

A HIGH-RESOLUTION MOLECULAR METHOD FOR IDENTIFICATION OF
SMALLTOOTH SAWFISH PREY

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The final copy of this thesis has been examined by the signatories, and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above-mentioned discipline

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Abstract

The foundation of food web analysis is a solid understanding of predator-prey associations. Traditional dietary studies of fishes have been by stomach content analysis. However, these methods are not applicable to Critically Endangered species such as the smalltooth sawfish (*Pristis pectinata*). Previous research using the combination of stable isotope signatures from fin clips and 18S rRNA gene sequencing of fecal samples identified the smalltooth sawfish as piscivorous at high taxonomic levels. Here, we present a high taxonomic resolution molecular technique for identification of prey using opportunistically acquired fecal samples. To assess potential biases, primer sets of two mitochondrial genes, 12S and 16S rRNA, were used alongside 18S rRNA, which targets a wider spectrum of taxa. In total, 19 fish species, from 7 orders and 11 families, native to the Gulf of Mexico were successfully identified, including one ray, the southern stingray (*Dasyatis americana*). Silver perch (*Bairdiella chrysoura*), bay anchovy (*Anchoa mitchilli*), tidewater mojarra (*Eucinostomus harengulus*), spotted seatrout (*Cynoscion nebulosus*), ladyfish (*Elops saurus*), and spot (*Leiostomus xanthurus*) were most prevalent in our analysis. The sawfish prey identified comprised diverse taxa, indicating that this species is a generalist piscivore. These findings and the molecular approach used will aid recovery planning for the smalltooth sawfish and has the potential to reveal previously unknown predator-prey associations from a wide range of taxa, being specifically desirable for use with rare and hard to sample species.

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INTRODUCTION

The smalltooth sawfish (*Pristis pectinata*) currently inhabits southwest Florida and the Florida Keys but was once widely distributed on both coasts of the Atlantic Ocean and in the Gulf of Mexico (1). Decades of human activity, including bycatch mortality in commercial and recreational fishing and loss of red mangrove (*Rhizophora mangle*) shorelines associated with residential and commercial development, have greatly reduced the size of the smalltooth sawfish population (1, 2). Thus, in 2003 the species was listed as endangered under the U.S. Endangered Species Act.

Despite recent expansion of knowledge about the smalltooth sawfish (3), its feeding ecology is poorly understood. To maximize the effectiveness of ongoing recovery planning, more detailed knowledge of the trophic ecology of smalltooth sawfish is needed to better understand what specific prey they rely on. Traditional dietary studies on piscivores typically involve lethal sampling for stomach content analysis, gastric lavage, stable isotopes, direct observation, and morphological hard part analysis of indigestible prey remains (4–7). Morphological characterization of prey remains found during gastric lavage or lethal sampling permits conclusions to be drawn about the number and size of fish prey consumed (8, 9); however, lethal sampling is not feasible for endangered species and gastric lavage, though considered non-invasive, may not be a permitted activity for endangered species (10). Therefore, alternative approaches to identify predator-prey associations are warranted. Recent research using the combination of stable isotope signatures from fin clips and 18S rRNA gene sequencing of fecal samples identified the smalltooth sawfish as piscivorous at low taxonomic resolution (11).

Here we present a high-resolution molecular method for identification of smalltooth sawfish prey using fecal samples collected over a six-year period. To assess potential biases that

might be caused by the selection of primer sets, two mitochondrial genes, 12S and 16S rRNA, were used together with 18S rRNA, a more evolutionarily conserved gene that targets a wider spectrum of taxa (11). These data will improve recovery planning for this Critically Endangered species and the method has the potential to reveal previously unknown predator-prey associations of a wide range of taxa, being specifically desirable for use with rare and hard to sample species.

MATERIALS AND METHODS

Smalltooth sawfish fecal sample collection and DNA extraction

From 2010 to 2015, 16 fecal samples were opportunistically obtained from primarily juvenile smalltooth sawfish in southwest Florida during ongoing field sampling or from necropsies (Table 1). All samples were stored at -20°C until analysis. DNA extractions were performed using Quick-DNA Fecal/Soil Microbe Kits (Zymo Research) according to manufacturer instructions. DNA was unable to be extracted from one sample due to an insufficient amount of feces.

High-throughput sequencing

DNA samples were sequenced using the Illumina MiSeq System (RTL, Lubbock, TX, USA). In addition to the four samples used in a previous analysis (11), 11 fecal samples (Table 1) were also amplified for the 18S rRNA gene following the same method. All 15 samples were then analyzed using a universal primer set for fish taxa (12) for the mitochondrial 12S and 16S rRNA genes. Resulting DNA sequences were clustered into OTUs at 97% similarity using the CD-Hit-Est clustering algorithm (13, 14). OTUs consisting of less than five sequences were considered inconsequential and omitted from further analysis (12S sequences omitted: $2.06 \pm 0.23\%$ [mean \pm

SE]; 16S: $0.65 \pm 0.08\%$; 18S: $1.28 \pm 0.06\%$), with the remainder entering our identification protocol using BLAST.

Sequencing error of 12S and 16S were estimated to be 2% by comparing OTU centroid sequences identified as smalltooth sawfish by BLAST and their respective similarity percentages with a reference sequence (GenBank Accession: KP400584) for each gene via multiple sequence alignment using the MUSCLE program within MEGA7 software (15) to manually check for chimeric sequences (Fig. 1). This estimated sequencing error was used in our sequence identification protocol. Sequencing error was not estimated for the 18S rRNA gene due to its low associated taxonomic resolution, with the taxonomic class of the highest scoring BLAST similarity result accepted for each OTU. When class was not applicable, the next highest taxonomic level was used. Taxonomy follows Page et al. (16) for fishes and Williams et al. (17) for crustaceans.

Mitochondrial 12S and 16S rRNA gene sequence identification protocol

Based on our estimation of sequencing error found in the host, BLAST results with a similarity score $\geq 98\%$ were accepted for each prey OTU. If this criterion was met and ties were present, OTUs would be moved to the next step of our identification protocol. The remaining OTUs along with those with ties were aligned with ~40 bp species signature sequences identified for all fish species documented in the sample collection area that either had reference sequences available in GenBank or were sequenced for the purpose of this study (Table 2) (18). This tag-sequencing strategy determined ~40 bp species signature sequences based on available data, with each sequence checked for specificity via BLAST. Exact matches were accepted as an accurate identification. If an exact match was not made with a tied OTU, the lowest shared taxonomic classification between the tied BLAST results was accepted (none were found higher than genus

level, and all were *Cynoscion* sp.). All results were checked against available fisheries data (19), with any dubious identifications relegated to the lowest viable taxonomic classification (none higher than genus level and all were *Menticirrhus* sp.).

Sanger sequencing for mitochondrial 12S and 16S rRNA genes of potential fish prey

Twenty-four fish species known from our sampling area were sequenced (Fig. 2; Table 2). Species selection was guided by preliminary results. Sixteen species were sequenced for the mitochondrial 12S rRNA gene and 23 species for the mitochondrial 16S rRNA gene using universal fish primer pairs (12S: Ac12Sf [ACTGGGATTAGATACCCCACTATG] and Ac12Sr [GAGAGTGACGGGCGGTGT]; 16S: Ac16Sf [CCTTTTGCATCATGATTTAGC] and Ac16Sr(C-) [CAGGTGGCTGCTTTTAGGC]) modified from Evans et al. (12) and sequenced as described previously (11).

Data deposit

High-throughput mitochondrial 12S and 16S rRNA, and 18S rRNA gene sequences of smalltooth sawfish fecal samples were deposited in the National Center for Biotechnology Information sequence read archive under accession number SAMN10130720. Fish species 12S and 16S rRNA gene sequences were deposited in GenBank under accession numbers MH715297–312 and MH715980-6002, respectively.

RESULTS

Smalltooth Sawfish fecal sample description

During field sampling from 2010 through 2015, 16 fecal samples were opportunistically obtained from primarily juvenile (< 2500 mm) smalltooth sawfish (780–4355 mm stretch total length; mean = 1397.5 mm) in a roughly equal proportion of males ($n = 8$) and females ($n = 7$) in southwest Florida from the Charlotte Harbor estuarine system and the Ten Thousand Islands National Wildlife Refuge (Fig. 2; Table 1).

18S rRNA analysis

In our analysis of fecal samples, DNA was unable to be extracted from one sample due to an insufficient amount of feces. The mean number of analyzed reads for each sample was $110,843 \pm 39,647$ (\pm SE; Table 3). After normalization (10,000 reads) and subsequent removal of host (smalltooth sawfish) sequences, four Kingdoms were identified: Animalia (91.3%), Chromalveolata (6.2%), Plantae (2.4%), and Fungi (0.1%; Fig. 3). All non-animal taxa were microscopic and considered to be of sediment or ambient water origin. Within Animalia, fishes comprised the majority of sequences (Actinopterygii: 83.1%; Elasmobranchii: 3.3%). In the majority of samples, Arthropoda sequences made up < 1% of Animalia sequences; however, considerable numbers were identified in one sample, SF4, with 99.2% of Animalia sequences and 100% of Arthropoda sequences identified as penaeid shrimp (Table 4), with 91.5% of these sequences exhibiting 99% similarity to reference sequences (*Litopenaeus setiferus*: JX403844.1; *Farfantepenaeus duorarum*: JX403828.1). SF4 was the smallest individual in our analysis (780 mm) and had no fish prey detected in our mitochondrial 16S rRNA gene analysis (Fig. 4).

Mitochondrial 12S and 16S rRNA gene analysis

Preliminary results revealed large amounts of unidentified sequences present in our samples and a lack of local fish species reference sequences, indicating the need for additional sequencing effort. Thus, we successfully sequenced 24 fish species, including nine orders and 13 families, known to inhabit our sampling area (18), yielding 16 and 23 additional fish species reference sequences for the mitochondrial 12S and 16S rRNA genes (hereafter 12S and 16S), respectively (Table 7). The high specificity of these sequences allowed closely related species to be distinguished in most cases, which was beneficial in determining the highest resolution (i.e., species level) identifications for OTUs with basic local alignment search tool (BLAST) results that did not meet our identification criterion (98% similarity) or exhibited ties. To provide an even more robust analysis, species signature sequences consisting of ~40 bp within 12S and 16S were identified for 150 and 128 species (Table 5), respectively, to conduct manual alignments of unidentified sequences during the final step of our identification protocol. This tag-sequencing strategy attributed to 17.4% and 2.8% of identified 12S and 16S sequences, respectively (Table 6).

