FEEDING ECOLOGY OF COASTAL SHARKS IN THE NORTHWEST GULF OF MEXICO

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

Feeding Ecology of Coastal Sharks in the Northwest Gulf of Mexico. (May 2015)

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The feeding ecology of three coastal shark species consisting of Atlantic Sharpnose (*Rhizoprionodon terraenovae*), Bonnethead (*Sphyrna tiburo*), and Atlantic Blacktip (*Carcharhinus limbatus*) was examined in the northwest Gulf of Mexico (GOM). A total of 601 (305 *R. terraenovae,* 239 *S. tiburo,* and 57 *C. limbatus*) sharks were collected through the recreational fishery offshore Galveston, Texas over 2013 and 2014. Stomach contents were examined for all individuals and quantified for short-term diet information (days) and stable isotopes of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) in muscle tissue were analyzed for a subset of samples to examine longer term (weeks to months) feeding patterns. Both *C. limbatus* and *R. terraenovae* stomach contents primarily consisted of teleost fish with % index of relative importance (IRIs) of 91.16 and 98.95%, respectively. Primary prey categories for R. *terraenovae* included unidentified Teleostei (87.40 %IRI), Panaeidae (3.56 %IRI), and Tuthoidea (2.92 % IRI). Dominant prey in *C. limbatus* consisted of Unidentified Teleostei (88.52 %IRI) and *Micropogonias undulatus* (7.46 %IRI), additional non-teleost fish prey categories including Crustacea and Cephalopoda accounted for 1.15 %IRI. *S. tiburo* had a diet primarily of crustaceans (87.20 % IRI), the top three contributors were unidentified Brachyura (48.91 %IRI), *Callinectes sapidus* (18.06 %IRI), and *C. similis* (3.32 % IRI). Stable isotope results revealed

similar trends as the stomach contents. Mean $\delta^{13}C$ was significantly enriched for *S. tiburo* (-16.84 ‰) relative to the other two species, which had similar mean $\delta^{13}C$ (*R. terraenovae* -17.07 ‰ and *C. limbatus* -17.06 ‰). Mean $\delta^{15}N$ was significantly enriched for *C. limbatus* (16.64 ‰) and similar between *R. terraenovae* (15.94 ‰) and *S. tiburo* (15.90 ‰). $\delta^{34}S$ was a useful tracer for benthic invertebrate consumption consisting of significantly depleted values for *S. tiburo* (15.45 ‰), relative to *R. terraenovae* (16.01 ‰) and *C. limbatus* (16.30 ‰). Collectively, both stomach contents and stable isotopes support unique feeding strategies of three common shark species that occupy similar habitats in the northwestern GOM.

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CHAPTER I

INTRODUCTION

Trophic interactions between predators and prey are important to understand ecosystem structure and health (Heithaus, Frid, Wirsing, & Worm, 2007). Analysis of the diets of predators can be used to identify targeted prey items that are important to the upper trophic levels in an ecosystem. They can also be used to quantify each prey item's relevance along with the overall predators' ecosystem impact (Cortés, 1999; Heithaus et al., 2007). Sharks can also play important roles as apex predators and exert a significant amount of top down control regulating prey density and diversity (Burgess et al., 2003; Myers, Baum, Shepherd, Powers, & Peterson, 2007). Along with significant ecosystem functions, sharks also provide a significant commercial and recreational fisheries impact, 97 million sharks harvested globally in 2010 (Worm et al., 2012). Sharks, in addition to being commercially and recreationally fished within the Gulf of Mexico, are vulnerable to longlining and trawling fisheries where they are often non-targeted bycatch (Hannan et al., 2013; Shepherd & Myers, 2005). Therefore, dietary composition and niche identification of sharks is important to evaluate their importance and in ecosystems where they are prone to vulnerability and exploitation.

Sharks within the order Carcharhiniformes comprise a large amount of the biomass within the northwest Gulf of Mexico (Burgess et al., 2003; Drymon, Powers, Dindo, Dzwonkowski, & Henwood, 2010). Three of the most abundant coastal Carcharhiniformes' in the Gulf of Mexico include, Atlantic Sharpnose (*Rhizoprionodon terraenovae*), Bonnethead (*Sphyrna tiburo*), and Blacktip (*Carcharhinus limbatus*) sharks. Distribution of these animals may vary due to their migratory nature, but they consistently appear to be abundant along the Texas coast (Burgess et al., 2003; Drymon et al., 2010). While similar in range, these species differ with respect to life history traits such as reproductive strategies, and assumed feeding patterns (D. M. Bethea, Carlson, Buckel, & Satterwhite, 2006; Castro, 1996; Cortés, Manire, & Hueter, 1996). East of the Mississippi, *C. limbatus* are known piscivores, targeting primarily sciaenids as juveniles and clupeids, along with other baitfish and larger teleosts, as they mature (Barry, Condrey, Driggers III, & Jones 2008; Branstetter, 1987; Castro, 1996). *R. terraenovae* are substantially more opportunistic, focusing on crustaceans such as shrimp, when they are juveniles, and clupeids and sciaenids and other teleost fishes along with cephalopods as they mature (D. M. Bethea et al., 2006; J. K. Carlson & Baremore, 2003). Bonnethead sharks are primarily benthic feeders, targeting stomatopods, shrimp, and cephalopods as juveniles and becoming focused on their main prey, blue crabs (*Callinectus sapidus*), as adults (Dana M. Bethea et al., 2007; Cortés et al., 1996). However, prey preference and ecological overlap of these three species within the northwestern Gulf of Mexico has yet to be fully quantified.

Looking at diet and foraging patterns where there is known species mixing can aid in understanding resource partitioning and trophic structure (Kinney, Hussey, Fisk, Tobin, & Simpfendorfer, 2011; Papastamatiou, Wetherbee, Lowe, & Crow, 2006). Few studies have been done using both stable isotope analysis and stomach contents to look at intraspecific variation in small scale environments (Drymon, Powers, & Carmichael, 2011). This combination of analysis offers useful information about short and long term feeding patterns, respectively (Kinney et al., 2011; Wells, Cowan, & Fry, 2008). Stomach contents can reveal what the animal has been feeding on over the past few hours to days (Cortés, 1997; Hynes, 1950; Hyslop, 1980), while stable isotope analysis can reveal the same behavior for weeks to months, both are depending upon species-specific tissue turnover rates (DeNiro & Epstein, 1978; Hussey et al., 2012; Post, 2002). Carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) stable isotopes within muscle tissue can be a powerful tool when combined to understand general feeding trends (del Rio, Wolf, Carleton, & Gannes, 2009; Gannes, O'Brien, & del Rio, 1997; B. J. Peterson & Fry, 1987). Carbon ratios are widely used to reveal the source(s) of primary production, and nitrogen ratios can reveal trophic level interactions along with individual trophic levels (DeNiro & Epstein, 1978; Hussey et al., 2012; Post, 2002). In addition, sulfur has been shown to reveal differences in pelagic versus benthic foraging strategies in teleost fishes (Fry et al., 2008; Kiyashkoa, Velivetskayab, & Ignatievb, 2011; Bruce J. Peterson, 1999; Thomas & Cahoon, 1993; Wells et al., 2008). Sulfur ($\delta^{34}S$) values tend to be lower in benthic zones due to the reduced uptake of the isotope in benthic invertebrates foraging on microbes at the bottom of the water column, while the values are higher in pelagic zones where the base of the food web is planktonic (Fry et al., 2008; Kiyashkoa et al., 2011; Bruce J. Peterson, 1999). Collectively, these three tracers have not been used with elasmobranchs to describe overall trophic and ecosystem structure within the marine food web.

