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Using DNA barcoding to improve taxonomic resolution of the diet of red snapper (*Lutjanus campechanus*) along the Atlantic coast of the southeastern United States

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Abstract—Predator–prey interactions are a vital yet often under-studied regulator of marine fish population and community structure. However, fish prey species often cannot be identified by using morphological characters because of degradation from digestion. Consequently, diet is often poorly known for piscivorous predators. The study described here combined visual inspection and molecular diet analysis to increase taxonomic resolution for prey found in stomachs of red snapper (*Lutjanus campechanus*) (number of stomachs=105) along the Atlantic coast of the southeastern United States. Overall, the diet of red snapper from this region was diverse with 42 invertebrate and 28 vertebrate taxa identified. Broadly, shrimp were the most important prey consumed according to indices of relative importance (39.95%), followed by fish (34.38%) and crab (19.04%) species. In total, 19 fish prey species were identified by using DNA barcoding, compared with 2 species identified when visual methods alone were used. Results of the use of increased taxonomic resolution do not indicate significant predation by red snapper on other managed fish species in the snapper-grouper complex, indicating that the rebuilding stock of red snapper in the region is not affecting other managed species through direct predation.

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The importance of predator–prey interactions on marine fish population and community dynamics has been recognized for decades (Sissenwine, 1984), yet it remains challenging to apply these interactions in fisheries management. Most of the world's stock assessment models focus on only the dynamics and fishing pressure of a single-species stock, but ecosystem processes significantly affect productivity of fisheries (Skern-Mauritzen et al., 2016). Multispecies population models and other ecosystem-based fisheries management approaches that consider ecological interactions have recently been implemented in several countries (Essington and Punt, 2011; Kruse et al., 2012). In the United States, for example, ecological reference points have been developed for fishery management plans for species off the Atlantic coast of the southeastern United States from North Carolina to Florida (SEDAR, 2020), and fishery management plans for species off the coasts of mid-Atlantic states from New York to North Carolina now aim to conserve vital prey resources for managed fish populations (MAFMC, 2017). Incorporation of ecological interactions in stock assessments and fishery management plans is appropriate for species in reef ecosystems off northwest Florida because many predators and their prey have high site fidelity, and high spatial overlap among multiple life stages of exploited species has been observed (Addis et al.[1]).

Diet studies are common and provide valuable ecological information on resource competition, habitat use, trophic structure, energy flow, and seasonal variability, all important factors for ecosystem-based fisheries management. Diet studies also provide estimates of natural mortality based on predation rates, and these estimates

[1] Addis, D. T., W. F. Patterson III, and M. A. Dance. 2012. Site fidelity and movement of reef fishes tagged at unreported artificial reef sites off NW Florida. Southeast Data, Assessment, and Review SEDAR31-RD33, 8 p. [Available from website.]

often exceed rates of fishing mortality (Tyrrell et al., 2011). Despite the importance of diet data to ecosystem modeling, collection and analysis of such data are fraught with challenges, and incorporation of diet data in fishery management plans and ecosystem-based fisheries management efforts has been limited.

One challenge is that prey found in stomachs can be difficult to identify because of loss of distinctive features from digestion and overlapping meristics among closely related taxa. For example, fish prey consumed by gag (*Mycteroperca microlepis*) can be 50–75% digested within 8 h of ingestion (Berens and Murie, 2008). Similarly, in other studies, species-level resolution for fish prey of red lionfish (*Pterois volitans*) (Harms and Appledoorn[2]) and gray snapper (*Lutjanus griseus*) (Longley and Hildebrand, 1941) could not be attained visually just 5 h after ingestion. Capture of predators immediately following predation is difficult; therefore, prey items are commonly in advanced stages of digestion upon examination. Calcified fish structures, such as otoliths, are less digestible than flesh and have been used to confirm species identification and even to infer prey size; however, otoliths of many species are difficult to discern, and small otoliths can be digested rapidly (Granadeiro and Silva, 2000).

For the reasons described in previous paragraphs, visual methods of diet analysis rarely provide complete taxonomic resolution and, as a result, are poor indicators of the species composition of the diets of piscivorous fish species. Poor taxonomic resolution of prey in stomachs of fish can obfuscate estimates of dietary specialization and overlap with concomitant species, which are known drivers of reef-fish community structure (Longenecker, 2007).

Results of recent studies indicate that taxonomic resolution in analysis of the diets of piscivorous predators can be improved significantly by using DNA barcoding that sequences the cytochrome oxidase I mitochondrial gene of prey items (Aguilar et al., 2017; Dahl et al., 2017). The main objective of our study was to improve resolution of diet composition for red snapper (*L. campechanus*), a commercially and recreationally valuable piscivorous predator along the Atlantic coast of the southeastern United States, by using DNA barcoding and visual methods of taxa identification. Until now, the diet of red snapper in this region has been poorly known. Although there have been similar studies in the Gulf of Mexico (e.g., Szedlmayer and Brewton, 2019), predator–prey interactions are known to be ecosystem-specific (Hanson and Chouinard, 2002) and often are not comparable across regions. Improving the resolution of diet composition for red snapper will elucidate potential effects of this species as a predator on other managed species and identify prey resources that could potentially limit population growth along the Atlantic coast of the southeastern United States.

Materials and methods

Collection of predator samples

During 2017 and 2018, samples of red snapper were collected through routine sampling of the Southeast Reef Fish Survey, a fishery-independent sampling program for which multiple organizations collaborate. The primary sampling gear used by the Southeast Reef Fish Survey during this collection period was a chevron trap (Smart et al.[3]), baited with Atlantic menhaden (*Brevoortia tyrannus*). Chevron traps were deployed for ~90 min during daylight hours in sets of 6 traps, at least 200 m apart along live bottom habitat. Samples for diet analysis were selected by using a size class (total weight: 0–2500 g, 2501–7500 g, or >7500 g) and a latitude (1° bins from 31°N to 34°N) in a stratified sampling design. The first 3 specimens in each combination of size class and latitude whose stomachs were not everted or visibly damaged and that contained prey items were retained from each trap. Similarly, additional samples were opportunistically collected with unstandardized hook-and-line gear.

