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Contribution to the Theme Section 'Biology and ecology of sawfishes'

Sympatric elasmobranchs and fecal samples provide insight into the trophic ecology of the smalltooth sawfish

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ABSTRACT: Growing concerns about the conservation of elasmobranchs have prompted a surge in research, because scientific studies that can support management actions are needed. Sawfishes are among the most threatened fishes worldwide and epitomize the challenge of conserving widely distributed, large-bodied marine fishes. We used a comparative approach to provide data on the trophic ecology of the smalltooth sawfish *Pristis pectinata* in the western Atlantic coastal waters of southwest Florida, USA. Specifically, we applied (1) stable isotope techniques to fin tissues of smalltooth sawfish and 2 sympatric elasmobranch species that have well-documented diets (i.e. bull shark *Carcharhinus leucas* and cownose ray *Rhinoptera bonasus*), and muscle tissue from a variety of known and potential prey species; and (2) an 18S rRNA gene sequencing technique to identify prey taxa in sawfish fecal samples. These analyses provided evidence that the smalltooth sawfish feeds primarily on teleost and elasmobranch fishes at all life stages even though sawfish move from estuarine to coastal habitats during their ontogeny. Although both sawfish and bull sharks occupy estuarine waters as juveniles and are piscivorous, the results also indicate that these species partition habitat. The cownose ray has been thought of as migratory throughout its range, but these data indicate that non-migratory, estuarine populations exist at lower latitudes. Collectively, these results will aid in the development of management decisions regarding these species and in improving long-term recovery planning for the smalltooth sawfish.

KEY WORDS: *Pristis pectinata* · *Carcharhinus leucas* · *Rhinoptera bonasus* · Stable isotopes · High-throughput sequencing · Habitat partitioning · Management · Ontogenetic habitat shifts

INTRODUCTION

Large predators, including many elasmobranchs, perform key roles in marine ecosystems through the regulation of community structure by top-down processes (Baum & Worm 2009, Ferretti et al. 2010). In recent decades, overfishing has had direct and indirect negative effects on elasmobranch populations globally, due in large part to their biological attributes such as slow growth rates, low fecundity, and late age at maturity (Stevens et al. 2000, Myers & Worm 2003, Ferretti et al. 2008, Hisano et al. 2011). Consequently, more elasmobranch species are being listed as threatened or endangered (Dulvy et al.

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2014). Understanding the ecological roles that elasmobranchs play in marine communities is crucial for developing sound management plans, especially for the most compromised populations.

Sawfishes (Pristidae) are among the most endangered fishes, and all 5 species are protected by state, federal, and international laws (Harrison & Dulvy 2014). Recent behavioral and sensory research has shown that sawfishes use ampullae on their toothed rostrum to sense prey-like electric fields and capture prey (Wueringer et al. 2012). However, other than anecdotes and some supporting data that suggest sawfish feed on schooling fishes and crustaceans and occasionally use their rostrum for defense (Breder 1952, Bigelow & Schroeder 1953, Thorson 1976, Thorburn et al. 2007, 2008, 2014), little is known about their diet or their interactions with other predators such as bull sharks *Carcharhinus leucas* (Thorburn & Rowland 2008, Thorburn et al. 2014). A small number of direct observations from field sampling, anglers, and necropsies of the smalltooth sawfish *Pristis pectinata* have indicated that this species feeds on fishes such as clupeids, carangids, mugilids, and dasyatids, the pinfish *Lagodon rhomboides*, and pink shrimp *Farfantepenaeus duorarum* (used as bait) (Poulakis et al. 2013).

Studies of movement patterns and habitat use of the smalltooth sawfish have shown that this species uses estuaries and coastal habitats in southwestern Florida, some of which have been heavily influenced by human activities, with much of the area designated as critical habitat for juveniles by the US government (Norton et al. 2012). To maximize the effectiveness of ongoing recovery planning, knowledge of the trophic ecology of the smalltooth sawfish in these habitats is needed to better understand what prey are important in these ecosystems and how sympatric elasmobranchs may also depend on the same prey resources. These data would improve our understanding of both the ecological role of the smalltooth sawfish in the ecosystem, and which members of the community this species interacts with.

Several techniques are commonly used to study the trophic ecology of organisms, including direct observation of feeding behavior, analysis of stomach contents and feces, and examination of chemical constituents such as fatty acids and stable isotopes (e.g. Hussey et al. 2012a, O'Rorke et al. 2012, Thorburn et al. 2014). In species with small populations, nonlethal techniques may be the only legal sampling methods, and these minimally invasive techniques include muscle biopsies, drawing blood, and fin clips (Hussey et al. 2012a). In addition, from the viewpoint of conservation, it is better to acquire samples without sacrificing organisms, especially for vulnerable or protected species (Heupel & Simpfendorfer 2010, Hammerschlag & Sulikowski 2011).

Analyses of the proportional abundances of stable isotopes of various elements in tissues of consumers and their potential prey as well as direct analyses of consumer fecal samples have been used to explore trophic ecology in multiple ecosystems (e.g. Hobson & Clark 1992a,b, O'Rorke et al. 2012, Tilley et al. 2013). For example, stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) can provide detailed insights not only on species-specific ecology, but also on community and ecosystem structure and function (e.g. basal resources and trophic level; Hussey et al. 2014). These techniques have revealed dietary resource partitioning among sympatric juvenile sharks in nurseries and between guild-level predator groups (Kinney et al. 2011, Heithaus et al. 2013). These multi-species, comparative studies can reveal the degree to which the niches of sympatric predators are unique (i.e. resource partitioning) or redundant (i.e. habitat partitioning). For example, predators may share food sources, but be separated by habitat type (e.g. mangroves vs. seagrass), environmental affinities (e.g. high vs. low salinity), or space (e.g. shorelines vs. open water). In addition, DNA sequencing (including high-throughput techniques) of consumer fecal samples can provide insights into specific prey types on a variety of taxonomic scales (Brown et al. 2012, O'Rorke et al. 2012). For example, 18S rRNA gene techniques have been used to study the diet of species for which there are research gaps or which would otherwise be difficult to study (O'Rorke et al. 2012). When used together, stable isotope and DNA-based techniques provide complementary information on trophic ecology.

Given the lack of published data and the limitations of sampling listed taxa such as sawfishes, aspects of trophic ecology of the smalltooth sawfish in southwest Florida were investigated by analyzing stable isotopes of carbon and nitrogen in fin tissues and DNA sequences in fecal samples. Specifically, our goals were to analyze data from multiple sources (i.e. smalltooth sawfish, 2 sympatric elasmobranchs, known and potential prey) in the ecosystem to determine (1) what broad prey types (e.g. invertebrates, fish) the smalltooth sawfish exploits from its nursery residency through adulthood in these waters by using fecal DNA and comparing isotopic signatures of smalltooth sawfish fins to those of sympatric species with well-characterized diets: the bull shark, which is piscivorous (Snelson et al. 1984, Cliff & Dud-

ley 1991, Thorburn & Rowland 2008) and the cownose ray *Rhinoptera bonasus*, which feeds on benthic invertebrates (Smith & Merriner 1985, Collins et al. 2007); (2) whether hypothesized ontogenetic diet shifts by smalltooth sawfish occur (e.g. early reliance on benthic infauna, later reliance on fish); and (3) whether resource or habitat partitioning could be occurring between the smalltooth sawfish and other elasmobranchs in the ecosystem.

