FINAL REPORT

Evaluating the Importance of Atlantic Chub Mackerel (*Scomber colias*) in the Diet of Highly Migratory Species in the Northwest Atlantic

Funding Opportunity-Mid-Atlantic Fishery Management Council Chub Mackerel RFP

Submitted To: Dr. Chris Moore
Executive Director Mid-Atlantic Fishery Management Council
800 North State Street, Suite 201, Dover, DE 19901
(302) 674-2331
cmoore@mamfc.org

Submitted By: School of Marine Sciences,
5706 Aubert Hall, RM 360,
University of Maine, Orono, ME 04469-5706

Principal Investigators:
Walter Golet, Ph.D. (PI)
Assistant Professor
School of Marine Sciences, University of Maine, Gulf of Maine Research Institute
350 Commercial Street, Portland Maine, 04101
walter.golet@maine.edu
1-207-228-1671

John Logan, Ph.D. (co-PI)
Aquatic Biologist
Massachusetts Division of Marine Fisheries
836 South Rodney French Boulevard, New Bedford, MA 02744
john.logan@state.ma.us
1-508-742-9722

Lisa Kerr, Ph.D. (co-PI)
Research Scientist
Gulf of Maine Research Institute
350 Commercial Street, Portland, Maine 04101
lkerr@gmri.org
207-228-1639

Joe Quattro, Ph.D. (co-PI)
Professor
Marine Science Program and Department of Biological Sciences, University of South Carolina, Columbia, South Carolina 29208
Quattro@biol.sc.edu
803-777-3240

Project Period: June 1, 2018 to June 30, 2021

Introduction

Highly migratory species (tunas, billfishes, sharks) support some of the most important commercial and recreational fisheries in the world. An elevated metabolism, pelagic lifestyle and large reproductive output require these species utilize large portions of ocean basins to satisfy their energetic demands. Migrations across large expanses of ocean are generally driven by oceanographic conditions that support prey aggregations or successful larval development. However, food webs in marine systems are complex and dynamic, especially for highly migratory species (HMS) that reside in offshore pelagic environments. Understanding foraging ecology is essential for interpreting changes in catch per unit effort (CPUE), changes in spatial and temporal distribution, energetic pathways, and recently management strategy evaluation where the abundance and composition of prey is explicitly considered in the assessment and management framework. Further, knowledge gaps for food webs can create barriers in management whereby a forage base with important energetic contributions to a top predator/s are not considered or where that forage base is exploited at levels which impact life history attributes of top predators. A fundamental understanding of the drivers associated with HMS distribution is essential for interpreting changes in CPUE, one of the most important inputs for stock assessment models worldwide. Further, a general understanding of function and energy flow in pelagic systems produces baseline data that can be used to assess large-scale ocean changes possibly linked to environmental drivers.

Despite the benefits to understanding foraging ecologyin pelagic systems, relatively little effort has been directed at or supported foraging ecology work in offshore marine systems. Given a general lack of sampling there is little information on the trophic ecology of these species, especially in the northwest Atlantic. This includes understanding the main vectors of energy flow, and a general understanding of trophic ecology in the offshore environment. This data gap is especially evident in the western Atlantic where existing diet data are limited relative to other ocean basins (Fig 1) (Young et al. 2015, Olson et al. 2016). Our understanding of food webs and large pelagic fish trophic ecology in other ocean basins has been greatly improved through cooperative sampling programs established with recreational and commercial fishing fleets in those regions (Nicol et al. 2013). Large- and small-scale sampling efforts have been combined to inform trophic structure and establish relative importance of dietary contributions. For example, long-term sampling efforts recently allowed researchers to identify decadal shifts in yellowfin tuna diet in the eastern Pacific (Olson et al. 2014). Basic information on foraging ecology in the Atlantic is lacking and expansion of sampling specifically in the western Atlantic would allow this region to be better represented in global diet and food web modeling analyses that seek to improve our understanding of climate change impacts to pelagic food webs and top predators (Young et al. 2015, Olson et al. 2016). Further, evaluation of the trophic ecology of highly migratory species allows us to identify regionally important prey species and apply ecosystem-based management principles to assess how different scales of fishing mortality on these potential prey bases may impact top pelagic predators.

Understanding foraging ecology for HMS is difficult given their highly migratory nature, the dynamics of ocean currents, water masses and prey aggregations. Large-scale shifts in distribution, however, can be attributed to changes in prey (Golet et al. 2015), especially if those predators rely heavily on one or two species for most of their energetic requirements (Chase

2002, Logan et al. 2015). These shifts can have pronounced effects on fish life history and trends in relative catch rates. Declining catch rates across multiple indices are often interpreted as a reduction in spawning stock biomass. However, those shifts in CPUE can also be artifacts of changes in distribution (Golet et al. 2015). Understanding food web dynamics is also important as we move towards an ecosystem approach to management. Understanding the importance and dynamics of lower trophic levels and their impact on upper predators, particularly economically important species (e.g. tunas/billfish) is a high priority. The Atlantic herring stock assessment has undergone a similar management strategy evaluation incorporating the importance of herring in the diets of top predators whereby managers allocate a certain proportion of the spawning stock biomass for ecosystem services.

Given their horizontal and vertical migratory patterns, HMS consumes a variety of different prey items, many of which are region-specific (Olson et al. 2016). Few, if any foraging studies can capture these regional differences in prey consumption in a single study. Rather, foraging ecology is often estimated during discrete time periods and geographic regions, but if done consistently and frequently enough, can be combined to inform assessment and management. While limited in the northwest Atlantic, foraging ecology of HMS has been examined from stomachs collected in recreational and commercial fisheries sporadically across three decades, predominantly south of Virginia. Most of these studies have relied on recreationally captured billfishes (marlins and swordfish) and tunas. Despite the temporal and spatial restrictions, these studies have provided important foraging data over relatively long time periods. For example, blue marlin diets examined over a decade from the Big Rock Tournament have been dominated by Scombridae often *Auxis* spp, and to a lesser degree *Scomber* species (Atlantic mackerel, king mackerel) (Ruderhaunsen et al 2010). Yellowfin tuna diet has been stable through time (Manooch and Mason 1983, Ruderhaunsen et al 2010) with the largest dietary contributions coming from Scombridae, Exocoetidae, and cephalopods (Ruderhaunsen et al 2010, Staudinger et al 2013). Information on the foraging ecology of bigeye tuna, swordfish, albacore tuna, and white and blue marlin in the western Atlantic is sparse and, in many cases, non-existent (Olson et al. 2016). All of the western Atlantic studies to date have used standard stomach contents analysis, i.e. visual assessment of dietary items. This method is useful for identifying intact prey, but the stomachs of HMS digest prey rapidly, especially the tunas which maintain elevated peritoneal temperatures. These elevated temperatures render most prey unidentifiable within hours often leaving a substantial amount of unidentifiable prey (Carey and Lawson 1973). While this method has been the standard for decades, complementary techniques (e.g. genetic barcoding) now allow for broader and more definitive identification of stomach contents, including species that cannot be identified using external morphological characteristics. Despite these advancements, all the diet studies in the western north Atlantic have utilized standard stomach contents analysis which in many cases can leave substantial amounts of prey unidentified or only coarsely identified to the family or genus level. For example, scombrids observed in stomach contents in published diet studies of pelagic predators in the western Atlantic were only identified to family level (Teffer et al. 2015) or predominantly to genus level (Rudershausen et al. 2010). Further, even though some data sets extend for ten years they are generally restricted to small geographic regions.

Here, we utilized a complementary set of techniques (visual ID and genetic barcoding) to examine the foraging ecology of HMS in the northwest Atlantic, specifically white and blue

marlin, yellowfin and bigeye tuna to quantify the importance of chub mackerel (*Scomber colias*) in the diets of these top-level predators in the mid-Atlantic region. This work built upon existing datasets and strengthened sampling in areas with limited or no sampling coverage.

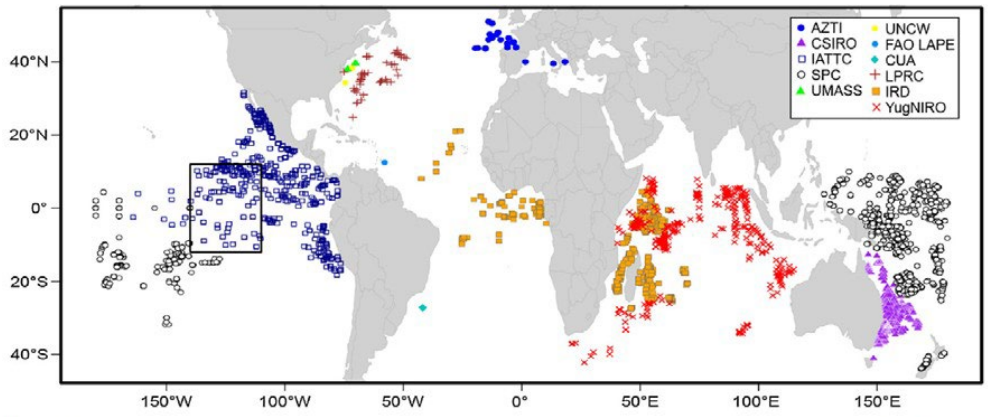


Fig. 1 Map showing the sampling locations where stomach contents and isotopic data for large pelagic predator fishes were compiled by CLIOTOP (Climate Impacts on Oceanic Top Predators) WG3 (Working Group 3: Trophic pathways in the open ocean ecosystems), and a sub-area (*black square*) in the eastern Pacific Ocean where yellowfin tuna data were selected to demonstrate the methods and analyses developed during the four workshops. Organization acronyms are defined as follows: *AZTI* Arrantzuarekiko Zientzia eta Teknika Iraskundea, *CSIRO* Commonwealth Scientific and Industrial Research Organization of Australia, *IATTC* Inter-American Tropical Tuna Commission, *SPC* Secretariat of the Pacific Community, *UMASS* University of Massachusetts, Amherst, *UNCW* University of North Carolina, Wilmington, *FAO LAPE* Food and Agriculture Organization of the United Nations Lesser Antilles Pelagics Ecosystem Project, *CUA* Centro Universitário de Araraquara, *LPRC* Large Pelagics Research Center, *IRD* Institut de Recherche pour le Développement, *YugNIRO* Southern Scientific Research Institute of Marine Fisheries and Oceanography

Adopted from Young et al. 2015

Scope of Work/Methods

Sample Collection

We evaluated the foraging ecology of marlins and tunas captured along the US east coast from the canyons off southern New England to South Carolina, with a specific emphasis on identifying if chub mackerel were an important prey species over a three-year period. Sampling HMS species is difficult, distributions can shift rapidly, elevated temperatures in the peritoneal cavity promote rapid digestion, and fish are known to regurgitate during the capture process. In fact, marlins often evert their stomachs completely outside their body when hooked. However, targeted sampling with specific protocols can enhance sampling opportunities and increase preservation of collected samples. Current regulations state US commercial fisheries (e.g. pelagic longline) cannot retain marlins in the Atlantic, all hooked marlins must be released immediately whether alive or dead at haul back. In keeping with recommendations through the International Commission for the Conservation of Atlantic Tunas (ICCAT), the US is allowed to harvest a combined 250 round-scale spearfish, white and blue marlin annually by recreational rod and reel only. Marlins harvested in US recreational fisheries, including tournaments, must meet Federal minimum size guidelines for each species and when directed those minimum sizes may exceed Federal minimums if self-imposed by tournament officials. Therefore, the availability of marlin samples is generally limited since many of these fish are released alive.

However, two large tournaments are held annually that retain marlins which provided us with sampling opportunities located within the geographic scope of this project. Those included the White Marlin Open held in Ocean City Maryland and the Mid-Atlantic, held in Ocean City

Maryland and Cape May New Jersey. Samples were collected from the White Marlin open in 2018 and 2019. We did not sample the White Marlin Open in 2020 due to travel restrictions imposed by PI Golet's academic institution related to Covid-19. The mid-Atlantic was sampled in 2018, 2019, and 2020 as PI Golet traveled independently of his institution to collect samples from the Cape May weigh station in 2020. Opportunistic sampling for Albacore tuna occurred at these tournaments but were not a directed sampling species. A summary of sampling by species is provided in Table 1. Bigeye and yellowfin tuna landed in commercial pelagic longline and recreational rod and reel fisheries were sampled from June to September in 2018 and June to November in 2019. Fish in this study were caught in the Gulf Stream current, the continental shelf, and slope canyons from Cape Hatteras to the international maritime border of Canada and the United States (Fig 2)

Sp.	2018	2019	2020
Blue marlin	6	10	1
White marlin	13	21	2
Roundscale spearfish	20	21	3
Albacore	0	25	6

Table 1 Stomachs sampled from *Kajikia albida, Makaira nigricans, Tetraturus geogii*, and *Thunnus alalunga*

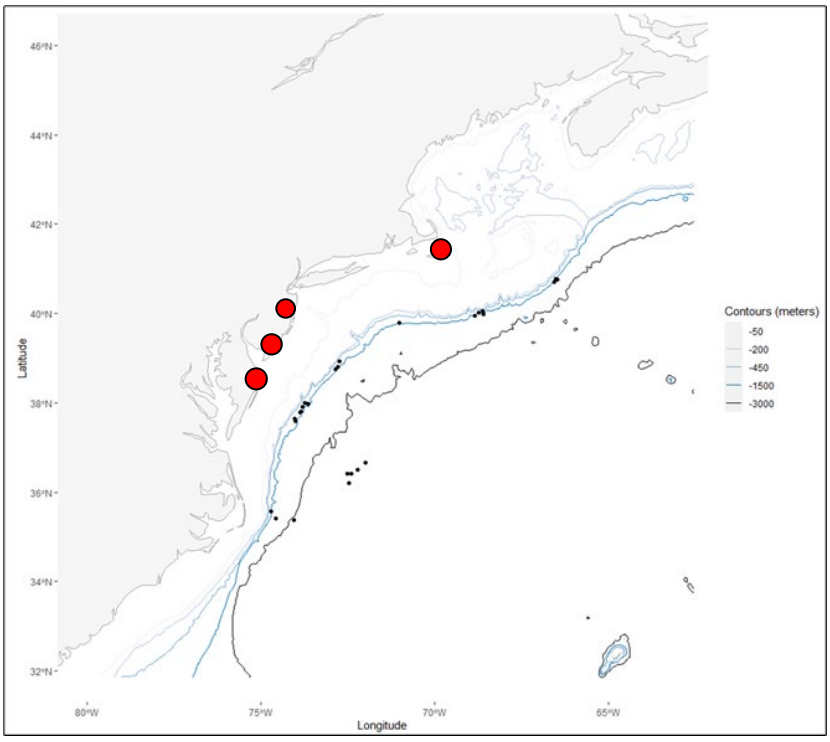


Fig 2. Geographic distribution of sample collection sites from this study for marlins and tunas. The small black dots represent pelagic longline sets. Note these are not precise locations where each fish was captured, rather the dots represent the set location. The red dots represent recreational sampling locations including Martha's Vineyard MA, Point Pleasant NJ, Cape May NJ, and Ocean City Maryland.