Red snapper were weighed to the nearest gram and measured to the nearest millimeter in total length (TL), fork length, and standard length. Stomachs were excised at sea from the esophagus to the pyloric sphincter, individually labeled and bagged, and placed in a freezer (–20°C) to halt digestion.

Processing of stomach contents

Frozen stomachs were immersed in water to thaw uniformly. Once thawed, all contents from individual stomachs were removed, with care taken to avoid scraping cells from the stomach itself. For examination of stomach contents of all fish captured in chevron traps, Atlantic menhaden were regarded as bait and discarded. Prey items of known bait species found in stomachs of fish captured with hook-and-line gear were also discarded (usually squid species or round scad, *Decapterus punctatus*). The remaining stomach contents were examined under a dissecting microscope and identified to the lowest taxonomic level possible, counted, and weighed (by wet weight to 0.001 g) by using a Sartorius[4] CPA223S analytical balance (Sartorius AG, Goettingen, Germany). A digestion code was assigned to each fish

[2] Harms, C. A., and R. S. Appeldoorn. 2013. Digestion rate analysis of fish prey items in lionfish (*Pterois volitans*). Poster presented at the 66th annual meeting of the Gulf and Caribbean Fisheries Institute; Corpus Christi, 4–8 November. Gulf Caribb. Fish. Inst., Marathon, FL.

[3] Smart, T. I., M. J. M. Reichert, J. C. Ballenger, W. J. Bubley, and D. M. Wyanski. 2015. Overview of sampling gears and standard protocols used by the Southeast Reef Fish Survey and its partners. Mar. Resour. Monit. Assess. Prect. Progr., MARAMP Tech. Rep. 2015-005, 14 p. [Available from website.]

[4] Mention of trade names or commercial companies is for identification purposes only and does not imply endorsement by the National Marine Fisheries Service, NOAA.

prey item to qualitatively assess digestion level (Smith et al., 2005; Fig. 1). Invertebrate prey were identified on the basis of morphology only, and digestion level was not assessed.

Molecular identification of fish prey

Muscle tissue from fish prey items that could not be morphologically identified to species level (digestion codes 2–3; Fig. 1) was retained for DNA barcoding. Tissue samples were rinsed with distilled water, preserved in a 2.5-mL vial containing 95% ethanol, and stored at −20°C. When possible, the most superficial layer was discarded to avoid contamination from the predator's stomach lining or other co-occurring prey items. Prey assigned a digestion code of 4 (Fig. 1) were not used for DNA barcoding because of predator contamination and low DNA yield in preliminary trials. Laboratory utensils were dipped in a 10% bleach solution and rinsed with distilled water between stomachs and between each prey item to prevent contamination. To isolate DNA, muscle tissue (~0.5 g) was blotted dry, placed in a microcentrifuge tube, and immersed in a 200-µL digestion solution, consisting of 145.5 µL nuclei lysis solution, 36.36 µL EDTA (0.5 M), 14.5 µL proteinase K (20 mg/mL), and 3.64 µL milli-Q water, and digested overnight at 55°C. The next morning, 180 mg of Promega Wizard SV lysis buffer (Promega Corp., Madison, WI) was added, and the sample was vortexed. Then DNA was isolated from the supernatant by using a Promega Wizard SV spin-column assembly according to the manufacturer's instructions.

A region of approximately 650 base pairs of the cytochrome oxidase I gene was amplified by using the universal fish primers Fish-F1 and Fish-R2 (Ward et al., 2005). A 25-µL polymerase chain reaction (PCR) volume consisted of 16.4 µL milli-Q water, 2.5 µL 1× 5PRIME HotMaster PCR Buffer (Qiagen Beverly Inc., Beverly, MA), 2.0 µL dNTPs, 2.0 µL $MgCl_2$, 0.3 µL each primer, 0.1 µL 5PRIME HotMaster *Taq* DNA polymerase (Qiagen Beverly Inc.), and 1.0 µL DNA template. The thermal cycler profile consisted of an initial denaturation at 94°C for 4 min, followed by 35 cycles of 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min, and a final extension at 72°C for 10 min (modified procedure from Ward et al., 2005). Each PCR contained a positive and negative control.

Polymerase chain reaction products were subjected to electrophoresis on a 1.5% agarose gel stained with ethidium bromide and then examined under ultraviolet light. Products were purified by using Affymetrix ExoSAP-IT (Thermo Fisher Scientific Inc., Waltham, MA) following the manufacturer's protocol. Samples were sent to Eurofins Genomics (Louisville, KY) for bidirectional Sanger sequencing with the same primers mentioned previously. Bidirectional sequences were aligned by using Sequencher, vers. 5.4 (Gene Codes, Ann Arbor, MI) and trimmed by using default criteria. Trimmed sequences were compared to their chromatograms and edited accordingly. Edited sequences were queried within the GenBank database of the National Center for Biotechnology Information (available from website) by using the Basic Local Alignment Search Tool (Altschul et al., 1990). A species was assigned to a prey item when a query sequence (minimum 300 base pairs) and a known reference sequence shared ≥98% similarity. If a species-level identification could not be assigned by using GenBank, the sequence was submitted to the Barcode of Life Database (available from website). Our edited sequences were deposited into GenBank (accession numbers MT580050–MT580051, MT582543–MT582580).

