Received Date: 21-Jan-2016
Accepted Date: 03-Oct-2016
Article Type: riginal Article
Received: January 21, 2016
Accepted: October 3, 2016

Combined gut-content and stable isotope trophic analysis of the pelagic stingray *Pteroplaytrygon violacea* (Bonaparte, 1832) diet from the western North Atlantic Ocean

By T. A. Weidner, A. C. Hirons, A. Leavitt, and D. W. Kerstetter

Nova Southeastern University Halmos College of Natural Sciences and Oceanography, Dania Beach, Florida, USA

Summary

The understanding of trophic relationships is vital for correctly modeling ecosystems and ecosystem effects of fisheries removals. The pelagic stingray is found in epipelagic sub-tropical and tropical waters worldwide and is a common bycatch in pelagic longline fisheries. Between August 2008 and November 2011, 156 specimens (81 males; 75 females) were collected during pelagic longline fishing operations in the U.S. South Atlantic Bight and Gulf of Mexico. Stomach content analyses found that the major prey items were cephalopod molluscs (59.18%), followed by actinopterygiian fishes (37.75%), and decapod crustaceans (35.71%). These concentrations of prey items found in the stomachs coincide with previous studies done in the Pacific Ocean. In contrast to previous studies that found high percentages of empty stomachs (63%), the current percentage of empty stomachs was much lower (25.6%), likely due to shorter times between collection and inspection. Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) was

performed on white muscle in order to correlate the trophic position with gut-content analysis. The $\delta^{13}C$ values ranged from -18.81 to -16.70‰, while the $\delta^{15}N$ ranged from 6.11 to 11.88‰. Modeling of stable isotope data suggest that while squid are occasionally an important part of the pelagic stingray diet, prey usually consist of shrimp and other pelagic crustaceans. Pelagic stingrays fed within two trophic levels, but their prey appeared to feed on different carbon sources than those found in other pelagic elasmobranchs. A deeper understanding of the pelagic stingray diet sources can help fisheries management as it begins to transition into ecosystem-based management.

Introduction

Pelagic waters have vast areas of oligotrophic, deep water offshore from the more turbid, nutrient-rich waters of the coastal zone. The lower productivity of oligotrophic pelagic waters results in potential overlap of prey items for predators and increased feeding competition relative to areas of higher biological production. Species found in the pelagic realm are also often difficult to study due to the high mobility of the animals and lack of access to study specimens. Thus, many organisms that are part of the bycatch complex in the pelagic longline fishery have little known about their life histories (Simpfendorfer et al., 2008; Cortes et al., 2010;).

Many bycatch organisms in commercial, pelagic longline fisheries are relatively understudied, which could lead to the potential depletion of an ecologically vital species. The International Union for Conservation of Nature (IUCN) lists approximately 47% of all pelagic elasmobranchs as ‘data deficient’; however, the pelagic stingray was recently moved from ‘data deficient’ to of ‘least concern’ with the caveat as long as the stock continues to be monitored through available pelagic observer data (Forselledo et al., 2008). A distinct lack of information on the pelagic food webs exists, specifically for those species that can both impact the larger, predatory fishes of economic import and alter the overall structure of the pelagic food web (Rooker et al., 2006). Limited data on feeding behaviors by the pelagic stingray contribute to the unknown effect that fisheries are exerting on the species.

Combining both stomach content and stable isotopes analyses will provide a better understanding of the food web interactions of the pelagic stingray.

Several methods have been used for assessing the diets of fishes. Stomach content analysis, traditionally the primary technique, can assess food web interactions between different species and construct food webs with comparative studies (e.g. Preti et al., 2001; Trites, 2001). Difficulties associated with stomach content identification likely result from high digestion rates, resulting in identification of partially digested material and mistakenly including the bait in the indices (Bowen, 1996).

Stable isotope analysis has become a widely used technique in combination with stomach content analysis to estimate trophic position. Use of biochemical techniques, such as stable isotope ratios, helps to alleviate biases such as unrecognizable prey items, stomach content 'snapshots,' and insufficient sampling numbers to provide adequate conclusions to trophic interactions (MacNeil et al., 2005). Stable isotope ratios of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) are transferred from prey to predator in a predictable way with $\delta^{13}C$ increasing 0.5-1‰ per trophic level and an increase of 3-4‰ in $\delta^{15}N$ (DeNiro and Epstein, 1978, 1981; Vander Zanden et al., 1999).

Relatively few studies have examined the diets of the cosmopolitan pelagic stingray *Pteroplaytrygon violacea*, a common bycatch species in commercial pelagic longline fisheries (e.g. Pacheco et al., 2011). Prior stomach content analyses on this species (reviewed in Table 1) demonstrated a variety of prey items, although the small sample sizes or shorter sampling periods of all these prior studies did not allow for assessments of seasonal or age-related diet shifts. Many of the prior studies also had >50% empty stomachs, thus further limiting their analyses.

The goal of the current study on the pelagic stingray diet composition and trophic position was to provide data on a common yet understudied elasmobranch bycatch species in the western North Atlantic pelagic longline fishery. Utilizing commercial fisheries and at-sea fisheries observers, the study achieved a much larger sample size from the western North Atlantic population over a larger seasonal representation. By combining traditional gut-content analysis with stable isotope analysis, including modeling of the stable isotope data, this project resulted in a more comprehensive understanding of the pelagic stingray role within the larger pelagic ecosystem.

Materials and Methods

Specimen Collection

Pelagic stingrays were collected opportunistically aboard U.S. domestic commercial pelagic longline vessels targeting thunnid tunas and swordfish *Xiphias gladius* in the western North Atlantic Ocean and Gulf of Mexico between approx. 25°N and 35°N and westward of 75°W. Gear configurations targeted depths of 50-65 m. If an onboard fisheries observer was present, the stingrays were brought onto the vessel, and the disk width (DW) measured and gender determined per presence or absence of claspers. Specimens were then retained whole in the fish hold on seawater/freshwater ice for the remainder of the trip (*ca.* 5 days). Other specimens were caught incidentally by other commercial pelagic longline vessels and retained as frozen whole individuals in the bait freezer until collected at the end of the trip.