The mean number of analyzed reads for all samples was $8,963 \pm 1,074$ (\pm SEM). Two samples (SF4, SF5) failed to sequence for our 12S analysis due to unsuccessful PCR amplicon generation. After removal of host sequences, our analysis identified $91.3 \pm 1.8\%$ of 12S and $88.9 \pm 4.9\%$ of 16S sample sequence reads at a minimum of genus (Table 4). Of 19 total fish taxa detected, including seven orders and 11 families, 17 were identified to species, with a mean of 15.1 ± 3.2 and 2.3 ± 0.3 fishes identified in 12S fecal samples and 6.8 ± 2.3 and 1.7 ± 0.4 fishes identified in 16S fecal samples. Silver perch (*Bairdiella chrysoura*), bay anchovy (*Anchoa mitchilli*), tidewater mojarra (*Eucinostomus harengulus*), spotted seatrout (*Cynoscion nebulosus*),

ladyfish (*Elops saurus*), and spot (*Leiostomus xanthurus*) were most prevalent in our analysis (Fig. 4; Fig. 5; Table 8).

The 16S primer set appeared to be less sensitive than the 12S primer set when comparing samples sequenced for both genes. In the 16S analysis, only three (all tidewater mojarra) of 26 identification instances were not corroborated by the other gene, whereas for 12S, 16 of 34 identification instances were not corroborated. Additionally, the 16S primer set was unable to identify any fish taxa for two samples (SF3, SF12), and striped mojarra (*Eugerres plumieri*) and pinfish (*Lagodon rhomboides*) were only identified using the 12S primer set (Fig. 4). While two seatrout species, sand seatrout (*C. arenarius*) and spotted seatrout, were identified in both analyses, species were not always distinguishable in our 12S analysis with some identifications relegated to *Cynoscion* sp., indicating multiple primer sets may be desirable to overcome potential biases.

DISCUSSION

Traditional dietary studies of fishes have mainly focused on stomach content analysis, using invasive methods that cannot be used to study Critically Endangered species such as the smalltooth sawfish. DNA-based prey identification can overcome many of these limitations, and a variety of molecular methods have been developed over the past decade to enable the investigation of feeding ecology at unprecedented resolution (20–23). Moreover, even heavily digested prey remains can be identified by these methods to track trophic links (24). Molecular techniques have been used to assess the diet of piscivores with a focus on marine predators such as pinnipeds (25), squids (26), and seabirds (27); however, this is the first study achieving species

level resolution for elasmobranchs. In addition, there has been limited application of these techniques for studying the feeding ecology of fishes more generally (28).

Application of high-throughput sequencing in feeding ecology studies

In the present study, we combined multiple molecular markers to identify fish prey to species. Mitochondrial 12S and 16S rRNA genes were used to target fishes, which were previously identified as the largest fraction of smalltooth sawfish prey taxa via the 18S rRNA gene (11). Using a set of high resolution mitochondrial genes expanded our results by accounting for differences between the two primer sets, with samples exhibiting variation in taxa detection and proportion between them. An example of the need for multiple genes occurred with silver perch and tidewater mojarra which were often detected together but exhibited drastic differences in sequence proportions between genes. In some samples, the silver perch was only detected by 12S while tidewater mojarra was only detected using 16S. At first, we thought this indicated an error in our analysis or sample preparation for Sanger sequencing, but after comparing identified query sequences of both species for both genes, our sequences of both species, and reference sequences of congeners, all sequences identified as one or the other species via multiple sequence alignment. This process indicated that both species' sequences were well differentiated from each other, were similar to other species within their respective genera, and met our threshold for valid identifications. Without the use of two overlapping high taxonomic resolution primer sets, there would have been undetected or undervalued taxa due to biases of individual primer sets and their respective genes.

We also used species-signature sequences consisting of around 40 bp to refine our data, increasing the number of successfully identified sequences and OTUs. However, in two samples,

we were unable to differentiate species in the genus *Cynoscion* for 12S OTUs. The only additional *Cynoscion* species not identified in the study was the silver seatrout (*C. nothus*), which is not known to use the estuary where these samples were collected (18, 19). In one sample, we were unable to differentiate species in the genus *Menticirrhus* for 16S OTUs. Although our identification protocol identified these as *M. littoralis*, this species is not known to use the estuary where this sample was collected (19), and relevant reference sequences of the two possible other *Menticirrhus* species (i.e., *M. americanus* and *M. saxatilis*) were absent from DNA databases. Further, Pompanon and colleagues (21) discussed various potential biases of this new technology that could hamper quantitative questions, such as differences in amplification efficiency or DNA survival during digestion. Because the methodology is progressing rapidly, these problems might be minimized in the near future; however, some issues, such as variation in prey size and the time the prey was consumed, are not easily addressed. For example, we were unable to rule out instances of secondary predation (i.e., consumption of a predator which has consumed the target prey), which has not been addressed in any vertebrate predator (20, 29). These challenges are generally present in feeding ecology analyses using traditional techniques and are not limited to molecular methods (30).

Links between diet, habitat use, and behavior

A high-throughput sequencing approach, able to detect trace amounts of prey, was first used on sawfish as a complementary method to assist studies using stable isotopes, a powerful and widely accepted technique in which trophic levels can be identified, but species level prey identification is impossible (11, 31–35). Poulakis and colleagues (11) studied the trophic ecology of the smalltooth sawfish in South Florida using a combination of stable isotopes and 18S rRNA

gene sequencing of fecal samples, providing evidence that the species feeds primarily on teleost and elasmobranch fishes. In the present study, we replicated and built on this technique with a larger sample size, resulting in successful identification of more prey and reaffirming the previous conclusion that this species is piscivorous. Fish prey items were detected in fecal samples from all individuals with the exception of one, which fed primarily on penaeid shrimp, which we speculate was scavenged discarded bait.

The fish prey we identified mirrored the habitats juvenile smalltooth sawfish are known to use within the Charlotte Harbor estuary (36). Sawfish preyed on species such as the tidewater mojarra that tends to be found along mangrove shorelines, silver perch and spotted seatrout that are typically found on offshore seagrass flats, and bay anchovies and pinfish that are found in both habitats (37). Acoustic tracking and monitoring in the study area has shown that smalltooth sawfish use all habitats available to them (38, 39), and the present study suggests that they feed on fishes in all of these habitats. Additionally, we did not observe an ontogenetic dietary shift in our data, which is a trait common amongst elasmobranchs (40, 41). No clear differences in prey were observed between juvenile age classes (less than 1 year old: <150 cm STL; greater than 1 year old: > 150 cm STL; (42), with fishes such as bay anchovy, ladyfish, and silver perch found in each. A dietary shift may become evident if more samples from larger juveniles and adults could be collected as these age classes are known to use more diverse habitats (Poulakis et al. 2017; Brame et al. 2019).

Although we know very little about sawfish feeding behavior and diet beyond anecdotal reports and previous stable isotope analysis (11), this study presents multiple new findings, such as the presence of anchovies, one of the smallest fish prey items identified in our analysis, and the southern stingray, which was anticipated by the 18S rRNA analysis that identified

Myliobatiformes in the diet (11). Recent behavioral experiments have shown captive sawfish feeding with rapid lateral swipes of their rostra in the water column and close to the bottom (43). Our data suggest that smalltooth sawfish are generalist piscivores that feed on pelagic and benthic fishes.

These findings and the molecular approach used will aid recovery planning for the smalltooth sawfish and has the potential to reveal previously unknown predator-prey associations from a wide range of taxa, being specifically desirable for use in protected species. Thus, maintaining healthy estuaries, including healthy fish populations, will be important for promoting recovery of the smalltooth sawfish population. Climate change and associated environmental impacts may destabilize current habitat for the species and alter habitat use, emphasizing the need for ecosystem level conservation (44).

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FIGURES

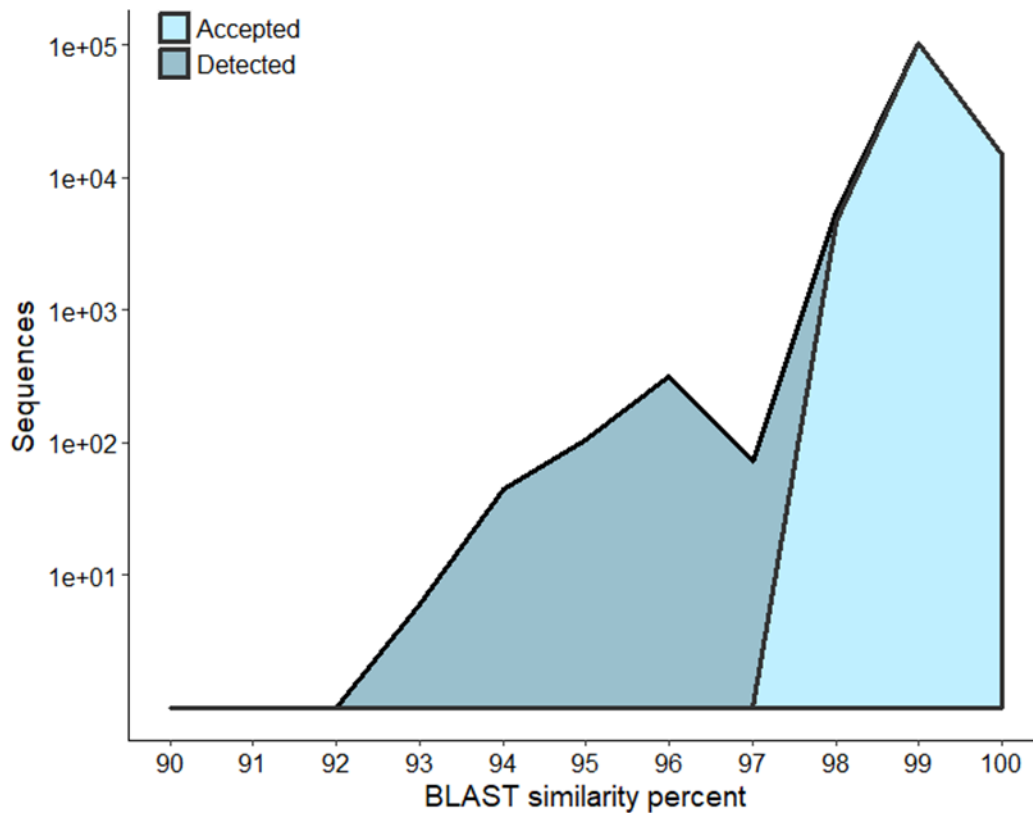


Figure 1. Estimation of smalltooth sawfish host sequencing errors determined using sequence distributions in Basic Local Alignment Search Tool similarity scores. Distribution of mitochondrial 12S and 16S rRNA sequences identified as smalltooth sawfish are shown as areas (dark blue, errors were detected and rejected; light blue, accepted) on a logarithmic scale with corresponding BLAST similarity scores. These distributions were used to estimate sequencing error, resulting in the conclusion that a BLAST similarity score of $\geq 98\%$ was accurate in our prey identification protocol.