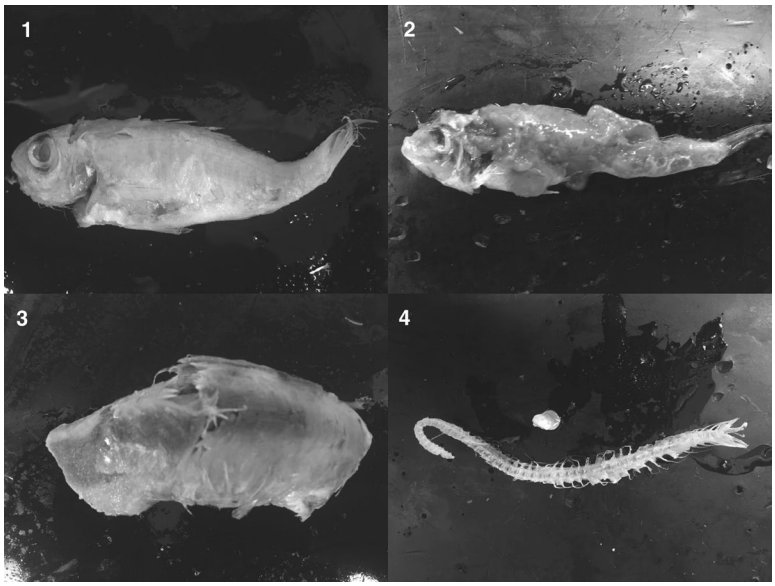

Figure 1

Images of prey items found in stomachs of red snapper (*Lutjanus campechanus*) collected along the Atlantic coast of the southeastern United States in 2017 and 2018, shown as examples of samples assigned to the 4 digestion codes used to qualitatively assess the condition of fish prey. The codes are (1) fresh, easy to identify visually; (2) partially digested: some skin or scales remain, many identifiable features remain; (3) mostly digested: most identifiable features absent, with some flesh attached to hard parts; and (4) severely digested: only hard parts (e.g., otoliths or vertebra) remain.

Data analysis

The relative contribution of prey items to the diet of red snapper examined in our study was described by using 3 traditional metrics: mean percentage by

weight (%W), mean percentage by number (%N), and frequency of occurrence (%FO) (Hyslop, 1980). Because samples were collected over a large spatial and temporal range, %W and %N analyses were modified to account for the patchy distribution of marine predators and their prey in space and time (Buckel et al., 1999). In our study, a *cluster* represented a set of traps or group of hook-and-line deployments consisting of various anglers and tackle configurations at a single location (reef patch). Indices of relative importance (IRI) (Cortés, 1997) were also calculated for each individual prey item and for prey aggregated into 10 broader taxonomic categories: amphipods, bivalves, bony fish species, cephalopods, copepods, crabs, polychaetes, shrimps, stomatopods, and tunicates. Differences in diet among size classes (300–500 mm TL, 501–700 mm TL, and 701–900 mm TL) and depths of capture (0–30 m, 31–60 m, and >60 m) for red snapper were also explored by using IRI.

A species accumulation curve was generated to evaluate whether sampling intensity sufficiently characterized the diet of red snapper. A linear model was used to assess the slope of the line fit to data for the last 5 stomachs (with an asymptotic slope <0.05 indicating a saturated curve because no additional prey taxa are expected to be encountered with additional sampling; Bizzarro et al., 2009). Species richness was extrapolated by using a first-order jackknife estimator to predict the number of prey species that would completely characterize the diet (Heltshe and Forrester, 1983). The species accumulation curve and extrapolated species richness were computed in R, vers. 3.5.2 (R Core Team, 2018) by using the package vegan, vers. 2.5-2 (Oksanen et al., 2018).

Results

Red snapper (319–854 mm TL; Fig. 2) were collected throughout the sampling area of the Southeast Reef Fish Survey from 31°N to 34°N (Fig. 3) and from depths of 23–72 m. In total, 105 stomachs from red snapper were analyzed: 86 specimens of red snapper were collected by using chevron traps and 19 specimens were captured by using hook-and-line gear. Generally, fish prey items were digested well (Fig. 4) such that only ~13% were visually identifiable to at least the genus (digestion codes 1–2; Fig. 1), with the majority of fish prey items (~71%) assigned a digestion code of 3. A total of 65 fish prey items from 48 stomachs were designated as *unidentified fish* after visual examination. For the 53 prey items from 30 stomachs subjected to molecular identification, the PCR success rate was ~89%. For ~87% of these prey items, usable sequences (mean length: 540 base pairs) were produced. Most specimens for whom DNA analysis resulted in failed PCR and sequencing reactions were in a late stage of digestion (digestion code 3), and the failed reactions were likely due to low DNA yield as evidenced by faint bands on agarose gels or low peaks on chromatograms. All PCR controls performed as expected.

DNA barcoding allowed 32 fish prey items that could not be identified visually to be identified to the species level, 6 unidentified items to be identified to the genus level, and 2 items to be identified to the family level (Suppl. Table). Therefore, by using DNA barcoding, 62% of all unidentified fish prey items in our study could be identified to at least the family level, and 58% and 49% of all unidentified fish prey items could be identified to the genus and species levels, respectively. Compared with the use of visual identification methods, use of DNA barcoding reduced the amount of unidentified fish prey items in the overall diet, with amount measured both by %FO, which decreased from 45.71% to 24.76%, and by %W, which decreased from 18.75% to 9.78%. All prey identified to species shared >99% sequence similarity with reference sequences in either the GenBank or Barcode of Life Database, except for 2 items identified as Atlantic midshipman (*Porichthys plectrodon*), which shared 98% sequence similarity with a reference sequence in GenBank (accession number KF930305.1). Sequences from the 2 most closely related fish prey items that were genetically identified to the species level, both as species in the genus *Synodus*, were 19.3% dissimilar.

