MANAGEMENT BRIEF

Genetic Identification of Species Responsible for Depredation in Commercial and Recreational Fisheries

J. Marcus Drymon*

Coastal Research and Extension Center, Mississippi State University, 1815 Popps Ferry Road, Biloxi, Mississippi 39532, USA; and Mississippi–Alabama Sea Grant, 703 East Beach Drive, Ocean Springs, Mississippi 39564, USA

Pearce T. Cooper and Sean P. Powers

Department of Marine Sciences, University of South Alabama, 5871 Drive North, Mobile, Alabama 36688, USA; and Dauphin Island Sea Lab, 101 Bienville Boulevard, Dauphin Island, Alabama 36528, USA

Molly M. Miller

Dauphin Island Sea Lab, 101 Bienville Boulevard, Dauphin Island, Alabama 36528, USA

Sharon Magnuson, Evan Krell, and Chris Bird

Genomics Core Laboratory, Texas A&M University–Corpus Christi, 6300 Ocean Drive, Unit 5800, Corpus Christi, Texas 78412, USA

Abstract

Depredation, the partial or complete removal of hooked fish (prey) by a nontarget predator species, is a cryptic interaction that negatively affects predators, prey, and fishing industries. However, these interactions are rarely observed, rendering positive identification of the predator nearly impossible. We therefore tested a genetic method for predator identification. Depredated remains from sharks and bony fish were sampled with buccal swabs. Genetic material was isolated from the swabs, which we hypothesized contained oral cells from the predator. A portion of the cytochrome-*c* oxidase subunit I locus was amplified using prey-specific blocking primers and sequenced in high depth using a metagenetics approach. We sequenced haplotypes from the remains of four sharks, where the predator was visually confirmed, and four bony fish, where the predator was unknown. For all interactions with known predators, our technique suggested the correct predator species. For all interactions where the predators were unknown, our technique suggested species previously confirmed as perpetrators in depredation events. Our findings provide a basis for the development of a genetic technique for predator identification, while highlighting challenges to be overcome before predator identification can be applied to large-scale fisheries.

Depredation, the partial or complete removal of hooked fish (prey) from fishing gear by nontarget predators (Gilman et al. 2008; Mandelman et al. 2008), is a conflict that produces a range of economic and conservation impacts. Financially, loss of marketable catch via depredation can be severe. For example, economic losses in demersal longline fisheries for Patagonian Toothfish *Dissotichus eleginoides* and Sablefish *Anoplopoma fimbria* resulting from depredation by sperm whale *Physeter macrocephalus* have been estimated to exceed US$3 million per year (Roche et al. 2007; Hanselman et al. 2018). The conservation-related consequences of depredation are complex, and perhaps even more critical. In tropical and subtropical pelagic longline fisheries targeting tunas *Thunnus* spp. and Swordfish *Xiphias gladius*, false killer whale *Pseudorca crassidens*, short-finned pilot whale *Globicephala macrorhynchus*, and Blue Shark *Prionace glauca* are commonly suspected predators. In these and similar instances, depredation poses injury or mortality risks to false killer whales and short-finned pilot whales protected by the

Marine Mammal Protection Act (Forney 2010) as well as Blue Sharks, which are listed on the International Union for Conservation of Nature Red List as near threatened (Dulvy et al. 2014) and have shown regional population declines of up to 80% over three generations (Dulvy et al. 2008).

The economic losses resulting from depredation often place stakeholders and the presumed predators at odds. This can lead to retaliatory action from fishermen defending their catch against protected and/or threatened species. For instance, between 1985 and 1988, eight killer whales *Orcinus orca* associated with Sablefish depredation events were killed in Alaska by fishermen using high-powered rifles and underwater explosives (Fraker 2013). As spatial overlap and competition for increasingly limited (and shared) resources intensify, so does the potential for similar human–predator interactions. When surveyed, the majority of participants in the Alaska longline fishery for Sablefish strongly agreed that whale depredation worsened between 1990 and 2010, and a similar percentage noted rising frustration as a result (Peterson and Carothers 2013). Likewise, in the Gulf of Mexico, anglers who experience depredation by sharks perceive sharks as a threat to their fishing opportunities, which translates into an unwillingness to support shark conservation and fisheries sustainability initiatives (Drymon and Scyphers 2017).