Section 1A: Stomach content analyses

Weights and DWs were recorded in the laboratory. Per the methods of Bowen (1996), the stomach was removed, weighed, and fixed in 10% deionized water-buffered formalin for approx. one month. The stomach was then transferred to 70% isopropyl or 70% ethanol for storage prior to examination.

During content analysis, the stomach was weighed, opened, and the contents emptied into a petri dish. The empty stomach was weighed and the contents sorted. Any identifiable material was recorded and placed into small vials for later identification to lowest taxon. Stomach contents were presented in the following indices: percentage by number, percentage by weight, percentage of occurrence, and by the index of relative importance (Cortés, 1997). Percentage by number (%N) is determined by the number of prey items of each prey type. The number of each prey type was then calculated to a percentage of the total number of prey items counted. Percentage by weight (%W) analyzes the weight of each prey item as a percentage of the total weight of prey items in an individual stomach. Percentage of weight suggests the relative importance of a given prey item to the overall ingested diet of

the individual consumer. The actual weights of prey items were used rather than reconstituted weights, in large part because length-weight morphometric relationships are unknown for most of the recovered prey items.

Percentage of occurrence (%O) quantifies the diet by compiling a total list of prey items found, then compared to the presence or absence of the prey item. High percentages of occurrence indicate that the given prey item is found in many individual specimens (Bowen, 1996). The index of relative importance (IRI) is calculated as:

$$IRI = \%O\ (\%W + \%N)$$

The IRI is then converted to a percentage (per Cortés, 1997):

$$\%IRI = 100*IRI / \Sigma IRI$$

Cumulative prey curves were used to determine whether an adequate number of stomachs had been examined to describe the diet sufficiently. The order in which stomachs were analyzed was randomized 999 times to minimize bias resulting from sampling order. Using the *Chao 1* estimator in Primer 7 (PRIMER-E Ltd.; Ivybridge, UK), the mean number of new prey categories found in the stomachs (with standard deviation) was plotted against the total number of stomachs analyzed. Randomizing the order, 999 bootstrap simulations of cumulative prey curves were constructed using the major categories of identifiable prey items. Per Ferry and Caillet (1996), the asymptote of the curve indicates the minimum sample size required to adequately describe the diet.

Section 1B: Stable isotope analyses

Muscle tissue samples were collected from the dorsal pectoral wing of each stingray captured during three seasons in 2008, 2009, 2011, and 2012. The white muscle tissue of the pectoral wing was chosen due to the lack of skeletal muscle generally available on the rays; the dorsal section of the wing allowed for a 2 cm^3 tissue sample. A total of 49 samples were collected (25 females, 24 males) and the samples were frozen in a -20°C standard freezer until processed. White muscle tissues were by dehydrating the samples at 60°C for 48-72 hours, ground, and homogenized with a Wig-L-Bug amalgamator, and pelletized before analysis using an isotope ratio mass spectrometer (Estrada et al., 2003). Stable carbon and nitrogen isotope ratios

($\delta^{13}C$ and $\delta^{15}N$) were used to determine dietary assimilation of prey items and also to help predict the potential trophic feeding level of the pelagic stingray (Vander Zander and Rasmussen, 2001; McCutchan et al., 2003). A generalized linear model (GLM) was used to assess statistical differences between sexes, seasons, and years, as GLMs are more accommodating to the unequal sample sizes across variables than ANOVAs. Significance was assessed at α=0.05.

The stable isotope values were then compared to literature values for potential prey; additional pelagic fish species' $\delta^{13}C$ and $\delta^{15}N$ were used due to the logistical difficulties associated with physically collecting some specimens (e.g. small pelagic shrimp, squid species, isopods) while aboard the cooperating commercial pelagic longline fishing vessels. Several studies have already reported values for some prey items, such as Atlantic herring, pelagic squids, and Atlantic flying fishes (e.g. Estrada et al., 2003; Rau et al., 1983), or co-occurring predators, such as mesopelagic fishes (Keller et al., 2015).

The trophic relationships between pelagic stingray and four main prey species were further assessed with the IsoSource mixing model set at 0.05 tolerance and for 5% increments (Phillips and Gregg, 2001). The $\delta^{13}C$ and $\delta^{15}N$ values for pelagic stingrays (both sexes combined) were obtained directly from this research. However, as stomach content items are rarely suitable for subsequent stable isotope analyses, $\delta^{13}C$ and $\delta^{15}N$ proxy values for prey were obtained from the literature for the western Atlantic longfin squid *Loligo pealeii* (from Abend and Smith, 1997), offshore-captured pink shrimp *Farfantepenaeus duorarum* (from Fry et al., 1999), as well as Sargassum-associated planehead filefish *Stephanolepis hispidus* and sargassum crab *Portunus sayi* (from Rooker et al., 2006). Although not included in %IRI and other gut-content analysis metrics, $\delta^{13}C$ and $\delta^{15}N$ values for *Sargassum natans* from Rooker et al. (2006) were also included in preliminary modeling. Prior to IsoSource modeling, the methods of Phillips et al. (2014) were used to correct $\delta^{13}C$ and $\delta^{15}N$ values with a diet-tissue discriminant factor (DTDF), which was calculated for each species individually using a trophic estimator function (Hobson et al., 1994).

Results

Section 1A: Stomach Content Analysis

A total of 156 stomachs were analyzed (males: n=85, DW mean=48.7 SD±3.8; females: n=71, DW mean=52.4 SD±6.5; see Table 2 for collection details). The cumulative prey curve showed a well-defined asymptote, and the associated bootstrap analysis in PRIMER 7 determined a 1% rate of increase for new prey items at only 32 individual stingrays (Fig. 1). We therefore conclude that the total sample size of 156 individuals was adequate to describe the diet of the pelagic stingray using the seven major prey categories listed in Table 1 (squid, octopus, shrimp, 'other crustaceans', monocanthid filefish, *Hippocampus* sp. seahorses, and 'other teleosts').