Several prey items could not be identified to species. Specimens of prey identified as *Ophidion* sp. were just below the a priori defined species identification threshold, with 97% similarity in GenBank to the bank cusk-eel

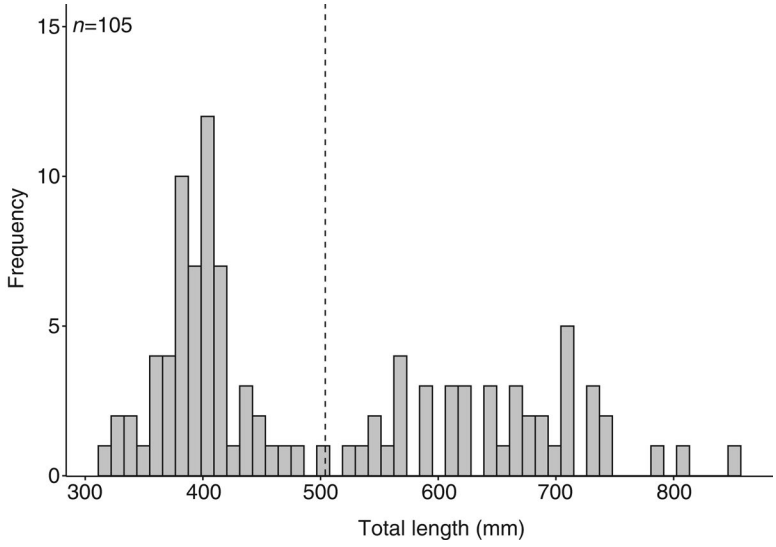

Figure 2

Size–frequency distribution of red snapper (*Lutjanus campechanus*) collected along the Atlantic coast of the southeastern United States in 2017 and 2018. The dashed line indicates the mean total length. *n*=sample size.

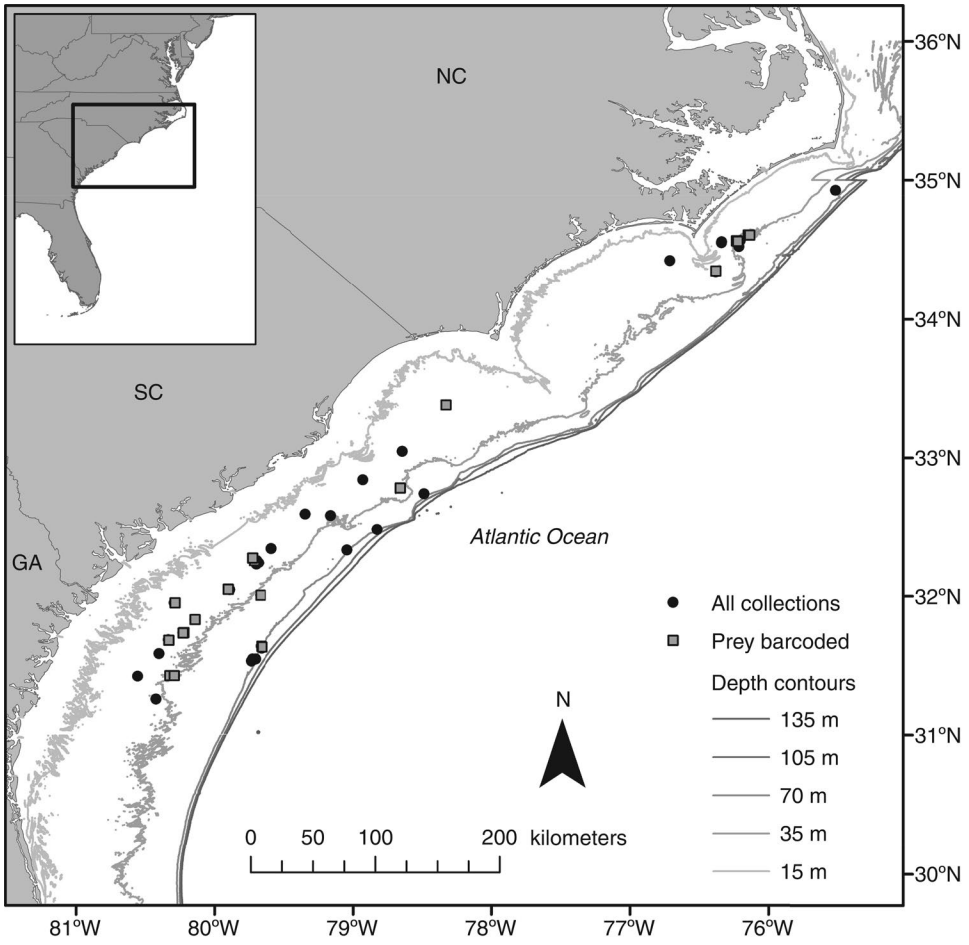

Figure 3

Map showing locations where red snapper (*Lutjanus campechanus*) were caught in 2017 and 2018 from Cape Hatteras, North Carolina (NC), to Saint Lucie, Florida. Black circles indicate capture locations for all red snapper collected. Gray squares indicate capture locations for red snapper from which prey items in stomach contents were sampled and identified with DNA barcoding. Depth contours are indicated by lines in various shades of gray. SC=South Carolina; GA=Georgia.

(*O. holbrookii*) (accession number GU702414.1). Ophidiidae is a paraphyletic family that has not been resolved well taxonomically. A neighbor-joining phylogeny generated in the Barcode of Life Database indicates that sequences from these specimens formed a monophyletic clade that includes bank cusk-eel and shorthead cusk-eel (*O. dromio*), both of which occur in the study area; therefore, we conservatively identified these specimens to genus. Specimens with sequences having top matches in GenBank to the scup (*Stenotomus chrysops*) (accession number HQ025017.1) or to the longspine porgy (*S. caprinus*) (accession number KJ012441.1) were identified conservatively as *Stenotomus* sp. because there is a difference of only one base pair (1%) between these reference sequences in GenBank and because both species occur within the study area.