Outside of the marlin tournaments, tuna sampling included rod and reel at five separate tournaments and repeated sampling of landings from a single charter vessel in New Jersey. Sea surface temperature (SST), gear dynamics, latitude and longitude were also recorded for samples obtained from 5 commercial longline trips for each haul and set. Fish sampled repeatedly from the charter vessel were caught along the shelf break canyons (personal comm) and exact locations of tunas landed in tournaments are unknown although fishing likely occurred at the shelf break and on the shelf itself according to spatial limitations of each tournament and previous studies of this fishery in the Mid-Atlantic Bight. Longlines sets occurred at dusk and were hauled at dawn. Hooks were baited with *Illex illecebrosus* (>150mm mantle length) and fished at depths of 20-50 meters.

Sets included five hooks between buoys, sections containing 120 hooks, with number of sections per set ranging from 3-10 (mean =7). Recreational fisheries operated during night and day mostly using surface trolled ballyhoo and artificial squid baits. In late summer and fall, rod and reel gear also targeted fish at depth by chunking or chumming butterfish baits. Stomachs were separated by respective sampling events to analyze spatial and temporal differences in diets of both predators (Table 2).

Sampling Event	Year	Gear	Mean CPUE	Latitude	Longitude	Ordinal days	Species	SST °C	Non-empty Stomachs
1	2018	LL	1.64	35.38-35.42	-74.57– -74.06	218-219	BET	28.2-30.0	1
			11.51				YFT		10
2	2019	LL	4.51	36.21-36.41	-72.52– -72.47	199-201	BET	29.4-29.5	15
			9.72				YFT		35
3	2018	RR		37.04-38.66	-74.62– -73.23	195-237	BET		14
							YFT		58
4	2019	RR		37.04-38.66	-74.62– -73.23	174	BET		0
							YFT		47
5	2019	RR		37.04-38.66	-74.62– -73.23	193-217	BET		0
							YFT		65
6	2018	RR		38.45-39.97	-73.49– -71.32	165-207	BET		9
							YFT		93
7	2018	RR		38.45-39.97	-73.49– -71.32	228-289	BET		5
							YFT		88
8	2019	RR		38.45-39.97	-73.49– -71.32	161-174	BET		12
							YFT		93
9	2019	LL	16.66	37.65-38.00	-74.03– -73.65	311-318	BET	17.6-20.8	82
			8.39				YFT		52
10	2018	RR		39.48-40.55	-71.83– -68.19	202	BET		7
							YFT		3
11	2019	LL	10.71	39.80-39.91	-71.03– -69.63	259-279	BET	22.5-23.3	9
			1.19				YFT		2
12	2019	LL	10.29	40.05-40.77	-68.63– -66.47	229-234	BET	26.4-28.2	33
			10.54				YFT		29

Table 2 Description of sea surface temperature (SST), capture gear, spatiotemporal ranges, longline CPUEs (fish/1,000 hooks), sampling events for non-empty stomachs collected between 2018-2019 for *Thunnus albacares* and *Thunnus obesus*.

Sex was recorded for each tuna and marlin (if available) and species were differentiated using liver, gonad and body morphology. Straight fork length (SFL) and curved fork length (CFL) were measured to the nearest 0.5 cm from tunas sampled at recreational ports and on longline vessels, respectively. Marlins were measured to the nearest .5cm (CFL) by tournament staff. In some cases, samples were obtained from recreational charter boats where SFL was immeasurable. Snout length (SL), the shortest distance from the tip of the rostrum to the eye was recorded and CFL was calculated using linear regressions from fish that had both SL and CFL measured. CFLs, both measured and calculated were then converted to SFL using standard equations. Stomachs from tunas caught in the commercial fishery were immediately eviscerated

from the esophagus, labeled internally and externally, then stored in Ziplock bags. Samples were then either frozen or stored on ice for the duration of the trip depending on vessel capability. Recreational tunas and marlins were put on ice after capture until sampling occurred on land at which point whole stomachs were kept on ice for transport until they could be frozen in lab. Stomachs were thawed and contents were rinsed into a 500-micron sieve. Depending on level of degradation, prey items were identified to the species level when possible or at least to phylum for heavily digested items. Fork length for chordates, mantle length for molluscs, carapace width for order Decapoda, and total length for all other arthropods were recorded when present. Every prey item was weighed to the nearest hundredth gram. Otoliths and beaks, loose and associated with tissue, were also used to identify prey items to the family level using references (Clarke 1986, Xavier and Cherel 2009). Loose hard parts not associated with tissue were not weighed but, compared separately based on number and occurrence due to temporal biases in digestion and accumulation. Items that were clearly used as bait from chumming, trolling, and longline with lacerations or bridles characteristic of each method were recorded but not involved in any statistical analyses.

DNA Extraction, Amplification and Sequencing

DNA was extracted from each sample using Qiagen DNeasy blood and tissue DNA extraction kits following the manufacturer's' protocol (QIAGEN Corporation, Maryland, USA) and then stored at -80℃. In our experience with genetic characterization of prey items from Atlantic bluefin tuna (*Thunnus thynuus*) stomachs (Butler et al. 2014), this extraction method consistently yields DNA of sufficient quality (and quantity) for subsequent PCR (Polymerase Chain Reaction). The presence and quality of DNA was confirmed by 1.5% agarose gel electrophoresis with ethidium bromide staining. The taxonomic identity of all identifiable teleostprey was verified primarily by PCR amplification and sequencing of the mitochondrial Cytochrome Oxidase I (COI) locus as described in Butler et al. (2015) and Hoffmayer et al. (2014) but using the LoboF1 and LoboR1 primers and conditions described in Lobo et al. (2013). For prey samples that failed to amplify with the 'Lobo' primer set, 'universal' 16s rRNA primers (16sar and 16sbr, Palumbi 1996) were used, a primer set, that in our experience, amplifies target DNA fragments much more consistently from degraded tissues (Helgoe and Quattro in preparation). PCR products were sequenced on an ABI 3130 automated sequencer using BigDye terminator sequencing (v 3.1, ThermoFisher Scientific, Waltham, Massachusetts, USA). DNA sequences obtained will be edited by eye using Sequencher (version 4.1; GenecodesCorporation, Michigan, USA) then parsed to analyses for taxonomic identification.

DNA Barcoding

DNA barcoding approaches were used to identify individual tissue samples to species wherever possible using three separate approaches to taxonomic identification. Approaches 1 and 2 used traditional 'BLAST' (Altschul et al. 1990) based searches using our unknown COI(or 16s) sequences as queries against those accessed in the 1) Barcode of Life Database (BOLD) and 2) GenBank sequence repositories. We used BLAST searches and the resulting homology scores (i.e., 'Max Score' and 'Identities' metrics) to identify samples to species. The third approach to sequence identity used the algorithms available in the software package SAP (Statistical Assignment Package). SAP assigned samples to a taxonomic group based on comparisons to a reference database. Specifically, SAP queried the NCBI onlinegenetic database, GenBank, for homologues and returned similar sequences with an identity of greater than 0.90 (we specified

the return of 100 most similar sequences that meet this criterion). SAP aligns the resultant sequences using ClustalW2 (Larkin et al. 2007) and used a Bayesian approach to calculate the probability of assignment to individual taxonomic categories (e.g., species, genus, family).

We considered taxonomic assignments with probabilities below 0.95 to be ambiguous and the next most proximal taxonomic category (e.g., family if genus wasambiguous) were used for identification (e.g., if identification to species cannot be confidently assigned, we defaulted to the next highest taxonomic rank receiving significant support). Taxonomic assignments, even with high probabilities, were assumed to be unreliable if sequence identity values between the queried sample sequence and the most similar homologueis less than 0.95.

Statistical Analyses

Calculated round weight using straight fork lengths and established length-weight equations (ICCAT) were used to determine repletion indices (g/kg) for both tuna species given they had sufficient sample sizes. Two-way ANOVAs were used to test differences in means between predator and gear. Left skewed values were log transformed to satisfy normality assumptions. Quantile regression was used to investigate predator/prey length relationships. 5th, 50th, and 95th quantile regressions were tested for significance with the package quantreg.

Number and weights of prey items by phylum, family, and species when available for predator species were summed and reported. Percent frequency of occurrence (*%FO$_i$*) and percent mean weight (*%MW$_i$*) were also reported and calculated as:

$$eq.1 \qquad \%FO_i = \frac{p_i}{p}\, x\, 100$$

$$eq.2 \qquad \%MW_i = \frac{1}{p}\sum_{j=1}^{p}\left(\frac{W_{ij}}{\sum_{i=1}^{n} W_{ij}}\right) x\, 100$$

Where $\%FO_i$ is the proportion of stomachs containing prey taxa (i) in all non-empty stomachs (p) and $\%MW_i$ is the proportion by weight of prey taxa (W_i) and the sum of weights of all prey taxa (n) in an individual stomach (j) divided by the number of all non-empty stomachs (p).

Sample size-based rarefaction and extrapolation curves using permutated frequency counts were plotted to investigate richness and diversity of prey families in each sampling event for tunas or by species for marlins. Observed richness and Shannon diversity were compared with extrapolated estimates double the sample sizes herein to assess sample size deficiencies. Analyses were conducted using the r iNEXT package.

For multivariate analyses prey was grouped into 14 guilds which was composed of consistently abundant prey families such as Ommastrephidae, Scombridae, Monacanthidae, Argonautidae, and other rare families grouped based on similar morphology, developmental stage, and/or habitat characteristics. Percent mass ($W_i / \sum_{i=1}^{n} W_i$) of prey guild in each individual stomach was used for ordination and non-parametric dimensional scaling (NMDS) was applied to visualize forage niche overlap between the tunas (Poland, Scharf, Staudinger et al. 2019). Analysis of similarity (ANOSIM) tests were also conducted using this matrix to explore the relationship of diets between predator, sampling events where n>28, latitude (≤36.41^{o}N, >36.41 and ≤38.66^{o}N, >38.66 and ≤39.97^{o}N, >39.97^{o}N), season (summer/fall), and capture gear. Prey guild *%MW$_i$* was

calculated for each predator specific sampling event where n>28 and Bray-Curtis and Chi-squared distances were calculated to visualize dissimilarities among sampling events. All analyses were conducted in Rstudio V3.4.3 using the vegan package.

Results

Bigeye and Yellowfin Tuna

Stomachs were collected from 199 bigeye and 606 yellowfin tuna, and more than 7,500 forage individuals were identified for these two tunas. Completely empty stomachs were limited occurring in only 2.53% of bigeye stomachs and 5.44% of yellowfin. Yellowfin tuna stomachs occasionally contained floating S*argassum spp*. and other macroalgae (15.8 %FO) while bigeye tuna largely did not (3.54 %FO). *Hirudinella ventricosa* and nematoda parasites occurred occasionally in both bigeye (12.6%) and yellowfin (12.2%) stomachs. No significant differences in SFL were detected for bigeye between gears ($t_{df(60.833)}$=-1.737, p=0.085), whereas mean SFL of yellowfin sampled by recreational (88.4 cm) and commercial gears (110.2 cm) differed significantly ($t_{df(37.761)}$=5.105, p< 0.001). For individuals whose sex could clearly be determined (BET-30.5%, YFT-22.6%) sex ratios were nearly 1:1 (BET-32F, 25M and YFT-62F, 69M).

Yellowfin and bigeye tuna lengths were well represented across years and gear categories (Fig 2). Length frequencies differed by sampling event and were not spatially homogenous (Fig 3). Length frequencies differed based on sampling event and were not uniform across Longline CPUEs also differed by sampling event with the largest values occurring in SE9 for bigeye and SE for yellowfin. Non-empty stomachs from 188 bigeye tuna (69.7-174.7 cm) and 574 yellowfin tuna (62.9-162.7 cm) contained prey associated with tissue from 57 families and at least 80 species. Prey identified exclusively from genetic barcoding included 5 families and 22 species. Hard part identification of loose otoliths and beaks revealed an additional 7 families not detected by genetic or gross identification methods (Table 4). Mean repletion values were 3.41 and 4.77 g kg^{-1} for yellowfin and bigeye tuna respectively. Log-transformed repletion indices of yellowfin and bigeye tuna captured with longline gear were significantly lower than those captured with rod and reel (TWO-WAY ANOVA, F=15.86, p<0.001, Fig. 4).

