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## SHORT COMMUNICATION

# DIET OF THE BLACKTIP SHARK (*CARCHARHINUS LIMBATUS*) IN THE NORTHWESTERN GULF OF MEXICO

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## INTRODUCTION

The Blacktip Shark (*Carcharhinus limbatus*) is a large species of requiem shark found across the globe in tropical and subtropical waters (Castro 1996, Burgess and Branstetter 2009). Blacktip Shark are among the most abundant shark species in the coastal regions of the Gulf of Mexico (GOM; Bethea et al. 2015) and are an important species both commercially and recreationally (de Silva et al. 2001, SEDAR 2018, NOAA Fisheries 2019). In 2018, the commercial landings of the Blacktip Shark in the GOM was estimated at 365.8 mt dressed weight (NOAA Fisheries 2019). Due to fishing pressures and nursery habitat degradation, the Blacktip Shark is classified as near threatened by the IUCN (Burgess and Branstetter 2009).

Understanding interactions among species is vital to building accurate ecosystem models, which inform both single species and ecosystem-based management strategies (Sagarese et al. 2017, Marshall et al. 2019). The most fundamental species interactions are predator-prey relationships. The Blacktip Shark is described as piscivorous, but also prey upon squid and crustaceans (Dudley and Cliff 1993); however, the specifics of their diet vary at regional scales (Barry et al. 2008). There have been several studies quantifying Blacktip Shark diets within the GOM (Hoffmayer and Parsons 2003, Bethea et al. 2004, Barry et al. 2008, Plumlee and Wells 2016) and their diets may differ among life stages (Barry et al. 2008). Prey accumulation curves of these previous studies did not reach an asymptote, indicating that further research is needed to fully characterize Blacktip Shark diets.

This study uses stomach contents analysis of opportunistically collected juvenile and adult Blacktip Shark, with the aims of further describing their diet within the northwestern GOM and comparing our results to those of previous studies. We also investigate differences in diet between different sexes and life stages.

## MATERIALS AND METHODS

Blacktip Shark specimens were collected opportunistically from recreational anglers at Galveston, TX, and Venice, LA, in the northwestern GOM from June–August 2016, February–September 2017, and July–August 2018. Bait type was not re-

corded; however, bait type was noted when stomach contents appeared to reflect bait. Upon collection, specimens were measured to the nearest cm using straight fork length (FL) and sex was recorded. Stomachs were immediately fixed in 10% formalin before being transferred to 70% ethanol for storage until processing.

Stomachs were weighed to the nearest 0.1 g, opened, and contents were sorted using metal sieves with mesh sizes of 1.27 cm, 1400  $\mu$ m, and 500  $\mu$ m. Contents were identified to the lowest possible taxon and weighed to the nearest 0.1 g. Dissociated otoliths were present in the stomach contents; these were weighed, identified to lowest possible taxon, and sorted into left and right otoliths where possible. From these otoliths the number of individuals was taken as the greater of either left or right otolith counts per taxon (Phillips et al. 2003). In several cases, stomach contents consisted of identifiable dissociated otoliths which clearly originated from unidentifiable teleost bodies in the same stomach (i.e. teleost bodies with empty saccules exposed), but the otoliths could not be directly matched to individual bodies. In these cases, weight for each taxon ( $Wb_{Ta}$ ) within a stomach was estimated according to the following equation:

$$\text{Equation 1: } Wb_{Ta} = WO_{Ta} + Wb_U \times \frac{N_{Ta}}{N_U},$$

where  $WO_{Ta}$  is the weight of the otoliths of the taxon,  $Wb_U$  is the weight of all unidentifiable teleost bodies,  $N_{Ta}$  is the number of individuals of the taxon within the stomach as determined by otolith identification, and  $N_U$  is the total number of all unidentifiable teleost bodies.

Stomach contents were analyzed using the standard metrics percent weight (% W), percent number (% N) and percent frequency of occurrence (% O; Cortés 1997). The index of relative importance (IRI; Pinkas et al. 1971) was calculated as below and converted to a percentage (% IRI) :

$$\text{Equation 2: } \text{IRI} = (\%W + \%N) \times \%O.$$

For comparison between sexes and life stage, teleost (unidentified Teleostei, Sciaenidae, Hemiramphidae, Carangidae, Trichiuridae, Ariidae, Clupeidae and Elopidae), elasmobranch

**TABLE 1.** Stomach contents of Blacktip Shark ( $n = 90$ ) captured in the northwestern Gulf of Mexico 2016-2018 by taxonomic group. Data were rounded to 2 decimal places post-processing. IRI – Index of Relative Importance.