Overall, the diet of red snapper captured along the Atlantic coast of the southeastern United States was diverse, with 42 invertebrate and 28 vertebrate taxa identified (Table 1). Generally, shrimp taxa composed the most important prey category consumed by red snapper in our study, on the basis of the IRI value (39.95%), followed by fish species (34.38%) and crab species (19.04%) (Fig. 5). More specifically, the most important prey taxa in the diet of red snapper, according to the IRI, were the brown rock shrimp (*Sicyonia brevirostris*), unidentified fish, portunid crabs, and the longspine swimming crab (*Achelous spinicarpus*), with IRI values of 32.58%, 28.85%, 7.14%, and 5.99%, respectively. Unidentified fish species composed the most dominant prey category by number (%N=12.97%) and occurred most frequently (%FO=24.76%), and the brown rock shrimp was the leading component of the diet by

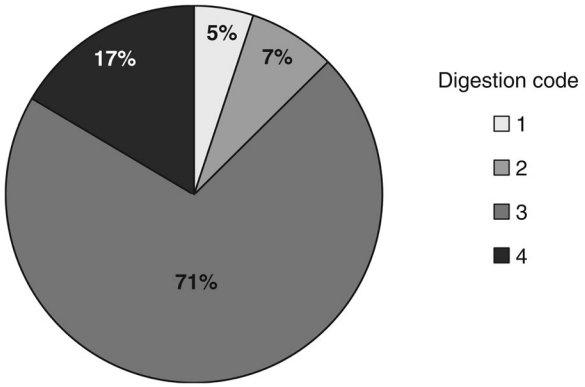

Figure 4

Proportion of fish prey found in stomachs of red snapper (*Lutjanus campechanus*) that were assigned to each of 4 digestion codes used to qualitatively assess the condition of fish prey. The codes are (1) fresh, easy to identify visually; (2) partially digested: some skin or scales remain, and many identifiable features remain; (3) mostly digested: most identifiable features absent, with flesh attached to vertebra; and (4) severely digested: only hard parts (e.g., otoliths or vertebra) remain. Red snapper were sampled along the Atlantic coast of the southeastern United States in 2017 and 2018.

weight (%W=21.25%). A total of 19 different species of fish were identified as prey. The most frequently consumed fish species were the bluespotted searobin (*Prionotus roseus*) (%FO=6.67%), snake eels (*Ophichthus* spp.) (%FO=3.81%), porgy species (*Stenotomus* spp.) (%FO=3.81%), the inshore lizardfish (*S. foetens*) (%FO=3.81%), and the tomtate (*Haemulon aurolineatum*) (%FO=2.86%).

Fish species formed the most important prey category, on the basis of IRI values across all size classes of red snapper, especially for the size classes of 300–500 mm TL (59.26%) and 701–900 mm TL (61.06%), in comparison with the size class of 501–700 mm TL (34.49%) (Fig. 6). Shrimp species composed a prey category that was more important in the diet of the size classes of 300–500 mm TL (IRI=30.14%) and 501–700 mm TL (IRI=28.85%) than in the diet of the size class of 701–900 mm TL (IRI=2.17%). Crab species were more important in the diet for the size classes of 501–700 mm TL (IRI=30.08%) and 701–900 mm TL (31.82%) than in the diet for the size class of 300–500 mm TL (IRI=8.08%). Fish species formed the most important prey category, according to the IRI, for red snapper captured at depths <30 m (74.12%) and at depths of 31–60 m (44.64%), but shrimp species were the dominate prey at depths >60 m (65.46%) (Fig. 7).

The slope of the fit of a linear model to data for the last 5 randomly sampled stomachs in the prey species accumulation curve was 0.32; therefore, the curve was not considered to reach an asymptote (Fig. 8). According to the first-order jackknife estimator, approximately 107 different prey species would be expected to have been identified, indicating that we taxonomically described ~64% of the diet of red snapper along the Atlantic coast of the southeastern United States.

Discussion

In our study, we used DNA barcoding to supplement visual identification of prey items to improve taxonomic resolution of the diet composition of red snapper along the Atlantic coast of the southeastern United States. We were able to identify a total of 19 fish prey species, using DNA barcoding. If we had relied on visual methods alone, we would have identified only 2 species and described only 10% of the total species richness of fish prey. Currently, the fishery management plan for the snapper-grouper complex in the Atlantic Ocean off the southeastern United States manages only 3 of these 19 species: the vermilion snapper (*Rhomboplites aurorubens*), the red porgy (*Pagrus pagrus*), and *Stenotomus* sp. The %W for both vermilion snapper and red porgy was <1% and for *Stenotomus* sp. was <6% in the diet of red snapper from the region in our study (Table 1).

Our findings agree with results of DNA barcoding studies done in the Gulf of Mexico that found negligible feeding on vermilion snapper (%W=1.01%), red porgy (%W=0.00%), and *Stenotomus* sp. (%W=1.31%) (Tarnecki and Patterson, 2015; Szedlmayer and Brewton, 2019). Further, feeding behavior related to ontogeny (i.e., size) of red snapper in our study was similar to observations of the diet of red snapper in the Gulf of Mexico, including that the largest fish consumed primarily fish and crab species (Wells et al., 2008). In other diet studies for red snapper in the Gulf of Mexico (McCawley and Cowan, 2007; Tarnecki and Patterson, 2015) significant consumption (>20%) of zooplankton, especially by larger fish, was observed. Such a level of consumption was not evident in our study, possibly because of under-representation of larger individuals in our study, seasonal abundance of zooplankton that did not coincide with the timing of our sampling, or other differences between ecosystems.

In our study, sequences sampled from most prey assigned to a species were >99% similar to reference sequences, exceeding the a priori sequence similarity threshold of 98% for species-level resolution. Given that Ward et al. (2005) reported average interspecific distances of 9.93% for marine fish species within the same genus and that sequences from the 2 most closely related species found in our study (both in the genus *Synodus*) were >19% dissimilar, we are confident that the 98% threshold for assigning a species in our study was appropriate and conservative for the goal of limiting false-positive species identifications. The inability to genetically distinguish between specimens in the genera *Ophidion* and *Stenotomus* in our study may be attributable to incomplete taxonomic coverage in the reference databases or to misidentification of the voucher specimens from which these reference sequences were generated (Stavrou et al., 2018).