The objectives of this study focus on ways to better understand the feeding ecology of three coastal shark species in the northwestern Gulf of Mexico. 1) To compare diets between the three species to quantify their most recent trophic position and analyze to observe any overlap. 2) In addition, bulk stable isotope analysis will be used to strengthen the understanding of partitioning between species and used to further identify niche behavior.

CHAPTER II

METHODS

Sample Collection

This study draws from a dataset of opportunistic samples gathered from the months of April through October in 2013 and 2014 from Galveston Bay, Texas. The samples were collected dockside from recreational fisherman, along with specimens (n=8) collected via bottom long line courtesy of the Texas Parks and Wildlife Department. Samples in 2013 were opportunistically collected from June through August. In 2014 subsamples were taken consisting of 25 samples per species per month, and a maximum of 10 samples per day for eight months from April to October. Sample location was assessed through angler interviews, and duration of trip and placed into categories of inshore (bay) or offshore (shelf). Inshore classification being bay or near shore trips, offshore was categorized by trips other than Galveston ship channel or Galveston bay. Each shark was sexed and three length measurements were taken to the nearest cm including total length, fork length, and precaudal length. Stomachs were removed from individuals at the dock and sealed via zip tie at the esophageal end and the anterior end of the scroll valve so that no contents were lost. Each specimen also had epaxial muscle tissue removed anterior to the primary dorsal fin. If the dorsal fin location could not be assessed after processing, tissue was removed from the dorsal portion of the vertebral column. All samples were then brought to the lab and prepared for storage.

Stomach Content and Stable Isotope Procedures

Tissue and vertebrae were immediately catalogued and frozen at - 20°C upon return to the lab. Stomachs were preserved via a 48-hour fixing process in 10% formalin, and then moved to a solution of 70% ethyl alcohol for longer term storage. Each stomach was measured for full wet weight, opened, and separated with a series of three metal mesh sieves sized 1.27 cm 1400μm, 500μm. All contents found within the stomachs were identified to lowest possible taxa, sorted, and weighed to the nearest 0.1 g.

Tissue samples were dried at 60°C for 48 hours in a Heratherm OGS180 drying oven (ThermoScientific). Then lipid extracted via the Dionex ASE 35, Accelerated Solvent Extractor, using 34mL cells with 3 cycles of 5 minute saturations with petroleum ether at 100°C and 1500 psi. Post-extraction the tissue was homogenized via Wig-L-Bug grinding mill, further dried at 60°C for 24 hours to remove any additional solvent, and encapsulated using 5x9 mm tin capsules, placed in a 96 plate well, and shipped for analysis. Stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ were performed using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (IRMS) (Sercon Ltd., Cheshire, UK), and $\delta^{34}S$ analysis was done using an Elementar vario ISOTOPE cube interfaced to a SerCon 20-22 IRMS (Sercon Ltd., Cheshire, UK). Heavy isotopes were compared to laboratory standards, nitrogen was compared via atmospheric N^2, carbon was compared via Vienna PeeDee Belemnite, and sulfur was compared via Vienna Canon Diablo Trilobite. All analysis was done through the Stable Isotope Facility at the University of California at Davis, CA USA. Stable isotope data was presented in delta notation, $\delta X = [(R_{sample}=R_{standard}) – 1] \times 1000$, where X is the heavy isotope, R_{sample} is the ratio of heavy to light isotope in the sample, and $R_{standard}$ is the ratio of heavy to light isotope in the reference standard. The need for lipid extraction was confirmed using replicate samples of extracted and non-extracted tissue for each species. Significant differences ($\alpha = 0.05$) were detected between mean carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios in both *C. limbatus* and *R. terraenovae*, while sulfur ($\delta^{34}S$) had no significant differences between extracted and non-extracted tissues, so the decision was made to fully extract all tissue to remove effects of high lipid concentration on isotopic ratios.

Data Analysis

Feeding patterns were investigated according to species, sex, year, month, and location (bay or shelf). Sharks were also separated into mature and immature for intraspecific analysis based upon von Bertalanffy growth curves (Branstetter, 1987; J. K. Carlson & Baremore, 2003; John K. Carlson & Parsons, 1997), yet due to a low comparable number between the two categories samples were placed into 10 cm incremented size bins for each species for interspecific analysis. *R. terraenovae* = maturity, Males= 73 cm, Females = 76 cm, *S. tiburo* = maturity, Males = 73 cm, Females = 80 cm, and *C. limbatus* = maturity, Males = 130 cm, Females = 153 cm. Analysis was done by organizing the taxonomic groups found within the stomachs into higher categories, highest level taxon was achieved at the subphylum and infraclass level (Teleostei, Crustacea, and Cephalopoda) while other taxon were grouped into Other (Echinodermata, Bivalva,and Phaeophyta). For further detail among groups, the group Teleostei was broken down into Family and the group Crustacea was broken down into Order. A percent index of relative importance (%IRI) was computed for prey items using (%W) weight, (%N) numerical quantity, and (%O) frequency of occurrence (Cortés, 1997; Pinkas, Oliphant, & Iverson, 1970) :

$$\mathrm{IRI} = (\%N + \%W) \times \%O$$

$$\%\mathrm{IRI} = (\mathrm{IRIprey\ item} / \mathrm{IRItotal}) \times 100$$

For analysis using stomach contents, (%W) weight was used to calculate difference between variables for individual sharks, because, it is a metric that is most used to quantify nutritional contribution (Rooker, 1995). Multiple analysis of variance (MANOVA) models were applied to all isotope ratios (δ^{13}C, δ^{15}N, and δ^{34}S) to assess differences among species, followed by analysis of variance (ANOVA) models to statistically compare the significant difference among species using individual isotope values using SYSTAT (Cranes Software International Ltd.). Linear regression tests were also run to determine any length effects correlating with δ^{13}C, δ^{15}N, and δ^{34}S isotope ratios. When length was determined to have significant effect, it was selected as a covariate and incorporated into analysis of covariate (ANCOVA) models which were used to determine inter and intraspecific differences using isotope ratios. Stomach contents by weight (%W) were analyzed using analysis of similarity (ANOSIM) and additional supplemental information was provided using similarity percentage analysis (SIMPER) to identify the most important prey items using PRIMER v6 (Clarke & Warwick, 2009). Stomachs containing contents with negligible weight and identifiable contents were excluded from all weight (%W) calculations, yet included on all IRI (%IRI) analysis. Further analysis using Shannon-Weiner diversity index (H´), incorporating both species evenness index (J´), and species richness (Gamma), to quantify the breadth of each species diet. Quadratic Discriminate Function Analysis (QDFA) was used to analyze classification success (using jackknife re-classification likelihood) for each species based upon δ^{13}C, δ^{15}N, and δ^{34}S isotope ratios, and percent weight (%W) of major taxonomic groups (Teleostei, Crustacea, Cephalopoda, and Other).