Strong economic and conservation-related incentives have prompted researchers to investigate strategies to mitigate cetacean and shark depredation and/or bycatch. Mitigation strategies have been designed to reduce cetacean and shark depredation by targeting sensory systems unique to those groups. Acoustic deterrent devices (e.g., Waples et al. 2013) produce an aversive stimulus that has shown promise in reducing cetacean depredation. Similarly, the unique electrosensory system in sharks has prompted researchers to investigate the use of electropositive metals (Brill et al. 2009; Tallack and Mandelman 2009; Hutchinson et al. 2012), permanent magnets (Rigg et al. 2009; O'Connell et al. 2011), and rare earth magnets (Robbins et al. 2011) as mechanisms to reduce shark bycatch and depredation. While promising, results to date are mixed (Godin et al. 2013; Favaro and Cote 2015), and further field-based work is needed to verify these approaches (Molina and Cooke 2012; Mitchell et al. 2018).

Depredation can best be mitigated if the predatory species is conclusively known (Mitchell et al. 2018). In terrestrial settings, livestock predators have been identified through analysis of salivary DNA recovered from bite wounds. Salivary DNA has been used to identify and determine the sex of coyotes *Canis latrans* consuming domesticated sheep *Ovis aries* (Williams et al. 2003; Blejwas et al. 2006) and to discriminate between wolf *C. lupus* or dog *C. familiaris* perpetrators in sheep depredation events (Sundqvist et al. 2008). Salivary DNA samples

isolated and sequenced from partially consumed salmonid carcasses have even been used to monitor populations of brown bear *Ursus arctos* (Wheat et al. 2016). In these cases, a panel of nuclear microsatellite markers was used to discriminate between species (Williams et al. 2003; Blejwas et al. 2006; Sundqvist et al. 2008) or individuals (Wheat et al. 2016), and nuclear Y chromosome markers were used to determine sex (Williams et al. 2003; Blejwas et al. 2006). Microsatellite panel development is time consuming, expensive, and requires that candidate predator species be taxonomically related (Tuler et al. 2015). Mitochondrial DNA is higher in copy number in all cells, and universal metazoan primers have been developed for markers within the mitochondrial genome (Leray et al. 2013). A mitochondrial-based approach may be superior in a marine environment, where cells transferred from the predator to the bite wound are likely to be less abundant and the diversity of potential predators is higher.

In the case of marine depredation, we suggest that most DNA isolated from a bite wound would be from the prey species. Therefore, we developed a two-part technique for a genetic method of predator identification from prey species remains. First, we designed blocking primers that aim to reduce amplification of the prey DNA. Second, we employed a metabarcoding approach where we used universal metazoan primers in amplification of a segment of the cytochrome-*c* oxidase subunit I (COI) locus and generated high levels of coverage for each sample using massively parallel sequencing on an Illumina MiSeq. The field portion of the study was conducted in the Alabama Reef Permit Zone, an area of the Alabama Shelf in the northern Gulf of Mexico, where tens of thousands of structures have been deployed to attract fish and enhance commercial and recreational fishing opportunities. Depredation in the commercial and recreational fisheries for Red Snapper *Lutjanus campechanus* by shark species is a pronounced problem in this area that has led to frustration among fishermen and reduced support for shark conservation (Drymon and Scyphers 2017). We discuss the applicability of, and improvements to, this method for predator identification based on the preliminary results of a trial run in a commercial and recreational fishery setting.