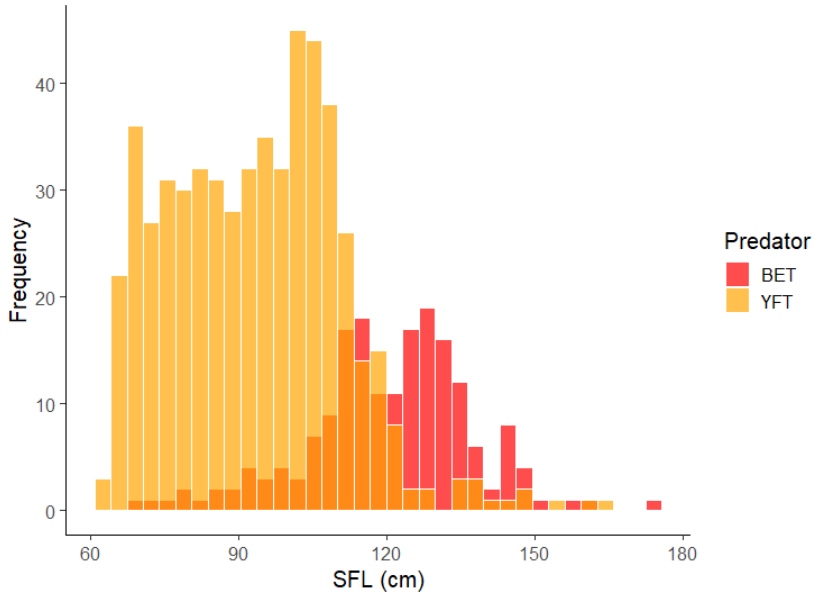


Figure 2. Length frequency histogram of bigeye and yellowfin tuna with non-empty stomachs sampled from June 2018 to November 2019.

Figure 3. Length frequency histograms of both predators in each sampling event.

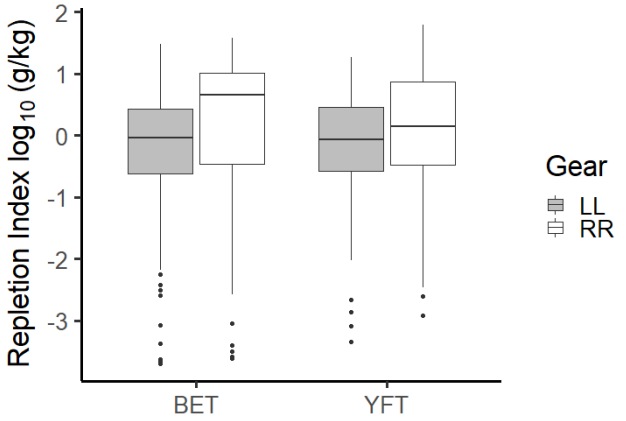


Fig 4. Log-transformed repletion indices of YFT and BET caught with longline and rod and reel gear.

Quantile regressions revealed significant positive relationships in prey/predator lengths for 5th and 95th quantiles for bigeye and 50th and 95th quantiles for yellowfin (Table 3). On average bigeye fed on larger chordate and mollusc prey than yellowfin tuna (Fig 5).

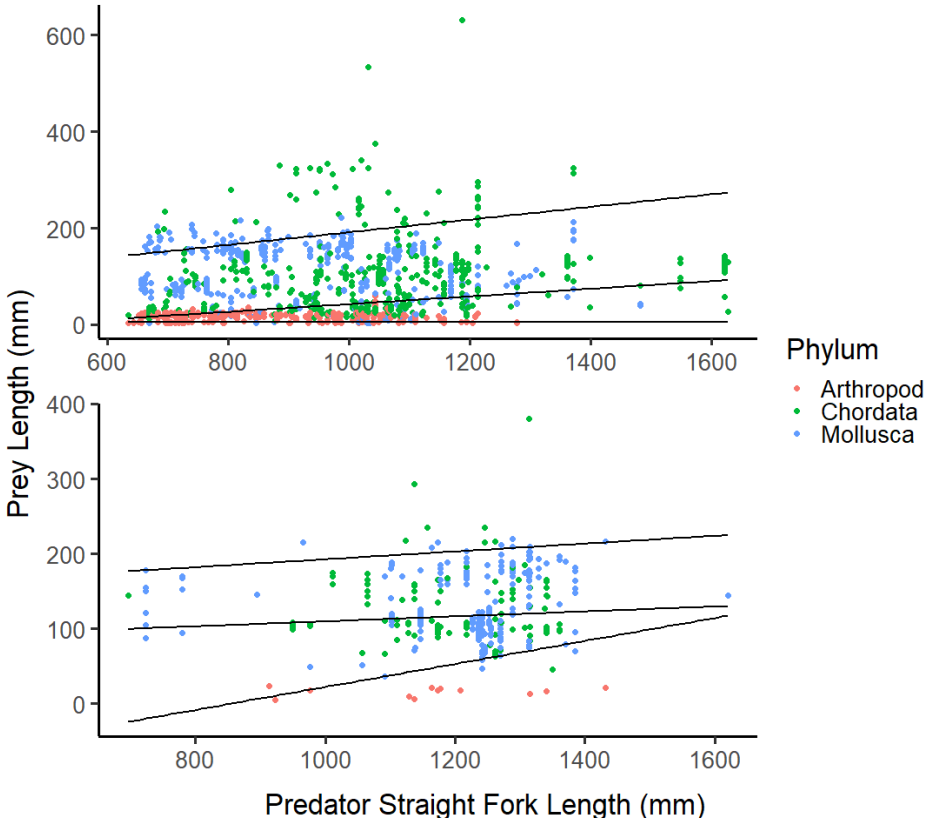


Figure 5. Predator/prey length relationships for yellowfin (top) and bigeye (bottom) with 5th, 50th, and 95th quantiles.

Predator	Quantile	β_0(SE)	β_1(SE)	p-value	Mean prey size (mm)		
					Arthropoda	Chordata	Mollusca
YFT					17.07	100.67	109.82
(n=630)	5th	3.48 (3.02)	0.002 (0.004)	0.68			
	50th	-37.1 (6.24)	0.08 (0.008)	<0.01			
	95th	60.0 (38.1)	0.13 (0.04)	<0.01			
BET					15.87	128.85	130.27
(n=332)	5th	-130.96 (64.19)	0.15 (0.05)	<0.01			
	50th	76.63 (41.10)	0.03 (0.03)	0.33			
	95th	140.20 (24.77)	0.05 (0.02)	0.012			

Table 3: Quantile regression parameter estimates, standard error (SE), and significant levels of each regression coefficient for both tuna predator-prey length relationships. Mean sizes by phylum are also reported.

YFT Prey Composition

Yellowfin tuna forage consisted of nearly equal proportions by weight, %FO, and %MW of chordates and mollusks (Table 4). Despite contributing the largest numbers of individuals, members of the phylum Arthropoda were less important comparatively but still contributed significant %MW and occurred frequently overall. Arthropoda prey were composed of sargassum associates and larvae such as *Idotea baltica*, *Portunus,* brachyuran megalopae, and *Cerataspis monstrosa*.

Amphipods from suborder Hyperiidea associated with the deep scattering layer dominated other Arthropod prey in terms of weight, %FO, and number. *Themisto spp.* was the most abundant yellowfin tuna prey genus by number herein and represented the third largest %MW overall. When this species occurred it often included tens to hundreds of individuals. *Phrosina semilunata and Phronima sp.* also occurred relatively often (7.84% and 6.97% respectively). Brachyuran megalopae were numerous and occurred frequently.

The phylum Chordata contained the greatest family richness of the three phyla. Species from the family Scombridae were the second most important prey family by $\%MW_i$ for yellowfin tuna diet overall. Those species included *Auxis rochei, Auxis thazard, Euthynnus alletteratus,* and *Scomber colias* with the former identified as the most frequently occurring scombrid (16.55 %FO). Occurring slightly more than scombrids, filter feeders from the family Salpidae were the most abundant chordate by number.

Ammodytes spp. occurred infrequently but contributed the third most weight of any chordate family and often included numerous individuals when present. Sargassum associates and other surface-dwelling chordates such as Monacanthids, Hemiramphids, Exocoetids, and Carangids were also of moderate importance (3.62-1.61% MW). Monacanthids such as *Aluterus monoceros* and *Stephanolepis hispidus* occurred frequently (9.23% FO) among chordates and were the fourth largest %MW contributor of yellowfin diet by family. *Selene setapinnis* often occurred with 10-30 individuals present. Apart from *Paralepis brevirostris* and *Nealotus tripes,* mesopelagic species from families such as Aleipisauridae, Bramidae, Myctophidae, and Nomeidae rarely occurred and were low in number. juvenile *Dactylopterus volitans*, *Canthigaster rostrata, and Sphoeroides maculatus* occurred frequently but did not contribute to significant $\%MW_i$. The remaining chordates were composed of rare fish species such as *Coryphaenus hippurus,* and *Luvarus imperialis*.

Tuethids and Octopods were preyed upon frequently by yellowfin tuna. *Illex illecebrosus* was by far the most frequently occurring (31.53% FO) and greatest contributor by weight (19.01% MW) of prey species in yellowfin tuna forage herein. Argonautids occurred frequently (10.98% FO) and Loliginids were significant in (360.60 g) weight respectively. All other mollusks were rare but included juvenile forms of the teuthid and octopod families Alloposidae, Brachioteuthidae, Vitrelledonellidae, Tremoctopodidae, Octopoteuthidae, and Lepidoteuthidae all of which spend the early life history in shallow pelagic depths. Two families of gastropods were also present but rare-Cavoliniidae and Atlantidae. Adult *Thysanoteuthis rhombus* was also observed as a rare prey item.

BET prey composition

The phylum Mollusca was more important by %MW and %FO than any phylum (Table 5). Arthropods were unimportant in comparison with yellowfin tuna and contributed a very small proportion of prey item weight, occurrence, and number. Arthropod species present were similar to yellowfin tuna forage, but none occurred more often than yellowfin tuna arthropod forage. Idoteids were the only Arthropod that occurred with any significance.

Phylum	Family	Species	Weight (g)	Number	%FO_i	%MW_i
Arthropoda			**670.33**	**3,904**	**46.86**	**12.16**
	Aristeidae		0.37	1	0.17	<0.01
	Euphausiidae		3.04	21	3.14	0.22
	Hyperiidae		452.00	3,033	21.60	5.81
		Themisto sp.	452.00	3,033	21.60	
	Idoteidae		23.44	129	7.32	0.93
		Idotea spp.	23.44	129	7.32	
	Lycaeidae		2.03	14	1.22	0.02
	Brachyura $_{(infraorder)}$		32.59	362	12.98	1.30
	Penaeidae		3.29	21	1.91	0.06
		Cerataspis petiti	3.29	21	1.91	
	Phronimidae		28.01	106	6.97	0.41
		Phronima sp.	28.01	106	6.97	
	Phrosinidae		84.54	186	7.84	1.28
		Phrosina semilunata	81.55	186	7.54	
	Portunidae		3.98	5	0.70	0.13
		Portunus sp.	3.07	3	0.52	
	Scyllaridae		0.28	1	0.17	<0.01
	Stomatopoda $_{(order)}$		5.17	25	3.14	0.31
	Unidentified Arthropoda		31.31	–	10.11	1.68
Chordata			**19,975.78**	**1,701**	**78.05**	**46.60**
	Alepisauridae		176.67	4	0.52	0.14
		Alepisaurus ferox	176.67	4	0.52	
	Ammodytidae		1,511.32	227	3.14	1.92
		Ammodytes sp.	1,511.32	227	3.14	
	Ariommatidae		7.35	4	0.52	0.06
		Ariomma bondi	7.35	4	0.52	
	Balistidae		43.39	26	2.61	0.48
	Bramidae		44.10	12	1.57	0.18
		Brama brama	2.33	1	0.17	
		Pterycombus brama	30.07	10	1.05	
		Pteraclis sp.	10.88	1	0.17	
	Carangidae		208.26	196	8.01	1.61
		Cranx crysos	65.00	3	0.52	
		Selene setapinnis	119.80	185	6.10	
		Seriola zonata	10.98	1	0.17	
		Trachurus lathami	9.31	3	0.52	
		Decapterus punctatus	0.73	1	0.17	
	Coryphaenidae		127.85	6	1.05	0.51
		Coryphaena hippurus	127.85	6	1.05	
	Cottidae		0.6	1	0.17	0.01
	Dactylopteridae		39.89	58	4.01	0.74
		Dactylopterus volitans	39.89	58	4.01	
	Exocoetidae		524.58	73	3.83	2.13
		Cheilopogon heterurus	99.59	19	0.70	
	Gempylidae		356.03	44	2.61	0.38
		Gempylus serpens	2.72	1	0.17	
		Nealotus tripes	294.23	38	1.39	
		Nesiarchus nasutus	15.40	4	0.17	
		Ruvettus pretiosus	3.26	1	0.17	
	Hemiramphidae		77.21	2	0.35	0.27
		Hemirampus brasiliensis	77.21	2	0.35	
	Luvaridae		25.1	2	0.35	0.03
		Luvarus imperialis	25.1	2	0.35	
	Monacanthidae		1,960.20	206	9.23	3.62
		Aluterus monoceros	1,575.88	128	3.14	
		Stephanolepis hispidus	62.92	7	1.05	
	Myctophidae		31.57	15	1.39	0.19
		Ceratoscopelus maderensis	5.68	4	0.17	
		Diaphus spp.	2.07	3	0.35	
	Nomeidae		6.22	3	0.35	0.01
		Cubiceps Pauciradiatus	6.22	3	0.35	
	Ogcocephalidae		1.5	4	0.35	0.02
	Paralepididae		115.16	37	2.61	0.62
		Arctozenous risso	12.91	1	0.17	
		Paralepis brevirostris	24.54	5	0.17	

	Phycidae		49.97	1	0.17	0.03
		Urophycis regia	49.97	1	0.17	
	Pricanthidae		5.09	4	0.70	0.16
		Priacanthus arenatus	5.09	4	0.70	
	Psychrolutidae		0.33	1	0.17	<0.01
	Salpidae		149.07	514	17.07	2.51
	Scomberesocidae		803.23	29	2.96	1.83
		Scomberesox saurus	736.60	29	2.79	
	Scombridae		11,964.72	134	16.55	11.51
		Auxis spp.	2,907.30	12	1.57	
		Auxis rochei	3,958.60	30	3.31	
		Auxis thazard	45.88	1	0.17	
		Euthynnus alletteratus	165.68	15	0.52	
		Scomber colias	151.27	2	0.35	
	Sparidae		71.76	1	0.17	0.14
	Syngnathidae		17.95	20	3.14	0.69
	Tetraodontidae		49.54	75	6.10	0.69
		Canthigaster rostrata	1.78	1	0.17	
		Sphoeroides maculatus	3.70	7	0.87	
	Trachipteriidae		0.16	1	0.17	0.17
	Zeiidae		17.6	1	0.17	0.07
	Unidentified Chordata		1,589.64	–	57.143	15.92
Mollusca			**20,782.51**	**875**	**79.27**	**41.24**
	Alloposidae		24.17	5	0.70	0.22
		Haliphron atlanticus	24.17	5	0.70	
	Argonautidae		139.00	137	10.98	1.25
	Atlantidae		1.67	7	0.88	0.01
	Brachioteuthidae		0.71	2	0.35	<0.01
	Cavoliniidae		1.74	8	1.74	0.05
	Lepidoteuthidae		1.22	2	0.35	<0.01
		Lepidoteuthis grimaldii	1.22	2	0.35	
	Loliginidae		360.60	48	2.10	0.82
		Loligo pealeii	360.60	48	2.10	
	Octopoteuthidae		1.17	2	0.35	0.01
	Ommastrephidae		16,198.62	648	31.53	19.01
		Illex illecebrosus	16,193.00	647	30.66	
		Orinthoteuthis antillarum	5.62	1	0.17	
	Thysanoteuthidae		10.41	3	0.52	0.03
		Thysanoteuthis rhombus	10.41	3	0.52	
	Tremoctopodidae		25.6	6	1.05	0.16
	Vitreledonellidae		0.82	7	1.05	0.03
	Unidentified Mollusca		4,016.83	–	68.99	19.66

Table 4: Phylum, families, and species of prey identified in 574 non-empty *Thunnus albacares* stomachs sampled from 2018-2019. Bolded families were identified exclusively through genetic barcoding while bolded species were either exclusively through genetic barcoding of gross identification and genetic barcoding.

