1.0 (5)	NA	NA	NA	NA	0.8 (5)	0.82 (22)
October 2010	0.50(2)	0.33(3)	1.0 (6)	NA	0.67 (19)*	NA	NA	0.60 (5)	1.0(4)	0.72 (39)
June 2011	NA	0.20(3)	0.0(7)	NA	0.07 (15)	0.0(1)	0.0(2)	NA	NA	0.04 (28)
Mean (Total N)	0.90 (10)	0.55 (11)	0.40 (25)	0.90 (10)	0.33 (34)	0.0(1)	0.0(2)	0.60 (5)	0.73(13)	0.51 (111)
		0.50 (46)		0.47 (44)		0.0 (3)		0.60(5)	0.73(13)	

* 7 of these samples were confiscated from restaurants by the Belize Fisheries Department and the market label was not known. These samples were not used to calculate proportions of mislabeling, but were used to calculate proportions of parrotfish.

Table A2: Summary of prices of fish fillets

Market Label	N	Minimum Price (US\$/lb)	Maximum Price (US\$/lb)	Mean Price (US\$/lb)
Snapper	62	4.00	6.23	5.39
Grouper	20	4.30	6.05	5.30
Snapper/Grouper	15	7.50	7.50	7.50
Snapper/Grouper/Hogfish	8	4.00	4.00	4.00
Cobia	2	3.00	3.00	3.00
Snook	3	2.25	2.50	2.33