METHODS

Field and genetic sampling.— In order to assess the applicability of a genetic method of predator identification in the field, we sampled as a commercial or recreational fisherman would if such a method were put into practice on a large scale. Specifically, depredated fish were collected opportunistically during two sampling programs designed to mimic the most common commercial and recreational angling gear types in the northern Gulf of Mexico. Shark depredation on sharks was documented

from bottom longline surveys in the northern Gulf of Mexico in 2017. Given the strength of the leader material (3-mm monofilament) and the size of the circle hook (15/0 circle hook), the predator was often captured along with the depredated species such that the predator could be positively and conclusively identified visually. Sampling details and further gear specifics for the bottom longline survey are described in Drymon et al. 2010. Depredation events on bony fish were also commonly observed in rod-and-reel surveys designed to mimic the recreational fishery. Depredated Red Snapper and Gray Triggerfish *Balistes capriscus* were sampled for genetic identification in 2017. In the rod-and-reel surveys, the identity of the predators could not be visually confirmed. To collect material for genetic identification, once the depredated specimen was brought on board, we swabbed the skin and flesh exposed by the bite wound with a cotton buccal swab. If swabbing could not be done on board, the specimen was placed on ice or frozen and swabbed as soon as possible. Swabs were then stored in 1× tris–EDTA buffer and frozen at −20°C.

Blocking primer design.— All of the prey species sampled in the bottom longline survey were Atlantic Sharpnose Shark *Rhizoprionodon terraenovae*, and the prey species sampled in the rod-and-reel survey were Red Snapper, with the exception of one Gray Triggerfish. To reduce the amplification of the prey species and increase amplification of the predator species, we designed blocking primers for Red Snapper (RSNblkCOIF 5′-CTACCCGC CCCTAGCAGGCAACCTA/3SpC3/) and Atlantic Sharpnose Shark (SPNblkCOIF 5′-CTATCCCCCATTAGC-TAGTAACATA/3SpC3/) that competitively annealed to the 5′ end of the forward COI priming site and prevented elongation via presence of the C3 spacer on the 3′ end of the primer. A competitive advantage was granted to the blocking primers by adjusting their molarity to 10× greater (10 μM) than the amplification primers (1 μM).

To assure primer specificity, we designed the Red Snapper blocking primer by aligning the forward universal degenerate primer sequence used in our reactions (mlCOIintF 5′-GGWACWGGWTGAACWGTWTAYC-CYCC-3′; Leray et al. 2013) to 18 COI haplotypes obtained from the National Center for Biotechnology Information (NCBI) GenBank database for Red Snapper and four comparison species (see Table A.1 in the Appendix for accession numbers). At the target annealing site, all 25 nucleotides in RSNblkCOIF were identical to all 10 Red Snapper haplotypes and had three to seven mismatches (Table A.1) with five haplotypes each of two common sympatric snapper species (Gray Snapper *Lutjanus griseus* and Lane Snapper *L. synagris*), five haplotypes of a common sympatric sciaenid species (Red Drum *Sciaenops ocellatus*), as well as five haplotypes of a commonly identified predator in depredation events (Sandbar

Shark *Carcharhinus plumbeus*). To ensure that RSNblkCOIF would block the amplification primer while also being specific, the 5′ end of RSNblkCOIF begins at the ninth nucleotide from the 3′ end of the amplification primer.

A similar approach was taken to design the blocking primer for Atlantic Sharpnose Shark. Ten haplotypes were used for each of four species (40 haplotypes total, see Table A.2 for accession numbers), including Atlantic Sharpnose Shark and the three most commonly observed predators in depredation events: Sandbar Shark, Blacktip Shark *Carcharhinus limbatus*, and Bull Shark *C. leucas*. At the annealing site of the blocking primer, there were no polymorphisms among the Atlantic Sharpnose Shark haplotypes and one to four mismatches with the other sharks (Table A.2). The one fixed mismatch with Sandbar Shark occurs at the 23rd position from the 5′ end of the primer, which should reduce the effective annealing temperature of the blocking primer and reduce annealing to Sandbar Shark DNA.