Chordates that contributed significant %MW included multiple species from the families Myctophidae and Gempylidae. To a lesser extent ammodytids, paralepidids, and monacanthids were also important among chordates. Salps also occurred relatively often (10.64 %FO). The remaining Chordate families were composed of rare dermersals and mesopelagics such as Phycidae, Scyliorhinidae, and Alepisauridae and epipelagics such as Ariomattidae, Centrolophidae, Hemiramphidae, Scombridae, and Sygnathidae.

The phylum Mollusca was the most dominant of the three contributing 66.95% by $\%MW_i$. to a greater extent than yellowfin tuna, *Illex illecebrosus* was the most important prey species for bigeye. Illex occurred in 45.75% of non-empty stomachs and accounted for 27.40% MW_i. Argonautids were second in importance of the molluscs occurring in 10.64% of bigeye stomachs. Neritic Loliginids and mesopelagic Histioteuthid and Gonatid squid occasionally occurred and contributed an intermediate proportion of mollusk weight. Other species of mollusk were rare but included adult *Lepidoteuthis grimaldii*, gastropods from families Atlantidae and Cavoliniidae, and juvenile brachioteuthids and octopoteuthids.

Phylum	Family	Species	Weight (g)	Number	$\%FO_i$	$\%MW_i$
Arthropoda			**17.03**	**47**	**15.96**	**0.18**
	Aristeidae		8.41	4	1.60	0.03
	Euphausiidae		0.18	4	1.60	0.01
	Hyperiidae		3.73	23	0.53	0.05
		Themisto sp.	3.73	23	0.53	
	Idoteidae		2.13	12	6.38	0.04
		Idotea spp.	2.13	12	6.38	
	Brachyura $_{(infraorder)}$		0.18	3	1.60	0.02
	Phronimidae		0.25	1	0.53	<0.01
		Phronima sp.	0.25	1	0.53	
	Phrosinidae		0.56	4	2.13	<0.01
		Phrosina semilunata	0.56	4	2.13	
	Unidentified Arthropoda		1.59	–	5.85	0.04
Chordata			**4,023.00**	**466**	**78.72**	**32.87**
	Alepisauridae		34.48	4	2.13	0.12
		Alepisaurus ferox	34.48	4	2.13	
	Ammodytidae		520.37	25	1.60	1.54
		Ammodytes sp.	520.37	25	1.60	
	Ariommatidae		4.85	1	0.53	0.01
		Ariomma bondi	4.85	1	0.53	
	Centrolophidae		4.26	1	0.53	0.02
		Hyperoglyphe perciformis	4.26	1	0.53	
	Gempylidae		676.06	112	19.68	3.74
		Gempylus serpens	2.13	1	0.53	
		Nealotus tripes	646.61	110	15.96	
		Nesiarchus nasutus	4.21	1	0.53	
	Monacanthidae		280.36	24	4.79	2.00
		Aluterus monoceros	238.74	18	3.19	
		Stephanolopis hispidus	10.74	1	0.53	
	Myctophidae		879.75	187	18.62	5.33
		Ceratoscopelus maderensis	267.41	73	3.19	
		Symbolophorus veranyi	313.99	79	10.11	
	Paralepididae		394.85	61	8.51	1.17
		Arctozenus risso	79.60	11	1.06	
		Magnisudis altlantica	15.37	1	0.53	
	Phycidae		48.09	2	1.06	0.12
		Urophycis regia	48.09	2	1.06	
	Salpidae		26.8	41	10.64	1.37
	Scomberesocidae		36.40	3	1.06	0.37
		Scomberesox saurus	36.40	3	1.06	
	Scombridae		274.49	2	1.06	0.33
		Auxis rochei	268.85	1	0.53	
	Scyliorhinidae		112.16	1	0.53	0.04
		Scyliorhinus retifer	112.16	1	0.53	
	Syngnathidae		0.69	2	0.53	0.05
	Unidentified Chordata		844.29	–	70.75	17.17
Mollusca			**19,152.44**	**560**	**90.96**	**66.95**
	Argonautidae		206.74	24	10.64	2.06
	Atlantidae		0.02	1	0.53	<0.01
	Brachioteuthidae		3.27	4	2.13	0.02
	Cavoliniidae		0.05	2	1.60	<0.01
	Gonatidae		24.46	18	3.19	0.15
		Gonatus steenstrup	24.46	18	3.19	
	Histioteuthidae		413.55	47	4.26	1.06
		Histioteuthis reversa	413.55	47	4.26	
	Lepidoteuthidae		30.44	1	0.53	0.06
		Lepidoteuthis grimaldii	30.44	1	0.53	
	Loliginidae		79.80	9	2.13	0.04
		Loligo pealeii	79.80	9	2.13	
	Octopoteuthidae		0.32	2	1.06	<0.01
	Ommastrephidae		13,820.03	452	45.75	27.40
		Illex Illecebrosus	13,820.03	452	45.75	
	Unidentified Mollusca		4,458.86	–	87.77	35.67

Table 5: Phylum and families of prey identified in 188 non-empty *Thunnus obesus* stomachs sampled in 2018-2019. Bolded families were identified exclusively through genetic barcoding while bolded species were identified either exclusively through genetic barcoding or gross identification and genetic barcoding.

Owing to rapid digestion by both predators a large portion of stomach contents remained unidentified. Unidentified Mollusca was twice by $\%MW_i$ that of unidentified Chordata for bigeye. Moreover, it is likely that most of the unidentified Mollusca tissue is composed of digested *Illex illecebrosus*, being that it is the most prevalent identifiable Mollusk for both

predators.

Gear comparisons

Ommastrephids were most abundant in tunas captured by rod and reel gear (Figure 6). Bigeye tuna ommastrephid prey was greater by $\%MW_i$ in longline capture gear than for yellowfin tuna in recreational fisheries. Species from the family Scombridae, flying fishes, pre-settled brachyuran megalopae, and *Themisto sp.* amphipods were most abundant for yellowfin tuna captured with rod and reel gear, while nyctopelagic fishes were abundant for bigeye tuna captured by longline. Neritic fishes such as *Ammodytes sp*. and rare demersals were most important for diets of bigeye tuna caught in recreational fisheries. Salps occurred more often in yellowfin tuna stomachs sampled from longline gear.

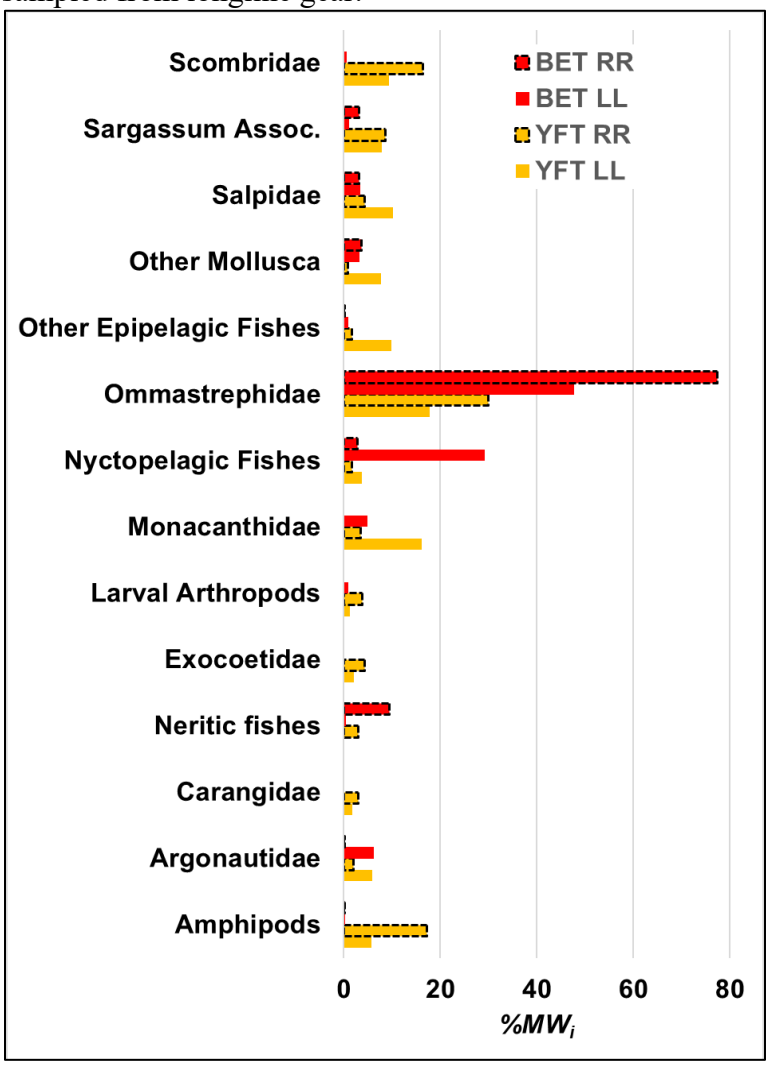
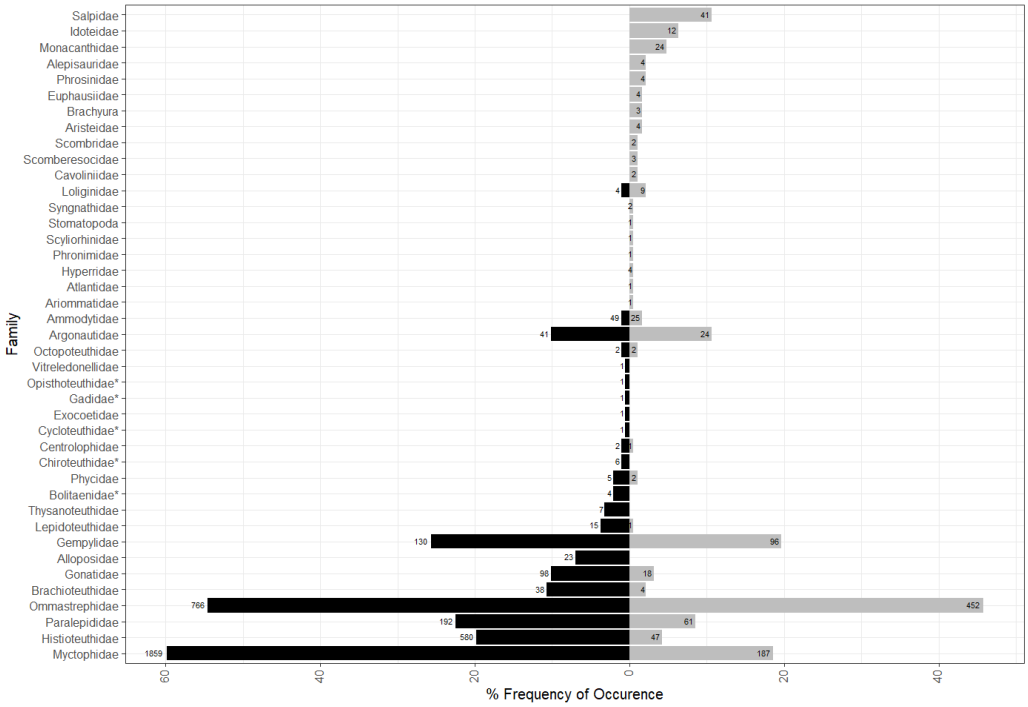


Figure 6. $\%MW_i$ of prey guilds for both predators and their capture gears.