APPENDIX B

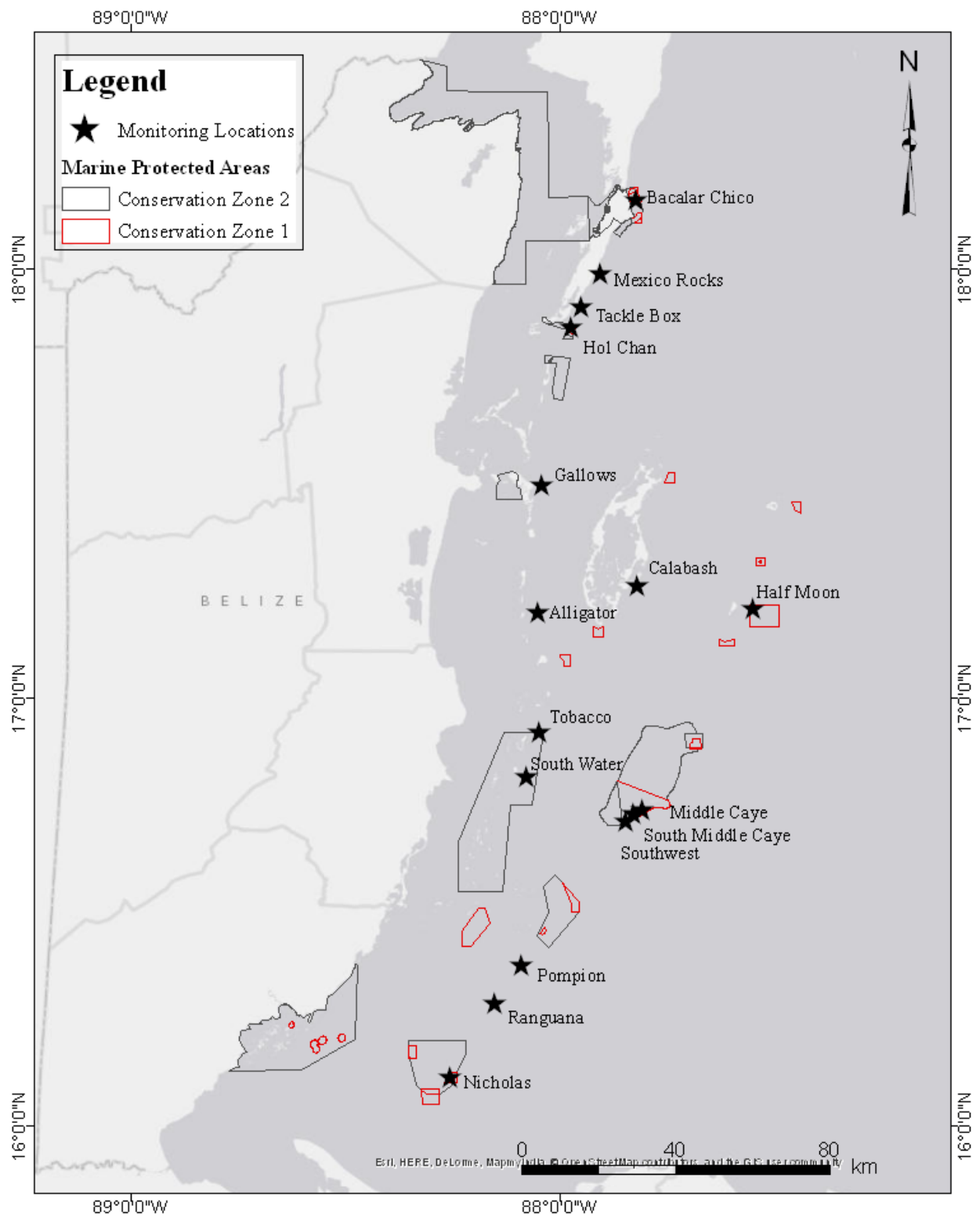
Table B1: Monitoring Site Details

Site	Latitude	Longitude	Reserve ID	Protection Status
Calabash	17.261470	-87.819700	No Status	None
Half moon	17.205600	-87.546790	Half Moon Caye National Monument	C1
Middle Caye	16.737030	-87.805360	Glover's Reef Marine Reserve	C2
South of Middle Caye	16.728750	-87.828670	Glover's Reef Marine Reserve	C2
Tobacco Caye	16.919110	-88.047570	No Status	None
South Water Caye	16.813460	-88.077560	South Water Caye Marine Reserve	C1
Alligator Caye	17.196600	-88.051150	No Status	None
Tackle Box	17.910560	-87.950830	No Status	None
Hol Chan	17.863430	-87.972380	Hol Chan Marine Reserve	C1
Mexico Rocks	17.987820	-87.903820	No Status	None
Bacalar Chico	18.162820	-87.822220	Bacalar Chico Marine Reserve	C1
Gallows	17.495920	-88.042550	No Status	None
Pampion Caye	16.373100	-88.089130	No Status	None
Ranguana Caye	16.285010	-88.150310	No Status	None
Nicholas Caye	16.112300	-88.255860	Sapadilla Cayes Marine Reserve	C1
Southwest Caye	16.71087	-87.8461	Glover's Reef Marine Reserve	C2

Table B2: Mean density (individual/m²), biomass (g/m²), and standard error (SE) from 2009 to 2013 by species.

Species	Density	SE	Biomass	SE
<i>S. atomarium</i>	0.004	0.003	0.029	0.016
<i>S. aurofrenatum</i>	0.102	0.021	6.973	0.713
<i>S. chrysopterum</i>	0.008	0.002	1.885	0.353
<i>S. coelestinus</i>	0.000	0.000	0.037	0.037
<i>S. coeruleus</i>	0.000	0.000	0.128	0.128
<i>S. iseri</i>	0.212	0.040	6.049	0.305
<i>S. rubripinne</i>	0.008	0.002	2.597	0.749
<i>S. taeniopterus</i>	0.047	0.015	1.625	0.633
<i>S. vetula</i>	0.003	0.002	0.308	0.073
<i>S. viride</i>	0.046	0.009	12.471	1.535

Figure B1: Monitoring Site Locations. Map created using ESRI ArcGIS 10.2.1.



Detailed description of covariates

Human population density

Humans within 50 km (maximum number of people that occurred within 50-km radius of each site) was used as the estimation for human population density. We chose 50 km as radius for the first measured variable because it is reasonable range of anthropogenic influence on Caribbean reefs (Mora 2008). Projection estimates of human population counts were obtained from the Gridded Population of the World V.3 at 0.25 degree resolution (SEDAC 2010) and calculated in ArcGIS v10.0.