Group	Class/Family	Lowest Taxonomic Group	% Weight	% Number	% Occurrence	% IRI
Mollusca						0.46
		Unidentified Mollusca	<0.01	0.34	1.72	0.01
	Gastropoda	Unidentified Gastropoda	<0.01	0.34	1.72	0.01
	Cephalopoda					0.44
		Unidentified Cephalopoda	0.31	2.38	8.62	0.36
		Unidentified Loliginidae	0.33	0.34	1.72	0.02
		<i>Loligo</i> sp.	0.51	0.34	3.45	0.04
		<i>Lolliguncula brevis</i>	0.19	0.68	1.72	0.02
Crustacea						0.65
		Unidentified Crustacea	<0.01	0.34	1.72	0.01
	Squillidae	<i>Squilla empusa</i>	0.49	0.68	1.72	0.03
	Penaeidae					0.61
		Unidentified Penaeidae	1.03	0.68	3.45	0.09
		<i>Penaeus</i> sp.	1.25	2.38	8.62	0.48
		<i>Penaeus duorarum</i>	1.39	0.34	1.72	0.05
Elasmobranchii						0.03
	Rhinopteridae	<i>Rhinoptera bonasus</i>	0.63	0.34	1.72	0.03
Teleostei						98.95
		Unidentified Teleostei	13.88	38.10	77.59	61.70
		Unidentified Perciformes	0.80	3.40	6.90	0.44
	Elopidae	<i>Elops saurus</i>	14.85	0.34	1.72	0.40
	Clupeidae					2.36
		Unidentified Clupeidae	0.15	0.68	3.45	0.04
		<i>Brevoortia patronus</i>	15.50	2.04	8.62	2.31
	Ariidae	Unidentified Ariidae	2.92	2.72	6.90	0.60
	Trichiuridae	Unidentified Trichiuridae	0.68	1.36	6.90	0.22
	Carangidae	<i>Chloroscombrus chrysurus</i>	3.91	4.42	6.90	0.88
	Hemiramphidae	<i>Hemiramphus</i> sp.	0.43	0.34	1.72	0.02
	Sciaenidae					32.33
		Unidentified Sciaenidae	8.07	5.44	13.79	2.85
		<i>Cynoscion</i> sp.	2.56	2.38	5.17	0.39
		<i>Cynoscion arenarius</i>	0.59	0.34	1.72	0.02
		<i>Cynoscion nothus</i>	3.33	1.02	5.17	0.34
		<i>Menticirrhus</i> sp.	3.96	0.68	3.45	0.24
		<i>Menticirrhus littoralis</i>	0.20	0.68	3.45	0.05
		<i>Micropogonias undulatus</i>	23.08	28.23	36.21	28.43

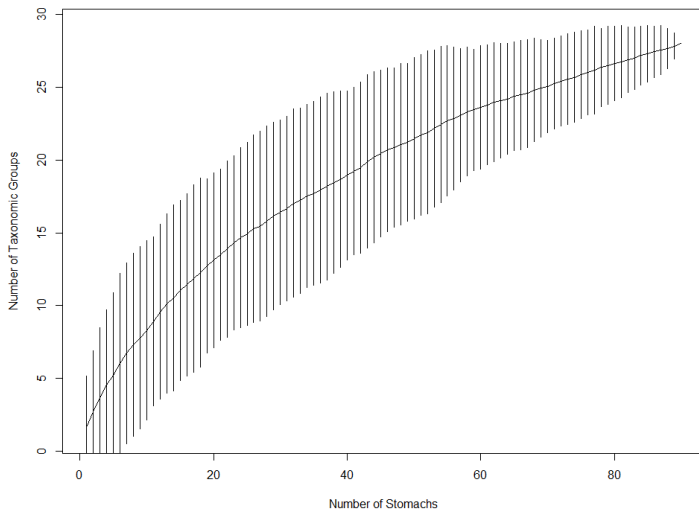
(Rhinopteridae) and crustacean (unidentified Crustacea, Penaeidae and Squillidae) prey were grouped into families, and mollusks (unidentified Mollusca, Gastropoda and Cephalopoda) were grouped into classes. Permutational multivariate analyses of variance (PERMANOVA) were used to test for differences in diet between sexes and life stages. Individuals were classified as either juvenile or adult based on length at 50% maturity ( $L_{50}$ ) for Blacktip Shark (female  $L_{50} = 119.2$  cm FL, male  $L_{50} = 105.8$  cm FL; Baremore and Passerotti 2013). Homoscedasticity of dispersions were tested using permutation tests for homogeneity of multivariate dispersions (PERMDISP). All analyses were performed on Bray–Curtis matrices of untransformed percent weight (% W) and percent number (% N). Where significant results were obtained, analysis of similarity