The species accumulation curve did not achieve saturation, indicating that sampling intensity in our study

Table 1

Diet composition for red snapper (*Lutjanus campechanus*) sampled along the Atlantic coast of the southeastern United States in 2017 and 2018, based on analysis of stomach contents. For prey found in stomachs, frequency of occurrence (%FO), mean percentage by number (%N), mean percentage by weight (%W), and index of relative importance (IRI) values are provided. Taxa of prey items were identified by using visual and molecular methods combined. An asterisk (*) denotes species identifications determined by using DNA barcoding only. Prey items that could not be identified to species are designated as unidentified (unid.) members of families or other taxa.

Phylum, class or other taxa	Scientific name	Common name	%FO	%N	%W	IRI (%)
Polychaeta						
	Amphinomidae	Unid. bristle worm	2.86	0.96	0.11	0.16
	Spionidae	Unid. spionid polychaete	0.95	0.18	0.00	0.01
	Polychaeta	Unid. polychaete	0.95	0.31	0.02	0.02
Mollusca						
	Pectinidae	Unid. scallop	0.95	0.14	0.00	0.01
	Tellinidae	Unid. tellin clam	1.90	0.31	0.01	0.03
Cephalopoda						
	Illex sp.	Unid. shortfin squid	0.95	1.35	1.35	0.13
	Loligo sp.	Unid. longfin squid	0.95	0.45	1.08	0.08
	Teuthida	Unid. squid	8.57	2.81	2.75	2.46
	Octopus sp.	Unid. octopus	0.95	0.18	0.88	0.05
	Octopoda	Unid. octopus	1.90	0.76	0.20	0.09
Copepoda						
	Calanoida	Unid. calanoid copepod	1.90	1.80	0.00	0.18
Stomatopoda						
	Squillidae	Unid. mantis shrimp	5.71	2.58	3.50	1.79
Amphipoda						
	Ampeliscidae	Unid. red-eyed amphipod	0.95	0.16	0.00	0.01
	Hyperiidea	Unid. hyperiid amphipod	0.95	1.80	0.00	0.09
Decapoda						
	Leptochela papulata	Light glass shrimp	5.71	5.24	0.43	1.67
	Mesopenaeus tropicalis	Salmon shrimp	1.90	0.86	0.62	0.15
	Penaeoidea	Unid. penaeoid shrimp	9.52	3.59	1.00	2.25
	Rimapenaeus constrictus	Roughneck shrimp	2.86	1.16	0.34	0.22
	Sicyonia brevirostris	Brown rock shrimp	21.90	7.58	21.25	32.58
	Sicyonia sp.	Unid. rock shrimp	9.52	2.61	3.18	2.84
	Sicyoniidae	Unid. rock shrimp	0.95	0.31	0.02	0.02
	Solenoceridae	Solenocerid shrimp	0.95	2.76	1.53	0.21
	Stomatopoda	Unid. mantis shrimp	3.81	0.51	0.02	0.10
	Decapoda	Unid. shrimp	0.95	0.12	0.01	0.01
	Achelous ordwayi	Redhair swimming crab	4.76	2.39	2.59	1.23
	Achelous spinicarpus	Longspine swimming crab	14.29	5.02	3.11	5.99
	Achelous sp.	Swimming crab	2.86	1.84	1.45	0.48
	Portunidae	Unid. swimming crab	11.43	6.77	5.33	7.14
	Albunea catherinae	Mole crab	0.95	0.08	0.46	0.03
	Calappa flammea	Flame box crab	3.81	3.03	4.44	1.47
	Ethusa mascarone	Stalkeye sumo crab	0.95	0.12	0.03	0.01
	Hepatus epheliticus	Calico box crab	1.90	0.29	0.60	0.09
	Hepatus pudibundus	Flecked box crab	0.95	0.14	0.19	0.02
	Inachinae	Unid. spider crab	0.95	0.58	0.33	0.04
	Ovalipes stephensoni	Coarsehand lady crab	4.76	1.67	3.83	1.35
	Ovalipes sp.	Unid. lady crab	3.81	2.62	2.64	1.03
	Pilumnus sp.	Unid. hairy crap	0.95	0.14	0.08	0.01
	Pinnixa sp.	Unid. pea crab	0.95	0.31	0.02	0.02
	Ranilia muricata	Muricate frog crab	0.95	0.14	0.41	0.03
	Raninidae	Unid. frog crab	0.95	0.12	0.01	0.01
	Brachyura	Unid. true crab	1.90	0.99	0.04	0.10

(*Continued on next page*)

Table 1 (*continued*)

Phylum, class or other taxa	Scientific name	Common name	%FO	%N	%W	IRI (%)
Urochordata						
	Urochordata	Unid. tunicate	3.81	1.49	5.76	1.43
Pisces						
	Actinopterygii	Unid. ray-finned fish	24.76	12.97	9.78	28.85
	Anguilliformes	Unid. eel	0.95	0.45	0.27	0.04
	*Ariosoma balearicum**	Bandtooth conger	1.90	1.43	2.80	0.42
	*Conger oceanicus**	Conger eel	0.95	0.12	0.02	0.01
	Ophichthidae	Unid. snake eel	1.90	0.84	0.97	0.18
	*Echiophis intertinctus**	Spotted spoon-nose eel	0.95	0.31	0.52	0.04
	*Ophichthus puncticeps**	Palespotted eel	0.95	0.31	0.72	0.05
	*Saurida brasiliensis**	Largescale lizardfish	0.95	0.12	0.06	0.01
	*Synodus foetens**	Inshore lizardfish	3.81	3.02	0.19	0.63
	*Synodus poeyi**	Offshore lizardfish	0.95	0.16	0.14	0.01
	*Bregmaceros cantori**	Striped codlet	1.90	0.25	0.06	0.03
	Ophidion sp.*	Unid. cusk-eel	3.81	2.02	1.57	0.71
	*Porichthys plectrodon**	Atlantic midshipman	0.95	0.90	1.27	0.11
	Carangidae	Unid. jack	0.95	0.68	0.58	0.06
	Decapterus punctatus	Round scad	0.95	1.35	1.35	0.13
	*Citharichthys macrops**	Spotted whiff	0.95	0.18	0.00	0.01
	*Syacium papillosum**	Dusky flounder	0.95	0.14	0.15	0.01
	*Halichoeres caudalis**	Painted wrasse	0.95	0.45	0.50	0.05
	Haemulidae	Unid. grunt	0.95	0.68	1.23	0.09
	*Haemulon aurolineatum**	Tomtate	2.86	0.58	0.30	0.13
	Rhomboplites aurorubens	Vermilion snapper	0.95	0.68	0.77	0.07
	*Pagrus pagrus**	Red porgy	0.95	0.31	0.01	0.02
	Stenotomus sp.	Unid. porgy	3.81	3.28	5.96	1.82
	*Sphoeroides dorsalis**	Marbled puffer	0.95	0.58	0.08	0.03
	*Serraniculus pumilio**	Pygmy sea bass	0.95	0.31	0.22	0.03
	*Prionotus roseus**	Bluespotted searobin	6.67	1.62	0.84	0.85