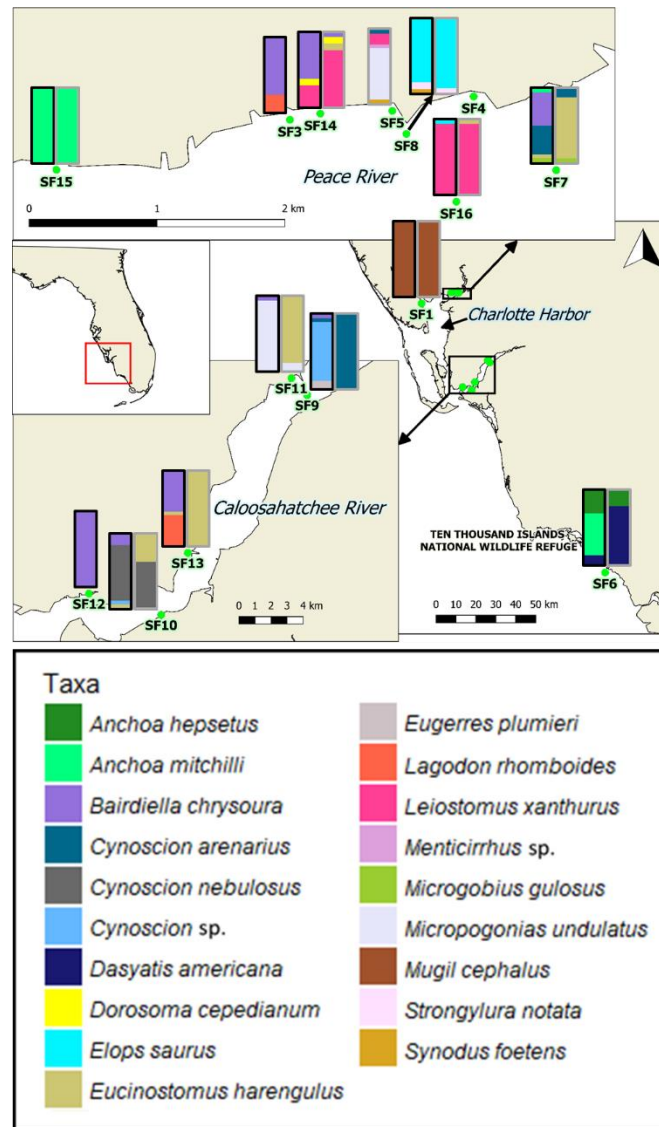


Figure 2. Map of smalltooth sawfish fecal sample collection locations in southwest Florida with stacked bars showing relative composition of fish prey taxa (minimum of 5%), after removal of host sequences. Each 100% stacked bar shows data from mitochondrial 12S (black border) and 16S (grey border) rRNA genes. No fish prey taxa were detected in 16S rRNA gene analysis of samples SF3, SF4, and SF12. DNA sequencing of 12S rRNA gene failed in SF4 and SF5. SF2 did not yield enough DNA for analysis from its extraction.

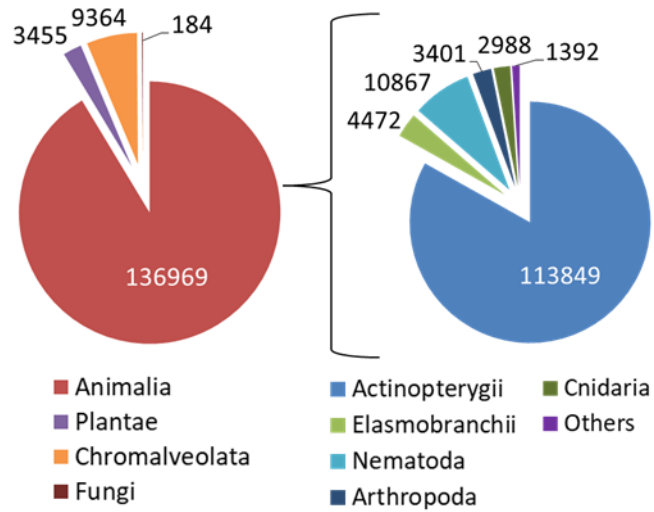


Figure 3. Abundance of normalized 18S rRNA gene sequence reads by Kingdom (left) and composition of Animalia (right) from smalltooth sawfish fecal samples after removal of host sequences. Animalia composed 91.3% of sequence reads, with fishes (Actinopterygii and Elasmobranchii) composing 86.4% of Animalia sequence reads. Others includes Mollusca and Platyhelminthes.

Order	Family	Taxa	Common Name	KEY																Frequency																
				12S present				16S present				Not present				12S & 16S present																				
				SF1	CH	SF3	PR	SF4*	PR	SF5*	PR	SF6	TI	SF7	PR	SF8	PR	SF9	CR	SF10	CR	SF11	CR	SF12	CR	SF13	CR	SF14	PR	SF15	PR	SF16	PR			
Aulopiformes	Synodontidae	<i>Synodus foetens</i>	Inshore Lizardfish																																2	
Beloniformes	Belonidae	<i>Strongylura notata</i>	Redfin Needlefish																																	1
Clupeiformes	Clupeidae	<i>Dorosoma cepedianum</i>	Gizzard Shad																																	1
Clupeiformes	Engraulidae	<i>Anchoa hepsetus</i>	Broad-striped Anchovy																																	1
Clupeiformes	Engraulidae	<i>Anchoa mitchilli</i>	Bay Anchovy																																	3
Elopiformes	Elopidae	<i>Elops saurus</i>	Ladyfish																																	2
Mugiliformes	Mugilidae	<i>Mugil cephalus</i>	Striped Mullet																																	1
Myliobatiformes	Dasyatidae	<i>Dasyatis americana</i>	Southern Stingray																																	1
Perciformes	Gerreidae	<i>Eucinostomus harengulus</i>	Tidewater Mojarra																																	6
Perciformes	Gerreidae	<i>Eugerres plumieri</i>	Striped Mojarra																																	1
Perciformes	Gobiidae	<i>Microgobius gulosus</i>	Clown Goby																																	1
Perciformes	Sciaenidae	<i>Bairdiella chrysoura</i>	Silver Perch																																	8
Perciformes	Sciaenidae	<i>Cynoscion arenarius</i>	Sand Seatrout																																	3
Perciformes	Sciaenidae	<i>Cynoscion nebulosus</i>	Spotted Seatrout																																	1
Perciformes	Sciaenidae	<i>Cynoscion</i> sp.	Seatrout																																	2
Perciformes	Sciaenidae	<i>Leiostomus xanthurus</i>	Spot																																	3
Perciformes	Sciaenidae	<i>Menticirrhus</i> sp.	Kingfish																																	1
Perciformes	Sciaenidae	<i>Micropogonias undulatus</i>	Atlantic Croaker																																	2
Perciformes	Sparidae	<i>Lagodon rhomboides</i>	Pinfish																																	2
				Total		1	2	0	5	3	5	3	4	4	3	1	3	4	1	3	4	1	3													

*No data present for mitochondrial 12S rRNA gene

CH = Charlotte Harbor; PR = Peace River; TI = Ten Thousand Islands National Wildlife Refuge; CR = Caloosahatchee River

Figure 4. Comparison of fish prey taxa detection in smalltooth sawfish fecal samples using mitochondrial 12S and 16S rRNA gene sequences. Location collected indicated next to sample name. SF2 did not yield enough DNA for analysis from its extraction.

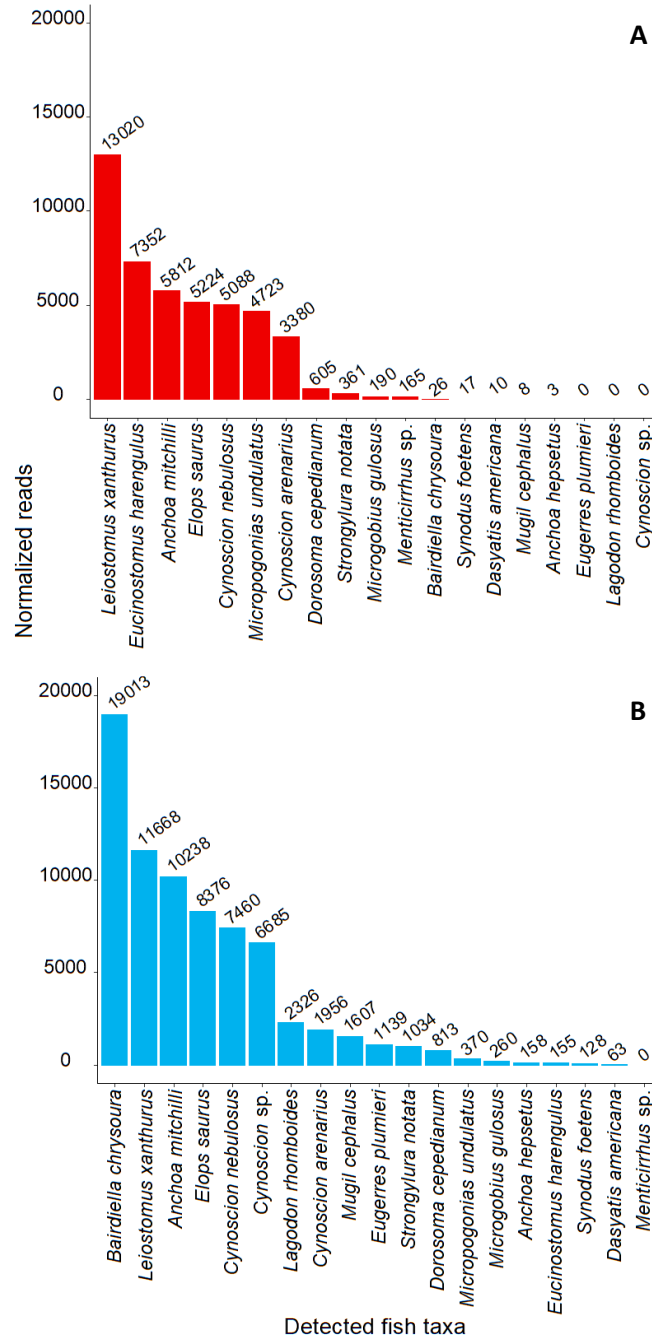


Figure 5. Normalized abundance of detected fish prey taxa in mitochondrial 16S (A) and 12S (B) rRNA gene sequence reads from smalltooth sawfish fecal samples. In the 16S rRNA gene analysis, 16 taxa were identified, and in the 12S rRNA gene analysis, 18 taxa were identified. The same taxa were included in both graphs for comparative purposes.

TABLES

Table 1. Collection data associated with smalltooth sawfish fecal samples. SF2 did not yield enough DNA for analysis from its extraction.

Sample identity	Date	Location	STL* (mm)	Sex
SF1†	7/31/2015	Upper Charlotte Harbor	4355	F
SF3	7/13/2015	Peace River	1020	F
SF4	4/9/2015	Peace River	780	M
SF5	5/13/2014	Peace River	825	F
SF6†	4/19/2015	Ten Thousand Islands	1690	F
SF7	5/28/2015	Peace River	1020	M
SF8	6/12/2014	Peace River	1556	F
SF9	4/23/2015	Caloosahatchee River	1447	M
SF10	9/22/2015	Caloosahatchee River	1235	M
SF11	5/22/2014	Caloosahatchee River	958	F
SF12‡	12/21/2010	Caloosahatchee River	2026	M
SF13‡	6/24/2010	Caloosahatchee River	1025	F
SF14‡	9/3/2010	Peace River	1380	M
SF15	5/1/2013	Peace River	835	M
SF16‡	4/3/2011	Peace River	810	M

*STL = stretch (maximum) total length; mean 1387 mm \pm SE 232 mm

†Sample obtained from necropsy

‡Sample used in previous 18S rRNA gene analysis (Poulakis et al., 2017)

Table 2. Fish species in sampling area sequenced for mitochondrial 12S and 16S rRNA genes.