MATERIALS AND METHODS

Study areas

The Charlotte Harbor estuarine system is one of the largest estuaries in Florida and is the northernmost region of the western Atlantic where the smalltooth sawfish *Pristis pectinata* is found. Estimated at 56 km long and 700 km^2 (Hammett 1990), it is a recognized nursery for smalltooth sawfish and has been designated as official juvenile critical habitat (Poulakis et al. 2011, Norton et al. 2012). Young-of-the-year and juvenile smalltooth sawfish occur most frequently in the mouths of the Peace and Myakka rivers in the northern portion of the estuarine system (~26° 55′ N, 82° 07′ W) and throughout the Caloosahatchee River (~26° 32′ N, 81° 58′ W) in the southern portion (Seitz & Poulakis 2002). Seagrasses such as shoal grass *Halodule wrightii* are present, but sparse in the sand-mud bottom regions of the estuary where the 3 focal elasmobranch species (i.e. smalltooth sawfish, bull shark *Carcharhinus leucas*, cownose ray *Rhinoptera bonasus*) are found. Red mangroves *Rhizophora mangle* have been identified as important shoreline vegetation for juvenile smalltooth sawfish, and these trees are present in the regions of the estuary where the species is typically found (Poulakis et al. 2011, Norton et al. 2012). Pine Island Sound (~26° 35′ N, 82° 10′ W) is the polyhaline portion of the estuarine system adjacent to the Caloosahatchee River, and tissue samples from this area were used for comparative purposes.

Florida Bay is a large lagoonal system located in southern Florida between the Florida Keys and the mainland, where it marks the southernmost portion of Everglades National Park (~25° 00′ N, 80° 50′ W; Sogard et al. 1989) and the southernmost region of the United States where the smalltooth sawfish is found. Shallow, seagrass-covered carbonate mud banks are a common feature in the bay, which is inhabited by the greatest concentration of adult smalltooth sawfish in its range (Poulakis & Seitz 2004, Waters et al. 2014). Florida Bay contains mud bottom, hard bottom, and macroalgal communities and has extensive red mangrove shorelines (Fernald & Purdum 1998).

Field sampling and sample collection

Fin tissue samples were collected from the 3 focal elasmobranch species from 2004 through 2012. For young-of-the-year and juvenile smalltooth sawfish, both random and directed sampling were conducted in the Charlotte Harbor estuarine system during the day using a 183 × 3 m center-bag haul seine and gill nets ranging in length from 30.5 to 183 m (detailed gear descriptions and sampling protocols may be found in Poulakis et al. 2011). For adult smalltooth sawfish, bottom longlines consisting of 4.0 mm monofilament mainline and 50 to 100 gangions were deployed during May and July 2011 in areas of Florida Bay where adults are known to occur (Poulakis & Seitz 2004, Waters et al. 2014). Gangions were terminated with 16/0 non-offset circle hooks baited with ladyfish *Elops saurus*, little tunny *Euthynnus alletteratus*, or Atlantic mackerel *Scomber scombrus*. Longlines were anchored and marked with a buoy at each end. Soak times for gill nets and longlines were typically 1 h and did not exceed 2 h. Bull sharks and cownose rays were sampled when encountered during the smalltooth sawfish sampling described above or when caught on longlines (50 gangions with 15/0 non-offset circle hooks) baited with striped mullet *Mugil cephalus* in and near Charlotte Harbor. Captured individuals were taken to the research vessel, measured (stretch total length [STL] or disc width [DW] for cownose rays), and using clean scissors, a ~2 g fin clip was taken from the free rear tip of either dorsal fin of smalltooth sawfish (usually the 2nd dorsal fin) and bull sharks or from the left pelvic fin of cownose rays. Fin tissue samples were either placed immediately in 95% ethanol or frozen (–20°C).

To investigate resource use by the 3 focal elasmobranchs as well as their habitat use patterns, muscle tissue from representative consumer species from 4 broad functional groups (i.e. molluscs, crustaceans, teleosts, elasmobranchs) were sampled in the greater Charlotte Harbor estuarine system (see Olin et al. 2013b for details). Briefly, samples were collected between 2006 and 2008 using shallow water (<10 m) longlines as described above, seines (21.3 m long with 3.2 mm stretch mesh, center bag), and trawls (6.1 m wide with 38 mm stretch mesh and a 3.2 mm

stretch-mesh liner). Upon collection, all species were measured; standard length for teleosts, STL for sharks, DW for stingrays, and carapace width for crabs (all to the nearest mm). White muscle tissue (~1 g) was excised from the dorsum, anterior to the first dorsal fin of teleosts and from the dorsal surface of stingrays. Oysters and various crustaceans were dissected prior to drying, and muscle tissue was retained. Muscle tissue samples were stored frozen (–20°C). Taxonomy follows Page et al. (2013) for fishes and Williams (1984) for crustaceans.

Stable isotope analysis

$\delta^{13}C$ and $\delta^{15}N$ were analyzed in samples of fin tissue of the 3 focal elasmobranchs and of muscle tissue of the representative consumer species. Although analyzing samples collected over many years for stable isotopes introduces the potential temporal bias of resource pools, such an approach can allow for detection of robust patterns that surpass short-term and small-scale isotope variation (e.g. Layman et al. 2005, Heithaus et al. 2013). For complete description of stable isotope sample preparation, lab analyses, and analytical precision, see Olin et al. (2013b, 2014). Briefly, ethanol was evaporated from fin tissue samples in a hood for 48 h. All fin samples were then rinsed in distilled water, dried in an oven at 60°C for 72 h and homogenized using scissors. Muscle tissues were freeze-dried for 48 h, then ground, and lipid extracted by twice agitating the ground tissue in a 2:1 chloroform:methanol solution for 24 h and decanting the solvent (modified method of Bligh & Dyer 1959). The relative abundances of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were determined on ~1350 to 1550 µg subsamples of fin tissue and on ~500 µg subsamples of muscle tissue on a Delta^{Plus} mass spectrometer (Thermo Finnigan) coupled with an elemental analyzer (Costech). Analytical precision, based on the standard deviation of 3 standards (NIST 8414, bovine muscle, and internal fish lab standard, n = 177), ranged from 0.06 to 0.09‰ for $\delta^{13}C$ and from 0.08 to 0.21‰ for $\delta^{15}N$. Lipid extraction was not undertaken on the fin samples because the lipid content (Hussey et al. 2011) and C:N ratios (2.99 ± 0.12; mean ± SD) were so low.

To prepare isotope data for analysis, a regression correction was applied to the $\delta^{13}C$ values of all 3 elasmobranchs following Olin et al. (2014) to account for the effects of storage in ethanol. Data were then evaluated for normality using Shapiro-Wilks tests and for homogeneity of variance through visual inspection of probability plots. A Welch *t*-test was used to test for differences between sexes in $\delta^{13}C$ and $\delta^{15}N$. Differences in $\delta^{13}C$ and $\delta^{15}N$ values among the 3 elasmobranchs were analyzed using ANOVA, followed by Tukey's honestly significant difference post hoc test.

Regression analysis of $\delta^{13}C$ and $\delta^{15}N$ versus size was performed on the 3 elasmobranch species (Olin et al. 2011). Evidence of maternal influence was observed for smalltooth sawfish and bull sharks, but was not observed for cownose rays. To account for this, samples from the smallest smalltooth sawfish and bull sharks were omitted, and life-stage specific size classes were created for further analysis: juveniles ranged from 1000 to 2500 mm STL for the smalltooth sawfish (Scharer et al. 2012) and from 1000 to 1700 mm STL for the bull shark (Wintner et al. 2002). Large juveniles and adults were combined for the smalltooth sawfish (>2500 mm STL) and the bull shark (>1700 mm STL). All cownose ray samples were included in the analysis and were divided into 3 size classes based on disc width: young-of-the-year (<500 mm DW), juvenile (500 to 699 mm DW), and adult (≥700 mm DW; Poulakis 2013).

To compare the inter- and intra-specific trophic diversity of the post-maternal influence size classes of the 3 elasmobranchs, we used community metrics developed by Layman et al. (2007) and augmented by Jackson et al. (2011) using the stable isotope Bayesian ellipses routine (SIBER) implemented through stable isotope analysis (Parnell et al. 2010) in the R statistical program version 3.1.2 (R Development Core Team 2015). Standard ellipse area (SEA) was calculated using the variance and covariance of bivariate isotope data, bootstrapped to 10 000 iterations (Jackson et al. 2012). The SEAs contain approximately 40% of the data and represent a core isotopic niche for size classes of each species. SEAs were then corrected (SEA_c) to minimize bias caused by sample size (Jackson et al. 2011, 2012). The SEA_c was used to calculate the degree of isotopic niche overlap, with associated 95% confidence intervals, representing a quantitative measure of dietary similarity between size classes. Ranges represent the range in $\delta^{13}C$ and $\delta^{15}N$ values in a size class and, therefore, quantify the total range of $\delta^{13}C$ and $\delta^{15}N$ values exploited by each size class.