CHAPTER III

RESULTS

A total of 601 (n=305 *R. terraenovae*, n=239 *S. tiburo*, and n=57 *C. limbatus*) stomachs were analyzed for this project. Of the 601, 85% (n=508) contained identifiable contents and were used for statistical analysis. Along with stomachs analyzed, 136 (n=50 *R. terraenovae,* n=50 *S. tiburo,* and n=36 *C. limbatus*) tissue samples were used for stable isotope analysis. Size ranges and sex ratios varied for collected samples of each species, *R. terraenovae* 62.6cm – 108cm (Figure 1) (48 females, 257 males), *S. tiburo* 62cm – 125.4cm (Figure 2) (166 females, 73 males), and *C. limbatus* 66.9cm – 184.4cm (Figure 3) (31 females, 26 males).

Stomach Contents

Among the three species, 54 unique taxa were discovered through stomach content analysis including, 16 orders (8 Teleostei, 3 Crustacea, 1 Cephalopoda, 4 other), 22 families (17 Teleostei, 4 Crustacea, 1 other), 21 genera (15 Teleostei, 5 Crustacea, 1 other), and 23 species (16 Teleostei, 6 Crustacea, 1 other). Samples from *R. terraenovae* (n= 253) contained 37 taxa, *S. tiburo* (n=221) contained 23 taxa, and *C. limbatus* (n=35) contained 14 taxa. Shannon-Weiner diversity index resulted in *R. terraenovae* with the largest gamma diversity (*R. terraenovae* = 37, *S. tiburo* = 23, *C. limbatus* = 14) yet *S. tiburo* had the highest J' (*R. terraenovae* = 0.52, *S. tiburo* = 0.62, *C. limbatus* = 0.32) evenness and H' (*R. terraenovae* = 1.99, *S. tiburo* = 2.39, *C. limbatus* = 1.22) diversity indices.

Both *C. limbatus* (Table 1.) and *R. terraenovae* (Table 2.) had a majority of their contents primarily consisting of unidentified teleost fish, comprising % IRIs' of 91.16 and 98.95%, respectively. Primary prey categories for R. *terraenovae* included unidentified Teleostei (87.40 %IRI), Panaeidae (3.56 %IRI), Tuthoidea (2.92 %IRI). Within identified Teleostei, for *R. terraenovae*, the largest contributing fish taxon was Sciaenidae (1.84 %IRI). *C. limbatus*, had primary prey categories of unidentified Teleostei (88.52 %IRI) and *Micropogonias undulatus* (7.46 %IRI), additional overall non-teleost prey categories (Crustacea, Cephalopoda, and Other) summed to 1.15 %IRI. *S. tiburo* (Table 3.) had a diet primarily of crustaceans (87.20 % IRI), with the top three contributors as unidentified Brachyuran (48.91 %IRI), *Callinectes sapidus* (18.06 %IRI), and *C. similis* (3.32 % IRI).

Stomach content analysis using ANOSIM was done by organizing the taxonomic groups into higher categories and comparing them by percent weight (%W). Highest level taxon was achieved at the subphylum and infraclass level (Teleostei, Crustacea, and Cephalopoda) while other higher taxon were grouped into Other (Echinodermata, Bivalva, and Phaeophyta). ANOSIM for among species analysis using percent weight (%W) of the highest taxon, revealed all species to be significantly different (R = 0.501, p-value = 0.001). SIMPER analysis showed %W of Crustacea was the most important contributor driving diet differences between *S. tiburo* and *C. limbatus* (93.27 Average Dissimilarity) and *S. tiburo* and *R. terraenovae* (75.52 Average Dissimilarity) with highest values associated with *S. tiburo*. High percent weight (%W) of Teleostei combined with low percent weight (%W) of Crustacea were important for *C. limbatus*, while higher percent weight (%W) of Cephalopoda was most important for *R. terraenovae* diet relative to *C. limbatus* (32.81 Average Dissimilarity). Analysis was also run within species using factors month, sex, maturity, location, and year. Only maturity was found to be significant, for both *C. limbatus* (R = 0.214 p-value = 0.044) and *S. tiburo* (R = 0.098 p-value = 0.037).

To infer further relationships analysis of individual teleost families were analyzed by weight (%W) in ANOSIM using stomachs containing teleost fish identified to family (n= 91; 72 = *R. terraenovae,* 8 = *S. tiburo,* 11 = *C. limbatus*). Analysis among species was found to be non-significant (R= 0, p-value= 0.485) indicating consumption of similar families among species. However, lack of statistical representation of many families which may be a reason for the lack of significance. There were several families where only one occurrence of a prey item (n=9) was found (Scombridae, Gobiidae, Ephippidae, Synodontidae, Megalopidae, Sparidae, Ophichthidae, Serranidae, Carangidae). Several families were also only found within one species (n=4), (Ariidae for *C. limbatus*; Lutjanidae, Trichiuridae, Engraulidae for *R. terraenovae*). Lastly, some families had a low comparable sample size, (n=2) for all but one species (Mugilidae and Paralichthyidae). Leaving only two families that were found across all three species (Clupeidae and Sciaenidae). Only location was found to have significant difference as a factor (R= 0.122, p-value= 0.012). SIMPER analysis shows Sciaenids and Clupeids accounted for more weight (%W) in the bay while Lujanids and Mugilids had higher weight for sharks caught out on the shelf. No other factors were found to have significant differences between Teleost families.

Further inquiry into crustacean weight (%W) using ANOSIM for individual crustacean orders (Brachyura, Dendrobranchiata, and Stomatopoda) was done using a subsample (n=250; 63 = *R. terraenovae,* 186 = *S. tiburo*) of sharks containing crustaceans identified to order, *C. limbatus* (n=1) was excluded from an analysis due to low applicable sample size. One-way ANOSIM comparing species revealed an overall significant difference between comparable species (R= 0.557, p-value= 0.001). SIMPER analysis revealed that higher Brachyura weight (%W) in *S. tiburo* and higher Dendrobranchiata weight (%W) in *R. terraenovae* were the largest contributing groups to the difference. Only month was found to be a significant factors using one-way ANOSIM (R= 0.189, p-value= 0.001). SIMPER analysis revealed a higher consumption of Dendrobranchiata weight (%W) across all species in April, May, and June versus a reduced consumption in July, August, September, and October which correlate with the peak collections of *R. terraenovae*.

Stable Isotope Analysis

Analysis with MANOVA for among species comparisons using all isotope ratios ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) indicated significant differences among species (df = 9,399, F-ratio = 29.697, p-value < 0.001). Individual ANOVAs were then analyzed using individual isotope ratio relationships among species. $\delta^{13}C$ was found to be significantly different by species, with highest values in *S. tiburo* (-16.89 ± 0.05‰ s.e.), followed by *C. limbatus* (-16.94 ± 0.04‰ s.e.), and *R. terraenovae* (-17.03 ± 0.03‰ s.e.). $\delta^{15}N$ was also found to be significantly different across species with highest values for *C. limbatus* (16. 43 ± 0.09‰ s.e.), followed by *R. terraenovae* (16.04 ± 0.08‰ s.e.), and lastly *S. tiburo* (15.91 ± 0.08‰ s.e.). $\delta^{34}S$ was found to be significantly different among species, with highest values in *C. limbatus* (16.79 ± 0.14‰ s.e.), followed by *R. terraenovae* (16.70 ± 0.16‰ s.e.), and *S. tiburo* (15.94 ± 0.16‰ s.e.) (Table 4).