Family	Species	Common name	Gene(s) sequenced	Accession #
Belontiidae	<i>Strongylura notata</i>	Redfin Needlefish	16S	MH716001
Dasyatidae	<i>Dasyatis americana</i>	Southern Stingray	12S, 16S	MH715301, MH715984
Dasyatidae	<i>Dasyatis sabina</i>	Atlantic Stingray	12S, 16S	MH715302, MH715985
Gerreidae	<i>Eucinostomus gula</i>	Silver Jenny	16S	MH715986
Gerreidae	<i>Eucinostomus harengulus</i>	Tidewater Mojarra	12S, 16S	MH715303, MH715987
Gerreidae	<i>Eugerres plumieri</i>	Striped Mojarra	16S	MH715988
Gobiesocidae	<i>Gobiesox strumosus</i>	Skilletfish	16S	MH715989
Gobiidae	<i>Bathygobius soporator</i>	Frillfin Goby	12S, 16S	MH715298, MH715981
Gobiidae	<i>Ctenogobius boleosoma</i>	Darter Goby	12S, 16S	MH715299, MH715982
Gobiidae	<i>Ctenogobius smaragdus</i>	Emerald Goby	12S, 16S	MH715300, MH715983
Gobiidae	<i>Gobionellus oceanicus</i>	Highfin Goby	12S, 16S	MH715304, MH715990
Gobiidae	<i>Gobiosoma longipala</i>	Twoscale Goby	12S, 16S	MH715305, MH715991
Gymnuridae	<i>Gymnura micrura</i>	Smooth Butterfly Ray	12S, 16S	MH715306, MH715992
Lutjanidae	<i>Lutjanus griseus</i>	Gray Snapper	12S	MH715307
Lutjanidae	<i>Lutjanus synagris</i>	Lane Snapper	12S, 16S	MH715308, MH715995
Mugilidae	<i>Mugil trichodon</i>	Fantail Mullet	12S, 16S	MH715311, MH715998
Rajidae	<i>Raja eglanteria</i>	Clearnose Skate	16S	MH715999
Sciaenidae	<i>Bairdiella chrysoura</i>	Silver Perch	12S, 16S	MH715297, MH715980
Sciaenidae	<i>Leiostomus xanthurus</i>	Spot	16S	MH715994
Sciaenidae	<i>Menticirrhus littoralis</i>	Gulf Kingfish	12S, 16S	MH715309, MH715996
Sciaenidae	<i>Micropogonias undulatus</i>	Atlantic Croaker	12S, 16S	MH715310, MH715997
Scorpaenidae	<i>Scorpaena brasiliensis</i>	Barbfish	12S, 16S	MH715312, MH716000
Sparidae	<i>Lagodon rhomboides</i>	Pinfish	16S	MH715993
Synodontidae	<i>Synodus foetens</i>	Inshore Lizardfish	16S	MH716002

Table 3. Analyzed sequence reads (SE: ± 39647) of 18S rRNA gene and relative abundance (SE: $\pm 7.0\%$) of host sequences.

Sample	Analyzed sequence reads	Smalltooth sawfish sequences (%)
SF1*	29493	57.2
SF3	23835	58.0
SF4	28667	88.9
SF5	20692	50.9
SF6*	28434	99.4
SF7	24764	70.0
SF8	33341	87.5
SF9	28583	74.5
SF10	29794	51.8
SF11	38248	97.7
SF12	334831	99.8
SF13	421995	86.9
SF14	156696	15.7
SF15	26773	22.0
SF16	436500	91.3
Total	1662646	
Mean	110843	70.1

*Sample obtained from necropsy

Table 4. Relative taxonomic abundance of 18S rRNA gene sequences (%) in smalltooth sawfish fecal samples after removal of host sequences.

Taxa	Classification	SF1	SF3	SF4	SF5	SF6	SF7	SF8	SF9	SF10	SF11	SF12	SF13	SF14	SF15	SF16
Actinopterygii	Class	7.67	96.22	0.47	89.01	48.78	82.71	95.92	97.60	92.43	55.08	44.94	48.42	99.60	95.87	77.20
Amoebozoa	Phylum								0.12							
Anthozoa	Class												18.63	0.03		
Asterales	Order								0.78							
Bacillariophyceae	Phylum								0.10							1.18
Bivalvia	Class								0.12							1.53
Chlorophyceae	Class	22.81	0.06		0.06				0.44					0.06		0.70
Chondrichthyes	Subclass	1.03	1.03		7.17	18.90	6.45	1.68	0.13	6.17	34.76		0.21	0.16	4.04	0.58
Chromadorea	Class	24.72	0.17							1.03		32.58	31.92			0.02
Conoidasida	Class	0.47	0.05		0.32			0.19			10.16	2.81	0.45			16.63
Coscinodiscophyceae	Class	11.55													0.01	
Dinophyceae	Phylum	16.58		19.81	3.31		10.84									
Enoplea	Class											8.43	0.13			
Gastrotricha	Phylum											11.24				
Haptophyceae	Class								0.41							
Hexanauplia	Class														0.03	
Katablepharidophyta	Class			23.07												
Labyrinthulomycetes	Class							0.12								0.04
Malacostraca	Class		2.47	56.65	0.13			0.17		0.33				0.12		
Malpighiales	Order	0.20														
Mammalia	Class															0.82
Maxillopoda	Class	6.87														
Myxosporea	Class	6.19				32.32		1.92		0.04					0.06	1.12
Spirotrichea	Class								0.30							
Poales	Order	0.57													0.02	
Saccharomycetes	Class	1.34														
Streptophyta	Class															0.18
Trematoda	Class												0.24			

Table 5. Mitochondrial 12S and 16S rRNA gene species-signature sequences identified for analysis, with those resulting from our sequencing effort denoted by bold text. Data are in alphabetical order by family. Mean = 40.0 bp; Min = 36 bp; Max = 44 bp.

Family	Species	Common name	Gene	GenBank Accession No.	Species specific sequence	Base pairs
Achiridae	<i>Achirus lineatus</i>	Lined Sole	12S	NC_023768	CTACAACAGCATAGCACGAATTTTGTGTTTAAACATACAA	40
			16S	NC_023768	CTCCCGGATTCTTCCCTCAAGGACATTTAATTAATATATA	40
	<i>Gymnachirus melas</i>	Naked Sole	16S	JQ939050	CTCCCGGCTTCTTCCAGACCGAGAGAGTTAGTCAAAAAGG	40
	<i>Trinectes maculatus</i>	Hogchoker	12S	NC_023769	CTACCACAGCACACTACGAATGTTATGTTGAAACACACAA	40
16S			NC_023769	CTCCCGGCTTCTTTCCTCACCACCCTCAACACACCCCA	40	
Acipenseridae	<i>Acipenser oxyrinchus</i>	Atlantic Sturgeon	12S	KP997217	CTGATACAGAAAATACAGAAATAACTGTGAAACCAGTG	40
			16S	KP997217	CTTAAGGCTTCTACTGCCACCCAGGTTATTACCAACAAAG	40
Albulidae	<i>Albula vulpes</i>	Bonefish	12S	X99180	CTGGTTCAGAATATTACGGATAATGCTTTGAAACAAGCAT	40
Amiidae	<i>Amia calva</i>	Bowfin	12S	NC_004742	CTAACTACAGAATACTACGGATGATTCTATGAAACATAGA	40
			16S	NC_004742	TTTAAGGATACTCAAACCACTACAGTGTTACCACCAAAG	40
Anguillidae	<i>Anguilla rostrata</i>	American Eel	16S	KJ564271	CTTATGTAATCTATAATCAAAAACATTACCGACCAAAAAGA	40
Aphredoderidae	<i>Aphredoderus sayanus</i>	Pirate Perch	12S	NC_004372	CTCAATGAGATTATTACTAATGGTAAATTGAAATATTAAC	40
			16S	NC_004372	CCTTTAATCTCTTAACCCCTAGACGATAATCCCAACAGG	40
Rivulidae	<i>Kryptolebias marmoratus</i>	Mangrove Rivulus	12S	AF283503	CTACACAGGGAACCACGGATAGTACAATGAAATGTGTACA	40
			16S	AF283503	TATTCCTTAATCAAAACTAATTTAATTTAACTAGACAAC	40
Ariidae	<i>Ariopsis felis</i>	Hardhead Catfish	12S	DQ990564	CTATAATAATAGAATACTACGGACGGCACTCTGAAATCAG	40
			12S	JX899751	CTATAATAATAGAATATTACGGACGGCACCCCTGAAACCAG	40
	<i>Bagre marinus</i>	Gafftopsail Catfish	16S	JX899751	CTCACACTCCTTGACTCACAACATTTTATTAATATATGAA	40
Atherinidae	<i>Atherinomorus stipes</i>	Hardhead Silverside	12S	AF150001	CTAACCCAGGTAATACGAAAAATGCAATGAAAAGCGTTAGA	40
Atherinopsidae	<i>Labidesthes sicculus</i>	Brook Silverside	16S	AY655511	CCCTAGACTTCTATAGTTTCGACTGGTACAACCTTTTCCC	40
	<i>Menidia beryllina</i>	Inland Silverside	12S	KX686069	CTGACCCAGGAAATCACGGACAGGGCCATGAAATTGGGCC	40
Balistidae	<i>Balistes capricus</i>	Gray Triggerfish	12S	AY700238	CTGCCCCAGGTAACCACGGACGATTTATTGAAAAATAAAT	40
Belonidae	<i>Strongylura marina</i>	Atlantic Needlefish	12S	AF231554	ACTACTAATAAGCAGAATTGGCACAGCCCAAAACGTCAGG	40
	<i>Strongylura notata</i>	Redfin Needlefish	12S	AF150002	CTAACTCAGGGTATACGAAAAATGTCATGAAAAACACAT	39
	16S	MH716001	CTATTAACTTTTTACCCTTAATTGGTCTCACCCTTAAAC	40		
<i>Strongylura timucu</i>	Timucú	12S	AF231570	CTAACCCAGGTAATACGAAAAATGCAATGAAAAAAGCAT	39	