To compare inter- and intra-specific resource use of the size classes of the 3 elasmobranchs, SEA_c was calculated using their $\delta^{13}C$ and $\delta^{15}N$ data adjusted with the diet-tissue discrimination factors developed for elasmobranch fin tissues (Caut et al. 2013; 1.06‰ $\Delta^{13}C$ and 0.49‰ for $\Delta^{15}N$). The adjusted SEA_c ellipses were graphed over the mean (±SD) stable isotope

data of the representative consumer species pooled by 4 functional groups (i.e. molluscs, crustaceans, teleosts, elasmobranchs). It was expected that the predator's isotope values would center on the prey resources that comprised the majority of their diet (Phillips & Gregg 2003, Fry 2006, Olin et al. 2013a). All statistical analyses were evaluated at a significance level of $\alpha = 0.05$.

18S rRNA gene analysis

From 2004 through 2012, 4 smalltooth sawfish fecal samples were opportunistically obtained during normal field sampling; 1 each from 3 males (810, 1380, and 2026 mm STL) and 1 female (1025 mm STL). Samples were frozen, and a portion (0.2 wet g) of each sample was used for DNA extraction to determine whether fecal DNA could be used to augment our stable isotope studies.

First, to elucidate how DNA extraction methods might influence the diversity and distribution of sequences, 2 methods were compared using 2 of the samples: (1) use of the PowerSoil DNA isolation kit (MoBio Laboratories) and (2) modified phenol-chloroform DNA extraction (Urakawa et al. 2010). The concentration and quality of DNA were tested using a NanoDrop spectrophotometer (Thermo Fisher Scientific). After normalization, the number of operational taxonomic units (OTUs) identified by 10 000 reads, OTU diversity, and OTU sequence distribution were compared between the 2 methods using a 2-tailed, paired Student's *t*-test.

After the concentration adjustment and DNA quality check, DNA samples were sequenced using the Illumina MiSeq System at the Research and Testing Laboratory (RTL). The 18S rRNA gene was amplified using universal primers (TAReuk454FWD1 [CCA GCA SCY GCG GTA ATT CC] and TAReukREV3 [ACT TTC GTT CTT GAT YRA]; Stoeck et al. 2010) to target all eukaryotes and develop an approach that would include all potential smalltooth sawfish prey. To analyze sequence data, the forward and reverse reads were taken in FASTQ format and merged using PEAR Illumina paired-end read merger (Zhang et al. 2014). The formatted FASTQ files were then converted into FASTA-formatted files for subsequent analyses. These reads were put through an RTL-developed quality trimming algorithm and sorted by length from longest to shortest. USEARCH was used for the prefix dereplication process and clustering of sequences (4% divergence), in which sequences less than 100 bp and single clusters were removed (Edgar 2010). The result of this step was the consensus sequence from each new cluster.

OTU selection was performed using the UPARSE OTU algorithm (Edgar 2013) to classify the large number of clusters into OTUs. Chimera (i.e. artificial sequences that arise when partial sequences from 2 species join) checking was performed on the selected OTUs using the UCHIME chimera-detection software executed in de novo mode (Edgar et al. 2011), and these sequences were removed. As a final step, each read was mapped to its corresponding cluster using the USEARCH global-alignment algorithm (Edgar 2010). Using the consensus sequence for each centroid as a guide, each sequence in a cluster was aligned to the consensus sequence and each base was corrected. The centroid sequence for each OTU was used to determine taxonomic information through a National Center for Biotechnology Information (NCBI), basic local alignment search tool (BLAST).

For phylogenetic analysis, the 2 largest OTUs, one associated with the Actinopterygii and the other with the Elasmobranchii, were scanned based on motif sequences (i.e. group-specific short fragments of 18S rRNA gene sequences) to identify groups of teleosts and rays. Since 18S rRNA sequences of elasmobranchs known from our study area were not previously reported, we sequenced the smalltooth sawfish, Atlantic guitarfish *Rhinobatos lentiginosus* and the 5 other rays that occur with the smalltooth sawfish in the study area (Poulakis et al. 2004): southern stingray *Dasyatis americana*, Atlantic stingray *D. sabina*, smooth butterfly ray *Gymnura micrura*, spotted eagle ray *Aetobatus narinari*, and cownose ray. We also attempted to determine the 18S rRNA gene sequences of 1 unidentified ray *Dasyatis* sp. (tail fixed in 10% formalin; smalltooth sawfish was a 1244 mm STL female) and 1 unidentified teleost (fin clip fixed in 95% ethanol; smalltooth sawfish was a 1698 mm STL female) that had both been partly swallowed and were exposed in the mouths of captured smalltooth sawfish. These prey samples were digested with Proteinase K until dissolved, and the DNA was extracted as described above (Urakawa et al. 2010).

PCR amplification of the 18S rRNA gene was carried out using a newly designed primer set, FISH18Sf (CCT GGT TGA TCC TGC CA) and FISH18Sr (ACG GAA ACC TTG TTA CG), which targets both Actinopterygii and Elasmobranchii. The thermal profiles used for the 18S rRNA amplification included an initial denaturing step consisting of 94°C for 5 min followed by 35 cycles of denaturation at 94°C

for 30 s, annealing at 45°C for 30 s, and elongation at 72°C for 30 s. The final extension step was 72°C for 10 min. After gene amplification, the PCR products were purified using the GeneJET PCR purification kit (Thermo Fisher Scientific). The forward primer (FISH18Sf) or a specially designed primer for sequencing (ATT GGA GGG CAA GTC TGG TGC C) was used for Sanger sequencing (GenScript).

The genetic distances of OTU centroids and reference 18S rRNA gene sequences were calculated using the Tamura-Nei method (Tamura et al. 2013) and visualized as phylogenetic trees (neighbor-joining, maximum-likelihood, and minimum-evolution methods) with bootstrap value supports using MEGA v.6.06 (Tamura et al. 2013). A total of 405 aligned positions were used in the final dataset and all ambiguous positions were removed for each sequence pair. The high-throughput 18S rRNA gene sequences for smalltooth sawfish were deposited into the Genbank sequence read archive under accession number SRP069750. The 18S rRNA gene sequences for smalltooth sawfish and the 6 other rays we analyzed (see above) were deposited under accession numbers KU705511–17. The 18S rRNA gene sequence of one of the partly swallowed prey samples found in the mouth of a smalltooth sawfish (i.e. Carangidae) was deposited under accession number KU695531.