may have been inadequate to completely characterize the diet of red snapper. However, because red snapper had a broad range of sizes, because the samples were collected from a large area in a range of depths, and because the species richness of the prey found in stomachs of the sampled red snapper was close to the predicted prey species richness, we are confident that the most important prey were accurately characterized. Further, the species accumulation curves used in investigations of the diets of marine fish species unlikely approach asymptote when a species-level taxonomic resolution is used for generalist predators (Preti et al., 2012). Therefore, only rare prey, which are likely opportunistically or inadvertently consumed and not considered significant diet components (Byron and Link, 2010), may be absent from this diet description. Red snapper persistently fed on few prey taxa, such as fish, portunid crab, and sicyoniid rock shrimp species, while sporadically feeding on a variety of other prey found over both reef and open sand habitats in our study, a result that is consistent with findings from a study in the Gulf of Mexico (Szedlmayer and Lee, 2004). The generalist use of resources by red snapper and our increased ability to identify prey items to the species level by using DNA barcoding likely explain the unsaturated species accumulation curve in our study.

We did not find evidence of significant predation by red snapper on other fish species managed as part of the snapper-grouper complex along the Atlantic coast of the southeastern United States, indicating that the rebuilding stock of red snapper in this region is not affecting other managed species through direct top-down control. However, competitive interactions have been identified as a major source of density-dependent mortality in marine systems (Hixon and Jones, 2005), and results from a seminal study (Pope, 1979) indicate that competitive interactions can have greater influence than direct predation on maximum sustainable yield in marine systems. Furthermore, competitive interactions may inhibit recovery of depleted stocks of marine fish species by limiting energy-rich prey necessary for reproductive success, even after fishing pressure is drastically reduced (Lambert and Dutil, 2000). Because of their broad diet, red snapper could be competing with other species. Therefore, additional studies on predator–prey interactions

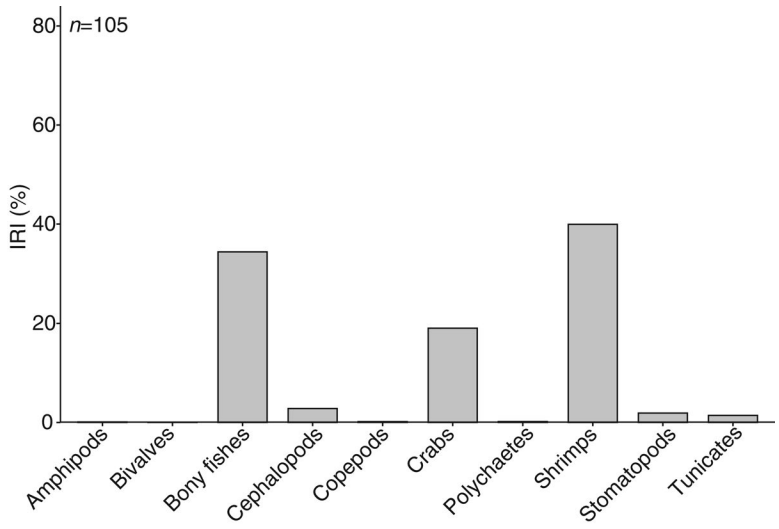

Figure 5

Generalized diet composition by prey category, according to index of relative importance (IRI) values based on examination of stomach contents of red snapper (*Lutjanus campechanus*) caught along the Atlantic coast of the southeastern United States in 2017 and 2018. Prey items were identified by using a combination of visual and DNA barcoding methods. *n*=number of stomachs analyzed.

of co-occurring predators within this ecosystem are necessary to elucidate competitive interactions between red snapper and other managed species in the region. Forage fish species, such as mackerel and anchovy species, that are extensively preyed upon by other fish species have recently been recognized as important ecosystem components and have been included in fishery management plans in the region and in bordering states (MAFMC, 2017; Federal Register, 2021). In our study, however, no specific prey emerged as strong candidates for management as potential bottom-up control mechanisms of population growth for red snapper in the region.

Conclusions

Our study addressed the critical need for detailed trophic information necessary to assess specific ecological effects of a rebuilding red snapper population along