Family	Species	Common name	Gene	GenBank Accession No.	Species specific sequence	Base pairs
	<i>Tylosurus crocodilus</i>	Houndfish	12S	AF231578	CTAAACCAGGGTATACGGAAAATACAATGAAAATACGTAT	39
Blenniidae	<i>Chasmodes saburrae</i>	Florida Blenny	12S	GQ865562	ACCCTCGTTAAACCTCACCCTTTTCTTGCCTATTCCCGCC	40
	<i>Hypsoblennius hentz</i>	Feather Blenny	12S	GQ865559	CTGAAATCAGGATATACGAATAATGTTTTGAAAAAGGCAC	40
Bothidae	<i>Bothus robinsi</i>	Twospot Flounder	12S	AF488509	GAGAAGGACAAGTCGTGTGCAGAATTGGCAAAGCCCAAGA	40
			16S	JQ939056	CCCCTAGTTTTTCCGCACCCCAGGGGAGTTATTACAGG	40
Callichthyidae	<i>Hoplosternum littorale</i>	Brown Hoplo	12S	HM114386	GCTACATTTTCTATTACAGAATATTACGAATAGCACTATG	40
Carangidae	<i>Caranx hippos</i>	Crevalle Jack	16S	DQ533167	CTCATGGCTTCTCCCTTCATATAATAATAACCACCTAATG	40
	<i>Chloroscombrus chrysurus</i>	Atlantic Bumper	12S	AY141387	CTACCCCTAGCGAACACGAATGATGCATTGAAACATGCAG	40
	<i>Hemicaranx amblyrhynchus</i>	Bluntnose Jack	16S	JQ939028	CTCATAGCTTCTCCTTAAACTGTGAGAGTTAGTCAAAGGG	40
	<i>Trachinotus carolinus</i>	Florida Pompano	12S	NC_024184	CTAAGATAGCGAACACGAATGATGCATTGAAACATAACAAC	40
			16S	NC_024184	CTCCCGGGTCTTCCCTCACCCCAATATGGTTTTTAACCA	40
	<i>Trachinotus falcatus</i>	Permit	16S	JQ939038	CTCCCGGCTTCTCCCTAAACCGCGAGAGTTAGTCAAAGGA	40
	Carcharhinidae	<i>Carcharhinus acronotus</i>	Blacknose Shark	12S	NC_024055	TGAAGGACCAAAAGTAAGCAAAAAGAATTAACCTCAAAA
16S				NC_024055	GCATTACAGAGCCAACCCGTCTCTGTGGCAAAAGAGTGGG	40
<i>Carcharhinus brevipinna</i>		Spinner Shark	12S	NC_027081	AGAGGAGATCAGAGTACTCCTCTGAAACTGGCTCTGGGAT	40
<i>Carcharhinus isodon</i>		Finetooth Shark	16S	AY830729	TTCACCATCTAAACAAGACTTACTCGTCAAAGAAATATAT	40
<i>Carcharhinus leucas</i>		Bull Shark	12S	NC_023522	CACCCTGTGAAGGATCAAAAAGTAAGCAAAAAGAATT	36
			16S	NC_023522	TTTTTACTATCTAGACAAGACTTACTCGTCAAAGAATTC	40
<i>Carcharhinus limbatus</i>		Blacktip Shark	16S	AY830732	TTTTTTACCATCTAAACAAGATTTTCTTGTTAAAGAAACC	40
<i>Galeocerdo cuvier</i>		Tiger Shark	12S	NC_022193	CCTGTGAAGGACTAAAAGTAAGCAAAAAGAATAAACTTC	40
			16S	NC_022193	ATTTTTAATATTTAAACAAGACTTACTCGTTAAAAGACCT	40
<i>Negaprion brevirostris</i>		Lemon Shark	12S	AY830756	TTCTAAAAATATACGAATGGTGAAGTGAACACACCTA	40
	16S		AY830756	ATTTTTTATTATCTAAACAAGACTTACTCGTCAAAGAAAC	40	
<i>Rhizoprionodon terraenovae</i>	Atlantic Sharpnose Shark	12S	AY830764	TTTTCAAAAATAAACGAATGGTAAACTGAAAAACACCTA	40	
		16S	AY830764	ATTTTTTACTACCTAAACAAGATTTACTCGTCAAAGGAAT	40	
Catostomidae	<i>Erimyzon sucetta</i>	Lake Chubsucker	12S	KM273816	CTATTCCAGAATAACACGAACAGCACTATGAAAAAATGCT	40
			16S	KM282468	GGTGAGCTACCCCGAGACAGCCTACATAGGGCCAACCCGT	40
Centrarchidae	<i>Enneacanthus gloriosus</i>	Bluespotted Sunfish	16S	AY742519	CTCCCGGCTCTTTTCTCATCTTAGTCTTTTCTCCCTACA	40
	<i>Lepomis gulosus</i>	Warmouth	12S	KM273835	TTAATAGAAAGTACACGAATGACTGACTGAAACGTCTTTC	40

Family	Species	Common name	Gene	GenBank Accession No.	Species specific sequence	Base pairs
			16S	AY742525	CTCCCGGCTTCTTACCTCAAACCCGTCGTAACCCCTTACA	40
	<i>Lepomis macrochirus</i>	Bluegill	12S	JN389795	TTAATAAAAAGAATACGAATGACTGACTGAAACGCTTTTC	40
			16S	JN389795	CTCTCGGATTCTTACCTCACTCTCGTCTAAACACCCAACA	40
	<i>Lepomis marginatus</i>	Dollar Sunfish	16S	AY742531	CTCTCGGGTCTTATTTCATCTCCGCCTAAGCACCAAGCA	40
	<i>Lepomis microlophus</i>	Redear Sunfish	16S	AY742535	CTCCCGGCTTCTTATTTCATGTCCGCTTTGACCCAACA	40
	<i>Lepomis punctatus</i>	Spotted Sunfish	12S	MF621732	TTAACAAAAAGAATACGAATGATTGACTGAAATGTCTTTTC	40
			16S	MF621732	CTCCCGGCTTCTTATTTCATATTGCTTTCACACCCAACA	40
	<i>Micropterus salmoides</i>	Largemouth Bass	12S	NC_014686	CAGGAATACGAACGATAAACTGAAATGTTTTCCAAAGG	40
			16S	NC_014686	CTCGCAGCTTCTTCACTACCCCTGTCTACTCCCTACAG	40
	<i>Pomoxis nigromaculatus</i>	Black Crappie	12S	NC_028298	TACATTAGGAATACGAACGACTGAAATCTTACGTC	40
			16S	NC_028298	CTCCCGGCTTCTTTTCTACCCTTGTCTAACACCCCTATA	40
Centropomidae	<i>Centropomus undecimalis</i>	Common Snook	12S	KC441979	CTACCACCAGCGCATACGAATAATGTATTGAAACATACTA	40
			16S	HQ731428	CCCCCGGCTTCTACATTCCTGACATCATAGTACATGATC	40
Clariidae	<i>Clarias batrachus</i>	Walking Catfish	12S	KC572134	CTACATCAGAATATTACGAACGGCACCTGAAAAAGTGCC	40
			16S	KC572134	CTCGCACTCTCAACTCAAAAACACACGTTAAAACACGAA	40
Clupeidae	<i>Brevoortia patronus</i>	Gulf Menhaden	16S	DQ912068	CCCGCCGCGCCCTCGCCACAACAGTTTTACTTAAACAAG	40
	<i>Dorosoma cepedianum</i>	Gizzard Shad	12S	NC_008107	CTGAAGCAGATTATTACGAAAAGTCATCTGAAATCGATG	40
			16S	NC_008107	CCCGTTTTACCCTTGGCCACCGCAGTCTTACTAAAATTAG	40
	<i>Dorosoma petenense</i>	Threadfin Shad	16S	NC_009580	CCCGTTTTACCCTCATCCGCCATAGTTTTACTCAAATTAG	40
	<i>Harengula jaguana</i>	Scaled Sardine	12S	NC_016667	GAGCCAGATGACTACGGAGAGTCGCCTGAAACCTGGCGAT	40
			16S	NC_016667	CCCGAAAGCCCTAGCCATTGCGGTCCCACCAAGATAAGG	40
	<i>Opisthonema oglinum</i>	Atlantic Thread Herring	12S	EU552701	CTGAAGCAGATTATTACGAAAAGTCACCTGAAATTAGTG	40
			16S	DQ912074	CCCGTTTAACCCTTATCCACCGCAGTTTCACTAAAATTAG	40
	<i>Sardinella aurita</i>	Spanish Sardine	12S	EU552700	CACAACAAATATCCGCCTGGGAACCTACGAGCGTTAGCTTA	40
			16S	DQ912067	CCCGCCGAACCCTCGTACACAACAGTTTTACTTAAATTAA	40
Coryphaenidae	<i>Coryphaena hippurus</i>	Dolphinfish	12S	KF814117	CTAACATAGTGAATACGAATAGTATATTGAAACATATACT	40
			16S	KF814117	CTCCAGCTTCTCTCTCACCTATTGTACACACAAAACAA	40
Cyprinidae	<i>Ctenopharyngodon idella</i>	Grass Carp	16S	NC_010288	CCTCGCACACCCCAAATCAAAAACATAACATCAAGACAAT	40
	<i>Notemigonus crysoleucas</i>	Golden Shiner	16S	NC_008646	CCTCGTATTACCCCAAACCAAAGAACATATCACTAAGGTA	40

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	<i>Opsopoeodus emiliae</i>	Pugnose Minnow	12S	NC_033926	CTATTATAGAATATTACGAATATGTAACATGAAATAGTGC	40
			16S	NC_033926	CTCGTACCCCTTAATCAAAAATATATTATTAAGACTACG	40
	<i>Pteronotropis hypselopterus</i>	Sailfin Shiner	12S	NC_033939	CTACTACAGAATACTACGGACATGCAACATGAAATAGTGC	40
			16S	NC_033939	AGCCTATATAATTTAGGGCTAACCCGTCTCTGTAGCAAAA	40
Cyprinodontidae	<i>Cyprinodon variegatus</i>	Sheepshead Minnow	12S	NC_028088	CTCATGGAGGAAATACGAATTGTGCCATGCAACAGCAC	38
			16S	NC_028088	ACCTTCTCCTATCAACCTGGCCTGCCCTAAGCCCAGAAA	40
	<i>Floridichthys carpio</i>	Goldspotted Killifish	12S	AF449407	CTCTTCGAGGAAATACGGATTGTGCCATGAAACAGCAC	38
	<i>Jordanella floridae</i>	Flagfish	12S	NC_011387	CTCATTGAGGAAATACGAATTGTGCCATGCAACAGCAC	38
			16S	NC_011387	TCTTACCTTCTTCCCTCATCTCTGGCTTGCCCTAAACCCA	40
Dasyatidae	<i>Dasyatis americana</i>	Southern Stingray	12S	MH715301	TTTCTAGAATACACGGACAGAAGCATGAAAACTTCTTAA	40
			16S	MH715984	ATTAATTTTTCAACTCGATAACAAAAAATTCTTTTAACAA	40
	<i>Dasyatis sabina</i>	Atlantic Stingray	12S	MH715302	TTTCTAGAGTACACGAACAAAAGCATGAAAACTTCTTGA	40
			16S	MH715985	ATTAATTTTTCAACTCGATAACAAAAATCTTTTTTAAACAAA	40
Diodontidae	<i>Chilomycterus schoepfi</i>	Striped Burrfish	12S	AY700256	CTTCAACAGGGAACACTACGAATGGCATTGAAATACATGC	40
	<i>Diodon holocanthus</i>	Balloonfish	12S	NC_009866	GCCCCCGGGGAACACTACGAGCACCAGCTTAAAACCCAAAG	40
			16S	NC_009866	TTTTGACTTCTTCCCCAAAAACCTTAATTAACCCACCA	40
Echeneidae	<i>Echeneis naucrates</i>	Sharksucker	16S	NC_022508	CCCTGTAATTTTCTTCTTCATATATTTACTTCTTAAAT	40
Elassomatidae	<i>Elassoma evergladei</i>	Everglades Pygmy Sunfish	12S	NC_003175	CTACTCAGCGAAAACGAACGACAAAATGAAATGTTTGTCT	40
			16S	NC_003175	CTTTTGGCTTCTCTAGTCATAGTAGATATTAGACCCCAAG	40
Eleotridae	<i>Dormitator maculatus</i>	Fat Sleeper	12S	KF415347	GACACAGTAAATACGAACGATGCGTTGAAACTAAGCATCT	40
			16S	KF415347	CTTTTGGTTTCTTTTGTCCAGCCTGGCTACGCCCACTTCA	40
Elopidae	<i>Elops saurus</i>	Ladyfish	12S	NC_005803	ACCAATAGTAAGCCTAATGAGCACAACTCAAAACGTCAGG	40
			16S	NC_005803	CCCACGACCTGCTTAAATCAAAAGAGCTAATTCTACCAAA	40
Engraulidae	<i>Anchoa hepsetus</i>	Striped Anchovy	12S	DQ912040	AAAAGTAAGCGAAATGGAAAATCTCCAGAACGTCAGGTCGA	41
			16S	DQ912075	CCTGCCTAGCCCAACTCACACCAGTTCTACAAAAGAAAGAC	40
	<i>Anchoa mitchilli</i>	Bay Anchovy	12S	DQ912042	CACGAAGTGGGAAGAAATGGGCTACATTGCCTAATCTAGGCTAC	44
			16S	DQ912077	GTCAAGTTATAGTGGTTGCCCAATAAATGAATACAAGTT	40
Ephippidae	<i>Chaetodipterus faber</i>	Atlantic Spadefish	12S	EF616894	CTGCCACAGAGAACACGCACGACATAATGAAAACAGATGT	40
			16S	DQ533172	CTCCCGGCTTCTTCCCTCCCTGGCACTCTGCCCCCGCGA	40