Intraspecific analysis was also done using ANOVA using month, sex, maturity, and year as factors for each of the isotope ratios. Location, which due to the low sample size from the shelf

(*S. tiburo,* n=1 and *C .limbatus*, n=0) was excluded from intraspecific analysis. δ^{13}C was also found to be higher for mature *S. tiburo* and *C. limbatus* as well as animals collected in 2013 relative to 2014. δ^{15}N was higher for female *R. terraenovae* relative to males; however, had no other significant effects between sex for the other two species. δ^{34}S had significant increases across all species in 2014 relative to 2013, and was higher in immature *S. tiburo* samples relative to mature (Table 5).

Regression analysis was run for each isotope and each species and compared to length (TL) to reveal ontogenetic patterns. *C. limbatus* length was found to have significant positive linear relationship between carbon (δ^{13}C) (y-intercept = -17.35; slope = 0.003) and nitrogen (δ^{15}N) (y-intercept = 15.39; slope = 0.008) and *S. tiburo* length was found to have a significant negative linear relationship between sulfur δ^{34}S (y-intercept = 18.82; slope = -0.029), while *R. terraenovae* showed no significant linear relationship (Table 6).

Quadratic Discriminate Function Analysis (QDFA) was then used to generate re-classification success for each species. Reclassification using weight (%W) was most useful in identifying specialization of feeding within the dataset (92% *S. tiburo,* 96% *C. limbatus,* and 17% *R. terraenovae*), while reclassification using isotope ratios yielded less accurate yet more consistent classification success (58% *S. tiburo*, 61% *C. limbatus*, and 38% *R. terraenovae*). Reclassification was improved over isotope ratios alone when combined with weight (%W) for *S. tiburo* (93%) and *C. limbatus* (76%) yet reduced for *R. terraenovae* (24%) due to higher dietary overlap (Figure 4.).

CHAPTER IV

DISCUSSION

Dietary and stable isotope analysis both yielded significantly different results for all three co-occurring shark species caught within the generalized region of Galveston Bay, TX indicating niche separation and resource partitioning. *S. tiburo* was found to be most unique with the vast majority of their diet consisting of benthic invertebrates. *C. limbatus* and *R. terraenovae* shared similar trends of teleost consumption, however *C. limbatus* was found to be a far more specialized feeder with almost exclusively teleost fish found within their stomachs. *R. terraenovae* was more generalized with a majority of teleost fish; however, their stomachs also included crustaceans and cephalopods.

C. limbatus has been categorized throughout several diet studies, including this one, as a large migratory piscivore. A full breadth of diet analysis was very challenging due to the high level of empty stomachs, which is consistent with previous studies averaging 56% (Hoffmayer & Parsons, 2003), 49% (Castro, 1996), and 60% (Barry et al., 2008) empty stomach as well as the low sample size in this study (n=57). However, our results from dietary analysis mirror the previously mentioned studies with almost exclusive teleost consumption indicating a specialized feeding strategy targeting fish. One clear result from the dietary analysis that differs from previous studies for *C. limbatus* is the preference for Atlantic croaker (*M. undulatus*) instead of the gulf menhaden (*B. patronus*) both of which are abundant in Galveston Bay (Rozas & Zimmerman, 2000) and in the nearshore and shoreline ecosystem along the Texas coast (Lewis et al., 2007). Previous studies indicated that *B. patronus* was the primary prey for *C. limbatus*

(Barry et al., 2008; Castro, 1996; Hoffmayer & Parsons, 2003), which may indicate a difference in dietary preference in the northwestern Gulf of Mexico compared with the southeastern United States and the northeastern Gulf of Mexico. Several unique prey items were found within *C. limbatus* that were not found within the other two shark species indicating a size class preference namely, fish belonging to Megalopidae and Ariidae. One stomach filled with approximately 58 grams of scales belonging to a *Megalops atlanticus*, with average scale diameters of 8 cm width, was found in a 1.3 meter female *C. limbatus*. This unique occurrence happened only once yet speaks to the difference in size class preference between the *C. limbatus* and the other two species.

Stable isotope analysis provided an excellent complement to dietary analysis for *C. limbatus* and compensated for data gaps resulting from a small sample size of stomachs. *C. limbatus* had significantly higher mean δ^{15}N indicative of higher trophic level feeding. *C. limbatus* also grows to a much larger size than both *R. terraenovae* and *S. tiburo* with a maximum size near 2 meters and has been shown to prey upon the other two species (Castro, 1996). *C. limbatus* has also been shown to be a longer lived animal with a slower maturation time relative to the other two species (Castro, 1996) which helps to explain the overall difference in feeding strategies corresponding to differences in life histories. Interspecific trends were detected via regression in *C. limbatus*, namely ontogenetic shifts in both δ^{13}C and δ^{15}N were both found to have significant increases with size corresponding with other studies suggesting feeding shift of *C. limbatus* relative to size (Castro, 1996). *C. limbatus* also are known to be highly migratory with regular seasonal migrations in the Gulf of Mexico and are thought to exhibit philopatry (Hueter, Heupel, Heist, &

Keeney, 2005) which may explain the increasing trend in δ^{13}C as the sharks begin to migrate from nursery areas as they grow.

S. tiburo is a highly specialized feeder that has been well established in its directed consumption of benthic invertebrates. This was evidenced by a large amount of stomach contents being crustaceans, the bulk of which were Greater Blue Crab (*Callinectes sapidus*). *C. sapidus* has been shown in several studies throughout the Gulf of Mexico and the Northwest Atlantic Ocean to be the primary diet of *S. tiburo* (Dana M. Bethea et al., 2007; Cortés et al., 1996). Our findings confirm a similar diet preference of *C. sapidus* with a focus on lesser blue crab (*C. similis*) and stomatopods. Another indicator of benthic specialization feeding strategies is evidence of the lower δ^{34}S for *S. tiburo* which when combined with generalized stomach contents provided 93% discrimination classification success relative to the other two species. Previous studies of *S. tiburo* observed ontogenetic diet shifts with increasing specificity towards larger *C. sapidus*, and moving away from other smaller prey items. Bethea et al (2007) and Cortés et al. (1996) found strong correlations between carapace length of *C. sapidus* and size of *S. tiburo* confirming changes in prey preference with size. Our findings did not show any correlation for diet preference changing with size for *S. tiburo*; however, δ^{34}S showed a negative relationship with size indicating an increase of specificity in benthic invertebrate consumption (Fry et al., 2008; Wells et al., 2008). Ontogenetic changes were also detected in δ^{13}C between immature and mature *S. tiburo*, which may also be linked to *C. sapidus*. *C. sapidus* spawn over several months from April to November, primarily in June through August, with larger females having several batches of eggs per season (Dickinson, Rittschof, & Latanich, 2006; Graham, Perry, Biesiot, & Fulford, 2012). When spawning, females move into offshore waters of higher salinity a corresponding pattern of older individuals are observed offshore (Dickinson et al., 2006)**.** This movement of prey can potentially drive larger *S. tiburo* further from the coast which is reflected in the increase in $\delta^{13}C$, which has been established as a useful tracer reflecting an offshore planktonic based food web vs. a nearshore terrestrial based food web (DeNiro & Epstein, 1978). *S. tiburo* was also shown to have a dietary contribution of vegetative material as similarly found in studies throughout the northwestern Atlantic Ocean. Cortés et al. (1998) found contribution from three species of sea grasses (*Thalassia testudinum*, *Syringodium filigorme*, and *Halodule wrightii*) in diets of *S. tiburo* in southwest Florida. An additional study from Bethea et al. (2007) found significant contributions from plant matter for the diets of *S. tiburo* although taxa were not elaborated upon. Findings from our study paralleled prior work done yet the primary vegetative matter found was strictly green algae (Chlorophyta) with no contributions from seagrasses or other angiosperms. These findings appear to be related to inadvertent bycatch and are correlated directly with *S. tiburo*'s primary prey *C. sapidus* along with similar benthic invertebrates as proposed by Cortés et al. (1998). This is further confirmed by the site related trends of algae rather than seagrass consumption due to Galveston Bay's low amount of seagrass beds and high amount of benthic colonial algae (Pulich & White, 1991).