Loose hard part comparisons

Loose otoliths and beaks occurred in 80.95% and 56.49% of bigeye and yellowfin tuna stomachs, respectively. Otolith and beak identification revealed that diets of both species were composed of greater species richness than prey with tissue alone (Figure 7). Families discovered

by loose hard parts alone included Bolitinaeidae, Chiroteuthidae, Cycloteuthidae, Opisthoteuthidae, Stromateidae, Merluccidae, and Gadidae. Species with small otoliths (Balistids, Monacanthids, Scombridae etc.) were rarely detected. Frequency of occurrences for hard parts were comparable to prey associated with tissue for yellowfin with slight exceptions of *Histiotuethis reversa* and *Haliphron atlanticus*, whereas deviations in frequency of occurrences between hard parts and prey associated with tissue were present for bigeye. Families that differed included Myctophidae, Histioteuthidae, Alloposidae, Gonatidae, Brachioteuthidae, and Paralepididae. Argonautid and Ommastrephid beaks occurred in similar percentages as prey associated with tissue. At least three species of myctophids, *Certoscopelus maderensis, Symbolophorus veranyi, and Diaphus spp.* contributed a minimum of 1,859 individuals to bigeye tuna diet. Myctophids were detected in bigeye tuna stomachs as loose otoliths 3-fold that of prey associated with tissue and were nearly 10-fold by number. *Histioteuthis reversa* were also numerous for bigeye tuna (580 lower beaks were detected). Nevertheless, ommastrephids occurred as beaks frequently and in high numbers for both predators (Figure 7).



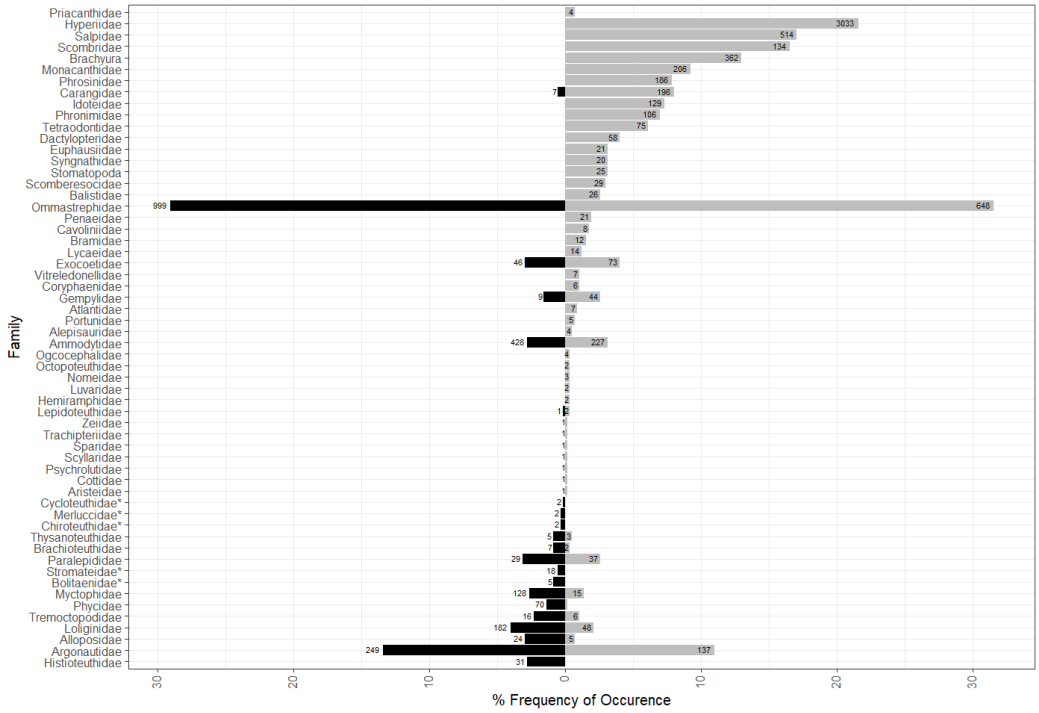


Figure 7. Number of individuals and frequency of occurrence comparisons between hard parts (black) and prey associated with tissue (grey) for families detected in non-empty stomachs of 188 *Thunnus obesus* (above) and 574 *Thunnus albacares* (below). Asterisks indicate exclusive identification by otoliths or beaks.

Spatial, Inter-, and Intra-annual comparisons

Family Accumulation Curves

Rarefaction and extrapolation curves revealed a range of richness and diversities across SEs for yellowfin prey tuna families (Fig 8). Observed richness varied across number of stomachs sampled and by sampling event. Sample sizes were particularly low for yellowfin tuna SE1, SE10, SE11 and for bigeye tuna in all sampling events except SE9 and SE12. Both observed richness and diversity were not comparable estimates of prey assemblage at these localities. SE with low sample sizes (YFT-SE1, SE10, SE11 and all BET except SE9 and SE12) were not included in further spatial analyses purely given the high probability of rare prey occurrence and the greater emphasis that our metrics would place on family importance in SEs with small sample sizes when compared to SEs with large sample sizes.

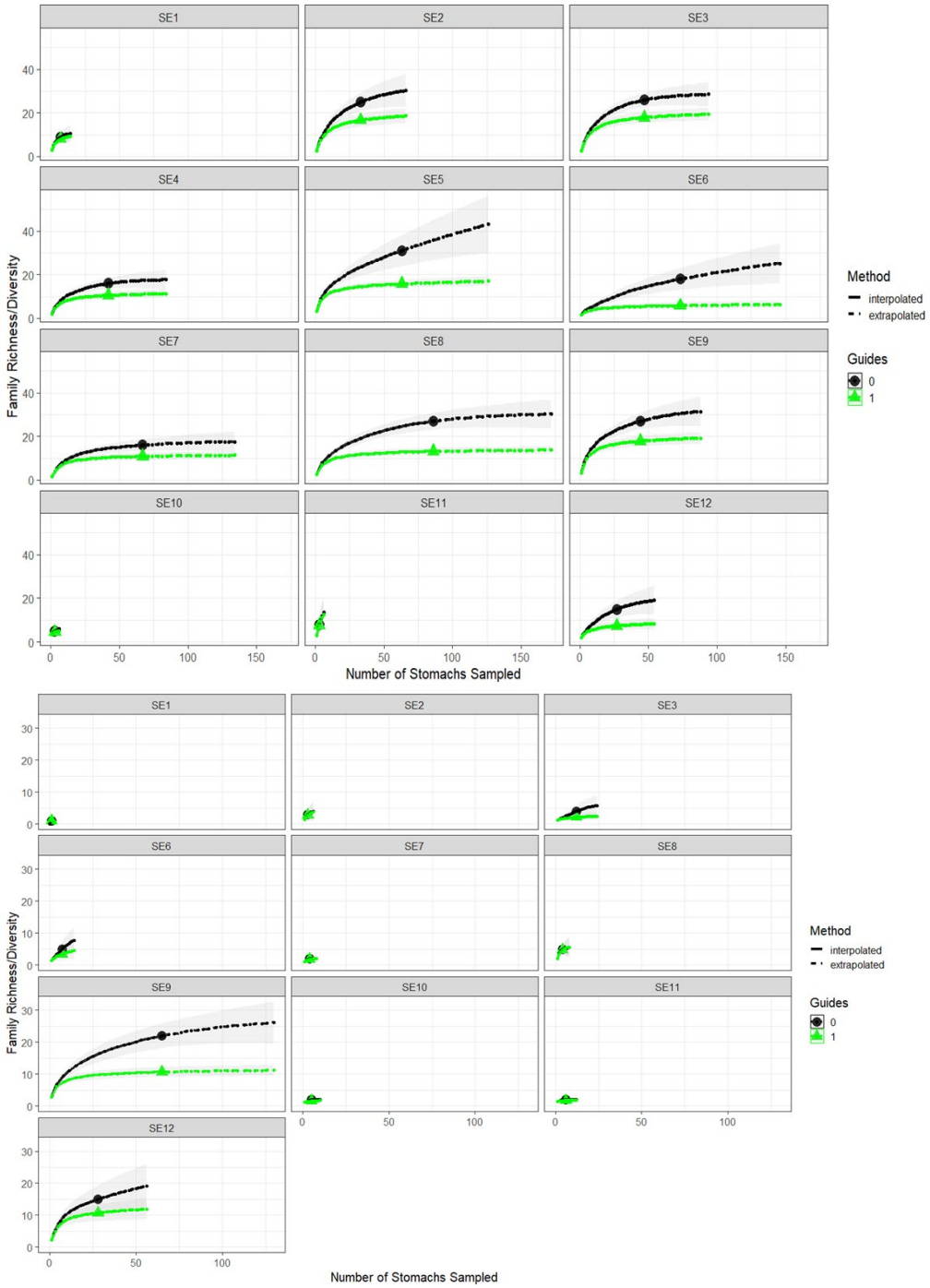
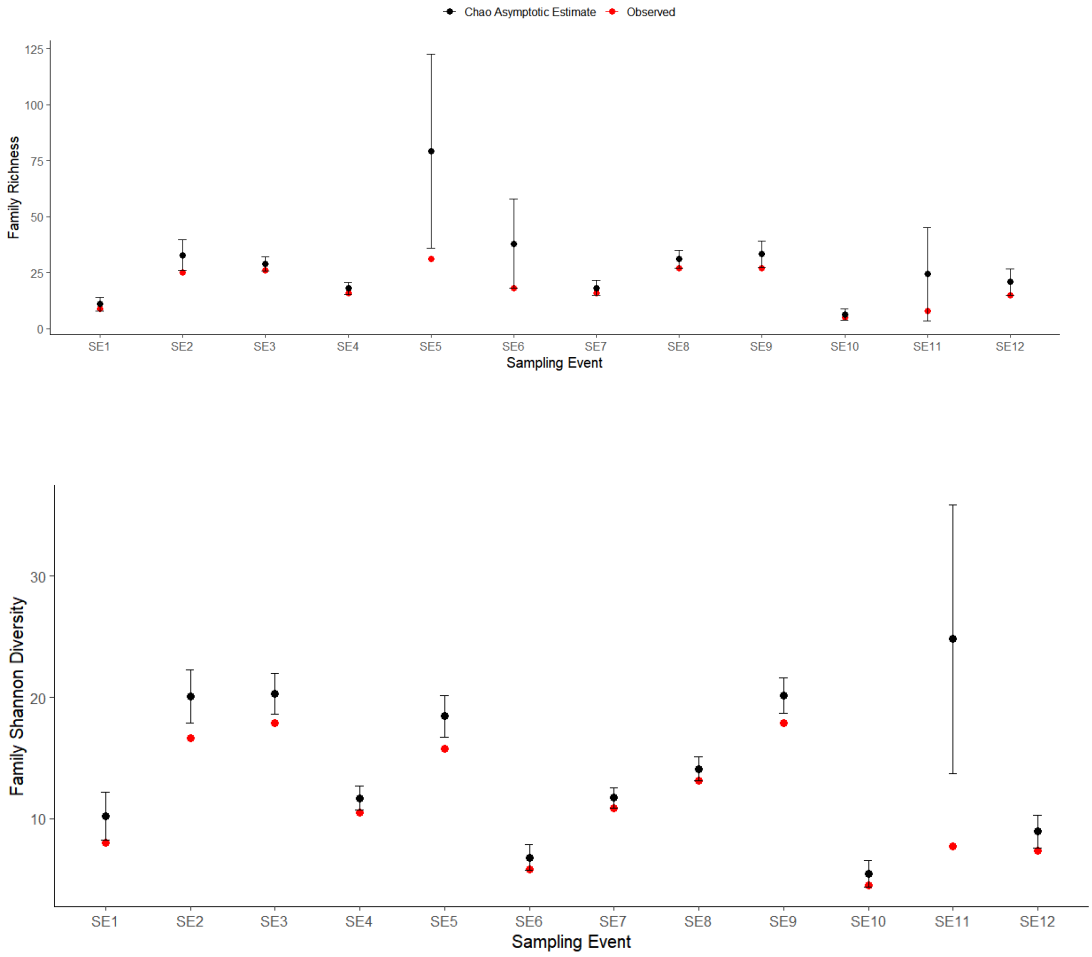


Figure 8. Sample-size based rarefaction and extrapolation curves by family and sampling event for *Thunnus albacares* (upper) and *Thunnus obesus* (lower). Shaded areas represent 95% confidence intervals based on reference data permutated 100 times. Shapes are observed richness and diversity, solid lines represent interpolated values, and dotted lines represent extrapolated values. Guides indicate parameter q of order 0 (species richness) and 1 (Shannon diversity). Observed richness was compared with richness at 2*n (double observed sample size) to determine if observed sample sizes reflected estimates of true diversity and richness (Chao et al. 2014)

In northern regions of the Mid-Atlantic bight asymptotic family diversity estimates and observed diversity was higher in 2019 (SE8) than in 2018 (SE9). Furthermore, yellowfin tuna forage families sampled largely in July of 2018 at this region (SE7) were less diverse and rich than in SE8. SE6 had considerably low diversity despite large sample sizes (Fig 9). Highest family diversity in bigeye tuna sampling events occurred in SE9 and SE12 owing to larger sample sizes (Fig 9).