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Fundulidae	<i>Fundulus grandis</i>	Gulf Killifish	12S	NC_012377	CTGCTTAAGGAAATACGGATTGTGTTATGAAAAATCACAT	40
			16S	NC_012377	CTCTTGCTTCTTATGCCAAATATGGATACCCAAACCTCA	40
	<i>Fundulus majalis</i>	Striped Killifish	12S	KX686099	CTGTCTAAGGAAATACGAATTGTATTCTGAAATGATTACA	40
Gerreidae	<i>Diapterus auratus</i>	Irish Pompano	12S	KJ468671	AACACCAGGGAATACGAATACTACGTTGAAACACGTA	40
	<i>Eucinostomus argenteus</i>	Spotfin Mojarra	12S	KJ622117	CTAATTCAGGGTATACGGATAATGAACTGAAACATTCATT	40
	<i>Eucinostomus gula</i>	Silver Jenny	12S	KT067840	CTAATACAGGGCACACGAACGATGACTTGAAATATACATT	40
			16S	MH715986	AAGAGTTATTCAAAGGGGGGACAGCCCCTTTGAAAGAAAA	41
	<i>Eucinostomus harengulus</i>	Tidewater Mojarra	12S	MH715303	CCGCCTATATACCGCCGTCGTAAGCTTACCCTGTGAAGGAA	41
			16S	MH715987	AAGAGTTATTCAAAGGGGGGACAGCCCCTTTGAAAGAAGAT	41
	<i>Eucinostomus jonesii</i>	Slender Mojarra	12S	KT067842	CTAATATAGGGCACACGGATAATGCACTGAAACGTGCGTA	40
	<i>Eugerres plumieri</i>	Striped Mojarra	12S	KT067857	CTGCAATCAGGGAATTACGGATAACGCGTTGAAATACGTG	40
			16S	MH715988	CTCTTGGCTTCTCTGCTCCGCGCCCTTCTACCCTGTCACCC	41
	<i>Gerres cinereus</i>	Yellowfin Mojarra	12S	KY815278	CTATTATAGGGAACACGGACAACGTAAGTAAATACGCGTT	40
			16S	JQ938957	CTCTCGGCTTCTTCCCAAACCTAGAGAGTTATTCAAAGGG	40
Ginglymostomatidae	<i>Ginglymostoma cirratum</i>	Nurse Shark	12S	NC_030189	TTTAACCAAAAAACACGGACAGTAAACTGAAAAATTACTT	40
			16S	NC_030189	ATTAATTTTTTATCACTAAACAAGACTATCTCATTAAAGT	40
Gobiesocidae	<i>Gobiesox strumosus</i>	Skilletfish	12S	KY656387	CTACTCAAGAGCACACGAAATAGGAATTGAAAAATCTCTT	40
			16S	MH715989	CCCAGGCATCTACAACCAAAAAAGGCCCCGCCTATTACA	40
Gobiidae	<i>Bathygobius soporator</i>	Frillfin Goby	12S	MH715298	CTGCCTCAGTACATACGGACGATGACCTGAAATTACATCCG	41
			16S	MH715981	CCTTTAATGTCTTAATTCCACCTCAGACTCGCTCACTAAT	41
	<i>Ctenogobius boleosoma</i>	Darter Goby	12S	MH715299	CTATAAAGTGAGCAGAGTTGGCAATGCCCAAAACGCCAGGT	41
			16S	MH715982	TACTCCAAGACAGCCTAATAATAGGGCAAACCCGTCTCTG	41
	<i>Ctenogobius smaragdus</i>	Emerald Goby	12S	MH715300	TTATAAAGTGAGCAGAGTTGGCAATGCCCAAAACGCCAGGT	41
			16S	MH715983	CTTTTGAGCTGCTCACCCACCGTATTATTGTACCCCCAGG	41
	<i>Gobionellus oceanicus</i>	Highfin Goby	12S	MH715304	CCGCCTATATACCGCCGTCGTAAGCTTACCCTGTGAAGGAA	41
			16S	MH715990	CTCTTTAATTATTTTTTCTCAAGGCAAGGCCTAACAAGGAT	41
	<i>Gobiosoma bosc</i>	Naked Goby	12S	AF491095	CTGTCCCAGTAAACACGGATCTTGCAATGAAACAAGCATT	40
			16S	AF491095	CTTTTGATGCTCCCACCCATAAAAAACACACTTCTCAAC	40
	<i>Gobiosoma longipala</i>	Twoscale Goby	12S	MH715305	CTGACACAGTGAAATACGAAAGATAGCTTGAACAACCATC	41

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			16S	MH715991	GAGCCCAGTTATAGCTGGTTGCCTGAGAAATGAATAGAAGT	41
	<i>Gobiosoma robustum</i>	Code Goby	12S	AF491087	CTGTATCAGTAAACACGGATTTTGCCTGAAATAAGCATC	40
			16S	AF491087	CTTTTGGATGCTCCCACCTTCTAGAAATACCTTTTCTCCC	40
	<i>Lophogobius cyprinoides</i>	Crested Goby	12S	KF415410	CTGGTACAGTGAATTTACGAACTTCATGCTCTGAAACAA	40
			16S	KF415410	CTTTTGAGCTGCTCACCCACCGTATTATTTACCCCCAGG	40
	<i>Microgobius gulosus</i>	Clown Goby	12S	AF491111	CTGAATCAGTAAAAACGGATTTTGCCTGAAATGAGCATC	40
			16S	AF491111	CTTTTCATTTCTTCTACCCACCCGCACCCGCCCCGGCTA	40
	<i>Microgobius thalassinus</i>	Green Goby	12S	AF491113	CTGAAACAGCAAACACGGATTATGCTGAAATAAGCATC	40
			16S	AF491113	GGCAATACCAGCCTCCGGCTAAGAGAAATTTAAGAGTTA	40
Gymnuridae	<i>Gymnura micrura</i>	Smooth Butterfly Ray	12S	MH715306	ATCTGAAGGAGGATTTAGCAGTAAGAGGGGACTATGAGAG	40
			16S	MH715992	TTAATTCTTCAAGCAAGCAATAAAAAACTTTTAACACAAG	40
Haemulidae	<i>Haemulon aurolineatum</i>	Tomtate	12S	EF095568	CTACTGCCAGTGAACACGAATAGTGCCTGAAATATGCAC	40
Hemiramphidae	<i>Hyporhamphus unifasciatus</i>	Atlantic Silverstripe Halfbeak	12S	AF231581	CTATCATAGGACATACGGATAATGTAATGAAACGTACATT	40
Ictaluridae	<i>Ameiurus catus</i>	White Catfish	12S	DQ421868	CTACAACACTAGAATATTAACGAATGGCACTATGAAAATTA	40
	<i>Ameiurus natalis</i>	Yellow Bullhead	12S	MF621735	CTATAACTAGAATATTAACGAACGGCACTATGAAAATTA	40
			16S	MF621735	CCCTCACCTCACAACACCTTTAAAATACACGAGCCCGAG	40
	<i>Ameiurus nebulosus</i>	Brown Bullhead	12S	MF621733	CTACAACACTAGAATATTAACGAATGGCATTATGAAAATTA	40
			16S	MF621733	CCCTCACCTCACCAACACCTTTATACTACACGAGCCCGAG	40
	<i>Ictalurus punctatus</i>	Channel Catfish	12S	NC_003489	ACCTAGAATATTACGAATGGCACCATGAAAATAATGCCTG	40
			16S	NC_003489	CTCGTACTCCTCATCTCACAATACTTTTATATTAACGAG	40
	<i>Noturus gyrinus</i>	Tadpole Madtom	12S	AY458874	CTACATCTAGAACATAAACGAATGGCGCCATGAAAATGCT	40
			16S	AY458874	CTCGCACTCCTCACCTCACAACACATAATTCTACGAATAT	40
Kyphosidae	<i>Kyphosus saltatrix</i>	Bermuda Chub	12S	KC136572	CTAACACAGTGAATTACGGACGATATACTGAAATTTATAT	40
			16S	KC136572	CTCCCTCACCTCGTCCGACCCCTCTGACACCTTAAG	40
Labridae	<i>Halichoeres bivittatus</i>	Slippery Dick	12S	AY279599	GTAATTATACTAAATACGGATGACATTCTGAAAAAATATC	40
	<i>Lachnolaimus maximus</i>	Hogfish	12S	AY279618	ATACCACATTGAATACGAATAGTAGAATGAAACATCTACT	40
Lepisosteidae	<i>Lepisosteus platyrhincus</i>	Florida Gar	12S	NC_029715	CTATGTCAGAATATTACGAACAACACCATGAAATTTGGTAT	40
			16S	NC_029715	CTCAAGACATGCTCCTAACCGCCCAAGTACTTACTTCAC	40
Lobotidae	<i>Lobotes surinamensis</i>	Atlantic Tripletail	12S	NC_026233	CTATTTTCAGAGAATACGGATGGTGTAATGAAACTTACACA	40