In contrast to previous studies, 40 stomachs were empty (25.6%; 22 M and 18 F), and 11.54% had unidentifiable digested material. Macroalgae (predominantly *Sargassum* sp.) were found in 4.09% of the stomachs. Parasitic nematodes were found in 1.36% of the stomachs. However, both the macroalgae and nematodes were assumed to be incidental or resident parasites versus an actively consumed prey item and, thus, excluded from subsequent analyses.

Prey items found in the stingray stomachs included mollusks, teleosts, and crustaceans. The %IRI and percent frequency of prey items are presented in Fig. 2. Mollusks (e.g. cephalopods) comprised the largest portion of the diet by %O: 59.2%, %N: 43.3%, %W: 14.9%, and %IRI: 70.3%. In particular, squid species had values of %O: 8.3%, %N: 42.7%, %W: 14.5%, and %IRI: 73.5%. However, of the 149 individual squids identified in the stomach contents, only 12 were identified by their mantle and appendages; the remaining percent of occurrence was determined from beaks found in the stingray stomachs.

Teleost fishes followed in dominance with values of %O: 37.8%, %N: 1.9%, %W: 5.5%, and %IRI: 5.7%. Unknown teleosts had index values of %O: 22.5%, %N: 12.0%, %W: 4.3%, and %IRI: 8.1%. Due to the advanced stages of digestion, *Hippocampus* sp. seahorses and monocanthid filefish were the only identifiable subcategory of teleosts. *Hippocampus* sp. had values of %O: 6.1%, %N: 2.0%, %W:

0.3%, and %IRI: 0.3%, while the filefish had values of %O: 9.1%, %N: 5.2%, %W: 0.9%, and %IRI: 1.2%.

Crustaceans comprised the smallest observed portion of the pelagic stingray diet. The index values were %O: 35.7%, %N: 31.0%, %W: 2.0%, and %IRI: 24.0%. Shrimp was the only identifiable crustacean prey, with values of %O: 24.5%, %N: 27.8%, %W: 2.0%, and %IRI: 16.1%.

Stable Isotope Analysis

Stingray disk widths ranged from 41-70 cm, which indicated that all samples were from reproductively mature adults (Neer, 2008). The dorsal muscle tissue was collected in various months over a four-year period, but samples were collected opportunistically and thus not consistently across seasons. While seasonality could not be tested due to inconsistent opportunistic sampling, inter-annual variability indicated no significant difference in either stable isotope among any of the years ($\delta^{15}N$: $F(3, 48) = 10.96$, $p < 0.0001$; $\delta^{13}C$: $F(3, 48) = 1.09$, $p = 0.364$).

The $\delta^{13}C$ ranged from -18.81 to -16.70‰ with a mean of -17.85 ± 0.44‰ and $\delta^{15}N$ ranged from 6.11 to 11.88‰ with a mean of 8.57 ± 1.25‰. The $\delta^{13}C$ values were similar between females and males with values ranging from -18.81 to -16.70‰ and -18.59 to -17.18‰, respectively. Female stingrays had a comparable range in $\delta^{15}N$ to males, 6.11 to 11.88‰ versus 6.47 to 10.95‰. There were no significant differences between male and female individuals for $\delta^{13}C$ [$F(1, 48) = 0.66$, $p = 0.421$], but there was a significant difference between sexes for $\delta^{15}N$ ($F(1, 48) = 0.00$, $p = 0.986$). Both stable isotope ratios indicated that the stingrays were foraging across two trophic levels, based on the fractionation values for both carbon and nitrogen (~0.5-1‰ and ~3‰ per trophic level, respectively). The C/N ratio ranged from 2.37 to 3.13 (mean: 2.77), indicating a diet of proteinaceous tissues not overtly rich in lipids. While there were no statistically significant differences, there were negative relationships between some of the different factors with the stable isotopes. For example, $\delta^{13}C$ had a negative relationship in the sample year 2009 (-0.310), and $\delta^{15}N$ had a negative relationship to the sample year 2011 (-0.557).

Although *Sargassum natans* was included in preliminary modeling, the number of solutions including this species was < 5%, and thus was subsequently dropped from the model. The values obtained from the IsoSource modeling for the remaining four prey items resulted in a relatively constrained diet polygon (Phillips

and Gregg, 2003; Fig. 3). Specifically, the modeling results suggested that although squid were collectively a large part of the pelagic stingray diet from the stomach content analyses, the overall contribution of squid to the broader assimilated diet was highly variable (0-70%). In contrast, the smaller pelagic crustaceans of shrimp and pelagic crabs contributed to a more consistent part of the assimilated diet (25-35% and 5-55%, respectively), while the Sargassum-associated planehead filefish contributed highly variable amounts to the overall diet (0-60%). To place the stable isotope-derived positioning of pelagic stingray within a larger ecosystem context, $\delta^{13}C$ and $\delta^{15}N$ stable isotope values of potential prey, related elasmobranch species, and co-occurring pelagic teleosts found in western North Atlantic pelagic waters were gathered from published literature for trophic comparisons (Table 3, Fig. 4).

