Genetic testing reveals some mislabeling but general compliance with a ban on herbivorous fish harvesting in Belize

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Abstract

Overfishing of herbivorous fishes is one of the primary causes of Caribbean coral reef decline. In Belize, herbivorous fishes comprised 28% of the catch from 2005 to 2008. In 2009, the Belize Fisheries Department implemented a national ban on herbivorous fish harvesting to mitigate high-macroalgal cover on much of the Belize Barrier Reef. However, compliance with this approach has not been evaluated. We assessed the proportion of herbivorous fish in local markets by genetically identifying fish fillets sold in five major towns in Belize from 2009 to 2011. We found that 5–7% of 111 fillets were identified as herbivorous fish and 32–51% were mislabeled. A 5–7% proportion of parrotfish in local markets suggests some ongoing parrotfish harvesting. However, our results suggest that the ban has reduced herbivorous fish harvesting and has the potential to help facilitate the restoration of coral reef ecosystems.

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 & Pauly 2008; Miller & Mariani 2010). 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 (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,

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Kingfish, Cobia, small tunas, Bonito, Pompano, Permit, and Hogfish (BMAF 2008). In Belize, the Nassau Grouper is protected from 1 December to 31 March and only individuals between 20 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; Graham *et al.* 2009; Stallings 2009; Paddack *et al.* 2009, 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 United States and other parts of the world (Jacquet & Pauly 2008; Miller & Mariani 2010; Tennyson *et al.* 1997). Marko *et al.* (2004) found that 77% of fish labeled as the overfished red snapper (*Lutjanus campechanus*) on the East Coast of the United States 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 United States, 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, personal communication, 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 (Table S1). We removed approximately 1 g 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 S1. 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 S1. 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 < 1e-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 (Thompson *et al.* 1997).

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 1,000 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 (Figure 1). Most samples were identified to species using BOLD and/or BLAST. We confirmed that a total of 69 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. 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 ± 25% (± 1 SE), 7 ± 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 ± 24% (± 1 SE), 5 ± 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 (Figure 2). We genetically identified fillets labeled as snapper or grouper to one of 11 families (Figure 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). 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). 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 (Table S1).

The proportion of parrotfish sold was significantly higher in supermarkets than in restaurants or cooperatives 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 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 (Table S1).

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 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 5–7% of fish fillets sold in local markets were illegal, parrotfish species and 32–51% were mislabeled. The proportion of mislabeling is similar to that in many parts of the world (Baker *et al.* 1996; Baker *et al.* 2007; Wong & Hanner 2008; Ardura *et al.* 2010; Garcia-Vazquez *et al.* 2011; Marko *et al.* 2011; Miller *et al.* 2011).

Low proportions of parrotfish were detected in Belize City (2–4%), Placencia (0%), and Punta Gorda (0%) whereas 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 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.