Isolation of DNA, library preparation, and sequencing.— After the bite wound was swabbed, DNA was isolated from the buccal swabs, which we hypothesize contained a small number of oral cells from the predator embedded in the superficial layers of the wound. Laboratory work involving DNA extractions, PCR amplification, and library preparation were performed by the Texas A&M University–Corpus Christi Genomics Core Lab. The DNA extraction was performed with an Omega E-Z 96 tissue kit (RNase treatment step included) in 200 μl of elution buffer. Polymerase chain reaction was performed to amplify a 313 bp fragment of COI from all metazoans in the sample. The PCR mastermix consisted of 3.8 μl nuclease-free water, 7.5 μl 2X DreamTaq Green Mastermix (ThermoFisher Scientific), 0.9 μl 10 μM blocking primer (either RSNblkCOIF for Red Snapper or SPNblkCOIF for Atlantic Sharpnose Shark), 0.9 μl 1 μM mlCOIintF primer (5′-/*barcode*/GGWACWGGWTGAAC WGTWTAYCCYCC-3′; Leray et al. 2013), 0.9 μl 1 μM jgHCO2198 primer (5′-/*barcode*/TAIACYTCIGGRTGIC-CRAARAAYCA-3′; Geller et al. 2013), and 1 μl DNA template. Both amplification primers had unique 5 bp barcodes on the 5′ end to identify each sample after pooling. The samples were amplified using a touchdown protocol that included an initial 3 min denaturation step at 95°C followed by 13 cycles of denaturation for 10 s at 95°C, annealing for 30 s at 62°C (–1°C per cycle) and elongation for 30 s at 72°C, followed by 27 cycles at an annealing temperature of 48°C, and a final 5 min elongation at 72°C (Leray et al. 2013). After PCR, all reactions were subjected to electrophoresis on a 1% agarose gel with Axygen 100 bp ladder, and the resulting gel image was scored based on presence of the target band (~313 bp) and three undesirable results indicating improper amplification: DNA smearing from high to low molecular weight, primer dimer, and nontarget amplification. Samples that did not amplify properly the first time

either had a DNA smear or primer dimer and were reamplified using a modified PCR protocol. If the DNA was smeared then the DNA template was reduced to 0.5 µl and the water was increased by 0.5 µl. Alternatively, if there was excessive primer dimer, the template DNA was increased to 2 µl and the water was reduced by 1 µl.

Samples that successfully amplified were moved forward to sequencing library preparation. Products were purified using AMPure XP beads (Beckman-Coulter) in a 0.8× reaction, and the concentration of DNA was quantified in duplicate with AccuBlue High Sensitivity dsDNA Quantitation Solution (Biotium) on a SpectraMax M3 plate reader (Molecular Devices). Next, 10 ng of DNA from each sample were pooled into one library and concentrated to a volume of 16.67 µl by lyophilization with a refrigerated centrivap (Labconco) and rehydration in nuclease-free water. Sequencing library preparation was completed using the TruSeq DNA PCR-Free Kit (Illumina), starting with the blunting step and using 0.33× reactions. Prior to sequencing, the library was adjusted to 2 nM using the Kapa Biosystems Library Quantification Kit on an ABI StepOnePlus real-time thermal cycler (Applied Biosystems) and checked for the desired fragment length distribution using an Advanced Analytical Fragment Analyzer and the High Sensitivity NGS kit. The completed library was sequenced on an Illumina MiSeq at New York University's Genome Technology Center using paired-end 250 bp sequencing with an estimated output of 18 million reads.