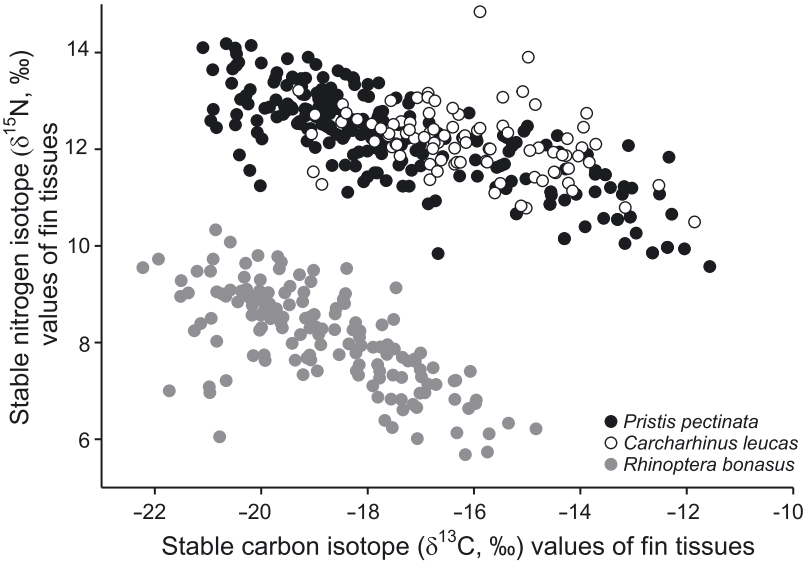

Fig. 1. Stable isotope values for fin tissue from all elasmobranchs sampled from waters of southwestern Florida

RESULTS

Stable isotopes

The 3 sympatric elasmobranchs exhibited a wide range of δ^{13}C (–22.2 to –11.6‰) and δ^{15}N (5.7 to 14.8‰) values (Fig. 1). No significant differences in δ^{13}C or δ^{15}N values were detected between sexes for any species (smalltooth sawfish *Pristis pectinata* δ^{13}C: t = –0.45, p = 0.656; δ^{15}N: t = –0.14, p = 0.886; bull shark *Carcharhinus leucas* δ^{13}C: t = 0.51, p = 0.611; δ^{15}N: t = 0.27, p = 0.785; cownose ray *Rhinoptera bonasus* δ^{13}C: t = 0.32, p = 0.742; δ^{15}N: t = 1.66, p = 0.337). Mean isotope values differed significantly among species (δ^{13}C: $F_{2,456}$ = 83.01, p < 0.001; δ^{15}N: $F_{2,456}$ = 1027.00, p < 0.001; Fig. 1); δ^{13}C and δ^{15}N values were lowest in cownose ray samples.

Intraspecific variation in isotopic values was associated with size for all 3 species (Fig. 2). For smalltooth sawfish, significant differences were detected between all size classes for both isotopes (δ^{13}C: $F_{2,216}$ = 138.00, p < 0.001; δ^{15}N: $F_{2,216}$ = 44.04, p < 0.001; Table 1). For bull sharks, significant size class differences were observed for δ^{13}C ($F_{2,88}$ = 5.24, p = 0.007) between young-of-the-year and juvenile size classes, but not between young-of-the-year and adults, suggesting maternal influence. No differences were detected for δ^{15}N values ($F_{2,88}$ = 2.76, p = 0.069; Table 1) from bull sharks. In cownose rays, differences were significant for δ^{13}C values ($F_{2,148}$ = 4.50, p = 0.013) and for δ^{15}N values ($F_{2,148}$ = 10.19, p < 0.001) (Table 1, Fig. 2); young-of-the-year individuals had the lowest isotope values.

Comparison of the isotopic niche within and among species indicated species-specific and size class (juvenile and large juvenile–adult only) differences. There was no overlap in SEA_c between size classes of smalltooth sawfish, suggesting that the positions of these size classes in isotope niche space were distinct (Fig. 3A, Table 2). The SEA_c of the 2 bull shark size classes overlapped by about 44% (Fig. 3A, Table 2). About 76% of the adult cownose ray SEA_c was contained within the SEA_c of the juvenile individuals (Fig. 3A, Table 2) and both size classes were clearly separated from smalltooth sawfish and bull shark. Comparisons among species showed that juvenile smalltooth sawfish δ^{13}C values were lower than bull shark values and the isotopic niche of large juvenile–adult smalltooth sawfish and bull sharks over-

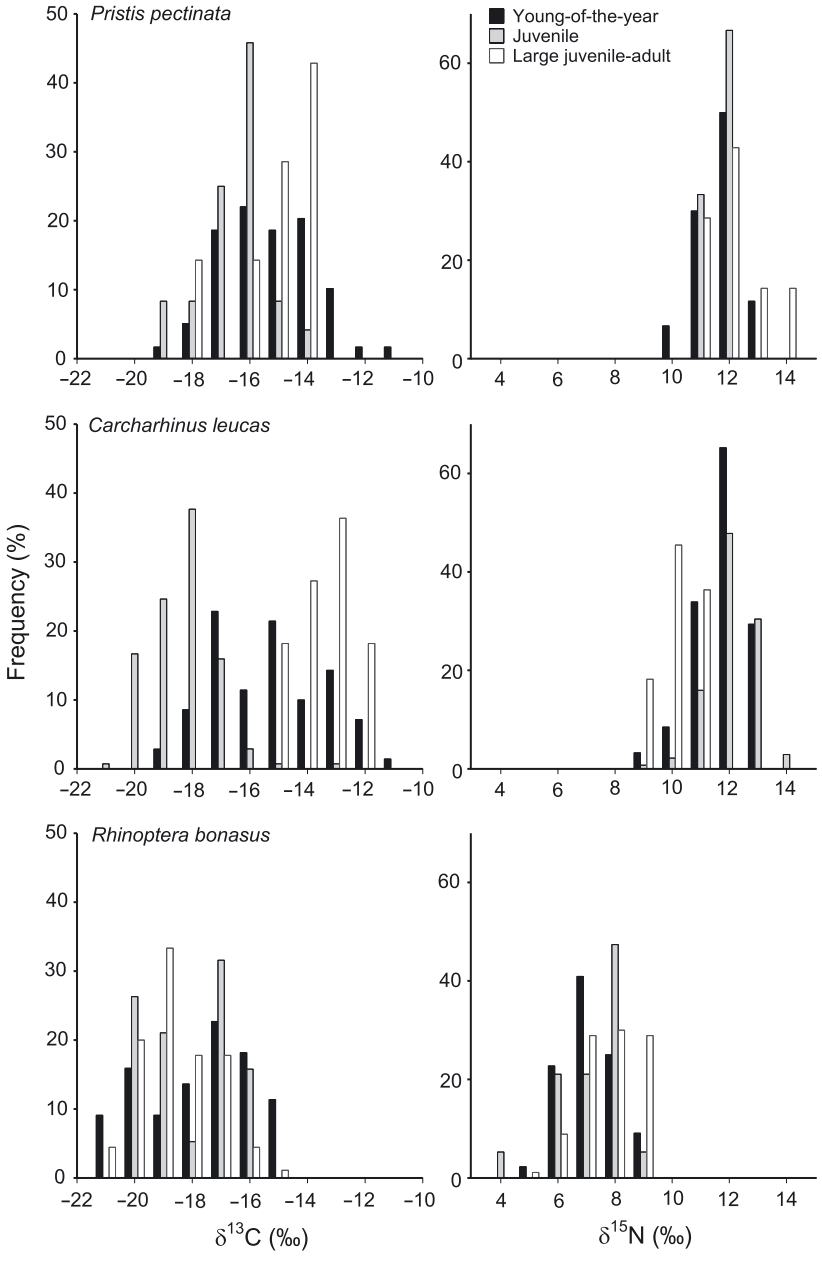

Fig. 2. Frequency distribution (%) of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios from fin tissues across size classes of smalltooth sawfish *Pristis pectinata*, bull shark *Carcharhinus leucas*, and cownose ray *Rhinoptera bonasus*

lapped (Fig. 3A). Ellipse areas were generally similar among size classes, except for the large juvenile–adult bull shark ellipse (Fig. 3B, Table 2). Based on the dispersion of $\delta^{15}N$ values, the isotopic area of large juvenile–adult bull sharks compared with that of juveniles suggests that large juvenile–adults were deriving their prey from multiple trophic levels.

For all 3 elasmobranch species, the carbon range was generally larger than that of nitrogen, suggesting that habitat influences stable isotopes more strongly than diet (Fig. 4). The adjusted ellipses of the juvenile size classes of smalltooth sawfish and bull sharks were similar in $\delta^{15}N$ position, suggesting feeding at the same trophic level — namely that of teleosts and other elasmobranchs. The $\delta^{13}C$ values among the largest individuals of smalltooth sawfish and bull sharks were more similar than those among juveniles and reflect ontogenetic movement out of the riverine systems (Fig. 4, Table 3).