Reef Area

Reef areas within 10 km radius of each site was calculated from the Global Distribution of Coral Reefs (2010) database as available at the Ocean Data Viewer United Nations Environment Program's World Conservation Monitoring Centre (UNEP-WCMC) (<http://data.unep-wcmc.org/datasets/13>). This database represents the global distribution of warm water coral reefs compiled mostly from the Millennium Coral Reef Mapping Project validated and un-validated maps as well as other sources acquired by UNEP-WCMC. Reef areas within the interest region were calculated in ArcGIS v10.0.

Reef structural complexity

For each transect set we visually estimated structural reef complexity on a scale of 0-5, where 0 was given to reefs with no vertical relief; 1, low and sparse relief; 2, low but widespread relief; 3, moderately complex relief; 4, very complex relief with numerous caves and fissures; and 5, reefs with exceptionally complex habitats, with numerous caves and

overhangs (Polunin and Roberts 1993). This topographic measure provided an assessment of reef complexity at the seascape level which is relevant to large and medium-sized fish (Polunin and Roberts 1993, Wilson et al. 2007). To minimize estimation subjectivity among observers, at least two divers estimated reef structural complexity for each transect set and the average was calculated to be used in the models. We evaluated the accuracy of the estimations among observers by comparing the standard deviations (SD) among transects per site and found that SDs were 0-0.7 in all cases, meaning that average estimation differences were never over 1 unit.

Mangrove Perimeter

Mangrove abundance was quantified as the perimeter covered by mangrove within 10 km radius of each site. Estimates of Caribbean mangrove distribution were obtained from the Global Distribution of Mangroves USGS (2011) database as available at the Ocean Data Viewer UNEP-WCMC (<http://data.unep-wcmc.org/datasets/21>). This database depicts the distributions of global mangroves based on Global Land Survey data and Landsat images. Landsat images (30 m resolution) were interpreted using unsupervised and supervised digital image classification techniques. Each image was atmospherically corrected, ground truth and validated with existing maps and databases.

Net primary productivity

We calculated mean oceanic net primary productivity ($\text{mg C m}^{-2} \text{ day}^{-1}$) for each site between 2002 and 2012 using remote-sensing. This was obtained from Aqua MODIS satellite monthly data combined in the vertical generalized production model (Behrenfeld and

Falkowski 1997) at a spatial resolution of 0.0833° (Oregon State University 2013). We used the mean of the last ten years period because primary productivity is inherently variable in time and established predatory communities may respond better to long term trends in primary productivity than to survey year or monthly mean values. Calculations were performed in ArcGIS 10.0.

Sea surface temperature

We used AHVRR Pathfinder Version 5.2 (PFV5.2) satellite data obtained from the US National Oceanographic Data Center and GHRSSST (NOAA 2013). The PFV5.2 data are an updated version of the Pathfinder Version 5.0 and 5.1 collections described in Casey et al. (2010). We calculated average monthly sea surface temperature (SST, 2002-2011) for each source 4 km² grid cell that corresponded to each reef site. We also calculated mean minimum monthly SST by selecting the lowest monthly average temperature per year to compute an average across years. Mean minimum monthly SST could be a better predictor of physiological constraints of some fish predator species (Jennings et al. 2008, Nadon et al. 2012). We used mean temperature of nine years because it may represent better the temperature regimen these top consumers experience overtime. All calculations were performed in ArcGIS 10.0.