Bioinformatics and operational taxonomic unit assignment.— Initial processing, read clustering, and operational taxonomic unit (OTU) assignment for the MiSeq library was conducted using the charybdis metabarcoding pipeline (https://github.com/cbirdlab/charybdis) on the Genomics Core Lab's high-performance computing cluster. The charybdis pipeline uses OBITOOLS version 1.2.9 (Boyer et al. 2016) with the addition of CROP version 1.33 (Hao et al. 2011), VSEARCH version 2.3.4 (Rognes et al. 2016), BLAST version 2.6.0 (Edgar 2010), and GENOMETOOLS version 1.5.9 (Gremme et al. 2013) together to cluster putative OTUs and assign them to taxa. Prior to this, for parallel processing, the raw read 1 and read 2 FASTQ files were divided into several smaller files using FASTQ SPLITTER version 0.1.2 (https://kirill-kryukov.c om/study/tools/fastq-splitter/). The read pairs were aligned and converted to FASTA format, using the functions illuminapairedend and obiconvert, respectively. The FASTA files were filtered using the function obigrep, removing read pairs with an alignment score lower than 40 or with less than 20 bp of overlapping sequence. Aligned read pairs were demultiplexed and assigned to samples (depredated swabs) according to the unique barcodes attached during PCR amplification using the function ngsfilter. All the sequences corresponding to each sample were sorted

into unique FASTA files for further processing. Duplicate read pairs were quantified and removed using the function obiuniq, leaving only the unique read pairs (variants) and their frequency. Singletons and variants that were likely to result from PCR errors were identified and removed using the function obiclean. Errors in PCR were defined as sequence variants that were, at most, half as frequent as a more abundant variant with one mismatch. Variants that differed in length from the expected 313 bp of COI by more than 15 nucleotides were filtered. Chimeric variants were identified and removed using the uchime_denovo function of VSEARCH. Variants were assigned to OTU using CROP with the block size set to 432 and the number of Markov chain–Monte Carlo iterations set to 10× the block size (4,320), as recommended in the CROP manual.

Each OTU was assigned to a taxon in a local database of COI sequences from NCBI's GenBank using the top hits of the BLAST alignment algorithm. A local database was created by downloading relevant sequences from the nucleotide database provided by NCBI (ftp://ftp.ncbi.nlm. nih.gov/blast/db/, downloaded in December of 2017) with an NCBI ENTREZ query targeting the following search terms: mitochondria, cytochrome, coi, co1, cox1, coxi, mitochondrial genome, and mitochondria genome. The database was additionally filtered to remove entries of uncertain origin with the following search terms: environmental samples[organism], metagenomes[orgn]. The filtered database had 7,692,226 entries. In the pipeline, when an OTU sequence was queried for top alignments using BLAST, if the top hit had an identity of over 97% and a query coverage of 100%, we allowed the OTU to be discriminated at the species level (Leray et al. 2013). All OTU's with identity scores less than 97% were described at the family level or a more general taxonomic group. In addition, we manually queried NCBI's complete nucleotide database using the Web-based BLASTn for those 10 OTU sequences with the 10 highest read counts. All had the same taxonomic match.

Analysis of read counts and assignment of a predator.— The output of the pipeline was a comma delimited table of read pair counts, where each row is an OTU and columns include a sample ID (a sequenced swab from a depredated fish), putative taxonomic assignment (the traditional full Linnaean hierarchy: KPCOFGS), OTU nucleotide sequence, BLAST coverage, and identity score. The identity of the predator was determined to be the OTU with the highest read count when the prey OTU, known contamination, and OTUs that are not possible predator candidates were removed. Any OTU with less than five reads was also excluded from analysis. Those OTUs that were excluded from consideration despite read counts above a candidate predator, and the reasons for exclusion, are documented in Table A.3. In other words, based on the metagenetic data

FIGURE 1. Shark depredation by sharks captured during fishery-independent bottom longline surveys. Atlantic Sharpnose Sharks were the most common prey, resulting from interactions with (**A**) Blacktip Sharks and (**B**) Bull Sharks, although larger species such as (**C**) Scalloped Hammerheads were also subject to depredation. [Color figure can be viewed at afsjournals.org.]

for each depredated sample, we chose from all potential predators whose genetic material was sequenced and assumed that the potential predator with the highest read count was the most likely predator for that particular sample. All other potential predator species in the sample and their read counts were also documented. In addition, we manually queried NCBI's complete nucleotide database using the Web-based BLASTn for those OTU sequences

TABLE 1. Gear (BL = bottom longline, RR = rod and reel), known prey species, known predator (visually confirmed on the bottom longline only), genetic ID, and read depth for the three most prevalent OTUs in each sample after known or likely contamination was removed. NA = not applicable. Removals due to known or likely contamination are shown in Table A.3.