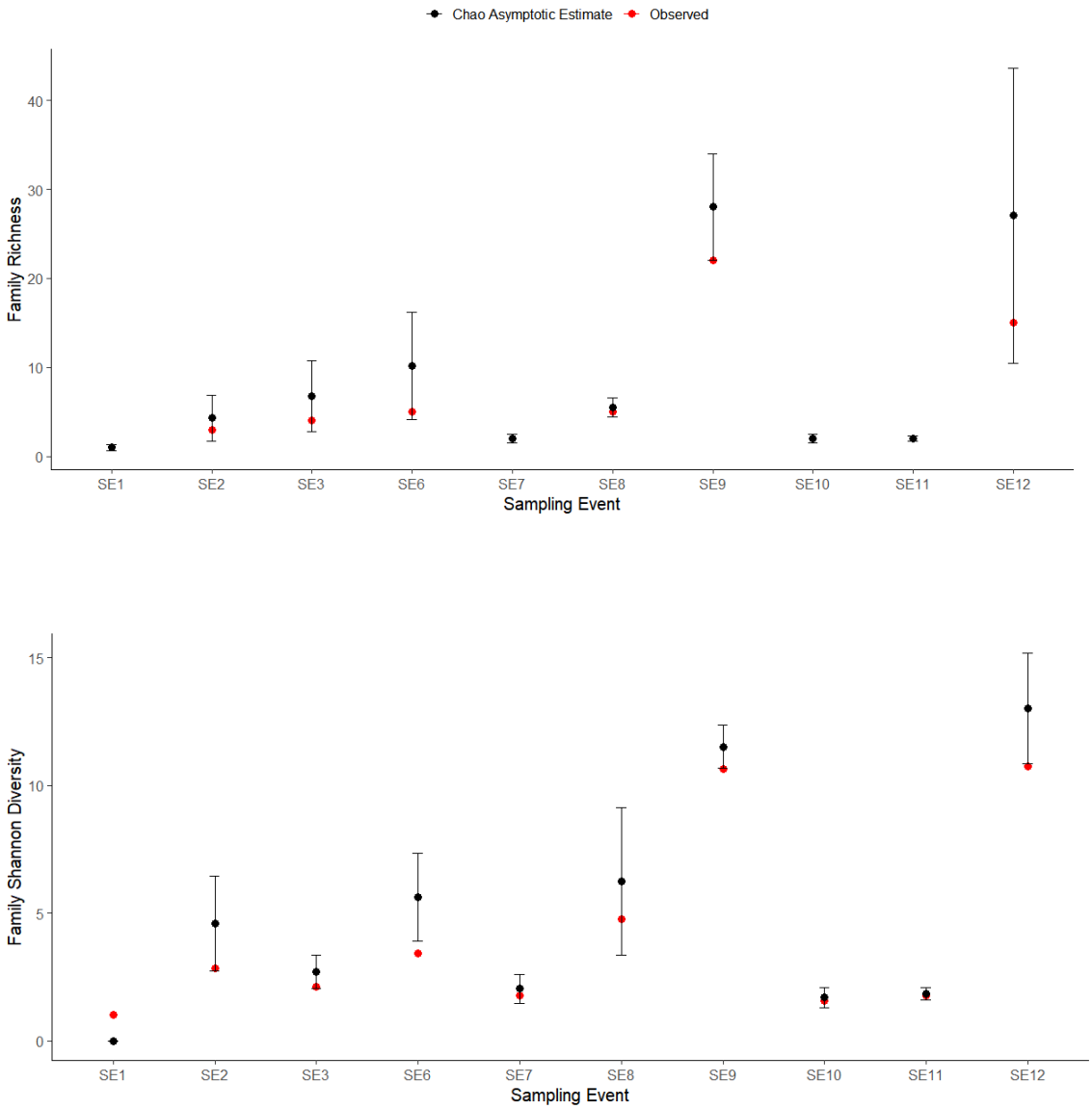


Figure 9. Observed values and asymptotic estimates of forage family richness and Shannon diversity for yellowfin (above) and bigeye (below). Error bars represent standard error. Sampling events with low sample sizes often resulted in low diversity. Low latitude sampling events constituted higher species diversity. Richness estimates for SE5 were significantly higher than observed richness, although observed Shannon diversity were comparable to those observed in SE3 in 2018.

Arthropod forage was not a primary item in any bigeye tuna sampling event (Figure 10) but contributed >15% MW_i in northern mid-Atlantic B ight sampling events for yellowfin tuna in both years (SE6, SE7, SE8) (Fig 10). Chordate forage was more variable across sampling events for both predators with lowest contributions occurring in SE6 for yellowfin tuna and SE10 and SE11 for bigeye tuna. Molluscs contributed large proportions of tuna prey in most SEs contributing >20% MW for all sampling events for both predator species where n>5.

Illex illecebrosus associated with tissue and beaks occurred for both species in all areas sampled and contributed the most significant *%MW* in most SE. In 2018 bigeye tuna sampling events with low sample sizes not included in further analyses, *Illex illecebrosus* dominated diet followed by *Ammodytes sp.* and paralepidids. One recreational sampling event occurred in June of 2019 (SE8, n=12) and contained *Lepidoteuthis grimaldii, Histioteuthis reversa,* and *Loligo pealeii* not observed in 2018. Southern New- England yellowfin tuna sampling events with low sample sizes included hyperiid amphipods and brachyuran megalopae in SE10 (n=3) and *Loligo pealeii* in SE11 (n=3).

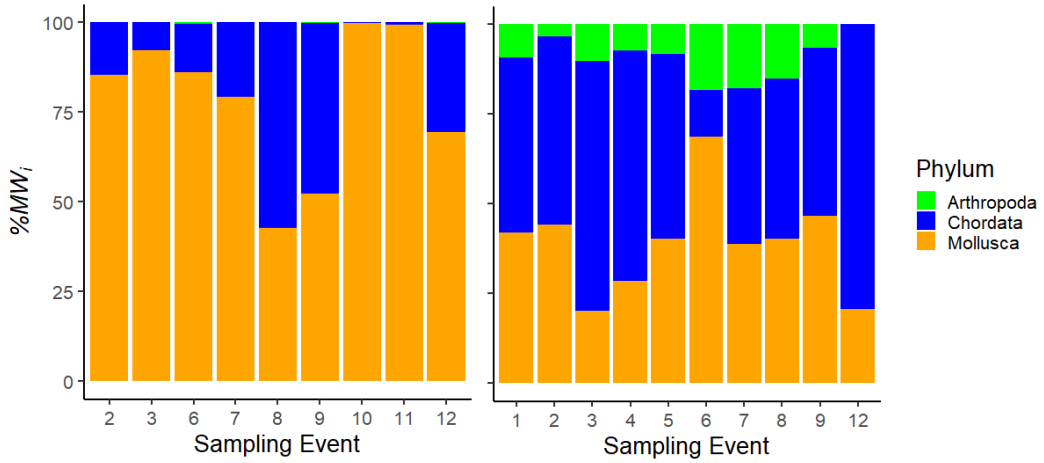


Figure 10. $\%MW_i$ of forage phyla found for bigeye (left) and yellowfin (right) in sampling events with sample sizes ≥ 5.

Ommastrephidae $\%MW_i$ was lowest among sampling events where n>28 yellowfin tuna SE3, SE4, and SE12 (13-15%) and occurred the least frequently in SE3 and SE4 (Figure 11). Ommastrephidae was highest by $\%MW_i$ and $\%FO_i$ in SE6 for yellowfin tuna (50% and 44% respectively). Ommastrephidae occurred nearly as much in SE5 as SE6 but was half that of SE6 in $\%MW_i$. Scombrids were least abundant for yellowfin tuna in SE6 by $\%FO_i$ and $\%MW_i$ (4% and 5%, respectively) but were the most important prey item for yellowfin tuna captured by rod and reel in a southern Mid-Atlantic Bight tournament sampled in June of 2019 (SE4). Here they accounted for 46% MW_i and occurred in 47% of stomachs.

Isopods, Brachyuran megalopae, and the hyperrid amphipods *Themisto sp., Phrosina semilunata,* and *Phronima sp.* occurred in many sampling events in yellowfin tuna. *Themisto sp.* was abundant by number, occurrence, and $\%MW_i$ in June of both years sampled in northern regions of the Mid-Atlantic Bight (SE6, SE7, SE8). *Phrosina semilunata* was most abundant during SE9 for yellowfin and bigeye where it occurred in 35% and 4% of stomachs respectively. *Phronima sp.* occurred frequently in SE4 (19% FO_i) and SE8 (18% FO_i).

Some prey taxa were abundant over a few sampling events and included *Ammodytes sp.* which occurred in all yellowfin and bigeye tuna sampling events captured with rod and reel in 2018 (SE3, SE6, SE7) except SE10. Argonautids occurred frequently in 2019 for yellowfin (SE2, SE5, SE9) and in one sampling event for bigeye (SE9). Carangids mostly in the form of *Selene*

setapinnis, occurred often (45%) and contributed the most MW_i (11%) in yellowfin tuna stomachs during SE5. Exocoetids also occurred specifically in yellowfin tuna sampling events. *Cheilopogon heterurus* occurred in 24% of stomachs sampled during SE3 and contributed 21% of MW_i. Other species of the family Exoceotidae occurred in SE12 (14% FO_i) and SE8 (4% FO_i). The majority of loose exocoetid otoliths were found in yellowfin tuna sampled during SE3. Species from the family Paralepididae occurred infrequently but were present in the majority of sampling events for both predators. Otoliths occurred more than twice as much as those identified with tissue and were three times as numerous in bigeye tuna stomachs, the majority of which occurred in SE9 and SE12. $\%FO_i$ was greatest for bigeye tuna in both sampling events with high sample sizes (SE9 and SE12) and in SE4 and SE9 for yellowfin tuna (Fig 11).

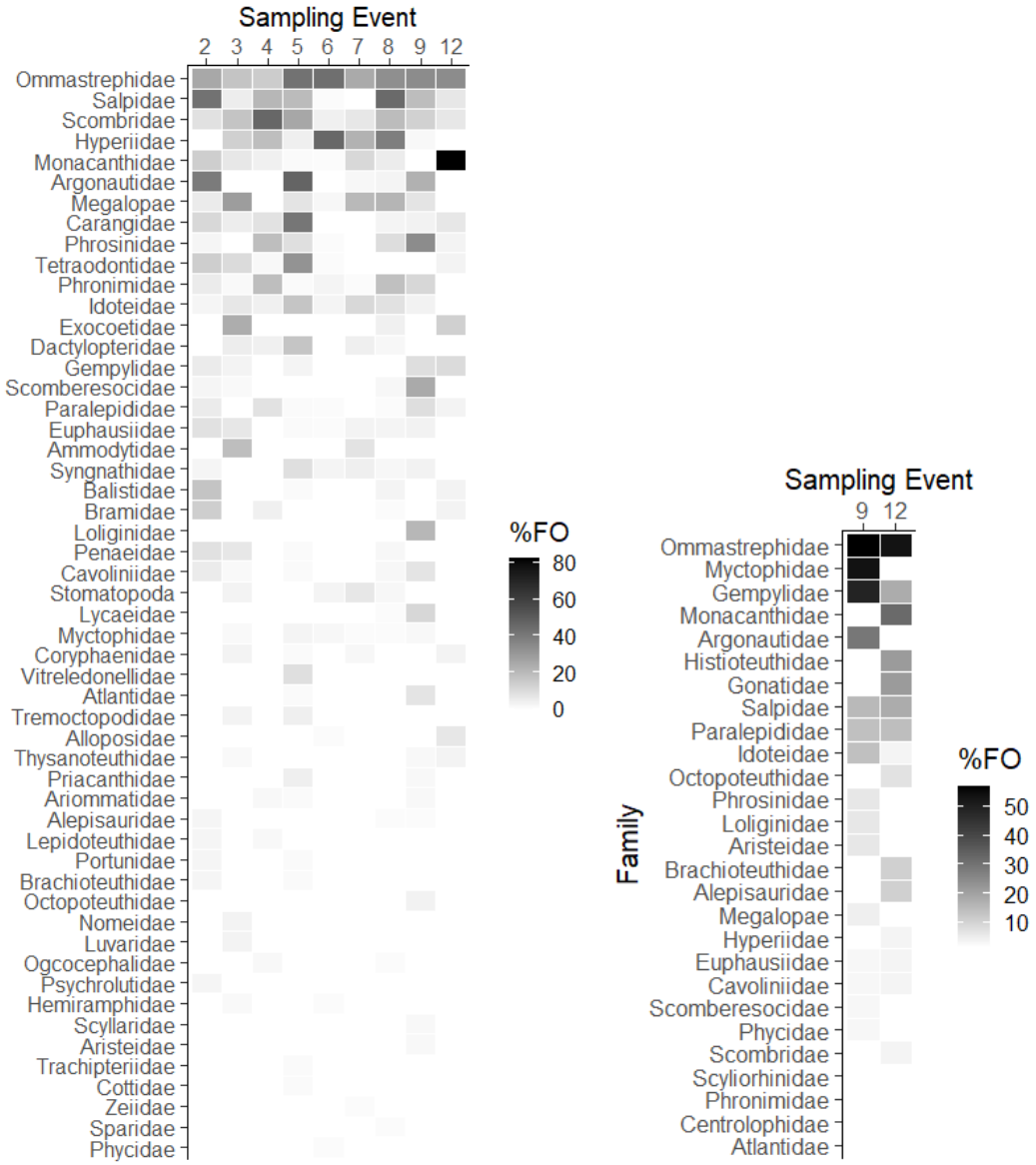


Figure 11. $\%FO_i$ of prey families between predator specific capture gear (left), yellowfin (middle) and bigeye (right) sampling events where n>28. Color scales are the same among $\%FO_i$ for yellowfin sampling events and predator specific capture gear comparisons.

Other moderately abundant species overall were largely only abundant in only one sampling event. Sampled from predators captured by longline gear these species included *Aluterus monoceros* (SE12), *Nealotus tripes* (SE9), *Scomberesox saurus* (SE9), *Gonatus steenstrupi* (SE12), *Histioteuthis reversa* (SE12), *Loligo pealeii* (SE9), *Symbolophorus veranyi* (SE9), *Ceratoscopelus madierensis* (SE9). *Aluterus monoceros* was the most important prey item for yellowfin tuna in SE12 occurring in 83% of stomachs and contributing 60% of MW_i. *Nealotus tripes* and myctophids were most abundant during SE9 for bigeye tuna. Myctophid individuals from loose otoliths were 11-fold greater in number (1,848) than myctophid individuals identified using measurable tissue in SE9.

Yellowfin tuna stomachs from this region also infrequently contained myctophid otoliths and greater than 100 individuals were present. *Loligo pealeii* were also found in SE9 in both predators but more so in yellowfin tuna occurring in 21% of stomachs sampled compared to the 6% of bigeye tuna. Loose beaks yielded 154 more individuals which was 3 times as many individuals than prey with measurable prey tissue revealed in this sampling event. Bigeye tuna forage species *Gonatus steenstrupi* and *Histioteuthis reversa* contained numerous loose beaks and contributed more $\%MW_i$ in SE12 than in any other sampling event. Individuals identified with loose beaks outnumbered those with measurable mass by 5 and 11 orders of magnitude and beaks occurred 3 and 4 times more often for the two squid species, respectively. Unlike *Gonatus steenstrupi*, *Histioteuthis reversa* occurred infrequently as beaks in other 2019 sampling events for both predators (SE8, SE9) and for yellowfin tuna in both years (all SE but SE4) but were not numerous.