Discussion

Stomach Content Analysis

Stomach content analysis supported previous reports from other diet studies on the pelagic stingray, finding small teleost fishes, crustaceans, and cephalopods (Table 1). However, the present study had a significantly lower rate of empty stomachs (25.6%) compared to 56.25% in Wilson and Beckett (1970) and 63% in Ribero-Prado and Amorim (2008). Aside from geographic locations, the difference in percentage of empty stomachs between the current study and previous studies could be due to the shorter time between capture and fixation of the stomachs in this study. Similar to Ribero-Prado and Amorim (2008), cephalopoda was the dominant prey taxa in the pelagic stingray diet (Fig. 1). The cephalopods in this study were identified as belonging to the families Loliginidae and Ommastrephidae. Crustacea were the next dominant prey taxa in percent number and percent index of relative importance. Similarly, Veras et al. (2009) found crustaceans, specifically the deepwater shrimp *Heterocarpus ensife*, to be a prominent prey item for pelagic stingrays caught in the southwestern equatorial Atlantic Ocean. Teleost fishes were the third most prevalent prey taxa, with unidentifiable teleosts occurring in highest abundance, followed by monocanthid filefishes and *Hippocampus* spp. seahorses, respectively.

While the opportunistic collection methods precluded true seasonal analyses, squids appeared more often in the stomach contents during the spring and summer months while crustaceans were more prominent prey items during the winter months. Straudinger (2006) reported that squid activity in offshore waters of the northwest Atlantic Ocean is pronounced during summer months due to stratification of the water column. The dietary shift could thus be due to the availability of prey items at different times of the year; for example, Neer (2008) reported that pelagic stingrays seasonally target schools of mating squids in the eastern Pacific Ocean. Alternatively, pelagic stingrays could be opportunistically depredating squid bait from pelagic longline sets, which may explain the wide range of estimated contributions by squid in the isotope modeling efforts; such depredation behaviors by odontocete whales have been described previously in longline fisheries (e.g. Hamer et al., 2012). Pelagic stingrays show a high rate of survival at gear retrieval in the pelagic longline fishery, especially with the circle hooks now required for the U.S. commercial fleet (Kerstetter and Graves, 2006).

Ontogenetic shifts in diet have been observed in aquaria-held pelagic stingrays, where younger age-classes fed predominately on crustaceans and shifted to a squid-dominated diet with increasing size (Mollet et al., 2002). This study suggests that the shift from a crustacean- to a squid-based diet may have been for caloric intake, where the larger rays were opting to eat squids rather than crustaceans (Mollet et al., 2002). However, due to the fishery-dependent gear selectivity of the pelagic longline gear used for specimen collection, the collecting vessels did not capture any juveniles and we could not address an ontogenetic diet shift with the western North Atlantic pelagic stingray population.

Pelagic Stingray Diets

Both stable isotope ratios indicated that the stingrays were foraging across two trophic levels, based on the fractionation values for both stable carbon and nitrogen isotopes. Female and male stingrays had similar $\delta^{13}C$ throughout the study, but the $\delta^{15}N$ suggested minor trophic differences between males and females. Based on fractionation factors of 0.5-1‰ and 3-4‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively (DeNiro and Epstein, 1978, 1981; Vander Zanden et al., 1999), potential prey likely had $\delta^{13}C$ of ~19‰ and 5.5-6‰ in $\delta^{15}N$. The western Atlantic longfin squid putatively resembles these values sufficiently for modeling. No shrimp stable isotope values

were located for the Mid-Atlantic Bight region for prey comparison; however, stable isotope values from the Gulf of Mexico shrimp species caught around western Florida had $\delta^{13}C$ of -17.5 to -14.5‰ and 8.3‰ in $\delta^{15}N$ (Fry, 1983; Fry et al., 1999). Although not done for the present study, concurrent sampling of prey items along with predators would provide the better sources of stable isotope data than the proxy values here and thus should be a priority for future combined gut-content/stable isotope diet studies.

The majority of elasmobranch species values taken from the literature appeared to have relied upon prey that utilized a similar carbon source (inshore vs. offshore) but were one to two trophic levels more enriched than the pelagic stingrays themselves (Rau et al., 1983; Estrada et al., 2003). Three nearshore elasmobranch species were incorporated into Fig. 4 to give reference that the pelagic stingrays were likely utilizing an offshore carbon source (Tilley et al., 2013). The teleost species with a similar carbon value, and potential source, to the pelagic stingrays were all more enriched trophically and could not have contributed significantly to their diets.

Sargassum sp. macroalgae was also found in pelagic stingray stomachs (4.1%). The stingrays were likely not feeding on the Sargassum directly, but ingesting it incidentally while preying upon organisms living in the aggregated mats, such as seahorses and small fishes, such as the sargassum pipefish. Rooker et al. (2006) examined the potential of Sargassum as the primary producer in pelagic systems, and reported the stable isotope ratios of Sargassum in addition to several upper trophic level predatory fishes (e.g. wahoo *Acanthocybium solandri*). The stable isotope ratios confirmed that Sargassum was not the carbon source for the Gulf of Mexico pelagic food web; instead, the largest fraction of organic matter in the pelagic system was from particulate organic matter rather than from the Sargassum itself. Our data are consistent with the finding of Rooker et al. (2006), in that pelagic stingrays are instead likely to be simply feeding opportunistically on the smaller species associated with the floating Sargassum mats of the pelagic waters.

The analysis of stomach contents has traditionally been the way to study the diet of an organism. However, stomach contents only provide a 'snapshot' picture of what an animal has recently ingested, which is why alternative techniques often prove valuable. For example, examining the $\delta^{13}C$ and $\delta^{15}N$ stable isotope values with the IsoSource mixing model suggest that the actual contributions of squid to the assimilated diet of the pelagic stingray is highly variable, but that the diet is actually

more consistent with feeding on pelagic crustaceans, such as shrimp and portunid swimming crabs. Since pelagic elasmobranchs are assumed to be intermittent feeders and typically found with prey items in advanced stages of digestion (Joyce et al., 2002; Wetherbee and Cortes, 2004), determination of an exact diet can prove to be difficult. By incorporating these additional techniques, such as stable isotope analysis of carbon and nitrogen, the understanding of the trophic interactions by a species in a given ecosystem can be better interpreted. Both techniques in combination provided a greater understanding of pelagic stingray diet composition, and confirmed previous studies of the opportunistic feeding style in the pelagic food web. As fisheries management shifts to a more ecosystem-based framework, understanding the trophic dynamics of middle-level predators such as the pelagic stingray will become vital for ensuring the longevity of high level, economically important predators.