18S rRNA gene analysis

After normalization (10 000 reads), OTU diversity and OTU sequence distribution were compared between the 2 DNA isolation techniques. The PowerSoil DNA isolation kit (MB) detected 5.9 ± 2.1 OTUs (mean ± SD), while the phenol-chloroform (P-C) method (MB1 and P-C1, and MB3 and P-C3) detected 7.6 ± 5.7 OTUs. There was no significant difference between the 2 methods (2-tailed paired Student's *t*-test: p = 0.73). The diversity and OTU distribution of sequences were also directly tested within the same pair of samples. Again, there was no significant difference between the 2 methods (2-tailed paired Student's *t*-tests: p = 0.13 and 0.29, respectively). Thus, we combined sequence reads from both extraction methods in subsequent analyses.

In total, 2.2 million 18S rRNA gene sequences were determined among 6 smalltooth sawfish fecal samples (n = 4 using MB, and n = 2 using P-C) (Fig. 5). After removing smalltooth sawfish sequences (86.3% of total counts) and unidentified sequences (1.8% of total), 4 kingdoms were identified: Animalia (90%), Chromalveolata (5%), Plantae (4%), and Fungi (1%) (Fig. 5A). All organisms identified in Chromalveolata, Plantae, and Fungi were microscopic and considered to be of sediment or ambient water origin. Among Animalia, 67% of sequences were identified as teleosts, while 4% were identified as rays. The contributions of Mollusca

Table 1. Mean (±SE; range) size (stretch total length or disc width; mm) and stable isotope values of elasmobranch fin tissues by size class. Superscript capital letters represent results of Tukey's post hoc tests for differences in isotope values among size class within each species. Values for size classes with the same letter were not significantly different ($\alpha = 0.05$)

Life stage	Length (mm)	n	No. of males	No. of females	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Smalltooth sawfish *Pristis pectinata*						
Young-of-the-year	851 ± 10.7 (671–996)	70	31	39	-15.7 ± 0.2^{A} (−11.6 to −21.1)	11.9 ± 0.1^{A} (9.6–14.2)
Juvenile	1445 ± 26.7 (1000–2187)	137	59	78	-18.8 ± 0.1^{B} (−13.9 to −21.1)	12.7 ± 0.1^{B} (9.8–14.2)
Large juvenile–adult	3604 ± 411.1 (2640–4090)	11	5	6	-13.9 ± 0.3^{C} (−12.4 to −16.0)	10.8 ± 0.2^{C} (9.9–11.9)
Bull shark *Carcharhinus leucas*						
Young-of-the-year	833.8 ± 11.2 (637–983)	59	28	31	-15.8 ± 0.2^{A} (−11.9 to −19.3)	12.1 ± 0.1 (10.5–13.2)
Juvenile	1446.7 ± 35.8 (1054–1664)	24	10	14	-17.0 ± 0.2^{B} (−14.3 to −19.1)	12.1 ± 0.1 (11.3–12.7)
Large juvenile–adult	1902.9 ± 91.8 (1750–2438)	7	3	4	-16.0 ± 0.5^{AB} (−14.1 to −18.4)	12.8 ± 0.5 (11.1–14.8)
Cownose ray *Rhinoptera bonasus*						
Young-of-the-year	378 ± 6.8 (314–493)	42	17	25	-18.3 ± 0.3^{A} (−14.8 to −21.7)	7.6 ± 0.1^{A} (5.7–9.5)
Juvenile	621 ± 15.2 (503–698)	19	11	8	-18.7 ± 0.3^{AB} (−16.3 to −20.9)	7.7 ± 0.3^{A} (6.0–9.0)
Adult	773 ± 3.4 (700–840)	89	30	59	-19.1 ± 0.1^{B} (−15.8 to −22.2)	8.4 ± 0.1^{B} (5.7–10.3)

(0.2% of total counts) were lower than for Nematoda (6%) and Cnidaria (9%); it is unlikely that any of these were consumed by smalltooth sawfish as prey (Fig. 5B). Considerable numbers of Arthropoda sequences were also found (14%), but 99.6% of these belonged to Harpacticoida copepods, while others were insects (0.1%) belonging to Diptera (i.e. fly) and Leucosiidae crabs (0.3%). The crab sequences were only found in 1 sample (P-C2) and formed 0.05% of total Animalia sequences. Thus, the high-throughput sequence data corroborated the evidence from stable isotope results that the smalltooth sawfish primarily consumes fishes (71% of total reads).

Based on the standard annotation pipeline, 85 OTUs were initially detected. We used the longest centroid sequence for each OTU for maximum-likelihood treeing and found that some OTUs positioned between Actinopterygii and Elasmobranchii in phylogenetic trees. Based on manual alignment and the BLAST search, these OTUs were identified as chimera sequences, even though standard chimera checking had been performed during initial sequence screening. As a result, a few sequences from teleosts were separated into front and rear parts, resulting in 91 OTUs, of which 7 were of teleost origin. Two OTUs were identified as Reeves shad *Tenualosa reevesii* (EU120031) with 100% identity (388 bp), while 5 other OTUs were identified as other teleost sequences with 100% identity (393 bp as longest) (Fig. 5C,D). Even though species such as *T. reevesii* do not occur in the study area, because 18S rRNA sequences are evolutionarily conserved, these matches likely represent other clupeids that do exist in the study area (e.g. scaled sardine *Harengula jaguana* or Atlantic thread herring *Opisthonema oglinum*; see Poulakis et al. 2004 for species list).

Using our knowledge of the ichthyofauna of the study area (Poulakis et al. 2004), we applied sequence matches from GenBank to identify possible teleost prey. The major teleost (OTU 19) showed 100% sequence identity with a wide range of teleost fish species that included striped mullet *Mugil cephalus*, but excluded species such as tarpon *Megalops atlanticus*. Clupeiform sequences (OTU 53) were found in a single sample (P-C2), while other teleost sequences were detected from all individuals (Fig. 5C,D). One DNA sequence from a prey fish sample that had been partly swallowed and was exposed in the mouth of a smalltooth sawfish belonged to the Carangidae (Fig. 5D). No information was obtained from the

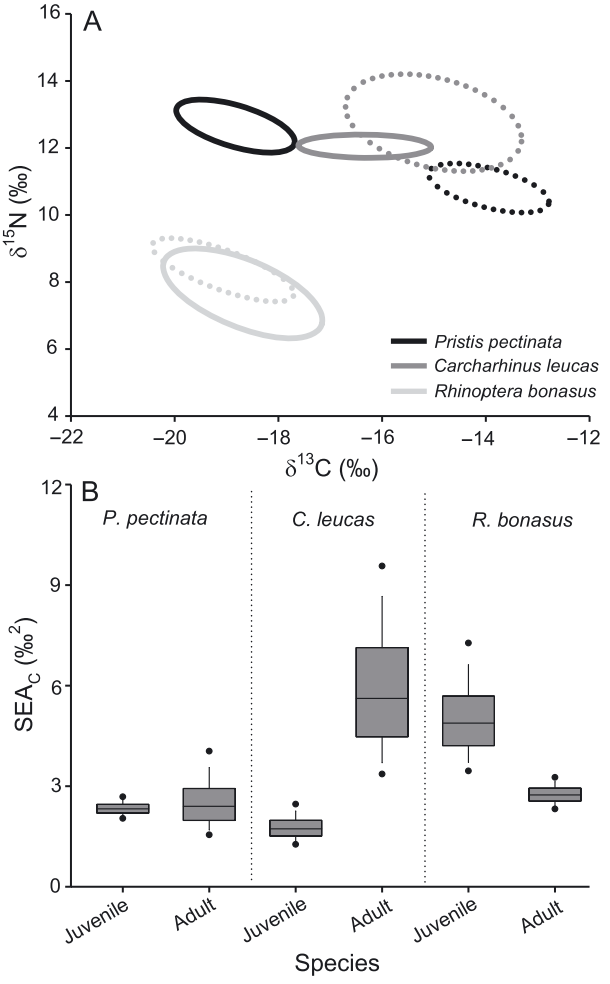

Fig. 3. (A) Standard ellipse areas corrected for sample size (SEA_c) of smalltooth sawfish *Pristis pectinata*, bull shark *Carcharhinus leucas*, and cownose ray *Rhinoptera bonasus*, juvenile (solid ellipses) and large juvenile–adult (dotted ellipses) size classes; (B) boxplots showing the 95% confidence intervals (black points) of the standard ellipse areas and the median, 10th, 25th, 75th and 90th percentiles for each taxon and size class. Adult: large juvenile–adult for smalltooth sawfish and bull shark

similarly exposed *Dasyatis* sp. tail due to the difficulty of gene amplification from the formalin-fixed sample.