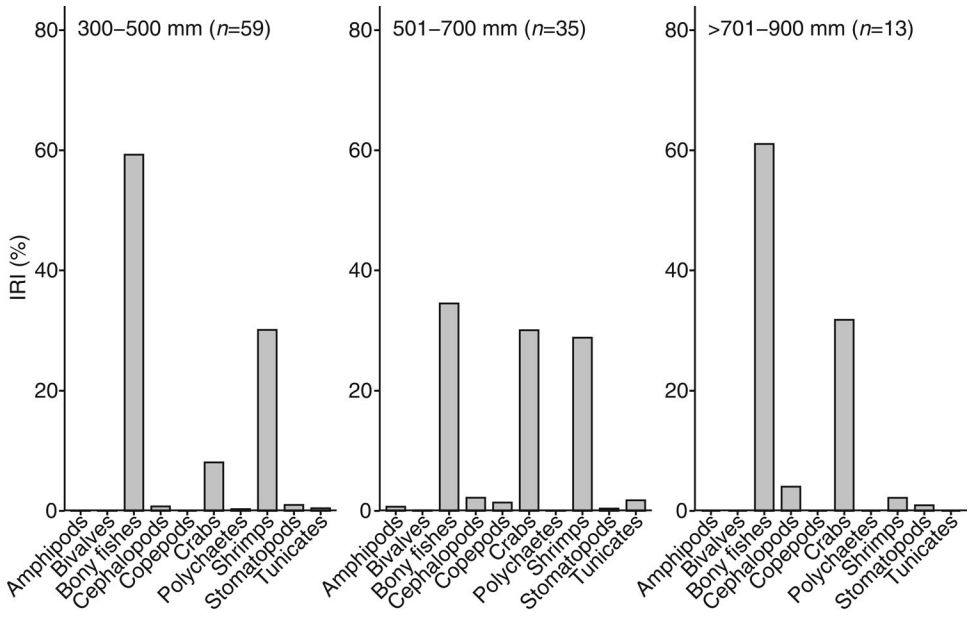

Figure 6

Generalized diet composition by size class and prey category, according to index of relative importance (IRI) values based on examination of stomach contents of red snapper (*Lutjanus campechanus*) caught along the Atlantic coast of the southeastern United States in 2017 and 2018. Prey items were identified by using a combination of visual and DNA barcoding methods. The size classes of red snapper are 300–500 mm in total length (TL), 501–700 mm TL, and 701–900 mm TL. *n*=number of stomachs analyzed.

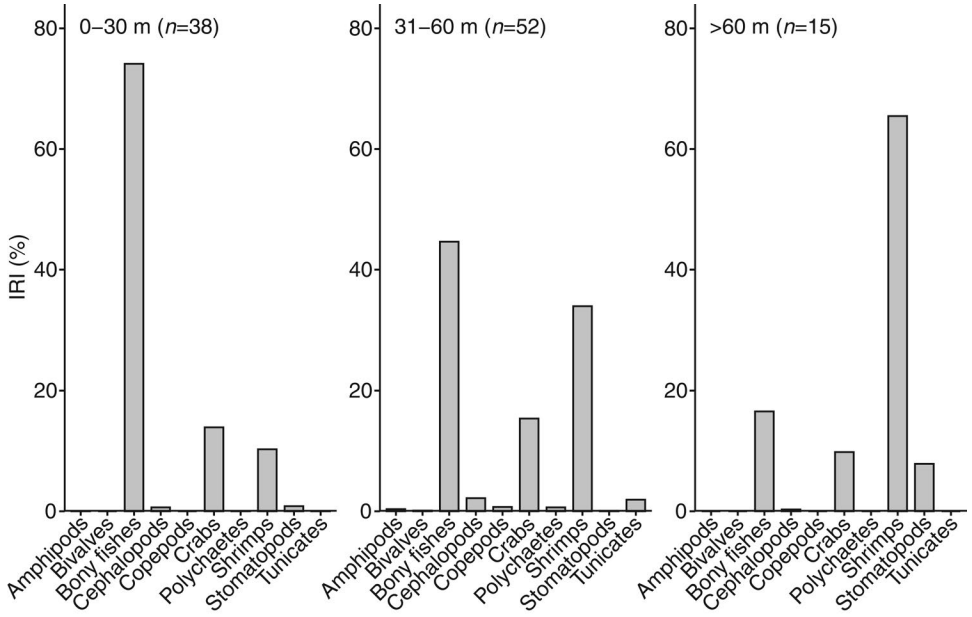

Figure 7

Generalized diet composition by depth and prey categories, according to index of relative importance (IRI) values based on examination of stomach contents of red snapper (*Lutjanus campechanus*) caught along the Atlantic coast of the southeastern United States in 2017 and 2018. Prey items were identified by using a combination of visual and DNA barcoding methods. The categories for depths at which red snapper were captured are 0–30 m, 31–60 m, and >60 m. *n*=number of stomachs analyzed.

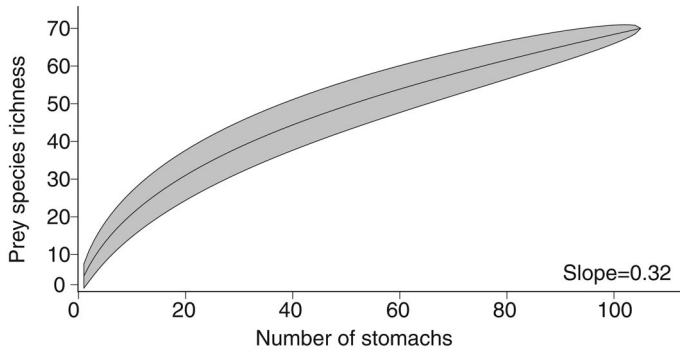

Figure 8

Species accumulation curve for prey found in stomachs of red snapper (*Lutjanus campechanus*) caught along the Atlantic coast of the southeastern United States in 2017 and 2018. Prey were identified by using a combination of visual and DNA barcoding methods. The shaded polygon represents the 95% confidence interval. The slope of a linear regression fit to data in the curve for the final 5 randomly sampled stomachs is given.

the Atlantic coast of the southeastern United States. Here, we provide results indicating that red snapper in this region are generalist predators that consume a wide range of invertebrate and vertebrate prey. Because the red snapper is a generalist predator, no individual prey species emerged as a resource that could potentially limit population growth and therefore merit management concern. Only minimal predation on other species of management concern was observed. Although we believe that the most significant prey of red snapper were documented in our study, additional sampling should be continued to further characterize the composition of prey species. Additional studies on spatial and temporal variability in the diet of red snapper and other co-occurring species would also significantly benefit the advancement of ecosystem-based fisheries management for the snapper-grouper complex in the region.

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