R. terraenovae was shown to be the most diverse feeder confirming the trends established in prior dietary studies (D. M. Bethea et al., 2006; Drymon et al., 2011; Hoffmayer & Parsons, 2003). Prey items identified in stomach contents reflected the highest level of species richness and found the most abundant prey items were also shared with the other two species of sharks. Two taxonomic groups found in *R. terraenovae* stomachs were specifically unique in abundance,, panaeid shrimp and cephalopods, both of which are found readily in abundance throughout Galveston Bay (Minello & Webb, 1997; Rozas & Zimmerman, 2000), yet were not found in large numbers in either other species. This generalization and overlap was also shown in the low classification success using discriminate analysis with both isotope ratios and stomach contents. Interspecific trends found in previous studies such as ontogenetic and site related effects were not shown due to the low level of sampling diversity and high level of bias associated with fisheries dependent data. Differences were found between sexes using $\delta^{15}N$ which may be an effect of sex related size differences found in *R. terraenovae* (J. K. Carlson & Baremore, 2003) indicating size related trophic shifts due to the mean length among females averaged four cm longer. Anecdotal evidence provided through collection abundance showed a decrease in *R. terraenovae* when the presence of *C. limbatus* and *S. tiburo* increased, indicating a seasonal peak and niche absence exploitation.

Establishing feeding patterns and niche separation among migratory predators is crucial to understanding ecosystem dynamics and predator interactions. Assumptions that group species together as predators, without conclusive evidence of prey and feeding ecology, do not allow for accurate estimates of species impact. This study suggest that all three of these shark species, feed on similar trophic levels but partition their environment based upon their individual niche preferences. These important distinctions between the species with significant range overlap can be used to provide estimates of their ecosystem impact throughout their range in the northwest Gulf of Mexico. Further study can be used to fully describe the relationships of each shark species ecological relationships via prey diversity and abundance. Mixing models can also be applied using isotope ratios in known prey items found in close proximity to each captured animal and estimating enrichment based upon prey contribution and would provide more accurate estimates of prey contribution. Accurate estimates of feeding niches and impacts can add crucial data to ecosystem models and provide insight into apex predator impact in near coastal ecosystems.

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APPENDIX

Table 1. %IRI table for *C. limbatus*

Taxonomic Group		Individual Taxon	% (W) Weight	% (N) Numerical Abundance	% (O) Frequency	% IRI
Teleostei	Unidentified Teleostei		34.51	61.06	60.00	88.52
	Sciaenidae	Unidentified Sciaenidae	0.00	0.88	2.86	0.04
		Cynoscion arenarius	16.99	0.88	2.86	0.79
		Micropogonias undulatus	14.92	13.27	17.14	7.46
	Ephippidae	*Chaetodipterus faber*	3.74	1.77	2.86	0.24
	Paralichthyidae		8.23	1.77	2.86	0.44
	Clupeidae	*Brevoortia patronus*	1.23	0.88	2.86	0.09
	Ariidae		3.29	4.42	5.71	0.68
	Megalopidae	*Megalops atlanticus*	14.63	0.88	2.86	0.68
Crustacea	Unidentified Crustacean		0.00	1.77	5.71	0.16
	Panaeidae		1.65	0.88	2.86	0.11
Other	Bivalvia		0.12	7.08	2.86	0.32
	Gastropoda		0.69	1.77	2.86	0.11
	Various Algae		0.00	2.65	8.57	0.35

Table 2. %IRI table for *R. terraenovae*

Taxonomic Group		Individual Taxon	% (W) Weight	% (N) Numerical Abundance	% (O) Frequency	%IRI
Teleostei	Unidentified Teleostei		28.98	50.87	74.70	87.40
	Sciaenidae	Unidentified Sciaenidae	2.54	5.95	11.07	1.38
		Menticirrhus	0.36	0.21	0.79	0.01
		Menticirrhus americanus	0.17	0.10	0.40	0.00
		Menticirrhus littoralis	0.06	0.10	0.40	0.00
		Cynoscion arenarius	1.89	0.31	1.19	0.04
		Micropogonias undulatus	2.04	2.67	5.93	0.41
		Bairdiella chrysoura	0.57	0.10	0.40	0.00

		Pogonias cromis	0.33	0.10	0.40	0.00
	Serranidae		0.00	0.31	1.19	0.01
	Lutjanidae	Unidentified Lutjanidae	5.78	0.21	1.19	0.10
		Lutjanus campechanus	8.87	0.21	0.79	0.11
	Scombridae		0.00	0.21	0.79	0.00
	Carangidae	Unidentified Carangidae	0.55	0.31	0.79	0.01
		Chloroscombrus chrysurus	0.53	0.10	0.40	0.00
	Trichiuridae	*Trichiurus lepturus*	1.83	0.72	2.77	0.10
	Gobiidae	*Gobioides broussonetii*	1.41	0.10	0.40	0.01
	Sparidae	*Archosargus probatocephalus*	2.11	0.10	0.40	0.01
	Paralichthyidae		0.23	0.51	1.98	0.02
	Clupeidae	Unidentified Clupeidae	10.02	1.54	4.74	0.80
		Brevoortia patronus	8.12	1.33	3.95	0.55
	Engraulidae		1.36	0.72	1.58	0.05
	Mugilidae		4.08	0.41	1.58	0.10
	Ariidae		3.65	0.10	0.40	0.02
	Ophichthidae	*Ophichthus gomesii*	0.68	0.10	0.40	0.00
	Synodontidae	*Synodus foetens*	1.61	0.10	0.40	0.01
Crustacea	Unidentified Crustacean		0.78	4.72	15.42	1.24
	Panaeidae		4.87	8.21	18.58	3.56
	Unidentified Brachayuran		1.46	8.51	5.93	0.87
	Portunidae	Unidentified Portunidae	1.02	0.92	2.77	0.08
		Callinectes similis	1.00	0.72	1.98	0.05
	Aethridae	*Hepatus epheliticus*	0.05	0.10	0.40	0.00
	Stomatopoda	Unidentified Stomatopod	0.46	1.03	3.56	0.08
		Squilla empusa	0.03	0.10	0.40	0.00
		Gibbesa neglecta	0.01	0.10	0.40	0.00
Cephalopoda	Tuthoidea		2.51	7.18	20.55	2.92
Other	Various Algae		0.03	0.92	3.56	0.05