Identification of Elasmobranchii required more detailed analysis due to the difficulty of separating smalltooth sawfish sequences from those of elasmobranch prey. The 18S rRNA gene sequence analysis of 6 local ray species (see '18S rRNA gene analysis') revealed that the smalltooth sawfish had an 18S rRNA gene sequence nearly identical to that of the Atlantic guitarfish *Rhinobatos lentiginosus* (1079/1080 bp identity with M97576). Thus, we could not distinguish Atlantic guitarfish from smalltooth sawfish sequences within the compared sequence region determined by high-throughput sequencing; however, this did not affect the analysis because Atlantic guitarfish do not occur with smalltooth sawfish in riverine habitats (Idelberger & Greenwood 2005). Five other ray sequences from species known to occur with smalltooth sawfish (Poulakis et al. 2004) were identical to each other within the compared sequence region, but distinguishable from those from reference skates and sharks (Fig. 5D). These ray sequences (100% identity) were found in fecal samples of 2 of the 4 individuals analyzed (Fig. 5C). Together with the *Dasyatis* sp. tail found in the mouth of a smalltooth sawfish, these data indicate that rays may be important prey for smalltooth sawfish.

DISCUSSION

Studying protected species is challenging given the limitations on the tools that can be used to minimize negative effects. This forces researchers to develop novel techniques or to apply tools in unique ways to address research questions. We used a nonlethal, comparative approach to provide insight into

Table 2. Comparison of stable isotope trophic diversity metrics resulting from isotopic niche analyses using the stable isotope Bayesian ellipses routine (SIBER) metric analyses (Jackson et al. 2011) adapted from community-level metrics developed by Layman et al. (2007) for the life-stage specific size classes of 3 elasmobranchs. n: sample size; NR: δ^{15}N range; CR: δ^{13}C range; SEA_c: standard ellipse area; SEA_c overlap fraction ranges from 0 to 1 and represents the amount of standard ellipse area overlap

Species	Life stage	n	NR	CR	SEA_c	SEA_c overlap within taxon	SEA_c overlap among taxa
Pristis pectinata	Juvenile	137	4.4	7.2	2.3	0.00	
	Large juvenile–adult	11	2.0	3.6	2.2		0.18
Carcharhinus leucas	Juvenile	24	1.4	5.1	1.4	0.44	
	Large juvenile–adult	7	3.7	4.6	7.3		0.25
Rhinoptera bonasus	Juvenile	19	4.7	4.6	5.2	0.76	
	Adult	90	4.6	6.5	2.7		0.00

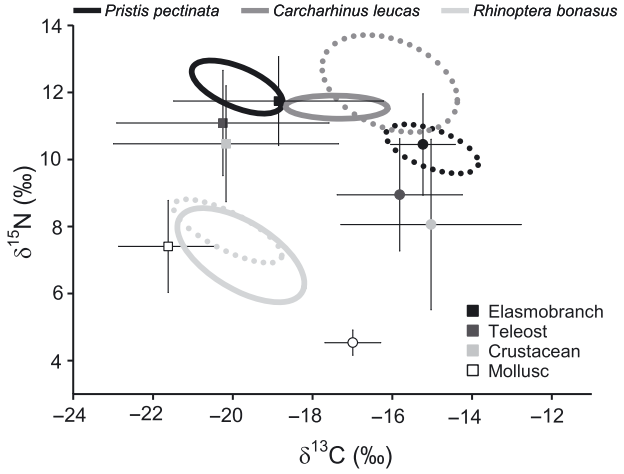

Fig. 4. Standard ellipse areas corrected for sample size (SEA_c) of the 3 elasmobranchs (juveniles: solid ellipse; large juvenile–adult: dotted ellipse), using $\delta^{13}C$ and $\delta^{15}N$ data corrected using diet-tissue discrimination factors of 1.06‰ for $\Delta^{13}C$ and 0.49‰ for $\Delta^{15}N$ (Caut et al. 2013), with stable isotope values of representative consumers (taxa means ± SD) sampled from the Caloosahatchee River (squares) and nearby polyhaline Pine Island Sound (circles) (see Tables 1 & 3 for stable isotope values of individual taxa)

the trophic ecology of the endangered smalltooth sawfish *Pristis pectinata*. Specifically, the application of stable isotope techniques on fin tissues of 2 sympatric elasmobranch species that have well-characterized diets (Snelson et al. 1984, Cliff & Dudley 1991, Collins et al. 2007), in conjunction with smalltooth sawfish, a variety of consumer species, and the 18S rRNA gene analysis has provided evidence to support the hypothesis that smalltooth sawfish feed primarily on fishes regardless of life stage. Further, this reliance on fish prey persists even though smalltooth sawfish and bull sharks *Carcharhinus leucas* move from estuaries to coastal habitats during their ontogeny (Simpfendorfer et al. 2005, Heupel & Simpfendorfer 2008, Scharer et al. 2012, Carlson et al. 2014, Waters et al. 2014). Moreover, our results support those of previous studies that suggested smalltooth sawfish and bull sharks partition their habitat while in the Charlotte Harbor estuarine system during their early life histories (see below). This realization has important implications for reaching informed management decisions and improving long-term recovery planning for the smalltooth sawfish.

The $\delta^{15}N$ values (used as a proxy for trophic level) of fin tissues presented here corroborate the known feeding behaviors of both bull sharks as piscivorous (Snelson et al. 1984, Cliff & Dudley 1991, Thorburn & Rowland 2008), and the cownose ray *Rhinoptera bonasus* as a consumer of invertebrates (Smith &

Table 3. Mean (±SE) muscle tissue stable isotope values of representative consumers in the Caloosahatchee River and Pine Island Sound areas of the Charlotte Harbor estuarine system in southwest Florida

Species	Caloosahatchee River			Pine Island Sound		
	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	n	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Molluscs						
Eastern oyster *Crassostrea virginica*	15	−21.6 ± 0.3	7.4 ± 0.4	5	−17.0 ± 0.3	4.5 ± 0.2
Crustaceans						
Blue crab *Callinectes sapidus*	21	−20.6 ± 0.6	10.7 ± 0.3	10	−14.0 ± 0.3	7.0 ± 0.3
Pink shrimp *Farfantepenaeus duorarum*	25	−19.8 ± 0.6	10.3 ± 0.4	6	−16.7 ± 1.2	9.8 ± 1.4
Teleosts						
Menhaden *Brevoortia* spp.	1	−17.8	11.0			
Atlantic spadefish *Chaetodipterus faber*	22	−20.5 ± 0.4	11.3 ± 0.3	1	−17.5	10.4
Atlantic thread herring *Opisthonema oglinum*	1	−20.9	9.9	2	−16.8 ± 0.2	9.1 ± 0.7
Pinfish *Lagodon rhomboides*	20	−20.0 ± 0.7	10.6 ± 0.5	6	−15.8 ± 1.0	7.1 ± 0.7
Gray snapper *Lutjanus griseus*	13	−17.9 ± 0.7	11.9 ± 0.3	8	−15.1 ± 0.3	9.3 ± 0.6
Lane snapper *Lutjanus synagris*	6	−16.5 ± 0.6	12.0 ± 0.1	5	−16.2 ± 0.4	9.3 ± 0.6
Hardhead catfish *Ariopsis felis*	49	−21.3 ± 0.3	11.9 ± 0.2	9	−15.6 ± 0.4	9.8 ± 0.4
Striped mojarra *Eugerres plumieri*	33	−20.8 ± 0.5	10.6 ± 0.3	14	−16.0 ± 0.5	8.7 ± 0.4
Tidewater mojarra *Eucinostomus harengulus*	15	−19.6 ± 1.0	9.6 ± 0.3			
Striped mullet *Mugil cephalus*	9	−19.9 ± 1.1	8.9 ± 0.2			
Crevalle jack *Caranx hippos*	4	−20.6 ± 0.2	13.7 ± 0.3			
Elasmobranchs						
Atlantic stingray *Dasyatis sabina*	10	−18.9 ± 0.8	11.8 ± 0.4			
Bonnethead shark *Sphyrna tiburo*				17	−15.3 ± 0.2	10.0 ± 0.3
Atlantic sharpnose shark *Rhizoprionodon terraenovae*				4	−15.9 ± 0.2	11.0 ± 0.4
Blacktip shark *Carcharhinus limbatus*				10	−14.9 ± 1.0	10.5 ± 0.6
Spinner shark *Carcharhinus brevipinna*				2	−14.9 ± 0.2	13.0 ± 0.2