Sample	Gear	Known prey species	Known predator (visually confirmed)	1st predator candidate		2nd predator candidate		3rd predator candidate	
				Genetic ID	Reads	Genetic ID	Reads	Genetic ID	Reads
1	BL	Atlantic Sharpnose Shark	Blacktip Shark	Blacktip Shark	24,804	Bull Shark	152	Sandbar Shark	0
2	BL	Atlantic Sharpnose Shark	Blacktip Shark	Blacktip Shark	171,735	Sandbar Shark	94,524	Scalloped Hammerhead	856
3	BL	Atlantic Sharpnose Shark	Blacktip Shark	Blacktip Shark	1,367	Sandbar Shark	158	NA	0
4	BL	Atlantic Sharpnose Shark	Blacktip Shark	Blacktip Shark	25,108	Sandbar Shark	447	Scalloped Hammerhead	322
5	RR	Red Snapper	Unknown	Sandbar Shark	324	Blacktip Shark	52	NA	0
6	RR	Red Snapper	Unknown	Bull Shark	175	Blacktip Shark	98	Sandbar Shark	66
7	RR	Red Snapper	Unknown	Sandbar Shark	51	NA	0	NA	0
8	RR	Gray Triggerfish	Unknown	Bull Shark	46	NA	0	NA	0

that correspond to potential predators for each sample. All had the same species-level match, identity scores above 99%, and coverage of 100%.

RESULTS

Trends from Field Sampling

On bottom longlines, Atlantic Sharpnose Sharks were the most commonly preyed upon species, often resulting from interactions with Blacktip Sharks (Figure 1A) and Bull Sharks (Figure 1B), although larger species like Scalloped Hammerhead *Sphyrna lewini* (Figure 1C) also experienced depredation. Red Snapper were commonly preyed upon during rod-and-reel sampling, although the identity of the predator during rod-and-reel sampling was not visually confirmed as in the bottom longline gear.

Molecular Identification of the Predator

Of the 13 depredation events that were sampled using buccal swabs, we successfully amplified and sequenced COI from 8. From bottom longline, four Atlantic Sharpnose Sharks were sampled, all of which had a known predator. From rod and reel, three Red Snapper and one Gray Triggerfish were sampled, all of which had an unknown predator. The gear type, depredated species, known predator (on bottom longline), species ID, and read counts for the three most prevalent potential predators in each sample are reported in Table 1. For each of the four depredated remains from the bottom longline, where the identity of the predator was visually confirmed, the OTU with the highest read count matches the confirmed identity of the predator. For each of the four depredated remains from rod-and-reel sampling, where the identity of the predator is unknown, the OTUs with the highest or second highest read count implicate a species that has been commonly observed in depredation events in our study area (e.g., Bull Shark or Sandbar Shark).

DISCUSSION

Catch-documented depredation events provided proof of concept for the development of a genetic technique for predator identification, while highlighting aspects of the approach that need to be refined. Notably, the use of a Red Snapper blocking primer successfully eliminated Red Snapper reads from depredated Red Snapper samples; however, Atlantic Sharpnose Shark reads were still present in abundance for depredated Atlantic Sharpnose Shark samples, despite the use of an Atlantic Sharpnose Shark blocking primer. Blocking primers have different efficacies for many reasons, including both sequence-specific annealing propensity at the temperature used in the themocycler protocol

and the initial abundance of blocking target sequences present. However, it is likely that Atlantic Sharpnose Shark reads would be in higher abundance without the use of blocking primers and thus potential predator target sequences would have received less read depth. We suggest future studies could optimize blocking primer concentrations using samples with known DNA concentrations. Furthermore, additional refinement of our proof of concept could include controlled experiments that offer hooked fish to captive predators, thereby increasing replication and providing a more robust test of our approach. Such an experiment could implement appropriate controls, such as cutting and swabbing an unbitten portion of the carcass.