Table 3. %IRI table for *S. tiburo*

Taxonomic Group		Individual Taxon	% (W) Weight	% (N) Numerical Abundance	% (O) Frequency	% IRI
Teleostei	Unidentified Teleostei		4.87	3.60	10.86	1.79
	Sciaenidae	Unidentified Sciaenidae	0.91	0.12	0.45	0.01
		Micropogonias undulatus	0.00	0.12	0.45	0.00
	Clupeidae	Unidentified Clupeidae	1.50	0.35	1.36	0.05
		Brevoortia patronus	1.91	0.35	1.36	0.06
	Mugilidae		0.72	0.23	0.45	0.01
Crustacea	Unidentified Crustacean		2.29	8.26	30.32	6.20
	Panaeidae	Unidentified Panaeidae	3.82	2.91	6.33	0.83
		Litopenaeus setiferus	1.00	0.12	0.45	0.01
	Unidentified Brachayuran		23.61	24.42	52.49	48.91
	Portunidae	Unidentified Portunidae	9.38	8.60	13.57	4.74
		Callinectes sapidus	31.45	13.26	20.81	18.06
		Callinectes similis	9.81	5.93	10.86	3.32
	Xanthidae		0.14	0.35	1.36	0.01
	Stomatopoda	Unidentified Stomatopod	2.09	8.84	20.81	4.41
		Squilla empusa	1.77	3.37	5.88	0.59
		Gibbesa neglecta	0.08	0.35	0.90	0.01
Cephalopoda	Tuthoidea	Unidentified Tuthoidea	0.06	7.09	10.41	1.44
		Loliginaidae	0.27	0.12	0.45	0.00
Other	Bivalva		0.00	1.74	1.81	0.06
	Gastropoda		0.00	0.58	1.36	0.02
	Various Algae		4.29	9.19	35.75	9.35
	Echinodermata	*Ophiolepis elegans*	0.02	0.12	0.45	0.00

Table 4. ANOVA Table for stable isotope ratios among species (α=0.05), **Bold figures** indicate significant differences (α=0.05).

Test	R^2	F-Ratio	p-value	Pairwise Tukey's HSD	p-value
$\delta^{13}C$	0.047	3.119	**0.047**	*R. terraenovae* vs. *S. tiburo*	**0.036**
				R. terraenovae vs. *C. limbatus*	0.325
				C. limbatus vs. *S. tiburo*	0.682
$\delta^{15}N$	0.124	9.453	**<0.001**	*R. terraenovae* vs. *S. tiburo*	0.440
				R. terraenovae vs. *C. limbatus*	**0.005**
				C. limbatus vs. *S. tiburo*	**<0.001**
$\delta^{34}S$	0.117	8.840	**<0.001**	*R. terraenovae* vs. *S. tiburo*	**0.001**
				R. terraenovae vs. *C. limbatus*	0.913
				C. limbatus vs. *S. tiburo*	**0.001**

Table 5. Mean differences (±SE) of isotope ratios among independent variables. **Bold figures** indicate significant differences using ANOVA (α=0.05).

Species	**Dependent Variable**	**$\delta^{13}C$ (‰)**	**$\delta^{15}N$ (‰)**	**$\delta^{34}S$ (‰)**
R. terraenovae	Maturity (Mature/ Immature)	-17.02 ± 0.03 / -17.04 ± 0.06	16.05 ± 0.09 / 16.01 ± 0.16	16.68 ± 0.19 / 16.79 ± 0.36
	Year (2013/2014)	-17.07 ± 0.03 / -16.98 ± 0.04	15.94 ± 0.12 / 16.15 ± 0.11	**16.15 ± 0.26 / 17.25 ± 0.12**
	Sex (Male/Female)	-17.04 ± 0.03 / -16.99 ± 0.06	**16.25 ± 0.07 / 15.33 ± 0.10**	16.77 ±0.16 / 16.46 ± 0.48
S. tiburo	Maturity (Mature/ Immature)	**-16.83 ± 0.04 / -17.33 ± 0.19**	15.94 ± 0.07 / 15.69 ± 0.47	**15.78 ± 0.16 / 17.15 ± 0.27**
	Year (2013/2014)	-16.84 ± 0.07 / -16.95 ± 0.07	15.90 ± 0.11 / 15.91 ± 0.12	**15.45 ± 0.20 / 16.43 ± 0.20**
	Sex (Male/Female)	-16.81 ± 0.04 / -16.93 ± 0.07	16.06 ± 0.08 / 15.83 ± 0.11	15.74 ± 0.24 / 16.13 ± 0.20
C. limbatus	Maturity (Mature/ Immature)	**-16.83 ± 0.07 / -17.00 ± 0.04**	16.67 ± 0.21 / 16.31 ± 0.08	16.71 ± 0.21 / 16.84 ± 0.19
	Year (2013/2014)	**-17.06 ± 0.05 / -16.89 ± 0.04**	16.64 ± 0.20 / 16.34 ± 0.09	**16.30 ± 0.18 / 17.01 ± 0.18**
	Sex (Male/Female)	-17.00 ± 0.05 / -16.89 ± 0.06	16.31 ± 0.08 / 16.54 ± 0.15	16.68 ± 0.24 / 16.90 ± 0.17

Table 6.Regression results for isotope ratios vs length estimating evidence for ontogenetic dietary shifting. **Bold figures** indicate significant relationships (α=0.05).

Species	**Dependent Variable**	**R^2**	**Relationship**	**p-value**
S. tiburo	$\delta^{13}C$	0.033	N/A	0.110
	$\delta^{15}N$	0.039	N/A	0.089
	$\delta^{34}S$	0.106	-	**0.012**
R. terraenovae	$\delta^{13}C$	0.015	N/A	0.192
	$\delta^{15}N$	0.000	N/A	0.934
	$\delta^{34}S$	0.000	N/A	0.695
C. limbatus	**$\delta^{13}C$**	0.091	+	**0.041**
	$\delta^{15}N$	0.107	+	**0.029**
	$\delta^{34}S$	0.000	N/A	0.982