(SIMPER) was carried out to determine contribution of taxonomic groups to dissimilarity. Diet was not compared between the 2 sampling locations due to the comparatively low number of samples from Venice, LA ( $n = 2$ ) compared to Galveston, TX ( $n = 68$ ). Statistical analyses were carried out in R version 4.0.0 (R Core Team 2020) using the vegan package (Oksanen et al. 2019).

## RESULTS

A total of 90 Blacktip Shark stomachs were processed, of which 56 (62%) contained identifiable contents. Due to a labelling error, 20 stomachs did not have associated sex, length or location data, and were excluded from statistical analyses. A total of 28 taxa were identified, of which 11 were identified to the species level (Table 1). The prey accumulation curves

did not reach asymptote (Figure 1) indicating that the diet of Blacktip Shark in the northwestern GOM was not fully characterised by our study.



**Figure 1.** Randomized prey accumulation curve for Blacktip Shark (*Carcharhinus limbatus*,  $n = 90$ ) captured in the northwestern Gulf of Mexico 2016–2018. Mean and standard deviation are plotted.

Stomach contents were dominated by teleosts (98.95% IRI), with minor contribution from crustaceans (0.65% IRI), mollusks (0.46% IRI), and elasmobranchs (0.03% IRI). Most teleosts were unidentifiable (61.70% IRI). The most important teleost family was Sciaenidae (32.33% IRI), particularly Atlantic Croaker (*Micropogonias undulatus*, 28.43% IRI), which was the most important prey item identified to species level, and the second most important overall after unidentified teleost. Gulf Menhaden (*Brevoortia patronus*) was another important species (2.31% IRI) which made up the majority of Clupeidae (2.36% IRI). The majority of crustaceans were penaeid shrimps (0.61% IRI), and the majority of mollusks were cephalopods (0.44% IRI). Cownose Ray (*Rhinoptera bonasus*) comprised the elasmobranch portion of the diet (0.03% IRI). To our knowledge, this study is the first to report the mantis shrimp *Squilla empusa* as a component of Blacktip Shark diets globally, and halfbeaks (*Hemiramphus* sp.) as a diet component in the northern GOM (Figure 2, Table 1). The stomach contents from 6 individuals were hypothesized to be discards from shrimp boats (11% of stomachs with contents), as they featured multiple taxa, including shrimp, in similar states of digestion.

Of the 47 individuals containing stomach contents available for statistical analysis, 27 were female and 20 were male (57% and 43%, respectively). There was no significant difference in the diets of males and females for either % W (PERMANOVA, pseudo- $F_{1,45} = 0.62$ , permuted P-value = 0.62) or % N (PERMANOVA, pseudo- $F_{1,45} = 0.40$ , permuted P-value = 0.75).

For the ontogenetic comparison, 32 individuals were classified as juveniles and 15 as adults (68% and 32%, respectively). There was no difference in diet between juveniles and adults for % W (PERMANOVA, pseudo- $F_{1,45} = 2.10$ , permuted P-

value = 0.09); however, there was for % N (PERMANOVA, pseudo- $F_{1,45} = 3.52$ , permuted P-value = 0.03). The SIMPER analysis indicated this difference was driven by greater proportions of unidentified teleosts, Clupeidae and Penaeidae in the diets of juveniles (38.88%, 5.10% and 3.72% dissimilarity, respectively) and greater proportions of Sciaenidae, Ariidae and cephalopods in the diets of adults (34.64%, 5.21% and 4.34% dissimilarity, respectively).