Counterintuitively, the power of our technique to identify a predator is also its greatest weakness. The primary difficulty with our method is that the target signal is essentially a contamination signal, a transfer of DNA from the predator to the prey; however, such miniscule contamination can also occur via transfer of DNA to the depredated remains, either from the researcher, the sampling environment (e.g., boat deck, measuring board, wet laboratory), or even the marine environment (e.g., environmental DNA). Given the importance of eliminating all potential sources of contamination, we recommend following best practices as described in Goldberg et al. (2016) and Deiner et al. (2017). A reduction in the required amount of sequencing will likely occur with improvements to our technique, particularly with more effective blocking primers and less contamination. With future developments, we believe that genetic identification of species responsible for depredation is possible and applicable in many scenarios; for example, this technique would work well if applied by trained fisheries observers aboard pelagic longline vessels, where depredation is common (Mandelman et al. 2008).

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Appendix: Detailed Data

TABLE A.1. Species, accession numbers, blocking primer mismatches, and mismatch positions used for the Red Snapper blocking primer.

Species	Accession number	Blocking primer mismatches	Mismatch positions
Sandbar Shark	EU398638.1	7	4,7,10,11,16,17,19
	FJ519148.1	7	4,7,10,11,16,17,19
	KF590222.1	7	4,7,10,11,16,17,19
	KP193151.1	8	1,4,7,10,11,16,17,19
	KP193317.1	8	1,4,7,10,11,16,17,19
Red Drum	KF461230.1	7	1,7,10,13,16,19,25
	KP112396.1	7	1,7,10,13,16,19,25
	KP112395.1	7	1,7,10,13,16,19,25
	KP112394.1	7	1,7,10,13,16,19,25
	KP112393.1	7	1,7,10,13,16,19,25
Gray Snapper	KF461196.1	6	1,7,11,16,19,22
	KF461198.1	5	1,7,11,19,22
	KF461197.1	5	1,7,11,19,22
	JQ842560.1	6	1,7,11,16,19,22
	JN021304.1	5	1,7,11,19,22
Lane Snapper	GU225378.1	4	1,7,19,25
	JQ839827.1	4	1,7,19,25
	KF461200.1	4	1,7,19,25
	JQ841932.1	4	1,7,19,25
	JN021308.1	3	7,19,25
Red Snapper	HQ162371.1	0	
	EU752115.1	0	
	FJ998466.1	0	
	MF041450.1	0	
	KF461194.1	0	
	KX119462.1	0	
	JN021303.1	0	
	KF461195.1	0	
	EU752113.1	0	
	KX119463.1	0	

TABLE A.2. Species, accession numbers, blocking primer mismatches, and mismatch positions used for the Atlantic Sharpnose Shark blocking primer.

Species	Accession number	Blocking primer mismatches	Mismatch positions
Blacktip Shark	JQ365263.1	4	1,7,19,23
	JQ365259.1	4	1,7,19,23
	JN989310.1	4	1,7,19,23
	KP193193.1	4	1,7,19,23
	KF461152.1	4	1,7,19,23
	HQ171642.1	4	1,7,19,23
	EU398620.1	4	1,7,19,23
	EU398622.1	4	1,7,19,23
	EU398621.1	4	1,7,19,23
	FJ519616.1	4	1,7,19,23
Bull Shark	KF646785.1	4	7,19,22,23
	JF493063.1	4	7,19,22,23
	KP193450.1	4	7,19,22,23
	EU818710.1	4	7,19,22,23
	FJ519800.1	4	7,19,22,23
	EF609311.1	4	7,19,22,23
	KF899808.1	4	7,19,22,23
	KF899812.1	4	7,19,22,23
	KM973110.1	4	7,19,22,23
	KM973108.1	4	7,19,22,23
Sandbar Shark	HQ171649.1	1	23
	JF493067.1	2	1,23
	FJ519152.1	1	23
	KP193212.1	2	1,23
	KP193409.1	2	1,23
	KP193317.1	2	1,23
	HQ171651.1	1	23
	JF493070.1	1	23
	JF493069.1	2	1,23
	KP193151.1	2	1,23
Atlantic Sharpnose Shark	KM987552.1	0	
	KF461226.1	0	
	HM991198.1	0	
	HM991199.1	0	
	FJ519634.1	0	
	FJ519583.1	0	
	FJ519282.1	0	
	FJ519274.1	0	
	KT075313.1	0	
	FJ519580.1	0	