Wave exposure

The log of wind driven wave exposure (J m⁻³) was extracted in ArchGIS 10.0 from the wave stress map for the Caribbean basin built by Chollett *et al.* (2012) and available at (<http://www.marinespatialecologylab.org/wp-content/uploads/2010/11/PECS1.png>). This

index does not include the influence of tides or swells, which are not generated by local wind, and it is an approximation of wave patterns in shallow areas (Chollett et al. 2012). Wave exposure has been a good predictor of spatial variation in reef building corals such as *Orbicella* sp. (former *Montastrea* sp.) (Chollett and Mumby 2012) and can partially explain beta diversity patterns of benthic communities (Harborne et al. 2011). Wave exposure may also directly affect the biomass and diversity of tropical reef fish (Friedlander et al. 2003) and the distribution and abundance of temperate reef fish by compromising swimming abilities (Fulton and Bellwood 2004). Alternatively, by modifying the distribution of foundation species like corals, wave exposure could affect fish species that depend on them. The detailed description of the wave exposure calculations and assumptions can be found in Chollett & Mumby (Chollett and Mumby 2012).

Macroalgal cover

Macroalgal cover was measured at each site using point intercepts along 6-8 transect lines (Lang et al. 2010). Six 10 m lead-core transect lines were laid on the benthos at each site, with each transect spaced 10 m apart. Macroalgae were identified to genus at 10 cm intervals along the 10 m transect lines. These transects were directly adjacent to the fish transect lines.

Predator Biomass

We performed visual fish censuses to estimate predatory reef fish species composition and density using a modification of the standard Atlantic and Gulf Rapid Reef Assessment (AGRRA) v5.4 techniques as described in the main text (Lang et al. 2010). Fish

biomass was calculated through the allometric weight-length relationship, $W = aTL^b$, where W is the weight of each individual (in grams), TL is the length of each fish (in cm) estimated from visual surveys, and the parameters a and b are species specifics (Froese and Pauly 2011).

APPENDIX C

Detailed description of covariates

Human population density

As accurate information of human impacts (i.e., fishing efforts, direct pollution, diving activities, etc.) is unreliable for most of our sites, we expected that human population density closest to our study sites would be an adequate surrogate for fishing activity levels. This was based on studies that demonstrated that the number of people per unit of reef area has been positively correlated with fishing pressure (Newton et al. 2007, Stallings 2009b, Ward-Paige et al. 2010, Williams et al. 2011, Nadon et al. 2012). We used the number of humans within 50 km of each site as this radius of influence has been adequate in detecting anthropogenic effects in the wider Caribbean, including Belize (Mora 2008). Projection estimates of human population counts for the year 2010 were obtained from the Gridded Population of the World V.3 at 0.25 degree resolution (SEDAC 2010) and calculated in ArcGIS v10.0.

Reef Area

Reef areas within 5 km and 10 km radius of each site was calculated from the Global Distribution of Coral Reefs (2010) database as available at the Ocean Data Viewer United Nations Environment Program's World Conservation Monitoring Centre (UNEP-WCMC) (<http://data.unep-wcmc.org/datasets/13>). This database represents the global distribution of warm water coral reefs compiled mostly from the Millennium Coral Reef Mapping Project validated and un-validated maps as well as other sources acquired by UNEP-WCMC. Reef areas within the interest region were calculated in ArcGIS v10.0.

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For each transect set we visually estimated structural reef complexity on a scale of 0-5, where 0 was given to reefs with no vertical relief; 1, low and sparse relief; 2, low but widespread relief; 3, moderately complex relief; 4, very complex relief with numerous caves and fissures; and 5, reefs with exceptionally complex habitats, with numerous caves and overhangs (Polunin and Roberts 1993). This topographic measure provided an assessment of reef complexity at the seascape level which is relevant to large and medium-sized fish (Polunin and Roberts 1993, Wilson et al. 2007). To minimize estimation subjectivity among observers, at least two divers estimated reef structural complexity for each transect set and the average was calculated to be used in the models. We evaluated the accuracy of the estimations among observers by comparing the standard deviations (SD) among transects per site and found that SDs were 0-0.7 in all cases, meaning that average estimation differences were never over 1 unit.