Acknowledgements

This project was supported by NOAA Contract #8404-S-006. C. France at the Smithsonian Institution Museum Support Center performed the stable isotope analysis. S. Bayse, C. Cross, and H. Keller (NSU) assisted with specimen collections as at-sea fisheries observers. Fishing vessels (F/V) *Kristin Lee*, *Shady Lady*, *Southern Lady*, *Janice Ann*, and *Dakota*, and their respective captains and crews assisted in the pelagic stingray specimen collections. C. Blanar (NSU) assisted with the PRIMER 7 prey curve analyses. C. Shea (FWC) assisted with statistical analyses.

References

Abend, A.G.; Smith, T.D., 1997: Differences in stable isotope ratios of carbon and nitrogen between long-finned pilot whales (*Globicephala melas*) and their primary prey in the western North Atlantic. ICES J. Mar. Sci.: J. Conseil, **54**(3), 500-503.

Bowen, S.H., 1996: Quantitative Description of the Diet. In: Fisheries Techniques, 2nd edn. Eds: B.R. Murphy and D.W. Willis. American Fisheries Society: Bethesda, MD. pp. 513-532 .

Cortés, E., 1997: A critical review of methods of studying fish feeding based on analysis of stomach contents: application to elasmobranch fishes. Can. J. Fish. Aquat. Sci. **54**(3), 726–738.

Cortés, E., 1999: Standardized diet compositions and trophic levels of sharks. ICES J. Mar. Sci. **56**, 707-717.

Cortés, E., Arocha, F., Beerkircher, L., Carvalho, F., Domingo, A., Heupel, M., Hotlzhausen, H.,Santos, M.N., Ribera, M.; Simpfendorfer, C., 2010: Ecological risk assessment of pelagic sharks caught in Atlantic pelagic longline fisheries. Aquat. Living Resour. **23**(1), 25.

Dávalos-Dehullu, E.; González-Navarro, E., 2003: Stomach content of one pelagic ray *Dasyatis violacea* (Bonaparte, 1832) (Rajiformes: Dasyatidae) from The Gulf of California, Baja California Sur, Mexico. Oceánides **18**(1): 43-44.

DeNiro, M.J.; Epstein, S., 1978: Influence of diet on the distribution of carbon isotopes in animals. Geochimica et Cosmochimica Acta **42**(5), 495-506.

DeNiro, M.J.,; Epstein, S., 1981: Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochimica Acta **45**(3), 341-351.

Estrada, J.A.; Rice, A.N.; Lutcavage, M.E.; Skomal, G.B., 2003: Predicting trophic position in sharks of the north-west Atlantic Ocean using stable isotope analysis. J. Mar. Biol. Ass. U.K. **83**, 1347-1350.

Ferry, L.A.; Caillet, G.M., 1996: Sample size and data analysis: are we characterizing and comparing diet properly? *In* Feeding Ecology and Nutrition in Fish, Symposium Proceedings, pp. 71-80. Eds: D. MacKinlay and K. Shearer. American Fisheries Society, San Francisco.

Forselledo, R.; Pons, M.; Miller, P.; Domingo, A., 2008: Distribution and population structure of the pelagic stingray, *Pteroplatytrygon violacea* (Dasyatidae), in the south-western Atlantic. Aquat. Living Resour. **21**(4), 357-363.

France, R.L., 1995: Carbon-13 enrichment in benthic compared to planktonic algae foodweb implications. Mar. Ecol.-Progr. Ser. **124**, 307-312.

Fry, B., 1983: Fish and shrimp migrations in the northern Gulf of Mexico analyzed using stable C, N, and S isotope ratios. Fish. Bull. NOAA **81**(4), 789-801.

Fry, B.; Mumford, P.L.; Robblee, M.B., 1999: Stable isotope studies of pink shrimp (*Farfantepenaeus duorarum* Burkenroad) migrations on the southwestern Florida shelf. Bull. Mar. Sci. **65**(2), 419-430.

Hamer, D.J.; Childerhouse, S.J.; Gales, N.J., 2012: Odontocete bycatch and depredation in longline fisheries: a review of available literature and of potential solutions. Mar. Mammal Sci. **28**(4), E345-E374.

Joyce, W.N.; Campana, S.E.; Natanson, L.J.; Kohler, N.E.; Pratt Jr., H.L.; Jenson, C.F., 2002: Analysis of stomach contents of the porbeagle shark (*Lamna nasus* Bonnaterre) in the northwest Atlantic. ICES J. Mar. Sci. **59**, 1263-1269.

Keller, H.R, 2011: Trophic study of escolar, snake mackerel, oilfish, and lancetfish in the Western North Atlantic Ocean using stomach content analysis and carbon and nitrogen stable isotope analysis. M.S. thesis, Nova Southeastern University, 106 p.

Keller, H.; Hirons, A.C.; Kerstetter, D.W., 2016: Combined stomach content and $\delta^{13}C/\delta^{15}N$ analyses of oilfish, escolar, snake mackerel, and lancetfish in the western North Atlantic. Mar. Ecol. **37**(4), 727-736.

Kerstetter, D.W.; Graves, J.E., 2006: Effects of circle versus J-style hooks on target and non-target species in a pelagic longline fishery. Fish. Res. **80**(2), 239-250.

Kohler, N.E., 1988: Aspects of the feeding ecology of the blue shark, *Prionace glauca,* in the Western North Atlantic. *ETD Collection for University of Rhode Island.* Paper AAI8811561.

MacNeil, M.A.; Skomal, G.B.; Fisk, A.T., 2005: Stable isotopes from multiple tissues reveal diet switching in sharks. Mar. Ecol.-Progr. Ser. **302** 199-206.