The remaining families included prey taxa that was not abundant by $\%MW_i$ or often occurred in only one or two sampling events. Demersals such as a single *Scyliorhinus retifer* in an SE9 bigeye stomach, Ogocephalidae (SE4 and SE8), and juvenile forms of Coryphaenus hippurus (SE3, SE6, SE7, SE12), Dactylopteridae (SE3, SE4, SE5, SE7, SE8), Trachipteridae (SE5), and Scyllaridae (SE9) represented a portion of these rare prey. Multiple *Urophycis regia* as otoliths and measurable tissue and a species of the family Aristeidae occurred in both predators' stomachs during SE9. Five yellowfin tuna stomachs sampled in 2019 (SE5) contained 126 phycid otoliths. Small tetraodontids, *Canthigaster rostrata* and *Spheoroides maculatus,* occurred frequently in low latitude sampling events (SE2, SE3, SE5) but were not important by weight. Species from the family Tetraodontidae occurred in 32% of yellowfin tuna stomachs during SE5 which was more than twice as often when compared to any other sampling event.

Dissimilarity distances and multivariate analyses

Under moderate stress non-metric dimensional scaling of $\%MW_i$ of prey guilds using individual predator stomachs for ordination revealed considerable overlap in dietary niches between yellowfin and bigeye tuna (Figure 12). Yellowfin tuna diet was much broader than bigeye tuna and the prey guilds not abundant in bigeye tuna diet included amphipods, sargassum associates, and family Exocoetidae reflects this. Nyctopelagic fishes found in bigeye tuna stomachs were a guild that distinguished predator diets.

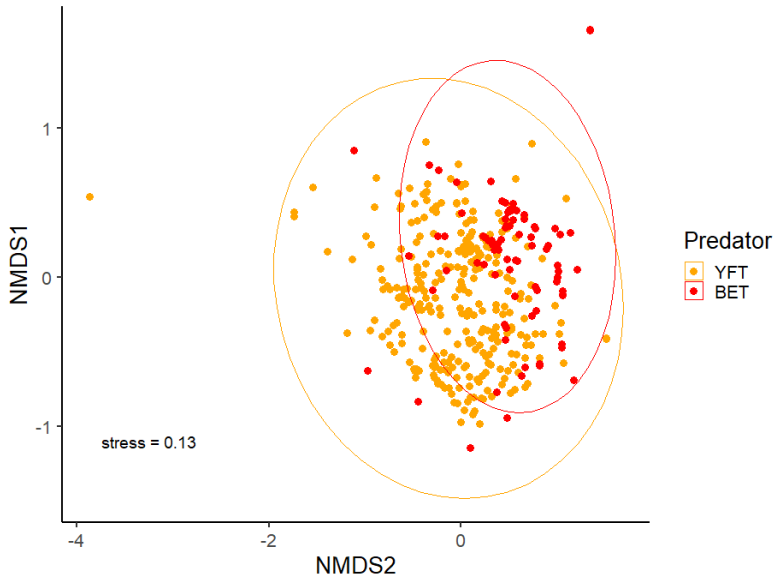


Figure 12. NMDS ordination scores for bigeye and yellowfin tuna. Ellipses represent 95% confidence intervals.

Significant differences were detected in ANOSIM results for predator species (R=0.05, p=0.0024) although low R-values indicate differences in diet between bigeye and yellowfin tuna herein were not as large compared to other factors. Differences between sampling events where n>28 (R=0.20, p<0.001) and latitude (R=0.11, p<0.001) had the largest between group rank dissimilarities. Other factors were significant but were less dissimilar for the two predators. Capture gear was not a significant factor that distinguished bigeye forage (R=0.003, p=0.443) but was significantly dissimilar for yellowfin (R=0.06, p<0.001). Forage dissimilarity was significant for bigeye tuna between summer and fall (R=0.10, p<0.001), but not for yellowfin tuna (R=0.007, p=0.39). Pairwise comparisons among interannually comparable yellowfin tuna sampling events, the northern regions of the Mid-Atlantic Bight (SE6 and SE8) in June were more similar (R=0.09) than southerly regions in July (SE3 and SE5, R=0.25). Pairwise comparisons of forage guilds in two longline sampling events where n>28 for both co-occurring tuna were significantly dissimilar during SE9 (R=0.27) and SE12 (R=0.23).

Marlins

A total of 97 marlins were sampled in 2018-2020, including 17 blue marlin, 36 white marlin, and 44 round-scale spearfish. A total of 874 prey items were extracted and identified from these stomachs. These samples were collected at the White Marlin Open and the Mid-Atlantic, held in Ocean City Maryland and Cape May New Jersey. Given the complexities regarding US regulations for marlin retention and self-imposed tournament regulations, the distribution of samples between years was not consistent (Table 6).

Sp.	2018	2019	2020
Blue marlin	6	10	1
White marlin	13	21	2
Roundscale spearfish	20	21	3
Albacore	0	25	6

Sp.	No. stomachs	Total prey weight (g)	n prey items
Blue marlin	17	7431.3	87
White marlin	36	7073.02	262
Roundscale spearfish	44	7594.55	525
UNK	3	761.98	19
Yellowfin tuna	2	769.389	86
Albacore	31	597.7445	197
Total	**103**	**23630.239**	**979**

Table 6 Stomachs sampled rom *Kajikia albida*, *Makaira nigricans*, and *Tetrapturus georgii*.

The bullet tuna, *Auxis rochei,* was the most commonly occurring prey species in the 97 marlin stomachs sampled and was most abundant in blue marlin (66% by MW_i) (Table 7). Scombrids were comparatively less abundant for roundscale spearfish (46% by MW_i) (Table 9) but were still the most important prey family and included *Scomber colias*. Scombrids found in blue marlin stomachs were on average larger size than in roundscale spearfish and white marlin. Roundscale spearfish and white marlin diets were largely composed of the shortfin squid, *Illex illecebrosus (*Tables 8+9*).* Sample sizes were low for blue marlin (n=16) and it is likely that the number of stomachs sampled were not sufficient to characterize full breadth of forage, but scombrids did occur in 12 of these stomachs, indicating directed foraging on bullet tunas. Species richness was higher in round scale spearfish and white marlin than in blue marlin diets. Small proportions of blue runner, lancetfish, moonfish, mahi, octopus, scads, and amphipods contributed to that increased richness.

Phylum	Family	Species	Weight (g)	Number	$\%FO_i$	$\%MW_i$
Arthropoda			**0.10**	**1**	**6.25**	**<0.01**
	Brachyura $_{(infraorder)}$		0.10	1	6.25	<0.01
Chordata			**7,277.84**	**64**	**93.75**	**91.39**
	Carangidae		3.72	1	6.25	0.20
		Caranx crysos	3.72	1	6.25	
	Hemiramphidae		10.66	2	12.5	0.54
	Luvaridae		0.35	1	6.25	0.02
		Luvarus imperialis	0.35	1	6.25	
	Myctophidae		9.58	3	6.25	0.52
	Scombridae		5,895.69	55	75.0	66.46
		Auxis rochei	4,039.22	44	62.5	
	Teradodontidae		44.19	2	6.25	0.79
		Sphoeroides maculatus	44.19	2	6.25	
	Unidentified Chordata		916.90	–	56.25	22.86
Mollusca			**268.16**	**5**	**37.5**	**8.61**
	Histioteuthidae		10.48	2	6.25	0.42
		Histioteuthis reversa	10.48	2	6.25	
	Ommastrephidae		249.04	3	18.75	7.90
		Illex Illecebrosus	249.04	3	18.75	
	Unidentified Mollusca		8.64	–	25	0.29

Table 7 Phylum, families and species of prey identified in 16 blue marlin (*Makaira nigricans*) stomachs sampled between 2018-2020. Bold species were identified through genetic barcoding.

Phylum	Family	Species	Weight (g)	Number	%FO_i	%MW_i
Arthropoda			**10.94**	**28**	**5.71**	**0.35**
	Brachyura $_{(infraorder)}$		2.05	3	5.71	0.24
	Euphausiidae		0.05	1	2.86	<0.01
	Hyperiidae		0.02	2	2.86	<0.01
	Phronimidae		0.36	2	2.86	<0.01
		Phronima sp.	0.36	2	2.86	
	Phrosinidae		8.26	20	2.86	0.09
		Phrosina semilunata	8.26	20	2.86	
	Unidentified Arthropoda		0.20	–	2.86	0.02
Chordata			**2,067.99**	**43**	**60.0**	**45.17**
	Alepisauridae		219.63	2	2.86	2.51
		Alepisaurus ferox	219.63	2	2.86	
	Belonidae		12.56	1	2.86	0.77
		Strongylura marina	12.56	1	2.86	
	Carangidae		200.62	11	5.71	8.04
		Caranx crysos	69.74	3	5.71	
	Echeneidae		55.48	1	2.86	2.44
		Remora brachyptera	55.48	1	2.86	
	Gempylidae		26.76	1	2.86	0.08
		Nealotus tripes	26.76	1	2.86	
	Hemiramphidae		105.23	3	8.57	2.90
		Hemiramphus brasiliensis	105.23	3	8.57	
	Paralepididae		3.04	7	2.86	0.03
		Lestidium atlanticum	3.04	7	2.86	
	Scombridae		1,343.16	17	20.0	11.64
		Auxis rochei	402.08	6	8.57	
		Scomber colias	835.35	8	8.57	
	Unidentified Chordata		91.18	–	45.71	14.92
Mollusca			**4,963.21**	**87**	**74.29**	**54.48**
	Alloposidae		1.65	2	5.71	0.44
		Haliphron atlantica	1.65	2	5.71	
	Argonautidae		8.68	2	2.86	1.61
	Cavoliniidae		0.04	1	2.86	<0.01
	Histioteuthidae		15.24	1	2.86	0.26
		Histioteuthis reversa	15.24	1	2.86	
	Ommastrephidae		4,581.56	80	51.43	34.89
		Illex Illecebrosus	4,581.56	80	51.43	
	Tremoctopodidae		0.32	1	2.86	0.02
	Unidentified Mollusca		366.05	–	62.86	19.10

Table 8 Phylum, families, and species of prey items identified in 35 white marlin (*Kajikia albida*) stomachs samples between 2018-2019. Bold species were identified through genetic barcoding.

Phylum	Family	Species	Weight (g)	Number	$\%FO_i$	$\%MW_i$
Chordata			**5,998.63**	**238**	**97.62**	**79.65**
	Alepisauridae		131.58	4	7.14	3.48
		Alepisaurus ferox	131.58	4	7.14	
	Ariommatidae		27.89	4	7.14	1.05
		Ariomma bondi	27.89	4	7.14	
	Carangidae		369.10	21	21.43	5.38
		Caranx crysos	187.76	7	7.14	
		Selar crumenophthalmus	42.54	4	9.52	
	Coryphaenidae		223.72	7	7.14	3.09
		Coryphaena hippurus	223.72	7	7.14	
	Gempylidae		10.34	1	2.38	0.22
		Nealotus tripes	10.34	1	2.38	
	Hemiramphidae		74.90	4	9.52	0.18
		Hemiramphus brasiliensis	74.90	4	9.52	
	Luvaridae		4.24	1	2.38	0.07
		Luvarus imperialis	4.24	1	2.38	
	Monacanthidae		4.25	2	4.76	0.16
		Aluterus monoceros	1.96	1	2.36	
	Myctophidae		61.10	12	16.67	1.37
	Nomeidae		9.97	6	7.14	0.21
		Cubiceps pauridactus	9.97	6	7.14	
	Paralepididae		1.70	2	4.76	0.02
		Lestidium atlanticum	1.70	2	4.76	
	Scombridae		4,087.69	174	88.10	45.91
		Auxis thazard	39.59	2	2.36	
		Auxis rochei	2,881.27	141	73.80	
		Euthynnus alletteratus	96.01	4	9.52	
		Sarda sarda	63.47	3	7.14	
		Thunnus atlanticus	9.48	1	2.36	
		Thunnus thynnus	7.14	4	9.52	
	Unidentified Chordata		992.15	–	76.19	16.85
Mollusca			**1,473.90**	**100**	**83.33**	**20.35**
	Argonautidae		28.13	3	7.14	0.20
	Histioteuthidae		1.82	4	9.52	0.18
		Histioteuthis reversa	1.54	2	4.76	
	Ommastrephidae		1,234.69	91	47.62	15.09
		Illex Illecebrosus	1,234.69	91	47.62	
	Tremoctopodidae		20.82	2	2.38	0.41
	Unidentified Mollusca		188.44	–	59.52	4.48

Table 9 Phylum, families, and species of prey species identified in 42 round-scale spearfish (*Tetrapturus georgii*) stomachs sampled between 2018-2019. Bold species were identified through genetic barcoding.

Interannual Diet Composition

Under optimal conditions, evaluating the foraging ecology of fishes should be completed over large spatial and temporal scales including across years to account for changes in the composition and abundance of prey. Despite limitations on the number of marlins that could be sampled across temporal and spatial scales, stomachs were collected over three years providing some insight into the dietary preferences for marlins off the mid-Atlantic during that time. Since so few marlins were collected during 2020 and only from one collecting site from one tournament (Covid restrictions), only two years of data were included in this inter-annual analysis. This analysis (albeit limited) suggests the diet of marlins collected off the Mid-Atlantic Bight was quite consistent between these two years. Scombrid and Ommastrephid species were similar by weight between years (Fig 13).