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Net primary productivity

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Sea surface temperature anomalies

We created a 29-year dataset (1982-2010) of annual frequency of weekly thermal stress anomalies (TSA) for each surveyed reefs using the National Oceanic and Atmospheric Administration's (NOAA) National Oceanographic Data Center (NODC) Coral Reef Temperature Anomaly Database (CoRTAD) Version 4.0 (Casey et al. 2010, Selig et al. 2010) (available at <http://data.nodc.noaa.gov/cortad/Version4>) (Fig.1). Temperature anomalies for this database were calculated from the Pathfinder Version 5.2 data temperature with a spatial resolution of ~ 4 km grid cell (Casey et al. 2010, Selig et al. 2010) and with a quality flag of four or better (Kilpatrick et al. 2001). We defined TSA as deviations of one-week where sea surface temperature (SST) was 1°C or greater than the mean maximum climatological week or the long term average warmest week from 1982 to 2010 (Selig et al. 2010). This threshold is generally accepted for environmental conditions that may cause bleaching and coral mortality (Glynn 1993, Liu et al. 2003). We calculated the long term (29

years) average and standard deviation annual-frequency TSA (weeks/year) for the grid cell that corresponded to each surveyed site to be used as fixed predictor in linear mixed effect models (Fig. 1).

Enforcement level

We used qualitative level of enforcement estimations published in the Healthy Reefs 2014 EcoAudit for Belize. Managers at each reserve were asked to score the overall level of enforcement as good, moderate, or inadequate. **Good**-regular patrols, overall satisfactory compliance and ecological integrity is thought to be maintained, **Moderate**-regular patrols conducted, but limited poaching occurs, legal outcomes are insufficient, and ecological integrity is slightly impacted, **Inadequate**-irregular patrols conducted, poaching persists, legal outcomes are insufficient, ecological integrity is impacted, and local community feedback demonstrates a high level of concern.

Protection Status

We classified the protection status of each site based on individual management plans for each MPA. Information regarding zoning of protected areas is provided by the Belize Fisheries Department (www.fisheries.gov.bz). Control sites had only national restrictions, in which fishing was unrestricted except for herbivorous fishes and Nassau grouper (see Belize National Statutory Instrument No. 49 of 2009). Conservation Zone 1 sites are in areas designated for recreation use and only non-extractive sports fishing is permitted. Conservation Zone 2 sites are within marine protected areas where limited artisanal fishing is

allowed, with special restrictions in place that include limited fishing licenses, banned use of traps, nets, and long-lines.

Wave exposure

The log of wind driven wave exposure (J m^{-3}) was extracted in ArcGIS 10.0 from the wave stress map for the Caribbean basin built by Chollett *et al.* (2012) and available at (<http://www.marinespatialecologylab.org/wp-content/uploads/2010/11/PECS1.png>). This index does not include the influence of tides or swells, which are not generated by local wind, and it is an approximation of wave patterns in shallow areas (Chollett *et al.* 2012). Wave exposure has been a good predictor of spatial variation in reef building corals such as *Orbicella* sp. (former *Montastrea* sp.) (Chollett and Mumby 2012) and can partially explain beta diversity patterns of benthic communities (Harborne *et al.* 2011). Wave exposure may also directly affect the biomass and diversity of tropical reef fish (Friedlander *et al.* 2003) and the distribution and abundance of temperate reef fish by compromising swimming abilities (Fulton and Bellwood 2004). Alternatively, by modifying the distribution of foundation species like corals, wave exposure could affect fish species that depend on them. The detailed description of the wave exposure calculations and assumptions can be found in Chollett & Mumby (Chollett and Mumby 2012).

Distance to Deep Water

The distance from each site to the 30 m contour line identified on NOAA bathymetry charts was calculated in ArcGIS 10.0.

Coral and Macroalgal cover

Macroalgal cover was measured at each site using point intercepts along 6-8 transect lines (Lang et al. 2010). Six 10 m lead-core transect lines were laid on the benthos at each site, with each transect spaced 10 m apart. Macroalgae were identified to genus at 10 cm intervals along the 10 m transect lines. These transects were directly adjacent to the fish transect lines.