Mollet, H.F.; Excurra, J.M.; O'Sullivan, J.B., 2002: Captive biology of the pelagic stingray, *Dasyatis violacea* (Bonaparte, 1832). Mar. Freshw. Res. **53**, 531-541.

McCutchan Jr., J.H.; Lewis Jr., W.M.; Kendall, C.; McGrath, C.C., 2003: Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos **102**, 378-390.

Moore, T.A., 2014: Trophic dynamics and feeding ecology within the Southeast Florida coastal pelagic fish community. M.S. thesis, Nova Southeastern University, 70 p.

Neer, J.A., 2008: The biology and ecology of the pelagic stingray, *Pteroplatytrygon violacea* (Bonaparte, 1832: In: Sharks of the Open Ocean. Eds. M. Camhi and E. Pikitch. Blackwell Scientific, New York. pp 152-159.

Pacheco, J.C., Kerstetter, D.W., Hazin, F.H., Hazin, H., Segundo, R.S. S.L., Graves, J.E., Carvalho, F.; Travassos, P.E., 2011: A comparison of circle hook and J hook performance in a western equatorial Atlantic Ocean pelagic longline fishery. Fish. Res. **107**(1), 39-45.

Preti, A.; Smith, S.E.; Ramon, D.A., 2001: Feeding habits of the common thresher shark (*Alopias vulpinus*) sampled from the California-based drift gill net fishery, 1998-1999. Cal. Coop. Ocean. Fish. pp. 145-152.

Rau, G.H.; Mearns, A.J.; Young, D.R.; Olson, R.J.; Schafer, H.A.; Kaplan, I.R., 1983: Animal $^{13}C/^{12}C$ correlates with trophic level in pelagic food webs. *Ecology* **64**(5), 1314-1318.

Ribeiro-Prado, C.C.; Amorim, A.F., 2008: Fishery biology on pelagic stingray *Pteroplatytrygon violacea* caught off southern Brazil by longliners settled in São Paulo State (2006-2007). Coll. Vol. Sci. Pap. ICCA*T* **62**(6), 1883-1891.

Rooker, J.R.; Turner, J.P.; Holt, S.A., 2006: Trophic ecology of Sargassum-associated fishes in the Gulf of Mexico determined from stable isotopes and fatty acids. Mar. Ecol.-Progr. Ser. **313**, 249-259.

Sequeira, A.E.; Sant'Anna, V.B., 2007: Data on the pelagic stingray, *Pteroplatytrygon violacea* (Bonaparte, 1832) (Myliobatiformes: Dasyatidae) caught in the Rio de Janeiro coast. Braz. J. Oceanogr. **55**(4), 323-325.

Simpfendorfer, C.; Cortes, E.; Heupel, M.; Brooks, E.; Baum, J.; McAuley, R.; Dudley, S.; Stevens, J.D.; Fordham, S.; Soldo, A., 2008: An integrated approach to determining the risk of overexploitation for data-poor pelagic Atlantic sharks. Expert Working Group Report, ICCAT.

Staudinger, M.D., 2006: Seasonal and size-based predation on two species of squid by four fish predators on the Northwest Atlantic continental shelf. Fish. Bull. NOAA. **104**, 605- 615.

Tilley, A.; López-Angarita, J.; Turner, J.R., 2013: Diet reconstruction and resource partitioning of a Caribbean marine mesopredator using stable isotope Bayesian modelling. PloS One **8**(11), e79560.

Trites, AW., 2001: Food webs in the ocean: who eats whom, and how much? In: Responsible Fisheries in the Marine Ecosystem. Eds: M. Sinclair and R. Valdimarsson. FAO, Rome and CABI Publishing, Wallingford. pp. 125-143.

Vander Zanden, J.M.; Rasmussen, J.B., 2001: Variation in δ15N and δ13C trophic fractionation: Implications for aquatic food web studies. Limnol. Oceanography **46**, 2061-2066.

Vander Zanden, J.M.; Casselman, J.M.; Rasmussen, J.B., 1999: Stable isotope evidence for food web consequences of species invasions in lakes. Nature **401**, 464-467.

Véras, D.P.; Vaske Júnior, T.; Hazin, F.H.V.; Lessa, R.P.; Travassos, P.E.; Tolotti, M.T.; Barbosa, T.M., 2009: Stomach contents of the pelagic stingray (*Pteroplatytrygon violacea*) (Elasmobranchii: Dasyatidae) from the tropical Atlantic. Braz. J. Oceanogr. **57**(4), 339-343.

Wetherbee, B.M.; Cortes, E., 2004: Food consumption and feeding habits. In: Biology of Sharks and Their Relatives. Eds: J.C. Carrier, J.A. Musick, M.R. Heithaus. CRC Press, Boca Raton, FL. pp. 223-244.

Wilson, P .C.; Beckett, J.S., 1970: Atlantic Ocean distribution of the pelagic stingray, *Dasyatis violacea*. Copeia 696-707.

Corresponding author: David W. Kerstetter
Nova Southeastern University Halmos College of Natural Sciences and Oceanography, 8000 North Ocean Drive, Dania Beach, FL 33004 USA
Email: kerstett@nova.edu

Table 1. Stomach contents in pelagic stingray *Pteroplaytrygon violacea*, including sample sizes, percent examined individuals with empty stomachs, and brief list of prey items. Sex listed in the prior publication is indicated in the sample size column (F or M); "U" = unsexed individual. Contents in the present study include only taxa observed in >20% of individual stingrays examined.