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			16S	NC_026233	CTTTGGGCTTCTTTACTCACTAAGCATCTTACAATTCACG	40
Loricariidae	<i>Pterygoplichthys disjunctivus</i>	Vermiculated Sailfin Catfish	16S	NC_015747	CTTGCACCTCCTCACCTCACAAAAGTTACCCTAACCTAT	40
Lutjanidae	<i>Lutjanus analis</i>	Mutton Snapper	12S	EF095569	CTAATATAGTGTAAATACGAACGATACACTGAAATACGTAT	40
	<i>Lutjanus griseus</i>	Gray Snapper	12S	MH715307	TCTCAAGAATATAACGAACAGAAATATGAAAAATTTCTTAA	40
			16S	JQ938964	CTCCCGGCTTCTTTCTAAACCGAGAGAGTTAATCAAAGGG	40
	<i>Lutjanus synagris</i>	Lane Snapper	12S	MH715308	TAACATAGTGAAATACGAACGATGCACTGAAATACGCATC	40
			16S	MH715995	CTCCCGGCTTCTTTCTTCCACCCCTAGTCTTTACCCCTTCT	41
Megalopidae	<i>Megalops atlanticus</i>	Tarpon	12S	NC_005804	CCCTCTCGAGTACACGAATAGAAACATGAAAACTTCTTA	40
			16S	NC_005804	CTCGAAGCCTGCCCTAAACCAAACCAACAACACCAAAGA	40
Mobulidae	<i>Manta birostris</i>	Giant Manta	12S	KF413894	CTCAACCCAGAATACTTGCGAACGACACAGTGAAACCTGA	40
			16S	KF413894	ATTAACCTTTTCAATCAGGTAATAAAAAATTTTAACTAAA	40
Monacanthidae	<i>Aluterus schoepfi</i>	Orange Filefish	12S	KT600932	CTACTTAGAGAACACGAATGATAAACTGAAAAACGTATCT	40
	<i>Stephanolepis hispidus</i>	Planehead Filefish	12S	KT600974	TTTACAAAAACAATACGAACGGTAAACTGAAAAACACCTA	40
Mugilidae	<i>Mugil cephalus</i>	Striped Mullet	12S	KP018403	CTTTGATAATTTATTACACCCATTATCCGCCAGGGAECTA	40
			16S	KP018403	CTAGTAATAGTCAAGCAAAAAGCATTTTAGTTTGACCCC	40
	<i>Mugil curema</i>	White Mullet	12S	NC_017889	TTTGATAGTTCAACCACGCCACTATCCGCCTGGGTACTA	40
			16S	KF375100	ACAACCTTCCAGGAGGGTAAAGATCATATTTTCATGAAGG	40
	<i>Mugil trichodon</i>	Fantail Mullet	12S	MH715311	CTAATACAGAGAATACGAATGATGAACTGAAATGTACATCT	41
			16S	MH715998	TTCTTATATTCTTCCCCCTTTTCGGTCATCATGGCCCTATC	41
Mullidae	<i>Mullus auratus</i>	Red Goatfish	16S	JQ938968	CCCACGGCTTCTCCTTATACCGCGGGAGTTAGTCAAAGGG	40
Muraenidae	<i>Gymnothorax saxicola</i>	Honeycomb Moray	12S	JX242931	CTGACCCAGAAATATCACGAACAGTGCAATGAAATACGCA	40
Myliobatidae	<i>Aetobatus narinari</i>	Spotted Eagle Ray	12S	JX978310	TCTCTATCAAGAACACACGAAAAGAAGCATGAAAACCCTC	40
Narcinidae	<i>Narcine bancroftii</i>	Lesser Electric Ray	12S	NC_034772	GAATAACAGTAAGCAAAAATGAATAAAATATTCAATACGTC	40
			16S	NC_034772	ATTAATTATTCAATTACGTACCATATAAGAATCTTAGACC	40
Ophichthidae	<i>Ophichthus gomesii</i>	Shrimp Eel	12S	AY430249	CTGAAAACAGAATACTACGAAAGGTGCCATGAAAACAAACAC	40
Ostraciidae	<i>Acanthostracion quadricornis</i>	Scrawled Cowfish	12S	AY700275	CTTTTCCAGTGAAATACGAACGATGTACTGAAATATACAT	40
Paralichthyidae	<i>Anclpsetta quadrocellata</i>	Ocellated Flounder	16S	JQ939064	CCCCTAATTTCCCACTAACTAGGAGTGTAACCTAAAGGG	40
	<i>Etropus crossotus</i>	Fringed Flounder	16S	JQ939068	CCCTTGGTTTTTCCCGAAACCAAGGGAGTTATTCATAGGG	40
	<i>Paralichthys albigutta</i>	Gulf Flounder	12S	AF316017	TAGAAGTAGCAAATACGAATGATACATTGAAATATGT	37

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			16S	JQ939111	CCCCTGGGTTCACCAACCAAGGGTGTAGTCAAAGGG	40
	<i>Paralichthys lethostigma</i>	Southern Flounder	12S	NC_029223	CTAAGACTAGCAAATACGAACGATGCATTGAAATATGCAA	40
			16S	NC_029223	CCCCTGGGTTCACCAACTCATTACTGTTATCACCTTCAG	40
	<i>Syacium papillosum</i>	Dusky Flounder	12S	AF488503	CTAATTCAGGGCACACGAAAAATATAATGAAAAACGTAT	39
Phycidae	<i>Urophycis floridana</i>	Southern Hake	12S	FJ215094	CTGCCCCAGAGAACTTATACGGATGGTATATTGAAATTA	40
			16S	FJ215198	ATAGCTAAAGGAGTTAGTCAGAAAGGGTACAGCTTTTCTG	40
Poeciliidae	<i>Gambusia holbrooki</i>	Eastern Mosquitofish	12S	NC_028274	CTTCCCCAGGAAACACGAATTGTGCTATGAAATAACACA	40
			16S	NC_028274	CTTTAATTATCTCGTACCCCGACGGCCTAACCAACAGCC	40
	<i>Heterandria formosa</i>	Least Killifish	12S	EF017473	CTCTCCTAGGAAAAACGAATTGTGTCATGAAACAACACAT	40
	<i>Poecilia latipinna</i>	Sailfin Molly	16S	NC_035305	CTCTACTTATCTCCCACCCAATAGTCCCACCACAAACCT	40
Polynemidae	<i>Polydactylus octonemus</i>	Atlantic Threadfin	16S	JQ938973	GATAGGAGTTCAGCCTTAAAGATTCTTTTGAACTTTAAG	40
Pomacentridae	<i>Abudefduf saxatilis</i>	Sergeant Major	12S	FJ616293	CTAAACCAGGAAATACGGACAATGTATTGAAACGTACATT	40
Pomatomidae	<i>Pomatomus saltatrix</i>	Bluefish	12S	NC_022507	CTAATGAAGCGAACACGAATGGTGCCTGAAAACGTGCG	40
			16S	NC_022507	CTTTGGGCTTCTCTCTCACTACAATTTATACTTACT	40
Pristidae	<i>Pristis pectinata</i>	Smalltooth Sawfish	12S	NC_027182	CTTTTAAGAAAAACGAACAGTACAATGAAAAATTACTCA	40
			16S	NC_027182	ATTAATTTATCTACCTGCCACAAATGGAACATTCTCATA	40
Rachycentridae	<i>Rachycentron canadum</i>	Cobia	12S	NC_011219	CTAAAAATAGTGAATACGAATAATACATTGAAACATGTAT	40
			16S	NC_011219	CTCCTGGCTTCTCAATTCATCTCAAAACAAATGACTCAAC	40
Rajidae	<i>Raja eglanteria</i>	Clearnose Skate	12S	KF317714	CACAAAAGTAAGCATAACGGATTTCTCCAAAACGTCAGG	40
			16S	MH715999	ATAATCCTTCACCTCGCCACCAAGATTACCAAAACAAGG	40
Rhinobatidae	<i>Rhinobatos lentiginosus</i>	Atlantic Guitarfish	12S	AY830717	CTATTAAGAAAAACGGATATTATAATGAAAAACTACTTA	40
			16S	AY830717	ATTAATTTACTCCAACCTGCCATAAATATTACTTATATAT	40
Rhinopteridae	<i>Rhinoptera bonasus</i>	Cownose Ray	12S	JX241047	CTCTTTAGAGTATACGGATAGAAACATGAAATCTTCTTA	40
Scaridae	<i>Nicholsina usta</i>	Emerald Parrotfish	12S	AY279624	ATGGAACAATGAATACGAATGGTTAATTGAAAGATTTCC	40
Sciaenidae	<i>Bairdiella chrysoura</i>	Silver Perch	12S	MH715297	CTAATGCAGAGGAAACGAATGATGTGCTGAAACGCACATC	40
			16S	MH715980	CTTCGGCTTCTCCCCTCACACCTGTCCTCCTTCCCCTTAG	41
	<i>Cynoscion arenarius</i>	Sand Seatrout	12S	AF128401	CTGAAATATATATCTGAAGGAGGATTTAGCGGTAAGCAT	40
			16S	AF128401	TCTCCCCTACTCCTGTTTTCTCCCATGATAACCAGAT	40
	<i>Cynoscion nebulosus</i>	Spotted Seatrout	12S	AF128402	CTAACATAGAGAAAACGAATGATATACTGAAACATATATC	40