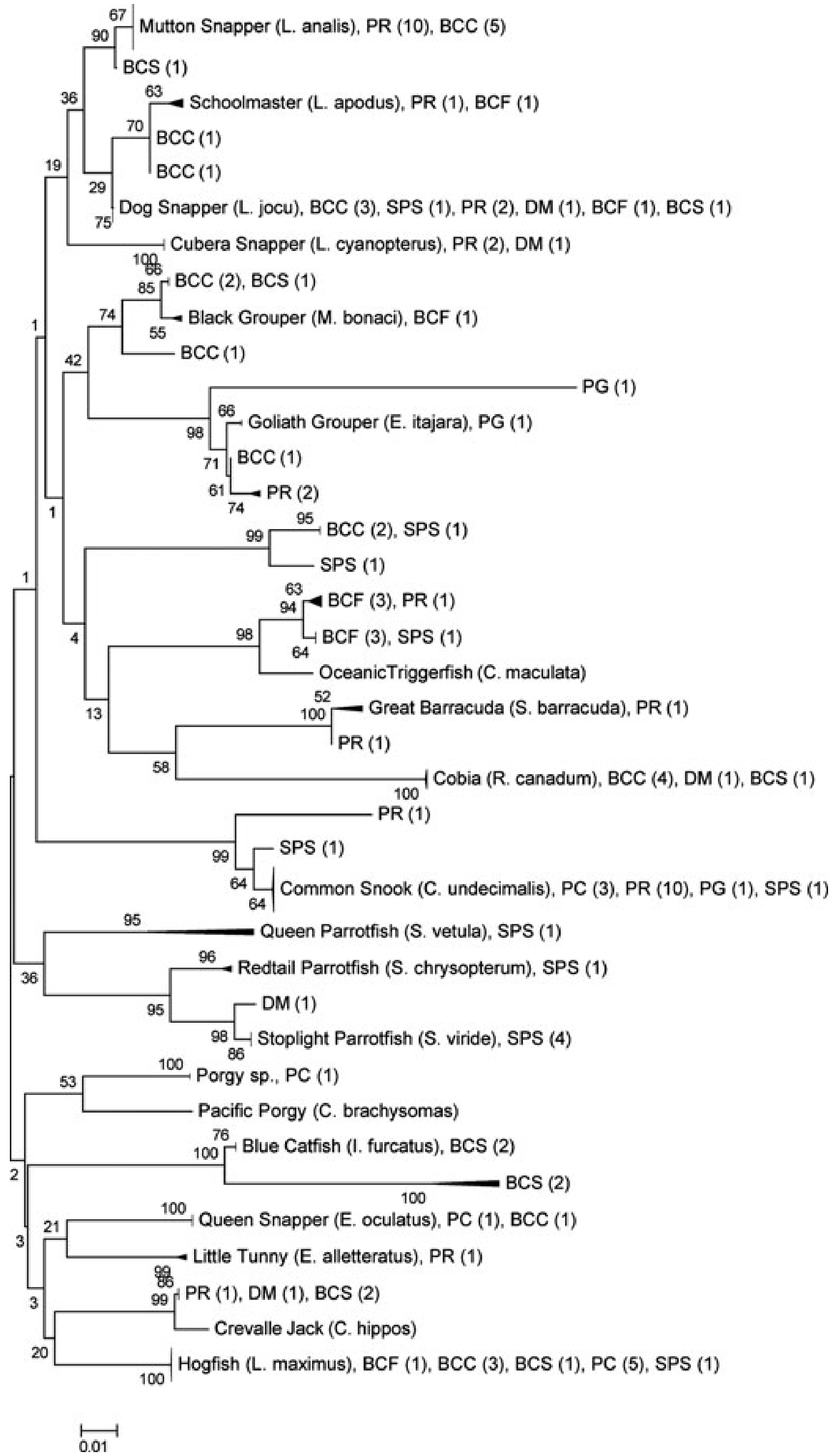

Figure 1 Evolutionary relationships of samples. The evolutionary history was inferred using the neighbor-joining method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown next to the branches. Tips are labeled by town 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.

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

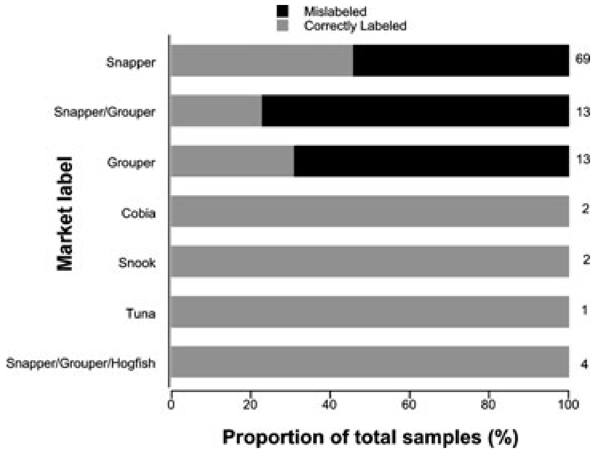

Figure 2 Proportion of mislabeling by market label. We pooled samples from each town/vendor to calculate the percentage of mislabeling. Sample size is listed at the end of each row.

95% (Noella Gray *et al.* 2010). 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 & 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 were not available to calculate a national average of harvested parrotfish before 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, 5–7% of fillets were parrotfish, which is signifcatly