Location	Sample Size	Empty	Contents	Reference
Western Atlantic	N=1	--	seahorse, shrimps, squids	Bigelow and Schroeder (1962)
NW Atlantic	N=1	--	decapod crustacean, heteropod	Scott and Tibbo (1968)
Western N Atlantic	N=16	56.25%	squids, seahorses, filefishes, coelenterate, barracudina, decapod crustacean, heteropod	Wilson and Beckett (1970)
Gulf of California	N=1 (F)	--	chub mackerel (*Scomber japonicus*)	Davalos-Dehullu and Gonzalez-Navarro (2003)
SW Atlantic	N=4 (2 M: 2 F)	50%	teleost fishes	Siqueira and Sant'Anna (2007)
SW Atlantic	N=157	63.05%	cephalopods, actinopterygiian fishes, crustaceans	Ribero-Prado and Amorim (2008)
Western S Atlantic	N=106 (69 M: 26 F: 11 U)	[not listed]	hyperiid amphipods, teleost fishes, crustaceans, pteropods	Veras et al. (2009)

Western N Atlantic and Gulf of Mexico	*N=156 (81 M: 75 F)*	*25.6%*	*Cephalopods, crustaceans,* Hippocampus *sp. seahorses, monocanthid filefish, teleosts*	*present study*

Table 2. Sampling details for pelagic stingray *Pteroplatytrygon violacea* specimens collected from western North Atlantic Ocean and Gulf of Mexico, 2008 to 2012, used in the present study.

Year	Total	Sex	Q1	Q2	Q3	Q4
2008	4	F				2
		M				2
2009	20	F	2		4	2
		M	5		2	5
2011	23	F				13
		M				10
2012	2	F	1			1
		M				

Table 3. Percent occurrence (%O), percent number (%N), percent weight (%W) and index of relative importance (%IRI) of prey items from 156 pelagic stingrays *Pteroplatytrygon violacea*, western North Atlantic Ocean and Gulf of Mexico. Three taxa of prey items were calculated along with the more specific families. Also included in the Table is the percent occurrence for partially digested material, macroalgae (*Sargassum* sp.), and empty stomachs.

Prey Item	**% O**	**% N**	**% W**	**%IRI**
Cephalopoda	**59.18**	**43.27**	**14.88**	**70.27**
Teuthida	59.18	43.26	14.51	74.00
Crustacean	**35.71**	**30.95**	**1.98**	**24.01**
Shrimp	24.49	27.79	1.97	16.10
unknown crustacean	11.22	3.15	0.01	0.78
Teleost	**37.76**	**1.92**	**5.50**	**5.72**
Hippocampus sp.	6.12	2.01	0.25	0.31
monocanthid filefish	9.18	5.16	0.92	1.23
unknown teleost	22.45	12.03	4.32	8.11
partially digested material	11.54			
macroalgae (*Sargassum* sp.)	4.09			
empty	25.64			

Table 4. Sources for $\delta^{15}N$ and $\delta^{13}C$ stable isotope data for pelagic stingray *Pteroplatytrygon violacea* males and females used in Figure 3, as well as additional prey and organic carbon sources, western Atlantic Ocean and Gulf of Mexico.

Figur	**n**	**$\delta^{13}C$**	**$\delta^{15}N$**	**Geographi**	**Source**

	Code	n	mean	mean	Location	
Pelagic elasmobranchs						
Pelagic stingray (F) *Pteroplatytrygon violacea*	a	10	-17.8	8.45	WNA	*present study*
Pelagic stingray (M) *Pteroplatytrygon violacea*	b	14	-17.85	8.69	WNA	*present study*
Shortfin mako *Isurus oxyrinchus*	c	1	-15.6	16.6	WSA	Bugoni et al. (2010)
Blue shark *Prionace glauca*	d	11	-17.4	12.9	WSA	Bugoni et al. (2010)
Mesopelagic elasmobranch						
Longfin mako *Isurus paucus*	e	1	-17.3	11.5	WNA	Keller (2011)
Pelagic teleosts						
Common dolphinfish *Coryphaena hippurus*	f	45	-16.7	9.26	WNA	Moore (2014)
Swordfish *Xiphias gladius*	g	2	-15.6	13.4	WSA	Bugoni et al. (2010)
Skipjack *Katsuwomus pelamis*	h	27	-16.87	8.68	WNA	Moore (2014)
King mackerel *Scomberomorus cavalla*	i	35	-18.41	13.18	WNA	Moore (2014)
Mesopelagic teleosts						
Pomfret, Family Bromidae	j	63	-18.1	10.6	WNA	Keller (2011)
Lancetfish *Alepisaurus* sp.	k	43	-18.22	9.61	WNA	Keller et al. (2016)
Escolar *Lepidocybium flavobrunneum*	l	40	-20.7	8.47	WNA	Keller et al. (2016)
Cape cigarfish *Cupiceps capensis*	m	2	-17	11.3	WNA	Keller (2011)
Small pelagic teleosts						
Planehead filefish *Monocanthus hispidus*	n	11	-18.45	10.2	GOM	Rooker et al. (2006)
Sargassum pipefish *Syngnatus pelagicus*	o	5	-16.95	11.4	GOM	Rooker et al. (2006)
Flyingfish, Family Exocetidae	p	3	-17.3	7	WNA	Keller (2011)
Pelagic invertebrates						
Sargassum crab *Portunis sayi*	q	6	-19.95	9.8	GOM	Rooker et al. (2006)

Argentine shortfin squid *Illex argentinus*	r	2 1	-17.2	14.2	WSA	Bugoni et al. (2010)
Brown grass shrimp *Leander tenucornis*	s	1 6	18.05	5.95	WNA	Hirons (unpubl. data)
Other						
Sargassum natans	t	--	-17.45	2.45	GOM	Rooker et al. (2006)
Sargassum fluitans	u	--	-16.6	2.8	GOM	Rooker et al. (2006)
POM (particulate organic matter)	v	--	-20.9	7.2	GOM	Rooker et al. (2006)
Green algae *Cladophora* sp.	w	--	-18.8	9.2	GOM	Rooker et al. (2006)

Figure 1. Cumulative prey curve for pelagic stingray *Pteroplatytrygon violacea* individuals (*n* = 156) collected in western North Atlantic Ocean and Gulf of Mexico. Black line = rarefaction curve for new prey items as a function of the number of samples using the *Chao 1* estimator in PRIMER 7. Grey shaded region = standard deviation of 0.95 around the rarefaction curve based on bootstrap simulations, which determined a 1% rate increase for new prey items in only 32 individuals.