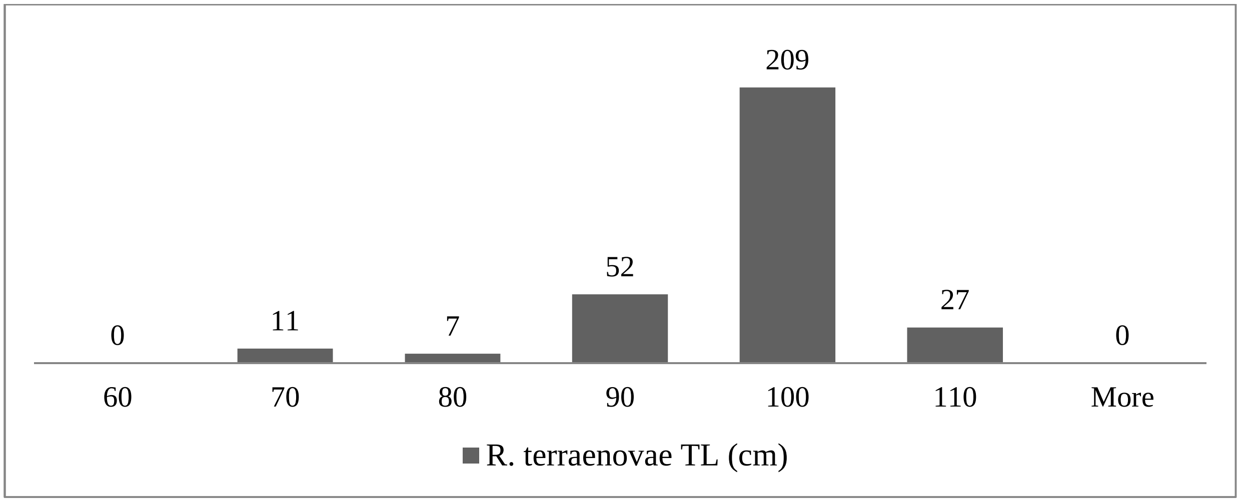

Figure 1. Size histogram for *R. terraenovae*

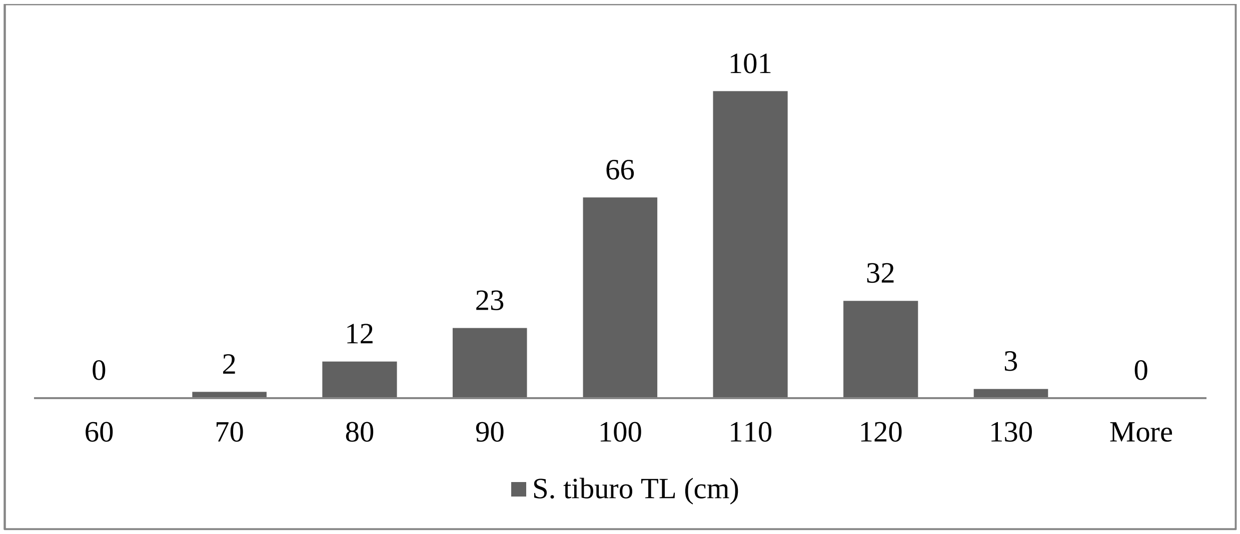

Figure 2. Size histogram for *S. tiburo*

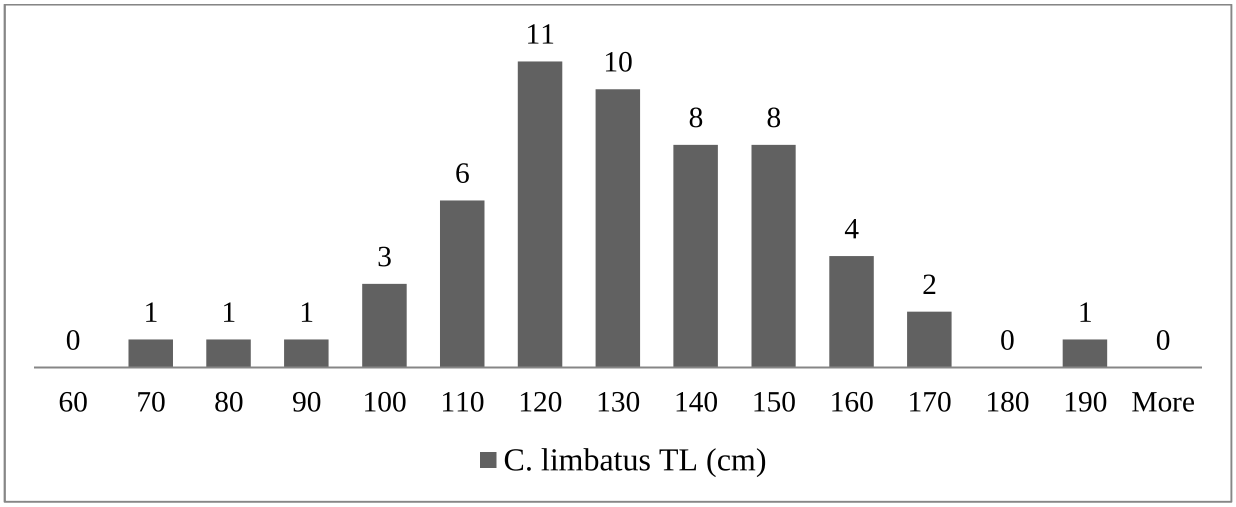

Figure 3. Size histogram for *C. limbatus*

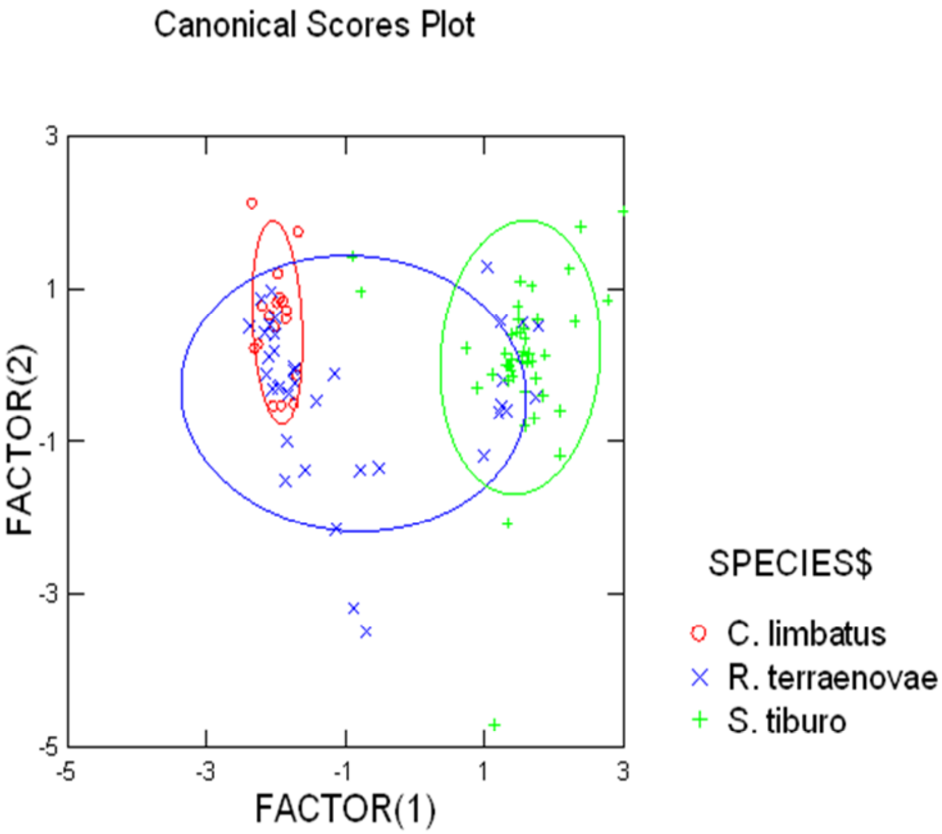

Figure 4. Canonical Scores Plots for differences among species using stomach contents (%W) and isotope ratios.