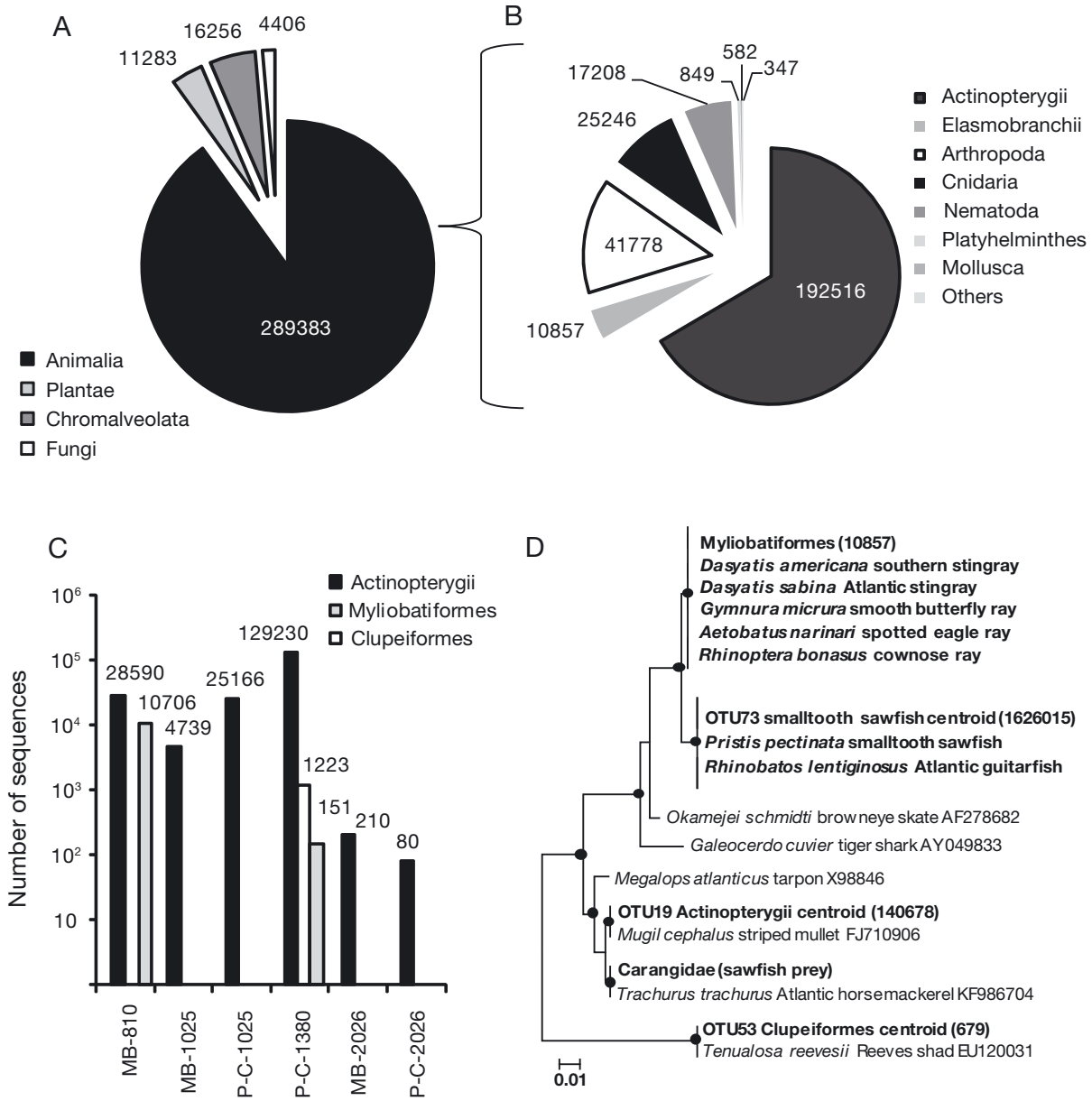

Fig. 5. Summary of 18S rRNA gene analysis of smalltooth sawfish *Pristis pectinata* fecal samples. (A) Sequence read composition by kingdom after removal of smalltooth sawfish DNA from fecal samples (values are numbers of counts); (B) sequence read composition of Animalia; (C) sequence types and numbers in each smalltooth sawfish fecal sample (note log scale on *y*-axis; letters and numbers on the *x*-axis refer to the DNA extraction method and stretch total lengths of smalltooth sawfish, respectively; see 'Materials and methods' for details). Identical operational taxonomic units (OTUs) identified by both methods were combined (see 'Results' for details); (D) neighbor-joining tree showing the genetic distances of 3 major OTU centroids (i.e. Elasmobranchii, Actinopterygii, Clupeiformes) and reference 18S rRNA gene sequences (bold: sequences determined in this study; non-bold: reference sequences from GenBank for comparison only; see 'Materials and methods' and 'Results' for details). Numbers in parentheses: count of sequences identified from smalltooth sawfish fecal samples. Nodes (black circles) have more than 70% bootstrap support by all treeing methods: neighbor-joining (1000 times), maximum-likelihood (100 times), and minimum-evolution (100 times). Bar: 0.01 estimated substitutions per site

Merriner 1985, Collins et al. 2007) at juvenile and adult life stages. Values of $\delta^{15}N$ in smalltooth sawfish were significantly higher than those in cownose rays, but not significantly different from those in bull sharks. However, complications in interpreting stable isotopes with respect to the diet of smalltooth sawfish and bull sharks during the first half of their first year do limit our inference. A controlled labora-

tory experiment evaluating isotopic turnover rates of fin tissues of growing neonate leopard sharks *Triakis semifasciata* estimated that fin tissues would reflect post-parturition diet at >190 d (Malpica-Cruz et al. 2012). Though we could not infer young juvenile diet directly using stable isotope values, our 18S rRNA gene analysis did include an 810 mm STL neonate smalltooth sawfish that ate fishes, suggesting that a dietary transition from benthic invertebrates to higher trophic-level prey (e.g. ontogenetic trophic level shift) does not occur. Additionally, a cursory evaluation of stomach contents from 36 neonate and larger young-of-the-year bull sharks from the Caloosahatchee River indicated a diet composed primarily of teleosts and elasmobranchs including catfishes and stingrays (J. A. Olin unpubl. data), further supporting the concept that the similarity of $\delta^{15}N$ values of the 2 species suggests a fish-based diet. Continued evaluation of fecal material via alternative mitochondrial gene analyses, or via isotopic analysis of structural components with higher rates of protein turnover (e.g. plasma) could provide further insight into dietary differences on short temporal scales (Hussey et al. 2012a, Matich & Heithaus 2014). Even with this consideration, our combined results do specify that the smalltooth sawfish feeds on teleost and elasmobranch fishes throughout its life.