Predator and Parrotfish Biomass

We performed visual fish censuses to estimate predatory reef fish species composition and density using a modification of the standard Atlantic and Gulf Rapid Reef Assessment (AGRRA) v5.4 techniques as described in the main text (Lang et al. 2010). Fish biomass was calculated through the allometric weight-length relationship, $W = aTL^b$, where W is the weight of each individual (in grams), TL is the length of each fish (in cm) estimated from visual surveys, and the parameters a and b are species specific (Froese and Pauly 2011).

Figure C1: Monitoring Site Locations

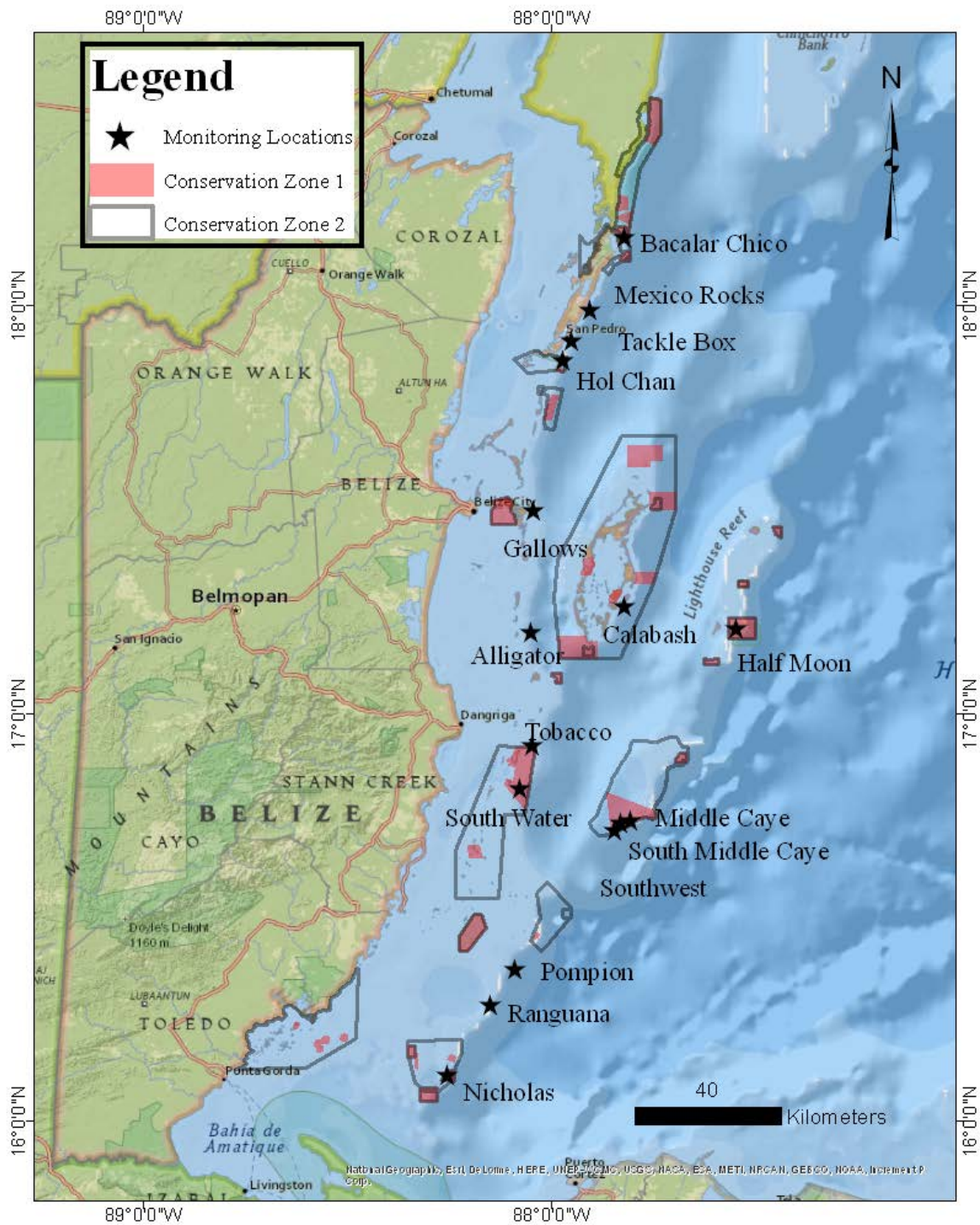
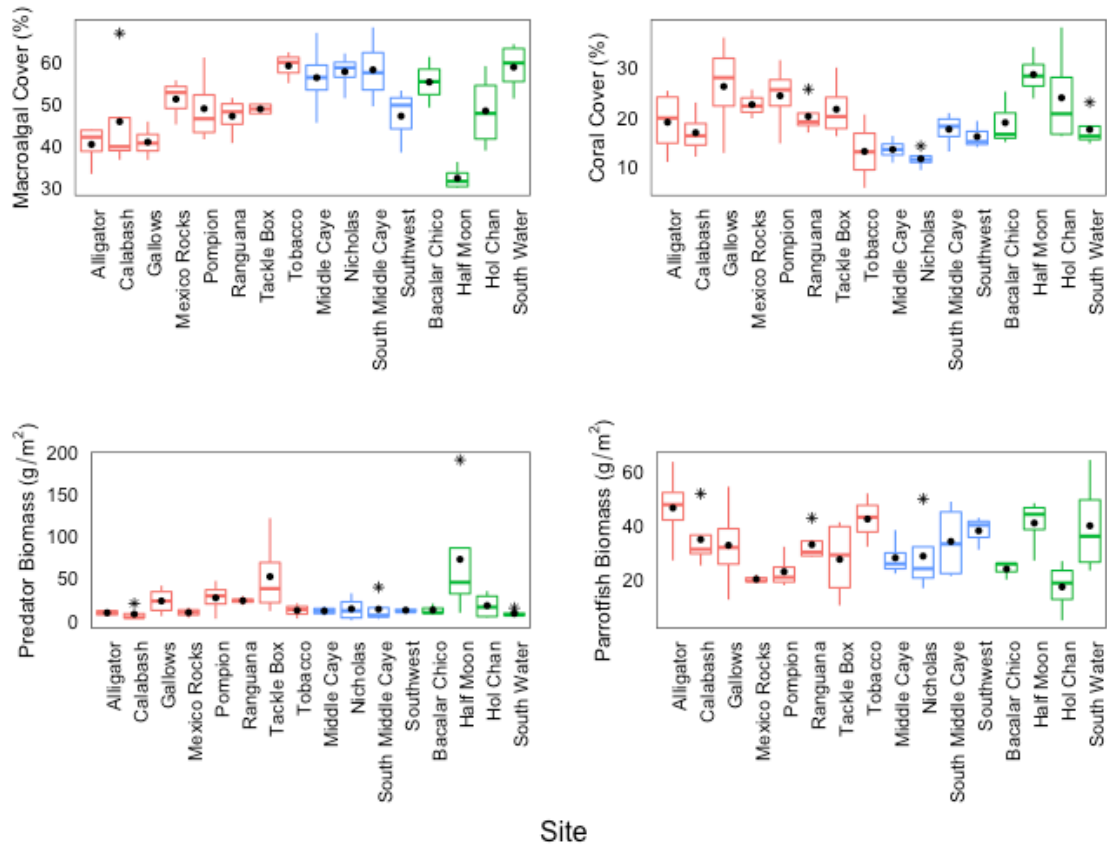


Figure C2: Comparison of parrotfish biomass, predatory reef fish biomass, macroalgal cover, and coral cover by site. Boxplots represent means per year. Red represents fished sites, blue represents C2 sites, and green represents C1 sites.



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