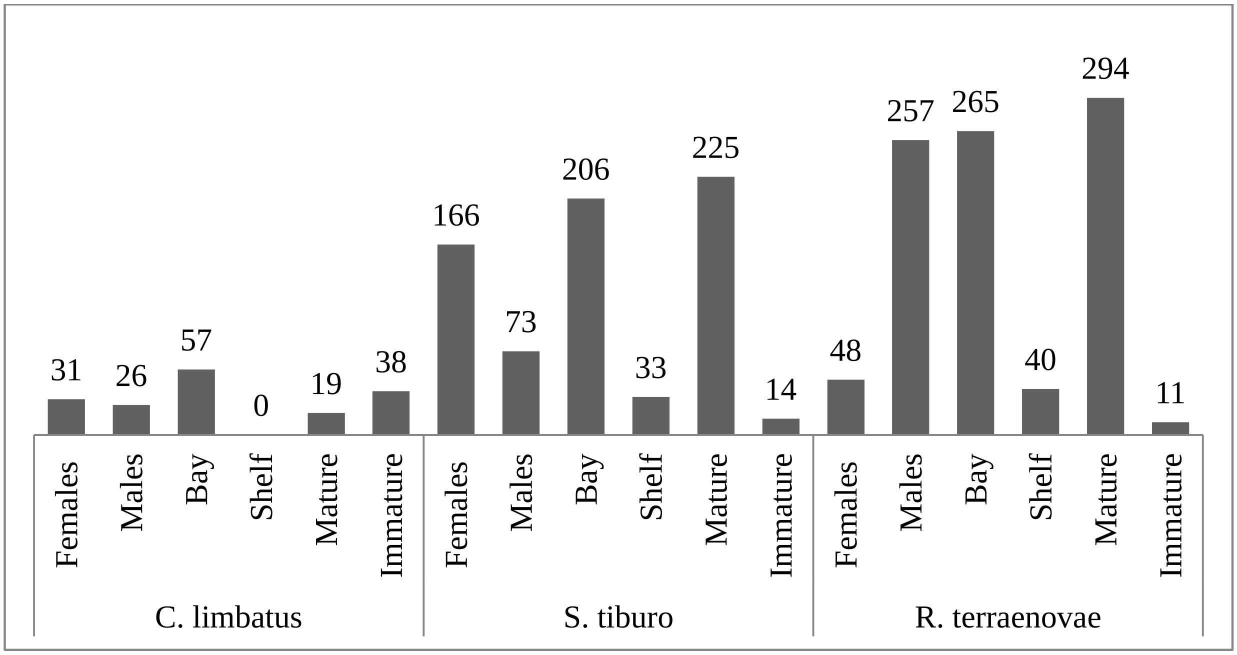

Figure 5. Sample demographics for each species, Sex, Location, and Maturity

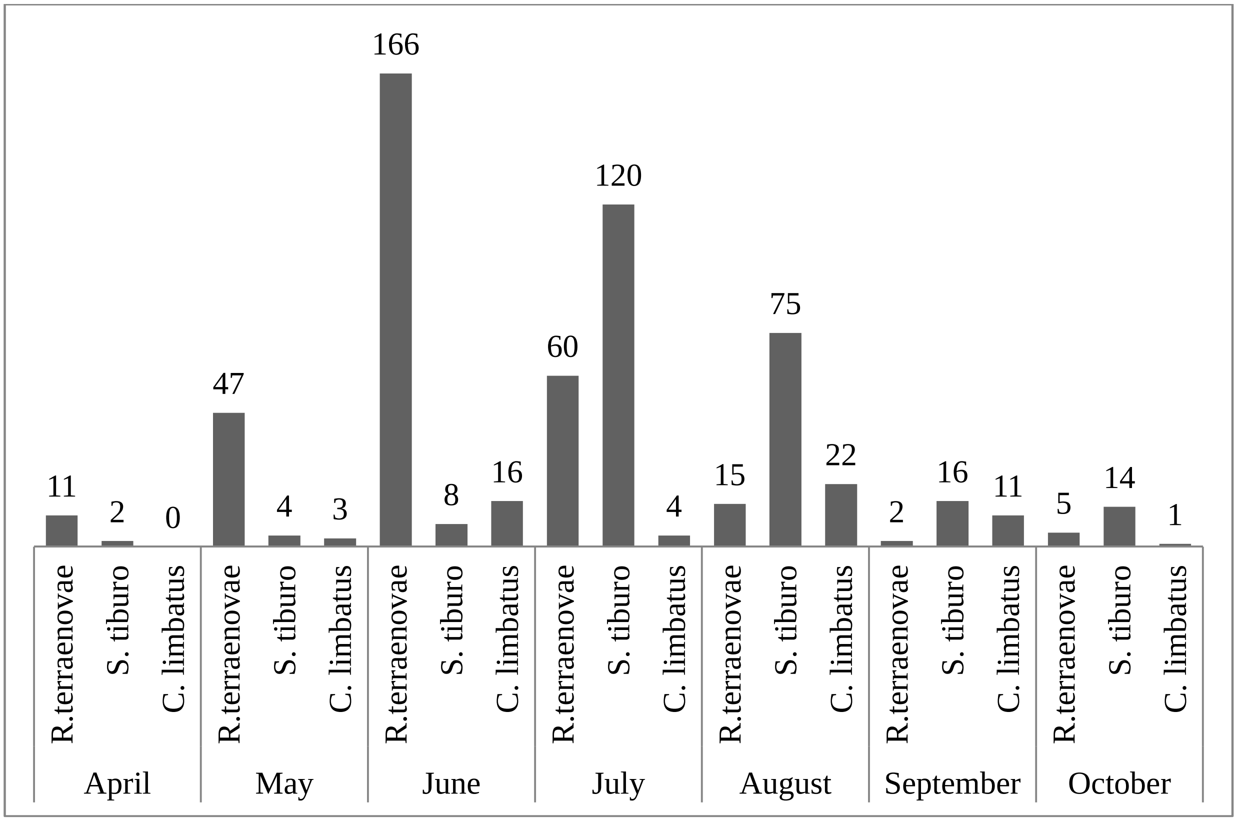

Figure 6. Monthly distribution for all shark species