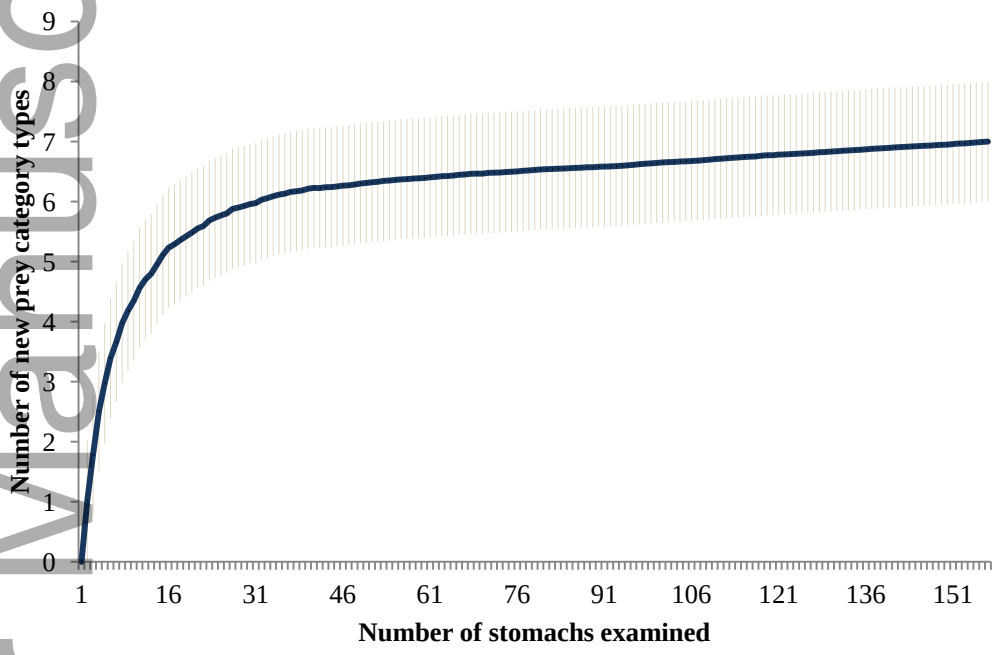

Figure 2. Percent occurrence of different items ingested by pelagic stingray *Pteroplatytrygon violacea* individuals (*n* = 156) collected in western North Atlantic Ocean and Gulf of Mexico. Squid, shrimp, and teleost fishes were the dominant prey items in stomachs. Numbers = percent occurrence (%O).

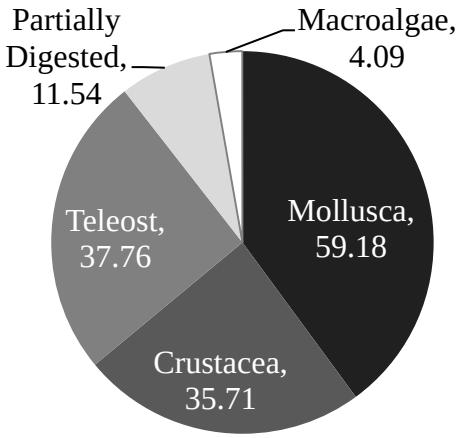

Figure 3. Mixing polygon for signatures of three food sources for pelagic stingray, western North Atlantic. Values corrected for trophic fractionation prior to modeling. Proxies for prey items from gut-content analyses included western Atlantic longfin squid *Loligo pealeii*, Gulf of Mexico offshore-captured pink shrimp *Farfantepenaeus duorarum*, as well as Gulf of Mexico Sargassum-associated planehead filefish *Stephanolepis hispidus* and sargassum crab *Portunus sayi*. Histograms within each sub-figure show distribution of feasible contributions from each prey source to the overall pelagic stingray diet (asterisk; males and females combined). Values in boxes = 1-99 percentile ranges calculated by IsoSource for these feasible distributions.

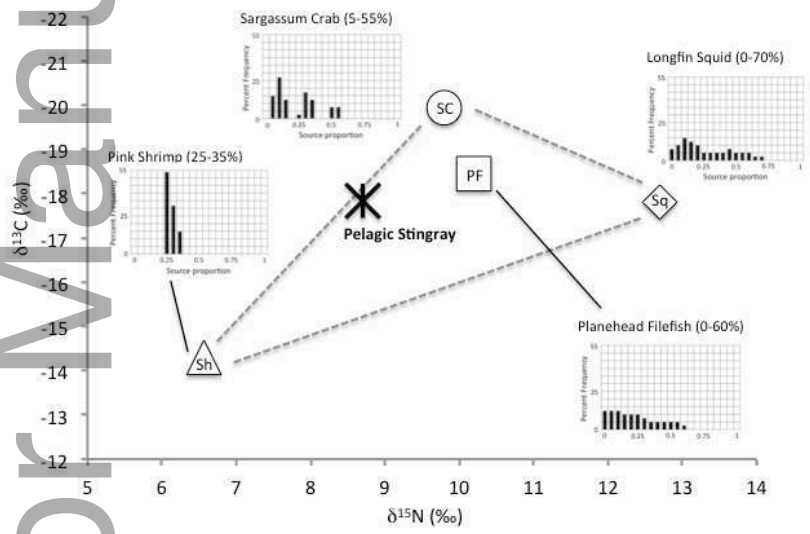

Figure 4. The $\delta^{13}C$ and $\delta^{15}N$, pelagic stingray *Pteroplatytrygon violacea* males and females (black outlined symbols, figure center). Additional pelagic fishes, elasmobranchs, potential prey items, and organic carbon sources from the western Atlantic Ocean and Gulf of Mexico are included for ecosystem reference (see Table 4 for sources of other stable isotope data and individual species codes). Grey arrows = expected trajectory of enrichment from organic carbon producers.