On the basis of $\delta^{13}C$ values (used as a proxy for basal resources), juvenile size classes of smalltooth sawfish and bull sharks appear to partition their habitat in estuarine nurseries (Snelson et al. 1984, Simpfendorfer et al. 2005, Heupel & Simpfendorfer 2008, Poulakis et al. 2011, 2013). In the Charlotte Harbor estuarine system, both species are born within the Caloosahatchee River and other rivers where they have affinities for different salinities (smalltooth sawfish: 18 to 30 psu, Poulakis et al. 2011; bull shark: 7 to 20 psu, Heupel & Simpfendorfer 2008). However, bull sharks have a tendency to move out of the Caloosahatchee River during their first year, while smalltooth sawfish remain in the river for as long as 3 yr (Simpfendorfer et al. 2005, 2011, Poulakis et al. 2013), likely explaining the lack of overlap between isotopic ellipses on the $\delta^{13}C$ axis. For the large juvenile–adult size classes, outside of the river this habitat partitioning becomes more apparent between smalltooth sawfish and bull sharks. This is supported by recent observations of large juvenile smalltooth sawfish originally tagged in the Charlotte Harbor estuary recaptured further to the south in the Ten Thousand Islands and the Florida Keys (G. R. Poulakis unpubl. data), the region in which the largest smalltooth sawfish in this study were caught and sampled. Data from studies using pop-up archival transmitting tags on larger individuals of both species after leaving their nurseries have shown that smalltooth sawfish remain almost exclusively in shallow coastal waters (<10 m; Carlson et al. 2014), and while bull sharks occasionally use similar habitats, they use deeper waters and make large-scale movements (Brunnschweiler et al. 2010, Carlson et al. 2010). These tagging data, combined with the relatively small range of $\delta^{13}C$ for smalltooth sawfish and the broader range for bull sharks in this study, suggest that these 2 species continue partitioning their habitat after leaving the nursery. In contrast, cownose rays have been thought of as migratory in the Gulf of Mexico, but growing evidence suggests that non-migratory, estuarine populations exist at lower latitudes such as Charlotte Harbor and Tampa Bay (Collins et al. 2008, Poulakis 2013, B. L. Winner unpubl. data). This estuarine residency is supported by our $\delta^{13}C$ data, which suggest that this ray species, particularly young individuals, are available year-round as possible prey for smalltooth sawfish and bull sharks.

The overall isotopic niche of smalltooth sawfish, based on $\delta^{15}N$ values, did not change between juvenile and large juvenile–adult size classes based on ellipse size, despite the decrease with size in both $\delta^{15}N$ and $\delta^{13}C$ ranges. The overall contracted diet breadth, based on nitrogen and carbon ranges, between juveniles and large juvenile–adult size classes of smalltooth sawfish was driven largely by a shift in habitat, as evidenced by the contraction of the carbon range in the largest size class. The decreased range in $\delta^{15}N$ in smalltooth sawfish with size might indicate dietary preferences, which could have negative consequences if abundances of preferred prey became low. Although $\delta^{15}N$ was lower in the large juvenile–adult size class of smalltooth sawfish, they likely continued to feed on higher trophic-level species (including teleost and elasmobranch fishes) and were just assimilating a lower baseline nitrogen signature, which was consistent with consumer isotope values from non-riverine portions of the estuary such as Pine Island Sound (see Fig. 4). There was nothing in the fecal data to suggest a different interpretation, such as a more diverse diet with size, at least over the juvenile size range we analyzed (810 to 2026 mm STL). It may be informative to analyze fecal samples from adult smalltooth sawfish to further explore ontogenetic diet shifts (e.g. incorporation of small sharks into the elasmobranch prey group).

Our data provided insights on ontogenetic diet shifts in bull sharks and cownose rays. The isotope

values in bull shark samples suggest that the expansion of diet breadth at larger size classes is driven by $\delta^{15}N$. This is not surprising given that in this species the home range expands with increasing size, and in light of stomach-content studies that have identified as many as 41 prey species in its diet (Cliff & Dudley 1991, Hussey et al. 2012b). Compared with that for juvenile cownose rays, the isotopic niche was smaller for adults, which suggests that their feeding may become more specialized as they grow. However, stomach-content data presented by Collins et al. (2007) showed little difference between the diets of immature and adult cownose rays in the study area. This discrepancy could have resulted from the different diet assessment techniques or the maturity index used to characterize individual cownose rays for stomach-content analyses. Regardless, the cownose ray does not feed at the same trophic level as smalltooth sawfish and bull sharks, and more research is needed to evaluate size-related diet shifts in these species.

Understanding how communities are structured and how resources are used within them requires knowledge of the roles members play at multiple trophic levels, including unique or redundant trophic roles among species and their life stages (Kinney et al. 2011, Heithaus et al. 2013, Hussey et al. 2015). Studies that have attempted to dissect these complex, interconnected systems have shown both high degrees of interspecific difference in the diets of sympatric predator species (i.e. resource partitioning) and substantial dietary similarity depending on the species and ecosystems studied (Heithaus 2001, Kinney et al. 2011, Kiszka et al. 2011). The present study provided data on resource use by smalltooth sawfish in Florida and allowed inferences regarding pathways of energy flow through the ecosystem. On the basis of known movement patterns of smalltooth sawfish and bull sharks (Snelson et al. 1984, Simpfendorfer et al. 2005, Heupel & Simpfendorfer 2008, Poulakis et al. 2011, 2013) as well as their mean isotopic values for $\delta^{13}C$ and $\delta^{15}N$ observed in the present study, both species used similar resources (i.e. did not exhibit resource partitioning) and instead occupied available habitats at different times during their similar life histories (i.e. exhibited habitat partitioning). As has been suggested by recent studies in Africa and Australia (Kiszka et al. 2011, Heithaus et al. 2013), these results highlight the need for a combination of multi-species dietary and behavioral data for accurate interpretation of resource use and community function.

Multiple lines of evidence support the contention that the entire Charlotte Harbor estuarine system, including the highly altered Caloosahatchee River, acts as a nursery for the 3 sympatric elasmobranch species examined in this study. Predation on these species is low, and food resources are sufficient to support consistent annual recruitment and subsequent interannual residence, including critical habitat for juvenile smalltooth sawfish (Collins et al. 2008, Heupel et al. 2010, Norton et al. 2012, Scharer et al. 2012, Poulakis 2013). However, it is important to note that the Caloosahatchee River is a freshwater-flow managed system that sometimes experiences extreme, unnatural fluctuations in salinity. In light of recent data on variability in nekton assemblages under different freshwater flow regimes (Olin et al. 2013b, 2015), restoring water flow from Lake Okeechobee to the south toward the Everglades, rather than diverting it mostly down the Caloosahatchee River (as occurs now), may benefit the ecosystem on which the smalltooth sawfish depends for recovery. Maintaining healthy estuarine food webs is especially important for elasmobranchs because many, including the smalltooth sawfish, have high interannual site fidelity (e.g. parturition site fidelity, natal philopatry) and as such would probably continue to use the same nurseries even if habitats or environmental conditions were to degrade (Keeney et al. 2003, Hueter et al. 2004, Feldheim et al. 2014, K. A. Feldheim et al. unpubl. data).

Given that smalltooth sawfish and bull sharks do not appear to exhibit resource partitioning, changes to the salinity regime in this estuarine system may alter this apparent balance and promote reduced habitat partitioning, raising the concern of increased competition for available prey resources until natural flow regimes can be restored. Given this possibility, establishment of long-term fisheries-independent monitoring and evaluation of the resulting data may become useful in determining the health and availability of the fish prey these species rely on. For example, our analysis suggests that rays are important prey for smalltooth sawfish. Thus, ensuring that ray populations are healthy may be important, especially because most ray species are not managed or protected from harvest. Expanding on the results of the present study by increasing our knowledge of specific prey contributions to smalltooth sawfish diet, through expansion of fish 18S rRNA sequence databases, mitochondrial gene analyses, and stable isotope mixing models is warranted and may uncover size-specific or spatially explicit differences among habitats in Florida. These data would guide management considerations and promote recovery from species-specific and ecosystem perspectives.

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