## DISCUSSION

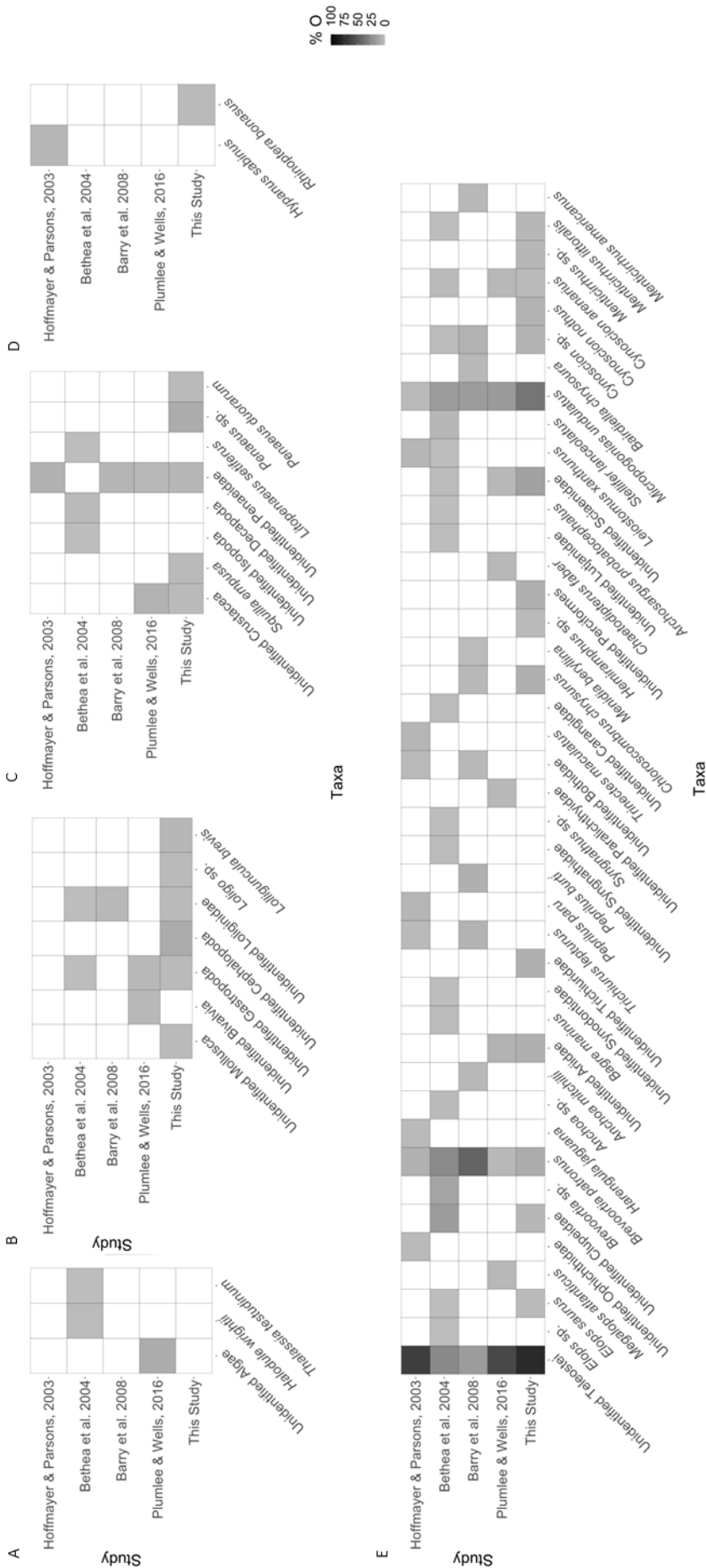
Our study supports previous findings of the primarily piscivorous diet of the Blacktip Shark in the northern GOM, along with minor consumption of crustaceans and cephalopods (Hoffmayer and Parsons 2003, Bethea et al. 2004, Barry et al. 2008, Plumlee and Wells 2016). The lack of asymptote in the prey accumulation curve, and the discovery of previously unreported species in stomach contents (Figure 2) highlights the importance of continued research into the diverse diet of the Blacktip Shark and possibly other species of sharks.

The most abundant prey species, Atlantic Croaker, is a common teleost in the northwestern GOM (Lewis et al. 2007). Given the low commercial and recreational interest in Atlantic croaker, it is unlikely that this food source is greatly impacted by human activity, though a growing fishery for juvenile Atlantic Croaker as live bait may have an impact in the future (VanderKooy 2017). The higher importance of Atlantic Croaker in our study and that of Bethea et al. (2004) compared to other studies (Hoffmayer and Parsons 2003, Barry et al. 2008, Plumlee and Wells 2016; Figure 2) is likely because both studies extrapolated identified otoliths to weights, which was not done in the other 3 studies.