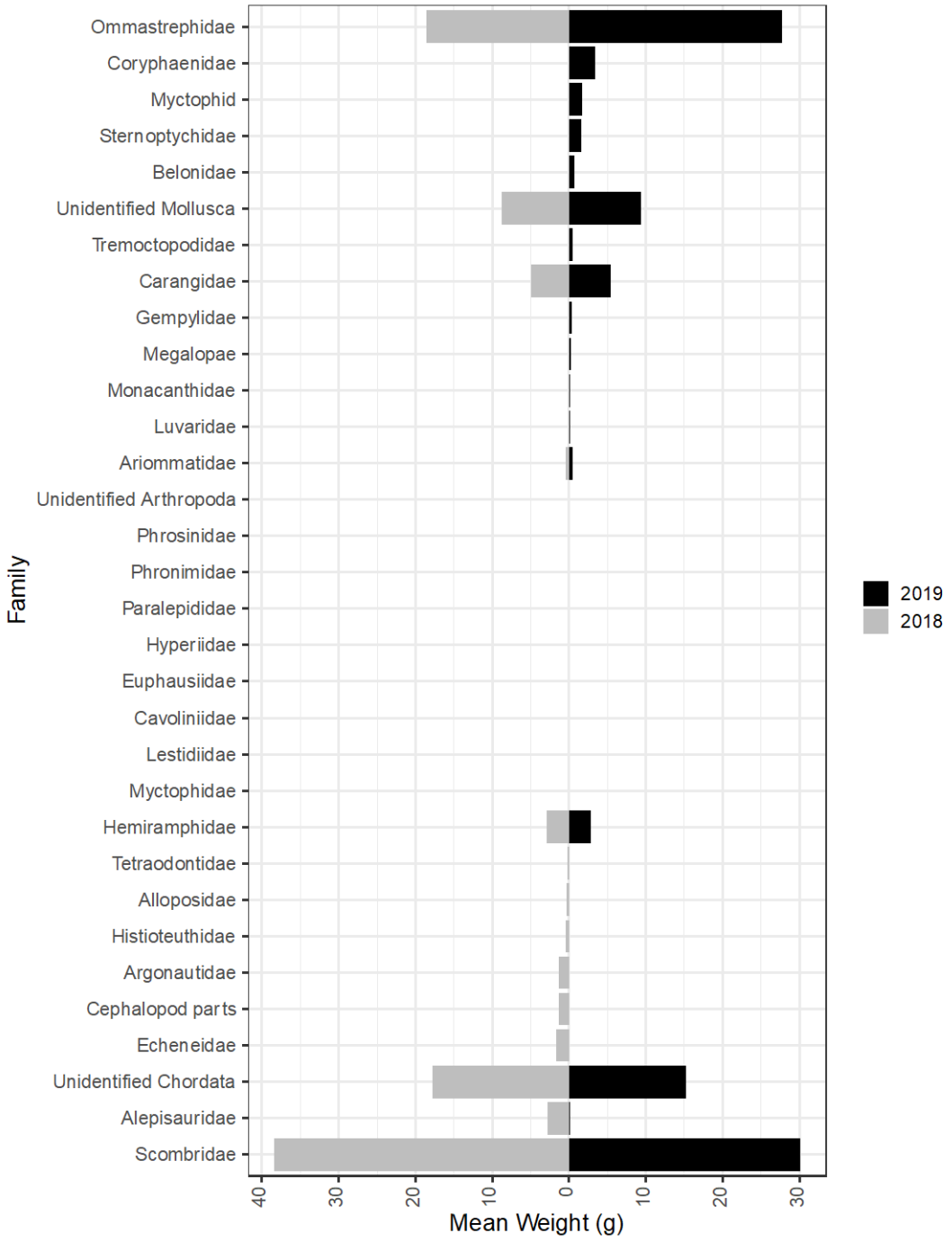


Fig 13 Interannual differences of *Kajikia albida, Makaira nigricans,* and *Tetrapturus georgii* prey by $\%MW_i$ from 2018-2019. Scombrids and Ommastrephids were the most abundant prey between years and their proportions remained similar across years.

Evaluation of sample size

One of the challenges conducting foraging ecology analysis is evaluating whether the samples collected capture the diversity and richness of the diet. These tools help to assess not only if the diet is adequately represented by the samples collected, but it also serves as a guide to restrict unnecessary sampling beyond what's needed to evaluate the diet. The application of these

sample-based rarefaction curves suggests the diversity of the diet for the three marlins species sampled is not adequate based on the slopes of the lines (Fig 14). This is not surprising given the limitations placed on the marlin fishery (≤ 250 marlins per year) in the US Atlantic. Despite these limitations, approximately 100 marlins were sampled for this study and while the curves suggest increasing sample size would improve understanding of species richness and diversity in the diet, achieving that given the current restrictions on the annual marlin harvest may not be possible. Adequately sampling these marlin stomachs may be challenging if the diets shift consistently on an inter-annually basis or even every few years.

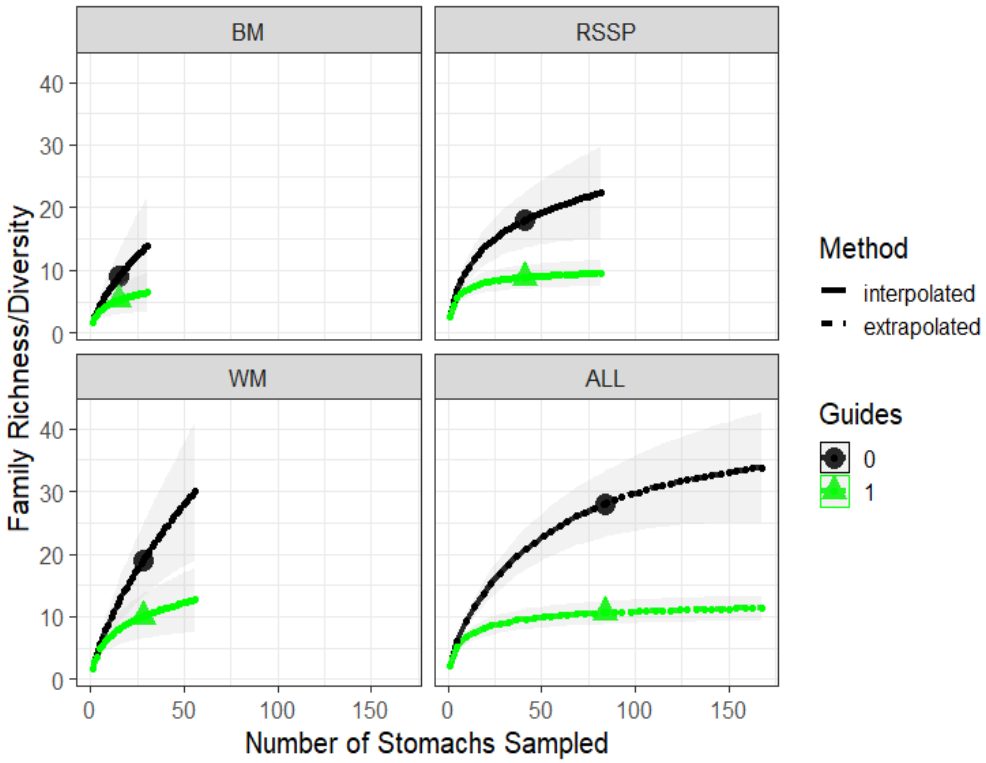


Fig 14. Sample-size based rarefaction and extrapolation curves by family and sampling event for *Makaira nigricans* (blue marlin upper left), *Tetrapturus georgii* (round-scale spearfish upper right), Kajikia *albida* (white marlin lower left), and *Thunnus alalunga* (albacore tuna lower right). Shaded areas represent 95% confidence intervals based on reference data permutated 100 times. Shapes are observed richness and diversity, solid lines represent interpolated values, and dotted lines represent extrapolated values.

Conclusion

The objective of this research project was to evaluate the foraging ecology of highly migratory species captured in the mid-Atlantic Bight region with the primary objective to identify the importance of chub mackerel. This research occurred between 2018 and 2020 and included dietary analysis on bigeye, yellowfin and albacore tuna, round-scale spearfish, blue and white marlin. Over 1,000 stomachs were collected during that time and >800 contained some dietary items useful for analysis across all these species. These samples were collected along the continental shelf break from southern New England to North Carolina and on occasion east of the Gulf Stream. Samples were collected from pelagic longline or rod and reel vessels. All

marlin stomachs were collected at the White Marlin Open or the Mid-Atlantic tournaments. Prey were identified using a combination of visual characteristics based on morphology, sagittal otolith, or beak morphology and when necessary genetic barcoding. While a diversity of prey items was identified across these different predators, two families, the Scombridae and the Ommastrephidae were the most dominant and represent the majority of prey consumed during the time period this study was conducted for marlins. Within the Scombridae, chub mackerel represented an exceptionally small fraction of consumed prey. In fact, over the course of this project and the thousands of prey items that were recovered and identified, only 10 of those were chub mackerel. One yellowfin tuna contained two chub mackerel with eight additional chub mackerel identified in two white marlin stomachs, one white marlin sampled in 2018 and one from 2019. This represents about 0.00026 of the diet for tunas and 0.0068 for marlins. In this study, and with the samples collected there is no indication that chub mackerel are a main dietary item for these HMS species. While the entirety of the marlin diet could be improved by larger sample sizes, given the restrictions in marlin landings and the consistency of the diet, the data do not support or indicate that chub mackerel are an important component of HMS diet at this time and in this region.

References

Altschul, Stephen, Gish, Warren, Miller, Webb, Myers, Eugene, and Lipman, David. 1990. "Basic local alignment search tool". Journal of Molecular Biology 215: 403–410.

C. M. Butler, J. M. Logan, J. M. Provaznik, E. R. Hoffmayer, M. D. Staudinger, J. M. Quattro, M. A. Roberts, G. W. Ingram Jr., A. G. Pollack and M. E. Lutcavage. 2014. Atlantic bluefin tuna Thunnus thynnus feeding ecology in the northern Gulf of Mexico: a preliminary description of diet from the western Atlantic spawning grounds. Journal of Fish Biology 86: 365–374.

Carey, F.G. and Lawson, K.D. 1973. Temperature regulation in free-swimming bluefin tuna. Com. Biochem. Physiol. 44A:375-392.

Chase B (2002) Differences in diet of Atlantic bluefin tuna (*Thunnus thynnus*) at five seasonal feeding grounds on the New England continental shelf. Fish Bull 100:168–180

Golet WJ, Galuardi B, Cooper AB, Lutcavage ME (2013) Changes in the Distribution of AtlanticBluefin Tuna (*Thunnus thynnus*) in the Gulf of Maine 1979-2005. PLoS ONE 8(9): e75480. doi:10.1371/journal.pone.0075480.

Hoffmayer, E. R., Franks, J. S., Driggers,W. B. III, McKinney, J. A., Hendon, J. M. & Quattro, J. M. (2014). Habitat, movements and environmental preferences of dusky sharks, Carcharhinus obscurus, in the northern Gulf ofMexico. Marine Biology 161, 911–924.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG. 2007. ClustalW and ClustalX version 2. Bioinformatics 23: 2947-2948.

Logan, J.M., Golet, W. & M.E. Lutcavage. 2015. Diet and condition of Atlantic bluefin tuna (*Thunnus thynnus*) in the Gulf of Maine, 2004-2008. Environmental Biology of Fishes 98(5): 1411-1430.

Manooch, C. S. III, and D. L. Mason. 1983. Comparative food studies of yellowfin tuna,*Thunnus albacares*, and blackfin tuna, *Thunnus atlanticus* (Pisces: Scombridae) from thesoutheastern and Gulf coasts of the United States. Brimleyana 9:33–52.

Nicol, S.J., Allain, V., Pilling, G.M., Polovina, J., Coll, M., Bell, J., Dalzell, P., Sharples, P., Olson, R., Griffiths, S., Dambacher, J.M., Young, J., Lewis, A., Hampton, J., Molina, J.J., Hoyle,S., Briand, K., Bax, N., Lehodey, P. and Williams, P. (2013) An Ocean Observation System for Monitoring the Effects of Climate Change on the Ecology and Sustainability of Pelagic Fisheriesin the Pacific Ocean. Climate Change, 119, 131-145. http://dx.doi.org/10.1007/s10584-012- 0598-y

Olson, R.J., L.M. Duffy, P.M. Kuhnert, F. Galván-Magaña, N. Bocanegra-Castillo, and V. Alatorre-Ramírez. 2014. Decadal diet shift in yellowfin tuna Thunnus albacares suggests broad-scale food web changes in the eastern tropical Pacific Ocean. *Marine Ecology Progress Series* 497: 157–178.

Olson, R.J., Young, J.W., Ménard, F., Potier, M., Goñi, N., Logan, J.M., and Galván-Magaña. (2016). Bioenergetics, trophic ecology, and niche separation of tunas. Advances in Marine Biology ISSN 0065-2881, http://dx.doi.org/10.1016/bs.amb.2016.06.002.

Rudershausen, PJ., Buckel, JA., Edwards, J. 2010. Feeding ecology of blue marlins, dolphin fish, yellowfin tuna, and wahoos from the north Atlantic Ocean and comparisons with other oceans. Trans. Amer. Fish. Soc. 139:1335-1359.

Staudinger, MD., Juanes, F., Salmon, B., Teffer, AK. 2013. The distribution, diversity, and importance of cephalopods in top predator diets from offshore habitats of the northwest Atlantic Ocean. Deep-Sea Research II 95:182-192.

Teffer, A.K., M.D. Staudinger, and F. Juanes. 2015. Trophic niche overlap among dolphinfish and co-occurring tunas near the northern edge of their range in the western North Atlantic. *Marine Biology* 162: 1823–1840.

Young, J.W., Olson, R.J.,Menard, F., Kuhnert, P.M., Duffy, L.M., Allain, V., Logan, J.M., Lorrain, A., Somes, C.J., Graham, B., Goñi, N., Pethybridge, H., Simier, M., Potier, M., Romanov, E., Pagendam, D., Hannides, C., Choy, C.A., 2015. Setting the stage for a global-scale trophic analysis of marine top predators: a multi-workshop review. Rev. Fish Biol. Fish. 25, 261–272.