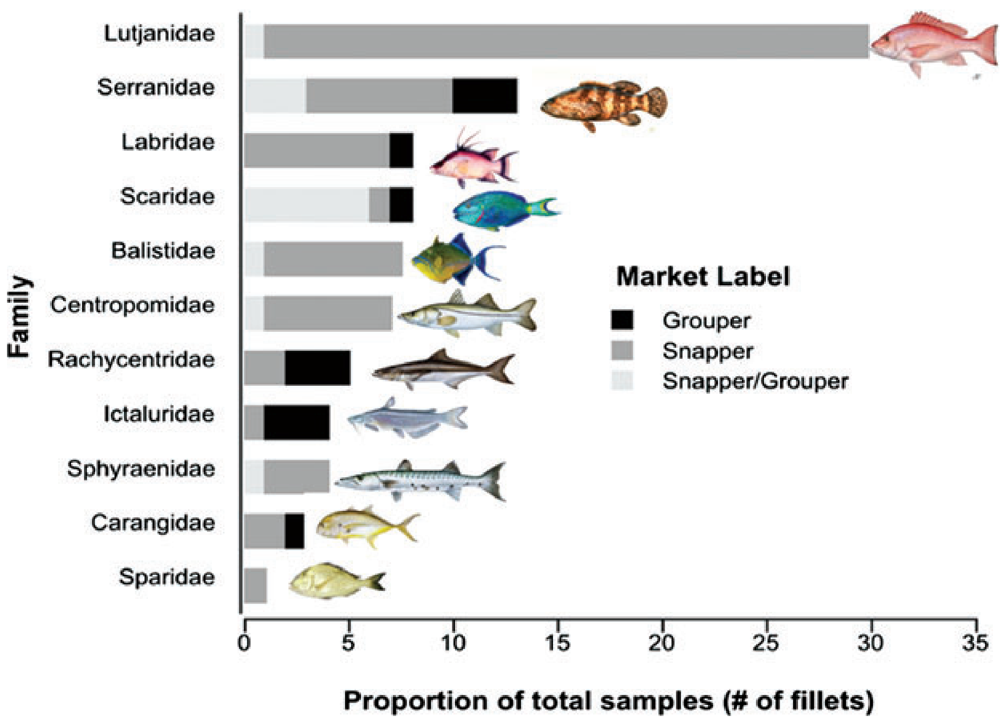

Figure 3 Species composition of samples labeled as snapper, grouper, and snapper/grouper.

Table 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% ±SE)	Total mislabeled fillets (Mean% ±SE)
Punta Gorda	2	3 (3)	0 ± 0	0 ± 0
Belize City	11	46 (28)	2 ± 10–4 ± 10[a]	50 ± 42–60 ± 42[a]
Placencia	4	44/37[b] (24/23)	0 ± 0	39 ± 32[a]–47 ± 35
Dangriga	1	5 (5)	20	60
San Pedro	3	13 (10)	33 ± 23[a]–43 ± 33	66 ± 24[a]–73 ± 25

[a]Mean calculation assumed that fillets identified as the same species at an individual vendor were cut from one fish.
[b]Seven 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 calculated proportions of parrotfish.

Table 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[b] (23/22)	0 ± 0	28 ± 32[a]–32 ± 33
Fish Market	4	15 (11)	7 ± 9–9 ± 10[a]	53 ± 20[a]–80 ± 20
Co-op	7	39 (20)	0 ± 0	34 ± 50[a]–54 ± 50
Supermarket	7	24 (19)	10 ± 25[a]–21 ± 25	33 ± 34[a]–50 ± 37

[a]Mean calculation assumed that fillets identified as the same species at an individual vendor were cut from one fish.
[b]Seven 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 calculated proportions of parrotfish.

Table 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[a]–64 ± 34
May 2010	5	22 (14)	14 ± 19[a]–23 ± 29	71 ± 23[a]–82 ± 17
October 2010[b]	6	39/32 (29/28)	5 ± 10–8 ± 17[a]	63 ± 20[a]–72 ± 23
June 2011	4	28 (17)	0 ± 0	4 ± 3–6 ± 4[a]

[a]Mean calculation assumed that fillets identified as the same species at an individual vendor were cut from one fish.
[b]Seven 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 calculated proportions of parrotfish.

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

Table 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[a]	50–60[a]
	Belize rural	24,305				
	Ambergris Caye/San Pedro	11,510	42.3	[b]	33[a]–43	66[a]–73
Stann Creek	Dangriga	9,096	7.3	≈30	20	60
	Stann Creek Rural	23,070				
	Placencia	750	12.4	≈25	0	39[a]–47

[a]Mean calculation assumed that fillets identified as the same species at an individual vendor were cut from one fish.
[b]data not available.

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; Graham *et al.* 2009; Stallings 2009; Paddack *et al*. 2009; Mumby *et al.* 2012). We identified most of the mislabeled fillet samples as Labridae (hogfish), Scaridae (parrotfish), and Balistidae (triggerfish; Figure 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 cooperatives 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 nontarget species (Wendy's Restaurant [local restaurant], personal communication, 2011). We found that snapper, grouper, and hogfish fillets were sold at an average of US$5.55 per pound, whereas 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.

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Supporting Information

Additional Supporting Information may be found in the online version of this article, including Supplementary Methods and tables.

Appendix S1: Supplementary Methods.
Table S1: Summary of Data Collection.
Table S2: Summary of prices of fish fillets.

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