Comparison of our results with those from a study of Blacktip Shark along the Florida Gulf coast (Heupel and Hueter 2002) emphasises the need for regionally specific analysis of Blacktip Shark diets. Heupel and Hueter (2002) report that Pinfish (*Lagodon rhomboides*) was the most abundant identifiable species in Blacktip Shark stomachs from Terra Ceia Bay, Florida, and did not report the occurrence of Atlantic Croaker. We did not find Pinfish in our study and this prey species was not found in other studies in the northern GOM (Hoffmayer and Parsons 2003, Bethea et al. 2004, Barry et al. 2008, Plumlee and Wells 2016; Figure 2), despite the species being common in the area (Lewis et al. 2007). It may be that Pinfish form part of the unidentified component of the northwestern GOM study results. The lack of Atlantic Croaker in Florida studies is unsurprising, as they are rare south of Tampa Bay (VanderKooy 2017). The switch to the consumption of Pinfish where Atlantic Croaker are not present may indicate that Blacktip Shark has an opportunistic general diet.

Our study suggests shrimp fishery discards may contribute to Blacktip Shark diets. Blacktip Shark feeding on shrimp fishery discards has been previously reported (Castro 1996), and the species is known to congregate around shrimp trawlers in the GOM. Shrimp fisheries are characterised by very high levels of bycatch (Karp et al. 2011, Scott-Denton et al. 2012), including species commonly found in Blacktip Shark





stomachs such as Atlantic Croaker and seatrout (*Cynoscion* spp.). More research is needed to understand and quantify the importance of shrimp bycatch to Blacktip Shark diets.

Gulf Menhaden have been previously reported to form a small but significant component of Blacktip Shark diets (Hoffmayer and Parsons 2003, Bethea et al. 2004, Barry et al. 2008, Plumlee and Wells 2016; Figure 2), which was verified in the present study. Identification of predators of Gulf Menhaden is crucial, as the menhaden (*Brevoortia* spp.) fishery is the second largest in the USA in terms of landings, of which the majority is Gulf Menhaden (National Marine Fisheries Service 2020). Despite their economic and ecological importance, the majority of expected predation on Gulf Menhaden remains unaccounted for (Sagarese et al. 2016). SIMPER analysis indicated that Clupeidae (primarily Gulf Menhaden) was a greater component of the diet of juvenile vs. adult Blacktip Shark. Similarly, 2 studies that found Gulf Menhaden to be a larger component of the Blacktip Shark diets (Bethea et al. 2004, Barry et al. 2008) studied neonates and juveniles exclusively. In comparison, this study, along with Plumlee and Wells (2016) studied juveniles and adults and found that Atlantic Croaker were the largest proportion of the identifiable diet (Figure 2). Hoffmayer and Parsons (2003) reported a high frequency of occurrence of Gulf Menhaden in Blacktip Shark stomachs, although life stage was not reported in this study. It is possible that a component of the unidentified teleosts in our study and others were Gulf Menhaden. The need to identify Gulf Menhaden in Blacktip Shark diets could be resolved using DNA metabarcoding.

There is still work to be done to fully quantify the contribution of different teleost species to the Blacktip Shark diet, and to fully realise the small but species rich non–teleost portion of the diet. As with other Blacktip Shark diet studies in the northern GOM (Hoffmayer and Parsons 2003, Bethea et al. 2004, Barry et al. 2008, Plumlee and Wells 2016), this study resulted in a large proportion of teleost material which could not be identified (61.70 % IRI in our study). DNA metabarcoding is increasingly used in diet studies (Berry et al. 2015, Harms–Tuohy et al. 2016) and offers a potential method for

**FIGURE 2.** Comparison of % frequency of occurrence (% O) of taxonomic groups of Blacktip Shark (*Carcharhinus limbatus*) prey across 5 studies in the northern Gulf of Mexico. Taxonomic groups are sorted into A. miscellaneous, B. Mollusca, C. Crustacea, D. Elasmobranchii and E. Teleostei. Where studies reported life stages separately (i.e., Bethea et al. 2004 and Barry et al. 2008), a weighted average was taken using number of stomachs with contents. Darker shading indicates a higher % O.

overcoming this problem. Fully understanding the diets of the Blacktip Shark is essential to building robust and accurate ecosystem models on which ecosystem-based fishery management can be based.

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