TABLE A.3. Sample number, gear used (BL = bottom longline, RR = rod and reel), known predator, prey species, excluded OTUs, read count, predator potential, and explanation of presence in each sample.

Sample	Gear	Known predator (visually confirmed)	Prey species	Top excluded OTUs	Reads	Possible predator?	Explanation of presence
1	BL	Blacktip Shark	Atlantic Sharpnose Shark	Atlantic Sharpnose Shark	1,139,704	Species too small to be a predator for this event	Prey
2	BL	Blacktip Shark	Atlantic Sharpnose Shark	Atlantic Sharpnose Shark	307,417	Species too small to be a predator for this event	Prey
3	BL	Blacktip Shark	Atlantic Sharpnose Shark	Atlantic Sharpnose Shark	363,342	Species too small to be a predator for this event	Prey
				Cyprinid OTU	3,281	No	Lab contamination
4	BL	Blacktip Shark	Atlantic Sharpnose Shark	Atlantic Sharpnose Shark	334,170	Species too small to be a predator for this event	Prey
5	RR	Unknown	Red Snapper	Atlantic Sharpnose Shark	14,582	Species too small to be a predator for this event	Lab contamination
				Gray Triggerfish	2,632	No	Field contamination (caught alongside prey)
				Cyprinid OTU	1,059	No	Lab contamination
				Siphonophore OTU	370	No	Field contamination
6	RR	Unknown	Red Snapper	Cyprinid OTU	245,171	No	Lab contamination
				Siphonophore OTU	9,242	No	Field contamination
				Polychete OTU	2,238	NO	Field or lab contamination
				Lesser Electric Ray *Narcine bancroftii*	2,119	NO	Lab contamination
				Atlantic Sharpnose Shark	485	Species too small to be a predator for this event	Lab contamination
				Fungal OTU	434	No	Lab contamination

TABLE A.3. Continued.

Sample	Gear	Known predator (visually confirmed)	Prey species	Top excluded OTUs	Reads	Possible predator?	Explanation of presence
7	RR	Unknown	Red Snapper	Gray Triggerfish	130,760	No	Field contamination (caught alongside prey)
				Pelagic sea slug (Pterotracheidae) OTU	1,217	No	Field contamination
				Gray Snapper	526	No	Field or lab contamination
				Lanternfish (Myctophidae) OTU	135	No	Field or lab contamination
				Striped Mullet *Mugil cephalus*	106	No	Field or lab contamination
				Atlantic Sharpnose Shark	90	Species too small to be a predator for this event	Lab contamination
				Hydrozoan OTU	88	No	Field or lab contamination
				Spider OTU	74	No	Lab contamination
				Cyprinid OTU	67	No	Lab contamination
				Cusk (Ophidiidae) OTU	63	No	Field or lab contamination
				Singlespot Frogfish *Fowlerichthys radiosus*	58	No	Field or lab contamination
8	RR	Unknown	Gray Triggerfish	Cyprinid OTU	288,617	No	Lab contamination
				Gray Triggerfish	273,120	No	Prey
				Atlantic Sharpnose Shark	904	Species too small to be a predator for this event	Lab contamination