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			16S	AF128402	TCTCCCCTCACCCCGTTTTTCTCGCCATGACAACCAAAT	40
	<i>Leiostomus xanthurus</i>	Spot	12S	KX686090	CTAATACAGAGAAAACGAATGATGTACTGAAATACACGTC	40
			16S	MH715994	CTACTCCAAGACAGCCTAATAATAGGGCAAACCCGTCTCTG	41
	<i>Menticirrhus americanus</i>	Southern Kingfish	12S	DQ874712	AGAAATGGGCTACATTCTCTCGAGCACTGAAAGAAGGAGG	40
	<i>Menticirrhus littoralis</i>	Gulf Kingfish	12S	MH715309	TAATATAGTGAAATACGAACGATACACTGAAATACGTATC	40
			16S	MH715996	TCCCGGATTTCTCCCGTCCATCCCAGTTTATACCCCTGCA	40
	<i>Menticirrhus saxatilis</i>	Northern Kingfish	12S	KX686072	AGAAATGGGCTACATTCTCTAACCTCAGAGAAAACAAATG	40
	<i>Micropogonias undulatus</i>	Atlantic Croaker	12S	MH715310	CTAATCTCAGAGAAAACGAATGATGCACTGAAACACGCATC	41
			16S	MH715997	CTCTCGGATTCTTCCCTCACCCTGTTCTCTCCCCCTTGA	41
	<i>Sciaenops ocellatus</i>	Red Drum	12S	NC_016867	CTAATACAGAGAATACGAATGATGTACTGAAATACACATC	40
			16S	NC_016867	CTCTCGGATTCTTCCCTCACCCCTGTCTTCTCTCCCCGA	40
Scombridae	<i>Euthynnus alletteratus</i>	Little Tunny	12S	NC_004530	CTACTATTAGCGAATACGAACGATGCACTGAAAACGTTCA	40
		Little Tunny	16S	NC_004530	TTCGCCATGGTCTTACCCCTACCGATACTCAAAAAGAAGTC	40
	<i>Scomberomorus cavalla</i>	King Mackerel	12S	NC_008109	CTATTCTAGCGAATACGAACGATAGTACTGAAAACGTACA	40
			16S	NC_008109	CTCCAGGCTTCTCTTCCACCGGGTTTAACCCCTGCCGA	40
	<i>Scomberomorus maculatus</i>	Spanish Mackerel	12S	DQ874689	CCCGTCGGTAAATACGAATGGTTTATTGAAAAATAAACT	40
Scorpaenidae	<i>Scorpaena brasiliensis</i>	Barbfish	12S	MH715312	TGAAATAGAGCATAACGAACGATGAACTGAAAACATCTG	40
			16S	MH716000	TTTCAGATTCTTTTTTAATTAAGTAATTCACCTTCTAAAA	40
Serranidae	<i>Centropristis striata</i>	Black Sea Base	12S	KX686079	CTATGATAGTGAATACGAATGATATCCTGAAACGGACATC	40
	<i>Diplectrum formosum</i>	Sand Perch	16S	FJ548768	CTCCCGGCTTCTTAATTCCATAATAAACCTTATCTATAG	40
	<i>Epinephelus itajara</i>	Atlantic Goliath Grouper	16S	FJ548765	TTCTTCTTCTCCAACCCCTCTGTCTCAACAGATATTTT	40
Sparidae	<i>Lagodon rhomboides</i>	Pinfish	12S	KX686070	CTTATACACAGGGAACACTACGAATGGTATACTGAAAACGTA	40
			16S	MH715993	TCCCGGCTTCTCACCTCAATTTTGGTTTATACTTCAATTA	40
Sphyraenidae	<i>Sphyraena barracuda</i>	Great Barracuda	12S	NC_022484	GCAAATAGTAGGCAAAGTTGGCACAGCCAAAACGTCAGG	40
			16S	NC_022484	CTCCCGGCTTCTCTTTCACACTTGACCAATGCTTTAAT	40
	<i>Sphyrna lewini</i>	Scalloped Hammerhead	12S	NC_022679	CTAACATTAGCGAATACGAACGCTGCATTGAAATATGCAA	40
			16S	NC_022679	ATTAATTTTTTATTACCCAAAACAAGACTTCTTATTAAAG	40
	<i>Sphyrna mokarran</i>	Great Hammerhead	12S	NC_035491	TTTACAAAACATATACGAATGGTAGACTGAAAACACCT	40
			16S	NC_035491	TTTTTATTATCTAAACAAGACTTTCTCGTCAAAGAAACCC	40

Family	Species	Common name	Gene	GenBank Accession No.	Species specific sequence	Base pairs
	<i>Sphyrna tiburo</i>	Bonnethead	12S	NC_028508	TTTTATTA AAAACATACGAACGGTAAACTGAAAAATACCT	40
			16S	NC_028508	TTTTTTTACCTAGACAAGACTTCCTTATTAAGGAATCC	40
Stromateidae	<i>Peprilus burti</i>	Gulf Butterfish	12S	AP012947	CTAGCTTAGCGAATACGAACGATGTAATGAAAATGTACAT	40
			16S	AP012947	CTCCCGTTTCTCCCTCACCACGGTCATACCCCTACCGA	40
Syngnathidae	<i>Anarchopterus criniger</i>	Fringed Pipefish	12S	KY065791	TCCATCGATGAACAACGAATGATTTATTGAAAAATAAACCC	40
	<i>Hippocampus erectus</i>	Lined Seahorse	12S	NC_022722	AGAACAACAGTGAGCAGAACTAGTAATAACCCAAAACGTC	40
			16S	NC_022722	CCATATTATACCCTAAAACCTACATAAAATTAATAATA	40
	<i>Hippocampus zosterae</i>	Dwarf Seahorse	12S	KY065837	TTACTAATGAATACGGATGGCATATTGAAACATAAACCT	40
	<i>Microphis brachyurus</i>	Opossum Pipefish	12S	NC_010273	TTTACCAACGCATAACGGACAACATGTTGAAACCACATAT	40
		Opossum Pipefish	16S	NC_010273	CTGATTTTACCCTCAAGCATTACTAAATTGTATTAAGCC	40
	<i>Syngnathus floridae</i>	Dusky Pipefish	12S	AF354957	GCTATTAAGCACACACGGAACCTCGTATTGAAACATACGT	40
	<i>Syngnathus louisianae</i>	Chain Pipefish	12S	KY065876	TCAACCGATAAACACGGATGATTAATTGAAAAATGAATCT	40
	<i>Syngnathus scovelli</i>	Gulf Pipefish	12S	AF354970	TTTGTCAACCGATAAACACGGATGATTGATTGAAAAATAA	40
Synodontidae	<i>Synodus foetens</i>	Inshore Lizardfish	12S	KC441961	CTTCCGCAGAGAAAAACGGAAGGGGCAGTAAACCTGCC	40
			16S	MH716002	CCCTCCCATTTTTACTCAAATTCTAATACCCATCAGACA	40
Tetraodontidae	<i>Lagocephalus laevigatus</i>	Smooth Puffer	12S	NC_015345	GGCTACATTCTGCCCAGAGAACAACGAATGATGTGTTG	40
			16S	NC_015345	TTTTTGGCTTCTTGCCTCACAAATAGTATTAATAAACAG	40
	<i>Sphoeroides spengleri</i>	Bandtail Puffer	12S	AY700284	AGAGATGGGCTACATTGCTCGAGTACTGAAAGAAGGAGG	40
Triakidae	<i>Mustelus norrisi</i>	Florida Smoothhound	12S	AY830755	TTTACCAAAAAACACGAATGGTGAACGAAAAACACGCCTA	40
			16S	AY830755	ATTAATTC TTTATTATCTAGACAAGAATTTCTCGTCAAAG	40

Table 6. Mitochondrial 12S and 16S rRNA gene sequence read information of successful identifications using the species-signature sequence method.

Sample	Analyzed sequence reads		Sequences identified by this method				OTUs identified by this method	
			12S		16S		12S	16S
	12S	16S	No.	%	No.	%		
SF1	5456	21202	75	1.4%	0	0	1	0
SF3	2993	3026	571	19.9%	0	0	16	0
SF4	ND*	16344	ND	ND	0	0	ND	0
SF5	ND	14967	ND	ND	1712	12.3%	ND	15
SF6	3675	24865	44	1.2%	17	0.1%	6	1
SF7	4199	11418	166	4.2%	335	3.0%	5	7
SF8	5958	12732	486	8.5%	30	0.2%	19	1
SF9	4783	8607	1458	31.0%	28	0.3%	3	1
SF10	7300	9213	1668	24.2%	2032	22.4%	17	8
SF11	9702	7080	428	4.4%	0	0	3	0
SF12	7371	8584	217	3.0%	0	0	4	0
SF13	16437	10169	231	1.4%	12	0.1%	7	1
SF14	4945	9795	1315	29.4%	248	2.8%	32	7
SF15	10896	4248	10698	98.5%	18	0.4%	4	1
SF16	933	4069	0	0	20	0.5%	0	1
Total	84648	166319						
Mean	6511	11088	1335	17.4%	297	2.8%	9	3
± SE	± 1112	± 1593	± 796	± 7.5%	± 168	± 1.6%	± 3	± 1

*ND = no data

Table 7. Analyzed sequence reads of mitochondrial 12S and 16S rRNA genes and relative percentages of sawfish host and fish prey sequences.

Sample	Analyzed sequence reads		Smalltooth sawfish sequences (%)		Fish prey sequence identified (%)†	
	12S	16S	12S	16S	12S	16S
SF1	5456	21202	82.3	99.9	91.0	75.0
SF3	2993	3026	11.3	100.0	95.4	
SF4	ND*	16344	ND	100.0	ND	
SF5	ND	14967	ND	31.4	ND	90.1
SF6	3675	24865	93.3	99.7	76.7	40.7
SF7	4199	11418	48.8	62.8	89.6	98.3
SF8	5958	12732	2.0	40.8	96.0	94.4
SF9	4783	8607	19.4	70.2	97.3	98.9
SF10	7300	9213	2.3	17.4	94.6	98.1
SF11	9702	7080	95.4	97.6	81.3	91.3
SF12	7371	8584	73.2	100.0	91.0	
SF13	16437	10169	90.1	98.1	87.5	97.4
SF14	4945	9795	0.5	31.9	90.4	84.4
SF15	10896	4248	0.7	41.3	99.7	99.1
SF16	933	4069	5.3	24.1	97.1	99.1
Total	84648	166319				
Mean	6511	11088	40.3	67.7	91.3	88.9
± SE	± 1112	± 1593	± 11.3	± 8.6	± 1.8	± 4.9

*ND = no data

†After removal of host sequences

Table 8. Relative taxonomic abundance of mitochondrial 12S and 16S rRNA gene sequences (%) in smalltooth sawfish fecal samples after removal of host sequences.

Species	SF1		SF3		SF4		SF5		SF6		SF7		SF8		SF9		SF10		SF11		SF12		SF13		SF14		SF15		SF16		
	12S	16S	12S	16S	12S*	16S	12S*	16S	12S	16S	12S	16S	12S	16S	12S	16S	12S	16S	12S	16S	12S	16S	12S	16S	12S	16S	12S	16S	12S	16S	
<i>Anchoa hepsetus</i>									30.9	21.2																					
<i>Anchoa mitchilli</i>									56.9		1.0																	100.0	100.0		
<i>Bairdiella chrysoura</i>			76.9								48.4				0.2		19.1		1.4		100.0		55.7		62.1		0.4				
<i>Cynoscio arenarius</i>								0.2			42.3	11.6			0.2	100.0															
<i>Cynoscion nebulosus</i>																	80.6	62.7													
<i>Cynoscion sp.</i>															85.1		0.1														
<i>Dasyatis americana</i>									12.2	78.8																					
<i>Dorosoma cepedianum</i>																										9.0	10.5				
<i>Elops saurus</i>													87.6	93.5																	1.4
<i>Eucinostomus harengulus</i>											2.6	83.2					0.2	37.3		89.9		1.6	100.0		10.8						3.8
<i>Eugerres plumieri</i>																	14.5														
<i>Lagodon rhomboides</i>			23.1																			42.7									
<i>Leiostomus xanthurus</i>								20.7																		28.9	78.3			98.6	96.2
<i>Menticirrhus sp.</i>							2.7																								
<i>Microgobius gulosus</i>											5.7	5.2																			
<i>Micropogonias undulatus</i>								76.1											98.6	10.1											
<i>Mugil cephalus</i>	100.0	100.0																													
<i>Strongylura notata</i>													11.0	6.5																	
<i>Synodus foetens</i>								0.3					1.4																		

*ND = no data